J Forensic Sci, Sept. 2005, Vol. 50, No. 5
Paper ID JFS2004317
Available online at: www.astm.org

Marilyn A. Menotti-Raymond,[1] Ph.D.; Victor A. David,[1] M.S.; Leslie L. Wachter,[2] B.S.; John M. Butler,[3] Ph.D.; and Stephen J. O'Brien,[1] Ph.D.

An STR Forensic Typing System for Genetic Individualization of Domestic Cat (*Felis catus*) Samples*

ABSTRACT: A forensic genotyping panel of 11 tetranucleotide STR loci from the domestic cat was characterized and evaluated for genetic individualization of cat tissues. We first examined 49 candidate STR loci and their frequency assessment in domestic cat populations. The STR loci (3–4 base pair repeat motifs), mapped in the cat genome relative to 579 coding loci and 255 STR loci, are well distributed across the 18 feline autosomes. All loci exhibit Mendelian inheritance in a multi-generation pedigree. Eleven loci that were unlinked and were highly heterozygous in cat breeds were selected for a forensic panel. Heterozygosity values obtained for the independent loci, ranged from 0.60–0.82, while the average cat breed heterozygosity obtained for the 11 locus panel was 0.71 (range of 0.57–0.83). A small sample set of outbred domestic cats displayed a heterozygosity of 0.86 for the 11 locus panel. The power of discrimination of the panel is moderate to high in the cat breeds examined, with an average P_m of 3.7E-06. The panel shows good potential for genetic individualization within outbred domestic cats with a P_m of 5.31E-08. A multiplex protocol, designed for the co-amplification of the 11 loci and a gender-identifying locus, is species specific and robust, generating a product profile with as little as 0.125 nanograms of genomic DNA.

KEYWORDS: forensic science, domestic cat, STR, forensic typing system, multiplex amplification, cat breeds

The use of DNA markers to identify sources of crime scene evidence has revolutionized forensic science. Since the application of variable number of tandem repeat loci (VNTR) (1) and the more robust short-tandem repeat (STR) loci to forensic analysis (2–5), DNA genotyping for human genetic identification at scenes of crime, war and human disaster has become routinely used in hundreds of laboratories throughout the world. Until recently, the genetic individualization of biological samples of non-human origin, an alternative biological target of forensic investigation, has been limited due to the lack of species-specific hypervariable locus probes and population databases required to compute match likelihoods. However, with isolation of polymorphic STR markers across eukaryotic genomes (2,6), and their incorporation into genetic linkage maps of domestic animal gene maps (7–15), the tools for forensic identification of animals that are integral to our daily lives are now available.

[1] Laboratory of Genomic Diversity, National Cancer Institute at Frederick, Frederick, MD 21702.
[2] Basic Research Program, SAIC-Frederick, Inc., National Cancer Institute at Frederick, Frederick, MD 21702.
[3] Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, MD 20899.

* The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names imply endorsement by the U.S. Government.

This publication has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under contract #NO1-CO-12400.

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.

Received 7 Aug. 2004; and in revised form 12 Nov. 2004 and 3 April 2005; accepted 9 April 2005; published 3 Aug. 2005.

Previously, we reported on the genetic individualization of a domestic cat hair associated with a human crime scene, which was part of the physical evidence introduced to court contributing to a second-degree murder conviction (16,17). This legal precedent for introducing animal genetic individualization in a homicide trial stimulated an interest from a number of forensic laboratories for feline testing and demonstrated the need for a formalized STR forensic typing system for the precise genetic individualization of domestic cat specimens. The current test for human identification utilizes tetranucleotide repeat STRs that minimize stutter band products generated during PCR amplification (18) which facilitates the interpretation of genotypes from mixed DNA samples. Cat hairs will likely be the most common felid forensic sample. Whereas cat hairs are discreet and would not intuitively suggest the possibility of a mixed sample, grooming practices of cats can involve the licking of one another, introducing the saliva of one cat to another. As a first step in the development of a formalized feline STR typing system, we report on initial polymorphism screenings of candidate tri and tetra-nucleotide STR loci in outbred domestic cats, an in-depth characterization of 22 highly polymorphic tetranucleotide STR in 28 cat breeds, and the selection of 11 loci as a forensic panel for the genetic individualization of domestic cat samples. Additionally, we present a protocol for the multiplex amplification of the 11-member panel and a gender-identifying sequence tagged site (STS) from the domestic cat Y-chromosome SRY gene.

Materials And Methods

Cat Breed Sample Collection

Blood and buccal swab samples of domestic cats, representing 28 breeds recognized by the Cat Fanciers' Association (CFA) (http://www.cfainc.org/) and/or The International Cat Association (TICA) (http://www.tica.org/ cat registries), were donated by

Copyright © 2005 by ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959.

private breeders (under request for anonymity). Samples included: 10 Abyssinian, 7 American Shorthair, 5 Bengal, 9 Birman, 9 Bombay, 5 British Shorthair, 8 Burmese, 5 Chartreux, 4 Colorpoint Shorthair, 10 Cornish Rex, 10 Devon Rex, 10 Egyptian Mau, 10 Exotic, 9 Havana Brown, 3 Japanese Bobtail, 5 Korat, 8 Maine Coon, 8 Manx, 7 Norwegian Forest Cat, 5 Oriental Shorthair, 8 Persian, 8 Ragdoll, 9 Russian Blue, 9 Scottish Fold, 9 Selkirk Rex, 6 Siamese, 10 Sphynx, 7 Tonkinese. Blood samples from outbred domestic cat samples (6) were obtained from the NIH cat colony, which originated from Liberty Labs (Waverly, NY).

DNA Extraction

DNA was extracted from blood and buccal samples using Qiagen Qiamp® DNA Blood Midi and Mini Extraction kits following the suggested protocols of the manufacturer.

Pedigree

Mendelian inheritance testing was performed for the new candidate STRs in DNA samples from a multi-generation pedigree (267 individuals) of outbred domestic cats maintained by the Nestlé-Purina PetCare Company which the Laboratory of Genomic Diversity is utilizing to generate a full genome genetic linkage map of microsatellites in the domestic cat.

Amplification of Individual STR Loci Used in Initial (Table 1) Screening Studies

PCR amplification of individual STR loci using fluorescently labeled primers (Invitrogen) was performed as described (17,19), with the exception that a "touchdown" amplification protocol was used as follows: one cycle at 95°C for 10 min; two cycles each of the following set of conditions with the annealing temperature reduced by 2°C in each subsequent cycle (range 60°C–50°C): 95°C for 15 s, annealing temperature (60°C–50°C) for 30 s, 72°C for 45 s; 28 cycles with an annealing temperature of 50°C; one cycle of 72°C for 30 min; hold at 4°C.

Conditions and Amplification of Cat STR-Multiplex:

PCR amplifications were performed in 20 μL reaction volumes containing 1X PCR Gold buffer (Applied Biosystems) with final reaction concentrations: 1.5 mM magnesium chloride ($MgCl_2$), 200 μM of four deoxyribonucleoside 5′-triphosphates (dATP, dCTP, dGTP, and dUTP), 2.0 Units *AmpliTaq* gold DNA polymerase (Applied Biosystems), 0.16 mg/mL bovine serum albumin (BSA, fraction V: Sigma), 4 μL cat STR primer mix- see final concentrations Table 6. Optimal DNA quantities with the standard 28 cycle PCR conditions are 1–5 ng genomic DNA (Coomber et al., In preparation). Thermal cycling conditions were performed with the GeneAmp 9700 (Applied Biosystems) using the following conditions in 9600-emulation mode (i.e. ramp speeds of 1°C/s). Full adenylation of all peaks should be observed under these PCR conditions. Cycling conditions: 1 cycle 90°C for 10 min, 28 cycles: 94°C for 1 min, 59°C for 1 min, 72°C for 1 min; 1 cycle of 60°C for 45 min; 25°C hold.

Allele Detection and Analysis

PCR products were diluted appropriately and 3 μL of each amplified product was combined with 0.22 μL GS500 LIZ (Applied Biosystems) and 8.78 μL Hi-Di™ formamide (Applied Biosystems). The samples were electrophoresed on an ABI 3100 Genetic Analyzer using the following parameters; Dye Set: G5, Run Module: GeneScan36_POP4DefaultModule using the 3100 POP™ 4 sieving polymer (Applied Biosystems), 1X Genetic Analyzer Buffer with EDTA and a 36 cm capillary array. Data were collected using the ABI 3100 Data Collection Software Version 1.0.1 and analyzed using GeneScan Version 3.7 Analysis Software and GenoTyper™ Version 2.5 Software.

