Canine DNA profiling in forensic casework - joining efforts for standardization

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Canine DNA profiling group

Introduction

Short Tandem Repeat (STR) analyses of canine cells have been applied successfully in a number of forensic cases and recently efforts were made for applying well characterized STR markers for forensic analyses. But the existence of great inter-laboratory variability indicates that the canine forensic field stil faces the urgent need for standardization and harmonization. In particular, the nature and selection of DNA markers need to be evaluated and allelic ladders as well as reference materials should be available to enable interlaboratory comparisons. Additionally a generally compatible allele nomenclature and an agreement on standardized statistical calculation methods adopted for the specific genetic characteristics of dog populations need thorough considerations

The Canine DNA Profiling Group (CaDNAP) developed two multiplex PCR assays containing thirteen highly polymorphic canine STR markers, SRY and the amelogenin marker. The markers were selected due to their variability to minimize the number of markers needed for unambiguous individualization. These were evaluated and characterized with respect to laboratory performance and population genetic features. The majority of markers have amplicons below 220 bp following the general trend in forensic molecular biology to reduce the amplicon size for successful typing of degraded DNA. These 13 canine STR markers have also been intensively characterized regarding their sequence structure. Repeat based nomenclatures have been established and allelic ladders were prepared for unambiguous allele calling. A validation of two PCR-multiplexes was performed following the revised validation guidelines for the human field [1].

Materials and methods

Multiplexes:

Fifteen canine specific STR markers were co-amplified in two PCR multiplex reactions - a Hepta-Plex (MP1) and a Nona-Plex (MP2), latter including two sex-specific markers, amelogenin and SRY (Fig. 1, Tab. 1). For both MP´s the total reaction volume was 20 µl including 1 X PCR Buffer II, 1.5 mM $MgCl_2$, 200 µM each dNTP, 2U AmpliTaq Gold Polymerase (Applied Biosystems (AB), and 0.25 mg/ml BSA (Serva, Heidelberg). The amplification process comprised an initial denaturation at 95°C for 11 min followed by 30 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.

Markers are described in detail (Fig. 2, Tab. 1) and in [2-6].

Allelic ladders:

The most frequent alleles of each marker were selected for allelic ladders and amplified using a singleplex PCR protocol. The following amplification strategy was practicable: initial denaturation step at 95°C for 11 min followed by 30 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 amplified alleles were then mixed to assamble the allelic ladder (Fig. 3).

For sequencing, ladder alleles were amplified using unlabeled primers and the following amplification strategy: 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.

Heterozygous amplicons were separated using a gel-electrophoreses system allowing a 4 bp resolution of the DNA fragments (Spreadex-Gel, Elchrome Scientific, Switzerland) and picked manually.

Electrophoresis was carried out on an ABI Prism 3100 Genetic Analyzer using POP6 and 36 cm capillary arrays.

Positive control:

The permanent canine cell line DH 82 [8] was subcloned (DH82-D3167) in order to make use of this renewable biological material as positive control that could be shared by different laboratories. For verification each allele of the positive control was sequenced and checked for the appropriate nomenclature (Fig. 4, 5).

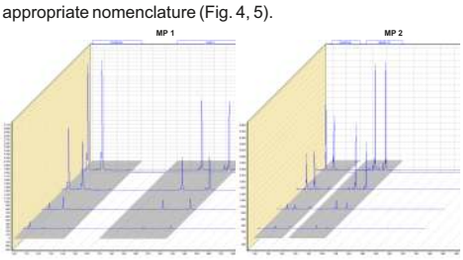

Fig. 6. Blue lane as example of results obtained from the sensitivity study for each multplex. Front to back: 20pg, 50pg, 250pg, 500pg.

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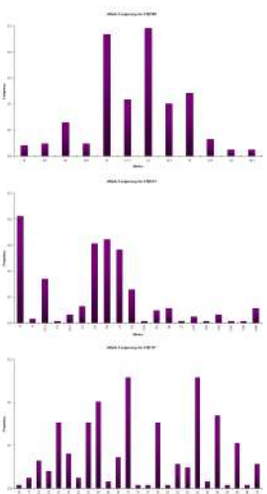

Fig. 2. Allelfrequency of 3 newly intensively characterized markers included in the multiplexes.

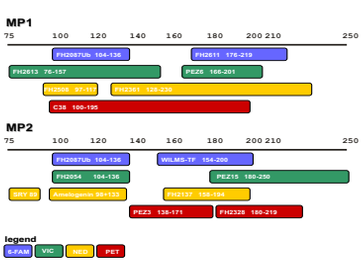

Fig. 1. Schematic representation of both canine STR multiplex reactions.

