

Duarte Nuno Vieira • Anthony Busuttil
Denis Cusack • Philip Beth
Editors



Acta Medicinae
Legalis et Socialis

L. Ganço^{1,2,3}, M. Carvalho¹, F. Balsa¹, A. M. Bento¹, A. Serra¹, M. J. Anjos¹,
A. Xufre⁴, F. Corte-Real^{1,5}

¹ Genetic Forensic Service Centre Branch of National Institute of Legal Medicine, Coimbra, Portugal

² Central Veterinary Hospital, Lisbon, Portugal

³ Egas Moniz Health Sciences Institute, Lisbon, Portugal

⁴ Dnatech, Lisbon, Portugal

⁵ Medicine Faculty of the University of Coimbra, Portugal

GENETIC IDENTIFICATION OF ANIMAL SAMPLES (*CANIS FAMILIARIS* AND *FELIS CATUS*) IN FORENSIC CONTEXT

Abstract: Pets live with people and place biological samples everywhere, which may be useful in a forensic context linking suspects and victims, to an occurrence.

There were analyzed samples of 63 unrelated dogs to the STR's markers PEZ1, FHC2054, FHC2010, PEZ5, PEZ20, PEZ12, PEZ3, PEZ6, PEZ8 and FHC2079, and 63 feline samples of unrelated animals for the FCA733, FCA723 and FCA731 markers.

Preliminary results show that it is possible to make genetic identification of individual animals of species under study, thus contributing to increase the potential of forensic samples of animal origin.

Keywords: Animal samples; STR's; *Canis familiaris*; *Felis catus*.

Introduction

In a criminal investigation, the biological collected samples are mostly human, but they are not the only forensic evidence.

Pets such as cats (*Felis catus*) and dogs (*Canis familiaris*), live with people and place biological samples such as hair, saliva and blood everywhere, which may be useful in a forensic context linking suspects and victims, to an occurrence.

There are three different types of animal DNA evidence:

- the animal as a witness (*e.g.* struggles of animals);
- the animal as an aggressor (*e.g.* animals involved in attacks on people);
- the animal as a victim (*e.g.* the remains of an animal lost or stolen).

The aim of this study is to implement techniques for individual animal identification through the analysis of short tandem repeats (STRs) for each specie under study, with different samples, namely hair and blood.

Materials and Methods

Blood samples of 63 unrelated dogs were extracted by the Chelex100® method (Walsh *et al.*, 1991) and hair samples were extracted by the DNA IQTM (Promega)

commercial kit. Amplification of PEZ1, FHC2054, FHC2010, PEZ5, PEZ20, PEZ12, PEZ3, PEZ6, PEZ8 and FHC2079 STRs was performed with the multiplex Canine StockMarks (Applied Biosystems) with the markers, according to manufacturer's instructions. The amplified product was applied in an automatic capillary electrophoresis sequencer ABI PRISM™ 310 Genetic Analyzer using the ROX 350 internal standard and analyzed with the GeneScan Analysis 3.1 software. The fragments sizes were compared with the described by Eichmann (2004).

For the feline samples blood of 63 unrelated animals was extracted by the Chelex100® method (Walsh *et al.*, 1991) and amplified for the FCA733, FCA723 and FCA731 markers in a multiplex reaction according Menotti-Raymond (2005). Detection of amplified product was performed as well as in dog samples using the Rox 500 internal standard

Results

The results presented in electropherograms (Figures 1 and 2) show that it can be possible to make genetic identification of *Canis familiaris* and *Felis catus* with the chosen markers.

Discussion

In a forensic context the limiting step can be the poor genetic material normally found in a crime scene.

The methods used in this study showed that it was possible to extract DNA from blood and hair leading to a good yield of DNA concentration (>3 ng/ul and <13ng/ul). All markers analysed appeared to be polymorphic, which is extremely important because it allows greater discrimination and thus a better individual identification.

Conclusion

The STRs markers used in this work showed that it is possible to make genetic identification of individual animals of both species under study, thus contributing to increase the potential of forensic samples of animal origin.

The studied markers proved to be highly informative and an important tool to assist in solving crime scene and casework related problems involving animal samples.

References

Walsh P.S., Metzger D.A., Higuchi R.; "Chelex 100 as a medium for a simple extraction of DNA for PCR-based typing from a forensic material"; Biotechniques; Vol 10, pp. 506-513; 1991.

MENOTTI-RAYMOND M.A. *et al*; “An STR Forensic Typing System for Genetic Individualization of Domestic Cat (*Felis catus*)”; Journal of Forensic Science; Vol. 50, n° 5, pp.1061 – 1069; 2005.

EICHMANN C. *et al*; “A Proposed nomenclature for 15 canine-specific polymorphic STR loci for forensic purposes”; International Journal of Legal Medicine; Vol.118, pp. 249 – 266; 2004.

StockMarks® Horse, Cattle and Dog Genotyping Kits – Protocol, Applied Biosystems.

