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JAMES COOK UNIVERSITY
AUSTRALIA

Characterisation of Cherax quadricarinatus densovirus; the first virus characterised from Australian freshwater crayfish

Volume 2

Thesis submitted by

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Bsc Science (Aquaculture)

Hons Class 1 (Microbiology & Immunology)

August 2016

For the degree of Doctor of Philosophy

in

Microbiology & Immunology

College of Public Health, Medical and Veterinary Science

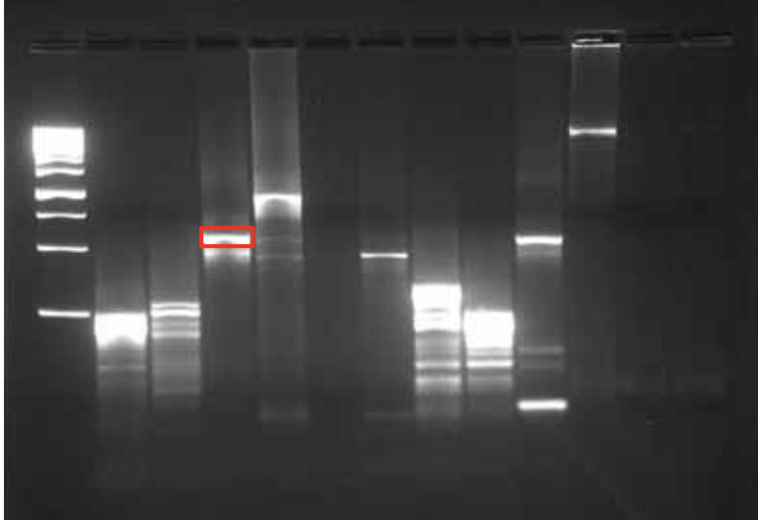
James Cook University

APPENDIX 3: Genome sequencing of Cherax quadricarinatus densovirus

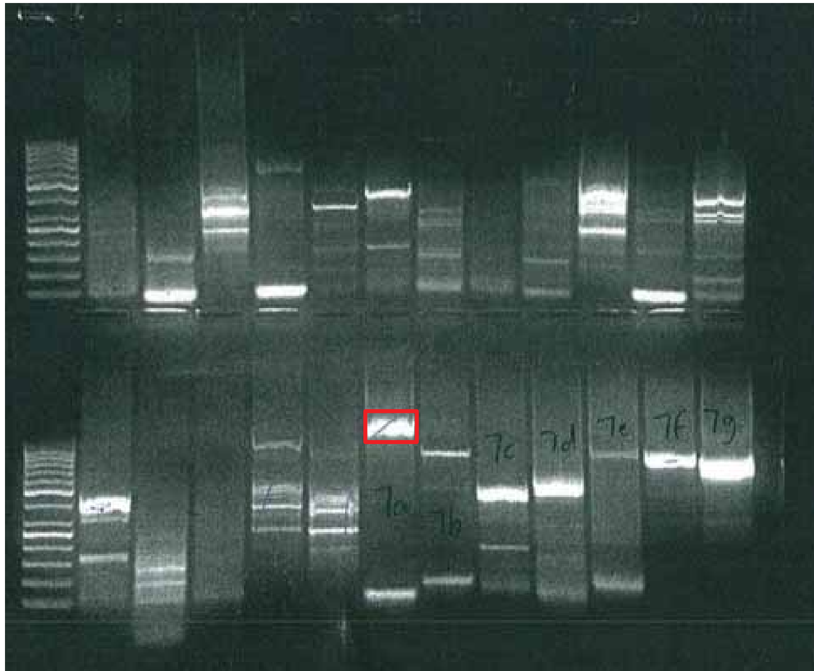
Appendix 3 presents an example of the worksheets and gel photos that detail the genome sequencing of Cherax quadricarinatus densovirus.

Presented on page 2 is a gel run at James Cook University using PCR product that generated at TAAHL in 2000/2001. The template originated from CqDV infected *Cherax quadricarinatus*. The primers used to generate the PCR product is unknown. The gel presented on page 3 presents PCR product amplified from CqDV infected *C. quadricarinatus* using HPV specific primers. These primers are presented in the table on page 3.

Page 5 presents work sheets used to applify the remainder of the CqDV genome. At the top of the worksheet, the date of testing and the name of the person who did the testing are recorded. Beneath this an accession number or code, this relates to the origin of the template that is used in the PCR. The accession numbers are recorded in a laboratory register (data not shown) that details the samples history, storage, assays carried out and date of sample destruction. The extraction method identifies the technique used to extract the DNA for PCR. On the left side of the main box is the tube ID, followed (to the right) by the sample name or description, this may include the primers for that particular sample, the gel lane identifies where on the gel the sample was run. Below the main box are the primer names, sequence and primer Tm. The PCR conditions are in a separate box labelled MASTER MIX. This box details the reagents used in the PCR, their concentration and volume. The PCR program is also recorded below this, including program used and the thermal cycler used. The electrophoresis conditions including the ladder are recorded at the bottom of this box. On a the next page is the gel photo that relates to the work sheet. In most instances, all of these products were cloned using either pGEM-T Easy Vector System (A1380, Promega, NSW), or One shot TOP10 chemically competent *E. coli* using the pCR4-TOPO TA vector (K4575-01, Life Technologies, VIC) as per manufacturer's instructions.



The PCR product on the above gel is from TAAHL that was stored 14 years ago amplified using unknown primers. This gel was run at James Cook University at the end of 2013. All of the bands on this gel were cloned using pGEM-T Easy Vector system. The PCR product in the red box was 626 nt long and had homology to densoviruses (see 5.3.1). From this sequence BgF3 and BgR3 primers were designed.



Position	Region required	Primer zone KC Id	Sequence	mer	Tm	GC %	note
	1-900nt		**NB: Area of hairpin formation first 200nt non coding. Promoting sequence from 144**				
	Selected:						
22	KC 17	Universal A	TAG CTC SGC GAT AAG CCT TA		58	45	
470	KC HPV 470F	Homologous 2	CGCAGTGT ATTTATmAGyTAtg		54	32	
570	KC HPV 570F	Homologous 3	GCAGATGwATTTTGGAGTGAT		55	38	
880	KC HPV 880 F	Homologous 4	tcc DRa Yaa Yca gat ggg ttg	21	55	38	NIL
	2500-3000		**NB 1600-2880 region is highly variable**				
1370	KC HPV 1370F		tgg aYc ata aYc ctc ttg tta c	22	58-59	38%	Nil
1557	KC HPV 1557 F		ggt gga tca taa ccc tct tgt	21	59	48	Nil
2908	KC HPV 2908 F		cag cmr tWa tga caa atg tYg	21	53-59	33	nil
3334	KC HPV 3334F		cag aYa Rga agt tYa aRa ttg gRt t	25	56-59	24	nil
	3600-5000		**NB: highly conserved and functional domains. Marked variation with mudcrab cf rest of HPVs.**				
3840	KC 3840F		cRc cta caa gaa Rag gag g	19	55-59	24	nil
check	KC HPV 4760F		cRt ggg gYa twg aca aca tDK	21	55	38	
	S.serrata 5700-END						
5035	KC HPV 5035F		aag agY gaR gac agY Ygg g	19	55-61	47	
5865	KC HPV 5865F		ccW KaW gYt caY aca caR Mg	18	55	47	
6105	KC HPV 6105F		gga acY aaR tat Kca gaY agR g	22	55	31	
6400	KC HPV 6400F		cgt gcg tgg ttt acc ctt	18	54	50	

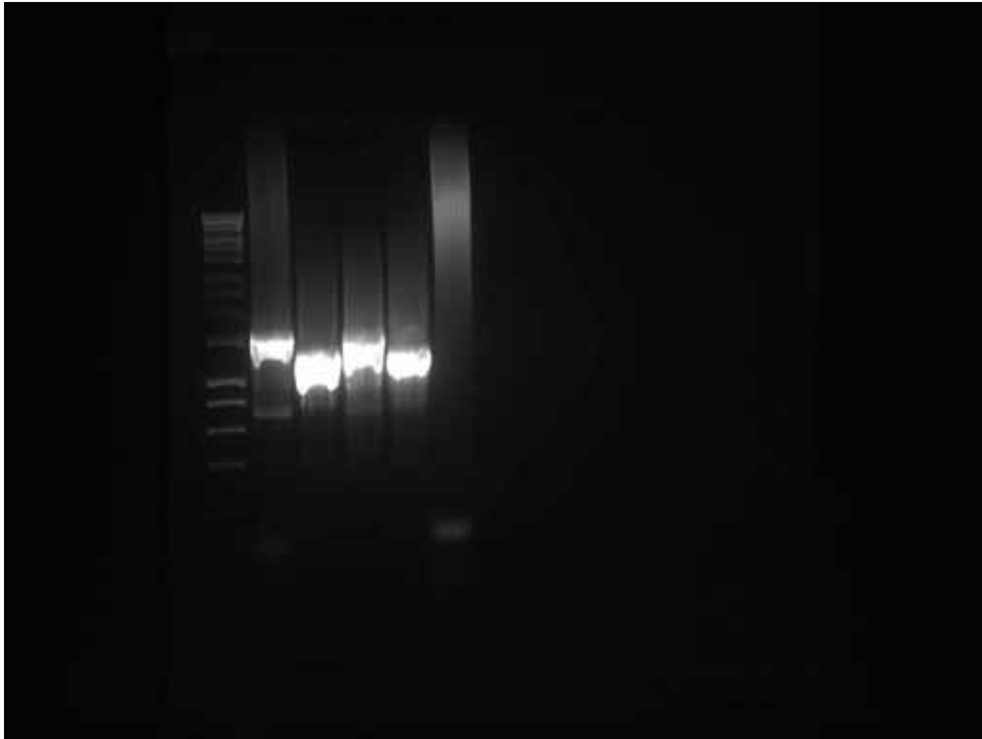
PCR product 7a-7g (bottom row of gel) were produced from HPV primers 470 F and 1370 R (above table). The product in the red box was cloned and sequenced. It was 1,595 nt and had homology with densovirus capsid proteins. From these two sequences, new viral specific primers were designed.

TESTED BY:_ Shaun Bochow____ DATE TESTED:______1/4/14________________

Sample Identification, Extraction and Results											
Accession: the Template came from A14-005 and VDNA4, the new accession number is A14-015 to denote the new round of PCR											
Extraction Method: Roche viral extraction											
Positive control :	Batch:			Positive =							
	Sample	Gel lane	Res ult	Id	Sample	Gel lane	Res ult	Id	Sample	Gel Lane	Res ult
L	Ladder 100bp Prom	1									
1	14 5-3 12RC (VDNA4)	2			Cut out 82 and 82.1						
2	13 5-3 12RC (VDNA4)	3			Cut out 83						
3	14 5-3 12RC (A14-005)	4			Cut out 84						
4	13 5-3 12RC (A14-005)	5			Cut out 85						
5	NTC	6									

Primer	Sequence	Tm
Primer 14 5-3 F	GTT ACA ATC TAT CGA CGT C	53 Tm
Primer 12 RC	TAA GAG GTG ACT CTG CTG G	57.3 Tm
Primer 13 F	GGA GAT GTG ATC CAG AGC TG	57.3 Tm

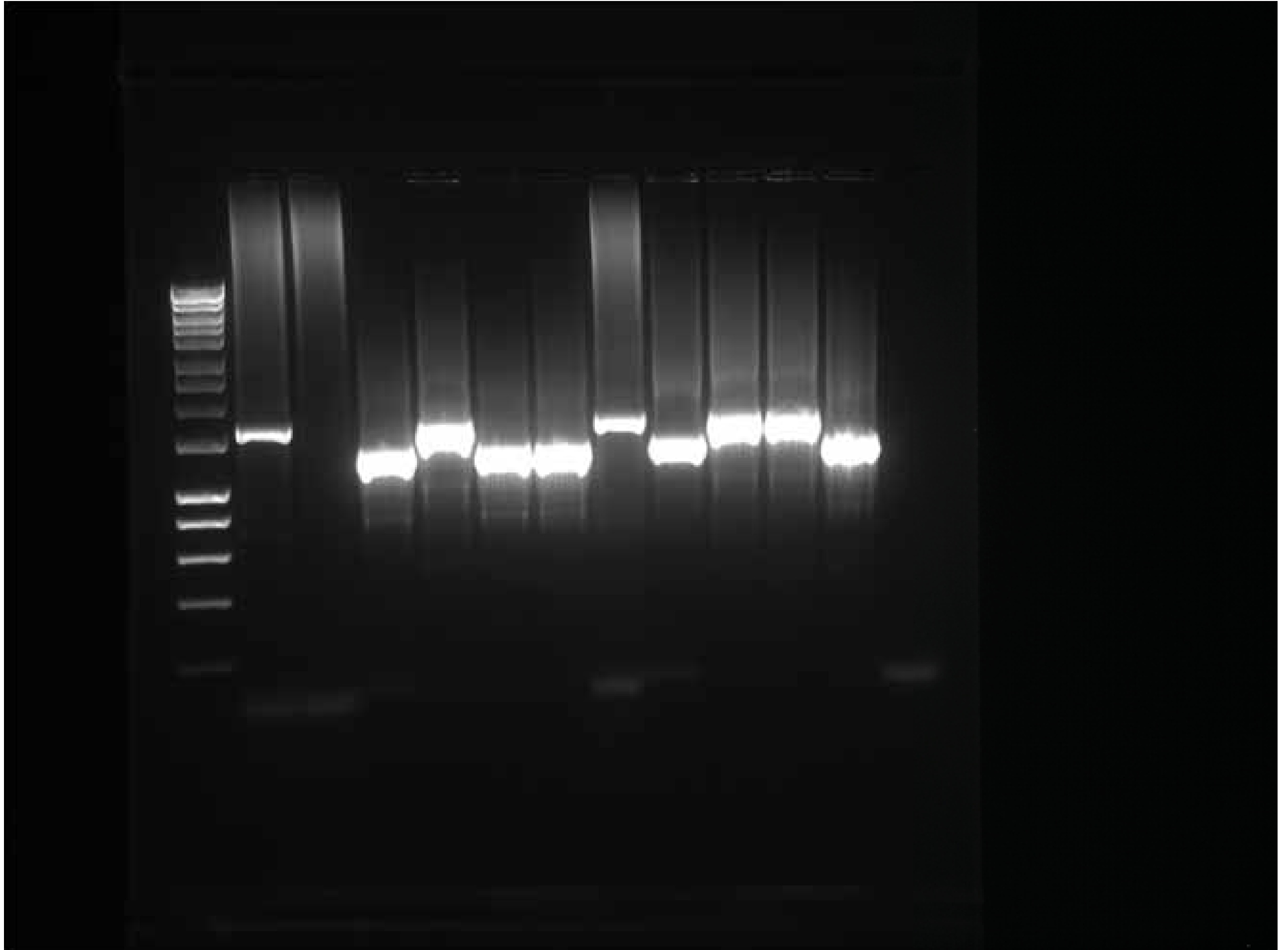
MASTER MIX				
BATCH OR EXPIRY DATE	Components MyFi	25 µL	X3(2 tubes)	TICK WHEN ADDED
	5x React buf (5µL)	5 µL	15	
	MyFi Pol (1µL)	1 µL	3	
	Primer F (1µL)	1 µL	3	
	Primer R (1µL)	1 µL	3	
	H_2O	16 µL	48	
	Dispense mastermix/tueb	**22ul/tube**		
	DNA template	1 µL		
Thermal Cycle MyFi: 94 °C/2 min; 45 X (95°C/30 sec, 53/57°C /20 sec, 72°C/5min); 72°C/5 min; Hold 12 °C				
Program = MyFi HV folder		Thermal cycler used: Biorad		
Run on 1% Gel @50v for 3.5hours, 6ul GelRed, 3ul Bioline hyperladder 1 100uM stock primer = 10ul of stock to 90ul of h2o = 10uM working sol., DNA-1.8-2.0, RNA-1.9-2.1				



TESTED BY:_ Shaun Bochow____ DATE TESTED:______2/4/14_________________

Sample Identification, Extraction and Results												
Accession: A14-015 this is a double PCR using the clean ups and PCR products from the previous PCR. The original template came from A14-005 and VDNA4												
Extraction Method: Roche viral extraction												
Positive control :	**Batch:**			**Positive =**								
	Sample	**Gel lane**	**Result**	**Id**	**Sample**	**Gel lane**	**Result**	**Id**	**Sample**	**Gel Lane**	**Result**	
L	Ladder bio hyp 1	1										
1a	14 5-3 12RC (82 clean up)	2		C82								
2a	14 5-3 12RC (82.1 clean up)	3										
3a	13 5-3 12RC (83 clean up)	4		C83								
4a	14 5-3 12RC (84 clean up)	5		C84								
5a	13 5-3 12RC (85 clean up)	6		C85R								
6a	13 5-3 12RC (85 clean up)	7		C85B								
6	14 5-3 12RC (1 original PCR template)	8		86d								
7	13 5-3 12RC (2 original PCR template)	9		87d								
8	14 5-3 12RC (3 original PCR template)	10		88dR								
8a	14 5-3 12RC (3 original PCR template)	11		88dB								
9	13 5-3 12RC (4 original PCR template)	12		89d								
10	NTC	13										
Primer 14 5-3 F		GTT ACA ATC TAT CGA CGT C				53 Tm						
Primer 12 RC		TAA GAG GTG ACT CTG CTG G				57.3 Tm						
Primer 13 F		GGA GAT GTG ATC CAG AGC TG				57.3 Tm						

