

**PAPER****CRIMINALISTICS**

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## A Population Genetic Database of Cat Breeds Developed in Coordination with a Domestic Cat STR Multiplex\*

**ABSTRACT:** A simple tandem repeat (STR) PCR-based typing system developed for the genetic individualization of domestic cat samples has been used to generate a population genetic database of domestic cat breeds. A panel of 10 tetranucleotide STR loci and a gender-identifying sequence tagged site (STS) were co-amplified in genomic DNA of 1043 individuals representing 38 cat breeds. The STR panel exhibits relatively high heterozygosity in cat breeds, with an average 10-locus heterozygosity of 0.71, which represents an average of 38 breed-specific heterozygosities for the 10-member panel. When the *entire* set of breed individuals was analyzed as a single population, a heterozygosity of 0.87 was observed. Heterozygosities obtained for the 10 loci range from 0.72 to 0.96. The power for genetic individualization of domestic cat samples of the multiplex is high, with a probability of match ( $p_m$ ) of 6.2E-14, using a conservative  $\theta = 0.05$ .

**KEYWORDS:** forensic science, domestic cat, simple tandem repeat, forensic typing system, population genetic database, cat breeds

The use of DNA markers to identify sources of crime scene evidence has revolutionized forensic science. The routine use of simple tandem repeat (STR) markers applied to genetic individualization of human samples has become the realization of the potential of samples of nonhuman origin. The identification of an individual pet or other animal may provide the critical piece of information in a criminal investigation and prosecution. With the majority of American households (55%) housing at least one cat or dog (73 million cats, 68 million dogs) (1), it is not unusual for animal specimens, particularly hair specimens, to be part of the physical evidence associated with a crime scene (2–7). A study on the transfer and persistence of animal hair has demonstrated that it is almost impossible to enter a house where a domestic animal lives without becoming a “carrier” of its hair (8). Hairs from a pet can be indicative of a perpetrator’s presence at a crime scene or provide evidence of a connection between victim and perpetrator. Since 1996, DNA extracted from cat hair and dog blood, hair, and saliva from crime scene evidence has contributed to physical evidence leading to conviction of defendants in Canada and numerous states (2,4,5,9,10). While the genetic individualization of animal specimens

is in its infancy, a set of guidelines has been proposed for animal genetic identity testing (11).

We have developed an STR typing system for genetic individualization of domestic cat (*Felis catus*) samples. Previously, we reported on the characterization of a panel of 22 tetranucleotide STR loci in 213 domestic cats representing 28 breeds, from which 11 loci were selected for a forensic typing panel (12). A multiplex amplification protocol was generated and optimized for the 11 loci (12,13) and a gender-identifying sequence tagged site (STS) from the cat Y-chromosome *Sex determining Region-Y* gene (SRY) (14). We report on a population genetic database for the multiplex, with which to compute composite match probabilities, that was generated from a panel of 1043 domestic cat samples representing 38 cat breeds recognized by the two largest domestic cat registries in the United States, the Cat Fanciers’ Association (CFA) and The International Cat Association (TICA).

### Materials and Methods

#### *Cat Breed Sample Collection*

Blood and/or buccal swab samples of 1043 individuals representing 38 cat breeds recognized by the CFA (<http://www.cfa.org>) or TICA (<http://www.tica.org/>) were obtained from cat breeders through request for samples in directed mailings or contact with cat breeders at cat shows organized by CFA or TICA. The identity of individual owners is withheld at the request for anonymity from many of the participants. We note that there are some distinctions in the breeds and breed standards recognized by these two largest cat registries in the United States (data not shown). Additional samples were part of the Laboratory of Genomic Diversity’s DNA stock collection of felid samples (15). The sample set consisted of 29 Abyssinian, nine American Curl, nine American Wirehair, 26