Population Genetic Analyses

Expected heterozygosities and allele frequencies were calculated using CERVUS (20) and Microsat ToolKit (21) where heterozygosity is computed as the unbiased expected heterozygosity according to equation 8.4 in Nei (22). Match probabilities were estimated using the software Powerstats (23) following Jones (24) without adjustment for sample size, given the size of the sample sets. Observed heterozygosity was computed as in Hedrick (25).

Results

We examined a total of 49 tri and tetra-nucleotide STR loci as candidates for a forensic panel for genetic individualization of domestic cat samples (Table 1). All loci have been mapped in either a radiation-hybrid (RH) (11) or genetic linkage map of the cat (10) relative to 579 Type I (coding genes) and 255 Type II (STR) loci, with the exception of locus FCA1058 which exhibited low retention frequency in the RH panel and has not been mapped in the genetic linkage map. All loci have demonstrated Mendelian inheritance patterns from genotype profiles exhibited in a family of domestic cats of several generations, 267 individuals and 483 potential informative meioses (data not shown) (Coomber et al., In preparation).

Table 1 summarizes the results of two sets of heterozygosity screenings: (1) An initial set of screenings was performed on all 49 loci in a set of outbred domestic cats ($n = 10$) (Seventeen of these 49 loci had been reported in an earlier study (10)). (2) Twenty-two loci which demonstrated high heterozygosity in outbred cats were then selected as candidates for screening in cat breeds, in order to select markers with the highest discriminating power for forensic analysis in cat breeds. (Heterozygosity values will be reported in this manuscript as expected heterozygosity, unless otherwise stated.) The data from both of these screenings are reported in Table 1. The 22 loci (Table 1, asterisk) were genotyped in a sample set of 28 cat breeds (3–10 animals/breed, $n = 213$), and a small sample set of outbred domestic cats ($n = 6$). Genotypes for the entire sample set are available at http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp. In subsequent tables we present measures of genetic diversity for the STR loci as analyzed in (1) the *entire* sample collection of individuals of recognized breed ($n = 213$) (2) the *individual* cat breeds, and (3) outbred domestic cats. Outbred domestic cats are individuals, which are not of a recognized breed. Allele frequencies, locus-specific allele sizes and distributions as observed in the individual breeds and across the complete set of breed individuals ($n = 213$) are presented in a supplementary table (Http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp).

The average expected heterozygosity that was observed across the complete set of individuals of recognized breed ($n = 213$) for the 22-locus set was relatively high at 0.80 (Table 2). Individual cat breeds demonstrated a range of heterozygosities for the 22 locus set, ranging from 0.51 to 0.80 (Table 2), while the average heterozygosity obtained for the sample set of outbred domestic cats for the 22-locus set was 0.81 (Table 2). Allelic diversity was high across the complete set of breed animals ($n = 213$), with an average

TABLE 1—*Forty nine cat tri and tetranucleotide repeat STR loci screened for inclusion in a DNA typing panel.*

STR*	STR Repeat	Chromosomal Assignment	Mapping Information[‡]	Range (bp)	Heterzygosity[§] (He)	Forward Primer[‖]	Reverse Primer
FCA 721	(GATA)9	A1	RH	176–192	0.71 (9)	CAATTTTTAATTGGGTTGGTGTT	CACTGCCGATAGAATGAAAAGA
FCA 722	(CA)8(CAA)10	A1	RH	199–211	0.69 (10)	GCTTTTGTTGATGGTGCGTA	ACAAATGGTGTGCCCTACAA
FCA 723*	(GGAA)8 G (GAAA)15	A1	RH	194–264	0.90 (211)	TGAAGGCTAAGGCACGATAGA	CGGAAAGATACAGGAAGGGTA
FCA 724	(GATA)8	A1	RH	202–218	0.65 (10)	GGTGATTAAACGTCAAAGGAGTC	GGTGCTGTGCCAGAGTTGTA
FCA 725*	(GATA)12	A1	RH	148–235	0.65 (211)	TGAATCACTATGTTGTACACCTGAAA	GAGGCTCAATTCTGATACCAAG
FCA 726	(GATA)11	A2	RH	229–245	0.66 (10)	GCACAGAGGATTCCCCATAA	GCCCCTGTTTGCTGTGTACT
FCA 727	(GTT)11	A2	RH	203–221	0.44 (8)	GCCATTTCTCAGCCATTGTT	TAGGAACACCGCTTGAGTGC
FCA 728	(GGAA)11	A2	RH	108–140	0.66 (10)	TTCAGCTTTTCCTCCTGACAA	CCTGCCTGTATTCCTCACAA
FCA 729	(CTAT)11/(CATC)11	A3	RH	158–194	0.77 (7)	AGCTCTCCAGTGGCTTCAGA	TTGTCCCTTCTCCCAGTCAC
FCA 730	(GATA)10	B1	RH	149–175	0.71 (6)	ATTGGGAATTGTAGCCAAGG	CTCCAAGTGGATGGAGCATT
FCA 731*	(CCAT)8/(CCAT)11	B1	RH	324–344	0.78 (211)	GATCCGTCTATCCATCTGTCC	ATGCTCCCCTGAAGCTGTAA
FCA 732*	(ATCT)7	B2	RH	230–262	0.67 (209)	CCTGTGGGTCGCTTCTACTT	AGAATTGCAAGGAGGCCACT
FCA 733*	(GATA)11	B2	RH	182–230	0.89 (195)	GAAGATGTGGGATAGATACACCAA	TGTTCAGAGGGCAAATTTCA
FCA 734	(GAAA)11	B3	RH	134–190	0.85 (9)	TGCTAGCTTCCTTCCTTGACA	ACTGGAGGGGTCAGGACTTT
FCA 735	(CCAT)6///(AC)15	B4	RH	101–109	0.62 (6)	TCAAGGCCAATTGTAGAGCA	TTCCATTCTCTATGGAATAGTCAGT
FCA 736*	(ATAC)10 (CA)3 (ATAG)14	B4	RH	196–280	0.89 (204)	ACCGAGCTCTGTTCTGGGTA	CAGACTGCACCACTGCCTAA
FCA 737	(GATA)10	C1	RH	197–205	0.64 (10)	TCTCCACTCTTCCCTCACTCA	CCACACCCTTTGGTCTCAGT
FCA 738*	(AAC)13	C1	RH	189–210	0.67 (210)	TCTTCACTGCTTCTGCCTCA	GTGGCCTGAGATGCTCTGAT
FCA 739	(GATC)9	C1	RH	191–235	0.85 (10)	GTGTCTGTATTTGTATCTGTATCTGT	AAAGGGAAGTGACCACTGGA
FCA 740*	(GATA)11	C1	RH	199–223	0.75 (209)	CACTTTCTGAAGGAGCAGCA	AACCAAATGGGAGTTTGTGG
FCA 741	(GAT)12	D1	RH	151–166	0.65 (7)	GCATGGAGAGGGTTTTAGCC	TTCGTCAAGTCCATGATAAATG
FCA 742*	(CTTT)11	D4	RH	123–175	0.87 (207)	TCAATGTCTTGACAACGCATAA	AGGATTGCATGACCAGGAAC
FCA 743	(GATA)10	D4	RH	204–220	0.50 (10)	CGACTCACACTCGCTGTCTC	CATTTGGTGGAGGTTTTGGT
FCA 744	(GATA)9	D4	RH	152–172	0.84 (10)	CATTGGGCCTACAGCCTACT	TCAACACCCTCACACCAATG
FCA 746	(GATA)10	D4	RH	178–194	0.66 (8)	GCCTCCCAGGATTATTTCAA	TGCGGTAAGGTGTTTCAAAG
FCA 747	(GATA)10	D4	RH	134–144	0.83 (9)	GCCTCTTTGGCAACCATTAG	TCTTGGAATTACTCCTGGTAAACA
FCA 748	(GATA)7/(GGAA)18	F2	RH	246–294	0.87 (10)	CCCACAATACCACTATGCTTCA	TTAGAGACTTTCCTTTGGTCTTTG
FCA 749*	(GATA)10/ (GATA)6	F2	RH	219–263	0.83 (208)	CTGGGGGAGGAGCTTACTTA	GATTTGAAAGAGGCCAGCAG
FCA 750*	(GATA)11///(GGAT)8	F2	RH	355–411	0.90 (127)	TTGGCCATGAGTTCAAGGTT	TTCTGTTTAAGGCAGTGTTTGC
FCA 1058	(GTTT)8	not mapped	low retention in RH	185–212	0.53 (6)	AACAGCCTCGTTCAAGGAAA	GAGTTCAAGGCCTGTGCTG
FCA 1059	(GAAA)9	A1	RH	121–141	0.74 (9)	TGAAAAGCATATGCAAAAGTTGA	TCTCCAAATTCCTATCTCACAAC
F115	(GAA)15	B2	GL	193–217	0.77 (10)		
F124*	(GAAA)15	E1	BOTH	211–265	0.89 (212)		
F141*[†]	(GAAA)13	A1	RH	278–336	0.89 (206)	GCAATGAGACTAGATGGAAGGA	GGCTCCAGATCCTTGTAGCA
F146	(GTT)9	A1	GL	145–158	0.73 (10)		
F164	(AAAC)9	C2	BOTH	146–166	0.65 (10)		
F27	(GAAA)14	B1	GL	97–129	0.71 (10)		
F37*	(TTTA)11	C1	BOTH	221–243	0.83 (164)		
F41*	(A)13(AAGG)11(AAAG)16	D2	BOTH	158–208	0.93 (211)		
F42*	(GAAA)13	A1	BOTH	202–238	0.80 (211)		
F49*	(TTG)8	D1	GL	110–122	0.57 (159)		
F53*	(GAAA)8	A1	GL	154–190	0.88 (211)		
F85*	(CTTT)10(CT)10(T)4(CTTT)15	B1	BOTH	216–316	0.94 (209)		
F98	(CAA)12	B4	BOTH	171–183	0.53 (10)		
FCA 391*	(GATA)10	B3	BOTH	240–268	0.62 (181)		
FCA 424	(GATA)11	C2	BOTH	171–187	0.68 (10)		
FCA 441*	(GATA)9	D3	BOTH	152–172	0.78 (210)		
FCA 453	(GATA)7	A1	BOTH	186–198	0.62 (10)		
FCA 559*	(GAAA)8 complex	B1	BOTH	134–202	0.76 (185)		

