A33 A Comparison of Mitochondrial DNA Species Identification Techniques of Questioned Animal Samples

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After attending this presentation, attendees will understand several PCR-based methods available for determining the origin species of biological material and the advantages and disadvantages of each.

This presentation will impact the forensic science community by introducing a variety of established DNA-based methods that are used to identify the species of origin of tissues or other biological materials and describing extensive experiments for their comparison regarding successful PCR amplification, sequencing, and identification of their species of origin.

It is not uncommon for legal authorities or forensic practitioners to be confronted with biological material of unknown origin. From these, two questions arise: (1) "Is the material human?"; or, (2) "What is the species of origin?" The former can be key in missing persons cases when a search turns up biological material, while the latter is important when material clearly animal in origin, but otherwise undefined, is encountered.

Modern DNA methodologies have allowed molecular biologists to identify regions of the animal genome that are similar enough among species such that they can be successfully PCR amplified using universal primers, while still varying enough between primers so that a more precise origin can be determined, be it species, genus, etc. The region between primers is sequenced, and the resultant sequence undergoes a query using Basic Local Alignment Search Tool (BLAST: NIH's on-line database of DNA sequences). This database has so much information available that a clean sequence virtually always results in matches in the database that are specific to a species. In this way, the species of origin of biological material is confirmed.

Multiple laboratories have developed and published protocols for molecular species identification. Virtually all are based on mitochondrial DNA loci that readily fit the conserved primer/variable internal region criteria needed for the assay. The first of these came from Alan Wilson's molecular evolution laboratory at UC Berkeley, and many others have followed, including the Michigan State University laboratory. MtDNA loci utilized the range from the most highly conserved mtDNA genes to the least conserved loci. Each of these species identification loci has its potential advantages and drawbacks, including overall universality, ease of amplification, ease of sequencing, and specificity.

In the current study, all mtDNA-based methods for animal species identification available, including those used in house, were compared based on the above criteria. The list of loci examined, their numerical location based on the human mtDNA reference sequence, and amplicon length, are displayed in Table 1.

Primer	Region	Base Pair Length
12S	1071-1199	128
16S 1	2489-2716	227
16S 2	2676-3007	331
Cytochrome Oxidase I	5926-6609	683
Cytochrome b 1	14724-15915	1191
Cytochrome b 2	14816-15173	357
Control Region 1	15995-16498	503
Control Region 2	15908-16498	590
Control Region 3	15735-16498	763

Sixty-six species were examined, consisting of 27 mammals, ten fish, seven birds, five reptiles, three amphibians, nine insects, one centipede, one millipede, one spider, one earthworm, and one crustacean. Amplification was attempted on each based on the loci listed in Table 1, and graded as either positive, weak positive, or negative. From there, a subset of species that produced amplicons, consisting of one white tail deer, bobcat, lake white fish, frog, bearded dragon, English sparrow, house centipede, click beetle, pill bug, and earthworm, was reamplified and the same primers were used for forward and reverse sequencing. Sequences were aligned and edited, and a BLAST search was conducted. The sequences were then scored for BLAST accuracy (correct species identified) and uniqueness (sequence differed from other species).

The most useful loci in regards to amplification of the widest range of species included 12S, 16S 1 and 2, and cytochrome oxidase I, all of which amplified in a large majority of species. In contrast, cytochrome b 1 faired poorly overall, most likely due to the large size of the amplicon. Of the three control region loci, CR 3 amplified in the most diverse set of species, while the other two were effective in mammals and birds, but less so in the remainder.

The best generated sequence data overall was 16S 1 and cytochrome b2. BLAST search results of quality sequences were consistent with the known species, although the small size of the 12S and 16S 1 amplicons often resulted in complete matches with related organisms, thus precise species identification was not possible. In other instances, it is

likely that the explicit species, or locus from that species, was not in the database at all. For instance, there are over 900 species of click beetle in North America, yet only one showed up in the database for 16S 1, and none for cytochrome b 2, indicating the specific species studied was not represented. This is a clear limitation of the technique in general; however, species most likely to be investigated by forensic scientists tend to be well represented in the database. Overall, utilizing mtDNA for species identification is an effective and widely applicable tool, while the locus, primers, and amplicon sizes utilized require careful consideration.

Species ID, Mitochondrial DNA, DNA Species ID

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