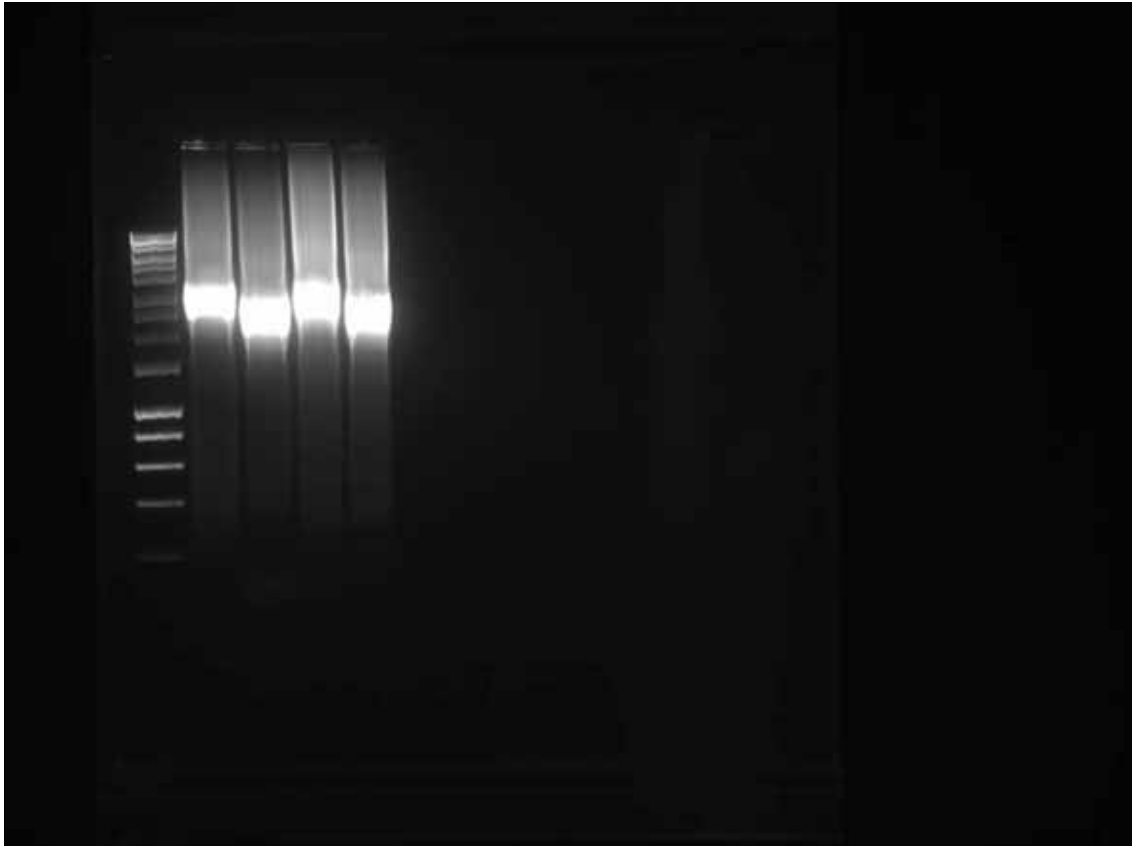
MASTER MIX				
BATCH OR EXPIRY DATE	**Components MyFi**	25 µL	**X12 (2 tubes)**	**TICK WHEN ADDED**
	5x React buf (5µL)	5 µL	60	
	MyFi Pol (1µL)	1 µL	12	
	Primer F (1µL)	1 µL	12	
	Primer R (1µL)	1 µL	12	
	H_2O	16 µL	192	
	Dispense mastermix/tueb	**24ul/tube**		
	DNA template	1 µL		
Thermal Cycle MyFi: 94 °C/2 min; 30 X (95°C/30 sec, 53/57°C /20 sec, 72°C/5min); 72°C/5 min; Hold 12 °C				
Program = MyFi HV folder		**Thermal cycler used: BioerTouch**		
Run on 1% Gel @50v for 3.5hours, 6ul GelRed, 3ul Bioline hyperladder 1 100uM stock primer = 10ul of stock to 90ul of h2o = 10uM working sol., DNA-1.8-2.0, RNA-1.9-2.1				



TESTED BY:_ Shaun Bochow____ DATE TESTED:______3/4/14_________________

Sample Identification, Extraction and Results												
Accession: the Template came from A14-005 and VDNA4, the new accession number is A14-015 to denote the new round of PCR												
Extraction Method: Roche viral extraction												
Positive control :	**Batch:**			**Positive =**								
	Sample	**Gel lane**	**Res ult**	**Cut out**	**Sample**	**Gel lane**	**Res ult**	**Id**	**Sample**	**Gel Lane**	**Res ult**
L	Ladder Bio hyper 1	1									
1	2A 5-3 11BRC (VDNA4)	2	54 Tm	90							
2	2A 5-3 12RC (VDNA4)	3	56 Tm	91							
3	2A 5-3 11BRC (A14-005)	4	54 Tm	92							
4	2A 5-3 12RC (A14-005)	5	56 Tm	93							
5	NTC										
Original Long PCR between contigs											
Primer 2A F		CGT GAG GAG ATT GAT TCA GA				56.4 Tm					
Primer 12 RC		TAA GAG GTG ACT CTG CTG G				57.3 Tm					
Primer 11B R		ATT GCC AAA CAG TAT GGT AC				54.3 Tm					

ßMASTER MIX				
BATCH OR EXPIRY DATE	**Components MyFi**	25 µL	**X3 (2 tubes)**	**TICK WHEN ADDED**
	5x React buf (5µL)	5 µL	15	
	MyFi Pol (1µL)	1 µL	3	
	Primer F (1µL)	1 µL	3	
	Primer R (1µL)	1 µL	3	
	H_2O	16 µL	48	
	Dispense mastermix/tueb	**24ul/tube**		
	DNA template	1 µL		
Thermal Cycle MyFi: 94 °C/2 min; 45 X (95°C/30 sec, 54/56°C /20 sec, 72°C/5min); 72°C/5 min; Hold 12 °C				
Program = MyFi HV folder		**Thermal cycler used: Biorad**		
Run on 1% Gel @50v for 3.5hours, 6ul GelRed, 3ul Bioline hyperladder 1 100uM stock primer = 10ul of stock to 90ul of h2o = 10uM working sol., DNA-1.8-2.0, RNA-1.9-2.1				



TESTED BY:_ Shaun Bochow____ DATE TESTED:______3/4/14_________________

Sample Identification, Extraction and Results

Accession: the Template came from A14-005 and VDNA4, the new accession number is A14-015 to denote the new round of PCR

Extraction Method: Roche viral extraction

Positive control : Batch: Positive =

	Sample	**Gel lane**	**Result**	**Cut out**	**Sample**	**Gel lane**	**Result**	**Id**	**Sample**	**Gel Lane**	**Result**
L	Ladder Bio hyper 1	1									
1	2A R (VDNA4)	2	56		Original Reverse PCR for ends						
2	2A R (A14-005)	3	56	94							
3	14RC (VDNA4)	4	53								
4	14RC (A14-005)	5	53	95							
5	13RC (VDNA4)	6	57								
6	13R (A14-005)	7	57	96							
7	12 3-5 (VDNA4)	8	53	97							
8	12 3-5 (A14-005)	9	53	98,99							
9	11B F (VDNA4)	10	54								
10	11B F (A14-005)	11	54	100							
11	NTC	12									

Primer	Sequence	Tm
Primer 2A R	TCT GAA TCA ATC TCC TCA CG	56.4 Tm
Primer 14 RC	GAC GTC GAT AGA TTG TAA C	53 Tm
Primer 13 R	CAG CTC TGG AT CACA TCT C	57.3 Tm
Primer 12 3-5	CAG CAG AGT CAC CTC TTA	53.9 Tm
Primer 11B F	GTA CCA TAC TGT TTG GCA ATG	54.3 Tm

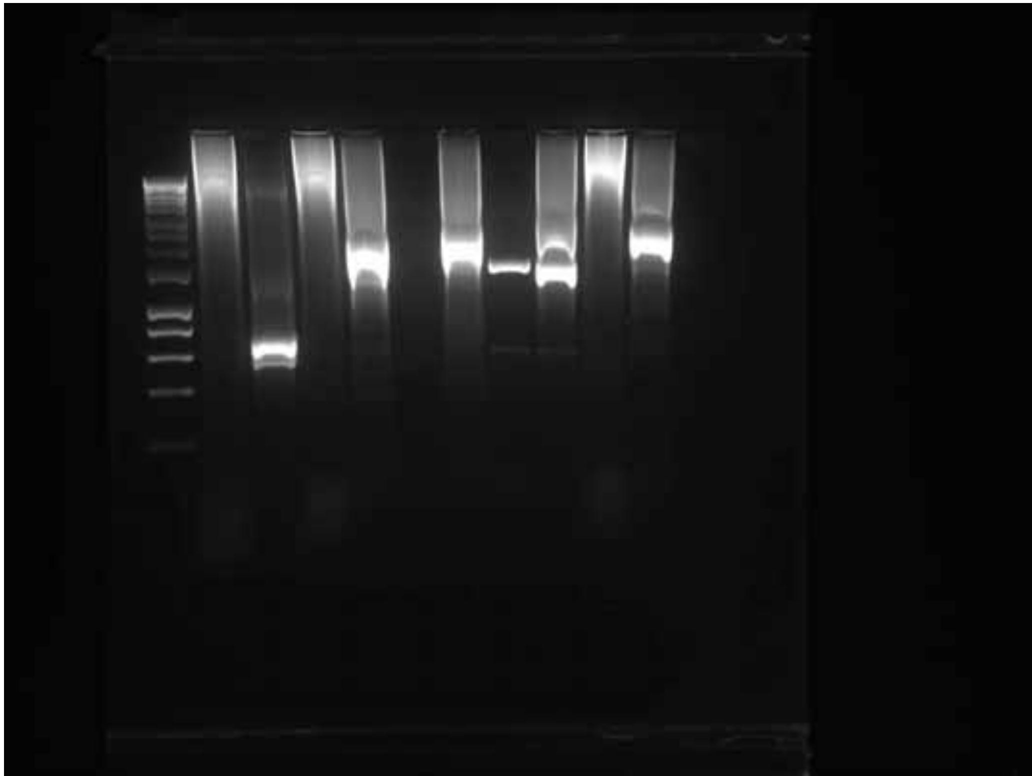
MASTER MIX

BATCH OR EXPIRY DATE	**Components MyFi**	25 µL	**X12**	**TICK WHEN ADDED**
	5x React buf (5µL)	5 µL	60	
	MyFi Pol (1µL)	1 µL	12	
	Primer F (1µL)	1 µL	12	
	Primer R (1µL)	1 µL	12	
	H_2O	16 µL	204	
	Dispense mastermix/tueb	**24ul/tube**		
	DNA template	1 µL		

Thermal Cycle MyFi: 94 °C/2 min; 45 X (95°C/30 sec, 56,53,57,54°C /20 sec, 72°C/5min); 72°C/5 min; Hold 12 °C

Program = MyFi HV folder Thermal cycler used: Biorad

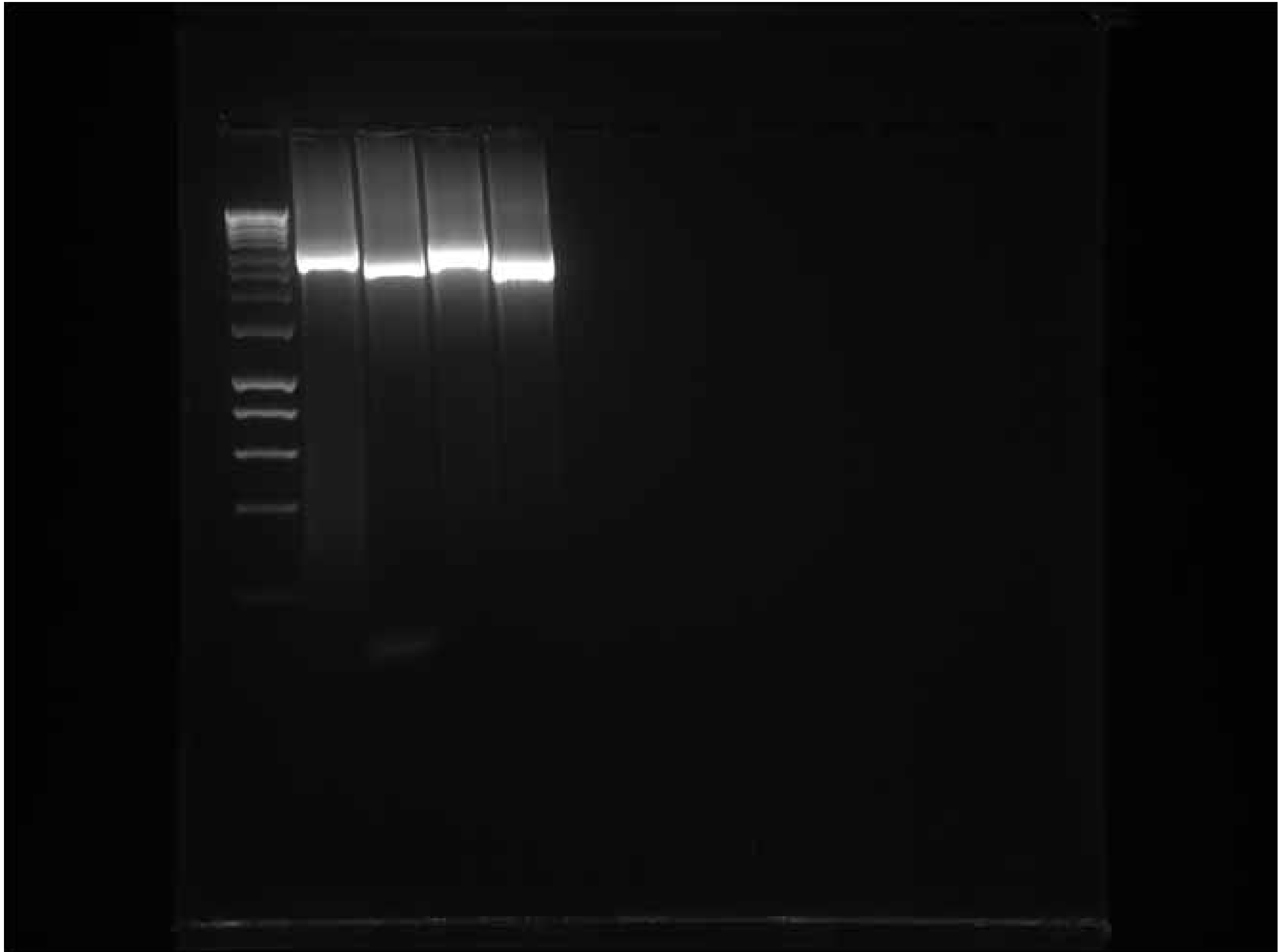
Run on 1% Gel @50v for 3.5hours, 6ul GelRed, 3ul Bioline hyperladder 1 100uM stock primer = 10ul of stock to 90ul of h2o = 10uM working sol., DNA-1.8-2.0, RNA-1.9-2.1



TESTED BY:_ Shaun Bochow____ DATE TESTED:______11/4/14_________________

Sample Identification, Extraction and Results											
Accession: the Template came from A14-005 and VDNA4, the new accession number is A14-015 to denote the new round of PCR This is a double PCR using cut outs as template											
Extraction Method: Roche PCR product clean up kit											
Positive control :	**Batch:**			**Positive =**							
	Sample	**Gel lane**	**Res ult**	**Cut out**	**Sample**	**Gel lane**	**Res ult**	**Id**	**Sample**	**Gel Lane**	**Res ult**
L	Ladder Bio hyper 1	1									
1*	2A F 11BR (clean up 90)	2	54 Tm	C90L							
2*	2A F 11BR (clean up 91)	3	56 Tm	C91							
3*	2A F 11BR (clean up 92)	4	54 Tm	C92							
4*	2A F 11BR (clean up 93)	5	56 Tm	C93							
5	NTC	6									
dPCR using clean ups as template Long PCR between contigs											
Primer 2A F	CGT GAG GAG ATT GAT TCA GA					56.4 Tm					
Primer 12 RC	TAA GAG GTG ACT CTG CTG G					57.3 Tm					
Primer 11B R	ATT GCC AAA CAG TAT GGT AC					54.3 Tm					