* 22 Loci selected for amplification in breed panel of 213 domestic cats; the 11 STRs in bold were tentatively selected for the DNA typing panel. DNA sequences for loci with FCA numbers above 700 and all F loci were submitted to GenBank and were assigned accession numbers. All other loci were previously submitted.

[†] Used published primers (10) for genotyping and used primers indicated for mapping in radiation hybrid panel. Unable to map first published primers in radiation hybrid panel due to amplification of product in hamster control.

[‡] RH-mapped in radiation hybrid panel (11), GL-mapped in genetic linkage map (10). Both-mapped in radiation hybrid panel and genetic linkage map.

[§] Heterozygosity (He)-Unbiased gene diversity (22); ( ), number of animals used for determination of He. [‖] Absent primers were previously published (10).

TABLE 2—*Population statistics for 22 STR loci in cat breeds and mixed breed domestic cats.*

Breed/Species	n	Loci	He*	Ho†	Av No Alleles/locus
Abyssinian	10	22	0.53	0.48	3.5
American Shorthair	7	22	0.70	0.61	4.5
Bengal	5	21	0.78	0.59	5.0
Birman	9	22	0.54	0.51	3.4
Bombay	9	22	0.58	0.51	3.4
British Shorthair	5	22	0.56	0.50	3.4
Burmese	8	22	0.65	0.59	4.2
Chartreux	5	22	0.67	0.55	3.9
Colorpoint Shorthair	4	21	0.70	0.54	3.5
Cornish Rex	10	22	0.61	0.54	4.1
Devon Rex	10	22	0.63	0.56	5.0
Egyptian Mau	10	22	0.54	0.46	3.6
Exotic	10	22	0.61	0.61	4.5
Havana Brown	9	22	0.51	0.48	3.6
Japanese Bobtail	3	22	0.65	0.55	3.0
Korat	5	22	0.63	0.64	3.5
Maine Coon	8	22	0.71	0.63	4.4
Manx	8	22	0.77	0.75	5.4
Norwegian Forest Cat	7	22	0.80	0.73	6.0
Oriental Shorthair	5	21	0.74	0.49	4.4
Persian	8	22	0.66	0.50	4.3
Ragdoll	8	22	0.68	0.57	4.5
Russian Blue	9	22	0.65	0.47	4.4
Scottish Fold	9	22	0.72	0.64	5.7
Selkirk Rex	9	22	0.70	0.69	5.3
Siamese	6	22	0.66	0.48	4.0
Sphynx	10	22	0.69	0.61	5.5
Tonkinese	7	22	0.67	0.55	4.4
Average	7.6	22	0.65	0.57	4.3
Complete breed set‡	213	22	0.80	0.57	14.3
Outbred domestic cats	6	22	0.81	0.67	5.6

* He-Unbiased gene diversity (22).
† Ho-Observed heterozygosity.
Complete breed set‡ = entire number of breed individuals analyzed as a unit.

of 14.3 alleles observed per locus (Table 2) (Http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp). The average number of alleles/locus observed within breeds was 4.3 alleles per locus, as compared to 5.6 alleles per locus in the sample set of outbred domestic cats (Table 2) (Http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp). The expanded allelic diversity in the complete set of breed individuals is likely a reflection of both the much larger sample size in this set ($n = 213$) relative to sampling size in the individual cat breeds (average = 7.6 cats/breed), and what appear to be reduced allelic ranges in individual breeds (Http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp). Though there were examples of distinctive allele sizes within some breeds (Http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp), a larger sample set will be necessary to confirm this observation.

Table 3 presents expected heterozygosities for the independent loci in (1) the complete set of breed individuals ($n = 213$), (2) the 28 individual cat breeds and (3) outbred domestic cats are presented. Locus-specific heterozygosities for the 22 markers across the complete set of breed individuals ($n = 213$) ranged from 0.57–0.95 (Table 3). Within the individual 28 cat breeds, locus specific heterozygosities were highly variable (Table 3). Additionally, there were marked differences among breeds in the distribution of alleles, size range and frequency of alleles for the 22 loci (Http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp). Three loci (FCA738, FCA742, F49) exhibited fixed allele sizes in a cat breed(s) (for sample sizes greater than 4 individuals). FCA738 was fixed in Birman, Chartreux and Selkirk Rex breeds, with the latter two breeds displaying a common fixed allele size (204 bp) (Http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp). Locus F49, which demonstrated the lowest average expected heterozygosity across the sample set (0.45), displayed a common fixed allele size in the Devon Rex, Tonkinese, Abyssinian and Korat breeds (118 bp) (Http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp). Locus FCA742 displayed a single allele size in the Havana Brown (127 bp) (Http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp). Individual breeds often exhibited reduced or compressed allelic size ranges relative to the complete range observed in the complete set of breed individuals (Http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp). The outbred domestic cat set of individuals, despite the small sample size, exhibited neither a fixed allele for any locus nor compressed allelic size ranges relative to the complete set of breed individuals. This suggests that the STR composite set will have high potential for genetic individualization in outbred domestic cats. Though there were examples of distinctive allele sizes within some breeds (Http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp), a larger sample set will be necessary to confirm this observation.

Selection of a Forensic Typing Panel of 11 STR Loci

A set of eleven highly polymorphic loci was selected as a forensic typing panel (Table I) from the 22 loci genotyped in cat breeds to optimize the potential to generate discriminating composite profiles. Selection criteria were based on identifying a panel of loci that were *both* unlinked and demonstrated high heterozygosity across multiple cat breeds. (Therefore, the loci with the 11 *highest* heterozygosities were not selected.) All loci selected are tetranucleotide repeat loci: six loci are simple tetranucleotide repeat loci (F53, FCA441, FCA733, FCA740, FCA742, F124; three are compound loci (FCA723, FCA731, FCA749), and two loci are complex (FCA736, F85) (Table 1) (GenBank Accession numbers AY988109–AY988153). The loci are well distributed across the cat's 18 autosomes, with 7 loci mapping to independent chromosomes (B2, B4, C1, D3, D4, E1 and F2), and four loci located on separate arms of the largest chromosomes A1 and B1 (26). Cat chromosomes, as opposed to being sequentially numbered like human chromosomes, are designated first by a letter which identifies chromosomes with similar centromere positions (27,28). We know from reference of closely mapped markers positioned in both the RH and genetic linkage maps (26) that the pairs of markers on chromosomes A1 and B1 are not genetically linked (i.e. recombination between them is greater than or equal to 50 percent).

