B82 Pyrosequencing-Based DNA Analysis for Species Identification

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Learning Overview: After attending this presentation, attendees will be aware of the development of a quick and robust biological assay for species identification using a pyrosequencing technique.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing a novel assay to identify species that were previously classified as non-human miscellaneous samples, as well as providing a novel real-time DNA quantitation assay that is vertebrate-specific.

In crime scenes, unknown biological samples are often human in origin, but some of them belong to other animals. Identifying the source of a biological sample in a crime scene, as well as in wildlife forensic cases, can be critical during investigation. Yet, forensic scientists often lack appropriate standardized protocols to categorize a non-human sample. While previous studies have shown the presence of potential genetic markers to differentiate species, there is still a lack of a quick, easy, and distinct method for species identification from tissue or body fluids.[1,2] Law enforcement agencies, police officers, prosecutors, and defense attorneys expect forensic personnel to have the capability to identify miscellaneous body fluids and tissue. However, no system proposed to date achieves this end using fast, easy, and inexpensive protocols.

With the emergence of novel techniques for DNA sequencing, older, less-specific procedures, such as the use of polyclonal/monoclonal anti-sera, can be updated and replaced.[3] In this study, next generation sequencing methods will be used to analyze a short mitochondrial amplicon that is known to be hypervariable between different species of mammals, birds, and fish. This study will utilize pyrosequencing as well as demonstrating the application on a novel massively parallel sequencer. The resultant sequences can be compared to a large database of known references for species identification.

For this purpose, silica-coated magnetic-particle technology was used to extract DNA from buccal swabs and livestock samples, followed by the SYBR® green-based quantitation method using in-house designed vertebrate-specific primers. Amplification and sequencing of 12s rRNA was then performed on the PyroMark® Q48 Autoprep instrument. Preliminary sequencing data of 30 different species that are commonly found in a household were aligned and compared. Some samples are from mammals, such as *Canis lupus familiaris, Felis catus,* and *Oryctolagus cuniculus;* birds, such as *Gallus gallus* and *Psittaciformes;* and fish, such as *Salmo salar* and *Thunnini.* Results show inter-species variability but intra-species concordance, confirmed with curated databases of known sequences. The goal of this study will be to provide law enforcement with a novel method that can be implemented in forensic DNA labs for a routine and user-friendly confirmatory test for species identification.

Reference(s):

1. Balitzki-Korte, B., K. Anslinger, C. Bartsch, and B. Rolf. Species identification by means of pyrosequencing the mitochondrial 12S rRNA gene. *International Journal of Legal Medicine* 119, no. 5 (2005): 291-294.
2. Yang, Li, Zongqing Tan, Daren Wang, Ling Xue, Min-xin Guan, Taosheng Huang, and Ronghua Li. Species identification through mitochondrial rRNA genetic analysis. *Scientific Reports* 4 (2014): 4089.
3. Kang'ethe, Erastus K., Joseph M. Gathuma, and Kaare J. Lindqvist. Identification of the species of origin of fresh, cooked and canned meat and meat products using antisera to thermostable muscle antigens by Ouchterlony's double diffusion test. *Journal of the Science of Food and Agriculture* 37, no. 2 (1986): 157-164.

Species Identification, Pyrosequencing, Miscellaneous Samples