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Mitochondrial DNA pseudogenes in the nuclear genome as possible sources of contamination

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Abstract. The discovery in the nuclear DNA of sequences homologous to mitochondrial DNA raises the possibility that accidental amplifications may occur when performing PCR analyses. This issue gains importance in forensics where mtDNA is commonly used for identification purposes. We analysed 19 NUMTs that encompass the D-loop, and verified that it is unlikely that the primers usually used in forensics for HVR amplification accidentally anneal to the NUMT sequences. We also performed PCR analysis with primers specifically designed for mtDNA and NUMT sequences with 97% homology to the coding region. No accidental amplification of the NUMT occurred. Moreover, the NUMT sequences only amplified with the highest annealing temperatures of the NUMT specific primers. We conclude that the high number of mtDNA molecules in the tissues allows its amplification to be ensured, even when the primers also anneal in NUMT sequences.

Keywords: Mitochondrial DNA; Nuclear mitochondrial insertion; Accidental amplification

1. Introduction

Shortly after the discovery of the mitochondrial genome, mitochondrial DNA-like sequences have been identified in the nuclear genome [1]. The presence of these nuclear mitochondrial insertions (NUMTs) may lead to accidental amplifications of nuclear fragments with primers specifically designed for mitochondrial DNA (mtDNA). Depending on the homology of each NUMT to the mtDNA, this problem may be more or less relevant. In this work, we focused on the NUMTs that may be a cause of contamination in forensic analyses.

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2. NUMT analysis

2.1. Study of annealing sites of primers used in forensic genetics

Following the report of the complete human genome sequence, various studies have been published describing and listing all NUMTs that exist in the different chromosomes. Out of the 247 NUMTs reported in one of these studies [1], we selected and analysed 19 that encompass the fragments of the D-loop that are usually used for forensic purposes, and identified the homologies to the primer annealing zones (Table 1).

We observed that none of the primers used for amplifying the Hypervariable Regions (HVRs) I and II in forensic studies [2] anneals completely in any NUMT. The highest homology was observed in three NUMTs (6, 11 and 17), where the annealing sites of both forward (AF) and reverse (BR) HVRI primers present one point substitution. We suggest that an accidental amplification of one of these NUMTs with the HVRI and HVRII primers is very unlikely to occur.

However, forensic and anthropological studies have been focusing more and more on the coding region, and much information is now obtained by using SNaPshot multiplexes or sequencing of various fragments outside the D-loop. The longest and most similar NUMT from Mishmar's study, with 97% homology to the region between 3914 and 9755 np of the Cambridge Reference Sequence (CRS), encompasses a target region for several analyses performed by forensic researchers. This high homology enables 11 primers used in a SNaPshot multiplex for mtDNA typing [3] to anneal perfectly to this NUMT.

Table 1
NUMTs analysed and its similarity to the hypervariable regions and primer annealing sites

Number	Accession number	% Similarity	Region similarity in CRS	Homology region	Subs/Del/Ins in annealing site			
					AF	AR	BF	BR
1	AL590396.13	72	12156–16563	HVRI	5	9	12	2
2	AC098817.3	71	11592–16567	HVRI	3	7	13	6
3	NT_005445.2	71	16434–6431	HVRII	6	6	12	14
4	AC013437.8	73	9840–16563	HVRI	2	8	14	2
5	AC097648.2	77	110–426	HVRII	–	10	8	9
6	AC097648.2	78	13029–16569	HVRI	1	9	10	1
7	AC002087.1	71	14164–16569	HVRI	5	8	13	6
8	AC099654.5	69	15755–16569	HVRI	2	9	9	3
9	AC099654.5	74	3819–16563	HVRI	3	10	8	6
10	AC022861.4	71	13552–16569	HVRI	2	8	11	4
11	AF227907.1	80	14360–16569	HVRI	1	8	9	1
12	AF227907.1	82	1–11116	HVRII	1	3	10	4
13	AP000676.5	66	15864–16475	HVRI	2	9	11	3
14	AL138955.14	71	13327–16560	HVRI	2	8	11	4
15	AC009140.6	72	13930–16562	HVRI	6	11	12	3
16	AC107940.6	82	11116–1	HVRII	1	3	10	4
17	AC107940.6	76	16569–15920	HVRI	1	8	9	1
18	AL391375.11	73	14685–16569	HVRI	3	9	12	4
19	AC025731.12	69	14422–16562	HVRI	4	10	15	4

Information about the NUMTs was obtained from Mishmar et al. [1], primers named according to Wilson et al. [2].

2.2. PCR analysis

In order to establish whether this is an issue that forensic investigators must take into account when studying mtDNA, we performed PCR with primers specifically designed for NUMT sequences that may be a source of accidental amplifications and compared the results to the ones obtained for mtDNA-targeted primers. The two sets of primers contained 2 different bases in both forward and reverse sequences but similar annealing temperature. We distinguished the NUMT from mtDNA by 2 substitutions on the amplified fragment. This analysis was made on samples from different tissues, such as blood, hair and buccal swabs, and in individuals belonging to different haplogroups.

After sequencing of the amplified fragments, we observed that both pairs of primers were amplifying mtDNA, and not the NUMT. We therefore decided to increase the primer specificity by rising the annealing temperature from 53 to 60 °C. The sequences obtained by amplification with the NUMT specific primers resulted in varying levels of mitochondrial and NUMT sequences. Most of the buccal swabs and blood samples presented both the sequences, although some of the hair samples presented only NUMT or mitochondrial sequences. Raising the primer annealing temperature to 62 °C almost all the sequences obtained were pure NUMT DNA, whereas the ones amplified using mtDNA specific primers remained pure mtDNA.

We also performed a PCR using primers that annealed perfectly to both NUMT and mtDNA. This reaction produced clean mtDNA sequences both at the highest and lowest annealing temperatures.

3. Conclusions

The analysis of the NUMTs that encompass the HVRs showed that the accidental amplification of the NUMT with the primers most commonly used in forensics is unlikely to occur. The PCR analysis allowed us to conclude that, even using primers specifically targeted to one of the most homologous NUMTs to mtDNA, mitochondrial sequences are predominantly obtained. This is probably due to the higher number of mtDNA molecules in the tissues when compared to nDNA. No accidental amplification of the NUMT occurred with primers that anneal in both kinds of sequences.

We therefore conclude that contamination of mtDNA with NUMT sequences in forensic analysis is not a worrying issue when using common routine samples and techniques.

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