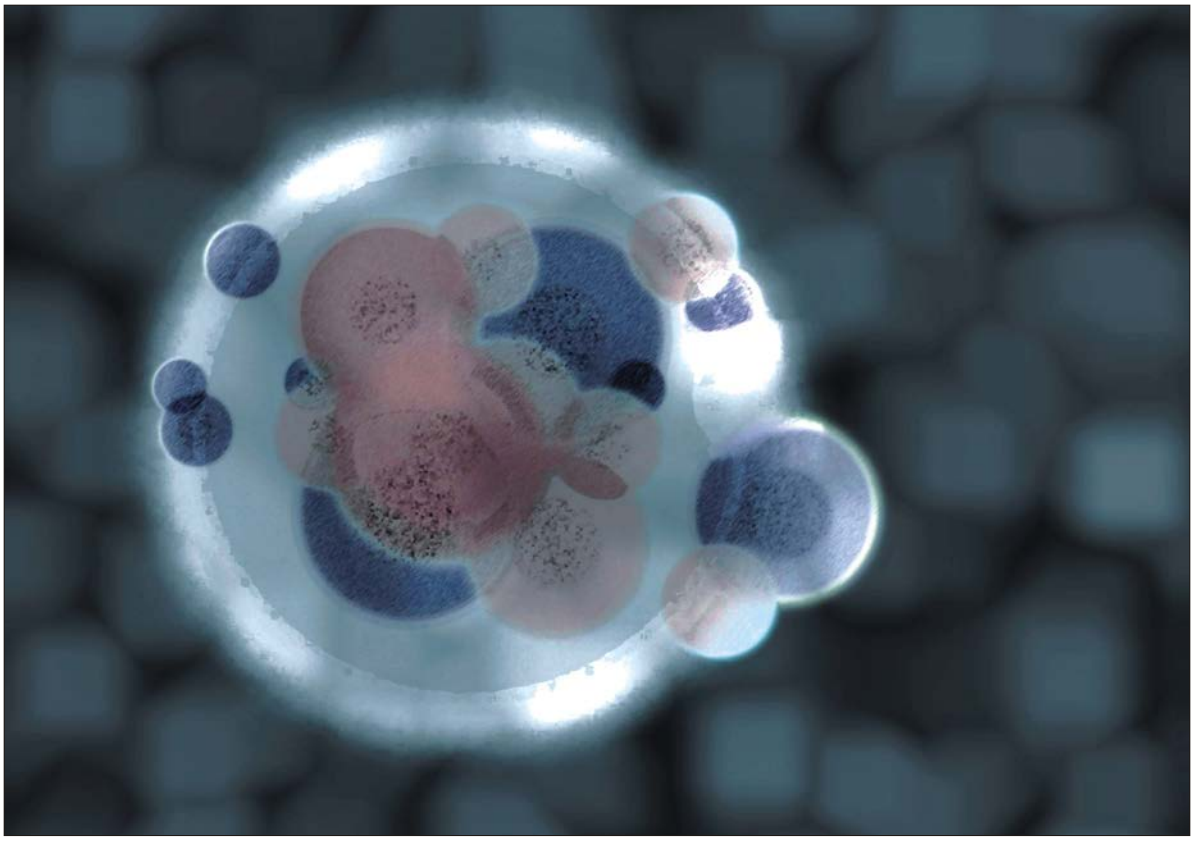

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Species Specificity Using Fifteen PCR-based Human STR Systems

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Abstract: Contamination of biological evidence with non-human DNA complicates forensic analysis and necessitates the use of sets of primers that show high specificity for human DNA. We report here the analysis of DNA from 15 vertebrate animals in addition to that of humans (males and females) using the PowerPlex® 16 system to assess its specificity for human euchromatin. The loci tested include, Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, Penta D, CSF1PO, D16S539, D7S820, D13S317 and D5S818. There were no STR PCR products observed for the 15 species regardless of the STR system. Amelogenin-PCR fragments were all below 100 bp and consist of only one fragment irrespective of the sex.

Key words: Short tandem repeats, PCR, DNA typing, animals

INTRODUCTION

In the last decade the forensic authorities worldwide have been extensively using Short Tandem Repeats (STRs) loci in forensic case work for their high discriminating power and individualization. STRs are short repetitive sequences of eukaryotic genome[1] that show highly polymorphic alleles that differ in length. STRs Differences in length are due to the number of repetitions of 3-7 base pairs sequence[2,3]. Nevertheless, forensic biological samples collected from crime scenes are always contaminated with biological materials from non-human origin. Such contamination might confuse proper DNA analysis and sheds doubt upon forensic technical and juricidial procedures. In a recent study by Buse *et al.*[4], two STR multiplexes (AmpFlSTR Profiler Plus and AmpFlSTR COfiler, PE Applied Biosystems, Foster City, CA) were evaluated for their species specificity. The authors reported either no or small amplification signals for nonhuman DNA at the amelogenin gene and various STR loci. In this study, we intented to determine the species specificity of another largely used STR multiplex system (PowerPlex® 16 system, Promega Co., WI, USA) which includes 15 STR loci and the gender-determining amelogenin gene using DNA from various domestic animals in order to validate the forensic reports.

