A Novel Method to Identify a Large Number of Mammalian Species In the UK From Trace Samples and Mixtures Without the Use of Sequencing

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INTRODUCTION:

Non-human biological evidence is encountered routinely in forensic casework. This evidence is often left unanalysed due to lack of a standard method. When analysis is undertaken it usually involves sequencing all or part of the mitochondrial genome, generally the 12S, 16S or cytochrome *b* gene, which is then compared to known sequences on GenBank. This technique is accepted, but has many problems including: being cost, time and labour intensive due to the extra step of sequencing products; mixtures cannot be separated; and degraded samples may not generate sufficient sequence data.

Therefore, ongoing research into the identification of non-human trace evidence is being undertaken throughout the forensic community to establish a quick, easy and cheap means to identify species.

Based on the variation in the amino acid sequences (Figure 1) it is possible to identify and locate areas where species specific primers may be developed (of high homology or variation). These primers will only react for the species for which they were designed. This would decrease the time and cost of the test by removing the sequencing step, allow separation of mixtures and allow identification of highly degraded samples since large fragments of intact DNA would not be required.

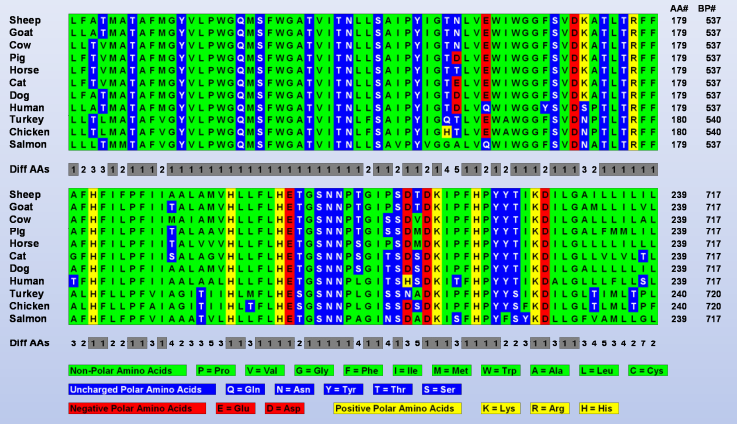


Figure 1: A section of the cyt*b* amino acid sequence. AA# is the amino acid number within cyt*b*; BP# is the equivalent base pair number within cyt*b*. Diff AAs is the different number of amino acids at each location.

MATERIALS AND METHODS:

Sequence Information and Primer Design

Cytochrome *b* sequence information were downloaded from the NCBI website and aligned using Clustal W (http://www.ebi.ac.uk/clustalw/). Universal primers were designed such that they will react with all mammalian species. Two of the universal primers used are modified versions of those given by Pääbo [1]. Species-specific primers were designed to only react with the species for which they were designed (three of which are from a previous study by Panvisavas [2]) and such that the size of the fragment could not be confused with any other fragments. Two species-specific reverse primers for each species was designed to react with one of three labelled universal forward primers. The species used in this study were: badger [*M. meles*], cat [*F. catus*], cow [*B. taurus*], dog [*C. familiaris*], donkey [*E. asinus*], fox [*V. vulpes*], goat [*C. hircus*], guinea pig [*C. porcellus*], harvest mouse [*M. minutus*], hedgehog [*E. europaeus*], horse [*E. caballus*], house mouse [*M. musculus*], human [*H. sapiens*], lamb [*O. aries*], pig [*S. scrofa*], rabbit [*O. cuniculus*], rat [*R. norvegicus*] and red deer [*C. elaphus*].

DNA Extraction

DNA was extracted from blood, tissue, hair, urine or buccal cells using the QIAamp® Micro Kit (Qiagen). Hair samples were allowed to digest in Proteinase K and DTT for up to 48 hours.

