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JAMES COOK UNIVERSITY AUSTRALIA

Thermal acclimation and light-harvesting complex expression in *Symbiodinium*

Thesis submitted by

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for the degree of Doctor of Philosophy Research Thesis (Biochemistry)

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Statement on the Contribution of Others

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	Technical support	Professor James Burnell [a, b] (Secondary advisor)
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Chapter No.	Details of publication	Nature and extent of the intellectual input of each author, including the candidate
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3	Gierz*, S. L., Gordon, B. R., Leggat, W. (2016) Integral light-harvesting complex expression in *Symbiodinium* within the coral *Acropora aspera* under thermal stress. *Scientific Reports*, 6, 25081. doi: 10.1038/srep25081.	Gierz, Gordon and Leggat designed thermal stress experiment. Gierz and Gordon performed experiment. Gierz performed cell density and chlorophyll pigment analysis. Gierz analyzed Imaging-PAM data. Gierz prepared samples, designed primers and performed quantitative PCR. Gierz and Leggat analyzed the data. Gierz developed figures and tables. Intellectual input into manuscript by Gierz and Leggat.

Thesis publications

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Abstract

Endosymbioses observed between photosynthetic dinoflagellates of the genus *Symbiodinium* and reef-building (Scleractinian) corals are crucial to the success of diverse reef ecosystems. Dysfunction of this symbiotic relationship can occur under a number of stressors (including elevated sea surface temperatures and ocean acidification), resulting in the expulsion of *Symbiodinium* from host cells or loss of photosynthetic pigments, a process known as coral bleaching. While ocean temperatures fluctuate on a daily basis, the mean ocean temperature is predicted to rise approximately 1 – 2 °C over the next century and is expected to lead to more mass coral bleaching events.

Within coral bleaching experiments, elucidation of sites of thermal sensitivity within *Symbiodinium* has focused on potential points where damage may originate. One of these potential sites are the integral light-harvesting protein complexes (LHCs), which bind chlorophylls and accessory pigment molecules with roles in light-harvesting by receiving and transferring light energy to photosystems, and photoprotection by dissipating excess energy under stress conditions. Little is known about the response of the diversified integral LHC gene family (acpPCs) in *Symbiodinium* to thermal stress, as only short term (24 h), light stress and dissociation experiments have been reported. Additionally, few studies have examined the broad transcriptional response of *Symbiodinium* to thermal stress conditions.

Therefore, the aims of this research were to examine the effect of extended thermal stress on *Symbiodinium* to determine variations in gene expression and morphology both *in vitro* and *in hospite* and to link this to observed physiological parameters. To achieve these aims thermal stress experiments were performed on cultured *Symbiodinium* sp. (clade F), and *in hospite* utilising *Acropora aspera* harbouring *Symbiodinium* clade C3. A targeted quantitative PCR approach was utilised to determine the expression of five integral LHC genes within *Symbiodinium in hospite* and a transcriptome approach was utilised to identify differentially expressed transcripts within *Symbiodinium* sp. (clade F) *in vitro.* Variations in *Symbiodinium*

morphology were characterized following exposure of *A. aspera* to thermal stress using confocal laser scanning microscopy.

Exposure of *Symbiodinium* sp. (clade F) cultures to a twenty-eight day thermal stress regime (~31 ℃) elicited a stress response measured as reduced cell growth from day four onwards ($p < 0.01$) and decreased dark-adapted yield on days fourteen ($p < 0.05$), nineteen ($p < 0.001$) and twenty-eight ($p < 0.001$). Whole transcriptome sequencing of *Symbiodinium* cells on days four, nineteen and twenty-eight identified 23,654 unique genes (FDR < 0.05), though 92.49% differentially expressed genes displayed ≤ 2-fold change in expression. The transcriptional response included differential expression of genes encoding photosynthetic machinery subunits, integral LHCs, fatty - acid desaturases, metabolic enzymes, and components of stress response pathways. The results indicate a shift in metabolism, from carbon fixation to fatty acid catabolism under thermal stress, supported by upregulation of β-oxidation, glyoxylate cycle and gluconeogenic enzymes and has not previously been quantified in *Symbiodinium*.

Exposure of *A. aspera* to a sixteen-day thermal stress regime elicited a bleaching response measured as reduced *Symbiodinium* density (day sixteen, $p < 0.001$) and significantly decreased dark-adapted yield (day sixteen, $p < 0.001$). The expression of five integral LHC genes in *Symbiodinium in hospite* were measured using quantitative PCR employing previously established reference genes. Of the five integral LHC genes quantified, three acpPC genes exhibited upregulated expression when corals were exposed to temperatures above 31.5 ℃ (acpPCSym_1:1, day sixteen (1.74-fold, $p < 0.001$); acpPCSym_15, day twelve (1.33-fold, $p < 0.05$); and acpPCSym_18, day ten (2.44-fold, $p < 0.05$) and day sixteen (2.08-fold, $p < 0.05$)). In contrast, acpPCSym_5:1 and acpPCSym_10:1 exhibited constitutive expression throughout the experiment. Interestingly, the three *acpPC* genes with increased expression cluster together in a phylogenetic analysis of light-harvesting complexes.

Variation in an assemblage of cellular and photophysiological variables in individual and populations of *Symbiodinium* sp. (clade C3) cells within *A. aspera* were characterized following exposure to a sixteen day thermal stress (approximately +0.7

℃ per d, maximum ~34 ℃). Coral branches were maintained across four aquaria, with two tanks per condition, and were sampled on days zero, eight, ten, twelve and sixteen. Specific physiological parameters such as *Symbiodinium* density, dark-adapted yield, effective quantum yield, chlorophyll pigment content, cellular morphology and chlorophyll *a* fluorescence intensity were measured to assess the cytological response to extended exposure at elevated temperatures below and above the bleaching threshold of *A*. *aspera*. A variety of responses among the *Symbiodinium* populations both within and between coral branches were identified in the parameters assayed. Further demonstrating that broad, multifaceted approaches are required when assessing coral bleaching cellular responses to ensure an accurate representation of holobiont health.

The results of this thesis provide insights into the molecular response of *Symbiodinium* exposed to thermal stress, below the bleaching thresholds. As in previous gene expression analyses, relatively small transcriptional changes were detected *in vitro* and *in hospite*, further supporting the hypothesis that other mechanisms of regulation (post-transcriptional or translational regulation) are critical in *Symbiodinium* stress responses. Quantification of multiple integral LHCs *in vitro* and *in hospite* identified genes with constitutive and inducible expression within the highly expanded family, providing potential insights into the functional purpose for LHC diversification in *Symbiodinium*. The implications for altered *Symbiodinium* gene expression and metabolism under thermal stress and the effect this may have on host - symbiont metabolite transfer is unknown, although, the results presented here provide preliminary data for studies investigating the molecular response of *Symbiodinium* under future temperature conditions.

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Abbreviations: ATM, serine/threonine protein kinase ATM; BRCA, breast cancer susceptibility homolog; CDCH2, cell division control protein; DLH1, meiotic recombination protein; DMC1, meiotic recombination protein; DNL, DNA ligase; EXO, exonuclease; FEN, flap endonuclease; GR1, protein gamma response 1; HOP2, homologous-pairing protein 2 homolog; MEI2, meiosis protein; MEI2-like, meiosis protein-like protein; MLH, DNA mismatch repair protein; MND, meiotic nuclear division protein; MSH, MutS protein homolog; MUS, crossover junction endonuclease; RA, DNA repair and recombination protein; RAD24, DNA damage checkpoint protein; RAD50, DNA repair protein; RD, DNA repair protein; RSPH, radial spoke head homolog; RTEL, regulator of telomere elongation helicase; XRCC, X-ray repair cross-complementing protein. Heatmap generated using the 'pheatmap' package. 76

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Abbreviations

ABH	Adaptive bleaching hypothesis
acpPC	Chlorophyll *a*-chlorophyll c_2-peridinin protein complexes
ATP	Adenosine triphosphate
BLAST	Basic Local Alignment Search Tool
CAB	Chlorophyll *a*/*b*-binding
CAC	Chlorophyll *a*/*c*-binding
CB	Chlorophyll-binding
cDNA	complementary deoxyribonucleic acid
Chl	Chlorophyll
Chl a	Chlorophyll *a*
Chl c	Chlorophyll *c*
CP	Chlorophyll-protein
Cytb_6f	Cytochrome b_6f complex
DEG	Differentially expressed gene
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTP	Deoxyribonucleotide triphosphate
ELIP	Early light-induced protein
EST	Expressed sequence tag
FCP	Fucoxanthin – chlorophyll a/c protein complex
Fd	Ferredoxin
F_m	Maximum chlorophyll fluorescence yield
FNR	Ferredoxin-$NADP^+$ reductase
F_o	Minimal chlorophyll fluorescence yield
F_v/F_m	Effective-quantum yield
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
Gb	Gigabase
GBR	Great Barrier Reef
HKG	Housekeeping gene
HLIP	High light-induced protein

HSP	Heat shock protein
Imaging-PAM	Imaging-Pulse-Amplitude Modulation
ITS2	Internal transcribed spacer region 2
kb	Kilobases
kDa	Kilodalton
LIL	Light harvesting-like
LHC	Light-harvesting complex
LHL	High intensity light-inducible LHC-like
LSU	Large subunit
mRNA	Messenger ribonucleic acid
miRNA	Micro-RNA
NADPH	Nicotinamide adenine dinucleotide phosphate
NCBI	National Centre for Biotechnology Information
NPQ	Non-photochemical quenching
OHP	One-helix protein
PAM	Pulse-amplitude modulation
PAR	Photosynthetically active radiation
PBS	Phosphate-buffered saline
PC	Plastocyanin
PCNA	Proliferating cell nuclear antigen
PCP	Peridinin – chlorophyll a protein complex
PCR	Polymerase chain reaction
PQ	Plastoquinone
PQH_2	Plastoquinol
PSBS	Subunit S of PS II
PS I	Photosystem I
PS II	Photosystem II
PSU	Photosynthetic unit
qPCR	Quantitative real-time polymerase chain reaction
RedCAP	Red lineage chlorophyll *a*/*b* binding-like protein
RC	Reaction centre

RNA	Ribonucleic acid
RNase	Ribonuclease
RNA-Seq	RNA-Sequencing
ROS	Reactive oxygen species
RuBisCo	Ribulose-1,5-bisphosphate carboxylase/oxygenase
SCP	Small chlorophyll-binding-like proteins
SEP	Stress-enhanced protein
smRNA	Small RNA
SST	Sea surface temperature
SSU	Small subunit

Chapter 1 Introduction

The success of coral reefs globally is largely dependent on the symbiosis between coral hosts and dinoflagellate endosymbionts belonging to the genus *Symbiodinium.* Primary production by *Symbiodinium* through photosynthesis provides up to 95 % of the coral's daily energy requirements (reduced organic carbon), which in turn receive nutrients from the host (Muscatine, 1990; Falkowski et al., 1993). Coral bleaching is a dysfunction of this symbiotic relationship and occurs under stress conditions (elevated sea surface temperature, ocean acidification, increased UV irradiance, eutrophication and disease) resulting in the expulsion of *Symbiodinium* from host cells or loss of the alga's photosynthetic pigments (Rosenberg and Ben-Haim, 2002; Hughes et al., 2003; Anthony et al., 2008; Leggat et al., 2011a). While ocean temperatures fluctuate on a daily basis, the mean ocean temperature is predicted to rise approximately 1 - 2 °C over the next century and is expected to lead to more mass coral bleaching events, though the effect of extended exposure (3 - 4 weeks) to elevated temperatures on coral reefs and their endosymbionts is largely unstudied (Hoegh-Guldberg, 1999; Parry et al., 2007). This thesis aims to characterize the cellular response and impact on photosynthetic integral light-harvesting complexes (LHCs) following exposure to thermal stress regimes in *Symbiodinium in vitro* and *in hospite*.

Dinoflagellates

Dinoflagellates are a large group of unicellular, flagellate protists with diverse ecological roles, with wide distributions in both marine and freshwater environments (Hackett et al., 2004a). More than 2,000 dinoflagellate extant species have been described (Taylor et al., 2007). Some dinoflagellate species are parasitic on marine organisms (invertebrates and vertebrates) (e.g., *Pfiesteria*) or are endosymbionts of marine invertebrates (e.g., *Symbiodinium* sp.) whereas, an estimated 1,555 species are described as free-living in marine environments (Taylor et al., 2007). Approximately half of the dinoflagellates are phototrophs, however, heterotrophic and mixotrophic species have been

identified (Hackett et al., 2004a; Taylor et al., 2007). Phylogenetic analysis of dinoflagellates (phylum Dinoflagellata) group them within the kingdom Alveolata, with sister phyla such as the Apicomplexa, Ciliates and Chromerida (Hackett et al., 2004a; Moore et al., 2008). Dinoflagellate life cycles vary, in some species asexual and sexual reproduction has been observed as well as motile and non-motile (cyst) stages (Wall and Dale, 1968; Taylor et al., 2007). Due to their varied distribution and associations, dinoflagellates have significant roles in primary production, are the causative agent for toxic algal blooms (red tides) and are critical to the survival of coral reefs (Hackett et al., 2004a).

Dinoflagellates have a number of characteristics and cellular traits that make them distinctive. The dinoflagellate nuclear genome or dinokaryon, is extremely large (3 - 200 pg DNA per cell) (Spector, 1984), utilizes nuclear proteins as opposed to histones (Rizzo, 1981) (though histone proteins have been detected in some dinoflagellate species (*Symbiodinium kawagutii*) (Lin et al., 2015)) and contains highly duplicated genes arranged in polycistronic or tandem arrays (Zhang et al., 2007; Bachvaroff and Place, 2008; Mendez et al., 2015). Throughout the cell cycle, chromosomes remain permanently condensed and the nuclear membranes remain intact (Hackett et al., 2004a). Regulation of gene expression in dinoflagellates is not fully resolved and typical eukaryotic gene regulatory mechanisms (e.g., TATA boxes and polyadenylation sites) are not always present (Hackett et al., 2004a). Some dinoflagellate genes have been shown to be under transcriptional regulation, for example peridinin-chlorophyll *a* binding protein expression in *Heterocapsa pygmaea* is regulated by growth irradiance (Triplett et al., 1993) and mitogen-activated protein kinase expression is linked with cell proliferation in *Pfiesteria piscicida* (Lin and Zhang, 2003). Select genes under either transcriptional or post-translational regulation mechanisms (or both), have been identified in dinoflagellates (Hackett et al., 2004a). Spliced leader RNA trans-splicing has also been identified in all major orders of dinoflagellates allowing the translation of polycistronically transcribed nuclear genes (Zhang et al., 2007). Sequencing of the dinoflagellate *Lingulodinium polyedrum* genome supports the arrangement of genes in tandem arrays though not of polycistronic transcription (Beauchemin et al.,

2012). Additionally, plastid-targeted nuclear-encoded polyproteins have been recorded in dinoflagellates (Hiller et al., 1995; Morse et al., 1995; Boldt et al., 2012) requiring post-translational cleavage to yield mature proteins.

Plastid genomes and characteristics vary among dinoflagellate lineages. Five divergent plastid types have been identified in dinoflagellates, though all dinoflagellates were derived from a peridinin-containing ancestor (Saldarriaga et al., 2001), with additional lineages resulting from tertiary or serial secondary endosymbiotic events (Delwiche, 1999; Yoon et al., 2002; Archibald and Keeling, 2004; Howe et al., 2008; Janouškovec et al., 2010). Peridinin-containing plastids are the most common in dinoflagellate species (e.g., *Amphidinium* sp., *Heterocapsa* sp., *Symbiodinium* sp.) and were likely derived from an endocytosed red alga (Saldarriaga et al., 2001; Yoon et al., 2002). They are generally surrounded by three membranes and contain the carotenoid pigment peridinin and chlorophylls *a* and c_2 (Saldarriaga et al., 2001). Fucoxanthin-containing plastids are surrounded by three membranes, contain chlorophylls $a/c_1 + c_2$ and the accessory pigment fucoxanthin (19'-hexanoyloxy-fucoxanthin and/or 19'-butanoyloxy-fucoxanthin) but lack peridinin (e.g., *Karenia brevis, Karenia mikimotoi* and *Karlodinium micrum*) (Yoon et al., 2002). The three other dinoflagellate types are the cryptophycean-like plastid (e.g., *Dinophysis* sp. (Schnepf and Elbrächter, 1988)), the fucoxanthin containing diatom-like plastid (e.g., *Peridinium foliaceum* (Chesnick et al., 1996)) and the prasinophyte-like plastid (e.g., *Lepidodinium viride* (Watanabe et al., 1990)). Within the peridinin-containing dinoflagellates the plastid genome is greatly fragmented and reduced to minicircles with the majority of genes having been transferred to the nucleus (Zhang et al., 1999; Hackett et al., 2004b; Koumandou et al., 2004) and a nuclear-encoded Form II ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) with similarity to an α-proteobacteria is found rather than the Form I RuBisCo (Morse et al., 1995; Whitney et al., 1995). Additionally, unique peptide import pathways are observed in dinoflagellates as the multi-membrane plastids require endoplasmic reticulum to Golgi to plastid transport (McFadden, 1999; Nassoury et al., 2003).

Symbiodinium

Dinoflagellates within the genus *Symbiodinium* (zooxanthellae) are one of the most ecologically important due to the symbiotic relationships they form. *Symbiodinium* spp. are photosynthetic, may be found free living in the water column and in sediments or in symbiotic associations with a variety of marine phyla including Cnidaria (e.g., corals and jellyfish), Mollusca, Porifera and Foraminifera (Baker, 2003; Coffroth and Santos, 2005; Stat et al., 2008; Pochon and Gates, 2010). Preliminary morphological, physiological and life cycle studies of isolated symbionts resulted in the initial classification as one single species *Symbiodinium microadriaticum* Freudenthal (Freudenthal, 1962). However, advances in molecular techniques have revealed that *Symbiodinium* are a taxonomically diverse species complex with great genetic diversity with hundreds of distinct types identified (Trench and Blank, 1987; Rowan and Powers, 1992; LaJeunesse, 2001; Pochon and Gates, 2010). Division of the diverse *Symbiodinium* genus has been accomplished via analysis of sequence variation in taxonomic markers (e.g., noncoding DNA fragments ITS1 and ITS2, and coding DNA fragments 5.8S, SSU, LSU and cp 23S rDNA) (LaJeunesse, 2001; Pochon and Gates, 2010). Currently nine divergent lineages (clades A – I) have been identified, with additional division into types (LaJeunesse, 2001; Pochon and Gates, 2010). Establishment of *in vitro* cultures of *Symbiodinium* has greatly advanced our understanding of their biology (McLaughlin and Zahl, 1959), though limitations exist as not all types are cultivable (Krueger and Gates, 2012) and functional differences may occur *in hospite* (e.g., growth and respiration rates) (Davy et al., 2012).

Observations of morphology in *Symbiodinium* have identified differences among types and in comparison with other dinoflagellates. Descriptions of *Symbiodinium in vitro* have identified two main alternating life stages, a motile flagellated cell known as the mastigote stage and a non-motile cell known as the coccoid stage (Freudenthal, 1962). *Symbiodinium in hospite* are coccoid and non-motile, becoming motile *ex hospite* potentially representing a dispersal or infection stage (Trench, 1979; Fitt et al., 1981). Variations in morphology

among *Symbiodinium* types include cell size (coccoid cells between 6 – 16 µm), the number of nuclear chromosomes and the number and size of peridinin-containing plastids (Blank and Huss, 1989; LaJeunesse, 2001; Jeong et al., 2014). The *Symbiodinium* mastigote stage is motile during the light photoperiod, has characteristic morphology of gymnodinioid dinoflagellates with transverse and longitudinal flagella, though the episome is athecate (Freudenthal, 1962; Fitt et al., 1981; Fitt and Trench, 1983). The coccoid cell stage is metabolically active and is not a dormant vegetative cyst as observed in other dinoflagellates (Fitt and Trench, 1983). Vegetative growth via asexual propagation has been observed in coccoid *Symbiodinium*, as yet sexual reproduction has not been documented, though genetic measures support the occurrence (Freudenthal, 1962; Blank, 1987; LaJeunesse, 2001; Chi et al., 2014; Lin et al., 2015; Wilkinson et al., 2015). Most dinoflagellates undergo mitosis at the mastigote stage, though in *Symbiodinium* this occurs at the coccoid stage under a diel cycle (Freudenthal, 1962; Fitt and Trench, 1983). Importantly, observations recorded *in vitro* potentially skew our understanding of *Symbiodinium* life cycles *in hospite* and in the environment, as these have different phenotypes and environmental conditions (e.g., nutrients).

Differences in physiological traits are observed among types across the diversified *Symbiodinium* genus (Chang et al., 1983; Trench and Blank, 1987; Iglesias-Prieto and Trench, 1994; Hennige et al., 2009). For example, *in vitro* experiments have shown pigment composition and both growth and photosynthesis rates differ among *Symbiodinium* types and at varied irradiances (Chang et al., 1983; Iglesias-Prieto and Trench, 1994; Hennige et al., 2009). Photophysiological differences determined by pulse-amplitude modulated fluorometry (i.e., maximum quantum yield and effective quantum yield of photosystem II), under control and thermal stress conditions among *Symbiodinium* types *in hospite* (Rowan, 2004; Sampayo et al., 2008) and *in vitro* (Tchernov et al., 2004; Takahashi et al., 2008; Hennige et al., 2009) have also been demonstrated. Differential stress tolerance is observed across the diverse *Symbiodinium* species complex, with both heat tolerant and heat sensitive types observed between and within clades (Rowan, 2004; Tchernov et

al., 2004). Differences in photoinhibition sensitivity have either been acquired independently by thermally tolerant types or have been acquired in the common ancestor of all *Symbiodinium* types and since lost in thermally sensitive species (Tchernov et al., 2004). Collectively, the functional and physiological diversity observed within the genus allows different types to occupy environmental niches and various photic zones contributing to the proliferation of coral reefs.

Coral reefs: Symbiosis, coral bleaching and acclimation

Symbioses between *Symbiodinium* and corals underpin the growth and productivity of coral reefs globally (Barnes, 1987). A coral reef's ability to flourish and dominate nutrient-poor environments is tightly linked with their associated endosymbiotic algae (Muscatine and Porter, 1977). Symbiotic relationships between *Symbiodinium* and host organisms are generally classed as facultative mutualistic endosymbiosis, as both partners benefit from the relationship, though some associations may be parasitic (Muscatine and Porter, 1977; Coffroth and Santos, 2005; Stat et al., 2008). Adding to the complexity of the symbiotic associations observed between coral colonies and *Symbiodinium* are the multitude of associated bacteria, fungi, Archaea and viruses that have also been identified, this is referred to as the "coral holobiont" (Rohwer et al., 2002). Numerous biological and environmental factors contribute to the health and productivity of each partner, these may also influence the relationships collapse and this will be explored in the proceeding sections.

Scleractinian corals are assemblages of multiple polyps that are connected and cover a calcium carbonate skeleton (Barnes, 1987). Within corals, *Symbiodinium* cells are located within host-derived vacuoles (called symbiosomes) within the endodermal cell layer, surrounding the gastrovascular cavity (Muscatine, 1967; Yellowlees et al., 2008; Davy et al., 2012). Population densities within symbiotic coral tissues range between 0.5×10^6 to 5×10^6 *Symbiodinium* cells cm^{-2} (Hoegh-Guldberg, 1999). *Symbiodinium* acquires nutrients such as inorganic carbon, nitrogen, phosphorus and other nutrients from host cells (Yellowlees et al., 2008; Gordon and Leggat, 2010; Davy et al.,

2012) and acquire light either incidentally from downwelling sunlight or as diffuse reflected light from coral skeleton scattering (Enríquez et al., 2005; Roth, 2014). The photosynthetic symbionts then fix or assimilate these and the metabolic products such as glucose, glycerol, lipids and amino acids are transported back to the host (Muscatine, 1967; Davies, 1984; Yellowlees et al., 2008; Gordon and Leggat, 2010). Within corals, between 5 % and 60 % of photosynthetically derived products from *Symbiodinium*, are translocated to the host and account for up to approximately 90 – 95 % of the hosts' daily energy requirements (Muscatine, 1990; Falkowski et al., 1993; Davy et al., 2012). Corals may then use the fixed carbon provided by *Symbiodinium* for growth and to enhance skeletal calcification (Goreau, 1959; Goreau and Goreau, 1959; Davies, 1984). Coral - *Symbiodinium* partnerships can be highly sensitive, some associations are resilient, while others are more susceptible and exposure to fluctuating environmental stresses may result in coral bleaching (Porter et al., 1989; Hoegh-Guldberg, 1999; Sampayo et al., 2008; Hume et al., 2015).

Corals in tropical and subtropical locations live within a temperature range that is close to their upper thermal threshold (Coles et al., 1976; Hughes et al., 2003). Increases in temperature above the summer maxima, as little as 1–2 °C over several weeks, or 3 °C to 4 °C over 1 - 2 days can induce coral bleaching (Coles et al., 1976; Jokiel and Coles, 1990). Coral bleaching, the paling or whitening of a coral colony, occurs either from the expulsion of *Symbiodinium* cells from the host and/or their photosynthetic pigments is a common response to environmental stress (Coles and Jokiel, 1978; Kleppel et al., 1989). Studies of the coral holobiont have focused on many environmental stressors implicated in the onset of coral bleaching including elevated sea-surface temperatures (SSTs) (Hughes et al., 2003), ocean acidification (Anthony et al., 2008) and disease (Rosenberg and Ben-Haim, 2002). The effect of high SSTs have been a key focus due to mass coral bleaching events (along the Great Barrier Reef in 1998 and 2002, approximately 42 % and 54 % of the reefs were bleached respectively (Berkelmans et al., 2004)), attributed to global climate change (Hoegh-Guldberg, 1999) with the 1998 bleaching event coinciding with an El

Niño Southern Oscillation event (Bruno et al., 2001). Recently in 2016, mass coral bleaching of the Great Barrier Reef has been recorded with 93 % of the 911 individual reefs surveyed displaying bleaching at varying severities also coinciding with elevated SSTs (Hughes et al., 2017). By 2050, a major decline in corals is expected with annual bleaching events and mass mortality predicted, due to rising SSTs and increased anthropogenic effects from pollution and farming (Parry et al., 2007). The coral stress response, and therefore the outcome of a bleaching event may be dictated by a number of factors, such as stress type (temperature, irradiance, sediment, nutrient, disease and other biotic factors), stress characteristic (intensity and duration), the physiology of the coral holobiont and its thermal history (Stambler, 2010; Ainsworth et al., 2016). The outcome of the coral bleaching event may vary, with the host being recolonized by *Symbiodinium*, disease outbreak or widespread coral mortality and reef degradation (Kleppel et al., 1989; Hoegh-Guldberg, 1999; Stambler, 2010).

Understanding the cellular mechanisms that underlie the process of coral bleaching and the influence of each of the holobiont partner's physiology has been the aim of many studies (Gates et al., 1992; Brown et al., 1995; Downs et al., 2002; Lesser, 2006; Ainsworth and Hoegh-Guldberg, 2008; Weis, 2008; Lesser, 2011; Downs et al., 2013). Collectively, the 'Oxidative theory of coral bleaching' is a popular hypothesis to describe the cellular mechanisms under temperature and light stress (Downs et al., 2002; Lesser, 2006; Lesser, 2011). Oxidative stress where lipids, proteins and DNA are damaged due to increased production and accumulation of reactive oxygen species (ROS) occurs in many organisms but is exacerbated in the coral symbioses by stresses such as elevated SSTs and increased irradiance (Downs et al., 2002; Lesser, 2006). ROS such as superoxide radicals, singlet oxygen, hydrogen peroxide and hydroxyl radicals are produced under normal cellular processes (e.g., mitochondrial and chloroplastic electron-transport, endoplasmic reticulum oxygenase reactions) are used as signal transduction molecules and antioxidant defense networks (e.g., enzymatic antioxidants (superoxide dismutase, catalase and peroxidases) and nonenzymatic antioxidants (ascorbic

acid and glutathione) exist to scavenge the free radicals and prevent damage (Lesser, 2006). Under stress conditions though, the production of ROS, exceeds the cells capacity to quench and mediate the removal and therefore oxidative stress and cellular damage may occur (Lesser, 2006). Within corals, the endosymbionts generate hyperoxic conditions whilst performing photosynthesis (Lesser, 2006). Under stress conditions, production of ROS in *Symbiodinium* exceeds the algal symbiont and hosts' antioxidant capacities (Downs et al., 2002; Lesser, 2006), with different stresses inducing different cellular pathologies (Downs et al., 2013). Expulsion of *Symbiodinium* from the host therefore removes the major source of ROS production and potentially ameliorates oxidative stress (Weis, 2008; Lesser, 2011). Mechanisms for the expulsion of *Symbiodinium* from host cells vary but may include, *in situ* degradation, exocytosis of symbionts and host cell responses such as detachment, apoptosis or necrosis (Gates et al., 1992; Ainsworth and Hoegh-Guldberg, 2008; Weis, 2008).

Elucidation of sites of thermal sensitivity within *Symbiodinium* have focused on potential points where damage potentially from oxidative stress results in photoinhibition (Iglesias-Prieto et al., 1992; Warner et al., 1996). These potential points include damage to the D1 protein of photosystem II (Warner et al., 1996), inhibition of the *de novo* synthesis of the D1 protein (Warner et al., 1999), the enzyme RuBisCo (Jones et al., 1998), thylakoid membrane integrity (Tchernov et al., 2004) and light-harvesting complexes (Takahashi et al., 2008). The light-harvesting complexes (also known as antenna proteins) have been shown to decrease energy transfer and dissociate from photosystem reaction centers following photoinhibition in order to protect cells during a stress event (Warner et al., 1996; Iglesias-Prieto and Trench, 1997; Hill and Ralph, 2006; Allakhverdiev et al., 2008). Decreasing the number of peripheral LHCs available to absorb and transfer energy is a proposed photoprotection mechanism, as this reduces the amount of light reaching the reaction centers and limits the risk of possible photodamage to the D1 reaction centre proteins (Hill and Ralph, 2006). Though multiple potential sites have been investigated, none of these

sites have conclusively been demonstrated as the initial site of thermal damage in *Symbiodinium*.

The complexity in understanding *Symbiodinium* associations is compounded by the extremely diverse species complex, the wide variety and flexibility of symbioses and the variability of stress responses across symbioses (Baker, 2003; Pochon et al., 2014). The abundance of a particular *Symbiodinium* type may be dictated by the host, its geographical location, the season and water depth, potentially influenced by both temperature and light environments (Rodriguez-Lanetty et al., 2001; Baker, 2003; LaJeunesse et al., 2003; Thornhill et al., 2006; Baker et al., 2015). Coral-*Symbiodinium* associations are established either via vertical transmission (maternal transfer of symbionts to oocytes) or via horizontal transmission (aposymbiotic oocytes/larvae acquire symbionts from the environment), though this does not determine the diversity of the *Symbiodinium* community (van Oppen, 2004). Within corals both host specific and symbiont specific associations are reported, ranging from strict specificity for either partner, to flexible, low specificity associations (LaJeunesse, 2001; Baker, 2003). Generalist associations are also reported between single host species and multiple *Symbiodinium* types (Baker, 2003; Coffroth and Santos, 2005; Pochon and Gates, 2010; Pochon et al., 2014). These generalist or facultative associations with multiple *Symbiodinium* types may occur in different proportions (e.g., a dominant type with multiple types in the background at low abundance levels) (Baker, 2003; LaJeunesse et al., 2003). Host physiological advantages may be gained from different *Symbiodinium* associations, as described earlier different types are functionally variable, having diverse physiological optima, stress tolerance levels and metabolic output (LaJeunesse et al., 2003; Tchernov et al., 2004; Thornhill et al., 2006; Loram et al., 2007; Stat et al., 2008; Cantin et al., 2009; Baker et al., 2015). Symbiont ‘shuffling’ and ‘switching’ describes how *Symbiodinium* communities *in hospite* may change as part of the ‘Adaptive bleaching hypothesis’ (ABH) (Buddemeier and Fautin, 1993; Kinzie III et al., 2001; Thornhill et al., 2006; Boulotte et al., 2016), though not all coral species display altered symbiont populations in response to environmental stresses (Sampayo

et al., 2008; Stat et al., 2009). The resilience of the holobiont may determine the survival of coral reefs under future climate conditions (Buddemeier and Fautin, 1993; Buddemeier and Smith, 1999; Gates and Edmunds, 1999; Palumbi et al., 2014).

Importantly over the next 50 years, if the thermal thresholds of coral holobiont's are exceeded, the bleaching, acclimation and adaptation responses will be critical in shaping the composition and appearance of coral reefs globally (Hoegh-Guldberg, 1999; Hughes et al., 2003; Hennige et al., 2010; Palumbi et al., 2014). The ABH describes mechanisms for improving survival and thermal tolerance by changing symbiont communities (Buddemeier and Fautin, 1993). Converse to the ABH, functional variation among corals (i.e., thermal tolerance) may also be gained via physiological variation of each partner of the holobiont through acclimation and adaptation, and has been demonstrated in two conspecific populations of *Symbiodinium* sp. C1 (Howells et al., 2012; Levin et al., 2016) and among six genotypes of *Acropora palmata* hosting the same isoclonal *Symbiodinium* 'fitti' strain (*sensu Symbiodinium* type A3) (Parkinson et al., 2015). Terms such as acclimation, photoacclimation, acclimatization, adaptation, photoadaptation and light-shade adaptation have been used interchangeably and ambiguously to describe responses observed (Falkowski and LaRoche, 1991). Here, acclimation refers to physiological processes that are altered to adjust to a changing environment, allowing survival of organisms across a range of conditions (Falkowski and LaRoche, 1991). Whereas, adaptation refers to long-term evolutionary processes where heritable traits are acquired via natural selection over generations. Within *Symbiodinium,* photoacclimation refers to the ability to alter photosynthetic phenotypes and processes in response to environmental conditions (Iglesias-Prieto and Trench, 1994; Hennige et al., 2009; Lichtenberg et al., 2016). Different *Symbiodinium* types exhibit diverse photo-acclimatory responses (e.g., increased size and number of photosynthetic units and increased cell size) under varied conditions, such as changes in the light environment (Chang et al., 1983; Iglesias-Prieto and Trench, 1994; Hennige et al., 2009). Few studies investigating mechanisms

of thermal acclimation and adaptation within *Symbiodinium* exist (Howells et al., 2012; Takahashi et al., 2013; Levin et al., 2016).

Symbiodinium genome, transcriptome and gene expression analyses

Development of molecular techniques and sequencing technologies has seen many advances in *Symbiodinium* genomics and transcriptomics. In 2007, the first expressed sequence tag (EST) dataset for *Symbiodinium* (clade C3; 5184 ESTs) was established, following host exposure to a variety of stresses (Leggat et al., 2007). In 2009, two further EST libraries were established using sanger sequencing, generated from a cultured *Symbiodinium* strain (CassKB8, clade A1; 2653 ESTs) (Voolstra et al., 2009b) and symbionts isolated from *Aiptasia pallida* (clade A3; 127 ESTs) (Sunagawa et al., 2009). These datasets provided initial data to understand *Symbiodinium* genes, allowed for the identification of orthologous genes between clade A1 and C3 and the identification of unique genes not found in other dinoflagellate EST analyses, though only a small number of genes were identified and annotated compared with the total estimated gene content (Leggat et al., 2007; Voolstra et al., 2009a; Leggat et al., 2011b). Early in 2012, two EST libraries were generated using 454 sequencing providing the first comprehensive *Symbiodinium* transcriptomes (CassKB8, clade A1; 57,676 ESTs and Mf10.5b, clade B1; 56,198 ESTs) (Bayer et al., 2012). Since 2012, improvements in high-throughput sequencing technologies and reductions in cost have allowed for the publication of three *Symbiodinium* nuclear genomes, *Symbiodinium minutum*, clade B1 (Shoguchi et al., 2013), the *Symbiodinium kawagutii* nuclear genome (clade F1) (Lin et al., 2015) and the *Symbiodinium microadriaticum* (clade A1) (Aranda et al., 2016) and a further thirteen sequencing projects (organelle genome and transcriptomes) representing clades A-D and F (summarized in Table 1.2) (summarised in Rosic et al., 2014b; Shinzato et al., 2014b; Xiang et al., 2015; Levin et al., 2016). *Symbiodinium* datasets have also been generated as an artifact of investigating host transcriptomes and the coral holobiont (Rosic et al., 2014a; Shinzato et al., 2014a; Mansour et al., 2016). Next-generation sequencing technology has also been employed recently as a tool for

genotyping *Symbiodinium* populations using species-specific markers (ITS2), due to the sensitivity of the method relative populations can be identified and quantified (Arif et al., 2014; Quigley et al., 2014; Boulotte et al., 2016).

Development of these genomic and transcriptomic data sets has greatly improved our understanding of the gene repertoire and transcriptional regulation in *Symbiodinium* (Bayer et al., 2012). *Symbiodinium* nuclear genomes contain large numbers of protein coding genes (*S. minutum* 41,925 genes; *S. kawagutii* 36,850 genes; *S. microadriaticum* 49,109 genes) and analysis of nuclear, mitochondrial and plastid genomes has provided an insight into gene duplication, gene content and editing mechanisms within *Symbiodinium* (Table 1.2) (Shoguchi et al., 2013; Barbrook et al., 2014; Mungpakdee et al., 2014; Lin et al., 2015; Shoguchi et al., 2015; Aranda et al., 2016). Differences are observed in the arrangement of genes between the available *Symbiodinium* genomes with expanded gene families (including regulator of chromosome condensation (RCC1), calcium channel and calmodulin families) identified in *S. kawagutii* and *S. minutum,* further supporting theories of gene duplication and identifying the arrangement of genes in tandem and clusters of unidirectional alignments (Shoguchi et al., 2013; Lin et al., 2015). Though differences are observed in the arrangement of genes between the two available *Symbiodinium* genomes (Lin et al., 2015). Basal reference transcriptomes have been established utilizing both cultured and symbiotic *Symbiodinium* (clades A-D and F) (Table 1.2), allowing for the estimation of sequence divergence and identification of orthologous genes among clades (Voolstra et al., 2009b; Ladner et al., 2012; Rosic et al., 2014b) and identification of regulatory small RNAs (smRNAs) (Baumgarten et al., 2013).

Given the number of *Symbiodinium* types that have been identified and the associations they form with a wide variety of hosts these “omic” datasets are providing a platform to improve our understanding of *Symbiodinium* and their symbioses. Stress and non-stress conditions have been employed to facilitate gene expression under various circumstances (Leggat et al., 2007; Baumgarten

et al., 2013) and to identify differentially expressed genes under stress conditions (Barshis et al., 2014; Rosic et al., 2014a; Xiang et al., 2015; Levin et al., 2016). The transcriptome of *Symbiodinium* sp. SSB01 was analyzed following exposure to different light levels (0 - 500 µmol photons m^{-2} s^{-1}) and nutrient supplemented media (IMK/ IMK with casein hydrolysate) for 24 h, revealing differential expression of multiple genes including cryptochrome 2 and RCC1 genes under different light levels (Xiang et al., 2015). Comparison of two type C1 *Symbiodinium* resulted in the identification of a set of DEGs following exposure to 32 °C (+5 °C) for nine and thirteen days that support differing thermo-tolerances observed between the two experimental types (Levin et al., 2016). Currently, there are six transcriptome studies exploiting stress conditions and examining the response of a wide variety of genes (Table 1.1), therefore, there still are many avenues to be explored in *Symbiodinium* genomics.

Table 1.1 Summary of *Symbiodinium* sequencing projects.

Species (strain)	Clade/ Type	Host/ Culture	Dataset/ Conditions	No. contigs/ Gene content	Sequencing technology	Citation
Genomes						
Symbiodinium minutum (Mf1.05b.01)	B1	Culture	Nuclear genome	41,925	454, Illumina	Shoguchi et al. (2013)
Symbiodinium sp.	C3	*Agaricia sp*.	Plastid genome	13 minicircles	Sanger	Barbrook et al. (2014)
Symbiodinium minutum (Mf1.05b.01)	B1	Culture	Plastid genome	109 (14 minicircles)	Sanger, Illumina	Mungpakdee et al. (2014)
Symbiodinium minutum (Mf1.05b.01)	B1	Culture	Mitochondrial genome		454, Illumina	Shoguchi et al. (2015)
Symbiodinium kawagutii	F1	Culture	Nuclear genome	36,850 70,985 ESTs	Sanger, 454	Lin et al. (2015)
Symbiodinium microadriaticum (CCMP2467)	A1	Culture	Nuclear genome	49,109	Illumina	Aranda et al. (2016)
Transcriptomes						
Symbiodinium sp.	C3	*Acropora aspera*	Varied stress conditions EST library	5184	Sanger	Leggat et al. (2007)
Symbiodinium sp. (CassKB8)	A1	Culture	EST library	2653	Sanger	Voolstra et al. (2009b)

Species (strain)	Clade/ Type	Host/ Culture	Dataset/ Conditions	No. contigs/ Gene content	Sequencing technology	Citation
Symbiodinium sp.	A3	*Aiptasia pallida*	EST library	127	Sanger	Sunagawa et al. (2009)
Symbiodinium sp. (CassKB8)	A1	Culture	Reference transcriptomes	57,676	454	Bayer et al. (2012)
Symbiodinium sp. (Mf1.05b)	B1	Culture		56,198		
Symbiodinium spp.	C	*Acropora hyacinthus*	Reference transcriptome	26,986	Illumina	Ladner et al. (2012)
Symbiodinium spp.	D			23,777		
Symbiodinium microadriaticum (CCMP2467)	A1	Culture	Varied stressors (< 24 h) smRNAs (miRNAs and siRNAs)	58,694	Illumina	Baumgarten et al. (2013)
Symbiodinium kawagutii	F1	Culture	5'-cap selective full length cDNA library	7,536	Sanger	Zhang et al. (2013)
Symbiodinium sp.	C3K	*A. hyacinthus*	DEG Thermal stress (+2.7 °C (mean 31.9 °C), 3 d) Reciprocal transplant	DEG identified using Ladner et al. (2012) as reference	Illumina	Barshis et al. (2014)
Symbiodinium sp.	D2					
Symbiodinium sp.	C3	*A. aspera*	DEG Thermal stress (+6 °C (30 °C), 1 & 3 d) Nutrient stress (ammonium + 20 µM)	DEG identified using DiffKAP method	Illumina	Rosic et al. (2014a)
Symbiodinium sp.	A2	Culture	Reference transcriptomes	29,846	Illumina	Rosic et al. (2014b)
Symbiodinium sp.	B2			42,233		
Symbiodinium sp.	C1			46,892		
Symbiodinium sp.	D1			42,885		

Species (strain)	Clade/ Type	Host/ Culture	Dataset/ Conditions	No. contigs/ Gene content	Sequencing technology	Citation
Symbiodinium sp.	C15	*Porites australiensis*	Reference transcriptome	26,627	Illumina	Shinzato et al. (2014a)
Symbiodinium spp. (SSB01)	B	Culture	Light stress (0 - 500 µmol photons m^{-2} s^{-1}) +/ - Nutrient supplement	59,669	Illumina	Xiang et al. (2015)
Symbiodinium aenigmaticum (mac04-487)	B19	Culture	DEG Species specific comparison of orthologous genes	45,343	Illumina	Parkinson et al. (2016)
Symbiodinium minutum (mac703, Mf1.05b, rt002 and rt351)	B1			51,199		
Symbiodinium pseudominutum (rt146)	B1			47,411		
Symbiodinium psygmophilum (HIAp, Mf10.14b.02, PurPFlex, rt141)	B19			50,745		
Symbiodinium sp.	C1	Culture	Thermal stress (+5 °C (32 °C), 9 & 13 d)	106,097	Illumina	Levin et al. (2016)
Symbiodinium sp.	C1			93,377		
Symbiodinium sp.		*Porites astreoides*	Host developmental stages	145,570	Illumina	(Mansour et al., 2016)

Table adapted from Shinzato et al. (2014b) and updated with current datasets available.

Targeted gene expression studies have also been employed to determine transcriptional changes in *Symbiodinium*. For example, quantitative-PCR (qPCR) has been used to determine changes in a variety of genes of interest related to stress responses (heat-shock proteins (Hsps) and antioxidant genes), metabolism genes and photosynthesis genes (Table 1.2) (Takahashi et al., 2008; Boldt et al., 2009; Leggat et al., 2011a; Rosic et al., 2011a; McGinley et al., 2012; McGinley et al., 2013; Ogawa et al., 2013; Krueger et al., 2015). The validation and establishment of suitable references for use in *Symbiodinium* has enabled the determination of differential gene expression under various conditions (Boldt et al., 2009; Rosic et al., 2011b). Although within these experiments significant changes have been observed in *Symbiodinium* physiology, and large fold changes in host gene expression have been recorded, differential gene expression in *Symbiodinium* occurs at a far smaller scale (± < 5-fold) (Leggat et al., 2011a; Rosic et al., 2011a; Ogawa et al., 2013). Further, studies linking the direct relationship between gene expression and protein expression in *Symbiodinium* under stress are limited (Takahashi et al., 2008; Putnam et al., 2013; Gierz, Boldt and Leggat, under review).

Table 1.2 Summary of *Symbiodinium* quantitative-PCR projects.

Symbiodinium strain	Clade/ Type	Conditions	Reference genes	Genes of Interest	Citation
Symbiodinium CS-73	A	Culture Thermal stress Range 25 – 34 °C, 24 h	ub52	acpPC	Takahashi et al. (2008)
Symbiodinium OTcH-1	A				
Symbiodinium sp.	C3	*A. aspera* Light levels 27.3 – 1540 µmol quanta m^{-2} s^{-1}, 1 – 9 d	β-actin Proliferating cell nuclear antigen 18S rRNA	acpPC Sym_1 acpPC Sym_9 acpPC Sym_10	Boldt et al. (2009)
Symbiodinium sp.	C3	*Acropora formosa*	-	PGPase	Crawley et al. (2010)
Symbiodinium sp.	C1	Culture Thermal stress 29 °C (+ 4 °C) 32 °C (+ 7 °C) Light levels 10 – 100 µmol quanta m^{-2} s^{-1}, 24 h	Actin Beta-Tubulin Calmodulin Cyclophin Cytochrome oxidase subunit 1 GAPDH poly(A) polymerase protein Ribosomal protein S4 S-adenosyl-L-methionine synthetase	Hsp90	Rosic et al. (2011b)
Symbiodinium sp.	C3	*Acropora millepora* Thermal stress 26 °C (+3 °C) 32 °C (+9 °C), 18 h			
Symbiodinium sp.	C3	*A. aspera* Thermal stress 21 – 34 °C, 8 d (+1 °C/ d, 6 d)	β-actin Proliferating cell nuclear antigen	Hsp70 Hsp90 GAPDH Glutamine synthetase	Leggat et al. (2011a)

Symbiodinium strain	Clade/ Type	Conditions	Reference genes	Genes of Interest	Citation
				Malonyl-CoA transferase α-ketoglutarate dehydrogenase	
Symbiodinium sp.	C3	*Acropora millepora* 32 °C (+0.1 °C/h), 18 h 32 °C (+0.4 °C/h), 5 d	Beta-Tubulin Ribosomal protein S4 S-adenosyl-L-methionine synthetase	Hsp40 Hsp70 Hsp90	Rosic et al. (2011a)
Symbiodinium sp.	A13	Culture Thermal stress 32 °C, 7 d (+1 °C/ d 28 to 32 °C)	β-actin PCNA S-adenosyl-L-methionine synthetase	psbA psaA	McGinley et al. (2012)
Symbiodinium sp.	A20				
Symbiodinium sp.	B1				
Symbiodinium sp.	F2				
Symbiodinium sp.	C1b-c	*Pocillopora verrucosa* Thermal stress 32 °C, 7 d (+1.5 °C/ d for 4 d)			
Symbiodinium sp.	D1				
Symbiodinium sp.		*Pocillopora damicornis* 25 or 29 °C, 9 d Elevated pCO_2 (415 or 635 µatm)	Solaris spike method	Hsp70 Ascorbate peroxidase RuBisCo (rbcL) Photosystem subunit III (PS I/III) PGPase	Putnam et al. (2013)
Symbiodinium sp.	A2	Culture 25 °C	Beta-Tubulin Ribosomal protein S4 S-adenosyl-L-methionine synthetase	Hemoglobin-1 Hemoglobin-2	Rosic et al. (2013)
Symbiodinium sp.	B2				
Symbiodinium sp.	C1				
Symbiodinium sp.	C3	*A. aspera* Thermal stress 30 °C, 1 – 3 d			

Symbiodinium strain	Clade/ Type	Conditions	Reference genes	Genes of Interest	Citation
		(+6 °C) Nutrient stress (ammonium + 20 µM)			
Symbiodinium sp.	A20	Culture Light stress 45 and 380 µmol quanta m^{-2} s^{-1}, 2 d Diel expression, 1 d	β-actin PCNA S-adenosyl-L-methionine synthetase	psbA psaA	McGinley et al. (2013)
Symbiodinium sp.	A13				
Symbiodinium sp.	D45	Culture			
Symbiodinium sp.	C3	*A. aspera* Thermal stress 14 d experiment + 4 – 6 °C, 4 d Elevated pCO_2 + 50 – 100 ppm	PCNA GAPDH Cyclophin	β-Carbonic Anhydrase PGPase RuBisCo Hsp70 Hsp90	Ogawa et al. (2013)
Symbiodinium sp.	B1	Culture Thermal stress 33 °C, 3 d (+8 °C)	Calmodulin Cytochrome oxidase subunit 1 S-adenosyl-L-methionine synthetase Beta-Tubulin	Hybrid ascorbate-cytochrome c peroxidase Catalase peroxidase Manganese superoxide dismutase	Krueger et al. (2015)
Symbiodinium sp.	C1	Culture Thermal stress 25 – 34 °C, 7d	PCNA β-Actin	acpPC Sym_1 acpPC Sym_10 acpPC Sym_17	Gierz et al. (2017) unpublished

Photosynthesis

Photoautotrophic organisms such as plants and algae are important primary producers, utilizing photosynthesis to satisfy metabolic requirements. Oxygenic photosynthesis occurs in organelles known as chloroplasts (plastids), in two stages, the light-dependent reactions and the light-independent reactions, converting light energy from the sun into chemical energy. Chloroplasts vary among lineages of photosynthetic organisms (green lineage (Chlorophytes (green algae) and Streptophytes (land plants), red lineage (Rhodophyta) and Glaucophyte lineage)), though are believed to be of cyanobacterial origin (Delwiche, 1999). An acquisition event or “primary endosymbiosis” between a nonphotosynthetic eukaryote and cyanobacterium resulted in the Chlorophyte, Rhodophyte and Glaucophyte lineages (Delwiche, 1999). Subsequent secondary and tertiary endosymbiotic events have attributed to the diversity observed in plastids among photosynthetic organisms (Cavalier-Smith, 1999; Delwiche, 1999). The Chromalveolate hypothesis, groups the Cryptophyta, Haptophyta, Stramenopiles (Heterokontophyta) and Alveolata, suggesting that all groups were derived from an ancestor that had undergone a secondary endosymbioses with a red algae (Cavalier-Smith, 1999). The hypothesis has been challenged following phylogenetic analysis, that support two monophyletic megagroups encompassing the diversity within the ‘chromalveolates’ as opposed to a singular monophyletic grouping (Burki et al., 2008; Kim and Graham, 2008; Burki et al., 2016).

Chloroplasts vary across the lineages of photosynthetic organisms in ultrastructure, pigment composition and gene content (Larkum and Vesk, 2003). Chloroplast structure generally includes a chloroplast membrane and thylakoids. The chloroplast membrane includes the outer membrane, an intermembrane space and an inner membrane, within the membranes are chloroplast translocon complexes (TIC, translocon on the inner chloroplast membrane and TOC, translocon on the outer chloroplast membrane) that are responsible for protein importation into the chloroplast (Finazzi et al., 2003; Soll and Schleiff, 2004). The chloroplast membrane can vary between two to four membranes, depending on the lineage, as a result of multiple endosymbiotic events (Delwiche, 1999). The inner membrane comprises the

stroma, within which the thylakoids, ribosomes and nucleoid are found. Embedded in the thylakoid membranes are the multisubunit protein complexes (photosystems) that are used in the light-dependent reactions and the light-harvesting protein complexes that bind photosynthetic pigments which absorb and transfer light energy (Green and Durnford, 1996; Allen et al., 2011; Minagawa, 2013), which will be discussed in further detail in the next section. The composition of subunits within protein complexes and pigment content varies between photosynthetic lineages (Larkum and Vesk, 2003; Allen et al., 2011). Pigment composition may include chlorophylls *a* and *b* or c_1/c_2 and carotenoids such as β-carotene, lutein, peridinin and fucoxanthin (Larkum and Vesk, 2003). Additionally plastids have their own genomes, which can also vary among photosynthetic organisms in structure, size and gene content, with many genes transferred to nuclear genomes (land-plant plastids typically contain >120 genes compared with 14 plastid-encoded genes within dinoflagellates) (Bachvaroff et al., 2004; Hackett et al., 2004b; Koumandou et al., 2004). Though variation is observed among organisms, the mechanisms of photosynthesis and chloroplastic electron transport are generally conserved.

In the light-dependent reactions, photons of light are absorbed by chlorophyll *a* molecules, transferred to reaction centre protein complexes (photosystem II (PS II) and photosystem I (PS I)) resulting in a series of redox reactions (Allen, 2003). The flow of electrons within the chloroplast electron transport chain follows two pathways: (1) linear electron flow from PS II (non-cyclic phosphorylation), (2) cyclic electron flow around PS I (Figure 1.1) (Allen, 2003; Minagawa, 2013). In linear electron flow (often referred to as “Z-scheme”), water is oxidized by the oxygen-evolving complex of PS II, the electrons then flow down an electron transport chain via the plastoquinone pool (PQ and PQH_2) to cytochrome b_6f complex (Cytb_6f) then to plastocyanin and then to PS I (P700) (Figure 1.1) (Allen, 2003; Finazzi et al., 2003). Excitation of PS I P700 reaction centre results in the transfer of electrons to ferredoxin (Fd), then to ferredoxin $NADP^+$ reductase (FNR) resulting in the reduction of $NADP^+$ to NADPH (Finazzi et al., 2003; Rochaix, 2011). Transfer along the chloroplast electron transport chain, generates a pH gradient across the thylakoid membrane which is used for the chemiosmotic synthesis of ATP (Allen, 2003; Minagawa, 2013). In cyclic electron flow, electrons flow around PS I where electrons

are transferred to Fd then either via FNR and the PQ pool or FNR and Cytb_6f are transferred back to PS I via plastocyanin (Finazzi et al., 2003; Foyer et al., 2012; Minagawa, 2013). Cyclic electron flow generates the chemiosmotic gradient required for ATP photophosphorylation, however, NADPH is not produced (Finazzi et al., 2003; Foyer et al., 2012). The Calvin cycle, carboxylation of ribulose-1, 5-bisphosphate by RuBisCo in the chloroplast stroma yields 3-phosphoglycerate consumes NADPH and ATP generated in the light-dependent reactions (Calvin and Benson, 1948; Foyer et al., 2012). 3-phosphoglycerate is further reduced to 3-phosphoglyceraldehyde, and the product is either used for the regeneration of ribulose-1,5-bisphosphate or exported to the cytosol and used to synthesis other organic compounds (glucose, starch, cellulose, amino acids and lipids) (Foyer et al., 2012).

Figure 1.1 Photosynthesis schematic, depicting the chloroplast electron transport chain throughout the light-dependent reactions. Linear electron flow from PS II to PS I (black arrows) through plastoquinone to Cytb_6f and plastocyanin. Electrons are passed via Fd to FNR, reducing $NADP^+$ to NADPH. Cyclic electron flow around PS I (blue arrows), electrons are passed to either via FNR and the PQ pool or FNR and Cytb_6f then to PC and back to PS I. Abbreviations: Cytb_6f, cytochrome b_6f complex; Fd, ferredoxin; FNR ferredoxin $NADP^+$ reductase; LHC, light-harvesting complex; PC, plastocyanin; PQ, plastoquinone; PQH_2, plastoquinol; PS I, photosystem I; PS II, photosystem II; Adapted from Finazzi et al. (2003).

Photosynthetic rates of carbon fixation may be influenced by external environmental factors (Ludlow, 1983). For example exposure to different light characteristics (i.e., spectra and intensity) may alter growth rates, photopigment composition and stress response in photosynthetic organisms (Ludlow, 1983; Su et al., 2014; Wu, 2016). Additionally, as the Calvin cycle enzyme RuBisCo is bifunctional (carboxylase/oxygenase), carbon dioxide and oxygen concentrations may alter enzyme activity as competition at the active site alters rates of carbon assimilation (Spreitzer and Salvucci, 2002; Portis and Parry, 2007; Peterhansel et al., 2010). Photorespiration, where RuBisCo uses oxygen as a substrate is not efficient (Peterhansel et al., 2010). Therefore, carbon-concentrating mechanisms (CCMs) exist to increase the concentration of carbon dioxide at the active site, thereby minimizing the oxygenation reaction (Badger et al., 1998). Across photosynthetic organisms RuBisCo activity is also regulated by pH, the enzyme RuBisCo activase (which is additionally regulated) and inorganic phosphate (Spreitzer and Salvucci, 2002). Temperature also influences the rate of carbon assimilation as enzymes have optimum temperature ranges for maximum efficiency and will denature when these temperatures are exceeded (Allakhverdiev et al., 2008). Additionally, under normal conditions photosynthetic electron transfer can generate ROS (e.g., reduction of O_2 to O_2^- by the Mehler reaction at PS I), which are mediated by regulatory mechanisms to prevent cellular damage (Asada, 2006; Lesser, 2006).

Under stress conditions (high irradiance and high temperatures), ROS formation can exceed the cells regulatory capacity resulting in oxidative stress (Müller et al., 2001; Asada, 2006; Latowski et al., 2011). Within photosynthetic cells, mechanisms such as the enzymatic antioxidant system (e.g., superoxide dismutase, catalase) and nonphotochemical quenching mitigate the potential damage caused by ROS (Müller et al., 2001; Latowski et al., 2011). Nonphotochemical quenching involves the conversion of excess excitation energy into heat (Müller et al., 2001). Mechanisms for the quenching of excess energy include direct dissipation via the xanthophyll cycle or indirectly via xanthophyll pigments interacting with photoprotective processes such as (1) aggregation-dependent light-harvesting complex (LHCII) quenching, (2) light-induced transformation and dissociation of antenna complex (LHCII) from trimers to monomers, or (3) charge transfer quenching between

chlorophyll *a* and zeaxanthin (Latowski et al., 2011). The xanthophyll cycle involves the transmembranous enzymatic conversion of carotenoid pigments called xanthophylls (Havaux and Tardy, 1996; Latowski et al., 2011). There are six types of xanthophyll cycles in photoautotrophs, for example in green plants, the xanthophyll violaxanthin is de-epoxidised to antheraxanthin and then to zeaxanthin, while diadinoxanthin is de-epoxidised to diatoxanthin in diatoms or dinoxanthin in dinoflagellates (Larkum and Howe, 1997; Latowski et al., 2011).

Light-harvesting protein complexes

Light-harvesting protein complexes, or antenna proteins, are an array of proteins that bind chlorophylls and accessory pigments for enhanced light capture and photoprotection via the dissipation of excess energy (Kuhlbrandt et al., 1994; Green and Durnford, 1996; Horton et al., 1996; Latowski et al., 2011). The photosynthetic light-harvesting complex system can be divided into two associated complexes, the core photosystem chloroplast-encoded light-harvesting antennae found in all photoautotrophs and the highly variable nuclear-encoded peripheral light-harvesting protein complexes (Green and Durnford, 1996). Core LHCs of plants, algae and cyanobacteria are highly conserved (Vanselow et al., 2009). Core LHCs bind chlorophyll *a* and β-carotene (Chl *a* binding), whereas the diversified peripheral LHCs bind chlorophyll *a,* additional chlorophylls (Chl *b* or Chl *c*), as well as, β-carotene, leutin and other accessory pigments (e.g., Chl *a/b* and Chl *a/c* binding proteins) (Table 1.3) (Green and Durnford, 1996; Vanselow et al., 2009). The core complex of PS II is comprised of the reaction center chlorophyll *a*-protein complex (encoded by *psbA*, *psbD*, *psbE*, *psbF*, *psbI* and *psbW*) and two internal light-harvesting chlorophyll-protein (CP) complexes CP47 and CP43 (encoded by *psbB* and *psbC*) (Green and Durnford, 1996). The PS I core light-harvesting complex is a single Chl-protein complex that comprises the reaction centre polypeptide (encoded by *psaA*) and internal antenna protein (encoded by *psaB*) (Green and Durnford, 1996). This high degree of conservation is not observed in the peripheral light-harvesting protein complexes (Green and Pichersky, 1994).

Peripheral antenna systems in photoautotrophs include phycobilisomes, LHC proteins, Light Harvesting-like (LHC-like) proteins and PS II subunit S (PsbS) (Green and Pichersky, 1994; Engelken et al., 2010; Heddad et al., 2012). Phycobilisomes were the first antenna systems in the photoautotrophs, and are still found in cyanobacteria, glaucophytes and red algae and phycobiliproteins in cryptophytes, but have been replaced by the integral thylakoid-membrane light-harvesting complexes (LHCs) in most photosynthetic eukaryotic lineages (Table 1.3) (Neilson and Durnford, 2010b). Antenna proteins within the extended LHC superfamily (LHCs and LHC-like) share a homologous chlorophyll-binding “LHC motif” (ExxxxRxAM) (Kuhlbrandt et al., 1994; Jansson, 1999; Neilson and Durnford, 2010b), that is hypothesised to have arisen from a cyanobacterial single transmembrane helix high light-induced protein (HLIP) (Engelken et al., 2010). The LHC-like protein family includes one-helix proteins (OHPs, also called HLIPs and SCPs (small Chl-binding-like proteins)), high light intensity-inducible LHC like 4 (LHL4) proteins, two-helix stress-enhanced proteins (SEPs) and three-helix early light-induced proteins (ELIPs) and like PSBS are hypothesised to have primary roles as photoprotective proteins (Table 1.3) (Montané and Kloppstech, 2000; Heddad and Adamska, 2002; Engelken et al., 2010; Neilson and Durnford, 2010a; Heddad et al., 2012). Whereas, the LHC proteins of photosynthetic eukaryotes have light harvesting/dissipation and photoprotective roles (Neilson and Durnford, 2010b). Studies of peripheral LHCs within green plants (Chl *a/b* binding light-harvesting proteins) have elucidated structural information which has further improved the understanding of light capture and transfer, evolution of LHCs, arrangement of peripheral LHCs within plastids and their photoprotective roles, though great differences are observed among photosynthetic eukaryotes (Green and Pichersky, 1994; Kuhlbrandt et al., 1994; Hoffman et al., 2011; Latowski et al., 2011).

Table 1.3 Antenna system distributions among photosynthetic organisms.

	Names/protein	Other names	Gene name	Lineage distribution
	Phycobilisomes			Cyanobacteria, Glaucophytes, Red algae
	Phycobiliproteins			Cryptophytes
LHC-like/ LIL proteins	OHP1/HLIP/SCP			Cyanobacteria, Glaucophytes, Red algae, Cryptophytes, Heterokontophytes, Green algae, Plants
	OHP2			Glaucophytes, Red algae, Heterokontophytes, Green algae, Plants
	SEP	LIL3		Glaucophytes, Red algae, Heterokontophytes, Green algae, Plants
	ELIP			Green algae, Plants
	LHL	LHL4		Green algae
	RedCAP			Red algae, Cryptophytes, Heterokontophytes, Haptophytes
	PSBS		*PsbS*	Green algae, Plants
LHC proteins	LHCI	CAB	*Lhca*	Green algae, Plants, Euglenophytes
	LHCII	CAB,CP24, CP26, CP29, Lhcbm, LHCPII, Lhcg	*Lhcb*	Green algae, Plants, Euglenophytes
	LHCQ		*Lhcq*	Green algae, Plants
	CAC		*Lhcc*	Cryptophytes
	Lhcp	CAC,PCP	*Lhcd*	Dinophytes (peridinin-containing)
	FCP	CAC, LHCF	*Lhcf*	Haptophytes, Heterokontophytes, Dinophytes (fucoxanthin-containing)
	LHCR	LhcaR	*Lhcr*	Red algae, Cryptophytes
	LHCZ	LHCZ	*Lhcz*	Chlorarachinophytes, Cryptophytes, Haptophytes, Heterokontophytes
	LI818/ LI818-like	LHCSR, LHCX	-	Green Algae, Chlorarachinophytes, Plants, Haptophytes, Heterokontophytes,

Table adapted from Hoffman et al. (2011), organization of LHC-like proteins from Heddad et al. (2012) (with additional data obtained from Jansson et al. (1999), Koziol et al. (2007), Engelken et al. (2010), Neilson and Durnford (2010b) and Sturm et al. (2013).

Peripheral LHC proteins in photoautotrophic organisms are encoded by an extended gene family (Table 1.3 and Figure 1.2) (Green and Pichersky, 1994; Green and Durnford, 1996; Jansson et al., 1999; Engelken et al., 2010; Neilson and Durnford, 2010b). Peripheral LHC polypeptides generally have three transmembrane α-helices and conserved chlorophyll and carotenoid binding sites with defined associations forming supercomplexes with photosystems (Jansson, 1999). The LHC superfamily proteins include the chlorophyll *a*/*b* binding (Chl *a/b* binding or CAB) proteins of plants and chlorophytes and euglenophytes (*Lhcb* and *Lhca* genes), the chlorophyll *a* and phycobilin binding proteins of red algae (rhodophytes) and cryptophytes (*Lhcr* genes), the chlorophyll *a*/*c* binding (Chl *a/c* binding or CAC) proteins of chromalveolates, including cryptophytes (*Lhcc* genes) and the fucoxanthin chlorophyll *a*/*c* binding proteins (FCPs/ CAC) of haptophytes and heterokonts (*Lhcf* genes), and the LI818/ LI818-like and LHCSR proteins of green algae, heterokonts and haptophytes (Table 1.3) (Grossman et al., 1990; Green and Pichersky, 1994; Kuhlbrandt et al., 1994; Jansson et al., 1999; Durnford, 2003; Koziol et al., 2007; Engelken et al., 2010; Hoffman et al., 2011). A new light-harvesting antenna called the red lineage chlorophyll *a*/*b*-binding like protein (RedCAP) has also recently been identified in red lineage eukaryotes including red algae, cryptophytes, haptophytes and heterokontophytes, hypothesised to participate in reorganization of light-harvesting systems in response to light (Engelken et al., 2010; Sturm et al., 2013). The distribution of peripheral LHC proteins reflects the patterns of inheritance of chloroplasts by endosymbiosis and subsequent reduction in photosynthetic organisms (Figure 1.2) (Neilson and Durnford, 2010b).

Figure 1.2 Distribution of peripheral light-harvesting complexes in eukaryotic organisms. Endosymbiotic events (blue arrows and symbols) and the acquisition of various antenna systems are depicted along the tree. Abbreviations: HLIP, High-Light Inducible Proteins; OHP, One Helix Proteins; SEP, Stress Enhanced Proteins; LIL, Light Harvesting-Like proteins; ELIP, Early Light Inducible Proteins; PsbS, Photosystem II subunit S; LHC, Light-Harvesting Complex; Peridinin-Chl protein, Peridinin-chlorophyll protein (Neilson and Durnford, 2010b).

Peripheral LHC proteins that bind chlorophyll *a*/*b* are found in chlorophytes, green plants, chlorarachinophytes and euglenophytes (Kuhlbrandt et al., 1994; Green and Durnford, 1996). In higher plants, LHCII associates with PS II, and is comprised of up to six pigment binding proteins (encoded by *Lhcb1-Lhcb6*) (Allen and Staehelin, 1992; Green and Durnford, 1996). The core of the LHCII is either a homo- or heterotrimeric apoprotein encoded by *Lhcb1-Lhcb3*, with each monomer binding eight chlorophyll *a*, six chlorophyll *b* and four carotenoids (Kuhlbrandt et al., 1994; Jansson, 1999; Liu et al., 2004). The three remaining LHCII associated genes encode monomeric CP complexes, CP29 (*Lhcb4*), CP26 *(Lhcb5*) and CP24 (*Lhcb6)* that have varied pigment-binding ratios (Allen and Staehelin, 1992; Jansson, 1999; Pan et al., 2011). Additionally, PS II-LHCII supercomplexes are assemblages of multiple trimers and monomers, with varied binding affinities to the PS II core and organization of LHCII proteins varies between species (Neilson and Durnford, 2010b). Associated with the PS I core is the LHCI antenna system. The gene content of LHCI varies across CAB organisms, though in higher plants six genes have been identified (*Lhca1-Lhca6*) (Jansson, 1999).The LHCI antenna system is a belt that surrounds the PS I reaction centre, that is formed by two dimers (heterodimers of *Lhca1/Lhca4* and *Lhca2/Lhca3*) (Ben-Shem et al., 2003). Two additional, minor LHCI proteins were identified in *Arabidopsis thaliana* (*Lhca5/Lhca6*) (Jansson, 1999), and are required for the formation of the full size NAD(P)H dehydrogenase – PS I supercomplex, mediating cyclic and chlororespiratory electron transport (Peng et al., 2009).

Though members of the higher plants, chlorophytes, euglenophytes and chlorarachinophytes share homology in CAB proteins, various differences are observed in the LHCs in these lineages (Koziol et al., 2007). Within the green alga *Chlamydomonas reinhardtii*, nine genes encoding proteins associated with the LHC II complex (*LhcbM1- M9*) and nine genes encoding LHCI antenna proteins have been identified (*Lhca1–Lhca9*) (Elrad and Grossman, 2004; Mozzo et al., 2010; Drop et al., 2014). The *C. reinhardtii,* LHCII heterotrimeric apoprotein is encoded by a combination of *LhcbM1*, *LhcbM2/7* and *LhcbM3*, the binding ratio of chlorophylls *a*/*b* are lower, CP24 (encoded by *Lhcb6* in higher plants) is absent and the absorption spectrum is blue shifted (Neilson and Durnford, 2010b; Drop et al., 2014). In the

euglenophyte *Euglena gracilis*, LHC proteins are detected, with LHCPII proteins sharing 65% homology to higher plant LHCII (Muchhal and Schwartzbach, 1992). Within *E. gracilis*, LHCPII proteins are encoded as concatenated genes, with multiple coding messenger RNAs (mRNAs) transcribed and translated as large polyproteins (Muchhal and Schwartzbach, 1992; Koziol and Durnford, 2008). These polyproteins are directed to the chloroplast post-translationally from the endoplasmic reticulum via the Golgi and are then cleaved to individual proteins at conserved decapeptide linking regions in the chloroplast (Muchhal and Schwartzbach, 1992; Kishore et al., 1993; Sulli and Schwartzbach, 1995; Durnford and Gray, 2006). The diversity observed in the antenna systems of chlorophyll *a*/*b*-containing organisms, indicates that LHCs were likely developed early in their evolution, however complexity has arisen to optimize light harvesting and photoprotection in the respective environmental niches (Green and Pichersky, 1994; Green and Durnford, 1996; Koziol et al., 2007).

Peripheral LHC proteins binding chlorophyll *a*/*c* are found in chromalveolates harboring complex plastids derived along the red lineage (Table 1.3) (Hoffman et al., 2011). Phylogenetic analysis of LHCs along the diversified red lineage of photosynthetic eukaryotes identifies four main groups: the LHCR, CAC, LHCZ and LI818/ LHCSR proteins (Table 1.3) (Koziol et al., 2007; Neilson and Durnford, 2010b). As mentioned previously, the red algal light-harvesting system consists of phycobilisomes, which associate predominately with PS II, and chlorophyll *a*-binding proteins (LHCR, *Lhcr*) that associate specifically with PS I (Wolfe et al., 1994; Tan et al., 1997; Busch et al., 2010; Neilson and Durnford, 2010b). Within cryptophyte algae however, associations of LHCR proteins with specific photosystems are unknown (Neilson and Durnford, 2010b).The CAC proteins of chromalveolates include the chlorophyll *a*/*c* binding proteins (*Lhcc* genes) of cryptophytes, and the fucoxanthin-chlorophyll *a*/*c* proteins (FCPs) (*Lhcf* genes) of haptophytes, heterokontophytes and dinoflagellates and the peridinin-chlorophyll binding proteins (PCP and acpPCs, *pcp* and *lhcd* genes) of dinoflagellates (Koziol et al., 2007; Neilson and Durnford, 2010b; Hoffman et al., 2011). The FCPs of haptophytes and heterokontophytes are the major LHCs, with dual light-harvesting and photoprotective roles (Durnford, 2003; Neilson and Durnford, 2010b; Nagao et al., 2014). Within heterokontophytes, FCPs

are encoded by a nuclear multi-gene family (23 *Lhcf* genes in *Cyclotella cryptica*) (Eppard and Rhiel, 2000) and specific *Lhcf* gene products have been identified associating with specific photosystems in diatoms (e.g., in *C. cryptica* FCP4 associates with PS I, while in *Cyclotella meneghiniana* FCPa (encoded by *Fcp2* and *Fcp6*) associates with PS II and FCPb (encoded by *Fcp4* and an undescribed protein) associates with PS I) (Veith and Büchel, 2007; Veith et al., 2009; Ikeda et al., 2013; Nagao et al., 2014). Specific associations of FCPs with either PS II or PS I have not been elucidated in haptophytes (Durnford, 2003; Neilson and Durnford, 2010b). The LHCZ and LI818/LHCSR clades contain both green and red lineage derived LHCs, whilst differential expression of LHCSR has been observed under light stress in green algae (Peers et al., 2009), heterokonts (Oeltjen et al., 2002) and haptophytes (Lefebvre et al., 2010) and potentially linked with photoprotection, no clear functional role has yet been assigned to the LHCZ type LHCs (Neilson and Durnford, 2010b).

As mentioned previously, divergent plastid lineages are found in dinoflagellates and the major light-harvesting system includes CAC binding proteins (Saldarriaga et al., 2001; Yoon et al., 2002; Durnford, 2003; Hoffman et al., 2011). The fucoxanthin containing dinoflagellates (e.g., *Karenia* sp. and *Karlodinium* sp.) arose following tertiary endosymbiosis through acquisition of a haptophyte plastid (Tengs et al., 2000; Yoon et al., 2002; Yoon et al., 2005). Resources established for fucoxanthin-dinoflagellates include EST libraries (Lidie et al., 2005; Yoon et al., 2005) and microarray data for *K. brevis* (Van Dolah et al., 2007) and the plastid genome of *Karlodinium veneficum* (Gabrielsen et al., 2011). However, limited information on FCPs in dinoflagellates are available (Durnford, 2003). Analysis of *K. brevis* ESTs identified FCPs as the highest expressed gene in the library with 50 copies identified (Lidie et al., 2005), though FCPs were then omitted from the diurnal- and circadian microarray study because of this (Van Dolah et al., 2007). Within peridinin-containing dinoflagellates (e.g., *Amphidinium carterae*, *H. pygmaea, Gonyaulax polyedra* and *Symbiodinium* sp.), two distinct unrelated forms of light-harvesting proteins are found, the peridinin-chlorophyll *a* protein complex (PCP, *pcp* (previously sPCP)) (Haxo et al., 1976; Prézelin and Haxo, 1976; Chang and Trench, 1982; Govind et al., 1990; Iglesias-Prieto et al., 1991; Weis et al., 2002) and the intrinsic chlorophyll *a*-

chlorophyll c_2 – peridinin protein complex (acpPC, *lhcd* or *acpPC* genes (previously ACP or *i*PCP) (Hiller et al., 1993; Iglesias-Prieto et al., 1993; ten Lohuis and Miller, 1998). Both PCPs and acpPCs are nuclear encoded and synthesized with N-terminal transit peptides for plastid targeting (Norris and Miller, 1994; Hiller et al., 1995; Sharples et al., 1996).

Differences between the two types of peridinin chlorophyll-binding proteins (PCP and acpPC) are observed among dinoflagellate species (Iglesias-Prieto et al., 1993; ten Lohuis and Miller, 1998; Durnford, 2003). Two forms of the water soluble PCP are found on the luminal periphery of thylakoid membranes, though sizes vary between species, the short homodimeric form (subunit molecular mass 14 – 16 kDa) and the monomeric long form (molecular mass of 30 – 35 kDa) (Haxo et al., 1976; Govind et al., 1990; Iglesias-Prieto et al., 1991; Norris and Miller, 1994; Sharples et al., 1996; Hiller et al., 2001; Weis et al., 2002). The structure of PCP in *A. carterae* has been resolved via X-ray crystallography (Hofmann et al., 1996; Schulte et al., 2009) and excitation energy transfer within PCP modeled using computational methods (Bricker and Lo, 2014). Studies of PCPs within the diverse *Symbiodinium* sp. complex have characterized the absorption and emission spectra and elucidated information such as the gene arrangement, pigment binding ratios and oligomerization state (Iglesias-Prieto et al., 1991; Weis et al., 2002; Reichman et al., 2003; Jiang et al., 2012; Jiang et al., 2015). Dinoflagellate PCPs share no significant sequence homology with LHCs, LHC-like proteins or phycobiliproteins, they are encoded by multigene families and organized in intronless tandem arrays (e.g., 5000 PCP gene copies in *Gonyaulax* genome (Le et al., 1997) and 36 ± 12 PCP copies/cell in *Symbiodinium* 203 (Reichman et al., 2003)) (Triplett et al., 1993; Norris and Miller, 1994; Sharples et al., 1996; Hiller et al., 1999; Weis et al., 2002). In contrast, dinoflagellate acpPCs are thylakoid membrane-bound complexes and share sequence similarity with chlorophyll *a*/*c* binding proteins of chromophytes (Hiller et al., 1993; Iglesias-Prieto et al., 1993; Hiller et al., 1995; Durnford, 2003). The intrinsic light-harvesting complex was identified as the major antenna system within *A*. *carterae*, where large transcripts (6.1 kb) encoding polyproteins that are post-translationally cleaved into ten different 19 kDa peptides are detected similar to the LHCPII of *E. gracilis* (Hiller et al., 1993; Hiller et al., 1995). Studies of LHCs have greatly improved our

understanding of plastid acquisition, plastid protein importation and expression of LHCs as nuclear-encoded polyproteins in CAB and CAC lineage organisms (Kishore et al., 1993; Hiller et al., 1995; Saldarriaga et al., 2001; Yoon et al., 2002; Nassoury et al., 2003; Durnford and Gray, 2006; Boldt et al., 2012).

Symbiodinium plastids and integral light-harvesting complexes

As previously described, *Symbiodinium* plastids are likely derived from endosymbioses along the red algal lineage, are surrounded by three membranes and contain chlorophylls *a* and c_2 and the accessory pigment peridinin (Delwiche, 1999; Saldarriaga et al., 2001). The *Symbiodinium* plastid genome like other dinoflagellates (e.g., *Heterocapsa* sp., *Amphidinium* sp.), is greatly reduced to genes on small plasmids known as 'minicircles' (Zhang et al., 1999; Koumandou et al., 2004), though unlike other dinoflagellates, each minicircle encodes a single gene (Barbrook et al., 2014; Mungpakdee et al., 2014). Characterization of the chloroplast gene repertoires from two *Symbiodinium* species (clade C3 and *S. minutum*) identified plastid-encoded and plastid associated nuclear-transferred genes (Barbrook et al., 2014; Mungpakdee et al., 2014). Within *Symbiodinium* sp. clade C3, thirteen minicircles were identified encoding single genes (12 genes and one partial gene sequence) whereas, within *S. minutum,* fourteen plastid-encoded genes were identified (Table 1.4) (Barbrook et al., 2014; Mungpakdee et al., 2014), consistent with the fourteen genes identified on minicircles in other dinoflagellate species (Koumandou et al., 2004). In *S. minutum*, a further 95 plastid-associated genes were identified in the nuclear genome (Mungpakdee et al., 2014). Some of these plastid-associated nuclear-encoded genes were highly duplicated, such as those encoding PS II proteins (e.g., 4 *psbF*, 6 *psbH,* 3 *psbJ*, 4 *psbL*, 6 *psbM,* 3 *psbO*, 2 *psbP*, 2 *psbU*, 4 *psbV* and 3 *psbY*) and integral light-harvesting complex proteins (acpPCs) (101 *lhcb* gene models) (Mungpakdee et al., 2014).

Table 1.4 Estimated type and size of plastid-encoded genes of *Symbiodinium* species.

Gene	*Symbiodinium* sp. clade C3 (kb)	*Symbiodinium minutum* (kb)
psbA	1.9	2.4
psbB	2.2	2.5
psbC	2.1	2.5
psbD	1.8	2.25
psbE	1.3	1.8
psbI	-	2.13
psaA	2.7	3.3
psaB	2.7	3.2
petB	1.7	2.3
petD	1.4	2.2
atpA	2.2	2.4
atpB	2.6	3
cp16S rRNA	1.4*	2.35
cp23S rRNA	2.8	2.5

Table adapted and generated using data from Barbrook et al. (2014); and Mungpakdee et al. (2014).
*Partial sequence.

Symbiodinium light-harvesting protein complexes are important for photoprotection and to maximise the photosynthetic potential of cells in diverse photic environments. In *Symbiodinium* the two types of peripheral light-harvesting complexes are found, the soluble peridinin chlorophyll *a*/*c* – protein complex (PCP) and the intrinsic membrane-bound chlorophyll *a*-chlorophyll c_2-peridinin protein complex (acpPC) (Iglesias-Prieto et al., 1993). *Symbiodinium* PCPs are well studied due to their unique features (Chang and Trench, 1982; Iglesias-Prieto et al., 1991; Norris and Miller, 1994; Iglesias-Prieto and Trench, 1996; Weis et al., 2002; Reichman et al., 2003; Jiang et al., 2012; Niedzwiedzki et al., 2013; Kanazawa et al., 2014; Jiang et al., 2015; Tanaka et al., 2016). However, the body of literature on *Symbiodinium* acpPCs is quite small (Iglesias-Prieto et al., 1993; Iglesias-Prieto and Trench, 1997;

Boldt et al., 2012; Jiang et al., 2014; Mungpakdee et al., 2014; Niedzwiedzki et al., 2014; Maruyama et al., 2015) and the effect of stress on *Symbiodinium* acpPCs is understudied (Reynolds et al., 2008; Takahashi et al., 2008; Boldt et al., 2009; Hill et al., 2012). Initial characterization of *Symbiodinium* acpPCs estimated a doublet apoprotein of molecular mass 17 - 22 kDa (Iglesias-Prieto et al., 1993), though recent studies estimate molecular mass from 18 - 65 kDa (Boldt et al., 2012). The acpPCs have been determined as the major light-harvesting protein in *Symbiodinium* as the majority of photosynthetic pigments are associated with the integral protein complexes (Iglesias-Prieto et al., 1993; Iglesias-Prieto and Trench, 1997). Analysis of the pigment composition of an acpPC fraction isolated from *Symbiodinium* sp. (CS-156), identified pigments in the molecular ratio of 4:6:6:1, chlorophyll *a*: chlorophyll c_2: peridinin: diadinoxanthin (Niedzwiedzki et al., 2014). This estimation differs to the molecular ratio of pigments within the peridinin-dinoflagellate *A. carterae* of 7:4:12:2 chlorophyll *a*: chlorophyll c_2: peridinin: diadinoxanthin (Hiller et al., 1993), though does not take into account the diversity of integral LHC genes detected within *Symbiodinium* species (Boldt et al., 2012; Maruyama et al., 2015).

Symbiodinium integral LHCs are plastid-targeted proteins that are encoded in the nucleus by an extremely diversified gene family (Boldt et al., 2012; Mungpakdee et al., 2014; Maruyama et al., 2015). Estimation of LHC gene content varies between these studies, 11 acpPC genes were reported within *Symbiodinium* sp. clade C3 using EST sequencing and 5' sequencing (Boldt et al., 2012), while genome and transcriptome sequencing of *S. minutum* (Mf1.05B.01, clade B1) (Shoguchi et al., 2013) revealed 101 acpPC gene models (Mungpakdee et al., 2014) and 80 acpPC gene loci (145 LHC subunits) (Maruyama et al., 2015). This difference reflects the methodologies employed and that not all *Symbiodinium* species encode the same subset of acpPC genes (Boldt et al., 2012; Maruyama et al., 2015). Through EST analysis of *Symbiodinium* sp. (clade C3 and A1.1) and northern blot analysis of *Symbiodinium* (clade C3 and C1) similarities of acpPCs with LHCs within green plants (LHCII), euglenophytes (*Euglena gracilis*) (Chl *a*/*b* binding protein) and dinoflagellates (*A. carterae*, *Amphidinium tamarense*, *Pyrocystis lunula* and *Heterocapsa triquetra*) (Chl *a/c* binding protein) were identified (Figure 3.1) (Boldt et al., 2012), which were further supported through analysis of integral LHCs within *S.*

minutum (Maruyama et al., 2015). These similarities include the arrangement of membrane spanning helices and conserved chlorophyll-binding residues, variation in cistron number within transcripts (monocistronic to polycistronic, containing between one to three acpPC peptides in *Symbiodinium* sp. C3 and one to nine LHC genes in *S. minutum*), with some separated by an SPLR cleavage motif and evidence that some acpPCs may be translated as polypeptides containing up to ten peptides (Boldt et al., 2012; Maruyama et al., 2015). Phylogenetic analysis of acpPC genes from *Symbiodinium* species has revealed that intra- and intergenic gene duplications of two LHC gene subfamilies (*Lhcr*-type and *Lhcf*-type) have given rise to the diverse gene family (Boldt et al., 2012; Maruyama et al., 2015) although specific associations of light harvesting proteins with photosystems within *Symbiodinium* have not been resolved (Boldt et al., 2012; Maruyama et al., 2015).

