B94 Identification of a Cooked Meat Sample By 12SrRNA Sequence Analysis

Varsha Srivastava, MSc, and Gudalore Venkatesh Rao, PhD, Centre for DNA Fingerprinting and Diagnostics, 7-18, E&F, ECIL Road, Nacharam, Hyderabad, AP, India*

Attendees will learn how the sequence of 12SrRNA gene adapted in this study proved to be usable for animal identification.

The trade in endangered animals requiring conservation is a problem in many parts of the world, particularly in Asia. Forensic science laboratories frequently encounter tissue or meat samples of wild animals lacking any morphological detail for identification purposes.

Identification methods such as blood grouping and biochemical polymorphism have proved useful but the discriminating power of these techniques is less than that of DNA markers. The fact that primers of broad utility could be found for the fast evolving mitochondrial DNA makes it likely that **universal primer** can also be designed for identification purposes.

Sequence information and comparison of the rRNA gene can be used as a tool in identification and classification of organisms. The maternal origin of mitochondrial markers makes this method preferable for analysis. Additionally, heteroplasmy does not exist in most of the organisms. As the copy number of mt-DNA is high and the test involves PCR amplification, only a few cells are required to carry out the analysis.

In the present study the knowledge regarding the sequence variation in ribosomal RNA gene was used and an attempt made to identify a meat sample.

A partial DNA sequence of l2SrRNA was used to identify the cooked meat. Genomic DNA was extracted from the tissue and blood samples using standard Phenol:Chloroform extraction procedures were followed in the laboratory. The samples were kept overnight and DNA was purified by precipitation with salt and washing with alcohol. The control species used included such domestic animals as cats, dogs, cattle, pigs, and humans. The universal primer of 12SrRNA region was used to amplify the sequence from the animal species. The sequence of the forward primer to amplify 12SrRNA region was: AAAAA GCTTC AAACT GGGATTAGATACCCCACTAT. The sequence of the reverse primer for the same region was: TGACT GCAGA GGGTG ACGGG CGGTG TGT. The MJ Research Thermal Cycler was used to amplify the samples. The conditions for amplification were: Initial denaturation at 95°C for 2 minutes. Denaturation at 95°C for 1 minute. Annealing at 58°C for I minute. Extension at 72°C for 1 minute. Number of total cycles was 30. Final extension was at 72°C for 10 minutes.

The PCR produced a single amplification product for each genomic template. The size of all PCR products tested showed no obvious differences when separated on a 2% Agarose gel with size being approximately 386 bp. Products were purified by treating the samples with shrimp alkaline phosphatase; exonuclease sequencing was performed with forward primer and Big Dye Terminator; and, cycle sequencing products were purified by salt precipitation, followed by three washes with 70 percent alcohol, and, final wash with absolute alcohol. Products were dissolved in 4.5 microlitre of sequencing dye. Finally, products were separated on 5 percent denatured polyacrylamide and were detected using a PE Applied Biosystems 377 DNA Sequencer.

Blast Software aligned the sequences. Identity of the meat sample with that of known sequence of 12SrRNA gene of s*us scrofa* was found to be 97%. The Genus s*us* is the Latin word for “pig” and Species name *scrofa* is also Latin for “breeding sow.” The sequence of 12SrRNA gene adapted in this study proved to be usable for animal identification.

Finally, the case with which homologous sequences can be gathered will facilitate a synergism between molecular and evolutionary biology, which will lead to insight into genetic structures and phylogenetic history.

12SrRNA, Mitochondria, Blast

 ** Presenting Author*