

Human Mitochondrial DNA HVSI Containing Poly-C Stretch

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Abstract

Determination of human mitochondrial DNA (mtDNA) sequence is essential for studying both normal variant polymorphism and variants disease related. Through direct sequencing can be obtained nucleotide sequence data of 16024-16383, the first hypervariable segment (HVSI) of the mtDNA control region. However, this method can not be applied to sampel containing poly-C stretch because of T16189C mutation. This thesis reported the nucleotide sequence of HVSI mtDNA for sampels containing poly-C stretch using cloning method. The strategy of this research included mtDNA amplification using Polymerase Chain Reaction (PCR) technique, recombinant DNA cloning to E.coli JM109, and sequencing using Dideoxy Sanger method. Electrophoresis of PCR product resulted one fragment 0.4 kb sized on agarose gel. Cloning this fragment into host cell has yielded white colony containing recombinant DNA. Based on the electrophoregraph of sequencing results have been obtained mtDNA HVSI nucleotide sequence of 1920 bp for four samples. The analysis showed that there are other mutation outside T16189C of three samples, they are XXAM, ESG, and GMR. The differentiation of poly-C stretch has also been investigated of 10[C], 11[C], 13[C] and 15[C] for C4B, GMR, ESG, and XXAM. Through this method can be obtained the nucleotide sequence that can not read by direct sequencing. Hence, the database of HVSI containing poly-C stretch has been added for the nomenclature of human mtDNA.