ROUTINE MITOCHONDRIAL DNA IDENTIFICATION

Tesson C., Penaud A., LE ROUX M.G., Millasseau A., Guibert V., Moisan J.P., Pascal O.
Laboratoire de Génétique Moléculaire . Hotel Dieu BP 1005 44035 Nantes Cedex France

DNA typing is a common technique in Forensic sciences used for the characterisation of body fluids. The analysis of genomic DNA may be limited if DNA is highly degraded or in low abundance (rootless hair and bones for example). On the other hand some biological tools (such as anti sera) could be used to exclude human origin but few could easily establish the animal origin. Mitochondrial DNA could be used to overcome these problems. Indeed mitochondrial DNA is composed of different areas, two of which are particulary interesting : the first one encodes the cytochrome b gene and show interspecies mutations. Amplification and sequencing of this region allows us to determine the animal origin (this technique is licenced from BioID compagny - St John's Canada). The second one encodes hypervariable regions with point mutations differing from one individual to another. We make routine use of these two techniques to resolve Forensic caseworks.

Materials and Methods

A/ DNA extraction

DNA extraction is performed routinely for caseworks applying techniques as previously described (Pascal 1991) except for hairs and bones. Hairs are dissolved by incubation in the presence of proteinase K and DTT followed by phenol chloroform purification, and bones using Guanidium thyiocyanate (Hoss 1993).

B/ Amplification:

*Animal identification : the primers used are those described by Bartlett (1992). In case of highly degraded DNA, shorter sequences are amplified.

*Mitochondrial Hypervariable regions :

the primers used are :

From 15 to 270 :	CAC CCT ATT AAC CAC TCA CG
	TGT GTG GAA AGT GGC TGT GC
from145 to 389	CTC ATC CTA TTA TTT ATC GC
	CTG GTT AGG CTG GTG TTA GG
From 1591 to 16258	TTA ACT CCA CCA TTA GCA CC
	TGG CTT TGG AGT TGC AGT TG
From 16140 to 16420	TAC TTG ACC ACC TGT AGT AC
	TGA TTT CAC GGA GGA TGG TG

38 cycles are performed.
Sequencing is performed with Sequenase^{R} (Amersham). Fragments are analysed either manually or in an IBI automatic sequencer.
C/ Interpretation :
Animal identification : the sequences are analysed by a specific comparison program by BioID in St John (Canada).
Human hypervariable regions : the sequences obtained are compared to the reference sequence (Anderson 1981) and the mutation frequencies used are those published by Piercy et al (1993).

RESULTS

Animal identification

Animal identification is a powerful tool used to ascertain the origin of biological samples, and may also be used to confirm or to disregard witness statements. We used, for example, this technique for a murder where four people of the same family were killed. An animal food bag, a bowl and a knife all containing blood stains, were found in the house of the suspect . The grandmother said that she had killed a mouse in the bag. Analysis of the DNA showed that the bloodstains found on the bag were from "mus domesticus" (mouse), the bloodstains from bowl were from "Gallus gallus" (chicken) and the bloodstains from the knife were from "Sus scrofa" (pig).

Human identification

In the past DNA, extracted from bones or from rootless hair have been very difficult to type. We currently use mitochondrial DNA for exclusion or identification.
Two main problems have to be considered :
-Contaminations
DNA is present in every nook of a molecular biology laboratory and we have to avoid the contamination of the sample by exogenous DNA and amplification products. Extractions from bones and hairs were performed in a separate room, using specific material set aside for these techniques (centrifuge, automatic pipettes, and so on). Preparation of the samples for PCR amplification is performed in a second room and sequencing in a third one. The cycle number is limited to 38. Negative extraction samples and negative amplification samples are included in each experiment. If contamination is seen in the negative samples, then we also sequence these PCR products. A single technician is appointed to Mitochondrial DNA typing and does not use other techniques.

INTERPRETATION:

Two major questions are in discussion:

a/ The fidelity of amplification : we use Sequenase^{R} sequencing kit, which is supposed to be the most faithful enzyme.

b/ The neomutation rate : which seems to be very low from one generation to the other. However no extensive work has been carried out which could permit us to confirm this assumption.

Therefore in order to compare (exclude or include) two samples from different origins we require at least three point mutations. In the case of identity, error risks are calculated using the mutation rate published by Piercy et al. Absence of point mutations are included in these calculations.

CONCLUSION

Mitochondrial DNA analysis is a very powerful technique which permit the tools of molecular biology to be used for legal purposes. Recently these techniques has been used successfully to identify Romanov family remains (Gill 1994 - Stoneking 1995). However these techniques are very long and a high degree of safety must be exercised in order to exclude contaminations. Personnel and materials must be perfectly adapted. Finally, the interpretation of results must be carried out carefully with regards to the fidelity of amplification and the neomutation rate.

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