



# Analysis of mitochondrial 12S rRNA gene sequence variation in four ethnically defined populations

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## Abstract

In this study, we have analysed the nucleotide sequence variation of the 12S rRNA mitochondrial gene (648–1601 bp) from five different populations (Spanish Caucasian, Autochthonous from the Basque Country, Chinese, New Guinea Highlander and Africans of Benin) by full sequencing of two overlapping PCR fragments using d-rhodamine cycle sequencing coupled with an ABI377 sequencer. Preliminary data indicate different patterns of sequence variation between Africans and non-Africans. Africans are much more polymorphic than non-African populations, which have only a very restricted subset of haplotypes. Furthermore, the greater part of Africans analysed showed two specific nucleotide substitutions (769G → A and 1018G → A) that were not observed in non-African individuals. In conclusion, the mtDNA 12SRNA gene in combination with other systems could be an interesting ethnic marker that could help to differentiate between African and non-African maternal lineages.

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*Keywords:* mtDNA 12S rRNA gene; Africans; Non-Africans; Ethnic marker

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## 1. Introduction

mtDNA control region sequencing has become a powerful tool to investigate the identity of biological evidence that cannot be analysed by the use of single-copy nuclear markers. However, the power of discrimination of mtDNA testing is very limited [1]. Targeting additional variation outside of the hypervariable regions will enhance the power

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and efficiency of mtDNA profiling. In this context, we have screened for the 12S rRNA gene sequence variation in four ethnically defined population groups.

## 2. Materials and methods

### 2.1. Sample preparation

EDTA blood samples or buffy coats of unrelated individuals were collected from an African population: Benin ( $n = 21$ ) and from four non-African populations: Madrid-Central Spain ( $n = 19$ ), Basque Country-North Spain ( $n = 20$ ), New Guinea Highlanders ( $n = 20$ ) and China ( $n = 21$ ). Genomic DNA was extracted as described previously [2] or by the standard phenol/chloroform extraction procedure.

### 2.2. PCR amplification

We have analysed the nucleotide sequence variation of the 12S rRNA mitochondrial gene (648–1601 bp) by full sequencing of two overlapping PCR fragments with two primer pairs:

F1: 5' TTATGTAGCTTACCTCCTCA/R1: 5' GATTAACTGTTGAGGTTTA  
F2: 5' TGAACACACAATAGCTAAGA/R2: 5' GAAATCTCCTAAGTGTAAGT

PCR amplification was carried out in a Thermal Cycler Gene Amp 2400 (Perkin Elmer, Applied Biosystems) with the following conditions: 95 °C for 10 min/95 °C for 15 s/60 °C for 30 s/72 °C for 30 s/72 °C for 10 min, 36 cycles.

### 2.3. Cycle sequencing

The purified PCR products were sequenced by cycle sequencing using ABI-PRISM d-rhodamine cycle sequencing ready reaction kit with Amplitaq DNA polymerase FS (Applied Biosystems). The sequencing products were analysed on an ABI377 sequencer. Sequences generated with direct and reverse primers were edited and compared with the Cambridge Reference Sequence [3].

### 2.4. Statistical analysis

Statistical evaluation was performed using the Arlequin software Ver. 2.000 [4].

## 3. Results and discussion

A total of 22 different haplotypes defined by 23 single nucleotide polymorphisms were found on the 12S rRNA gene (648–1601 bp) in a sample of 102 individuals from four ethnically defined population groups (Table 1).

