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Species identification of animal specimens by cytochrome b gene

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Abstract

Five animal specimens of two cases suspected to be part of illegal trades in wildlife products were from the Council of Agriculture (COA) for species identification. There were two furs, one penis, one testis and one section of an organ. The specimens were stale, but PCR amplifications of mitochondrial cytochrome b gene were successful following our methods. After PCR amplification and sequencing of the cytochrome b gene, the sequences were compared with those registered in the DNA bank. The results showed that the similarities of all specimens were not less than 99.7 %. Therefore, the results of species identification for these animal specimens were definite and reliable. The sources of fur-1 and fur-2 were cats (*Felis catus*); and penis, testis and the section of an organ were from cattle (*Bos taurus*). All specimens tested were not from the conservation animals.

Keywords: species identification, cytochrome b gene, forensic science

Introduction

Certain species of animals are protected under the Convention of International Trade in Endangered Species (CITES), and any trading of these species is prohibited or under strict control. In Taiwan, the Wildlife Conservation Act was legislated for animal conservation. It's essential to identify the protected conservation animals unambiguously. On occasion, a dispute or deception regarding processed products of animals arises between consumer and seller over content thought to be unrepresentative of the advertised product. Therefore, species identification of animal specimens is important for forensic science.

DNA tests are the most popular methods for species identification of animal specimens at present. In our laboratory, the system for animal species identification was established based on DNA sequencing of mitochondrial cytochrome b gene [1]. This method was used to successfully identify the origin of rhinoceros horns [2], endangered turtles [3], shahtoosh [4] and meat products [5]. In this report, the system was adopted to identify the species of animal specimens from the Council of Agriculture (COA) for two cases suspected to be illegal trading in wildlife products.

Materials and methods

Case reports

From 2006 to 2007, a total of five animal specimens of two cases were from COA for species identification.

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These specimens included two furs (fur-1 and fur-2), one penis, one testis and one section of an organ. They were stale and some had developed molds on their surfaces.

DNA extraction

All specimens were processed by washing and drying before DNA extraction. For DNA extraction, approximately 4 cm^2 of each fur sample and 50 mg of the other samples was collected respectively. The collected samples were cut into small pieces or pulverized and DNA was extracted by the modified salt/chloroform method [3, 4]. The resulting DNA was dissolved in 40 µl of ddH_2O.

PCR amplification and DNA sequencing

Initially, DNA from all specimens was amplified with the primer pair L14724/H15149. These primers were designed according to the report of Irwin DM et al. [6] and if the products of the first PCR amplification were not enough for DNA sequencing, the nested PCR amplification was performed. The primer pair L14908/H15119rab were used in the secondary amplification. These primers were designed according to our previous study (unpublished data). Sequences of all the primers are listed in Table 1. PCR amplifications were performed in a reaction mixture of 50 µl, which contained 10 µl of isolated genomic DNA or 5 µl of the first PCR products, 0.15 µM each of primers, reaction buffer (10 mM Tris-HCl, pH 8.3, 2.5 mM $MgCl_2$, 50 mM KCl, 0.01%(w/v) gelatin), 200 µM dNTP, and 1 unit of VioTaq DNA polymerase (Viogene, Taipei, Taiwan). Both of the PCR amplifications were performed at 94 ^oC for 5 minutes and for 35 cycles according to the program of 94 ^oC for 45 seconds, 50 ^oC for 45 seconds and 72 ^oC for 1 minute, and extensions at 72 ^oC for 30 minutes in a thermal cycler (GeneAmp PCR System 2400, Applied Biosystems, Foster, CA, USA).

Sequencing of the PCR products was performed using both the forward and reverse primers of PCR amplification and the BigDye Terminator kit (ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit). The cycle sequencing products were separated with a POP-7™ polymer (Applied Biosystems) and detected by an ABI 3730 DNA Analyzer.

Sequence analysis

Sequences were compared with those registered in the EMBL databank by the Fasta program accessed through the website: www.ebi.ac.uk/fasta33/nucleotide.html

Results and discussion

After the first PCR amplification there were four specimens with the predicted size products and enough DNA for further sequencing. These specimens were fur-2, penis, testis and the section of an organ. The other specimen, fur-1, also contained the predicted size products and enough DNA for sequencing after the secondary PCR amplification. All the PCR products were successfully sequenced and the sequences were compared with those registered in EMBL databank. The results are showed in Table 2. With the exception of the fur-2 specimen, all others displayed 100.0 % similarity with the cytochrome b gene of the species with the highest homology. The sequence of fur-2 displayed 99.7 % similarity with the cytochrome b gene of *Felis catus*,

Table 1 Sequences of primer pairs and their predicted size of amplification products

	Primers*	Sequences	Size
First PCR	L14724 H15149	5’ -CGAAGCTTGATATGAAAAACCATCGTTG-3’ 5-AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3’	486bp
Secondary PCR	L14908 H15119rab	5’-GGCCTATTCTTAGCCATACAC -3’ 5’-TAACCCATAAATGCTGTGGC -3’	251bp

***** Numbering is according to the human mtDNA sequence [7].

Table 2 The similarity and species with the highest homology compared with DNA sequences registered in EMBL database

Specimen	Species with the highest homology (accession number)	Query coverage	Similarity
Fur-1	*Felis catus* (AB194812)	100.0 %	100.0 %
Fur-2	*Felis catus* (AB004237)	100.0 %	99.7 %
Penis	*Bos Taurus* (AY676858)	100.0 %	100.0 %
Testis	*Bos Taurus* (DQ124387)	100.0 %	100.0 %
Section of organ	*Bos Taurus* (AY676858)	100.0 %	100.0 %

which is the most similar species. The similarities of all five specimens were not less than 99.7 %. Therefore, the results of species identification for these animal specimens obtained by this method were definite and reliable. The sources of fur-1 and fur-2 were cats (*Felis catus*); the penis, testis and the section of an organ were from cattle (*Bos taurus*). All the specimens were not removed from the conservation animals .

The results of this report identified the species of the suspicious animal specimens and provided proofs for judgment in the investigation of conservation and endangered species of law enforcement.

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