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Research Article

# PCR/ESI-TOF-MS Based Method for Detecting Polymorphisms of mtDNA in Chinese Han Population and Testing of a Special Maternity Case

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**Abstract**

Base compositions of 12 amplicons in HV1 derived from primers covering coordinates15924-16428 and base compositions of 12 amplicons in HV2 derived from primers covering coordinates 31-576 were determined using ESI-TOF-MS based PLEX-ID system. Eight segments in HV1 and ten segments in HV2 were found to be polymorphic. Highly polymorphic information was available in the regions of 16124-16250 and 138-340. After the assay was applied to a special maternity duo case, the false mother was excluded as the biological mother despite the fact that they shared at least an allele at 46 autosomal STR loci.

## INTRODUCTION

Due to maternal inheritance, mitochondrial DNA (mtDNA) is valuable for testing of special kinship, e.g. relationships between maternal individuals, half-sibling sisters, aunt-nephew, maternal uncle-nephew and grandmother-granddaughter. In addition, mtDNA can be used for detection of problematic samples such as old and degraded bones, teeth and hair shafts. Identification of the remains of Russia's last Tsar Nicholas II is a typical case about mtDNA application [1].

In forensic laboratories, mtDNA detection was routinely performed by Sanger sequencing, but the technique was only for analysis of part of mtDNA hypervariable regions (16024-16365 in HV1 and 73-340 in HV2). If the technology of higher resolving power can be used, more information about mtDNA in wider range will be gathered.

In this study, multiplex PCR amplification followed by electrospray Ionisation time-of-flight mass spectrometry (ESI-TOF MS) was used to detect the polymorphisms of mtDNA by characterizing the base compositions of HV1 and HV2, nucleotide positions 15924-16428 [Figure 1] and 31-576 [Figure 2] [2], respectively. This assay allows for maximum discrimination of the hypervariable segments of mtDNA without targeting specific nucleotide positions.

## MATERIALS AND METHODS

### Sample collection

Blood samples were collected from twelve unrelated Chinese Han individuals with informed consent.

### DNA extraction and quantification

Genomic DNA was extracted from blood samples using QIAamp Mini Blood kit (Qiagen, German) and quantified using the Quantifiler Human DNA Quantification kit (Life Technologies, USA) and then the DNA concentration was adjusted to 10ng/μL.

### mtDNA amplification and detection

American Abbott company provided technical service for the mtDNA analysis [3,4]. Primer sequences were reported by Hall TA et al in 2009 [5]. Firstly, 8 triplex PCR systems were used to amplify 12 segments of mtDNA HV1 coordinates 15924-16428 and 12 segments of HV2 coordinates 31-576 (the adjacent segments overlap 2-22 bp, shown in [Figure 3]. Then the PCR products were separated by ESI-TOF MS and the base compositions of HV1 and HV2 regions were determined by IBIS Track software (Abbott, USA) on the PLEX-ID platform.

During the test, known sample which had been sequenced was used as positive control. PCR amplifications and clean-up were conducted in Mastercycler (Eppendorf, German) on 96-well

15901 AAACTAATAC ACCAGTCTTG TAA**ACCGGAG ATGAAAACCT TTTTCCAAGG ACAAATCAGA**
**15961 GAAAAAGTCT TTAACTCCAC CATTAGCACC CAAAGCTAAG ATTCTAATTT AAACTATTCT**
**16021 CTGTTCTTTC ATGGGGAAGC AGATTTGGGT ACCACCCAAG TATTGACTCA CCCATCAACA**
**16081 ACCGCTATGT ATTTCGTACA TTACTGCCAG CCACCATGAA TATTGTACGG TACCATAAAT**
**16141 ACTTGACCAC CTGTAGTACA TAAAAACCCA ATCCACATCA AAACCCCCTC CCCATGCTTA**
**16201 CAAGCAAGTA CAGCAATCAA CCCTCAACTA TCACACATCA ACTGCAACTC CAAAGCCACC**
**16261 CCTCACCCAC TAGGATACCA ACAAACCTAC CCACCCTTAA CAGTACATAG TACATAAAGC**
**16321 CATTTACCGT ACATAGCACA TTACAGTCAA ATCCCTTCTC GTCCCCATGG ATGACCCCCC**
**16381 TCAGATAGGG GTCCCTTGAC CACCATCCTC CGTGAAATCA ATATCCCG**CA CAAGAGTGCT

**Figure 1** Revised Cambridge Reference Sequence of HV1 portion of the mtDNA control region (nucleotide positions 15924-16428 were underlined).

