



horses used for meat production are likely to prove beneficial and may provide valuable insight into the genetics underlying body composition in horses.

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Whole-genome shotgun sequence assembly enables rapid gene characterization in the tropical fish barramundi, *Lates calcarifer*

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Source description: Barramundi, *Lates calcarifer*, is a commercially important fish farmed throughout Australia and South-East Asia. Despite an increasing availability of genetic resources for the species (e.g. microsatellite and SNP markers,¹ linkage² and BAC-based³ maps and transcriptomic assemblies^{4–6}), the complete characterization of genes is still reliant on laborious molecular methods (e.g. genome walking/RACE PCR cloning/Sanger sequencing).

We provide a publicly available comprehensive genomic sequence resource for the Australian barramundi comprising ~84% of the genome (~590/700 Mb total genome size⁷) based on whole-genome shotgun assembly (WGS).

This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession LBLR00000000. The version described in this paper is version LBLR01000000. The genome assembly comprises 22775 contigs (N50 = 310513 bp) *de novo* assembled from 161 599 806 Illumina 100-bp paired-end reads (NCBI SRA accession SRR1973498). Sequence coverage represents ~46× coverage of the species genome, whereby 98.7% of the sequence assembly has more than 30 reads at any nucleotide position. Among other features, this genome assembly resource enables for rapid identification and characterization of *de novo* gene sequences including intron–exon boundaries key for optimal RT-qPCR primer design and identification of CpG sites within promoter regions for targeted epigenetic studies.

To illustrate the high utility of this resource, 10 partially published⁶ or novel genes involved in the sexual development pathways could be rapidly identified within the WGS based on NCBI BLASTN and then fully and accurately characterized (*DMRT1*, *CYP19A1*, *NR5A1*, *ESR1*, *FOXL2*, *AMH*, *SOX8*, *SOX9*, *GPR54* and *KISS1*; GenBank accessions KR232516, KR492506–KR492514). A high gene coverage of 51 ± 11 (mean \pm SD) reads at any nucleotide position confirmed the reliability of WGS. Furthermore, data allowed for the full reconstruction of the mitochondrion genome (mtDNA) of this Australian individual (GenBank accession KR349919), based on the previously published Asian *L. calcarifer* mtDNA reference genome⁸ (GenBank accession DQ010541). A total of 264 863 sequences mapped against DQ010541 yielded 1596 ± 137 (mean \pm SD) reads at any nucleotide position (minimum and maximum coverage of 721 and 1960 reads respectively). Pairwise similarity of mtDNA between our Australian (16 517 bp) and the Asian (16 535 bp) reference was 98.2%, with the largest differences observed for the *ND5* (93.4%), *CR* (93.5%) and *COX2* (94.7%) genes.

Next-generation sequencing and *de novo* genome assembly: Purified DNA extracted from a previously genotyped individual,⁹ homozygous for 14 of 16 microsatellites, was sequenced at the Australian Genome Research Facility (Illumina HiSeq 2000 platform). Due to high read quality [mean Q score at any position (1–100 bp) > 32; FASTQC¹⁰], *de novo* genomic assembly was performed on untrimmed reads using VELVET¹¹ (optimum k-mer = 57 bp, insert size = 330 bp, minimum contig length = 200 bp). GENEIOUS (Biomatters) was used for BLAST searches, sequence alignment, read mapping and gene characterization.

Concluding remarks: Due to the barramundi's small genome size, WGS provided a relatively cheap (~US\$ 3K), rapid and robust genomic reference library. This genomic resource published herein will facilitate future genetic studies for the commercially important barramundi. Further, it will contribute to the full characterization of the species' genome when combined with other sequencing, mapping and annotation strategies.

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