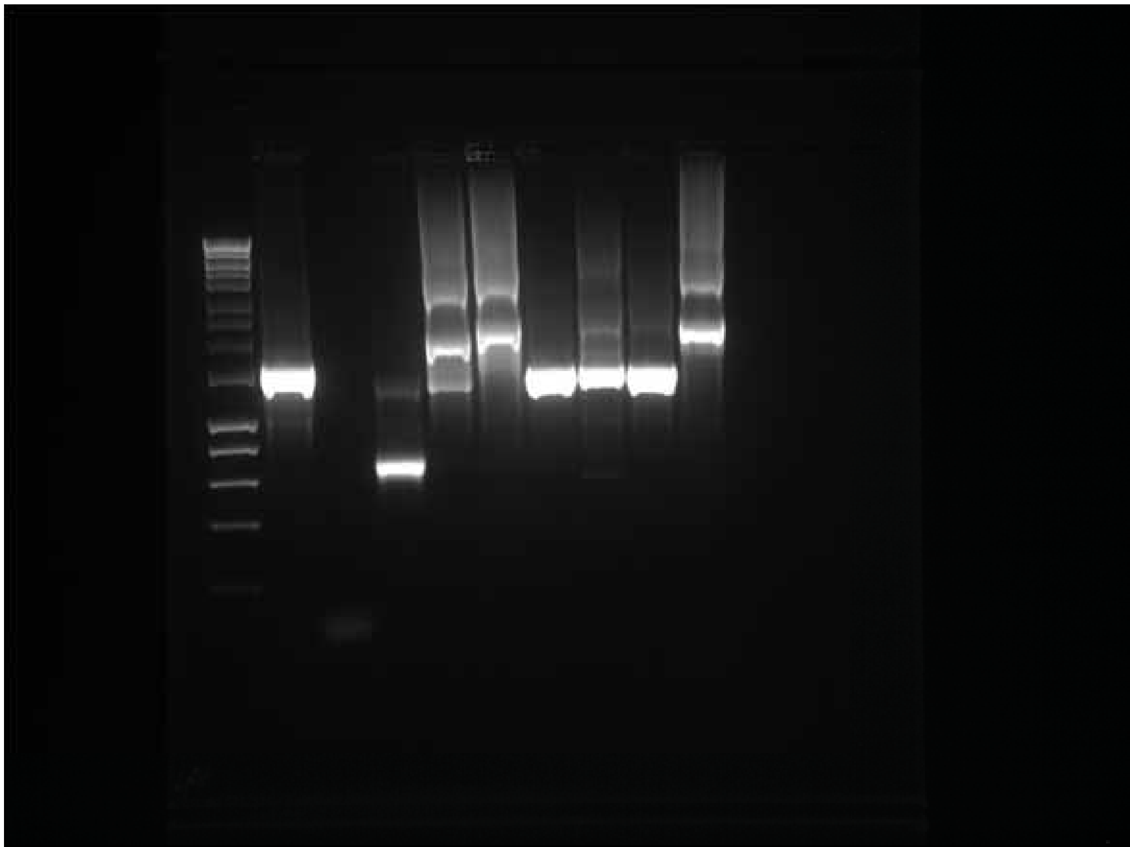
MASTER MIX				
BATCH OR EXPIRY DATE	**Components MyFi**	25 μL	**X6**	**TICK WHEN ADDED**
	5x React buf (5μL)	5 μL	30	
	MyFi Pol (1μL)	1 μL	6	
	Primer F (1μL)	1 μL		
	Primer R (1μL)	1 μL		
	H_2O	16 μL	96	
	Dispense mastermix/tueb	**24ul/tube**		
	DNA template	1 μL		
Thermal Cycle MyFi: 94 °C/2 min; 30 X (95°C/30 sec, 54/56°C /20 sec, 72°C/5min); 72°C/5 min; Hold 12 °C				
Program = MyFi HV folder		**Thermal cycler used: BioTouch**		
Run on 1% Gel @50v for 3.5hours, 6ul GelRed, 4ul Bioline hyperladder 1 100uM stock primer = 10ul of stock to 90ul of h2o = 10uM working sol., DNA-1.8-2.0, RNA-1.9-2.1				



TESTED BY:_ Shaun Bochow____ DATE TESTED:______11/4/14_________________

Sample Identification, Extraction and Results											
Accession: the Template came from A14-005 and VDNA4, the new accession number is A14-015 to denote the new round of PCR The template for this PCR is clean up 90 (2A 5-3 11bRC REV) this is a nested PCR to get seq from the middle of this seq											
Extraction Method: Roche PCR clean up kit											
Positive control :	**Batch:**			**Positive =**							
	Sample	**Gel lane**	**Res ult**	**Cut out**	**Sample**	**Gel lane**	**Res ult**	**Id**	**Sample**	**Gel Lane**	**Res ult**
L	Ladder Bio hyper 1	1									
N1	14 5-3 12RC (clean up 90)	2	54	C90S							
	NTC	3									
Nested PCR using 90 as template											
Primer 12 RC		TAA GAG GTG ACT CTG CTG G				57.3 Tm					
Primer 14 5-3 F		GTT ACA ATC TAT CGA CGT C				53 Tm					

MASTER MIX				
BATCH OR EXPIRY DATE	**Components MyFi**	25 µL	**X2**	**TICK WHEN ADDED**
	5x React buf (5µL)	5 µL	10	
	MyFi Pol (1µL)	1 µL	2	
	Primer F (1µL)	1 µL	2	
	Primer R (1µL)	1 µL	2	
	H_2O	16 µL	32	
	Dispense mastermix/tueb	**24ul/tube**		
	DNA template	1 µL		
Thermal Cycle MyFi: 94°C/2 min; 30 X (95°C/30 sec, 54°C /20 sec, 72°C/5min); 72°C/5 min; Hold 12°C				
Program = MyFi HV folder		**Thermal cycler used: BioTouch**		
Run on 1% Gel @50v for 3.5hours, 6ul GelRed, 3ul Bioline hyperladder 1 100uM stock primer = 10ul of stock to 90ul of h2o = 10uM working sol., DNA-1.8-2.0, RNA-1.9-2.1				



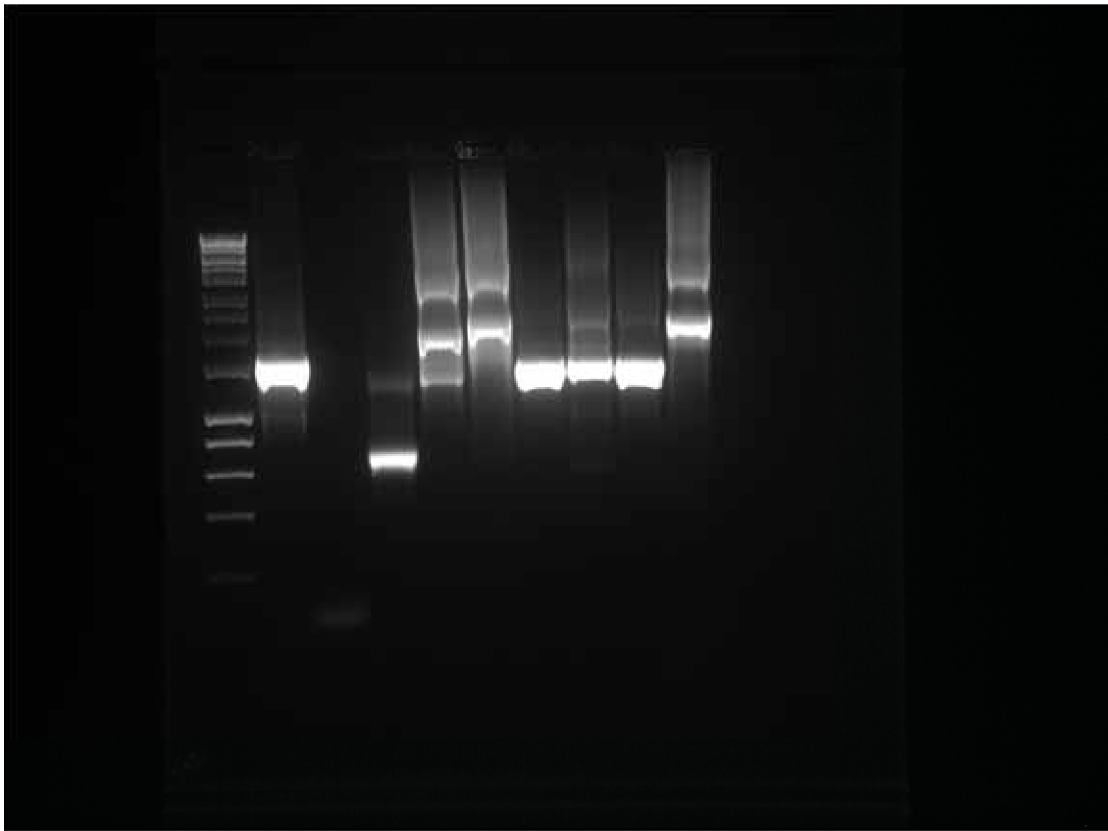
	Aquatic Pathobiology Working Group	*FORM: CqDV PCR*
	CQDV PCR	

TESTED BY:_ Shaun Bochow____ DATE TESTED:______11/4/14_________________

Sample Identification, Extraction and Results												
Accession: the Template came from A14-005 and VDNA4, the new accession number is A14-015 to denote the new round of PCR The template used for these PCR is the cuts outs												
Extraction Method: Roche PCR clean up kit												
Positive control :	**Batch:**				**Positive =**							
	Sample	**Gel lane**	**Res ult**	**Cut out**	**Sample**	**Gel lane**	**Res ult**	**Id**	**Sample**	**Gel Lane**	**Res ult**
1x	2A R REV (clean up 94)	4	56	C94	dPCR Reverse PCR for ends						
2x	14RC (clean up 95)	5	53	C95.1.2.3							
3x	13R (clean up 96)	6	57	C96.1.2							
4x	12 3-5 (clean up 97)	7	53	C97							
5x	12 3-5 (clean up 98)	8	53	C98							
6x	12 3-5 (clean up 99)	9	53	C99							
7x	11B F (clean up 100)	10	54	C100							
8x	NTC	11									

Primer	Sequence	Tm
Primer 2A R	TCT GAA TCA ATC TCC TCA CG	56.4 Tm
Primer 14 RC	GAC GTC GAT AGA TTG TAA C	53 Tm
Primer 13 R	CAG CTC TGG AT CACA TCT C	57.3 Tm
Primer 12 3-5	CAG CAG AGT CAC CTC TTA	53.9 Tm
Primer 11B F	GTA CCA TAC TGT TTG GCA ATG	54.3 Tm

MASTER MIX				
BATCH OR EXPIRY DATE	**Components MyFi**	25 μL	**X9**	**TICK WHEN ADDED**
	5x React buf (5μL)	5 μL	45	
	MyFi Pol (1μL)	1 μL	9	
	Primer F (1μL)	1 μL		
	Primer R (1μL)	1 μL		
	H_2O	16 μL	144	
	Dispense mastermix/tueb	**24ul/tube**		
	DNA template	1 μL		
Thermal Cycle MyFi: 94 °C/2 min; 30 X (95°C/30 sec, 56,53,57,54°C /20 sec, 72°C/5min); 72°C/5 min; Hold 12 °C				
Program = MyFi HV folder		**Thermal cycler used: Biorad**		
Run on 1% Gel @50v for 3.5hours, 6ul GelRed, 3ul Bioline hyperladder 1 100uM stock primer = 10ul of stock to 90ul of h2o = 10uM working sol., DNA-1.8-2.0, RNA-1.9-2.1				

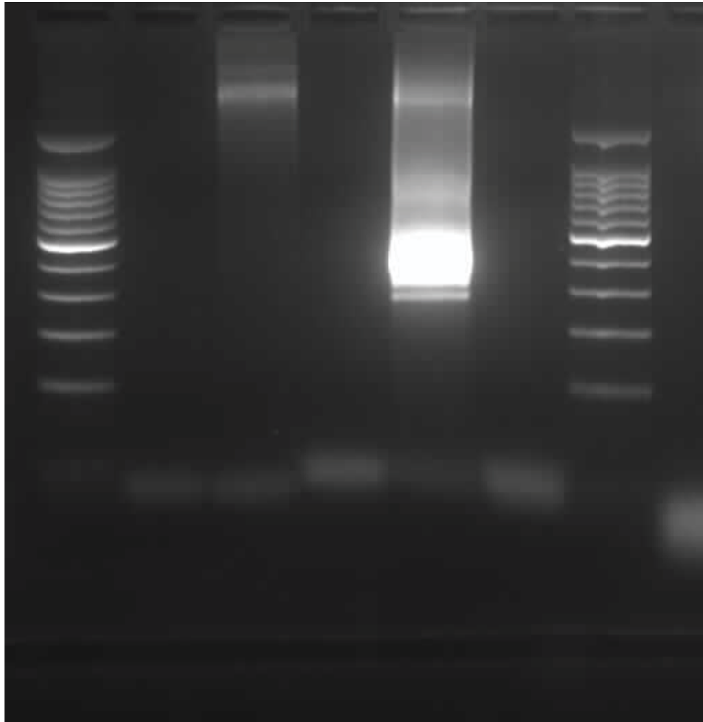


	Aquatic Pathobiology Working Group	*FORM: CqDV PCR*
	CQDV PCR	

TESTED BY:_ Shaun Bochow____ DATE TESTED:______3/6/14_________________

Sample Identification, Extraction and Results											
Accession: the viral Template came from A14-005 and VDNA4, the new accession number is A14-023 to denote the new round of PCR											
Extraction Method: Roche viral extraction											
Positive control :	**Batch:**			**Positive =**							
	Sample	**Gel lane**	**Res ult**	**Id**	**Sample**	**Gel lane**	**Res ult**	**Id**	**Sample**	**Gel Lane**	**Res ult**
	Second comb										
L	Ladder 100bp prom	1									
1	VDNA4 (CqPV 5 F)	2									
2	A14-005 (CqPV 5 F)	3									
3	VDNA4 (CqPV 5 RcF)	4									
4	A14-005 (CqPV 5 RcF)	5	103								
5	NTC	6									
	Expect ~500bp										
	5' end										
Primer CqPV 5 F	CGA CAC CTC TCA CGT AGT CTC Tm 63.3										
Primer CqPV 5 RcF	CTC TAG TGC ACT CTC CAC AGC Tm 63.3										

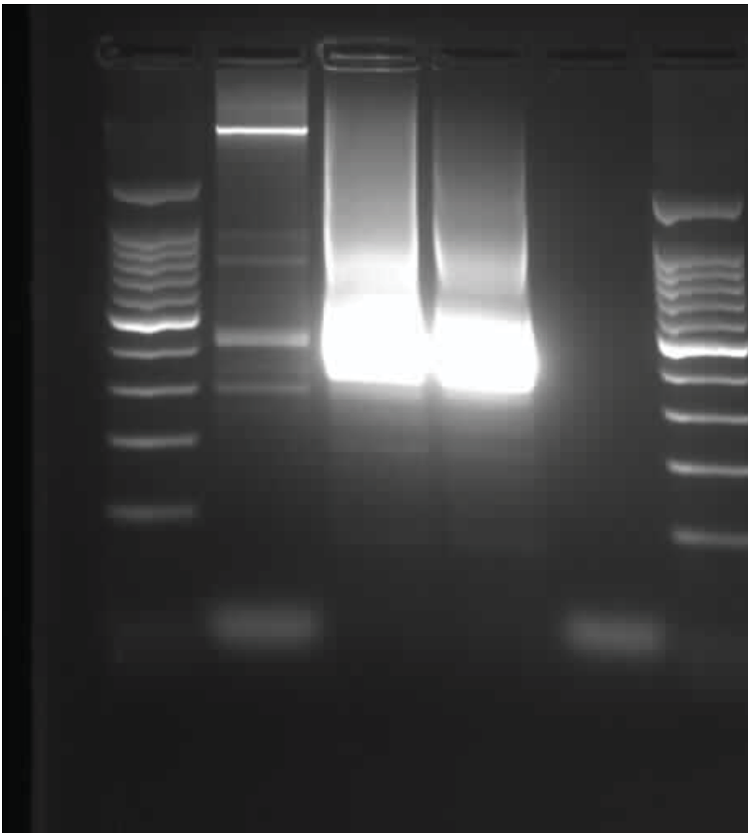
MASTER MIX				
BATCH OR EXPIRY DATE	**Components MyFi**	25 µL	**X3(2 tubes)**	**TICK WHEN ADDED**
	5x React buf (5µL)	5 µL	15	
	MyFi Pol (1µL)	1 µL	3	
	Primer F (1µL)	1 µL	3	
	Primer R (1µL)	0 µL	0	
	H_2O	17 µL	51	
	Dispense mastermix/tueb	**24ul/tube**		
	DNA template	1 µL		
Thermal Cycle MyFi: 94 °C/2 min; 45 X (95°C/30 sec 62.5°C /20 sec, 72°C/1min); 72°C/7 min; Hold 12 °C				
Program = MyFi HV folder		**Thermal cycler used: Biorad**		
Run on 2% Gel @109v for 50 min, 6ul GelRed, 2µl Promega 100bp ladder, 1µl loading dye, 4µl H_2O, 100uM stock primer = 10ul of stock to 90ul of h2o = 10uM working sol., DNA-1.8-2.0, RNA-1.9-2.1				