1 GATCACAGGT CTATCACCCT ATTAACCACT **CACGGGAGCT CTCCATGCAT TTGGTATTTT**
**61 CGTCTGGGGG GTATGCACGC GATAGCATTG CGAGACGCTG GAGCCGGAGC ACCCTATGTC**
**121 GCAGTATCTG TCTTTGATTC CTGCCTCATC CTATTATTTA TCGCACCTAC GTTCAATATT**
**181 ACAGGCGAAC ATACTTACTA AAGTGTGTTA ATTAATTAAT GCTTGTAGGA CATAATAATA**
**241 ACAATTGAAT GTCTGCACAG CCACTTTCCA CACAGACATC ATAACAAAAA ATTTCCACCA**
**301 AACCCCCCCT CCCCCGCTTC TGGCCACAGC ACTTAAACAC ATCTCTGCCA AACCCCAAAA**
**361 ACAAAGAACC CTAACACCAG CCTAACCAGA TTTCAAATTT TATCTTTTGG CGGTATGCAC**
**421 TTTTAACAGT CACCCCCCAA CTAACACATT ATTTTCCCCT CCCACTCCCA TACTACTAAT**
**481 CTCATCAATA CAACCCCCGC CCATCCTACC CAGCACACAC ACACCGCTGC TAACCCCATA**
**541 CCCCGAACCA ACCAAACCCC AAAGACACCC CCCACA**GTTT ATGTAGCTTA CCTCCTCAAA

**Figure 2** Revised Cambridge Reference Sequence of HV2 portion of the mtDNA control region (nucleotide positions 31-576 were underlined).