Within individual cat breeds, average heterozygosities for the entire 11 locus set were moderately high, ranging from 0.57 (Abyssinian, Birman, Havana Brown) to 0.83 (Norwegian Forest Cat, Bengal) (Table 4). An overall average heterozygosity of 0.71 was obtained for the entire 11 locus set in the 28 breeds, as compared to an average heterozygosity of 0.86 obtained in the sample of outbred domestic cats (Table 4). Locus specific heterozygosities for the 11 independent loci ranged from 0.75 (FCA740) to 0.95 (locus F85) (Table 5). Within the entire set of breed individuals ($n = 213$), an average locus heterozygosity of 0.86 was obtained for the entire panel of STR loci. The number of alleles/locus observed for the 11 independent loci in the entire set of 213 animals averaged 15.6 alleles/locus (Table 5), ranging from 6 alleles in locus FCA731 to 32 alleles in the complex locus F85 (Http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp).

Match Probabilities

The probability of match (P_m) of each locus was calculated for the individual breeds according to the formula of Jones (24), essentially

TABLE 3—*Locus specific heterozygosities observed in cat breeds/species.*

			Locus Name										
Breed/Species	N		FCA391	**FCA441***	FCA559	**FCA723***	FCA725	**FCA731***	FCA732	**FCA733***	**FCA736***	FCA738	**FCA740***
Abyssinian	10	ABY	0.47	0.60	0.10	0.42	0.19	0.44	0.62	0.60	0.28	0.51	0.68
American Shorthair	7	ASH	0.65	0.50	0.78	0.79	0.74	0.76	0.65	0.68	0.71	0.40	0.69
Bengal	5	BEN	0.78	0.89	0.53	0.93	0.71	0.80	0.53	1.00	1.00	0.80	0.38
Birman	9	BIR	0.68	0.50	0.59	0.29	0.11	0.43	0.47	0.58	0.84	0.00	0.50
Bombay	9	BOM	0.61	0.66	0.37	0.48	0.50	0.47	0.50	0.61	0.51	0.50	0.61
Brithish Shorthair	5	BSH	0.46	0.20	0.00	0.62	0.53	0.78	0.25	0.75	0.62	0.64	0.71
Burmese	8	BUR	0.68	0.70	0.26	0.75	0.33	0.23	0.77	0.68	0.70	0.40	0.58
Chartreux	5	CHA	0.61	0.73	0.43	0.93	0.71	0.87	0.53	0.61	0.64	0.00	0.69
Colorpoint Shorthair	4	CSH	0.46	0.82	0.61	0.46	0.73	0.71	0.61	0.83	0.93	0.54	0.25
Cornish Rex	10	CRE	0.58	0.80	0.62	0.83	0.53	0.68	0.42	0.67	0.66	0.44	0.61
Devon Rex	10	DRE	0.39	0.50	0.81	0.69	0.10	0.74	0.36	0.36	0.90	0.76	0.70
Egyptian Mau	10	MAU	0.27	0.69	0.77	0.60	0.31	0.10	0.40	0.57	0.68	0.10	0.43
Exotic	10	EXO	0.31	0.62	0.46	0.82	0.42	0.57	0.20	0.39	0.55	0.56	0.70
Havana Brown	9	HAV	0.46	0.64	0.59	0.81	0.11	0.21	0.14	0.76	0.77	0.31	0.34
Japanese Bobtail	3	BOB	0.53	0.00	0.60	0.87	0.73	0.93	0.33	0.53	0.60	0.53	0.80
Korat	5	KOR	0.38	0.80	0.78	0.73	0.73	0.51	0.56	0.73	0.84	0.71	0.20
Maine Coon	8	MCC	0.63	0.76	0.49	0.73	0.68	0.78	0.62	0.91	0.67	0.43	0.80
Manx	8	MAX	0.60	0.77	0.53	0.75	0.63	0.67	0.71	0.82	0.89	0.68	0.78
Norwegian Forest Cat	7	WEG	0.71	0.74	0.64	0.89	0.82	0.86	0.51	0.86	0.91	0.67	0.66
Oriental Shorthair	5	OSH	0.50	0.60	0.71	0.89	0.62	0.82	0.80	0.82	0.96	0.61	0.47
Persian	8	PER	0.14	0.70	0.60	0.88	0.57	0.50	0.49	0.68	0.54	0.58	0.78
Ragdoll	8	RAG	0.60	0.78	0.17	0.83	0.34	0.82	0.77	0.74	0.92	0.59	0.58
Russian Blue	9	RUS	0.44	0.75	0.26	0.87	0.66	0.75	0.47	0.75	0.67	0.67	0.66
Scottish Fold	9	SFO	0.35	0.62	0.68	0.75	0.78	0.59	0.45	0.82	0.78	0.48	0.78
Selkirk Rex	9	SRE	0.44	0.62	0.71	0.67	0.70	0.72	0.61	0.86	0.58	0.00	0.78
Siamese	6	SIA	0.64	0.53	0.76	0.68	0.71	0.30	0.59	0.88	0.82	0.62	0.17
Sphynx	10	SPH	0.20	0.75	0.77	0.88	0.47	0.66	0.62	0.81	0.82	0.44	0.84
Tonkinese	7	TOK	0.82	0.74	0.62	0.66	0.53	0.53	0.47	0.87	0.89	0.44	0.62
Average	7.6		0.51	0.64	0.54	0.73	0.54	0.62	0.52	0.72	0.74	0.48	0.60
Complete breed set[‡]	213	Hetz.	0.62	0.78	0.76	0.90	0.65	0.78	0.67	0.89	0.89	0.67	0.75
Outbred domestic cats	6	FCA	0.67	0.71	0.91	0.88	0.83	0.84	0.44	0.96	0.91	0.85	0.83

		Locus Name										
Breed/Species	N	**FCA742***	**FCA749***	FCA750	F37	F41	F42	F49	**F53***	**F85***	**F124***	F141
Abyssinian	10	0.70	0.70	0.71	0.71	0.83	0.68	0.00	0.54	0.73	0.63	0.43
American Shorthair	7	0.89	0.82	0.46	0.53	0.81	0.73	0.53	0.89	0.77	0.85	0.85
Bengal	5	0.87	0.51	NA[†]	0.79	0.80	0.67	0.73	0.93	0.93	0.93	0.96
Birman	9	0.46	0.61	0.44	0.59	0.78	0.65	0.47	0.63	0.68	0.75	0.82
Bombay	9	0.78	0.74	0.61	0.78	0.78	0.58	0.20	0.58	0.79	0.56	0.58
Brithish Shorthair	5	0.62	0.84	0.82	0.33	0.89	0.36	0.33	0.64	0.71	0.51	0.76
Burmese	8	0.78	0.88	0.87	0.75	0.78	0.68	0.25	0.67	0.80	0.87	0.89
Chartreux	5	0.82	0.73	0.87	0.20	0.80	0.86	0.73	0.64	0.87	0.76	0.78
Colorpoint Shorthair	4	0.79	0.75	NA	0.61	0.93	0.75	0.60	0.75	0.86	0.75	0.86
Cornish Rex	10	0.77	0.52	0.47	0.57	0.86	0.62	0.27	0.65	0.85	0.78	0.19
Devon Rex	10	0.74	0.55	0.79	0.80	0.89	0.76	0.00	0.77	0.81	0.60	0.76
Egyptian Mau	10	0.85	0.61	0.87	0.77	0.51	0.66	0.50	0.67	0.78	0.55	0.11
Exotic	10	0.68	0.70	0.56	0.61	0.75	0.50	0.71	0.85	0.84	0.76	0.83
Havana Brown	9	0.00	0.40	0.41	0.00	0.81	0.69	0.67	0.78	0.84	0.76	0.71
Japanese Bobtail	3	0.87	0.60	1.00	0.53	0.87	0.53	0.00	0.80	0.87	0.80	0.87
Korat	5	0.56	0.20	0.82	0.73	0.71	0.78	0.00	0.78	0.80	0.73	0.78
Maine Coon	8	0.76	0.34	1.00	0.59	0.83	0.83	0.62	0.67	0.83	0.81	0.77
Manx	8	0.77	0.88	0.89	0.76	0.85	0.77	0.67	0.83	0.94	0.88	0.84
Norwegian Forest Cat	7	0.86	0.74	0.89	0.84	0.88	0.84	0.77	0.75	0.96	0.93	0.90
Oriental Shorthair	5	0.93	0.89	NA	0.43	0.84	0.89	0.51	0.82	0.84	0.84	0.80
Persian	8	0.71	0.68	0.50	0.54	0.90	0.79	0.77	0.62	0.93	0.83	0.83
Ragdoll	8	0.68	0.60	0.83	0.68	0.92	0.80	0.41	0.82	0.62	0.73	0.82
Russian Blue	9	0.68	0.80	0.53	0.41	0.65	0.61	0.54	0.78	0.76	0.80	0.69
Scottish Fold	9	0.86	0.80	0.88	0.62	0.87	0.69	0.66	0.75	0.93	0.85	0.78
Selkirk Rex	9	0.82	0.84	0.79	0.64	0.90	0.66	0.65	0.81	0.88	0.86	0.86
Siamese	6	0.71	0.88	0.80	0.20	0.88	0.74	0.43	0.71	0.80	0.82	0.89
Sphynx	10	0.62	0.83	0.82	0.79	0.86	0.44	0.49	0.85	0.66	0.84	0.80
Tonkinese	7	0.60	0.96	0.68	0.68	0.84	0.62	0.00	0.54	0.82	0.89	0.84
Average	7.6	0.72	0.69	0.73	0.59	0.82	0.68	0.45	0.73	0.82	0.77	0.75
Complete breed set[‡]	213	0.87	0.83	0.91	0.83	0.93	0.80	0.57	0.88	0.95	0.89	0.89
Outbred domestic cats	6	0.80	0.89	0.83	0.80	0.88	0.87	0.44	0.82	0.94	0.87	0.88

