B40 Species Identification of Degraded Bone Fragments Using the 12S rRNA Gene

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After viewing this presentation, attendees will be presented with an assay that allows for the reliable species identification of degraded bone fragments using the 12S rRNA gene located in the mitochondrial genome.

This presentation will impact the forensic community by demonstrating a protocol for the species identification of small skeletal fragments to aid in missing person investigations.

In many missing persons cases, such as those regularly undertaken at the Armed Forces DNA Identification Laboratory (AFDIL), small skeletal fragments of unknown and potentially non-human origin are sometimes encountered. When initial DNA testing of these fragments is unsuccessful, it is important to determine if the amplification failed because the fragment is simply too degraded or the fragment is from a non-human source. In order to address the question, a set of universal PCR primers targeting a 307-base pair region of the mitochondrial cytochrome *b* gene[1,2] was recently tested and validated at AFDIL. However, when the assay was implemented in routine practice, it showed limited success due to the large amplicon size. As a result, an assay targeting a smaller fragment was necessary.

Published[3] vertebrate primers targeting a short (140 bp) yet variable portion of the mitochondrial 12S rRNA gene were further investigated for species identification. The amplification was optimized for aged, degraded skeletal remains and then tested under these conditions for sensitivity, mixture detection and effectiveness on degraded animal bones. The optimized assay yielded visible amplification products from as little as 10pg of input DNA on agarose gels, and produced sequencing results from approximately 1pg of input DNA. Experiments evaluating various mixture ratios proved the optimized protocol to be sensitive to minor components present at approximately 10% of the total input DNA quantity. Finally, DNAs results from a variety of known vertebrate species were successfully tested to confirm that the small amplicon provided sufficient information to classify the samples.

To complete the developmental validation of the assay, the 12S protocol was used to evaluate 20 bone samples submitted blindly by the Central Identification Laboratory (CIL). Initial testing correctly identified 13 samples (65%) at the species level and classified an additional 3 samples were correctly classified as non-human. Only 20% of the samples either failed to amplify or produced molecular data that differed significantly from the anthropological classifications. New bone fragments were submitted by CIL in order to re-evaluate for any the failed or and non-concordant samples for additional testing samples. Results from the subsequent testing produced informative data for these remaining 20%. In total, proved the 12S assay proved to be informative for all of the 20 samples. Successful identifications were obtained for 95% of the samples. The sequence data from the remaining unidentified skeletal element was a mixture between the authentic dog DNA and contaminant human DNA and a single inconclusive classification. In comparison, the validated cyt *b* protocol was less successful, with the majority (60%) of the same samples either failing to amplify or providing inconclusive results.

Through a variety of tests, the 140-base pair 12S mtDNA protocol optimized at AFDIL has been shown to be a robust and reliable assay that will be helpful in verifying human versus non-human origin of aged, degraded skeletal fragments.

The opinions and assertions contained herein are solely those of the authors and are not to be construed as official or as views of the United States Department of Defense or the United States Department of the Army.

References:

1 Parson W, Pegoraro K, Niederstätter H, Föger M, Steinlechner M. Species identification by means of the cytochrome b gene. Int J Legal Med. 2000 Dec; 114(1-2): 23-28.

2 Irwin D, Kocher T, Wilson A. Evolution of the cytochrome *b* gene in mammals. J Mol Evol. 1991; 32: 128-144.

3 Balitzki-Korte B, Anslinger K, Bartsch C, Rolf B. Species identification by means of pyrosequencing the mitochondrial 12S rRNA gene. Int J Legal Med. 2005 Sep; 119(5): 291-4.

Mitochondrial DNA, Species Identification, Degraded

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