Investigation of integral light-harvesting protein complexes within *Symbiodinium* under experimental conditions has shown diverse responses. Under low sub-saturating light levels (low, 40 µmol quanta m^{-2} s^{-1}; high, 250 µmol quanta m^{-2} s^{-1}) cellular concentrations of acpPC protein were increased in three cultured *Symbiodinium* species compared to high sub-saturating light levels (Iglesias-Prieto and Trench, 1997). Whereas in *Symbiodinium* (clade C3) within *A. aspera,* the expression of three acpPC genes remained unchanged under light stress (573 – 1540 µmol quanta m^{-2} s^{-1}) (Boldt et al., 2009). However, under short-term thermal stress, expression of one acpPC transcript and acpPC protein expression varied between two cultured *Symbiodinium* clade A species (CS-73 and OTcH-1) of varied thermal tolerances (Takahashi et al., 2008). Differential levels of dissociation of integral light harvesting complexes has also been displayed in *Symbiodinium* within three different corals (*Acropora millepora*, *Pavona decussata* and *Pocillopora damicornis*) under temperature stress (Hill et al., 2012) and in *Symbiodinium* clade A3 under light stress (Reynolds et al., 2008). Due to the variability in the quantified responses of integral light-harvesting protein complexes in *Symbiodinium* under stress, the light harvesting and/or photoprotective functional roles and the high-diversification of the gene family, the acpPCs were therefore investigated further in this thesis.

Coral study species: *Acropora aspera*

Acroporids are major reef forming corals that dominate the Indo-Pacific region inhabiting reef flats and lagoons (0 - 5 m) (Veron, 2000). *Acropora aspera* is a branching scleractinian (stony coral) species that forms colonies 1 - 3 m in diameter and a variety of colour morphs exist, including blue, light blue and brown, and creamy-brown (Dove, 2004). *A. aspera* was chosen as it has previously been used as a study species (Dove, 2004; Leggat et al., 2011a; Ogawa et al., 2013; Rosic et al., 2014a), symbioses are formed with *Symbiodinium* clade C3, a basal *Symbiodinium* clade (LaJeunesse et al., 2003) and an EST library is available for *Symbiodinium* C3 (Leggat et al., 2007). Field work was conducted on Heron Island, Great Barrier Reef, Australia (23°44'S; 151°91'E), where branches of *A. aspera* (tan morph) were collected from the reef flat at low tide and taken to aquaria at the Heron Island Research Station for experimentation.

Research objectives

Short periods of high temperature are highly stressful to corals (Berkelmans et al., 2004), with studies emulating these acute conditions in an attempt to understand mechanisms of coral bleaching (Iglesias-Prieto et al., 1992; Ralph et al., 2001; Ralph et al., 2005; Takahashi et al., 2008). These studies, though extremely valuable in providing an insight into bleaching processes, employ harsh experimental conditions that are not reflective of future long-term predicted SSTs or coral bleaching induced by moderate thermal stress over long periods of time (Berkelmans et al., 2004; Fujise et al., 2014). The primary objectives of this research were to use molecular techniques to investigate the effect of thermal stress on *Symbiodinium*, in an attempt to approximate the cellular response under future temperature conditions. Gene expression studies were linked with physiological measurements and used to investigate the response of *Symbiodinium* culture isolates and whilst in symbiosis with a scleractinian coral, *A. aspera*. Previous targeted gene expression studies have found differential expression of integral LHCs under short-term thermal stress in *Symbiodinium* cultures (Takahashi et al., 2008 and Gierz, Boldt and Leggat, under review), this thesis additionally focuses on determining the expression of integral

light-harvesting complexes in *Symbiodinium* under thermal stress *in vitro* and *in hospite*.

In the first data chapter (Chapter 2), the transcriptional response of *Symbiodinium* clade F *in vitro* to thermal stress was determined. Cultures of *Symbiodinium* clade F were exposed to a twenty-eight day thermal stress, throughout which cell physiology was assessed and linked to changes in gene expression. Next generation sequencing methodologies (RNA-Seq) were used to determine the transcriptional response of *Symbiodinium* cultures. This experimental regime, paired with the use of sequencing technology is the first of its kind, providing an insight into the cellular response under future temperature conditions.

Chapter 3 investigates alterations of photophysiology in *Symbiodinium* C3 in symbiosis with *A. aspera* under thermal stress. *A. aspera* were subjected to a simulated bleaching event over a sixteen day period. *Symbiodinium* photophysiology, cell density and chlorophyll content were measured. The expression of five genes encoding integral light-harvesting protein complexes from three distinct LHC families found in *Symbiodinium* within a coral host was determined using quantitative PCR. LHC expression has been linked to thermal stress in *Symbiodinium* and subsequently coral bleaching, which has large implications for coral reef survival. Integral LHC expression had not previously been determined under thermal stress in symbiosis. This study also employed an extended thermal stress regime (16 d), allowing for the identification of changes in LHC expression at temperatures experienced in a natural bleaching event

In Chapter 4, the cellular physiology of *Symbiodinium* within *A. aspera* exposed to thermal stress for a sixteen day period was assessed. In general, assessment of coral bleaching relies on mesurements of symbiont density or dark-adapted yield of PS II. However, in depth analysis of individual cells and populations of *Symbiodinium* isolated from branches of *A. aspera* identified variable responses over the multiple physiological parameters assayed, at temperatures below the bleaching threshold. Further illustrating that broad, multifaceted approaches are required to accurately assess the variety of responses elicited in *Symbiodinium* under bleaching conditions.

Finally, Chapter 5 discusses the findings of these experiments, the implications of elevated SST on coral reefs and future directions.

Chapter 2 Transcriptomic analysis of thermally stressed *Symbiodinium* reveals differential expression of stress and metabolism genes

This study describes the transcriptome response of *Symbiodinium* sp. (clade F) cultures exposed to thermal stress (four, nineteen and twenty-eight days) at future temperature conditions and to link with physiological changes observed. Within the differentially expressed genes, transcripts with significance to the stress response of *Symbiodinium* were detected. The results indicate a shift in metabolism, from carbon fixation to fatty acid catabolism, supported by upregulation of β-oxidation, glyoxylate cycle and gluconeogenic enzymes, this to our knowledge, has not previously been reported in *Symbiodinium*. The implications of altered metabolic processes from exposure to thermal stress found in this study, on coral – *Symbiodinium* associations has not been explored. This study provides an important reference for understanding the mechanisms of coral bleaching at future temperature conditions.

Abstract

Endosymbioses between dinoflagellate algae (*Symbiodinium* sp.) and scleractinian coral species form the foundation of coral reef ecosystems. The coral symbiosis is highly susceptible to elevated temperatures, resulting in coral bleaching, where the algal symbiont is released from host cells. This experiment aimed to determine the transcriptional changes in cultured *Symbiodinium*, to better understand the response of cellular mechanisms under future temperature conditions. Cultures were exposed to elevated temperatures (average 31 °C) or control conditions (24.5 °C) for a period of twenty-eight days. Whole transcriptome sequencing of *Symbiodinium* cells on days four, nineteen and twenty-eight were used to identify differentially expressed genes under thermal stress. A large number of genes representing 37.01% of the transcriptome (~23,654 unique genes, FDR < 0.05) with differential expression were detected at no less than one of the time points. Consistent with previous studies of *Symbiodinium* gene expression, fold changes across the transcriptome were low, with 92.49% differentially expressed genes at ≤ 2-fold change. The transcriptional response included differential expression of genes encoding stress response components such as the antioxidant network and molecular chaperones, cellular components such as core photosynthesis machinery, integral light-harvesting protein complexes and enzymes such as fatty-acid desaturases. Differential expression of genes encoding glyoxylate cycle enzymes were also found, representing the first report of this in *Symbiodinium*. As photosynthate transfer from *Symbiodinium* to coral hosts provides up to 90% of a coral's daily energy requirements, the implications of altered metabolic processes from exposure to thermal stress found in this study on coral-*Symbiodinium* associations are unknown and should be considered when assessing the stability of the symbiotic relationship under future climate conditions.

Introduction

Unicellular dinoflagellates (genus *Symbiodinium*) form symbiotic relationships with reef-building corals and other marine invertebrates. The success of coral reef ecosystems is due to mutualistic nutrient exchange between host and endosymbionts (Yellowlees et al., 2008). Exposure to stressors (e.g., elevated temperature) has been attributed as causing coral bleaching, the dysfunction of the symbiotic relationship, resulting in the expulsion of *Symbiodinium* from the coral host. Coral bleaching is the release of either the *Symbiodinium* cells from host tissue or the loss of their photosynthetic pigments (Iglesias-Prieto et al., 1992). Depending on the degree of the bleaching event the result may vary, with the host being recolonised by *Symbiodinium*, disease outbreak or widespread coral mortality (Hoegh-Guldberg, 1999).

Experimentation on *Symbiodinium* and the coral holobiont has focused on many environmental factors implicated in the onset of coral bleaching including elevated seawater temperatures, acidification, eutrophication (nutrient stress) and disease. The effect of high sea-surface temperatures have been a key focus due to mass coral bleaching events (approximately 42% GBR reefs bleached in 1998 and ~54% reefs bleached in 2002 (Berkelmans et al., 2004)), attributed to global climate change (Hoegh-Guldberg, 1999) with the 1998 bleaching event coinciding with an El Niño Southern Oscillation event (Bruno et al., 2001; Fujise et al., 2014). Modeling of bleaching patterns have shown that short periods of high temperature are highly stressful to corals (Berkelmans et al., 2004) with studies emulating these acute conditions in an attempt to understand mechanisms of coral bleaching (Iglesias-Prieto et al., 1992; Ralph et al., 2001; Ralph et al., 2005; Takahashi et al., 2008). These studies though extremely valuable in providing an insight into bleaching processes, employ experimental conditions that are not reflective of future long-term predicted sea-surface temperatures or coral bleaching induced by moderate thermal stress over long periods of time (Berkelmans et al., 2004; Fujise et al., 2014; Ainsworth et al., 2016). Additionally mechanisms of thermal acclimation within *Symbiodinium* are also unknown, with only two recent studies investigating the effect

of moderate thermal stress on photobleaching (Takahashi et al., 2013; Fujise et al., 2014).

Dinoflagellates have a number of cellular traits and features that make them unique. Dinoflagellates have large nuclear genomes, the chromosomes remain permanently condensed through the cell cycle and contain highly expressed genes with elevated copy numbers and tandem repeats (Hackett et al., 2004a). Approximately half of the dinoflagellates are photosynthetic, having acquired a variety of plastids via endosymbiotic events (Delwiche, 1999; Hackett et al., 2004a). In general, the plastids are surrounded by three envelope membranes and have unique chloroplast genome structure, having been reduced to single gene minicircles, with the majority of genes transferred to the nucleus (Hackett et al., 2004a; Barbrook et al., 2014; Mungpakdee et al., 2014).

The genus *Symbiodinium* is a taxonomically diverse species complex, divided into nine phylogenetically distinct clades (A-I) (Pochon and Gates, 2010). Additionally, intra-cladal diversity exists subdividing the genus further. Associations between *Symbiodinium* exist with many taxa including ciliates, platyhelminthes and a variety of marine invertebrates such as cnidarians, molluscs, poriferans and foraminiferans (Baker, 2003; Coffroth and Santos, 2005; Stat et al., 2008; Venn et al., 2008; Pochon et al., 2010). *Symbiodinium* associations may either exist as mixed populations, at intra-clade or inter-clade level, or as host – symbiont specific interactions (Baker, 2003; Coffroth and Santos, 2005; Pochon and Gates, 2010). Mixed *Symbiodinium* populations may occur in different proportions, with a single dominant species with multiple species detected at low abundances (Baker, 2003). Therefore, understanding the different *Symbiodinium* strains may provide an insight into the complexity of symbiotic interactions that are observed. Development of high throughput sequencing technologies has seen many advances in *Symbiodinium* genomics and transcriptomics (summarised in Shinzato et al. (2014b)). These include publication of three *Symbiodinium* nuclear genomes, *Symbiodinium minutum* (clade B1) (Shoguchi et al., 2013), the *Symbiodinium kawagutii* (clade F1) nuclear genome (Lin et al., 2015) and the *Symbiodinium microadriaticum* (clade A1) nuclear genome (Aranda et al., 2016) and a further thirteen sequencing projects representing

organelle genomes and transcriptomes of various *Symbiodinium* sp. (clades A-D and F) (Table 1.1). The *Symbiodinium minutum* (clade B) genome has identified a large number of protein-coding genes (41,925), attributed to duplication events due to the presence of highly repetitive gene clusters (Shoguchi et al., 2013; Shinzato et al., 2014b). Further, expansions of regulator of chromosome condensation family protein (RCC1) provide a possible molecular basis for permanently condensed chromatin observed in dinoflagellates (Shoguchi et al., 2013; Shinzato et al., 2014b). Other expanded multi-copy gene families identified include ion channel proteins and the chlorophyll *a*/*b*-binding proteins (*lhcb*, PF00504) (Shoguchi et al., 2013). Study of the *S. minutum* mitochondrial genome (Shoguchi et al., 2015) has shown that it is greatly expanded and fragmented, whereas, the plastid genome is greatly reduced with most (all but 14 located on DNA minicircles) plastid-associated genes being transferred to the nucleus (Mungpakdee et al., 2014). Mechanisms for RNA editing in *Symbiodinium* have also been revealed from studying the plastid genome (Mungpakdee et al., 2014). *Symbiodinium* transcriptomes and EST datasets have been published using different sequencing technologies representing various clades that associate with a variety of hosts (summarised in Leggat et al., 2011b; Rosic et al., 2014b; Shinzato et al., 2014b; Xiang et al., 2015; Levin et al., 2016). Due to the interest in studying establishment of symbiosis, gene expression studies have focused on genes associated with these molecular mechanisms (Voolstra et al., 2009a; Mohamed et al., 2016).

Symbiodinium genome and transcriptome data have allowed for studies of specific gene families of interest (Shinzato et al., 2014b). Integral light-harvesting complex (LHC) gene families (chlorophyll *a*-chlorophyll c_2 peridinin protein complex (*acpPC*)) in *Symbiodinium* have been investigated due to their unique gene arrangement (annotated as chlorophyll *a*/*b* binding proteins in some instances) (Takahashi et al., 2008; Boldt et al., 2012; Maruyama et al., 2015; Xiang et al., 2015). Gene-mining has revealed high diversification of the integral light-harvesting gene family (*acpPC*) in *Symbiodinium* species (Boldt et al., 2012; Maruyama et al., 2015), supporting theories of intra- and intergenic duplication from ancestral LHC gene(s) possibly of red algal origin (Engelken et al., 2010; Boldt et al., 2012; Maruyama et al., 2015). The functional purpose for the highly redundant multicopy gene family has been

hypothesised as photoprotective (Boldt et al., 2012; Maruyama et al., 2015; Xiang et al., 2015), and differential expression of *acpPC* genes in *Symbiodinium* has been detected under thermal stress in targeted gene expression studies (Takahashi et al., 2008; Gierz et al., 2016 (Chapter 3)). This study investigates the expression of *Symbiodinium* sp. (clade F) light-harvesting *acpPC* genes under thermal stress at the transcriptome level.

Whilst these next-generation sequencing projects have generated large quantities of data and provide basal reference transcriptomes, some of these studies lack comparison between stress and non-stress conditions, which is one of the aims of this study. Recently, the transcriptional response of cultured *Symbiodinium* (strain SSB01) maintained under different light intensities with different growth conditions (Xiang et al., 2015), and of two type C1 *Symbiodinium* cultures exposed to a thirteen day thermal stress (32 °C) (Levin et al., 2016) have been published, and are providing insights into *Symbiodinium* transcriptomes under stress conditions. Previous studies of *Symbiodinium* using targeted gene expression analysis (quantitative-PCR), demonstrate that alteration of gene expression generally occurs at low fold changes, with regulation hypothesised to occur instead through translational or post-translational mechanisms (Leggat et al., 2011a; Rosic et al., 2011a; Krueger et al., 2015; Gierz et al., 2016 (Chapter 3)). This study employed Illumina RNA-Seq and used four biological replicates at each time point per condition to ensure a robust analysis. Samples were selected for sequencing to encapsulate stages of exposure to elevated temperatures at beginning (day four), middle (day nineteen) and end (day twenty-eight) of the experimental period. This experimental design has allowed us to investigate the potential effects of exposure to elevated temperatures on *Symbiodinium* molecular processes.

Methods

Culture conditions and experimental design

Cultures of *Symbiodinium* sp. (clade F (ITS2 rDNA region) (Appendix A)) were obtained from the Australian Institute of Marine Science (AIMS). Cells were grown in ASP-8A media (Blank, 1987), in 75 cm^2 vented tissue culture flasks at 25 °C. Cultures were maintained in light cabinets (Thermoline Scientific refrigerated incubator, Sanyo) at an irradiance of 80 – 100 µmol quanta m^{-2} s^{-1} measured using a LI-193SA Underwater Spherical Quantum Sensor with a LI-250A Light Meter (LI-COR® Inc., NE, USA).

Over a period of twenty-eight days cultures of *Symbiodinium* were exposed to control temperatures (24.5 - 25 °C) or thermal treatment temperatures of approximately (30 - 31.5 °C) (Figure 2.1). In both treatment and controls daily fluctuations in temperature reflect 12: 12 h light: dark photoperiod. Temperatures in each incubator were recorded every ten minutes with HOBO® temperature/alarm pendant data loggers (Onset, Massachusetts, USA). To maintain an adequate number of cells throughout the experiment fifteen flasks were designated to each treatment with five replicate culture flasks sampled at each time point per treatment (Appendix B Figure 6.1). Samples were taken at ten hours into the light photoperiod.

Figure 2.1 Experimental temperatures cultured *Symbiodinium* were exposed to. Temperature of control (solid line) and heated treatment (dashed line) during the twenty-eight day thermal experiment, moving average displayed.

Symbiodinium density and chlorophyll pigment analysis

On days one, four, seven, nineteen and twenty-eight, five replicate culture flasks were taken from each treatment for cell number approximation and pigment quantification. Cell numbers were determined using a Neubauer haemocytometer, with replicate cell counts performed (n = 4). *Symbiodinium* cells were pelleted by centrifugation at 4,500 g for 3 min for chlorophyll *a* and *c* quantification. Chlorophylls were extracted in 90% acetone for 20 h in the dark at 4 °C, measured on a DU-650 spectrophotometer (Beckman, USA) at 630 and 664 nm light wavelengths and and pigment content quantified using the equations of Jeffrey and Humphrey (1975).

Imaging-Pulse-amplitude modulated fluorometry

Imaging-Pulse-amplitude modulated (PAM) fluorometry (MAXI Imaging-PAM and ImagingWin software, Walz, Effeltrich, Germany) were used to measure photosynthetic efficiency of *Symbiodinium* cultures. The preprogrammed Induction Curve + Recovery kinetic recording type was used to examine the ability of *Symbiodinium* to dissipate excess light energy and recover from light stress after exposure to elevated temperature. *Symbiodinium* cells were aseptically transferred

to 15 mL falcon tubes and pelleted by centrifugation at 4,500 g for 3 min. Cell pellets were resuspended in approximately 250 microliters ASP-8A media and transferred to a 96-well plate and dark-adapted for twenty minutes prior to analysis. Following dark-adaption, minimum chlorophyll fluorescence (F_o) were determined using blue measuring light (intensity 2), and maximum chlorophyll fluorescence (F_m) were determined by applying a pulse (0.72 s) of saturating light (intensity 5, ~2,800 µmol quanta m^{-2} s^{-1}) allowing calculation of the dark-adapted maximal quantum yield of PS II (F_v/F_m). For the Induction Curve, actinic illumination (254 µmol quanta m^{-2} s^{-1}, intensity 6) was switched on and fifteen saturating pulses of photosynthetically active radiation (~2800 µmol quanta m^{-2} s^{-1} (intensity 5, 0.72 s)) were applied at 20 s intervals for 5 min. During the Recovery phase, a further sixteen saturation pulses were applied within a 14 min period without actinic illumination, time between each pulse exponentially increased. Imaging-PAM fluorometry were used to determine photo-kinetic parameters, such as the maximal quantum yield of PS II (F_v/F_m), effective quantum yield at the end of the induction curve and non-photochemical quenching (NPQ) at the beginning of the recovery phase. Light levels were measured using a LI-190SA Quantum Sensor with a LI-250A Light Meter (LI-COR® Inc., NE, USA).

Data analysis

Statistics software package SPSS statistics (v19.0, IBM, USA) were used for all statistical analyses of physiological parameters. A generalized linear model with 'day' and 'treatment' as main effects and 'day x treatment' as an interaction were used for pairwise comparison of cell density, chlorophyll *a* and *c*, and imaging-PAM with sequential Bonferroni post hoc test to determine significant differences between control and treatment. The generalized linear model approach was chosen as samples taken at each time point were considered independent.

RNA isolation and sequencing

For total RNA isolation, 15 mL of cells were pelleted by centrifugation at 4,500 g for 3 min. Cells were then transferred to a screw cape tube and centrifuged at 8,050 g for 3 min. Pellets were snap frozen in liquid nitrogen and stored at -80 °C. Total RNA

were extracted using the RNeasy Plant Mini Kit (Qiagen, USA). *Symbiodinium* cells were first lysed using the FastPrep®-24 sample preparation system (MP Biomedicals, Australia). Four hundred and fifty microliters of Buffer RLT containing 1% β-mercaptoethanol were used to resuspend cells and were transferred to a lysing matrix D tube (MP Biomedicals, Australia), and shaken three times for 40 seconds at 4.5 M s^{-1} to lyse the cells. Total RNAs were isolated from cells using the Purification of Total RNA from Plant cells and tissues protocol. The optional on-column DNase Digestion was performed using the RNase-Free DNase set (Qiagen, USA) as per the manufacturer's protocol.

Concentrations of isolated total RNAs were checked using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, USA) and quality were assessed using a Bioanalyzer (Agilent) prior to library generation. RNA-Seq libraries were prepared by the Australian Genome Research Facility (AGRF, Melbourne, Australia), using the Illumina TruSeq RNA sample preparation kit V2 (Illumina) and the standard Illumina protocols. Multiplexed sequencing were performed on the twenty-four libraries by AGRF on an Illumina HiSeq 2000 platform on two lanes, generating over 370 million 100 bp single-end reads (Appendix C Table 6.1).

RNA-Seq analysis

Image analyses were performed in real time by the HiSeq Control Software (HCS) v1.4.8 and Real Time Analysis (RTA) v1.18.61, running on the instrument computer. The Illumina CASAVA (Consensus Assessment of Sequence and Variation) 1.8.2 pipeline was used to generate the sequence data. Sequencing reads were filtered according to their multiplexing tags and multiplexing tags were removed. The sequenced library were mapped against a *Symbiodinium* reference transcriptome generated from the same culture (unpublished Bobeszko, Forêt and Leggat). Briefly, for the *Symbiodinium* reference transcriptome (BioProject number PRJNA371519), paired-end reads were trimmed, removing sequence adaptors and low-quality regions using libngs (https://github.com/sylvainforet/libngs) with a minimum quality of 20 and a minimum size of 75 bp. Trimmed reads were then assembled with Trinity (Grabherr et al., 2011) and the resulting assembly clustered using CD-HIT-EST (Fu

et al., 2012) using 90% similarity and a word size of 8. TransDecoder (Haas et al., 2013) and Blast2GO (Conesa et al., 2005) were used to predict protein coding sequences.

The single-end sequenced library were mapped using the ArrayStar application and QSeq module of the DNAStar Lasergene Genomics Suite (Version 11) (DNASTAR, Inc., USA) with all parameters set to defaults. QSeq parameters were set to default, read counts were normalized via RPKM (reads per kilo base of exon model per million mapped reads) (Mortazavi et al., 2008) and processed genes defined as 'use sequences as genes'. The RPKM method standardizes molar concentration of transcripts by determining transcript length (in kilobases) and the read abundances by dividing each read count by the library size (in millions) to normalize (Mortazavi et al., 2008). The RPKM normalization method is accepted in RNA-Seq analysis as it removes technical biases introduced by sequence-length and library-size (Li et al., 2015; Conesa et al., 2016), and is suitable for the single-end reads generated from sequencing. Statistically significant expression changes between the control and treatment data sets were determined using the student's t-test with multiple test correction by Benjamini-Hochberg false discovery rate (FDR < 0.05). The results for each time point comparison (day four, 9,471 DEG; day nineteen, 12,701 DEG; and day twenty-eight, 13,269 DEG (Figure 2.9 and Appendix D Table 6.2)), uniquely differentially expressed at any time point (23,654 DEG) and differentially expressed at every time point (2,798 DEG) were exported and saved in Microsoft Excel (Microsoft, USA) and used for subsequent analysis.

Nucleotide fasta files for candidate transcripts were generated using the 'SeqinR' package (http://seqinr.r-forge.r-project.org/) (Charif and Lobry, 2007), venn diagrams were drawn with the 'VennDiagram' package (http://cran.r-project.org/package=VennDiagram) (Chen and Boutros, 2011), using R version 3.3.2 (http://www.r-project.org/) (R Core Team, 2014). Distribution of gene ontology (GO) terms within the sequences that were differentially expressed at all time point (2,798 DEG) were determined using Blast2GO (v3.3.5) (Conesa et al., 2005). Briefly, nucleotide sequences were imported into Blast2GO and GO terms generated using default parameters for the blast (blastx), mapping and annotation steps (Conesa and

Götz, 2008). To visualize GO term distributions, combined gene ontology annotation graphs were produced (default settings) and used to generate graph level pie charts of biological processes (Figure 2.10), molecular function (Appendix D Figure 6.2) and cellular component (Appendix D Figure 6.3), sequences were filtered by GO terms and a cut-off ontology level of 3 were applied, slices smaller than 2% were grouped in the “other” slice. To further analyze the distribution of GO terms within the biological process category data were split into three sets, significantly upregulated at all time points (1,428 DEG), significantly downregulated at all time points (1,331 DEG) or significantly expressed (up and down) at all time points (39 DEG) (Appendix D Figure 6.4). Sequences with GO terms contributing to biological process categories were selected/filtered and used to further identify genes and pathways differentially expressed over all time points. Heatmaps were drawn with the ‘pheatmap’ package (http://cran.r-project.org/package=pheatmap) (Kolde, 2015).

Data deposition

The Illumina sequenced read data reported in this article have been deposited into the National Center for Biotechnology Information (NCBI) Sequence Read Archive under the accession number SRA467551, which is associated with BioProject number PRJNA342240.

Data included in Appendices E, F, G and two additional tables relevant to this analysis are available on the James Cook University Tropical Data Hub.

Results

Physiological responses of *Symbiodinium* to thermal stress

Symbiodinium cell densities were significantly decreased in thermally stressed cultures from day four onwards of the experiment (Figure 2.2). Flasks sampled on day one were sampled again on day fourteen, day four again on day nineteen and day seven again on day twenty-eight.

Figure 2.2 *Symbiodinium* cell density exposed to control conditions (solid line) and heated treatment (dashed line). *Error bars* represent ± s.e.m., n = 5, some *error bars* obscured by data point markers. The statistical difference (sequential Bonferroni post hoc) between treatment and control is indicated as *$p < 0.05$ or **$p < 0.01$.

Maximum quantum yield of photosystem II, F_v/F_m, was measured on sampling days throughout the experiment. For cultures maintained at control temperatures dark-adapted yield ranged between 0.536 and 0.616 (average 0.577, ± 0.003 SE) (Figure 2.3). Analysis of the dark-adapted yield found that there were significant interactions between treatment and day ($p < 0.001$, df = 5) (Figure 2.3) and for days ($p < 0.001$, df = 5) and treatments ($p < 0.001$, df = 1). Sequential Bonferroni *post hoc* analysis found that F_v/F_m decreased in the heated treatment on day fourteen ($p < 0.05$), day

nineteen ($p < 0.01$), and day twenty-eight ($p < 0.01$) of the experiment (Figure 2.3). *Symbiodinium* effective quantum yield at the end of the induction phase were also determined (Figure 2.4). This measurement is taken after the cells were exposed to a phase of actinic light with periodic saturating pulses. Cells maintained at ~ 31°C only exhibited decreased effective quantum yield on day four ($p < 0.01$) (Figure 2.4). Non-photochemical quenching (NPQ) a proxy for cell protective mechanisms was measured over the course of the experiment. Analysis of NPQ found that there were significant effects in the interaction between day and treatment ($p < 0.001$, df = 5) and between days ($p < 0.001$, df = 5) (Figure 2.5). NPQ values did vary slightly from controls in thermally stressed cells, though it only significantly decreased on day nineteen ($p < 0.001$) (Figure 2.5).

Figure 2.3 Dark-adapted yield (F_v/F_m) of *Symbiodinium* cells during the experiment. Dark–adapted yield of control treatments (solid line) and heated treatments (dashed line). Error bars represent ± s.e.m., $n = 5$, some *error bars* obscured by data point markers. The statistical difference (sequential Bonferroni post hoc) between treatment and control is indicated as *$p < 0.05$ or **$p < 0.01$.

Figure 2.4 Effective quantum yield of *Symbiodinium* cells at the end of the induction phase. Control treatments (solid line) and heated treatments (dashed line). *Error bars* represent ± s.e.m., $n = 5$, some *error bars* obscured by data point markers. The statistical difference (sequential Bonferroni post hoc) between treatment and control is indicated as $*p < 0.05$ or $**p < 0.01$.

Figure 2.5 Non-photochemical quenching of *Symbiodinium* cells at the first data point of the recovery phase control treatments (solid line) and heated treatments (dashed

line). *Error bars* represent ± s.e.m., *n* = 5, some *error bars* obscured by data point markers. The statistical difference (sequential Bonferroni post hoc) between treatment and control is indicated as *$p < 0.05$ or **$p < 0.01$.

Chlorophyll *a* content per *Symbiodinium* cell were increased in treatment cells compared to control cells over the experimental period (Figure 2.6). Analysis of chlorophyll *a* content found that there were significant interaction day x treatment ($p < 0.001$, df = 5) (Figure 2.6) and differences between day ($p < 0.001$, df = 5) and treatment ($p < 0.001$, df = 1). Though chlorophyll *c* content were increased in thermally stressed cells no significant differences were found between control and treatment cells (Figure 2.7). No significant differences were found in the ratio of chlorophyll *c* to chlorophyll *a* in *Symbiodinium* cells throughout the experiment (Figure 2.8).

Figure 2.6 *Symbiodinium* pigment concentrations. Chlorophyll *a* per *Symbiodinium* cell subjected to control conditions (solid line) and heated treatment (dashed line). *Error bars* represent ± s.e.m., n = 5, some *error bars* obscured by data point markers. The statistical difference (sequential Bonferroni post hoc) between treatment and control is indicated as *$p < 0.05$ or **$p < 0.01$.

Figure 2.7 *Symbiodinium* pigment concentrations. Chlorophyll *c* per *Symbiodinium* cell subjected to control conditions (solid line) and heated treatment (dashed line). *Error bars* represent ± s.e.m., n = 5, some *error bars* obscured by data point markers. The statistical difference (sequential Bonferroni post hoc) between treatment and control is indicated as $*p < 0.05$ or $**p < 0.01$.

Figure 2.8 Ratio of Chl *a* to Chl *c* per *Symbiodinium* cell subjected to control conditions (solid line) and heated treatment (dashed line). *Error bars* represent ±

s.e.m., n = 5, some *error bars* obscured by data point markers. The statistical difference (sequential Bonferroni post hoc) between treatment and control is indicated as *$p < 0.05$ or **$p < 0.01$.

Differential gene expression at a pre-bleaching temperature threshold

Differential gene expression in *Symbiodinium* in response to elevated temperature were determined at day four, nineteen and twenty-eight. Overall, the number of unique DEGs detected throughout the thermal stress accounts for 37.01% of the transcriptome (FDR < 0.05). Though a large number of differentially regulated genes were detected, only 2.78% (1,776 contigs) were unique genes with ≥ 2-fold change in expression. Comparison of gene expression between all time points identified 2,798 common transcripts differentially expressed (both up and down) under elevated temperature at all time points (Figure 2.9) and these were further analyzed. Gene Ontology (GO) analysis of these common transcripts identified biological processes including 409 genes encoding proteins for cellular metabolic processes, 204 genes encoding proteins involved in cellular component organization and 133 genes encoding proteins associated with response to stress (Figure 2.10).

Figure 2.9 Thermal stress-induced differential gene expression. Venn diagram illustrates the differential expression of 35,441 genes (FDR < 0.05) of *Symbiodinium* sp., after exposure to thermal stress for four, nineteen and twenty-eight days. Venn diagram generated using the VennDiagram package in R.

Figure 2.10 Visualization of the distribution of biological process GO classifications for the 2,798 genes differentially expressed at all time points in *Symbiodinium* exposed to thermal stress (FDR < 0.05). GO annotation graph produced using Blast2GO, GO categories displayed at ontology level 3 and slices smaller than 2% grouped into the 'other' term, numbers displayed represent the number of sequences assigned to each ontology category.

Stress response

Antioxidant genes important in stress responses were detected with differential expression in thermally stressed cells. Superoxide dismutase (SOD) transcripts displayed increased expression throughout the experiment. Manganese SOD (*MnSOD*) expression were significantly increased at day nineteen (comp78421_c0, 1.166 up, $p < 0.03$), whereas, copper/zinc SOD (*CuZnSOD*) were significantly upregulated at day four (comp47575_c0, 1.243 up, $p < 0.03$) and for transcript comp28011_c0 at day nineteen (1.187 up, $p < 0.02$) and day twenty-eight (1.136 up, $p < 0.03$) (Figure 2.11 and Appendix E Table 6.3). Additionally, a single transcript encoding both ubiquitin and NiSOD domains (comp24219_c0) was downregulated on days nineteen (1.304 down, $p < 0.04$) and twenty-eight (1.197 down, $p < 0.005$) (Figure 2.11 and Appendix E Table 6.3). A further three transcripts encoding *NiSODs* and two transcripts encoding *MnSODs* were annotated (Appendix F Table 6.4), though no significant expression changes were detected under the conditions used.

Two transcripts encoding catalase peroxidase (*KatG*) exhibited significantly increased expressed in thermally stressed cells (comp80428_c0, day four, 1.215 up, $p < 0.001$ and comp61565_c0, day twenty-eight, 1.110 up, $p < 0.03$) (Figure 2.11 and Appendix E Table 6.3). Nine transcripts encoding ascorbate peroxidases (*APX*), four peroxiredoxin (*Prx*) genes, twenty-seven thioredoxin (*Trx*) genes and ten glutathione S–transferase (*GST*) transcripts were detected with significantly different expression throughout the thermal stress (Figure 2.11 and Appendix E Table 6.3).

Transcripts encoding forty-one heat shock proteins (HSPs) (*HSP90*, *HSP70*, *HSP20*), heat shock transcription factors (*HSTF*) and molecular chaperones (*DNAJ*) were detected with differential expression through all time points. In the downregulated data set, nine transcripts encoding *HSPs*, *DNAJs* and heat shock-related proteins (*HRPs*) were detected with significantly decreased expression at all three time points (Figure 2.11 and Appendix E Table 6.3). In the upregulated data set, two *HSP70* transcripts (comp71407_c0 and comp76430_c0) were detected with significantly increased expression at all time points (Figure 2.11 and Appendix E Table 6.3). Further, chloroplast targeted heat shock proteins exhibited no difference in expression patterns compared with cytosolic associated heat shock proteins.

Day Four
Day Nineteen
Day Twenty-eight
mnsod (comp78421_c0)
cuznsod (comp47575_c0)
cuznsod (comp28011_c0)
nisod (comp24219_c0)
katg (comp80428_c0)
katg (comp61565_c0)
apx (comp20095_c0)
apx (comp21891_c0)
apx (comp36166_c0)
apx (comp40867_c0)
apx (comp43143_c0)
apx (comp65681_c0)
apx (comp66617_c0)
apx (comp88508_c0)
apx (comp90586_c0)
prx (comp115094_c0)
prx (comp42154_c0)
prx (comp63575_c0)
prx (comp77639_c0)
trx (comp102079_c0)
trx (comp106802_c0)
trx (comp147613_c0)
trx (comp18594_c0)
trx (comp20192_c1)
trx (comp86111_c0)
trx (comp73859_c0)
trx (comp109991_c0)
trx (comp110338_c0)
trx (comp12847_c0)
trx (comp18562_c0)
trx (comp31838_c0)
trx (comp31956_c0)
trx (comp34679_c0)
trx (comp35649_c0)
trx (comp41119_c0)
trx (comp44208_c0)
trx (comp56148_c0)
trx (comp61747_c0)
trx (comp62517_c0)
trx (comp66152_c0)
trx (comp66165_c0)
trx (comp73552_c0)
trx (comp77231_c0)
trx (comp8010_c0)
trx (comp93578_c0)
trx (comp95736_c0)
gst (comp14913_c0)
gst (comp21843_c0)
gst (comp66650_c0)
gst (comp67710_c0)
gst (comp72345_c0)
gst (comp94166_c0)
gst (comp118633_c0)
gst (comp44743_c0)
gst (comp26118_c0)
gst (comp79594_c0)
hsp90 (comp178345_c0)
hsp90 (comp36567_c0)
hsp90 (comp44695_c1)
hsp90 (comp59092_c0)
hsp90 (comp80804_c0)
hsp90 (comp80807_c0)
hsp90 (comp37367_c0)
hsp90 (comp24798_c0)
hsp90 (comp80701_c0)
hsp70 (comp76430_c0)
hsp70 (comp26160_c0)
hsp70 (comp14362_c0)
hsp70 (comp287558_c0)
hsp70 (comp71407_c0)
hsp70 (comp72074_c0)
hsp70 (comp127611_c0)
hsp70 (comp83195_c0)
hsp70 (comp76833_c0)
hsp70 (comp45518_c0)
hsp70 (comp80358_c1)
hsp70 (comp80854_c0)
hsp70 (comp76252_c0)
hsp70 (comp79597_c0)
hsp70 (comp80127_c1)
hsp70 (comp61160_c0)
hsp70 (comp103222_c0)
hsp70 (comp90410_c0)
hsp70 (comp25006_c0)
hsp70 (comp69759_c0)
hsp70 (comp72110_c0)
hsp20 (comp82297_c0)
hsp20 (comp18274_c0)
hsp20 (comp286632_c0)
hsp20 (comp18722_c0)
hstf (comp4906_c0)
dnaj (comp47270_c0)
dnaj (comp64766_c0)
hrp (comp177335_c0)
hrp (comp50529_c0)
hrp (comp88823_c0)
hrp (comp137746_c0)
4
2
0
−2
−4

Figure 2.11 Heatmap illustration of differentially expressed stress response genes (FDR < 0.05) in *Symbiodinium* exposed to thermal stress at days four, nineteen and twenty-eight. Data are expressed as fold-changes relative to control; only significant data are shown ($p < 0.05$), non-significant data denoted as white boxes. Differential expression of antioxidant defenses (enzymatic and nonenzymatic antioxidants) and molecular chaperones. Abbreviations: CuZnSOD, copper-zinc superoxide dismutase; MnSOD, manganese superoxide dismutase; NiSOD, nickel superoxide dismutase; KatG, catalase peroxidase; APX, ascorbate peroxidase; Prx, peroxiredoxin; Trx, thioredoxin; GST, glutathione S-transferase; HSP90, heat shock protein 90; HSP70, heat shock protein 70; HSP20, heat shock protein 20; HRP, heat shock-related protein; DNAJ, chaperone DnaJ; HSTF, heat stress transcription factor. Heatmap generated using the 'pheatmap' package.

DNA damage repair and proteasomal degradation pathways were differentially regulated in thermally stressed *Symbiodinium* cells. Nine DNA repair RAD proteins (*RAD5*, *RAD16* and *RAD23-1*) were annotated with differential expression over the course of the experiment (Figure 2.12 and Appendix E Table 6.3). A single *RAD50* DNA repair transcript (comp72013_c0) displayed significantly increased expression at all time points (Figure 2.12 and Appendix E Table 6.3). DNA photolyases (*PHR*) and cryptochrome transcripts (cryptochrome DASH (*CRYD*) and cryptochrome 2 (*CRY2*) showed varied expression, six transcripts were detected with significantly increased expression and six transcripts displayed significantly decreased expression over the experiment (Figure 2.12 and Appendix E Table 6.3). Ubiquitin proteasome pathway (UPP) components were detected with significant fold changes, including ubiquitination enzymes for conjugation (E1, E2 and E3 enzymes) and deubiquitination (*DUBs*) and ubiquitin-like modifiers (*SUMO*, *NEDD8*, *ISG15*, *APG8* and *APG12*) (Appendix G Table 6.5). Though a large number (> 260) of UPP enzymes and modifiers displayed significant fold changes (Appendix G Table 6.5), only five transcripts were detected with significantly decreased expression at all time points and fifteen transcripts displayed significantly increased fold change at all time points (Figure 2.12 and Appendix E Table 6.3).

Apoptosis-like transcripts were detected in thermally stressed *Symbiodinium* cells. Seventeen transcripts encoding three metacaspase 1 isoforms (*MCA1*, *MCA1A* and *MCA1B*) were detected with differential expression in thermally stressed cells (Figure 2.12 and Appendix E Table 6.3). Three detected metacaspase contigs (two *MCA1*

isoforms and one *MCA1B* isoform) were significantly downregulated at all time points (Figure 2.12 and Appendix E Table 6.3). One transcript (comp71271_c0) encoding a *MCA1A* isoform displayed significantly increased expression at all time points (Figure 2.12 and Appendix E Table 6.3). A further thirteen metacaspase transcripts were detected with differential expression over the experiment, ten trancripts were significantly upregulated at at least one time point, and three were significantly downregulated at at least one time point (FDR < 0.05) (Figure 2.12 and Appendix E Table 6.3). Six transcripts encoding apoptosis-inducing factor homologs (*AIFB* and *AIFM3*) were detected, one *AIFM3* transcript and three *AIFB* transcripts displayed increased gene expression and two displayed decreased expression across the experiment though not all time points were significantly different to controls (Figure 2.12 and Appendix E Table 6.3).

Transcripts encoding anti-apoptosis proteins were also differentially expressed in thermally stressed cells. Three transcripts encoding inhibitors of apoptosis (*IAP*, Baculoviral IAP repeat-containing protein isoforms) were detected with increased expression on day four (*BIRC2*; comp13582_c0, 1.596 up, $p < 0.02$) and day nineteen (*BIRC3*; comp12814_c0, 1.862 up, $p < 0.04$ and comp40288_c0, 1.482 up, $p < 0.02$) (Figure 2.12 and Appendix E Table 6.3). One further apoptosis inhibitor 1 (*IAP1*, comp115062_c0) displayed decreased expression on day nineteen (1.183 down, $p < 0.02$) and day twenty-eight (1.197 down, $p < 0.03$) (Figure 2.12 and Appendix E Table 6.3). Transcripts encoding suppressors of apoptosis, Bax inhibitor 1 (*BI1*) family genes, were detected in the *Symbiodinium* transcriptome including thirteen protein lifeguard isoforms (*LFG1-LFG4*) (Figure 2.12 and Appendix E Table 6.3). In thermally stressed cells nine *BI1* family genes were detected with differential expression including three *LFG1*, three *LFG2,* two *LFG3* and a *BI1*-like protein (Figure 2.12 and Appendix E Table 6.3). Interesting three transcripts encoding the *LFG4* isoform did not exhibit altered expression under the conditions used (Appendix F Table 6.4).

Day Four
Day Nineteen
Day Twenty-eight
rad5 (comp101963_c0)
rad5 (comp78533_c0)
rad5 (comp220535_c0)
rad5 (comp35209_c0)
rad5 (comp21516_c0)
rad16 (comp73860_c0)
rad16 (comp165954_c0)
rad23-1 (comp73891_c0)
rad50 (comp72013_c0)
phr (comp67078_c0)
phr (comp72290_c0)
phr (comp56353_c0)
phr (comp45980_c0)
phr (comp74421_c0)
phr (comp137408_c0)
cryd (comp71866_c0)
cryd (comp118057_c0)
cryd (comp75893_c0)
cryd (comp18731_c0)
cry2 (comp76815_c0)
cry2 (comp41340_c0)
e3 upl (comp101171_c0)
e3 upl (comp110281_c0)
e3 upl (comp31775_c0)
ubb (comp70849_c0)
ulp (comp107763_c0)
e3 upl (comp100427_c0)
e3 upl (comp125847_c0)
e3 upl (comp21112_c0)
e3 upl (comp34342_c0)
e3 upl (comp56396_c0)
e3 upl (comp59743_c1)
e3 upl (comp66718_c0)
e3 upl (comp77019_c0)
ube3a (comp77215_c0)
ubp (comp44366_c0)
ubp (comp56934_c0)
ubp (comp60887_c0)
ubp (comp71649_c0)
url40 (comp91016_c0)
rad18 (comp72509_c0)
mca1 (comp24052_c0)
mca1 (comp41409_c0)
mca1b (comp33972_c0)
mca1a (comp71271_c0)
mca1 (comp21702_c0)
mca1 (comp71741_c0)
mca1 (comp25905_c0)
mca1 (comp95898_c0)
mca1 (comp76592_c0)
mca1 (comp54046_c0)
mca1 (comp77002_c0)
mca1a (comp96039_c0)
mca1a (comp140896_c0)
mca1b (comp67553_c0)
mca1 (comp78275_c0)
mca1 (comp125073_c0)
mca1 (comp52996_c0)
aifm3 (comp86663_c0)
aifb (comp80309_c0)
aifb (comp46418_c0)
aifb (comp80341_c1)
aifb (comp94854_c0)
aifb (comp97752_c0)
birc2 (comp135820_c0)
birc3 (comp128141_c0)
birc3 (comp40288_c0)
iap (comp115062_c0)
lfg1 (comp41335_c0)
lfg1 (comp41927_c0)
lfg2 (comp82136_c0)
lfg2 (comp20899_c0)
lfg2 (comp18631_c0)
lfg2 (comp24363_c0)
lfg3 (comp94830_c0)
lfg3 (comp103654_c0)
bi1l (comp99453_c0)
4
2
0
−2
−4

Figure 2.12 Heatmap illustration of differentially expressed stress response genes (FDR < 0.05) in *Symbiodinium* exposed to thermal stress at days four, nineteen and twenty-eight. Data are expressed as fold-changes relative to control; only significant data are shown ($p < 0.05$), non-significant data denoted as white boxes. Differential expression of stress related transcripts including genes encoding DNA damage repair proteins, selected ubiquitin proteasome pathway components, metacaspases and anti-apoptosis proteins. Abbreviations: PHR, DNA photolyase; CRYD, cryptochrome DASH; E3 UPL, E3 ubiquitin-protein ligase; UBE, ubiquitin-protein ligase 3A; UBP, ubiquitin carboxyl-terminal hydrolase; URL40, ubiquitin ribosomal protein L40; UBB, polyubiquitin-B; ULP, ubiquitin-like specific protease; MCA, metacaspase; AIF, apoptosis-inducing factor; BIR, baculoviral IAP repeat-containing protein; IAP, inhibitor of apoptosis; LFG, protein lifeguard; BI1L, Bax inhibitor-like protein. Heatmap generated using the 'pheatmap' package.

Photosynthesis related genes

Nine transcripts encoding polypeptide subunits of PS II (*psbC*, *psbF*, *psbH*, *psbK*, *psbO*, *psbP* and *psbY*) were detected with significant changes in expression (Figure 2.13). Genes encoding PS II D1 protein, PS II D2 protein and CP47 reaction centre protein (encoded on plastid mini-circles by *psbA, psbD* and *psbB* respectively) were annotated in the transcriptome (Appendix F Table 6.4), though no significant fold changes were detected under the experimental conditions used (FDR < 0.05). One transcript encoding *psbF* (comp42202_c0), which encodes cytochrome b559 β forming part of the reaction centre core of PS II, displayed significant downregulation at all time points (Figure 2.13). Seven transcripts encoding polypeptide subunits of PS I (*psaA*, *psaC*, *psaD*, *psaF*, *psaJ* and *psaL*) were detected with significant changes in expression (Figure 2.13). Plastid minicircle genes encoding integral membrane peptide subunits of PS I were annotated in the transcriptome, expression of the PS I P700 chlorophyll *a* apoprotein A1 gene (*psaA*) were decreased at day twenty-eight (3.306 down fold change, $p < 0.02$) (Figure 2.13), no significant differences in expression were detected for the PS I P700 chlorophyll *a* apoprotein A2 (*psaB*) (Appendix F Table 6.4). Differential expression of transcripts encoding ferredoxin-NADP+ reductase (*petH* (comp42084_c0)) and ferredoxin (*petF* (comp68408_c0 and comp35855_c0)) were detected with significant differences in expression under thermal stress (Figure 2.13 and Appendix E Table 6.3).

psbc (comp20334_c0)
psbf (comp42202_c0)
psbh (comp79713_c0)
psbk (comp42379_c0)
psbo (comp80077_c0)
psbp (comp21047_c0)
psbp (comp110020_c0)
psbp (comp98847_c0)
psby (comp81492_c0)
psaa (comp20374_c0)
psac (comp81124_c0)
psad (comp74159_c0)
psaf (comp80131_c1)
psaj (comp79899_c0)
psal (comp78356_c0)
psal (comp40074_c0)
peth (comp42084_c0)
petf (comp68408_c0)
petf (comp35855_c0)
rbcl (comp68158_c0)
rcbl (comp79516_c0)
rbcl (comp63174_c0)
rbcl (comp78141_c0)
rbcl (comp71061_c0)
rbcl (comp79880_c0)
rbcl (comp55807_c0)
rbcl (comp80942_c0)
rbcl (comp80955_c0)
zep (comp79868_c0)
zep (comp88413_c0)
vde (comp106814_c0)
vde (comp86229_c0)
vde (comp42301_c0)
cb (comp20639_c0)
cb (comp78907_c0)
cb (comp66799_c0)
cb (comp81913_c0)
cb (comp25880_c0)
cb (comp108755_c0)
cb (comp39540_c0)
ccac (comp74084_c0)
ccac (comp23_c0)
fcp (comp74174_c1)
fcp (comp79970_c0)
fcp (comp49189_c0)
fcp (comp75958_c0)
fcpa (comp108983_c0)
fcpb (comp41609_c0)
fcpb (comp116163_c0)
fcpb (comp80251_c0)
fcpb (comp40433_c0)
fcpb (comp74012_c0)
fcpb (comp104344_c0)
fcpb (comp60532_c0)
fcpb (comp49803_c0)
fcpe (comp82434_c0)
fcpe (comp78034_c0)
fcpe (comp79363_c0)
fcpe (comp71870_c0)
fcpe (comp78124_c0)
fcpe (comp47350_c0)
fcpe (comp76716_c0)
fcpe (comp78175_c0)
fcpe (comp80062_c0)
fcpe (comp80141_c0)
fcpe (comp79645_c1)
fcpe (comp73249_c0)
fcpe (comp18050_c0)
fcpe (comp116919_c0)
fcpe (comp71239_c0)
fcpf (comp80391_c0)
fcpf (comp62255_c0)
fcpf (comp79914_c0)
fcpf (comp41586_c0)
fcpf (comp68073_c0)
fcpf (comp79305_c0)
fcpf (comp44655_c0)
fcpf (comp71241_c0)
fcpf (comp73165_c0)
fcpf (comp79645_c0)
fcpf (comp40015_c0)
fcpf (comp70173_c0)
lh18 (comp76981_c0)
lh18 (comp89863_c0)
lh18 (comp80141_c1)
lh18 (comp78161_c0)
li818 (comp70226_c0)
Day Four
Day Nineteen
Day Twenty−eight
4
2
0
−2
−4

Figure 2.13 Expression heatmaps of differentially expressed photosynthesis, metabolism and growth genes (FDR < 0.05) in *Symbiodinium* after exposure to thermal stress for four, nineteen and twenty-eight days. Data are expressed as fold-changes relative to control; only significant data are shown ($p < 0.05$), non-significant data denoted as white boxes. Differential expression of photosynthesis related genes. Abbreviations: psb, photosystem II protein; psa, photosystem I protein; peth, ferredoxin-nadp reductase; petf, ferredoxin; rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; zep, zeaxanthin epoxidase; vde, violaxanthin de-epoxidase; cb, chlorophyll binding protein; ccac, caroteno-chlorophyll a-c binding protein; fcp, fucoxanthin-chlorophyll a-c binding protein; lh18, light-harvesting complex i protein; li818, chlorophyll a-b binding protein l1818. Heatmap generated using the 'pheatmap' package.

Plastid-targeted genes were differentially expressed in thermally stressed *Symbiodinium* cells. For example nine transcripts encoding the unique Form II ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCo) enzyme (*rbcL*, large subunit) were differentially expressed under thermal stress (Figure 2.13 and Appendix E Table 6.3). Expression of RuBisCo transcripts were temporally varied, six isoforms displayed significantly increased expression at day twenty-eight (largest 1.465 up fold change). Additionally, a single RuBisCo isoform (comp68158_c0) with significant differential expression at all time points were identified (Figure 2.13 and Appendix E Table 6.3).

Genes involved in photoprotective mechanisms in *Symbiodinium* including non-photochemical quenching were differentially expressed under thermal stress. In *Symbiodinium*, the xanthophyll cycle involves the de-epoxidation and epoxidation reactions of diadinoxanthin/ diatoxanthin for energy dissipation and to regulate the amount of energy reaching the photosystem reaction centers. Three violaxanthin de-epoxidase (*vde*) transcripts were detected with significant changes in expression at time points throughout the thermal stress (Figure 2.13 and Appendix E Table 6.3). Two zeaxanthin epoxidase (*zep*) transcripts (comp79868_c0 and comp88413_c0), displayed significantly increased fold changes through all time points (Figure 2.13 and Appendix E Table 6.3).

Expression of the nuclear-encoded light harvesting complex (LHC) proteins responsible for enhancing light transfer to core photosystems and photoprotection were determined in thermally stressed cells. A transcript encoding the extrinsic

water-soluble LHC peridinin-chlorophyll *a*-binding protein (PCP) was detected in the *Symbiodinium* transcriptome (comp80938_c0) (Appendix F Table 6.4), though no significant fold changes were detected under the experimental conditions used (FDR < 0.05). Fifty-four transcripts encoding the *Symbiodinium* specific intrinsic membrane-bound LHC isoforms (chlorophyll *a*-chlorophyll c_2-peridinin protein (acpPC)) were detected with significant fold changes during the thermal stress (PFAM ID PF00504.16. Blast annotations shown, *CB*, chlorophyll binding protein; *CCAC*, caroteno-chlorophyll a-c binding protein; *FCP*, fucoxanthin-chlorophyll a-c binding protein; *LH18*, Light-harvesting complex I protein; *LI818*, chlorophyll a-b binding protein L1818) (Figure 2.13 and Appendix E Table 6.3). Of these fifty-four intrinsic LHC transcripts, fourteen were detected with significant fold changes at all time points (thirteen were upregulated and one transcript displayed mixed expression) (Figure 2.14). The remaining forty light-harvesting protein complex transcripts detected displayed predominately increased expression at all time points (85% up fold change), though not all were significantly different to controls (Figure 2.13 and Appendix E Table 6.3). Additionally, a further twenty-nine transcripts annotated in the transcriptome as light-harvesting protein complexes displayed no change in expression under the experimental conditions (Appendix F Table 6.4).

Metabolism and growth

Genes encoding enzymes for fatty acid desaturation and fatty acid β-oxidation were detected with differential expression in *Symbiodinium* cells exposed to thermal stress. Seven delta-fatty acid desaturase transcripts were detected with differential expression in thermally stressed cells (Figure 2.14). Five contigs were annotated as delta-5 desaturases (isoforms *fad5A*, *fad5B* and *fad5C*) and two were annotated as palmitoyl-monogalactosyldiacylgycerol delta-7 desaturase. Throughout the experiment two palmitoyl-monogalactosyldiacylgycerol delta-7 desaturase (*fad7*) contigs were downregulated (comp72598_c0, day four, 1.399 down, $p < 0.04$; comp21243_c0, day nineteen, 1.565 down $p < 0.01$, day twenty-eight, 1.210 down $p < 0.01$) whereas, three of the delta-5 desaturase contigs were significantly upregulated at day nineteen and day twenty-eight (Figure 2.14 and Appendix E Table 6.3). Representatives of all genes involved in the β-oxidation of fatty acids

were differentially expressed over the course of the experiment. Six transcripts encoding acyl-CoA dehydrogenases (*ACADs*) were detected with differential expression over the course of the experiment, with five displaying significantly increased fold changes on day twenty-eight (Figure 2.14). Transcripts encoding enoyl-CoA hydratases (3 *ECH*), fatty acid oxidation complex subunit alpha (1 *FADJ*) and peroxisomal bifunctional enzymes (1 *MFEA* and 2 *ECHP*) were all detected with increased expression in thermally stressed cells (Figure 2.14 and Appendix E Table 6.3). One transcript, encoding a probable 3-hydroxyacyl-CoA dehydrogenase (*HCDH*), was detected with decreased expression on day twenty-eight (comp107722_c0, 1.102 down $p < 0.02$) (Figure 2.14). Three transcripts encoding β-ketothiolases (*FADA*), responsible for the final cleavage step of the β-oxidation pathway were detected with significantly increased expression on day twenty-eight (Figure 2.14). One of the β-ketothiolases (comp79906_c0) was annotated as a peroxisomal associated transcript, and displayed significantly increased expression on day nineteen (1.276 up, $p < 0.01$) and day twenty-eight (1.169 up, $p < 0.03$) (Figure 2.14). Further, three transcripts encoding peroxisome membrane proteins (*PMP34*), two peroxisome adenine nucleotide carrier isoforms (*PNC1* and *PNC2*) and two peroxisome ATP-binding cassette sub-family D members were all detected with significant increases in expression under these conditions (Appendix E Table 6.3).

Glyoxylate cycle and gluconeogenic pathway enzymes were detected with differential expression in thermally stressed cells. Transcripts encoding enzymes of the glyoxylate cycle including citrate synthase (*CS*), aconitase (*acnB*), isocitrate lyase (*aceA*), malate synthase (*aceB*) and malate dehydrogenase (*MDH2*) were detected with differential expression in thermally stressed *Symbiodinium* cells (Figure 2.14). Specifically, four transcripts encoding isocitrate lyases (aceA) were detected with significant changes in expression under thermal stress (Figure 2.14 and Appendix E Table 6.3). Two of the isocitrate lyase contigs displayed decreased expression on day four, whereas on day twenty-eight three of the isocitrate lyase contigs displayed significantly increased fold change compared to controls (Figure 2.14). Two malate synthase (*aceB*) transcripts were detected with decreased

expression, comp85614_c0 on day four (1.171 down, $p < 0.04$) and comp96309_c0 on day nineteen (1.259 down, $p < 0.002$) and day twenty-eight (1.263 down, $p < 0.008$) (Figure 2.14). Three transcripts encoding succinate dehydrogenase flavoprotein subunits were also detected with significantly increased expression (comp25945_c0 on day twenty eight, 1.294 up, $p < 0.009$), and comp72308_c0 on day nineteen, 1.433 up, $p < 0.02$ and day twenty-eight, 1.287 up, $p < 0.004$, and comp337315_c0 on day nineteen, 31.456 up, $p < 0.02$ and day twenty-eight 25.270 up, $p < 0.03$) (Figure 2.14) In addition, three contigs encoding phosphoenolpyruvate carboxykinase (*PEPCK*) were detected with differential expression in thermally stressed cells (Figure 2.14 and Appendix E Table 6.3).

Serine/threonine-protein kinases are crucial components of diverse signaling pathways and for regulation of cell proliferation, meiosis and apoptosis. One hundred and seventy-seven transcripts representing more than twenty serine/threonine-protein kinase families were detected with significant changes in expression in thermally stressed cells (Appendix E Table 6.3). Nine transcripts encoding three classes of aurora kinases (*Aurora-A*, *Aurora-B* and *Aurora-C* (*AurK*)) and two aurora-related kinases (*ARKs*) were detected with significant fold changes throughout the experiment (Figure 2.14 and Appendix E Table 6.3). Twenty-two never-in-mitosis A serine/threonine kinase (*Nek*) transcripts representing seven Nek families were detected with differential expression (Figure 2.14 and Appendix E Table 6.3). One transcript encoding a cyclin-dependent kinase (*CDK5*, comp29198_c0) was detected with significantly decreased expression at all time points (Figure 2.14 and Appendix E Table 6.3).

Differential regulation of cellular component biosynthesis was detected in thermally stressed *Symbiodinium* cells. Transcripts encoding lipid biosynthetic acyl carrier protein (*acp*) (comp62787_c0), CDP-diacylglycerol-serine O-phosphatidyltransferase (*pss*) (comp44746_c0) and two phosphatidylserine decarboxylase proenzymes (*psd*) exhibited decreased expression in thermally stressed cells (Figure 2.14 and Appendix E Table 6.3). Four magnesium-chelatase subunit H (*chlH*) transcripts were detected with significantly increased expression over the experiment, whereas, two magnesium-chelatase subunit I (*chlI*) transcripts exhibited decreased expression on

day twenty-eight (comp46484_c0, 1.173 down, $p < 0.001$ and comp39765_c0, 1.181 down, $p < 0.05$) (Figure 2.14 and Appendix E Table 6.3). Additionally, one transcript encoding a magnesium-chelatase subunit D (*chlD*) (comp84001_c0) (Appendix F Table 6.4), displayed no changes in expression under the conditions used here (FDR < 0.05).

Day Four
Day Nineteen
Day Twenty-eight
31.456
25.270
fad5a (comp215171_c0)
fad5b (comp140217_c0)
fad5b (comp57362_c0)
fad5c (comp60315_c0)
fad5c (comp87976_c0)
fad7 (comp72598_c0)
fad7 (comp21243_c0)
acad (comp101969_c0)
acad (comp36804_c0)
acad (comp37569_c0)
acad (comp46814_c0)
acad (comp78877_c0)
acad (comp62055_c0)
ech (comp25743_c0)
ech (comp27873_c0)
ech (comp96336_c0)
fadj (comp74423_c0)
mfea (comp14670_c0)
echp (comp77958_c0)
echp (comp78677_c0)
hcdh (comp107722_c0)
fada (comp78395_c0)
fada (comp79891_c0)
fada (comp79906_c0)
cs (comp27261_c0)
cs (comp82087_c0)
cs (comp94429_c0)
acnb (comp45820_c0)
acea (comp169919_c0)
acea (comp203351_c0)
acea (comp72338_c0)
acea (comp79153_c0)
aceb (comp85614_c0)
aceb (comp96309_c0)
mdh2 (comp102494_c0)
mdh2 (comp10299_c0)
mdh2 (comp127234_c0)
mdh2 (comp25855_c0)
mdh2 (comp47624_c0)
sdh (comp25945_c0)
sdh (comp337315_c0)
sdh (comp72308_c0)
pepck (comp114921_c0)
pepck (comp19221_c0)
pepck (comp88707_c0)
aurkb (comp42268_c0)
aurkb (comp43256_c0)
aurkb (comp53954_c0)
aurkc (comp76653_c0)
aurkaa (comp99904_c0)
aurkab (comp134026_c0)
aurkab (comp21024_c0)
ark1 (comp48941_c0)
ark1 (comp46241_c1)
nek1 (comp43847_c0)
nek1 (comp44719_c0)
nek1 (comp54331_c0)
nek1 (comp65528_c0)
nek1 (comp68026_c0)
nek1 (comp30155_c0)
nek1 (comp24271_c0)
nek1 (comp109521_c0)
nek1 (comp130708_c0)
nek2 (comp58032_c1)
nek3 (comp163489_c0)
nek4 (comp14102_c0)
nek4 (comp68822_c0)
nek4 (comp134332_c0)
nek5 (comp32274_c0)
nek5 (comp109786_c0)
nek5 (comp40381_c0)
nek5 (comp12853_c0)
nek5 (comp13966_c0)
nek5 (comp102834_c0)
nek6 (comp27360_c0)
nek8 (comp57105_c0)
cdk5 (comp29198_c0)
acp (comp62787_c0)
pss (comp151799_c0)
psd (comp44050_c0)
psd (comp44746_c0)
chlh (comp51082_c0)
chlh (comp158198_c0)
chlh (comp31503_c0)
chlh (comp71167_c0)
chli (comp46484_c0)
chli (comp39765_c0)
4
2
0
−2
−4

Figure 2.14 Expression heatmaps of differentially expressed metabolism and growth genes (FDR < 0.05) in *Symbiodinium* after exposure to thermal stress for four, nineteen and twenty-eight days. Data are expressed as fold-changes relative to control; only significant data are shown ($p < 0.05$), non-significant data denoted as white boxes. Differential expression of fatty acid desaturases, fatty acid β-oxidation enzymes, glyoxylate cycle enzymes, selected serine/threonine-protein kinases and cellular component biosynthesis genes. High expression levels of a SDH transcript are denoted numerically.Abbreviations: fad, delta fatty acid desaturase; ACAD, acyl-CoA dehydrogenase; ECH, enoyl-CoA hydratase; FADJ, fatty acid oxidation complex subunit; MFEA, peroxisomal multifunctional enzyme A; ECHP, peroxisomal bifunctional enzyme; HCDH, 3-hydroxyacyl-CoA dehydrogenase; FADA, β-ketothiolase; CS, citrate synthase; acnB, aconitase; aceA, isocitrate synthase; aceB, malate synthase; MDH2, malate dehydrogenase; SDH, succinate dehydrogenase (ubiquinone) flavoprotein subunit; PEPCK, phosphoenolpyruvate carboxykinase; Heatmap generated using the 'pheatmap' package.

Meiosis-specific and meiosis-related genes previously annotated in *Symbiodinium* (Chi et al., 2014; Levin et al., 2016) were detected in the transcriptome and in the repertoire of differentially expressed transcripts. Eight meiosis-specific transcripts representing four genes (three *Dmc1*, two *Hop2*, one *Mnd1* and two *Msh4)* were annotated with differential expression in thermally stressed cells (Figure 2.15 and Appendix E Table 6.3). Twenty meiosis-related transcripts representing seventeen genes were also annotated with differential expression in thermally stressed *Symbiodinium* cells (Figure 2.15 and Appendix E Table 6.3). Additionally, fifty-two transcripts encoding protein MEI2 and MEI2-like isoforms were detected with differential expression (Figure 2.15 and Appendix E Table 6.3). *MEI2* genes have been annotated in *Shizosaccharomyces pombe* and *MEI2-like* proteins have been annotated in *Arabidopsis thaliana* and *Oryza sativa subsp. japonica*. Two radial spoke head homologs (*Rsph1*) were detected with differential expression (Figure 2.15 and Appendix E Table 6.3), previously detected around the chromosomes during metaphase in male gametes undergoing meiotic division. However, *Rsph1* has also been implicated in axenomal central pair regulating dynein activity for flagella and cilia movement.

dlh1 (comp116145_c0)
dmc1 (comp122198_c0)
dmc1 (comp151318_c0)
hop2 (comp147597_c0)
hop2 (comp43393_c0)
mnd1 (comp52471_c0)
msh4 (comp151677_c0)
msh4 (comp191937_c0)
atm (comp34940_c0)
atm (comp53928_c0)
brca1 (comp45520_c0)
brca2 (comp79139_c0)
cdc2h (comp76876_c0)
dnl (comp71994_c0)
exo (comp21276_c0)
fen (comp84040_c0)
gr1 (comp66751_c0)
mlh3 (comp51373_c0)
msh2 (comp79008_c0)
mus (comp31875_c0)
rhp (comp58263_c0)
rd231 (comp73891_c0)
rad24 (comp338383_c0)
rad50 (comp72013_c0)
ra54b (comp1992_c0)
rtel (comp55030_c0)
rtel (comp65380_c0)
xrcc5 (comp69445_c0)
mei2 (comp82451_c0)
mei2 (comp72679_c0)
mei2 (comp11213_c0)
mei2 (comp11663_c0)
mei2 (comp3090_c0)
mei2-like 1 (comp93139_c0)
mei2-like 1 (comp79032_c0)
mei2-like 2 (comp74450_c0)
mei2-like 2 (comp33650_c0)
mei2-like 2 (comp77579_c0)
mei2-like 2 (comp102634_c0)
mei2-like 2 (comp216824_c0)
mei2-like 2 (comp59081_c0)
mei2-like 2 (comp90032_c0)
mei2-like 2 (comp70257_c0)
mei2-like 2 (comp73286_c0)
mei2-like 2 (comp53430_c0)
mei2-like 3 (comp97757_c0)
mei2-like 3 (comp74366_c0)
mei2-like 3 (comp41180_c0)
mei2-like 3 (comp147342_c0)
mei2-like 3 (comp55332_c0)
mei2-like 3 (comp55889_c0)
mei2-like 3 (comp77201_c0)
mei2-like 3 (comp89398_c0)
mei2-like 3 (comp49964_c0)
mei2-like 3 (comp65334_c0)
mei2-like 3 (comp19571_c0)
mei2-like 3 (comp25019_c0)
mei2-like 4 (comp60896_c0)
mei2-like 4 (comp32962_c0)
mei2-like 4 (comp80393_c0)
mei2-like 4 (comp79803_c0)
mei2-like 4 (comp56101_c0)
mei2-like 4 (comp52203_c0)
mei2-like 4 (comp133629_c0)
mei2-like 4 (comp135921_c0)
mei2-like 4 (comp55194_c0)
mei2-like 4 (comp15254_c0)
mei2-like 4 (comp139859_c0)
mei2-like 5 (comp31235_c0)
mei2-like 5 (comp20863_c0)
mei2-like 5 (comp95583_c0)
mei2-like 5 (comp28619_c0)
mei2-like 5 (comp55231_c0)
mei2-like 5 (comp51440_c0)
mei2-like 5 (comp106586_c0)
mei2-like 5 (comp57820_c0)
mei2-like 5 (comp20572_c0)
mei2-like 5 (comp20280_c0)
mei2-like 5 (comp78683_c0)
mei2-like 6 (comp134993_c0)
rsph (comp78297_c0)
rsph (comp77736_c0)
Day Four
Day Nineteen
Day Twenty-eight
4
2
0
−2
−4

Figure 2.15 Differential expression of meiosis-specific, meiosis-related and RNA binding proteins. Expression heatmaps of differentially expressed genes (FDR < 0.05) in *Symbiodinium* after exposure to thermal stress for four, nineteen and twenty-eight days. Data are expressed as fold-changes relative to control; only significant data are shown ($p < 0.05$), non-significant data denoted as white boxes. Abbreviations: ATM, serine/threonine protein kinase ATM; BRCA, breast cancer susceptibility homolog; CDCH2, cell division control protein; DLH1, meiotic recombination protein; DMC1, meiotic recombination protein; DNL, DNA ligase; EXO, exonuclease; FEN, flap endonuclease; GR1, protein gamma response 1; HOP2, homologous-pairing protein 2 homolog; MEI2, meiosis protein; MEI2-like, meiosis protein-like protein; MLH, DNA mismatch repair protein; MND, meiotic nuclear division protein; MSH, MutS protein homolog; MUS, crossover junction endonuclease; RA, DNA repair and recombination protein; RAD24, DNA damage checkpoint protein; RAD50, DNA repair protein; RD, DNA repair protein; RSPH, radial spoke head homolog; RTEL, regulator of telomere elongation helicase; XRCC, X-ray repair cross-complementing protein. Heatmap generated using the ‘pheatmap’ package.

Discussion

Predictions for future climate conditions estimate the sea surface temperature to rise between 1.1 - 6.4 °C depending on emissions scenarios (Solomon et al., 2007). The implications of this change for coral reefs and the marine ecosystems they support are unknown. However, this exposure of coral reefs to elevated temperatures may have catastrophic consequences and result in the loss of many species. Through examining the physiological response of *Symbiodinium* cultures to elevated temperatures, and linking this to molecular processes that are altered under thermal stress we may begin to understand how reefs may be affected in the future. In this study, we exposed cultured *Symbiodinium* sp. (clade F) to thermal stress (twenty-eight days, ~ 30 - 31.5 °C) (Figure 2.1) and generated a library of contigs that represent the transcriptome under future temperature conditions.

Analysis of the *Symbiodinium* transcriptome revealed more than thirty-seven percent were differentially expressed under thermal stress (FDR < 0.05). A large number of DEGs (23,654 unique contigs) were detected, of which 2,798 were differentially expressed at all time points (Figure 2.9). The majority of DEGs, (21,878 genes, (92.49%)), displayed a ≤ 2-fold change in expression. This is reflective of previous targeted expression studies in *Symbiodinium* where relatively small changes in gene expression were determined (Leggat et al., 2011a; Rosic et al., 2011a; McGinley et al., 2012; Ogawa et al., 2013; Gierz et al., 2016 (Chapter 3)), due to this it has been hypothesised that translational or post-translational regulation may be critical in *Symbiodinium* cellular responses. Biological process GO visualization revealed that distribution of combined time point DEGs (2,798 transcripts) were relatively even in assigned terms between those up and downregulated transcripts (Appendix D Figure 6.4). Under these conditions regulation of molecular processes in this *Symbiodinium* strain is variable, with components of many pathways exhibiting dissimilar expression patterns.

Over the course of the experiment temperature significantly affected *Symbiodinium* density and photosynthetic efficiency. Previously, growth rates of six cultured *Symbiodinium* strains (representing clades A, B, C, D and F) with various thermal

tolerances where determined at different temperatures (25 °C, 30 °C and 33 °C) (Karim et al., 2015). Showing that increased temperatures can alter growth rates and photosynthetic efficiency differently, resulting in the classification of three categories depending on the thermal tolerance of the strain (Karim et al., 2015). In this study, *Symbiodinium* cell density were decreased from day four compared to controls (Figure 2.2), and the photosynthetic ability of thermally stressed cells were maintained until day fourteen (Figure 2.3). The slight but significantly depressed dark-adapted yield (day fourteen, nineteen and twenty-eight) (Figure 2.3) indicates that cells exposed to elevated temperatures were exhibiting altered photosynthetic efficiencies in response to thermal stress. However, as complete loss of photosynthetic efficiency did not occur (Figure 2.3), this strain is categorized as photosynthetically tolerant though growth response were highly sensitive to elevated temperature (Karim et al., 2015).

Analysis of chlorophyll pigments in *Symbiodinium* found significantly increased chlorophyll content in a manner seen previously (McBride et al., 2009; Ogawa et al., 2013; Gierz et al., 2016 (Chapter 3)). Chlorophyll *a* concentration were significantly higher in thermally stressed cells on days nineteen and twenty-eight (Figure 2.6). Comparison of growth rates and chlorophyll content in *Symbiodinium californium* found cultures exhibiting low growth rates (incubated at 5, 10 and 30 °C) also showed an increased chlorophyll *a* content, whereas those actively growing had reduced chlorophyll *a* content (McBride et al., 2009). In phytoplankton, variation of chlorophyll *a*-specific absorption has been attributed to packaging of chlorophylls within different pigment-protein complexes (chlorophyll *a*-chlorophyll c-peridinin (ACP) versus PS I) (Bissett et al., 1997). It is possible that the increased chlorophyll *a* content observed in thermally stressed *Symbiodinium* cells may be due to changes in the specific pigment-protein complexes within the chloroplasts. Analysis of chlorophyll *c* content (Figure 2.7) and the ratio of chlorophyll *c* to chlorophyll *a* (Figure 2.8) found that there were no significant differences between controls and thermally stressed cells. Biosynthesis of chlorophyll involves the ATP-dependent insertion of a magnesium ion into protoporphyrin IX, catalyzed by three magnesium chelatase subunits (Von Wettstein et al., 1995). Six transcripts encoding two of the three magnesium-chelatase subunits (two *chlI* and four *chlH*) were differentially

expressed in thermally stressed cells (Figure 2.14 and Appendix E Table 6.3). However, expression of these subunits were not consistent with *chlI* subunits detected with significant decreased fold changes, no changes detected in a *chlD* subunit, and four *chlH* subunit with significantly increased expression during exposure to increased temperatures (Figure 2.14 and Appendix E Table 6.3). The implications for the variable expression of these subunits critical for chlorophyll biosynthesis requires further investigation.

Measurements of photosynthetic ability are often employed as indicators of *Symbiodinium* cell health (Buxton et al., 2012; Hill and Takahashi, 2014). In this study, Induction Curve + Recovery kinetic recording type were used to determine the ability of cells to respond to light stress. Significant decreases in effective quantum yield at the end of the induction phase were detected on day four (Figure 2.4) and may be indicative of an early photosynthetic response to elevated temperatures. Throughout the remainder of the experiment no significant difference in effective quantum yield between controls and thermally stressed cells were detected after the recovery phase, though values were slightly depressed in treatment cells (Figure 2.4). NPQ were found to be significantly decreased in treated cells on day nineteen, and may indicate that the photoprotective mechanisms of cells were impacted by exposure to thermal stress (Figure 2.5). Though little changes were observed in NPQ, we detected xanthophyll cycle enzyme genes (*vde* and *zep*) with significantly different expression (Figure 2.13 and Appendix E Table 6.3). *Vde* and *zep* are responsible for the epoxidation and de-epoxidation of dinoxanthin/ diadinoxanthin as a photoprotective mechanism by dissipating excess energy.

Differential expression of the *Symbiodinium* antioxidant network

Photobleaching in *Symbiodinium* induced by thermal stress and high solar irradiance has been linked to oxidative damage resulting from the production of reactive oxygen species (ROS) (Murata et al., 2007; Takahashi et al., 2008; Krueger et al., 2014). ROS can be deleterious to cells resulting in oxidative damage to lipids, proteins and DNA but may also function as second messengers for signal transduction (Lesser, 2006). Defenses against ROS in *Symbiodinium* and other photoautotrophs include

an antioxidant network of enzymes such as SODs, catalases and peroxidases as well as nonenzymatic antioxidants such as glutathione, thioredoxins (*Trx*), peroxiredoxins (*Prx*) and carotenoids (via the xanthophyll cycle) (Lesser, 2006; Bayer et al., 2012; Krueger et al., 2014; Krueger et al., 2015). Comparison of *Symbiodinium* types has shown that the antioxidant network and the antioxidant response to thermal stress can vary between strains of different thermal tolerance with implications for photosynthesis and cell viability (Krueger et al., 2014). Within *Symbiodinium* cells exposed to thermal stress transcripts encoding CuZnSOD, MnSOD, KatG and ZEP were all significantly upregulated whereas, expression of APX and VDE transcripts varied (Figure 2.11, Figure 2.13 and Appendix E Table 6.3). Within the *Symbiodinium* transcriptome assembly, three transcripts encoding prokaryotic-like nickel superoxide dismutase (NiSOD) and one transcript encoding both ubiquitin and NiSOD domains were also identified. Genes containing ubiquitin/NiSOD and NiSOD domains have also been identified in the antioxidant gene repertoire of *Symbiodinium* sp. CassKB8 and *Symbiodinium* sp. Mf1.05b (Bayer et al., 2012) and two C1 type *Symbiodinium* (Levin et al., 2016) and may represent genes acquired from prokaryotes by lateral gene transfer. Within *Symbiodinium* sp. CassKB8 and *Symbiodinium* sp. Mf1.05b a high number of Trx domain containing genes (106 and 73 *Trx* genes respectively (PF00085.14)) were identified (Bayer et al., 2012), within this transcriptome assembly 85 Trx domain containing genes were identified with twenty-seven *Trx* genes differentially expressed under thermal stress (Figure 2.11 and Appendix E Table 6.3). Trx superfamily proteins (Trx and Trx-like proteins) have roles in the oxidative stress response by regulating the redox state, aid in the repair of damaged photosystems (Nishiyama et al., 2011) and regulate many photosynthetic enzymes in plants (including Calvin cycle enzymes such as glyceraldehyde 3-phosphate dehydrogenase, phosphoribulokinase and RuBisCo activase) (Hisabori et al., 2007). Differential expression of components of the antioxidant network implicated in protecting cells from ROS and regulating photosynthetic processes were detected here in thermally stressed *Symbiodinium*. Given that we did not observe a loss in photosynthetic ability of cells, but did observe a shift in metabolism, and don't know the mechanism of regulation of RuBsiCo form II in *Symbiodinium* the effect of cell

redox state on photosynthesis and CO_2 fixation via the Calvin cycle under thermal stress requires further investigation.

Cell cycle in thermally stressed *Symbiodinium*

The life cycle of *Symbiodinium* has predominately been considered asexual, deduced by studying morphological transitions and direct observations of mitotic growth. During the vegetative growth phase, haploid cells undergo a diel cycle of mitosis (Fitt and Trench, 1983; Santos and Coffroth, 2003). Progression of the cell cycle in *Symbiodinium* has been shown to halt when fatty acid syntheses were inhibited by addition of cerulenin (interpreted from decreased free fatty acid and phosphatidylethanolamine (PE) content) (Wang et al., 2013). In prokaryotic and eukaryotic cells, PEs are structural components of membranes and *de novo* synthesis of PE occurs via the CDP-ethanolamine pathway a branch of the Kennedy pathway (Gibellini and Smith, 2010). Mutant *Escherichia coli* cells deficient in PE due to defective CDP-ethanolamine pathway genes (*pss* and *psd)*, showed reduced transcription of flagella genes, resulting in decreased motility and chemotaxis compared to wild type cells (Shi et al., 1993). In this study, components of the CDP-ethanolamine pathway were differentially expressed in thermally stressed cells, one CDP-diacylglycerol-serine-O-phosphatidyltransferase transcript (encoded by *pss*) displayed significantly decreased expression on day nineteen (comp44746_c0) and two phosphatidylserine decarboxylase transcripts (encoded by *psd*) were significantly decreased (comp151799_c0 on day four, and comp44050_c0 on day nineteen and twenty-eight) (Figure 2.14 and Appendix E Table 6.3). Therefore, in thermally stressed *Symbiodinium,* decreased expression of CDP-ethanolamine pathway genes for PE synthesis could impact the cell cycle due to reduced glycerophospholipid content available for cellular processes.

Mitotic kinases implicated in cell cycle regulation and DNA damage responses were identified with differential expression in thermally stressed *Symbiodinium.* For example, aurora kinases regulate cell proliferation by controlling M-phase events, such as mitotic spindle attachment (Aurora-A) (Nigg, 2001) and NimA-related kinases (Neks) have roles in cell cycle control regulating establishment of the mitotic

spindle, chromosome condensation, response to DNA damage and flagella/cilia development (Nigg, 2001; Fry et al., 2012). Additionally cell division control protein homologs and cyclin dependent kinases were also detected with differential expression in thermally stressed cells (Figure 2.14 and Appendix E Table 6.3). Expression of these cell cycle regulators were not consistent across the gene families identified. Of the nine differentially expressed transcripts encoding *nek1* genes in thermally stressed cells, four contigs were significantly increased and three were significantly decreased on day twenty-eight (Figure 2.14 and Appendix E Table 6.3). Therefore, although a large number of mitotic kinases exhibited differential expression, linking the expression of these cell cycle regulatory proteins in thermally stressed cells to the observed physiological response is not feasible with the current data.

