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Letter to the Editor

Identification of endangered petrel species from poor quality feather debris using cytochrome *b* sequences

Keywords: Petrel; Feather debris; Cloning; Cytochrome *b*

Dear Sir,

Identification of a specimen from a degraded biological sample is always a challenge for any forensic lab. Several methodologies have been put forward to deal with this problem most of them based in a careful DNA extraction method [1]. DNA recovered is most of the times contaminated with exogenous DNA of unknown origin [2]. Worst of all, most of the times, contaminant DNA is at much higher quantity than the sample's own DNA making it extremely difficult to quantify the relative DNA amount of different origins present in the sample [2]. We report here a case study where the main components present in a contaminated biological sample were identified. *Calonectris diomedea* is *a* highly protected petrel species from the archipelago of Madeira hunted almost to extinction. Feather debris suspected of belonging to *C. diomedea borealis* were found on board of a fisherman's boat and collected by the police for morphological identification. The analysis failed due to the fact that the debris was of extremely poor quality and the debris was sent to our lab for DNA identification. The debris material was composed of 5 mg bristles and two 35 mm long down feathers. The material was collected 18 months prior to the analysis. The debris was contaminated with organic material from other sources and had been handled by humans. We used a protocol that allowed us to successfully amplify a DNA fragment from the cytochrome *b* gene. Amplification products were found to be contaminated with fragments belonging to several exogenous biological sources. Bristles and down feathers were vigorously washed three times in sterile water. Any treatment to wash feathers and bristles with bleach or any other abrasive product could also affect the quantity of the presumably already low amount of petrel DNA present in the sample. DNA extraction was performed using Dneasy Tissue Kit (Qiagen). In order to capture all possible available DNA, we add 5 mg polyA carrier of carrier DNA at a concentration of 25 μg/ml (SIGMA) to the extraction buffer. Amplification of cytochrome *b* gene was performed in a 25 μl reaction volume using primers L14841 and H15149 described in Kocher et al. [3]. Each reaction contained 0.25× buffer, 2.5 mM $MgCl_2$, 0.3 mM of each dNTP, 0.25 mM of each primer and 1 U FirePol DNA Polymerase I (Solis Biodyne): 35 cycles of 30 s at 94 °C, 30 s at 52 °C, 30 s at 72 °C. PCR products were purified with 1 U shrimp alkaline phosphatase and 1 U exonuclease I (Amersham). Amplified products were cloned into pCR2.1 vector–TA Cloning Kit (Invitrogen) according to the manufacturer's protocol. Fifty-four clones from the amplification products of bristles and each of the two down feathers were selected for screening. Plasmids with inserts were directly sequenced with BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). MtDNA was successfully amplified from feathers and bristles but resulted in a collection of fragments of the cytochrome *b* gene, but clearly belonging to different species as confirmed by the output from direct sequencing of the amplification product. Therefore, we decided to clone the collection of amplified fragments and randomly analyse them. Each of the 54 clones was sequenced and all inserts were identified as a cytochrome *b* gene fragment of expected length and sequence. Only two types of inserts were recovered and all clones derived from either the same contaminant source (*Homo sapiens*, 30%) or the same petrel (*C. diomedea borealis*, 70%). The bird sequence was identical to GenBank accession numbers AJ004161–AJ004174. The human sequence was identical to the Cambridge Reference Sequence, with a variant at nucleotide 15154 (C → A). None of the contamination material were recovered in the clones analysed, probably been effectively eliminated in the simple wash step performed prior to DNA extraction. However, human DNA was the primary contamination source and the previous human manipulation of the debris with naked hands left a visible and strong trace that was not easily eliminated. Cloning amplified products turned out to be a satisfactory method to randomly separate possible sequences present in an amplification mixture. Obviously, this experiment does not allow us to guarantee if the number of clones recovered from both human and petrel correspond to the amount of DNA present in the debris or to the amount of sequences present in the amplification mixture.

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