Abstract

Title of thesis: INSERTION AND EVOLUTION OF AN ENDOGENOUS RETOVIRUS INTO *KIT* IS RESPONSIBLE FOR MULTIPLE PHENOTYPES AT THE *WHITE* LOCUS IN THE DOMESTIC CAT

Andrea Coots Wallace, Master of Science, 2014

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The *Dominant White* locus (*W*) in the domestic cat demonstrates pleiotropic effects exhibiting complete penetrance for absence of coat pigmentation and incomplete penetrance for deafness and iris hypopigmentation. I preformed linkage analysis using a pedigree segregating *White* to identify *KIT* (Chr. B1), as the feline *W* locus. Segregation and sequence analysis of the *KIT* gene in two pedigrees (P1 and P2) revealed the remarkable retrotransposition and evolution of a feline endogenous retrovirus (FERV1) as responsible for two distinct phenotypes of the *W* locus, Dominant White, and White Spotting. The retrotransposition interrupts a DNase I hypersensitive site in *KIT* intron 1 that was previously demonstrated to regulate temporal and tissue specific expression of *KIT* in mice. A large

population-genetic survey of cats (*n*=269), supports our findings and demonstrates statistical significance of the FERV1 LTR and full-length element with Dominant White ($p < 0.0001$) and White Spotting ($p< 0.0001$), respectively.

ENDOGENOUS RETROVIRUS INSERTION IN KIT ONCOGENE DETERMINES WHITE AND WHITE SPOTTED IN DOMESTIC CATS

By

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Dedication

I would like to dedicate this work to the husband whom I adore – my fabulous Ben. You are the love of my life, my rock, and my forever friend. I usually can't believe you put up with me... thank you for letting me work this out and always being there for me in every way, your patience is incomprehensible.

I would also like to dedicate this work to my loving and supportive parents who allowed me to learn and grow through all life's experiences and yet were always there when I needed help, understanding, and love. And to my lovely sister Amanda, whom I can talk to about anything – and can always provide the most logical answer and a good laugh.