TESTED BY:_ Shaun Bochow____ DATE TESTED:______6/6/14_________________

Sample Identification, Extraction and Results											
Accession: the viral Template came from A14-005 and cut outs, the new accession number is A14-023 to denote the new round of PCR											
Extraction Method: Roche viral extraction											
Positive control :	**Batch:**				**Positive =**						
	Sample	**Gel lane**	**Res ult**	**Id**	**Sample**	**Gel lane**	**Res ult**	**Id**	**Sample**	**Gel Lane**	**Res ult**
	Gel 2										
L	Ladder Prom 100bp	1									
1	A14-005 2.5ul (CqPV 5 RcF)	2	108. 1, 108. 2								
2	103 PCR TLT 1ul	3	109								
3	103 cut out 1ul	4	110								
4	NTC	5									
	Expect ~500bp										
	5' end										
Primer											
Primer CqPV 5 RcF		CTC TAG TGC ACT CTC CAC AGC Tm 63.3									

MASTER MIX				
BATCH OR EXPIRY DATE	**Components MyFi**	50 µL	**X5**	**TICK WHEN ADDED**
	5x React buf (5µL)	10 µL	15	
	MyFi Pol (1µL)	2 µL	10	
	Primer F (1µL)	2 µL	10	
	Primer R (1µL)	0 µL	0	
	H_2O	34 µL	170	
	Dispense mastermix/tueb	**48ul/tube**		
	DNA template	1 µL		
Thermal Cycle MyFi: 94 °C/2 min; 35 X (95°C/30 sec 62.5°C /20 sec, 72°C/1min); 72°C/7 min; Hold 12 °C				
Program = 5 prime end		**Thermal cycler used: Biorad**		
Run on 2% Gel @109v for 50 min, 6ul GelRed, 2µl Promega 100bp ladder, 1µl loading dye, 4µl H_2O, 100uM stock primer = 10ul of stock to 90ul of h2o = 10uM working sol., DNA-1.8-2.0, RNA-1.9-2.1				



	Aquatic Pathobiology Working Group	*FORM: CqDV PCR*
	CQDV PCR	

TESTED BY:_ Shaun Bochow____ DATE TESTED:______7/6/14_________________

Sample Identification, Extraction and Results

Accession: the viral Template came from VDNA6, the new accession number is A14-023 to denote the new round of PCR

Extraction Method: Roche viral extraction

Positive control : Batch: Positive =

	Sample	**Gel lane**	**Result**	**Id**	**Sample**	**Gel lane**	**Result**	**Id**	**Sample**	**Gel Lane**	**Result**
	Gel 1 Comb 1										
CqPV 5 RcF (tubes 1-5) 1) Add **1µl** of primer to 1µl sample and **10µl** of H_2O Add **2µl** of primer to 1µl sample and **9µl** of H_2O Add **3µl** of primer to 1µl sample and **8µl** of H_2O Add **4µl** of primer to 1µl sample and **7µl** of H_2O Add **5µl** of primer to 1µl sample and **6µl** of H_2O 2) heat samples to 95°C for 6 minutes (Biorad block B) 3) take out and put on ice (PCR ice rack) 4) heat normal master mix to 95°C for 2 minutes (Biorad block A) 5) cool to 58°C and for 1 minute (hit pause button Biorad block A) 6) open lid to Biorad block A and add 12µl to M.M 7) heat to 72°C for 1 minute 8) Normal PCR for 30 cycles				**CqPV qCr5-3 (tubes 6-10)** 1) Add **1µl** of primer to 1µl sample and **10µl** of H_2O Add **2µl** of primer to 1µl sample and **9µl** of H_2O Add **3µl** of primer to 1µl sample and **8µl** of H_2O Add **4µl** of primer to 1µl sample and **7µl** of H_2O Add **5µl** of primer to 1µl sample and **6µl** of H_2O							
L	Ladder Prom 100bp	1									
1	CqPV 5 RcF VDNA6	2		6	CqPV F VDNA6	7					
2	CqPV 5 RcF VDNA6	3		7	CqPV F VDNA6	8					
3	CqPV 5 RcF VDNA6	4	**105.1, 105.2**	8	CqPV F VDNA6	9					
4	CqPV 5 RcF VDNA6	5	**106**	9	CqPV F VDNA6	10					
5	CqPV 5 RcF VDNA6	6	**107**	10	CqPV F VDNA6	11					

Primer CqPV 5 RcF	CTC TAG TGC ACT CTC CAC AGC	Tm 56.3
Primer CqPV F	GGA ATA TTA CGT TCA GCA AGT CC	Tm 53.5

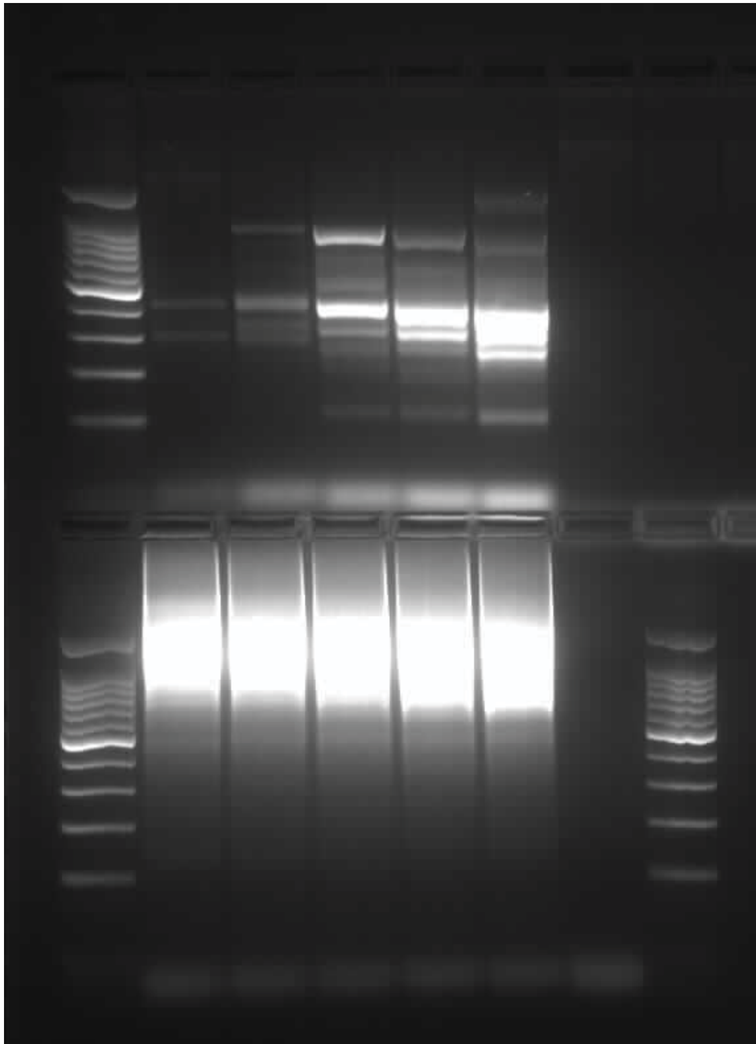
MASTER MIX

BATCH OR EXPIRY DATE	**Components MyFi**	25 µL	**X6**	**TICK WHEN ADDED**
	5x React buf (5µL)	5 µL	30	
	MyFi Pol (1µL)	1 µL	6	
	Primer F (1µL)	0 µL		
	Primer R (1µL)	0 µL		
	H_2O	7 µL	42	
	Dispense mastermix/tueb	**24ul/tube**		
	DNA template	1 µL		

Thermal Cycle: 94 °C/2 min; 58 °C/1 min; 35 X (95°C/30 sec 58°C/20 sec, 72°C/1 min); 72°C/7 min; Hold 12 °C

Program = Ends Iced **Thermal cycler used: Biorad**

Run on 2% Gel @109v for 50 min, 6ul GelRed, 2µl Promega 100bp ladder, 1µl loading dye, 4µl H_2O, 100uM stock primer = 10ul of stock to 90ul of h2o = 10uM working sol., DNA-1.8-2.0, RNA-1.9-2.1

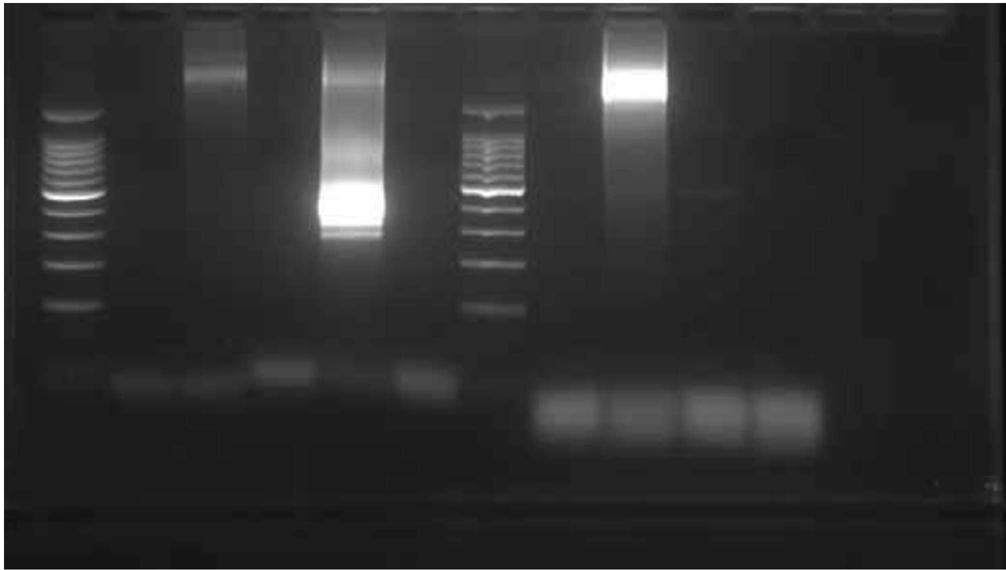


	Aquatic Pathobiology Working Group	*FORM: CqDV PCR*
	CQDV PCR	

TESTED BY:_ Shaun Bochow____ DATE TESTED:______4/6/14_________________

Sample Identification, Extraction and Results											
Accession: the viral Template came from A14-005 and VDNA4, the new accession number is A14-023 to denote the new round of PCR Clean up C85B was used as control											
Extraction Method: Roche viral extraction											
Positive control : **Batch:** **Positive = Clean up C85B**											
	Sample	**Gel lane**	**Res ult**	**Id**	**Sample**	**Gel lane**	**Res ult**	**Id**	**Sample**	**Gel Lane**	**Res ult**
	Second comb										
L	Ladder	7									
1	VDNA4 (2444 F – 2904 RC R)	8									
2	A14-005 (2444 F – 2904 RC R)	9	104								
3	Clean up C85B (2444 F – 2904 RC R)	10									
4	NTC	11									
	Expect 480bp										
	Gap filler two seq or less										
Primer 2444 F		CTC CTG TGT ATC GTA CAC CTG TM 58.4									
Primer 2904 RC R		GAA TGA TAG CGC TAC CGC T Tm 57.3									

MASTER MIX				
BATCH OR EXPIRY DATE	**Components MyFi**	25 µL	**X5**	**TICK WHEN ADDED**
	5x React buf (5µL)	5 µL	25	
	MyFi Pol (1µL)	1 µL	5	
	Primer F (1µL)	1 µL	5	
	Primer R (1µL)	1 µL	5	
	H_2O	16 µL	80	
	Dispense mastermix/tueb	**24ul/tube**		
	DNA template	1 µL		
Thermal Cycle: 94 °C/2 min; 45 X (95°C/30 sec 57°C /20 sec, 72°C/45sec); 72°C/7 min; Hold 12 °C				
Program = Gap filler **Thermal cycler used: Genetouch**				
Run on 2% Gel @109v for 50 min, 6ul GelRed, 2µl Promega 100bp ladder, 1µl loading dye, 4µl H_2O, 100uM stock primer = 10ul of stock to 90ul of h2o = 10uM working sol., DNA-1.8-2.0, RNA-1.9-2.1				

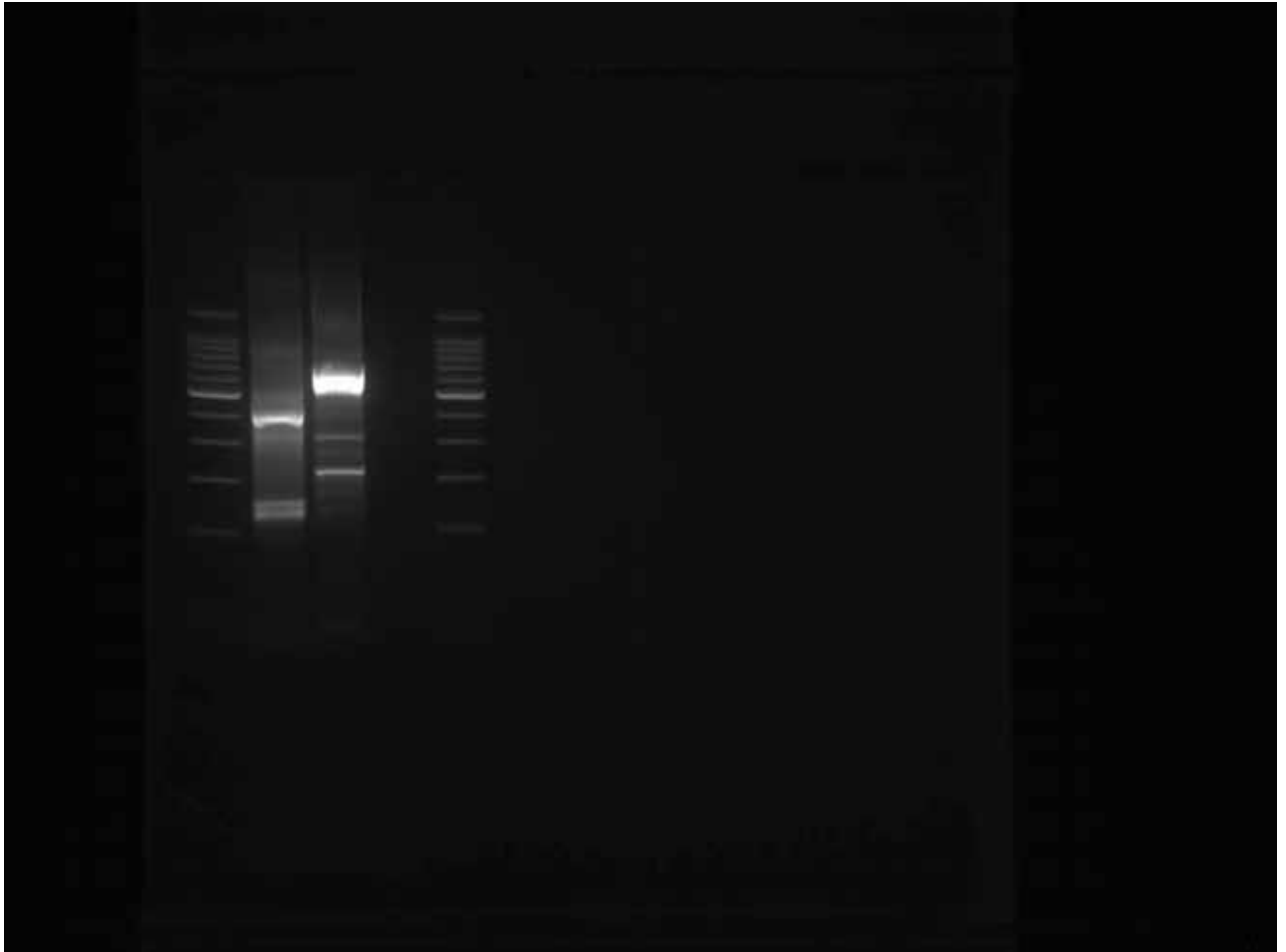


	Aquatic Pathobiology Working Group	FORM: CqDV PCR
	CQDV PCR	

TESTED BY:_ Shaun Bochow____ DATE TESTED:______4/8/14_________________

Sample Identification, Extraction and Results											
Accession: the viral Template came from A14-036.1, the new accession number is A14-034 to denote the new round of PCR											
Extraction Method: Roche viral extraction											
Positive control :	**Batch:**			**Positive =**							
	Sample	**Gel lane**	**Res ult**	**Id**	**Sample**	**Gel lane**	**Res ult**	**Id**	**Sample**	**Gel Lane**	**Res ult**
L	Hyper ladder 1kb	1									
1	A14-036 CqPV 5 RcF (5' end left)	2	115								
2	A14-036 2A R (5' end left)	3	116, 117								
3	NTC	4									
Primer CqPV 5 RcF		CTC TAG TGC ACT CTC CAC AGC				TM 63.3					
Primer 2A R		TCT GAA TCA ATC TCC TCA CG				TM 56.4					