* The 11 loci selected for the DNA typing panel.
[†] NA-no amplification; loci which failed to amplify were not included in calculations of average heterozygosity.
[‡] Complete breed set-the entire 213 individuals analyzed as a single unit.

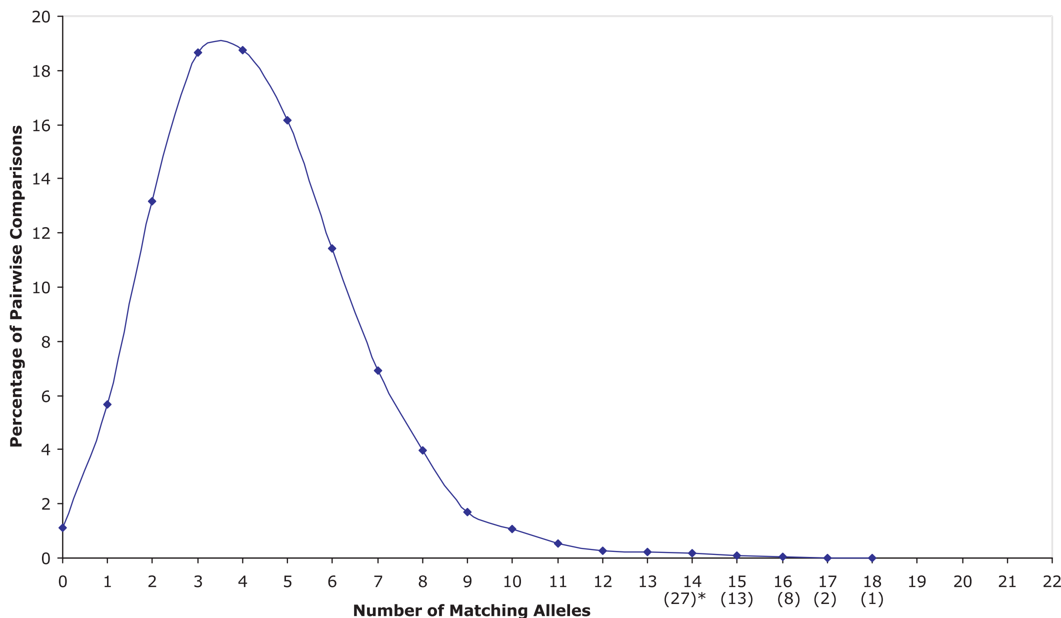

FIG. 1—*Number of Matching Alleles for All Pairwise Comparisons Between 213 Breed Cats Typed with 11 Proposed Core STR Loci. *For comparisons which had 14–18 matching alleles, the number of comparisons is indicated in parenthesis.*

TABLE 4—*Population statistics for 11 STR loci in cat breeds and mixed breed cats.*

Breed/Species	n	Loci	He*	Ho†	Av No Alleles/locus	Pm‡
Abyssinian	10	11	0.57	0.52	3.9	2.4E-06
American Shorthair	7	11	0.76	0.70	4.9	6.8E-08
Bengal	5	11	0.83	0.62	5.6	5.8E-07
Birman	9	11	0.57	0.59	3.5	6.0E-06
Bombay	9	11	0.62	0.56	3.9	1.1E-06
British Shorthair	5	11	0.64	0.47	3.5	4.8E-06
Burmese	8	11	0.69	0.62	4.7	1.7E-07
Chartreux	5	11	0.75	0.66	4.4	9.4E-07
Cornish Rex	10	11	0.71	0.71	4.6	1.1E-07
Colorpoint Shorthair	4	11	0.72	0.58	3.6	2.4E-05
Devon Rex	10	11	0.67	0.55	5.2	1.7E-07
Egyptian Mau	10	11	0.59	0.54	3.9	1.2E-06
Exotic	10	11	0.68	0.72	5.2	1.1E-07
Havana Brown	9	11	0.57	0.57	4.0	3.1E-06
Japanese Bobtail	3	11	0.70	0.58	3.4	4.7E-05
Korat	5	11	0.63	0.65	3.5	1.1E-05
Maine Coon	8	11	0.73	0.69	5.1	3.1E-08
Manx	8	11	0.82	0.80	6.2	4.6E-09
Norwegian Forest Cat	7	11	0.83	0.81	6.6	7.3E-09
Oriental Shorthair	5	11	0.81	0.61	5.2	3.0E-07
Persian	8	11	0.71	0.51	5.0	8.3E-08
Ragdoll	8	11	0.74	0.61	4.8	2.7E-08
Russian Blue	9	11	0.75	0.58	5.3	3.7E-08
Scottish Fold	9	11	0.77	0.73	6.3	6.5E-09
Selkirk Rex	9	11	0.77	0.81	5.8	4.9E-09
Siamese	6	11	0.66	0.45	4.2	9.5E-07
Sphynx	10	11	0.78	0.64	6.3	3.3E-09
Tonkinese	7	11	0.74	0.63	5.1	8.4E-08
Average	7.6	11	0.71	0.63	4.8	3.7E-06
Complete breed set§	213	11	0.85	0.63	15.6	
Outbred domestic cats	6	11	0.86	0.75	6.0	5.3E-08

* He-Unbiased gene diversity (22).
† Ho-Observed heterozygosity.
‡ Pm-Probability of match (24).
§ Complete breed set = entire 213 individuals analyzed as a single unit.

TABLE 5—*Summary statistics for 11 member STR cat forensic panel.*

Locus	Alleles*	Size Range† (bp)	He Breed‡	He (213)§	Breed Range He‖
FCA441	8	20	0.64 ± 0.19	0.78	0–0.89
FCA723	20	70	0.73 ± 0.16	0.90	0.29–0.93
FCA731	6	20	0.62 ± 0.22	0.78	0.10–0.93
FCA733	16	48	0.72 ± 0.15	0.89	0.36–1.0
FCA736	23	81	0.74 ± 0.17	0.89	0.28–1.0
FCA740	7	24	0.60 ± 0.19	0.75	0.17–0.84
FCA742	15	52	0.72 ± 0.18	0.87	0.00–0.93
FCA749	14	44	0.69 ± 0.18	0.83	0.2–0.96
F53	11	36	0.73 ± 0.17	0.88	0.54–0.93
F85	32	100	0.82 ± 0.08	0.94	0.62–0.96
F124	20	54	0.77 ± 0.11	0.89	0.51–0.93
Average	15.6	49.9	0.71 ± 0.16	0.85	

* Alleles-Number of alleles observed across complete data set of breed individuals ($n = 213$).
† Size range variation observed between largest and smallest products.
‡ He breed-Heterozygosity averaged for 28 breeds.
§ He (213)-Heterozygosity in composite breed set (entire 213 individuals analyzed as a single unit).
§ Breed range He-range in heterozygosity observed in 28 breeds.

the product for 11 loci of the sum of the frequency of observed genotypes squared ($P_1 = \sum P_i^2$) (Table 4). The probability that two unrelated individuals will match by chance at all 11 loci ranged within the 28 breeds from 4.7×10^{-5} observed in the Japanese Bobtail breed to 3.3×10^{-9} observed in the Sphynx breed (Table 4). Within the small set of six outbred domestic cat, a P_m of 5.3×10^{-8} was calculated (Table 4).

