

Reproducibility of mtDNA analysis between laboratories: a report of the European DNA profiling group (EDNAP)

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Abstract

The aim of this collaborative exercise was to determine whether uniformity of mtDNA sequencing results could be achieved among different EDNAP laboratories. Laboratories were asked to sequence mtDNAHV1 region (16024–16365) from three bloodstains, proceeding in accordance with the protocol and strategies currently used in each individual laboratory. Cycle sequencing was used by 11 laboratories and solid phase single stranded sequencing was used by one laboratory. Different PCR strategies and PCR conditions were used by the different laboratories. Three laboratories used semi-nested PCR, two nested PCR, three direct amplification of HV1 and four amplification of overlapping fragments covering the HV1 region. Despite the

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diversity of methodologies used, all the laboratories reported the same results. The successful result of this exercise shows that PCR based mtDNA typing by automated sequencing is a valid, robust and reliable means of forensic identification despite the different strategies and methodologies used by the different laboratories. © 1998 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Mitochondrial DNA (mtDNA) in humans displays considerable sequence variation between individuals [4,7]. Much of the variation is within the non-coding region, which contains the origin of replication for one strand, both origins of transcription, and the D-loop region [2]. This sequence variation is specifically concentrated in two hypervariable regions usually designated as HV1 and HV2 [5]. Due to the high copy number per cell (1000–10 000) mtDNA analysis is especially appropriate when studying degraded samples. In forensic casework mtDNA analysis is particularly important for the individualisation of certain types of evidence, notably hair shafts, which contain little or no genomic DNA.

MtDNA variation can be analysed by a variety of strategies [1,3,4,8–10] but the combination of PCR amplification with direct DNA sequencing is usually the ultimate choice for identification.

A potential drawback to mtDNA sequencing is the labour intensive nature of the technique. For routine forensic analysis the process must be highly automated to maximise sequence throughput and minimise errors in data handling as well as laboratory errors. The variety of strategies for PCR and sequencing can complicate matters further.

The aim of this collaborative exercise was to determine whether uniformity of mtDNA sequencing results could be achieved among different laboratories using the whole range of different methodologies employed.

2. Material and methods

2.1. Samples

EDNAP laboratories were asked to sequence the mtDNA HV1 region (16024–16365) from a total of three bloodstains previously submitted to the laboratories in 1995 for a previous EDNAP exercise (samples 1, 3 and 5). Analysis of samples proceeded in accordance with the protocol and strategies currently used in each individual laboratory. Laboratories were asked to fill out a short questionnaire about their sequencing strategy, in addition to submitting results, and also to state whether this technique was used routinely and whether screening methods were used prior to sequencing.

A total of 12 laboratories submitted results, and representatives of these laboratories are named as authors of this paper.

2.2. Extraction and quantification

Different extraction procedures were reported, including extraction with chelating resins and classical phenol–chloroform procedures.

Nine out of 12 laboratories reported the use of quantification procedures. Quantiblot™ was the most commonly used method after extraction (laboratories 1, 3, 11, 12). Agarose minigels were used by the majority of laboratories for post PCR DNA screening (laboratories 2, 3, 4, 5, 6, 11 and 12). One lab used miniaturised polyacrylamide gels and silver staining (laboratory 1) and another used Picogreen dsDNA quantitation kit (Molecular Probes Europe) (laboratory 7).

2.3. Primers and PCR strategy

Primers and PCR strategies are summarised in Table 1.

PCR strategies can be classified into four groups. Laboratories 1, 4 and 6 used semi-nested PCR, laboratory 2 and 11 nested PCR, laboratories 5, 7 and 10 used direct amplification of HV1 and laboratories 3, 8, 9 and 12 amplified overlapping fragments covering the HV1 region.

In general, different amplification conditions were used by the different laboratories (data not shown). The participants reported using either Taq polymerase or AmpliTaqGold™.

Negative and positive controls were used by all the groups.