Documentation of *Symbiodinium* sexual recombination has been difficult, as cytological evidence of karyogamy has not been found (Chi et al., 2014). However, investigation of population genetic patterns and advances in genomic data has potentially identified a collection of meiotic genes in *Symbiodinium* (Chi et al., 2014; Wilkinson et al., 2015; Levin et al., 2016). In this study we identified a number of these meiosis-specific and meiosis-related transcripts (Figure 2.15 and Appendix E Table 6.3), providing further support for the occurrence of sexual recombination in *Symbiodinium.* Additionally the occurrence of sexual recombination may be restricted to specific conditions, i.e. to free-living symbionts, or be inactivated by symbiotic conditions (Chi et al., 2014), reducing the opportunities for cytological observation. Recently, analysis of the transcriptional response of two type C1 *Symbiodinium* to heat stress (32 °C), identified upregulation of two mutS homolog genes (*Msh4* and *Msh5*) and no significant changes in other *Msh* genes (*Msh1*, *2*, *3*, and *6*), suggesting these genes are essential to meiosis lending support to adaption (Levin et al., 2016). In this study, two transcripts annotated as *Msh4* were significantly downregulated on day nineteen (comp151677_c0 and comp191937_c0), one transcript annotated as *Msh2* exhibited significant upregulation on days four and nineteen (comp79008_c0) (Appendix E Table 6.3), whereas, three transcripts annotated as *Msh5* (comp169914_c0, comp201605_c0 and comp32251_c0) and an *Msh6* transcript (comp43641_c0) displayed no change

in expression (Appendix F Table 6.3). In this study exposure of *Symbiodinium* to thermal stress resulted in reduced growth rate potentially inducing a cell cycle phase conducive to meiotic-like division. However, differential expression of these genes may also be for repair of DNA damage or the stabilization of DNA in thermally stressed *Symbiodinium*.

Genes encoding RNA binding proteins containing the RNA recognition motif (RRM) were detected with differential expression. In thermally stressed *Symbiodinium* cells, RRM containing genes were detected with similarity to the *MEI2* gene identified in *Schizosaccharomyces pombe* and the MEI2-like gene families identified in *A. thaliana* and *Oryza sativa*. In *S. pombe*, accumulation and localization of the MEI2 protein to meiRNA results in pre-meiotic DNA synthesis and entry into meiosis I (Anderson et al., 2004; Jeffares et al., 2004). MEI2-like genes have been identified in various eukaryotes including *Chlamydomonas reinhardtii,* with single copies identified in ascomycete fungi, alveolates, and entamoebidae and gene families identified in plants, however, they are not found in metazoans (Anderson et al., 2004; Jeffares et al., 2004). Analysis of conserved orthologs between clade C3 and A1 *Symbiodinium* identified an RNA-binding protein MEI2 homologue with hits to the alveolate *Plasmodium falciparum* genome (Voolstra et al., 2009b). Recently, two MEI2-like proteins (MEI2-like 2 and MEI2-like 4) were identified with differential expression between species in the comparison of clade B *Symbiodinium* species (Parkinson et al., 2016). The MEI2-like genes share conserved RRM domains with MEI2 genes (Anderson et al., 2004; Jeffares et al., 2004), though functionally are believed to be involved in cell development during vegetative growth in *A. thaliana* as well as regulating meiosis (Kaur et al., 2006). RNA binding proteins have also been implicated in chromatin organization and remodeling (Kaur et al., 2006) and MEI2-like gene knockout in *Plasmodium yoelli* prevents a post-transcriptional regulatory mechanism inhibiting liver schizont stage maturation (Dankwa et al., 2016). The function of these RNA binding proteins in thermally stressed *Symbiodinium* is unclear, but with fifty-two transcripts displaying differential expression further studies are needed.

The molecular response to stress can vary with many pathways in place to minimize damage and re-establish cellular homeostasis. The type and degree of stress can ultimately alter the fate of the cell with various cellular functions targeted during stress responses such as cell cycle control, molecular chaperoning, protein repair, protein degradation and DNA repair, however, if cellular function cannot be regained cell death (apoptosis) may occur (Kültz, 2005). Here within *Symbiodinium* exposed to thermal stress DEGs encoding RAD DNA repair proteins, DNA photolyases, ubiquitin proteasome pathway components, molecular chaperones (heat-shock proteins (HSPs) and DNAJ), pro-apoptosis (metacaspases and apoptosis-inducing factors) and anti-apoptosis (IAPs and Bax1, protein lifeguard) were differentially expressed (Figure 2.12 and Appendix E Table 6.3). Previous studies of *Symbiodinium* have identified HSPs (HSP70 and HSP90) and HRPs (Leggat et al., 2007; Rosic et al., 2014b) and quantified their expression in response to stress (Leggat et al., 2011a; Rosic et al., 2011a; Barshis et al., 2014), however, under the various conditions used expression patterns of these molecular chaperones varied**.** Exposure of *Symbiodinium* to thermal stress, elicited components of stress response pathways detected here using gene expression analysis (Figure 2.11, Figure 2.12 and Appendix E Table 6.3), which were reflected by decreased cell growth within the physiological parameters measured. By understanding the roles of molecular chaperones in maintaining cellular functions including protein folding and the effect of the proteasomal repair and degradation pathways we may improve our understanding of the stress response of *Symbiodinium* cells.

Photosynthesis in thermally stressed *Symbiodinium*

Understanding of the photosynthetic machinery gene homologs and their organization has recently advanced with sequencing of the *Symbiodinium* chloroplast and nuclear genomes (Shoguchi et al., 2013; Barbrook et al., 2014; Mungpakdee et al., 2014). Previously, characterization of *Symbiodinium* photosystem subunit proteins and genes has examined PS II core proteins *psbA* (D1 protein) and *psbD* (D2 protein), PS II manganese-stabilizing protein (or PsbO protein) encoded by *psbO* and PS I core protein *psaA* (P_{700} protein) (Iglesias-Prieto and Trench, 1997; Warner et al., 1999; Iida et al., 2008; Takahashi et al., 2008;

McGinley et al., 2012; Castillo-Medina et al., 2013; Gierz et al., 2016 (Chapter 3)). Photoinhibition of PS II and decreased expression of PS II D1 protein (D1 protein content and *psbA* gene expression) have been observed in thermally stressed *Symbiodinium* cells (Takahashi et al., 2008; McGinley et al., 2012; Gierz et al., 2016 (Chapter 3)). In this study, exposure of *Symbiodinium* to thermal stress resulted in a slight decrease in dark-adapted yield (Figure 2.3), a measurement of the PS II activity, though PS II core proteins encoded by *psbA* and *psbD* showed no significant changes under these conditions. However, transcripts encoding various subunits of photosystem II (PS II), the cytochrome b_6f complex, photosystem I (PS I), ATP synthase, cytochrome c_6, phycocyanin beta, ferredoxin (FRX) and ferredoxin-NADP (+) reductase (FNR) were detected with differential expression under thermal stress conditions (Figure 2.13 and Appendix E Table 6.3). Notably four transcripts encoding two extrinsic proteins of the PS II oxygen-evolving complex (*psbO* and *psbP*) were significantly upregulated in thermally stressed cells (Figure 2.13 and Appendix E Table 6.3). PsbO homologs are found in higher plants, green algae, red algae, diatoms and cyanobacteria and stabilize the Mn cluster and may be critical for the recruitment and assembly of PS II (Ifuku and Noguchi, 2016). PsbP homologs have been annotated in plants, green algae and cyanobacteria, have high calcium ion binding affinity and may aid in stabilizing the PS II-light-harvesting complex II supercomplexes in higher plants (Ifuku et al., 2011; Ifuku and Noguchi, 2016).

Biosynthesis of photosynthetic complexes is a controlled process relying on synthesis, insertion and coordination of each subunit for successful assembly (Rochaix, 2011). Assembly of PS I in *Chlamydomonas reinhardtii* relies on the insertion of the scaffold anchor protein PsaB, followed by PsaA forming the chlorophyll *a*-protein complex I and finally by PsaC, after which the other subunits are incorporated (Rochaix, 2011). Photosynthetic ATP synthesis relies on the generation of a proton gradient, either by linear electron flow from PS II to PS I (producing reduced NADP) through the cytochrome b_6f complex or cyclic electron flow (CEF) through PS I and the cytochrome b_6f complex (Rochaix, 2011). Additionally, CEF around PS I in *Symbiodinium* has been demonstrated to increase under short moderate heat stress, proposed to alleviate photoinhibition by dissipating excess energy (qE) (Aihara et al., 2016). In this study, expression of the PS I P_{700}

gene (*psaA*) varied over the experiment but significantly decreased on day twenty-eight (Figure 2.13 and Appendix E Table 6.3). Previous targeted studies of *psaA* across multiple strains of *Symbiodinium* also found thermal stress resulted in decreased gene expression (McGinley et al., 2012).This change in *psaA* expression could therefore impair assembly of new PS I complexes in *Symbiodinium* exposed to thermal stress, potentially disrupting photoprotection via CEF and the synthesis of ATP and reduction of NADP, which are required for cellular metabolic processes including carbon fixation via the Calvin cycle.

In *Symbiodinium*, the integral LHC family has been studied due to their proposed functional roles of enhancing light capture and photo-protection by dissipating excess energy (Iglesias-Prieto et al., 1993; Takahashi et al., 2008). Intrinsic LHCs have therefore been implicated in the stress response of *Symbiodinium* cells (Takahashi et al., 2008; Maruyama et al., 2015; Xiang et al., 2015; Gierz et al., 2016 (Chapter 3)). High diversification of the integral light-harvesting gene family (*acpPC*) in *Symbiodinium* has been shown following analysis of the *Symbiodinium minutum* genome (Maruyama et al., 2015) and analysis of the *Symbiodinium* sp. C3 *acpPC* gene repertoire (Boldt et al., 2012). The expression of *acpPC*s have been characterized in *Symbiodinium* between two strains of varied thermal tolerance (Takahashi et al., 2008), within a coral host under thermal stress (Gierz et al., 2016 (Chapter 3)) and under light stress (Xiang et al., 2015). In this study, we identified fifty-four differentially expressed transcripts encoding *acpPCs* in thermally stressed cells (Appendix E Table 6.3), of these LHC genes fourteen were significantly expressed at every time point throughout the experiment (Figure 2.14). The functional associations of *Symbiodinium* LHCs cannot be determined from this study, though the varied expression of *acpPC* genes following exposure to thermal stress may be indicative of specific gene function.

Fatty acid desaturases

Fatty acids have been quantified in *Symbiodinium* in attempts to link thermal tolerance to thylakoid membrane lipid composition (Tchernov et al., 2004) and to develop lipid biomarkers for stress (Kneeland et al., 2013). Analysis of lipid content

has shown that thermally tolerant species possess different ratios of polyunsaturated fatty acid (PUFA) compared to thermally sensitive strains (Tchernov et al., 2004). However, in multiple clades the lipid composition of whole cells versus enriched chloroplast fractions (Díaz-Almeyda et al., 2011) and the lipid profile (Kneeland et al., 2013) has shown that PUFA desaturation cannot be used to estimate thermal sensitivity of *Symbiodinium*. Fractionation of chloroplasts has shown that between clades lipid composition can differ, and desaturation and isomerization of these fatty acids can alter the melting point of thylakoid membranes, with increased membrane fluidity observed in thermally stressed *S. microadriaticum* A1 (Díaz-Almeyda et al., 2011). Further studies of *Symbiodinium* sp. type C1 and subtype D1 identified decreases in the desaturation ratio and in the fatty acid-to-sterol ratio in cells incubated above 30 °C (Kneeland et al., 2013). However, as total fatty acids were saponified, the sources of change in the lipid profile (storage lipids versus membrane lipids) could not be discerned (Kneeland et al., 2013). In this study *Symbiodinium* lipid content were not quantified, so the lipid profiles cannot be estimated. However, DEGs encoding fatty acid desaturase were detected in thermally stressed *Symbiodinium* cells (Figure 2.14 and Appendix E Table 6.3). Recently, transcriptome analysis of multiple *Symbiodinium* clade B strains revealed differences in expression of fatty acid metabolism and biosynthesis pathway genes potentially related to membrane composition, energy storage and varied growth rates between species (Parkinson et al., 2016).

Previously, in clade C and D type *Symbiodinium*, orthologs of the palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase were annotated in each assembly, with significantly elevated d_N/d_s along the clade D lineage (Ladner et al., 2012). As described in the clade C and D analysis, these orthologs are very similar to the palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase (*ADS3*) in *Arabidopsis thaliana* which are involved in the desaturation of hexadecatrienoic acid ($16{:}3\Delta^{7,10,13}$) (Ladner et al., 2012). As mentioned previously, the lipid composition of thylakoids determined that the ratios of fatty acids (C16, C18 and C22) can change under thermal stress influencing thylakoid membrane fluidity (Díaz-Almeyda et al., 2011). In this study, two classes of fatty acid delta desaturases involved in PUFA biosynthesis were detected with differential expression in *Symbiodinium* exposed to thermal

stress (Figure 2.14). Without having quantified the lipid content of fractionated thylakoids in this study we cannot identify if the increased delta-5 desaturase activity and decreased delta-7 desaturase activity were specific for membrane lipids or storage lipids. Though the differential expression of fatty acid desaturase enzymes detected has not previously been reported, and future studies in *Symbiodinium* may benefit from pairing assays of both fatty acid desaturase expression and lipid content quantification.

Lipid catabolism in thermally stressed *Symbiodinium*

Analysis of DEGs detected multiple transcripts encoding the four enzymes of the β-oxidation pathway and two enzymes of the glyoxylate cycle (Figure 2.14). DEGs encoding the four enzymes (acyl CoA dehydrogenase, enoyl CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase and β-ketothiolase) of the fatty acid β-oxidation pathway (Figure 2.14 and Appendix E Table 6.3) were detected in thermally stressed *Symbiodinium*. Additionally enzymes targeted for both mitochondrial and peroxisomal β-oxidation were annotated, in mammalian cells β-oxidation occurs in both the mitochondria and peroxisomes, whereas, in plant cells β-oxidation is restricted to peroxisomes (Poirier et al., 2006). The peroxisomal β-oxidation pathway in mammalian cells is used for very-long-chain fatty acids and their derivatives that are otherwise slowly degraded in the mitochondria (Poirier et al., 2006), and in plants has been linked to the metabolism of fatty acids from membrane lipids supplying acetyl-CoA to the glyoxylate cycle (Cornah and Smith, 2002). As mentioned previously, fatty acid content of *Symbiodinium* sp. C1 and *S. microadriaticum* A1 thylakoid membranes were significantly modified under thermal stress (Díaz-Almeyda et al., 2011), it is possible that in this study we are detecting DEGs of the β-oxidation pathway for the modification of membrane lipid content by removing free fatty acids in thermally stressed cells or the use of storage lipids.

In plants, bacteria, protists and fungi degradation of fatty acids by β-oxidation generates acetyl-CoA, which may then enter the glyoxylate cycle to produce substrates for gluconeogenesis (Cornah and Smith, 2002). Glyoxylate cycle enzymes have been identified in yeast and plants under starvation (Cornah and

Smith, 2002), in a number of corals from genome and transcriptome analyses (Meyer et al., 2009; DeSalvo et al., 2010; Kenkel et al., 2013; Shinzato et al., 2014b) in dinoflagellates (*Karenia brevis* and *Amphidinium carterae*) (Bachvaroff et al., 2004; Butterfield et al., 2013), and recently in the *S. kawagutii* genome (Lin et al., 2015). The glyoxylate cycle in plants and filamentous fungi occurs in glyoxysomes, which have also been identified in *Symbiodinium* by transmission electron microscopy (Sammarco and Strychar, 2013). It is also possible that the peroxisomal β-oxidation transcripts identified may instead be glyoxysomal-associated enzymes in *Symbiodinium*. Two enzymes of the glyoxylate cycle, isocitrate lyase (aceA) and malate synthase (aceB) (Kornberg and Madsen, 1958) were detected with differential expression in thermally stressed *Symbiodinium* (Figure 2.14 and Appendix E Table 6.3). Four of the five differentially expressed transcripts encoding isocitrate lyase orthologs were significantly upregulated on day twenty-eight (Figure 2.14), this enzyme cleaves isocitrate into glyoxylate and succinate (Kornberg and Madsen, 1958). However, the contigs encoding malate synthase were downregulated in thermally stressed cells (Figure 2.14 and Appendix E Table 6.3). In gluconeogenesis, the conversion of malate/oxaloacetate to phosphoenolpyruvate is catalyzed by phosphoenolpyruvate carboxykinase (PEPCK) (Pilkis and Granner, 1992). In thermally stressed *Symbiodinium* cells we detected contigs encoding orthologs of *Dictyostelium discoideum* and *Cucumis sativus* PEPCKs (Figure 2.14 and Appendix E Table 6.3). Of the three *PEPCKs* annotated all displayed significantly decreased expression on day four and the two *D. discoideum* orthologs were later significantly upregulated on day twenty-eight (Figure 2.14 and Appendix E Table 6.3), indicating that the gluconeogenic pathway were in use in thermally stressed *Symbiodinium* on day twenty-eight. This shift to gluconeogenic metabolism could be a mechanism to reduce free fatty acids in cells from membrane remodeling or potentially be indicative of mobilization of fatty acid stores due to inhibition of photosynthesis induced by thermal stress.

Success of the coral-dinoflagellate symbiosis is largely reliant on the exchange of metabolic products (Davy et al., 2012). Though still unclear due to the difficulties of studying the symbiotic relationship, the translocation of photosynthates from symbionts to host cells (including glycerol, glucose, alanine and organic acids such

as citrate, succinate, fumarate, malate and glycolate) is estimated to account for up to 60% of photosynthetically fixed carbon (Davy et al., 2012). Although the behavior of isolated *Symbiodinium* can vary from that of cells in symbiosis (Sutton and Hoegh-Guldberg, 1990; Davy et al., 2012), the implications of altered metabolism from exposure to thermal stress may ultimately influence the stability of the symbiotic relationship.

This study provides an overview of the transcriptome response of *Symbiodinium* exposed to thermal stress and highlights differential expression of key genes. This study is the first of its kind to employ a moderate thermal stress regime, for a period of twenty-eight days reflective of future temperature conditions, and provides an assessment of physiological parameters that are paired with RNA-Seq analysis. Our results provide a basis for further studies as the transcriptome analysis provides documentation of differentially expressed genes in *Symbiodinium* exposed to thermal stress for an extended time period. Surprisingly, despite small fold changes a large proportion (23,654 genes) of the transcriptome exhibited altered expression. The longitudinal approach used here has also allowed us to identify genes that display consistently altered expression and those that are only transiently differentially expressed. Differential expression of key stress, photosynthesis, metabolism and cell cycle genes were detected. Differential expression of glyoxylate cycle enzymes reported here, represents the first instance of this in *Symbiodinium*. The implications for the change in *Symbiodinium* metabolism under extended thermal stress and the effect this may have on *Symbiodinium*-host interactions is unknown, though future studies investigating impacts of extended thermal stress should aim to incorporating metabolomics.

Chapter 3 Integral light-harvesting complex expression in *Symbiodinium* within the coral *Acropora aspera* under thermal stress

This study characterizes the expression of five genes encoding integral light-harvesting complex (LHC) proteins from three distinct LHC families found in *Symbiodinium* within a coral host. LHC expression has been linked to thermal stress in *Symbiodinium* and subsequently coral bleaching, which has large implications for coral reef survival. This is the first instance where the expression of multiple LHCs has been characterized in *Symbiodinium* in a coral under thermal stress. We provide data supporting differential expression of LHCs across the light-harvesting phylogeny, which may indicate functional purpose for the observed LHC hyperdiversity. This study employed a thermal stress regime, allowing us to identify changes in LHC expression at temperatures experienced in a natural bleaching event.

Abstract

Coral reef success is largely dependent on the symbiosis between coral hosts and dinoflagellate symbionts belonging to the genus *Symbiodinium*. Elevated temperatures can result in the expulsion of *Symbiodinium* or loss of their photosynthetic pigments and is known as coral bleaching. It has been postulated that the expression of light-harvesting protein complexes (LHCs), which bind chlorophylls (Chl) and carotenoids, are important in photobleaching. This study explored the effect a sixteen-day thermal stress (increasing daily from 25-34 ℃) on integral LHC (chlorophyll *a*-chlorophyll c_2-peridinin protein complex (acpPC)) gene expression in *Symbiodinium* within the coral *A. aspera.* Thermal stress leads to a decrease in *Symbiodinium* photosynthetic efficiency by day eight, while symbiont density was significantly lower on day sixteen. Over this time period, the gene expression of five *Symbiodinium* acpPC genes was quantified. Three acpPC genes exhibited up-regulated expression when corals were exposed to temperatures above 31.5 ℃ (acpPCSym_1:1, day sixteen; acpPCSym_15, day twelve; and acpPCSym_18, day ten and day sixteen). In contrast, the expression of acpPCSym_5:1 and acpPCSym_10:1 was unchanged throughout the experiment. Interestingly, the three acpPC genes with increased expression cluster together in a phylogenetic analysis of light-harvesting complexes.

Introduction

Photosynthetic eukaryotic dinoflagellates belonging to the genus *Symbiodinium* form symbiotic relationships with a variety of marine taxa. Endosymbiotic associations observed between these photosynthetic dinoflagellates and corals are generally classed as mutualistic, as both the host and symbiont benefit from the relationship (Coffroth and Santos, 2005; Stat et al., 2008). The photosynthetic symbiont acquires nutrients such as inorganic carbon, nitrogen and phosphate from host cells (Yellowlees et al., 2008), in turn symbionts then provide up to 90 % of the energy required by corals to grow and reproduce. These relationships between scleractinian corals and *Symbiodinium* are critical for the proliferation of reefs, supporting diverse marine ecosystems. Coral bleaching, the loss of photosynthetic pigments or endosymbionts from host cells occurs under stress conditions such as elevated SSTs of only a few degrees above long-term maxima (Goreau and Hayes, 1994; Fujise et al., 2014). While ocean temperature fluctuations occur on a daily basis, the mean sea surface temperature is predicted to rise by approximately 1-2 °C over the next century and is expected to lead to more mass bleaching events (Hoegh-Guldberg, 1999; Solomon et al., 2007).

Experimentation on *Symbiodinium* and the coral holobiont has focussed on many environmental factors implicated in the onset of coral bleaching including elevated SSTs, eutrophication and disease. The effect of high SSTs have been a key focus due to mass coral bleaching events (~42 % GBR reefs bleached in 1998 and ~54 % reefs bleached in 2002 (Berkelmans et al., 2004)), attributed to global climate change (Hoegh-Guldberg, 1999) with the 1998 bleaching event coinciding with an El Niño Southern Oscillation event (Bruno et al., 2001; Fujise et al., 2014).

Differential thermal stress sensitivity is observed across the diverse *Symbiodinium* species complex, with both heat tolerant and heat sensitive species observed within the same clade (Tchernov et al., 2004). Differences in photoinhibition sensitivity have either been acquired independently by thermally tolerant types or have been acquired in the common ancestor of all *Symbiodinium* types and since lost in thermally sensitive species (Tchernov et al., 2004). Elucidation of sites of thermal

sensitivity within *Symbiodinium* has focussed on potential points where damage results in a decrease in photosynthetic efficiency (Iglesias-Prieto et al., 1992; Warner et al., 1996). These potential points include damage to the D1 protein of photosystem II (PS II) (Warner et al., 1996), inhibition of the *de novo* synthesis of the D1 protein (Warner et al., 1999), the enzyme RuBisCo (Jones et al., 1998), thylakoid membrane integrity (Tchernov et al., 2004), the carbon concentrating mechanism (Leggat et al., 2004) and LHCs (Takahashi et al., 2008). However, none of these sites have conclusively been demonstrated as the initial site of thermal damage.

LHCs, also called antenna proteins, are found in photoautotrophic organisms and are an array of protein, chlorophyll and accessory pigment molecules with roles in light-harvesting and photoprotection (Kuhlbrandt et al., 1994; Horton et al., 1996). In *Symbiodinium* the light-harvesting system can be divided into two associated complexes, the highly conserved core LHCs, and variable periphery LHCs (Iglesias-Prieto et al., 1993). Studies of green plant LHCs have elucidated structural information, which has further improved the understanding of light capture and transfer, the arrangement of peripheral LHCs and their evolution in photosynthetic eukaryotes (Green and Pichersky, 1994; Kuhlbrandt et al., 1994). Peripheral LHCs may be categorized within the large gene super-family based on associated pigments into three related groups of pigment binding proteins (Green and Durnford, 1996; Boldt et al., 2012). The first group binds chlorophylls *a* and *b*, the second binds chlorophylls *a* and *c* and the third group binds chlorophyll *a* and phycobilins. The chlorophyll *a/c* lineage LHCs are additionally divided into the fucoxanthin-chlorophyll *a/c* (FCP) and peridinin–chlorophyll *a/c* (PCP/acpPC) complexes, which are found in dinoflagellates (Green and Durnford, 1996; Boldt et al., 2012; Maruyama et al., 2015).

In *Symbiodinium* two types of peripheral LHCs are found, PCP and acpPC (Iglesias-Prieto et al., 1993). Dinoflagellate PCPs share no sequence similarity with other known LHCs and are water soluble complexes found on the luminal periphery of thylakoid membranes (Hiller et al., 1993). In contrast, dinoflagellate acpPCs are integral thylakoid membrane complexes that share sequence similarity with the chlorophyll *a*/*c* subfamily of LHCs (Hiller et al., 1993). Further, characterisation of

Symbiodinium C3 acpPCs and *Symbiodinium* A1.1 LHCs cluster sequences with three clades within the Chl *a*/*c* binding LHC family, indicating high diversity of these proteins within species (Boldt et al., 2012). Dinoflagellate and *Symbiodinium* PCPs are well studied due to their unique features (Norris and Miller, 1994; Hofmann et al., 1996; Hiller et al., 2001; Jiang et al., 2012). In *Symbiodinium* the LHCs have been shown to decrease energy transfer and dissociate from the photosystem reaction centres following photoinhibition in order to protect cells during stress events (Warner et al., 1996; Iglesias-Prieto and Trench, 1997; Hill and Ralph, 2006; Allakhverdiev et al., 2008; Hill et al., 2012). Decreasing the number of peripheral LHCs available to absorb and transfer energy is a proposed photoprotection mechanism, as this reduces the amount of light reaching the reaction centres and limits the risk of possible photodamage to the D1 reaction centre proteins (Hill and Ralph, 2006). Further, kinetic studies have revealed that LHC proteins disassociate and reattach to thylakoid membranes under increased light levels to ameliorate stress (Hill et al., 2012). However, few studies have examined the effect of thermal stress on *Symbiodinium* acpPC expression (Takahashi et al., 2008; Hill et al., 2012).

Targeted studies of *Symbiodinium* transcript levels have shown that changes in gene expression occur on a relatively small scale. Quantitative-PCR has been used to determine changes in a variety of genes of interest related to stress response (Boldt et al., 2009; Leggat et al., 2011a; Rosic et al., 2011a; Ogawa et al., 2013). The validation of housekeeping genes (HKGs) for use in *Symbiodinium* has allowed for a reference to be established in order to determine differential gene expression under various conditions (Boldt et al., 2009; Rosic et al., 2011b). Although significant changes have been observed in *Symbiodinium* physiology and large fold changes in host gene expression have been recorded in cells exposed to stress, changes in *Symbiodinium* gene expression occur at a far smaller scale (± < 5-fold) (Leggat et al., 2011a; Rosic et al., 2011a; Ogawa et al., 2013).

This study focussed on the response of acpPC genes in *Symbiodinium* cells during a thermal stress event (sixteen days) in *A. aspera.* We find that the response of five acpPC genes in *Symbiodinium* under stress varies across a LHC phylogeny. This is

the first study to investigate the effect of thermal stress on gene expression patterns in the LHC superfamily within a coral host.

Figure 3.1 Phylogenetic analysis of with LHCs from Chl *a/b* and Chl *a/c* containing organisms. Chl *a/b* binding protein complexes cluster together while the Chl *a/c* binding protein complexes form a second cluster. *Symbiodinium* sp. C3 acpPC sequences and *Symbiodinium* type A1.1 LHCs are found throughout the four clades (Clade 1-3b) of the Chl *a/c* binding protein complexes. Reproduced from Boldt et al. (2012).

Methods

Thermal stress experimental design

Coral fragments (*n* = 312) were collected (under Great Barrier Reef Marine Park Authority permit G13/36402.1) from four different colonies of *Acropora aspera* (tan morph, approximately 78 from each colony) on the reef flat of Heron Island at low tide in May 2013. Heron Island colonies of *A. aspera* have been demonstrated to associate only with *Symbiodinium* clade C3 (LaJeunesse et al., 2003; Dove, 2004; Ogawa et al., 2013). Coral fragments were taken to the Heron Island Research Station and placed in a holding tub supplied with filtered water from the reef flat. Fragments were randomly selected and placed upright in racks. Nubbin racks were then transferred to eight 65 L replicate tanks and allowed to acclimate for five days, at the end of this period tissue regrowth was observed on the cut section of the nubbins. Each of the replicate tanks were supplied with a flow of sand-filtered water pumped from the reef flat into two sump tanks, each sump tank then supplied water to four experimental tanks forming a semi-closed system.

The eight tanks were assigned to one of two treatments, control conditions (ambient temperature) and thermal-stress treatment conditions. The control conditions remained at the ambient seawater temperature (~ 24°C) for the duration of the experiment (Figure 3.2). Fluctuations observed in control conditions are natural daily temperature fluctuations (Figure 3.2). The thermal-stress treatment temperature was increased by 0.7 ℃ for eleven days (25-32.3 ℃), and then held at 33 ℃ for three days and then at 34 ℃ for a further three days (Figure 3.2) to simulate a bleaching event. To achieve the required temperatures for the thermal treatment a three hundred Watt Eheim Jager (Eheim, Deisizou, Germany) heater was used in the heated sump as well as four 25W Aqua One glass heaters. To ensure diurnal temperature variation in thermal treatment tanks the Eheim heater was turned off overnight to reflect natural fluctuations. Temperatures in each tank were recorded every 10 minutes with HOBO® temperature/alarm pendant data loggers (Onset, Massachusetts, USA). Light levels were monitored over the course of the

experiment, every 10 minutes with Odyssey Photosynthetic Active Radiation (PAR) recorders (Dataflow Systems Limited, New Zealand).

Figure 3.2 Temperature of ambient (solid line) and heated treatment (dashed line) during the sixteen-day thermal experiment. Values represent the average of 4 replicate tanks at control and treatment temperatures.

Imaging-Pulse Amplitude-Modulated Fluorometry

Imaging-PAM fluorometry (MAXI Imaging-PAM, Walz, Effeltrich, Germany) was used to measure photosynthetic efficiency of *Symbiodinium* within *A. aspera*. Imaging-PAM analysis was performed on the first day and every day from the third day following sunset. Nubbins were dark-adapted for twenty minutes prior to imaging-PAM analysis. Three replicate nubbins from each of the eight replicate tanks were designated for Imaging-PAM analysis and used throughout the experiment. Corals were measured in the same order at each imaging-PAM analysis. Dark-adapted yield and maximal fluorescence were determined using a weak pulse of light, followed by a saturating pulse of 2,700 μmol quanta m^{-2} s^{-1} of photosynthetically active radiation (PAR) for 800 ms. Induction + Recovery curves were used to examine the photosynthetic efficiency and ability of symbionts to recover from light

stress throughout the experiment. The Induction + Recovery analysis utilised fifteen pulses of saturating light (2,700 μmol quanta m^{-2} $s^{-1)}$ with a constant actinic light (111 μmol quanta m^{-2} s^{-1}) over a 5-minute period, followed by a dark recovery period of 14 min. Data from the Induction + Recovery curve was used to determine photo-kinetic parameters, such as F_v/F_m and NPQ. Following analysis, nubbins were returned to experimental tanks.

Pigment Quantification and *Symbiodinium* Density

At 14:00 h on days zero, eight, ten, twelve and sixteen, three replicate nubbins were taken from each replicate tank (*n* = 9 - 12) and were stripped of tissue using a Waterpik™ dental irrigator using seawater (Johannes and Wiebe, 1970). On day zero the blastate was centrifuged at 3,076 g for 5 min immediately following tissue removal. On days eight, ten, twelve and sixteen the blastate was homogenised with an immersion blender for 5 s and centrifuged at 3,076 g for 3 min to pellet algal cells. Total pelleted cells were resuspended in 50 mL of seawater. Aliquots (1 mL) were taken for cell density approximation and morphological analysis (see Chapter 4). The remaining cells were centrifuged at 3,076 g for 3 min to pellet cells and stored at -80 ℃ for chlorophyll *a* and *c* quantification. Chlorophyll was extracted in 90% acetone for 20 h in the dark at 4 ℃ and quantified using the equations of Jeffrey and Humphrey (1975). Cell number was determined using a Neubauer haemocytometer, with replicate cell counts performed (*n* = 5). Surface area of waterpiked nubbins was determined using the wax dipping method (Stimson and Kinzie III, 1991).

Gene Expression Analysis

At 2 pm on days five, eight, ten, twelve and sixteen, three replicate nubbins were taken from each replicate tank (total twelve nubbins per treatment) and snap-frozen in liquid nitrogen and stored at -80 ℃ for later mRNA isolation. Coral branches that had been snap-frozen in liquid nitrogen were crushed with a hydraulic press before transfer to a mortar chilled with liquid nitrogen and ground finely with a chilled pestle. The powder was then divided into cryotubes and stored at -80 ℃. Messenger RNA was isolated from cells using the Dynabeads® mRNA DIRECT™ kit as per the protocol outlined in Leggat et al. (2011a). Extracted mRNA was quantified

spectrophotometrically using the Nucleic acid: RNA-40 setting on a NanoDrop-1000 (NanoDrop Technologies, Wilmington USA). DNase treatment of 0.1 µg of mRNA per reaction was carried out using RQ1 RNase-Free DNase (Promega) in a total volume of 9 µl. cDNA was reverse transcribed from DNase treated mRNA isolated from *Symbiodinium* cells for quantitative real-time-PCR (qPCR). Reverse transcription (RT) was performed using the SuperScript™ III First-Strand Synthesis SuperMix for RT-PCR (Invitrogen). The 2x RT Reaction Mix contains the following components oligo $(dT)_{20}$ (2.5 µM), random hexamers (2.5 $ng.\mu l^{-1}$), 10 mM $MgCl_2$, and dNTPs. Template cDNA dilution series were prepared to optimize quantification accuracy. For analysis, cDNA was diluted 1:40 prior to use as a template in the qPCR analysis. Quantitative RT-PCR was performed using a Rotor-Gene™ 6000 (Corbett Life Science, Australia). The qPCR was performed in a final volume of 15 µl, containing 7.5 µl of GoTaq® qPCR Master Mix (Promega, USA), 4 µl of diluted template and gene specific primers (266 nM). qPCR conditions were as follows: 95℃ for 2 min; 40 cycles of 95℃ for 15 s and 61℃ for 60 s. All qPCR were followed by a melt curve analysis from 55 to 95℃ to ensure single product amplification (Appendix H, Figure 6.5). Each Rotor-Disc™-100 included three technical replicates for ten biological samples with three genes. Non-template controls for each primer set were performed in triplicate in each run.

HKGs used in our analysis were selected from previously established reference genes, which included Proliferating Cell Nuclear Antigen (PCNA) (Boldt et al., 2009), *cyclophin* (Cyc) (Rosic et al., 2011b), *glyceraldehyde 3-phosphate dehydrogenase* (GAPDH) (Rosic et al., 2011b), *S4 ribosomal protein* (Rp-S4) (Rosic et al., 2011b) and *S-adenosyl-L-methionine synthetase* (SAM) (Rosic et al., 2011b). Five acpPC (*acpPCSym_1:1*, *acpPCSym_5:1*, *acpPCSym_10:1*, *acpPCSym_15* and *acpPCSym_18*) *Symbiodinium* C3 primers were designed against acpPC sequences from an EST library (Leggat et al., 2007) obtained from the NCBI Genbank database (www.ncbi.nlm.nih.gov). Primers were designed for acpPC genes in *Symbiodinium* using the software DNASTAR Primer Select (Lasergene 11) (Table 3.1). The *psbA* primers were previously established for use in *Symbiodinium* (McGinley et al., 2012). Relative expression analysis was performed using qBASE plus 2.5 software (Biogazelle; http://www.biogazelle.com/products/qbasePLUS) (Vandesompele et al.,

2002; Hellemans et al., 2007). Validation of HKGs (expression stability and the optimum number of genes) in this experiment was performed using geNorm (qBASE plus) (Vandesompele et al., 2002; Hellemans et al., 2007).

Table 3.1 Primer sequences and amplification efficiency used for quantitative PCR for *Symbiodinium*

Gene name	Forward primer	Reverse primer	Reaction efficiency
acpPCSym_1:1	AGTGGAGTGAACCAGGAAGCAA	AACCAATCGCACCGACCAAGAG	1.05
acpPCSym_5:1	GGCGACTGCACCAAGGAGGACT	GAACACATCGGGCCAGAGCATACC	1.13
acpPCSym_10:1	GGAAACCCTAGCCGAGTGG	CTTGACATTTCCGAGAGCCTTCC	1.00
acpPCSym_15	GGGTGCCATTGAGTCTGTCC	TTAAGCCAAGGTCTCCCGCATTCT	0.96
acpPCSym_18	TCCCCTGGGCTTCTCTGATAC	GTTCTGCCACAAAGCCAATAGTT	1.01
psbA	TGCAGAAACTGCAGGAGATATTAGCC	TACTCCAAGGGCAGTGAACC	0.95
Cyc	ATGTGCCAGGGTGGAGACTT	CCTGTGTGCTTCAGGGTGAA	0.97
GAPDH	GGTGGTTGATGGCCAGAAGAT	CACCAGTGGATTCGCAAACA	1.06
PCNA	GAGTTTCAGAAGATTTGCCGAGAT	ACATTGCCACTGCCGAGGTC	1.00
Rp-S4	CCGCACAAACTGCGTGAGT	CGCTGCATGACGATCATCTT	0.99
SAM	GCCTACATTTGCCGACAGATG	AATGGCTTGGCAACACCAAT	1.03

Data analyses

Statistics software package (SPSS Statistics v 22.0, IBM, USA) was used for all statistical analyses. A generalized linear model with ‘day’ and ‘treatment’ as main effects and ‘day × treatment’ as an interaction was used for pairwise comparisons of cell density, Chl *a* and *c*, imaging-PAM and gene expression data. The sequential Bonferroni *post hoc* test was used to adjust for the false discovery rate (or type I error).

Results

Symbiodinium density

Over a period of sixteen days, branches of *A. aspera* were exposed to temperature increasing from ambient levels (~25 °C) to a bleaching temperature of ~34 °C (Figure 3.2). As has previously been found, this temperature increase led to a significant decrease in *Symbiodinium* cell densities ($p < 0.001$) over the course of the experiment, with average densities of 6.0×10^5 cells cm^{-2} in the treatment corals on day sixteen, compared to 1.6×10^6 cells cm^{-2} in the control corals on the same day (Figure 3.3).

Figure 3.3 *Symbiodinium* cell density per cm^2 in *A. aspera. A. aspera* nubbins subjected to control conditions (solid line) and heated treatment (dashed line). *Error bars* represent ± s.e.m., n = 9-12, some *error bars* obscured by data point markers. The statistical difference (*post hoc* sequential Bonferroni analysis) between treatment and control is indicated as $*p < 0.05$ or $**p < 0.01$.

Chlorophyll pigment content

Chorophyll *a* content per *Symbiodinium* cell increased over the experimental period in the heated treatments from day eight of the experimental period (Figure 3.4).

Analysis of the chlorophyll *a* content found that there were significant differences between day ($p < 0.001$, df = 5) and treatment ($p < 0.05$, df = 1) but not in the interaction treatment × day ($p > 0.05$, df = 5) (Figure 3.4). Similarly chlorophyll *c* content per *Symbiodinium* cell increased from day ten onwards in the experiment period (Figure 3.5). Analysis of the chlorophyll *c* content found that there were significant differences between day ($p < 0.01$, df = 5) and treatment ($p < 0.01$, df = 1) but not in the interaction treatment × day ($p > 0.05$, df = 5) (Figure 3.5). The ratio of chlorophyll *c* to chlorophyll *a* was unchanged between control and treatment conditions throughout the experiment (Figure 3.6).

Figure 3.4 *Symbiodinium* Chl *a* pigment concentrations in *A. aspera A. aspera* nubbins subjected to control conditions (solid line) and heated treatment (dashed line). *Error bars* represent ± s.e.m., n = 9-12, some *error bars* obscured by data point markers. The statistical difference (*post hoc* sequential Bonferroni analysis) between treatment and control is indicated as $^{*}p < 0.05$ or $^{**}p < 0.01$.

Figure 3.5 *Symbiodinium* Chl *c* pigment concentrations in *A. aspera*. *A. aspera* nubbins subjected to control conditions (solid line) and heated treatment (dashed line). *Error bars* represent ± s.e.m., *n* = 9-12, some *error bars* obscured by data point markers. The statistical difference (*post hoc* sequential Bonferroni analysis) between treatment and control is indicated as *$p < 0.05$ or **$p < 0.01$.

Figure 3.6 Ratio of Chl *c* to Chl *a* per *Symbiodinium* cell in *A. aspera* nubbins. *A. aspera* nubbins subjected to control conditions (solid line) and heated treatment

(dashed line). *Error bars* represent ± s.e.m., n = 9-12, some *error bars* obscured by data point markers. The statistical difference (*post hoc* sequential Bonferroni analysis) between treatment and control is indicated as *$p < 0.05$ or **$p < 0.01$.

Chlorophyll fluorescence and photosynthetic efficiency

Maximum quantum yield of photosynthesis (F_v/F_m) was measured following sunset during the experiment. For corals maintained at control temperatures, F_v/F_m was between 0.602 and 0.692 (average 0.657) (Figure 3.7). Analysis of F_v/F_m found that there was a significant effect between day and treatment ($p < 0.001$, df = 5) and differences between day ($p < 0.001$, df = 5) and treatment ($p < 0.001$, df = 1) (Figure 3.7). A sequential Bonferroni *post hoc* analysis found that F_v/F_m decreased in the heated treatment on days eight, ten, twelve and sixteen of the experiment compared to controls from the same days ($p < 0.01$) (Figure 3.7).

Figure 3.7 *Symbiodinium* F_v/F_m within *A. aspera* during the experiment. *A. aspera* nubbins exposed to control conditions (solid line) and heated treatment (dashed line). Values represent average obtained from twelve biological replicates across four replicate tanks. *Error bars* represent ± s.e.m., n = 12, some *error bars* obscured by data point markers. The statistical difference (*post hoc* sequential Bonferroni analysis) between treatment and control is indicated as *$p < 0.05$ or **$p < 0.01$.

Non-photochemical quenching (NPQ) was also measured over the course of the experiment. As with F_v/F_m, there were significant interaction effects between treatment × day ($p < 0.001$, df = 5), and between day ($p < 0.001$, df = 5) and treatment ($p < 0.05$, df = 1) (Figure 3.8). *Post hoc* analysis demonstrated NPQ was significantly increased by heating on days ten, twelve and fourteen before declining to zero on the final day of the experiment (Figure 3.8).

Figure 3.8 *Symbiodinium* NPQ within *A. aspera* at the last point of the induction phase during the Imaging-PAM analysis. Values represent average obtained from twelve biological replicates across four replicate tanks. *Error bars* represent ± s.e.m., $n = 12$, some *error bars* obscured by data point markers. The statistical difference (*post hoc* sequential Bonferroni analysis) between treatment and control is indicated as $*p < 0.05$ or $**p < 0.01$

Gene expression under thermal stress

The expression of five acpPC genes (acpPCSym_1:1, acpPCSym_5:1, acpPCSym_10:1, acpPCSym_15 and acpPCSym_18) from three distinct LHC clades (Figure 3.9) was determined. Over the course of the experiment three acpPC genes were found to have significant increases in gene expression, acpPCSym_1:1 on day sixteen (1.74-fold, $p = 0.001$), acpPCSym_15 on day twelve (1.33-fold, $p = 0.014$) and acpPCSym_18 on days ten (2.44-fold, $p = 0.012$) and sixteen (2.08-fold,

p = 0.020) (Figure 3.9a, d and e). These three genes belong to two distinct LHC clades (Figure 3.1), both acpPCSym_15 and acpPCSym_18 belong to Clade 1, while acpPCSym_1:1 belongs to Clade 2. The largest fold change seen in these genes was a 2.44-fold increase in acpPCSym_18 compared to control. For the remaining two acpPCs (acpPCSym_5:1 and acpPCSym_10:1) which both belong to Clade 3b (Figure 3.1), no significant changes in gene expression were detected (Figure 3.9b and c). In addition to the five acpPCs the expression of the *psbA gene* was also determined. During the course of the experiment no significant differences in *psbA* expression between control and treatment conditions were found (Figure 3.9f).

Figure 3.9 Relative expression of *Symbiodinium* genes of interest when exposed to thermal stress. Values expressed as relative expression of treatment (dashed line) to control (solid line) for each time point: **a** acpPCSym_1:1, **b** acpPCSym_5:1, **c** acpPCSym_10:1, **d** acpPCSym_15, **e** acpPCSym_18 and **f** *psbA*. *Error bars* represent ± s. e. m., n = 4-10, some *error bars* obscured by data point markers. The statistical differences (*post hoc* sequential Bonferroni analysis) between treatment transcript abundance and control is indicated as *$p < 0.05$ or **$p < 0.01$.

Discussion

This study investigated the effect of increased temperatures on the expression of five acpPC genes in *Symbiodinium* under thermal stress in *A. aspera* and is the first to examine the expression of acpPC genes from different LHC Clades. The sixteen-day thermal regime was selected to enable sampling at temperatures leading up to, and inclusive of, a bleaching event and is the first experiment to investigate differential expression of integral antenna proteins in *Symbiodinium* within a coral host under thermal stress. Quantitative PCR was used to quantify the expression of fiveacpPCgenes that are dispersed though out three clades of the Chl *a*/*c* lineage of the LHC phylogeny (Figure 3.9).

Over the course of the experiment temperature significantly effected *Symbiodinium* density and physiology. Symbiont cells decreased to approximately half the density in thermally stressed corals compared to control corals (Figure 3.3) as has been found in variety of other studies (Middlebrook et al., 2008; Ogawa et al., 2013; Fujise et al., 2014). In addition chlorophyll *a* and chlorophyll *c* levels were elevated over the course of the experiment (Figure 3.4 and Figure 3.5) in a manner seen before in this species (Gierz and Leggat, unpublished data; Ogawa et al., 2013). However, a statistical difference was only observed on day sixteen in chlorophyll *c* (Figure 3.5), this is consistent with other studies where an increase in chlorophyll pigments were observed (Ogawa et al., 2013). In corals, heat-related increases in chlorophyll *a* have previously been recorded at low symbiont densities (Fitt et al., 1993; Jones, 1997), though in other experiments *Symbiodinium* pigmentation may be unchanged or decreased (Dove et al., 2006; Strychar and Sammarco, 2012). Increases in Chl pigments have been attributed to repackaging of chlorophylls in the chloroplast membrane, with evidence that specific pigment-protein complexes may absorb more light at specific wavelengths (Bissett et al., 1997). In phytoplankton, Chl *a*-specific absorption of different pigment-protein complexes from the same organism can be highly variable (Bissett et al., 1997). Therefore, it is possible that the increases in *Symbiodinium* pigments observed in heated corals may be attributed to alterations in the type of pigment-protein complexes expressed under thermal stress.

Imaging-Pulse-amplitude modulated (PAM) fluorometry analysis demonstrated that *Symbiodinium* cells exposed to elevated temperatures exhibited decreased photosynthetic efficiency (Figure 3.7), this is consistent with previous studies demonstrating the response of cells to elevated temperatures. Decreases in dark-adapted yield occurred throughout the experiment despite small changes in symbiont density, F_v/F_m levels of ~ 0.00 were recorded on day sixteen despite cell density being approximately five hundred thousand per cm^2, indicating cells were incapable of photosynthesis at the end of the stress period. Increased NPQ response in cells at days eight, ten and twelve (Figure 3.8) illustrates that the cells were dissipating excess light energy. However, this NPQ response was not present on day sixteen of thermal stress indicating that the symbionts had passed a threshold where photosynthetic processes were no longer functioning. Together, the photosynthetic efficiency results, *Symbiodinium* densities and changes to pigment levels, demonstrate that in this experiment *Symbiodinium* were subjected to the full range of temperatures that are seen in a bleaching event, with responses from initial thermal stress through to *Symbiodinium* expulsion. As such it is reasonable to conclude that acpPC expression patterns are representative of what would be seen in a natural bleaching event.

Expression of acpPC genes was found to vary in *Symbiodinium* cells throughout the experiment. Functionally little is known about the diversity of LHCs, for example whether complexes only associate with specific photosystems, are some more efficient at light capture or energy transfer, are others favoured for photoprotection or do some display increased stability under high temperatures. Characterisation of *Symbiodinium* acpPCs has shown that there is large diversity within the gene super-family(Boldt et al., 2012). Analysis of the *Symbiodinium* genome has provided more of an insight into the diversification of the LHC family (Maruyama et al., 2015), reinforcing theories on gene duplication and deletion events leading to the current structure of the *Symbiodinium* genome. The complexity observed in the integral LHC family has been attributed to multiple rounds of intra- and inter-genic gene duplication events (Green and Pichersky, 1994; Maruyama et al., 2015). A large gene super-family encodes integral LHCs, and a significant level of sequence similarity has been detected between the protein complexes (Boldt et al., 2012;

Maruyama et al., 2015). Phylogenetic analysis of LHCs and LHC-like protein super-families indicate that the ancestor is most likely a central group of two-helix stress-enhanced proteins that had previously evolved from a gene-duplication event of the high-light induced proteins of cyanobacteria (Green, 2003; Engelken et al., 2010). However, based upon sequence divergence, it is reasonable to assume that different clades of acpPC may have different functions. As such, the differences in expression between those acpPC (Figure 3.9a-e) from Clade 1 and 2 versus Clade 3b (Figure 3.1), may be indicative of functional roles, with Clade 1 and 2 possibly being involved in stress response while those of Clade 3b are constitutively expressed under the conditions used here.

Some ways in which acpPC may functionally vary is in the binding of varied pigment ratios, specificity for association to photosystems and response to stress events. For example it has been found that a variety of acpPC transcripts are missing key chlorophyll and pigment binging residues (Boldt et al., 2012). In addition, it is not clear to which photosystems different acpPCs bind. In green plants, ten highly conserved genes encoding Chl *a*/*b* binding proteins have been identified, associated with photosystem I (PS I) are four pigment - protein complexes (encoded by genes *Lhca1, Lhca2, Lhca3* and *Lhca4*), and associated with PS II are six pigment – protein complexes (encoded by genes *Lhcb1, Lhcb2*, *Lhcb3*, *Lhcb4*, *Lhcb5*, *Lhcb6*) (Green and Pichersky, 1994). This can be contrasted to *Symbiodinium* where there is high sequence diversity coupled with high copy number, and as yet it is not clear which proteins bind to PS I or PS II (Boldt et al., 2012; Maruyama et al., 2015). It has been suggested that this sequence diversity allows for functional diversity such as, stress response (Boldt et al., 2012), attachment/dissociation (Hill et al., 2012) and enhanced photoprotection (Reynolds et al., 2008). As such, it will only be with the linkage of more transcriptome and genome studies, and the analysis of chlorophyll and accessory pigments binding residues, linked to functional studies, that we will be able to elucidate the reason for the expansion of this gene family in dinoflagellates.

Core photosystem genes, *psaA* and *psbA* have previously been investigated in *Symbiodinium* under thermal stress. Decreases of *psaA* and *psbA* are hypothesised to significantly impair the mechanisms associated with coping with thermal stress

(McGinley et al., 2012). In this study, the expression of the *psbA* gene, which encodes the core PS II D1 protein, was also quantified (Figure 3.9f). Over the course of the experiment *psbA* expression increased on days eight and ten (Figure 3.9f) although expression in treatment samples was not statistically significant. However, on day sixteen, *psbA* expression decreased (Figure 3.9f), potentially to reduce light absorption to limit the amount of energy captured under stress conditions as a photoprotective mechanism.

As in previous studies, investigating transcript abundance in *Symbiodinium* very small changes in gene expression were observed in this study. In the five acpPC genes quantified, the largest observed change was a 2.44 fold increase (acpPCSym_18) on day ten of the thermal stress experiment. In *Symbiodinium in hospite*, these small changes in transcripts have been observed previously (Boldt et al., 2009; Leggat et al., 2011a; Rosic et al., 2011a; Ogawa et al., 2013) and it is postulated that regulation is most likely post-translational and not at the transcriptional level (Bachvaroff and Place, 2008; Leggat et al., 2011a; McGinley et al., 2012).

This study exploited a bleaching experiment to investigate the effect of thermal stress on photosynthetic genes. Quantitative PCR was used to determine the expression of five integral LHC genes. Three LHC genes (acpPCSym_1:1, acpPCSym_15 and acpPCSym_18) were found to have increased expression over the duration of the experiment and interestingly, grouped in Clade 1 and Clade 2 of the LHC phylogeny. Additionally, two LHC genes (acpPCSym_5:1 and acpPCSym_10:1) grouped with Clade 3b did not exhibit differences in expression. Though transcriptional changes were detected, expression changes observed were less than 2.5 fold throughout the experiment. This is consistent with previous studies where small-scale changes in gene expression were also observed. Given that we currently do not know how the diverse range of LHCs are associated with the photosystems, both PS II and PS I, their specific functional roles (e.g., light harvesting efficiency versus photoprotection), or the importance of *Symbiodinium* photosynthesis to the survival of corals, it is imperative that future research focuses on the specific roles of LHCs.

Chapter 4 Characterization of *Symbiodinium* isolated from thermally stressed *Acropora aspera* demonstrates importance of broad approaches when assessing coral bleaching responses

This study aimed to characterize physiological and structural changes of individual *Symbiodinium* sp. (clade C3) isolated from *Acropora aspera*. Confocal laser scanning microscopy was used to assess morphological changes and a methodology was developed to quantify chlorophyll *a* fluorescence in thermally stressed *Symbiodinium* sp. (clade C3) cells. The data presented within this chapter highlight and reinforce the importance of assessing and integrating multiple physiological parameters within *Symbiodinium*, as single specific parameters (i.e., cellular density, dark-adapted yield, chlorophyll *a* fluorescence) are not comprehensive to accurately describe cellular condition. In addition, we find that from analysis of single cells, a variety of responses within the total *Symbiodinium* population both within and between, coral branches can be identified reinforcing the concept that the *Symbiodinium* population within a coral displays a variety of responses on a cellular level.

Abstract

Mutualistic endosymbioses between scleractinian corals and *Symbiodinium* species underpin the success of coral reefs globally. Coral bleaching is the dysfunction of this symbiosis; and is characterized either by a loss of symbionts from host tissues or by the loss of photosynthetic pigments within *Symbiodinium* cells and occurs as the relationship is extremely sensitive to stressors such as elevated temperatures, ocean acidification and eutrophication. Whilst our understanding of the complex associations corals form and the processes of coral bleaching have improved, there are still many complexities to be resolved, including tipping points and the mechanisms underlying bleaching responses. Therefore, to further improve our understanding of coral bleaching processes, this study characterized the cellular physiology and morphology of *Symbiodinium* sp. (clade C3) within *A. aspera* exposed to a sixteen-day thermal stress, where temperatures were increased daily (approximately +0.7 ℃) from ~25 ℃ to a maximum of, and then held at, 34 ℃ for four days. For all measurements of *Symbiodinium* cellular physiology and morphology, a total of twelve replicate coral branches from two ambient and two heated aquaria (n = 3) were sampled per day. Coral branches maintained in heated aquaria displayed significant declines in *Symbiodinium* density on day sixteen ($p < 0.001$) following exposure to 34 ℃ for four days. Photophysiological measurements however, identified significant declines in the dark-adapted maximal quantum yield of photosystem II (PS II) (F_v/F_m) of *Symbiodinium* from day nine onwards in coral branches maintained in both heated aquaria (two days at > 32 ℃) ($p < 0.05$). Additionally, short-term light stress experiments identified significant differences in the effective quantum yield of PS II (F_v/F_m) of *Symbiodinium* in coral branches between ambient and heated aquaria from day six onwards ($p < 0.001$), where maximum temperatures on days five and six averaged 31.2 ℃. Indicating that the physiology and stress response mechanisms of zooxanthellae in *A. aspera* were compromised from six days exposure to increasing moderate temperatures, 2.5 -3 ℃ below the bleaching threshold of this species. Confocal laser scanning microscopy of *Symbiodinium* cells isolated from coral branches on days ten, twelve and sixteen identified significant proportional changes in the population of healthy

versus degrading cells on all days quantified ($p < 0.001$) a parameter that could not be assessed from cell density approximations alone. However, significant differences in the quantified intensity of chlorophyll *a* fluorescence were only determined on day sixteen ($p < 0.001$). Furthermore, measurements of chlorophyll *a* and *c* pigment content, dark-adapted yield, effective quantum yield of PS II, morphological composition and chlorophyll *a* fluorescence were all found to have tank effects, this difference is attributed to daily differences in irradiance due to the positioning of the heated aquaria. This study further demonstrates the ability to detect significant differences in the physiological condition of *Symbiodinium*, between individual cells and among populations, and reinforces the importance of assessing and integrating multiple cellular parameters to accurately describe condition when characterizing coral bleaching responses.

Introduction

Coral reefs thrive in oligotrophic environments due to the symbioses formed between coral hosts and dinoflagellates of the genus *Symbiodinium* (=zooxanthellae) (Muscatine and Porter, 1977). Mutualistic associations of *Symbiodinium* have also been documented with a variety of other marine taxa including cnidarians, ciliates, molluscs, poriferans and foraminiferans (Muscatine, 1967; Baker, 2003; Coffroth and Santos, 2005; Pochon and Gates, 2010; Mordret et al., 2016). Perpetuation of the relationship between heterotrophic hosts and photoautotrophic endosymbionts is based largely on nutrient exchange between both partners; resulting in accelerated skeletal calcification in corals whilst providing inorganic nutrients, dissolved organic matter and a protected environment for *Symbiodinium* (Goreau, 1959; Goreau and Goreau, 1959; Muscatine and Porter, 1977; Davies, 1984; Yellowlees et al., 2008). Corals in tropical and subtropical waters are often exposed to temperatures close to upper thermal thresholds and perturbations in environmental conditions (i.e., elevated sea surface temperatures (SSTs), increased light intensity, reduced salinity (Hoegh-Guldberg and Smith, 1989)) can significantly impact the coral - algal symbiosis, and may result in coral bleaching (Coles et al., 1976; Jokiel and Coles, 1990; Hughes et al., 2003).

Coral bleaching, the paling or whitening of a coral colony, is characterized either by the expulsion of *Symbiodinium* cells from the host or alteration in the composition of photosynthetic pigments (Coles and Jokiel, 1978; Kleppel et al., 1989). Incidents of coral bleaching due to elevated SSTs, coincide with increases in temperatures above the summer maxima, as little as 1 – 2 °C over several weeks, or 3 °C to 4 °C over 1 - 2 days (Coles et al., 1976; Jokiel and Coles, 1990). Forecasts for rising SSTs indicate increases in SST of between 1.1 °C to 6.4 °C by 2100 (Solomon et al., 2007), leading to predictions of annual mass bleaching events by as early as 2050, as corals become continuously exposed to temperatures close to their thermal thresholds (Hoegh-Guldberg, 1999; Parry et al., 2007). The outcome of a bleaching event may be influenced by stress type (e.g., temperature), stress characteristic (e.g., intensity and duration) (Stambler, 2010) and the thermal history of the coral holobiont (Middlebrook et al., 2008; Ainsworth et al., 2016). Therefore, the post-

bleaching response is variable, from host survival with repopulation of resident *Symbiodinium* communities (Sampayo et al., 2008; Stat et al., 2009), shifts in *Symbiodinium* populations by switching or shuffling (Buddemeier and Fautin, 1993; Kinzie III et al., 2001; Boulotte et al., 2016), decreased coral reproduction (Szmant and Gassman, 1990), disease outbreak (Bruno et al., 2007; Muller et al., 2008) or widespread coral mortality (Hoegh-Guldberg, 1999). Understanding the cellular responses of the coral holobiont to various stress types and stress characteristics has been the aim of many studies, to further characterize the biology of coral bleaching and improve our knowledge of the variable responses that are observed (Yonge and Nicholls, 1931; Hoegh-Guldberg and Smith, 1989; Gates et al., 1992; Brown et al., 1995; Downs et al., 2002; Dove, 2004; Lesser, 2011; Downs et al., 2013; Fujise et al., 2014).

Since early observations of coral bleaching (Mayer, 1914; Boschma, 1925), studies have aimed to pinpoint the mechanism that trigger coral bleaching (Yonge and Nicholls, 1931), though the initial molecular tipping point(s) are yet to be fully resolved (summarised in Downs et al., 2013). Temperature induced coral-bleaching experiments have been used to describe the fate of associated *Symbiodinium* populations (Yonge and Nicholls, 1931; Steele and Goreau, 1977), as debate between theories of expulsion and *in situ* symbiont digestion exist (summarised in Downs et al., 2013). For example, studies by Gates et al. (1992), Brown et al. (1995) (1992), and Ainsworth and Hoegh-Guldberg (2008), proposed, and investigated, a range of release mechanisms that may be active under thermal induced bleaching in cnidarians, including exocytosis of *Symbiodinium*, *in situ* degradation, apoptosis of host gastrodermal cell layers and host-cell detachment. Additionally, the ‘oxidative theory of coral bleaching’ has been developed to describe cellular processes that occur within the symbiont and host, that may catalyze the breakdown of the coral-algal symbiosis triggering these various release mechanisms (Downs et al., 2002; Lesser, 2006). The oxidative theory of coral bleaching, describes an imbalance in the production of harmful reactive oxygen species (ROS) by the photosynthetic algae due to stressors, that exceeds the cells amelioration capabilities, leading to cellular damage within the symbiont and host cells (Downs et al., 2002; Lesser, 2006).

Whilst these triggers and mechanisms have been proposed and debated, definitive initiating factors of coral bleaching are still unresolved.

Experiments to elucidate coral bleaching tipping points generally utilize measurements of host and/ or symbiont physiological parameters as indicators of holobiont health. For example, measurements of cellular density, rates of expulsion, mitotic indices, photosynthetic pigments, respiration rates and photosynthetic rates are all common parameters used for assaying *Symbiodinium* within the coral holobiont at the population level (Hoegh-Guldberg and Smith, 1989; Bhagooli and Hidaka, 2004; Dove, 2004; Hill and Ralph, 2007). Microscopy studies of *Symbiodinium* were initially used for morphological descriptions and life cycle analysis for species identification, utilizing light microscopy, transmission electron microscopy and scanning electron microscopy (Freudenthal, 1962; Trench, 1971; Steele and Goreau, 1977; Fitt et al., 1981; Blank and Huss, 1989; LaJeunesse, 2001). Though these microscopy technologies have also been used to characterize cellular physiology of *Symbiodinium* under stress conditions (Titlyanov et al., 1996; Franklin et al., 2004; Strychar et al., 2004; Pasaribu et al., 2016) and to identify the histopathology of bleaching cnidarians (Gates et al., 1992; Brown et al., 1995; Downs et al., 2013). Additional imaging methods used in *Symbiodinium* studies include flow cytometry, epifluorescence microscopy and confocal laser scanning microscopy allowing visualization of morphology with molecular probes and chlorophyll autofluorescence under stress and non-stress conditions (Lesser and Shick, 1990; Franklin et al., 2004; Strychar et al., 2004; Ainsworth and Hoegh-Guldberg, 2008; Kazandjian et al., 2008; Bay et al., 2011; Castillo-Medina et al., 2011; Jeong et al., 2012; Fujise et al., 2014; Jeong et al., 2014).

This study characterizes the physiological parameters of individual, and populations of *Symbiodinium* within *Acropora aspera* exposed to a sixteen day thermal stress regime. Classic physiological parameters such as cell density, chlorophyll pigment content and photosynthetic efficiency of PS II were used to assess *Symbiodinium* viability and an additional methodology was developed using confocal laser scanning microscopy to quantify chlorophyll *a* fluorescence in isolated cells. We find that individual *Symbiodinium* cells demonstrate a variety of responses both when isolated

from within a coral branch and when compared between coral branches maintained under the same temperature conditions. The variable nature of the assessed physiological parameters identified between *Symbiodinium* cells, highlights the importance of integrating multiple physiological measurements to accurately characterize cellular condition when describing coral-algal symbioses and coral bleaching.

Methods

This analysis used samples collected from the thermal stress of *A. aspera* coral branches, used for the targeted quantitative analysis of integral light-harvesting complexes within *Symbiodinium* sp. C3 (Gierz et al., 2016 (Chapter 3)). Within this chapter experimental design for the thermal stress of the coral *A. aspera* is as per Chapter 3, methodologies relevant to the data presented in this chapter are specified. As such, there is overlap with supporting data presented in Chapter 3, for symbiont density estimations, chlorophyll pigment content quantification and the dark-adapted yield of PS II. The data presented here are a subset of the experiment used in Chapter 3, using two aquaria under each experimental condition.

Thermal stress experiment and environmental data

Acropora aspera nubbins (tan morph) containing *Symbiodinium* sp. (clade C3) (Dove, 2004) were exposed to thermal stress for a period of sixteen days at the Heron Island Research Station (HIRS) (experimental protocol outlined in Chapter 3 and (Gierz et al., 2016)). Temperatures in each tank were recorded every 10 min with HOBO® temperature/alarm pendant data loggers (Onset, Massachusetts, USA) (Figure 4.1). Four Odyssey Integrating Photosynthetic Active Radiation (PAR) recorders (Dataflow Systems Limited, New Zealand), were calibrated using a LI-193SA Underwater Spherical Quantum Sensor with a LI-250A Light Meter (LI-COR® Inc., NE, USA) and used to monitor light levels in two control and two treatment tanks every 10 minutes over the course of the experiment (Figure 4.2). PAR recorders were submerged and fixed upright in each of the four aquaria adjacent coral branches, unfortunately flooding of one of the control tank instruments due to a housing fault resulted in corruption of collected data. Presented are the light levels from one control tank and the averaged values from the two treatment tank PAR sensors (Figure 4.2).

Figure 4.1 Temperatures recorded during the sixteen-day thermal experiment, ambient aquaria (solid line, black; treatment tank 1, blue; treatment tank 2) and heated aquaria (dashed line, black; heated tank 1, red; heated tank 2). Figure reproduced and adapted with additional data from Gierz et al. (2016).

Figure 4.2 Photosynthetically active radiation recorded within aquaria during the sixteen-day thermal experiment. Data collected from PAR sensors within one ambient aquaria (solid line) and the average of two heated aquaria (dashed line) are shown. Values represent the running average light levels recorded in aquaria.

Daily environmental data for the experimental period (06/05/2013 – 22/05/2013) were obtained from the Integrated Marine Observing System (IMOS) through the Australian Institute of Marine Science (AIMS) data centre (see http://www.data.aims.gov.au), for average rain accumulation from the Heron Island Weather Station (Platform, Relay Pole 6, Vaisala WXT520) and average daily PAR from the Heron Island Above water Light (PAR) sensor (Platform, Relay Pole 8, LI-192SA Underwater Quantum Sensor, LI-COR® Inc) (Figure 4.3). The declines in daily ambient temperatures and PAR on days eight and eleven (Figure 4.1 and Figure 4.2) correspond with days where rain accumulated and decreased PAR was recorded (Figure 4.3).

Figure 4.3 Environmental data obtained from the Integrated Marine Observing System (IMOS). Bars show the daily-recorded rain accumulation (mm) taken from Heron Island IMOS relay pole 6 and the dotted line shows the daily average PAR, data taken from IMOS relay pole 8 (http://www.data.aims.gov.au).

Experimental sampling

At 14:00 h on days zero, eight, ten, twelve and sixteen, three replicate nubbins were taken from two replicate tanks (n = 6) and were stripped of tissue using a Waterpik™ dental irrigator filled with seawater (Johannes and Wiebe, 1970). On day zero the blastate was centrifuged at 3,076 g for 5 min immediately following tissue removal. On days eight, ten, twelve and sixteen the blastate was homogenised with an immersion blender for 5 s and centrifuged at 3,076 g for 3 min to pellet

Symbiodinium cells. Total pelleted cells were resuspended in fifty millilitres of seawater. Aliquots were taken for morphological assessment via confocal laser scanning microscopy (1 mL), cell density approximation (1 mL) and the remaining *Symbiodinium* cell suspension (48 mL) were centrifuged at 3,076 g for 3 min to pellet cells and stored at -80 ℃ for chlorophyll pigment quantification.

Paraformaldehyde fixation of *Symbiodinium* cells

For analyses of cell morphology via confocal laser scanning microscopy, one millilitre of *Symbiodinium* cells (approximately 0.69 -1.78 $\times 10^6$ cells mL^{-1}) were centrifuged at 10,625 g for 2 min. Pelleted cells were resuspended in 4 % paraformaldehyde (EM grade) (Electron Microscopy Sciences, USA) in 3X phosphate-buffered saline (PBS) (Sigma Aldrich, USA) and incubated for 12 h in darkness at 4 ℃. Following fixation, *Symbiodinium* cells were centrifuged at 10,625 g for 2 min, washed twice in 3X PBS and resuspended in two hundred microlitres 3X PBS and stored in the dark at 4 ℃ until observation.

Symbiont densities and chlorophyll pigment quantification

In hospite Symbiodinium cell density per cm^2 coral skeleton was determined for each sample. Per coral nubbin, five replicate cell counts were performed using a Neubauer haemocytometer under a light microscope, and the surface area of each waterpiked nubbin was determined using the wax dipping method (Stimson and Kinzie III, 1991). Chlorophyll pigments were extracted in 90% acetone for 20 h in the dark at 4 ℃, and then measured on a DU-650 spectrophotometer (Beckman, USA) at 630 and 664 nm in a quartz cuvette. Chlorophyll pigment compositions of each sample were determined using the equations of Jeffrey and Humphrey (1975) and these were normalised to the corresponding symbiont densities.

Imaging-pulse amplitude-modulated fluorometry

Imaging-pulse amplitude-modulated (PAM) fluorometry (MAXI Imaging-PAM, and ImagingWin software, Walz, Effeltrich, Germany) was used to measure photosynthetic efficiency of *Symbiodinium* within the coral *A. aspera.* Imaging-PAM analysis was performed on the first day and every day from the third day following

sunset at 18:30 h, nubbins were dark-adapted for twenty minutes prior to imaging-PAM analysis. Three replicate nubbins from each of the eight replicate tanks were designated for Imaging-PAM analysis and used throughout the experiment. Dark-adapted yield and maximal fluorescence were determined using a weak pulse of light, followed by a saturating pulse of 2,700 μmol quanta m^{-2} s^{-1} of photosynthetically active radiation (PAR) for 800 ms. The preprogrammed Induction Curve + Recovery kinetic recording type was used to examine the ability of *Symbiodinium* to acclimate to and recover from short-term light stress after exposure to elevated temperatures. Following dark-adaption, minimum chlorophyll fluorescence (F_o) was determined using blue measuring light (intensity 2), and maximum chlorophyll fluorescence (F_m) were determined by applying a pulse (0.72 s) of saturating light (intensity 5, ~2700 µmol quanta m^{-2} s^{-1}), allowing calculation of the dark-adapted maximal quantum yield of PS II (F_v/F_m). For the Induction Curve, actinic illumination (111 µmol quanta m^{-2} s^{-1}, intensity 6) was switched on and fifteen saturating pulses of photosynthetically active radiation (~2700 µmol quanta m^{-2} s^{-1} (intensity 5, 0.72 s)) were applied at 20 s intervals for 5 min (Figure 4.4). During the Recovery phase, a further sixteen saturation pulses were applied within a 14 min period without actinic illumination, time between each pulse exponentially increased. Following analysis, coral nubbins were returned to experimental tanks. Light levels were measured using a LI-190SA Quantum Sensor with a LI-250A Light Meter (LI-COR® Inc., NE, USA). Data obtained from the Induction + Recovery curves were used to determine the effective quantum yield (F_v/F_m) of PS II for *Symbiodinium in hospite*. Presented here are the data from nubbins from four tanks (two control and two heated aquaria) that correspond to the tanks and samples used for morphological and confocal microscope analysis.

Figure 4.4 Profile of the photosynthetically active radiation (PAR) that coral nubbins were exposed to throughout the Imaging PAM Induction + Recovery curve analysis.

Confocal laser scanning microscopy

Symbiodinium cells sampled on days ten, twelve and sixteen were observed using an inverted Zeiss LSM710 confocal microscope with the META microscope spectroscopy attachment (Zeiss, Germany), equipped with a Plan-Apochromat 100x/1.40 Oil DIC M27 oil immersion objective (Zeiss, Germany). To eliminate bias *Symbiodinium* cell suspensions were analyzed blind and randomly. Ten microliters of each paraformaldehyde fixed cell suspension extracted from each *A. aspera* nubbin were placed on coverglass bottom culture dishes with 12mm wells (ProSciTech, Australia) for imaging. Unstained cell suspensions were prepared for profiling of chlorophyll *a* auto-fluorescence and transmitted light (T-PMT) imaging and a randomly selected control cell suspension was used to determine and set the digital offset, detector gain and pinhole for all images. Imaging of chlorophyll *a* fluorescence was performed using channel mode, where a HeNe laser provided excitation at 633nm and fluorescence was monitored at 647-721nm. For all cell suspensions, between 5-10 tile scan experiments were performed (10 x 10 grid, 457.78 μM^2, 5120 pixels x 5120 pixels) ensuring no overlapping of imaged areas. Confocal images were processed with ZEN 2009 software (Zeiss, Germany).

Characterization of *Symbiodinium* cell condition

Qualitative assessment of *Symbiodinium* morphology was performed to determine the proportion of healthy and degenerating *Symbiodinium* cells isolated from *A. aspera.* Observations of *Symbiodinium* morphology were made using bright field tile scan images, taken with the T-PMT setting on the confocal microscope. Differentiation between healthy zooxanthellae and degenerating zooxanthellae were made using morphological criteria, determined from previous descriptions of zooxanthellae morphology and ultrastructure that were isolated or expelled from coral hosts as described in Titlyanov et al. (1996), Bhagooli and Hidaka (2004), Franklin et al. (2004) and Strychar et al. (2004). Healthy zooxanthellae were circular, with intact cell walls, whereas, degenerating zooxanthellae generally displayed variable cell size and shape, vacuolization, lipid droplets, indistinct subcellular organization and accumulation bodies. Differences in the cellular pathologies of *Symbiodinium* were observed among heated samples from day ten, twelve and sixteen. For the purpose of quantification, degrading cells were treated as degenerate irrespective of morphological differences observed among days.

For each replicate *A*. *aspera* branch (n = 36), the morphological characteristics of between twenty-eight to five hundred and forty-eight *Symbiodinium* cells isolated per coral nubbin were visualized. This variation is attributed to the change in symbiont density in thermally stressed corals and the proportion of cells that were quantified in the focal plane of each tile scan. The tile scan imaging technique employed in this analysis produced a 10 x 10 grid of microscope that was used to systematically count cells. For each tile scan processed, the total number of *Symbiodinium* cells that were completely within the frame of the micrograph and in the focal plane were counted. To prevent the double counting of cells over joined scans, cells per scan overlapping the top or right were counted as ”in” and cells overlapping the bottom or left of each joined image were counted as “out”. Counted cells were then sorted as per the healthy/ degrading criteria, and the total number of each population of cells recorded. The proportion of dividing *Symbiodinium,* identified as doublet cells in the mitotic phase of cytokinesis, was also recorded. A pellet of degraded zooxanthellae particles similar to those observed in Titlyanov et al. (1996) was observed in the cell

suspension isolated on day ten, from heated tank two nubbin three, and eight symbiosomes (each containing two endosymbionts) (Kazandjian et al., 2008) were recorded throughout all samples, though due to the homogenization steps taken when extracting symbionts from coral tissue these parameters cannot be quantified in this analysis.

Quantitative analysis of chlorophyll *a* fluorescence intensity

For quantification of chlorophyll *a* fluorescence, each confocal image file was exported from ZEN 2009 as a tagged image file with RGB and ch1 single channel. ImageJ freeware (Schneider et al., 2012) was used to measure average chlorophyll fluorescence intensities in *Symbiodinium* cells isolated from *A*. *aspera* exposed to control and thermally stressed conditions. To eliminate the influence of the imaging depth on the fluorescence intensity only cells with corresponding focussed cell structures in transmission-PMT images were used for quantification of chlorophyll fluorescence, as mentioned all hardware parameter settings (i.e., objective, zoom, gain and offset) remained unchanged between samples. For all quantification measurements, the same region of interest (ROI) area was used and for each image analyzed three background regions were selected and between three – six cells were selected over a minimum of five tile scans with a total of twenty-five *Symbiodinium* cells analyzed per *A. aspera* nubbin, totalling one hundred and fifty cells per treatment per time point. Within ImageJ, measurements of area, integrated density and mean gray value were selected. Background correction of fluorescence intensity was performed by averaging the mean gray value for all background ROIs, and applying this value to all integrated density values, as per methodology described in Lichocka and Schmelzer (2014). These values were then utilised to determine the average chlorophyll *a* fluorescence intensity of *Symbiodinium* sp. (clade C3) cells isolated from *A. aspera*.

Region of Interest	Area	Mean	Integrated Density
1	32400	0.513	16633
2	32400	0.548	17769
3	32400	0.585	18951
4	32400	27.818	901305
5	32400	26.315	852602
6	32400	23.391	757859
7	32400	26.588	861440
8	32400	34.209	1108373

Region of Interest	Area	Mean	Integrated Density
1	32400	0.236	7640
2	32400	0.194	6276
3	32400	0.212	6874
4	32400	10.957	355020
5	32400	10.435	338099
6	32400	14.81	479837
7	32400	21.482	696033
8	32400	13.222	428407
9	32400	13.459	436063

Figure 4.5 Demonstration of the methodology used to determine chlorophyll *a* fluorescence intensity in *Symbiodinium* cells isolated from *A. aspera.* Examples of chlorophyll *a* fluorescence intensity measurements for *Symbiodinium* cells isolated on day sixteen, (A) from control tank one nubbin one and (B) heated tank two nubbin three are provided. Measurements for specific regions of interest (yellow boxes) were made using ImageJ, numbers indicate regions of interest in each adjacent data set. The first three regions of interest in each frame were used for background correction.

Statistical analysis

Statistics software package (SPSS Statistics v 22.0, IBM, USA) was used for all statistical analyses. A generalized linear model with ‘Day’ and ‘Condition’ as main effects and ‘Day × Condition’ and ‘Day × Condition × Tank’ interactions were used for pairwise comparison of *Symbiodinium* cell density, chlorophyll pigment composition and dark-adapted yield of PS II. This generalized linear model approach was also used to analyze the effective quantum yield of PS II data obtained from the Induction + Recovery curves. Two data points were selected for this analysis, the

last data point of the Induction phase (0:05:03 h:mm:ss) and the last data point of the Recovery curve (0:19:17 h:mm:ss), these specific data point were selected to characterize the symbiont populations response to short-term light stress and ability to recover from light stress respectively. As significant ‘Day × Condition’ and ‘Day × Condition × Tank’ interactions were detected, differences between Conditions or Tanks were determined using the sequential Bonferroni test for *post hoc* comparisons.

Trends within data for the proportion of degrading *Symbiodinium* cells and chlorophyll *a* fluorescence intensity were analyzed using a generalized linear model with ‘Day’ and ‘Condition’ as main effects and ‘Day × Condition’ and ‘Day × Condition × Tank’ as interactions and Nubbin nested within the ‘Day × Condition × Tank’ interaction. Significant differences were determined between conditions and tanks using the sequential Bonferroni test for *post hoc* comparisons.

Results

Symbiodinium density and chlorophyll pigment content

Analysis of *Symbiodinium* densities within *A. aspera* branches identified significant mains effect of Day ($p < 0.001$, df = 4) and Condition ($p < 0.001$, df = 1), and in the 'Day × Tank' interaction ($p < 0.001$, df = 4), however no significant tank effects were observed. On day sixteen, a significant decline of *Symbiodinium* cells *in hospite* was observed in nubbins maintained in heated aquaria ($p < 0.001$), here branches had been exposed to temperatures of approximately 34 °C for 4 d (Figure 4.6). The average symbiont density on day sixteen in control nubbins was 1.6×10^6 cells cm^{-2}, whereas treatment coral densities averaged approximately 0.7×10^6 cells cm^{-2} (Figure 4.6).

Figure 4.6 *Symbiodinium* cell density per cm^2 within *A. aspera*. Coral branches subjected to control conditions (solid lines, control tank 1; open triangles, control tank 2; closed triangles) and heated conditions (dashed lines, heated tank 1; open circles, heated tank 2; closed circles). *Error bars* represent ± s.e.m., $n = 3$, some *error bars* obscured by data point markers. Uppercase letters indicate statistically distinct groups (*post hoc* sequential Bonferroni, $p < 0.05$) between control and heated conditions on the same day.

Exposure of *A. aspera* branches to thermal stress over sixteen days altered the chlorophyll *a* and *c* pigment content of *Symbiodinium* cells (Figure 4.7). Analysis of

chlorophyll *a* pigment content identified significant main effects of Day ($p < 0.001$, df = 4), Condition ($p < 0.01$, df = 1) and the 'Day × Condition × Tank' interaction ($p < 0.01$, df = 10), though no significant effects were observed in the interaction 'Day × Condition' ($p > 0.05$, df = 4). A sequential Bonferroni *post hoc* test identified significantly increased chlorophyll *a* content in *Symbiodinium* cells incubated in heated tank one on day twelve (control tank 2, $p < 0.01$) and on day sixteen (control tank 1, $p < 0.05$) (Figure 4.7A). Additionally, on day twelve a significant difference in chlorophyll *a* content was identified in coral branches maintained in each of the heated aquaria, where chlorophyll *a* pigment content in heated tank one was determined at 0.92 pg $cell^{-1}$ and at 0.55 $cell^{-1}$ in heated tank two ($p < 0.05$) (Figure 4.7A). Analysis of chlorophyll *c* content identified significant main effects of Day ($p < 0.001$, df = 4) and Condition ($p < 0.001$, df = 1) and significant effects in the 'Day × Condition' ($p < 0.001$, df = 4) and 'Day × Condition × Tank' interactions ($p < 0.001$, df = 10). Increased chlorophyll *c* content was observed in *Symbiodinium* cells isolated from corals exposed to temperatures above 32 °C in heated tank one on days twelve (control tank 1, $p < 0.05$; control tank 2, $p < 0.01$) and sixteen (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) (Figure 4.7B). Additionally, significant differences in the chlorophyll *c* content were identified among replicate corals maintained in each of the heated aquaria as nubbins sampled from heated tank two were not significantly different to control tanks one or two over the sixteen day experiment ($p > 0.05$), but were significantly different to nubbins sampled from heated tank one on days twelve ($p < 0.01$) and sixteen ($p < 0.001$). Significant differences in the ratios of chlorophyll *c* to chlorophyll *a* content were identified between Day ($p < 0.01$, df = 4) as a main effect and in the interaction 'Day × Condition' ($p < 0.05$, df = 4) (Figure 4.7C). No significant differences in Condition ($p > 0.05$, df = 1) as a main effect or in the 'Day × Condition × Tank' interaction ($p > 0.05$, df = 10) were detected. Though a significant 'Day × Condition' interaction was identified ($p < 0.05$, df = 4), pairwise comparisons (sequential Bonferroni *post hoc* test) determined no significant differences ($p > 0.05$) in the ratios of chlorophyll *c* to chlorophyll *a* content of *Symbiodinium* in coral branches maintained in ambient of heated aquaria on any specific day (Figure 4.7C).

Figure 4.7 Physiological measurements taken for *Symbiodinium* within *A. aspera*. (A) *Symbiodinium* chlorophyll *a* pigment content in *A. aspera*. (B) *Symbiodinium* chlorophyll *c* pigment content in *A. aspera.* (C) Ratio of chlorophyll *c* to chlorophyll *a* per *Symbiodinium* cell. *A. aspera* branches subjected to control conditions (solid lines, control tank 1; open triangles, control tank 2; closed triangles) and heated conditions (dashed lines, heated tank 1; open circles, heated tank 2; closed circles). *Error bars* represent ± s.e.m., *n* = 3, some *error bars* obscured by data point markers. Lowercase letters indicate statistically distinct groups (*post hoc* sequential

Bonferroni, $p < 0.05$) among tanks on the same day. Figure reproduced and adapted with additional data from Gierz et al. (2016) (Chapter 3).

