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Species identification by cytochrome *b* gene: Casework samples

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Abstract. This study presents species identification of biological material of unknown origin by sequence analysis of the cytochrome *b* gene. This method has been applied to our forensic casework samples involved in criminal investigations, where the forensic evidence was important to solve the cases. © 2006 Published by Elsevier B.V.

Keywords: Forensic casework; Mitochondrial DNA; Cytochrome *b* gene; Species identification

1. Introduction

In routine casework (paternity tests, criminal cases and human remains identification), sometimes it is necessary to identify the exact species of the biological material. Conventional methods and immunological procedures have several drawbacks; they are mostly used for the general discrimination between human and non-human samples. In particular cases, the detailed information of the species origin assumes a primary significance in a legal investigation.

Recently, the study of Parson [1] proposed the identification of vertebrate species by nucleotide sequence analysis of the cytochrome *b* (cytb) gene. The cytb gene is one of the 37 genes within the circular mitochondrial genome [2]. This gene is ideal for species identification as it shows limited variability within and much greater variation between species.

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The above-mentioned study shows the applicability of the cytb gene as a method of species identification in the forensic field. In our cases reports, proceeding from our routine casework, the method has been applied.

2. Material and methods

2.1. DNA extraction

DNA was extracted using the organic method and negative controls were included.

2.2. Amplification and purification of the PCR product

Ctyb was amplified under the conditions described by Parson [1]. The PCR was carried out in a thermocycler GeneAmp® PCR System 9700 (Applied Biosystems). The success of the PCR reaction was visualized using 1.5% agarose gel electrophoresis, stained with ethidium bromide, under UV light. PCR products were purified with MinElute™ PCR Purification Kit (Qiagen) according to the manufacturer protocol. Negative controls of DNA extraction and negative PCR control were subjected to amplification. All samples were also amplified using the AmpF*l*STR® Identifiler™ PCR Amplification Kit (Applied Biosystems).

2.3. Sequencing

PCR product was cycle sequenced using the PCR primers separately, in a cycle sequencing reaction using BygDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems), and removal of unincorporated dye terminators was accomplished by DyeEx™ 2.0 Spin Kit (Qiagen), all according to the manufacturer recommendations. Each strand was sequenced using the same primers employed for PCR. Electrophoretic separation and detection of the sequencing reaction products were performed using an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems).

2.4. Sequence similarity

After sequence analysis, all the obtained sequences were confirmed and aligned manually to identify species, using the online BLAST search engine of the National Center for Biotechnology Information (NCBI) [3]. Sequences where a similarity of the query is found are displayed according to the degree of sequence match.

3. Results and discussion

In the studied samples, the amplification of the STR loci failed. Sequence data of a ~ 358 bp fragments of the cytb gene were obtained. The sequences were compared to those in the database of GenBank and the maximum scoring segment pair was found. The information content within the nucleotide sequence of the cytb gene enabled the identification of the biological material (at species level), in the two cases.

3.1. Case report 1

The first case reports the missing body of an 8-year-old girl. The police search leads the investigation to a homicide case, with cadaver concealment. The alleged perpetrators, according to the thesis of the investigation, might have thrown the body in a pigsty. The detailed inspection of the

pigsty showed tiny fragments with high contents of calcium and phosphorus inducing the human origin (bones) of the material.

As the STR analysis performed failed, the evidence samples were analysed as described above. The obtained nucleotide sequence was submitted to the BLAST search, based on the cytb sequences present in the GenBank databases. The result of the search provided a list of database entries which are sorted by decreasing sequence similarity to the submitted sequence. The cytb sequence of *Sus scrofa* (pig) was 100% identical to our sequences.

This result ruled out the suspicion, based on the collected evidences, that the girl might have been eaten by the pigs.

3.2. Case report 2

The second case reports the finding of a piece of intestine in a forest. The anatomic study could not determine the origin of the biological material. Our laboratory was asked to continue the identification process.

The material was studied as previously reported. The STR analysis performed was negative. The sequence was submitted to the BLAST search. The cytb sequence of *Sus scrofa* (pig) was 100% identical to our sequence.

This result excludes the hypothesis of the probable human origin of the intestine.

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