PERFORMANCE OF DIFFERENT DNA POLYMERASES IN THE AMPLIFICATION AND SEQUENCING OF THE MTDNA CONTROL REGION

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The performance of eight different polymerases in the PCR amplification for the sequencing of the human mtDNA control region was tested. The polymerases used were Taq (Promega), Taq (Qiagen), Pfu (Stratagene), AmpliTaq Gold® (Perkin), Taq Qiagen + Q sol, Taq Plus Precision (Stratagene), Pfu Turbo® (Strategene) and Expand high Fidelity (Roche-Boehringer). The sensitivity assays were performed with 0.1 to 100 pf human genomic DNA.

Taq Plus Precision, Pfu Turbo®, Qiagen + Q solution and Expand high Fidelity gave the best sensitivity threshold for the amplification of the 1333 bp mtDNA.

The eight Polymerases gave sufficient 400 bp HV1 PCR product for the sequencing reaction using the nested PCR method with 0.1 pg genomic DNA.

Using the standard PCR, the sensitivity limit for the amplification of the 400 bp HV1 mtDNA fluctuated between 0.1 and 0.4 pf for all Polymerases except the Taq Plus Precision which needed 0.4 to 12.5 pg genomic DNA.

Considering the sequencing reaction with the HV1 standard PCR product, the Taq Qiagen, Taq Quiagen + Q solution and Expand high fidelity gave a high quality sequencing data using 25 pg genomic DNA whereas 100 pf was required with the other Polymerases.

The HV1 mtDNA PCR success rates of 15 forensic evidence samples were between 53.3 and 93.3%. The best results were achieved using the TaqQiagen + Q solution, the Pfu Turbo and the Expand high Fidelity PCR System.

In conclusion, it seemed to be important to test the performance of different polymerases to optimize the PCR product in order to obtain high quality sequencing results particularly for the mtDNA typisation of forensic samples with highly degraded or very little DNA.