## B85 A Single Step Multiplex PCR to Identify Mammalian Species in the United Kingdom

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After attending this presentation, attendees will be introduced to a new technique, based on the cytochrome b gene of the mitochondrial genome, to determine mammalian species present from trace evidence without the need to sequence the products.

This presentation will impact the forensic community and/or humanity by allowing for the identification of evidence that could not pre- viously be analyzed by conventional means. In addition, the test will allow for the quick identification of species from highly degraded or powdered samples such a Traditional Chinese Medicine, will be able to identify the components of a mixture, and can also be expanded to include other animals such as those found on the CITES appendices.

Genes within the mitochondrial genome have many advantages for species testing when compared to those in the nuclear genome. The high copy number of the mitochondrial genome per cell relative to the one in the nucleus will allow for detection of mitochondrial DNA from trace biological materials. Mitochondrial DNA will also be able to survive degra- dation longer than nuclear DNA due to the strong protein membrane. These factors are important as frequently the sample to be tested is poor quality or may be as a powder with no morphological characteristic. The trade in Traditional Chinese Medicines is one such example where the sample to be analyzed will be pulverized bone or hair and conventional DNA testing may not be possible.

The further reason to use the mitochondrial genome is that genes on the mitochondrial genome have a higher evolutionary rate when compared to their counterparts in the nuclear genome and this increases the genetic variation. For a gene to be of value in species testing it must exhibit little intra-species variation and sufficient inter-species variation to permit dif- ferentiation of closely related species. The cytochrome *b* gene is one of the most commonly used genes used for both taxonomic purposes and species identification. Within the cytochrome *b* gene there are domains of highly conserved DNA sequences, which permit the design of universal primers. These universal primers will bind to any mammalian species known to date. In proximity to these highly conserved regions there are domains of sequence that show greater variability allowing for the design of species specific primers. The universal primer in conjunction with the species-spe- cific primer will produce a product of a particular size only if the species is present. Fluorescent dyes are attached to the universal primers to allow for designation of species with overlapping size. By attaching the dyes to the universal primers the cost of the test is reduced.

A multiplex has been designed for 15 mammalian species using uni- versal and species-specific primers. These include donkey, horse, sheep, goat, cow, pig, cat, human, rabbit, red deer, rat, guinea pig, dog, fox and hedgehog. For each species there are multiple species-specific markers leading to unambiguous identification. There is scope for the addition of many more mammalian species and with the expansion to other gene loci on the mitochondrial genome more markers will be introduced to the mul- tiplex reaction. The test has already been used in two criminal cases in the UK. In one case it was alleged that dog and human would be present on a sample taken from a victim of an alleged assault. Direct sequencing of the cytochrome b gene would have produced a mixture if both species were present, but the multiplex test was able to identify the presence of human but indicate that no dog was present. In a second case it was alleged that a male had illegally killed a red deer. Blood on the trousers of the accused male was tested and found to be a mixture of human and red deer sup- porting the allegation.

The ultimate goal of the test will be to identify the species present from any biological trace material for both common and protected species. The same approach can be used for the identification of CITES protected species.

**Species Identification, Cytochrome b, Mitochondrial DNA**

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