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The MeowPlex: A New DNA Test Using Tetranucleotide STR Markers for the Domestic Cat

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INTRODUCTION

With over 65 million cats in the United States today (Pet Food Institute, Washington, D.C.), a large percentage of U.S. households have a cat and thus an abundance of cat hair. With the appropriate DNA tools, cat hairs could be used by a forensic laboratory to provide a link between the perpetrator of a crime and a crime scene. An assailant may unknowingly carry clinging cat hairs from a victim's cat away from the scene of a crime, or hair from the perpetrator's cat may be left at the scene (1). Either scenario may provide a crucial link and help solve an important case. In the most famous case to date, DNA analysis of hair from a cat named Snowball was used to link a murder suspect to a crime scene—a result that led to a criminal conviction (2).

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Evidence from domestic cat hairs may play an increasingly larger role in the future due to a new cat DNA test that has recently been developed through a collaboration between the National Cancer Institute's (NCI) Laboratory of Genomic Diversity and the National Institute of Standards and Technology (NIST). For several years, the Laboratory of Genomic Diversity has been working towards the development of this cat STR typing system. The goals of this project are the selection of polymorphic tetranucleotide STR loci with characteristics similar to the 13 STR loci in use today for the FBI's Combined DNA Index System (CODIS), the creation of a robust multiplex STR assay for cats, and the development of population databases for various cat breeds.

In the Snowball case, a set of 10 dinucleotide repeat markers was used with singleplex PCR amplification (2,3). While a reliable match was made in that particular case, the high degree of stutter with dinucleotide repeats and the consumption of precious DNA with singleplex amplifications are undesirable. Because many cat hairs yield very little DNA suitable for STR analysis, multiplex PCR will be critical for obtaining substantial results in future cases.

SELECTION OF CAT STR MARKERS

Hundreds of STR markers have been mapped in the cat genome using techniques described previously (4). Initially, 22 potentially polymorphic tetranucleotide loci were selected for further characterization and possible inclusion in a final forensic panel. These STR markers were tested across approximately 200 cat DNA samples representing 29 different breeds (i.e., 5–10 samples per breed; ref. 5). From these initial studies, 11 highly polymorphic tetranucleotide loci were selected for inclusion in a multiplex assay.

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Table 1. Cat STR Markers Used in the MeowPlex Assay. Results obtained from typing over 200 cats representing 29 different breeds (5).

Locus Identifier	Dye Label	Chromosomal Location	Observed Heterozygosity (range across breeds)	Alleles Observed	Repeat Motif
F53	Blue	A1	0.53–0.93	9	Simple repeat [AAGA]
C08	Blue	B2	0.44–1.00	17	Compound repeat [ATAG][ATAC]
B04	Blue	A1	0.35–0.93	22	Compound repeat [AAGG][AAAG]
G11	Blue	B1	0.10–0.80	6	Simple repeat [ATCC]
FCA441	Green	D3	0.20–0.89	10	Simple repeat [ATAG]
D09	Green	B4	0.43–0.93	26	Compound repeat [ACAT][ATAG]
F124	Green	E1	0.51–0.93	20	Compound repeat [AGGA][AGAA]
C12	Green	F2	0.20–0.96	14	Complex repeat [AGAT][ACAT]
C09	Yellow	D4	0.00–0.94	15	Simple repeat [CTTT]
F85	Yellow	B1	0.66–0.98	33	Complex repeat [TTTC][TCTC]
D06	Yellow	C1	0.17–0.94	22	Simple repeat [TATC]
SRY	Green	Y	N/A	+/–	Gender ID

Selection criteria included Mendelian inheritance patterns, lack of linkage between loci (i.e., located on different chromosomes or well-spaced on the same chromosome), high heterozygosity across multiple breeds, no cross-species amplification, single target amplification, and absence of null alleles caused by sequence variation in primer binding regions. A mutation rate of less than 1 in 1,000 meioses was also a desirable characteristic, just as it is for human STR markers.

