Relevant aspects for forensic STR analysis of canine DNA: Repeat based nomenclature, allelic ladders and PCR multiplexes

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As the dog is deemed to be our most popular pet, it can also be considered as the most interesting animal species from a forensic point of view. Canine saliva as well as dog hairs can remain everywhere where contact between dogs and humans have taken place. As 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 minimum guidelines, such as common used STR markers and a reliable nomenclature. These enable exchange of data and international collaborations. The majority of canine STR markers described in the literature were reported by the estimated fragment size as determined by electrophoresis of the PCR-products. The lack of a uniform harmonized nomenclature makes the application of these markers difficult. Here we present a set of 8 forensically useful STR markers (FH2087Us, FH2611, PEZ15, FH2054, PEZ2, PEZ6, WILMS-TFs, and FH2328I) co-amplified in two newly designed multiplexes (Figs.1c, d) The sequence structure of selected alleles of these markers was the basis for the implementation of a repeat based nomenclature (Figs. 2a - h) [1;2]. The nomenclature of the alleles is adopted from the recommendations of the International Society of Forensic Genetics (ISFG) for human STR analysis. Additionally, allelic ladders containing the common alleles for all markers used in both multiplexes are shown, which allow an unequivocal allele designation of unknown samples (Figs. 1a,b).

Material and methods

Multiplexes: 8 canine-specific STR markers were co-amplified in two multiplex PCR reactions (MP1 and MP2 see Figures 1c and d). For both MP´s the total reaction volume was 25 µl including 1 X PCR Buffer II, 1.5 mM $MgCl_2$, 200 µM each dNTP, 2 U AmpliTaq Gold Polymerase (Applied Biosystems (AB), Foster City, CA, USA) and 0.25 mg/ml BSA (Serva, Heidelberg). The concentration of each primer was as listed below (sequences are listed in Table 1): MP1: 120nM (FH2087Us, FH2611), 140nM (PEZ2, PEZ6) und 200nM (FH2328I); MP2: 160nM (FH2087Us), 120nM (PEZ15), 60nM (FH2054) und 160nM (WILMS-TFs). Amplification was performed on a Gene Amp 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. 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).

Allelic ladders: Buccal swabs were taken from 131 randomly selected dogs as described in detail in [1]. The alleles were amplified with unlabeled primers using a singleplex PCR protocol. For most of the alleles the following amplification strategy was practicable: Initial denaturation step at 95°C for 11 min followed by 35 cycles of 95°C for 15 sec, 58°C for 30 sec, 72°C for 60 sec and a final incubation at 72°C for 30 min. The heterozygous amplicons were separated using a gel-electrophoreses system allowing a 4 bp resolution of the DNA fragments (Spreadex-Gel, Elchrome Scientific, Switzerland) and were picked manually.

