2018

2018 - DNA Barcoding of Feather Samples for Bird Identification - Poster Presentation

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DNA Barcoding of Feather Samples for Bird Identification

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ABSTRACT

Feathers and down are degraded during manufacturing processes, however small quantities of DNA present in the feathers may remain. This project will investigate whether mitochondrial DNA can be purified, amplified, and sequenced from processed down and feather samples. The target genes for amplification using Polymerase Chain Reaction (PCR) are the 16s ribosomal small subunit (16s) and Cytochrome Oxidase I (COI). Amplified target DNA will be purified, sequenced, and analyzed to determine the identity of the feather's source.

We have extracted DNA from multiple processed feathers of varying types and sizes, as well as successfully amplified the COI and 16s regions. We were able to identify the source species of the samples through analysis of the amplified DNA sequences.

Our next task is to design an DNA assay to identify specific breeds of birds from feather samples.

OBJECTIVE

This project aims to produce a DNA Assay to determine the identity of the bird species present in the down and feathers used in manufactured products.

BACKGROUND

- Products with feather and down filling, such as down jackets, vary in price due to their softness or their ability to retain heat. Because these traits depend on the feather's origin, clients and manufacturers would want to know the identity of their source. The current method of species determination is visual inspection, though this method is subjective and limited to broad categories. A DNA based species identification test would yield more accurate results.
- Due to the degradation of the samples during processing, we will target mitochondrial DNA (mtDNA), which have high copy numbers compared to nuclear DNA. mtDNA is also unique due to its rapid change in sequences among various species, making its use in DNA barcoding widespread throughout various biological fields.
- The 16s and COI regions of mtDNA serve as genetic markers in DNA barcoding, as they are sufficiently variable among species and conserved interspecies. This allows for quick and easy differentiation and identification of various organisms.

METHODS

Samples

- Pre-washed and Fully-washed feather and down samples received from client.

DNA Purification

- DNA obtained through digestion and purification of feathers using Qiagen DNeasy Blood and Tissue Kit.
- Digestion of samples completed overnight.

Polymerase Chain Reaction (PCR)
Amplification of Target DNA Sequences

COI Forward Primer 5'-AACCTWGCCCAACGCY-3'
COI Reverse Primer 5'-RTGGTGGGCTCAKAC-3'

COI primers are degenerate. The COI gene is not identical in all birds. Each of the COI primers are a mix of 4 sequences, allowing amplification of the COI gene from different species.

"W" is A or T "Y" is C or T
"R" is A or G "K" is G or C

16s Forward Primer 5'-ATAAGACGAGAAGACCC-3'
16s Reverse Primer 5'-GATTGCGCTGTTATCCC-3'

Gel Electrophoresis
Confirm amplification of DNA

- Confirm amplification of target DNA by fragment size.
- SYBR Safe DNA Gel Stain was used instead of ethidium bromide. EtBr requires UV light for illumination, and UV light creates thymine dimers in DNA. Dimers would prevent DNA sequencing.

Gel Extraction

- The DNA bands are cut out of the agarose gel and purified using Zymoclean Gel DNA Recovery Kit. This removes excess primers, dNTPs, buffer components, and enzymes.

DNA Sequencing

- DNA is shipped to Sequetech in California to be sequenced using the Sanger Sequencing Method.

Data Analysis

- Species identification by comparing sample DNA sequences against the NCBI database using BLAST (Basic Local Alignment Search Tool.)

Results

- We extracted varying quantities of mtDNA from both pre-washed and fully-washed (processed) feather samples.
- The 16s and COI genes were successfully amplified in the purified DNA from fully-washed feathers. (Figure 1)
- After sequencing, we identified the sources of the feather samples through analysis using NCBI BLAST. (Figures 2 and 3.)

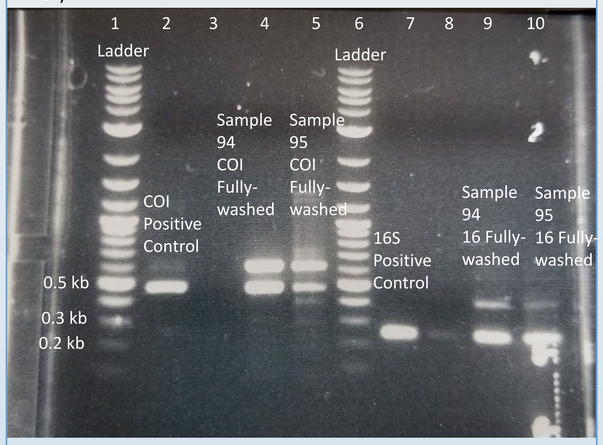

Figure 1. Agarose gel with amplified 16S and COI sections of samples. The COI amplified samples were found at about 0.5 kilobases (kb) while 16S amplified samples appear at around 0.22 kb. Lanes 3 and 8 contained negative controls for the feather samples. Bands from non-target regions appear above target DNA bands.