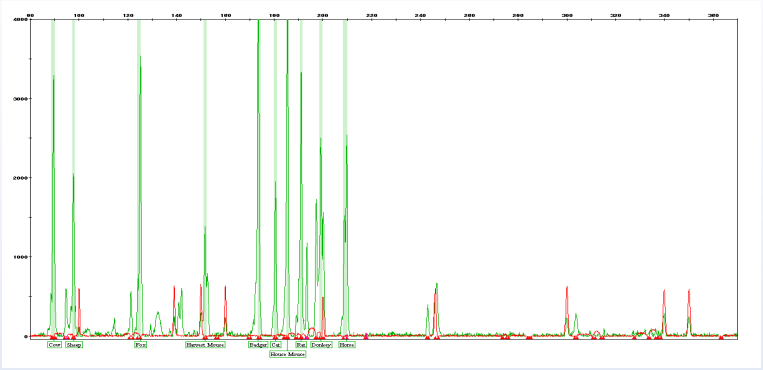
Multiplex PCR

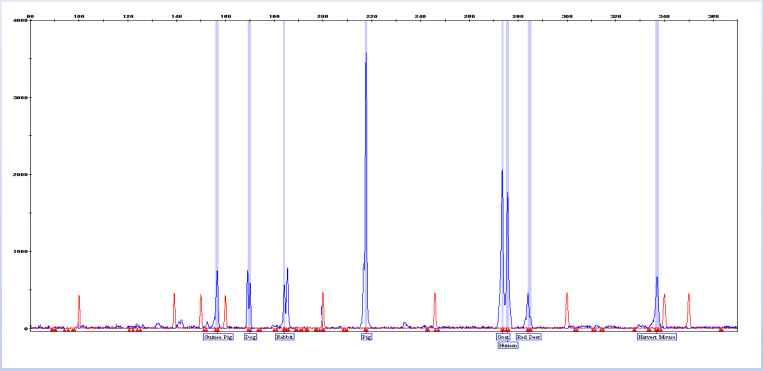
The primers were multiplexed together into one PCR. Final PCR volume was 20 µL containing: GeneAmp® 10X PCR Gold Buffer (100 mM TRIS-HCl, 15 mM MgCl2, pH 8.0), dNTPs (200 µM), 1.5 units AmpliTaq Gold® (Applied Biosystems, Foster City, CA), Universal and Species-specific primers (final concentrations ranging from 26.5 nM to 2.5 µM), sterile H_2O, and 40,000 copies of template DNA (equivalent to 680 fg). The PCR cycle proceeded for 35 cycles at 95 °C for 30 seconds, 60 °C for 30 seconds and 72 °C for 45 minute followed by a final extension step of 20 minutes at 72 °C.

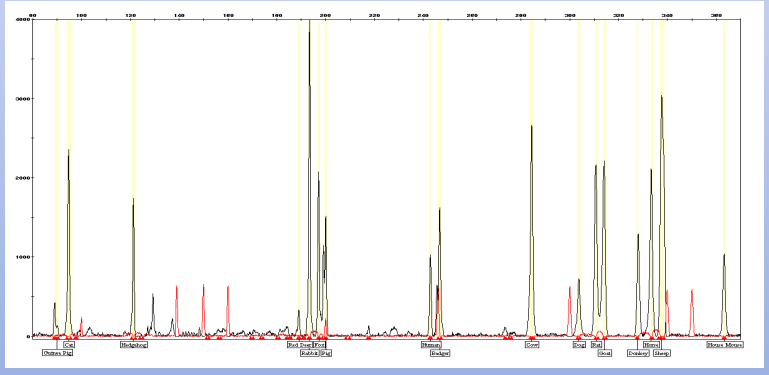
Validation and Creating a Ladder

The multiplex was analysed for run and sample variation and also tested with 29 species not included in the test. For each species: One sample was analysed 10 times for run variation and several (from 2 – 14) different individuals were analysed from each species included in the multiplex. Based on the results a ladder was created to identify all peaks using GeneMapper v3.2 (Applied Biosystems, Foster City, CA).

RESULTS:







CONCLUSIONS:

Primers reacted with the animal for which they were designed resulting in peaks of expected size (in bp) that were easily distinguishable from the other species tested. Similarly sized peaks such as goat and human (in blue) can be easily distinguished even in the case of a mixture. The ladder identifies all peaks making analysis quick and easy and the test has proven sensitive to 17 fg of template DNA.

The test is simple, fast, cheap and the results are easily interpreted making the test ideal for use in forensic casework. Potentials include identification of: components in food, which may have religious implications; dog and fox mixtures (contrary to fox hunting laws in the UK); dog or cat hair used in fur coats; and any other mammalian components in mixtures. The test also has the potential for more species to be added at a later time, including CITIES listed species, and expansion to include other genes on the mitochondrial genome.

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REFERENCES:

[1] Pääbo, S., Gifford, J.A. and Wilson, A.C.,Nucleic Acids Research, 1988. 16(20): p. 9775-9787.

[2] Panvisavas, N., Species testing by DNA analysis, 2004, Strathclyde University: Glasgow.