As an empirical study of the proportion of allelic matches between composite profiles of unrelated individuals using the 11 members STR panel, we determined the number of matching alleles for all pair-wise comparisons of individuals in the breed sample set. The distribution of matches is represented by a skewed normal distribution, with the number of matches ranging from 0–18 alleles (Fig. 1). The average number of matching alleles was 3.2.

TABLE 6—*Cat multiplex primer sequences and final concentrations.*

STR Marker	Final Concentration (uM)	Dye	Primer	Primer Sequence (5′–3′)	Size Range* (bp)
FCA733	0.8	6-FAM	F	GATCCATCAATAGGTAAATGGATAAAGAAGATG	128–226
			R	6FAM-TGGCTGAGTAATATTCCACTGTCTCTC	
FCA723	0.8	6-FAM	F	6FAM-TGAAGGCTAAGGCACGATAGATAGTC	243–317
			R	GCCACCCAGGTGTCCTGCTTC	
FCA731	1.6	6-FAM	F	6FAM-ATCCATCTGTCCATCCATCTATT	337–401
			R	GGTCAGCATCTCCACTTGAGG	
SRY	0.04	VIC	F	VIC-TGCGAACTTTGCACGGAGAG	96–97
			R	GCGTTCATGGGTCGTTTGACG	
FCA 441	0.3	VIC	F	GTGTCTTGATCGGTAGGTAGGTAGATATAG	113–137
			R	VIC-ATATGGCATAAGCCTTGAAGCAAA	
FCA736	0.1	VIC	F	VIC-CCGAGCTCTGTTCTGGGTATGAA	164–222
			R	GTGTCTTTCTAGTTGGTCGGTCTGTCTATCTG	
F124	1.1	VIC	F	VIC-TGTGCTGGGTATGAAGCCTACTG	255–367
			R	GTGTCTTCCATGCCCATAAAGGCTCTGA	
F53	0.8	PET	F	PET-CCTATGTTGGGAGTAGAGATCACCT	115–272
			R	GTGTCTTGAGTGGCTGTGGCATTTCC	
FCA 749	1.1	PET	F	PET-GAGGAGCTTACTTAAGAGCATGCGTTC	276–416
			R	GTGTCTTAAACCTATATTCGGATTGTGCCTGCT	
FCA742	1.1	NED	F	NED-AAATTTCAATGTCTTGACAACGCATAAG	122–175
			R	GCCAGGAACACCATGTTGGGCTA	
F85	1.3	NED	F	NED-TAAATCTGGTCCTCACGTTTTC	183–301
			R	GCCTGAAAATGTATCCATCACTTCAGAT	
FCA740	1.1	NED	F	NED-CCAAGGAGCTCTGTGATGCAAA	308–336
			R	GTTCCCACAGGTAAACATCAACCAA	

* Size range of PCR products generated in a sample set of 1043 domestic cats of recognized breed (Menotti-Raymond et al. In preparation).
Allele sizes and ranges differ from those presented in Table 5, as these products were generated from primer pairs redesigned for multiplex amplification and in a much larger sample set.

The largest number of matching alleles (18) was observed between individuals of the same breed (Abyssinian2498: Abyssinian2499); 13 alleles was the greatest number of matching alleles observed between individuals in different breeds, Colored Point Shorthair 621: Exotic 1938. These two breeds would not be considered to be closely related (29).

Development and Optimization of an STR Multiplex Amplification Protocol

Hair specimens are likely to be the most common sample from domestic cats associated with crime scenes. Our preliminary analyses have demonstrated that cat hair roots are a poor source of genomic DNA, yielding at most 30 nanograms of DNA from the very best fresh plucked guard hair roots (30). Hairs which are aged, shed, or originate from the undercoat (fine wool hairs) prove to be a much poorer source of DNA (30). To maximize information derived from hairs and other trace specimens collected at crime scenes, we developed a multiplex PCR reaction to assess simultaneously the 11 cat STR markers (31) with the addition of a gender-identifying STS from the SRY gene on the cat Y chromosome (32). PCR primers were designed that generate amplification products in a size range from 100 to 400 base pairs, with the SRY STS product detectable at 96 base pairs (31) (Table 6). Four fluorescent tags have been used to label products in order to eliminate the "overlap" of alleles of adjacent loci. Size ranges for the eleven loci, determined from a population genetic database of 1043 animals representing 38 cat breeds (Menotti-Raymond et al. In preparation), and fluorescent tags which include three primer pairs labeled in blue (6-FAM)(FCA733, FCA723, FCA731), four primer pairs labeled in green (VIC) (FCA441, FCA736, F124, SRY), three primer pairs labeled in yellow (NED) (FCA742, F85, FCA740), and two labeled in red (PET) (F53, FCA749), are illustrated in Fig. 2. We note that product sizes and ranges reported for the multiplex (Table 6) differ from those of Table 5, as these products were generated with new primer pairs and in a much larger sample set.

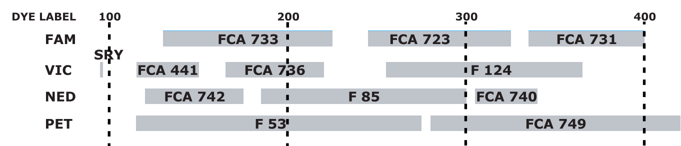

FIG. 2—*Fluorescent dyes and size ranges for domestic cat 12-plex as observed in a 1043 member domestic cat genetic data base generated from 38 cat breeds (Menotti-Raymond et al., in preparation). Locus designations have been renamed relative to Butler et al. (31). FCA 733 = C08; FCA 723 = B04; FCA 731 = G11; FCA 736 = D09; FCA 742 = C09; FCA 740 = D06; FCA 749 = C12.*

The "meowplex" is amplified using the same thermal cycling conditions used in commercial STR kits for the genotyping of human DNA, namely 28 cycles of PCR with an annealing temperature of 59°C. The use of common amplification conditions and PCR setup and performance should assist in easing the adoption of the cat multiplex by forensic DNA laboratories already performing human STR typing.

The 12-locus multiplex is robust and generates an easily interpretable product profile (Fig. 3). While we agree that the balance across all loci is not visually perfect, in practice, we found that the multiplex worked well for over 1200 domestic cats surveyed under these conditions (manuscript In preparation). Due to variation in primer quality, future investigators will likely need to adjust primer concentrations from those recommended here, to obtain balanced signal strengths.

Initial sensitivity assays demonstrate full product profiles are generated with between 1–2 ng of genomic DNA (Coomber et al. In preparation). Reliable profiles are obtained with concentrations of genomic DNA as low as 0.125 ng (Coomber et al. In preparation). These results are similar to those observed with the STR

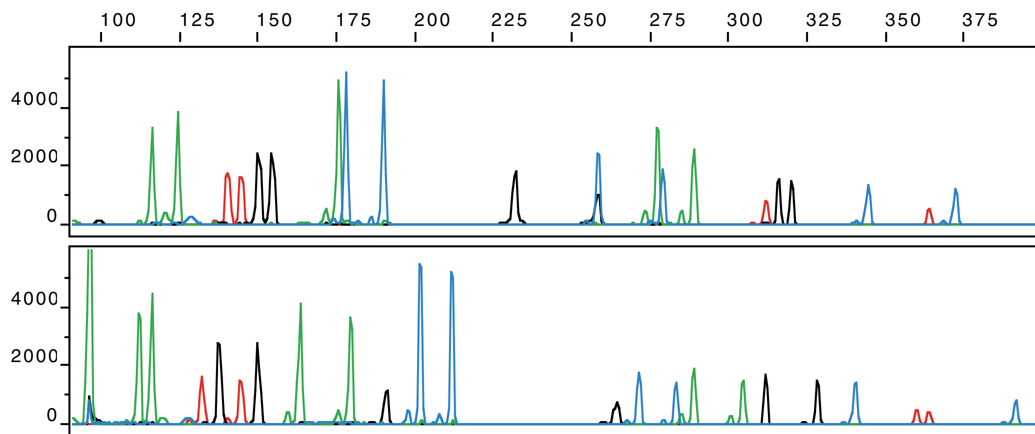

FIG. 3—*Chromatogram from 2 domestic cats amplified with multiplex panel and electrophoresed on an ABI 3100. The top panel is amplified from 5 ng of female domestic cat DNA and the bottom panel is amplified from 5 ng of male domestic cat DNA. The X-axis represents size of PCR products in base pairs; the Y-axis represents fluorescence units.*

CODIS loci used in human DNA identification, which require a minimal amount of 0.2 ng and an optimal amount of 1–2.5 ng of sample DNA (33,34). The size of the haploid cat genome has been estimated to be approximately 3.1 pg based on an average of several independent research studies (35). Therefore, approximately 20 whole genome equivalents are required to obtain a reliable profile from the "meowplex." Allele dropout, or the stochastic amplification of one allele of a heterozygote, was observed at DNA concentrations below 0.06 ng for all 11 STR loci (Coomber et al., In preparation). Details regarding electrophoresis running conditions and performance on different platforms for the multiplex can be obtained in Butler et al. (31).

