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IF NOT HUMAN, THEN WHAT? SPECIES IDENTIFICATION BY PCR OF THE CYTOCHROME B REGION

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The PCR technique can be used to detect the presence of non-primate DNA in a sample; this has forensic application when the source of a biological sample is in question.

It has been shown that the conserved region of the mitochondrially coded cytochrome b gene can be amplified in over a hundred different species using a standard set of primers [1]. In this study we use a common forward primer, based on the known DNA sequence of the mitochondrial cytochrome b gene, and variable length reverse primers (FAM ™ dye labeled) to amplify DNA from six different domestic animal species. Resolution of the labeled PCR products on an ABI 3130-xl ® Genetic Analyser shows fragments of specific lengths, corresponding to each of the different species targeted. The fragment sizes are 154, 223, 271, 330, 394 and 433 bp for goat, bird, cattle, sheep, pig and horse DNA respectively.

The robustness of this technique was examined using 50 different samples of DNA for each of the different species tested. We included different breeds of the same species, which also gave unambiguous results. Only sheep primers cross-react with goat DNA. Therefore, in the presence of goat DNA two fragments of different sizes are produced, being 154 and 330 bp. None of the primers cross-react with DNA from humans, cats, dogs and alpacas. Overall, the sensitivity of these tests varied from 1 ng of template DNA for cattle down to 10 pg of DNA for goats and birds. We have successfully combined these primers into a highly efficient multiplex format. Combining the primers into a single reaction enables the rapid and accurate identification of goat, bird, cattle, sheep, pig and horse DNA in a sample or the identification of mixtures of these species, down to a ratio of 100:1.

Results of the study show that it is possible to reliably discriminate between each of the species tested using this technique, based on the variability in lengths of PCR products generated. The identification of the species of origin of a sample can serve as a guide to identifying the appropriate panel of STR markers to use for further individualization, especially in situations in which biological evidence from an animal may be linked to a crime scene. The identification of mixtures of these species may also be of significance in product contamination or substitution cases.

References

[1] Kocher, T.D. et al (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings National Academy of Sciences USA 86: 6196-6200.