Photosynthetic efficiency of *Symbiodinium* PS II

Significant differences were found in the photosynthetic responses of thermally stressed *Symbiodinium in hospite* (Figure 4.8 and Figure 4.9). The dark-adapted yield (F_v/F_m) of *A. aspera* branches maintained under control conditions (control tanks one and two) averaged 0.651 (± 0.001 SE, range 0.589 – 0.689) over the course of the experiment (Figure 4.8). Corals exposed to daily increases in temperature (ranging between 24 – 31 °C) from the beginning of the experiment until the eighth day, did not display significant changes in dark-adapted yield (Figure 4.8). However, declines in the dark-adapted yield of PS II were found in *Symbiodinium in hospite* from day nine onwards in nubbins maintained in heated aquaria (Figure 4.8). Analysis of dark-adapted yield found that there were significant main effects of Day ($p < 0.001$, df = 15) and Condition ($p < 0.001$, df = 1) and significant effects in the 'Day × Condition' ($p < 0.001$, df = 15) and 'Day × Condition × Tank' interactions ($p < 0.001$, df = 32). On day nine, coral branches had experienced two days' exposure at maximal temperatures of approximately 32 °C and an average decline of 0.157 in the dark-adapted yield were observed between treatments and controls. A sequential Bonferroni *post hoc* test identified significant differences in the dark-adapted yield of PS II in *Symbiodinium* within *A. aspera* on day nine in both heated tanks one (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) and two (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$). On day ten however, an average decline of 0.103 for the maximal dark-adapted yield was recorded for *in hospite Symbiodinium* maintained in heated aquaria. A sequential Bonferroni *post hoc* test identified significant differences on day ten in heated tank one (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) and heated tank two (control tank 1, $p < 0.05$), though the difference in dark-adapted yield between control tank two and heated tank two was not significant ($p > 0.05$) and no significant differences were observed between the heated aquaria ($p > 0.05$). Overall *in hospite* zooxanthellae maintained in heated tank one exhibited slightly decreased dark-adapted yield compared with those from branches maintained in heated tank two, a statistical difference in dark-adapted yield among

corals maintained in the two heated tanks was detected on day twelve ($p < 0.001$) (Figure 4.8). By day sixteen, the dark-adapted yield of PS II of symbionts in both of the heated treatment tanks had reached 0.00 (Figure 4.8).

Figure 4.8 Dark-adapted maximum quantum yield (F_v/F_m) of *in hospite Symbiodinium* of *A. aspera* during experimental stress period. *A. aspera* branches exposed to control conditions (solid lines, control tank 1; open triangles, control tank 2; closed triangles) and heated conditions (dashed lines, heated tank 1; open circles, heated tank 2; closed circles). Values represent the averages of each replicate tank, corresponding to the samples used for morphologic analysis. *Error bars* represent ± s.e.m., $n = 3$, some *error bars* obscured by data point markers. Lowercase letters indicate statistically distinct groups (*post hoc* sequential Bonferroni, $p < 0.05$) among tanks on the same day. Figure adapted with additional data from (Gierz et al., 2016).

Induction + Recovery curves for effective quantum yield of PS II were used to estimate the ability of *in hospite* zooxanthellae to respond to short-term light stress in both control and heated conditions (Figure 4.9). Analysis of effective quantum yield of PS II found that there were significant main effects of Day ($p < 0.001$, df = 9) and Condition ($p < 0.001$, df = 1) and significant effects in the 'Day × Condition' ($p < 0.001$, df = 9) and 'Day × Condition × Tank' interactions ($p < 0.001$, df = 20) for both data points within the Induction + Recovery curves analyzed. *Symbiodinium* within *A. aspera* nubbins maintained under control and heated conditions displayed no

change in the effective quantum yield of PS II on days zero (Figure 4.9A) or five (Figure 4.9B) when exposed to short –term light stress at the end of the induction phase or at the end of the recovery curve. On day five, the average temperature of heated tank one between 09:00 h and 18:00 h was 30.1 °C (± 0.5 °C SD) and a maximum temperature of 31.1 °C was recorded at 09:20 h, and the average temperature of heated tank two between 09:00 h and 18:00 h was 30.3 °C (± 0.5 °C SD) and a maximum temperature of 31.4 °C was recorded at 09:50 h (Figure 4.1).

Exposure of heated coral branches on day six to short term light-stress in the induction phase reduced the photosynthetic ability of *Symbiodinium* cells (Figure 4.9C). A sequential Bonferroni *post hoc* test identified significant declines in the effective quantum yield of PS II in *Symbiodinium* on day six in both heated tanks one (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) and two (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) at the end of the induction phase, though no significant differences were detected between controls or heated coral branches at the end of the recovery phase. On day six, the average temperature of heated tank one between 09:00 h and 18:00 h was 30.4 °C (± 0.2 °C SD) and a maximum temperature of 31.1 °C was recorded at 13:30 h, and the average temperature of heated tank two between 09:00 h and 18:00 h was 30.5 °C (± 0.3 °C SD) and a maximum temperature of 31.1 °C was recorded at 10:20 h.

On day seven, *Symbiodinium* within heated coral branches exposed to short term light-stress displayed reduced effective quantum yield at the end of the induction phase in both heated tank one (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) and two (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) (Figure 4.9D). A significant decline in the effective quantum yield of PS II at the end of the recovery phase was detected in coral branches maintained in heated tank one compared with those in control tank one ($p < 0.05$) (Figure 4.9D). No significant differences were detected between heated tank one and control tank two or with heated tank two at the end of the recovery phase on day seven ($p > 0.05$) (Figure 4.9D). On day seven, the average temperature of heated tank one between 09:00 h and 18:00 h was 31.4 °C (± 0.2 °C SD) and a maximum temperature of 31.8 °C was recorded at 10:50 h, and the average temperature of heated tank two between 09:00 h and 18:00 h was

31.4 °C (± 0.2 °C SD) and a maximum temperature of 31.9 °C was recorded at 10:50 h (Figure 4.1).

On days eight and ten, sequential Bonferroni *post hoc* tests identified significant declines in the effective quantum yield of PS II in *Symbiodinium* in both heated tank one (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) and heated tank two (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) at the end of the induction phase and at the end of the recovery curve (Figure 4.9E, F). No significant differences were detected between coral branches maintained in each heated aquaria at the end of the induction phase ($p > 0.05$) or at the end of the recovery curve ($p > 0.05$) on days eight or ten (Figure 4.9E, F).

On day twelve (Figure 4.9G), significant declines in the effective quantum yield of PS II in *Symbiodinium* at the end of the induction phase were detected in both heated tank one (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) and heated tank two (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$). Additionally, a tank effect was observed for coral branches maintained in heated tank one and heated tank two ($p < 0.001$). At the end of the induction phase following short-term light stress, *in hospite Symbiodinium* maintained in heated tank one had an effective quantum yield of 0.057 (± 0.02 SE) and *in hospite Symbiodinium* maintained in heated tank two had an effective quantum yield of 0.114 (± 0.006 SE) (Figure 4.9G). Significant declines in the effective quantum yield of PS II at the end of the recovery phase were detected in coral branches maintained in heated tank one compared with those in control tanks (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) and in heated tank two with those in control tanks (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) (Figure 4.9G). Tank also influenced the effective quantum yield of PS II at the end of the recovery phase for coral branches maintained in heated tank one and heated tank two ($p < 0.001$). At the end of the recovery curve, *Symbiodinium* within coral branches maintained in heated tank one had an effective quantum yield of 0.211 (± 0.02 SE) and *Symbiodinium* within coral branches maintained in heated tank two had an effective quantum yield of 0.343 (± 0.02 SE) (Figure 4.9G). On day twelve, the average temperature of heated tank one between 09:00 h and 18:00 h was 33.6 °C (± 0.4 °C SD) and a maximum temperature of 34.3 °C was recorded at 10:30 h, and

the average temperature of heated tank two between 09:00 h and 18:00 h was 33.8 °C (± 0.6 °C SD) and a maximum temperature of 34.9 °C was recorded at 10:40 h (Figure 4.1).

On days thirteen and fourteen, sequential Bonferroni *post hoc* tests identified significant declines in the effective quantum yield of PS II in *Symbiodinium* at the end of the induction phase in both heated tank one (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) and heated tank two (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) (Figure 4.9H, I). Significant declines in the effective quantum yield of PS II in *Symbiodinium* at the end of the recovery curve in both heated tank one (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) and heated tank two (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) were also detected (Figure 4.9H, I). No significant differences were detected between coral branches maintained in each heated aquaria at the end of the induction phase ($p > 0.05$) or at the end of the recovery curve ($p > 0.05$) on days thirteen or fourteen (Figure 4.9H, I).

Figure 4.9 Induction + Recovery curves for effective quantum yield of PS II for *Symbiodinium* within the coral *A. aspera*. Coral nubbins were dark-adapted prior to analysis and measurements taken at 18:30 h. Effective quantum yield of PS II

measurements for days (A) zero, (B) five, (C) six, (D) seven, (E) eight, (F) ten, (G) twelve, (H) thirteen, (I) fourteen and (J) sixteen of the thermal stress. *A. aspera* nubbins exposed to control conditions (solid lines, control tank 1; open triangles, control tank 2; closed triangles) and heated conditions (dashed lines, heated tank 1; open circles, heated tank 2; closed circles). Values represent average obtained from three biological replicates per tank. *Error bars* represent ± s.e.m., $n = 3$, some *error bars* obscured by data point markers. Lowercase letters indicate statistically distinct groups (*post hoc* sequential Bonferroni, $p < 0.05$) among tanks on the same day.

By day sixteen, the effective quantum yield of PS II of the zooxanthellae in heated tanks one and two had reached 0.000 (Figure 4.9J). Significant differences in the effective quantum yield of PS II were determined between control conditions and heated conditions at the end of the induction phase (heated tank one (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) and heated tank two (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$)) and at the end of the recovery curve (heated tank one (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) and heated tank two (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$)) (Figure 4.9J). No significant differences were detected between coral branches maintained in each heated aquaria at the end of the induction phase ($p > 0.05$) or at the end of the recovery curve ($p > 0.05$) (Figure 4.9J).

Morphologies of *Symbiodinium*

Morphological variation between healthy and degenerating zooxanthellae differed between days sampled (Figure 4.10). Degrading *Symbiodinium* cells isolated on day ten display small amounts of lipid droplets, though pyrenoid and starch caps remain uniform. On day twelve, lipid droplets and accumulation bodies are evident in *Symbiodinium* cells, irregularities in pyrenoid and starch caps and indistinct organization of subcellular organelles are observed. On day sixteen, degraded cells with indistinct morphologies and irregularities in shape and size of pyrenoids and starch caps if visible are observed.

Figure 4.10 Bright field confocal laser scanning micrographs depicting cellular pathologies of *Symbiodinium* isolated from *A. aspera*. (A) Healthy *Symbiodinium*

cells isolated from coral branches maintained in control tank one on day ten. *Symbiodinium* cells maintained in heated tank one undergoing various stages of degradation are depicted, isolated from coral branches on (B) day ten, (C) day twelve and (D) day sixteen. Abbreviations: py, pyrenoid; sc, starch cap; cn, cnidoblast; ld, lipid droplet; ab, accumulation body. Scale bars indicate 10 µm.

Analysis of *Symbiodinium* morphological variation found that there were significant main effects of Day ($p < 0.001$, df = 2) and Condition ($p < 0.001$, df = 1) and significant effects in the 'Day × Condition' ($p < 0.001$, df = 2) and 'Day × Condition × Tank' interactions ($p < 0.001$, df = 6) and in the nested 'Nubbin (Day × Condition × Tank)' term ($p < 0.001$, df = 24). A sequential Bonferroni *post hoc* test identified significantly increased proportions of degrading zooxanthellae on day ten and twelve in heated tank one (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) and in heated tank two (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$). A significant tank effect was also identified between heated aquaria on days ten ($p < 0.001$) and twelve ($p < 0.001$), where the quantified population of degrading *Symbiodinium* cells in coral branches sampled from heated tank one was estimated at 40 % (± 2.9 % SE) on day ten and 96 % (± 1.3 % SE) on day twelve, and 21 %(± 2.1 % SE) on day ten and 81 % (± 2.8 % SE) on day twelve in heated tank two. On day sixteen, approximately 92 % (± 3 % SE) of the *Symbiodinium* population isolated from coral branches in heated tank one and qualitatively assessed were classified as degrading, differing significantly to controls ($p < 0.001$) (Figure 4.11). The population of *Symbiodinium* isolated from coral branches maintained in heated tank two were determined to be approximately 91 % degraded (± 1.25 % SE), this population estimate was significantly different to cells isolated from coral branches maintained in control aquaria ($p < 0.001$), though was not significantly different to those zooxanthellae assessed from branches maintained in heated tank one ($p > 0.05$) (Figure 4.11).

Figure 4.11 Morphological composition of *Symbiodinium* cells isolated from the coral *A. aspera* exposed to thermal stress. Bars show the observed percentages of healthy-looking (open) and degenerate (shaded) morphologies of *Symbiodinium*, in ambient aquaria (control tank one (grey shaded bars); control tank two (blue shaded bars)) and heated aquaria (heated tank one (orange shaded bars); heated tank two (yellow shaded bars). *Error bars* represent ± s.e.m., n = 28 – 548 *Symbiodinium* cells per *A. aspera* branch visualized, some *error bars* obscured by data point markers. Lowercase letters indicate statistically distinct groups (*post hoc* sequential Bonferroni, $p < 0.05$) among tanks on the same day.

Frequency of proliferating *Symbiodinium*

The population of proliferating *Symbiodinium* cells isolated from *A. aspera* branches was counted using bright field microscopy images (

Figure 4.12). Analysis of the proliferating zooxanthellae populations found that there were significant main effects of Condition ($p < 0.001$, df = 1) and significant effects in the 'Day × Condition' interaction ($p < 0.05$, df = 2) and in the nested 'Nubbin (Day × Condition × Tank)' term ($p < 0.01$, df = 24). No significant main effect of 'Day' ($p > 0.05$, df = 2) or 'Day × Condition × Tank' interactions ($p > 0.05$, df = 6) were identified. Pairwise comparisons (sequential Bonferroni *post hoc* test) determined significant decreases in the frequencies of proliferating zooxanthellae between heated and control aquaria conditions on day ten ($p < 0.05$) and day twelve ($p < 0.01$) (Figure 4.12).

Figure 4.12 Frequency of proliferating *Symbiodinium* identified in cell suspensions isolated from *A. aspera*. *Symbiodinium* isolated from coral branches in control aquaria (control tank one (grey shaded boxes); control tank two (blue shaded boxes)) and heated aquaria (heated tank one (orange shaded boxes); heated tank two (yellow shaded boxes). Uppercase letters indicate statistically distinct groups (*post hoc* sequential Bonferroni, $p < 0.05$) between control and heated conditions on the same day.

The effect of thermal stress on *Symbiodinium* chlorophyll *a* fluorescence

The fluorescence intensity of chlorophyll *a* in *Symbiodinium* cells isolated from *A. aspera* branches was quantified using confocal laser scanning microscopy (Figure 4.14). Analysis of chlorophyll *a* fluorescence intensity found that there were significant main effects of Day ($p < 0.001$, df = 2) and Condition ($p < 0.001$, df = 1) and significant effects in the 'Day × Condition' ($p < 0.001$, df = 2) and 'Day × Condition × Tank' interactions ($p < 0.001$, df = 6) and in the nested 'Nubbin (Day × Condition × Tank)' term ($p < 0.001$, df = 24). A sequential Bonferroni *post hoc* test identified significant declines in the quantified chlorophyll *a* fluorescence in *Symbiodinium* cells on day sixteen in both heated tank one (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) and in heated tank two (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) (Figure 4.14). Additionally, a tank effect was identified for chlorophyll *a* fluorescence in *Symbiodinium* isolated from coral branches maintained in heated tank one and heated tank two ($p < 0.01$) (Figure 4.14).

Figure 4.13 Confocal laser scanning micrographs of *Symbiodinium* isolated from *A. aspera* coral branches maintained in control (A, C, E) and heated aquaria (B, D, F). For each panel, bright field micrographs are displayed on the left and the corresponding chlorophyll *a* autofluorescence micrographs are displayed on the right. Representative micrographs of *Symbiodinium* populations isolated on day ten, from control tank one nubbin three (A) and heated tank two nubbin three (B), on day twelve, from control tank two nubbin three (C) and heated tank two nubbin three (D) and on day sixteen from control tank one nubbin one (E) and heated tank one nubbin three (F) are shown. Asterisks indicate cells classified as degrading zooxanthellae in morphological analysis from bright field micrographs (black asterisks), and

corresponding cells are depicted in the chlorophyll a fluorescence images (white asterisks) and arrows indicate dividing zooxanthellae. Scale bars indicate 10 µm.

Figure 4.14 Quantified chlorophyll *a* fluorescence intensity of *Symbiodinium* cells isolated from the coral *A. aspera*. Box and whisker plot of chlorophyll *a* fluorescence intensity of *Symbiodinium* isolated from coral branches in control aquaria (control tank one (grey shaded boxes); control tank two (blue shaded boxes)) and heated aquaria (heated tank one (orange shaded boxes); heated tank two (yellow shaded boxes) (n = 75). Boxes are medians with 25th and 75th quartiles, and whiskers show the range of data. Lowercase letters indicate statistically distinct groups (*post hoc* sequential Bonferroni, $p < 0.05$) among tanks on the same day.

Discussion

Rising sea temperatures are the greatest threat to the health and survival of coral reefs and the diverse ecosystems they support (Hoegh-Guldberg, 1999; Hughes et al., 2003; Parry et al., 2007; van Hooidonk et al., 2013). Incidents of thermal induced coral bleaching coincide with short periods of high temperatures or extended periods of moderate increases above the summer maxima (Coles et al., 1976; Coles and Jokiel, 1978; Hoegh-Guldberg, 1999). However, as SSTs rise from climate change, the bleaching thresholds of corals decrease, and the frequency and severity of bleaching events are expected to increase (van Hooidonk et al., 2013; Ainsworth et al., 2016). Therefore, efforts to understand how constituents of the coral holobiont respond to stress are imperative for developing strategies to preserve and protect coral reefs in the future.

This study characterizes the physiology and cellular morphologies of *Symbiodinium* sp. (clade C3) within *A. aspera* branches exposed to gradual increases in temperature (approximately + 0.7 ℃ per day for 12 d) over a sixteen day period. The extended thermal stress experiment was implemented to ensure sampling of *in hospite Symbiodinium* populations at temperatures below and above the bleaching threshold of *A. aspera* to improve understanding of the pathology of coral-algal symbioses at ecologically relevant temperatures. Here, analysis of a set of physiological parameters, including cell density (Figure 4.6), chlorophyll pigment content (Figure 4.7), dark-adapted maximum quantum yield (F_v/F_m) (Figure 4.8), effective quantum yield of PS II (Figure 4.9), qualitative assessment of morphology (Figure 4.11) and chlorophyll *a* fluorescence intensity (Figure 4.14), reveals differences can be resolved among *Symbiodinium* populations, both within and between coral branches. These observed and quantified differences in physiology are of significant importance for assessing coral bleaching and stress responses, as this further demonstrates a range of responses are occurring within symbiont populations, though *Symbiodinium* health is commonly only assessed at the whole population level (i.e., cell density or dark-adapted yield of PS II).

Exposure of *A. aspera* coral branches to gradual increases in water temperatures over sixteen days, from ambient reef flat temperatures of ~25 ℃ to a maximum of ~34 ℃ (Figure 4.1) above the bleaching threshold, significantly altered the cellular pathologies of *in hospite Symbiodinium*. No significant differences in *Symbiodinium* densities within *A. aspera* were observed on days zero, eight, ten or twelve ($p > 0.05$) (Figure 4.6), where coral branches had been exposed to daily increases in water temperatures to a maximum of 34 ℃ on day twelve (Figure 4.1). For coral branches maintained under heated conditions, a significant decline in *Symbiodinium* density was observed on day sixteen (Figure 4.6, $p < 0.001$), at which point coral branches had been maintained at approximately 34 ℃ for 4 d, a response similar to previous thermal experiments conducted on the same species (Middlebrook et al., 2008; Ogawa et al., 2013).

Chlorophyll pigment content was quantified in *Symbiodinium* isolated from coral branches on days zero, eight, ten, twelve and sixteen and elevated pigment content was identified in *Symbiodinium* cells isolated from coral branches maintained only in heated tank one (Figure 4.7). For coral branches in heated tank one compared to controls, significant increases in chlorophyll *a* pigment content were identified on days twelve and sixteen (Figure 4.7A), though the quantified pigment content was only significantly different to control tank two on day twelve ($p < 0.01$) and control tank one on day sixteen ($p < 0.05$). For coral branches in heated tank one compared to control aquaria, significant increases in chlorophyll *c* pigment content were identified on days twelve (control tank 1, $p < 0.05$; control tank 2, $p < 0.01$) and sixteen (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) (Figure 4.7B). Previous studies have demonstrated temperature dependent increases in chlorophyll pigment content in *Symbiodinium* from *A. aspera* (Ogawa et al., 2013), and analysis of recovering *Montastrea annularis* identified increases in chlorophyll *a* content in a density dependent manner (Fitt et al., 1993), where chlorophyll *a* content was a function of symbiont density. In this study though, no significant differences were detected in *Symbiodinium* densities between coral branches maintained in the two heated aquaria ($p > 0.05$) (Figure 4.6), though differences in the chlorophyll pigment content were detected in *Symbiodinium* between coral branches maintained in

heated tanks one and two (Figure 4.7), on day twelve for chlorophyll *a* pigment content ($p < 0.05$), and on days twelve ($p < 0.01$) and sixteen ($p < 0.001$) for chlorophyll *c* pigment content. The differences in chlorophyll *a* and *c* pigment content, between heated aquaria reported here are therefore independent of *Symbiodinium* density, and are instead attributed to the synergistic effect of light and temperature. Although temperatures in each heated aquaria remained relatively unchanged (Figure 4.1), branches in the two heated tanks received variable direct sunlight over each light period due to the arrangement of experimental aquaria at HIRS (personal observations). As light loggers were not placed in each aquaria, data supporting this cannot be provided, though previous studies conducted at HIRS have also reported the influence of variable light irradiance on experimental samples attributed to environmental factors (e.g., direct sunlight) and tank locations (Dove, 2004). Additionally, on day eleven elevated rain accumulation (46.5 mm) and decreased PAR (~ 70 µmol photons m^{-2} s^{-1}) (Figure 4.3) were recorded via the IMOS at HIRS, as a consequence slightly lower maximal temperatures were achieved in heated aquaria on day eleven (32.6 ℃) than day ten (33.3 ℃) (Figure 4.1), and the effect of decreased irradiance and therefore shading of coral branches maintained in heated aquaria prior to a temperature increase on day twelve (heated tank one maximum 33.9 ℃, heated tank two maximum 34.4 ℃) are unknown, though are of interest given theories of priming and acclimation in reducing the severity of coral bleaching (Middlebrook et al., 2008; Ainsworth et al., 2016).

The dark-adapted maximal quantum yield and effective quantum yield of PS II are the most commonly reported parameters when describing *Symbiodinium* photophysiology in response to stress (Jones et al., 1998; Dove, 2004; Hill and Ralph, 2007; Leggat et al., 2011a; Fujise et al., 2014). Continued exposure of coral branches to temperatures above 31.5 ℃ resulted in significant declines in *Symbiodinium* dark-adapted maximal photosynthetic efficiency from day nine onwards ($p < 0.05$) (Figure 4.8). On day ten, analysis of dark-adapted yield of *Symbiodinium* within corals maintained in heated tank one found significant declines with control tank one ($p < 0.001$) and control tank two ($p < 0.001$) and in heated tank two significant declines were identified compared with corals maintained in control

tank one ($p < 0.05$) (Figure 4.8). On day twelve, the dark-adapted yield of PS II of *Symbiodinium* in corals maintained in heated aquaria both significantly differed to control aquaria, heated tank one (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$) and heated tank two (control tank 1, $p < 0.001$; control tank 2, $p < 0.001$). A significant tank effect was detected on day twelve in dark-adapted maximal photosynthetic efficiency of *Symbiodinium* in coral branches maintained in both heated aquaria ($p < 0.001$) (Figure 4.8). The differences in dark-adapted yield between heated tanks one and heated tanks two on day twelve and the non-significant decline of dark-adapted yield in heated tank one on day ten compared to heated tank two are again attributed to the synergistic effect of temperature and light as each tank experienced variable irradiance over the course of each natural light period. Though on all other days, no significant differences in the dark-adapted yield of PS II were observed between heated aquaria ($p > 0.05$) (Figure 4.8). On day sixteen, values of 0.00 were obtained for dark-adapted yield of PS II in *Symbiodinium* (Figure 4.8), as symbiont densities of approximately 0.7×10^6 cells cm^{-2} were reported (Figure 4.6) in thermally stressed *A. aspera* branches, this indicates cells remaining in coral branches were incapable of photosynthesis.

The response of thermally stressed *in hospite Symbiodinium* to short-term light stress was assessed using the kinetic type Induction + Recovery curve and measurements of effective quantum yield of PS II (Figure 4.9). Significant declines in the photosynthetic ability of thermally stressed *Symbiodinium* following short-term light stress were observed by day six of thermal stress, following exposure to average temperatures of 30.4 ℃ and maximal temperatures of 31.1 ℃ in both heated tanks (Figure 4.9C). This response at the end of the induction phase on day six, demonstrates that even though photophysiological sensitivity through measurements of dark-adapted yield were not evident in coral branches maintained in heated aquaria until day nine (Figure 4.8), exposure to short-term light stress demonstrated decreased stress response abilities three days prior. This further demonstrates that assessment of dark-adapted yield alone is not an appropriate tool to accurately describe *Symbiodinium* health under thermally-induced bleaching conditions.

Analysis of effective quantum yield on day seven, demonstrated that continued exposure of coral branches to thermal stress in heated tank one significantly altered the recovery of *Symbiodinium* following short-term light stress (control tank one, $p < 0.05$) (Figure 4.9D). This difference was not observed in *Symbiodinium* in corals maintained in heated tank two ($p > 0.05$). On day seven, between the two heated aquaria the daily average temperature recorded, was the same at 31.4 °C (± 0.2 °C SD)), and corals maintained in heated tank two experienced a maximum temperature 0.1 °C higher than those in heated tank one (Figure 4.1). As there is disparity between the two treatments in maximal temperature and the quantified effective quantum yield, it is hypothesized again that varied exposure to irradiance and synergistic temperature and light exposure likely reduced the stress response abilities of coral branches maintained in heated tank one. Additionally, on day twelve, significant differences in effective quantum yield were detected in corals between heated and control aquaria at the end of the induction phase ($p < 0.001$) and at the end of the recovery curve ($p < 0.001$) (Figure 4.9G). As previously suggested, differences in shading between heated aquaria, and therefore the synergistic effect of light and thermal stress on corals maintained in heated tank one are likely the reason for this observed difference in effective quantum yield between coral branches in the two heated aquaria (Figure 4.9).

Within the thermal stress experiment conducted here on *A. aspera*, the photosynthetic ability and physiology of *Symbiodinium* cells expelled from host tissues into the water column were not analyzed. Previous studies have characterized the post-bleaching photosynthetic viability and physiology of *in hospite* and expelled *Symbiodinium* populations (Bhagooli and Hidaka, 2004; Hill and Ralph, 2007). Studies of *Galaxea fascicularis* identified a significant decrease in dark-adapted yield of PS II (F_v/F_m) of expelled versus *in hospite* symbionts following short exposure (24 h) to elevated temperatures of 28 °C, however this decline was not observed in branches maintained at 30 °C and 32 °C (Bhagooli and Hidaka, 2004). Whereas, the effective quantum yield of PS II in symbionts of *Pocillopora damicornis* maintained for 36 h at 32 °C displayed significant declines compared to controls, and declines were also observed for expelled versus *in hospite* zooxanthellae (Hill and Ralph, 2007). Continued exposure (96 h) of expelled *Symbiodinium* to elevated

temperatures (28 °C, 30 °C and 32 °C), also resulted in significant declines of dark-adapted yield and effective quantum yield of PS II in all treatments by 72 h. In both of the experiments discussed above, the morphological condition of cells was assessed, demonstrating increased populations of degrading and heat-damaged *Symbiodinium* in a temperature and temporal dependent manner (Bhagooli and Hidaka, 2004; Hill and Ralph, 2007). Given the significant differences in dark-adapted yield (Figure 4.8) and effective quantum yield of PS II (Figure 4.9) of *Symbiodinium* within *A. aspera*, between control and heated tanks, and within heated tanks in this experiment, future studies will benefit from analyzing both *in hospite* and expelled zooxanthellae as in previous studies, under a similar thermal regime (Figure 4.1) employed in this study.

Morphological variation of cells has previously been documented in both *in vitro* and *in hospite Symbiodinium* exposed to thermal stress (Titlyanov et al., 1996; Bhagooli and Hidaka, 2004; Franklin et al., 2004; Hill and Ralph, 2007; Pasaribu et al., 2015). As in the temporal and starvation experiments on three hermatypic coral species (*Porties cylindrica*, *Seriatopora caliendrum* and *Stylophora pistillata*) in Titlyanov et al. (1996) and the temporal thermal stress of *Stylophora* species (Franklin et al., 2004), *Symbiodinium* from *A. aspera* maintained in heated aquaria differed in morphological appearance among cell suspensions isolated on days ten, twelve and sixteen (Figure 4.10). Previous studies have also characterized stages of apoptosis and necrosis in thermally stressed *Symbiodinium* (Strychar et al., 2004; Hauff et al., 2014) though in this study quantification of specific organelle size, and ultrastructure alterations in thermally stressed *Symbiodinium* could not be completed, due to the resolution of single cells in the confocal laser scanning microscope images and irregularities in cell shapes prevented measurements of cell size and volume. In this study, the proportion of degrading versus healthy *Symbiodinium* on each day were quantified, regardless of variation in morphology observed on each day.

Quantification of the proportions of healthy and degrading *Symbiodinium* cells isolated from *A. aspera* on days ten, twelve and sixteen found significant variations between coral branches maintained in ambient and heated aquaria (Figure 4.11, $p < 0.001$). Additionally, on days ten and twelve assessment of the morphologies of

Symbiodinium isolated from coral branches maintained within heated aquaria found significant differences in the population of healthy versus degrading cells between the two heated tanks (Figure 4.11, $p < 0.001$). This variability in the proportions of degrading morphologies in coral branches between heated tanks on days ten and twelve is attributed to variable irradiance each of the tanks were exposed to, further reflecting patterns previously identified in chlorophyll pigment content (Figure 4.7) and the photophysiology of PS II (Figure 4.8 and Figure 4.9). No significant difference in the proportions of degrading *Symbiodinium* cells were identified among coral branches between heated aquaria on day sixteen ($p > 0.05$), indicating that exposure to temperatures exceeding 34 °C for four days caused significant increases in degraded cell populations regardless of irradiance.

Within this study, sampling of *in hospite Symbiodinium* for morphological analysis were performed at 14:00 h, to obtain samples following exposure to maximum daily temperatures and peak irradiances. However, as cell degradation *in hospite* has been demonstrated to occur at night (Titlyanov et al., 1996), and studies have shown no clear diel patterns for symbiont expulsion (summarised in Davy et al., 2012), future studies of cell viability will benefit greatly from sampling throughout the diel cycle at critical periods identified in thermally induced coral bleaching. Additionally, analysis of *in hospite* and expelled *Symbiodinium* populations were not performed in this study, and this has prevented comparisons of morphological variation between these populations. This, combined with the homogenization method for sampling of *Symbiodinium*, has also inhibited quantification of cell packets within host tissues containing degraded *Symbiodinium* particles (Titlyanov et al., 1996) and comparisons of mechanisms of cell expulsion from host tissues (i.e., release of *Symbiodinium* by exocytosis, host autophagic degradation, host cell detachment) (Gates et al., 1992; Hanes and Kempf, 2013) under an extended thermal stress regime. Therefore, in order to fully characterize morphological variation among *Symbiodinium* populations and between individual cells, and encapsulate all aspects of the coral bleaching process, future studies should aim to characterize *in situ Symbiodinium* populations, isolated *Symbiodinium* populations and populations expelled from coral branches.

Understanding the regulation of cnidarian- symbiont biomass under both ambient and stress conditions is particularly important, as measurements of *Symbiodinium* proliferation may be used as indicators of holobiont health (Obura, 2009; Davy et al., 2012; Dimond et al., 2013). However, measurements of proliferation frequencies are compounded by numerous factors such as the variation of symbiont cell specific densities across different host species (Chang et al., 1983; Muscatine et al., 1998), disparities between host and symbiont mitotic indices (Dimond et al., 2013) and variability in *Symbiodinium* mitotic indices and mitosis diel - phase periodicity between *in hospite* and cultured strains (Wilkerson et al., 1988; Falkowski et al., 1993; Davy et al., 2012). For example, mitotic indices of cultured *Symbiodinium* may range from 2 – 5 days, whereas *in hospite* this may exceed ten to seventy days, representing proliferation frequencies of ~1 % - >14 % across various cnidarian-algal symbioses (Fitt and Trench, 1983; Wilkerson et al., 1988; Davy et al., 1996; Davy et al., 2012). As yet cellular mechanisms underlying homeostatic control of host - *Symbiodinium* biomass are unknown, though proposed regulatory processes include expulsion and/ or degradation of excess symbionts and control of symbiont and host cell proliferation to maintain dynamic equilibrium (Baghdasarian and Muscatine, 2000; Davy et al., 2012).

In this study, analysis of proliferating *Symbiodinium* in coral branches identified significant declines in frequencies between control and heated conditions on day ten ($p < 0.05$) and day sixteen ($p < 0.01$) (Figure 4.12). Where on days ten and sixteen, coral branches maintained under heated conditions exhibited lower frequencies of proliferating cells than those in control aquaria (Figure 4.12), likely due to the extended exposure to increased temperatures influencing the physiological state of *Symbiodinium* cells and therefore inhibiting mitosis. However, on day twelve, no significant differences in the frequencies of proliferating *Symbiodinium* were identified ($p > 0.05$) (Figure 4.12). In fact on day twelve, *Symbiodinium* isolated from coral branches that were maintained in heated tank two, exhibited increased frequency of proliferation compared with controls ($p > 0.05$) (Figure 4.12). Although higher temperatures were experienced on day twelve then ten, when these observations of proliferation were made, it is possible that the milder conditions experienced on day eleven (decreased temperatures, increased rainfall and

decreased PAR, (Figure 4.1 and Figure 4.3)) have resulted in the promotion of *Symbiodinium* proliferation to increase and maintain algal density. Given that diel periodicity is regularly observed in *Symbiodinium* mitosis (Fitt and Trench, 1983), this is a feasible possibility, though from the data collected host influence on this cannot be determined. Additionally, as coral branches were randomly sampled from over each of the *A. aspera* colonies, and samples were homogenized prior to analysis, the influence of *in hospite* location both within branches and within colonies cannot be assessed. As previous studies have indicated that branch tips display increased *Symbiodinium* and host cell division, and skeletal accretion (Gladfelter, 1983; Wilkerson et al., 1988), future thermal recovery studies may benefit from targeting these specific regions when assessing proliferation.

Chlorophyll *a* fluorescence intensity was quantified in individual *Symbiodinium* cells isolated from *A. aspera* branches under control and heated conditions. Although *Symbiodinium* within coral branches exposed to thermal stress displayed photophysiological differences from as early as day six (e.g., induction + recovery curve analysis of effective quantum yield of PS II, Figure 4.9), variation in the intensity of chlorophyll *a* fluorescence were not detected until day sixteen ($p < 0.001$, Figure 4.14). Further, on day sixteen, individual *Symbiodinium* cells isolated from coral branches maintained in both heated tanks displayed significant differences in chlorophyll *a* fluorescence intensity ($p < 0.01$, Figure 4.14). This variation in chlorophyll *a* fluorescence intensity between *Symbiodinium* populations from coral branches maintained in either heated aquaria on day sixteen was not observed in measurements of cell density (Figure 4.6), quantified chlorophyll *a* pigment content (Figure 4.7A), photophysiological parameters (Figure 4.8 and Figure 4.9) or morphology measurements (Figure 4.11). However, this difference between heated *Symbiodinium* populations was reflected in the measurements of chlorophyll *c* pigment content on day sixteen ($p < 0.001$) (Figure 4.7B). Disparity between decreases in chlorophyll *a* fluorescence intensity determined via confocal microscopy on day sixteen in heated tanks (Figure 4.14) and increases in chlorophyll *a* and *c* pigment content in *Symbiodinium* isolated from coral branches maintained in heated tank one (Figure 4.7A) cannot be explained, though as chlorophyll *a* fluorescence intensity was quantified using specific excitation and emission

wavelengths and chlorophyll pigments were assayed via published methodologies (Jeffrey and Humphrey, 1975), these measurements are considered valid assessments of physiology in *Symbiodinium.* Although fine scale variability could be determined among individual cells both between and within treatments, this methodology should not be used diagnostically to verify the viability of thermally stressed symbionts in coral bleaching experiments.

Understanding the multitude of physiological responses individual cells and populations of *Symbiodinium* undergo is critical to develop appropriate methods to assess the viability of the coral holobiont under bleaching conditions. This study has shown that common approaches of assessing *Symbiodinium* at the population level, with limited physiological measurements (e.g., cell density and/ or dark-adapted yield of PS II) risk inaccurately describing cellular condition by broadly categorizing responses. Here it has been demonstrated, that fine scale differences among individual and between populations of *Symbiodinium* cells can be identified over a variety of physiological parameters, strengthening the notion that the *Symbiodinium* population within a coral displays a host of responses on a cellular level. Additionally, the synergistic effect of light and temperature in treatment corals was hypothesized to have significantly influenced *Symbiodinium* physiology at pre-bleaching temperatures. This deserves further investigation as understanding responses to sustained increases in sea surface temperatures with variable irradiances, will shape how governments and management bodies make informed decisions to preserve coral reef health globally.

Chapter 5 General Discussion

Over the next century, climate change is predicted to have significant environmental impacts (Parry et al., 2007). Reef building corals in tropical and subtropical waters experience SSTs that are close to their thermal limits, where small increases of 1 – 2 °C above average SSTs can induce coral bleaching (Hoegh-Guldberg, 1999; Marshall and Baird, 2000; Hoegh-Guldberg et al., 2007). Throughout the past 35 years, incidents of mass coral bleaching have been correlated with periods of elevated SSTs (Hoegh-Guldberg, 1999; Hughes et al., 2017), and under future climate conditions, global warming (with increases of between 1.1 °C to 6.4 °C depending on the IPCC scenario) and ocean acidification are predicted to considerably alter the growth and composition of coral reefs (Hoegh-Guldberg et al., 2007; Solomon et al., 2007; Ainsworth et al., 2016). Various scenarios have been proposed to estimate the responses of corals under future climate conditions, and identify the potential ecological and economic impacts of global reef decline (Hoegh-Guldberg et al., 2007; Ainsworth et al., 2016). However, few studies have characterized the molecular responses or investigated exposure of corals and/ or their symbiotic partners (*Symbiodinium* sp.) to moderate thermal stress (Putnam et al., 2013; Fujise et al., 2014; Levin et al., 2016). Additionally, as the *Symbiodinium* genus is genetically diverse, with differing stress tolerances and varied host associations (Baker, 2003), it is hoped that in understanding how representative strains of the extended *Symbiodinium* genus responds to thermal stress, we may better understand how this influences the thermal threshold of corals and ultimately their stress responses.

By characterizing the physiological responses of the coral holobiont symbiont and pairing this with gene expression data, the molecular response in two *Symbiodinium* sp. (clade C3 and clade F) was determined. Exposure to moderate thermal stress induced changes in the cellular physiology of *Symbiodinium in vitro* (Chapter 2) and *in hospite* (Chapter 3 & 4). Analysis of gene expression data from *Symbiodinium* exposed to thermal stress identified differential expression of genes using broad (Chapter 2) and targeted (Chapter 3) transcriptional analysis methods. The results provide preliminary data for studies investigating the molecular response of

Symbiodinium under future temperature conditions. By elucidating mechanisms essential to coral survival under future climate conditions we will greatly improve our understanding of coral tipping points and acclimation processes.

The research presented within this thesis documents experiments contributing to our understanding of *Symbiodinium* biology and transcriptomics. The analysis of differentially expressed genes presented in Chapter 2 is the first of its kind in (1) experimental design utilizing an extended thermal stress regime, (2) is paired with the characterization of physiological variables as quantification of the stress response and (3) provides an analysis of the transcriptional response using RNA-Seq within *Symbiodinium* sp. (clade F) to thermal stress. The analysis of multiple integral LHCs using qPCR presented in Chapter 3 is the first of its kind in (1) experimental design, using a thermal stress regime in a coral symbiosis reflecting temperatures experienced leading up to a natural bleaching event, (2) is paired with measurements of physiological variables to characterize the stress response (3) provides an analysis of integral LHCs within *Symbiodinium* within a coral host under thermal stress. Additionally, Chapter 4 provides an insight into the variability of cytological parameters among individual cells and between populations of *Symbiodinium* under a thermal stress regime within a coral host. Highlighting the need to employ multiple techniques and broad approaches when assessing the physiology and cumulative effects of coral bleaching experiments.

Currently, the majority of our knowledge of the effects of thermal stress on coral reefs comes from experiments using short exposures to high temperatures (1 – 3 d, + 6 – 9 °C (~32 - 34 °C)) to induce coral bleaching (Table 1.1 and Table 1.2) (Ralph et al., 2001; Barshis et al., 2014; Fujise et al., 2014). These experimental protocols are often used to simulate short periods of high temperatures experienced on reefs and are essential in understanding the mechanisms underlying coral bleaching, though are not reflective of predicted climate conditions or bleaching resulting from cumulative stress periods. One of the aims for this thesis was to subject *Symbiodinium* sp. to thermal stress regimes that will replicate possible future temperature conditions, where the baseline temperature will be higher, but below the current bleaching threshold. This aim was achieved through implementation of

thermal stress experimental protocols in both field and lab experiments. Sampling (28 d, heat stress at 30 - 31.5 °C (Figure 2.1)) of cultured *Symbiodinium* sp. (clade F) and sampling of *Symbiodinium* sp. C3 *in hospite* during an incremental stress (16 d, 25 - 34 °C (Figure 3.2)) experiment allowed for elucidation of the molecular responses following exposure to moderate thermal stress. The data gained from these experiments contributes to the development and improvement of experimental protocols for future studies in attempts to gain a better understanding of coral reef acclimatory processes.

Assessment of *Symbiodinium* found significant changes in physiological variables in *in vitro* and *in hospite* experiments (Chapters 2, 3 and 4). Measurements of cell densities displayed significant differences between controls and treatments, though the mechanisms underlying this variation differ between the experiments. In the thermal stress performed on cultured *Symbiodinium* sp. (clade F), the difference in density is attributed to decreased growth in thermally treated cells (Figure 2.2), whereas decreased *Symbiodinium* sp. (clade C3) densities in *A. aspera* (Figure 3.3 and Figure 4.6) is attributed to a characteristic bleaching response from the host. These results are consistent with previous studies that have identified similar results in culture (Krueger et al., 2014; Karim et al., 2015) and field experiments (Middlebrook et al., 2008; Ogawa et al., 2013) under thermal stress. Significant differences in chlorophyll pigment content were also detected in thermally treated *Symbiodinium* under both *in vitro* and *in hospite* conditions. In cultured *Symbiodinium* sp. (clade F) a significant increase in chlorophyll *a* content was determined on days nineteen and twenty-eight (Figure 2.6) whereas, in *Symbiodinium* sp. (clade C3) *in hospite* a significant increase in chlorophyll *c* content was recorded on day sixteen (Figure 3.5). Significant increases in chlorophyll content have previously been recorded in *Symbiodinium* photoacclimation experiments in *Acropora yongei* (Roth et al., 2010) and in thermally stressed *Symbiodinium* sp. (clade C3) within *A. aspera* (Ogawa et al., 2013). The differences observed between increases in chlorophyll *a* or *c* pigment content here may be attributed to (1) different experimental and environmental conditions, (2) distinct *Symbiodinium* types used with (3) varied photoacclimation responses potentially by expression of different proportions of pigment binding proteins, such as the core antenna proteins or

integral LHCs (e.g., acpPCs). Characterization of pigment binding ratios for integral LHCs in dinoflagellates has identified differences between species (e.g., in *Symbiodinium* sp. (CS-156)) a ratio of 4:6:6:1 of chlorophyll *a*: chlorophyll c_2: peridinin: diadinoxanthin was determined (Niedzwiedzki et al., 2014), while, in *A. carterae* a ratio of 7:4:12:2 was determined (Hiller et al., 1993)). Though characterization of pigment binding ratios of acpPCs in *Symbiodinium* sp. (CS-156) did not take into account the diversified gene family and the relationship between each of the proteins expressed and their corresponding pigment content.

Photophysiology measurements were used in these experiments to assess the photosynthetic performance and estimate the health of thermally stressed *Symbiodinium*. PAM fluorometry has become a popular method to assess the photophysiology of *Symbiodinium in vitro* and *in hospite* (Warner et al., 1996; Ralph et al., 2001; Bhagooli and Hidaka, 2003; Ralph et al., 2005; Krämer et al., 2012). For example, photophysiology measurements have been used to determine the viability of *Symbiodinium* sp. allowing identification of differing thermal tolerances among clades in both culture experiments (Krueger et al., 2014; Karim et al., 2015) and in field studies (e.g., between *Symbiodinium* C and *Symbiodinium* D in *Pocillopora verrucosa* (Rowan, 2004)) (Warner et al., 1996). Thermal stress of cultured *Symbiodinium* sp. (clade F) (Chapter 2) resulted in a slight but significant decline in F_v/F_m at day fourteen, nineteen and twenty-eight (Figure 2.3), indicating a photoacclimatory response as cumulative stress at moderate temperature induced changes in the photophysiology of *Symbiodinium* though capactiy of PS II was retained. In previous experiments conducted on *A. aspera* harbouring *Symbiodinium* sp. (clade C3) measurements of the maximum photochemical efficiency of PS II (F_v/F_m) via PAM fluorometry, representing the maximum capacity of PS II, detected significant changes when coral nubbins were incubated at temperatures exceeding 32 °C (Middlebrook et al., 2008; Leggat et al., 2011a; Ogawa et al., 2013). Concordant with previous studies, exposure of *A. aspera* nubbins (Chapter 3) to thermal stress at temperatures below bleaching thresholds (30.5 - 32 °C) resulted in decreases in the maximum capacity of PS II (Figure 3.7). Additionally, in depth analysis of effective quantum yield of PS II using Induction + Recovery curve kinetics in *Symbiodinium* sp. (clade C3) within *A. aspera* (Chapter 4, Figure 4.9), revealed

that exposure to thermal stress compromised the light-stress response following just one day exposure to temperatures exceeding 31 °C (Figure 4.1). Exposure of this coral species to these temperatures are considered below the bleaching-threshold, however this methodology revealed impacted stress response three days prior to signs of photodamage assessed via dark-adapted yield of PS II (Figure 4.8). These photophysiological data, along with previous studies of *A. aspera* harbouring *Symbiodinium* sp. (clade C3) provide an insight into potential physiological changes that may be observed if SSTs rise and persist under future climate scenarios.

The two transcriptional resources generated within this research further contribute to our knowledge of *Symbiodinium* gene expression. As highlighted in the introduction, advances in sequencing technologies have greatly contributed to improving our understanding of *Symbiodinium* sequence divergence and gene arrangement (Table 1.1). Additionally, studies of gene expression within *Symbiodinium* (summarised in Table 1.1 and Table 1.2) have progressed in recent years with the development of reference genes (Boldt et al., 2009; Rosic et al., 2011b) and use of technologies such as qPCR (Takahashi et al., 2008; Crawley et al., 2010; Leggat et al., 2011a) and next generation sequencing (e.g., RNA-Seq) (Barshis et al., 2014; Xiang et al., 2015; Levin et al., 2016). RNA-Seq analysis identified a relatively large percentage (~37.01%) of unique genes with differential expression (FDR < 0.05) across the experiment (Appendix E **Error! Reference source not found.**). Within *Symbiodinium* transcriptional studies few changes in gene expression have previously been identified, though in this RNA-Seq study a large number per time point were identified. For example, 9,471 DEGs (FDR < 0.05) were identified in *Symbiodinium* sp. (clade F) incubated at ~31 °C on day four (Appendix E **Error! Reference source not found.**). Though relatively small fold-changes in *Symbiodinium* gene expression analysis *in vitro* and *in hospite* were identified (e.g., Figure 2.13 and Figure 3.9), further supporting hypotheses that other mechanisms of regulation (post-transcriptional or translational regulation) are also critical in *Symbiodinium* stress responses (Leggat et al., 2011a; Ogawa et al., 2013; Putnam et al., 2013; Barshis et al., 2014; Lin et al., 2015; Levin et al., 2016). The transcriptional data presented here highlights the importance of (1) high biological replication for statistical power and (2) use of sensitive quantitative technologies

such as RNA-Seq and qPCR to improve our understanding of molecular responses. One of the drawbacks faced in the RNA-Seq analysis presented here was the coverage of gene annotation in the reference transcriptome (~45%), further improvements in gene annotations will improve identification of key genes and allow for the quantification of specific genes expression using more targeted approaches (e.g., qPCR or targeted next-generation sequencing) at lower costs (e.g., MiSeq).

Within *Symbiodinium* and dinoflagellates mechanisms of gene regulation are still unresolved, with various studies investigating transcriptional (see project summaries in Chapter 1, Table 1.1 and Table 1.2) and post-transcriptional regulation (Baumgarten et al., 2013; Lin et al., 2015; Dagenais-Bellefeuille et al., 2017; Liew et al., 2017). For example, sets of potential regulatory small RNAs (smRNAs) (i.e.. miRNAs, siRNAs) have been identified within *S. microadriaticum* (8 miRNAs and 13 siRNAs) (Baumgarten et al., 2013) and *S. kawagutii*. (102 mature miRNAs) (Lin et al., 2015). Though as yet the functionality of these smRNAs in *Symbiodinium* are yet to be established in *in vitro* experiments. Recently in the dinoflagellate *L. polyedrum*, it was demonstrated that miRNAs do not influence the circadian synthesis of the Luciferin Binding Protein (LBP) at the translational level (Dagenais-Bellefeuille et al., 2017). As although LBP mRNA expression remained constant, RNA interference did not alter LBP expression (Dagenais-Bellefeuille et al., 2017). Within *L. polyedrum*, a repertoire of fifty-three miRNAs were identified, though expression levels were lower than of those in higher plants (Dagenais-Bellefeuille et al., 2017). Recent studies of *S. microadriaticum* have provided evidence of RNA editing of nuclear-encoded genes (Liew et al., 2017) complementing previous observations of RNA editing of the S. *minutum* plastid genome (Mungpakdee et al., 2014), suggesting that this may influence the acclimatory potential of symbionts under stress and potentially impact host survival. Future applications of techniques developed in the study of *L. polyedrum* miRNAs in *Symbiodinium* species, and functional studies targeting RNA-editing may greatly improve our understanding of the mechanisms controlling expression at the transcriptional and translational level.

Previous studies have investigated the influence of stress on metabolic processes in *Symbiodinium* and the coral holobiont using targeted gene expression. In targeted

studies on multiple *Symbiodinium* types, the effects of both thermal stress and ocean acidification on the carbon fixation pathways were investigated by studying enzymes of the Calvin cycle (RuBisCo) and photorespiratory cycle (phosphoglycolate phosphatase (PGPase)) (Crawley et al., 2010; Ogawa et al., 2013; Putnam et al., 2013) and relating this to physiological variables. In the thermal stress experiment (Chapter 2), differential expression of multiple transcripts encoding the enzyme RuBisCo were detected in *Symbiodinium in vitro* (Appendix E Table 6.3), while no significant differences in expression of PGPase transcripts were detected. Expression of RuBisCo is of particular interest due to its rate-limiting role in carbon fixation, the Form II structure (Whitney et al., 1995) and as yet unlike higher plants where Rubisco activase regulates enzyme activity no homolog has been identified for regulation in *Symbiodinium* (Lilley et al., 2010). Though RuBisCo has recently been identified as a target of miRNAs following analysis of the *S. kawagutii* genome (Lin et al., 2015). Therefore, expression of RuBisCo miRNAs should be further investigated in concert with gene and protein expression data, as this may be a regulatory mechanism of this key enzyme.

Analysis of transcriptional data from cultured *Symbiodinium* sp. (clade F) (Chapter 2) identified groups and individual genes with differential expression imperative in the cellular response to thermal stress. Specifically, genes encoding enzymes of the glyoxylate cycle were detected with significant changes in expression for the first time in *Symbiodinium* (Figure 2.14). Within the *S. kawagutii* genome, genes encoding enzymes of major metabolic pathways including the TCA cycle, the Calvin cycle and the glyoxylate cycle have been identified (Lin et al., 2015). Previous analysis of dinoflagellate metabolism using EST data had identified transcripts encoding enzymes of the glyoxylate cycle (isocitrate lyase in *A. carterae* and malate synthase in *K. brevis*), though no transcripts encoding isocitrate lyase or malate synthase were identified in the *Symbiodinium* sp. C3 EST library included in the analysis (Butterfield et al., 2013) and differential expression of glyoxylate cycle enzyme genes in other *Symbiodinium* transcriptome analyses (Table 1.1) has not been described. Homologs of isocitrate lyase and malate synthase have also been identified in cnidarian genomes (e.g., *Nematostella vectensis* (Kondrashov et al., 2006) and *Acropora digitifera* (Shinzato et al., 2011)) and differential expression of

these enzymes has been detected in *Acropora palmata* (DeSalvo et al., 2010; Polato et al., 2013) and *Porites astreoides* (Kenkel et al., 2013) under thermal stress, indicating that the glyoxylate cycle is extremely important in the coral stress response as well.

Differential expression of genes relating to fatty acid biosynthesis and β-oxidation were detected in cultured *Symbiodinium* sp. (clade F) (Chapter 2) exposed to thermal stress. Previous *Symbiodinium* culture studies have examined the role of lipids in thermal tolerance and investigated how fatty acid content of cells and membranes alter under thermal stress (Tchernov et al., 2004; Díaz-Almeyda et al., 2011; Kneeland et al., 2013). Additionally characterization of the fatty acid compositions between healthy, stressed and bleached coral colonies (Bachok et al., 2006; Papina et al., 2007) and characterization of fractionated lipids in field experiments (feeding/light stress (Treignier et al., 2008) and feeding/thermal stress (Tolosa et al., 2011)) have provided an insight into how lipid concentrations may alter within both hosts and symbionts under stress conditions. Further, compositional analysis of PUFAs within corals and *Symbiodinium* have determined specific biosynthetic pathways and provided further insights into lipid transfer between members of the holobiont (Imbs et al., 2010; Imbs et al., 2014). Given that between the culture studies various conclusions have been drawn regarding differences between *Symbiodinium* clades, limited thermal stress field experiments and in the culture experiment conducted here (Chapter 2) differential expression of multiple lipid related enzymes were identified, future studies investigating *Symbiodinium* under future temperature conditions should aim to include lipid analyses when characterizing the stress response.

How the differential expression of catabolic pathway constituents and glyoxylate cycle enzyme genes translates to functional changes in *Symbiodinium in vitro* and within a coral symbiosis is relatively unknown and deserves further investigation. Early analyses on cnidarian symbioses identified metabolite transfer between hosts and *Symbiodinium* using radiolabeling methods and paper chromatography (Muscatine and Hand, 1958; Muscatine, 1967; Trench, 1971; summarised in Gordon and Leggat, 2010). This allowed for the targeted identification of metabolites

produced and transferred under various conditions (i.e., *in vitro* versus *in hospite*), and development of newer nontargeted metabolomic profiling (using H^1 nuclear magnetic resonance spectroscopy and liquid chromatography-mass spectrometry) in the coral holobiont has allowed for the identification and quantification of a wide variety of compounds (Gordon et al., 2013). Recent metabolomic studies using ^{13}C labelling of a thermally stresed cnidarian symbiosis model (*Aiptasia pulchella* and *S. minutum*, ITS2 type B1, 7 d at 32 °C) (Hillyer et al., 2017), have provided strong support of catabolic processes identified in the transcriptome study undertaken in Chapter 2. However, short isotope labelling incubation periods (5 h) were hypothesized to have prevented the identification of catabolic intermediates (Hillyer et al., 2017), though previous studies of *Symbiodinium* from various invertebrate hosts (Tytler and Trench, 1986) also supports the expression of β-carboxylation and catabolic enzymes (i.e., PEPCK). Application of these nontargeted metabolomic approaches combined with transcriptomics in future studies will provide a broad analysis approach to determine the molecular response and functional changes of cnidarian symbioses under stress conditions.

Characterizing the cellular response of *Symbiodinium* to stress has been the aim of many studies investigating the tipping points of coral bleaching. Many enzymatic antioxidants (SODs, KatG and APX) have been identified in *Symbiodinium* and are critical in reducing damage caused by ROS to alleviate photobleaching (Lesser, 2006; Krueger et al., 2015). Previous studies of multiple *Symbiodinium* species have identified four SOD metalloforms (CuZn, Mn, Fe and NiSOD) (Matta et al., 1992; Lesser, 2006; Krueger et al., 2015; Lin et al., 2015; Levin et al., 2016). Within the transcriptome analysis performed here (Chapter 2), three SOD metalloforms (CuZn, Mn and Ni) were identified (Appendix E Table 6.3). Both CuZn and MnSOD metalloforms were detected with significant increases in expression throughout exposure to thermal stress (Figure 2.11 and Appendix E Table 6.3), indicating that these enzymatic antioxidants are important in the stress response of *Symbiodinium* sp. (clade F) at the moderate temperature used. In plants, SOD metalloforms may be isolated to specific organelles/subcellular locations, for example within the cytosol (CuZn), mitochondria (CuZn and Mn), peroxisomes (CuZn and Mn) and chloroplasts (CuZn, Mn and Fe) (Alscher et al., 2002). As yet subcellular localization of specific

SODs within *Symbiodinium* have not been resolved (Krueger et al., 2015). Bioinformatic predictions have been performed on a number of *Symbiodinium* transcriptome datasets, identifying mitochondrial and secretory signal peptides for MnSOD isoforms and chloroplastic signal peptides for the FeSOD isoform analyzed, though CuZn/NiSODs were not included in the analyses (Krueger et al., 2015). Future studies incorporating bioinformatics analyses of SOD metalloforms identified in recent *Symbiodinium* sequencing projects (i.e., Chapter 2, Lin et al., 2015; Aranda et al., 2016; Levin et al., 2016), and subcellular localisation experiments incorporating immunohistochemistry with SOD metalloform-specific antibodies, organelle specific superoxide dismutase tracking dyes with confocal laser scanning microscopy will be critical in expanding our understanding of *Symbiodinium* responses to oxidative stress.

Within the studies performed for this thesis, genes encoding integral LHCs in two *Symbiodinium* species were investigated to improve our understanding of their functionality and responses during thermal stress. Previous studies had identified large numbers of phylogenetically related integral LHC genes in *Symbiodinium* species (Boldt et al., 2012; Maruyama et al., 2015), though few studies had explored the effect of stress on gene expression (Takahashi et al., 2008; Boldt et al., 2009; Xiang et al., 2015). In these stress studies, comparison of two cultured clade A *Symbiodinium* species (CS-73 and OTcH-1) determined a decrease in expression of an acpPC gene (via qPCR) in the thermally sensitive CS-73 species (Takahashi et al., 2008), whilst light stress did not alter the expression of the three acpPC genes quantified in *Symbiodinium* sp. C3 *in hospite* (via qPCR) (Boldt et al., 2009). Though high light exposure (500 µmol photons m^{-2} s^{-1}) induced increased expression of an acpPC transcript (via RNA-Seq) in a cultured *Symbiodinium* clade B strain (SSB01), with authors proposing that this transcript may have photoprotective functionality (Xiang et al., 2015).Therefore, due to the proposed roles of light-harvesting complexes in enhancing light-capture and photoprotection, the small number of studies investigating differential expression of integral LHCs under stress and the highly expanded gene family identified in *Symbiodinium* species, these antenna proteins were chosen as genes of interest.

Within the differential expression analysis performed on cultured *Symbiodinium* sp. clade F (Chapter 2), ninety-six CB proteins (PF00504_Chloroa_b-bind) were identified in the reference transcriptome (Appendix E Table 6.3), supporting previous studies that had identified high diversification of the gene family (Boldt et al., 2012; Maruyama et al., 2015). Additionally, analysis of integral LHCs in *Symbiodinium in vitro* (Chapter 2) and *in hospite* (Chapter 3) under thermal stress identified genes with constitutive and inducible expression within the highly expanded gene family, further supporting theories for the functional diversification of the integral LHCs. For example, of the ninety-six genes encoding CB proteins in *Symbiodinium* sp. (clade F) (Chapter 2), fifty-four displayed differential expression under thermal stress throughout the experiment (Figure 2.13). Supporting the RNA-Seq analysis, three of the five integral LHC genes assayed via qPCR in *Symbiodinium* sp. C3 within *A. aspera* (Chapter 3) displayed inducible expression in thermally stressed cells (Figure 3.9), providing the first report of differential expression in field analyses of integral LHCs within *Symbiodinium*. Additionally, further exploration of the regulation of the highly expanded LHC superfamily in light of advances in smRNAs (Baumgarten et al., 2013), identification of LHCs as target genes of miRNAs in *S. kawagutii* (Lin et al., 2015) and RNA editing of nuclear (Liew et al., 2017) and plastid genomes (Mungpakdee et al., 2014), may also greatly improve our understanding of the roles of these antenna proteins within *Symbiodinium*.

Analysis of the extended light-harvesting complex protein superfamily across many species has resolved structural and functional divergence among lineages of photosynthetic eukaryotes (Table 1.3) (Green and Pichersky, 1994; Engelken et al., 2010; Neilson and Durnford, 2010a; Hoffman et al., 2011; Boldt et al., 2012; Maruyama et al., 2015). The various classes of antenna proteins (e.g., LHCs, LHC-like proteins and PSBS proteins), are hypothesized to have evolved and diversified from common ancestral stress-enhanced proteins that replaced cyanobacterial phycobilisomes, through multiple endosymbiotic events (Green and Pichersky, 1994; Engelken et al., 2010; Neilson and Durnford, 2010b). Recent phylogenetic analyses have identified a new class of antenna protein, chlorophyll *a*/*b*-binding-like proteins (RedCAPs) within red algae and diatoms, organisms that contain secondary plastids derived from the “red lineage” of photosynthetic eukaryotes (Engelken et al., 2010;

Sturm et al., 2013). Within the diatom *Phaeodactylum tricornutum*, RedCAP expression was demonstrated to occur in a diurnal and light dependent manner (Sturm et al., 2013), in a similar fashion to the diurnal regulation of FCPs in the diatom *C. cryptica* (Oeltjen et al., 2002). Further analysis of two diatom species (*Chaetoceros gracilis* and *Thalassiosira pseudonana*), identified both FCP-PS I supercomplexes and RedCAP sequence proteins bound to PS I (Ikeda et al., 2013). Though dinoflagellate plastids are likely derived from a tertiary endosymbioses of red algal origin (Delwiche, 1999; Yoon et al., 2005), no sequence similarity with RedCAP proteins identified in diatoms (Sturm et al., 2013) were found within the *Symbiodinium* sp. (clade F) transcriptome (Chapter 2) used for differential expression analysis in thermally stressed cells.

Unlike higher plants, specific associations of integral LHC proteins have not been reported with PS II or PS I in *Symbiodinium*. Though proposed in Chapter 3, it is still unclear if functionally specific integral LHCs exist within the highly diversified family (i.e., have greater photoprotective roles versus light harvesting roles). However, recent analysis of the *S. minutum* LHC gene repertoire has identified sequence similarities with *lhcr* and *lhcf* domains derived from haptophyte and heterokontophytes lineages, that display specific interactions with either photosystems (Veith and Büchel, 2007; Maruyama et al., 2015). Evidence for such interactions of integral LHCs are yet to be reported in *Symbiodinium.* Recent photo-kinetic measurements have elucidated dissociation/ reassociation of light-harvesting proteins from photosystems in *in hospite Symbiodinium* (Hill et al., 2012), though as yet the sequence/structures of these specific proteins have not been resolved. It is hoped that integration of the methodologies used for photokinetic measurements with protein sequencing and phylogenetics may provide future studies with a greater understanding of specific LHCs and whether specialized associations are formed within *Symbiodinium*.

Given the variability detected among individual *Symbiodinium* cells and between populations within thermally stressed coral branches in Chapter 4, this deserves more investigation. Currently, with advances in technologies and methodologies for studies of individual *Symbiodinium* cells and microenvironments within coral studies

(Barott et al., 2015; Lichtenberg et al., 2016; Wangpraseurt et al., 2016) this may become a reality. For example, studies of *Symbiodinium* photosynthesis *in hospite* along a depth gradient, has demonstrated variability in photo-acclimation (effective quantum yield of PS II and relative electron transport) along natural light gradients (Lichtenberg et al., 2016). Therefore, applying the methodologies from studying microenvironments within corals, in corporation with extended thermal stress studies and microscopy studies, will ensure comprehensive approaches are taken when assessing coral bleaching physiological responses.

Extensive studies conducted both for this thesis and by other researchers, are demonstrating that the ability of constituents of the holobiont to acclimate to rising SSTs will be critical to the survival of coral reefs close to their upper thermal thresholds (Hoegh-Guldberg, 1999; Hennige et al., 2010; Palumbi et al., 2014; Ainsworth et al., 2016). Repopulation of coral colonies by resident background communities of resilient *Symbiodinium* species have been demonstrated after stress events (Buddemeier and Fautin, 1993; Boulotte et al., 2016). Though the ability of constituents of the coral holobiont to adapt to future environmental changes through assisted evolution are under debate and being tested experimentally (van Oppen et al., 2015; Chakravarti et al., 2016). Questions arise regarding adaptation in *Symbiodinium*, as although meiosis gene repertoire's have been identified in a number of species (Chapter 2; Chi et al., 2014; Levin et al., 2016), this is yet to be observed cytologically or linked to influencing physiological fitness. However, if *Symbiodinium* adaptation can be influenced, perhaps selective breeding of species with higher thermal tolerances to also express relevant genes may improve photosynthate transfer, light-harvesting efficiency and photoprotection abilities under stress conditions.

Corals of the Great Barrier Reef and Indo-Pacific regions are found in association predominately with clade C *Symbiodinium* though minor but widespread clade D associations also reported (Baker, 2003; Stat and Gates, 2011). Whereas, corals of the Caribbean are commonly associated with members of *Symbiodinium* clades A, B and C (LaJeunesse, 2002; Baker, 2003). The number of genomic and transcriptomic resources available for *Symbiodinium* have dramatically increased, predominately for

clade A, B and C phylotypes (Table 1.1 and Table 1.2) and through understanding how diverse *Symbiodinium* types respond to environmental stress we will improve our understanding of how reefs may respond and change in the future (Baker, 2003; Rowan, 2004; Jones and Berkelmans, 2010; Stat and Gates, 2011; Shinzato et al., 2014b). For the analyses performed within this thesis two *Symbiodinium* species were selected, a widely studied clade C3 and a less studied clade F type. Analysis of differential gene expression *in vitro* under thermal stress was performed on cultured *Symbiodinium* genotyped as clade F (Chapter 2 and Appendix C). Clade F *Symbiodinium* were initially reported as clade C though have now been resolved as a closely related sister clade and symbiotic associations with various sub-clades (F2 - F5) have been reported among cnidarians and foraminiferans (Rodriguez-Lanetty et al., 2002; Baker, 2003; Lien et al., 2012; Pochon et al., 2014). This transcriptome analysis therefore documents differential gene expression and provides an insight into the cellular response of a previously unstudied *Symbiodinium* phylotype, phylogenetically related to clade C *Symbiodinium* species. Investigation of the expression of integral LHCs *in hospite* under thermal stress was performed on *A. aspera*. harbouring *Symbiodinium* sp. Clade C3 (Chapter 3). *A. aspera* was chosen as previous studies have established an EST library for *Symbiodinium* sp. clade C3 (Leggat et al., 2007), analyzed the integral LHC gene complement (Boldt et al., 2012) and investigated acpPC gene expression in different light environments (Boldt et al., 2009), and therefore provided a good candidate species to further investigate acpPC expression under thermal stress conditions.

What is evident in studying the acclimation and stress response of the coral holobiont is that comprehensive approaches encompassing many techniques are required. Studies of gene expression in *Symbiodinium* in these studies (Chapter 2 and Chapter 3) and in previous studies have identified relatively small fold changes leading to hypotheses of other regulatory mechanisms controlling molecular responses (Leggat et al., 2011a; Rosic et al., 2011a; Ogawa et al., 2013). Attempts to assay both gene and protein expression have identified differential responses in RuBisCo expression in *Symbiodinium in hospite* (Putnam et al., 2013) and integral LHCs (acpPCs) in *Symbiodinium* sp. (clade C1) *in vitro* (Gierz, Boldt and Leggat, under review). However, in *Symbiodinium* difficulties in developing assays (e.g.,

qPCR and immunoblotting) exist due to sequence divergence among species within the genus (Gierz, unpublished data), though as mentioned availability of sequence information for different clades through genomes and transcriptomes will greatly resolve and improve this issue. Application of newer metabolomic analysis methods in coral - *Symbiodinium* research also provides potential insights to the stress response of the coral holobiont, at a scale not previously achieved through radioactive carbon labeling methods (Muscatine, 1967; Trench, 1971; Gordon and Leggat, 2010; Gordon et al., 2013; Hillyer et al., 2017). Additionally, the influence of bacterial communities associated with coral reef communities under both ambient and stress conditions represents another factor that needs to be considered when studying the health of the coral holobiont (Bourne and Webster, 2013; Hernandez-Agreda et al., 2016), especially due to their linked functional roles in nutrition (Lesser et al., 2007), nutrient cycling (Raina et al., 2009) and disease (Thurber et al., 2009). Through development and implementation of multifaceted approaches, future research encompassing physiological variables, gene expression analyses, lipid analysis, proteomics and metabolomics in both laboratory and field studies will greatly improve our understanding of mechanisms governing responses in the coral holobiont.

To extend on the research conducted within this thesis future studies should aim to address the following questions:

- Are specific integral LHCs in *Symbiodinium* associated with specific photosystems?
- Do specific integral LHCs in *Symbiodinium* have defined light-harvesting versus photoprotective roles?
- How does the expression of integral LHCs (genes and proteins) vary over various temporal scales? More data are required between time points used in the studies here.
- How does the expression of integral LHCs vary across photic environments? Across a depth gradient, on a micro- (e.g., colony) and/or macro-scale (e.g., reef).
- What key mechanisms of regulation are present in *Symbiodinium*?

- How do transcriptional, epigenetic, translational or post-translational regulatory mechanisms contribute to the molecular response of *Symbiodinium*?
- Do *Symbiodinium* types *in vitro* with differing thermal tolerances exhibit similar metabolic responses under thermal stress?
  Suggest to target clade C and clade D *Symbiodinium*, due to their abundance and distribution in tropical and subtropical corals.
- What molecular responses are observed in intact host - *Symbiodinium* associations maintained under moderate thermal stress?
- Does moderate thermal stress influence coral colony accretion?
- Do *Symbiodinium* types *in hospite* exhibit shifts in metabolic genes under thermal stress as observed in *in vitro* experiments?
- Does moderate thermal stress alter the microbiome of a coral? If there is a change is it beneficial or detrimental?
- What effect do other synergistic stressors (e.g., high irradiance and elevated carbon dioxide) combined with thermal stress have on coral-*Symbiodinium* acclimatory processes?

In conclusion, this thesis assessed cellular responses of the coral holobiont symbiont (*Symbiodinium*) to further improve our understanding of mechanisms underlying acclimation processes. In these studies, I have described physiological data and detailed gene expression in two *Symbiodinium* types exposed to thermal stress. Differential expression of key metabolic and stress response genes were identified using relatively new transcriptomic approaches that will contribute to experimental design and potentially measures of health within the coral holobiont in future studies. In laboratory and field studies, differential expression of genes encoding the extremely diversified integral LHCs were detected under thermal stress, demonstrating that the gene family encodes genes with constitutive, facultative and inducible expression. Additionally, the field experiment analyzing integral LHC expression *in hospite* constitutes fundamental research to address questions surrounding the functional purpose for the diversification of antenna systems in *Symbiodinium*. The data presented provide critical observations of thermal

acclimation and integral LHCs in *Symbiodinium* that has furthered knowledge of the impacts of rising SSTs expected with global climate change.

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Chapter 6 Appendices

Appendix A

Symbiodinium genotyping sequences from transcriptome

ITS2 rDNA region

>comp80962_c1_seq1 len=4582 path=[1932:0-3175 12001:3176-3194 12020:3195-3195 12021:3196-3360 5293:3361-3361 5294:3362-3385 5318:3386-4581]

TCGACCAGAGGCTACAAACCTTGGAGACCTGATGCGGTTATGGGTACGACCAGGGGTGTGAATAAATCTGTCCTTCGGCT
TTTCAAGGGCAGTTGAGAACGCACCAGACACCTCGGAAGACAAGGTGCTTTACCCGCAACTTAACCCTATCTCCAGATAAT
CTGATTCCAGGGTAAACTGCGGTTAAGAAGAAAAGACAACACTTCCTAGGGCTCCCCCCTGCGTCGCCGAATTCTCTTACG
TTGCCGTACAGTATCCACGACCTGGTTGGGGGAGGTTAACCCCATTCCCTTTCGAGGATCGAAGCAAAGCTTGCATATGC
GGAACATCCCTCTCTCTTAGGATCGACTAACCCATGTCCAATTGCTGTTCACATGGAACCCTTCTCCGCTTCAGCCTTCAAA
GTTCTCATTTGAATATTTGCTACTACCACCAAGATCTGCACCAGAGGCTGATTCGCTCAGGCTCACGCCACAAGCTGCATC
GCAACCCCCGCGCCCTCCTACACACTGAGACATGATCACGTCAGTGGTTGGGCATCGGTCGCCCGCTTGAGCGCCATCC
ATTTTCGGGGCTAGTCCATTCGGCAGGTGAGTTGTTACACACTCCTTAGCGGATTTCGACTTCCATGACCACCGTCCTGCT
GTCAAAATGAACCAACACCCTTTGTGGTATCTGATGAGCGTGCAGTTAAGCACCTTAGCCCAACGTTAGGTTCATCCCTCA
TCGCCAGTTCTGCTTACCAAAAACGGCCCACTTAGAGTTGTCATTTATCCCAGAAGTTCAGTGAAGCAACCACTGTGGATC
TTACCCATTTAAAGTTTGAGAATAGGTCGAAGACAACACGCCCCCAATTCCTCTAATCATTCGCTTTACCTGATAAAACTGTT
CAACTCCAGCTATCCTGAGGGAAACTTCGGAAGGAACCAGCTACTAGATGGTTCGATTAGTCTTTCGCCCCTATACCCACG
TATGACGAACGATTTGCACGTCAGTACCGCTACGAGCCTCCATCAGAGTTTCCCCTGACTTTGCCCTTCGCAGGCATAGTT
CACCATCTTTCGGGTCCTAACACACATGCTCAAACTCAAACTTCTCCTAATTGATCGGTCGGTTGCCGGTGCAAAATCCCA
GCAGTCACCTTCATTGCGCCTTTAGGCTTGCTACCCAAGAACTTGCAGGCGTGCTAGACTCCTTGGTCCGTGTTACAAGAC
GGGTCGAAAAAAACCAATGAGTCAACTAGCCACGCTTGTCTGAATCCCTAACAGCCCAGAGACGCATCCGCAAATGTGCC
CTGCGAGCTGAAGGCCATCCTCAAACAGGTGTGGCAAGTGACGCGCAAGCTAAGAAACACAGCAGACAACTATTCACGCT
TAAGCACACAGCTTGCGCTGATGTGCCCAAGGCTCACAGCAGCCTACAGCAATCTCAGCAAGACGTGTGGTTCTCTTCGC
TTCCCTTTCAGCGATTTCAAGCACTTTTAACTCTCTTTCCAAAGTCCTTTTCATCTTTCCCTCATGGTACTTGTTCACTATCG
GTCTCGAGCCCGTATGTAGCTTTAGATGAAATTTACCACCTTATTTACGCTCCAATTCCGAGGAGCGTGACTCTGAGAACA
AGTGCTGTGGACAGCGGACATCAAGCGAAACACAGGATTCTCACCCGGGCACACGCTCTATTCCAAGAGGCTTACGCTCG
AGCCGCCGATAGCACTATGCCTGCAGGCTACATCCCAAAACCGAAGCTTCAGTTTCCAGCCTGAGCTAATCCCTGTTCATT
CGCCATTACTGAGGGAATCCTATTTAGTTCCTTTTCCTCCGCTTACTTATATGCTTAAATTCAGCGGGTTCACTTGTCTGACT
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Appendix B

Figure 6.1 Experimental sampling regime.

Appendix C

Table 6.1 Data yield from 100bp single end Illumina sequencing.

Sample Name	Single Reads	Data Yield (bp)
155067_2256	13,885,466	1.39 Gb
155068_2257	16,185,953	1.62 Gb
155069_2258	15,546,631	1.55 Gb
155070_2259	14,968,272	1.50 Gb
155071_2306	15,666,239	1.57 Gb
155072_2307	15,146,061	1.51 Gb
155073_2308	15,495,172	1.55 Gb
155074_2309	16,806,380	1.68 Gb
155075_5256	15,255,290	1.53 Gb
155076_5257	15,701,830	1.57 Gb
155077_5258	15,239,405	1.52 Gb
155078_5259	16,245,041	1.62 Gb
155079_5306	14,298,140	1.43 Gb
155080_5307	15,036,676	1.50 Gb
155081_5308	14,945,041	1.49 Gb
155082_5309	15,159,478	1.52 Gb
155083_62511	13,867,274	1.39 Gb
155084_62512	16,080,178	1.61 Gb
155085_62513	15,808,218	1.58 Gb
155086_62514	15,197,970	1.52 Gb
155087_63011	16,099,122	1.61 Gb
155088_63012	15,605,572	1.56 Gb
155089_63013	16,306,510	1.63 Gb
155090_63014	15,970,870	1.60 Gb
Total	370,516,789	37.05 Gb

Appendix D

Table 6.2 Illumina statistics from Arraystar and Qseq

Samples compared	Statistics	Statistics post-hoc	R^2 (Linear Correlation)	No. genes at 99% confidence	No. genes at 95% confidence	No. genes at 90% confidence	No. genes at 2-fold change	No. genes at 4-fold change	No. genes at 8-fold change	No. of genes at 2-fold change and 99% confidence	No. of genes at 4-fold change and 99% confidence	No. of genes at 8-fold change and 99% confidence
25C Day 4 vs 30C Day 4	Student's t-test	FDR (Benjamini Hochberg)	0.9291	1352	9471	15790	3361	830	213	151	17	10
25C Day 19 vs 30C Day 19	Student's t-test	FDR (Benjamini Hochberg)	0.9111	3058	12701	19240	4142	1199	380	462	106	32
25C Day 28 vs 30C Day 28	Student's t-test	FDR (Benjamini Hochberg)	0.9171	3961	13269	19319	3882	1164	373	398	102	36

Figure 6.2 Visualization of the distribution of molecular function GO classifications for the 2,798 genes differentially expressed at all time points in *Symbiodinium* exposed to thermal stress (FDR < 0.05). GO annotation graph produced using Blast2GO, GO categories displayed at ontology level 3 and slices smaller than 2% grouped into the 'other' term, numbers displayed represent the number of sequences assigned to each ontology category.

Figure 6.3 Visualization of the distribution of cellular component GO classifications for the 2,798 genes differentially expressed at all time points in *Symbiodinium* exposed to thermal stress (FDR < 0.05). GO annotation graph produced using Blast2GO, GO categories displayed at ontology level 3 and slices smaller than 2% grouped into the 'other' term, numbers displayed represent the number of sequences assigned to each ontology category.

Figure 6.4 Distribution of biological process GO terms of differentially expressed transcripts in thermally stressed *Symbiodinium*. Data displayed for 2,798 transcripts that were differentially expressed (FDR < 0.05) at day four, nineteen and twenty-eight. Transcripts that displayed increased expression at all time points (light grey bars), transcripts that displayed decreased expression at all time points (dark grey bars) and transcripts that displayed mixed expression at all time points (black bars) are shown.

Appendix E

Table 6.3 Annotations, protein sequences and expression values (fold change) of differentially expressed genes within *Symbiodinium* sp. (clade F) under thermal stress.

