Midi- and mini-primer sets for mtDNA control region sequence analysis from highly degraded forensic samples

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Introduction

Mitochondrial DNA (mtDNA) analysis is a powerful tool for forensic identification testing of highly degraded skeletal remains due to its stability and the large number of genome copies per cell. As highly degraded samples contain populations of intact DNA molecules that are severely restricted in size, the mini-primer set amplification strategy which attempts to target and preferentially amplify authentic human mtDNA sequences with small PCR products was suggested by the Armed Forces DNA Identification Laboratory (AFDIL). These mini-primer can recover reliable mtDNA sequences from highly degraded skeletal remains and dramatically increase the PCR amplification success rate when compared with those consisting of approximately 250 bp. However, the length heteroplasmy by mutation at nucleotide position 16189 hinders the interpretation of mtDNA sequencing results when using AFDIL primer sets. Also, due to the high nucleotide variability of the control region sequences, primer binding sites can be affected by mutations which lower or inhibit PCR yield. To facilitate mtDNA control region sequence analysis, we propose here improved midi- and mini-primer sets by modifying primer sets of the AFDIL.

Primer design

To develop a modified midi- and mini-primer sets, some primers were used as the same as in previous reports by AFDIL, and the other were designed using the Primer3 program (http://frodo.wi.mit.edu/primer3/input.htm). Among the primers of AFDIL, the primers of which efficiencies are prone to be influenced by frequent mutations or by PCR conditions were preferentially redesigned. The primers which produce amplicons highly overlapping with adjacent PCR products were also newly designed. The typical default parameters for candidate primer design were as follows : primer size ranges from 10 to 27 nucleotide with 20 nucleotides as the optimum, primer T_m values from 48℃ to 63℃ with 60℃ as the optimum, and the primer GC% ranges from 20% to 80%. After primer designing, screening PCR amplifications were performed to qualify the candidate primers using DNAs belonging to the mtDNA haplogroup D4. Through screening PCR amplifications, the final set of primers was selected out of a few candidate primer pairs with high amplification efficiency to have relatively short primer length and high T_m.

PCR amplification

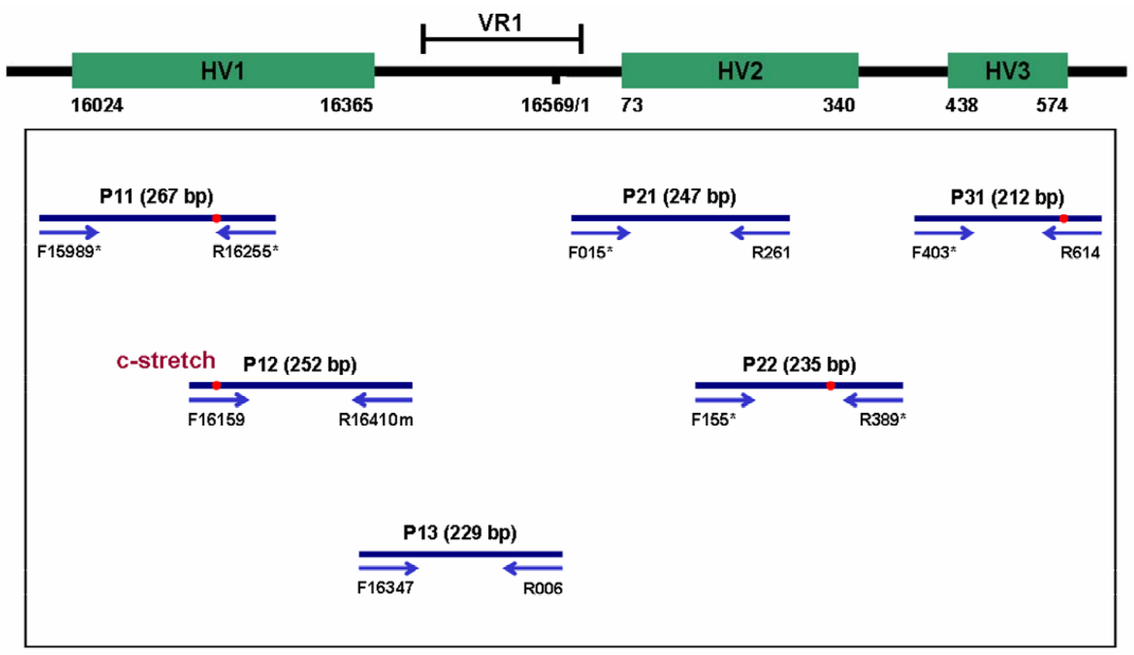
PCR reaction: Total 25 μL reaction contained 2 μL of degraded DNA, 2.5 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA, USA), 2.5 μL of Gold ST*R 10X buffer (Promega, Madison, WI, USA) and 0.6 μM of each forward and reverse primer.