To examine species specificity of the multiplex, the 11 tetranucleotide STR loci were examined in a range of North American mammalian species including badger, beaver, brown bear, chipmunk, cow, coyote, deer, dog, domestic cat, ferret, fox, goat, guinea pig, hamster, horse, human, mink, mole, mouse, ocelot, otter, pig, possum, puma, rabbit, raccoon, sheep, skunk, wolf, and two prokaryotes, *Sacchromyces cervesiae* and *Escherichia coli*. The multiplex displayed a high degree of specificity for DNA in the felid family with PCR products observed in ocelot, puma and domestic cat and products for two loci generated from brown bear (data not shown, Coomber et al. In preparation), another member of the Carnivore order. Under the standard amplification conditions, no products were observed in any other mammalian species or the prokaryotes.

Discussion

We have developed a tetranucleotide STR forensic typing system for the genetic individualization of domestic cat samples, which includes an 11 member STR panel, and a gender-identifying STS, which can be amplified in a single reaction. We elected to generate a population genetic profile and future genetic database of STR loci in cat breeds, as factors associated with the generation and propagation of breeds (founder effects, small effective population sizes, the use of popular sires, inbreeding, artificial selection), could have a strong influence on the genetic profiles of breeds and generation of population sub-structure. Although the majority of domestic cats maintained as pets in the United States are outbred in nature (approximately 97%) (http://www.cfainc.org/), an assumption cannot be made *a priori* that an evidence sample came from an outbred domestic cat. An STR panel developed for forensic analysis of cat samples must have adequate resolution for genetic individualization within the reduced gene pools of cat breeds.

The majority of cat breeds recognized by the two largest cat registries in the United States (CFA, TICA) have arisen within the last 100 years, as the result of artificial selection at loci determining coat color, pattern, hair length and other morphological characteristics. What is known about the generation of cat breeds is largely anecdotal in nature (29,36,37). Many breeds have been generated with a small number of founding animals, followed by inbreeding to fix alleles, which give rise to desired characteristics. The impact of these practices on a genetic level is evident in the present breed database. Observed heterozygosities were generally lower than expected heterozygosities (Tables 2, 4). Allele size ranges often appeared reduced in many of the cat breeds, or within a tight size range relative to the range observed in the entire set of breed individuals ($n = 213$) or the small sample set of outbred domestic cats (Http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp). Some breeds were fixed for a particular allele size, or did not amplify for a specific locus, suggesting a fixed "null" allele (Http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp). Additionally, distinctive patterns in locus-specific heterozygosities, allele frequencies and distributions were observed across the breeds (Table 3, Http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp). Similar allelic patterns and distributions have been observed in dog, sheep and cattle breeds (38–40), and are likely a reflection of factors associated with the generation and propagation of breeds (38, 41–43). An expanded breed sample set will offer additional information relative to these observations.

Our 11-member STR panel was selected from unlinked tetra nucleotide STR loci exhibiting high heterozygosity values in the 22-locus set examined in 28 breeds, in order to maximize the potential for genetic individualization. The cat breeds examined exhibit moderate to good levels of heterozygosity compared to those values observed in a small sample set of outbred domestic cats (Table 4), suggesting the panel has good potential for genetic discrimination of individuals within the 28 cat breeds examined. Across the entire sample set of 213 breed individuals examined, an average of 15.6 alleles per locus and locus heterozygosity of 0.85 was obtained for the panel of 11 STR (Table 4). The 11-member average heterozygosity of 0.86, and P_m obtained for outbred domestic cats (5.3×10^{-8}) suggest that the STR panel will have good potential for genetic discrimination across the more genetically diverse population of outbred domestic cats.

We believe that the match probabilities observed for this sample set of breed cats, ($P_m = 4.7 \times 10^{-5} - 3.3 \times 10^{-9}$) (Table 4) are influenced by the current sample sizes for many of the breeds (7.6 individuals/breed). The probability of match (P_m), as computed according to Jones (24), is the product for 11 loci of the sum of the frequencies of *observed* genotypes squared. The breeds which exhibit the lowest potential for genetic individualization in the sample set ($P_m = 10^{-5}$), the Japanese Bobtail, Colorpoint Shorthair and Korat, were observed in cat breeds represented by the smallest sample sizes, 3, 4, and 5 individuals respectively. With only 3–5 genotypes possible, this leads to a misrepresentation of the true number of genotypes in the breeds and their frequencies. However, if we compute P_m as the product for the 11 loci of all *possible* genotypes and their frequencies *expected* by populations in Hardy-Weinberg equilibrium, we observe P_m values with much greater potential for genetic individualization (data not shown).

A robust multiplex amplification has been developed (31), which co-amplifies the 11 STRs and a gender-identifying STS from the Y chromosome *SRY* gene (32). The multiplex is currently being genotyped in a sample collection of approximately 1050 cats representing 38 cat breeds recognized in the United States to generate a population genetic database with which to compute match probabilities. Additionally, a quantitative PCR assay has been developed for the quantification of genomic DNA extracted from domestic cat samples. The assay, targets highly repetitive genomic SINE elements (which occupy approximately 10% of the cat nuclear genome (44). The feline assay monitors product accumulation through a fluorescent dye, which binds to double-stranded DNA (i.e., SYBR® Green I) at the completion of each PCR cycle. Utilization of double stranded DNA binding dye maximizes sensitivity of the assay, as it detects the *population* of SINE elements amplified. As primers were designed in a region (polymerase region) that exhibited a high degree of sequence conservation across species, the assay is not species specific. However, we do not think that the lack of species specificity deters from the utility of the assay, as DNA mixtures are unlikely to be an issue with feline samples. The assay can be performed rapidly and is highly sensitive, detecting as little as 10 fg of feline genomic DNA (45).

As a cautionary note to researchers who would like to use the cat multiplex to amplify DNA in other members of the family Felidae, we advise amplification of the independent loci before attempting the multiplex. We have observed size-range overlap with adjacent loci, loci that fail to amplify (likely due to differences in primer target sites between species), and the need to adjust primer concentrations to achieve a balanced product profile (Menotti-Raymond et al., In preparation).

Acknowledgments

This project was supported by an Interagency Grant Number 1999-IJ-R-A079 awarded by the National Institute of Justice, Office of Justice Programs, US Department of Justice to the National Cancer Institute's Laboratory of Genomic Diversity. Points of view in this document are those of the author and do not necessarily represent the official position or policies of the US Department of Justice. The authors gratefully acknowledge the National Institute of Justice for funding of this research. We thank Alan Tereba, Promega Corporation, for modifying his software program, "Tools for Analysis of Population Statistics," to accommodate our data set. We thank the Nestlé-Purina PetCare Company for DNA samples used in Mendelian inheritance testing. We gratefully acknowledge the Cat Fanciers' Association (CFA) and The International Cat Association (TICA) for their considerable help in facilitating sample collection from cat breeds. We also thank the hundreds of independent cat breeders who provided us with blood and buccal swab samples of their cats and pedigrees. Without the help of these individuals and organizations, this research would not have been possible. Amy Snyder was a dedicated technician in this research project.