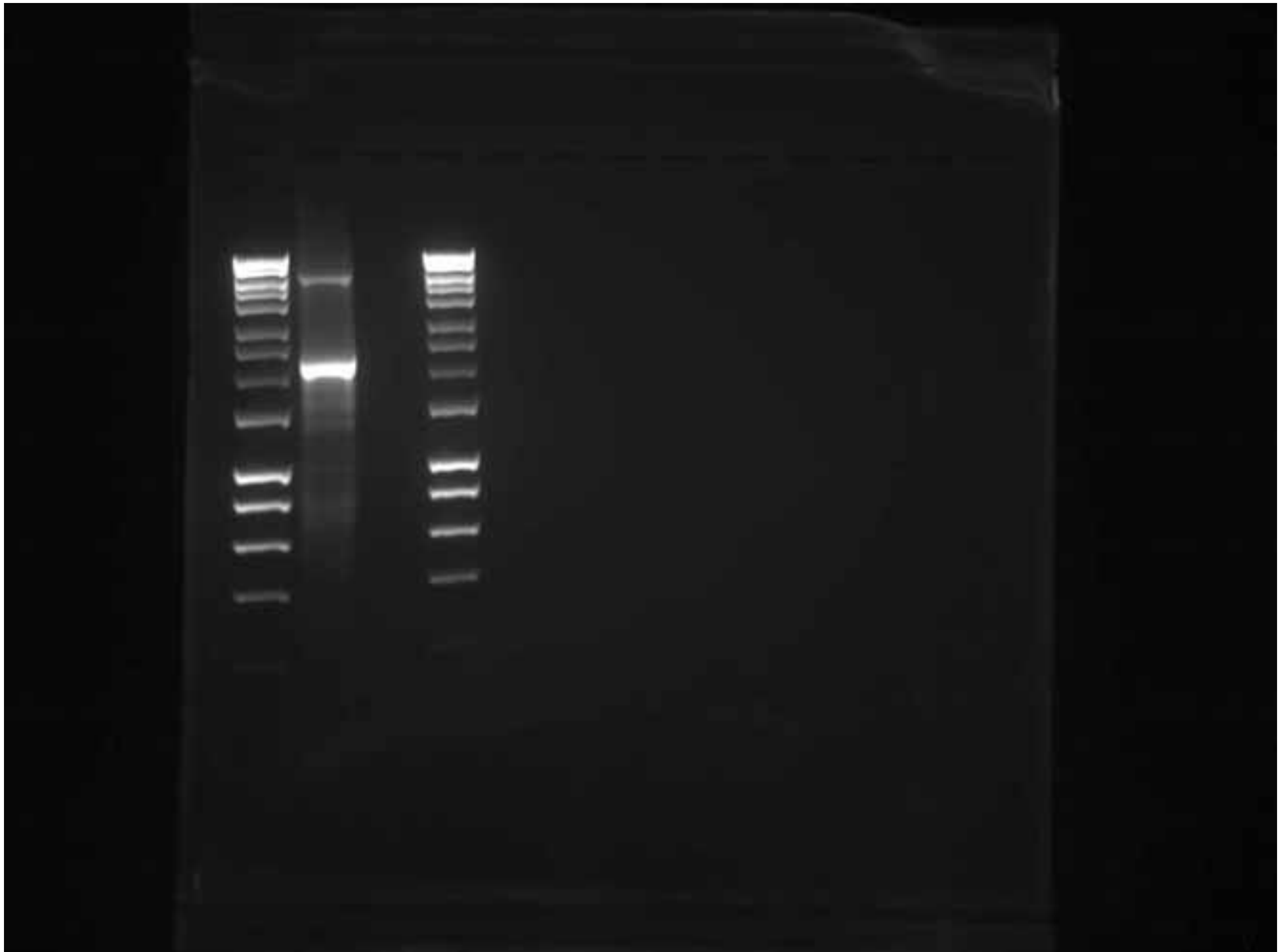
MASTER MIX					
BATCH OR EXPIRY DATE	**COMPONENTS**	25 μL	One reaction (12.5 μL)	**TOTAL (X 3)**	**TICK WHEN ADDED**
	Reaction Buffer (5x, Bioline)	5 μL	2.5μL	μL	
	$MgCl_2$ (50 mM) **(increased by 0.5ul)**	1 μL	0.25 μL	μL	
	Mango Taq polymerase (5U/μL, Bioline)	0.5 μL	0.25 μL	μL	
	dNTP's (10mM each)	0.5 μL	0.25 μL	μL	
	H_2O (nuclease free)	15.0 μL	7.25 μL	μL	
	Total to add to each tube		11.5ul/tube	**μL**	
	Primer F (10pmol/μL)	1 μL	0.5 μL	μL	
	Primer R (10pmol/μL)	1 μL	0.5 μL	μL	
	Total to add to each tube			**μL**	
		25ul/tube			
	Dispense Master Mix/tube	24 μL	11.5 μL	**24/**	
	Add DNA template	1 uL	1 uL	**1/**	
Thermal Cycle: 94 °C/2 min; 35 X (95°C/30 sec (60, 56)°C/20 sec, 72°C/15sec); 72°C/7 min; Hold 12 °C					
Program = 5' left **Thermal cycler used: Biorad**					
Run on 2% Gel @109v for 50 min, 6ul GelRed, 2μl Promega 100bp ladder, 1μl loading dye, 4μl H_2O, 100uM stock primer = 10ul of stock to 90ul of h2o = 10uM working sol., DNA-1.8-2.0, RNA-1.9-2.1					



TESTED BY:_ Shaun Bochow____ DATE TESTED:______6/8/14_________________

Sample Identification, Extraction and Results											
Accession: the viral Template came from A14-036 and, the new accession number is A14-034 to denote the new round of PCR											
Extraction Method: Roche viral extraction											
Positive control :	**Batch:**		**Positive = Clone**								
	Sample	**Gel lane**	**Res ult**	**Id**	**Sample**	**Gel lane**	**Res ult**	**Id**	**Sample**	**Gel Lane**	**Res ult**
L	Hyperladder 1kb	1									
1	A14-036 Primer 11B F (3' right)	2	120, 121								
2	NTC	3									
Primer 11B F		GTA CCA TAC TGT TTG GCA ATG				Tm 57.4					
Primer						Tm					

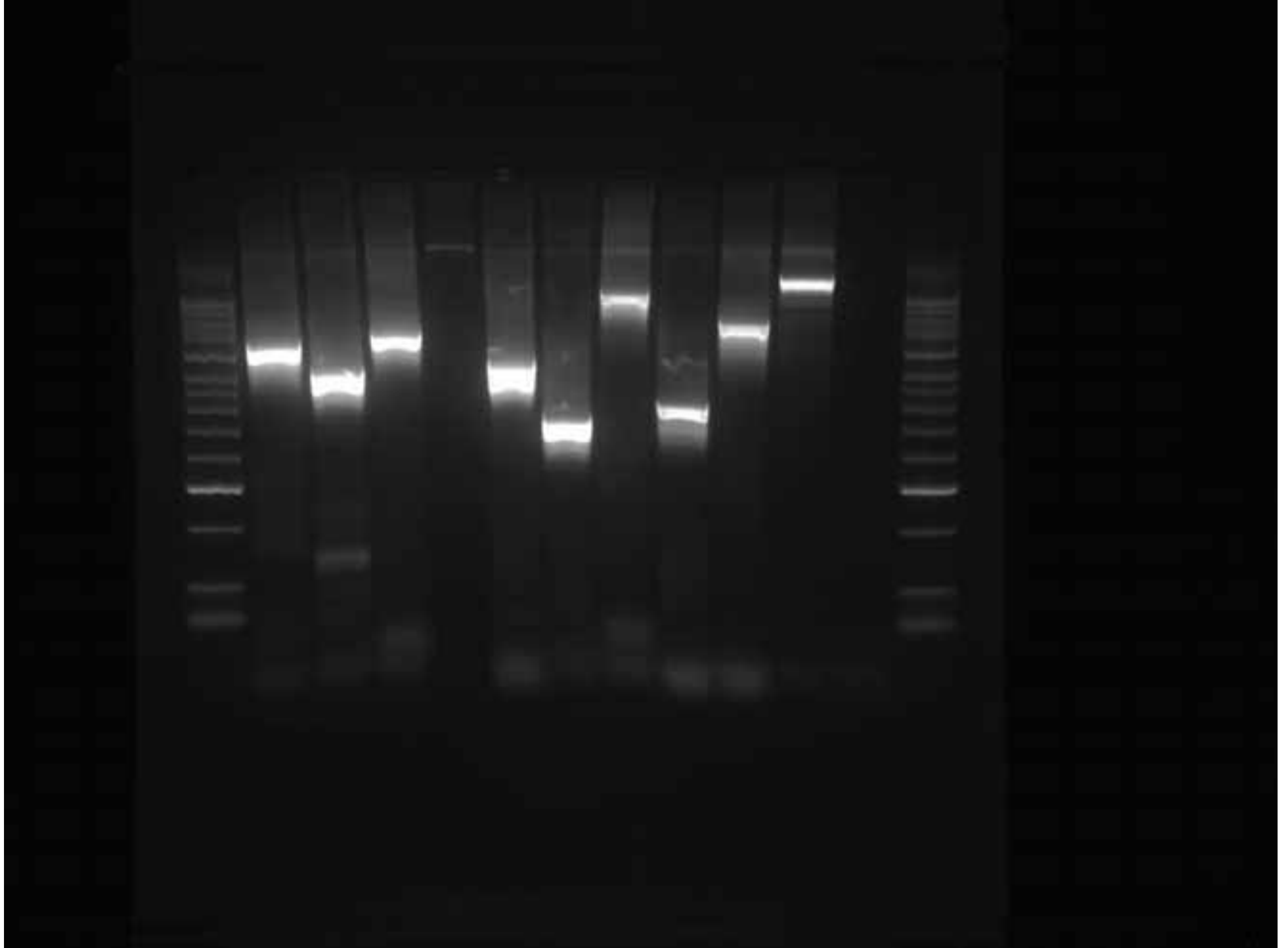
MASTER MIX				
BATCH OR EXPIRY DATE	**Components MyFi**	25 µL	**X1**	**TICK WHEN ADDED**
	5x React buf (5µL)	5 µL	5	
	MyFi Pol (1µL)	1 µL	1	
	Primer F (1µL)	1 µL	1	
	Primer R (1µL)	1 µL	0	
	H_2O	17 µL	17	
	Dispense mastermix/tueb	**24ul/tube**		
	DNA template	1 µL		
Thermal Cycle MyFi: 94 °C/2 min; 35 X (95°C/30 sec 57°C/20 sec, 72°C/45sec); 72°C/7 min; Hold 12 °C				
Program = 3 Prime end		**Thermal cycler used: Biorad**		
Run on 1% Gel @50v for 3.5hours, 6ul GelRed, 2ul Bioline hyperladder 1kb; 100uM stock primer = 10ul of stock to 90ul of h2o = 10uM working sol., DNA-1.8-2.0, RNA-1.9-2.1				



TESTED BY:_ Shaun Bochow____ DATE TESTED:______12/8/14_________________

Sample Identification, Extraction and Results											
Accession: the viral Template came from A14-036, the new accession number is A14-034 to denote the new round of PCR											
Extraction Method: Roche viral extraction											
Positive control :	**Batch:**			**Positive =**							
	Sample	**Gel lane**	**Result**	**Id**	**Sample**	**Gel lane**	**Result**	**Id**	**Sample**	**Gel Lane**	**Result**
L	HyperLadder 50bp										
1 (122)	A14-036 CqPV 5 F - BgF3R (expect 1054)	1	30sec/60 °C								
2 (123)	A14-036 Primer 2A F- BgF3R (expect 788)	2	20sec/56 °C								
3 (124)	A14-036 Primer 2A F - BgR3R (expect 1135)	3	40sec/56 °C								
Primer CqPV 5 F	CGC TGT GGA GAG TGC ACT AGA G					TM 60.5					
Primer 2A F	CGT GAG GAG ATT GAT TCA GA					Tm 56.4					
Primer BgR3	CAC ATT CCG TTG CTG AAG GTG					Tm 61.3					
Primer BgF3 R	GGA TGT ACT TGG CTC AAC GG					TM 60.5					

MASTER MIX					
BATCH OR EXPIRY DATE	**COMPONENTS**	25 µL	One reaction (12.5 µL)	**TOTAL (X 4)**	**TICK WHEN ADDED**
	Reaction Buffer (5x, Bioline)	5 µL	2.5µL	20 µL	
	$MgCl_2$ (50 mM) (**increased by 0.5ul)**	1 µL	0.25 µL	4 µL	
	Mango Taq polymerase (5U/µL, Bioline)	0.5 µL	0.25 µL	2 µL	
	dNTP's (10mM each)	0.5 µL	0.25 µL	2 µL	
	H_2O (nuclease free)	15.0 µL	7.25 µL	60 µL	
	Total to add to each tube		11.5ul/tube	**µL**	
	Primer F (10pmol/µL)	1 µL	0.5 µL	0 µL	
	Primer R (10pmol/µL)	1 µL	0.5 µL	0 µL	
	Total to add to each tube			**µL**	
		25ul/tube			
	Dispense Master Mix/tube	24 µL	11.5 µL	**24/**	
	Add DNA template	1 uL	1 uL	**1/**	
Thermal Cycle: 94 °C/2 min; 35 X (95°C/30 sec Tm°C/20 sec, 72°C/Xsec); 72°C/7 min; Hold 12 °C					
Program = 5' end of genome		**Thermal cycler used: Biorad**			
Run on 2% Gel @100v for 1.5 hour, 6ul GelRed, 3µl Hyperladder 50bp, 100uM stock primer = 10ul of stock to 90ul of h2o = 10uM working sol., DNA-1.8-2.0, RNA-1.9-2.1					



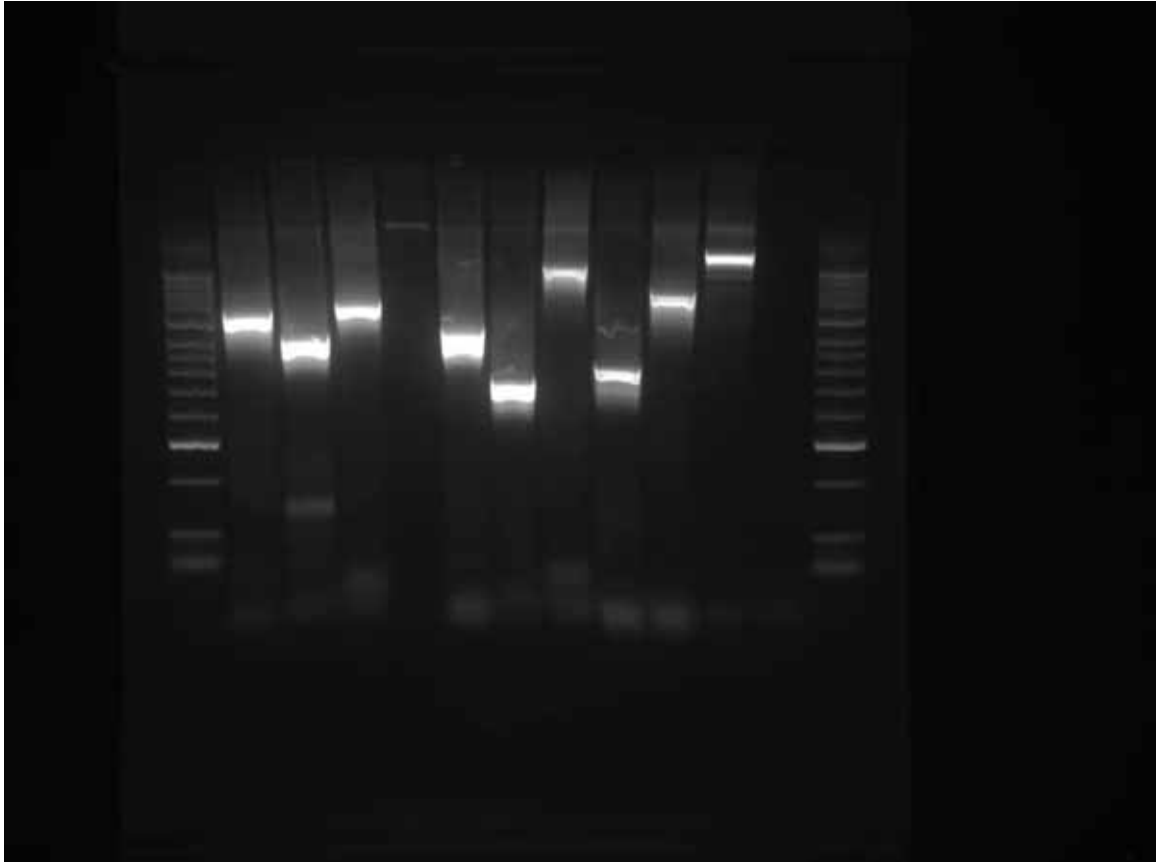
	Aquatic Pathobiology Working Group	*FORM: CqDV PCR*
	CQDV PCR	