Cycling condition: 95˚C 11 min; 40 cycles of 95˚C 20 sec, 50˚C 20 sec, 72˚C 30 sec; 72˚C 7 min using the PTC-200 DNA engine (MJ Research, Waltham, MA, USA).

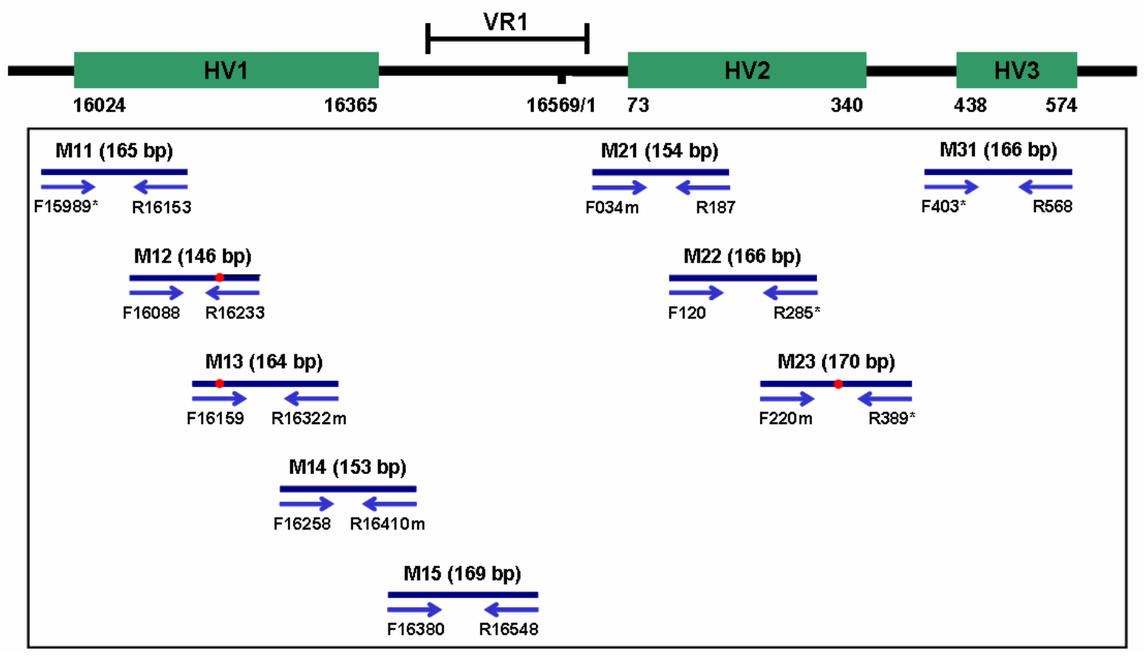
➡ **Primer sequences and detailed protocol are available at http://forensic.yonsei.ac.kr.**

Results

1. Midi-primer set



2. Mini-primer set



Primer schemes for the amplification of mtDNA control regions from highly degraded forensic samples.

(Primers identical to those of AFDIL are indicated with an asterisk (*) and the poly C-stretches are indicated by red circle.)

Discussion

❖ The midi-primer set consists of six overlapping PCR products ranging from 212 bp to 267 bp and the mini-primer set consists of nine overlapping PCR amplicons of 170 bp or less, so as to encompass three hypervariable (HV) regions and variable region 1 (VR1).

❖ Due to the adjusted T_m of each primer, all of the amplicons were well amplified under the same condition, and the primers which produce amplicons showing too much or too little overlapping with adjacent PCR products for direct sequencing of amplicons were also redesigned.

❖ Modified primer sets allow efficient amplification of mtDNA being less affected by HV1 length heteroplasmy.

❖ Particularly they are universally applicable to every population groups, because the first three nucleotides of the 3’ end of each redesigned primer are located at the nucleotide positions with a low mutation frequency (1.0% or less) in the world three major population groups (i.e. Europeans, Asians, and Africans).

❖ To amplify mtDNA control region for forensic casework, primer pairs can be chosen depending on the level of degradation of sample and the desired amplicon. Hence, these midi- and mini-primer sets will be useful for mtDNA control region sequence analysis from highly degraded forensic samples.