#component	trans	prot	TopBlastHit	Pfam	eggnog	gene_ontolo	prot_seq	F-TEST (ANOVA) P	F-TEST (ANOVA) F	Day 4 25C and 30C Fold change	Student's t-test P value Day 4 25C and 30C	Student's t-test T value Day 4 25C and 30C	Day 19 25C and 30C Fold change	Student's test Day 19 25C and 30C P value	Student's t-test Day 19 25C and 30C T value	Day 28 25C and 30C Fold change	Day 28 Student's t-test 25C and 30C P value	Day 28 Student's t-test 25C and 30C T value	Standard deviation Day 4 25C	Standard deviation Day 4 30C	Standard deviation Day 19 25C	Standard deviation Day 19 30C	Standard deviation Day 28 25C	Standard deviation Day 28 30C
comp78421_	comp	m.15	sp|Q59094|	PF00081.17	COG0605^	GO:0042597	GLAGVLGLESAQAYD	0.00033	10.074	1.082 up	0.493	-1.105	1.166 up	0.0319	-4.38	1.076 up	0.414	-1.269	0.07951	0.19203	0.09496	0.0364	0.08406	0.14588
comp28011	comp	m.6		PF00080.15	.	.	MAFGRWSISLFLVGL	0.00005	13.802	1.139 up	0.063	-3.623	1.187 up	0.0241	-4.856	1.136 up	0.0269	-4.579	0.08119	0.06451	0.0724	0.07208	0.05169	0.0618
comp47575_	comp	m.9	sp|Q27666|	PF00080.15	.	GO:0005737	SRCGSPNLGAMGNC	0.00453	5.898	1.243 up	0.0323	-4.745	1.100 up	0.527	-0.979	1.068 up	0.446	-1.183	0.11805	0.06031	0.16934	0.22554	0.09911	0.12816
comp24219_	comp	m.6	sp|Q8SWD	PF00240.18	COG5272^	GO:0005737	QLEDGRTLSDYNIQK	3.87E-	33.374	1.152 down	0.104	2.928	1.304 down	0.0384	4.098	1.197 down	0.00499	8.577	0.03009	0.13673	0.13303	0.13172	0.04149	0.04407
comp80428_	comp	m.16	sp|B3E099|	PF00141.1	COG0376^	GO:0004096	HDLWSLVSIMALRSM	1.02E-	28.14	1.215 up	0.00152	-22.555	1.087 down	0.275	1.707	1.019 down	0.689	0.638	0.0187	0.01656	0.10615	0.09485	0.06902	0.05229
comp61565_	comp	m.12	sp|Q3IQZ9|	PF00141.1	COG0376^	GO:0004096	PGRRVRSARSVSSRA	1.81E-	38.256	1.071 up	0.281	-1.76	1.005 up	0.916	-0.181	1.110 up	0.0268	-4.588	0.07705	0.0829	0.06111	0.06258	0.05943	0.02823
comp20095_	comp	m.5	sp|Q7XJ02|	PF00141.1	COG0376^	GO:0009570	LAQAFPAFFCLTVCAS	1.00E-	42.385	1.229 down	0.013	6.96	1.248 down	0.0157	5.721	1.091 down	0.0537	3.587	0.05056	0.06916	0.03953	0.1049	0.0493	0.05075
comp21891_	comp	m.5	sp|Q6ZJJ1|A	PF00141.1	COG0376^	GO:0009941	GCGPIMIRLSFSDAGV	0.0118	4.711	1.092 down	0.514	1.054	1.451 down	0.0434	3.918	1.212 down	0.196	2.081	0.16043	0.18143	0.18049	0.20647	0.06972	0.25823
comp36166_	comp	m.8	sp|Q0JEQ2|	PF00141.1	COG0376^	GO:0009941	MGNTACCQTEAIDGS	0.0186	4.182	1.181 up	0.0453	-4.133	1.190 up	0.166	-2.259	1.006 up	0.919	-0.181	0.09999	0.05959	0.1952	0.10818	0.09896	0.03242
comp40867_	comp	m.8	sp|Q0JEQ2|	PF00141.1	COG0376^	GO:0009941	VKPCQRSSSSPGSEP	0.00007	13.277	1.036 down	0.245	1.911	1.125 down	0.00465	9.812	1.181 down	0.0311	4.348	0.02754	0.04699	0.01648	0.03072	0.04289	0.10209
comp43143_	comp	m.8	sp|Q42564|	PF00141.1	COG0376^	GO:0009941	AMIRGQQVCLFLLGLA	2.99E-	52.76	1.201 down	0.00322	13.978	1.152 down	0.0106	6.709	1.175 down	0.00892	6.817	0.03352	0.01764	0.01745	0.05865	0.03682	0.05755
comp65681_	comp	m.12	sp|Q7XZP5|	PF00141.1	COG0376^	GO:0016021	MASMSWFHVIFGIFLR	0.00004	14.678	1.252 up	0.0417	-4.271	1.144 up	0.137	-2.471	1.064 up	0.223	-1.943	0.13261	0.07432	0.11102	0.11209	0.08851	0.0293
comp66617_	comp	m.13	sp|Q0JEQ2|	PF00141.1	COG0376^	GO:0009941	MGNTQCCKAVDTSPD	0.00159	7.393	1.005 up	0.942	-0.137	1.052 down	0.119	2.628	1.146 down	0.00937	6.678	0.05682	0.08898	0.04721	0.03039	0.03896	0.04439
comp88508_	comp	m.17	sp|Q0JEQ2|	PF00141.1	COG0376^	GO:0009941	FSLPQNPSFCACAFC	0.013	4.595	1.149 up	0.0179	-6.086	1.155 up	0.0697	-3.274	1.032 up	0.741	-0.535	0.03627	0.05543	0.0633	0.11011	0.11294	0.12688
comp90586_	comp	m.17	sp|Q7XJ02|	PF04324.10	COG0376^	GO:0009570	PDGTKLLGGILVGDIS	0.00002	15.903	1.019 up	0.619	-0.81	1.170 up	0.0445	-3.875	1.131 up	0.0309	-4.361	0.04074	0.05378	0.05277	0.1048	0.06793	0.045
comp11509	comp	m.1	sp|Q9GLW9|	PF08534.5^	.	GO:0005739	VALQPWELSPSNKVL	0.00202	7.032	1.483 up	0.205	-2.119	1.510 up	0.0364	-4.175	1.407 up	0.0195	-5.133	0.43374	0.31763	0.27572	0.07302	0.14612	0.12501
comp42154_	comp	m.8	sp|P0CB50|	PF00578.16	COG0450^	GO:0005739	FWLKFWLKSNFGSES	2.01E-	37.555	1.100 down	0.101	2.965	1.176 down	0.0196	5.25	1.215 down	0.00565	8.173	0.03103	0.08783	0.06216	0.06436	0.05389	0.04277
comp63575_	comp	m.12	sp|O08709|	PF00578.16	COG0450^	GO:0016023	MGCCGGKKAPKKPP	1.98E-	25.059	1.292 up	0.0161	-6.35	1.286 up	0.0263	-4.712	1.360 up	0.012	-6.108	0.09926	0.06086	0.07171	0.13636	0.14116	0.0344
comp77639_	comp	m.15	sp|P30044|P	PF08534.5^	COG0678^	GO:0031410	MPRQGVLSRGLVLLS	1.49E-	26.315	1.072 down	0.037	4.477	1.169 down	0.00743	7.789	1.215 down	0.0163	5.463	0.01708	0.04187	0.02904	0.05023	0.07834	0.06723
comp102079	comp	m.1	sp|Q39362|T	PF08534.5^	.	GO:0005737	FAFGPSRLPSYASIAF	0.00306	6.435	1.227 up	0.304	-1.669	1.131 up	0.538	-0.954	1.244 down	0.0147	5.682	0.28438	0.21208	0.20903	0.3094	0.08943	0.06587
comp106802	comp	m.4	sp|Q98TX1|	PF00085.15	.	GO:0005737	FTHICQTHLCFLLVIAA	0.00052	9.229	1.269 up	0.0722	-3.422	1.272 up	0.0331	-4.32	1.222 up	0.102	-2.81	0.15232	0.13168	0.10584	0.12133	0.06851	0.19426
comp147613	comp	m.2	sp|P34723|T	PF00085.15	.	GO:0009055	ASFVVPHRFPGGRLP	0.006	5.532	1.343 up	0.0463	-4.091	1.228 up	0.275	-1.708	1.105 up	0.354	-1.443	0.17473	0.11369	0.30371	0.16902	0.18795	0.06911
comp18594_	comp	m.4	sp|O64628|T	PF02114.11	COG0526^	GO:0045454	MGYYEKNIQVLNDVL	0.00106	8.043	1.396 up	0.0196	-5.854	1.250 up	0.0915	-2.933	1.401 up	0.204	-2.038	0.09361	0.13534	0.16671	0.1433	0.43785	0.19057
comp20192_	comp	m.5	sp|O81332|T	PF13833.1^	.	GO:0009507	MARLRVQGHGWLAR	0.0119	4.7	1.081 up	0.316	-1.624	1.214 up	0.0383	-4.103	1.027 up	0.733	-0.552	0.07215	0.11916	0.06462	0.12068	0.08001	0.11587
comp86111	comp	m.16	sp|Q5JMR	PF00085.15	COG0526^	GO:0009570	FVHGISCNMPSMLRA	0.00021	10.902	1.071 up	0.726	-0.583	1.370 up	0.0234	-4.908	1.233 up	0.0807	-3.077	0.17315	0.29665	0.16952	0.07499	0.08696	0.17691
comp73859_	comp	m.14	sp|Q6Y657|	PF00578.16	COG0208^	GO:0005971	AVLAKDSNCLRSPNL	0.00001	17.158	1.126 up	0.0636	-3.609	1.084 down	0.0695	3.278	1.181 down	0.028	4.507	0.03604	0.08853	0.0471	0.05419	0.07593	0.07486
comp109991	comp	m.7	sp|Q5R6T1	PF00085.15	.	GO:0005788	TACMSILWLNVLFRAL	1.22E-	27.279	1.217 up	0.0124	-7.157	1.144 up	0.256	-1.786	1.043 down	0.622	0.774	0.044	0.06595	0.11191	0.18646	0.0764	0.14091
comp11033	comp	m.7	sp|Q50KB1	PF00085.15	.	GO:0005783	PPKPVALMWRLLVLP	3.24E-	22.979	1.171 up	0.0404	-4.325	1.239 up	0.0138	-6.008	1.085 up	0.135	-2.489	0.0363	0.09938	0.08568	0.05769	0.06715	0.06752
comp12847_	comp	m.1	sp|P38658|E	PF00085.15	.	GO:0005788	GEMLGFQVVAMLRFM	0.0018	7.203	1.286 up	0.149	-2.49	1.353 up	0.0201	-5.195	1.162 up	0.064	-3.36	0.28794	0.04838	0.15434	0.06666	0.07065	0.10821
comp18562_	comp	m.4	sp|Q71A37|	PF02114.11	COG0526^	GO:0045454	SPLSAGCLRVAIVAMA	0.00024	10.631	1.104 up	0.442	-1.235	1.082 down	0.531	0.97	1.409 down	0.0113	6.231	0.17246	0.15414	0.18099	0.14998	0.10077	0.12288
comp31838_	comp	m.7	sp|Q6AUC	PF00085.15	NOG23798	GO:0005576	RRSLRPIAMAVCCAR	0.00097	8.174	1.193 up	0.332	-1.566	1.744 up	0.0298	-4.491	1.603 up	0.0545	-3.567	0.22376	0.2376	0.16527	0.31711	0.37703	0.06011
comp31956_	comp	m.7	sp|Q50KB1	PF00085.15	.	GO:0005783	WEQQSQTNYLAMGA	0.00004	14.752	1.068 down	0.222	2.025	1.213 down	0.015	5.819	1.201 down	0.0159	5.508	0.06274	0.07114	0.02781	0.09184	0.06651	0.06967
comp34679_	comp	m.7	sp|P73920|T	PF13728.1	COG0526^	GO:0016021	FGPFGPFGSYVATFA	0.026	3.813	1.129 down	0.208	2.103	1.029 down	0.812	0.389	1.223 down	0.028	4.508	0.10812	0.12667	0.18029	0.11621	0.09886	0.08287
comp35649_	comp	m.7	sp|Q503L9|	PF00578.16	NOG27311	GO:0005829	MGSGASAPKAPADAL	0.00001	16.869	1.243 up	0.0351	-4.576	1.313 up	0.0218	-5.048	1.184 up	0.0159	-5.505	0.11734	0.07134	0.10091	0.11882	0.07966	0.03869
comp41119	comp	m.8	sp|Q50KB1	PF00085.15	.	GO:0005783	SNWSVRWPLGAFRV	0.0266	3.79	1.036 up	0.421	-1.292	1.020 down	0.786	0.437	1.103 down	0.0304	4.386	0.06564	0.0449	0.1191	0.05603	0.05083	0.04036
comp44208_	comp	m.9	sp|Q5R5L3|	PF00226.26	.	GO:0005788	MPAVQRKGAKAAKDR	0.00009	12.675	1.582 down	0.0474	4.054	1.458 down	0.0239	4.871	1.315 down	0.0222	4.91	0.05406	0.3224	0.08941	0.20484	0.10393	0.12308
comp56148_	comp	m.11	sp|Q50KB1	PF00085.15	.	GO:0005783	YQPESIPRISPSLAMK	0.00426	5.981	1.309 up	0.15	-2.483	1.207 up	0.0158	-5.706	1.089 up	0.469	-1.126	0.24984	0.18859	0.08427	0.04466	0.09839	0.19733
comp61747_	comp	m.12		PF00085.15	.	.	FPKLTLTGAKKAQKT	2.19E-	36.963	1.094 down	0.0246	5.3	1.426 down	0.00595	8.64	1.273 down	0.00807	7.09	0.04344	0.02309	0.08977	0.07768	0.05802	0.07944
comp62517_	comp	m.12	sp|Q29RV1|	PF00085.15	COG0526^	GO:0005788	SWCQLPSPCRDDTW	5.55E-	47.268	1.493 up	0.0382	-4.426	2.047 up	0.00233	-14.573	1.519 up	0.00216	-12.675	0.08518	0.24729	0.06483	0.12622	0.09154	0.02608
comp66152_	comp	m.12	sp|Q503L9|	PF00578.16	NOG27311	GO:0005829	MSLIELLGSTVLGKGC	1.27E-	40.657	1.215 down	0.0246	5.3	1.202 down	0.0103	6.777	1.255 down	0.0156	5.553	0.05854	0.08853	0.07398	0.02631	0.10853	0.04732
comp66165_	comp	m.12	sp|Q92249|	PF00085.15	COG0526^	GO:0005788	MVHDAVIQHGIEVMH	2.35E-	36.51	1.074 up	0.253	-1.877	1.099 down	0.0209	5.125	1.048 down	0.204	2.038	0.02475	0.10826	0.03126	0.04329	0.05815	0.03241
comp73552_	comp	m.14	sp|Q43116|	PF00085.15	.	GO:0005788	LLEVMFSQGPSKASQ	0.00078	8.525	1.124 up	0.119	-2.761	1.168 up	0.304	-1.599	1.274 up	0.0105	-6.415	0.10954	0.05444	0.21941	0.17655	0.10621	0.02542
comp77231_	comp	m.15		PF00085.15	.	.	FLCPLSWFVSADLDV	0.0018	7.205	1.455 down	0.0453	4.135	1.043 up	0.826	-0.361	1.037 down	0.863	0.295	0.14797	0.2163	0.14097	0.30699	0.24251	0.26228
comp8010_c	comp	m.16	sp|Q17770|	PF00085.15	COG0526^	GO:0005783	SFSLVSLSAAMIRALC	0.00001	18.126	1.152 down	0.081	3.263	1.167 up	0.0273	-4.65	1.142 up	0.113	-2.69	0.11632	0.04711	0.05358	0.08008	0.10143	0.101
comp93578_	comp	m.17	sp|Q50KB1	PF00085.15	.	GO:0005783	LKPDWDKLASKWNKP	4.07E-	76.005	1.534 down	0.0278	5.046	1.041 up	0.673	-0.661	1.644 down	0.0177	5.313	0.20184	0.13866	0.12756	0.12554	0.06654	0.2619
comp95736_	comp	m.17	sp|O23184|	PF13499.1^	COG5126^	GO:0005509	RFPFLEVMSSIGEPIP	0.00081	8.459	1.111 up	0.0958	-3.035	1.156 up	0.106	-2.767	1.181 up	0.0218	-4.944	0.07093	0.07091	0.11014	0.10383	0.07767	0.05893

comp14913_	comp	m.2	sp|P0ACA9	PF02798.15	COG0625^	GO:0004364	AMERLLLLVLGAIAVL	0.00007	13.196	1.284 up	0.0452	-4.138	1.673 up	0.0198	-5.225	1.316 up	0.0455	-3.805	0.10089	0.14228	0.1804	0.21981	0.1835	0.09908
comp21843_	comp	m.5	sp|P46428|		NOG122057	GO:0004364	YRLAAAASPLIAGVGM	1.28E-	40.605	1.672 up	0.0135	-6.86	2.424 up	0.00519	-9.197	1.941 up	0.00748	-7.291	0.16882	0.13512	0.22085	0.16858	0.19693	0.17373
comp66650_	comp	m.13	sp|O35660|	PF02798.15	NOG05174^	GO:0005737	FGSSSFLLVPCQPVP	1.50E-	26.294	1.398 up	0.0135	-6.846	1.457 up	0.0186	-5.353	1.117 up	0.0581	-3.482	0.10731	0.09217	0.16842	0.11319	0.04035	0.08283
comp67710_	comp	m.13	sp|P15214|	PF02798.15	.	GO:0005737	MASQPLEVTLYHYPLT	0.00892	5.039	1.025 up	0.822	-0.39	1.259 up	0.0429	-3.933	1.187 up	0.0989	-2.836	0.17888	0.05278	0.15564	0.06678	0.15933	0.07111
comp72345_	comp	m.14	sp|Q08862|	PF02798.15	NOG26641	GO:0005737	MTDGEVIARATTRKIS	1.51E-	26.259	1.095 up	0.233	-1.968	1.239 up	0.0168	-5.564	1.238 up	0.00357	-9.832	0.11385	0.0713	0.04302	0.10269	0.05388	0.03239
comp94166_	comp	m.17	sp|P46436|	PF02798.15	.	GO:0004364	MGSTLRRQCRKALSF	0.00287	6.528	1.111 up	0.347	-1.515	1.383 up	0.0421	-3.962	1.212 up	0.0589	-3.464	0.1295	0.15439	0.20046	0.12569	0.11621	0.11034
comp11863	comp	m.1	sp|P46436|	PF13417.1^	.	GO:0004364	LKGFGEAIRLALYIGN	0.00154	7.443	1.313 up	0.00635	-9.75	1.100 down	0.437	1.2	1.000 down	0.999	0.006	0.07521	0.02936	0.13004	0.19125	0.35088	0.09655
comp44743_	comp	m.9	sp|P08010|	PF02798.15	NOG30008	GO:0005737	GSSWLPIPAVSNGLVT	7.45E-	44.774	1.124 up	0.0409	-4.303	1.130 down	0.0152	5.794	1.178 down	0.018	5.281	0.02768	0.07367	0.04214	0.04449	0.08098	0.03877
comp26118	comp	m.6	sp|Q9C6C8|	PF02798.15	COG0625^	GO:0005737	DAMAGKVVLVGTAKA	0.00278	6.572	1.343 down	0.0223	5.531	1.053 down	0.77	0.468	1.099 down	0.413	1.271	0.15048	0.03225	0.18126	0.26699	0.05354	0.20781
comp79594_	comp	m.16	sp|P46436|	PF02798.15	.	GO:0004364	SHLSSHLFLVASGFLS	0.00001	17.713	1.199 down	0.0863	3.176	1.624 down	0.0131	6.131	1.595 down	0.0229	4.852	0.1557	0.05487	0.04205	0.22431	0.14845	0.2347
comp178345	comp	m.4	sp|Q04619|	PF00183.13	COG0326^	GO:0005829	EMFNELSEDKESYKK	0.00082	8.442	1.403 down	0.0948	3.049	1.919 up	0.0307	-4.441	1.477 down	0.298	1.635	0.28511	0.14644	0.13891	0.40008	0.33015	0.60401
comp36567_	comp	m.8	sp|Q9DGQ	PF03234.9^	.	GO:0005737	FGSHGNVAMSGFDYS	2.48E-	24.077	1.501 down	0.00597	10.126	1.522 down	0.0295	4.51	1.491 down	0.00578	8.094	0.05743	0.10065	0.05586	0.2631	0.11057	0.08991
comp44695_	comp	m.9	sp|O44001|	PF13589.1^	.	GO:0005737	IFSYQFTSFVLMAPAE	3.99E-	50.191	1.447 down	0.00613	10.021	1.479 down	0.0067	8.177	1.496 down	0.0044	9.024	0.05819	0.08911	0.07047	0.11881	0.09496	0.08714
comp59092_	comp	m.11	sp|O44001|	PF00183.13	.	GO:0005737	FYKSLSNDWEDHLAV	3.65E-	33.714	1.665 down	0.00821	8.629	1.286 down	0.0197	5.237	1.156 down	0.0519	3.629	0.14399	0.09145	0.12193	0.06649	0.09516	0.06566
comp80804_	comp	m.16	sp|O44001|	PF13589.1^	.	GO:0005737	MAETFAFNADIQQLM	0.00001	17.072	1.246 down	0.0126	7.07	1.189 down	0.0204	5.174	1.139 down	0.0687	3.27	0.0589	0.06789	0.0754	0.06094	0.11128	0.03004
comp80807_	comp	m.16	sp|O44001|	PF13589.1^	.	GO:0005737	LPISQAQQNVISFEWS	6.52E-	20.337	1.367 down	0.00354	13.035	1.213 down	0.0197	5.238	1.107 down	0.23	1.911	0.05455	0.04266	0.07428	0.07673	0.14154	0.06134
comp37367_	comp	m.8	sp|P54651|H	PF13589.1^	COG0326^	GO:0005829	KHFGSSTDIWSTFES	6.72E-	30.214	1.290 down	0.0234	5.413	1.084 down	0.0659	3.345	1.145 down	0.00983	6.558	0.04301	0.12913	0.05279	0.04623	0.02078	0.05607
comp24798_	comp	m.6	sp|O44001|	PF13589.1^	.	GO:0005737	KTNSTLTIEDSGIGMT	9.65E-	28.398	1.365 down	0.0131	6.936	1.163 down	0.103	2.798	1.073 down	0.302	1.62	0.05535	0.11731	0.14506	0.05897	0.06732	0.10679
comp80701_	comp	m.1	sp|P36183|E	PF13589.1^	.	GO:0005788	RSSSVMFRLWAVLAL	0.00025	10.601	1.141 down	0.0186	5.98	1.149 down	0.0519	3.657	1.074 down	0.255	1.804	0.03329	0.05424	0.0715	0.08313	0.09635	0.0638
comp76430_	comp	m.1	sp|Q9I8F9|H	PF00012.15	COG0443^	GO:0005524	PSPEEKSYAVGIDLGT	0.00000	28.138	1.162 up	0.0469	-4.075	1.604 up	0.0131	-6.133	1.466 up	0.00329	-10.134	0.0957	0.04675	0.19125	0.11378	0.10434	0.03128
comp26160_	comp	m.6	sp|P30722|D	PF00012.15	.	GO:0009507	AQIAHRRLQCLAVEM	0.00025	10.565	1.193 up	0.0377	-4.442	1.234 up	0.081	-3.081	1.184 up	0.115	-2.671	0.11097	0.03074	0.12172	0.15572	0.16517	0.07773
comp14362_	comp	m.2	sp|Q05746|	PF00012.15	.	GO:0005524	NVLRIINEPTAAAIAYG	3.12E-	23.132	1.103 up	0.348	-1.512	1.251 up	0.0179	-5.437	1.533 up	0.00139	-16.773	0.1625	0.09293	0.05845	0.10376	0.03435	0.06501
comp287558	comp	m.7	sp|A9W6R7|	PF00012.15	COG0443^	GO:0005524	FDLVGIPPAPRGVPQI	0.0122	4.667	3.099 up	0.473	-1.154	3.919 up	0.0384	-4.098	3.035 up	0.0311	-4.348	2.79292	0.44329	0.86516	0.41997	0.66332	0.32095
comp71407_	comp	m.1	sp|O59855|	PF00012.15	.	GO:0005829	MQGRQPMAARHEWT	2.64E-	82.663	2.332 up	0.00167	-26.689	1.744 up	0.00591	-8.675	1.702 up	0.00632	-7.835	0.08075	0.0432	0.1758	0.05803	0.18523	0.06419
comp72074_	comp	m.1	sp|Q16956|	PF00012.15	.	GO:0005788	MQYLIALGGLVVLVSA	0.00084	8.395	1.159 up	0.046	-4.105	1.285 up	0.0336	-4.297	1.034 up	0.619	-0.783	0.0618	0.08387	0.161	0.04962	0.04871	0.11374
comp127611	comp	m.1	sp|Q03685|	PF00012.15	.	GO:0005788	MWRATVAFAALVATG	0.00004	14.267	1.408 down	0.045	4.147	1.049 up	0.671	-0.664	1.368 up	0.0455	-3.806	0.08753	0.2214	0.20146	0.06126	0.15463	0.1808
comp83195_	comp	m.1	sp|Q42434|	PF00012.15	.	GO:0005788	IVLVGGSTRIPKVQKL	6.92E-	30.075	1.055 down	0.274	1.789	1.017 up	0.714	-0.578	1.162 up	0.0346	-4.194	0.07324	0.04829	0.06507	0.05867	0.04273	0.09463
comp76833_	comp	m.1	sp|Q03685|	PF00012.15	.	GO:0005788	AQDFGSRDSSGLNNS	3.67E-	22.483	1.045 down	0.0946	3.053	1.035 down	0.485	1.078	1.089 up	0.00542	-8.313	0.01546	0.03874	0.08034	0.04528	0.00647	0.02896
comp45518_	comp	m.9	sp|Q9S7C0|	PF00012.15	COG0443^	GO:0005829	ETPRRRPKKAMVTKN	0.00006	13.563	1.199 down	0.045	4.145	1.076 down	0.148	2.389	1.078 up	0.0653	-3.336	0.04194	0.1194	0.03631	0.08127	0.04017	0.05173
comp80358_	comp	m.1	sp|Q9LTX9|	PF00012.15	COG0443^	GO:0048046	KYTRSQGVGSFATLS	7.12E-	29.938	1.112 down	0.0112	7.507	1.097 down	0.0256	4.757	1.062 down	0.0374	4.083	0.0316	0.02585	0.04846	0.02865	0.03039	0.03
comp80854_	comp	m.1	sp|Q05746|	PF00012.15	.	GO:0005524	MAKKTAIGIDLGTTYS	1.22E-	40.983	1.208 down	0.00259	15.544	1.252 down	0.00833	7.406	1.046 down	0.158	2.313	0.02299	0.02654	0.04639	0.07437	0.03361	0.04539
comp76252_	comp	m.1	sp|P41753|H	PF00012.15	.	GO:0005524	ATTVPVDEIVAAINQS	3.64E-	33.743	1.173 down	0.136	2.605	1.282 down	0.0213	5.092	1.361 down	0.00151	15.497	0.0632	0.1658	0.06905	0.12284	0.04489	0.03585
comp79597_	comp	m.1	sp|F4JMJ1|	PF00012.15	COG0443^	GO:0009507	MVLPGRPWIVFLAGAI	4.91E-	31.916	1.048 down	0.169	2.34	1.221 down	0.00621	8.451	1.205 down	0.0211	4.995	0.02568	0.05248	0.03216	0.06036	0.06467	0.08635
comp80127_	comp	m.1	sp|Q9S9N1|	PF00012.15	.	GO:0005618	QKIEAKNGLENYCFT	8.33E-	43.757	1.091 down	0.149	2.49	1.235 down	0.0183	5.392	1.161 down	0.00887	6.835	0.04327	0.09184	0.0478	0.10277	0.05503	0.03097
comp61160_	comp	m.1	sp|F4JMJ1|	PF00012.15	COG0443^	GO:0009507	MGIPRPVLTLLLVLAL	0.00066	8.803	1.095 down	0.187	2.225	1.105 down	0.263	1.755	1.156 down	0.022	4.926	0.07962	0.08748	0.05792	0.15409	0.05232	0.06713
comp103222	comp	m.2	sp|Q8T869|	PF00012.15	COG0443^	GO:0005788	LKPFLVQELQQNLGIH	1.37E-	40.13	1.391 down	0.0241	5.354	1.114 down	0.195	2.079	1.248 up	0.0771	-3.129	0.14681	0.10061	0.09459	0.11744	0.15746	0.13048
comp90410_	comp	m.1	sp|O24581|	PF00012.15	.	GO:0005618	QGGSRRFTKIPRQNS	0.00025	10.583	1.233 down	0.0378	4.441	1.122 down	0.0967	2.866	1.040 up	0.436	-1.209	0.11109	0.07901	0.07612	0.08846	0.07122	0.06395
comp25006_	comp	m.6	sp|P0CB32|	PF00012.15	COG0443^	GO:0008180	FSCAEMPDGPPADDE	0.00001	16.901	1.189 down	0.0416	4.275	1.383 down	0.0284	4.573	1.147 down	0.0261	4.635	0.09273	0.07144	0.07668	0.18986	0.04917	0.07038
comp69759_	comp	m.1	sp|P37900|H	PF00012.15	.	GO:0005739	FDPENFSGRSQQFTM	8.69E-	43.458	1.154 down	0.0171	6.196	1.190 down	0.012	6.36	1.087 down	0.0358	4.146	0.04924	0.04549	0.06027	0.05153	0.04162	0.04136
comp72110_	comp	m.1	sp|O59838|	PF00012.15	COG0443^	GO:0005829	ELCHFAPGHAISLFDR	3.20E-	23.026	1.125 down	0.0554	3.81	1.176 down	0.0161	5.666	1.105 down	0.164	2.273	0.04754	0.07622	0.06565	0.05037	0.03914	0.12122
comp82297_	comp	m.1		PF00011.16	.	.	MDAESFNALGPMPPA	0.00003	14.956	1.142 down	0.0327	4.723	1.216 down	0.051	3.684	1.115 down	0.0617	3.41	0.054	0.06077	0.1201	0.09596	0.07244	0.0581
comp18274_	comp	m.4		PF00011.16	.	.	SDLSPIVPLRRLQIHRL	0.00019	11.123	1.067 down	0.415	1.308	1.337 down	0.0228	4.952	1.208 down	0.0322	4.295	0.07408	0.12353	0.03809	0.16526	0.09341	0.08673
comp286632	comp	m.7	sp|O74984|	PF00011.16	COG0071^	GO:0005739	MLTTRFPAFGNSIFDL	1.09E-	41.785	3.526 down	0.0066	9.585	1.399 up	0.434	-1.209	1.660 down	0.0638	3.364	0.12782	0.3572	0.27057	0.75549	0.2213	0.37431
comp18722_	comp	m.4		PF00011.16	.	.	VRAAWGNPGTACAFI	0.00001	17.401	1.234 down	0.0228	5.484	1.178 down	0.126	2.563	1.372 down	0.00283	10.922	0.05733	0.09478	0.05333	0.17704	0.07777	0.03102
comp4906_c	comp	m.9	sp|Q6K6S5|	PF00447.12	COG5169^	GO:0005737	YKGSLLTFIRQLHFYG	2.18E-	24.627	1.283 up	0.31	-1.646	3.113 up	0.0162	-5.649	3.097 up	0.00816	-7.055	0.42367	0.112	0.23806	0.52903	0.23862	0.39602
comp47270_	comp	m.9	sp|Q04960|	PF00226.26	.	GO:0005886	MFFGGFPGGDFPGM	3.49E-	33.985	1.265 down	0.0109	7.604	1.218 down	0.0163	5.628	1.138 down	0.0171	5.368	0.05888	0.06716	0.04877	0.08874	0.06956	0.00466
comp64766_	comp	m.1	sp|Q8WW22	PF00226.26	COG0484^	GO:0016020	AAQSAEAEAMSTCSS	0.00005	14.023	1.268 down	0.0377	4.447	1.210 down	0.0182	5.404	1.235 down	0.0415	3.937	0.10101	0.11678	0.0834	0.05846	0.06825	0.13909
comp177335	comp	m.4	sp|Q08168|	PF13414.1^	.	GO:0005737	TEEEQEEQTLLLEDAV	0.00111	7.968	1.181 down	0.288	1.734	1.104 down	0.389	1.328	1.595 up	0.0306	-4.377	0.15704	0.22889	0.09852	0.19168	0.25788	0.16803
comp50529_	comp	m.1	sp|Q08168|	PF13414.1^	.	GO:0005737	QLEEARFTQERQKRE	2.63E-	35.807	1.428 down	0.0483	4.023	1.390 down	0.00988	6.872	2.001 down	0.00178	14.107	0.10691	0.23219	0.11517	0.07681	0.11089	0.08854
comp88823_	comp	m.1	sp|Q08168|	PF13414.1^	.	GO:0005737	MAEQWTIKVKIASMD	7.30E-	19.939	1.387 down	0.0126	7.067	1.367 down	0.0285	4.566	1.319 down	0.0138	5.81	0.03225	0.12964	0.14202	0.13758	0.05333	0.12684
comp137746	comp	m.2	sp|Q08168|	PF13414.1^	.	GO:0005737	KEAKEAKATGPVELE	0.00001	17.488	2.177 down	0.0175	6.144	1.287 down	0.116	2.659	1.547 down	0.0232	4.83	0.15233	0.33219	0.12107	0.2456	0.14635	0.21602
comp101963	comp	m.1	sp|Q6BIP2|	PF00176.18	COG0553^	GO:0005737	PERPFKLLDNGSDVA	3.70E-	22.448	1.068 up	0.129	-2.667	1.102 up	0.161	-2.288	1.154 up	0.0115	-6.201	0.05963	0.04032	0.06938	0.10128	0.0592	0.03079
comp78533_	comp	m.1	sp|Q6CJM4|	PF00271.26	COG0553^	GO:0005737	MKTRGRILQQFCETD	0.00888	5.044	1.113 up	0.599	-0.855	1.295 up	0.174	-2.207	1.474 up	0.0182	-5.261	0.23753	0.27514	0.21401	0.26212	0.19628	0.08297
comp220535	comp	m.5	sp|Q6BIP2|	PF00176.18	COG0553^	GO:0005737	RFRPDEAAPKSLAVD	0.00038	9.806	1.027 up	0.918	-0.191	1.082 up	0.67	-0.666	1.852 up	0.00706	-7.478	0.3805	0.1364	0.23767	0.24529	0.22649	0.07272
comp35209_	comp	m.7	sp|Q4IJ84|R	PF13920.1^	COG0553^	GO:0005737	NTAEVPKIAVKQHLRL	0.0103	4.874	1.409 up	0.0389	-4.393	1.353 up	0.198	-2.065	1.259 up	0.0667	-3.308	0.20334	0.09767	0.37304	0.19875	0.18184	0.08538
comp21516_	comp	m.5	sp|P36607|R	PF00176.18	COG0553^	GO:0005737	LAEAPARPAGFLLDLH	2.89E-	23.444	1.118 down	0.105	2.918	1.122 down	0.0613	3.438	1.155 down	0.0215	4.965	0.07861	0.07813	0.04543	0.08531	0.04787	0.06891
comp73860_	comp	m.1	sp|P79051|R	PF00176.18	.	GO:0000109	KREEMEEGGDAYAD	2.21E-	24.572	1.317 down	0.0741	3.387	1.126 up	0.0824	-3.06	1.289 up	0.017	-5.38	0.07888	0.22137	0.05464	0.09765	0.11602	0.07134
comp165954	comp	m.3	sp|P31244|R	PF00176.18	COG0553^	GO:0031463	KGPALVVCPMAAVNQ	0.00002	16.465	1.625 down	0.167	2.354	1.189 up	0.256	-1.786	1.322 up	0.0136	-5.84	0.09589	0.58736	0.11879	0.25328	0.09385	0.10128
comp73891_	comp	m.1	sp|Q84L33|	PF11976.3^	COG5272^	GO:0005634	MKITVRPIKGESFFVE	0.00078	8.529	1.028 down	0.762	0.511	1.154 down	0.0495	3.728	1.219 down	0.0164	5.456	0.12144	0.10594	0.06714	0.08836	0.03625	0.09837
comp72013_	comp	m.1	sp|Q9JIL8|R	PF13476.1^	COG0419^	GO:0000781	LALPHLAMTTISKLGIQ	9.55E-	42.746	1.263 up	0.0245	-5.318	1.255 up	0.00699	-7.993	1.328 up	0.00137	-17.082	0.11838	0.04525	0.0137	0.08106	0.0399	0.02661
comp67078_	comp	m.1	sp|P34205|P	PF00875.13	.	GO:0003904	DARRCRDVNGVKLQE	5.09E-	21.23	1.460 up	0.0185	-5.993	1.505 up	0.0155	-5.747	1.325 up	0.0167	-5.411	0.07645	0.16546	0.09773	0.18068	0.14866	0.02214
comp72290_	comp	m.1	sp|O48652|	PF00875.13	COG0415^	GO:0003914	KISASFVQRARAIMKS	6.67E-	30.258	1.502 up	0.0179	-6.078	1.454 up	0.00414	-10.407	1.423 up	0.0149	-5.651	0.15139	0.12043	0.06254	0.08288	0.07541	0.16394
comp56353_	comp	m.1		PF00875.13	.	.	EAMLHVFPRAVALGD	0.00011	12.304	1.159 up	0.0358	-4.542	1.225 up	0.0372	-4.144	1.346 up	0.0618	-3.407	0.0419	0.08405	0.13136	0.05231	0.20055	0.15276
comp45980_	comp	m.9	sp|P12768|P	PF03441.9^	.	GO:0003904	MKMYIPTIKHCIQDKR	0.0852	2.611	1.199 up	0.549	-0.969	1.314 up	0.0345	-4.256	1.166 down	0.393	1.327	0.42692	0.33436	0.12832	0.13386	0.0946	0.32051
comp74421_	comp	m.1	sp|Q28811|	PF00875.13	.	GO:0003904	PDLGLMIGRRDLPSF	2.18E-	36.99	1.649 down	0.0118	7.302	2.025 down	0.00663	8.22	2.005 down	0.00672	7.629	0.09722	0.17231	0.11312	0.22045	0.09144	0.24673

comp137408	comp	m.2	.	PF00875.13	.	.	STGLTPAAELAKISSE	0.037	3.442	1.217 down	0.329	1.576	1.138 down	0.555	0.915	1.339 down	0.00816	7.053	0.11224	0.34219	0.37935	0.15448	0.10017	0.06557
comp71866_	comp	m.1	sp|Q5IFN2|	PF00875.13	COG0415^	GO:0009507	SLLRPAMNVVCAWLR	0.00072	8.669	1.024 up	0.871	-0.289	1.364 up	0.124	-2.585	1.385 up	0.00983	-6.561	0.15617	0.18015	0.30606	0.16316	0.1031	0.09955
comp118057	comp	m.1	sp|Q84KJ5|	PF03441.9^	COG0415^	GO:0009507	PLLPPEERQFFRWRG	0.00038	9.796	1.265 down	0.201	2.142	1.154 down	0.0644	3.375	1.559 down	0.0264	4.615	0.24573	0.19999	0.06629	0.10333	0.1097	0.25519
comp75893_	comp	m.1	sp|Q84KJ5|	PF00875.13	COG0415^	GO:0009507	LALMPGKERVNVVWF	1.51E-	59.856	1.996 down	0.00243	16.474	1.930 down	0.00322	12.022	1.823 down	0.00939	6.673	0.09946	0.06909	0.07428	0.13929	0.24011	0.09934
comp18731_	comp	m.4	sp|Q5IFN2|	PF00875.13	COG0415^	GO:0009507	AARSAALWLRCDLRL	1.49E-	39.594	1.044 down	0.182	2.259	1.161 down	0.0357	4.207	1.058 down	0.267	1.752	0.03955	0.03851	0.04409	0.09303	0.06877	0.06376
comp76815_	comp	m.1	sp|Q96524|	PF03441.9^	COG0415^	GO:0005634	EPLPPLRRLVSPKDW	0.00018	11.268	1.445 up	0.0185	-5.988	1.581 up	0.0281	-4.593	1.143 up	0.308	-1.596	0.15807	0.08078	0.27824	0.07352	0.13347	0.2026
comp41340_	comp	m.8	sp|Q96524|	PF00875.13	COG0415^	GO:0005634	RRAKASPETPGGCVV	6.05E-	20.604	1.004 up	0.943	-0.136	1.447 down	0.0104	6.752	1.008 down	0.948	0.121	0.0822	0.05998	0.03761	0.15345	0.19292	0.06913
comp72509_	comp	m.1	sp|Q6CHI1|	PF13920.1^	COG5432^	GO:0005634	SPQRHSGTRERGRK	1.20E-	27.344	1.229 up	0.0193	-5.881	1.276 up	0.0347	-4.25	1.365 up	0.0122	-6.079	0.02864	0.09709	0.11886	0.11531	0.13002	0.0704
comp101171	comp	m.7	sp|Q9ZU51|	PF13639.1^	NOG32841	GO:0046872	NGVAVVAMPLRVEGI	0.00003	15.434	1.325 down	0.0369	4.488	1.365 down	0.0103	6.791	1.261 down	0.0405	3.969	0.08395	0.16079	0.07665	0.10797	0.15064	0.07601
comp110281	comp	m.7	sp|F8W2M1|	PF00632.20	.	GO:0005783	EARNQSVLAMTMPNR	1.07E-	27.902	1.328 down	0.0283	5.004	1.546 down	0.0039	10.793	1.201 down	0.0372	4.092	0.08107	0.14255	0.05481	0.10281	0.10615	0.07395
comp31775_	comp	m.7	sp|Q810L3|	PF00498.21	NOG25320	GO:0016605	LQQTVKALLEDGIFPG	8.39E-	66.718	2.248 down	0.0054	10.518	2.871 down	0.00536	9.052	2.237 down	0.00158	15.092	0.07483	0.20927	0.14033	0.30552	0.06653	0.13887
comp70849_	comp	m.1	sp|Q8MKD1|	PF00240.18	COG5272^	GO:0005737	FFHRQNPGCKSKAQL	0.00003	15.299	1.358 down	0.0275	5.071	1.620 down	0.043	3.93	1.678 down	0.00798	7.115	0.07198	0.15867	0.06846	0.34782	0.07663	0.19552
comp107763	comp	m.5	sp|O13769|	PF02902.14	COG5160^	GO:0005634	ARRLFVERSGGSAAK	1.59E-	39.137	1.762 down	0.00528	10.646	1.715 down	0.00697	8.006	1.402 down	0.0187	5.204	0.09235	0.12279	0.07485	0.17958	0.16724	0.08497
comp100427	comp	m.2	sp|Q9FPH0|	PF13920.1^	NOG23581	GO:0016874	RSLKERQRVAAWPRS	0.00002	15.65	1.260 up	0.0215	-5.635	1.343 up	0.00998	-6.848	1.303 up	0.0514	-3.643	0.08285	0.0846	0.07698	0.09772	0.14412	0.15264
comp125847	comp	m.1	sp|Q8VYC8|	PF13639.1^	COG5243^	GO:0016021	GGSTVLHLAAHLGGS	9.48E-	65.272	1.240 up	0.0145	-6.651	1.400 up	0.00696	-8.019	1.361 up	0.0562	-3.525	0.07232	0.05919	0.09505	0.07512	0.20965	0.14063
comp21112_	comp	m.5	sp|Q6WWW	PF00632.20	COG5021^	GO:0005622	PPVMIVSSDMDWLVQ	6.76E-	45.571	1.369 up	0.0406	-4.317	1.281 up	0.0488	-3.747	1.307 up	0.0134	-5.873	0.09957	0.18524	0.06329	0.17995	0.1094	0.0734
comp34342_	comp	m.7	sp|Q8GY23|	PF00632.20	COG5021^	GO:0005622	PSLQSICSWLEFFLEV	0.00006	13.596	1.142 up	0.0114	-7.437	1.188 up	0.0205	-5.164	1.246 up	0.0368	-4.107	0.04361	0.02802	0.07196	0.06453	0.08191	0.13105
comp56396_	comp	m.1	sp|Q9JI90|R	PF00097.20	NOG26670	GO:0005737	REVRDVTQPWVCPIC	1.25E-	61.911	1.380 up	0.00442	-11.857	1.741 up	0.00302	-12.605	1.518 up	0.00765	-7.226	0.06679	0.04101	0.07488	0.10258	0.1036	0.13069
comp59743_	comp	m.1	sp|O95714|	PF00569.12	COG5021^	GO:0005814	MDEEMEVVEIRICIGE	7.94E-	29.365	1.290 up	0.0225	-5.512	1.343 up	0.0182	-5.399	1.290 up	0.00433	-9.071	0.03992	0.12738	0.0625	0.14472	0.0738	0.03372
comp66718_	comp	m.1	sp|Q5ZIJ9|M	PF00023.25	COG0666^	GO:0005737	MRRLLLMLQLLFAQF	1.07E-	41.94	1.189 up	0.0161	-6.354	1.267 up	0.0411	-3.999	1.580 up	0.00153	-15.426	0.0451	0.06442	0.14582	0.08977	0.08272	0.02202
comp77019_	comp	m.1	sp|Q3UIR3|	PF13920.1^	NOG84763	GO:0005737	EPLEPLELLERDRTDR	4.53E-	32.426	1.205 up	0.0315	-4.792	1.513 up	0.0186	-5.359	1.523 up	0.00283	-10.933	0.07553	0.0833	0.13505	0.17744	0.10519	0.03572
comp77215_	comp	m.1	sp|Q05086|	PF00632.20	COG5021^	GO:0005737	LNCAKGAMRAPAEQR	1.89E-	37.971	1.248 up	0.0157	-6.414	1.468 up	0.0158	-5.7	1.520 up	0.00319	-10.305	0.06861	0.0727	0.13921	0.13564	0.1095	0.04219
comp44366_	comp	m.9	sp|Q24574|	PF00443.24	COG5077^	GO:0005634	TEELLVQQAIAESLER	3.10E-	23.155	1.411 up	0.0122	-7.21	1.543 up	0.00736	-7.827	1.527 up	0.0239	-4.775	0.11677	0.0736	0.05662	0.14976	0.13332	0.21865
comp56934_	comp	m.1	sp|Q80U87|	PF00443.24	COG5533^	GO:0005829	MAMRLLRRSSSSNTA	2.27E-	124.763	1.984 up	0.00151	-22.749	2.031 up	0.00276	-13.384	1.833 up	0.00332	-10.078	0.07387	0.04587	0.1353	0.07106	0.11232	0.13237
comp60887_	comp	m.1	sp|A5PMR2|	PF13423.1^	COG5560^	GO:0048471	SGSVGVRSSSWSGV	3.91E-	22.237	1.200 up	0.0374	-4.458	1.496 up	0.022	-5.025	1.504 up	0.00786	-7.153	0.10743	0.04969	0.14726	0.17858	0.11565	0.11721
comp71649_	comp	m.1	sp|Q84WU2|	PF00443.24	COG5077^	GO:0005829	RKENLPPPAEHELEFL	1.91E-	25.213	1.241 up	0.0134	-6.878	1.426 up	0.0141	-5.943	1.267 up	0.00935	-6.691	0.03679	0.083	0.08013	0.15284	0.08202	0.06118
comp91016_	comp	m.1	sp|P0DJ25|	PF00240.18	.	GO:0005634	EIFGLRWRAAVVDFT	3.87E-	50.498	1.343 up	0.0104	-7.844	1.341 up	0.024	-4.866	1.485 up	0.00393	-9.455	0.08633	0.06573	0.11859	0.12756	0.06747	0.10029
comp24052_	comp	m.6	sp|Q4PEQ5|	PF10283.4^	NOG68179	GO:0004197	RARQLWKGAMANFM	2.79E-	23.584	1.128 down	0.014	6.752	1.318 down	0.0151	5.8	1.194 down	0.0182	5.252	0.04896	0.0163	0.0851	0.10818	0.07068	0.06716
comp41409_	comp	m.8	sp|O74477|	PF00656.17	NOG68179	GO:0005829	MGKVCSVLSGRPMA	2.29E-	55.536	1.199 down	0.0212	5.672	1.315 down	0.00644	8.335	1.311 down	0.00137	17.087	0.04514	0.08078	0.03311	0.08902	0.02499	0.03834
comp33972_	comp	m.7	sp|Q7S4N5|	PF00656.17	NOG68179	GO:0004197	MLSCCAAPDTSAAEM	2.43E-	123.403	1.367 down	0.00584	10.249	1.717 down	0.00188	16.92	1.659 down	0.00217	12.654	0.06649	0.05769	0.01276	0.09139	0.10107	0.05585
comp71271_	comp	m.1	sp|A1CQZ0|	PF01442.13	NOG68179	GO:0004197	SHKEDVVQKLQVVRE	4.37E-	74.877	1.373 up	0.00794	-8.758	1.540 up	0.0033	-11.834	1.520 up	0.00364	-9.774	0.04887	0.09233	0.06209	0.08503	0.09096	0.08374
comp21702_	comp	m.5	sp|A3LSY7|	PF00656.17	NOG68179	GO:0004197	MKACKGLVVSCSYPQ	1.98E-	25.056	1.110 up	0.031	-4.821	1.191 up	0.0273	-4.651	1.123 up	0.101	-2.817	0.03932	0.04863	0.05059	0.09595	0.11083	0.04443
comp71741_	comp	m.1	sp|Q4PEQ5|	PF00656.17	NOG68179	GO:0004197	ITSYMVAFLGRFLAPR	6.85E-	20.164	1.138 up	0.0305	-4.847	1.157 up	0.0959	-2.877	1.297 up	0.0194	-5.146	0.02903	0.07146	0.08729	0.11757	0.12793	0.07071
comp25905_	comp	m.6	sp|Q7XJE6|	PF00656.17	NOG68179	GO:0004197	LGRLNNWRESYRRLS	0.00096	8.177	1.316 up	0.0237	-5.396	1.209 up	0.0801	-3.094	1.093 up	0.289	-1.665	0.13121	0.06669	0.16362	0.0681	0.06688	0.1392
comp95898_	comp	m.1	sp|Q4PEQ5|	PF00656.17	NOG68179	GO:0004197	QQKALQFTMGGVNS	7.97E-	44.211	1.079 up	0.147	-2.512	1.404 up	0.00396	-10.648	1.396 up	0.00386	-9.507	0.04029	0.07767	0.07586	0.05217	0.08573	0.05414
comp76592_	comp	m.1	sp|Q75B43|	PF01442.13	NOG68179	GO:0004197	APNSERGAETEREME	0.00032	10.144	1.196 up	0.054	-3.851	1.229 up	0.0496	-3.724	1.110 up	0.112	-2.695	0.09734	0.09315	0.12832	0.09571	0.07033	0.08789
comp54046_	comp	m.1	sp|Q75B43|	PF00656.17	NOG68179	GO:0004197	LSNHTLLVQISQWHR	0.00026	10.522	1.025 up	0.636	-0.775	1.340 up	0.0269	-4.673	1.235 up	0.0644	-3.354	0.03541	0.08652	0.10796	0.14523	0.13301	0.12402
comp77002_	comp	m.1	sp|A5D9W7|	PF00656.17	NOG68179	GO:0004197	MVLTIARRPCECWRV	0.0128	4.615	1.075 up	0.048	-4.037	1.015 down	0.602	0.811	1.014 down	0.464	1.139	0.04002	0.03305	0.04657	0.03077	0.02183	0.02992
comp96039_	comp	m.1	sp|Q4WJA1|	PF00656.17	NOG68179	GO:0004197	PSRERAVEGMAMGN	0.0157	4.377	1.208 up	0.0267	-5.137	1.008 down	0.916	0.181	1.009 down	0.913	0.194	0.04756	0.09493	0.05154	0.11985	0.10851	0.08801
comp140896	comp	m.2	sp|A1CQZ0|	PF00656.17	NOG68179	GO:0004197	MVKRAVCVGCNYPSK	0.00276	6.584	1.024 down	0.795	0.443	1.198 up	0.217	-1.967	1.348 up	0.0212	-4.986	0.11579	0.10898	0.22213	0.14609	0.05729	0.16345
comp67553_	comp	m.1	sp|Q7S4N5|	PF00656.17	NOG68179	GO:0004197	LAGAVNDAFLIAETLQ	4.77E-	21.46	1.220 up	0.0233	-5.44	1.294 up	0.0506	-3.696	1.236 up	0.0228	-4.854	0.06177	0.08577	0.10638	0.17127	0.09683	0.08089
comp78275_	comp	m.1	sp|O74477|	PF00656.17	NOG68179	GO:0005829	MQRKSREERVKIASV	0.00101	8.108	1.027 down	0.757	0.522	1.001 up	0.978	-0.05	1.082 down	0.0387	4.037	0.08067	0.12368	0.09898	0.04199	0.04132	0.03848
comp125073	comp	m.1	sp|O74477|	PF00656.17	NOG68179	GO:0005829	AAEARIVQPAQAAAG	0.00002	15.966	1.368 down	0.0278	5.046	1.475 down	0.0106	6.71	1.271 down	0.111	2.705	0.06667	0.16666	0.11529	0.12114	0.20712	0.15013
comp52996_	comp	m.1	sp|A5D9W7|	PF00656.17	NOG68179	GO:0004197	FIGLKTAMAFLKMLPF	0.00022	10.848	1.271 down	0.159	2.416	1.620 down	0.00826	7.43	1.526 down	0.0605	3.431	0.19612	0.20969	0.11593	0.14723	0.09011	0.34435
comp86663_	comp	m.1	sp|Q96NN9|	PF00355.21	COG0446^	GO:0005783	SAMSAWSQCRRWLS	0.00025	10.555	1.215 up	0.0203	-5.769	1.164 up	0.0368	-4.161	1.109 up	0.131	-2.526	0.02595	0.09422	0.05449	0.09036	0.09689	0.06828
comp80309_	comp	m.1	sp|Q54NS8|	PF00070.22	COG0446^	GO:0050660	GSSFVFFVRCCGAAF	9.48E-	19.053	1.251 up	0.0305	-4.853	1.177 up	0.0306	-4.443	1.279 up	0.0149	-5.65	0.03837	0.1275	0.08636	0.06126	0.08908	0.08865
comp46418_	comp	m.9	sp|Q54NS8|	PF07992.9^	COG0446^	GO:0050660	LAQAFLLIPAAFAKKH	2.32E-	36.597	1.208 up	0.0445	-4.163	1.137 up	0.0555	-3.567	1.136 up	0.0124	-6.038	0.02335	0.12892	0.0766	0.07086	0.0452	0.04114
comp80341_	comp	m.1	sp|Q54NS8|	PF00070.22	COG0446^	GO:0050660	FGSSFVFFVRCCGAA	0.00001	18.508	1.091 up	0.116	-2.798	1.131 up	0.0232	-4.923	1.057 up	0.0225	-4.885	0.01292	0.08943	0.02832	0.06658	0.02769	0.01826
comp94854_	comp	m.1	sp|Q54NS8|	PF00070.22	COG0446^	GO:0050660	GGQFTGNFCARELKK	0.0185	4.187	1.135 down	0.0499	3.97	1.032 down	0.751	0.505	1.062 down	0.422	1.246	0.06906	0.06161	0.11832	0.13975	0.14005	0.01911
comp97752_	comp	m.1	sp|Q54NS8|	PF00070.22	COG0446^	GO:0050660	MGKAAKPRCLIIGAQF	0.00024	10.67	1.074 down	0.639	0.769	1.261 down	0.00764	7.692	1.171 down	0.0116	6.19	0.12642	0.23946	0.05997	0.06321	0.05548	0.04879
comp135820	comp	m.2	sp|Q13490|	PF13920.1^	NOG24334	GO:0035631	QDTVRSSRGQHPEAP	0.00562	5.615	1.596 up	0.0223	-5.536	1.784 up	0.0816	-3.072	1.323 up	0.293	-1.652	0.14336	0.19716	0.52629	0.13719	0.32035	0.37062
comp128141	comp	m.1	sp|Q13489|	PF13920.1^	NOG24334	GO:0005737	QAVSTFQTHLGFFPL	0.00009	12.689	1.047 up	0.827	-0.379	1.862 up	0.0387	-4.087	1.580 up	0.063	-3.381	0.29052	0.20282	0.40574	0.16829	0.29783	0.25243
comp40288_	comp	m.8	sp|Q13489|	PF13920.1^	NOG24334	GO:0005737	MVRMPPHRCVEWLE	2.31E-	24.38	1.168 up	0.2	-2.15	1.482 up	0.0204	-5.168	1.221 up	0.142	-2.437	0.13818	0.15679	0.10134	0.19519	0.11718	0.2054
comp115062	comp	m.1	sp|Q24306|I	PF07647.12	NOG24334	GO:0005622	QAVTLDVPAELIKDPR	0.00012	12.178	1.197 down	0.236	1.953	1.183 down	0.0228	4.952	1.197 down	0.0252	4.688	0.08604	0.25199	0.05442	0.08165	0.06603	0.08932
comp41335_	comp	m.8	sp|Q7Z429|L	PF01027.15	COG0670^	GO:0016021	APYAFGATSGPALPQ	6.54E-	20.323	1.093 up	0.0433	-4.201	1.240 down	0.0298	4.489	1.202 down	0.0631	3.379	0.04689	0.03924	0.10073	0.09506	0.07904	0.13635
comp41927_	comp	m.8	sp|Q7Z429|L	PF01027.15	COG0670^	GO:0016021	SRFLSQFRHRFLVPK	0.00165	7.333	1.149 down	0.41	1.323	1.404 down	0.035	4.234	1.359 down	0.0103	6.468	0.21084	0.21904	0.10582	0.20567	0.02406	0.13488
comp82136_	comp	m.1	sp|Q5R4I4|L	PF01027.15	.	GO:0030054	MAEMTESTPLDPAPQ	1.32E-	40.421	1.140 down	0.032	4.761	1.143 down	0.157	2.32	1.264 down	0.0109	6.33	0.07004	0.03814	0.15296	0.06759	0.0614	0.08742
comp20899_	comp	m.5	sp|Q9BWQ8	PF01027.15	COG0670^	GO:0030054	MEELQGEEQVLVNSN	0.0935	2.522	1.003 up	0.98	-0.051	1.014 down	0.915	0.184	1.202 down	0.0489	3.706	0.13695	0.10131	0.22158	0.06007	0.06479	0.1279
comp18631_	comp	m.4	sp|Q7Z429|L	PF01027.15	COG0670^	GO:0016021	YPVPAACPPYTGAAA	0.00005	13.865	1.040 up	0.58	-0.898	1.255 down	0.0362	4.185	1.137 down	0.0403	3.978	0.08893	0.09332	0.08458	0.13235	0.05706	0.07388
comp24363_	comp	m.6	sp|O88407|L	PF01027.15	COG0670^	GO:0030054	RAFEVALVDIAMAATT	0.00001	17.412	1.166 up	0.0822	-3.242	1.361 up	0.00469	-9.777	1.232 up	0.0329	-4.263	0.04882	0.1278	0.03826	0.08275	0.12976	0.05686
comp94830_	comp	m.1	sp|Q8BJZ3|	PF01027.15	COG0670^	GO:0010008	LTTATLGKSFEQPIWM	0.00003	15.443	1.346 up	0.0236	-5.399	1.203 up	0.117	-2.651	1.003 up	0.966	-0.08	0.12422	0.09898	0.07481	0.18742	0.0845	0.07171
comp103654	comp	m.2	sp|Q969X1|	PF01027.15	COG0670^	GO:0010008	AFTAAPISSKMSQKN	0.00002	15.805	1.011 up	0.889	-0.252	1.205 up	0.0458	-3.835	1.259 up	0.0456	-3.8	0.10877	0.07254	0.07501	0.11873	0.15928	0.07258
comp99453_	comp	m.1	sp|Q94A20|	PF01027.15	COG0670^	GO:0016021	MMDLEGQRFAELQGI	0.00235	6.813	1.229 down	0.0336	4.665	1.278 down	0.0151	5.807	1.037 down	0.788	0.44	0.10338	0.07503	0.07669	0.0952	0.21764	0.10501
comp20334_	comp	m.5	sp|P49472|P	PF00421.14	.	GO:0009535	MPLLSSLKRRTLVGS	5.35E-	21.045	1.333 up	0.142	-2.547	1.601 down	0.348	1.452	2.711 down	0.0311	4.35	0.22638	0.23529	0.76552	0.5386	0.20953	0.62752
comp42202_	comp	m.8	sp|Q06J12|P	PF00283.14	.	GO:0009535	PRPRQPFWLKNCRH	0.00001	17.464	1.126 down	0.0494	3.989	1.249 down	0.0463	3.819	1.261 down	0.0142	5.755	0.0074	0.08608	0.09824	0.13674	0.0874	0.07696
comp79713_	comp	m.1	.	PF00737.15	.	.	SSHFCQSRHNAPQNS	1.32E-	26.88	1.074 up	0.462	-1.183	1.051 up	0.278	-1.697	1.205 up	0.0248	-4.717	0.09686	0.14678	0.02057	0.08247	0.03424	0.10913

comp42379_	comp	m.8	sp|P49513|P	PF02533.10	.	GO:0009507	DLRAQAAPFERQPLK	0.00003	15.384	1.253 up	0.0594	-3.708	1.220 up	0.014	-5.969	1.092 up	0.166	-2.258	0.15252	0.08728	0.07106	0.06474	0.10421	0.04528
comp80077_	comp	m.1	sp|P85194|P	PF01716.13	.	GO:0009535	MARSGAVVGVLLAAA	1.11E-	41.598	1.064 up	0.192	-2.196	1.214 up	0.0164	-5.618	1.082 up	0.168	-2.248	0.04032	0.07161	0.03969	0.09167	0.05348	0.08697
comp21047_	comp	m.5	sp|P82538|P	PF01789.11	NOG08775	GO:0009507	SHSERRLRVRGPLQI	1.38E-	40.114	1.317 up	0.0103	-7.87	1.372 up	0.00637	-8.374	1.370 up	0.00913	-6.763	0.07958	0.06243	0.10326	0.03491	0.06952	0.11519
comp110020	comp	m.7	.	PF01789.11	.	.	PTGVASTMATVSAPM	7.23E-	19.967	1.527 up	0.00639	-9.711	1.546 up	0.0113	-6.518	1.278 up	0.145	-2.415	0.08811	0.0899	0.1467	0.1253	0.24629	0.15908
comp98847_	comp	m.1	.	PF01789.11	.	.	VVRSLSRKMQARSAR	0.00061	8.932	1.092 up	0.413	-1.313	1.286 up	0.0428	-3.937	1.248 up	0.00403	-9.328	0.10882	0.16063	0.17389	0.0623	0.03643	0.05813
comp81492_	comp	m.1	.	PF06298.6^	.	.	LAQVFLVRFACITRCA	3.43E-	34.091	1.068 up	0.215	-2.065	1.172 down	0.251	1.805	1.398 down	0.0188	5.195	0.07784	0.04934	0.06515	0.24638	0.13287	0.13055
comp20374_	comp	m.5	sp|Q9XQV3|	PF00223.14	.	GO:0009535	QEKSSHPHEQKILAKI	1.26E-	27.094	1.413 up	0.0604	-3.684	1.683 down	0.296	1.625	3.306 down	0.0155	5.566	0.16114	0.21775	0.81158	0.44324	0.11212	0.60972
comp81124_	comp	m.1	sp|A0T0L2|P	PF12837.2^	.	GO:0009535	RRPRLAMSRSLMMVA	3.20E-	23.031	1.224 up	0.0451	-4.144	1.172 up	0.0742	-3.195	1.050 up	0.502	-1.047	0.09184	0.10708	0.09645	0.10616	0.06239	0.12171
comp74159_	comp	m.1	sp|P49481|P	PF02531.11	.	GO:0009535	TTRGAMTILAQAPGP	2.49E-	54.709	1.175 up	0.0354	-4.562	1.244 up	0.012	-6.359	1.091 up	0.028	-4.507	0.04101	0.09391	0.04415	0.08907	0.03673	0.04271
comp80131_	comp	m.1	sp|P51193|P	PF02507.10	.	GO:0009535	SREGGTGLHRSNPA	8.27E-	29.168	1.191 up	0.0331	-4.691	1.221 up	0.0162	-5.643	1.159 up	0.0161	-5.487	0.05087	0.09527	0.05914	0.08371	0.03917	0.06746
comp79899_	comp	m.1	.	PF01701.13	.	.	MARLGGLLLLVAGALL	0.00118	7.864	1.106 up	0.0307	-4.839	1.042 up	0.45	-1.167	1.008 down	0.858	0.305	0.03235	0.051	0.10078	0.01977	0.05166	0.06084
comp78356_	comp	m.1	sp|Q85FP8|	PF02605.10	.	GO:0009535	LRSHFGSRSRTHSYL	1.29E-	40.558	1.179 up	0.0255	-5.22	1.254 up	0.0182	-5.404	1.203 up	0.007	-7.501	0.03279	0.08522	0.08802	0.08304	0.04146	0.05781
comp40074_	comp	m.8	sp|Q85FP8|	PF02605.10	.	GO:0009535	FGSRLKVTPLLLSGRV	0.00073	8.634	1.046 up	0.237	-1.952	1.100 up	0.0863	-3.004	1.098 up	0.0427	-3.896	0.05764	0.03573	0.03385	0.08556	0.05746	0.0387
comp42084_	comp	m.8	sp|P41344|F	PF00175.16	COG0369^	GO:0048046	GVCSNHICDMSPGDD	0.00002	15.943	1.076 up	0.189	-2.213	1.059 up	0.453	-1.157	1.137 up	0.0264	-4.612	0.04266	0.08576	0.09118	0.11105	0.0685	0.04277
comp68408_	comp	m.1	sp|P10770|F	PF00111.22	.	GO:0009507	SRRSILKKRIWHPDTT	0.0261	3.809	1.275 up	0.0316	-4.784	1.017 down	0.915	0.183	1.063 up	0.746	-0.525	0.06493	0.13163	0.12054	0.25006	0.28636	0.18268
comp35855_	comp	m.7	sp|Q92J08|F	PF00111.22	COG0633^	GO:0051537	MQLTFFRTLPWRLVG	1.55E-	26.148	1.085 down	0.164	2.376	1.408 down	0.0864	3.003	1.587 down	0.00548	8.271	0.09567	0.026	0.09611	0.31461	0.03456	0.1575
comp68158_	comp	m.1	sp|Q5ENN5|	PF00016.15	.	GO:0031969	VVHCHVGSCIFAFFPL	2.24E-	24.498	1.286 down	0.029	4.957	1.207 up	0.0442	-3.89	1.347 up	0.00919	-6.743	0.0471	0.13909	0.11626	0.07736	0.08368	0.09651
comp79516_	comp	m.1	sp|Q5ENN5|	PF02788.11	.	GO:0031969	QGSKGVTQFFSSLIVP	8.77E-	28.882	1.172 down	0.0185	5.998	1.206 down	0.0231	4.933	1.048 up	0.272	-1.735	0.03118	0.07005	0.08091	0.07406	0.0223	0.07573
comp63174_	comp	m.1	sp|Q41406|	.	.	GO:0009507	VRACAPCLGPSFSLP	0.00044	9.534	1.289 down	0.039	4.388	1.106 up	0.358	-1.421	1.237 up	0.0228	-4.863	0.07112	0.15129	0.16662	0.12143	0.07654	0.10086
comp78141_	comp	m.1	sp|Q5ENN5|	.	.	GO:0031969	PGSPLPRAIFVNSVGY	5.87E-	71.043	1.002 up	0.965	-0.086	1.228 down	0.0114	6.5	1.031 down	0.44	1.197	0.06472	0.0769	0.06587	0.06342	0.02994	0.06741
comp71061_	comp	m.1	sp|Q5ENN5|	.	.	GO:0031969	APAPAAATPSMPQMA	1.09E-	41.775	1.004 down	0.954	0.112	1.097 up	0.218	-1.958	1.233 up	0.0234	-4.807	0.07539	0.08352	0.07707	0.11331	0.0825	0.09522
comp79880_	comp	m.1	sp|Q5ENN5|	.	.	GO:0031969	GPDPHTPGSPLPRAIF	4.38E-	21.783	1.009 up	0.908	-0.212	1.145 up	0.135	-2.492	1.465 up	0.00539	-8.334	0.10132	0.08034	0.11365	0.10903	0.07663	0.1078
comp55807_	comp	m.1	sp|Q42813|	PF02788.11	.	GO:0009507	VPNSNRTLWRRRSDI	0.0136	4.543	1.103 down	0.328	1.582	1.073 up	0.326	-1.523	1.196 up	0.0421	-3.918	0.14071	0.11052	0.09243	0.09723	0.12127	0.05183
comp80942_	comp	m.1	sp|Q41406|	PF00016.15	.	GO:0009507	GNNQGMGDVEYGKIY	0.00002	16.129	1.012 down	0.735	0.566	1.028 up	0.746	-0.516	1.241 up	0.0135	-5.867	0.04875	0.03641	0.12619	0.09426	0.05895	0.08843
comp80955_	comp	m.1	sp|Q41406|	PF02788.11	.	GO:0009507	AAAFVGASVAPAKKE	1.43E-	26.491	1.037 down	0.344	1.526	1.009 down	0.875	0.266	1.167 up	0.0186	-5.215	0.05433	0.04436	0.06369	0.07376	0.05909	0.0623
comp79868_	comp	m.1	sp|Q40412|	PF01494.14	.	GO:0031969	MAPRTPARTLLLAVLG	4.70E-	32.183	1.197 up	0.0377	-4.447	1.325 up	0.0148	-5.841	1.477 up	0.00265	-11.332	0.10528	0.05075	0.09303	0.10344	0.06978	0.07087
comp88413_	comp	m.1	sp|O81360|	PF01494.14	.	GO:0009507	GAVAGAVVAAAAGRR	7.30E-	19.934	1.111 up	0.035	-4.583	1.320 up	0.0208	-5.136	1.179 up	0.0117	-6.16	0.05045	0.04314	0.03911	0.15098	0.05298	0.05632
comp106814	comp	m.4	sp|Q9SM43|	PF07137.6^	.	GO:0009535	HCLLQSRGCEPFPMA	7.38E-	19.896	1.436 up	0.00629	-9.82	1.561 up	0.0506	-3.697	1.437 up	0.00202	-13.153	0.05401	0.09179	0.24759	0.24435	0.05459	0.0579
comp86229_	comp	m.1	sp|Q40593|	PF07137.6^	.	GO:0009535	SSHIGLREQWRCTPK	0.0047	5.849	1.103 down	0.137	2.591	1.109 down	0.0303	4.459	1.013 down	0.756	0.505	0.07568	0.07906	0.05256	0.04147	0.04815	0.06058
comp42301_	comp	m.8	sp|Q40593|	.	.	GO:0009535	GLPAMEAQRPVPTSE	2.38E-	24.264	1.199 up	0.0686	-3.495	1.158 up	0.0283	-4.579	1.162 up	0.0873	-2.983	0.08122	0.12657	0.09213	0.00821	0.1059	0.10013
comp20639_	comp	m.5	.	PF00504.16	.	.	IVLFLGHYEGYFWRQ	8.99E-	65.886	1.666 up	0.013	-6.952	1.509 up	0.00471	-9.715	1.257 up	0.00934	-6.695	0.08158	0.19576	0.05801	0.10759	0.03297	0.093
comp78907_	comp	m.1	sp|P10708|C	PF00504.16	.	GO:0009535	FWLKGCSSSAQTTSE	4.08E-	75.843	1.193 up	0.0163	-6.316	1.191 up	0.00361	-11.341	1.149 up	0.00544	-8.302	0.01375	0.07959	0.0214	0.03908	0.03831	0.02985
comp66799_	comp	m.1	.	PF00504.16	.	.	LIEDEKSIWNQFRKAE	5.52E-	47.327	1.253 up	0.0421	-4.252	1.360 up	0.0103	-6.78	1.348 up	0.00125	-18.069	0.05927	0.14124	0.09334	0.09205	0.02749	0.03903
comp81913_	comp	m.1	.	PF00504.16	.	.	MFEGEGAKRAPGDLN	1.89E-	37.976	1.416 up	0.0208	-5.715	1.450 up	0.0133	-6.093	1.153 up	0.142	-2.435	0.05867	0.16577	0.12994	0.11906	0.12584	0.11237
comp25880_	comp	m.6	.	PF00504.16	.	.	KRLKRVSVAKKAGKT	5.91E-	46.667	1.266 down	0.014	6.751	1.043 up	0.567	-0.889	1.201 down	0.0227	4.865	0.05916	0.08165	0.09967	0.09704	0.07054	0.08279
comp108755	comp	m.6	sp|P27524|C	PF00504.16	.	GO:0009535	MSLSFTSPAAFQAGP	0.00001	17.795	1.129 up	0.227	-1.999	1.404 up	0.00626	-8.433	1.324 up	0.0101	-6.512	0.12513	0.12409	0.09529	0.06649	0.02872	0.12109
comp39540_	comp	m.8	sp|P13869|C	PF00504.16	.	GO:0009535	SSSFFKAVCLVSHVR	7.60E-	29.592	1.038 up	0.526	-1.025	1.442 up	0.00822	-7.448	1.262 up	0.0346	-4.194	0.02802	0.10385	0.0555	0.13069	0.1373	0.08277
comp74084_	comp	m.1	sp|P55738|C	PF00504.16	.	GO:0009535	IGMFFQDGLTGSAWG	1.68E-	25.773	1.184 up	0.0106	-7.745	1.287 up	0.0219	-5.038	1.227 up	0.00919	-6.74	0.03848	0.05013	0.08597	0.11615	0.03398	0.08097
comp23_c0	comp	m.6	sp|P55738|C	PF00504.16	.	GO:0009535	KTAGATFFNASRSLP	2.49E-	24.064	1.052 up	0.652	-0.739	1.777 up	0.0157	-5.712	1.598 up	0.0101	-6.497	0.13016	0.15068	0.14229	0.2532	0.08487	0.19013
comp74174_	comp	m.1	sp|Q39709|F	PF00504.16	.	GO:0009535	RAVQQLSEGPYVETE	3.35E-	34.272	1.380 up	0.0188	-5.959	1.581 up	0.0125	-6.271	1.340 up	0.00478	-8.701	0.04911	0.14815	0.10763	0.1812	0.05862	0.07759
comp79970_	comp	m.1	sp|Q39709|F	PF00504.16	.	GO:0009535	LAQALSQPGLSARVN	2.06E-	24.875	1.204 up	0.0387	-4.403	1.263 up	0.0264	-4.709	1.212 up	0.00947	-6.654	0.01023	0.12139	0.08672	0.11403	0.04846	0.06813
comp49189_	comp	m.1	sp|Q40297|F	PF00504.16	.	GO:0009535	RRSRQQRRAAAAEAE	1.03E-	28.09	1.176 up	0.0423	-4.246	1.184 up	0.0358	-4.2	1.148 up	0.0291	-4.449	0.0637	0.08997	0.1057	0.0482	0.03113	0.08431
comp75958_	comp	m.1	sp|Q39709|F	PF00504.16	.	GO:0009535	MSRISMAVAAIGLCSL	3.10E-	34.786	1.118 up	0.156	-2.441	1.278 up	0.00904	-7.149	1.043 up	0.376	-1.377	0.05801	0.11859	0.0446	0.08844	0.07372	0.05005
comp108983	comp	m.6	sp|Q40297|F	PF00504.16	.	GO:0009535	GAQEVNYKVHHSASV	0.00054	9.147	1.172 up	0.311	-1.641	1.379 up	0.0387	-4.087	1.132 up	0.0894	-2.956	0.19779	0.19787	0.21399	0.07585	0.10327	0.06403
comp41609_	comp	m.8	sp|Q40296|F	PF00504.16	.	GO:0009535	GSAWGDWAQYTDSP	4.29E-	32.761	1.280 up	0.034	-4.639	1.385 up	0.0176	-5.473	1.125 up	0.018	-5.277	0.05107	0.14524	0.08686	0.14829	0.0465	0.04487
comp116163	comp	m.1	sp|Q40296|F	PF00504.16	.	GO:0009535	KVEPPWEASQELGVT	0.00003	15.577	1.644 up	0.0383	-4.42	1.543 up	0.0224	-4.985	1.485 up	0.0386	-4.042	0.3055	0.11048	0.20396	0.1467	0.15942	0.23332
comp80251_	comp	m.1	sp|Q40296|F	PF00504.16	.	GO:0009535	FATIGMLWPDVFGKF	4.03E-	22.113	1.193 up	0.0114	-7.429	1.184 up	0.0506	-3.695	1.115 up	0.0547	-3.56	0.01902	0.06587	0.08802	0.09835	0.03174	0.08265
comp40433_	comp	m.8	sp|Q40296|F	PF00504.16	.	GO:0009535	WANYTASPLRAFENE	6.08E-	20.584	1.140 up	0.061	-3.668	1.364 up	0.0313	-4.409	1.328 up	0.0156	-5.554	0.07763	0.06832	0.13682	0.15052	0.12316	0.08103
comp74012_	comp	m.1	sp|Q08585|F	PF00504.16	NOG27499	GO:0009535	TKVGDEQGFRKLRTS	6.77E-	30.185	1.176 down	0.207	2.105	1.608 up	0.0118	-6.406	1.569 up	0.011	-6.297	0.20831	0.08064	0.19639	0.08543	0.07688	0.19167
comp104344	comp	m.3	sp|Q40296|F	PF00504.16	.	GO:0009535	GSSGVSGVWLCAKA	0.255	1.597	1.091 up	0.651	-0.742	1.249 up	0.0347	-4.248	1.066 down	0.656	0.704	0.20688	0.26928	0.11704	0.09551	0.21857	0.14715
comp60532_	comp	m.1	sp|Q40296|F	PF00504.16	.	GO:0009535	SAWGDWANHADSPL	0.00005	14.078	1.211 down	0.0839	3.213	1.176 up	0.0485	-3.756	1.205 up	0.157	-2.325	0.08425	0.14997	0.10273	0.07137	0.22283	0.06562
comp49803_	comp	m.1	sp|Q40296|F	PF00504.16	.	GO:0009535	PGKTWQRFCGPSGK	0.00135	7.653	1.027 down	0.867	0.298	1.338 up	0.102	-2.812	1.365 up	0.0101	-6.506	0.03213	0.25978	0.10945	0.27832	0.11675	0.07364
comp82434_	comp	m.1	sp|Q40301|F	PF00504.16	.	GO:0009535	ATALALTAGSIAFVAP	2.09E-	56.522	1.525 up	0.00451	-11.633	1.535 up	0.00673	-8.143	1.421 up	0.00403	-9.375	0.04831	0.0929	0.05928	0.1399	0.01837	0.10677
comp78034_	comp	m.1	sp|Q40301|F	PF00504.16	.	GO:0009535	FFLLKSAPRKDTCVG	7.82E-	44.386	1.349 up	0.0148	-6.579	1.459 up	0.00619	-8.485	1.312 up	0.00462	-8.826	0.0408	0.12507	0.09616	0.08526	0.04101	0.07896
comp79363_	comp	m.1	sp|Q40301|F	PF00504.16	.	GO:0009535	LWLMHQVPTNPPGKL	2.90E-	35.176	1.403 up	0.0266	-5.143	1.509 up	0.0082	-7.459	1.346 up	0.00379	-9.582	0.03959	0.18616	0.08148	0.13679	0.06695	0.05957
comp71870_	comp	m.1	sp|Q41093|F	PF00504.16	NOG27499	GO:0009535	GLPALGAGRFVSASS	4.73E-	32.138	1.336 up	0.0344	-4.615	1.119 up	0.219	-1.956	1.259 up	0.0288	-4.463	0.12176	0.13419	0.12237	0.11271	0.12651	0.07933
comp78124_	comp	m.1	sp|Q40301|F	PF00504.16	.	GO:0009535	QFSMKDSTETGEPGN	3.27E-	22.938	1.150 up	0.0044	-11.878	1.021 down	0.8	0.411	1.257 up	0.00999	-6.525	0.02976	0.01643	0.12736	0.0724	0.07623	0.06693
comp47350_	comp	m.9	sp|Q41093|F	PF00504.16	NOG27499	GO:0009535	SAWGDWSLYTASPLR	1.13E-	96.483	1.301 up	0.00647	-9.651	1.067 up	0.313	-1.57	1.031 down	0.394	1.323	0.05134	0.05971	0.10681	0.05352	0.02601	0.06261
comp76716_	comp	m.1	sp|Q40301|F	PF00504.16	.	GO:0009535	GRLAMMAIIGMFFQD	9.74E-	18.969	1.164 up	0.0517	-3.917	1.326 up	0.0153	-5.769	1.199 up	0.00966	-6.606	0.04188	0.10379	0.0735	0.12085	0.0197	0.07699
comp78175_	comp	m.1	sp|Q40301|F	PF00504.16	.	GO:0009535	YTESPLRAFENELGV	5.64E-	20.863	1.107 up	0.0609	-3.671	1.210 up	0.0336	-4.295	1.136 up	0.0131	-5.922	0.01719	0.07834	0.08885	0.09223	0.05306	0.0325
comp80062_	comp	m.1	sp|Q40301|F	PF00504.16	.	GO:0009535	AATGAAALMAASAFV	0.00069	8.722	1.059 up	0.194	-2.181	1.206 up	0.0498	-3.719	1.151 up	0.0439	-3.855	0.02826	0.07077	0.09919	0.10659	0.04514	0.09565
comp80141_	comp	m.1	sp|Q40301|F	PF00504.16	.	GO:0009535	LWLMHQVPTNPPGKL	7.12E-	20.025	1.079 up	0.229	-1.989	1.349 up	0.0315	-4.396	1.340 up	0.00547	-8.275	0.0313	0.10588	0.11019	0.16313	0.07981	0.06392
comp79645_	comp	m.1	sp|Q40301|F	PF00504.16	.	GO:0009535	KHGRVAMYATMGYIV	0.00032	10.135	1.055 up	0.294	-1.706	1.311 up	0.0432	-3.923	1.236 up	0.0197	-5.119	0.06971	0.05862	0.09983	0.17293	0.07955	0.08917
comp73249_	comp	m.1	sp|Q41093|F	PF00504.16	NOG27499	GO:0009535	TMSTVMCQGGWQDS	0.00001	17.539	1.017 up	0.816	-0.402	1.544 up	0.0281	-4.592	1.450 up	0.00451	-8.933	0.04525	0.11793	0.15696	0.22377	0.03074	0.11622
comp18050_	comp	m.4	sp|Q40301|F	PF00504.16	.	GO:0009535	FWLKPTATHRASSVP	0.00002	16.202	1.001 up	0.99	-0.029	1.255 up	0.0443	-3.884	1.018 up	0.828	-0.363	0.01971	0.17492	0.08482	0.1459	0.10151	0.10013
comp116919	comp	m.1	sp|Q40301|F	PF00504.16	.	GO:0009535	LKLPAAQEVHLVPPAV	0.00173	7.262	1.018 down	0.796	0.442	1.219 up	0.0174	-5.503	1.284 up	0.0757	-3.152	0.09712	0.06672	0.07598	0.07132	0.17609	0.14691