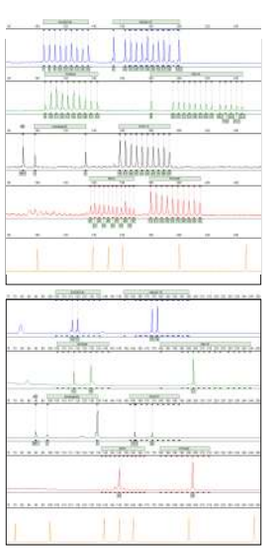

Fig. 3 (above). Electropherogram of MP 2 ladder.
Fig. 4 (below). Electropherogram of MP 2: mixture of 500 pg canine DNA and 10 ng human DNA. The X-Amelogenin peak is higher as human DNA gives signal at this marker allel.

Results

Multiplexes:

The two multiplexes were successfully validated according to the revised SWIGDAM guidelines [1].

For both multiplexes an annealing temperature of 58°C was found to give best results. We did not observe major artefacts effecting the interpretation of the STR-profiles obtained.

Full profiles (signals above 100 RFU) for each multiplex were obtained using at least 50 pg of DNA (Fig. 6).

22 animal species were tested using the multiplexes for positive amplification results. The wolf samples gave full profiles for each multiplex whereas partial profiles were obtained from the fox, the black-backed jackal and the raccoon dog. Only amelogenin-X showed a signal in almost all tested animals including the human sample (Fig. 4).

Allelic ladder:

Ladders for both multiplexes were generated (Fig. 3). For verification each allele presented in the ladders was sequenced and checked for correct nomenclature that was established according to the recommendations for animal DNA forensic and identity testing [7].

Marker	Chrom. Location	Repeat basic structure	Nomenclature	Alleles (n)	Het (exp)
FH2087Ub	CFA25	$(GAAA)_n$	[2]	9	0.834
FH2611	CFA36	$(GAAA)_n(GGAA)_n(GAGA)_n$	[2]	19	0.873
WILMS-TF	CFA18	$(GAAA)_n$	[2]	25	0.902
FH2054	CFA12	$(GATA)_n$	[2]	10	0.839
PEZ6	CFA27	$(GAAA)_n$	[3]	22	0.880
FH2613	CFA02	$(GAAA)_n$	[this study]	20	0.873
PEZ15	CFA16	$(GAAA)_n$	[2]	21	0.889
FH2508	CFA23	$(GAAA)_n$	[this study]	12	0.841
FH2361	CFA33	$(TTTC)_n$	[4]	25	0.834
FH2137	CFA03	$(GAAA)_n$	[5]	25	0.922
C38	CFA38	$(TTCT)_n$	[4]	27	0.901
PEZ3	CFA19	$(AAA)_n(GAA)_n(GCA)_m(GAA)_l(GCA)_l$	[3]	12	0.848
FH2328	CFA29	$(GAAA)_n$	[3]	13	0.860
Amelogenin	CFAX		[6]		
SRY	CFAY		[2]		

Table 1. Summary of the 15 markers investigated. Chromosomal location of each marker. The repeat structure and the repeat based nomenclature are according to the recommandations of [7]. The number of alleles detected within a marker does not correspond with the alleles included in the ladders. Expected Heterozygosity (HETexp) for the 15 canine STRs are shown in this table.

FH2087Ub
Allel 10 $(GAAA)_8(GCAA)_1$
CACATTCACTGATGCATTTCGCTAAAAATAAGTAAAATTGTTTTAGCAAAAAAGAAAGAAAGAAAGAAAGAAA
GAAAGAAAGAAAGAAAGCAAGCAGAGAGGAAGGAGAGACAGAAAAAGAG
Allel 11 $(GAAA)_{10}(GCAA)_1$
CACATTCACTGATGCATTTCGCTAAAAATAAGTAAAATTGTTTTAGCAAAAAAGAAAGAAAGAAAGAAAGAAA
GAAAGAAAGAAAGAAAGCAAGCAGAGAGGAAGGAGAGACAGAAAAAGAG

FH2611
Allel 21 $(GAAA)_{14}(GGAA)_1(GAGA)_4$
GAAGCCTATGAGCCAGATCATTCTCAATGTGACCATTATGGCTATTGTTGCTGAGGGTGGAAGTAGGAAGAA
AGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAGGAAGGAAGGAAGAGAGA
GAGAGAGAGACGGAGGGTGGAAGGAAGAAGGAAGGAAGGAGAGAAGGAAGGCATCATCTAACA

SRY
GATGGCTCTAGAGAATCCCCAAATGCAAAACTCAGAGATCAGCAAGCAGCTGGGGTACCAGTGGAAAATGCT
TACAGAAGCCGAAAAATGGCC

Fig. 5. Examples of allele sequences from the positive control DH82-D3167.
underlined: primer sequences, blue: repeat region.

References
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