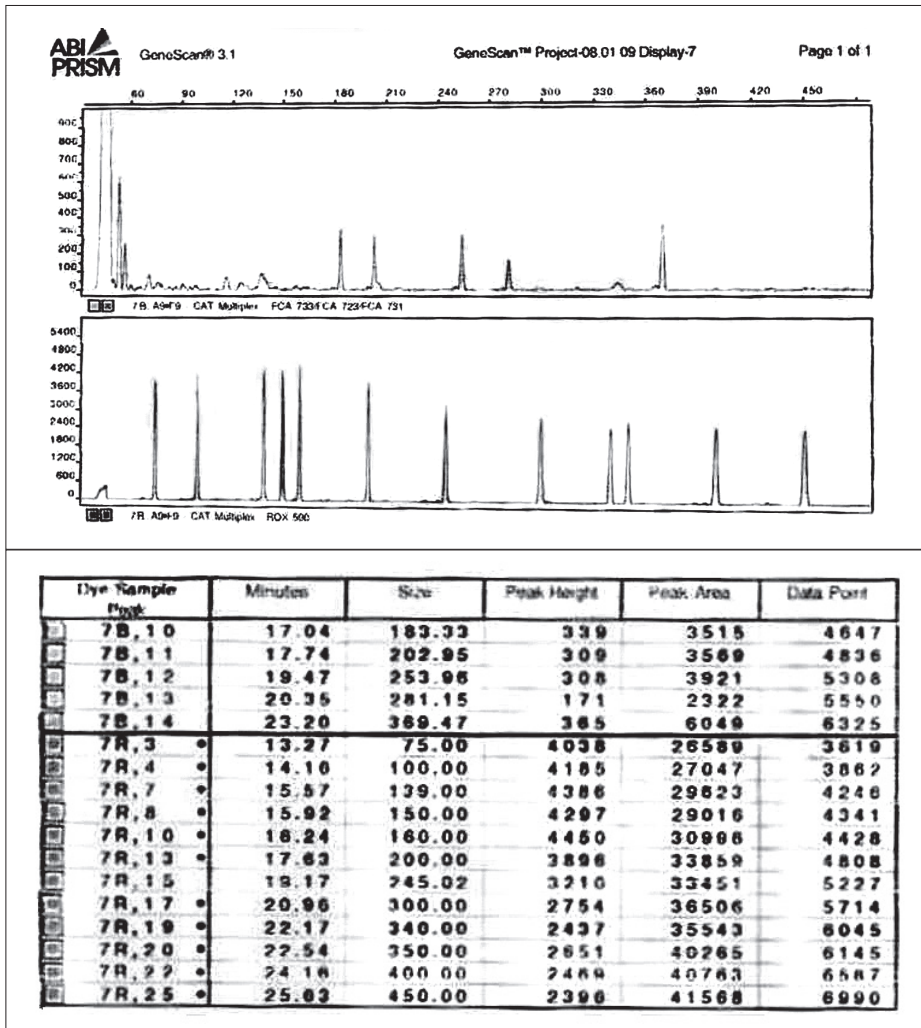
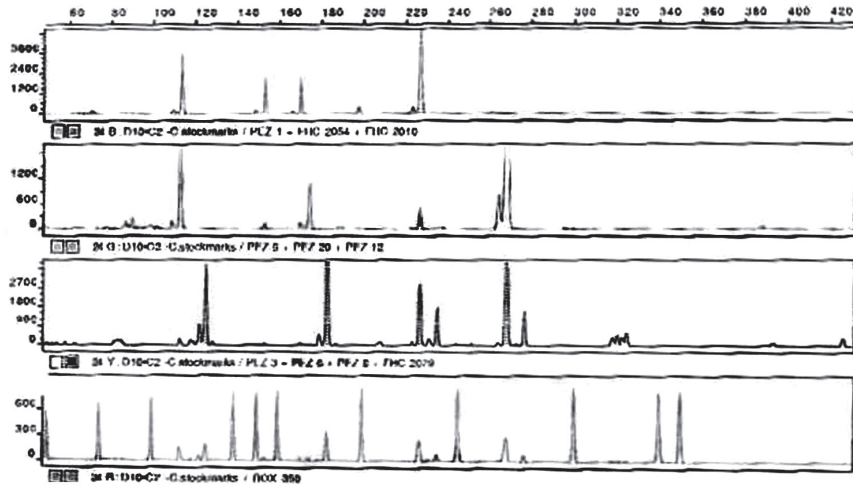


Figure 1 (a,b) – Felid electropherogram for the STR’s FCA733, FCA723 and FCA731.



Dyn/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
24B_5	14.12	113.90	3605	25861	3850
24B_7	15.37	153.52	2258	14623	4182
24B_9	15.89	170.43	2184	14108	4333
24B_12	17.69	227.06	7183	66107	4823
24G_17	14.10	113.26	5250	34650	3844
24G_20	16.03	174.95	1114	9021	4372
24G_24	18.93	268.44	5311	77976	5163
24Y_17	14.52	125.78	3853	26153	3959
24Y_23	16.30	183.35	6432	45850	4445
24Y_29	17.70	227.42	2929	30603	4826
24Y_31	17.95	235.54	1793	12836	4894
24Y_35	18.93	268.44	3984	48415	5163
24Y_37	19.17	276.58	1603	12422	5228
24R_1	11.78	50.00	597	3674	3212
24R_2	12.80	75.00	696	4299	3489
24R_3	13.64	100.00	764	4607	3718
24R_6	14.95	139.00	831	4975	4076
24R_7	15.27	150.00	833	5065	4164
24R_8	15.57	160.00	831	5172	4244
24R_10	16.84	200.00	861	5615	4592
24R_13	18.25	245.45	843	6170	4976
24R_16	19.85	300.00	859	6836	5412
24R_17	20.94	340.00	828	7145	5710
24R_18	21.26	350.00	829	7338	5797

Figure 2 (a,b) – Canid electropherogram for the STR's PEZ1, FHC2054, FHC2010, PEZ5, PEZ20, PEZ12, PEZ3, PEZ6, PEZ8 and FHC2079.