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SPECIES IDENTIFICATION OF FELINE DNA BASED ON ANALYSIS OF CYTOCHROME *B*

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Abstract

A method for species identification of biological material from *Felis Catus* (the domestic cat) was developed in this study. The analysed primers flanked a cytochrome *b* fragment of mtDNA belonging to *Felis Catus* (NC_001700). Appropriate primers were selected based on their specificity (analysed using FastPCR software) and length of the PCR product obtained. Forward and reverse primers with the lowest homology to DNA of the other species studied in the laboratory were chosen for further analysis. Cross-reaction of the chosen primers gave no falsely positive results. A PCR product of 286 bp was obtained for feline DNA. The method developed in this study enables the potential content of feline components to be determined rapidly and conclusively.

Key words: cytochrome *B*, PCR, cat, species identification, mtDNA

Species identification is a routine analysis performed in laboratories. It is used in animal feed analysis to identify banned animal components. The method is also applied to analyse tissues of animals that fell victim to road accidents or provide illegal products used in folk medicine. The majority of species identification methods has been developed for farm animals (Bottero et al., 2003; Lahiff et al., 2001), while detection of material from domestic animals has undeservedly received less attention from scientists. The above reasons indicate the need for developing methods for species identification of domestic animals. The present paper discusses a method for species identification of cats based on mtDNA analysis.

Material and methods

Hair samples from four cats, blood samples from a dog, bovine, sheep, pig, hen, duck, goose, turkey, red deer, roe deer and goat, and meat samples from a walleye pollack and a sutchi catfish were investigated. DNA was isolated from meat and blood

using the Wizard kit (Promega) according to the manufacturer's protocol, and from hair using proteinase K. Primers (Table 1) flanking a fragment of the cytochrome *b* locus of the feline mtDNA genome (*Felis Catus* NC_001700), designed using Primer3 software, were chosen for the analysis (Rozen and Skaletsky, 1998).

Table 1. Sequence of primers designed for identification of feline mtDNA (*Felis Catus*)

Forward	Reverse	Amplified fragment	Product length (bp)
F1: 5'-ttacacgccaacggagcttcta-3'	R1: 5'-ctaggatggagagtactagggcta-3'	15281=>15935	665
F1: 5'-ttacacgccaacggagcttcta-3'	R2: 5'-tggatcggagaattgcgtatgcga-3'	15281=>15887	607
F3: 5'-atctcagccttagcaggagtacac-3'	R2: 5'-tggatcggagaattgcgtatgcga-3'	15602=>15887	286

Species specificity of the proposed primers was analysed using FastPCR software (Kalendar, 2009).

The primers selected for further analysis were used for PCR with a thermal programme of 95°C for 3 min and 28 × (94°C for 1 min, 62°C for 1 min, 72°C for 1 min) 72°C for 15 min. The optimum reaction mixture was: 1 × Buffer; dNTPmix – 0.6 mM; AmpliTaq Gold polymerase – 1.25 U; MgCl2 – 1.5 mM, each primer – 0.8 pmol/µl, DNA – 1.5 µl. Total volume of the reaction mixture was 25 µl.

Results

The results of in silico cross-reactions of the analysed primers with the DNA of animal species other than cats are presented in Table 2.

Table 2. Specificity of primers proposed for species identification of feline DNA

Analysed species	Forward 1	Forward 3	Reverse 1	Reverse 2
Cattle	88%			
Pig				
Sheep				
Hen			88%	
Goose			82%	
Turkey			82%	
Duck				
Red deer				82%
Roe deer				
Goat			88%	82%
Dog			88%	
Walleye pollack				

From these primers, the most species specific pair of Forward3 and Reverse 2 was selected for further analysis.

The PCR results were analysed electrophoretically with a 2.5% agarose gel. The size of DNA fragments was determined as the absolute number of base pairs (bp) by comparison with known size marker (25bp DNA).

Figure 1 presents the result of PCR analysis. A positive result of 286 bp was only obtained for DNA isolated from feline hair. DNA from the other species did not yield the reaction product.