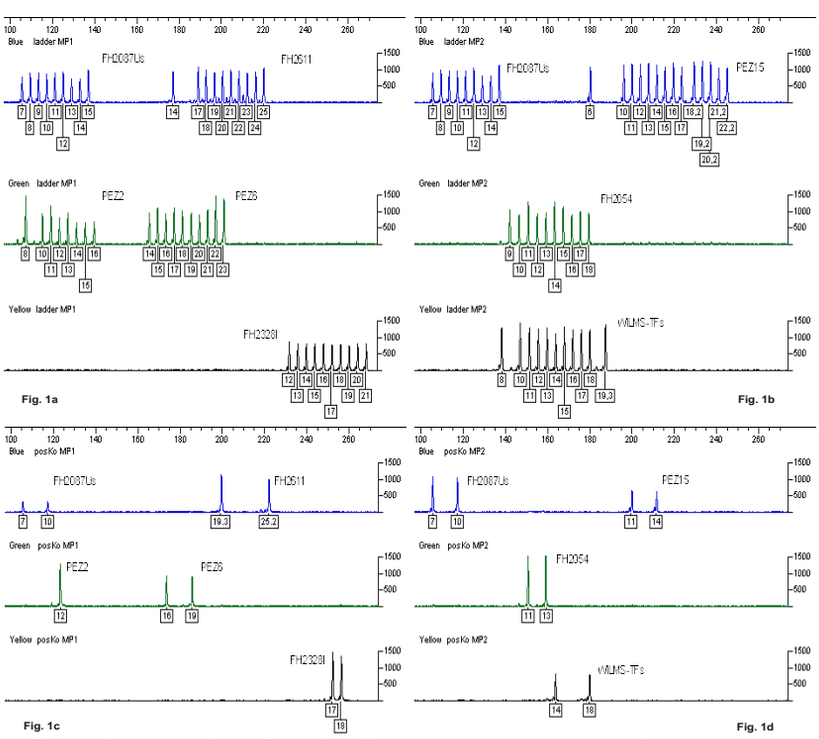

Fig. 1a Fig. 1b Fig. 1c Fig. 1d

Fig. 1a - d: Genotyper plots of the amplification of a dog DNA with both multiplexes and their corresponding ladders. The locus FH2087Us was included in both multiplexes and served as internal control. Peaks are labelled with respect to a repeat-based nomenclature (for details see [1].

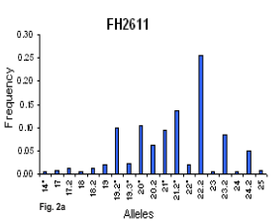

Fig. 2a

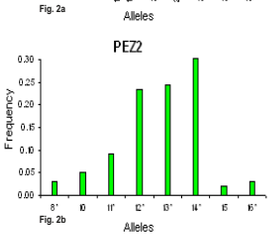

Fig. 2b

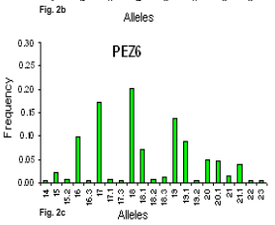

Fig. 2c

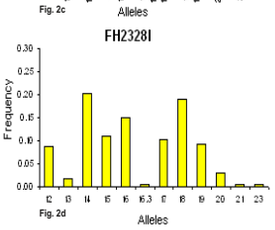

Fig. 2d

Marker	Primer Sequence (5´-3´)	Label	Chrom. location	MP
FH2087Us*	Fs-CACATTCACTGATGCATTTCGC Rs-CTCTTTTTCTGTCTCTCCTTCCTCTG	6-FAM	CFA25	MP1/2
FH2611	Fs-GAAGCCTATGAGCCAGATCA Rs-TGTTAGATGATGCCTTCCTTCT	6-FAM	CFA36	MP1
PEZ2	F-TCCTCTCTAACTGCCTATGC R-GCCCTTGAATATGAACAATGACACTGTATC	TET	no entry	MP1
PEZ6	F-ATGAGCACTGGGTGTTATAC R-ACACAATTGCATTGTCAAAC	TET	CFA27	MP1
FH2328I*	FI-GCTCTATGTGTCACTGCTATGA RI-CCTACCAGGTAGTTTTCAGAAAT	HEX	CFA29	MP1
PEZ15	F-CAGTACAGAGTCTGCTTATC R-CTGGGGCTTAACTCCAAGTTC	6-FAM	CFA16	MP2
FH2054	F-GCCTTATTCATTGCAGTTAGGG R-ATGCTGAGTTTTGAACTTTCCC	TET	CFA12	MP2
WILMS-TFs*	Fs-CACTGTTCTGTGGTTTGCAGGAG Rs-CCAGAGATTTTCCTTTTCTTAAGGG	HEX	CFA18	MP2

* s=short, l=long

Table1: Primer sequences, flourescent labels, chromosomal location and multiplex assemble of the 8 investigated markers. The chromosomal locations were taken from the literature.