References

1. Jeffreys AJ, Brookfield JF, Semeonoff R. Positive identification of an immigration test-case using human DNA fingerprints. Nature 1985;317: 818–9. [PubMed]
2. Weber JL, May PE. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 1989;44:388–96. [PubMed]
3. Kimpton CP, Oldroyd NJ, Watson SK, Frazier RR, Johnson PE, Millican ES, et al. Validation of highly discriminating multiplex short tandem repeat amplification systems for individual identification. Electrophoresis 1996;17:1283–93. [PubMed]
4. Micka KA, Sprecher CJ, Lins AM, Theisen Comey C, Koons BW, Crouse C, et al. Validation of multiplex polymorphic STR amplification sets developed for personal identification applications. J Forensic Sci 1996;41:582–90. [PubMed]
5. Edwards A, Hammond HA, Jin L, Caskey CT, Chakraborty R. Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics 1992;12:241–53. [PubMed]
6. Tautz D. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res 1989;17:6463–71. [PubMed]
7. Williams JL, Eggen A, Ferretti L, Farr CJ, Gautier M, Amati G, et al. A bovine whole-genome radiation hybrid panel and outline map. Mamm Genome 2002;13:469–74. [PubMed]
8. Swinburne J, Gerstenberg C, Breen M, Aldridge V, Lockhart L, Marti E, et al. First comprehensive low-density horse linkage map based on two 3-generation, full-sibling, cross-bred horse reference families. Genomics 2000;66:123–34. [PubMed]
9. Crawford AM, Dodds KG, Ede AJ, Pierson CA, Montgomery GW, Garmonsway HG, et al. An autosomal genetic linkage map of the sheep genome. Genetics 1995;140:703–24. [PubMed]
10. Menotti-Raymond M, David VA, Lyons LA, Schaffer AA, Tomlin JF, Hutton MK, et al. A genetic linkage map of microsatellites in the domestic cat (*Felis catus*). Genomics 1999;57:9–23. [PubMed]
11. Menotti-Raymond M, David VA, Agarwala R, Schaffer AA, Stephens R, O'Brien SJ, et al. Radiation hybrid mapping of 304 novel microsatellites in the domestic cat genome. Cytogenet Genome Res 2003;102:272–6. [PubMed]
12. Barendse W, Vaiman D, Kemp SJ, Sugimoto Y, Armitage SM, Williams JL, et al. A medium-density genetic linkage map of the bovine genome. Mamm Genome 1997;8:21–8. [PubMed]
13. Vaiman D, Schibler L, Bourgeois F, Oustry A, Amigues Y, Cribiu EP. A genetic linkage map of the male goat genome. Genetics 1996;144:279–305. [PubMed]
14. Breen M, Lindgren G, Binns MM, Norman J, Irvin Z, Bell K, et al. Genetical and physical assignments of equine microsatellites–first integration of anchored markers in horse genome mapping. Mamm Genome 1997;8:267–73. [PubMed]
15. Marklund L, Johansson Moller M, Hoyheim B, Davies W, Fredholm M, Juneja RK, et al. A comprehensive linkage map of the pig based on a wild pig-Large White intercross. Anim Genet 1996;27:255–69. [PubMed]
16. Menotti-Raymond MA, David VA, O'Brien SJ. Pet cat hair implicates murder suspect. Nature 1997;386:774. [PubMed]
17. Menotti-Raymond M, David VA, Stephens JC, Lyons LA, O'Brien SJ. Genetic individualization of domestic cats using feline STR loci for forensic applications. J Forensic Sci 1997;42:1039–51. [PubMed]
18. Hauge XY, Litt M. A study of the origin of 'shadow bands' seen when typing dinucleotide repeat polymorphisms by the PCR. Hum Mol Genet 1993;2:411–5. [PubMed]
19. David VA, Menotti-Raymond M. Automated DNA detection with fluorescence-based technologies. In: Hoelzel AR, editor. Molecular genetic analysis of populations. Oxford, New York, Tokyo: Oxford University Press, 1998:337–70.
20. Marshall TC, Slate J, Kruuk LE, Pemberton JM. Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 1998;7:639–55. [PubMed]

21. Park SDE. Trypanotolerance in West African cattle and the population genetic effects of selection. Dublin: University of Dublin, 2002.
22. Nei M. Molecular evolutionary genetics. New York: Columbia University Press, 1987.
23. Tereba A. Tools for analysis of population statistics. Madison, WI: Promega Corporation, 2002.
24. Jones DA. Blood samples: probability of discrimination. J Forensic Sci Soc 1972;12:355–9. [PubMed]
25. Hedrick PW. Genetics of populations. Boston: Science Books International, 1983.
26. Menotti-Raymond M, David VA, Chen ZQ, Menotti KA, Sun S, Schäffer AA, et al. Second generation integrated genetic linkage/radiation hybrid maps of the domestic cat. J Hered 2003;94:95–106. [PubMed]
27. Wurster-Hill DH, Centerwall WR. The interrelationships of chromosome banding patterns in canids, mustelids, hyena, and felids. Cytogenet Cell Genet 1982;34:178–92. [PubMed]
28. Wurster-Hill DH, Gray CW. The interrelationships of chromosome banding patterns in procyonids, viverrids, and felids. Cytogenet Cell Genet 1975;15:306–31. [PubMed]
29. Fogle B. The new encyclopedia of the cat. New York, NY: Dk, 2001.
30. Menotti-Raymond M, David V, O'Brien SJ. DNA yield from single hairs (wool and guard/shed and plucked), success rate in amplifying STR and mtDNA targets, estimating DNA yield using multicopy target. Eleventh International Symposium on Human Identification. Biloxi, MS: http://www.promega.com/geneticidproc/ussymp11proc/abstracts/menotti_raymond.pdf, 2000.
31. Butler JM, David VA, O'Brien SJ, Menotti-Raymond M. The MeowPlex: a new DNA test using tetranucleotide STR markers for the domestic cat. Profiles in DNA 2002;5:7–10.
32. Murphy WJ, Sun S, Chen Z, Yuhki N, Hirschmann D, Menotti-Raymond M, et al. A radiation hybrid map of the cat genome: implications for comparative mapping. Genome Res 2000;10:691–702. [PubMed]
33. Micka KA, Amiott EA, Hockenberry TL, Sprecher CJ, Lins AM, Rabbach DR, et al. TWGDAM validation of a nine-locus and a fourlocus fluorescent STR multiplex system. J Forensic Sci 1999;44:1243–57. [PubMed]
34. PE Corporation AmpFlSTR® Profiler Plus™ PCR Amplification Kit User's Manual. Foster City, CA: Perkin Elmer Corporation.
35. Gregory TR. Animal genome size database. http://www.genomesize.com/. Last accessed: March 2005, 2005.
36. Budiansky S. The character of cats. New York: Viking, 2002.
37. Vella CM, shelton LM, Mcgonagle JJ, Stanglein TW. Robinson's genetics for cat breeders and veterinarians. Edinburgh: Butterworth Heinemann, 2003.
38. Parker HG, Kim LV, Sutter NB, Carlson S, Lorentzen TD, Malek TB, et al. Genetic structure of the purebred domestic dog. Science 2004;304:1160–4
39. Buchanan FC, Adams LJ, Littlejohn RP, Maddox JF, Crawford AM. Determination of evolutionary relationships among sheep breeds using microsatellites. Genomics 1994;22:397–403. [PubMed]
40. Hansen C, Shrestha JN, Parker RJ, Crow GH, McAlpine PJ, Derr JN. Genetic diversity among Canadienne, Brown Swiss, Holstein, and Jersey cattle of Canada based on 15 bovine microsatellite markers. Genome 2002;45:897–904. [PubMed]
41. Pihkanen S, Vainola R, Varvio S. Characterizing dog breed differentiation with microsatellite markers. Anim Genet 1996;27:343–6. [PubMed]
42. Zajc I, Mellersh CS, Sampson J. Variability of canine microsatellites within and between different dog breeds. Mamm Genome 1997;8:182–5. [PubMed]
43. Zajc I, Sampson J. Utility of canine microsatellites in revealing the relationships of pure bred dogs. J Hered 1999;90:104–7. [PubMed]
44. Yuhki N, Beck T, Stephens RM, Nishigaki Y, Newmann K, O'Brien SJ. Comparative genome organization of human, murine and feline MHC class II region. Genome Res 2003. [PubMed]
45. Menotti-Raymond M, David V, Wachter L, Yuhki N, O'Brien SJ. Quantitative polymerase chain reaction-based assay for estimating DNA yield extracted from domestic cat specimens. Croatian Med J 2003;44:327–31.

Additional information and reprint requests:
Marilyn Menotti-Raymond, Ph.D.
NCI-Frederick
Building 560, Room 11-38
Frederick, MD 21702-1201