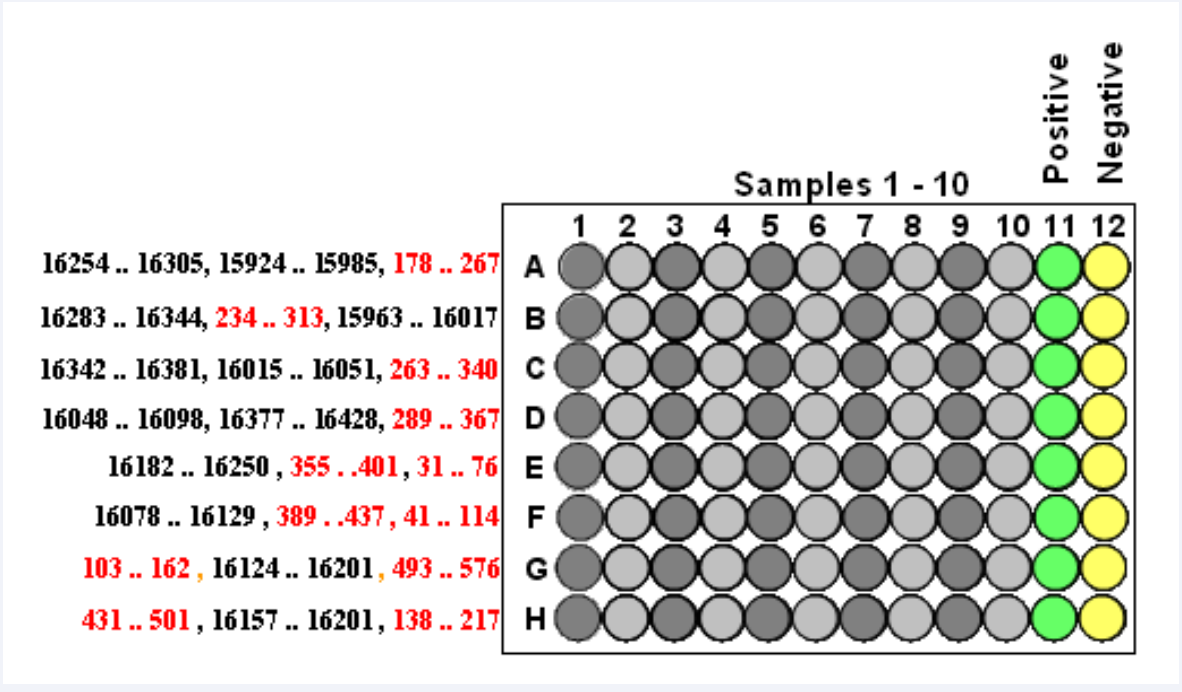


**Figure 3** Eight triplex PCR system and amplified segments set up.

**Case application**

(M) and the boy (B) shared at least an allele at each of the 46

autosomal STRs detected, but she was adamant that she had not given birth to the child. In order to define the facts, mtDNA and SNP detection was followed.

## RESULTS AND DISCUSSION

### Techniques for mtDNA detection

Various technologies for mtDNA detection have been reported, such as Sanger sequencing, high-resolution melt (HRM) profiling, denaturing high-performance liquid chromatography (DHPLC), exo-DNA polymerase and RFLP analysis, single strand conformation polymorphism (SSCP) analysis, denaturing gradient Gel electrophoresis assay, pyrosequencing, SNapShot and next-generation sequencing. For example, detection of mitochondrial heteroplasmy in cerebrospinal fluid using DHPLC was reported by Conley in 2003 [6]; Quantitation of heteroplasmy in mitochondrial DNA mutations by primer extension using Vent(R) (exo-) DNA polymerase and RFLP analysis was reported by Jacobi in 2001 [7]. In 1997 Nollau found that SSCP analysis could be used for detecting heteroplasmic mtDNA if the level of heteroplasmy was above 10% [8]; In 2000 Tully confirmed that the sensitivity of denaturing gradient Gel electrophoresis assay was about 1% [9]. It was worth mentioning that above techniques were only for detection of part of mtDNA hypervariable regions (16024-16365 in HV1 and 73-340 in HV2) because of the limitation of discrimination power.

In this work we applied ESI-TOF-MS assay on the PLEX-ID platform and more discriminating information than that from Sanger sequencing was obtained. ESI-TOF-MS assay for HV1 and HV2 were designed to read bases 15924-16428 and 31-576 respectively, while the Sanger sequencing for HV1 and HV2 were designed to read bases 16024-16365 and 73-340, which means that ESI-TOF-MS assay spans regions larger than Sanger sequencing. Although the PLEX-ID platform for analyzing of mtDNA is now no longer available, the comparative analysis of the ESI-TOF-MS technology and the traditional fluorescence-based capillary electrophoresis for typing of amplicons has demonstrated the potential advantages of the mass-spectrometric technique [10].

### Polymorphism of mtDNA HV1

8 out of 12 segments of HV1 (15924-16428) showed SNP and/or poly-C length polymorphisms [Table 1]. It could be seen that

