

Molecular method for identification of fallow venison

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SUMMARY

Reliable and simple identification of species origin of meat and meat products can be carried out by applying species-specific primers that target particular short DNA fragments. The aim of our research was to design species-specific primers for the amplification of mitochondrial DNA control region of fallow deer (*Dama dama*) and to optimize the polymerase chain reaction protocol (PCR protocol) for such primers. Primers are used for the identification of fallow deer and the differentiation of fallow deer from other species, using only DNA extraction, PCR and electrophoresis. The application of primers was tested on eight fallow deer samples, three red deer and three roe deer samples.

Key words: primer, mitochondrial DNA, fallow deer, *Dama dama*

INTRODUCTION

The identification of species through DNA barcodes plays an increasingly important role in the control of food products, especially food products containing game meat. Game meat is considered a delicacy. It is characterized by distinctive and intense flavour, low level of fat and cholesterol, and high level of polyunsaturated fatty acids. Furthermore, game animals are not treated with hormones and steroids (Fajardo et al., 2008b). Therefore game meat commands higher market price than meat of farm animals. Consequently, foodstuffs containing game are often incorrectly labelled in order to generate greater profit (Brodmann et al., 2001). In addition to being illegal, false labelling of type and amount of meat in food products may cause health problems for consumers sensitive to allergens that are omitted from food labels, and raise issues arising from religious dietary restrictions (Fajardo et al., 2007b). All of the above emphasizes a necessity to establish reliable and simple method for the identification of species

origin of farm and game animals from which the meat in foodstuffs originated (Pfeiffer et al., 2004).

Over the past two decades, polymerase chain reaction (PCR) and DNA sequencing have become effective and economically efficient principal research methods commonly used in molecular genetics by a substantial number of researchers (Awise, 2004). DNA analysis is therefore the most reliable method of identifying species that is currently employed. Such identification is based on DNA extraction, PCR amplification of a specific DNA sequence (usually a part of mitochondrial DNA), sequencing of PCR product, and analysis of nucleotide sequences. Nucleotide sequences are analysed using applicable software and compared with reference sequences that represent a basis for determining a particular species (Awise, 2004). When species-specific primers that replicate DNA sequence only for one particular species are used, the identification can be performed by confirming the amplification of PCR product using only gel-electrophoresis, without determining the precise order of nucleotides by sequencing.

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The aim of our research was to design species-specific primers for the identification of fallow deer (*Dama dama*) and the development of genetic method for differentiation of fallow deer from other members of the Cervidae present in Croatia - namely red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*). This method was devised to identify and distinguish the tissue of fallow deer from other species by using only DNA extraction, PCR and electrophoresis. This not only bypasses the sequencing, which remains inaccessible to a large number of laboratories, but also facilitates, accelerates and reduces the cost of identification process.

MATERIALS AND METHODS

Species-specific primers were designed using the Primer Blast application (Rozen and Skaletsky, 2000) and were based on the mitochondrial DNA sequence of fallow deer downloaded from the GenBank database (GenBank No: AF291895.1). The following software parameters were used:

PCR template: AF291895.1

Primer Pair Specificity Checking Parameters

Organism: *Homo sapiens*, *Cervus elaphus*, *Capreolus capreolus*, *Sus scrofa*, *Rupicapra rupicapra*

Database: NR

The efficiency of primers was tested on a total of 14 muscle samples, consisting of eight fallow deer (*Dama dama*), three red deer (*Cervus elaphus*) and three roe deer samples (*Capreolus capreolus*). Samples originated from animals hunted during the routine implementation of wildlife management programs in Croatia and were stored in 96% ethanol at -20°C. DNA was extracted from muscle samples, using a commercial ChargeSwitch® gDNA Tissue Kit, Invitrogen, according to manufacturer's protocol.

