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Relevant aspects for forensic STR analysis of canine DNA: Repeat-based nomenclature and sensitive PCR multiplexes

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Abstract. Eight polymorphic canine STR markers were co-amplified in two newly designed PCR multiplex reactions (FH2087Us, FH2611, PEZ2, PEZ6, FH2328l, PEZ15, FH2054, WILMS-TFs). The sequence structure of selected alleles of these markers was the basis for the implementation of a repeat-based nomenclature. The PCR multiplexes amplify short amplicon lengths which makes the assay sensitive to the analysis of degraded DNA. © 2005 Elsevier B.V. All rights reserved.

Keywords: Canine microsatellite; STR; PCR multiplex; Forensic

1. Introduction

Forensic identity testing of canine DNA using short tandem repeats (STRs) is becoming commonplace in resolving criminal cases. It has become increasingly important to have a set of commonly used STR markers and a reliable nomenclature, which enables exchange of data and international collaborations. The sequence structure of selected alleles of forensically useful STRs was the basis for the implementation of a repeat-based nomenclature [1,2] that is adopted from the recommendations of the International Society of Forensic Genetics (ISFG) for the nomenclature of human STRs [3,4]. We describe two newly designed robust PCR multiplexes sensitive to degraded DNA for 8 polymorphic canine STR markers.

2. Material and methods

8 canine-specific STR markers were co-amplified in two multiplex PCR reactions (MP1 and MP2). For both MPs, the total reaction volume was 25 μl including 1× PCR

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Table 1
Primer sequences, fluorescent labels and concentration as used in the 2 canine STR multiplexes

Marker	Primer Sequence (5′–3′)	Label	Concentration (nM)	MP
FH2087Us[a]	Fs-CACATTCACTGATGCATTTCGC Rs-CTCTTTTTCTGTCTCTCCTTCCTCTG	6-FAM	120/160	MP1/2
FH2611	F-GAAGCCTATGAGCCAGATCA R-TGTTAGATGATGCCTTCCTTCT	6-FAM	120	MP1
PEZ2	F-TCCTCTCTAACTGCCTATGC R-GCCCTTGAATATGAACAATGACACTGTATC	TET	140	MP1
PEZ6	F-ATGAGCACTGGGTGTTATAC R-ACACAATTGCATTGTCAAAC	TET	140	MP1
FH2328l[a]	Fl-GCTCTATGTGTCACTGCTATGA Rl-CCTACCAGGTAGTTTTCAGAAAT	HEX	200	MP1
PEZ15	F-CAGTACAGAGTCTGCTTATC R-CTGGGGCTTAACTCCAAGTTC	6-FAM	120	MP2
FH2054	F-GCCTTATTCATTGCAGTTAGGG R-ATGCTGAGTTTTGAACTTTCCC	TET	60	MP2
WILMS-TFs[a]	Fs-CACTGTTCTGTGGTTTGCAGGAG Rs-CCAGAGATTTTCCTTTTCTTAAGGG	HEX	160	MP2

[a] s = short, 1 = long.

Buffer II, 1.5 mM $MgCl_2$, 200 μM each dNTP, 2 U Ampli*Taq* Gold Polymerase (Applied Biosystems (AB), Foster City, CA, USA) and 0.25 mg/ml BSA (Serva, Heidelberg). The primer sequences, fluorescent labels and reaction concentrations are given in Table 1. The amplification was performed on a GeneAmp PCR System 9600 (Perkin Elmer, Norwalk, CT, USA) comprising initial denaturation at 95 °C for 11 min followed by 29 cycles of 95 °C for 30 sec, 58 °C for 45 sec 72 °C for 90 sec and a final incubation at 72 °C for 60 min. The amplification products were subjected to capillary electrophoresis on an ABI Prism 3100 Genetic Analyzer. The data were analyzed using GeneScan Analysis Version 3.7 and Genotyper Version 2.5 (both AB). The DNA concentration was measured using the "PicoGreen dsDNA Quantitation Kit" (Molecular Probes, Eugene, OR, USA).

3. Results

The 8 canine-specific STRs FH2087Us, FH2611, PEZ2, PEZ6, FH2328l, PEZ15, FH2054, WILMS-TFs were amplified in two multiplex STR reactions. The marker FH2087Us was included in both assays to serve as an internal sample control. Primers were designed in a way that for both multiplexes an annealing temperature of 58 °C gave best results. Full profiles were observed for both multiplexes (peak heights above 100 RFUs) using as much as a minimum of 200 pg of template DNA for amplification. For all investigated loci, the observed stutter peak heights were lower than 10% of the main allele peak height enabling unambiguous allele calling and interpretation of mixed STR profiles. The amplification products reside in category ranges below 280 bp, the majority of alleles (6 of 8 loci) are found below 220 bp, rendering the two STR multiplexes a useful tool for the investigation of severely degraded canine DNA, such as found in dog hair or minute amounts of canine saliva.

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