MATERIALS AND METHODS

Blood samples were collected from 15 different non-human species and humans (Table 1) and were stored at -20°C until further use. DNA extraction was carried out using the Wizard® Genomic DNA Purification Kit (Promega Co., WI, USA) according to the manufacturer's protocol. Polymerase chain reaction was conducted according to the PowerPlex® 16 system technical manual (Promega Co., WI, USA). Briefly, amplification was carried out using 2.5 ng of template DNA and products were loaded on the ABI Prism 310 Genetic Analyzer and DNA Sequencer (Applied Biosystem, CA, USA), using ILS-600 (Promega Co., WI, USA) as internal line standard according to the manufacturer's recommendations. Gene scan analysis was performed on the raw data using the Gene scan Software 2.0.2 (Applied Biosystem, CA, USA).

RESULTS AND DISCUSSION

All of STR loci have been well-characterized with regard to their chromosomal location, repeat sequence and allelic polymorphism (PowerPlex® 16 system Technical Manual, Promega Co., WI, USA). The aim of this study was to evaluate the species specificity of sixteen STR systems including Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, Penta D, CSF1PO,

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Table 1: Results of Amplification of human and nonhuman DNAs

Animals	Amelogenin Mean size ±SEM[a]	STR amplification (+/-)
Camel Female	89.290±0.010	-[b]
Camel Male	89.215±0.005	-
Donkey Female	99.005±0.005	-
Donkey Male	99.635±0.045	-
Horse Female	99.690±0.010	-
Horse Male	99.580±0.010	-
Cattle Female	99.795±0.005	-
Cattle Male	89.440±0.010	-
Dog Female	99.085±0.015	-
Dog Male	99.785±0.005	-
Cat Female	88.195±0.005	-
Cat Male	88.240±0.010	-
Rat Female	88.135±0.005	-
Rat Male	88.140±0.010	-
Mouse Female	88.195±0.005	-
Mouse Male	88.275±0.005	-
Goat Female	88.335±0.005	-
Goat Male	88.205±0.005	-
Sheep Female	99.885±0.005	-
Sheep Male	99.060±0.010	-
Rabbit Female	-	-
Rabbit Male	-	-
Mule	99.520±0.010	-
Chicken Female	88.560±0.000	-
Chicken Male	88.755±0.005	-
Turkey Female	88.280±0.010	-
Turkey Male	88.865±0.005	-
Lizard Female	88.860±0.010	-
Lizard Male	88.880±0.010	-
Human Female	105.985±0.005	+[c]
Human Male	106.000±0.020	+
	111.995±0.015	+

a : Standard error of the mean, b: No amplification products for any of the 15 STR loci tested, c : Positive amplification for each of the 15 STR loci tested.

D16S539, D7S820, D13S317, D5S818 and the amelogenin gene.

The amplification of human amelogenin always gave the expected PCR fragments depending on the sex of the donor (Table 1). No anomalies in the size of the PCR products were observed in any human sample. Inconsistent with a previous study by Buel *et al.*[5] amplifications of animal amelogenin produced a single fragment below 100 bp in size regardless of sex (Table 1). In their study, Buel *et al.*[5] reported positive PCR sex amplifications in the size of 102-103 bp for various domestic and wild animals DNA using previously described primers[6]. The use of those primers along with the possibility of occurrence of troubleshoots in PCR amplification or electrophoresis might deeply affect the interpretation of the real origin of the sample. Present results indicate that the amelogenin primer set provided in the PowerPlex® 16 system Kit (Promega Co., WI, USA) is highly specific for differentiating human biological sample from non-human samples.

Except for humans, no PCR products for the tested fifteen STR loci were observed in any of the species indicating the high specificity of the primer sets used (Table 1). However, an earlier study by Crouse and Schumm[7] and the more recent study by Buse *et al.*[4] have shown that animal DNAs from primate origin exhibited non-allelic amplified fragments for some human-derived STR primer pairs. This necessitates a full and an extensive research on animals from primate and non-primate origin to determine the species specificity for all and future STR loci of forensic value.

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