Control Region of Mitochondral DNA amplification and sequencing

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Abstract

The mtDNA fragment (15869-740) was amplified using primers for polymerase chain reaction (PCR) : L15869F and H719R (L15869 F 5' AAAATACTCAAATGGGCCTGTC 3', H719R 5' CGTGGTGATTTAGAGGGTGAAC 3'). The 20 µl PCR reactions contained 2.0 µl 5×buffer, 1.6 µl 2.5 mM dNTP mix, 0.8 µl each of reverse (R) and forward (F) PCR primers (8 pM each), 0.2 µl of KOD Enzyme (1.0 U/µl), and 20 ng of template DNA. PCR was performed under the following cycle conditions: initial denaturation of 94 °C for 5 minutes; followed by 35 cycles of 94 °C denaturation for 30 seconds, 55 °C annealing for 30 seconds, and 72 °C elongation for 40 seconds; followed by a final extension at 72 °C for 10 minutes. The production fragment was sequenced with the following primers: L15869F and 80R for HVSI, 16539F and H719R for HVSII. The purified PCR products were sequenced by ABI 377 DNA automatic sequencer.

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