

Appendix A – Genotyping procedures.

DNA was extracted from blood samples by using a Chelex (BioRad, USA) resin based extraction method described in Walsh et al. (1991). The genotype data for the included individuals was obtained by Polymerase Chain Reaction (PCR) amplification of 13 microsatellite loci: Ase18, Pdo μ 1, Pdo μ 3 (Neumann and Wetton 1996), Pdo μ 5, Pdo10 (Griffith et al. 2007), Pdo16, Pdo17, Pdo19, Pdo22, Pdo27, Pdo40, Pdo44, Pdo47 (Dawson et al. 2012).

The 10 μ L PCR reactions were carried out on a GeneAmp PCR system 9700 machines (Applied Biosystems, USA) in two multiplex panels using the QIAGEN Multiplex PCR Kit (QIAGEN, Hilden, Germany) with fluorescent-labelled forward primers and non-labelled but pigtailed (GTTT- was added at the 5' end) reverse primers. Multiplex panel 1 included markers Ase18, Pdo μ 1, Pdo μ 3, Pdo μ 5, Pdo10, Pdo40, and multiplex panel 2 included markers Pdo16, Pdo17, Pdo19, Pdo22, Pdo27, Pdo44, Pdo47. For each sample, 1 μ L of the PCR products was mixed with 0.5 μ L size ladder (GeneScan LIZ 600, Applied Biosystems, USA) in addition to 10 μ L Hi-Di Formamide (Applied Biosystems, USA). The amplified microsatellite alleles were separated by electrophoresis and detected in an automated 16-capillary ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, USA). Individual alleles at each microsatellite locus were scored using the software Genemapper 4.0 (Applied Biosystems, USA).

References

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