TESTED BY:_ Shaun Bochow____ DATE TESTED:______13/8/14_________________

Sample Identification, Extraction and Results											
Accession: the viral Template came from A14-036, the new accession number is A14-034 to denote the new round of PCR											
Extraction Method: Roche viral extraction											
Positive control :	**Batch:**			**Positive =**							
	Sample	**Gel lane**	**Res ult**	**Id**	**Sample**	**Gel lane**	**Res ult**	**Id**	**Sample**	**Gel Lane**	**Res ult**
L											
1 (125)	A14-036 BgF3- Primer 13 R (expect 838)	5	20sec/57 °C								
2 (126)	A14-036 BgR3 F - Primer 13 R (expect 495)	6	15sec/57 °C								
Primer BgF3		CCG TTG AGC CAA GTA CAT CC				TM 60.5					
Primer 13 R		CAG CTC TGG ATC ACA TCT C				TM 57.3					

MASTER MIX					
BATCH OR EXPIRY DATE	**COMPONENTS**	25 µL	One reaction (12.5 µL)	**TOTAL (X 3)**	**TICK WHEN ADDED**
	Reaction Buffer (5x, Bioline)	5 µL	2.5µL	15 µL	
	$MgCl_2$ (50 mM) (**increased by 0.5ul)**	1 µL	0.25 µL	3 µL	
	Mango Taq polymerase (5U/µL, Bioline)	0.5 µL	0.25 µL	1.5 µL	
	dNTP's (10mM each)	0.5 µL	0.25 µL	1.5 µL	
	H_2O (nuclease free)	15.0 µL	7.25 µL	45 µL	
	Total to add to each tube		11.5ul/tube	**µL**	
	Primer F (10pmol/µL)	1 µL	0.5 µL	0 µL	
	Primer R (10pmol/µL)	1 µL	0.5 µL	0 µL	
	Total to add to each tube			**µL**	
		25ul/tube			
	Dispense Master Mix/tube	24 µL	11.5 µL	**24/**	
	Add DNA template	1 uL	1 uL	**1/**	
Thermal Cycle: 94 °C/2 min; 35 X (95°C/30 sec 57°C/20 sec, 72°C/Xsec); 72°C/7 min; Hold 12 °C					
Program = Mid HG		**Thermal cycler used: Biorad**			
Run on 2% Gel @100v for 1.5 hours, 6ul GelRed, 3µl HyperLadder 50bp, 100uM stock primer = 10ul of stock to 90ul of h2o = 10uM working sol., DNA-1.8-2.0, RNA-1.9-2.1					

Primer BgR3F	CAC CTT CAG CAA CGG AAT GTG	TM 61.3



TESTED BY:_ Shaun Bochow____ DATE TESTED:______11/8/14_________________

Sample Identification, Extraction and Results

Accession: the viral Template came from A14-036, the new accession number is A14-034 to denote the new round of PCR

Extraction Method: Roche viral extraction

Positive control : **Batch:** **Positive =**

	Sample	**Gel lane**	**Result**	**Id**	**Sample**	**Gel lane**	**Result**	**Id**	**Sample**	**Gel Lane**	**Result**
L	Hyperladder 50bp										
1 (127)	A14-036 Primer 13 F – Primer 11B_RC_Reverse (expect 1825)	7	40sec/54 °C								
2 (128)	A14-036 Primer 13_F – 2444_R (expect 581)	8	15sec/57 °C								
3 (129)	A14-036 2444_F – Primer 11B_RC_Reverse (expect 1271)	9	40sec/54 °C								
4	NTC										

Primer 13 F	GGA GAT GTG ATC CAG AGC TG	TM 57.3
Primer 11B R	ATT GCC AAA CAG TAT GGT AC	TM 54.3
Primer 2444 R	CAG GTG TAC GAT ACA CAG GAG	TM 61.3
Primer 2444 F	CTC CTG TGT ATC GTA CAC CTG	TM 61.3

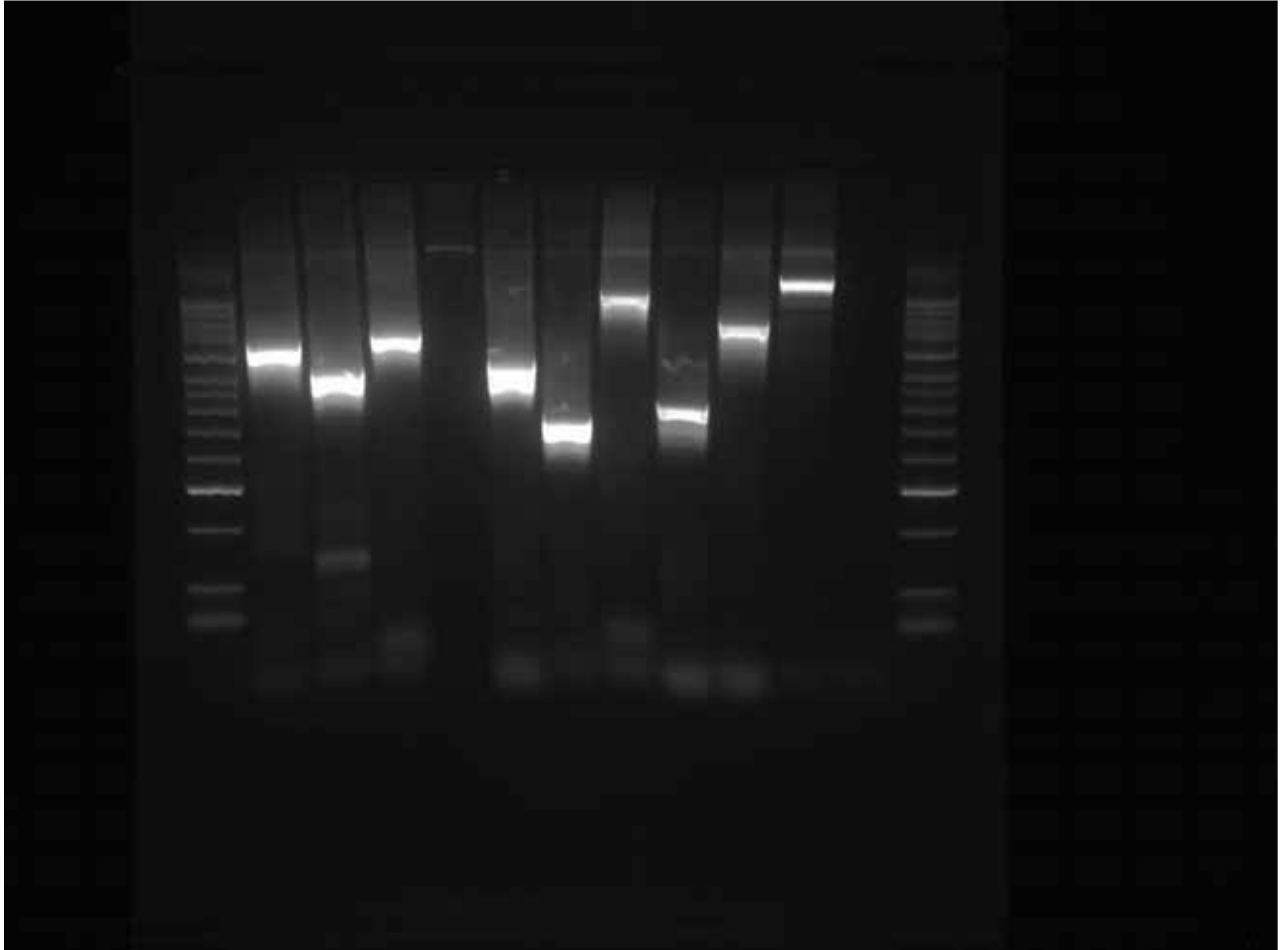
MASTER MIX

BATCH OR EXPIRY DATE	**COMPONENTS**	25 µL	One reaction (12.5 µL)	**TOTAL (X 4)**	**TICK WHEN ADDED**
	Reaction Buffer (5x, Bioline)	5 µL	2.5µL	20 µL	
	$MgCl_2$ (50 mM) **(increased by 0.5ul)**	1 µL	0.25 µL	4 µL	
	Mango Taq polymerase (5U/µL, Bioline)	0.5 µL	0.25 µL	2 µL	
	dNTP's (10mM each)	0.5 µL	0.25 µL	2 µL	
	H_2O (nuclease free)	15.0 µL	7.25 µL	60 µL	
	Total to add to each tube		11.5ul/tube	**µL**	
	Primer F (10pmol/µL)	1 µL	0.5 µL	0 µL	
	Primer R (10pmol/µL)	1 µL	0.5 µL	0 µL	
	Total to add to each tube			**µL**	
		25ul/tube			
	Dispense Master Mix/tube	24 µL	11.5 µL	**24/**	
	Add DNA template	1 uL	1 uL	**1/**	

Thermal Cycle: 94 °C/2 min; 35 X (95°C/30 sec Tm°C/20 sec, 72°C/Xsec); 72°C/7 min; Hold 12 °C

Program = 3' half HG **Thermal cycler used: Biorad**

Run on 2% Gel @100v for 1.5 hours, 6ul GelRed, 3µl HyperLadder 50bp, 100uM stock primer = 10ul of stock to 90ul of h2o = 10uM working sol., DNA-1.8-2.0, RNA-1.9-2.1

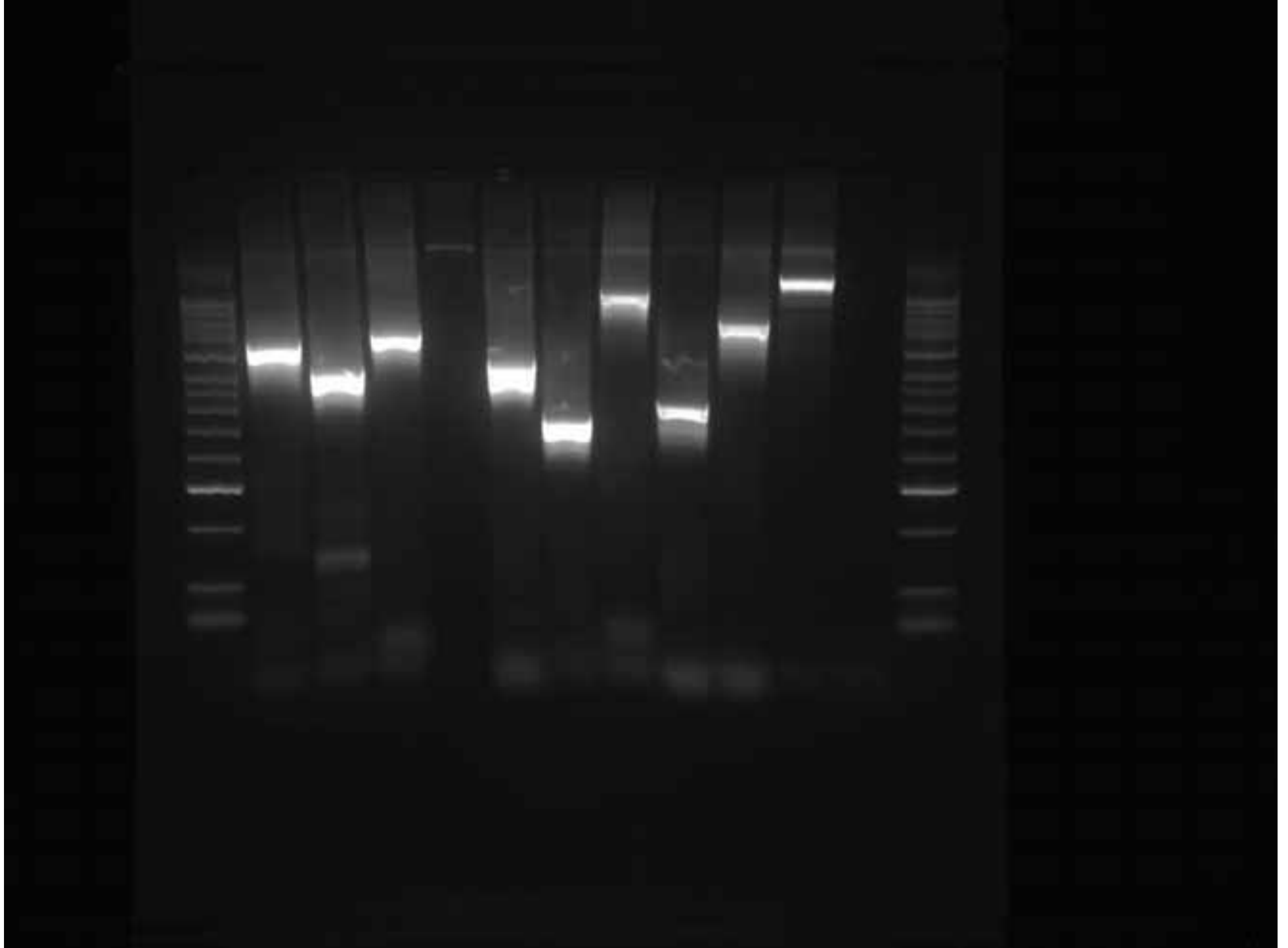


	Aquatic Pathobiology Working Group	*FORM: CqDV PCR*
	CQDV PCR	

TESTED BY:_ Shaun Bochow____ DATE TESTED:______13/8/14_________________

Sample Identification, Extraction and Results											
Accession: the viral Template came from A14-036, the new accession number is A14-034 to denote the new round of PCR											
Extraction Method: Roche viral extraction											
Positive control :	**Batch:**			**Positive = Clone**							
	Sample	**Gel lane**	**Res ult**	**Id**	**Sample**	**Gel lane**	**Res ult**	**Id**	**Sample**	**Gel Lane**	**Res ult**
L	Hyperladder 50bp										
1 (130)	A14-036 Primer 11B F (3' right)	10									
2	NTC	11									
Primer 11B F		GTA CCA TAC TGT TTG GCA ATG				Tm 57.4					
Primer						Tm					