Table 1
Primers and PCR strategies used by the different participating laboratories

Laboratory	Primers	PCR Strategies
Lab. 1	L-1599741-00408 L-15997/H-16401	Seminested PCR
Lab. 2	L-15933/H-00576 L-15997/H-16401	Nested PCR
Lab. 3	L-15997/H-16236 L-16159/H-16395	Overlapping fragments
Lab. 4	L-15997/H-00408 L-15997/H-16401	Seminested PCR
Lab. 5	L-1597841-16428	Direct PCR
Lab. 6	L-1599741-16239 L-1599741-16401	Seminested PCR
Lab. 7	F-15971/R-16410	Direct PCR
Lab. 8	L-1599041-16239 L-1615941-16401	Overlapping fragments
Lab. 9	L-1597141-16225 L-16140/H-16420	Overlapping fragments
Lab. 10	L-1599741-16401	Direct PCR
Lab. 11	L-1592841-16498 L-1599541-16400	Nested PCR
Lab. 12	L-1597441-16225 L-1615941-16420	Overlapping fragments

Some laboratories reported the use of Microcon 100 (Amicon), QIAquick (Qiagen) and Microspin 300 (Pharmacia) for purification before sequencing.

2.4. Sequencing methodology

Cycle sequencing was used by 11 laboratories and solid phase single stranded sequencing was used by one laboratory (no. 2).

In general each template was sequenced in both, forward and reverse directions.

Sequencing was performed in all cases using automated sequencers, including ABD 377 (Perkin–Elmer) (Laboratories 1, 2, 3, 4, 5, 10, 11, and 12), ABD 373 (Perkin–Elmer) (Laboratory 6 and 7), Pharmacia ALF (Laboratory 1 and 8) and ALF express (Laboratory 8).

Analysis of the sequence was performed in all cases using commercial software such as Sequence NavigatorTM, ABD Seq Ed or ClustalW [11].

3. Results and discussion

The results are summarised in Table 2. Sample 1 (EDNAP sample 1-1995) had five substitutions relative to the Anderson sequence: One AG transition at nucleotide position (np) 16129, two T–C transitions (np 16223 and np 16278) and one C–T transition (np 16311). In addition a G–A transition (np 16391) was observed but not formally reported because it was out of the region requested for sequencing in this exercise.

Sample 2 (EDNAP Sample 3-1995) showed two differences in relation to the reference sequence [2]: a G–A transition in np 16293 and a C–T transition at np 163 11.

Sample 3 (EDNAP sample 5-1995) showed no differences in relation to the reference sequence.

Despite the diversity of methodologies used, all the laboratories reported the same results. The additional variation in sample 1 (16391 G–A) was reported by the majority of laboratories (10/12 participating laboratories). The variation was seen by the other two laboratories but not reported since it was not requested.

PCR amplification of overlapping fragments (4 laboratories) and semi-nested PCR (four laboratories) were the most commonly used strategies. There was no uniformity in the primers used as different primers were selected by the different laboratories and only 3 labs coincided in their choices (Lab. 1, 4 and 6).

Table 2
Results of the EDNAP 1997 mtDNA exercise

	16129	16223	16278	16293	16311	*16391
And.	G	C	C	A	T	A
1	A	T	T	–	C	G
3	–	–	–	G	C	–
5	–	–	–	–	–	–

Base changes from the reference sequence published by Anderson et al., 1981 [2].

*Out of the region requested for sequencing.

In general, cycle sequencing was preferred (11/12 laboratories) with only one laboratory using solid phase sequencing (laboratory 2). Automated sequencers and sequence analysis software were used in all cases.

Most of the laboratories reported using mtDNA analysis in casework (laboratories 1, 2, 5, 7, 8, 10, 11 and 12). Two other laboratories (laboratories 3 and 4) reported not using mtDNA routinely in casework, and no information was provided by a further two labs. Most of the laboratories using mtDNA analysis in casework did not include screening procedures for analysing mtDNA. Three laboratories used screening procedures; laboratory 1 used single stranded conformational polymorphism analysis (SSCP) or SSCP-RE (SSCP of restriction enzymes fragments; [3]). Laboratory 2 used a minisequencing approach [9]. Laboratory 4 reported using Amplification Created Restriction Site [6] in a single case. MtDNA typing reliability and efficiency are primarily dependent on the quality of the DNA extract and quality control measures are used to limit external contamination. Methods to detect heteroplasmy, some aspects of the nomenclature and statistics may need further discussion and refinement. The successful result of this exercise using three bloodstains shows that PCR based mtDNA typing by automated sequencing is a valid, robust and reliable means of forensic identification despite the different strategies and methodologies used by the different laboratories.

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