Marker	Alleles found (n)	STR class	Repeat basic structure	HET	PIC
WILMS-TFs	24	Compound	$(GAAA)_n$	0.897	0.889
PEZ15	20	Compound	$(GAAA)_n$	0.887	0.877
PEZ6	21	Complex	$(GAAA)_n$	0.881	0.870
FH2611	19	Compound	$(GAAA)_n(GGAA)_n(GAGA)_n$	0.871	0.860
FH2087Us	11	Compound	$(GAAA)_n$	0.857	0.842
FH2054	10	Compound	$(GATA)_n$	0.837	0.818
PEZ2	8	Simple	$(GGAA)_n$	0.782	0.749
FH2328I	12	Compound	$(GAAA)_n$	0.859	0.843

Table 2: Summary of the 8 markers investigated. The classification of the STR loci is depending on the complexity of the polymorphic region and a repeat based nomenclature was indtroduced as described in more detail in [1]. The number of alleles detected within a marker does not correspond with the alleles included in the ladders. Each ladder does imply only the main alleles of each marker. Heterozygosity (HET), Polymorphism information content (PIC) for the 8 canine STRs are shown in this table.

Fig.2a-h: Allelfrequency of the 8 investigated markers. n = 131 except for the marker FH2328I (n=113) *Allel sequences are listed in [1].

References:

(1) Eichmann C, Berger B, Parson W. A proposed nomenclature for 15 canine-specific polymorphic STR loci for forensic purposes. Int J Legal Med 2004; 118(5):249-266.
(2) Hellmann A, Rohleder U, Eichmann C, Pfeiffer I, Parson W, Schleenbecker U. A proposal to the allele nomenclature of six canine specific STR loci in forensics. Submitted 2005.

Re-amplification of all single alleles as well as of all ladders had to be performed individually. The amplification products were re-amplified using fluorescent labeled primers. Amplification was performed on a Gene Amp PCR System 9600 comprising initial denaturation at 95°C for 11 min followed by 30 cycles of 95°C for 15 sec, 58°C for 30 sec (45 sec for ladders) 72°C for 60 sec (90 sec for ladders) and a final incubation at 72°C for 30 min (60 min for ladders).

Results

Multiplexes: For both multiplexes an annealing temperature of 58°C was found to give best results. Full profiles were observed for both multiplexes (peak heights above 100 RFUs) using a minimum of 200 pg of template DNA for amplification. We did not observe major artefacts affecting the interpretation of the STR-profiles.
For all investigated loci, the stutter peaks were lower than 10% of the main allele peak as known from human-specific STRs.

Allelic ladders: Ladders for the 8 markers, including all main alleles of each marker, were generated (see Figures 1a and b). The sequence structure of selected alleles of these markers was the basis for the implementation of a repeat based nomenclature as described in [1;2] according to the recommendations of the International Society of Forensic Genetics (ISFG) for the nomenclature of human STRs. For verification, each allele presented in the ladders was sequenced and checked for correct nomenclature.

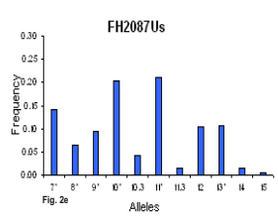

Fig. 2e

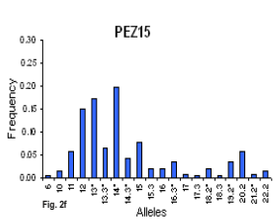

Fig. 2f

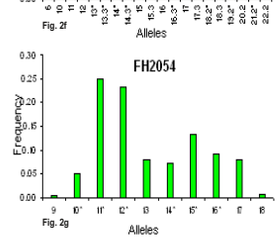

Fig. 2g

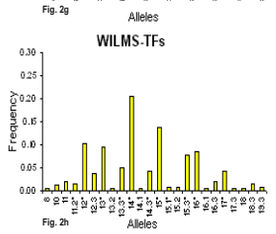

Fig. 2h

Fig.2a-h: Allelfrequency of the 8 investigated markers. n = 131 except for the marker FH2328I (n=113) *Allel sequences are listed in [1].

Acknowledgments:

This work was supported in part by a grant from the Bundeskriminalamt Wiesbaden, Germany. We thank Harald Niederstaetter for helpful assistance and discussion.