

Supplementary Material For:

Fast, cost-effective development of species-specific microsatellite markers by genomic sequencing

Jawad Abdelkrim^{1,2}, Bruce C. Robertson^{1,3}, Jo-Ann L. Stanton⁴, Neil J. Gemmell^{1,2}

¹*School of Biological Sciences, University of Canterbury, Christchurch, New Zealand,* ²*Centre for Reproduction and Genomics, Department of Anatomy & Structural Biology, University of Otago, Dunedin, New Zealand,*

³*Department of Zoology, University of Otago, Dunedin, New Zealand,* and ⁴*Anatomy Otago Genomics Sequencer Unit, Department of Anatomy & Structural Biology, University of Otago, Dunedin, New Zealand*

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Touchdown PCR protocol designed for the amplification of the microsatellite markers developed for the blue duck

PCR amplifications were performed using the same protocol for all loci and resulted in similar PCR efficiencies. All reactions were conducted in a final volume of 15 μ L containing 0.4 μ M each reverse and fluorescent dye-labeled M13 (-21) primer, 0.2 μ M forward primer with the M13 (-21) universal sequence added, 1 \times buffer, 2 mM MgCl₂, 0.2 mM each dNTP, 4mM tetramethylammonium chloride (TMAC), 1 unit *Taq* polymerase and 25 ng DNA. PCR amplifications were done on a PTC 225 thermal cycler (MJ Research, Waltham, MA, USA) as follows: 94°C for 3 min; 38 cycles of 94°C for 15 s, annealing temperature T for 30 s (with T decreasing from 59°C to 53°C every two cycles, then 51°C for 4 cycles and 49°C for 26 cycles), and 72°C for 20 s; and a final extension of 72°C for 20 min.