PCR amplification and restriction fragment length polymorphism analysis of four SNPs (C5178A, A10398G, G13708A, and C13928G) in the mtDNA coding region

Feng-ling Xu; Mei Ding; Jun Yao; Zhang-sen Shi; Bao-jie Wang

Abstract

The four SNPs (C5178A, A10398G, G13708A, and C13928G) in the mtDNA coding region were detected using PCR-RFLP analysis. The primers listed in able 2 were used to amplify target fragments. The mismatch method was applied to generate an HpyCH4Ⅲ artificial restriction endonuclease site in the amplified fragment that included the C13928G SNP. The 20 μl PCR reactions contained 2.0 μl 10×buffer, 2 μl 2.5 mM dNTP mix, 2 μl each of R and F PCR primers (10

pM each), 0.2 μl of rTaq Enzyme (5 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 30 cycles of 94 °C denaturation for 30 seconds, annealing at 61-65°C (Table 2) for 30 seconds, and elongation at 72 °C for 30 seconds; followed by a final extension at 72 °C for 5 minutes. PCR products were digested with restriction enzymes (S1 Table), and fragments were detected on 6% polyacrylamide gel.

Table 2. Primers used for the analysis of mtDNA polymorphisms in the hypervariable region and the coding region.

Locus	Annealing Temperature (°C)	Primer Sequences (5′ → 3′)
Hypervariable Regions	□	L15869 F 5' AAAATACTCAAATGGGCCTGTC 3'
		H719 R 5' CGTGGTGATTTAGAGGGTGAAC 3'
		16539 F 5' ACACGTTCCCCTTAAATAAGAC 3'
		80 R 5' AGCGTCTCGCAATGCTATCG 3'
5178	61°C	5178 F 5' ATCTCTCCCTCACTAAACGTAAGCCTT 3'
		5178 R 5' TTAGTATAAAAGGGGAGATAGGTAGGAGTAGC 3'
10398	64°C	10398 F 5' GCCCTCCTTTTACCCCTACCA 3'
		10398 R 5' GGGAGGATATGAGGTGTGAGCGAT 3'
13708	65°C	13708 F 5' TCATCGCTACCTCCCTGACAAG 3'
		13708 R 5' ATGCTAGGGTAGAATCCGAGTATGTT 3'
13928	61°C	13928 F 5' TATTCGCAGGATTTCTCATTACTAACAACATTTC 3'
□	□	13928 R 5' AAAATATATAAGGATTGTGCGGTGTGTGACG 3' [a]

[a] Mismatched base.

Citation: Feng-ling Xu; Mei Ding; Jun Yao; Zhang-sen Shi; Bao-jie Wang PCR amplification and restriction fragment length polymorphism analysis of four SNPs (C5178A, A10398G, G13708A, and C13928G) in the mtDNA coding region. **protocols.io**

dx.doi.org/10.17504/protocols.io.ipgcdjw

Published: 28 Jun 2017

Protocol