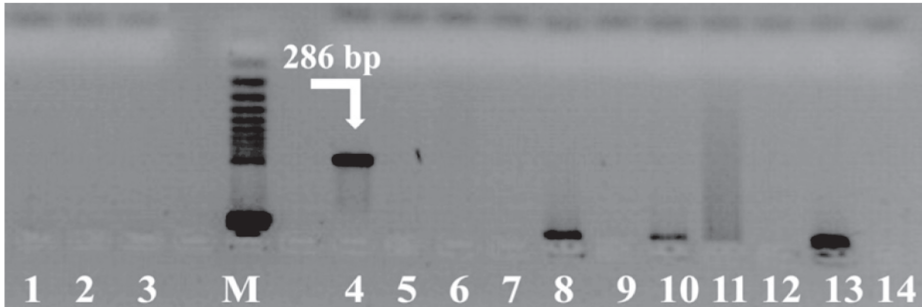

Figure 1. PCR reaction. Lanes contain a PCR product, in which the matrix was DNA isolated from: cattle (1), pig (2), sheep (3), cat (4), hen (5), goose (6), turkey (7), duck (8), red deer (9), roe deer (10), goat (11), dog (12), walleye pollack (13). In lane 14, negative control of the PCR reaction. M – size marker (25bp)

Discussion

Methods for species identification of farm animals are widely discussed in the literature, but methods concerning domestic animals such as cats have received little study. However, this species deserves attention due to the possible illegal use of cat byproducts as pet foods and illegal manufacture of products from cat lard. A fragment encoding 12s-RNA or cytochrome *b* is most often used for the analysis. The former is used for simultaneous identification of material derived from cats, dogs and rats in a multiplex reaction. DNA from each of these species is amplified by a separate pair of primers and results in PCR products of different size (Martin et al., 2007). Material derived from these species can also be identified based on analysis of cytochrome *b* polymorphism. In this case one pair of primers is used and the final species detection is obtained using restriction enzymes (Fumiere et al., 2006). Cytochrome *b* is used for species differentiation of several species of Felidae. An important contribution to this research was made by Hsieh (Hsieh et al., 2001), who described a procedure for identification of several Felidae species threatened with extinction in Taiwan. A method for identification of feline, canine and human components in a multiplex reaction is used in routine analysis, especially in criminological research (Nakaki et al., 2007). As can be seen, each of the analytical variants is designed to address a different research problem. Likewise, the method described in the present study is aimed to identify fe-

line component only where it is the subject of our interest, while reducing the time and cost of analysis. It uses cytochrome *b* polymorphism among mammals, fish and birds. The FastPCR analysis of species specificity detected if the proposed primers amplify the DNA of other species than the cat or not. In silico analysis of these properties followed by cross-reactions is highly important due to the potential presence of components from different species in the material studied. These tests make it possible to eliminate primers that give falsely positive results. Another advantage of this method is the size of the PCR product. The fact that it is only 286 bp in length suggests that it can be amplified using even partly degraded material (Rodríguez et al., 2004).

The method elaborated in this study enables the potential content of feline components to be determined rapidly and conclusively. It can also be developed to identify material composed of several species in a multiplex reaction.

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MAŁGORZATA NATONEK-WIŚNIEWSKA

Identyfikacja gatunkowa kociego DNA na podstawie analizy cytochromu *b*

STRESZCZENIE

Przedmiotem pracy było opracowanie metody identyfikacji gatunkowej materiału biologicznego pochodzącego od gatunku *Felis Catus* (kot domowy). Analizowane startery flankowały fragment cyto-

chromu *b* mtDNA należącego do *Felis Catus* (NC_001700). Wyboru odpowiednich starterów dokonano kierując się analizą ich specyficzności wykonaną programem FastPCR oraz długością otrzymanego produktu PCR. Do dalszych badań wyznaczono forward i rewers o najmniejszej homologii do DNA innych gatunków badanych w laboratorium. Przy zastosowaniu wytypowanych starterów przeprowadzono reakcje krzyżową, w wyniku której nie otrzymano wyników fałszywie pozytywnych. Dla DNA kociego uzyskano produkt PCR o wielkości 286 pz. Z przedstawionej pracy wynika, że opracowana metoda pozwala w szybki i jednoznaczny sposób określić potencjalną zawartość kocich komponentów.