comp71239_	comp	m.1	sp|Q41093|F	PF00504.16	NOG27499	GO:0009535	GLPALGAGRFVSASS	0.00001	18.692	1.085 up	0.345	-1.522	1.104 up	0.137	-2.472	1.396 up	0.011	-6.309	0.14031	0.06876	0.09854	0.06045	0.04351	0.14646
comp80391_	comp	m.1	sp|Q40300|F	PF00504.16	.	GO:0009535	GDWANYTASPLRAFE	0.00001	17.361	1.103 up	0.026	-5.186	1.240 up	0.0401	-4.037	1.162 up	0.00732	-7.347	0.02944	0.04603	0.10257	0.11471	0.04601	0.03718
comp62255_	comp	m.1	sp|Q41094|F	PF00504.16	NOG27499	GO:0009535	MSRLGVAAVAVGALA	6.38E-	30.493	1.163 up	0.0319	-4.769	1.185 up	0.019	-5.316	1.129 up	0.029	-4.452	0.05231	0.07546	0.06622	0.06432	0.05625	0.05562
comp79914_	comp	m.1	sp|Q40300|F	PF00504.16	.	GO:0009535	MASTSRSTIIAATGAA	1.82E-	38.195	1.095 down	0.0493	3.991	1.253 up	0.0289	-4.543	1.222 up	0.0228	-4.861	0.02914	0.0595	0.08501	0.11557	0.08004	0.08834
comp41586_	comp	m.8	sp|Q40300|F	PF00504.16	.	GO:0009535	PLRASTQDVLFKGGA	0.00008	12.986	1.539 down	0.0319	4.764	1.126 down	0.17	2.231	1.213 up	0.0144	-5.719	0.07432	0.25069	0.13366	0.07593	0.05826	0.07857
comp68073_	comp	m.1	sp|Q40300|F	PF00504.16	.	GO:0009535	WANYTASPLRAFENE	0.00013	11.841	1.203 up	0.0202	-5.785	1.216 up	0.0603	-3.459	1.042 up	0.437	-1.205	0.03559	0.08516	0.0912	0.13589	0.08272	0.05621
comp79305_	comp	m.1	sp|Q40300|F	PF00504.16	.	GO:0009535	LAMMAIIGMFFQDGLT	0.00020	11.053	1.113 up	0.0431	-4.211	1.228 up	0.0594	-3.478	1.115 up	0.0893	-2.957	0.0293	0.06752	0.11464	0.12658	0.07142	0.07979
comp44655_	comp	m.9	sp|Q40300|F	PF00504.16	.	GO:0009535	KKIARMASSNKTAQIA	0.00019	11.118	1.368 down	0.0392	4.375	1.271 up	0.09	-2.953	1.072 up	0.639	-0.741	0.14061	0.15189	0.1756	0.15624	0.2512	0.11068
comp71241_	comp	m.1	sp|Q40300|F	PF00504.16	.	GO:0009535	PFSCAVPFRSRFHSN	1.03E-	28.077	1.117 up	0.173	-2.316	1.587 up	0.0107	-6.657	1.419 up	0.0086	-6.907	0.07621	0.11591	0.05672	0.19214	0.08865	0.11659
comp73165_	comp	m.1	sp|Q40300|F	PF00504.16	.	GO:0009535	SPLRAFEEELGVQAP	0.00011	12.354	1.011 down	0.846	0.339	1.383 up	0.041	-4.001	1.302 up	0.0203	-5.061	0.05347	0.08581	0.1817	0.14745	0.11851	0.09285
comp79645_	comp	m.1	sp|Q40300|F	PF00504.16	.	GO:0009535	RFPGYCSPSEGVKFA	4.20E-	21.943	1.127 up	0.12	-2.755	1.104 down	0.116	2.666	1.231 down	0.0118	6.138	0.06376	0.10818	0.07648	0.0758	0.05829	0.0786
comp40015_	comp	m.8	sp|Q40300|F	PF00504.16	.	GO:0009535	QGFCQDPHRPSAAAP	0.00486	5.806	1.139 down	0.11	2.857	1.115 up	0.207	-2.016	1.160 up	0.0484	-3.72	0.04579	0.12396	0.11898	0.10153	0.08501	0.07834
comp70173_	comp	m.1	sp|Q40300|F	PF00504.16	.	GO:0009535	IAGTGSTKSSALRGVR	0.00026	10.534	1.088 down	0.518	1.044	1.276 up	0.09	-2.953	1.214 up	0.0112	-6.274	0.13034	0.19354	0.21427	0.10549	0.08361	0.03192
comp76981_	comp	m.1	sp|P08976|L	PF00504.16	.	GO:0009507	AVATAAVALVASRKS	0.00001	18.734	1.198 up	0.0727	-3.413	1.424 up	0.0207	-5.145	1.213 up	0.0267	-4.593	0.03712	0.14883	0.08772	0.17787	0.05975	0.10585
comp89863_	comp	m.1	sp|P08976|L	PF00504.16	.	GO:0009507	IEAIYKVPTAGWLQIFA	0.0305	3.646	1.086 up	0.114	-2.819	1.060 up	0.0437	-3.906	1.001 up	0.986	-0.034	0.03235	0.07846	0.02682	0.03418	0.07447	0.05216
comp80141_	comp	m.1	sp|P08976|L	PF00504.16	.	GO:0009507	HGRIAMLAFVGLVVPE	0.00004	14.64	1.156 up	0.13	-2.654	1.269 up	0.00906	-7.139	1.032 up	0.522	-0.997	0.04806	0.15084	0.06387	0.07229	0.08333	0.04314
comp78161_	comp	m.1	sp|P08976|L	PF00504.16	.	GO:0009507	PSRAMAKSVTLSALG	0.00002	16.372	1.145 up	0.0304	-4.859	1.288 up	0.0522	-3.65	1.151 up	0.0431	-3.882	0.01749	0.07883	0.08913	0.17912	0.04127	0.09619
comp70226_	comp	m.1	sp|Q03965|L	PF00504.16	.	GO:0009535	TFDVKWLRESELKHG	6.00E-	46.558	1.269 up	0.0306	-4.844	1.366 up	0.00621	-8.462	1.081 up	0.013	-5.939	0.08281	0.11524	0.09333	0.051	0.01771	0.03357
comp215171	comp	m.5	sp|Q9Y1W0|	PF00173.23	COG3239^	GO:0016021	LVSNHEGGRRVIEQM	8.69E-	43.456	1.031 down	0.845	0.341	1.492 up	0.00565	-8.859	1.569 up	0.00722	-7.403	0.10344	0.23837	0.06392	0.11367	0.11689	0.13112
comp140217	comp	m.2	sp|O96099|F	PF00173.23	COG3239^	GO:0016021	MELFTAAEVARHNKP	7.03E-	20.07	1.421 down	0.0159	6.385	1.139 down	0.284	1.67	1.059 up	0.495	-1.064	0.09627	0.12662	0.08601	0.20812	0.1329	0.085
comp57362_	comp	m.1	sp|O96099|F	PF00487.19	COG3239^	GO:0016021	VWTNIADADPDAKHV	0.00022	10.805	1.415 down	0.085	3.196	1.236 up	0.0325	-4.349	1.541 up	0.0269	-4.579	0.09866	0.29767	0.13686	0.03358	0.24409	0.1217
comp60315_	comp	m.1	sp|Q1ZXQ5|	PF00173.23	COG3239^	GO:0016021	MTAAMTMSDIKSDMT	1.76E-	38.459	1.391 up	0.0237	-5.39	2.031 up	0.0067	-8.18	1.608 up	0.00304	-10.544	0.06822	0.16306	0.05307	0.24424	0.04182	0.12322
comp87976_	comp	m.1	sp|Q1ZXQ5|	PF00173.23	COG3239^	GO:0016021	IGTLSAQLTNEKAATF	6.80E-	45.513	1.240 down	0.023	5.464	1.325 down	0.00498	9.39	1.055 down	0.0505	3.663	0.04869	0.10275	0.03781	0.07801	0.01438	0.04033
comp72598_	comp	m.1	sp|Q949X0|	PF00487.19	COG1398^	GO:0031969	APLFPRPSSRPKRWH	0.00023	10.74	1.399 down	0.0434	4.198	1.250 down	0.0767	3.152	1.114 down	0.297	1.638	0.10219	0.20707	0.17883	0.09987	0.17288	0.08017
comp21243_	comp	m.5	sp|Q949X0|	PF00487.19	COG1398^	GO:0031969	MARRSISLLAPAVAIVL	1.48E-	39.658	1.106 down	0.213	2.074	1.565 down	0.00578	8.784	1.210 down	0.011	6.303	0.1252	0.06532	0.08363	0.12111	0.05887	0.06457
comp101969	comp	m.1	sp|Q5ZHT1|	PF02771.11	COG3173^	GO:0005777	MEGEVSTDAMGYILD	0.00012	12.144	1.216 up	0.0669	-3.535	1.306 up	0.0283	-4.579	1.254 up	0.0205	-5.045	0.14654	0.06372	0.12036	0.11792	0.098	0.085
comp36804_	comp	m.8	sp|P33224|A	PF02770.14	COG1960^	GO:0005737	MESKPSASAVPTLPL	5.71E-	20.817	1.270 up	0.0465	-4.085	1.482 up	0.0142	-5.94	1.365 up	0.0057	-8.145	0.10112	0.13526	0.14162	0.12834	0.09314	0.05911
comp37569_	comp	m.8	sp|P45857|A	PF02771.11	COG1960^	GO:0003995	FGSSFKGFKLPQLPQ	4.20E-	112.024	1.089 up	0.0502	-3.959	1.069 up	0.19	-2.109	1.180 up	0.00307	-10.511	0.03089	0.05467	0.06664	0.0627	0.03827	0.02461
comp46814_	comp	m.9	sp|Q9VSA3|	PF00441.19	COG1960^	GO:0005811	ITFDDVKVPKGNLLGK	1.15E-	62.945	1.300 up	0.0265	-5.153	1.244 up	0.00988	-6.874	1.352 up	0.0215	-4.967	0.14232	0.03766	0.01905	0.08994	0.14075	0.10491
comp78877_	comp	m.1	sp|P45867|A	PF02771.11	COG1960^	GO:0003995	MVSPAMLKGMNTEVY	5.09E-	72.814	1.207 up	0.0467	-4.08	1.457 up	0.00423	-10.249	1.532 up	0.00273	-11.136	0.00704	0.1332	0.08523	0.06304	0.10684	0.02846
comp62055_	comp	m.1	sp|Q0NXR6|	PF02771.11	COG1960^	GO:0005739	LEFLHYPATLLLAMAK	0.00001	18.008	1.130 down	0.0396	4.36	1.039 down	0.275	1.707	1.004 down	0.948	0.12	0.05497	0.05997	0.04612	0.04696	0.08334	0.0482
comp25743_	comp	m.6	sp|P53526|E	PF00378.15	COG1024^	GO:0004300	MPTFGPLGDGTDAEN	0.00102	8.095	1.142 up	0.242	-1.928	1.347 up	0.0167	-5.578	1.203 up	0.0986	-2.84	0.12167	0.15827	0.09987	0.11742	0.1734	0.07345
comp27873_	comp	m.6	sp|Q1ZXF1|	PF00378.15	COG1024^	GO:0005759	PLARMLSPAFRRSVP	0.00054	9.17	none	1	0.002	1.341 up	0.0292	-4.523	1.026 up	0.825	-0.369	0.20926	0.13522	0.15627	0.10341	0.11693	0.17272
comp96336_	comp	m.1	sp|O53163|	PF00378.15	COG1024^	GO:0005886	CDSFQWCSEAGQTLI	0.00003	15.25	1.152 up	0.0309	-4.829	1.018 up	0.838	-0.339	1.082 up	0.228	-1.919	0.02647	0.0804	0.13364	0.07899	0.04688	0.11016
comp74423_	comp	m.1	sp|B5BBA1|	PF00378.15	COG1250^	GO:0016507	MSMAKASSRLARAW	1.59E-	26.038	none	0.999	-0.007	1.276 up	0.0137	-6.031	1.169 up	0.0286	-4.473	0.11716	0.09224	0.03285	0.11192	0.05962	0.08156
comp14670_	comp	m.2	sp|Q9NKW1	PF00106.20	COG1028^	GO:0005777	MASWRLDDKVAIVTG	0.00077	8.554	1.196 up	0.281	-1.758	1.238 up	0.071	-3.251	1.428 up	0.0338	-4.227	0.16146	0.24599	0.15151	0.11426	0.228	0.08569
comp77958_	comp	m.1	sp|Q6NYL3|	PF00378.15	COG1250^	GO:0005777	GFPPFPRFHSSAMPA	5.47E-	20.962	1.134 down	0.0513	3.928	1.081 up	0.0563	-3.546	1.221 up	0.00937	-6.685	0.04788	0.07948	0.06232	0.01325	0.0616	0.06067
comp78677_	comp	m.1	sp|Q6NYL3|	PF00378.15	COG1250^	GO:0005777	AQAFCTHRGVVKSSP	4.92E-	31.909	1.079 down	0.0383	4.42	1.172 up	0.0799	-3.096	1.315 up	0.00927	-6.715	0.03697	0.03345	0.10558	0.10377	0.11338	0.03208
comp107722	comp	m.5	sp|P41938|H	PF02737.13	COG1250^	GO:0005759	MAMASMEDQLKVAVI	0.0653	2.869	1.165 down	0.315	1.627	1.054 down	0.478	1.098	1.102 down	0.0215	4.963	0.02907	0.27054	0.13137	0.04336	0.03091	0.04744
comp78395_	comp	m.1	sp|O32177|F	PF00108.18	COG0183^	GO:0003988	MDFKPLSAYIVDACRT	5.47E-	20.962	1.134 down	0.0513	3.928	1.081 up	0.0563	-3.546	1.221 up	0.00937	-6.685	0.04788	0.07948	0.06232	0.01325	0.0616	0.06067
comp79891_	comp	m.1	sp|A1TZR8|	PF00108.18	COG0183^	GO:0005737	QDRTHLSIYFHHDTD	4.92E-	31.909	1.079 down	0.0383	4.42	1.172 up	0.0799	-3.096	1.315 up	0.00927	-6.715	0.03697	0.03345	0.10558	0.10377	0.11338	0.03208
comp79906_	comp	m.1	sp|Q921H8|	PF00108.18	COG0183^	GO:0005739	KVNQLTAIFSLRPNCP	1.59E-	26.038	none	0.999	-0.007	1.276 up	0.0137	-6.031	1.169 up	0.0286	-4.473	0.11716	0.09224	0.03285	0.11192	0.05962	0.08156
comp27261_	comp	m.6	sp|P20901|C	PF00285.16	.	GO:0005737	AFSKKVMSCLFSKGQ	0.018	4.22	1.029 up	0.404	-1.338	1.107 up	0.0151	-5.799	1.013 up	0.806	-0.406	0.04802	0.04125	0.04675	0.02037	0.07364	0.06108
comp82087_	comp	m.1	sp|Q553V1|	PF00285.16	COG0372^	GO:0005759	MHQAFRLHGRQRRA	1.81E-	88.663	1.081 up	0.0212	-5.67	1.119 up	0.0327	-4.34	1.223 up	0.00213	-12.854	0.0166	0.03621	0.03048	0.06833	0.03516	0.02851
comp94429_	comp	m.1	sp|Q17GM7|	PF00285.16	COG0372^	GO:0005759	FPTRSEHIQRQRLVAA	6.64E-	45.713	1.184 up	0.0623	-3.641	1.377 up	0.000925	-26.557	1.272 up	0.00326	-10.17	0.05903	0.1205	0.00908	0.03358	0.01473	0.06677
comp45820_	comp	m.9	sp|P36683|A	PF11791.3^	COG1049^	GO:0005829	MMRAVRRLPVLKAVS	1.53E-	59.677	1.130 up	0.0311	-4.812	1.300 up	0.00791	-7.568	1.488 up	0.00317	-10.368	0.01956	0.07106	0.0565	0.08258	0.07077	0.08516
comp169919	comp	m.3	sp|P25248|A	PF00463.16	.	GO:0009514	ARLQADIMGTETVLIA	3.71E-	22.441	1.361 up	0.417	-1.303	2.865 up	0.0548	-3.586	5.243 up	0.00775	-7.194	0.48084	0.48612	0.44182	0.72261	0.45579	0.48369
comp203351	comp	m.5	sp|Q6BRY4|	PF00463.16	COG2224^	GO:0005777	PVQVVQMAKYLECVY	5.85E-	20.729	2.496 up	0.0946	-3.052	2.563 up	0.0739	-3.2	3.207 up	0.0142	-5.748	0.57004	0.65041	0.41702	0.73907	0.14746	0.5662
comp72338_	comp	m.1	sp|A8NR45|	PF00463.16	.	GO:0005777	NKVDQLFRAQLFHDR	0.00231	6.839	1.216 down	0.0227	5.494	1.122 down	0.188	2.123	1.039 down	0.537	0.962	0.06626	0.07897	0.12033	0.10177	0.09559	0.06485
comp79153_	comp	m.1	sp|P25248|A	PF00463.16	.	GO:0009514	RISCAHHVLGSNSSSL	1.16E-	27.501	1.118 down	0.0343	4.621	1.041 down	0.37	1.385	1.105 up	0.0182	-5.261	0.05773	0.03984	0.07813	0.03017	0.03639	0.04116
comp85614_	comp	m.1	sp|Q3K5N4|	PF01274.17	COG2225^	GO:0005737	FWLEIDREHSLGIHSG	0.00197	7.071	1.171 down	0.0375	4.457	1.027 down	0.514	1.01	1.084 down	0.211	2.004	0.0832	0.05963	0.03969	0.06686	0.11405	0.02326
comp96309_	comp	m.1	sp|A6WDF2|	PF00682.14	COG0119^	GO:0003852	FGLRVAAQCVPGLVE	1.43E-	39.877	1.048 down	0.36	1.473	1.259 down	0.00212	15.405	1.263 down	0.00852	6.928	0.06216	0.06879	0.02741	0.0335	0.09238	0.03085
comp102494	comp	m.1	sp|Q9LKA3|	PF00056.18	COG0039^	GO:0048046	AMGARCSCLEGLKG	0.00009	12.612	1.038 up	0.477	-1.143	1.292 up	0.0157	-5.719	1.148 up	0.0555	-3.542	0.06735	0.06668	0.0781	0.10319	0.10073	0.05113
comp10299_	comp	m.2	sp|Q9LKA3|	PF00056.18	COG0039^	GO:0048046	CVCGAAGGIGQPLCL	9.64E-	65.069	1.199 up	0.0184	-6.001	1.009 up	0.944	-0.122	1.148 up	0.205	-2.03	0.07755	0.04058	0.10251	0.1936	0.15901	0.11565
comp127234	comp	m.1	sp|O24047|	PF00056.18	.	GO:0005737	LPMIASGAIFGPSRRV	0.00041	9.668	1.137 down	0.423	1.287	1.041 down	0.57	0.882	1.083 down	0.0298	4.414	0.14093	0.25262	0.1	0.08862	0.04197	0.03096
comp25855_	comp	m.6	sp|P83373|	PF00056.18	.	GO:0005759	RLFSFSRPLKQPPICA	2.24E-	55.744	1.092 up	0.126	-2.689	1.080 up	0.0397	-4.052	1.035 up	0.454	-1.163	0.04747	0.08195	0.03429	0.0435	0.07245	0.04847
comp47624_	comp	m.9	sp|Q9ZP06|	PF00056.18	COG0039^	GO:0048046	FWLKACLDTPSVLQR	2.78E-	35.454	1.138 down	0.116	2.797	1.038 up	0.377	-1.364	1.140 down	0.00863	6.9	0.09555	0.09414	0.02927	0.0734	0.03071	0.04564
comp25945_	comp	m.6	sp|Q6ZDY8|	PF00890.19	COG1053^	GO:0005618	HAYDTVKGADWLGD	0.00039	9.757	1.038 down	0.598	0.857	1.237 up	0.167	-2.254	1.294 up	0.00927	-6.721	0.09763	0.07923	0.10625	0.25128	0.10401	0.03809
comp72308_	comp	m.1	sp|O82663|	PF00890.19	COG1053^	GO:0005618	LKAPKAPVTTAILPPA	5.76E-	20.78	1.217 up	0.0564	-3.785	1.433 up	0.0212	-5.105	1.287 up	0.0042	-9.174	0.05323	0.14048	0.07913	0.18735	0.01319	0.07842
comp337315	comp	m.7	sp|P21914|D	PF13534.1^	COG0479^	GO:0005875	SSKATKIYPLPHMHVV	0.00097	8.158	8.125 up	0.207	-2.105	31.456 up	0.0276	-4.627	25.270 up	0.0352	-4.168	2.69264	0.99812	2.09718	0.47594	2.13857	0.6513
comp114921	comp	m.1	sp|P42066|P		.	GO:0005737	MASRAPAMRSLARAC	0.00053	9.206	1.581 down	0.0231	5.45	1.157 up	0.221	-1.943	1.050 up	0.733	-0.551	0.14047	0.19799	0.17824	0.12327	0.19859	0.1656
comp19221_	comp	m.4	sp|Q75JD5|	PF01293.15	COG1866^	GO:0045335	TTAMASRSVMRSLTR	1.49E-	26.336	1.352 down	0.0156	6.439	1.029 down	0.548	0.93	1.208 up	0.0137	-5.829	0.08968	0.10134	0.05909	0.06794	0.07326	0.05879
comp88707_	comp	m.1	sp|Q75JD5|	PF01293.15	COG1866^	GO:0045335	GAKTGRSPLDKRVVM	8.83E-	28.845	1.388 down	0.00646	9.676	1.112 up	0.113	-2.694	1.191 up	0.00687	-7.559	0.06868	0.06976	0.09956	0.05533	0.05234	0.04188
comp42268_	comp	m.8	sp|Q7YRC6|	PF07714.12	COG0515^	GO:0000775	AGSAQSVATDGLAWP	2.79E-	35.443	1.314 up	0.0101	-7.926	1.373 up	0.00599	-8.622	1.189 up	0.0261	-4.631	0.0884	0.04597	0.08108	0.06859	0.0562	0.09256
comp43256_	comp	m.9	sp|A4IGM9|	PF00536.25	COG0515^	GO:0000785	SSIGSNGSCTTVIVEF	4.18E-	21.966	1.033 up	0.162	-2.392	1.253 up	0.0268	-4.679	1.194 up	0.00692	-7.535	0.02788	0.02773	0.06355	0.12371	0.03748	0.05693
comp53954_	comp	m.1	sp|Q6NW76|	PF12796.2^	COG0515^	GO:0005694	AAAADHKPIFWWITAV	0.00006	13.779	1.135 down	0.233	1.972	1.156 down	0.0768	3.149	1.220 down	0.0354	4.163	0.06875	0.17235	0.01449	0.13234	0.0689	0.11969

comp76653_	comp	m.1	sp|Q9UQB9|	PF00069.20	COG0515^	GO:0032133	WRMNSMEAWSTSCG	3.60E-	22.56	1.228 down	0.0387	4.401	1.041 up	0.432	-1.212	1.036 up	0.515	-1.015	0.08593	0.104	0.0518	0.08216	0.08053	0.06146
comp99904_	comp	m.1	sp|Q91820|	PF00069.20	.	GO:0005813	VAPKAETAESLTRMP	8.12E-	29.266	1.275 up	0.052	-3.908	1.238 up	0.00672	-8.154	1.238 up	0.00667	-7.648	0.07269	0.16417	0.07139	0.02529	0.05116	0.06229
comp134026	comp	m.2	sp|Q91819|	PF00069.20	.	GO:0005813	SGPAGPASWRPHLS	0.00305	6.44	1.067 down	0.573	0.915	1.206 up	0.148	-2.384	1.301 up	0.018	-5.275	0.05322	0.19911	0.20855	0.09129	0.12749	0.06681
comp21024_	comp	m.5	sp|Q91819|	PF07714.12	.	GO:0005813	MSENMEVQKDGGKE	0.00303	6.451	1.019 up	0.743	-0.548	1.060 down	0.0925	2.92	1.144 down	0.0276	4.533	0.08752	0.05251	0.04264	0.03898	0.03189	0.07966
comp48941_	comp	m.9	sp|O59790|	PF07714.12	COG0515^	GO:0032133	MQRIVHPTQAAAWQ	1.11E-	27.711	1.204 up	0.0165	-6.28	1.267 up	0.0238	-4.876	1.331 up	0.00332	-10.087	0.0707	0.04788	0.08434	0.11191	0.04875	0.06593
comp46241_	comp	m.9	sp|O59790|	PF00069.20	COG0515^	GO:0032133	PCLKRSDWASPHGTC	0.00003	15.489	1.003 down	0.925	0.175	1.311 down	0.063	3.401	1.444 down	0.0162	5.474	0.0554	0.03502	0.0731	0.21828	0.09605	0.16831
comp43847_	comp	m.9	sp|Q96PY6|	PF00069.20	COG0515^	GO:0031965	RGSRGKETAMPPHLD	6.41E-	46.039	1.415 down	0.0161	6.345	1.214 down	0.0126	6.228	1.335 down	0.00266	11.319	0.10515	0.11799	0.07757	0.04552	0.01905	0.07133
comp44719_	comp	m.9	sp|P51954|N	PF00069.20	COG0515^	GO:0005813	APLLMEESDDGLPVD	3.80E-	22.342	1.933 down	0.00701	9.302	1.588 down	0.0359	4.196	1.388 down	0.018	5.279	0.16209	0.12476	0.12569	0.29255	0.12954	0.12426
comp54331_	comp	m.1	sp|Q96PY6|	PF00069.20	COG0515^	GO:0031965	MVYAREDVENVLAAR	0.00001	18.478	1.315 down	0.0571	3.766	1.057 up	0.489	-1.071	1.116 up	0.00284	-10.913	0.09564	0.18708	0.09404	0.11712	0.01801	0.02287
comp65528_	comp	m.1	sp|Q96PY6|	PF00069.20	COG0515^	GO:0031965	FVQRAGRWSLWMGE	0.00004	14.423	1.002 down	0.97	0.075	1.044 up	0.367	-1.395	1.198 up	0.00705	-7.478	0.06934	0.0913	0.04778	0.0761	0.06049	0.03485
comp68026_	comp	m.1	sp|Q96PY6|	PF00069.20	COG0515^	GO:0031965	KGPGGYRHVDTVAIW	0.00273	6.598	1.085 up	0.0942	-3.058	1.151 up	0.145	-2.411	1.163 up	0.0207	-5.026	0.0505	0.0584	0.14785	0.08092	0.06688	0.05517
comp30155_	comp	m.7	sp|Q96PY6|	PF00069.20	COG0515^	GO:0031965	DLQRVRHISTGRFGL	0.00033	10.033	1.182 up	0.0122	-7.195	1.008 up	0.893	-0.229	1.021 down	0.711	0.595	0.04457	0.05046	0.08013	0.06577	0.08194	0.06246
comp24271_	comp	m.6	sp|Q96PY6|	PF00069.20	COG0515^	GO:0031965	LGQRGSPLQDAYPKQ	1.98E-	25.05	1.117 down	0.111	2.846	1.244 down	0.0178	5.455	1.142 down	0.00818	7.045	0.05653	0.09736	0.083	0.08085	0.03109	0.04464
comp109521	comp	m.6	sp|P51954|N	PF00069.20	COG0515^	GO:0005813	PTFFCFVHADLPPCPI	0.00303	6.449	1.151 down	0.111	2.845	1.173 down	0.00531	9.113	1.088 down	0.408	1.286	0.11697	0.08197	0.04027	0.03054	0.13083	0.13783
comp130708	comp	m.2	sp|P51954|N	PF00069.20	COG0515^	GO:0005813	KKSDLGPSWVLCGCP	0.00090	8.297	1.011 down	0.905	0.219	1.233 up	0.144	-2.42	1.271 up	0.048	-3.73	0.05158	0.14099	0.23271	0.0918	0.16049	0.0938
comp58032_	comp	m.1	sp|P51955|N	PF00069.20	COG0515^	GO:0005813	KRAKLRSLRSLCLDICI	0.0176	4.246	1.044 down	0.472	1.157	1.131 down	0.0188	5.336	1.127 down	0.164	2.273	0.02367	0.10617	0.03954	0.05407	0.1253	0.08704
comp163489	comp	m.3	sp|Q9R0A5|	PF00069.20	COG0515^	GO:0030424	LSYWIVLKFYPGGDV	0.022	3.997	1.035 up	0.891	-0.248	1.218 up	0.548	-0.929	1.403 up	0.0233	-4.816	0.25816	0.31901	0.31773	0.52628	0.12756	0.15786
comp14102_	comp	m.2	sp|P51957|N	PF00069.20	COG0515^	GO:0005634	MSWEGLSHTQLPAPS	0.00042	9.62	1.226 down	0.0411	4.295	1.056 up	0.494	-1.057	1.179 up	0.0789	-3.103	0.10917	0.08278	0.07302	0.12969	0.14805	0.03946
comp68822_	comp	m.1	sp|Q9Z1J2|	PF00069.20	COG0515^	GO:0005634	SFLVTMALSLDLEEEP	0.00003	14.917	1.500 down	0.0225	5.517	1.454 down	0.0421	3.96	2.041 down	0.0267	4.594	0.06514	0.20209	0.10334	0.25283	0.06578	0.44339
comp134332	comp	m.2	sp|Q9Z1J2|	PF00069.20	COG0515^	GO:0005634	MTSSGTTLPDWGYEE	0.00283	6.545	1.165 down	0.0473	4.058	1.190 down	0.117	2.651	1.097 up	0.443	-1.191	0.07006	0.08316	0.14874	0.1176	0.16144	0.15775
comp32274_	comp	m.7	sp|Q6P3R8|	PF00069.20	COG0515^	GO:0005524	MHHFQLIRGLGRGSG	0.0203	4.085	1.335 down	0.308	1.654	1.589 down	0.0356	4.207	1.275 down	0.215	1.985	0.40188	0.30479	0.26708	0.17245	0.27917	0.21805
comp109786	comp	m.6	sp|Q6P3R8|	PF00069.20	COG0515^	GO:0005524	PVAVAAASTAVAFGS	0.00018	11.197	1.016 down	0.839	0.355	1.008 down	0.922	0.169	1.143 up	0.0101	-6.516	0.05807	0.11616	0.10756	0.0914	0.03013	0.05112
comp40381_	comp	m.8	sp|Q6P3R8|	PF00069.20	COG0515^	GO:0005524	VEFAATRDAAPERMN	1.06E-	27.959	1.250 down	0.0156	6.428	1.455 down	0.0106	6.693	1.193 down	0.00849	6.938	0.07024	0.0719	0.07624	0.14266	0.04345	0.05949
comp12853_	comp	m.1	sp|Q6P3R8|	PF00069.20	COG0515^	GO:0005524	TLHPDAFKNEHAPSD	0.00146	7.528	1.401 up	0.0428	-4.222	1.306 up	0.0777	-3.133	1.147 up	0.123	-2.593	0.20935	0.09682	0.17519	0.17292	0.11975	0.09493
comp13966_	comp	m.2	sp|Q6P3R8|	PF00069.20	COG0515^	GO:0005524	MPYRVGDTLGAFTVE	0.0102	4.886	1.303 up	0.0294	-4.934	1.151 up	0.203	-2.035	1.143 up	0.282	-1.694	0.09422	0.12293	0.12416	0.15644	0.18326	0.13625
comp102834	comp	m.1	sp|Q6P3R8|	PF00069.20	COG0515^	GO:0005524	MVMDRSVLATRLHGR	0.00032	10.122	1.236 down	0.0895	3.126	1.190 down	0.0274	4.639	1.014 down	0.831	0.356	0.07543	0.18063	0.1019	0.03678	0.08162	0.084
comp27360_	comp	m.6	sp|Q9LT35|	PF07714.12	COG0515^	GO:0005524	MPLESRAVEGSPRLP	0.00015	11.628	1.359 down	0.0296	4.914	1.454 down	0.0189	5.319	1.170 down	0.158	2.313	0.06023	0.17009	0.09398	0.18025	0.1017	0.16738
comp57105_	comp	m.1	sp|Q86SG6|	PF00069.20	COG0515^	GO:0005737	GLPLHIDGCGDKLASV	0.00001	17.962	1.307 up	0.139	-2.571	1.828 up	0.0124	-6.276	1.905 up	0.014	-5.788	0.13716	0.26812	0.14214	0.23819	0.28911	0.14057
comp29198_	comp	m.7	sp|Q03114|	PF00069.20	COG0515^	GO:0030424	QVAQPTKFWWVWGH	2.35E-	24.305	1.551 down	0.027	5.11	1.263 down	0.0222	5.006	1.176 down	0.0032	10.264	0.12972	0.21121	0.07318	0.11299	0.0361	0.02782
comp62787_	comp	m.1	sp|Q1XDK6|	PF00550.20	.	GO:0009507	TSAGTRGSSENHPPR	2.66E-	23.781	1.047 down	0.341	1.536	1.211 down	0.063	3.401	1.284 down	0.0117	6.167	0.03786	0.07898	0.04729	0.15586	0.0826	0.08306
comp151799	comp	m.3	sp|Q8RGF2|	.	COG0688^	GO:0004609	PGFTLCSHVSMGCRG	0.00251	6.717	1.889 down	0.046	4.105	1.082 down	0.653	0.702	1.070 down	0.743	0.53	0.2057	0.39703	0.09586	0.31308	0.25945	0.26423
comp44050_	comp	m.9	sp|Q8BSF4|	PF02666.10	COG0688^	GO:0005739	MKRFQSLRRFKWSD	1.67E-	25.823	1.108 down	0.125	2.7	1.416 down	0.00562	8.873	1.528 down	0.0104	6.437	0.08574	0.06925	0.03674	0.10699	0.18192	0.05506
comp44746_	comp	m.9	sp|P96282|P	PF01066.16	COG1183^	GO:0005576	MPEEVKASSRAPQWL	0.00457	5.888	1.129 down	0.146	2.514	1.274 down	0.0294	4.514	1.041 down	0.685	0.646	0.02603	0.13764	0.04025	0.14974	0.13963	0.11453
comp51082_	comp	m.1	sp|O50314|	PF02514.11	COG1429^	GO:0005524	SVAVVLGLLGARPVT	0.0015	7.479	1.253 up	0.115	-2.809	1.467 up	0.0117	-6.433	1.440 up	0.116	-2.662	0.16794	0.16055	0.0609	0.16105	0.38334	0.09891
comp158198	comp	m.3	sp|O50314|	PF02514.11	COG1429^	GO:0005524	WLAVQPPLGIPGDPM	0.00112	7.95	1.320 up	0.112	-2.833	1.333 up	0.0349	-4.239	1.363 up	0.0439	-3.857	0.1806	0.21781	0.14688	0.12964	0.17372	0.15403
comp31503_	comp	m.7	sp|Q9FNB0|	PF11965.3^	.	GO:0009706	PLLESAMSSQSQVSM	0.00007	13.255	1.296 down	0.0484	4.021	1.061 up	0.619	-0.774	1.245 up	0.0161	-5.488	0.16761	0.08209	0.2012	0.09521	0.10536	0.04717
comp71167_	comp	m.1	sp|Q9FNB0|	PF02514.11	.	GO:0009706	PASGGNFQDSLLKLV	0.00006	13.418	1.093 down	0.0951	3.045	1.093 up	0.222	-1.939	1.190 up	0.0148	-5.661	0.05696	0.06265	0.07938	0.10694	0.07865	0.04117
comp46484_	comp	m.9	sp|Q9TL08|	PF01078.16	.	GO:0009507	DLSSPFFHHRLVKEIL	1.60E-	25.994	1.037 up	0.238	-1.944	1.143 down	0.0442	3.889	1.173 down	0.00181	13.951	0.04808	0.02641	0.07274	0.06752	0.02485	0.02181
comp39765_	comp	m.8	sp|Q9TL08|	PF01078.16	.	GO:0009507	KGVTRWHIDPQKMLP	0.00001	17.926	none	0.998	0.008	1.049 down	0.483	1.085	1.181 down	0.0493	3.694	0.05088	0.07621	0.07245	0.10753	0.11199	0.06613
comp116145	comp	m.1	sp|Q96449|	PF08423.6^	.	GO:0005634	MAAVTVESSQKRKAS	3.20E-	79.456	2.836 down	0.00151	23.381	4.457 down	0.00433	10.151	3.067 down	0.00364	9.767	0.05472	0.11644	0.30751	0.29316	0.15812	0.29095
comp122198	comp	m.1	sp|Q61880|	PF08423.6^	COG0468^	GO:0000781	SQSVAFAMRKDLLSIK	4.17E-	32.943	2.130 down	0.0109	7.61	2.124 down	0.00305	12.478	1.884 down	0.0224	4.894	0.16391	0.23522	0.09683	0.14492	0.36635	0.07385
comp151318	comp	m.3	sp|P50265|D	PF08423.6^	COG0468^	GO:0005634	ATAPAMVDHALVEAD	0.00023	10.784	1.621 down	0.119	2.763	3.303 down	0.0271	4.662	2.454 down	0.0309	4.359	0.2865	0.41542	0.38653	0.63055	0.30268	0.51156
comp147597	comp	m.2	sp|O35047|	PF07106.8^	NOG27444	GO:0005634	TAPAAPATVAVQPAE	0.00215	6.942	4.902 down	0.0304	4.861	3.272 down	0.152	2.355	1.733 down	0.128	2.555	0.70507	0.62715	1.09334	0.95608	0.5048	0.36185
comp43393_	comp	m.9	sp|O35047|	PF07106.8^	NOG27444	GO:0005634	KAKSEAKGAKDVKKL	6.47E-	30.42	2.224 down	0.015	6.545	3.299 down	0.0031	12.401	1.784 down	0.0491	3.7	0.2691	0.22754	0.1383	0.24087	0.34741	0.28839
comp52471_	comp	m.1	sp|Q9BWT6|	PF03962.10	COG5124^	GO:0005634	MSKRKGMSFDEKKVT	0.00002	16.55	1.474 up	0.0177	-6.11	1.398 up	0.0401	-4.037	1.353 up	0.0186	-5.218	0.10518	0.15009	0.13169	0.20021	0.12642	0.10952
comp151677	comp	m.3	sp|O15457|	PF00488.16	COG0249^	GO:0000795	RGMQWYKVAVRTAL	0.00065	8.83	1.416 down	0.123	2.723	1.961 down	0.00571	8.826	1.495 down	0.215	1.985	0.25663	0.26545	0.11616	0.18709	0.23512	0.53538
comp191937	comp	m.4	sp|O15457|	PF05192.13	COG0249^	GO:0000795	LERRHFDEAEGQSLL	0.00067	8.778	2.140 down	0.113	2.829	3.070 down	0.0161	5.659	2.180 down	0.0825	3.051	0.41339	0.65698	0.27112	0.50368	0.46761	0.56985
comp34940_	comp	m.7	sp|Q9M3G7|	PF02260.15	COG5032^	GO:0005634	GLGCLGTCGLFRRCA	3.76E-	22.384	1.423 up	0.0186	-5.975	1.581 up	0.0506	-3.695	1.311 up	0.0457	-3.796	0.14478	0.09001	0.2706	0.23428	0.18228	0.09571
comp53928_	comp	m.1	sp|Q9M3G7|	PF00454.22	COG5032^	GO:0005634	NTVRQLTEQKIQRIDE	0.00002	16.307	1.319 up	0.0471	-4.065	1.562 up	0.013	-6.163	1.157 up	0.347	-1.465	0.11502	0.15946	0.16355	0.13003	0.20625	0.20111
comp45520_	comp	m.9	sp|Q8RXD4|	.	NOG27449	GO:0005634	NKLSQQRRMLHSGD	5.69E-	47.048	1.109 up	0.0413	-4.283	1.149 down	0.0188	5.339	1.044 up	0.295	-1.645	0.06129	0.03342	0.06687	0.03459	0.03814	0.066
comp79139_	comp	m.1	sp|O35923|	PF09103.5^	NOG33129	GO:0033593	PVGHVGFMTAGGKA	0.00009	12.807	1.688 down	0.00789	8.787	1.408 down	0.0311	4.42	1.338 down	0.128	2.548	0.13989	0.1	0.14389	0.17089	0.07676	0.32076
comp76876_	comp	m.1	sp|Q9XZD6|	PF00069.20	COG0515^	GO:0005737	LDVLLQAALHLQPWA	0.00055	9.137	1.014 down	0.915	0.199	1.233 down	0.0298	4.491	1.191 down	0.121	2.612	0.18767	0.07469	0.10902	0.07959	0.06902	0.18052
comp71994_	comp	m.1	sp|Q54CR9|	PF04675.9^	COG1793^	GO:0005694	DHGILMSASLTSCDAE	0.00003	15.448	1.074 up	0.209	-2.097	1.090 down	0.232	1.89	1.165 down	0.0117	6.158	0.06726	0.07242	0.03665	0.12775	0.05906	0.04092
comp21276_	comp	m.5	sp|Q9W6K2|	PF00752.12	.	GO:0005634	RTSASIPGSAHDPMGI	5.31E-	21.076	1.051 down	0.21	2.092	1.259 down	0.0303	4.461	1.050 down	0.478	1.104	0.02804	0.06322	0.04524	0.14193	0.11319	0.06289
comp84040_	comp	m.1	sp|C5YUK3|	PF00752.12	COG0258^	GO:0005739	MGIKGLVKFLQENAP	0.00061	8.946	1.035 down	0.207	2.106	1.106 down	0.115	2.67	1.159 down	0.0115	6.209	0.02257	0.04179	0.09281	0.05735	0.03889	0.05684
comp66751_	comp	m.1	sp|Q9ZRT1|	PF08573.5^	NOG14552	GO:0005634	MLHSNDVQDAVSAED	1.21E-	136.224	1.518 up	0.00164	-20.856	1.467 up	0.00259	-13.954	1.582 up	0.00232	-12.096	0.0245	0.05236	0.07847	0.01109	0.09133	0.0603
comp51373_	comp	m.1	sp|Q9UHC1|	PF08676.6^	COG0323^	GO:0005712	PGPGPGRFFFARSGS	0.0103	4.865	1.408 down	0.0465	4.086	1.100 down	0.463	1.133	1.016 down	0.939	0.14	0.12792	0.20524	0.21312	0.11969	0.14007	0.3085
comp79008_	comp	m.1	sp|O13396|	PF05188.12	COG0249^	GO:0032301	MAFDSEVQENAVCCI	0.00011	12.331	1.106 up	0.0374	-4.46	1.107 up	0.00923	-7.091	1.044 up	0.22	-1.961	0.05014	0.04176	0.0289	0.02972	0.05943	0.0255
comp31875_	comp	m.7	sp|Q6BJ48|	PF02732.10	COG1948^	GO:0005634	TPCMAKPAVQQELEE	0.00003	15.224	1.015 down	0.925	0.175	1.672 up	0.0222	-5.004	1.630 up	0.00656	-7.709	0.22077	0.1225	0.21481	0.20442	0.12942	0.12937
comp58263_	comp	m.1	sp|O74803|	PF00627.26	COG5272^	GO:0005829	PAAPAAPAAPAADAP	0.00002	16.483	1.102 up	0.0455	-4.127	1.129 down	0.181	2.165	1.014 up	0.901	-0.219	0.06273	0.02687	0.14474	0.07368	0.17508	0.08878
comp73891_	comp	m.1	sp|Q84L33|	PF11976.3^	COG5272^	GO:0005634	MKITVRPIKGESFFVE	0.00078	8.529	1.028 down	0.762	0.511	1.154 down	0.0495	3.728	1.219 down	0.0164	5.456	0.12144	0.10594	0.06714	0.08836	0.03625	0.09837
comp338383	comp	m.7	sp|P42656|R	PF00244.15	COG5040^	GO:0032153	ARKDKMAEGSDAYPE	0.0332	3.555	1.126 down	0.822	0.39	3.940 up	0.0435	-3.913	7.260 up	0.269	-1.745	0.43496	0.76646	0.92638	0.4051	3.25938	0.34414
comp72013_	comp	m.1	sp|Q9JIL8|R	PF13476.1^	COG0419^	GO:0000781	LALPHLAMTTISKLGIQ	9.55E-	42.746	1.263 up	0.0245	-5.318	1.255 up	0.00699	-7.993	1.328 up	0.00137	-17.082	0.11838	0.04525	0.0137	0.08106	0.0399	0.02661
comp1992_c	comp	m.5	sp|Q6PFE3|	.	COG0553^	GO:0005634	FGLPPRVEVVLRLRLA	0.0122	4.673	1.879 down	0.135	2.61	2.689 down	0.0456	3.841	2.224 down	0.217	1.976	0.4803	0.50582	0.19328	0.71769	0.52674	1.04217
comp55030_	comp	m.1	sp|P0C928|	PF06733.10	COG1199^	GO:0005634	ADNDGQIRQACRQAR	0.0553	3.032	1.257 down	0.0414	4.28	1.185 up	0.391	-1.322	1.120 up	0.593	-0.838	0.06161	0.14168	0.25886	0.26713	0.18808	0.34294
comp65380_	comp	m.1	sp|B4PZB4|	PF06733.10	COG1199^	GO:0005634	VLVEFPYEAYDCQLE	0.00001	18.009	1.226 down	0.0153	6.491	1.257 down	0.0114	6.491	1.087 up	0.22	-1.96	0.03926	0.0817	0.04291	0.09227	0.09641	0.07717

comp69445_	comp	m.1	sp|Q54LY5|	PF03731.10	NOG29974	GO:0005958	MAWRCKEAIVLVIDVG	0.00178	7.22	1.038 down	0.526	1.024	1.150 down	0.041	4.001	1.110 down	0.164	2.277	0.0443	0.0966	0.08516	0.05449	0.08901	0.09912
comp82451_	comp	m.1	sp|P08965|		NOG26286	GO:0005829	NLGYAFVNMVDSKAV	0.00006	13.448	1.040 down	0.676	0.688	1.476 down	0.0474	3.786	1.301 down	0.025	4.701	0.11281	0.12386	0.06214	0.29062	0.12983	0.09622
comp72679_	comp	m.1	sp|P08965|	PF04059.7^	NOG26286	GO:0005829	SSCLGSNLPVPPHRE	0.00022	10.86	1.109 down	0.304	1.671	1.586 up	0.0119	-6.378	1.124 up	0.288	-1.672	0.06711	0.16625	0.11899	0.17147	0.1938	0.05782
comp11213_	comp	m.8	sp|P08965|	PF04059.7^	NOG26286	GO:0005829	RNEAPQVPPGVLPQA	0.00002	16.731	1.421 down	0.0282	5.013	1.290 down	0.0915	2.933	1.202 down	0.0228	4.859	0.04261	0.19774	0.2261	0.10819	0.06276	0.08986
comp11663_	comp	m.1	sp|P08965|	PF04059.7^	NOG26286	GO:0005829	MVTMQEDFSGDRTD	0.00003	15.172	1.129 down	0.456	1.198	1.201 down	0.145	2.41	1.298 down	0.0144	5.727	0.18584	0.227	0.16787	0.14187	0.08829	0.09747
comp3090_c	comp	m.7	sp|P08965|	PF04059.7^	NOG26286	GO:0005829	QESGVGRGFGAFRA	2.08E-	56.557	1.802 down	0.00831	8.566	1.225 down	0.0444	3.879	1.082 down	0.461	1.145	0.0723	0.18487	0.10203	0.11192	0.19444	0.04331
comp93139_	comp	m.1	sp|Q64M78|	PF04059.7^	NOG26286	GO:0000166	SSDLWRKVQPLRKRP	2.21E-	24.573	1.266 down	0.0242	5.34	1.306 down	0.0107	6.661	1.090 down	0.0523	3.62	0.06632	0.1089	0.06681	0.09462	0.05216	0.04498
comp79032_	comp	m.1	sp|Q64M78|	PF04059.7^	NOG26286	GO:0000166	PAAQMFHAPAAEQLP	0.00143	7.558	1.091 up	0.532	-1.009	1.117 up	0.305	-1.596	1.227 up	0.0414	-3.939	0.1303	0.21371	0.12587	0.15578	0.13136	0.07254
comp74450_	comp	m.1	sp|Q64M78|	PF04059.7^	NOG26286	GO:0000166	MPKGSGGKVSSTKQ	0.00002	16.705	1.276 down	0.0967	3.021	1.123 up	0.225	-1.924	1.253 up	0.00595	-8.017	0.06706	0.22321	0.08188	0.15433	0.05455	0.0605
comp33650_	comp	m.7	sp|Q64M78|	PF04059.7^	NOG26286	GO:0000166	TKLSEGAIARCFSVSA	0.0202	4.088	1.110 down	0.716	0.606	1.228 up	0.158	-2.315	1.658 up	0.0433	-3.875	0.39767	0.30022	0.16637	0.19537	0.1409	0.3493
comp77579_	comp	m.1	sp|Q64M78|	PF04059.7^	NOG26286	GO:0000166	MAMGPEVAGRLILRN	7.02E-	45.247	1.417 down	0.0416	4.271	1.232 down	0.0144	5.908	1.300 down	0.00612	7.932	0.08653	0.21945	0.0704	0.07372	0.08872	0.03568
comp102634	comp	m.1	sp|Q64M78|	PF04059.7^	NOG26286	GO:0000166	MALMQPVLTLPVEGS	0.00013	11.887	1.069 down	0.166	2.362	1.203 down	0.0444	3.88	1.079 up	0.116	-2.665	0.04248	0.06982	0.03335	0.13378	0.0185	0.08045
comp216824	comp	m.5	sp|Q64M78|	PF04059.7^	NOG26286	GO:0000166	QTIQEEAPAKGDEAP	2.61E-	54.143	1.994 down	0.0201	5.795	1.017 up	0.909	-0.195	1.041 down	0.791	0.436	0.12197	0.32127	0.21883	0.13898	0.16558	0.20945
comp59081_	comp	m.1	sp|Q652K6|	PF04059.7^	NOG24559	GO:0000166	KNKTRRSQRRRTSSL	0.00001	17.499	1.245 down	0.0544	3.839	1.192 down	0.0382	4.105	1.198 down	0.045	3.819	0.14326	0.08138	0.09194	0.08311	0.11789	0.06915
comp90032_	comp	m.1	sp|Q6EQX3|	PF04059.7^	NOG26286	GO:0000166	SSILALWRPLCIASSF	4.79E-	48.555	1.554 down	0.00738	9.094	1.820 down	0.0044	10.088	1.460 down	0.00922	6.733	0.08582	0.11048	0.06034	0.16034	0.14909	0.0641
comp70257_	comp	m.1	sp|Q6EQX3|	PF04059.7^	NOG26286	GO:0000166	MAHNKAGVVIKNTFLE	1.24E-	27.18	1.463 down	0.0236	5.403	1.169 down	0.277	1.697	1.046 up	0.518	-1.008	0.17299	0.10721	0.23584	0.12246	0.11742	0.05708
comp73286_	comp	m.1	sp|Q6EQX3|	PF04059.7^	NOG26286	GO:0000166	DLDDVPMMHTHSQK	0.00033	10.06	1.158 up	0.0187	-5.968	1.096 up	0.0681	-3.303	1.036 down	0.585	0.856	0.04004	0.05879	0.05959	0.05367	0.0691	0.09882
comp53430_	comp	m.1	sp|Q6EQX3|	PF04059.7^	NOG26286	GO:0000166	VQVHTMTMAMEYEE	5.14E-	31.671	2.588 down	0.00956	8.097	3.797 down	0.00833	7.406	3.242 down	0.00937	6.684	0.11666	0.31816	0.08939	0.51206	0.1135	0.49496
comp97757_	comp	m.1	sp|Q6EQX3|	PF04059.7^	NOG26286	GO:0000166	PAFPSLYLAQVLGAAL	2.61E-	23.866	1.142 down	0.0118	7.323	1.002 down	0.975	0.056	1.176 up	0.0289	-4.458	0.04227	0.03086	0.06707	0.11048	0.09844	0.03641
comp74366_	comp	m.1	sp|Q6EQX3|	PF04059.7^	NOG26286	GO:0000166	QVSFSEGLDHSGTAT	2.09E-	56.481	1.475 down	0.0122	7.207	1.454 down	0.00342	11.632	1.239 down	0.0146	5.704	0.06404	0.14198	0.0807	0.04621	0.07708	0.07642
comp41180_	comp	m.8	sp|Q6EQX3|	PF04059.7^	NOG26286	GO:0000166	MGESLTSLVLKRVPP	0.00163	7.351	1.069 down	0.27	1.802	1.111 down	0.045	3.86	1.111 down	0.0388	4.033	0.04859	0.09537	0.05141	0.06021	0.03782	0.0655
comp147342	comp	m.2	sp|Q6ZI17|O	PF04059.7^	NOG26286	GO:0005634	MDLTEVRFEELDANS	7.11E-	45.16	1.513 down	0.0156	6.441	1.095 down	0.375	1.37	1.197 down	0.068	3.283	0.14725	0.11302	0.13906	0.13156	0.15644	0.02552
comp55332_	comp	m.1	sp|Q6ZI17|O	PF04059.7^	NOG26286	GO:0005634	YMPCKEHRNVPAGFA	1.03E-	28.111	1.407 down	0.0188	5.955	1.020 down	0.738	0.532	1.018 up	0.814	-0.39	0.09328	0.13696	0.06522	0.08773	0.10574	0.08913
comp55889_	comp	m.1	sp|Q6ZI17|O	PF04059.7^	NOG26286	GO:0005634	SEPVKMFSSLPEPDQ	3.62E-	22.539	1.362 down	0.00593	10.166	1.114 down	0.16	2.301	1.037 down	0.579	0.868	0.0562	0.06741	0.03627	0.13119	0.10288	0.06746
comp77201_	comp	m.1	sp|Q6ZI17|O	PF04059.7^	NOG26286	GO:0005634	MLPENPARHTVRACI	2.74E-	35.53	1.400 down	0.00818	8.639	1.261 down	0.0194	5.266	1.397 down	0.0122	6.071	0.09781	0.05548	0.04881	0.11771	0.08597	0.1337
comp89398_	comp	m.1	sp|Q6ZI17|O	PF04059.7^	NOG26286	GO:0005634	RCRTRLLTYSLNRSL	2.63E-	23.826	1.200 down	0.064	3.601	1.209 down	0.0145	5.889	1.050 down	0.38	1.366	0.06889	0.12915	0.04854	0.07954	0.09678	0.03872
comp49964_	comp	m.1	sp|Q6ZI17|O	PF04059.7^	NOG26286	GO:0005634	MAYTMDMHASMPGL	1.79E-	38.336	1.461 down	0.0301	4.883	1.353 down	0.00442	10.042	1.158 down	0.023	4.841	0.143	0.17277	0.06224	0.06089	0.05552	0.06805
comp65334_	comp	m.1	sp|Q6ZI17|O	PF04059.7^	NOG26286	GO:0005634	STLLLLGQSHDWWLV	5.48E-	20.957	1.197 down	0.0596	3.703	1.265 down	0.017	5.55	1.174 down	0.0422	3.912	0.1352	0.03715	0.06084	0.10638	0.0471	0.10865
comp19571_	comp	m.4	sp|Q6ZI17|O	PF04059.7^	NOG26286	GO:0005634	MESTGANSPTGGTSP	0.00028	10.401	1.007 down	0.888	0.255	1.059 up	0.271	-1.723	1.080 up	0.0457	-3.798	0.05615	0.06633	0.05463	0.08054	0.02184	0.05452
comp25019_	comp	m.6	sp|Q6ZI17|O	PF04059.7^	NOG26286	GO:0005634	MSQTAKSKRNQRES	7.30E-	19.937	1.258 down	0.0256	5.211	1.072 down	0.384	1.343	1.063 down	0.337	1.5	0.01381	0.12671	0.10314	0.10983	0.07381	0.09156
comp60896_	comp	m.1	sp|Q6ZI17|O	PF04059.7^	NOG26286	GO:0005634	LASIAQTNVLMAPVDG	7.60E-	19.794	1.549 down	0.0297	4.908	1.025 up	0.818	-0.377	1.047 up	0.0242	-4.752	0.08184	0.24394	0.1672	0.09384	0.02008	0.01981
comp32962_	comp	m.7	sp|Q75M35|	PF04059.7^	NOG30140	GO:0000166	MEPWILVDRVASGQA	0.00002	16.723	1.768 down	0.0408	4.307	1.062 up	0.58	-0.858	1.089 up	0.562	-0.906	0.15747	0.34797	0.14666	0.14005	0.24087	0.13057
comp80393_	comp	m.1	sp|Q75M35|	PF04059.7^	NOG30140	GO:0000166	AGDAMDGETEASAG	5.49E-	31.284	1.046 down	0.0244	5.321	1.188 down	0.0119	6.398	1.092 down	0.0291	4.449	0.01804	0.0167	0.02576	0.07365	0.05045	0.02752
comp79803_	comp	m.1	sp|Q75M35|	PF04059.7^	NOG30140	GO:0000166	MSRKGSWKGGGKG	0.00001	17.952	1.025 up	0.61	-0.832	1.156 up	0.0631	-3.4	1.273 up	0.0409	-3.955	0.06415	0.05705	0.08596	0.08821	0.15265	0.08858
comp56101_	comp	m.1	sp|Q75M35|	PF04059.7^	NOG30140	GO:0000166	VDRSTILWQFLPLKFT	0.00086	8.359	1.425 down	0.0499	3.971	1.057 up	0.515	-1.008	1.126 up	0.0283	-4.491	0.15276	0.20743	0.11129	0.11494	0.05108	0.05726
comp52203_	comp	m.1	sp|Q75M35|	PF04059.7^	NOG30140	GO:0000166	MPSTWAKKSWEKWE	0.00085	8.376	1.131 down	0.0886	3.138	1.221 down	0.0119	6.38	1.138 down	0.226	1.928	0.04404	0.10452	0.05811	0.06919	0.01384	0.19391
comp133629	comp	m.2	sp|Q75M35|	PF04059.7^	NOG30140	GO:0000166	MTVPREWSMEQSVS	8.09E-	19.586	1.088 down	0.529	1.017	1.408 up	0.00439	-10.091	1.334 up	0.0148	-5.656	0.06192	0.23257	0.0603	0.07726	0.13388	0.06133
comp135921	comp	m.2	sp|Q75M35|	PF04059.7^	NOG30140	GO:0000166	MQMPTAGLKLQHLES	3.73E-	33.598	1.770 down	0.0403	4.331	2.118 down	0.00496	9.416	2.104 down	0.0129	5.963	0.19683	0.3258	0.14093	0.18185	0.17669	0.31359
comp55194_	comp	m.1	sp|Q8VWF5|	PF04059.7^	NOG26286	GO:0000166	FLLLRTVCGPIAPIAMA	0.00028	10.334	1.515 down	0.0294	4.931	1.571 down	0.0301	4.473	1.758 down	0.0534	3.592	0.09056	0.2256	0.06391	0.28464	0.04672	0.45123
comp15254_	comp	m.3	sp|Q8VWF5|	PF04059.7^	NOG26286	GO:0000166	PDAPGSSFRFSDNID	2.28E-	36.701	1.751 down	0.011	7.584	1.642 down	0.0107	6.684	1.272 down	0.0392	4.019	0.04927	0.20736	0.10083	0.18898	0.11656	0.1276
comp139859	comp	m.2	sp|Q8VWF5|	PF04059.7^	NOG26286	GO:0000166	PLTFGKRLWQSTLCC	3.05E-	34.87	1.770 down	0.049	4.002	1.513 down	0.0108	6.632	1.387 down	0.00293	10.739	0.12787	0.3914	0.08359	0.15977	0.07219	0.05047
comp31235_	comp	m.7	sp|Q8VWF5|	PF04059.7^	NOG26286	GO:0000166	REPREPREPGPQETA	4.28E-	32.784	1.461 down	0.00956	8.09	1.317 up	0.0388	-4.083	1.254 up	0.0234	-4.812	0.07183	0.1147	0.15596	0.11661	0.06918	0.11721
comp20863_	comp	m.5	sp|Q8W4I9|	PF04059.7^	NOG26286	GO:0000166	MAVVCVPVVVVPVFQ	7.99E-	44.19	1.329 down	0.0125	7.112	1.528 down	0.0105	6.727	1.168 down	0.025	4.702	0.0527	0.10266	0.12267	0.13449	0.08556	0.04222
comp95583_	comp	m.1	sp|Q8W4I9|	PF04059.7^	NOG26286	GO:0000166	PPQADFNGYGYMPE	6.54E-	30.364	1.094 up	0.162	-2.395	1.203 up	0.00558	-8.895	1.237 up	0.011	-6.307	0.05387	0.09432	0.02051	0.05637	0.03152	0.09217
comp28619_	comp	m.7	sp|Q9LYN7|	PF04059.7^	NOG26286	GO:0000166	FGGYPHICGIQRVMR	3.97E-	22.179	1.309 down	0.0386	4.409	1.485 down	0.018	5.425	1.259 down	0.0401	3.984	0.12572	0.12357	0.14243	0.15511	0.10796	0.1274
comp55231_	comp	m.1	sp|Q9LYN7|	PF04059.7^	NOG26286	GO:0000166	MIHAARGLSRKPTSS	0.00002	16.048	1.440 down	0.0654	3.57	1.511 down	0.00728	7.855	1.331 down	0.00625	7.874	0.10742	0.27489	0.05135	0.14285	0.094	0.04658
comp51440_	comp	m.1	sp|Q9LYN7|	PF04059.7^	NOG26286	GO:0000166	QEASLDSSSVSRAFQ	3.26E-	34.452	1.432 down	0.0295	4.925	1.353 down	0.0239	4.867	1.495 down	0.00562	8.186	0.13435	0.16212	0.1162	0.13647	0.06764	0.12478
comp106586	comp	m.4	sp|Q9LYN7|	PF04059.7^	NOG26286	GO:0000166	MPGSCTRCRQPKEY	0.00186	7.158	1.300 up	0.0233	-5.435	1.066 up	0.503	-1.037	1.313 up	0.174	-2.211	0.13537	0.03375	0.09567	0.15081	0.34872	0.07362
comp57820_	comp	m.1	sp|Q9SVV9|	PF04059.7^	NOG28655	GO:0000166	NSGIDSLSGAMTRVL	0.00056	9.083	1.238 down	0.0247	5.292	1.067 up	0.352	-1.44	1.052 up	0.505	-1.038	0.10861	0.04216	0.10873	0.07416	0.0651	0.12779
comp20572_	comp	m.5	sp|Q9SVV9|	PF04059.7^	NOG28655	GO:0000166	MECDYDYSPQEGQW	4.98E-	21.303	1.096 down	0.0493	3.992	1.103 down	0.29	1.65	1.014 up	0.874	-0.274	0.04801	0.04604	0.10698	0.13447	0.06458	0.13637
comp20280_	comp	m.5	sp|Q9SVV9|	PF04059.7^	NOG28655	GO:0000166	ACRADGFLPPATLST	8.88E-	28.816	1.056 down	0.134	2.62	1.148 down	0.0227	4.958	1.179 down	0.0053	8.41	0.04677	0.03919	0.06166	0.05149	0.0312	0.04736
comp78683_	comp	m.1	sp|Q9SVV9|	PF04059.7^	NOG28655	GO:0000166	CNPDHQVSWRPVFN	3.14E-	23.107	1.250 down	0.0219	5.579	1.357 down	0.0107	6.664	1.135 down	0.032	4.308	0.05559	0.10135	0.08534	0.10097	0.07721	0.03559
comp134993	comp	m.2	sp|Q9SVV9|	PF04059.7^	NOG28655	GO:0000166	LWGKKLIVRSGTQRT	4.77E-	48.612	1.737 down	0.013	6.956	1.485 down	0.0184	5.375	1.258 down	0.0155	5.562	0.12893	0.18931	0.11599	0.17808	0.07067	0.09619
comp78297_	comp	m.1	sp|Q8WYR4	PF02493.15	COG4642^	GO:0005829	MNEVKVASISKQLSF	0.0156	4.378	1.099 down	0.23	1.984	1.184 down	0.251	1.806	1.179 down	0.00539	8.344	0.10998	0.0832	0.08871	0.25504	0.02957	0.04895
comp77736_	comp	m.1	sp|Q8VIG3|	PF02493.15	COG4642^	GO:0005694	MADLTHLVQQAQHRT	0.00001	16.759	1.406 up	0.106	-2.907	1.539 up	0.0328	-4.335	1.668 up	0.0101	-6.518	0.23252	0.24589	0.16623	0.23396	0.18187	0.13535

Appendix F

Table 6.4 Annotations for *Symbiodinium* genes of interest identified in the analysis, including plastid-associated, antioxidant and meiosis-related genes. List adapted from previous *Symbiodinium* transcriptional studies (Chi et al., 2014; Mungpakdee et al., 2014; Krueger et al., 2015).

Gene	#component	trans_derive	prot_id	TopBlastHit	Pfam	SignalP	TmHMM	eggnog	gene_ontolo	prot_seq
NiSOD	comp37467_c0	comp37467	m.81797	.	PF09055.6^Sod_Ni^Nickel-containing	.	.	.	.	MRMLRFARALARPGAMAASGLAASYATQKNDRDERFLMRQASQVVQC
NiSOD	comp46363_c0	comp46363	m.95485	.	PF09055.6^Sod_Ni^Nickel-containing	.	.	.	.	NIQKESTLHLVLRLRGGHCQVPCGIFDDPKLVADVKEAVATIKKAMVQIGE
NiSOD	comp85582_c0	comp85582	m.168620	.	PF09055.6^Sod_Ni^Nickel-containing	.	.	.	.	LTGRLIFPRKGEMLSSIVRRLPVQATLRPAVRFHCQVPCGIFTDELRVQA
MnSOD	comp45584_c0	comp45584	m.94209	sp|Q59094|SODM_ACIAD`Q59094`Q:5-239,H:1-228`50.21%ID`E:1e-	PF00081.17^Sod_Fe_N^Iron/manganese	.	.	COG0605^	GO:004259	LGSSLLKPLKMLRATLSLSWLSLAFGETFTLPALPYEYDALEPYIDEQTMR
MnSOD	comp79931_c0	comp79931	m.162595	sp|Q59094|SODM_ACIAD`Q59094`Q:35-258,H:3-226`51.1%ID`E:2e-	PF00081.17^Sod_Fe_N^Iron/manganese	.	.	COG0605^	GO:004259	MAPRRFRGAATLVVGCLLVCCLERGFVSPTNATRRSLAAGFASGLAGVL
LFG4	comp44166_c0	comp44166	m.91625	sp|Q9DA39|LFG4_MOUSE`Q9DA39`Q:19-237,H:29-	PF01027.15^Bax1-I^Inhibitor of apoptosis-	.	.	COG0670^	GO:000013	MCDIESNSFGHFEGIGAEQVRLGFIRKVYGIVACQVSVTALAAALACGPL
LFG4	comp47216_c0	comp47216	m.96858	sp|Q9DA39|LFG4_MOUSE`Q9DA39`Q:135-288,H:91-	PF01027.15^Bax1-I^Inhibitor of apoptosis-	.	.	COG0670^	GO:000013	SRVLASWLNLAPALLTALCEDRRASHVRHRVQFLWTLRGHRSRAGAFGI
LFG4	comp61307_c0	comp61307	m.120410	sp|Q9DA39|LFG4_MOUSE`Q9DA39`Q:6-241,H:12-	PF01027.15^Bax1-I^Inhibitor of apoptosis-	.	.	COG0670^	GO:000013	MVSTMDIETTPNFGDLKGISDSQIRLGFIRKVYGIVCTQVVATAVFAALFC
psbA	comp80975_c0	comp80975	m.165615	sp|Q9MSC2|PSBA_HETRO`Q9MSC2`Q:13-342,H:19-	PF00124.14^Photo_RC^Photosynthetic reaction	.	.	.	GO:000953	MKNTSYYQLNLLGNVMGFVLSTTNRLYIGCFGILMFPLLTLATIAYITAFILA
psbD	comp40619_c0	comp40619	m.85736	sp|A0T0T0|PSBD_THAPS`A0T0T0`Q:18-357,H:12-	PF00124.14^Photo_RC^Photosynthetic reaction	.	.	.	GO:000953	MKLIYSTRFMASKAYLPGSLTLLDDWLKRDRFVFIGWSGLLLFPTAYLAA
psbB	comp40570_c0	comp40570	m.85662	sp|P49471|PSBB_ODOSI`P49471`Q:5-497,H:3-	PF00421.14^PSII^Photosystem II protein^5-	.	.	.	GO:000953	MSAVLPWFRVHIVVLNDPGRLISSHIMHTALVAGWSALMLLYELITIDPTDP
psaB	comp80362_c2	comp80362	m.163672	sp|Q9XQV2|PSAB_HETTR`Q9XQV2`Q:9-679,H:15-	PF00223.14^PsaA_PsaB^Photosystem I	.	.	.	GO:000953	KNVAKIISTDSFVSLNGRCATSRYLQVMGSIHDIESYFGIDNTLSLNLQIFT
PCP	comp80938_c0	comp80938	m.165472	sp|P51874|PCP_SYMSP`P51874`Q:1-201,H:1-202`80.2%ID`E:1e-	PF02429.10^PCP^Peridinin-chlorophyll A	.	.	.	GO:000950	MVRGARKAIAVGVAVAVACGLQKHLNFVPGPRHAAPVAAAAASMMMAP
psb31 lhcb	comp117553_c	comp11755	m.12065	sp|Q40300|FCPF_MACPY`Q40300`Q:25-118,H:7-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	GVVLLSAARSTRSRPLRSCLRAEKSPEIKGPKVKPPPKVEPPWEASQEL
	comp128884_c	comp12888	m.19116	sp|Q40301|FCPE_MACPY`Q40301`Q:37-198,H:23-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	RRGRVRRCAEAQEAQEAQAQEEVAMEVKDVELEEEEAPAKRKLRERR
	comp14596_c0	comp14596	m.28447	sp|Q40300|FCPF_MACPY`Q40300`Q:76-246,H:25-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	MTRRAAAGATSAAIAGMAAVSTAFVCPVARTSHTEGATALRGASSASSS
	comp16076_c0	comp16076	m.35325	sp|Q41094|FCPF_PHATR`Q41094`Q:205-347,H:34-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	NOG27499	GO:000953	AEAMLSESWVLVPTQAGVSSRTFLQVGSPSPKQPQVKADGARSSYKDL
	comp199727_c	comp19972	m.50743	sp|Q40301|FCPE_MACPY`Q40301`Q:67-152,H:3-88`45.35%ID`E:1e-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	RICPRFSSMATGRRVACGAAFTGAALVTAPSFVAPSSIPRVETSVQRSTA
	comp21083_c0	comp21083	m.54250	sp|Q40300|FCPF_MACPY`Q40300`Q:28-215,H:18-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	PLRASTQDVLFKGGAGSVDGGMGAGSFGGSKSTSTSSSSATVSSTDLG
	comp22058_c0	comp22058	m.57176	sp|Q9SQL2|CB24_PEA`Q9SQL2`Q:122-292,H:57-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	SGVSNKAARDLRLQSSFFNSRLGMTGRRVATSAAVTAAATTLAACANIQA
	comp23903_c0	comp23903	m.61750	sp|P27525|CB4B_SOLLC`P27525`Q:140-293,H:73-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	VAPWGSAGPAVAQSRPTRVCADVPTARCLPPARAVPAWKTSRSMQCSL
	comp26316_c0	comp26316	m.66889	sp|Q40300|FCPF_MACPY`Q40300`Q:144-298,H:23-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	YQIIFFSIFLRTMGRVKGAAVLAVGGGLCLAPSFVPLLSSSSEVRAGGHV
	comp33725_c0	comp33725	m.77287	.	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	.	FSYFNKPRSLEAMLLLIGSQPKHGAMRPIGNGQVRPQAHQVSWANPGP
	comp43779_c0	comp43779	m.90999	.	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	.	LQILLVAGLIETQLFKDQSFGGFGYAKYGEPGNFGTGYWGRKIQDPAERK
	comp45013_c0	comp45013	m.93113	sp|Q40301|FCPE_MACPY`Q40301`Q:3-173,H:35-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	LRAFENELGVQAPVGYFDPMGLSKDGDVETFRRRREAELKNGRVAMFA
	comp47390_c0	comp47390	m.97178	sp|Q41093|FCPE_PHATR`Q41093`Q:1-158,H:33-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	NOG27499	GO:000953	FEGELGVQPPVGFWDPLGLSSDGDVEVFKRRREVELKHGRISMYACLR
	comp59287_c0	comp59287	m.116367	sp|Q40300|FCPF_MACPY`Q40300`Q:114-281,H:26-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	GPVAAVHAARRVCWARPQAVPFSMAETFVSFGQAPTPSSVRAPHSGVQ
	comp60604_c0	comp60604	m.118907	sp|Q40296|FCPB_MACPY`Q40296`Q:106-213,H:44-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	AAAPFNKMAVTRCLLLAGMAFAVVGSCFMLPLGGRHTTRTSLRARGGE
	comp60876_c0	comp60876	m.119487	sp|Q03965|L181_CHLMO`Q03965`Q:135-301,H:54-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	FFVAERIETQNSSSSGSRATRRHGPFEAMWKSRCIRCRLQVVIIVVFMVF
	comp613_c0	comp613_c	m.120606	sp|Q40301|FCPE_MACPY`Q40301`Q:2-103,H:26-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	YADSPLRAEADFSKELGVTPPLGFWDPLGFSKFENTELAKLQFKRRRIVE
	comp63379_c0	comp63379	m.124355	sp|Q40300|FCPF_MACPY`Q40300`Q:101-246,H:33-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	MVQTGKCVALAAGSAATMALMGAPAFTSAPNTVSESHVPVRTYGAAQS
	comp74009_c0	comp74009	m.147263	sp|Q40296|FCPB_MACPY`Q40296`Q:29-159,H:25-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	LRGRSAGQASTGSSMLSTVGVAALGVTVAALRRDANAARKVERCAFEN
	comp74245_c0	comp74245	m.147706	sp|Q40301|FCPE_MACPY`Q40301`Q:277-441,H:35-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	QEIGSGLIFVTVEGILFILSFLFVPMAKSMSLLAAGAAGAYLCSEAFVPGV
	comp78268_c0	comp78268	m.157967	sp|Q40300|FCPF_MACPY`Q40300`Q:89-274,H:9-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	SRGVVSVALSPQGDPCGESPHLRLRFRNSILYILFKMASSRVSAASAAAG
	comp79755_c0	comp79755	m.162089	sp|Q40297|FCPA_MACPY`Q40297`Q:122-282,H:28-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	MAPRNTMLAAAGLAAGGYVATQAFVPSASGPAVSTEAVANLRGASAAPS
	comp79905_c0	comp79905	m.162506	sp|Q40296|FCPB_MACPY`Q40296`Q:91-233,H:40-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	MASTSRVSALAAGATGAALFTGATFVSAPSSTPKLRANSAVASSASKPLA
	comp79910_c0	comp79910	m.162529	sp|Q40296|FCPB_MACPY`Q40296`Q:127-221,H:44-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	LAQAFWPQNRPSVCPIFSRGSSPVSVAIVSIMTKASKTMAVAAAATAATM
	comp81566_c0	comp81566	m.165947	.	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	.	DILGWSDILIEDEKSIWNQFRKAENQELNNGRLAMMGITGLIAQDVLFGD
	comp84119_c0	comp84119	m.167594	sp|Q40300|FCPF_MACPY`Q40300`Q:77-262,H:27-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	ARLAVGAVGLAAPVFVFPGAAPRPSQLRGNSAVQERAPQGGSTCSTLG
	comp88341_c0	comp88341	m.170591	sp|Q40301|FCPE_MACPY`Q40301`Q:60-228,H:31-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	MSLTAPWIFHGPTAVLPARRAVQSAASVRSFPSPCLGVTVAVCASVLPAR
	comp93457_c0	comp93457	m.174452	sp|Q03965|L181_CHLMO`Q03965`Q:21-97,H:139-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	RFPGDKFQAVANSFEAHDKMVEAGYMLPFLGAIGTFELYSLWLLFKGWE
	comp93797_c0	comp93797	m.174678	sp|Q40296|FCPB_MACPY`Q40296`Q:104-205,H:44-	PF00504.16^Chloroa_b-bind^Chlorophyll A-B	.	.	.	GO:000953	FGSRTPSGSKAFSDLTAMPSMSKLSVAVGAVGAGLATTAFVAPAAKSTST
PM34	comp63751_c0	comp63751	m.125076	sp|O43808|PM34_HUMAN`O43808`Q:27-330,H:6-291`28.3%ID`E:6e-	PF00153.22^Mito_carr^Mitochondrial carrier	.	.	NOG25862	GO:000577	RAAEPAIQPCEFHKQFPRMHIDQESAAFIALVQASAGAAGAIVASAALQP
PM34	comp109038_c	comp10903	m.6328	sp|O43808|PM34_HUMAN`O43808`Q:4-284,H:3-291`35%ID`E:1e-	PF00153.22^Mito_carr^Mitochondrial carrier	.	.	NOG25862	GO:000577	ALFSVCSFEHAVSGSTGTVLATLLLFPLERLKTLLQVDPEAYHGLLDVLRR
PM34	comp64032_c0	comp64032	m.125661	sp|O43808|PM34_HUMAN`O43808`Q:16-271,H:11-	PF00153.22^Mito_carr^Mitochondrial carrier	.	.	NOG25862	GO:000577	LTTVSAQSVAMPSPALSGLAGLIASMLSVVLLYPLDQAGVVSQASGEHAN
PNC1	comp76187_c0	comp76187	m.152523	sp|B6ZJZ9|PNC1_SOYBN`B6ZJZ9`Q:13-290,H:10-	PF00153.22^Mito_carr^Mitochondrial carrier	.	.	.	GO:001602	MANISVWEKVWQEVRSGLVSSVGAEAALFPLDTIKLQQQVYGGTAASVL
PNC2	comp102587_c	comp10258	m.1751	sp|Q8VZS0|PNC2_ARATH`Q8VZS0`Q:15-347,H:3-	PF00153.22^Mito_carr^Mitochondrial carrier	.	.	.	GO:001602	GFPWKTQSSQTFRAMDASIEGFVKALGGGVGALFSTTVLYPLEVTKTKV
ABCD3	comp183207_c	comp18320	m.44547	sp|P28288|ABCD3_HUMAN`P28288`Q:83-348,H:63-	PF06472.10^ABC_membrane_2^ABC	.	.	COG4178^	GO:000582	VSELHGWGVLVNSLEKVPWKRLVFTMVGTLLVLPVVMMVVVSIVLCREA
ABCD3	comp6552_c0	comp6552	m.128496	sp|P55096|ABCD3_MOUSE`P55096`Q:1-75,H:580-652`44%ID`E:4e-		.	.	COG4178^	GO:001602	AFARLFYHNPKFVVLDECTNGISPDVEHAIYNRCTTLGMAIFSISHKIELKE
ChlD	comp84001_c0	comp84001	m.167517	sp|O07345|CHLD_SYNE7`O07345`Q:88-784,H:11-	PF01078.16^Mg_chelatase^Magnesium	.	.	COG1239^	GO:000552	MEPSSWIPVRHLQPSDVHTTGSRRTFKPPRVSGLSLGAGSLLAIPFAASK
psbC	comp20334_c0	comp20334	m.51612	sp|P49472|PSBC_ODOSI`P49472`Q:2-452,H:16-	PF00421.14^PSII^Photosystem II protein^17-	.	.	.	GO:000953	MPLLSSLKRRTLVGSRYAWWSGNARFIELSGKFLGAHLAHAALILVWAGA
psbE										
psbf	comp21180_c0	comp21180	m.54555	sp|Q1XD97|PSBF_PORYE`Q1XD97`Q:107-149,H:2-	PF00283.14^Cytochrom_B559^Cytochrome	.	.	.	GO:000953	FGSSDFSSCTPSACGDSTSHSEAMASYNVILALFCAGCVSMAFVAPAPS
	comp41176_c0	comp41176	m.86632	sp|Q1XD97|PSBF_PORYE`Q1XD97`Q:87-128,H:2-	PF00283.14^Cytochrom_B559^Cytochrome	.	.	.	GO:000953	MALREWRAFSHVVSFLALCSSVVAFVPPVHTSTKYQAAVSVPGAAEMPE
	comp42202_c0	comp42202	m.88296	sp|Q06J12|PSBF_BIGNA`Q06J12`Q:121-158,H:14-	PF00283.14^Cytochrom_B559^Cytochrome	.	.	.	GO:000953	PRPRQPFWLKNCRHCAAWELSRSWIRRLASSPAMRVLLFLASALTACAF
	comp65892_c1	comp65892	m.129167	sp|Q1XD97|PSBF_PORYE`Q1XD97`Q:116-158,H:2-	PF00283.14^Cytochrom_B559^Cytochrome	.	.	.	GO:000953	MSCLLIFGVLGSSDFSSCTPSACGDSTSHSEAMASYNVILALFCAGCVS
	comp85409_c0	comp85409	m.168500	sp|Q06J12|PSBF_BIGNA`Q06J12`Q:117-144,H:14-	PF00283.14^Cytochrom_B559^Cytochrome	.	.	.	GO:000953	GKHDFLIYFPQSGLKTRSQALWSFLDVAMLRVLLFTSLCSAAVAFVAPAS
	comp90316_c0	comp90316	m.172079	sp|Q1XD97|PSBF_PORYE`Q1XD97`Q:81-123,H:2-	PF00283.14^Cytochrom_B559^Cytochrome	.	.	.	GO:000953	MAMRSVLSLAVFVLPVVAFVAPANSRPEMTREVRVTLSPEPVVEASSSW