**Table 1**: Polymorphisms of mtDNA HV1.

| Primer | Amplicon | Base composition | Frequency | Primer | Amplicon | Base composition | Frequency |
|---|---|---|---|---|---|---|---|
| 2901 | 15924-15985 | A24 G10 C14 T14 | 0.0833 | 2893 | 16182-16250 | A23 G5 C30 T10 | 0.0588 |
| | | A25 G9 C14 T14 | 0.9167 | | | A23 G5 C31 T10 | 0.0588 |
| 2925 | 15963-16017 | A22 G4 C13 T16 | 1.0000 | | | A23 G5 C32 T10 | 0.0588 |
| 2899 | 16015-16051 | A7 G10 C5 T15 | 1.0000 | | | A23 G5 C33 T10 | 0.0588 |
| 2898 | 16048-16098 | A15 G7 C16 T13 | 1.0000 | | | A24 G5 C26 T14 | 0.0588 |
| 2897 | 16078-16129 | A15 G8 C13 T16 | 0.0833 | | | A24 G5 C27 T13 | 0.5296 |
| | | A15 G8 C14 T15 | 0.7500 | | | A24 G5 C28 T12 | 0.0588 |
| | | A16 G7 C14 T15 | 0.1667 | | | A24 G5 C28 T13 | 0.0588 |
| 2896 | 16124-16201 | A26 G7 C29 T15 | 0.0588 | | | A24 G5 C29 T13 | 0.0588 |
| | | A26 G7 C30 T15 | 0.0588 | 2892 | 16254-16305 | A18 G4 C21 T9 | 0.1667 |
| | | A26 G7 C31 T15 | 0.0588 | | | A18 G4 C22 T8 | 0.2500 |
| | | A26 G7 C32 T15 | 0.0588 | | | A18 G4 C23 T7 | 0.2500 |
| | | A26 G8 C27 T17 | 0.1177 | | | A18 G4 C24 T6 | 0.2500 |
| | | A27 G7 C27 T17 | 0.3531 | | | A19 G3 C23 T7 | 0.0833 |
| | | A27 G7 C28 T16 | 0.0588 | 2891 | 16283-16344 | A24 G5 C18 T15 | 0.0833 |
| | | A28 G6 C27 T17 | 0.0588 | | | A24 G5 C19 T14 | 0.1667 |
| | | A28 G6 C28 T16 | 0.0588 | | | A24 G5 C20 T13 | 0.3334 |
| | | A28 G6 C29 T16 | 0.0588 | | | A24 G5 C21 T12 | 0.0833 |
| | | A28 G6 C30 T16 | 0.0588 | 2891 | 16283-16344 | A25 G4 C17 T16 | 0.0833 |
| 2895 | 16157-16201 | A16 G1 C20 T7 | 0.0555 | | | A25 G4 C18 T15 | 0.2500 |
| | | A16 G1 C21 T7 | 0.0555 | 2890 | 16342-16381 | A8 G5 C17 T10 | 0.7500 |
| | | A16 G1 C22 T7 | 0.0555 | | | A8 G5 C18 T9 | 0.2500 |
| | | A16 G1 C23 T7 | 0.0555 | 2889 | 16377-16428 | A12 G9 C20 T11 | 1.0000 |
| | | A16 G2 C18 T9 | 0.0555 | | | | |
| | | A16 G2 C19 T8 | 0.0555 | | | | |
| | | A17 G1 C19 T7 | 0.0555 | | | | |
| | | A17 G1 C19 T8 | 0.3892 | | | | |
| | | A17 G1 C20 T7 | 0.1113 | | | | |
| | | A17 G1 C21 T7 | 0.0555 | | | | |
| | | A17 G1 C22 T7 | 0.0555 | | | | |

16124-16250 in HV1 was high informative for forensic purpose.

## Polymorphism of mtDNA HV2

10 out of 12 segments of HV2 (31-576) showed SNP and/or poly-C length polymorphisms [Table 2]. It was found that 138-340 in HV2 was high polymorphic.

## Case application

The assay was applied to the special duo case in which the false mother could not be excluded as the biological mother because they shared at least an allele at 46 STR loci. To reach accurate conclusion, mtDNA detection was carried out as supplement to autosomal STR loci. mtDNA profiles clearly excluded the mother