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American Shorthair, 11 Balinese, 13 Bengal, 43 Birman, 16 Bobtail, 21 Bombay, 13 British Shorthair, 50 Burmese, 21 Chartreux, 13 Colorpoint Shorthair, 41 Cornish Rex, 57 Devon Rex, 18 Exotic, 49 Havana, 13 Javanese, 11 Korat, 21 Egyptian Mau, 19 Himalayan, 29 Manx, 43 Maine Coon Cat, 67 Norwegian Forest Cat, 19 Ocicat, 33 Oriental Shorthair, 51 Persian, 43 Ragdoll, 23 Russian Blue, 41 Scottish Fold, 35 Siamese, 14 Singapura, 24 Somali, 26 Sphynx, 28 Selkirk Rex, 19 Tonkinese, 17 Turkish Angora, and 28 Turkish Van. Pedigrees were requested from cat breeders to prevent the inclusion of first- and second-order relatives, whenever possible. Blood samples from outbred domestic cat samples (16) were obtained from the NIH cat colony, which originated from individuals obtained 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 manufacturer’s suggested protocols (Qiagen, Valencia, CA). DNA was quantified using a Hoefer DyNA Quant 200 Flurometer (Amersham BioSciences, Piscataway, NJ). A portion of each sample was diluted to a standard concentration of 2.5 ng/μL with sterile distilled water (Quality Biological, Gaithersburg, MD).

*Amplification and Electrophoresis and Genotyping of the STR Multiplex*

Samples were amplified and electrophoresed as described in Menotti-Raymond et al. (12). A fraction of samples (c. 96) was amplified from DNA bound to FTA® paper (Life Technologies, Gaithersburg, MD). For each sample, a 2-mm “punch,” generated with apparatus supplied by the manufacturer, was placed in a 96-well polymerase chain reaction (PCR) plate and washed following the manufacturers’ protocol. Each punch was then hydrated with 2 μL of distilled water, followed by the addition of 15 μL of PCR mix. Genotypes were analyzed using GeneScan Version 3.7 Analysis Software and GenoTyper™ Version 2.5 Software (Applied Biosystems, Foster City, CA). Alleles were assigned to “bins” using the Allelogram software application (<http://tech.groups.yahoo.com/group/allelogram/files>). The Allelogram software assigned the alleles into bins with a size range of ±0.5 base pairs (bp). The alleles are designated by their size in base pairs and not by the number of repeats, which is the common terminology used for alleles in the human STR multiplex. We have not yet correlated each bin with a specific number of repeats by sequencing representatives of each bin. The entire sample set was genotyped two times by two independent investigators in the same laboratory using the same equipment. Results were compared and discrepancies between “calls” were examined. In cases where there were discrepancies between calls, the two people making the calls looked at the data together and came to a consensus decision as to the correct call. There were no discrepancies that could not be resolved.

*Population Genetic Analyses*

Observed heterozygosities are the proportions of individuals heterozygous at a locus, and the averages ( $H_o$ ) of these over loci are shown in Table 1. Expected heterozygosities, or gene diversities, are calculated as one minus the sum of squares of observed allele frequencies at a locus, with the whole quantity being multiplied by  $n/(n-1)$ , where  $n$  is the number of individuals genotyped at the locus. The averages ( $H_e$ ) over loci of these expected heterozygosities are also shown in Table 1, and the differences between  $H_o$  and

TABLE 1—Heterozygosities and inbreeding coefficients for 10 STR loci in 38 cat breeds.

Cat Breed	<i>n</i>	Average $H_e^*$	Average $H_o^\dagger$	Average No Alleles/Loci	$f^\ddagger$
Abyssinian	29	0.64	0.65	5.5	-0.03
American Curl	9	0.83	0.74	7.0	0.10
American Shorthair	26	0.78	0.77	8.5	0.02
American Wirehair	9	0.77	0.77	6.2	0.00
Balinese	11	0.55	0.52	4.5	0.07
Bengal	13	0.79	0.68	7.6	0.15
Birman	43	0.59	0.52	5.8	0.12
Bombay	21	0.71	0.64	6.0	0.10
British Shorthair	13	0.67	0.61	5.2	0.08
Burmese	50	0.69	0.57	8.3	0.18
Chartreux	21	0.75	0.73	6.8	0.03
Colorpoint Shorthair	13	0.70	0.63	6.1	0.10
Cornish Rex	41	0.72	0.70	7.5	0.03
Devon Rex	57	0.65	0.62	9.0	0.05
Egyptian Mau	21	0.70	0.65	6.4	0.07
Exotic	18	0.77	0.73	7.4	0.05
Havana	49	0.53	0.51	5.5	0.03
Himalayan	19	0.73	0.69	7.6	0.05
Japanese Bobtail	16	0.77	0.71	6.5	0.07
Javanese	13	0.68	0.69	5.3	-0.03
Korat	11	0.53	0.51	4.4	0.04
Maine Coon Cat	43	0.80	0.75	10.8	0.05
Manx	29	0.84	0.79	10.1	0.07
Norwegian Forest Cat	67	0.85	0.82	14.4	0.03
Ocicat	19	0.64	0.56	5.1	0.13
Oriental Shorthair	33	0.72	0.59	8.6	0.19
Persian	51	0.77	0.69	10.3	0.10
Ragdoll	43	0.76	0.72	9.2	0.06
Russian Blue	23	0.70	0.64	7.5	0.08
Scottish Fold	41	0.82	0.78	10.5	0.05
Selkirk Rex	28	0.80	0.83	8.5	-0.03
Siamese	35	0.62	0.56	6.4	0.10
Singapura	14	0.57	0.45	4.0	0.23
Somali	24	0.66	0.54	6.3	0.16
Sphynx	26	0.78	0.71	8.6	0.09
Tonkinese	19	0.69	0.62	6.1	0.10
Turkish Angora	17	0.81	0.83	7.9	-0.04
Turkish Van	28	0.75	0.63	8.5	0.17
Average	27	0.71	0.66	7.4	0.07
Outbred cats	24	0.85	0.72	11.7	0.12