Table 1  
Observed haplotypes and frequencies

| Observed haplotype                                | No. of individuals | Haplotype                                   | Frequency |
|---|--------------------|---|-----------|
| <i>Africans of Benin (n = 21)</i>                 |                    |   |           |
| 1   | 1                  | 709A, 750G, 769A, 789A, 1018A, 1438G        | 0.0476    |
| 2   | 2                  | 731G, 750G, 769A, 1018A, 1438G              | 0.0952    |
| 3   | 3                  | 709A, 710C, 750G, 769A, 825A, 1018A         | 0.1428    |
| 4   | 1                  | 1438G                                       | 0.0476    |
| 5   | 3                  | 750G, 769A, 1018A, 1438G                    | 0.1428    |
| 6   | 2                  | 750G, 769A, 825A, 1018A, 1048T, 1438G       | 0.0952    |
| 7   | 1                  | 750G, 769A, 870T, 1018A, 1438G              | 0.0476    |
| 8   | 1                  | 709A, 750G, 769A, 1018A, 1438G, 1442A       | 0.0476    |
| 9   | 1                  | 709A, 750G, 769A, 825A, 1018A, 1420C, 1438G | 0.0476    |
| 10  | 6                  | 750G, 1438G                                 | 0.2857    |
| <i>Chinese (n = 21)</i>                           |                    |   |           |
| 10  | 14                 | 750G, 1438G                                 | 0.666     |
| 11  | 2                  | 709A, 750G, 1438G                           | 0.0952    |
| 12  | 1                  | 709A, 750G, 961C, 1438G, 1598A              | 0.0476    |
| 13  | 1                  | 750G, 752T, 1107C, 1438G                    | 0.0476    |
| 14  | 1                  | 750G, 1438G, 1520C                          | 0.0476    |
| 15  | 1                  | 750G, 1005C, 1438G                          | 0.0476    |
| 16  | 1                  | 750A, 1391C, 1438G                          | 0.0476    |
| <i>New Guinea Highlanders (n = 20)</i>            |                    |   |           |
| 10  | 12                 | 750G, 1438G                                 | 0.571     |
| 11  | 4                  | 709A, 750G, 1438G                           | 0.19      |
| 17  | 5                  | 750G, 1438G, 1462A                          | 0.238     |
| <i>Spanish Caucasians (n = 19)</i>                |                    |   |           |
| 10  | 13                 | 750G, 1438G                                 | 0.6842    |
| 11  | 2                  | 709A, 750G, 1438G                           | 0.1053    |
| 18  | 1                  | 709A, 750G, 930A, 1438G                     | 0.0526    |
| 19  | 1                  | 750G, 769A, 1018A, 1438G, 1442A             | 0.0526    |
| 20  | 1                  | 750G, 1189C, 1438G                          | 0.0526    |
| 21  | 1                  | Cambridge Reference Sequence                | 0.0526    |
| <i>Autochthonous from Basque Country (n = 20)</i> |                    |   |           |
| 10  | 16                 | 750G, 1438G                                 | 0.8       |
| 11  | 1                  | 709A, 750G, 1438G                           | 0.05      |
| 20  | 1                  | 750G, 1189C, 1438G                          | 0.05      |
| 22  | 2                  | 750G  | 0.1       |

As you can see in Table 2, the results clearly indicated a different pattern of haplotype variation between the high gene diversity (0.8905) in Africans and the restricted set of variability (0.6095–0.3632) that predominates throughout non-Africans, suggesting a common and recent African origin of all non-African human populations. The New Guinea Highlanders and the Autochthonous Basque Country population samples showed the most restricted variation. This may be attributed to a population bottleneck in the case of New Guinea [5] and in the case of Basques could reflect the postulated strong isolation of this European population.

Table 2  
Diversity parameters

|                                  | Benin           | China           | New Guinea      | Madrid          | Basque Country  |
|----------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| No. of individuals               | 21              | 21              | 20              | 19              | 20              |
| No. of haplotypes                | 10              | 7               | 3               | 6               | 4               |
| No. of polymorphic sites         | 13              | 8               | 2               | 8               | 3               |
| Gene diversity                   | 0.8905          | 0.5619          | 0.6095          | 0.538           | 0.3632          |
| Nucleotide diversity             | 0.00329         | 0.00097         | 0.00074         | 0.00107         | 0.0004          |
| Mean pairwise differences (S.D.) | 3.143<br>(1.69) | 0.924<br>(0.66) | 0.705<br>(0.55) | 1.017<br>(0.71) | 0.389<br>(0.38) |

On the other hand, it is interesting to stress that the greater part of the Africans analysed (14/21, 67%) showed two specific nucleotide substitutions (769A and 1018A) that were not observed in non-Africans populations except for one Spanish Caucasian (Haplotype no. 19) that showed an African haplotype for the mtDNA control region (73G, 146C, 150T, 152C, 182T, 195C, 198T, 204A, 260A, 263G, 315.1C, 16114A, 16129A, 16148T, 16213A, 16223T, 16278T, 16311C, 16355T, 16362C).

In conclusion, the mtDNA 12SRNA gene in combination with other nuclear and mitochondrial systems could be an interesting ethnic marker that could help to differentiate between African and non-African lineages.

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