**Table 2:** Polymorphisms of mtDNA HV2.

| Primer | Amplicon | Base composition | Frequency | Primer | Amplicon | Base composition | Frequency |
|---|---|---|---|---|---|---|---|
| 2902 | 31-76 | A5 G16 C10 T15 | 0.9167 | 2907 | 263-340 | A25 G6 C34 T14 | 0.3125 |
| | | A5 G16 C11 T15 | 0.0833 | | | A25 G6 C35 T14 | 0.1875 |
| 2903 | 41-114 | A12 G25 C19 T18 | 0.9167 | | | A25 G6 C36 T14 | 0.3125 |
| | | A12 G25 C20 T18 | 0.0833 | | | A26 G5 C34 T14 | 0.0625 |
| 2904 | 103-162 | A10 G10 C17 T23 | 0.2143 | | | A26 G5 C35 T14 | 0.0625 |
| | | A10 G10 C18 T22 | 0.3571 | | | A26 G5 C36 T14 | 0.0625 |
| | | A10 G10 C19 T21 | 0.3571 | 2923 | 289-367 | A27 G6 C35 T12 | 0.3750 |
| | | A11 G10 C18 T21 | 0.0715 | | | A27 G6 C36 T12 | 0.2500 |
| 2905 | 138-217 | A23 G9 C17 T31 | 0.0667 | | | A27 G6 C37 T12 | 0.3750 |
| | | A23 G9 C18 T30 | 0.3333 | 2910 | 355-401 | A21 G3 C14 T9 | 1.0000 |
| | | A23 G9 C19 T29 | 0.3333 | 2916 | 389-437 | A11 G7 C12 T19 | 1.0000 |
| | | A23 G9 C20 T28 | 0.0667 | 2912 | 431-501 | A20 G1 C34 T16 | 0.6154 |
| | | A24 G8 C19 T29 | 0.1333 | | | A20 G1 C35 T15 | 0.3846 |
| | | A24 G9 C19 T28 | 0.0667 | 2913 | 493-576 | A26 G6 C45 T5 | 0.4615 |
| 2906 | 178-267 | A31 G15 C14 T29 | 0.0769 | | | A27 G6 C46 T5 | 0.3078 |
| | | A31 G15 C15 T28 | 0.2308 | | | A27 G6 C49 T5 | 0.0769 |
| | | A32 G15 C14 T29 | 0.3847 | | | A27 G6 C50 T5 | 0.0769 |
| | | A32 G15 C15 T28 | 0.1538 | | | A28 G6 C47 T5 | 0.0769 |
| | | A33 G14 C14 T29 | 0.0769 | | | | |
| | | A33 G14 C16 T27 | 0.0769 | | | | |
| 2908 | 234-313 | A29 G6 C29 T16 | 0.1332 | | | | |
| | | A29 G6 C31 T16 | 0.0667 | | | | |
| | | A30 G6 C29 T16 | 0.2667 | | | | |
| | | A30 G6 C30 T16 | 0.2667 | | | | |
| | | A30 G6 C31 T16 | 0.2667 | | | | |

**Table 3**: mtDNA differences between the detected mother and boy.

| mt DNA HV | | | mt DNA HV | | |
|---|---|---|---|---|---|
| **Primer** | **Segment** | **Base composition** | **Primer** | **Segment** | **Base composition** |
| 2896 | 16102..16224 | M:A45 G13 C41 T24 | 2908 | 204..330 | M:A42 G16 C38 T32 |
| | | B:A44 G13 C43 T22<br>B:A44 G13 C44 T22<br>B:A44 G13 C45 T22<br>B:A44 G13 C46 T22 | | | B:A42 G16 C39 T32<br>B:A42 G16 C40 T32 |
| 2893 | 16154..16268 | M:A44 G7 C45 T19 | 2907 | 239..363 | M:A44 G10 C49 T23 |
| | | B:A43 G7 C48 T16<br>B:A43 G7 C49 T16<br>B:A43 G7 C50 T16<br>B:A43 G7 C51 T16 | | | B:A43 G11 C50 T23<br>B:A43 G11 C51 T23 |
| 2892 | 16231..16338 | M:A40 G9 C39 T20 | 2923 | 262..390 | M:A47 G10 C53 T20 |
| | | B:A41 G8 C40 T19 | | | B:A47 G10 C54 T20<br>B:A47 G10 C55 T20 |
| 2891 | 16256..16366 | M:A38 G8 C40 T25 | 2913 | 464..603 | M:A43 G10 C62 T23 |

**Table 3**: mtDNA differences between the detected mother and boy.

| mt DNA HV | | | mt DNA HV | | |
|---|---|---|---|---|---|
| **Primer** | **Segment** | **Base composition** | **Primer** | **Segment** | **Base composition** |
| | | B:A37 G9 C41 T24 | | | B:A44 G10 C63 T23 |
| 2890 | 16318..16402 | M:A20 G14 C31 T20 | | | |
| | | B:A20 G14 C30 T21 | | | |

**Table 4:** SNP differences between the detected mother and boy.

| SNP locus | Mother(M) | Boy(B) |
|---|---|---|
| rs 1109037 | A | G |
| rs 214955 | G | A |
| rs 7229946 | G | A |
| rs 985492 | C | T |
| rs 9951171 | G | A |

as the biological parent because the two persons had clear differences at 9 of the 24 fragments [Table 3]. The conclusion was confirmed by additional typing of SNP [Table 4]. Later on, investigation confirmed that the putative mother was in fact the boy's paternal aunt. The mtDNA detection results meant that the mass spectrometry-based method for mitochondrial profiling could have promising prospects.

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