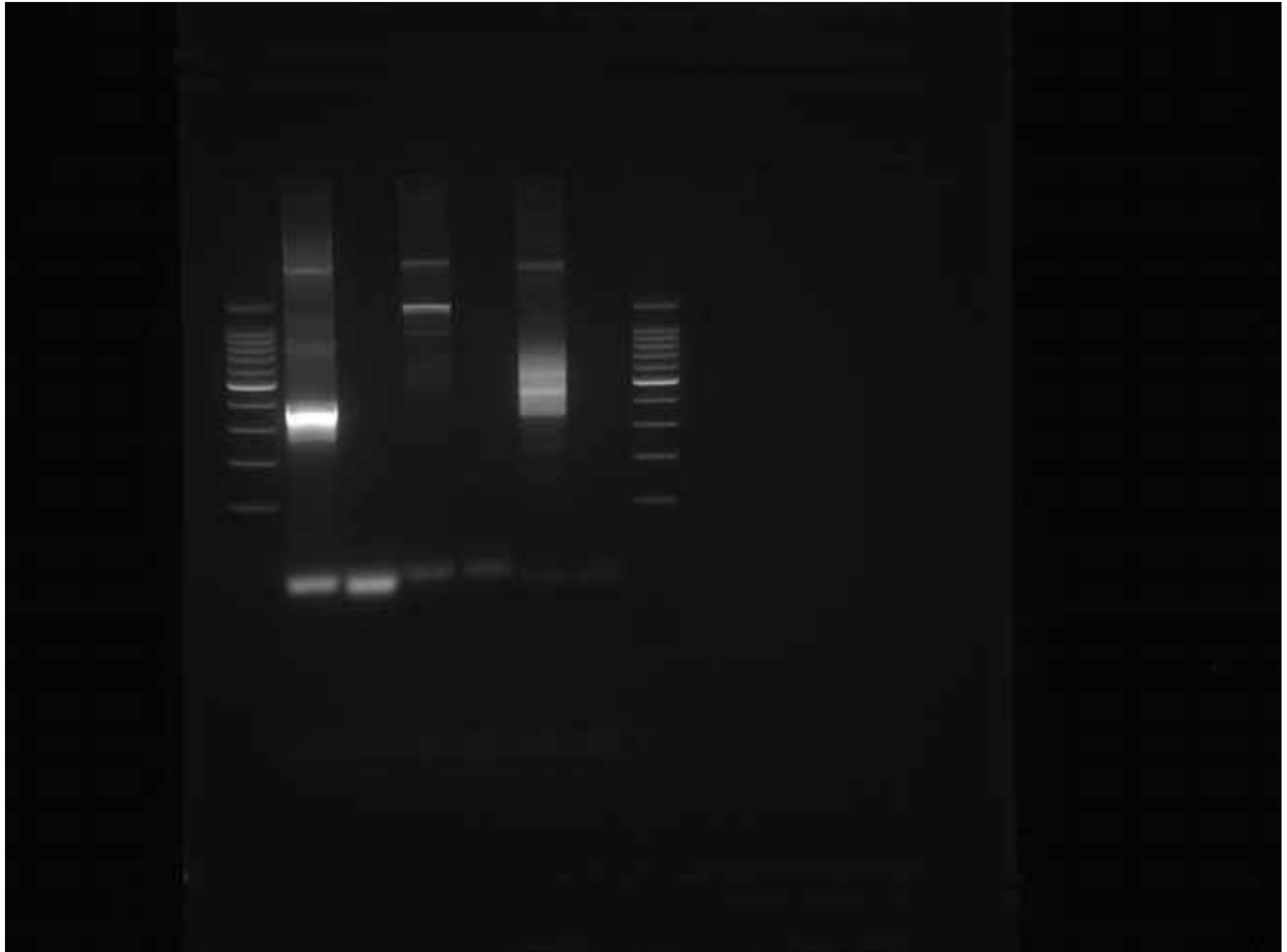
MASTER MIX				
BATCH OR EXPIRY DATE	**Components MyFi**	25 µL	**X1**	**TICK WHEN ADDED**
	5x React buf (5µL)	5 µL	5	
	MyFi Pol (1µL)	1 µL	1	
	Primer F (1µL)	1 µL	1	
	Primer R (1µL)	0 µL	0	
	H_2O	17 µL	17	
	Dispense mastermix/tueb	**24ul/tube**		
	DNA template	1 µL		
Thermal Cycle MyFi: 94 °C/2 min; 35 X (95°C/30 sec 57°C/20 sec, 72°C/45sec); 72°C/7 min; Hold 12 °C				
Program = 3 Prime end		**Thermal cycler used: Biorad**		
Run on 2% Gel @100v for 1.5 hours, 6ul GelRed, 3µl HyperLadder 50bp, 100uM stock primer = 10ul of stock to 90ul of h2o = 10uM working sol., DNA-1.8-2.0, RNA-1.9-2.1				



TESTED BY:_ Shaun Bochow____ DATE TESTED:______4/9/14_________________

Sample Identification, Extraction and Results											
Accession: the viral Template came from A14-036.1, the new accession number is A14-050 to denote the new round of PCR											
Extraction Method: Roche viral extraction											
Positive control :	**Batch:**			**Positive =**							
	Sample	**Gel lane**	**Result**	**Id**	**Sample**	**Gel lane**	**Result**	**Id**	**Sample**	**Gel Lane**	**Result**
L	Promega 100bp	1									
1	4552 F – 4923 R A14-036.1	2	131								
2	NTC	3									
L	Promega 100bp	8									
Primer 4552 F		GTA CTA GGT TGC CGT AAA CC TM 58.4 224									
Primer 4923 R		ACG CAG ATA AGA AAT CTG CTG Tm 57.4 205									

MASTER MIX					
BATCH OR EXPIRY DATE	**COMPONENTS**	25 μL	One reaction (12.5 μL)	**TOTAL (X 3)**	**TICK WHEN ADDED**
	Reaction Buffer (5x, Bioline)	5 μL	2.5μL	15 μL	
	$MgCl_2$ (50 mM)	0.5 μL	0.25 μL	1.5 μL	
	Mango Taq polymerase (5U/μL, Bioline)	0.5 μL	0.25 μL	1.5 μL	
	dNTP's (10mM each)	0.5 μL	0.25 μL	1.5 μL	
	H_2O (nuclease free)	15.5 μL	7.25 μL	46.5 μL	
	Total to add to each tube		11.5ul/tube	**μL**	
	Primer F (10pmol/μL)	1 μL	0.5 μL	3 μL	
	Primer R (10pmol/μL)	1 μL	0.5 μL	3 μL	
	Total to add to each tube			**μL**	
		25ul/tube		**24/ tube**	
	Dispense Master Mix/tube	24 μL	11.5 μL	**24/**	
	Add DNA template	1 uL	1 uL	**1/**	
Thermal Cycle: 94 °C/2 min; 35 X (95°C/30 sec 57°C/20 sec, 72°C/15sec); 72°C/7 min; Hold 12 °C					
Program = VP-A		**Thermal cycler used: Biorad**			
Run on 2% Gel @200v for 30 min, 6ul GelRed, 2μl Promega 100bp ladder, 1μl loading dye, 4μl H_2O, 100uM stock primer = 10ul of stock to 90ul of h2o = 10uM working sol., DNA-1.8-2.0, RNA-1.9-2.1					

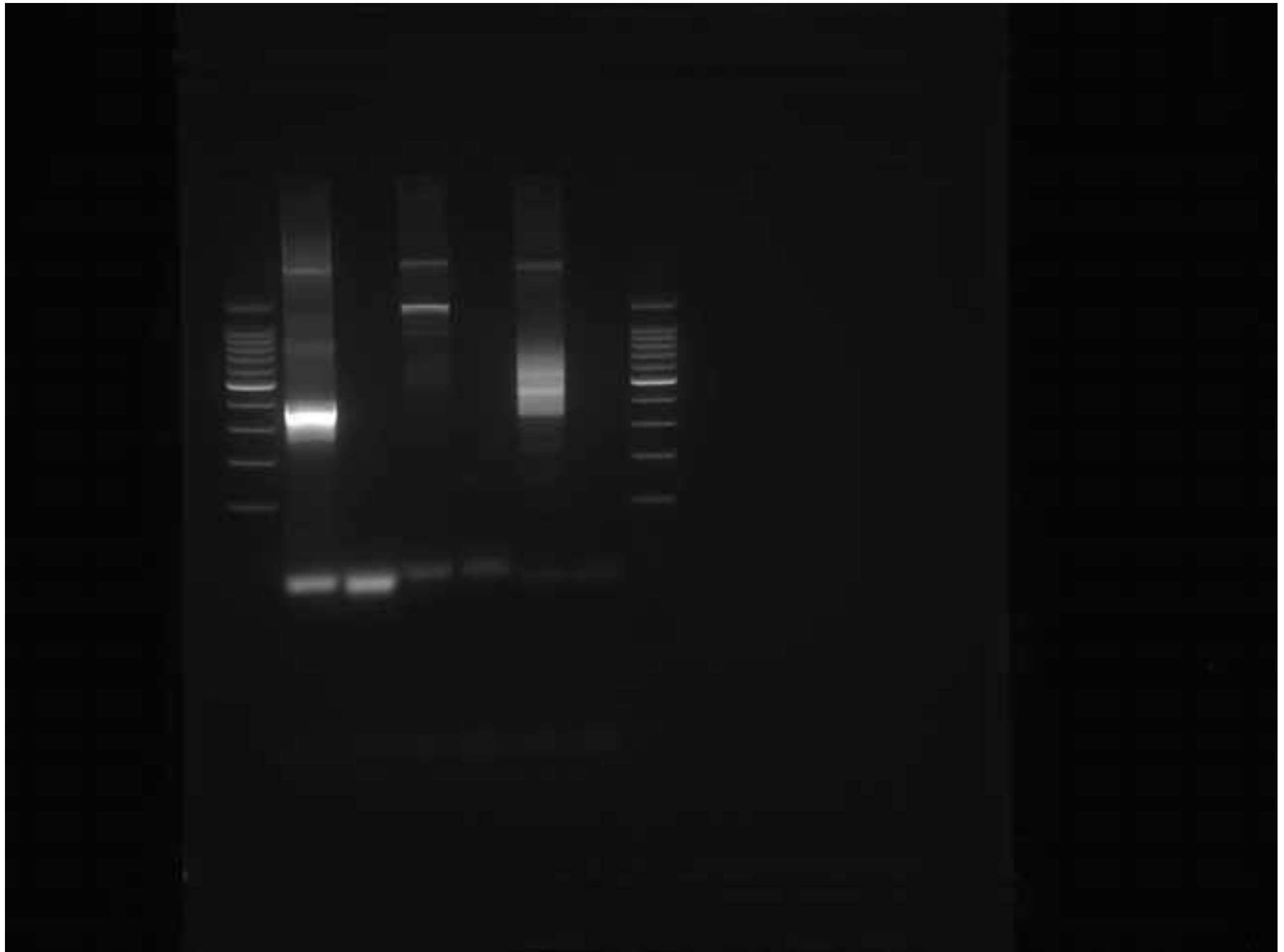


	Aquatic Pathobiology Working Group	*FORM: CqDV PCR*
	CQDV PCR	

TESTED BY:_ Shaun Bochow____ DATE TESTED:______4/10/14_________________

Sample Identification, Extraction and Results											
Accession: the viral Template came from A14-036.1, the new accession number is A14-050 to denote the new round of PCR											
Extraction Method: Roche viral extraction											
Positive control :		**Batch:**			**Positive =**						
	Sample	**Gel lane**	**Res ult**	**Id**	**Sample**	**Gel lane**	**Res ult**	**Id**	**Sample**	**Gel Lane**	**Res ult**
L	Promega 100bp	1									
1	4552 F A14-050	4	132								
2	NTC	5									
L	Promega 100bp	8									
Primer 4552 F		GTA CTA GGT TGC CGT AAA CC				TM 58.4					
Primer 4923 R		ACG CAG ATA AGA AAT CTG CTG				Tm 57.4					

MASTER MIX					
BATCH OR EXPIRY DATE	**COMPONENTS**	25 µL	One reaction (12.5 µL)	**TOTAL (X 3)**	**TICK WHEN ADDED**
	Reaction Buffer (5x, Bioline)	5 µL	2.5µL	15 µL	
	$MgCl_2$ (50 mM)	0.5 µL	0.25 µL	1.5 µL	
	Mango Taq polymerase (5U/µL, Bioline)	0.5 µL	0.25 µL	1.5 µL	
	dNTP's (10mM each)	0.5 µL	0.25 µL	1.5 µL	
	H_2O (nuclease free)	15.5 µL	7.25 µL	46.5 µL	
	Total to add to each tube		11.5ul/tube	**µL**	
	Primer F (10pmol/µL)	1 µL	0.5 µL	3 µL	
	Primer R (10pmol/µL)	1 µL	0.5 µL	3 µL	
	Total to add to each tube			**µL**	
		25ul/tube		**24/ tube**	
	Dispense Master Mix/tube	24 µL	11.5 µL	**24/**	
	Add DNA template	1 uL	1 uL	**1/**	
Thermal Cycle: 94 °C/2 min; 35 X (95°C/30 sec 57°C/20 sec, 72°C/15sec); 72°C/7 min; Hold 12 °C					
Program = VP-A		**Thermal cycler used: Biorad**			
Run on 2% Gel @200v for 30 min, 6ul GelRed, 2µl Promega 100bp ladder, 1µl loading dye, 4µl H_2O, 100uM stock primer = 10ul of stock to 90ul of h2o = 10uM working sol., DNA-1.8-2.0, RNA-1.9-2.1					

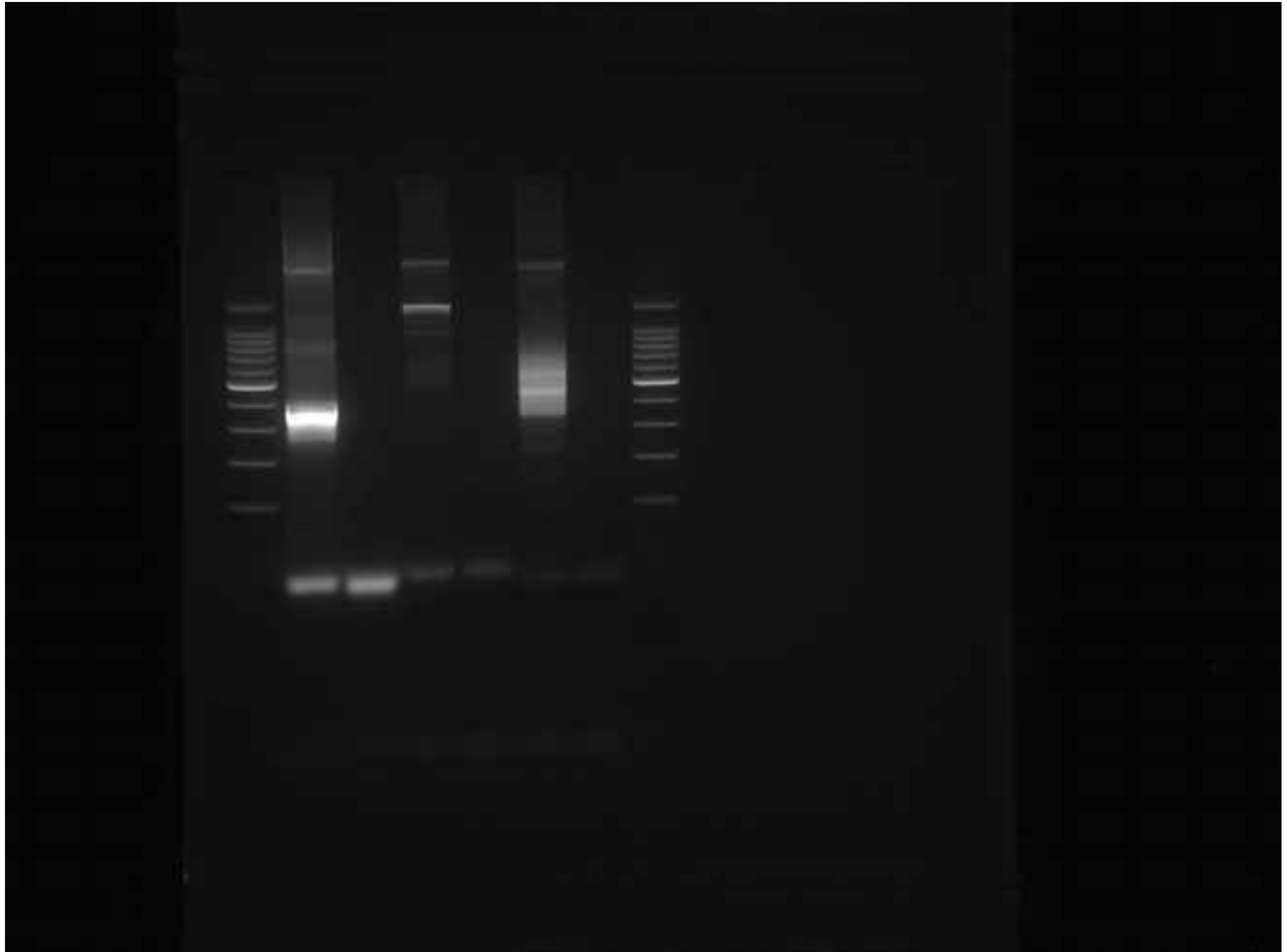


	Aquatic Pathobiology Working Group	*FORM: CqDV PCR*
	CQDV PCR	

ESTED BY:_ Shaun Bochow____ DATE TESTED:______4/10/14__________________

Sample Identification, Extraction and Results											
Accession: the viral Template came from A14-036.1, the new accession number is A14-050 to denote the new round of PCR											
Extraction Method: Roche viral extraction											
Positive control :	**Batch:**			**Positive =**							
	Sample	**Gel lane**	**Res ult**	**Id**	**Sample**	**Gel lane**	**Res ult**	**Id**	**Sample**	**Gel Lane**	**Res ult**
L	Promega 100bp	1									
1	5 P hP CqDV1 A14-036	6									
2	NTC	7									
L	Promega 100bp	8									
Primer 5 P hP CqDV1		CCA GTA GTA GTG AAG AAA GC Tm 56.4									
Primer											

MASTER MIX					
BATCH OR EXPIRY DATE	**COMPONENTS**	25 µL	One reaction (12.5 µL)	**TOTAL (X)**	**TICK WHEN ADDED**
	Reaction Buffer (5x, Bioline)	5 µL	2.5µL	15 µL	
	$MgCl_2$ (50 mM)	0.5 µL	0.25 µL	1.5 µL	
	Mango Taq polymerase (5U/µL, Bioline)	0.5 µL	0.25 µL	1.5 µL	
	dNTP's (10mM each)	0.5 µL	0.25 µL	1.5 µL	
	H_2O (nuclease free)	15.5 µL	7.25 µL	46.5 µL	
	Total to add to each tube		11.5ul/tube	**µL**	
	Primer F (10pmol/µL)	1 µL	0.5 µL	3 µL	
	Primer R (10pmol/µL)	1 µL	0.5 µL	3 µL	
	Total to add to each tube			**24 µL**	
		25ul/tube			
	Dispense Master Mix/tube	24 µL	11.5 µL	**24/**	
	Add DNA template	1 uL	1 uL	**1/**	
Thermal Cycle: 94 °C/2 min; 35 X (95°C/30 sec 56°C/20 sec, 72°C/15sec); 72°C/7 min; Hold 12 °C					
Program = 5 prime	**Thermal cycler used: Biorad**				
Run on 2% Gel @200v for 30 min, 6ul GelRed, 2µl Promega 100bp ladder, 1µl loading dye, 4µl H_2O, 100uM stock primer = 10ul of stock to 90ul of h2o = 10uM working sol., DNA-1.8-2.0, RNA-1.9-2.1					

