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Sequencing of mitochondrial HV1 and HV2 DNA with length heteroplasmy

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Abstract

This study presents a fast method for sequencing the poly C/G regions in HV1 and HV2 in the mitochondrial DNA (mtDNA).

Keywords: Mitochondrial DNA; Length heteroplasmy; Junction primers

1. Introduction

Sequencing of mitochondrial DNA (mtDNA) with length heteroplasmy in poly C/G stretches [1] in general give ambiguous results in the DNA region 3′ of the homopolymeric region. Molecular cloning of single DNA fragments followed by sequencing may be used in cases with length heteroplasmy [2]. These methods are, however, time consuming and presently not of practical use in forensic casework. We have sequenced the DNA regions 3′ of two homopolymeric mtDNA regions in HV1 and HV2 using PCR and sequencing primers placed at the junctions between the homopolymeric regions and the 3′ parts of the H- and L-strands.

2. Materials and methods

DNA was extracted from blood with phenol/chloroform from 15 samples with length heteroplasmy. HV1 mtDNA was amplified with the primers 5′CAC CAT TAG CAC CCA AAG CT′3 and 5′TGA TTT CAC GGA GGA TGG TG′3, Fig. 1a, and HV2 with the

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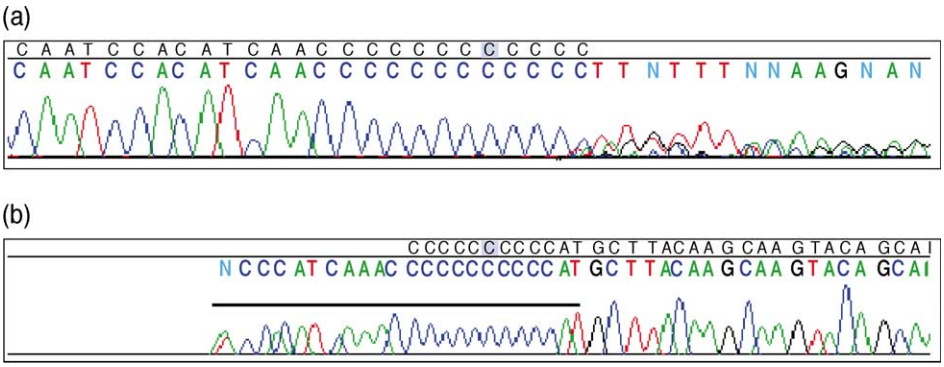

Fig. 1. (a) Sequence of the poly C/G and the flanking regions in HV1 obtained with conventional sequencing primers, L15997-21M13 and H16401M13Rev. (b) Sequence of the poly C/G and the 3′ region in HV1 obtained with junction primer, L16195-21M13 and H16401M13Rev. The marked area corresponds to the position of the junction primer.

primers 5′CTC ACG GGA GCT CTC CAT GC′3 and 5′CTG TTA AAA GTG CAT ACC GCC A′3, Fig. 2a. Six samples showed heteroplasmy in the homopolymeric region in HV1 consisting of poly C/G beginning in position 16184. Eight samples showed heteroplasmy in the homo-polymeric region in HV2 consisting of poly C/G beginning in position 303. One sample showed length heteroplasmy in both HV1 and HV2. For each region, junction primers complementary to the junction between the homopolymeric region and the 3′part of the DNA strands were designed for both the L- and H-strands. The primers used in the second amplification for obtaining sequence results for the L-strand in HV1 were 5′TGT AAA ACG ACG GCC AGT ATC CAC ATC AAA CCC CCC CCC CCAT′3 (L16195-21M13) and 5′CAG GAA ACA GCT ATG ACC TGA TTT CAC GGA GGA TGG TG′3 (H16401M13Rev), Fig. 1b. The primers used in the second amplification for obtaining sequence results for the L-strand were 5′TGT AAA ACG ACG GCC AGT CCC CCC

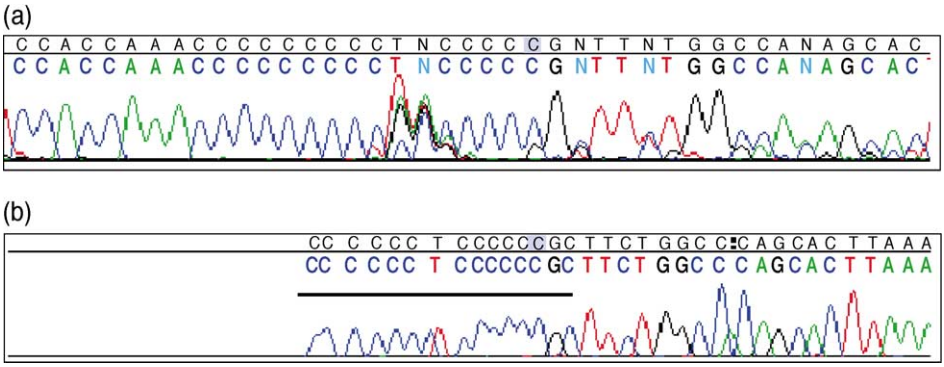

Fig. 2. (a) Sequence of the poly C/G and the flanking regions in HV2 obtained with conventional sequencing primers, L48-21M13 and H408M13Rev. (b) Sequence of the poly C/G and the 3′ region in HV1 obtained with junction primer L317-21M13 and H408M13Rev. The marked area corresponds to the position of the junction primer.

CCT CCC CCC GC′3 (L317-21M13) and 5′CAG GAA ACA GCT ATG ACC CTG TTA AAA GTG CAT ACC GCC A′3 (H408M13Rev), Fig. 2b. In order to obtain sequence results from the H-strand to complete the sequencing, the primers used for the second amplification in HV1 were 5′CAG GAA ACA GCT ATG ACC AAG CAT GGG GGG GGG GGT T′3 (H16182M13Rev) and 5′TGT AAA ACG ACG GCC AGT CAC CAT TAG CAC CC AAA GCT′3 (L15997-21M13) and the primers used for the second amplification in HV2 were 5′CAG GAA ACA GCT ATG ACC GGG GGA GGG GGG GGG TTT G′3 (H299M13Rev) and 5′TGT AAA ACG ACG GCC AGT CTC ACG GGA GCT CTC CAT GC′3 (L48-21M13). PCR-products were purified by MicroSpin S-300 HR columns (APB) and sequenced using the Applied Biosystems BigDye Primer and Terminator Cycle Sequencing kits. The extension products were analysed on either ABI 377- or ABI 310-sequencer.

3. Results and discussion

Sequencing of DNA regions 3′ to the homopolymeric regions with length heteroplasmy with junction primers gave unambiguous sequencing results in both directions in all 32 sequences from the 15 heteroplasmic samples. The first nucleotides corresponding to the 3′ end of the primer could not be typed. Thus, the use of the junction primers described here facilitates both forward and reverse sequencing in samples with length heteroplasmy in a way so fast and simple that it may be used in forensic casework. Special primers must be designed for rare mtDNA types with other sequences of the first two nucleotides 3′ of a poly C/G region.

4. Conclusion

This study shows that it is possible to sequence both mtDNA strands 3′ to homopolymeric C/G-stretches in HV1 and HV2 in individuals with length polymorphism.

References

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