psbH	comp25167 c0	comp25167	m.64084	.	PF00737.15^PsbH^Photosystem II 10 kDa	.	.	.	.		MSPKFNILNLLLFGLLACVLRSGINFVNGPVTQRNVSVSAAAQGYFPVGN
	comp79708 c0	comp79708	m.161946	.	PF00737.15^PsbH^Photosystem II 10 kDa	.	.	.	.		AIAGRADGSRPVSFFQIIHRAIPSALPAMARQSSVMIYGLATCLCLWALSS
	comp79713_c0	comp79713	m.161958	.	PF00737.15^PsbH^Photosystem II 10 kDa	.	.	.	.		SSHFCQSRHNAPQNSRDLSLEMASRSSCVLILAACLCLWALSSQMSFVN
psbJ	comp74163 c0	comp74163	m.147531	sp|Q1KVW7|PSBJ SCEOB`Q1KVW7`Q:90-127,H:5-	PF01788.12^PsbJ^PsbJ^94-127^E:6.1e-13	.	.	.	GO:000953		RCSLCSGSRTQTAAPFSRPEGELQLMARSSNLLVLAVVACATYMLLPSE
psbK	comp42379_c0	comp42379	m.88608	sp|P49513|PSBK_ODOSI`P49513`Q:69-102,H:7-40`79.41%ID`E:3e-	PF02533.10^PsbK^Photosystem II 4 kDa	.	.	.	GO:000950		DLRAQAAPFERQPLKMARSSGLLVLVAALAALCMLQSAFVPSSKPQVSN
	comp79724_c1	comp79724	m.162006	sp|P49513|PSBK_ODOSI`P49513`Q:73-110,H:7-44`78.95%ID`E:2e-	PF02533.10^PsbK^Photosystem II 4 kDa	.	.	.	GO:000950		KKAQSSFSCCSARRGFEAMARSSGLLVLLAAVAALCMLQSAFVGPSSKP
psbL	comp79045 c0	comp79045	m.160105	sp|Q4G382|PSBL EMIHU`Q4G382`Q:66-102,H:4-38`75.68%ID`E:6e-	PF02419.12^PsbL^PsbL protein^67-	.	.	.	GO:000953		MARRPTLFVLAAAACVWLFSSSISSPEQEVFVVQTPALRGQGAFTGNTV
psbM	-										
psbN	comp81722_c0	comp81722	m.166026	sp|A0T0P7|PSBN_THAPS`A0T0P7`Q:101-136,H:8-	PF02468.10^PsbN^Photosystem II reaction	.	.	.	GO:000953		FGSSCPLAHAQRIPFFLRSIAKTMALRSRLLPFVALLAALCCIFKATAFTGA
psbO	comp80077_c0	comp80077	m.162992	sp|P85194|PSBO_HELAN`P85194`Q:51-323,H:51-	PF01716.13^MSP^Manganese-stabilising	.	.	.	GO:000953		MARSGAVVGVLLAAAAFGSCFLAGVPTQPPRAPTTPNHVDSLGAAPTQ
	comp84020 c0	comp84020	m.167539	sp|P12853|PSBO CHLRE`P12853`Q:95-168,H:49-	PF01716.13^MSP^Manganese-stabilising	.	.	NOG05777	GO:000953		QGLQIFSPKSWRVFERPMARASIVGLTLALAAVGSCFLGGVPTQPPRAP
psbP	comp21047_c0	comp21047	m.54132	sp|P82538|PPL1_ARATH`P82538`Q:57-227,H:71-	PF01789.11^PsbP^PsbP^59-226^E:4.3e-22	.	.	NOG08775	GO:000950		SHSERRLRVRGPLQICAVLGLCLTALQQAVQLFLSPRQPEPIVQRRQLLA
	comp110020_c	comp11002	m.7109	.	PF01789.11^PsbP^PsbP^85-244^E:4.8e-14	.	.	.	.		PTGVASTMATVSAPMAMAPPARPARPARPALRLQRTLERPSPKVMLGAP
	comp144250 c	comp14425	m.27588	.	PF01789.11^PsbP^PsbP^229-307^E:1.2e-06	.	.	.	.		PAWKLDGAAMVPRTEAVRPKRRHGVLVIACCLPSLCFVPSLGKGRSEQR
	comp29552_c0	comp29552	m.72009	.	PF01789.11^PsbP^PsbP^11-61^E:8e-07	.	.	.	.		SREPQEISILGEPQASLERLLRESIAPEGSKKEVEAISAEKIQKGGNTYY
	comp30306_c0	comp30306	m.73108	.	PF01789.11^PsbP^PsbP^144-266^E:8.4e-06	.	.	.	.		SGNKSGNKSGNKSGNKWLYLVQRLFTDMGKAAESRRRDSTYWAVLAG
	comp98847_c0	comp98847	m.178170	.	PF01789.11^PsbP^PsbP^179-246^E:8e-06	.	.	.	.		VVRSLSRKMQARSARPARPLVGLCVAFLAARFCTVELELFVSPKAQPSE
psbQ	-										
psbR	-										
psbS	-										
psbTc	-										
psbTn	-										
psbU	comp23626 c0	comp23626	m.61017	sp|Q84XB6|PSBU PHATR`Q84XB6`Q:20-78,H:69-	PF06514.6^PsbU^Photosystem II 12 kDa	.	.	NOG14297	GO:000953		ALPPAMARAIGRSEERTEMKMVDINNASVTEYQQFKGLYPSGAAIISGNG
	comp78207 c0	comp78207	m.157812	sp|Q84XB6|PSBU PHATR`Q84XB6`Q:71-188,H:30-	PF06514.6^PsbU^Photosystem II 12 kDa	.	.	NOG14297	GO:000953		MARVLLAAALLAIPASAYVAPRAGAASPQSVQLPASQVQLEQLDLEEPS
psbw	-										
psbX	-										
psbY	comp79800 c0	comp79800	m.162200	.	PF06298.6^PsbY^Photosystem II protein Y	.	.	.	.		AKTHSAAPDASNAAIFTSASITDLKRRFLRSGMARSSSIVAPLLVLASVAL
	comp80056 c0	comp80056	m.162923	.	PF06298.6^PsbY^Photosystem II protein Y	.	.	.	.		SHFYISLHRGSQASLSLWRSVMARSSSIVAPLLVLASVALAVFSFGPDAF
	comp81492 c0	comp81492	m.165906	.	PF06298.6^PsbY^Photosystem II protein Y	.	.	.	.		LAQVFLVRFACITRCAAIVMAQRRSPVAVLALVVAAIFVGQQCSAFLPSP
psbZ	-										
psb27	-										
psb28	-										
psb29	-										
psb30/ycf	-										
psaA	comp20374 c0	comp20374	m.51644	sp|Q9XQV3|PSAA HETTR`Q9XQV3`Q:20-688,H:5-	PF00223.14^PsaA PsaB^Photosystem I	.	.	.	GO:000953		QEKSSHPHEQKILAKITNIFRYVNATLWSKAGHFNKALSKGAKTTTWIWN
psaC	comp81124 c0	comp81124	m.165720	sp|A0T0L2|PSAC PHATC`A0T0L2`Q:86-165,H:2-79`80%ID`E:5e-	PF12837.2^Fer4 6^4Fe-4S binding domain^88-	.	.	.	GO:000953		RRPRLAMSRSLMMVACAVALTARFAADAFVPLVGLGRAAPAPSTSFAA
	comp69267 c0	comp69267	m.136542	sp|A0T0L2|PSAC PHATC`A0T0L2`Q:108-187,H:2-79`80%ID`E:3e-	PF12837.2^Fer4 6^4Fe-4S binding	.	.	.	GO:000953		LAQVSVLSQALCPQTCGQSKPSNCPAMQQLLLCCAAAAAALVARSAAF
psaD	comp74159 c0	comp74159	m.147518	sp|P49481|PSAD ODOSI`P49481`Q:118-254,H:5-	PF02531.11^PsaD^PsaD^121-247^E:4.5e-47	.	.	.	GO:000953		TTRGAMTILAQAPGPSWHVHRVFHNIVMAPRVVGLAAAVLGVAAVTFVA
psaE	comp82042 c0	comp82042	m.166182	sp|P12352|PSAE CHLRE`P12352`Q:50-134,H:15-96`53.41%ID`E:5e-	PF02427.12^PSI PsaE^Photosystem I reaction	.	.	NOG08807	GO:000953		RSGRSGSEVFSLMARSRMGRLLALSLLAFGLGCLSCLNFVAPLRPSSTV
	comp82271 c0	comp82271	m.166319	sp|Q9ZFU3|PSAE MASLA`Q9ZFU3`Q:65-127,H:3-	PF02427.12^PSI PsaE^Photosystem I reaction	.	.	.	GO:000953		ILAQAQGFSENRPRHAVSTAMARVTRALALLVLGLFALLAAPSFTALRAE
	comp41173 c0	comp41173	m.86625	sp|P12352|PSAE CHLRE`P12352`Q:44-129,H:14-96`51.69%ID`E:2e-	PF02427.12^PSI PsaE^Photosystem I reaction	.	.	NOG08807	GO:000953		LKPLSTGPASLSKMAMRLRGLVLLAVAAALGLWNSTSFVGNGLPGRTSS
psaF	comp80131 c1	comp80131	m.163132	sp|P51193|PSAF PORPU`P51193`Q:134-280,H:29-	PF02507.10^PSI PsaF^Photosystem I reaction	.	.	.	GO:000953		SREGGTGLHRSNPAMARPGTLLCVAGVSLLAASLAFVGTPAGSHASSE
	comp79493 c0	comp79493	m.161374	sp|P51193|PSAF PORPU`P51193`Q:120-266,H:29-	PF02507.10^PSI PsaF^Photosystem I reaction	.	.	.	GO:000953		MARRVSLLALAAAAAAFAACCAFVGPSVGRSQGRQVELGRAATPQGAL
psaG	-										
psaH	-										
PsaI	-										
psaJ	comp79899 c0	comp79899	m.162488	.	PF01701.13^PSI PsaJ^Photosystem I reaction	.	.	.	.		MARLGGLLLLVAGALLGSHVSTLLFANAPTQSLRSQTATRAVDERDEGL
psaK	-										
psaL	comp78356 c0	comp78356	m.158196	sp|Q85FP8|PSAL CYAME`Q85FP8`Q:250-387,H:5-	PF02605.10^PsaL^Photosystem I reaction	.	.	.	GO:000953		LRSHFGSRSRTHSYLPQVGWLPPAMREFLNVSTTLTLAAIGACTLLLAG
	comp40074 c0	comp40074	m.84861	sp|Q85FP8|PSAL CYAME`Q85FP8`Q:247-342,H:5-	PF02605.10^PsaL^Photosystem I reaction	.	.	.	GO:000953		FGSRLKVTPLLLSGRVAAMRDFLNMSTCLMLAVLGACTILLTGDVMAFTT
psaM	-										
psaN	-										
psaP	-										
psaX	-										
lhca	-										
petA	comp80702 c0	comp80702	m.164707	sp|Q95AG0|CYF CHLSU`Q95AG0`Q:127-429,H:6-	PF01333.14^Apocytochr F C^Apocytochrome	.	.	.	GO:000953		QLRPRSARNVRRALCVAMALAPAFTVISPGATPTVTTLAQEASTVRRNV
petB	comp81072 c0	comp81072	m.165702	sp|Q9XQU7|CYB6 HETTR`Q9XQU7`Q:2-218,H:3-	PF00033.14^Cytochrom B N^Cytochrome b(N-	.	.	.	GO:000953		MWLYDWFEERLEIQCIADDILGKLVPPHVNIFYCFGGVVSLLFLFQVISGL
	comp125686 c	comp12568	m.17209	.	PF00033.14^Cytochrom B N^Cytochrome b(N-	.	.	.	.		LLWWHKQTGIAMLVALIVRIFLRLRSGIPPRFPGHPIVQMIETQSLRLFYLF
	comp24726 c0	comp24726	m.63286	sp|P00175|CYB2 YEAST`P00175`Q:68-138,H:94-164`50.7%ID`E:5e-	PF00096.21^zf-C2H2^Zinc finger, C2H2	.	.	COG1304^	GO:000582		MAMANGTRAFLGCGYCTEAFPERNQLEVHIKMIHPGQPYRCIPAPLRHR
	comp76463 c0	comp76463	m.153177	sp|Q9V4N3|CYB5 DROME`Q9V4N3`Q:362-448,H:2-	PF00173.23^Cyt-b5^Cytochrome b5-like	.	.	COG5274^	GO:000578		MVPNDMHPSSIQDGKMHHEGEKFHSARGTGAISKNLPLSEVNRHCIPED
	comp79650 c2	comp79650	m.161771	sp|O99256|CYB PLACH`O99256`Q:1-338,H:9-347`48.53%ID`E:7e-	PF13631.1^Cytochrom B N 2^Cytochrome	.	.	.	GO:001602		MKSHLQSYPCPLIINYFWNLGFLLGITILLQIISGIFLGLHYTSDINSAYFSIF
	comp81481 c0	comp81481	m.165902	.	PF00033.14^Cytochrom B N^Cytochrome b(N-	.	.	.	.		LWWHKQTGVAMLIAIVMRIFFRMRSGIPPRFPGNKAVQFIETLSLRAFYG
	comp82272 c0	comp82272	m.166320	.	PF01292.15^Ni hydr CYTB^Prokaryotic	.	.	.	.		FTYSRALRHLHLIMAVGIFGAVGTAQAAQHCEGQTKKQLLWWHHKQTGIA
petC	comp132892 c	comp13289	m.21425	sp|P83794|UCRI MASLA`P83794`Q:88-255,H:10-	PF08802.5^CytB6-F Fe-S^Cytochrome B6-F	.	.	.	.		SIFAWPSHHHVESRFGPKSQTLQASEPKARLMVPLRQHLSMKSAQGFC
	comp78558 c0	comp78558	m.158754	sp|Q69S39|UCRIA ORYSJ`Q69S39`Q:7-225,H:6-	PF08802.5^CytB6-F Fe-S^Cytochrome B6-F	.	.	COG0723^	GO:000994		KEQPRNVATMTTKTRMLRGVAVLAAGMLATCAFVGPQVPVSRGELSTM
	comp57371 c0	comp57371	m.112912	sp|Q7XYM4|UCRIA BIGNA`Q7XYM4`Q:98-216,H:134-	PF00355.21^Rieske^Rieske [2Fe-2S]	.	.	.	GO:000953		MAASAKSSLLPWLCLALVCFLRPWSGFVSPLRVDPTERRQLHLRAQRE
	comp72033 c0	comp72033	m.142537	.	PF08802.5^CytB6-F Fe-S^Cytochrome B6-F	.	.	.	.		LKRQIHRADGTFTFFRPCVPAMAKSTREMRGLALLVTLAICGLSYTFVAP
	comp102929 c	comp10292	m.2006	.	PF00355.21^Rieske^Rieske [2Fe-2S]	.	.	.	.		LAQVQYCVFSLDLGTATEIASMAASKQRSQKLFPLLLLLCMVAAWKQSS
	comp112633 c	comp11263	m.8783	sp|Q9FYC2|PAO ARATH`Q9FYC2`Q:23-282,H:80-	PF00355.21^Rieske^Rieske [2Fe-2S]	.	.	COG4638^	GO:000970		RRKAVVTGVERKSKVARAPKPKASFNWEKQWYPVLPLSMLEGSGPEPI
	comp120241 c	comp12024	m.13709	.	PF00355.21^Rieske^Rieske [2Fe-2S]	.	.	.	.		MAAMAAMAPILDPDPSYCWVPTEIQRCSDLVDGQKWIESFEAMLKDES
	comp12130 c0	comp12130	m.14409	sp|Q9FYC2|PAO ARATH`Q9FYC2`Q:86-512,H:88-	PF00355.21^Rieske^Rieske [2Fe-2S]	.	.	COG4638^	GO:000970		SSLAMIGPSWANPRGSDPRWVRIHGEPPRCRILPGPGSHSTLRFAVAPL
	comp125879 c	comp12587	m.17325	sp|Q8W496|PTC52 ARATH`Q8W496`Q:94-536,H:67-	PF00355.21^Rieske^Rieske [2Fe-2S]	.	.	COG4638^	GO:000994		KDKKEEIKEEKEGKEVKEGSAEKEDAPGEKKDSDDDAEAEKEAKARKA
	comp13143 c0	comp13143	m.20636	sp|Q6N063|OGFD2 HUMAN`Q6N063`Q:21-308,H:53-	PF00355.21^Rieske^Rieske [2Fe-2S]	.	.	NOG24819	GO:000550		VALGCNVDDVAMASLPDETGAGRDFDALFAELRSDLGEDNFRNFLVALS
	comp13848 c0	comp13848	m.24559	sp|Q3ED68|Y1295 ARATH`Q3ED68`Q:126-343,H:141-	PF13806.1^Rieske 2^Rieske-like [2Fe-2S]	.	.	NOG24819	GO:000550		NHTKTRKTRMVDYSKWDKLQISDEEDAKPNHTKTRKTRMVDYSKWDKL
	comp169835 c	comp16983	m.39195	sp|Q8TAC1|RFESD HUMAN`Q8TAC1`Q:5-132,H:26-	PF00355.21^Rieske^Rieske [2Fe-2S]	.	.	NOG45762	GO:005153		DSQALAAKERLHLQVEGRYVSVLRGKAGALHCLDSICYHTGGPLTIGDIE
	comp198078 c	comp19807	m.50120	sp|Q8TAC1|RFESD HUMAN`Q8TAC1`Q:48-101,H:41-	PF00355.21^Rieske^Rieske [2Fe-2S]	.	.	NOG45762	GO:005153		MSCQSQAAMADTVDIESELWHLALSAQELASLPSEPSMEVRKVAGHCIL
	comp26951 c0	comp26951	m.67974	sp|Q9FYC2|PAO ARATH`Q9FYC2`Q:5-465,H:79-	PF00355.21^Rieske^Rieske [2Fe-2S]	.	.	COG4638^	GO:000970		AAPAGSAEAWRERHWFPIASTLELDPKRPTPVRLDGLDLVVWQVPGEE
	comp27790 c0	comp27790	m.69074	sp|Q9ZWM5|CAO CHLRE`Q9ZWM5`Q:12-352,H:281-	PF00355.21^Rieske^Rieske [2Fe-2S]	.	.	COG4638^	GO:000970		MADASEPLLPAAKGAKDVSGGLLHSRLPLDVRKQWYAIALSDDLPKAKA
	comp342184 c	comp34218	m.77937	sp|P22944|NIR EMENI`P22944`Q:25-129,H:899-	PF13806.1^Rieske 2^Rieske-like [2Fe-2S]	.	.	COG1251^	GO:005153		NNFANKDVSKKAKNKETKSLQTKGIEFVDEREQKRPADWPKHQQFYPK
	comp34856 c0	comp34856	m.78696	sp|Q17938|DAF36 CAEEL`Q17938`Q:66-459,H:15-	PF00355.21^Rieske^Rieske [2Fe-2S]	.	.	.	.		SLVFFQRHPGAMDPEWLRSCWPWMGSSALGVCGLLRLMEPSKTETSE
	comp4184 c0	comp4184	m.87761	sp|Q8TAC1|RFESD HUMAN`Q8TAC1`Q:26-93,H:26-	PF13806.1^Rieske 2^Rieske-like [2Fe-2S]	.	.	NOG45762	GO:005153		MAEAVTRASASDAAGDWVRVADSQALAAKERLHLQVEGRYVSVLRGKA

	comp41915 c0	comp41915	m.87899	.	PF00355.21^Rieske^Rieske [2Fe-2S]	.	.	.	.	.	.	NDVLIGKTEAGALFCVGNLCPHIGTPMSEGADVIGDVIVCPLHGSSFKVT
	comp53395 c0	comp53395	m.106589	sp|Q17938|DAF36 CAEEL`Q17938`Q:73-161,H:58-	PF00355.21^Rieske^Rieske [2Fe-2S]	.	.	.	.	.	.	VFLGVTLNHHGFFVPMAPRADDVQTVASGFLTAGVSTLCDSKLGRYAIG
	comp70975 c0	comp70975	m.140143	.	PF00355.21^Rieske^Rieske [2Fe-2S]	.	.	.	.	.	.	MQRMHHWTLPAAVCGAAGLATWALLRRRVAGWQLVGRRLNLEPGPC
	comp71736 c0	comp71736	m.141864	sp|Q17938|DAF36 CAEEL`Q17938`Q:3-361,H:63-	PF00355.21^Rieske^Rieske [2Fe-2S]	.	.	.	.	.	.	SSRFKALRQKHFPPGFPNTWHAVCNAADLTDGQVKSISALGTELVAFRG
	comp77370 c1	comp77370	m.155610	sp|P51133|UCRI3 TOBAC`P51133`Q:150-356,H:56-	PF00355.21^Rieske^Rieske [2Fe-2S]	.	.	.	.	.	GO:001602	MLPRLLARSAPVLRVRPHVAVLRLQTRCFAHPTNTHLMRDNDEMAPSS
	comp86663 c0	comp86663	m.169389	sp|Q96NN9|AIFM3 HUMAN`Q96NN9`Q:83-611,H:75-	PF00355.21^Rieske^Rieske [2Fe-2S]	.	.	.	.	COG0446^	GO:000578	SAMSAWSQCRRWLSRGFARTAPARTRPRASWNGASAVDVPVVERWD
	comp96857 c0	comp96857	m.176821	sp|P0ABR7|YEAW ECOLI`P0ABR7`Q:71-276,H:19-	PF00355.21^Rieske^Rieske [2Fe-2S]	.	.	.	.	COG4638^	GO:005153	FHGGLLGYSRCLQDLCVAMNIARRAMMQVHRRALWHACRIPRTLSTIPG
petD	comp41018 c0	comp41018	m.86361	sp|O78415|PETD GUITH`O78415`Q:2-145,H:14-157`53.47%ID`E:2e-	PF00032.12^Cytochrom B C^Cytochrome b(C-	.	.	.	.	.	GO:000953	MLLAKLSSGMGHNSYGEPAWPNDIAYIFPVLIYGLGVLILGLSVACPLEIV
petG	comp22582 c0	comp22582	m.58517	sp|A6YG69|PETG LEPTE`A6YG69`Q:83-113,H:2-32`67.74%ID`E:5e-	PF02529.10^PetG^Cytochrome B6-F complex	.	.	.	.	.	GO:000953	LENHLGSTAVVPMARSRAALVILAIMACVAMNAVSFVAPPKAEVPVSALA
	comp71571 c0	comp71571	m.141487	sp|A6YG69|PETG LEPTE`A6YG69`Q:95-125,H:2-32`67.74%ID`E:6e-	PF02529.10^PetG^Cytochrome B6-F complex	.	.	.	.	.	GO:000953	SSFDQIKQRQFRLDSTGLFFEAMAMRSRTILPALACLLACVFFGNAFVSA
	comp89231 c0	comp89231	m.171277	sp|A6YG69|PETG LEPTE`A6YG69`Q:83-114,H:2-33`65.62%ID`E:3e-	PF02529.10^PetG^Cytochrome B6-F complex	.	.	.	.	.	GO:000953	SQLPGQKNCALIGPPSGAMARSRTILAPLVTLACLASLSWTFIAPRPAPEI
petL	-											
petM	-											
petN	comp20460 c0	comp20460	m.52005	.	PF03742.9^PetN^PetN^85-105^E:6.1e-09	.	.	.	.	.	.	SSFASAPVFFSTPLTPAMAPARRSPLAFGFLAFAMLAAPAFLGPLVQAPL
	comp21232 c0	comp21232	m.54683	.	PF03742.9^PetN^PetN^98-118^E:7.2e-09	.	.	.	.	.	.	KSSRWFIVPLTVRQITPVTIRRKDMAAARRSPLAVLLCVAASCYILCSLSL
	comp81143 c0	comp81143	m.165737	.	PF03742.9^PetN^PetN^91-111^E:6.6e-09	.	.	.	.	.	.	WLKPIFADRVLRDQAVPVTMTRRSPLALVLLATLALAAWPAFLEPIGRAV
petO	-											
ccsA	comp78886 c0	comp78886	m.159631	sp|Q9XIA4|CCS1 ARATH`Q9XIA4`Q:143-600,H:114-	PF05140.9^ResB^ResB-like family^144-	.	.	.	.	COG1333^	GO:000953	IVLGIVFDAISMASMMPLQLRPLGIRARDTRAQQVGAPMPASVVASGGS
petJ	comp122441 c	comp12244	m.15120	sp|Q85FS2|CYC6 CYAME`Q85FS2`Q:64-148,H:20-	PF13442.1^Cytochrome CBB3^Cytochrome C	.	.	.	.	.	GO:000954	LGRSSGMRAPSQNPAHPELRDAYRVEDLESREATDHWWLPFVAGVVV
	comp132570 c	comp13257	m.21267	sp|P00072|CYC FAGES`P00072`Q:13-122,H:8-109`35.14%ID`E:4e-	PF13442.1^Cytochrome CBB3^Cytochrome C	.	.	.	.	.	GO:000575	PPRPPPELVAEIPFNVRRGQAIFKKYCSQCHTFKTEGRGTIRGPNLFGVV
	comp137065 c	comp13706	m.23766	sp|P00072|CYC FAGES`P00072`Q:30-139,H:8-109`35.14%ID`E:7e-	PF13442.1^Cytochrome CBB3^Cytochrome C	.	.	.	.	.	GO:000575	WISGCFLDGMAATPPPPPRPPPELVAEIPFNVRRGQAIFKKYCSQCHT
	comp198494 c	comp19849	m.50300	sp|P00040|CYC SCHGR`P00040`Q:2-102,H:7-107`74.26%ID`E:2e-	PF00034.16^Cytochrom C^Cytochrome c^3-	.	.	.	.	.	GO:000575	MSAEKGAKIFKTKCSQCHTVEAGGAHKQGPNLHGLWGRQSGQADGFN
	comp37975 c0	comp37975	m.82436	sp|P07497|CYC6 SYNP6`P07497`Q:209-279,H:23-	PF13442.1^Cytochrome CBB3^Cytochrome C	.	.	.	.	COG2010^	GO:003197	DGFTAVMAVMAPLFALALIWPVAAENCVDSPEVGLLQTQVGLPQGLSSL
	comp57191 c0	comp57191	m.112603	.	PF13442.1^Cytochrome CBB3^Cytochrome C	.	.	.	.	.	.	MAQPGARIMPQGRRWLRPAPAALLTLALCSQVAWVLPNLAPPFLSLRS
	comp68226 c0	comp68226	m.134193	sp|P07497|CYC6 SYNP6`P07497`Q:143-241,H:29-	PF13442.1^Cytochrome CBB3^Cytochrome C	.	.	.	.	COG2010^	GO:003197	MVKDLRRRVAYRAGLILLVAAALPHVFCGPFSEISRADRQRNNARLPPLE
	comp79476 c0	comp79476	m.161337	sp|Q3MDW2|CYC6 ANAVT`Q3MDW2`Q:55-161,H:5-	PF13442.1^Cytochrome CBB3^Cytochrome C	.	.	.	.	COG2010^	GO:003197	MSRVASIVCAVSLVLYGASCFILPASTPRGAPQQGAGAVAQPSASSVAP
	comp81080 c0	comp81080	m.165707	sp|Q4N594|CYC THEPA`Q4N594`Q:1-112,H:1-111`71.43%ID`E:2e-	PF13442.1^Cytochrome CBB3^Cytochrome C	.	.	.	.	COG3474^	GO:000575	MPVPEPDVEVPSGDTKKGAKLFKAKCAQCHTIEQGGNTKQGPPLWGLI
	comp97922 c0	comp97922	m.177543	sp|P00110|CYC6 BUMFI`P00110`Q:104-188,H:1-85`71.76%ID`E:2e-	PF13442.1^Cytochrome CBB3^Cytochrome C	.	.	.	.	.	GO:000954	FGLPLALSPAQLPMSRLLRILLVAVAYQGHAFVAVQEESTVPVLRRPELG
	comp102660 c	comp10266	m.1784	.	PF00111.22^Fer2^2Fe-2S iron-sulfur cluster	.	.	.	.	.	.	APAGLARVKTGPQMVRRKVPRWAPDGGGGVDLMVADKGIGGELDTLT
	comp130602 c	comp13060	m.20143	sp|Q54FB7|XDH DICDI`Q54FB7`Q:25-645,H:16-695`30.21%ID`E:9e-	PF00111.22^Fer2^2Fe-2S iron-sulfur cluster	.	.	.	.	COG4630^	GO:000577	SNFGTNDQQLGQTVKIAMTFAPPDRKDPLQFFLNGELVRVYDPDPRQM
	comp143851 c	comp14385	m.27370	sp|P10770|FER PERBI`P10770`Q:40-132,H:1-93`53.76%ID`E:5e-	PF00111.22^Fer2^2Fe-2S iron-sulfur cluster	.	.	.	.	.	GO:000950	ARLCRPMLLLWTTYGLTFLWTGVSRPGAVRRKWRQVGRQYKVTLESEE
	comp20516 c0	comp20516	m.52184	sp|P42577|FRIS LYMST`P42577`Q:165-326,H:7-168`53.09%ID`E:8e-	PF00111.22^Fer2^2Fe-2S iron-sulfur cluster	.	.	.	.	.	GO:000573	MSRQVRRAGLLGVTLLAASRCFVSPLHRAAPGRRDVARRYKVTLETPS
	comp209634 c	comp20963	m.53853	sp|Q12553|XDH EMENI`Q12553`Q:15-138,H:33-157`56.35%ID`E:5e-	PF00111.22^Fer2^2Fe-2S iron-sulfur cluster	.	.	.	.	COG4630^	GO:000577	PAERAGGLKVGEVPWRTQLSFYVNGRPVKVQDAEPHHTLLWFLRERLG
	comp251561 c	comp25156	m.64053	sp|Q12553|XDH EMENI`Q12553`Q:17-118,H:34-131`43.14%ID`E:1e-	PF00111.22^Fer2^2Fe-2S iron-sulfur cluster	.	.	.	.	COG4630^	GO:000577	APQLPQPLKERFGAPEEASLRFWVNGEEKVVTSGQFPVTTSLAEYLRYH
	comp35855 c0	comp35855	m.79879	sp|Q92J08|FER2 RICCN`Q92J08`Q:122-239,H:3-	PF00111.22^Fer2^2Fe-2S iron-sulfur cluster	.	.	.	.	COG0633^	GO:005153	MQLTFFRTLPWRLVGSRAFVSAQRSGRLLRCSPAIRFATSGPPRGPYQ
	comp36257 c0	comp36257	m.80419	sp|Q12184|ADRX YEAST`Q12184`Q:47-159,H:56-	PF00111.22^Fer2^2Fe-2S iron-sulfur cluster	.	.	.	.	COG0633^	GO:000575	SSCLGHGAPRATFRDRVCEAMLRAARLLARPALGRLHAVQSMQSMLRP
	comp41979 c0	comp41979	m.87975	sp|Q8W3D9|PORB ORYSJ`Q8W3D9`Q:78-414,H:85-	PF00106.20^adh short chain	.	.	.	.	COG1028^	GO:000994	SSIRGSNSEDFGPPGFGQVFHFQFQLKISIMFMLRCGLVLILQALLQPAH
	comp44869 c0	comp44869	m.92888	.	PF13180.1^PDZ 2^PDZ domain^134-	.	.	.	.	.	.	IRWRGAAVLAASSLCLSGLGTTWIGSRHTGVQNGDVRMVGLAASKKAAI
	comp47174 c0	comp47174	m.96796	sp|Q8LB02|DHSB2 ARATH`Q8LB02`Q:10-276,H:1-	PF13085.1^Fer2 3^2Fe-2S iron-sulfur cluster	.	.	.	.	COG0479^	GO:000574	KKLRFRAFRVTFAALASPGFTRASMALAALRSLAPRSLASRGFAAMAKP
	comp68408 c0	comp68408	m.134654	sp|P10770|FER PERBI`P10770`Q:90-178,H:1-89`88.76%ID`E:3e-	PF00111.22^Fer2^2Fe-2S iron-sulfur cluster	.	.	.	.	.	GO:000950	SRRSILKKRIWHPDTTRMASQSRVLPALLAAACWAALLRSFAWSDAETF
	comp71033 c0	comp71033	m.140268	sp|Q8LB02|DHSB2 ARATH`Q8LB02`Q:2-214,H:63-	PF13085.1^Fer2 3^2Fe-2S iron-sulfur cluster	.	.	.	.	COG0479^	GO:000574	LKPFLQEYNIDLKSCGPMILDALIKIKDEVDTTLTFRRSCREGICGSCAMNI
	comp73625 c0	comp73625	m.146302	sp|Q6GX84|FIGL1 RAT`Q6GX84`Q:234-498,H:393-	PF00111.22^Fer2^2Fe-2S iron-sulfur cluster	.	.	.	.	COG0464^	GO:000573	GAATPSSSSTAKCGEKVTVHFLDPNMDGLPRRMSVKAPVGSTLVDVAK
	comp79225 c1	comp79225	m.160632	sp|P10770|FER PERBI`P10770`Q:73-165,H:1-93`87.1%ID`E:3e-	PF00111.22^Fer2^2Fe-2S iron-sulfur cluster	.	.	.	.	.	GO:000950	MASQSRVLPVLVVAACCAALLRSFLAPSADTFVAPQQSVHLGCKALSKG
	comp86536 c0	comp86536	m.169299	sp|Q9AKH1|FER2 RICRI`Q9AKH1`Q:65-171,H:4-	PF00111.22^Fer2^2Fe-2S iron-sulfur cluster	.	.	.	.	.	GO:005153	RWVSGLLRQKISAVTAVHPMISALRRASLRQTGLLNSTTAKHVAFRGVQ
petH	comp42084 c0	comp42084	m.88118	sp|P41344|FENR1 ORYSJ`P41344`Q:1-138,H:178-	PF00175.16^NAD binding 1^Oxidoreductase	.	.	.	.	COG0369^	GO:004804	GVCSNHICDMSPGDDVLITGPTGAEMLLPEDPEANIIMLATGTGIAPMRS
	comp72819 c0	comp72819	m.144457	sp|Q00598|FENR CYAPA`Q00598`Q:122-436,H:75-	PF00175.16^NAD binding 1^Oxidoreductase	.	.	.	.	.	GO:003406	GRGLLLQREDQQRTVIQLQEGKPSSMSNKTDSSVKMVVAGAAAAIAAG
	comp78561 c0	comp78561	m.158770	sp|Q00598|FENR CYAPA`Q00598`Q:79-443,H:38-	PF00175.16^NAD binding 1^Oxidoreductase	.	.	.	.	.	GO:003406	IKRIMNWFGGTWLNLTFALCLAWKILEAMAQTVVGAALLGAAGTAFLASP
	comp81322 c0	comp81322	m.165824	sp|P41344|FENR1 ORYSJ`P41344`Q:14-131,H:172-	PF00175.16^NAD binding 1^Oxidoreductase	.	.	.	.	COG0369^	GO:004804	GEDKPDKAGTAFPENEVYRGVCSNHICDMSVGDDVLITGPTGAEMLLPT
	comp81660 c0	comp81660	m.165994	sp|P41344|FENR1 ORYSJ`P41344`Q:1-123,H:181-	PF00175.16^NAD binding 1^Oxidoreductase	.	.	.	.	COG0369^	GO:004804	SNHICDMSVGDDVLITGPTGAEMLLPTDPEANIIMLATGTGIAPMRSYLRL
	comp40081 c0	comp40081	m.84884	sp|Q00598|FENR CYAPA`Q00598`Q:71-247,H:44-	.	.	.	.	.	.	GO:003406	SAQACLLGALPSFASFVPMIGATVGSTAAGSGAGTTFVVPPPSGHARLL
	comp40829 c0	comp40829	m.86070	sp|Q00598|FENR CYAPA`Q00598`Q:6-204,H:141-	.	.	.	.	.	.	GO:003406	MEGGGLRLYPISSSRMGDDQRSKTLSLYVESQGAGFSNLKVGDKLNITG
	comp83803 c0	comp83803	m.167367	sp|Q8W493|FNRL2 ARATH`Q8W493`Q:10-106,H:85-	.	.	.	.	.	COG0369^	GO:004804	VVHGKEIPWNLFSPKAPYQGKVVANDVHPQTLTEPTGDANWETTHVTF
ftrC	comp105282 c	comp10528	m.3741	sp|O49856|FTRC SOYBN`O49856`Q:30-171,H:1-	PF02943.10^FeThRed B^Ferredoxin	.	.	.	.	.	GO:000950	GAGHAGHAPWPLQRCTVQMAPYRTGASTVVATLATLAATAVADRWVR
	comp200673 c	comp20067	m.51049	.	PF02943.10^FeThRed B^Ferredoxin	.	.	.	.	.	.	APVPGVSLAAALQMEGFWANARLQSMALEKQMLEMNKDRVKRAMDVM
trxY	comp15561 c0	comp15561	m.33053	sp|Q5JMR9|TRXY ORYSJ`Q5JMR9`Q:102-203,H:63-	PF00085.15^Thioredoxin^Thioredoxin^101-	.	.	.	.	COG0526^	GO:000957	PAPATEAVDAPPAEPAQAPQAVPEAETGSTGSTGSTSAAAGDFVAQCR
	comp86111 c0	comp86111	m.168977	sp|Q5JMR9|TRXY ORYSJ`Q5JMR9`Q:89-184,H:7-	PF00085.15^Thioredoxin^Thioredoxin^85-	.	.	.	.	COG0526^	GO:000957	FVHGISCNMPSMLRAVQSDPRSAKLILSHSGVRLWVVRFAVTLACAHLL
trxF	comp20192 c1	comp20192	m.51350	sp|O81332|TRXF MESCR`O81332`Q:232-342,H:78-	PF13833.1^EF-hand 8^EF-hand domain	.	.	.	.	.	GO:000950	MARLRVQGHGWLARAAMVSFLLAFINGLFSGLPFLTPRGARWLRNTIPT
atpA	comp71230 c0	comp71230	m.140750	sp|Q1ACM8|ATPA CHAVU`Q1ACM8`Q:17-461,H:26-	PF00006.20^ATP-synt ab^ATP synthase	.	.	.	.	.	GO:000953	MNPNPSPFPIILLVTIEKDILIYGVIISVGCGVVTLEGLLMAFISEVFTTQSSS
atpB	comp20436 c0	comp20436	m.51912	sp|Q1KVT0|ATPB SCEOB`Q1KVT0`Q:295-663,H:121-	PF00006.20^ATP-synt ab^ATP synthase	.	.	.	.	.	GO:000953	KLPTQRVKLYNHACVCSCVGPVVDVKGQSDLFYRERYMANILASGYKD
atpC	comp69905 c0	comp69905	m.137854	sp|Q06908|ATPG ODOSI`Q06908`Q:49-225,H:60-	PF00231.14^ATP-synt^ATP synthase^48-	.	.	.	.	.	GO:000953	KTQAMARGSWLSVLAVAALVSLSRWVQLDFVGQMQRPTTPKVVRCAG
	comp74056 c0	comp74056	m.147343	sp|B1XHY7|ATPG SYNP2`B1XHY7`Q:71-390,H:5-	PF00231.14^ATP-synt^ATP synthase^70-	.	.	.	.	COG0224^	GO:004526	ILAQGRPIKNRTETAHPAVHPLPNMARGVFGLALVAFGLLSLNRYWSAV
atpD	comp68660 c1	comp68660	m.135209	sp|P51243|ATPD PORPU`P51243`Q:81-232,H:14-	PF00213.13^OSCP^ATP synthase delta	.	.	.	.	.	GO:000953	EQETLCTLLIKGPTMARGRVIMACAALACTLVSMKSAFTPSLPGNMGMA
	comp87940 c0	comp87940	m.170258	sp|Q4G398|ATPD EMIHU`Q4G398`Q:1-62,H:97-158`38.71%ID`E:1e-	PF00213.13^OSCP^ATP synthase delta	.	.	.	.	.	GO:000953	YVQSMYYKQSITPIRVTSAQRLTGDQLQKIEQKMKGKVGTTDVKLVAEV
atpE	comp45056 c0	comp45056	m.93172	sp|Q1XDM7|ATPE PORYE`Q1XDM7`Q:88-218,H:3-	PF02823.11^ATP-synt DE N^ATP synthase,	.	.	.	.	.	GO:000953	LVLGIDRVPRPAVGTLDTSIFRNRKAMARSSPVVSLMLLGLVALCGQRFL
	comp73529 c0	comp73529	m.146097	sp|Q1XDM7|ATPE PORYE`Q1XDM7`Q:77-203,H:3-	PF02823.11^ATP-synt DE N^ATP synthase,	.	.	.	.	.	GO:000953	RTAKFPIRSPPRNAAKMARSPVLSFLLFGVLALLGKRSMETFVGVTSRPN
	comp87766 c0	comp87766	m.170127	sp|P51260|ATPE PORPU`P51260`Q:54-181,H:3-129`41.41%ID`E:1e-	PF02823.11^ATP-synt DE N^ATP synthase,	.	.	.	.	.	GO:000953	MSRLCGQKAGRRDRWFLRVLAVVFAATRWEECFAMASERPVGSEPGD
atpF	comp18404 c0	comp18404	m.44941	sp|A5GND0|ATPF SYNPW`A5GND0`Q:32-189,H:10-	PF00430.13^ATP-synt B^ATP synthase B/B'	.	.	.	.	COG0711^	GO:001602	AAAVAAATAVSAPAMAEEGGGLLDFGKVELGGGFALNLNIPDINLINISILI
	comp60659 c0	comp60659	m.119035	sp|A5GND0|ATPF SYNPW`A5GND0`Q:72-229,H:10-	PF00430.13^ATP-synt B^ATP synthase B/B'	.	.	.	.	COG0711^	GO:001602	MARRNILAVAFVAAVAAFALRWSSTAFLPGNQPMLRAGAMASAGALAA
	comp78743 c0	comp78743	m.159263	sp|Q1XDP2|ATPX PORYE`Q1XDP2`Q:58-154,H:14-	PF00430.13^ATP-synt B^ATP synthase B/B'	.	.	.	.	.	GO:000953	MARRSPLAFVLALLALLCAPAFLMPGKMGATVTEEKSQLLAAGGALMVA
atpG	-											
atpH	comp86788 c0	comp86788	m.169470	sp|A0T0E7|ATPH PHATC`A0T0E7`Q:62-113,H:5-56`63.46%ID`E:5e-	PF00137.16^ATP-synt C^ATP synthase	.	.	.	.	.	GO:000953	MAAPRSLLLTAAVLCSAGFCAFVAAPRPAAAAHPQVSAQTAAVSAMALV
atpI	comp71890 c0	comp71890	m.142189	sp|A6MVW9|ATPI RHDSA`A6MVW9`Q:121-302,H:62-	PF00119.15^ATP-synt A^ATP synthase A	.	.	.	.	.	GO:000953	QVEDTLDNQSRGCPLPKMLRSRSTLLRCAAGVALCVALQAALSFVAPAS
apcA	-											
apcB	-											
acpD	-											
acpE	-											
acpF	-											
cpcA	-											
cpcB	-											
	comp38227 c0											
	comp62547 c0	comp62547	m.122736	sp|P07125|CPCE NOSS1`P07125`Q:41-190,H:74-	PF13646.1^HEAT 2^HEAT repeats^27-	.	.	.	.	COG1413^	GO:003008	CPWASPKPAMVAADGIVGSPNVAGQIAELVQKLQSSRARDRWNAADGL
	comp27200 c0	comp27200	m.68321	sp|Q02178|MPEV SYNPY`Q02178`Q:249-459,H:62-	PF13646.1^HEAT 2^HEAT repeats^27-	.	.	.	.	.	GO:003008	TMKAAPKAAGYVEDPAEVAADQLCAWDANTRRFGLQSLGLLGPAAAEH
cfxQ	comp147899 c	comp14789	m.29425	sp|P51228|CFXQ PORPU`P51228`Q:45-229,H:31-	PF00004.24^AAA^ATPase family associated	.	.	.	.	.	GO:000950	SAALPSMFKQRYQVVMQRREDKHEIIMLDSGVPSRELLLGCEAEEMLAS

	comp34265 c0	comp34265	m.77992	sp|P51228|CFXQ PORPU`P51228`Q:1-130,H:85-	PF00004.24^AAA^ATPase family associated	.	.	.	GO:000950	TAARIYASILYHLGLIPNNRLVEVQRGDLVGQYIGLTEHKTKCKIREAMGG
rbcL	comp156844 c	comp15684	m.33545	sp|Q41406|RBL2 SYMSP`Q41406`Q:74-123,H:290-339`72%ID`E:5e-	PF00016.15^RuBisCO large^Ribulose	.	.	.	GO:000950	MNEVHPSAESQKGFLKRPAAPSSRQVRILSYPFPVQLSSASSLLVLGGC
	comp55807 c0	comp55807	m.110371	sp|Q42813|RBL2 LINPO`Q42813`Q:65-534,H:61-	PF02788.11^RuBisCO large N^Ribulose	.	.	.	GO:000950	VPNSNRTLWRRRSDIRTHLPLRWQNGKIAVALWLTRCCSLRRWGLRGA
	comp80942 c0	comp80942	m.165491	sp|Q41406|RBL2 SYMSP`Q41406`Q:1-521,H:110-	PF00016.15^RuBisCO large^Ribulose	.	.	.	GO:000950	GNNQGMGDVEYGKIYDFYLPPAFLRLYDGPSVNVEDMWRILGRGTTNG
	comp80955 c0	comp80955	m.165540	sp|Q41406|RBL2 SYMSP`Q41406`Q:1-507,H:487-	PF02788.11^RuBisCO large N^Ribulose	.	.	.	GO:000950	AAAFVGASVAPAKKENVVARQALDQSSRYADLSLDEDTLIRNGKHVLVA
	comp41717 c0	comp41717	m.87552	sp|Q43088|RBCMT PEA`Q43088`Q:79-434,H:84-	PF09273.6^Rubis-subs-bind^Rubisco LSMT	.	.	.	GO:000950	PSAFLPLAFSGLAFLVPWPSRSARSAAWNARAAAAQSRLPLRALGDSEE
	comp37410 c0	comp37410	m.81755	sp|Q5ENN5|RBL2 HETTR`Q5ENN5`Q:34-100,H:618-	.	.	.	.	GO:003196	PASPVTVPETAPVAAAFVGASAQAPAKTRQPMGFAYSIQQDVYADFLFT
	comp63174 c0	comp63174	m.123968	sp|Q41406|RBL2 SYMSP`Q41406`Q:118-159,H:500-	.	.	.	.	GO:000950	VRACAPCLGPSFSLPFSVSRRLRSLGTMAQRPSNLVLAGAAAGGLALLN
	comp66074 c0	comp66074	m.129499	sp|Q5ENN5|RBL2 HETTR`Q5ENN5`Q:86-149,H:618-	.	.	.	.	GO:003196	GNALNHPETMQPDTHAPGSPLPRSYYAAEVGYLVDGTDMLRAGNLSVQ
	comp67923 c0	comp67923	m.133494	sp|Q43088|RBCMT PEA`Q43088`Q:66-313,H:84-	PF00856.23^SET^SET domain^65-283^E:8.8e-	.	.	.	GO:000950	KKSGQSAQGYSNGGSGGAGATDGSKTDVTADAKKETWKSVPTLLQRR
	comp68158 c0	comp68158	m.134014	sp|Q5ENN5|RBL2 HETTR`Q5ENN5`Q:35-138,H:9-	.	.	.	.	GO:003196	VVHCHVGSCIFAFFPLAFELCSTMTQRSSNLVLAGAAAGGLALLNAASD
	comp71061 c0	comp71061	m.140340	sp|Q5ENN5|RBL2 HETTR`Q5ENN5`Q:40-160,H:618-	.	.	.	.	GO:003196	APAPAAATPSMPQMAPPVAAAFVGTSAQVSGTVQRQPAGFAYSIQKDV
	comp74965 c0	comp74965	m.149583	sp|Q43088|RBCMT PEA`Q43088`Q:80-327,H:84-	PF00856.23^SET^SET domain^79-297^E:5.3e-	.	.	.	GO:000950	ENTVCKALSDAMAKKKSGQSAQGYSNGGSGGAGATDGSKTDVTADAK
	comp76011 c0	comp76011	m.152086	sp|Q43088|RBCMT PEA`Q43088`Q:12-250,H:75-	.	.	.	.	GO:000950	MDLATFSRVTLADFASTGRGAALLCDVKQGDVLLEVPLDRCWTAEAAR
	comp78141 c0	comp78141	m.157645	sp|Q5ENN5|RBL2 HETTR`Q5ENN5`Q:107-236,H:609-	.	.	.	.	GO:003196	PGSPLPRAIFVNSVGYLPDGTPLNAAGNALNHPETMQPDSHAPGSPLPR
	comp79516 c0	comp79516	m.161418	sp|Q5ENN5|RBL2 HETTR`Q5ENN5`Q:112-171,H:618-	.	.	.	.	GO:003196	QGSKGVTQFFSSLIVPSPKDMVQSKMSCAVAVAAFGLVLLNGVNFVAPN
	comp79880 c0	comp79880	m.162438	sp|Q5ENN5|RBL2 HETTR`Q5ENN5`Q:115-235,H:618-	.	.	.	.	GO:003196	GPDPHTPGSPLPRAIFVNSVGYLPDGTAMNAAGNALNHPERMQPDMHV
	comp93928 c0	comp93928	m.174772	sp|Q43088|RBCMT PEA`Q43088`Q:30-305,H:20-304`25.4%ID`E:1e-	PF00856.23^SET^SET domain^88-273^E:1.7e-	.	.	.	GO:000950	MTRSRRAGAIQAVVVVAACAYLARTWCAPGRPVTVKNPRRTLRMAIAS
acpP	comp29314 c0	comp29314	m.71642	sp|P52414|ACP4 CUPLA`P52414`Q:23-111,H:37-132`40%ID`E:1e-	PF00550.20^PP-binding^Phosphopantetheine	.	.	.	GO:000950	WACALALASMALAMPWVGSRAQRRSIPRSLRRSSASEAVTDEVIRIIKQE
	comp62787 c0	comp62787	m.123153	sp|Q1XDK6|ACP PORYE`Q1XDK6`Q:72-146,H:4-79`53.95%ID`E:3e-	PF00550.20^PP-binding^Phosphopantetheine	.	.	.	GO:000950	TSAGTRGSSENHPPRQHVPSATSRPHLIMARSGLVTLLCCLVFLRTLTFV
carA	comp152063 c	comp15206	m.31334	sp|Q91437|PYR1 SQUAC`Q91437`Q:1-258,H:26-274`56.2%ID`E:4e-	PF00988.17^CPSase sm chain^Carbamoyl-	.	.	.	GO:000573	EVVFNTGIVAYPESLTDPSYAGQILVITYPLVGNYGVPSDEKDELNLPRW
	comp40009 c0	comp40009	m.84742	sp|Q91437|PYR1 SQUAC`Q91437`Q:1-827,H:109-	PF00988.17^CPSase sm chain^Carbamoyl-	.	.	.	GO:000573	EEHEVPGIFGVDTRAITKRLRMEGSALGAVVMGSDSAPALVDPNLRNLV
	comp58692 c0	comp58692	m.115240	sp|P07259|PYR1 YEAST`P07259`Q:10-393,H:3-409`51.59%ID`E:2e-	PF00988.17^CPSase sm chain^Carbamoyl-	.	.	COG0458^	GO:001602	PPRPDNDGKTLAGTVDEFASWEAAAKTPMELRLQSGECWRGWNFGAK
	comp80861 c0	comp80861	m.165197	sp|O93937|PYR1 EMENI`O93937`Q:36-1551,H:58-	PF00988.17^CPSase sm chain^Carbamoyl-	.	.	COG0458^	GO:001659	RKSRMGLDFVNGGGPMDTKPVNGETKDLLQWLNDRRKCDLELANGTM
	comp8614 c0	comp8614	m.169001	sp|P07259|PYR1 YEAST`P07259`Q:56-115,H:20-79`76.67%ID`E:7e-	PF00988.17^CPSase sm chain^Carbamoyl-	.	.	COG0458^	GO:001602	EGELQKQCGRRFMSELPDSPEKMGWDVVGFDGVEPIDVKPSNEQPAD
gltB	comp177569 c	comp17756	m.42189	sp|P55037|GLTB SYNY3`P55037`Q:13-238,H:28-	PF00310.16^GATase 2^Glutamine	.	.	COG0069^	GO:005153	PTKMSYKGFDAIPEAQGLYDPRNEKDSCGVGMVADLTGKPARDIVDGA
	comp219992 c	comp21999	m.56996	sp|P51375|GLTB PORPU`P51375`Q:2-203,H:376-568`50%ID`E:1e-	PF00310.16^GATase 2^Glutamine	.	.	.	GO:000957	YMGASLDRNGLRPARYFQLHDGTCVVSSETGILESKIFPPDMFKSKGRL
ilvb	comp95230 c0	comp95230	m.175641	sp|P36620|ILVB SCHPO`P36620`Q:65-628,H:88-	PF02776.13^TPP enzyme N^Thiamine	.	.	COG0028^	GO:000594	PICQRQQARPMKAIRALCGHAQVTRFVKPRAARAVSRCHVYTLQARSW
glmS	comp65514 c0	comp65514	m.128461	sp|Q8KG38|GLMS CHLTE`Q8KG38`Q:1-643,H:1-	PF13522.1^GATase 6^Glutamine	.	.	COG0449^	GO:000573	MCGIFAYIGDKEAAALLITALKRLEYRGYDSAGIGIHGVPLKVRKKVGKVA
	comp68297 c0	comp68297	m.134371	sp|Q8KG38|GLMS CHLTE`Q8KG38`Q:36-435,H:258-	PF01380.17^SIS^SIS domain^100-223^E:1.9e-	.	.	COG0449^	GO:000573	LKTRNQMGRLAQGQASAAMVSMQAPLQAPDVGRETMLTDIWETPEVL
chlI	comp39765 c0	comp39765	m.84464	sp|Q9TL08|CHLI NEPOL`Q9TL08`Q:95-440,H:9-354`58.79%ID`E:3e-	PF01078.16^Mg chelatase^Magnesium	.	.	.	GO:000950	KGVTRWHIDPQKMLPSGSTPMLAVRCGTAARPAVLTSQGSSISTPLSRS
	comp46484 c0	comp46484	m.95703	sp|Q9TL08|CHLI NEPOL`Q9TL08`Q:107-451,H:6-	PF01078.16^Mg chelatase^Magnesium	.	.	.	GO:000950	DLSSPFFHHRLVKEILHCGLFFHLPIMFSVDSPATMAFVANPGIQQPRPS
cobA										
preA	comp32433 c0	comp32433	m.75721	sp|P31171|PREA CYAPA`P31171`Q:16-151,H:193-	PF00348.12^polyprenyl synt^Polyprenyl	.	.	.	GO:000984	ACHAAALLSRPQSSPDAEECQNCYRFGAYIGLAFQVVDDILDFIATEEEL
secA	comp163668 c	comp16366	m.36658	sp|Q8DHU4|SECA THEEB`Q8DHU4`Q:29-178,H:2-	PF07517.9^SecA DEAD^SecA DEAD-like	.	.	COG0653^	GO:000573	KNRAWRRRSQREQRLAKVTRKLFGNMEQLNPYFYESPEKQTQKRYEPQ
	comp204348 c	comp20434	m.51907	sp|A5GI02|SECA SYNPW`A5GI02`Q:2-195,H:135-	PF07517.9^SecA DEAD^SecA DEAD-like	.	.	COG0653^	GO:000573	TNDYLARRDAAWVGRVLRFLGLTVGVVQAEMSSAQKREAYRCDVTYVT
	comp240070 c	comp24007	m.62018	sp|Q7V9M9|SECA PROMA`Q7V9M9`Q:1-124,H:60-	PF07517.9^SecA DEAD^SecA DEAD-like	.	.	COG0653^	GO:000573	ETLEALLPEAFALVREASKRTLRLRHFDVQILGGIALHEGQIAEMATGEGK
	comp3717 c0	comp3717	m.81472	sp|Q2JJ09|SECA SYNJB`Q2JJ09`Q:1-157,H:318-	PF01043.15^SecA PP bind^SecA preprotein	.	.	COG0653^	GO:000573	EFTGRPVEGRSWSDGLQQAIEAKEGVPISKEAIVLASISYQCLFRLYQKL
	comp43302 c0	comp43302	m.90177	sp|Q47RW8|SECA2 THEFY`Q47RW8`Q:180-254,H:93-	PF07517.9^SecA DEAD^SecA DEAD-like	.	.	COG0653^	GO:000573	TFFGAVKDMHSNESFRRAGENQNLDEIVGRLTSVSGLDGTEYHELVAAL
	comp53252 c0	comp53252	m.106387	sp|Q8DHU4|SECA THEEB`Q8DHU4`Q:102-720,H:10-	PF07517.9^SecA DEAD^SecA DEAD-like	.	.	COG0653^	GO:000573	ELCAMALNTAWVPAPLTGSTQASAPTASRHSGAGPLELSAQQGVSRTT
	comp57243 c0	comp57243	m.112695	sp|Q8DHU4|SECA THEEB`Q8DHU4`Q:1-643,H:96-	PF07517.9^SecA DEAD^SecA DEAD-like	.	.	COG0653^	GO:000573	AEMKTGEGKTIVALLPTFLAALEDKGGVYVVTPNDYLARRDAENVGQVL
	comp66068 c0	comp66068	m.129480	sp|Q6NHD5|SECA2 CORDI`Q6NHD5`Q:47-575,H:50-	PF07517.9^SecA DEAD^SecA DEAD-like	.	.	COG0653^	GO:000573	EAIQSAASVAPASSEHWDQQCKDQLPQILASVSAYFTISKSGKAFKELAR
	comp73373 c0	comp73373	m.145725	sp|Q8YMS8|SECA NOSS1`Q8YMS8`Q:1-904,H:18-	PF07517.9^SecA DEAD^SecA DEAD-like	.	.	COG0653^	GO:000573	EPQVQAINALEETMKAKSDDDLRALTADLQQRAQSGTALEELLPEAFAL
	comp184175 c	comp18417	m.45008	.	PF07517.9^SecA DEAD^SecA DEAD-like	.	.	.	.	SEIISEFKVQPDEASASFVPGNLRRGYDLYKEAYQAHLDAWQEAGMDD
	comp233013 c	comp23301	m.60128	.	PF07517.9^SecA DEAD^SecA DEAD-like	.	.	.	.	RRKVCYPGKGRLADPNQLLKRPHVVQTLACLRMIGYDTRQNALQNHLL
	comp344463 c	comp34446	m.78220	.	PF07517.9^SecA DEAD^SecA DEAD-like	.	.	.	.	NKRDLANQLIQVETGGGKSLILGAAATIFAMLGFYVRDACYSDYLSSRDA
	comp9800 c0	comp9800	m.177614	.	PF07517.9^SecA DEAD^SecA DEAD-like	.	.	.	.	NSVQTLIIRKVMNCPQLGPFDFADLDICLRLVDRTEGGANQVFLSRATVL
secY	comp24033 c0	comp24033	m.62073	sp|Q54XK2|SC61A DICDI`Q54XK2`Q:6-469,H:3-	PF10559.4^Plug translocon^Plug domain of	.	.	COG0201^	GO:000578	MGEGSFRFLTLIKPVLWLVPEVNAPEHRVPFKEKVCWTAISLFVFLVCCQ
	comp84123 c0	comp84123	m.167599	sp|Q54XK2|SC61A DICDI`Q54XK2`Q:26-489,H:3-	PF10559.4^Plug translocon^Plug domain of	.	.	COG0201^	GO:000578	FESEETLPGKRCCTSKVRGAATMGKFRLLDLVKPCMCVLPEVSTPDRRI
	comp93886 c0	comp93886	m.174743	sp|Q54XK2|SC61A DICDI`Q54XK2`Q:29-318,H:3-	PF10559.4^Plug translocon^Plug domain of	.	.	COG0201^	GO:000578	SSQVLSSSRGLWAVGDRRTCAMSMQSGSFRFLNLIKPVMCVLPEVEAP
sufB	-									
tatC	comp73003 c0	comp73003	m.144836	sp|P54086|TATC SYNY3`P54086`Q:42-284,H:15-	PF00902.13^TatC^Sec-independent protein	.	.	COG0805^	GO:001602	MEAAKKPTAAESRLALEQATTGGSYQNLTADQRGLVNGFFFPDEEELD
DMC1	comp116145 c0	comp11614	m.11045	sp|Q96449|DMC1 SOYBN`Q96449`Q:34-308,H:29-300`56%ID`E:3e-	PF08423.6^Rad51^Rad51^96-310^E:1.7e-	.	.	.	GO:000563	MAAVTVESSQKRKASAMGTMAVVEADDGEEVVPYSLIDKLQESGINAAD
	comp122198 c	comp12219	m.14958	sp|Q61880|DMC1 MOUSE`Q61880`Q:2-288,H:49-	PF08423.6^Rad51^Rad51^36-289^E:4.8e-	.	.	COG0468^	GO:000078	SQSVAFAMRKDLLSIKGLSDQKVDKIIEAARKSSEVGFVTCTQLVSKMKS
	comp151318 c0	comp15131	m.30975	sp|P50265|DLH1 CANAX`P50265`Q:24-179,H:10-	PF08423.6^Rad51^Rad51^83-177^E:2.4e-	.	.	COG0468^	GO:000563	ATAPAMVDHALVEADDEDVPYSLIDKLQESGINAADLKKLKDAGFNTSQS
	comp45562 c0	comp45562	m.94171	sp|Q08327|RECA ACEPO`Q08327`Q:88-396,H:22-	PF00154.16^RecA^recA bacterial DNA	.	.	.	GO:000573	HQTQKEPSMQPLPMLAPSLHAPPSPQIGAAAPPSGLGWKACHLLPMTG
	comp73268 c0	comp73268	m.145439	sp|C0QI90|RECA DESAH`C0QI90`Q:45-338,H:40-	PF08423.6^Rad51^Rad51^37-83^E:2.9e-	.	.	COG0468^	GO:000573	VAKAPAVPRSSLAFAAVTGAIAWKTWTARRQRAPQQVEKMNTKNFLST
HOP1										
HOP2	comp147597 c	comp14759	m.29301	sp|O35047|HOP2 MOUSE`O35047`Q:53-111,H:16-	PF07106.8^TBPIP^Tat binding protein 1(TBP-	.	.	NOG27444	GO:000563	TAPAAPATVAVQPAEKEAAKVAAQPKVVAKAKSEAKGAKDVKKLSKDE
	comp43393 c0	comp43393	m.90322	sp|O35047|HOP2 MOUSE`O35047`Q:24-218,H:16-	PF07106.8^TBPIP^Tat binding protein 1(TBP-	.	.	NOG27444	GO:000563	KAKSEAKGAKDVKKLSKDEMEAKVLEYMRQQNRPYNSQNVFDNLHGA
MER3	-									
MND1	comp52471 c0	comp52471	m.105284	sp|Q9BWT6|MND1 HUMAN`Q9BWT6`Q:1-207,H:1-	PF03962.10^Mnd1^Mnd1 family^17-205^E:8e-	.	.	COG5124^	GO:000563	MSKRKGMSFDEKKVTLLAAMQGEASFFTLKELETLGKSKGVIPQAVKDV
	comp13506 c0	comp13506	m.22662	sp|Q8CI78|RMND1 MOUSE`Q8CI78`Q:59-344,H:190-	PF02582.9^DUF155^Uncharacterised ACR,	.	.	COG1723^	GO:000573	MWSVRGQRQGCAMRALLYRRLGRSMATAIATPGRSRNEASEAMISADL
MSH4	comp151677 c	comp15167	m.31126	sp|O15457|MSH4 HUMAN`O15457`Q:10-403,H:418-	PF00488.16^MutS V^MutS domain V^225-	.	.	COG0249^	GO:000079	RGMQWYKVAVRTALRLRHALSALPHLADALSPKSRRRQEASAGHLDDL
	comp191937 c	comp19193	m.48088	sp|O15457|MSH4 HUMAN`O15457`Q:1-160,H:240-	PF05192.13^MutS III^MutS domain III^74-	.	.	COG0249^	GO:000079	LERRHFDEAEGQSLLEEAIVVGLQQADFSSKFAAAASFTALWRYIESSSD
MSH5	comp169914 c	comp16991	m.39237	sp|O43196|MSH5 HUMAN`O43196`Q:2-214,H:306-	.	.	.	COG0249^	GO:000079	QLHRELRMMQDLPRLLARMHRCYNFDNMVDWRSLVTTLGHMSAALGL
	comp201605 c	comp20160	m.51252	sp|F4JEP5|MSH5 ARATH`F4JEP5`Q:29-212,H:72-	PF05192.13^MutS III^MutS domain III^141-	.	.	.	GO:004307	LGQRHLGAVSAPRQGLCVLPSRSPAELVETAARPLTQDDDGNEPFPTTL
	comp32251 c0	comp32251	m.75466	sp|O43196|MSH5 HUMAN`O43196`Q:1-302,H:527-	PF00488.16^MutS V^MutS domain V^18-	.	.	COG0249^	GO:000079	AELDVLLAYTAAAVKYRWVRPKLSEDVKRLHIVKGRHPLVEAASTAHHG
REC8	-									
RED1										
SPO11	comp184174 c	comp18417	m.45005	sp|Q9M4A1|SPO12 ARATH`Q9M4A1`Q:1-168,H:132-	PF09664.5^DUF2399^Protein of unknown	.	.	COG1697^	GO:000569	ELFACQSIMDKALRDAVGALQISRPHLGIFTAEKGLLAGDVTFQDPTCYV
	comp348249 c	comp34824	m.78675	sp|Q9Y5K1|SPO11 HUMAN`Q9Y5K1`Q:15-91,H:110-	PF04406.9^TP6A N^Type IIB DNA	.	.	COG1697^	GO:000078	KLERLLRGSSCQRLRFRRIMALMDSVHGLLRSGRTATPRELYYRHVNIF
	comp47522 c0	comp47522	m.97422	sp|Q9Y5K1|SPO11 HUMAN`Q9Y5K1`Q:41-193,H:217-	.	.	.	COG1697^	GO:000078	GDVTFQDPTCYVSRAAQGASGTSIGEAMLEDDKCIQVSEKAKWVLVVE
ZIP1	-									
ATM	comp34940 c0	comp34940	m.78766	sp|Q9M3G7|ATM ARATH`Q9M3G7`Q:1-127,H:3728-	PF02260.15^FATC^FATC domain^97-	.	.	COG5032^	GO:000563	GLGCLGTCGLFRRCAEAAMALLRQNGSLVTAVVEVFVHDPIYAWSCLTP
	comp53928 c0	comp53928	m.107420	sp|Q9M3G7|ATM ARATH`Q9M3G7`Q:20-532,H:3248-	PF00454.22^PI3 PI4 kinase^Phosphatidylinosi	.	.	COG5032^	GO:000563	NTVRQLTEQKIQRIDEDVAKAQKKGTTAAVQKEVKDLQEQKGKLEKHRK
	comp56093 c0	comp56093	m.110844	sp|Q9M3G7|ATM ARATH`Q9M3G7`Q:42-503,H:3150-	PF00454.22^PI3 PI4 kinase^Phosphatidylinosi	.	.	COG5032^	GO:000563	RWEQARCLYALKSPEGMSIAKALAAECRQGIQGPRAGSSNWAAQVLSC
ATR	-									
BRCA1	comp45520 c0	comp45520	m.94070	sp|Q8RXD4|BRCA1 ARATH`Q8RXD4`Q:576-646,H:779-	.	.	.	NOG27449	GO:000563	NKLSQQRRMLHSGDAPHRKNLLAACNDFDRLLKSFVERPPGSTGLRAV
BRCA2	comp79139 c0	comp79139	m.160405	sp|O35923|BRCA2 RAT`O35923`Q:146-321,H:2527-	PF09103.5^BRCA-2 OB1^BRCA2,	.	.	NOG33129	GO:003359	PVGHVGFMTAGGKAVAADEGRRQRAQALFATLNETIDEQGLFPKPAQD
CDC2	comp12391 c0	comp12391	m.16067	sp|P93101|CDC2 CHERU`P93101`Q:423-583,H:47-	PF00069.20^Pkinase^Protein kinase	.	.	.	GO:000552	ASASAASSPEQPSGANDRTSAAEQGGADERQPEAEMFPDGDPKEDAP
	comp74657 c0	comp74657	m.148802	sp|Q9XZD6|CDC2H PLAVI`Q9XZD6`Q:19-304,H:1-	PF00069.20^Pkinase^Protein kinase	.	.	COG0515^	GO:000573	QVVPRVPALGSRRGARAGMDQYQKIEKVGEGTYGIVYKAQDSAGEVFA
	comp76876 c0	comp76876	m.154200	sp|Q9XZD6|CDC2H PLAVI`Q9XZD6`Q:19-303,H:1-	PF00069.20^Pkinase^Protein kinase	.	.	COG0515^	GO:000573	LDVLLQAALHLQPWAVRGMEQYQKIEKVGEGTYGVVYKAQDSGGKVYA

DNA2	-										
EXO1	comp21276 c0	comp21276	m.54775	sp|Q9W6K2|EXO1 XENLA`Q9W6K2`Q:14-214,H:1-	PF00752.12^XPG N^XPG N-terminal	.	.	.	GO:000563	RTSASIPGSAHDPMGIQGLLPFLRSYVKKTHIETFQGTTIGVDAMCWMH	
FEN1	comp55945 c0	comp55945	m.110601	sp|B4LM90|FEN1 DROVI`B4LM90`Q:9-329,H:66-	PF00752.12^XPG N^XPG N-terminal	.	.	COG0258^	GO:000573	EDAVKRTEGLFRRSVRLLELGIKPIFVFDGEAPEMKSHVLQQRAKNRAR	
	comp57535 c0	comp57535	m.113130	sp|B4FHY0|FEN1 MAIZE`B4FHY0`Q:2-297,H:1-286`45.79%ID`E:2e-	PF00752.12^XPG N^XPG N-terminal	.	.	.	GO:000573	AMGIKGLFPYLVEAAPKAYNTSELKRYTGRRLAVDASAWMYQFLTIVRT	
	comp84040 c0	comp84040	m.167552	sp|C5YUK3|FEN11 SORBI`C5YUK3`Q:1-347,H:1-344`53.3%ID`E:3e-	PF00752.12^XPG N^XPG N-terminal	.	.	COG0258^	GO:000573	MGIKGLVKFLQENAPKSVKEIPSQAAYTGRLIAIDASMCLYQFLIMIRENR	
ku70	comp88861 c0	comp88861	m.171004	sp|O93257|XRCC6 CHICK`O93257`Q:59-624,H:56-	PF03731.10^Ku N^Ku70/Ku80 N-terminal	.	.	NOG30531	GO:000569	FRSCDQDLSGSFRCFSLGFHCAPMADFDPTDYDGQNADDPLNEGEDEL	
ku80	comp69445 c0	comp69445	m.136934	sp|Q54LY5|XRCC5 DICDI`Q54LY5`Q:5-719,H:7-771`24.09%ID`E:1e-	PF03731.10^Ku N^Ku70/Ku80 N-terminal	.	.	NOG29974	GO:000595	MAWRCKEAIVLVIDVGKSMQETFAAANDGSKVTRLQTALGVAQRLVQH	
LIG4	comp71994 c0	comp71994	m.142452	sp|Q54CR9|DNLI4 DICDI`Q54CR9`Q:18-820,H:137-	PF04675.9^DNA_ligase_A N^DNA ligase N	.	.	COG1793^	GO:000569	DHGILMSASLTSCDAESFHRFCLFLESLHLKKKTEKLQAVEHFLKRFDGT	
MLH1	comp31033 c0	comp31033	m.73959	sp|Q9JK91|MLH1 MOUSE`Q9JK91`Q:1-565,H:1-555`38.63%ID`E:5e-	PF02518.21^HATPase_c^Histidine kinase-,	.	.	COG0323^	GO:000571	MSDMAGRRIVQLEEAVINRIAAGEVVVRPANALKELIENSLDAKSQRINV	
	comp90298 c0	comp90298	m.172055	sp|P40692|MLH1 HUMAN`P40692`Q:2-240,H:558-		.	.	COG0323^	GO:000571	RECAYQRLLRLIGGIGCITLVEPLPLKDLVQLGLRDPESGYDKLPGSDIRA	
MLH3	comp195219 c	comp19521	m.48945	sp|Q9UHC1|MLH3 HUMAN`Q9UHC1`Q:5-173,H:1-	PF02518.21^HATPase_c^Histidine kinase-,	.	.	COG0323^	GO:000571	MHCPVIQKLPKDVIEKLRGNVVIPSVARAAEEMVLNAVEADASVVEVELQ	
	comp51373 c0	comp51373	m.103570	sp|Q9UHC1|MLH3 HUMAN`Q9UHC1`Q:150-344,H:1192-	PF08676.6^MutL C^MutL C terminal	.	.	COG0323^	GO:000571	PGPGPGRFFFARSGSRAAARKRTLRALVLQEEKHIQAERQAQDAMATQ	
MMS4	-										
MPH1	comp18375 c0	comp18375	m.44795	sp|O94235|MPH1 SCHPO`O94235`Q:42-323,H:308-	PF00069.20^Pkinase^Protein kinase	.	.	COG0515^	GO:000077	VAAAKSSSPRNAESPCHTPQLDVSLENSEPSERAEQAKSSKVVLVNGIP	
	comp49249 c0	comp49249	m.100253	sp|O94235|MPH1 SCHPO`O94235`Q:289-562,H:308-	PF00069.20^Pkinase^Protein kinase	.	.	COG0515^	GO:000077	RAGRPVGFVYFLVEQVSEFNSEARKLAMTGRSYIKRNLRQAAGDVRQIL	
MRE11	comp93271 c0	comp93271	m.174312	sp|Q9UVN9|MRE11 COPC7`Q9UVN9`Q:18-498,H:21-	PF00149.23^Metallophos^Calcineurin-like	.	.	.	GO:000563	HAGSVPAGCMADELPDADTLRILVLTDTHVGYSEKDKVRGKDAMDSLEE	
MSH2	comp79008 c0	comp79008	m.159967	sp|O13396|MSH2 NEUCR`O13396`Q:33-725,H:160-	PF05188.12^MutS_II^MutS domain II^11-	.	.	COG0249^	GO:003230	MAFDSEVQENAVCCITATGSGDQFGWAVFSEDLRAFRICEYLEDVHLSR	
MSH3	-										
MSH6	comp43641 c0	comp43641	m.90747	sp|O04716|MSH6 ARATH`O04716`Q:18-881,H:385-	PF01624.15^MutS_I^MutS domain I^13-	.	.	COG0249^	GO:003230	QKAEYQKAQGHNTPMLMQYWKLKSAHFDKVAFFKVGKFYEIFYYDAFIA	
MUS81	comp169014 c	comp16901	m.38784	sp|Q640B4|MUS81 XENTR`Q640B4`Q:107-177,H:152-		.	.	COG1948^	GO:000563	SSTAMAKIWNADLAHAAEVRVWYHQSKGSSKEFMWRKAHEAILAVEKEI	
	comp31875 c0	comp31875	m.75053	sp|Q6BJ48|MUS81 DEBHA`Q6BJ48`Q:33-123,H:359-	PF02732.10^ERCC4^ERCC4 domain^38-	.	.	COG1948^	GO:000563	TPCMAKPAVQQELEEIEISSESESARDVESRSQVVLLVDERERLRNTEP	
	comp49096 c0	comp49096	m.100017	sp|Q551H0|MUS81 DICDI`Q551H0`Q:254-488,H:637-	PF02732.10^ERCC4^ERCC4 domain^256-	.	.	COG1948^	GO:000563	RTNCKKRLAKALKDAAKGKTSKASRPPPLRIPRKGKGSGKGGRGRGRA	
	comp18003 c0	comp18003	m.43316	sp|Q941I6|PMS1 ARATH`Q941I6`Q:15-348,H:20-	PF02518.21^HATPase_c^Histidine kinase-,	.	.	.		LLETKGSRDGAMNRLAKPVVHEICTNQVVVTLQACVKELVENSLDAKAT	
	comp74225 c0	comp74225	m.147644	sp|O75943|RAD17 HUMAN`O75943`Q:2-407,H:95-	PF03215.10^Rad17^Rad17 cell cycle	.	.	COG0470^	GO:000565	CHHPRNESSLVVAKKKIAEVREFLFQPNGRRLLILKGPPGSGKASVLHAL	
RAD23	comp104290 c	comp10429	m.3092	sp|Q40742|RAD23 ORYSJ`Q40742`Q:1-94,H:1-96`39.58%ID`E:1e-	PF13881.1^Rad60-SLD 2^Ubiquitin-2 like	.	.	COG5272^	GO:000563	MKLQVRPLKGETFDLEVEADWTVEAVKAAIAGMKPDLPAELQKVMHKG	
	comp45467 c0	comp45467	m.93975	sp|Q84L33|RD23A ARATH`Q84L33`Q:25-376,H:2-	PF11976.3^Rad60-SLD^Ubiquitin-2 like Rad60	.	.	COG5272^	GO:000563	DLERGVGPQPKVVVSSCVCAAMAQKITCRPLKGDTFDVEVNGEASVAD	
	comp47260 c0	comp47260	m.96944	sp|Q84L31|RD23C ARATH`Q84L31`Q:1-241,H:160-	PF00627.26^UBA^UBA/TS-N domain^16-	.	.	COG5272^	GO:000563	PAPPAPPAGPQAPVENEIAIQSLCDMGFPPELVRQCLRAAFNNPDRAVE	
	comp58263 c0	comp58263	m.114425	sp|O74803|RHP23 SCHPO`O74803`Q:182-304,H:255-	PF00627.26^UBA^UBA/TS-N domain^25-	.	.	COG5272^	GO:000582	PAAPAAPAAPAADAPLDACDPVPREEASVQSLMEMGFPRDQVEAALRA	
	comp64013 c0	comp64013	m.125638	sp|Q84L33|RD23A ARATH`Q84L33`Q:24-226,H:1-	PF11976.3^Rad60-SLD^Ubiquitin-2 like Rad60	.	.	COG5272^	GO:000563	ASWRESAAGHLADLKTCLKSDLGMKITVRPIKGESFFVEVPETDTIESLK	
	comp71433 c0	comp71433	m.141204	sp|A3KMV2|RD23A BOVIN`A3KMV2`Q:1-171,H:1-	PF11976.3^Rad60-SLD^Ubiquitin-2 like Rad60	.	.	COG5272^	GO:000563	MSIEVTLKLLSGEKFQVKANPEEKISDLKAKVSEARPELPVECQKLIYSGK	
	comp73891 c0	comp73891	m.146990	sp|Q84L33|RD23A ARATH`Q84L33`Q:1-284,H:1-	PF11976.3^Rad60-SLD^Ubiquitin-2 like Rad60	.	.	COG5272^	GO:000563	MKITVRPIKGESFFVEVPETDTIESLKQAIAAAKAEYPQSRQKLLHNGRVL	
	comp74225 c0	comp74225	m.147644	sp|O75943|RAD17 HUMAN`O75943`Q:2-407,H:95-	PF03215.10^Rad17^Rad17 cell cycle	.	.	COG0470^	GO:000565	CHHPRNESSLVVAKKKIAEVREFLFQPNGRRLLILKGPPGSGKASVLHAL	
RAD24	comp338383 c	comp33838	m.77436	sp|P42656|RAD24 SCHPO`P42656`Q:16-142,H:6-	PF00244.15^14-3-3^14-3-3 protein^16-	.	.	COG5040^	GO:003215	ARKDKMAEGSDAYPEREECVYNARLAEQAERYEDMVTYMKKVAKSEK	
RAD50	comp174345 c	comp17434	m.41071	sp|Q9SL02|RAD50 ARATH`Q9SL02`Q:38-176,H:2-	PF13476.1^AAA 23^AAA domain^3-40^E:4.4e-	.	.	COG0419^	GO:000078	ISKLGIQGIRSFNHERMEIIEFEKPLTLIVGPNGSGKTTISKLGIQGIRSFNH	
	comp72013 c0	comp72013	m.142491	sp|Q9JIL8|RAD50 RAT`Q9JIL8`Q:8-1326,H:1-1308`27.11%ID`E:1e-	PF13476.1^AAA 23^AAA domain^13-	.	.	COG0419^	GO:000078	LALPHLAMTTISKLGIQGIRSFNHERMEIIEFEKPLTLIVGPNGSGKTTIIEC	
RAD51	comp22115 c0	comp22115	m.57373	sp|Q91918|RA51A XENLA`Q91918`Q:79-387,H:29-	PF08423.6^Rad51^Rad51^131-385^E:4.4e-	.	.	.	GO:000563	PRSSIFWFIMEQQVQVRQNKRARREAVLEDSSVVEAPAAQPAPAVAAA	
	comp42250 c0	comp42250	m.88386	sp|Q8R2J9|RA51C CRIGR`Q8R2J9`Q:45-358,H:18-	PF08423.6^Rad51^Rad51^110-358^E:4e-	.	.	.	GO:000573	KSGLVDMTHTCSGPSISMDAKLPASAQALDVRILHSIPTPVADALISRGC	
	comp48957 c0	comp48957	m.99758	sp|O35719|RA51B MOUSE`O35719`Q:55-369,H:15-	PF08423.6^Rad51^Rad51^136-325^E:2.7e-	.	.	COG0468^	GO:000563	PKPCAALPIGSLKVHMAYTANAMVYKSAQGFGDPPLDRLFSDAPEPVLG	
	comp30656 c0	comp30656	m.73523	sp|O55230|RA51D MOUSE`O55230`Q:56-240,H:85-	PF06745.8^KaiC^KaiC^58-118^E:1.2e-	.	.	COG0468^	GO:000563	VRCSMAKGNASNTSWDVCDRILADAGEAEKPQPMTGEQLLFSEDGAML	
RAD52	-										
RAD54	comp120768 c	comp12076	m.14076	sp|A4PBL4|RAD54 ORYSJ`A4PBL4`Q:22-344,H:235-	PF00176.18^SNF2_N^SNF2 family N-terminal	.	.	COG0553^	GO:000563	PPADDPEHRKDVIVDFDVSCKLREHQRIGVQFLFDCLMGLKDFNGCGCI	
	comp132825 c	comp13282	m.21380	sp|A4PBL4|RAD54 ORYSJ`A4PBL4`Q:63-297,H:115-	PF00176.18^SNF2_N^SNF2 family N-terminal	.	.	COG0553^	GO:000563	AFLTSSSFALDSVSVWSPCATIMAPKRAARAPGGRELVSAPEASASLVQ	
	comp187852 c	comp18785	m.46281	sp|B3MMA5|RAD54 DROAN`B3MMA5`Q:13-123,H:574-	PF00271.26^Helicase_C^Helicase conserved	.	.	COG0553^	GO:000563	EGLNTPGPEGTDVLLLSSKAGGTGLNLIGANRLILFDPDWNPANDAQAM	
	comp1992 c0	comp1992	m.50601	sp|Q6PFE3|RA54B MOUSE`Q6PFE3`Q:3-153,H:517-	-	.	.	COG0553^	GO:000563	FGLPPRVEVVLRLRLAPTQVAAYQALLADLHSGPQNMAKALKAMMALRL	
	comp218207 c	comp21820	m.56376	sp|B3MMA5|RAD54 DROAN`B3MMA5`Q:1-77,H:608-	-	.	.	COG0553^	GO:000563	QAMARIWRQGQTKPCFIYRFVLAGTLEEKICQRQQVKTDLAQITVDGQD	
	comp47752 c0	comp47752	m.97831	sp|O12944|RAD54 CHICK`O12944`Q:3-457,H:274-	PF00176.18^SNF2_N^SNF2 family N-terminal	.	.	COG0553^	GO:000563	REVEDAGIDLVVCDEAHKLKNDEAATTKCISALPAKRRLLISGTPIQNSLE	
REC114	-										
RTEL1	comp14045 c0	comp14045	m.25634	sp|A8WS58|RTEL1 CAEBR`A8WS58`Q:62-443,H:486-	PF13307.1^Helicase_C 2^Helicase C-terminal	.	.	COG1199^	GO:000563	FSSQLELAATLFGQLAAAVRRPELYVARAHPGGQGQQAHLHLWLMSAE	
	comp158111 c	comp15811	m.34123	sp|P0C928|RTEL1 DANRE`P0C928`Q:136-392,H:4-	PF13245.1^AAA 19^Part of AAA domain^162-	.	.	COG1199^	GO:000563	WPWEMDEVDQALEFEPCPGGFFDDLDDQDLLAAALRAEEQLGQSAAN	
	comp180901 c	comp18090	m.43576	sp|B3MSG8|RTEL1 DROAN`B3MSG8`Q:94-142,H:11-		.	.	COG1199^	GO:000563	GVCGMQRVRQRRGKLTKAKPDLAKEKAALHRVLGEPVIQLEARPPGVQ	
	comp33497 c0	comp33497	m.76988	sp|Q0VGM9|RTEL1 MOUSE`Q0VGM9`Q:42-245,H:6-	PF06733.10^DEAD 2^DEAD 2^133-	.	.	COG1199^	GO:000563	ATWRRRERRERASALRFWEVQLPPPVVGYDLQVERLCLSTSVNGLEIH	
	comp49452 c0	comp49452	m.100559	sp|Q5RJZ1|RTEL1 RAT`Q5RJZ1`Q:5-608,H:32-642`25.56%ID`E:1e-	PF06733.10^DEAD 2^DEAD 2^70-	.	.	COG1199^	GO:000563	AAAAMEKSTAALVESPTGTGKTLALLSACLAFQWHRFKSWKDAGHLPS	
	comp55030 c0	comp55030	m.109112	sp|P0C928|RTEL1 DANRE`P0C928`Q:11-659,H:163-	PF06733.10^DEAD 2^DEAD 2^6-126^E:2.2e-	.	.	COG1199^	GO:000563	ADNDGQIRQACRQARQQKSCQFHSGLLSSDLPELAANKLRGELWDIED	
	comp65380 c0	comp65380	m.128183	sp|B4PZB4|RTEL1 DROYA`B4PZB4`Q:1-702,H:9-	PF06733.10^DEAD 2^DEAD 2^83-240^E:1e-	.	.	COG1199^	GO:000563	VLVEFPYEAYDCQLEYMRAVVRSLENGENALLESPTGTGKTLCILCASLG	
	comp66200 c0	comp66200	m.129754	sp|B3MSG8|RTEL1 DROAN`B3MSG8`Q:10-271,H:501-	PF13307.1^Helicase_C 2^Helicase C-terminal	.	.	COG1199^	GO:000563	ASRALREGPLEARHIVHPWQLFAAVLPCADRCNAPIVSTYGNWQSEEFV	
SAD1	comp61767 c0	comp61767	m.121273	sp|Q95LV7|SUN3 MACFA`Q95LV7`Q:205-342,H:124-	PF07738.8^Sad1_UNC^Sad1 / UNC-like C-	.	.	.		AGAPGATAPAAHGRRGLGLMAGVAMLAFACLRPIQWTWPKSNVGSAR	
SAE2	comp12897 c0	comp12897	m.19178	sp|Q28GH3|SAE2 XENTR`Q28GH3`Q:13-366,H:15-	PF00899.16^ThiF^ThiF family^15-148^E:9.7e-	.	.	COG0476^	GO:000563	VKAVVGEERFAMIQSAKLLVVGAGGIGCELLKNLVLSGFREIEVVDLDTID	
	comp24876 c0	comp24876	m.63522	sp|Q9UBT2|SAE2 HUMAN`Q9UBT2`Q:1-345,H:175-	PF10585.4^UBA_e1_thiolCys^Ubiquitin-	.	.	COG0476^	GO:000563	IRSTPEKPVHCLTWAKNLFDLCFGPEDESNLLADLAAEMRKFQAQESVD	
	comp66751 c0	comp66751	m.130968	sp|Q9ZRT1|GR1 ARATH`Q9ZRT1`Q:367-430,H:519-	PF08573.5^SAE2^DNA repair protein	.	.	NOG14552	GO:000563	MLHSNDVQDAVSAEDPGGLSTTPASVRGISLQQAADILVRAVREAQCCE	
SGS1	-										
SLX1	comp118352 c	comp11835	m.12561	sp|B6JY16|SLX1 SCHJY`B6JY16`Q:20-123,H:3-115`44.25%ID`E:1e-	PF01541.19^GIY-YIG^GIY-YIG catalytic	.	.	.	GO:000563	SSSSSSSSDKSEESEEGRGYCCYVLRSFSGSRTYTGITTDLSRRLRQHN	
	comp95401 c0	comp95401	m.175788	sp|A7STV9|SLX1 NEMVE`A7STV9`Q:3-258,H:9-259`31.09%ID`E:2e-	PF01541.19^GIY-YIG^GIY-YIG catalytic	.	.	NOG29674	GO:000563	MQAFYGCYLLQSLSNNRRTYIGFTMDPSRRLRQHNGEITAGANRTKRW	
SLX4	comp45593 c0	comp45593	m.94221	.	PF09494.5^Slx4^Slx4 endonuclease^117-	.	.	.	.	MQFFGLKPSGSRQFMIRRLHDIVDYIDGGTAWLQSIREAPTPQKPTKAS	
SMC5	-										
SMC6	-										
XRCC4	-										
XRS2	-										
YEN1	-										

Appendix G

Table 6.5 Annotations for *Symbiodinium* Ubiquitin proteasome pathway components identified in the analysis.