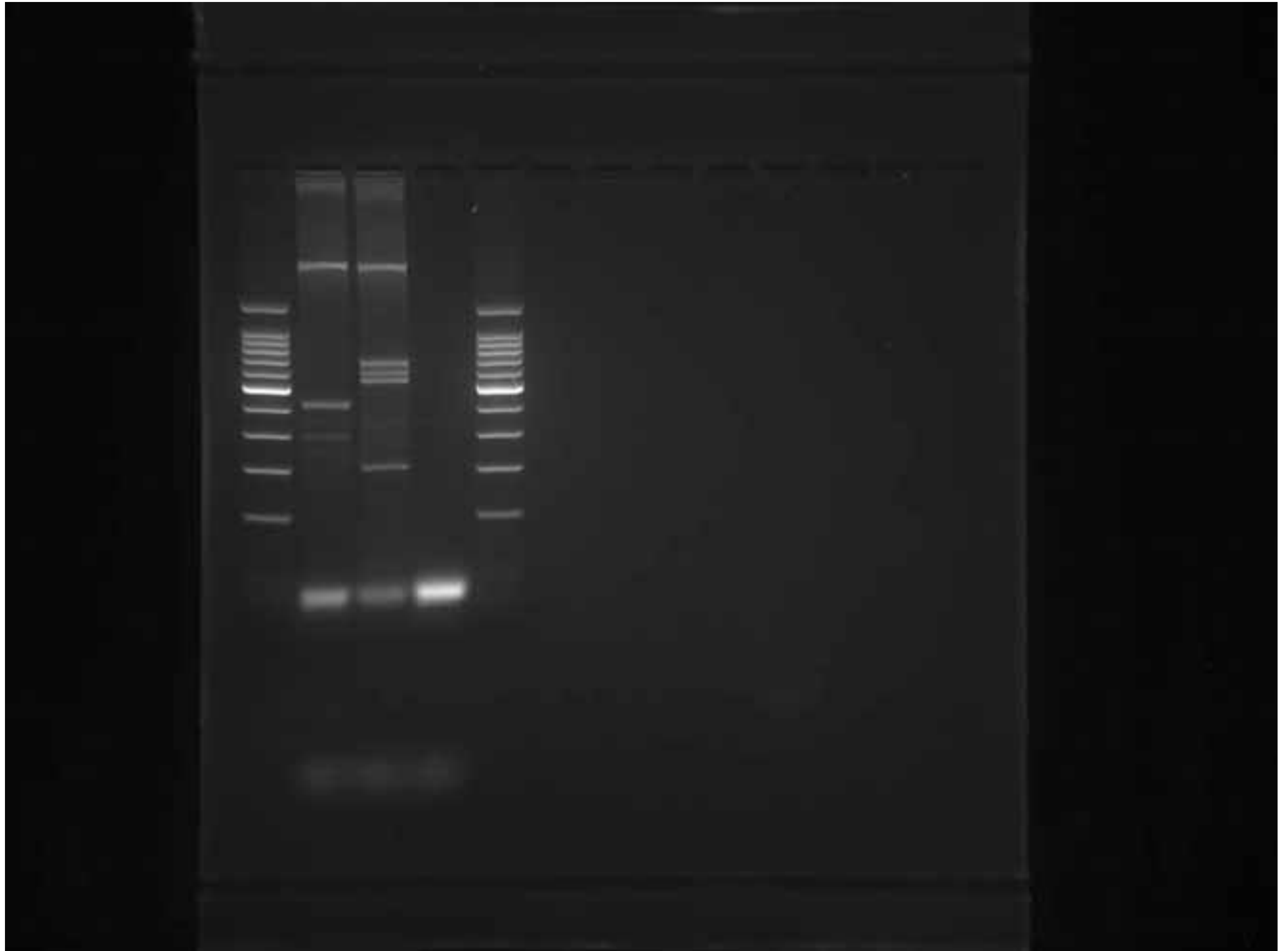


	Aquatic Pathobiology Working Group	*FORM: CqDV PCR*
	CQDV PCR	

ESTED BY:_ Shaun Bochow____ DATE TESTED:______6/10/14_________________

Sample Identification, Extraction and Results											
Accession: the viral Template came A14-036, the new accession number is A14-050 to denote the new round of PCR											
Extraction Method: Roche viral extraction											
Positive control :	**Batch:**			**Positive =**							
	Sample	**Gel lane**	**Result**	**Id**	**Sample**	**Gel lane**	**Result**	**Id**	**Sample**	**Gel Lane**	**Result**
L	Promega 100bp	1									
1	A14-036.1 CqPV 5 RcF	2	133								
2	A14-036.1 2A R	3	134, 135								
3	NTC	4									
L	Promega 100bp	5									
Primer CqPV 5 RcF	CTC TAG TGC ACT CTC CAC AGC					Tm 63.3					
Primer 2A R	TCT GAA TCA ATC TCC TCA CG					Tm 56.4					

MASTER MIX					
BATCH OR EXPIRY DATE	**COMPONENTS**	25 µL	One reaction (12.5 µL)	**TOTAL (X 3)**	**TICK WHEN ADDED**
	Reaction Buffer (5x, Bioline)	5 µL	2.5µL	15 µL	
	$MgCl_2$ (50 mM)	1 µL	0.25 µL	3 µL	
	Mango Taq polymerase (5U/µL, Bioline)	0.5 µL	0.25 µL	1.5 µL	
	dNTP's (10mM each)	0.5 µL	0.25 µL	1.5 µL	
	H_2O (nuclease free)	15 µL	7.25 µL	45 µL	
	Total to add to each tube		11.5ul/tube	**µL**	
	Primer F (10pmol/µL)	1 µL	0.5 µL	µL	
	Primer R (10pmol/µL)	1 µL	0.5 µL	µL	
	Total to add to each tube			**22 µL**	
		25ul/tube			
	Dispense Master Mix/tube	24 µL	11.5 µL	**24/**	
	Add DNA template	1 uL	1 uL	**1/**	
Thermal Cycle: 94 °C/2 min; 35 X (95°C/30 sec (60,56°C)/20 sec, 72°C/15sec); 72°C/7 min; Hold 12 °C					
Program = 5 prime	**Thermal cycler used: Biorad**				
Run on 2% Gel @200v for 30 min, 6ul GelRed, 2µl Promega 100bp ladder, 1µl loading dye, 4µl H_2O, 100uM stock primer = 10ul of stock to 90ul of h2o = 10uM working sol., DNA-1.8-2.0, RNA-1.9-2.1					



APPENDIX 4: Examples of quantitative real-time SYBR PCR

Appendix 4 presents an examples of the worksheets, melt report and quantitation report from runs on the Corbett Rotor-Gene 6000.

At the top of the worksheet, the date of testing and the name of the person who did the testing are recorded. Beneath this an accession number or code, this relates to the origin of the template that is used in the qPCR. The accession numbers are recorded in a laboratory register (data not shown) that details the samples history, storage, assays carried out and date of sample destruction. The extraction method identifies the technique used to extract the DNA for PCR. On the left side of the main box is the rotor position, followed (to the right) by the sample name or accession number. The PCR conditions are in a separate box labelled MASTER MIX. This box details the reagents used in the qPCR, their concentration and volume. The qPCR program is also recorded below this, including the melt conditions. Below this is the primer names, sequence and Tm of the selected primers. In a separate box is the dilution series of the clone used to generate the standard curve. The colony forming units are calculated to copies per reaction. The melt data and quantification data

TESTED BY:____Shaun Bochow ______ DATE TESTED:_____23/01/16_______________

Sample Identification, Extraction and Results											
Accession Number/s: A16-002 – Realtime tissue tropism A13-001 samples diluted in teaching lab 10-4											
Extraction Method: Roche viral nucleic acid extraction											
Positive control : C15-109a 1					**Batch:**			**Positive Position:**			
Id	**Sample**	**Result**		**Id**	**Sample**	**Result**		**Id**	**Sample**	**Rotor pos.**	**Result**
1	C15-109a 1 10-2			25	A13-001-21 muscle 10-4			49			
2	C15-109a 1 10-3			26	A13-001-21 gill 10-4			50			
3	C15-109a 1 10-4			27	A13-001-21 epithelium 10-4			51			
4	C15-109a 1 10-5			28	A13-001-23 heart 10-4			52			
5	C15-109a 1 10-6			29	A13-001-23 atn glnd 10-4			53			
6	C15-109a 1 10-7			30	A13-001-23 pleopod 10-4			54			
7	C15-109a 1 10-8			31	A13-001-23 muscle 10-4			55			
8	C15-109a 1 10-9			32	A13-001-23 gill 10-4			56			
9	C15-109a 1 10-10			33	A13-001-23 epithelium? Not 70ul 10-4			57			
10	A13-001-15 heart 10-4			34	A13-001-24 heart 10-4			58			
11	A13-001-15 atn glnd 10-4			35	A13-001-24 atn glnd 10-4			59			
12	A13-001-15 pleopod 10-4			36	A13-001-24 pleopod 10-4			60			
13	A13-001-15 muscle 10-4			37	A13-001-24 muscle 10-4			61			
14	A13-001-15 gill 10-4			38	A13-001-24 gill 10-4			62			
15	A13-001-15 epithelium 10-4			39	A13-001-24 epithelium 10-4			63			
16	A13-001-18 heart 10-4			40	A15-025.3			64			
17	A13-001-18 atn glnd 10-4			41	NTC			65			
18	A13-001-18 pleopod 10-4			42	Ntc			66			
19	A13-001-18 muscle 10-4	Not 45ul?		43	Ntc			67			
20	A13-001-18 gill 10-4			44				68			
21	A13-001-18 epithelium 10-4			45				69			
22	A13-001-21 heart 10-4			46				70			
23	A13-001-21 atn glnd 10-4			47				71			
24	A13-001-21 pleopod 10-4			48				72			

SYBR MASTER MIX 5mM MgCl							
BATCH OR EXPIRY DATE	**COMPONENTS**	20 µL	**DIL**	**STOCK P**	**H_2O**	**TOTAL (X 44)**	**TICK WHEN ADDED**
	SensiFast SYBER no Rox Buffer (2x)	10 µL				440	
	Primer F (20pmol/µL) (1:5 dilution of 100mmol stock)	1 µL	44/5	8.8-44 =	35.2	P 8.8	
	Primer R (20pmol/µL) (1:5 dilution of 100mmol stock)	1 µL	44/5	8.8-44 =	35.2	P 8.8	
	50 mM MgCl2 diluted	0.8 µL				35.2	
	H_2O (nuclease free)	4.7 µL		206.8+35.2+35.2		277.2	
	Dispense Master Mix/tube	17.5 µL					
	Add DNA template	2.5 µL					
Thermal Cycle: Hold 1: (95 x 3mins) Cycling: (40 x 95C x30s, 60Cx 15s. Acquire on Green at 60C) Hold 2: (60 °C 5 min), Melt: (Ramp from 79C to 89C acquire every 0.5C, Hold for 90s on 1st step, Hold for 5s on next steps, Melt A Green) Gain optimisation on all samples							
Program =	**Thermal cycler used: Corbett Rotorgene 6000**						

CqDV_5 n: CGC TGT GGA GAG TGC ACT AGA GGC	Tm 63
CqDV_2A n: TCT GAA TCA ATC TCC TCA CGA TCG C	Tm 58
Reference:	

Calculated dilutions of for standards using clone C15-109a1			
Dilution	CFU/ µL	CFU in 1ul of extract	C15-109a1 x 2.5ul copies/reaction
Neat	$8.57x10^{7}$	85700000	214250000
10^{-1}	$8.57x10^{6}$	8570000	21425000
10^{-2}	$8.57x10^{5}$	857000	2142500
10^{-3}	$8.57x10^{4}$	85700	214250
10^{-4}	$8.57x10^{3}$	8570	21425
10^{-5}	$8.57x10^{2}$	857	2142.5
10^{-6}	$8.57x10^{1}$	85.7	214.25
10^{-7}	8.57	8.57	21.425
10^{-8}	0.857	0.857	2.1425
10^{-9}	0.0857	0.0857	.21425
10^{-10}	0.00857	0.00857	.021425
10^{-11}			
10^{-12}			

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Melt Report

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Quantitation Report

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TESTED BY:____Shaun Bochow ______ DATE TESTED:_____24/01/16_______________

Sample Identification, Extraction and Results

Accession Number/s: A16-008 – Realtime PCR negative organs tissue tropism

Extraction Method: Roche viral nucleic acid extraction

Positive control : C15-109a 1 **Batch:** **Positive Position:**

Id	Sample	Result		Id	Sample	Result		Id	Sample	Rotor pos.	Result
1	C15-109a 1 10-2 n			25	A16-008.3.6			49			
2	C15-109a 1 10-3 n			26	A16-008.4.1			50			
3	C15-109a 1 10-4 n			27	A16-008.4.2			51			
4	C15-109a 1 10-5 n			28	A16-008.4.3			52			
5	C15-109a 1 10-6 n			29	A16-008.4.4			53			
6	C15-109a 1 10-7 n			30	A16-008.4.5			54			
7	C15-109a 1 10-8 n			31	A16-008.4.6			55			
8	A16-008.1.1			32	A16-008.5.1			56			
9	A16-008.1.2			33	A16-008.5.2			57			
10	A16-008.1.3			34	A16-008.5.3			58			
11	A16-008.1.4			35	A16-008.5.4			59			
12	A16-008.1.5			36	A16-008.5.5			60			
13	A16-008.1.6			37	A16-008.5.6			61			
14	A16-008.2.1			38	**A15-025.3 neg**			62			
15	A16-008.2.2			39	A15-057 10^{-2}			63			
16	A16-008.2.3			40	A15-057 10^{-3}			64			
17	A16-008.2.4			41	A15-057 10^{-4}			65			
18	A16-008.2.5			42				66			
19	A16-008.2.6			43				67			
20	A16-008.3.1			44				68			
21	A16-008.3.2			45				69			
22	A16-008.3.3			46				70			
23	A16-008.3.4			47				71			
24	A16-008.3.5			48				72			

SYBR MASTER MIX 5mM MgCl

BATCH OR EXPIRY DATE	COMPONENTS	20 µL	DIL	STOCK P	H_2O	TOTAL (X 45)	TICK WHEN ADDED
	SensiFast SYBR no Rox Buffer (2x)	10 µL				450	
	Primer F (20pmol/µL) (1:5 dilution of 100mmol stock)	1 µL	45/5	9-45 =	36	P 9	
	Primer R (20pmol/µL) (1:5 dilution of 100mmol stock)	1 µL	45/5	9-45 =	36	P 9	
	50 mM MgCl2 diluted	0.8 µL				36	
	H_2O (nuclease free)	4.7 µL		211.5+36+36		283.5	
	Dispense Master Mix/tube	17.5 µL					
	Add DNA template	2.5 µL					

Thermal Cycle: Hold 1: (95 x 3mins) Cycling: (40 x 95C x30s, 60Cx 20s. Acquire on Green at 60C) Hold 2: (60 °C 5 min), Melt: (Ramp from 79C to 89C acquire every 0.5C, Hold for 90s on 1st step, Hold for 5s on next steps, Melt A Green) Gain optimisation on all samples

Program = **Thermal cycler used: Corbett Rotorgene 6000**

CqDV_5 n: CGC TGT GGA GAG TGC ACT AGA GGC Tm 63

CqDV_2A n: TCT GAA TCA ATC TCC TCA CGA TCG C Tm 58

Reference:

Calculated dilutions of for standards using clone C15-109a1			
Dilution	CFU/ µL	CFU in 1ul of extract	C15-109a1 x 2.5ul copies/reaction
Neat	$8.57x10^7$	85700000	214250000
10^{-1}	$8.57x10^6$	8570000	21425000
10^{-2}	$8.57x10^5$	857000	2142500
10^{-3}	$8.57x10^4$	85700	214250
10^{-4}	$8.57x10^3$	8570	21425
10^{-5}	$8.57x10^2$	857	2142.5
10^{-6}	$8.57x10^1$	85.7	214.25
10^{-7}	8.57	8.57	21.425
10^{-8}	0.857	0.857	2.1425
10^{-9}	0.0857	0.0857	.21425
10^{-10}	0.00857	0.00857	.021425
10^{-11}			
10^{-12}			

Melt Report

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