STR, simple tandem repeat.

\* $H_e$ -unbiased gene diversity.

† $H_o$ -observed heterozygosity.

‡Inbreeding coefficient.

$H_e$  lead to the estimated within-population inbreeding coefficients  $F_{IS}$  also shown in Table 1.

F statistics were calculated from the method of Cockerham and Weir (17). One thousand bootstrapping replicates were performed. The nominal confidence interval was 95%. Match probabilities were calculated according to Weir (18). For Appendix 4, an exact test for Hardy–Weinberg was performed utilizing a program by Weir (18).

Appendices can be accessed at the following website; <http://www.cstl.nist.gov/strbase/catSTRs.htm>.

**Results**

A panel of 10 felid-specific STR loci, amplified as a multiplex (12), has been used to generate a population genetic database of domestic cat breeds with which to compute composite match probabilities. An STS amplified from the Y-chromosome SRY gene is included in the multiplex for gender identification of samples (12). The multiplex has been amplified in a panel of 1043 samples representing 38 cat breeds recognized by the two largest cat registries in

TABLE 2—Heterozygosities and inbreeding coefficients for 10-member STR multiplex.

Locus	Alleles	Cat Chromosome	Size Range (bp)	$H_e$ Cat Breeds	$H_o$ Cat Breed	$H_e$ (Outbred)	$f$
F53	34	A1	115–272	0.91	0.73	0.89	0.20
FCA723	26	A1	243–317	0.90	0.75	0.87	0.16
FCA731	17	B1	337–401	0.89	0.70	0.81	0.21
F85	52	B1	183–301	0.96	0.61	0.95	0.36
FCA733	24	B2	128–226	0.90	0.73	0.89	0.18
FCA740	10	C1	308–336	0.72	0.55	0.71	0.24
FCA441	12	D3	113–137	0.78	0.61	0.77	0.22
FCA742	22	D4	122–175	0.88	0.67	0.90	0.24
F124	30	E1	255–367	0.89	0.72	0.90	0.19
FCA749	22	F2	276–416	0.84	0.69	0.83	0.18
Average	22.6			0.87	0.68	0.85	0.22

STR, simple tandem repeat.

$H_e$  breed-Nei's unbiased heterozygosity (36) averaged for 38 breeds.

$H_o$  breed-observed heterozygosity averaged for 38 breeds.

$H_e$  (outbred)-heterozygosity in outbred sample set ( $n = 24$ ).

the United States, the CFA and TICA. Sample sizes for each locus in each breed are presented in Appendix 1. A small sample set of outbred domestic cats ( $n = 24$ ) was genotyped to determine how well the multiplex would perform in the outbred cat population. We define "outbred" cats as individuals that are not a recognized breed, commonly identified as an "alley cat." Caution should be taken not to draw too many conclusions relative to the outbred cat population based on this small sample set, which was not the intent of this paper.