The first step in the analysis of primer efficiency was the optimization of PCR protocol. The goal of such optimization process was to determine the optimum temperature at which primers bind to the species-specific DNA chain. That is why we triggered a series of PCR reactions at a temperature gradient between 57°C and 7°C. Temperature of 92°C for the activation of polymerase was applied for 2 minutes, the denaturation of templates was at 94°C during 30 seconds, the binding of primers was performed at a temperature between 57°C and 71°C for 30 seconds, the chain was extended by applying a temperature of 72°C for 2 minutes, with the final extension achieved by applying a temperature of 72°C for 10 minutes. To prepare a PCR mixture, a commercial kit Platinum® PCR SuperMix, Invitrogen was used. The amplification of mtDNA control region sequence was performed in 10 µL of solution composed of 1 µL of DNA, 1 µL 2 µM primer solution and 9 µL

Platinum® PCR SuperMix, Invitrogen. The PCR reaction was carried using the GeneAmp PCR System 2700 (Applied Biosystems). Electrophoresis on 1.5% agarose gel verified whether the amplification of PCR products occurred. Electrophoresis was performed at a room temperature, at a constant voltage of 90 V, applied for 30 minutes.

RESULTS AND DISCUSSION

By using the Primer-blast tool (Rozen and Skaletsky, 2000) we have designed species-specific primers suitable to amplify mtDNA control region sequence of fallow deer (*Dama dama*). Primers bind at loci 399 and 641 of the fallow deer mtDNA control region, producing a 243 base pairs (bp) long sequence (Table 1).

Table 1. Nucleotide sequence of fallow deer species-specific primers

	Nucleotide sequence of the primer (5'→3')
Forward primer	TCGCTCCGGGCCCATAGACT
Reverse primer	GCTCCGGGTCCGGGCCTTAG

The optimization of PCR reaction at a temperature gradient between 57°C and 71°C confirmed that the optimal specific binding of primers is achieved at a temperature of 70°C. Next, we examined the efficiency of primers designed to distinguish fallow deer species from other members of the *Cervidae* present in Croatia, on eight DNA samples of fallow deer, three red deer samples and three roe deer samples. Six out of eight fallow deer samples (75 %) produced a PCR product, while samples of red deer and roe deer did not produce any PCR products (Figure 1). By verifying the DNA extraction we found that DNA extraction failed in the two samples of fallow deer that did not produce PCR products.



Figure 1. Depiction of agarose gel after electrophoresis of PCR products in samples of fallow deer (L1-L6), red deer (J1-J3) and roe deer (S1-S3)

Most research studies analysing meat and meat product using molecular genetic methods are conducted on farm animals (Girish et al., 2004). There are a considerably lower number of studies regarding the identification of game meat, although various markets record a growing demand for game meat. Fajardo et al. (2007a) designed three species-specific primers for the differentiation of wild Bovidae members, chamois (*Rupicapra rupicapra*), ibex (*Capra ibex*) and mouflon (*Ovis musimon*). The same team of scientists (Fajardo et al. 2008a) also conducted a study of species-specific primers for

the differentiation of wild boar (*Sus scrofa scrofa*) and domestic pig (*Sus scrofa domestica*) by targeting the D-loop of mitochondrial DNA control region and nuclear melanocortin receptor 1 (MC1R) genes. They proved that the use of species-specific primers that target specific short DNA fragments is a reliable method of food control, especially in heat-treated samples or foodstuffs containing meat of two or more different animal species (sausages, pates, minced meat, etc.) (Montiel-Sosa et al., 2000). This method also proved to be cheaper, quicker and more suitable for the routine analysis of a large number of samples (Herman, 2001).

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— Međunarodna manifestacija **Šokolijada najznačajnija je gastronomska manifestacija grada Nina** i u Zadarskoj županiji, a uvrštena je u 100 najznačajnijih manifestacija u Hrvatskoj. Nadaleko poznata ninska manifestacija započela je i to podjelom svježeg mesa svinjske vratine. Ove godine prijavio se rekordni broj natjecatelja, sveukupno 106, stoga je podjela organizirana u dva dijela, a meso su osigurali mesnica Lukin iz Zadra i mesnica Poljak iz Nina. Organizator manifestacije, Turistička zajednica grada Nina, nabavila je 803 kg svježeg mesa svinjske vratine za 106 natjecatelja koji dolaze iz različitih mjesta. Šokolijada dakle započinje podjelom mesa u siječnju, a završava u srpnju kad se odabiru najbolji šokolari te se završni dani manifestacije održavaju 18. i 19.



srpnja ove godine.

— Na prvoj manifestaciji, održanoj 2003. godine sudjelovalo je 10 natjecatelja, lani ih je bilo 79, a ove godine rekordnih 106!