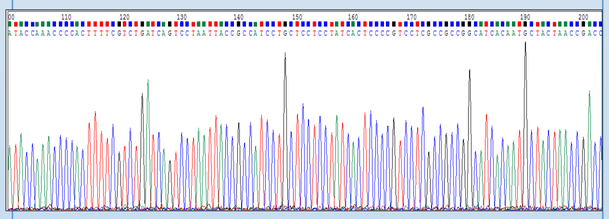

Figure 2. Chromatogram of Sample 94 COI Forward Primer Amplified DNA Sequence. Each peak depicts a marker indicating a certain nucleotide present at a given location in the sequence.

Sequencing Data

Strand	Chicken Meat (Control)	Fully-washed
16S Forward	*G. gallus*	*A. platyrynchos*
16S Reverse	*G. gallus*	*A. platyrynchos*
COI Reverse	*G. gallus*	*A. platyrynchos*
COI Reverse	*G. gallus*	*A. platyrynchos*

Figure 3. Table showing the results of sequence analysis via NCBI BLAST. Fully-washed feather sequences were compared in the NCBI database. Control samples were known to be *G. gallus* (Chicken.) Analysis of the feather sequences showed a 99% match to *A. platyrynchos* (Mallard Duck.)

Conclusions

It is possible to extract and purify DNA from processed feathers in suitable quantities and qualities which allowed for amplification and sequencing. We were able to determine the origin species of the samples by analyzing the sequencing data.

Current Work

We are designing an assay to determine bird breeds from pre-washed down and feather samples.

We have designed three primer sets to amplify the D-loop gene in the mitochondrial DNA of *A. platyrhyncos* into three fragments:

Fragment 1 Forward Primer 5'-ATAGTACGGCGAAGGATTC-3'
Fragment 1 Reverse Primer 5'-CATTCACTCTGGGTCCG-3'
Fragment 2 Forward Primer 5'-AACCAACTATCCTACATGCACG-3'
Fragment 2 Reverse Primer 5'-TAACTGAATGTGGTCAAAGTGC-3'
Fragment 3 Forward Primer 5'-CCTTCCCAGTGACTTCG-3'
Fragment 3 Reverse Primer 5'-GGATACTTGCATGTATATGTCTAGC-3'

While the Fragment 1 and 2 primers sets amplified two thirds of the D-loop gene, the Fragment 3 primer set did not amplify their target DNA sequences after multiple attempts.

However, current experiments show amplification of the D-loop gene in two halves when Fragment 1 Forward Primer is used with Fragment 2 Reverse Primer (1F2R) and Fragment 2 Forward Primer is used with Fragment 3 Reverse Primer (2F3R) (Figure 4.) We are currently preparing more samples for sequencing.

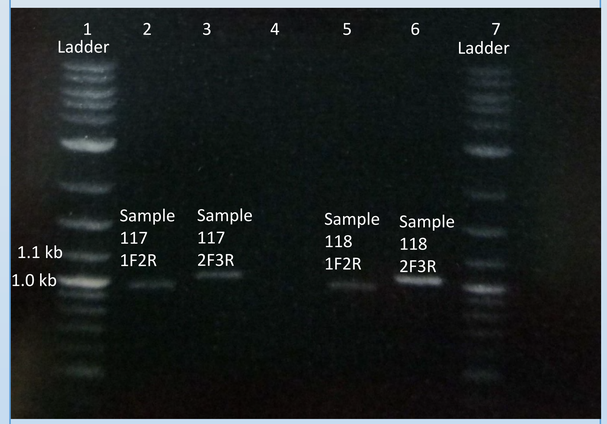

Figure 4. Amplification of the D-loop gene using two combinations of the three designed primer sets. Samples amplified with the 1F2R combination had a length of 1.0 kb while samples amplified with 2F3R had a length of 1.1 kb. Lane 4 is empty.

References

- Sarri, C., Stamatis, C., Sarafidou, T., Galara, I., Godosopoulos, V., Kolovos, M., . . . Mamuris, Z., (2014) A new set of 16s rRNA universal primers for identification of animal species. *Food Control*, 43(September), 35-41.
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