Cat breeds exhibit moderate to high locus heterozygosities, with a 10-locus average heterozygosity of 0.71, ranging in the 38 breeds from 0.53 to 0.85 (Table 1). Outbred domestic cats exhibited a 10-locus average of 0.85. Inbreeding coefficients for the 38 breeds and the outbred sample are presented in Table 1. We note that 12 of the 38 breeds have observed heterozygosities greater than that seen for the outbred sample, suggesting similar levels of inbreeding for the breeds and outbreds. We would not read too much into this observation, however, because of the small sample sizes for all 39 samples. Additionally, the outbred samples were obtained from two pedigrees maintained in an animal colony, which to the best of our knowledge represented unrelated individuals. However, some of the founders were obtained from a commercial supplier of laboratory animals (see Materials and Methods), and individuals may not have been completely unrelated.

The observed levels of inbreeding did not produce statistically significant departures from Hardy-Weinberg equilibrium (HWE), indicating that the levels were not significantly different from zero within breeds.

Heterozygosities for the individual loci utilizing all of the cats of recognized breed as a single data set are quite high, ranging from 0.72 to 0.96 (Table 2), and are not significantly different than heterozygosities obtained for the outbred cat sample set. Inbreeding coefficients per locus (Table 2) are quite variable, likely reflecting the small sample sizes of the individual breeds. The inbreeding coefficients are additionally high across the 10 loci (0.16–0.36) (Table 2), because they were generated from the entire 1043 member data set of 38 breeds that demonstrate marked allele frequency differences among breeds (Appendices 2, 3) and therefore exhibit the Wahlund effect (19). The cat breeds additionally demonstrated considerable variation in both allelic size ranges and distributions (Appendix 2).

Genotype frequencies observed for nine loci demonstrated expectations consistent with populations in HWE. One locus  $p$ -values for the 10 loci across the 38 populations largely demonstrated values of 0.05 or greater (Appendix 4). The exception was locus F85, for

TABLE 3—One-locus match probabilities.

Locus	Observed	Expected				
		$\theta = 0.000$	$\theta = 0.001$	$\theta = 0.005$	$\theta = 0.010$	$\theta = 0.050$
FCA723	0.020	0.019	0.019	0.020	0.022	0.038
FCA733	0.021	0.020	0.020	0.021	0.023	0.039
FCA731	0.028	0.024	0.024	0.026	0.028	0.044
FCA441	0.087	0.085	0.086	0.088	0.090	0.111
F124	0.024	0.022	0.023	0.024	0.026	0.042
FCA742	0.035	0.027	0.027	0.028	0.030	0.048
F85	0.009	0.003	0.003	0.003	0.004	0.013
FCA740	0.093	0.112	0.112	0.115	0.118	0.145
F53	0.016	0.014	0.015	0.016	0.017	0.032
FCA749	0.045	0.046	0.047	0.049	0.051	0.071

which 19 of the 38 breeds and the outbred sample set were statistically out of HWE.

One-locus match probabilities were constructed for the 10 loci, which examined only the 827 individuals that had complete 10-locus profiles. All 341,551 possible pairs were examined at each locus to see whether they had the same genotype at that locus (Table 3, Appendix 5). The proportions ranged generally from 1 to 10%, in contrast to human data that generally shows around 10% (Appendix 6). Appendix 7 lists all pairs that demonstrated at least 12 matching pairs of alleles out of 20. All but one of these pairs is for cats in the same breed, and almost all of them from the Havana breed, the breed that exhibited the lowest 10-locus heterozygosities. This reflects the differences of allele frequencies among breeds and breed-specific alleles. Another possibility is relatedness of individuals within breeds. The Havana is one of two breeds that demonstrated the lowest 10-locus heterozygosity (0.53) in the 38 cat breeds. Appendix 8 demonstrates genotypic profiles of individuals with at least 14 pairs of matching alleles. Of note, a very high degree of homozygosity is exhibited; one individual (HAV790) is homozygous at all 10 loci. For each locus, the proportion of pairs that had the same genotype was found ("Observed"; Table 3, Appendix 5) and compared to the match probabilities calculated according to the standard forensic match probability equations of Evett and Weir (20). One-locus match probabilities, at increasing values of  $\theta = 0.000$ –0.05, are presented in Table 3. Utilizing a  $\theta = 0$ , the expected values were impressively close to the observed values (Table 3).