The cat STR loci selected for the forensic panel are listed in Table 1 along with their characteristics as determined in the initial study. The F85 locus is the most polymorphic and has alleles that span a range of more than 100bp. It should be noted that the names of the cat STR markers listed in Table 1 are not finalized and thus may change as they receive "FCAxxx" designations.

Cats have 18 pairs of autosomes and the sex chromosomes X and Y. Cat autosomes are designated A1, A2, A3, B1, B2, B3, B4, C1, C2, D1, D2, D3, D4, E1, E2, E3, F1, and F2, based on chromosome morphology, rather than the purely numerical format from largest to smallest used with human chromosomes. The forensic panel contains 11 STRs on 9 different autosomes. The markers F53 and B04 are on chromosome A1 while F85 and G11 map to chromosome B1. The cat STR loci that map to the same chromosome are greater than 50 centiMorgans apart based on cross-reference to tightly linked markers that are mapped in the radiation hybrid and genetic linkage maps of the cat (4,6). Thus, scientists should be able to combine the allele frequencies for each individual locus using the product rule to obtain a high power of genetic discrimination.

THE MULTIPLEX STR ASSAY

All 11 cat STR markers supplied to NIST from NCI were incorporated into a multiplex assay dubbed the "MeowPlex". A gender identification marker was also included in the multiplex through the addition of primers specific for the SRY gene on the cat Y chromosome (6). The PCR products from this 12plex multiplex amplification fall in the size range of 100bp to 400bp and use three dye colors. Primers were designed to leave approximately 20bp between each locus in the same dye color to reduce potential overlap if new alleles are discovered in the future. The allele size ranges and dye colors for the MeowPlex assay are illustrated in Figure 1. Four loci are labeled in blue, five in green, and three in yellow.

The use of familiar amplification conditions and PCR set-up and performance should ease the adoption of the MeowPlex assay by forensic DNA laboratories already performing human STR typing.

Primers amplify well using the same thermal cycling conditions used in common commercial STR kits, namely 28 cycles of PCR with an annealing

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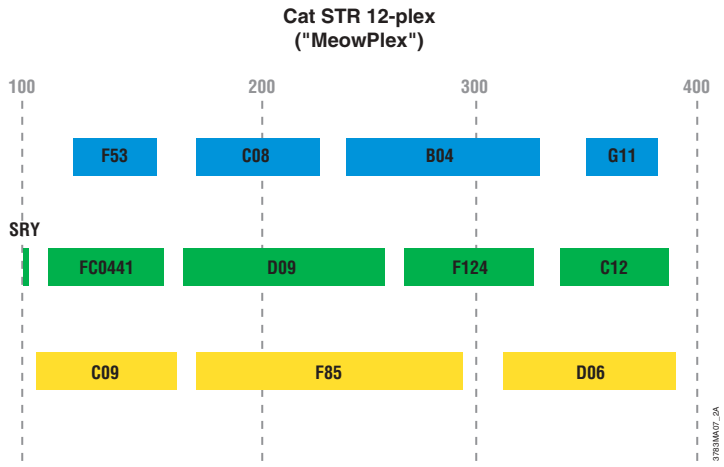

Figure 1. Schematic description of allele ranges and dye colors in the newly-developed cat STR 12plex (MeowPlex).

temperature of 59°C. The use of familiar amplification conditions and PCR set-up and performance should ease the adoption of the MeowPlex assay by forensic DNA laboratories already performing human STR typing. The MeowPlex typically requires 1–5ng of extracted DNA for population samples (Figure 2). The assay sensitivity has not been tested extensively at subnanogram levels but should be similar to commercial human STR kits.