								F-TEST (ANOVA) P	F-TEST (ANOVA) F	Day 4 25C and 30C Fold change	Student's t-test P value Day 4 25C	Student's t-test T value Day 4 25C	Day 19 25C and 30C Fold change	Student's test Day 19 25C and 30C	Student's t-test Day 19 25C and 30C	Day 28 25C and 30C Fold change	Day 28	Day 28	Standard deviation Day 4 25C	Standard deviation Day 4 30C	Standard deviation Day 19 25C	Standard deviation Day 19 30C	Standard deviation Day 28 25C	Standard deviation Day 28 30C
comp70849_c0	comp70	m.139851	sp|Q8MKD1	PF00240.18	COG5272^	GO:000573	FFHRQNPG	0.0000333	15.299	1.358 down	0.0275	5.071	1.620 down	0.043	3.93	1.678 down	0.00798	7.115	0.07198	0.15867	0.06846	0.34782	0.07663	0.19552
comp94301_c0	comp94	m.175015	sp|P0CG55|	PF11976.3^	.	GO:000573	AILAQERLT	4.17E-07	32.939	1.105 up	0.0152	-6.514	1.114 up	0.0131	-6.149	1.019 down	0.658	0.702	0.02008	0.03951	0.02032	0.04648	0.02483	0.07506
comp63607_c0	comp63	m.124834	sp|P0CG70|	PF00240.18	.	GO:000573	MIERMFDA	0.000029	15.678	1.048 up	0.535	-1.002	1.336 down	0.0051	9.31	1.289 down	0.0267	4.594	0.07976	0.11034	0.04615	0.07718	0.06991	0.14337
comp41564_c0	comp41	m.87312	sp|P0CG68|	PF00240.18	.	GO:000573	IEVSRFFIL	0.0000359	15.1	1.060 up	0.238	-1.948	1.227 up	0.0193	-5.282	1.173 up	0.0901	-2.947	0.05729	0.06599	0.08101	0.07744	0.12641	0.09203
comp94528_c0	comp94	m.175206	sp|P22589|U	PF13881.1^	.	GO:000573	LGSKIASA	0.0000318	15.423	1.172 up	0.376	-1.424	1.375 down	0.0813	3.077	1.395 down	0.013	5.938	0.14317	0.28938	0.09844	0.28209	0.09555	0.13077
comp60239_c0	comp60	m.118272	sp|P20973|U	PF00899.16	.	GO:000552	SDLAYRVL	2.35E-06	24.301	1.299 down	0.0149	6.566	1.144 up	0.0552	-3.573	1.255 up	0.0128	-5.967	0.04903	0.10424	0.04962	0.09693	0.09816	0.04999
comp18287_c0	comp18	m.44384	sp|Q7ZVX6|	PF00899.16	COG0476^	GO:001688	RESKVQVIR	0.0000122	18.249	1.329 up	0.0124	-7.14	1.205 up	0.0725	-3.225	1.091 up	0.176	-2.196	0.08399	0.07869	0.08325	0.14523	0.07735	0.08543
comp24246_c0	comp24	m.62549	sp|Q9D906|	PF00899.16	COG0476^	GO:000573	PLAMAQQA	0.000453	9.496	1.042 up	0.411	-1.32	1.317 up	0.023	-4.934	1.196 up	0.102	-2.802	0.084	0.03528	0.05668	0.15117	0.0906	0.16111
comp65163_c0	comp65	m.127798	sp|Q5U300|	PF02134.16^	COG0476^	GO:000552	QEFKRKA	5.09E-06	21.225	1.037 down	0.452	1.208	1.117 up	0.126	-2.571	1.162 up	0.0231	-4.838	0.07959	0.03536	0.06353	0.10716	0.07682	0.04655
comp105433_c0	comp10	m.3832	sp|P62840|	PF00179.21^	.	GO:000552	MEVLKADL	0.00136	7.639	1.260 up	0.0391	-4.382	1.117 down	0.276	1.701	1.077 down	0.485	1.086	0.12211	0.09161	0.15254	0.11062	0.13764	0.14265
comp57269_c0	comp57	m.112745	sp|Q9ZVX1|	PF00179.21^	NOG238109	GO:000582	WTAEPLQY	8.93E-06	19.25	1.229 down	0.0099	7.975	1.023 down	0.668	0.671	1.146 down	0.0798	3.09	0.04397	0.06028	0.08897	0.04901	0.10735	0.06921
comp77326_c0	comp77	m.155512	sp|P35129|	PF00179.21^	COG5078^	GO:000552	MALKRIQK	3.03E-07	34.912	1.157 up	0.0141	-6.749	1.149 up	0.0747	-3.185	1.005 up	0.821	-0.377	0.02735	0.0562	0.12102	0.03457	0.03267	0.02157
comp232587_c0	comp23	m.60012	sp|P62840|	PF00179.21^	.	GO:000552	ANCSAGPV	2.56E-06	23.954	1.039 down	0.926	0.172	3.575 up	0.0147	-5.853	2.413 up	0.0299	-4.413	0.5881	0.28114	0.35218	0.52008	0.35578	0.45327
comp43440_c0	comp43	m.90389	sp|P25867|	PF00179.21^	COG5078^	GO:000587	SCAMARPFI	0.0000295	15.631	1.057 down	0.338	1.544	1.148 down	0.0145	5.889	1.185 down	0.00458	8.857	0.04119	0.09656	0.06039	0.03095	0.05253	0.01796
comp65595_c0	comp65	m.128645	sp|Q6IE24|U	PF13423.1^	NOG291259	GO:000422	MFHGPGP	0.00003	15.581	1.391 up	0.0628	-3.626	1.704 up	0.0301	-4.472	1.698 up	0.00755	-7.264	0.2463	0.09211	0.219	0.26554	0.07197	0.19784
comp85253_c0	comp85	m.168377	sp|P23566|	PF00179.21^	COG5078^	GO:000582	MSTAATRR	1.82E-08	57.905	1.083 up	0.0829	-3.231	1.226 up	0.0164	-5.611	1.367 up	0.00539	-8.344	0.0396	0.06001	0.08105	0.06702	0.07638	0.07655
comp87175_c0	comp87	m.169730	sp|O74196|	PF00179.21^	.	GO:000552	MALRRIMKE	8.22E-06	19.528	1.038 down	0.676	0.687	1.319 down	0.0364	4.177	1.318 down	0.00503	8.555	0.11271	0.1103	0.10326	0.16105	0.01355	0.09228
comp112714_c0	comp11	m.8816	sp|O00102|	PF00179.21^	COG5078^	GO:000582	HLQLSIPHE	0.0238	3.909	1.062 up	0.405	-1.337	1.139 up	0.0472	-3.794	1.135 up	0.256	-1.797	0.05467	0.1188	0.04639	0.08774	0.18796	0.07889
comp52595_c0	comp52	m.105469	sp|O42646|	PF05139.9^	.	GO:000578	IVLASNKFR	0.00893	5.037	1.090 down	0.395	1.365	1.155 up	0.0435	-3.914	1.144 up	0.06	-3.443	0.03467	0.17924	0.06644	0.083	0.08441	0.07486
comp102516_c0	comp10	m.1695	sp|P33296|	PF00179.21^	COG5078^	GO:000578	MEAQSASA	0.00145	7.534	1.034 up	0.145	-2.528	1.117 up	0.187	-2.13	1.174 up	0.045	-3.819	0.03861	0.00571	0.05063	0.14219	0.0551	0.10852
comp26970_c0	comp26	m.67997	sp|Q6DG60|	PF00179.21^	COG5078^	GO:000573	MDSGARRL	0.000117	12.234	1.044 down	0.513	1.056	1.217 up	0.0561	-3.552	1.263 up	0.0183	-5.247	0.07726	0.08884	0.05906	0.14835	0.08877	0.09318
comp41873_c0	comp41	m.87814	sp|P70711|U	PF00179.21^	COG5078^	GO:000552	LKRASGSS	6.66E-06	20.259	1.181 down	0.0704	3.457	1.202 down	0.0503	3.706	1.300 down	0.0149	5.649	0.08673	0.10869	0.03767	0.13837	0.06175	0.11903
comp80793_c0	comp80	m.164979	sp|P15731|	PF00179.21^	.	GO:000050	MSLRASRH	7.48E-07	29.673	1.054 up	0.351	-1.502	1.008 down	0.878	0.26	1.259 up	0.0169	-5.396	0.07686	0.06604	0.09507	0.02956	0.05985	0.10813
comp85859_c0	comp85	m.168813	sp|Q9VM35|	.	NOG272407	GO:000552	YDTKSPHM	0.000445	9.525	1.021 up	0.568	-0.926	1.114 down	0.0771	3.144	1.238 down	0.0268	4.586	0.05784	0.03346	0.05074	0.08576	0.07826	0.1097
comp80791_c0	comp80	m.164968	sp|P35132|	PF00179.21^	.	GO:001688	MGASACS	0.00165	7.334	1.322 up	0.0965	-3.025	1.199 up	0.164	-2.267	1.345 up	0.0169	-5.392	0.25474	0.07982	0.17106	0.15626	0.11086	0.11374
comp81338_c0	comp81	m.165827	sp|Q6GPW2|	PF11976.3^	.	GO:000563	MAEGGDA	2.98E-07	35.023	1.104 up	0.0249	-5.263	1.021 down	0.804	0.403	1.114 down	0.186	2.137	0.05017	0.02097	0.04359	0.14822	0.13059	0.06581
comp82048_c0	comp82	m.166185	sp|Q9NGP4|	PF00179.21^	COG5078^	GO:000563	MASFASKR	1.54E-06	26.164	1.520 up	0.0124	-7.154	1.243 up	0.0274	-4.637	1.067 up	0.219	-1.961	0.05128	0.16099	0.12647	0.04923	0.03634	0.08949
comp89402_c0	comp89	m.171404	sp|Q6P8D9|	PF00179.21^	COG5078^	GO:000552	MIKIFGVGR	0.00521	5.714	1.000 down	0.997	0.012	1.048 up	0.494	-1.056	1.191 down	0.0138	5.811	0.10907	0.13522	0.11104	0.0656	0.0619	0.06093
comp76019_c0	comp76	m.152108	sp|Q5UQ40|	PF13519.1^	.	GO:000015	MSSLFCLG	0.000975	8.166	1.158 up	0.148	-2.503	1.158 up	0.0547	-3.587	1.269 up	0.0355	-4.156	0.09835	0.13788	0.08764	0.07914	0.14925	0.07252
comp110281_c0	comp11	m.7245	sp|F8W2M1|	PF00632.20^	.	GO:000578	EARNQSVL	1.07E-06	27.902	1.328 down	0.0283	5.004	1.546 down	0.0039	10.793	1.201 down	0.0372	4.092	0.08107	0.14255	0.05481	0.10281	0.10615	0.07395
comp118846_c0	comp11	m.12825	sp|Q84ME1|	PF13920.1^	NOG332026	GO:004687	GTTGTTGT	0.0195	4.131	1.287 up	0.129	-2.664	1.203 up	0.0456	-3.842	1.082 up	0.54	-0.954	0.23361	0.14251	0.09802	0.09909	0.07695	0.22723
comp84213_c0	comp84	m.167670	sp|F1N6G5|	PF13857.1^	.	GO:000578	MAAARTRR	0.0000986	12.606	1.062 up	0.195	-2.178	1.148 up	0.00923	-7.092	1.067 up	0.418	-1.258	0.05959	0.05404	0.03921	0.04044	0.08537	0.12377
comp27178_c0	comp27	m.68279	sp|Q7TMY8|	PF09409.5^	COG5021^	GO:000573	MADTTMSD	0.000157	11.588	1.083 up	0.182	-2.255	1.110 up	0.141	-2.435	1.242 up	0.0261	-4.633	0.03711	0.09536	0.08016	0.09493	0.09313	0.09789
comp100427_c0	comp10	m.274	sp|Q9FPH0|	PF13920.1^	NOG235818	GO:001687	RSLKERQR	0.0000293	15.65	1.260 up	0.0215	-5.635	1.343 up	0.00998	-6.848	1.303 up	0.0514	-3.643	0.08285	0.0846	0.07698	0.09772	0.14412	0.15264
comp101171_c0	comp10	m.746	sp|Q9ZU51|	PF13639.1^z	NOG328417	GO:004687	NGVAVVAM	0.0000317	15.434	1.325 down	0.0369	4.488	1.365 down	0.0103	6.791	1.261 down	0.0405	3.969	0.08395	0.16079	0.07665	0.10797	0.15064	0.07601
comp125847_c0	comp12	m.17311	sp|Q8VYC8|	PF13639.1^z	COG5243^	GO:001602	GGSTVLHL	9.48E-09	65.272	1.240 up	0.0145	-6.651	1.400 up	0.00696	-8.019	1.361 up	0.0562	-3.525	0.07232	0.05919	0.09505	0.07512	0.20965	0.14063
comp21112_c0	comp21	m.54313	sp|Q6WWW	PF00632.20^	COG5021^	GO:000562	PPVMIVSSD	6.76E-08	45.571	1.369 up	0.0406	-4.317	1.281 up	0.0488	-3.747	1.307 up	0.0134	-5.873	0.09957	0.18524	0.06329	0.17995	0.1094	0.0734
comp31775_c0	comp31	m.74904	sp|Q810L3|C	PF00498.21	NOG253207	GO:001660	LQQTVKALL	8.39E-09	66.718	2.248 down	0.0054	10.518	2.871 down	0.00536	9.052	2.237 down	0.00158	15.092	0.07483	0.20927	0.14033	0.30552	0.06653	0.13887
comp34342_c0	comp34	m.78075	sp|Q8GY23|	PF00632.20^	COG5021^	GO:000562	PSLQSICS	0.0000648	13.596	1.142 up	0.0114	-7.437	1.188 up	0.0205	-5.164	1.246 up	0.0368	-4.107	0.04361	0.02802	0.07196	0.06453	0.08191	0.13105
comp56396_c0	comp56	m.111397	sp|Q9JI90|R	PF00097.20	NOG266709	GO:000573	REVRDVTQ	1.25E-08	61.911	1.380 up	0.00442	-11.857	1.741 up	0.00302	-12.605	1.518 up	0.00765	-7.226	0.06679	0.04101	0.07488	0.10258	0.1036	0.13069
comp59743_c1	comp59	m.117271	sp|O95714|H	PF00569.12^	COG5021^	GO:000581	MDEEMEV	7.94E-07	29.365	1.290 up	0.0225	-5.512	1.343 up	0.0182	-5.399	1.290 up	0.00433	-9.071	0.03992	0.12738	0.0625	0.14472	0.0738	0.03372
comp66718_c0	comp66	m.130870	sp|Q5ZIJ9|	PF00023.25^	COG0666^	GO:000573	MRRLLLML	1.07E-07	41.94	1.189 up	0.0161	-6.354	1.267 up	0.0411	-3.999	1.580 up	0.00153	-15.426	0.0451	0.06442	0.14582	0.08977	0.08272	0.02202
comp72509_c0	comp72	m.143720	sp|Q6CHI1|	PF13920.1^	COG5432^	GO:000563	SPQRHSGT	1.20E-06	27.344	1.229 up	0.0193	-5.881	1.276 up	0.0347	-4.25	1.365 up	0.0122	-6.079	0.02864	0.09709	0.11886	0.11531	0.13002	0.0704
comp77019_c0	comp77	m.154631	sp|Q3UIR3|	PF13920.1^	NOG84763^	GO:000573	EPLEPLELL	4.53E-07	32.426	1.205 up	0.0315	-4.792	1.513 up	0.0186	-5.359	1.523 up	0.00283	-10.933	0.07553	0.0833	0.13505	0.17744	0.10519	0.03572
comp101094_c0	comp10	m.694	sp|Q5M807|	PF13639.1^z	NOG300581	GO:000578	RFPAALMF	0.000161	11.534	1.282 up	0.0184	-6.006	1.218 up	0.0305	-4.448	1.101 up	0.224	-1.937	0.07091	0.09643	0.08102	0.09957	0.10113	0.10321
comp103583_c0	comp10	m.2507	sp|Q9LYW5|	PF13920.1^	NOG332026	GO:004687	WLKLHVLR	0.0000197	16.784	1.274 up	0.0257	-5.208	1.416 up	0.013	-6.153	1.126 up	0.221	-1.952	0.09554	0.09424	0.08651	0.13862	0.0763	0.15901
comp105690_c0	comp10	m.4002	sp|Q9LT17|	PF00787.19	NOG313539	GO:004687	KLSRLKTW	7.34E-06	19.915	1.229 down	0.0404	4.324	1.139 up	0.0418	-3.974	1.097 up	0.19	-2.113	0.07993	0.11246	0.02516	0.09137	0.06686	0.10767
comp15419_c0	comp15	m.32356	sp|Q8GY23|	PF00632.20^	COG5021^	GO:000562	SHRALDPA	0.0000618	13.714	1.269 up	0.036	-4.53	1.373 up	0.0429	-3.934	1.264 up	0.0723	-3.209	0.05441	0.14169	0.16777	0.1611	0.20104	0.06301
comp18673_c0	comp18	m.45997	sp|Q6PAV2|	PF00415.13^	COG5021^	GO:000573	QVEQGPRG	1.26E-06	27.115	1.264 up	0.0019	-18.507	1.330 up	0.0109	-6.611	1.179 up	0.0656	-3.328	0.02365	0.02799	0.06193	0.10818	0.12571	0.06778
comp62245_c0	comp62	m.122162	sp|Q8BT14|	.	COG5175^	GO:000573	KIYQLGLP	2.47E-06	24.094	1.385 down	0.0154	6.467	1.337 down	0.00619	8.471	1.030 down	0.71	0.598	0.02891	0.14247	0.0675	0.07235	0.08271	0.12178

comp64175_c0	comp64	m.125932	sp|Q7YRZ8|	PF13920.1^	.	GO:000573	QSPSWRQ	1.96E-07	37.721	1.187 down	0.0345	4.608	1.120 up	0.0148	-5.843	1.103 up	0.194	-2.091	0.10116	0.03727	0.04069	0.03898	0.09876	0.09259
comp73266_c0	comp73	m.145429	sp|Q8R516|		COG0666^	GO:000576	HLSWTKQP	6.45E-06	20.369	1.337 up	0.0109	-7.638	1.422 up	0.0112	-6.535	1.191 up	0.26	-1.783	0.01506	0.10875	0.0788	0.13428	0.17608	0.22191
comp79403_c0	comp79	m.161135	sp|Q6PAV2|	PF13540.1^	COG5021^	GO:000573	AGRYHSLLL	0.0000155	17.52	1.389 up	0.0124	-7.135	1.409 up	0.0121	-6.338	1.241 up	0.0848	-3.02	0.09996	0.0879	0.13607	0.07672	0.10926	0.17547
comp92274_c0	comp92	m.173537	sp|Q91XF4|	PF13414.1^	COG5540^	GO:000573	EPPATAET	3.14E-06	23.111	1.327 up	0.0234	-5.416	1.332 up	0.00361	-11.319	1.037 up	0.491	-1.073	0.07573	0.1308	0.05378	0.04956	0.05649	0.08242
comp124817_c0	comp12	m.16675	sp|Q8L649|	PF12678.2^	NOG327333	GO:004687	LHAMAQSR	6.21E-07	30.626	1.417 down	0.0222	5.555	1.166 down	0.064	3.381	1.321 down	0.0135	5.868	0.0479	0.17469	0.08697	0.09822	0.09327	0.10053
comp16287_c0	comp16	m.36274	sp|Q9VR91|	PF13540.1^	COG5021^	GO:000581	LQAALHGG	0.00104	8.065	1.432 up	0.0407	-4.314	1.105 up	0.326	-1.525	1.205 up	0.031	-4.354	0.17146	0.16861	0.14062	0.12748	0.06547	0.10535
comp27607_c0	comp27	m.68853	sp|Q9H992|	PF12906.2^	NOG124954	GO:001687	TGVKVVAST	5.65E-08	47.121	1.548 down	0.0492	3.996	1.009 down	0.9	0.214	1.137 down	0.0359	4.142	0.10702	0.29705	0.07932	0.1047	0.07732	0.04551
comp71955_c0	comp71	m.142348	sp|O15164|	PF00643.19	COG5076^	GO:000582	SWQLATHG	0.0000498	14.254	1.372 down	0.0291	4.952	1.136 down	0.118	2.638	1.247 down	0.0579	3.488	0.14994	0.10731	0.05828	0.12712	0.13857	0.11969
comp72904_c0	comp72	m.144648	sp|C5FHU9|	PF01466.14^	.	GO:001656	LKPSWLGL	1.05E-08	64.078	1.303 up	0.0116	-7.352	1.237 up	0.0541	-3.605	1.551 up	0.00307	-10.503	0.07364	0.0733	0.04788	0.16356	0.08139	0.08915
comp89443_c0	comp89	m.171435	sp|P0C8K8|	PF00097.20	.	GO:001687	SIPCAVRTA	0.000147	11.719	1.233 down	0.0355	4.557	1.626 down	0.0559	3.556	1.894 down	0.0203	5.061	0.06783	0.11421	0.03405	0.39342	0.03902	0.36219
comp107550_c0	comp10	m.5240	sp|Q9UKV5|	PF13639.1^z	COG5243^	GO:003042	PAMSCFGT	0.000621	8.926	1.608 down	0.0265	5.15	1.275 down	0.161	2.293	1.114 up	0.538	-0.96	0.07552	0.2554	0.07573	0.29646	0.20988	0.24961
comp137632_c0	comp13	m.24084	sp|Q63HN8|	.	NOG86922^	GO:000573	PRVMWSG	0.0015	7.477	1.381 down	0.0269	5.116	1.094 down	0.559	0.905	1.060 up	0.572	-0.884	0.09912	0.15285	0.23442	0.16686	0.15901	0.10894
comp148734_c0	comp14	m.29824	sp|Q6WWW	.	COG5021^	GO:000562	VKPERSKV	0.0021	6.978	1.409 down	0.027	5.113	1.268 down	0.258	1.777	1.002 down	0.982	0.044	0.11562	0.15563	0.17535	0.34364	0.14162	0.12634
comp156541_c0	comp15	m.33409	sp|Q15034|H	PF00415.13^	COG5021^	GO:000573	WTMFAATA	0.0158	4.365	1.177 down	0.0191	5.919	1.005 up	0.98	-0.047	1.291 up	0.0709	-3.231	0.05586	0.05675	0.16753	0.30003	0.20176	0.10717
comp212156_c0	comp21	m.54647	sp|Q6PAV2|	PF00415.13^	COG5021^	GO:000573	PRRVACGN	1.35E-06	26.771	1.392 down	0.0172	6.193	1.091 down	0.465	1.129	1.179 up	0.109	-2.727	0.08817	0.12648	0.18692	0.12354	0.13418	0.1124
comp41610_c0	comp41	m.87380	sp|Q5XIK5|	PF02825.15^	NOG298024	GO:000582	FGSSPFVTF	0.000135	11.91	1.205 up	0.00915	-8.233	1.025 down	0.483	1.084	1.050 up	0.504	-1.04	0.0572	0.03205	0.05339	0.0414	0.13714	0.01248
comp61728_c0	comp61	m.121197	sp|Q99PP7|	PF00643.19^	COG5076^	GO:000563	MSIASSPSQ	0.0000399	14.825	1.294 up	0.0177	-6.106	1.034 up	0.734	-0.54	1.021 down	0.644	0.731	0.05643	0.10827	0.16847	0.07005	0.03973	0.07473
comp63526_c0	comp63	m.124677	sp|Q9Y252|	PF13920.1^z	COG5540^	GO:003042	RRFFPHFRI	0.000822	8.447	1.354 down	0.0482	4.029	1.288 down	0.0709	3.254	1.234 down	0.0718	3.217	0.09326	0.19631	0.19678	0.10855	0.15974	0.10095
comp65631_c0	comp65	m.128703	sp|Q9D0C1|	PF13639.1^z	NOG235630	GO:000582	LPVMQLQQ	0.000203	11.047	1.318 down	0.027	5.113	1.163 down	0.0761	3.163	1.025 up	0.748	-0.52	0.13731	0.07366	0.1128	0.07989	0.11477	0.08301
comp67544_c0	comp67	m.132696	sp|Q9NVW2|	PF13639.1^z	COG5540^	GO:000573	MSVTDAWIII	0.00192	7.111	1.240 up	0.0354	-4.56	1.018 up	0.879	-0.256	1.150 up	0.285	-1.682	0.10444	0.08776	0.09182	0.18752	0.19266	0.14342
comp7169_c0	comp71	m.141792	sp|Q5GLZ8|	PF13540.1^	COG5021^	GO:000573	GRPEATEV	0.0684	2.823	1.251 down	0.0296	4.916	1.051 up	0.762	-0.486	1.049 down	0.779	0.461	0.09658	0.08981	0.23385	0.18344	0.20143	0.22237
comp73290_c0	comp73	m.145511	sp|Q6GPV5|	PF13639.1^z	.	GO:001687	RTAVSSHK	0.0000127	18.13	1.271 down	0.0375	4.457	1.047 down	0.457	1.149	1.005 up	0.955	-0.104	0.06434	0.14177	0.10849	0.04362	0.09311	0.11855
comp77825_c0	comp77	m.156759	sp|Q9FKG6|	PF00069.20^	COG0515^	GO:000015	MNDQKAQQ	0.000125	12.078	1.284 up	0.0175	-6.143	1.071 up	0.297	-1.622	1.088 up	0.254	-1.808	0.09809	0.06518	0.06899	0.10128	0.06158	0.12005
comp100561_c0	comp10	m.337	sp|Q7XI08|X	PF13920.1^z	COG0666^	GO:001687	CNPPNKMP	4.20E-06	21.953	1.121 up	0.0569	-3.772	1.421 up	0.00621	-8.461	1.348 up	0.0229	-4.85	0.077	0.04253	0.10254	0.06219	0.11121	0.13876
comp109366_c0	comp10	m.6605	sp|Q9SCQ2|	PF00632.20^	COG5021^	GO:000562	LCLRFRPKE	0.0000901	12.815	1.143 up	0.402	-1.345	1.521 up	0.0165	-5.602	1.416 up	0.00133	-17.779	0.11506	0.26449	0.1293	0.17312	0.02224	0.05194
comp115062_c0	comp11	m.10365	sp|Q24306|I	PF07647.12^	NOG243347	GO:000562	QAVTLDVP	0.00012	12.178	1.197 down	0.236	1.953	1.183 down	0.0228	4.952	1.197 down	0.0252	4.688	0.08604	0.25199	0.05442	0.08165	0.06603	0.08932
comp12353_c0	comp12	m.15852	sp|Q8GY23|	PF00632.20^	COG5021^	GO:000562	FLRVVRTLT	7.51E-08	44.712	1.197 up	0.0623	-3.641	1.261 up	0.0405	-4.022	1.329 up	0.00946	-6.658	0.03425	0.1386	0.11888	0.11697	0.11844	0.03496
comp18836_c0	comp18	m.46481	sp|Q20798|	PF13639.1^z	COG5243^	GO:000578	SELIGSAREI	0.000016	17.407	1.220 down	0.074	3.39	1.476 down	0.0112	6.545	1.234 down	0.0554	3.543	0.0166	0.16916	0.14637	0.08977	0.06454	0.15925
comp21446_c0	comp21	m.55148	sp|Q80V91|	PF13923.1^z	NOG84763^	GO:000573	MLDFQFWS	4.89E-07	31.949	1.092 up	0.103	-2.949	1.153 up	0.0392	-4.07	1.297 up	0.00887	-6.835	0.03262	0.08044	0.0512	0.08698	0.08166	0.07374
comp22114_c0	comp22	m.57371	sp|Q8LBL5|	.	NOG251630	GO:000573	LHVYLRCN	2.31E-06	24.382	1.098 up	0.0783	-3.31	1.323 up	0.0223	-4.998	1.330 up	0.00163	-14.744	0.06418	0.0505	0.11998	0.10862	0.03201	0.04574
comp22570_c0	comp22	m.58497	sp|Q86Y13|	PF13639.1^z	NOG126093	GO:000573	MTSGHHALI	4.21E-06	21.946	1.296 down	0.054	3.851	1.515 down	0.0208	5.138	1.470 down	0.0122	6.073	0.10995	0.1608	0.1133	0.20423	0.08471	0.16229
comp24737_c0	comp24	m.63309	sp|Q9LT17|	PF13920.1^z	NOG313539	GO:004687	MEGRLENL	0.000105	12.477	1.113 up	0.27	-1.806	1.327 up	0.02	-5.21	1.284 up	0.0447	-3.828	0.08991	0.14705	0.10443	0.1171	0.08025	0.17105
comp27180_c0	comp27	m.68285	sp|Q9SIZ8|	PF00023.25^	NOG303191	GO:001602	LTPMHIAAR	8.43E-06	19.439	1.148 down	0.245	1.913	1.778 down	0.0213	5.096	1.586 down	0.00207	13.018	0.15424	0.14034	0.13714	0.29588	0.05042	0.08898
comp28501_c0	comp28	m.70148	sp|Q80SY4|	PF12796.2^	COG0666^	GO:000581	HRLPLAFLI	0.0000119	18.342	1.056 up	0.515	-1.051	1.392 down	0.0216	5.07	1.474 down	0.00635	7.824	0.10187	0.10989	0.06148	0.17812	0.12671	0.06661
comp300900_c0	comp30	m.72777	sp|Q9VR91|	PF06701.8^	COG5021^	GO:000581	KKAILKEGD	0.000137	11.872	1.025 up	0.97	-0.075	1.608 up	0.0394	-4.063	3.739 up	0.00786	-7.157	0.60374	0.77872	0.30467	0.14475	0.44599	0.28958
comp36497_c0	comp36	m.80713	sp|Q08109|	PF12678.2^z	COG5243^	GO:000083	MGALPSAM	7.79E-06	19.716	1.276 down	0.143	2.539	1.580 down	0.00643	8.339	1.321 down	0.00726	7.379	0.15141	0.23244	0.07438	0.13994	0.02876	0.10527
comp41532_c0	comp41	m.87279	sp|Q8GYT9|	PF13639.1^z	NOG317826	GO:001602	LKCPQWAP	0.000201	11.066	1.021 down	0.866	0.299	1.434 down	0.0345	4.259	1.322 down	0.00253	11.643	0.13116	0.16017	0.04335	0.24043	0.05375	0.04356
comp46330_c0	comp46	m.95421	sp|Q9WTV7|	PF13639.1^z	COG5540^	GO:000573	MGCCDSKP	7.84E-07	29.43	1.090 down	0.149	2.491	1.232 up	0.0119	-6.377	1.224 up	0.0113	-6.239	0.06813	0.07347	0.08285	0.04599	0.07039	0.06204
comp46963_c0	comp46	m.96510	sp|Q5RCV8|	PF12678.2^z	.	GO:000578	MVPPTACG	7.05E-06	20.056	1.094 down	0.436	1.252	1.455 down	0.00468	9.765	1.621 down	0.00933	6.695	0.16731	0.12472	0.09364	0.05958	0.08758	0.18909
comp61749_c0	comp61	m.121230	sp|Q20798|	PF13639.1^z	COG5243^	GO:000578	FRSHICRAV	3.87E-06	22.278	1.022 down	0.693	0.653	1.275 down	0.00648	8.316	1.121 down	0.0106	6.401	0.09436	0.03258	0.01351	0.08339	0.04064	0.03195
comp63557_c0	comp63	m.124736	sp|Q4U2R1|	.	COG5021^	GO:000581	MSKMEGYQ	0.000012	18.3	1.122 down	0.285	1.742	1.427 down	0.0249	4.802	1.204 down	0.0246	4.728	0.16337	0.09901	0.06202	0.20466	0.08441	0.07622
comp66212_c0	comp66	m.129771	sp|Q9H992|	PF12906.2^	NOG124954	GO:001687	MSAQAPGP	1.94E-06	25.152	1.088 down	0.142	2.548	1.263 up	0.0249	-4.803	1.160 up	0.0269	-4.582	0.08351	0.04848	0.11255	0.08372	0.04018	0.08458
comp66667_c0	comp66	m.130744	sp|Q8C863|I	PF09409.5^	COG5021^	GO:000593	PAVPVSDS	0.000212	10.959	1.035 up	0.777	-0.481	1.309 up	0.0161	-5.654	1.418 up	0.0136	-5.837	0.06992	0.19742	0.09379	0.10084	0.11842	0.1257
comp71559_c0	comp71	m.141464	sp|O14326|	PF02825.15^	COG5021^	GO:000562	FANLAPHW	2.89E-06	23.442	1.162 up	0.0508	-3.942	1.522 up	0.0212	-5.1	1.451 up	0.0031	-10.472	0.10881	0.01872	0.15465	0.18056	0.06221	0.08164
comp72847_c0	comp72	m.144510	sp|Q9LVW9|	PF01485.16^	NOG313429	GO:001687	LVYFAMDD	4.54E-06	21.653	1.526 up	0.0897	-3.123	1.991 up	0.00736	-7.829	2.042 up	0.00469	-8.787	0.26286	0.28914	0.19719	0.16002	0.0804	0.22037
comp75712_c0	comp75	m.151418	sp|Q15034|	PF13540.1^	COG5021^	GO:000573	SVVVHPST	5.37E-06	21.028	1.150 up	0.0887	-3.137	1.075 up	0.0453	-3.849	1.131 up	0.00402	-9.336	0.05971	0.11406	0.03712	0.03992	0.03006	0.02346
comp76728_c0	comp76	m.153868	sp|Q9LT17|	PF12678.2^z	NOG313539	GO:004687	HGRRRREA	2.03E-06	24.947	1.162 up	0.0705	-3.457	1.256 up	0.0224	-4.983	1.272 up	0.0174	-5.342	0.08694	0.09037	0.08094	0.10467	0.08394	0.09944
comp81624_c0	comp81	m.165977	sp|Q8GYT9|	PF12678.2^z	NOG317826	GO:001602	ACVLYKPN	6.46E-06	20.363	1.017 down	0.79	0.454	1.120 down	0.0293	4.52	1.143 down	0.0501	3.675	0.06466	0.09066	0.05649	0.04575	0.0729	0.0759
comp86109_c0	comp86	m.168975	sp|E7FAM5|	PF01436.16^	.	GO:000093	MTQVSEKA	3.94E-06	22.208	1.068 down	0.28	1.765	1.431 down	0.0163	5.634	1.406 down	0.00558	8.209	0.07769	0.07622	0.01577	0.18307	0.0852	0.08418
comp922_c0	comp92	m.173552	sp|Q6NRV8|	PF13639.1^z	.	GO:000573	LTLWTTVAL	0.000777	8.539	1.245 up	0.235	-1.958	1.798 up	0.0499	-3.716	1.426 up	0.021	-5.01	0.243	0.21393	0.34462	0.29796	0.10867	0.17326
comp94671_c0	comp94	m.175302	sp|Q8LPN7|	PF12678.2^z	NOG235630	GO:001687	MDPGLLGA	5.46E-07	31.316	1.133 down	0.103	2.943	1.218 down	0.0129	6.18	1.278 down	0.00266	11.301	0.10031	0.07024	0.05832	0.07131	0.04761	0.04075
comp97502_c0	comp97	m.177270	sp|O60103|	PF12906.2^	COG5183^	GO:003017	AEIECKVMS	2.13E-06	24.734	1.058 up	0.203	-2.131	1.233 up	0.00464	-9.82	1.278 up	0.0132	-5.912	0.06488	0.04064	0.02747	0.05526	0.03793	0.11362
comp101589_c0	comp10	m.1026	sp|Q8NA82|	PF12906.2^	NOG246774	GO:001687	GPPFIVQNN	0.000523	9.231	1.020 up	0.642	-0.761	1.154 up	0.0288	-4.553	1.033 down	0.519	1.004	0.06436	0.04147	0.06438	0.06403	0.06326	0.07175
comp108989_c0	comp10	m.6296	sp|Q8RWB8|	PF00632.20^	COG5021^	GO:000562	SSQASAEK	0.0000174	17.141	1.181 down	0.0976	3.01	1.239 down	0.0156	5.737	1.100 down	0.265	1.76	0.03778	0.15539	0.07931	0.07301	0.11013	0.11167
comp110767_c0	comp11	m.7585	sp|Q5M807|	PF13920.1^z	NOG300581	GO:000578	QHFGQVLF	0.000331	10.076	1.265 up	0.139	-2.575	1.552 up	0.0132	-6.119	none	1	-0.004	0.16193	0.20777	0.05273	0.20045	0.14766	0.18397
comp111287_c0	comp11	m.7943	sp|Q9R1W3|	PF13639.1^z	COG5540^	GO:000578	RLRPLQVSL	0.0000645	13.608	1.254 down	0.0713	3.441	1.220 down	0.0314	4.404	1.025 down	0.679	0.658	0.12915	0.13914	0.06709	0.11222	0.09214	0.05974
comp12484_c0	comp12	m.16695	sp|Q86Y13|	PF13920.1^z	NOG126093	GO:000573	GGPCGRNR	0.000994	8.135	1.384 down	0.0573	3.763	1.466 down	0.0113	6.509	1.287 down	0.185	2.143	0.22399	0.1098	0.12444	0.11555	0.29643	0.16712
comp14000_c0	comp14	m.25337	sp|D3ZVM4|	PF01436.16^	.	GO:000093	AWDAMAPT	0.000173	11.382	1.024 up	0.673	-0.696	1.510 up	0.0265	-4.703	1.137 up	0.185	-2.144	0.03099	0.09582	0.20166	0.15307	0.14487	0.09615
comp142571_c0	comp14	m.26709	sp|Q9SKC3|	PF01485.16^	NOG327249	GO:001687	PLGGLQEV	0.00522	5.712	1.218 up	0.194	-2.182	1.621 up	0.0159	-5.686	1.189 up	0.432	-1.219	0.15866	0.20721	0.21255	0.12241	0.23572	0.33625
comp144310_c0	comp14	m.27641	sp|Q9SKC4|	PF01485.16^	NOG327249	GO:001687	ICFGEDGVL	0.0609	2.937	1.298 up	0.356	-1.486	1.442 up	0.0401	-4.037	1.124 up	0.303	-1.616	0.43164	0.26593	0.21016	0.15635	0.20309	0.04881
comp173679_c0	comp17	m.40809	sp|Q84RR0|	.	NOG327249	GO:001687	PPELHRAR	0.213	1.763	3.574 up	0.468	-1.168	2.195 up	0.0428	-3.937	1.710 up	0.327	-1.53	3.13783	0.23295	0.40526	0.40993	0.92049	0.41972
comp17390_c0	comp17	m.40910	sp|Q8IVU3|	PF13540.1^	COG5021^	GO:000573	VLLRSDGS	0.0000198	16.773	1.308 up	0.0508	-3.944	1.623 up	0.0152	-5.794	1.202 up	0.0621	-3.401	0.17523	0.08969	0.16521	0.17602	0.09191	0.12628
comp17711_c0	comp17	m.42026	sp|Q6TEM9|	PF13920.1^z	NOG149394	GO:000573	WIPFSSLTL	0.0000746	13.262	1.022 down	0.756	0.523	1.215 down	0.0303	4.46	1.021 up	0.509	-1.03	0.08877	0.08093	0.06108	0.11075	0.05674	0.01882
comp178237_c0	comp17	m.42530	sp|O95714|	PF00569.12^	COG5021^	GO:000581	KKAKKLAK	0.0000472	14.388	1.613 up	0.0679	-3.512	2.105 up	0.00963	-6.965	1.408 up	0.193	-2.096	0.37061	0.13169	0.3029	0.05832	0.24423	0.40321
comp21922_c0	comp21	m.56734	sp|Q9JI90|R	PF05773.17^	NOG266709	GO:000573	NRCHECST	0.00838	5.115	1.159 up	0.0972	-3.014	1.157 up	0.0277	-4.62	1.003 down	0.975	0.059	0.06924	0.12316	0.04943	0.0767	0.06376	0.16991

comp219462_c0	comp21	m.56799	sp|Q8GY23|	PF14377.1^	COG5021^	GO:000562	DAVVLEALP	0.00243	6.767	1.190 down	0.448	1.221	1.752 up	0.0329	-4.329	1.279 up	0.186	-2.141	0.20239	0.35841	0.29077	0.2352	0.20538	0.2616
comp40887_c0	comp40	m.86178	sp|Q6PAV2|	PF00632.20^	COG5021^	GO:000573	MQQYVTYV	0.00017	11.412	1.080 down	0.207	2.104	1.265 up	0.0244	-4.839	1.152 up	0.114	-2.679	0.0625	0.08584	0.11535	0.07969	0.15203	0.01573
comp48858_c0	comp48	m.99590	sp|Q99ML9|	PF12678.2^z	NOG291583	GO:000573	LNYCYFRS	0.000783	8.526	1.204 up	0.226	-2.005	2.068 up	0.0245	-4.83	1.219 up	0.242	-1.856	0.11486	0.24235	0.34589	0.2624	0.17901	0.25057
comp48940_c0	comp48	m.99732	sp|P36096|T	PF13920.1^z	COG5540^	GO:000579	SHPTAAAS	0.0284	3.717	1.010 down	0.929	0.167	1.265 up	0.0262	-4.723	1.095 up	0.458	-1.153	0.15578	0.10589	0.06449	0.12867	0.21489	0.07483
comp58743_c0	comp58	m.115324	sp|P0CH30|	PF13504.1^L	.	GO:001687	SLSFAFAAG	0.00581	5.574	1.111 up	0.614	-0.823	1.290 up	0.0178	-5.455	1.253 up	0.285	-1.684	0.14712	0.34041	0.1123	0.07474	0.26397	0.28264
comp59070_c0	comp59	m.115942	sp|Q9NWF9	PF01485.16^	NOG330241	GO:000573	PMATVDVD	8.40E-06	19.453	1.062 down	0.472	1.157	1.172 up	0.0197	-5.238	1.159 down	0.0763	3.142	0.11986	0.0937	0.0424	0.07647	0.09514	0.0975
comp73032_c0	comp73	m.144915	sp|Q4FE47|	PF12796.2^	NOG317888	GO:000573	AEICCAMSK	0.00634	5.462	1.087 up	0.269	-1.81	1.253 up	0.0222	-5.007	1.075 up	0.424	-1.242	0.08455	0.10447	0.08992	0.09426	0.09088	0.14228
comp76622_c0	comp76	m.153566	sp|Q9VR91|	.	COG5021^	GO:000581	MLQLHVAL	0.000681	8.766	1.209 up	0.0569	-3.774	1.254 up	0.0163	-5.626	1.272 up	0.0805	-3.08	0.05619	0.13405	0.06159	0.09883	0.20378	0.09806
comp76692_c0	comp76	m.153754	sp|Q8IVU3|	PF13540.1^	COG5021^	GO:000573	MALHSVLLR	0.00432	5.964	1.244 up	0.152	-2.471	1.250 up	0.0116	-6.452	1.248 up	0.157	-2.325	0.18111	0.17959	0.08593	0.05079	0.19664	0.19272
comp76785_c0	comp76	m.153987	sp|Q9DBU5|	PF13639.1^z	COG5540^	GO:003042	KTEKNMGN	0.0000765	13.204	1.019 down	0.837	0.359	1.218 down	0.0158	5.696	1.141 down	0.0855	3.01	0.13035	0.08172	0.07112	0.07064	0.07233	0.10387
comp94517_c0	comp94	m.175194	sp|F1RCR6|	PF00632.20^	.	GO:000565	AGAAGAAE	4.32E-07	32.72	1.262 up	0.108	-2.885	1.190 up	0.0291	-4.531	1.210 up	0.0669	-3.304	0.1253	0.19642	0.09326	0.06028	0.12773	0.10734
comp104178_c0	comp10	m.2970	sp|Q8K243|	.	NOG252602	GO:000563	MQKARAEL	0.00274	6.594	1.113 up	0.202	-2.138	1.131 up	0.215	-1.976	1.219 up	0.0282	-4.497	0.07209	0.12626	0.10858	0.14481	0.09219	0.08784
comp105896_c0	comp10	m.4142	sp|Q9ERV1|	PF00642.19^	COG5084^	GO:000562	SSWLKMAR	0.00048	9.389	1.073 down	0.511	1.06	1.180 down	0.133	2.507	1.338 down	0.0137	5.832	0.14604	0.12608	0.12369	0.14601	0.06943	0.12645
comp127078_c0	comp12	m.18038	sp|Q9ZT50|	PF13639.1^z	NOG316107	GO:000573	MTSVIPADV	0.00561	5.617	1.130 down	0.375	1.427	1.111 up	0.394	-1.315	1.376 up	0.0128	-5.981	0.11003	0.22186	0.22451	0.05735	0.07567	0.13431
comp142589_c0	comp14	m.26718	sp|Q9VE61|	PF13639.1^z	NOG294567	GO:001687	LEAWYLQG	5.18E-07	31.626	1.615 down	0.095	3.046	1.374 down	0.0535	3.62	1.326 down	0.0103	6.469	0.15379	0.42743	0.15157	0.20331	0.03731	0.12023
comp143554_c0	comp14	m.27230	sp|Q9LYZ7|	PF00632.20^	COG5021^	GO:000562	HDRQTREV	0.0866	2.595	1.087 down	0.534	1.005	1.076 up	0.557	-0.91	1.229 down	0.0112	6.26	0.22057	0.09473	0.20028	0.12311	0.03799	0.0873
comp18224_c0	comp18	m.44062	sp|Q803I8|S	PF12678.2^z	COG5243^	GO:000578	KYPFATSG	8.12E-06	19.573	1.135 down	0.0729	3.41	1.098 down	0.2	2.056	1.277 down	0.00573	8.12	0.03433	0.10199	0.08587	0.09968	0.02723	0.08268
comp199132_c0	comp19	m.50560	sp|Q6WWW|	.	COG5021^	GO:000562	EAETPQGM	0.00248	6.737	1.129 down	0.545	0.977	1.192 up	0.193	-2.092	1.544 up	0.0245	-4.737	0.16927	0.31713	0.14967	0.19104	0.19606	0.17808
comp21147_c0	comp21	m.54434	sp|Q5GLZ8|	PF13540.1^	COG5021^	GO:000573	RACVAVEP	0.00315	6.395	1.041 down	0.32	1.606	1.052 down	0.282	1.68	1.098 down	0.0278	4.519	0.03456	0.06442	0.07993	0.03607	0.03816	0.04658
comp24141_c0	comp24	m.62313	sp|Q9LFH6|	PF13920.1^z	NOG332026	GO:004687	VRQGWGV	0.00959	4.95	1.191 up	0.0505	-3.952	1.015 down	0.887	0.242	1.138 down	0.0494	3.692	0.04982	0.11784	0.07869	0.16757	0.05818	0.08289
comp25763_c0	comp25	m.65563	sp|Q93Z92|	PF12678.2^z	NOG288486	GO:001602	MQRADTAS	2.47E-06	24.092	1.023 down	0.512	1.058	1.082 down	0.229	1.904	1.156 down	0.0129	5.964	0.05968	0.02028	0.09608	0.07193	0.05143	0.04772
comp258260_c0	comp25	m.65742	sp|Q6PAV2|	PF13540.1^	COG5021^	GO:000573	PQPASFVEL	0.0689	2.816	2.373 down	0.271	1.8	1.477 down	0.363	1.406	1.980 down	0.0204	5.051	0.7126	1.18822	0.23412	0.76612	0.13023	0.36796
comp28830_c0	comp28	m.70741	sp|Q6PAV2|	PF13540.1^	COG5021^	GO:000573	TDLGICHVT	0.00228	6.857	1.105 up	0.438	-1.247	1.325 up	0.161	-2.289	1.470 up	0.023	-4.841	0.16004	0.16852	0.32198	0.1491	0.22005	0.06619
comp30588_c0	comp30	m.73446	sp|Q8LPN7|	PF13920.1^z	NOG235630	GO:001687	MSGPPPFF	0.000316	10.169	1.070 down	0.284	1.746	1.023 down	0.752	0.503	1.166 down	0.0362	4.131	0.02536	0.11013	0.03968	0.12754	0.09303	0.05374
comp35711_c0	comp35	m.79694	sp|Q5FWP4	PF00498.21^	.	GO:001660	MAENFDDA	0.000317	10.163	1.115 up	0.16	-2.408	1.100 down	0.184	2.148	1.155 down	0.0489	3.706	0.09318	0.09203	0.04911	0.1186	0.05414	0.09893
comp37652_c0	comp37	m.82024	sp|Q15751|	.	COG5021^	GO:000582	YLRRARMF	0.0782	2.694	1.105 up	0.525	-1.026	1.127 down	0.245	1.835	1.211 down	0.0314	4.335	0.23056	0.16305	0.16136	0.09775	0.10898	0.06675
comp39004_c0	comp39	m.83599	sp|E7FAM5|	PF01436.16^	.	GO:000093	AELVCGGA	0.0163	4.333	1.228 up	0.171	-2.331	1.076 up	0.496	-1.053	1.204 up	0.0338	-4.228	0.14582	0.20829	0.16518	0.1166	0.10642	0.06967
comp41720_c0	comp41	m.87558	sp|Q8GYT9|	PF13639.1^z	NOG317826	GO:001602	MAWLSRLP	0.000251	10.62	1.047 up	0.465	-1.175	1.041 down	0.341	1.473	1.222 down	0.0261	4.629	0.07489	0.08499	0.06863	0.03994	0.12325	0.02116
comp44555_c0	comp44	m.92320	sp|Q9VR91|	.	COG5021^	GO:000581	FAAILADGR	0.105	2.418	1.179 up	0.66	-0.723	1.400 up	0.312	-1.572	1.622 up	0.0159	-5.513	0.51981	0.40335	0.518	0.3375	0.19975	0.15576
comp44624_c0	comp44	m.92442	sp|Q1L721|	PF04564.10^	.	GO:000562	MGLEDHEL	0.000263	10.526	1.012 up	0.856	-0.318	1.123 down	0.18	2.169	1.178 down	0.0077	7.207	0.02703	0.11183	0.12104	0.09587	0.06333	0.01707
comp44801_c0	comp44	m.92765	sp|Q6PAV2|	PF13540.1^	COG5021^	GO:000573	MEGSSMEV	0.000657	8.827	1.205 down	0.432	1.262	1.217 up	0.283	-1.673	1.404 up	0.0201	-5.086	0.25285	0.34428	0.18172	0.28695	0.17076	0.0891
comp44985_c0	comp44	m.93079	sp|Q86YT6|	PF13637.1^	COG0666^	GO:000581	GADLPPLH	0.00742	5.264	1.164 down	0.625	0.798	1.068 down	0.887	0.241	1.495 up	0.0381	-4.058	0.23773	0.49737	0.73023	0.30085	0.25798	0.12404
comp58435_c0	comp58	m.114752	sp|Q9LN71|	PF13639.1^z	NOG277561	GO:001602	PSYSPSES	0.0000775	13.171	1.162 up	0.0742	-3.386	1.235 up	0.0525	-3.643	1.247 up	0.00704	-7.483	0.10802	0.06868	0.02545	0.16559	0.08059	0.0281
comp60181_c0	comp60	m.118171	sp|Q641J8|	PF12678.2^z	.	GO:000563	SQSTVLETL	7.08E-07	29.971	1.043 up	0.314	-1.631	1.097 down	0.337	1.486	1.249 down	0.016	5.499	0.05749	0.0489	0.10618	0.14667	0.09357	0.07018
comp65069_c0	comp65	m.127574	sp|Q13434|	PF13639.1^z	.	GO:001687	MGPAIGPG	0.00466	5.862	1.079 up	0.402	-1.344	1.047 down	0.624	0.763	1.277 down	0.0107	6.371	0.08195	0.14301	0.12856	0.122	0.07552	0.08103
comp65596_c0	comp65	m.128649	sp|O60103|	PF12906.2^	COG5183^	GO:003017	MPMTFRQS	0.0000312	15.473	1.320 down	0.101	2.968	1.157 down	0.293	1.638	1.341 down	0.0083	6.996	0.23861	0.12639	0.15178	0.20799	0.07057	0.09851
comp66046_c0	comp66	m.129424	sp|Q6WWW|	PF02825.15^	COG5021^	GO:000562	AEGPVSGV	0.000031	15.497	1.072 up	0.171	-2.332	1.211 up	0.105	-2.78	1.326 up	0.0269	-4.577	0.07784	0.03749	0.1127	0.16378	0.15718	0.08424
comp66429_c0	comp66	m.130223	sp|Q9LY41|	PF13639.1^z	NOG296717	GO:001602	ERLGSEVA	0.0000144	17.741	1.100 up	0.227	-2.001	1.051 up	0.419	-1.246	1.205 up	0.00849	-6.937	0.03223	0.1344	0.05992	0.10045	0.06277	0.04571
comp67519_c0	comp67	m.132633	sp|Q8BZZ3|	PF00397.21^	COG5021^	GO:000573	GAPEPAVS	2.18E-07	36.969	1.050 down	0.312	1.638	1.254 down	0.0576	3.516	1.114 down	0.0156	5.549	0.05277	0.06958	0.14719	0.114	0.0403	0.03961
comp69150_c0	comp69	m.136327	sp|Q5R9W1|	PF12906.2^	.	GO:003017	RILCSSDDM	0.000585	9.032	1.119 down	0.136	2.603	1.079 down	0.155	2.332	1.165 down	0.0199	5.096	0.0438	0.11687	0.07053	0.06277	0.06737	0.05453
comp69337_c0	comp69	m.136698	sp|Q90972|	PF12861.2^z	COG5540^	GO:001602	DLTRVLFAV	0.0021	6.975	1.003 down	0.962	0.094	1.089 down	0.345	1.462	1.244 down	0.0174	5.341	0.0751	0.05407	0.11088	0.1269	0.07918	0.08749
comp72922_c0	comp72	m.144685	sp|A8Y4B2|	PF13639.1^z	COG5243^	GO:000578	MVPGVDGL	0.000217	10.909	1.056 up	0.533	-1.007	1.170 down	0.125	2.574	1.480 down	0.0249	4.713	0.0572	0.14775	0.14349	0.10221	0.08483	0.22463
comp77286_c0	comp77	m.155372	sp|Q15751|	.	COG5021^	GO:000582	GDSSQVQR	0.021	4.049	1.388 up	0.258	-1.854	1.366 up	0.0946	-2.892	1.352 up	0.0182	-5.262	0.47379	0.19025	0.20681	0.23266	0.07788	0.14617
comp79591_c0	comp79	m.161600	sp|P40072|	PF13639.1^z	COG5574^	GO:000573	MFATLRKR	0.000276	10.427	1.140 down	0.16	2.406	1.233 down	0.073	3.216	1.268 down	0.0175	5.327	0.10354	0.11875	0.10987	0.15277	0.12815	0.01447
comp80621_c0	comp80	m.164439	sp|F1RCR6|	PF00632.20^	.	GO:000565	VRLPPGKR	0.00022	10.886	1.031 up	0.478	-1.142	1.124 up	0.0539	-3.609	1.155 up	0.0224	-4.894	0.04348	0.0644	0.06418	0.06842	0.0563	0.06393
comp88896_c0	comp88	m.171040	sp|Q9CY62|	PF12678.2^z	NOG294567	GO:001687	VTLLNTVPS	0.0000361	15.085	1.116 up	0.192	-2.191	1.257 down	0.066	3.342	1.374 down	0.00449	8.944	0.09931	0.10621	0.07692	0.1821	0.07522	0.06974
comp92503_c0	comp92	m.173727	sp|P0CH30|	PF12861.2^z	.	GO:001687	SVPAWRHP	0.0000267	15.905	1.070 down	0.532	1.01	1.122 down	0.284	1.67	1.279 down	0.0237	4.789	0.16189	0.10921	0.12427	0.15649	0.11121	0.09875
comp94028_c0	comp94	m.174839	sp|Q4FE47|	PF00096.21^	NOG317888	GO:000573	DGASEASA	0.00393	6.09	1.009 down	0.763	0.508	1.075 up	0.0843	-3.033	1.088 down	0.0265	4.605	0.04632	0.02406	0.04574	0.05254	0.0399	0.03506
comp44366_c0	comp44	m.91974	sp|Q24574|	PF00443.24^	COG5077^	GO:000563	TEELLVQQA	3.10E-06	23.155	1.411 up	0.0122	-7.21	1.543 up	0.00736	-7.827	1.527 up	0.0239	-4.775	0.11677	0.0736	0.05662	0.14976	0.13332	0.21865
comp56934_c0	comp56	m.112186	sp|Q80U87|	PF00443.24^	COG5533^	GO:000582	MAMRLLRR	2.27E-10	124.763	1.984 up	0.00151	-22.749	2.031 up	0.00276	-13.384	1.833 up	0.00332	-10.078	0.07387	0.04587	0.1353	0.07106	0.11232	0.13237
comp60887_c0	comp60	m.119515	sp|A5PMR2|	PF13423.1^	COG5560^	GO:004847	SGSVGVRS	3.91E-06	22.237	1.200 up	0.0374	-4.458	1.496 up	0.022	-5.025	1.504 up	0.00786	-7.153	0.10743	0.04969	0.14726	0.17858	0.11565	0.11721
comp71649_c0	comp71	m.141672	sp|Q84WU2|	PF00443.24^	COG5077^	GO:000582	RKENLPPP	1.91E-06	25.213	1.241 up	0.0134	-6.878	1.426 up	0.0141	-5.943	1.267 up	0.00935	-6.691	0.03679	0.083	0.08013	0.15284	0.08202	0.06118
comp58141_c0	comp58	m.114210	sp|O57429|	PF00443.24^	COG5560^	GO:004847	MDARISIVL	0.0000255	16.035	1.178 down	0.0295	4.92	1.153 down	0.0321	4.366	1.105 down	0.196	2.082	0.03618	0.08946	0.08073	0.04928	0.11002	0.08513
comp92740_c0	comp92	m.173879	sp|Q80U87|	PF00443.24^	COG5533^	GO:000582	VGGSCPST	0.0000395	14.851	1.166 down	0.0462	4.096	1.267 down	0.0135	6.058	1.097 down	0.155	2.34	0.06167	0.08959	0.09765	0.05695	0.03899	0.10803
comp173873_c0	comp17	m.40897	sp|Q84WU2|	PF00443.24^	COG5077^	GO:000582	NSMDSETD	0.0000204	16.679	1.820 down	0.0164	6.302	1.156 down	0.402	1.294	1.031 up	0.805	-0.407	0.24197	0.12897	0.17566	0.27339	0.13463	0.17078
comp45313_c0	comp45	m.93686	sp|Q9FPT1|	PF00443.24^	COG5077^	GO:000582	AELTQDTG	0.0000241	16.202	1.098 down	0.0231	5.462	1.102 down	0.301	1.61	1.015 down	0.722	0.574	0.03408	0.03578	0.14472	0.0971	0.04749	0.05906
comp57395_c0	comp57	m.112944	sp|Q9FPT1|	PF13423.1^	COG5077^	GO:000582	DDVSDSLG	1.42E-06	26.56	1.167 down	0.0148	6.576	1.116 down	0.168	2.244	1.019 down	0.464	1.14	0.02037	0.06484	0.13177	0.052	0.0197	0.04553
comp78843_c0	comp78	m.159525	sp|Q9C585|	PF06337.7^	COG5560^	GO:000823	MSLTNGDV	6.17E-07	30.668	1.244 down	0.026	5.182	1.068 down	0.13	2.527	1.002 down	0.948	0.119	0.0349	0.11684	0.04655	0.05971	0.04328	0.0399
comp42444_c0	comp42	m.88703	sp|Q9FPS4|	PF00443.24^	COG5077^	GO:000823	GTCCELEG	5.55E-08	47.271	1.112 down	0.08	3.28	1.401 down	0.0151	5.799	1.256 down	0.0107	6.363	0.07478	0.05722	0.15801	0.05681	0.06241	0.08249
comp56666_c0	comp56	m.111801	sp|A5PMR2|	PF00443.24^	COG5560^	GO:004847	VESCHCVR	1.67E-06	25.799	1.113 up	0.198	-2.161	1.522 up	0.011	-6.587	1.644 up	0.00705	-7.478	0.14023	0.03326	0.12167	0.1384	0.19041	0.02482
comp66662_c0	comp66	m.130734	sp|Q9SB51|	PF00443.24^	COG5533^	GO:001602	MGNLIIPGE	0.000279	10.409	1.106 down	0.0665	3.544	1.101 down	0.0345	4.259	1.131 down	0.05	3.677	0.04659	0.06774	0.036	0.05504	0.05705	0.07826
comp69790_c0	comp69	m.137632	sp|A3AF13|	PF00443.24^	COG5077^	GO:000563	AEVMAPKR	1.44E-06	26.483	1.061 up	0.11	-2.864	1.174 up	0.0213	-5.09	1.157 up	0.00659	-7.684	0.05751	0.01828	0.02853	0.08664	0.04668	0.02923
comp71602_c0	comp71	m.141561	sp|Q9SJA1|	PF01753.13^	COG5533^	GO:001602	MPENCAAS	0.000011	18.586	1.031 up	0.757	-0.521	1.399 up	0.00533	-9.096	1.256 up	0.0066	-7.679	0.15073	0.08174	0.08713	0.0614	0.04993	0.06978
comp29938_c0	comp29	m.72596	sp|Q67XW5|	PF01753.13^	COG5533^	GO:001602	FVGPSGLH	0.0217	4.011	1.389 down	0.234	1.966	1.362 up	0.0223	-5	1.012 up	0.965	-0.083	0.23124	0.42439	0.16906	0.05734	0.11027	0.42359
comp342810_c0	comp34	m.78003	sp|P51784|	PF00443.24^	COG5560^	GO:000573	ELDESLNV	0.0161	4.346	1.264 up	0.784	-0.467	4.795 up	0.0087	-7.277	3.163 up	0.311	-1.587	0.97643	1.0721	0.52284	0.33623	1.8511	0.98001
comp53293_c0	comp53	m.106442	sp|Q99LG0|	PF00443.24^	COG5207^	GO:000563	FSAEKTHH	0.013	4.597	1.021 up	0.891	-0.248	1.298 up	0.0446	-3.873	1.011 up	0.887	-0.245	0.1111	0.22603	0.06457	0.18335	0.0723	0.11408

comp105448_c0	comp10	m.3845	sp|Q9SJA1|	PF01753.13^	COG5533^	GO:001602	RCDILIRRIM	0.0481	3.173	1.162 down	0.428	1.272	1.194 down	0.326	1.525	1.406 down	0.046	3.788	0.19572	0.28055	0.30791	0.13642	0.16073	0.20385
comp19105_c0	comp19	m.47724	sp|Q949Y0|	PF00443.24^	NOG286607	GO:000950	VERDLCGL	0.000089	12.842	1.100 down	0.103	2.952	1.149 down	0.0582	3.504	1.191 down	0.0475	3.745	0.04009	0.08484	0.10479	0.04691	0.11576	0.06933
comp19768_c0	comp19	m.49948	sp|B2GUZ1|	PF06337.7^	.	GO:000573	GSVPALAM	0.00569	5.6	1.028 up	0.144	-2.53	1.003 up	0.98	-0.047	1.072 up	0.0484	-3.719	0.02797	0.01521	0.09498	0.1767	0.04311	0.03285
comp50640_c0	comp50	m.102409	sp|Q8K387|	PF13423.1^	COG5560^	GO:000823	PQPVTEME	0.0000127	18.139	1.121 up	0.135	-2.616	1.202 up	0.178	-2.181	1.378 up	0.00983	-6.558	0.06942	0.10573	0.05349	0.23811	0.13261	0.04876
comp76721_c0	comp76	m.153844	sp|Q8NFA0|	PF00443.24^	COG5210^	GO:000579	HGIDGDWV	0.00209	6.983	1.065 up	0.459	-1.191	1.110 up	0.164	-2.27	1.150 up	0.0459	-3.79	0.05271	0.14448	0.10573	0.08191	0.10318	0.02734
comp7786_c0	comp77	m.156885	sp|Q9MAQ3|	PF06337.7^	COG5560^	GO:000823	IDRSNSVQE	6.35E-06	20.427	1.195 down	0.172	2.321	1.326 up	0.0598	-3.469	1.324 up	0.00375	-9.635	0.16571	0.14796	0.10337	0.21078	0.05435	0.06415
comp97336_c0	comp97	m.177173	sp|Q67XW5|	PF01753.13^	COG5533^	GO:001602	LQMGRVDI	0.000293	10.312	1.158 up	0.133	-2.631	1.212 up	0.055	-3.58	1.150 up	0.0243	-4.748	0.08289	0.13897	0.05769	0.14429	0.04137	0.07448
comp80802_c0	comp80	m.165017	sp|Q8BWR4|	PF00443.24^	COG5077^	GO:000823	KLMAAVAP	5.93E-07	30.88	1.017 up	0.409	-1.326	1.097 up	0.189	-2.114	1.202 up	0.0106	-6.39	0.03496	0.01265	0.07117	0.10579	0.0644	0.05255
comp16400_c0	comp16	m.36797	sp|Q96FW1|	PF10275.4^	NOG267426	GO:000573	MEDQEAVG	0.0000669	13.521	1.530 up	0.0185	-5.992	1.654 up	0.029	-4.539	1.417 up	0.0668	-3.306	0.13597	0.15326	0.16362	0.27513	0.15367	0.26273
comp115688_c0	comp11	m.10757	sp|Q7ZX21|	PF02338.14^	.	GO:000484	PTASSSSTF	1.56E-06	26.102	1.101 up	0.144	-2.53	1.389 up	0.0117	-6.433	1.413 up	0.0126	-6	0.09321	0.05883	0.10154	0.10705	0.145	0.08147
comp38385_c0	comp38	m.82932	sp|Q8LG98|	PF10275.4^	NOG267426	GO:000823	RPGQSFSL	0.000222	10.868	1.111 down	0.22	2.035	1.229 down	0.0148	5.846	1.209 down	0.0623	3.396	0.07367	0.13085	0.04903	0.08939	0.05096	0.15359
comp47786_c1	comp47	m.97896	sp|Q2TBG8|	PF01088.16^	NOG327708	GO:000573	MAVDMEKI	0.000859	8.372	1.093 up	0.362	-1.468	1.252 down	0.0969	2.864	1.318 down	0.025	4.706	0.03235	0.17217	0.20942	0.08628	0.05905	0.15906
comp57906_c0	comp57	m.113793	sp|Q4VA72|	PF01398.16^	NOG322509	GO:007053	VNTWQQCF	0.0543	3.052	1.251 up	0.426	-1.279	1.398 up	0.0347	-4.249	1.085 up	0.361	-1.421	0.48935	0.13294	0.18018	0.13902	0.16289	0.03361
comp32350_c0	comp32	m.75603	sp|Q10169|	PF00240.18^	COG5272^	GO:000582	PRQVPVDA	0.00261	6.664	1.095 up	0.305	-1.665	1.105 down	0.252	1.802	1.243 down	0.0211	4.993	0.13684	0.08056	0.13009	0.0953	0.11178	0.05782
comp91016_c0	comp91	m.172637	sp|P0DJ25|	PF00240.18^	.	GO:000563	EIFGLRWRA	3.87E-08	50.498	1.343 up	0.0104	-7.844	1.341 up	0.024	-4.866	1.485 up	0.00393	-9.455	0.08633	0.06573	0.11859	0.12756	0.06747	0.10029
comp163156_c0	comp16	m.36421	sp|P0C224|	PF00240.18^	COG5272^	GO:000563	MELEVDGIA	0.0000918	12.769	2.394 down	0.0134	6.874	1.920 down	0.0652	3.36	2.175 down	0.039	4.027	0.20741	0.30225	0.2838	0.4834	0.24416	0.50054
comp265372_c0	comp26	m.67290	sp|P0CH07|	PF11976.3^	.	GO:002262	MQIFVKTLT	3.82E-07	33.451	3.786 up	0.0917	-3.093	9.333 up	0.00939	-7.036	7.889 up	0.00279	-11.037	1.23115	0.16327	0.58969	0.70093	0.31559	0.43817
comp20362_c0	comp20	m.51637	sp|P69061|	PF11976.3^	COG1998^	GO:000563	MRLFVRDV	0.0003	10.266	1.053 up	0.536	-0.998	1.014 up	0.92	-0.173	1.244 down	0.00934	6.692	0.11335	0.10063	0.11003	0.22247	0.09013	0.02769
comp46344_c0	comp46	m.95457	sp|Q42202|	PF11976.3^	COG5272^	GO:000563	GTWLKYFP	1.09E-06	27.819	1.063 up	0.193	-2.19	1.151 down	0.248	1.82	1.326 down	0.00959	6.626	0.05643	0.05797	0.05997	0.2161	0.12082	0.02306
comp79554_c1	comp79	m.161513	sp|P37164|	PF11976.3^	COG5272^	GO:000584	FWLKPWAV	0.0000257	16.018	1.000 down	0.992	0.023	1.037 down	0.492	1.062	1.265 down	0.00766	7.222	0.06204	0.10038	0.08566	0.05337	0.07534	0.05637
comp81042_c0	comp81	m.165689	sp|P79781|	PF11976.3^	COG5272^	GO:000565	SSWLGRLG	0.000813	8.464	1.056 up	0.41	-1.323	1.016 up	0.863	-0.288	1.167 down	0.0252	4.692	0.07991	0.09067	0.10245	0.13007	0.08736	0.03762
comp81293_c0	comp81	m.165810	sp|P37164|	PF11976.3^	COG5272^	GO:000584	ILAQVLRVA	0.000159	11.552	1.000 down	0.991	0.027	1.124 down	0.208	2.008	1.314 down	0.00458	8.855	0.08482	0.0474	0.13944	0.09399	0.05764	0.06795
comp81526_c0	comp81	m.165925	sp|Q42202|	PF11976.3^	COG5272^	GO:000563	ILAQGKVVG	0.00102	8.102	1.098 up	0.0553	-3.816	1.074 down	0.237	1.87	1.191 down	0.0276	4.532	0.02697	0.0654	0.04058	0.10357	0.09547	0.05729
comp77215_c0	comp77	m.155193	sp|Q05086|	PF00632.20^	COG5021^	GO:000573	LNCAKGAM	1.89E-07	37.971	1.248 up	0.0157	-6.414	1.468 up	0.0158	-5.7	1.520 up	0.00319	-10.305	0.06861	0.0727	0.13921	0.13564	0.1095	0.04219
comp125968_c0	comp12	m.17391	sp|Q05086|	PF00632.20^	COG5021^	GO:000573	ALLLSPVLA	0.0000564	13.941	1.004 down	0.972	0.069	1.051 down	0.573	0.875	1.187 up	0.0445	-3.835	0.17038	0.08511	0.13088	0.0995	0.06567	0.11132
comp46738_c0	comp46	m.96131	sp|Q05086|	PF00632.20^	COG5021^	GO:000573	VMEASACP	0.000028	15.766	1.055 up	0.401	-1.348	1.224 up	0.0946	-2.893	1.320 up	0.00604	-7.979	0.04048	0.10764	0.18341	0.08395	0.09226	0.04013
comp107763_c0	comp10	m.5421	sp|O13769|	PF02902.14^	COG5160^	GO:000563	ARRLFVER	1.59E-07	39.137	1.762 down	0.00528	10.646	1.715 down	0.00697	8.006	1.402 down	0.0187	5.204	0.09235	0.12279	0.07485	0.17958	0.16724	0.08497
comp75037_c0	comp75	m.149783	sp|Q8L7S0|	PF02902.14^	COG5160^	GO:001692	MAEPICIDL	0.000184	11.251	1.194 up	0.0391	-4.382	1.278 up	0.0137	-6.017	1.251 up	0.0613	-3.417	0.07274	0.09184	0.07856	0.08773	0.09044	0.16651
comp77666_c0	comp77	m.156364	sp|O13769|	.	COG5160^	GO:000563	MSTDAVEL	0.0000259	15.991	1.213 up	0.0502	-3.961	1.501 up	0.0211	-5.11	1.284 up	0.0121	-6.091	0.06684	0.12409	0.12392	0.19337	0.07835	0.08892
comp32307_c0	comp32	m.75556	sp|Q0WKV8|	PF02902.14^	COG5160^	GO:001692	QVQVWRQ	1.71E-06	25.714	1.233 down	0.0829	3.231	1.251 down	0.161	2.29	1.299 down	0.017	5.379	0.04443	0.18217	0.23274	0.16101	0.09084	0.10703
comp22390_c0	comp22	m.58111	sp|Q8H715|	PF02991.11^	.	GO:000042	MAPKVRKA	0.0257	3.826	1.127 down	0.025	5.256	none	1	0.004	1.061 up	0.238	-1.875	0.0425	0.05022	0.1339	0.13636	0.02427	0.08907
comp50661_c0	comp50	m.102441	sp|Q8VYK7|	PF02991.11^	NOG249730	GO:000042	EKADSEMA	0.00339	6.294	1.830 down	0.0425	4.232	1.742 down	0.127	2.558	1.653 down	0.155	2.336	0.21215	0.35347	0.18457	0.5987	0.39623	0.47867
comp25803_c0	comp25	m.65676	sp|Q6FXR8|	PF02991.11^	.	GO:000042	FGSLAQDG	1.28E-06	27.023	1.050 down	0.651	0.742	1.288 down	0.0299	4.482	1.413 down	0.00735	7.336	0.09812	0.16278	0.10312	0.12662	0.12019	0.06407
comp29860_c0	comp29	m.72489	sp|Q1SF86|	PF04110.8^	.	GO:000573	DDIWAQVL	0.00247	6.744	1.289 up	0.0938	-3.063	1.496 up	0.0216	-5.063	1.231 up	0.345	-1.473	0.16067	0.17732	0.20368	0.10636	0.40049	0.08067
comp30847_c0	comp30	m.73721	sp|Q9VTF9|	PF03152.9^	COG5140^	GO:000050	AAAAMDFD	1.56E-06	26.107	1.110 down	0.338	1.543	1.143 down	0.182	2.156	1.315 down	0.00716	7.436	0.14205	0.136	0.14602	0.10406	0.05446	0.09133
comp70248_c0	comp70	m.138588	sp|Q55BK0|	PF00179.21^	COG5140^	GO:004316	LMEWEVDM	0.0000218	16.487	1.198 up	0.0302	-4.878	1.101 up	0.247	-1.825	1.229 up	0.0319	-4.312	0.10263	0.03048	0.14702	0.04078	0.11341	0.07944
comp33203_c0	comp33	m.76613	sp|Q55BK0|	PF03152.9^	COG5140^	GO:004316	MFGGGLFG	3.58E-06	22.585	1.178 up	0.128	-2.678	1.042 down	0.22	1.947	1.097 down	0.0351	4.172	0.14072	0.10752	0.04899	0.03774	0.05547	0.03304
comp44587_c0	comp44	m.92368	sp|P62975|	PF11976.3^	COG5272^	GO:002262	MNVESFRL	2.92E-06	23.397	1.056 up	0.115	-2.81	1.014 up	0.802	-0.407	1.163 down	0.0287	4.468	0.00937	0.05565	0.06151	0.08293	0.03763	0.09035
comp62097_c0	comp62	m.121880	sp|P69317|	PF00240.18^	.	GO:000573	MRIYVTKVS	4.71E-06	21.504	1.179 up	0.0314	-4.796	1.086 up	0.133	-2.508	1.024 up	0.39	-1.335	0.05847	0.08058	0.07569	0.05877	0.04684	0.02461
comp82802_c0	comp82	m.166728	sp|P69317|	PF11976.3^	.	GO:000573	RSPKPREK	0.000931	8.241	1.215 up	0.0596	-3.702	1.082 down	0.59	0.837	1.337 down	0.00471	8.778	0.09888	0.11579	0.2376	0.13236	0.06203	0.07288
comp24219_c0	comp24	m.62468	sp|Q8SWD4|	PF00240.18^	COG5272^	GO:000573	QLEDGRTL	3.87E-07	33.374	1.152 down	0.104	2.928	1.304 down	0.0384	4.098	1.197 down	0.00499	8.577	0.03009	0.13673	0.13303	0.13172	0.04149	0.04407
comp93716_c0	comp93	m.174634	sp|O08623|	PF00569.12^	NOG278569	GO:000577	MMSMIATV	0.000549	9.143	1.121 up	0.0356	-4.553	1.085 up	0.348	-1.452	1.137 up	0.11	-2.725	0.07124	0.01494	0.10957	0.12147	0.06243	0.12143
comp68218_c0	comp68	m.134172	sp|P22856|	PF02338.14^	.	GO:000823	RVSGTKKA	4.43E-07	32.562	1.009 down	0.909	0.211	1.152 down	0.0174	5.5	1.253 down	0.00824	7.02	0.07131	0.10263	0.05123	0.05383	0.08158	0.04461
comp81389_c0	comp81	m.165848	sp|P52285|	PF03931.10^	COG5201^	GO:000593	MPEGDKVL	3.20E-06	23.029	1.030 down	0.594	0.867	1.153 down	0.0458	3.835	1.197 down	0.0112	6.269	0.06829	0.07103	0.07197	0.07938	0.07527	0.0353
comp116200_c0	comp11	m.11093	sp|P53152|	PF00179.21^	NOG239185	GO:000573	PRGTWPTG	0.000113	12.297	1.261 down	0.0499	3.97	1.125 down	0.0606	3.454	1.034 down	0.807	0.404	0.16475	0.03645	0.08183	0.05517	0.09992	0.22233
comp85488_c0	comp85	m.168545	sp|P55034|	PF13519.1^	COG5148^	GO:000582	LKSGSRTG	0.0000163	17.356	1.022 up	0.786	-0.463	1.029 up	0.598	-0.819	1.101 down	0.0145	5.713	0.06223	0.12609	0.05586	0.08701	0.02324	0.04313
comp51411_c0	comp51	m.103627	sp|P61237|	PF03226.9^	NOG300891	GO:000573	EGLTAQLAK	0.00014	11.822	1.097 down	0.661	0.72	1.397 up	0.00962	-6.964	1.369 up	0.00567	-8.159	0.13833	0.34597	0.08211	0.11178	0.06216	0.09226
comp89373_c0	comp89	m.171384	sp|Q3TIX9|	PF02148.14^	NOG259163	GO:000563	FLWVNFVV	4.28E-07	32.778	1.087 down	0.0856	3.186	1.128 down	0.00515	9.232	1.169 down	0.00378	9.575	0.05825	0.04895	0.01643	0.03414	0.03753	0.02839
comp91746_c0	comp91	m.173124	sp|Q6NZ09|	PF04683.8^	NOG288027	GO:000573	FGSRCVEIC	0.000389	9.771	1.328 up	0.0233	-5.426	1.053 up	0.438	-1.199	1.034 up	0.644	-0.73	0.06469	0.13645	0.09913	0.07659	0.05014	0.12485
comp110066_c0	comp11	m.7125	sp|Q4KM30|	PF05903.9^	NOG236523	GO:000573	TLTNIKRVN	0.000638	8.88	1.087 up	0.393	-1.372	1.334 up	0.0297	-4.495	1.260 up	0.041	-3.952	0.15742	0.08083	0.10068	0.15556	0.12433	0.11454
comp136429_c0	comp13	m.23394	sp|Q9UII4|H	PF13540.1^	COG5021^	GO:000582	KTAHSVAV	0.0014	7.595	1.115 up	0.435	-1.255	1.303 up	0.0569	-3.533	1.301 up	0.0158	-5.52	0.13994	0.20999	0.05664	0.20905	0.12681	0.05411
comp139432_c0	comp13	m.25020	sp|Q9UII4|H	PF00415.13^	COG5021^	GO:000582	TQGRLFAW	0.0006	8.986	1.133 down	0.375	1.427	1.019 down	0.781	0.448	1.328 up	0.0294	-4.434	0.07531	0.24218	0.07951	0.09859	0.12658	0.13453
comp14376_c0	comp14	m.27335	sp|F2Z461|	PF13540.1^	.	GO:000573	YTQVSAGA	0.00742	5.265	1.273 up	0.169	-2.342	1.369 up	0.135	-2.489	1.321 up	0.0227	-4.87	0.28847	0.0748	0.33599	0.1415	0.14608	0.07716
comp152540_c0	comp15	m.31507	sp|F2Z461|	PF13540.1^	.	GO:000573	TVLLCSDG	0.0146	4.455	1.145 up	0.656	-0.732	1.521 up	0.0412	-3.992	1.736 up	0.0761	-3.144	0.2922	0.45013	0.18678	0.239	0.48418	0.14815
comp15689_c0	comp15	m.33578	sp|Q6ICB0|	PF05903.9^	NOG236523	GO:000573	NIFAFAESP	0.0281	3.729	1.082 up	0.307	-1.655	1.181 up	0.0364	-4.176	1.117 up	0.37	-1.394	0.06032	0.12403	0.08874	0.07396	0.14232	0.18073
comp16685_c0	comp16	m.37948	sp|Q6GLM5|	PF13499.1^	.	GO:000573	APRAMCAG	0.000386	9.789	1.064 down	0.574	0.913	1.286 up	0.0285	-4.572	1.176 up	0.116	-2.658	0.17092	0.09789	0.12476	0.09857	0.15101	0.0915
comp17590_c0	comp17	m.41549	sp|Q9UII4|H	PF13540.1^	COG5021^	GO:000582	CPKAMGRR	2.62E-07	35.864	1.542 down	0.0227	5.495	1.126 down	0.0746	3.188	1.120 down	0.104	2.785	0.08617	0.21072	0.07461	0.07785	0.10551	0.05295
comp18216_c0	comp18	m.44020	sp|Q5ZIV7|	PF05903.9^	NOG267736	GO:000573	AELDSQVM	0.0000866	12.908	1.373 up	0.00985	-7.998	1.301 up	0.0411	-3.998	1.114 up	0.198	-2.072	0.08654	0.07487	0.13299	0.13562	0.05933	0.13936
comp182921_c0	comp18	m.44408	sp|Q54QS0|	PF09743.4^	NOG277822	GO:001687	MEAIRALQQ	0.0117	4.713	1.161 up	0.47	-1.163	1.509 up	0.00892	-7.179	1.285 up	0.379	-1.367	0.30581	0.21099	0.08732	0.1405	0.46503	0.25537
comp26711_c0	comp26	m.67620	sp|Q6GLM5|	PF00036.27^	.	GO:000573	MGNSGLLG	0.0000968	12.649	1.005 down	0.967	0.08	1.146 up	0.026	-4.733	1.191 up	0.0447	-3.83	0.05457	0.19312	0.06434	0.0532	0.08963	0.09698
comp29168_c0	comp29	m.71368	sp|F2Z461|	PF13540.1^	.	GO:000573	MAAGRYHS	0.000317	10.159	1.458 up	0.013	-6.977	1.484 up	0.0782	-3.125	1.178 up	0.0664	-3.314	0.10991	0.11065	0.10811	0.34873	0.1335	0.05035
comp37232_c0	comp37	m.81537	sp|Q7T347|	PF07910.8^	NOG293056	GO:000578	KPLNPHKD	0.406	1.174	1.039 down	0.889	0.252	1.302 up	0.0219	-5.036	1.012 up	0.966	-0.081	0.31823	0.31589	0.0822	0.127	0.28335	0.32431
comp37958_c0	comp37	m.82413	sp|Q6ASW7|	PF02037.22^	NOG237400	GO:000563	SHHQVTMG	4.03E-07	33.143	1.252 down	0.0292	4.943	1.161 down	0.0265	4.702	1.304 down	0.0168	5.41	0.07854	0.10534	0.03833	0.08323	0.09338	0.1067
comp44062_c0	comp44	m.91476	sp|Q94451|	PF11789.3^z	NOG125513	GO:000563	KPQSVLEM	0.00784	5.196	1.233 up	0.127	-2.686	1.129 down	0.41	1.271	1.381 down	0.0298	4.413	0.14269	0.17465	0.09745	0.2589	0.08492	0.19369
comp46620_c0	comp46	m.95939	sp|Q6ICB0|	PF05903.9^	NOG236523	GO:000573	QWTFCPVL	0.000253	10.602	1.221 down	0.0491	3.999	1.030 up	0.653	-0.703	1.002 down	0.951	0.114	0.0788	0.12111	0.02508	0.12099	0.06133	0.03933
comp50855_c0	comp50	m.102767	sp|Q9UII4|H	PF13540.1^	COG5021^	GO:000582	TVLLRSDGS	0.00339	6.292	1.447 up	0.161	-2.397	1.611 up	0.017	-5.548	1.309 up	0.205	-2.031	0.26626	0.35717	0.19243	0.15649	0.25276	0.28729

comp58773_c0	comp58	m.115389	sp|D3ZF42|	PF02902.14^	.	GO:000563	MELAELMQ	3.89E-10	113.998	1.977 down	0.00166	21.303	2.501 down	0.0024	14.474	1.878 down	0.00439	9.028	0.03269	0.08636	0.06335	0.17143	0.09678	0.17676
comp61960_c0	comp61	m.121665	sp|Q5PQ09|	PF05903.9^	.	GO:000573	MTWTVPPR	0.0209	4.05	1.097 up	0.317	-1.618	1.186 up	0.0153	-5.772	1.040 up	0.605	-0.813	0.15266	0.06391	0.03576	0.07769	0.10046	0.09703
comp66875_c0	comp66	m.131220	sp|Q6DC39|	PF05903.9^	NOG267736	GO:000573	LAFCVAMR	0.0017	7.292	1.268 up	0.05	-3.968	1.370 up	0.0422	-3.958	1.258 up	0.202	-2.049	0.08791	0.14869	0.07767	0.21641	0.29684	0.1297
comp68968_c1	comp68	m.135935	sp|Q5PQ09|	PF05903.9^	.	GO:000573	MTFDGVRQ	0.0000181	17.03	1.154 down	0.0766	3.342	1.225 down	0.00838	7.386	1.165 down	0.03	4.405	0.06685	0.10465	0.0611	0.05082	0.05714	0.0824
comp72388_c0	comp72	m.143425	sp|O94451|	PF11789.3^z	NOG125513	GO:000563	AMAGIPVKP	0.00069	8.742	1.005 up	0.941	-0.14	1.022 up	0.851	-0.311	1.165 down	0.0342	4.211	0.05359	0.10987	0.06068	0.1988	0.05585	0.08858
comp75630_c0	comp75	m.151224	sp|Q5FVJ8|	PF02902.14^	NOG251510	GO:000823	MALVSWKT	0.00133	7.675	1.134 up	0.37	-1.442	1.294 up	0.0483	-3.763	1.075 up	0.66	-0.696	0.15568	0.19859	0.10876	0.16549	0.13071	0.27361
comp76352_c0	comp76	m.152931	sp|F2Z461|	PF13540.1^	.	GO:000573	LRSDGHAV	1.75E-07	38.539	1.299 up	0.013	-6.962	1.404 up	0.0049	-9.493	1.210 up	0.0228	-4.858	0.03971	0.10115	0.01051	0.10275	0.06188	0.09537
comp76486_c0	comp76	m.153231	sp|Q5R6L5|	PF10363.4^	.	GO:000563	KRTIDSEWI	0.0000347	15.194	1.045 down	0.473	1.153	1.103 up	0.142	-2.427	1.134 up	0.0208	-5.025	0.06726	0.08864	0.07435	0.0911	0.04359	0.05802
comp80213_c0	comp80	m.163330	sp|Q5PQ09|	PF00169.24^	.	GO:000573	GEAKADAIE	7.22E-06	19.975	1.225 down	0.111	2.844	1.540 down	0.00471	9.712	1.235 down	0.0495	3.691	0.04191	0.20238	0.05369	0.1166	0.09726	0.13392
comp85359_c0	comp85	m.168474	sp|Q4KM30|	PF05903.9^	NOG236523	GO:000573	APPSTVIML	0.000128	12.024	1.041 up	0.461	-1.185	1.178 up	0.0872	-2.991	1.307 up	0.00402	-9.374	0.03786	0.09167	0.1026	0.1209	0.07712	0.0291
comp86650_c0	comp86	m.169382	sp|Q3APG8|	PF00164.20^	COG0048^	GO:001593	FWLKPIFRL	7.19E-07	29.879	1.483 up	0.0245	-5.314	1.478 up	0.00677	-8.123	1.383 up	0.0083	-6.997	0.08395	0.1971	0.06077	0.12488	0.10432	0.08373
comp89713_c0	comp89	m.171617	sp|Q6ICB0|	PF05903.9^	NOG236523	GO:000573	MAKVTAYV	0.000039	14.884	1.183 up	0.00296	-14.655	1.077 up	0.283	-1.675	1.194 down	0.019	5.178	0.02585	0.02073	0.09455	0.08648	0.04924	0.086
comp90764_c0	comp90	m.172455	sp|Q9ZU75|	PF00179.21^	COG5078^	GO:001688	PSLCLVHD	0.000293	10.306	1.113 up	0.108	-2.883	1.038 down	0.582	0.856	1.118 down	0.014	5.784	0.04855	0.09577	0.04036	0.12152	0.03664	0.04224

Appendix H

Figure 6.5 Melt curve analysis of reaction products from qPCR assay. Melt curve analysis for HKGs, PCNA (maroon line), *cyc* (red line), *SAM* (orange line), *Rp-S4* (yellow line), GAPDH (green line). Melt curve analysis for genes of interest, acpPCSym_1:1 (blue line), acpPCSym_5:1 (purple line), acpPCSym_10:1 (pink line), acpPCSym_15 (black line), acpPCSym_18 (grey line) and *psbA* (brown line).