The frequency of "null" alleles, a phenomenon that occurs when a locus fails to amplify because of lack of complementarity between primer and target site (21) or deletion of the target site,

can be difficult to assess. We have had the opportunity to examine directly the incidence of null alleles in our database. A comparison of the genotypes of individuals generated in both this study and a previous study which utilized a different set of primers, prior to the design of primers compatible for multiplex amplification (12,13), demonstrated the existence of null alleles in the database for loci F53 and F85 at a frequency of 0.01 and 0.12, respectively (data not shown).

An STS for the Y-chromosome SRY gene (14) was included in the multiplex for gender identification of samples. Amplification of this locus is extremely robust (22). Identical profiles were obtained for this locus across the 1043-member data set in two separate amplifications. Additionally, SRY genotyping confirmed the gender status of 886 of the 892 individuals for which we had pedigrees or records (458 females, 434 males). The six discrepancies observed, ostensibly three males and three females, were samples obtained from cat breeders which were mailed to us, and for which we had no visual contact to confirm the gender of the individual or the possibility of sample mix-up.

Whereas all the loci in the multiplex panel are tetranucleotide repeat loci, nine loci exhibited alleles that differ in size by either 2 bp (FCA441, FCA723, FCA733, FCA740, FCA742, F53, F85, F124) or 1 bp (FCA441, FCA123, FCA740, FCA742, F53, F85). Alleles differing by 1 bp have been reported because of the addition of a nontemplated +A product, often seen as an artifact of PCR amplification (23). However, in genotyping the 10 loci in a large multigeneration cat pedigree of 256 individuals (22,24), we have confirmed the identity of alleles differing by 1 bp in segregation analyses of heterozygous individuals (data not shown).

**Discussion**

We have elected to generate this first population genetic database in cat breeds, as opposed to outbred domestic cats. Many factors associated with the generation of breeds, including artificial selection for desired morphological characteristics, the widespread use of popular sires, founder effects, population bottlenecks, and small effective population sizes are recognized in population genetics as contributing factors to population substructure. Although almost all domestic cats maintained as pets in the United States are outbred in nature (*c.* 97%) (<http://www.cfa.org>), an assumption cannot be made *a priori* that an evidence sample came from an outbred domestic cat; additionally, there are millions of cats of recognized breed in the U.S.A. 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 and match probabilities should be generated informed from a knowledge base of population substructure in cat breeds.

Cat breeds exhibit moderate to high locus heterozygosities for the multiplex, with a 10-locus average ranging in the 38 breeds from 0.53 to 0.85 (Table 1). The small sample set of outbred cats demonstrated a heterozygosity of 0.85 for the 10-locus set (Table 1). The individual loci, as examined across the entire data set of breed cats, exhibited heterozygosities ranging from 0.72 to 0.96 (Table 2). The multiplex exhibits good potential for genetic individualization of domestic cat samples within cat breeds and in outbred domestic cats. The individual feline markers appear to be more discriminating than the CODIS markers utilized in human forensic applications (Appendix 6). Match probabilities generated for each locus demonstrate that utilization of  $\theta = 0$  generates one-locus match probabilities very close to what is observed in the data set (Table 3). In computing match probabilities utilizing the feline panel, we recommend taking a conservative approach utilizing a  $\theta$

large enough that the expected values are always larger than the observed values (Table 3). Taking  $\theta = 0.05$  appears to be sufficiently conservative for single-locus calculations (Table 3). Utilizing a  $\theta = 0.05$ , the power for genetic individualization of domestic cat samples of the 10-locus multiplex is relatively high, with a probability of match ( $p_m$ ) of 6.2E-14.

Genotype frequencies observed for nine loci demonstrated expectations largely consistent with populations in HWE (Appendix 4). The exception was locus F85, for which 19 of the 38 breeds and the outbred sample set were statistically out of HWE (Appendix 4). Careful considerations need to be made as to how this locus is to be used in forensic calculations.

Variation within populations is described by the within-population inbreeding coefficient  $F_{IS}$ , whereas variation among populations (or breeds) contributes to the total inbreeding coefficient  $F_{IT}$  and the total co-ancestry or population structure coefficient  $F_{ST}$ . This last parameter is also written as theta ( $\theta$ ) in the forensic literature. If there is random mating within populations,  $F_{IT}$  and  $F_{ST}$  are expected to be equal and  $F_{IS}$  equal to zero. The nonsignificant  $F_{IS}$  value is consistent with highly significant  $F_{IT}$  and  $F_{ST}$  values shown in Table 4. Alleles within individuals ( $F_{IT}$ ) or between pairs of individuals ( $F_{ST}$ ) within a population appear to be correlated when compared to alleles from different populations.