The current dye labels used for the MeowPlex include 6 FAM (blue), VIC (green), and NED (yellow). When analyzed with the ABI PRISM® 310 Genetic Analyzer, these dyes are compatible with filter set F (4-dye) if the GS500 ROX size standard is used, or with filter set G5 (5-dye) if the GS500 LIZ size standard is used. Matrix standards are also commercially available to create the appropriate color separation matrices on a variety of instruments. The

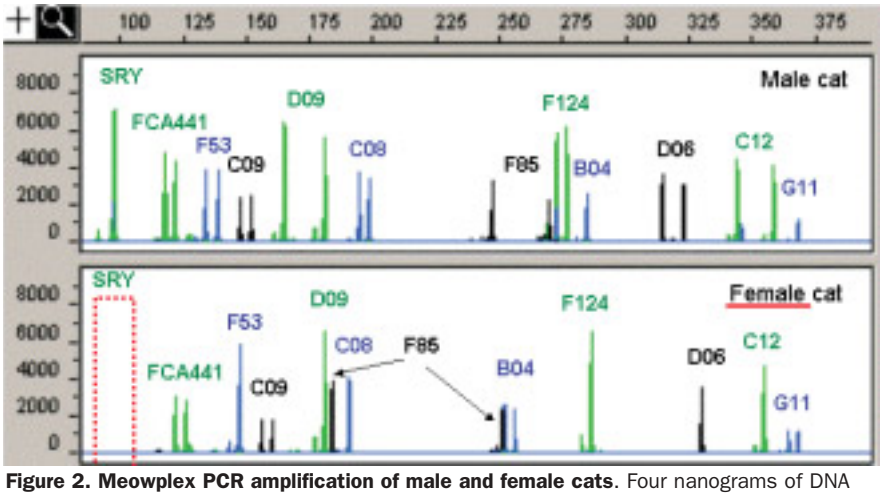

Figure 2. Meowplex PCR amplification of male and female cats. Four nanograms of DNA isolated from cat hair was amplified per reaction.

MeowPlex amplification products have been successfully analyzed using the ABI PRISM® 377 DNA Sequencer and ABI PRISM® 310, 3100, and 3700 Genetic Analyzers at NIST and NCI.

SPECIES SPECIFICITY WITH MEOWPLEX PRIMERS

DNA samples from 29 different species including humans have been tested with the MeowPlex in order to determine species specificity (Menotti-Raymond *et al.*, in preparation). Preliminary tests demonstrated that the MeowPlex is specific to individuals in the family Felidae (i.e., domestic cats and other felids such as pumas and ocelots). Monomorphic PCR products were observed in dog, wolf, and otter samples. No amplification is observed with human DNA using the MeowPlex primers.

On average, about 10ng of nuclear DNA can be recovered from the root of a single cat hair.

POPULATION STUDIES

Population studies are underway at the Laboratory of Genomic Diversity where DNA samples from over 1,200 cats representing the 37 different breeds recognized by the two largest cat breed registries in the United States (the Cat Fanciers Association and the International Cat Association) have been collected. An eight-generation pedigree has been obtained for most of the cats in the database so that levels of inbreeding and relatedness may be determined. Based on a preliminary population

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screen of 29 breeds across 10 of the 11 cat STRs (excluding G11), random match probabilities in the range of 10^{-7} to 10^{-13} are observed. Thus, the MeowPlex has good potential for genetic individualization of cats across all breeds. This fact will be important as only ~3% of cats are purebreds.

RECOVERY OF DNA FROM CAT HAIR ROOTS

Cat hair morphology and age appear to be associated with genotyping success. Preliminary results from experiments conducted at NCI indicate that more than 50% of the time STR results can be obtained from cat hairs containing roots. On average, about 10ng of nuclear DNA can be recovered from the root of a single cat hair (5).

CONCLUSION

A 12plex PCR multiplex has been developed for genotyping 11 STRs and a gender identification marker in domestic cats. The MeowPlex works under similar conditions and has similar sensitivities to human STR kits and is specific to cats and close relatives. Ongoing population studies should greatly enhance use of this nonhuman evidence. A commercial cat DNA test, in a format similar to current human STR analyses, could be available in the future to aid forensic DNA laboratories with analysis of this valuable evidence.

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Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that any of the materials, instruments, or equipment identified are necessarily the best available for the purpose.

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