The numerical strength of a match between a crime-scene cat hair and a particular cat is calculated by supposing that the source cat is unknown, and likely of unknown breed. We advocate the use of the combined database for match probability calculations, along with a value of theta, or  $F_{ST}$ , that will accommodate allele frequency differences among breeds. The results shown in Table 3 suggest a theta value of 0.05.

We have dropped one locus, FCA736, from our original proposed multiplex of 11-loci (12) because of a high incidence of null alleles (~46%), concentrated generally in breeds of Southeast Asian ancestry (Balinese, Birman, Burmese, Colorpoint Shorthair, Javanese, Korat, Ocicat, Oriental Shorthair, Siamese, Singapura, Tonkinese) and the Russian Blue breed. Sequence analysis of FCA736 alleles, generated from the original primers, identified a deletion in the new multiplex-designed primer site of 12 bp that would prevent primer binding (data not shown). Because of size constraints relative to adjacent loci, it was impossible to redesign the primers to eliminate the null alleles.

The earliest fossil records that have been linked to the domestication of the cat date to *c.* 9500 years ago from Cyprus (16). We have recently reported on a Fertile Crescent origin for the domestication of *F. catus* from the Near Eastern wild cat, *Felis sylvestris lybica* (25). Cat breeds are relatively recent in origin from an evolutionary standpoint, within hundreds of years (26,27), and

TABLE 4—Population structure.

Locus	$f = F_{IS}$	$F = F_{IT}$	Theta = $F_{ST}$
FCA723	0.037	0.167	0.135
FCA733	0.037	0.189	0.158
FCA731	0.049	0.217	0.177
FCA441	0.075	0.219	0.156
F124	0.058	0.196	0.146
FCA742	0.058	0.244	0.197
F85	0.233	0.367	0.174
FCA740	0.064	0.244	0.193
F53	0.034	0.203	0.174
FCA749	0.032	0.181	0.154
Average	0.069	0.224	0.166

Upper and lower bounds for  $F_{IS}$ : 0.112, 0.041;  $F_{IT}$ : 0.264, 0.193;  $F_{ST}$ : 0.178, 0.154.



what is known about the history of cat breeds is generally anecdotal in nature. The majority of breeds recognized in the United States by the two largest cat registries (CFA and TICA) have received breed recognition only within the last 100 years (27). These breeds represent recent phylogenetic lineages that capture different combinations of coat color, hair length and texture, patterning, and distinctive morphological traits, many with homologous counterparts in coat color genes of the mouse and other domestic species (27). Several of these phenotypic variants of coat color and hair length have been mapped and characterized in the domestic cat (24,28–32). An interesting note in regard to coat color, which was a product of the generation of this data set, was our observation of the near fixation of FCA742 in the Havana breed, which led to the first application of association mapping in the cat. The fixation of FCA742 in individuals of the Havana cat breed was because of artificial selection for a specific allele of the closely linked tyrosinase-related protein 1 gene (*TYRPI*) (33) and led to the identification and characterization of *TYRPI* mutations causative of “chocolate” and “cinnamon” coat color variants in the Havana and several other cat breeds (29).

We have utilized this data set in a prior publication to generate a phylogenetic neighbor-joining tree of the 38 cat breeds based on distance matrices generated from proportion of shared alleles algorithm (Dps) from composite genotypes (34). The hierarchical branching order of the different breeds was generally unresolved, providing little evidence that one breed was “older” than the rest, though, it did offer support for several of the breed associations identified by STRUCTURE (34). This is not surprising given the extremely short history of these breeds. Of interest is that 96% of individuals in the data set assigned to their registered breed or population (34). Thus, a potential application of the multiplex would be to estimate for an unknown sample the breed from which an evidentiary sample was derived.

Initial validation studies of the multiplex (22) have been conducted following the recommendations of Budowle et al. (11), which examine reproducibility of the typing system, species specificity, sensitivity, ability to identify DNA mixtures and patterns of Mendelian inheritance. Mutation rates calculated for all of the loci based on inheritance patterns observed in a multigeneration pedigree used to generate a genetic linkage map of the cat (35) demonstrate rates within the range of sex-averaged rates observed for CODIS loci (22).

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