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Chapter 1: An Introduction

Chapter 1.1: History

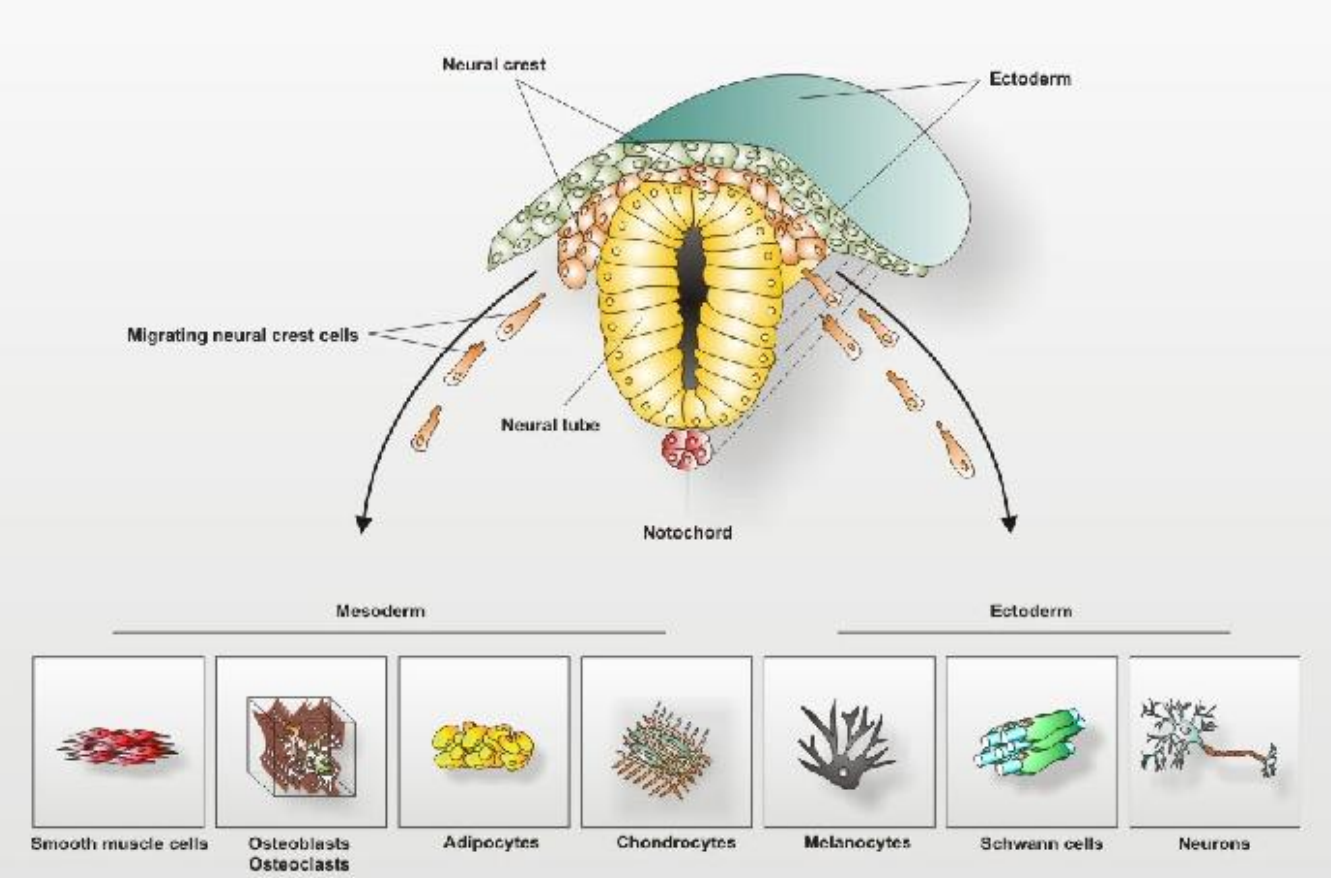
The congenitally deaf white cat has long been of interest to biologists because of the unusual co-occurrence of a specific coat color, iris pigmentation, and deafness, attracting the attention of Charles Darwin, among others (BAMBER 1933; BERGSMA and BROWN 1971; DARWIN 1859; WILSON and KANE 1959; WOLFF 1942). Multiple reports support the syndromic association of these phenotypes in the cat, as the action of a single autosomal dominant locus, *Dominant White* (*W*), with pleiotropic effects, exhibiting complete penetrance for suppression of pigmentation in the coat and incomplete penetrance for deafness and hypopigmentation of the iris (BERGSMA and BROWN 1971; GEIGY *et al.* 2006; WHITING 1919).

Chapter 1.2: Non-Feline Models

This phenotypic co-occurrence of deafness and hypopigmentation occur in multiple mammalian species, including the mouse, dog, mink, horse, rat, Syrian hamster, human (CHABOT *et al.* 1988; CLARK *et al.* 2006; FLOTTORP and FOSS 1979; HAASE *et al.* 2007; HAASE *et al.* 2009; HILDING *et al.* 1967; HODGKINSON *et al.* 1998; HUDSON and RUBEN 1962; KARLSSON *et al.* 2007; MAGDESIAN *et al.* 2009; RUAN *et al.* 2005; TSUJIMURA *et al.* 1991), and alpaca (Belinda Appleton, personal communication). In humans, the

combination is observed in Waardenburg syndrome Type 2 (W2), which exhibits distinctive hypopigmentation of skin and hair and is responsible for 5% of the cases of human congenital sensorineural deafness *(LIU et al. 1995).* Causal mutations for W2 have been characterized in six genes (*MITF, EDN3, EDNRB, PAX3, SOX10 and SNAI2*) (PINGAULT *et al. 2010*), with most individuals exhibiting mutations in only one of these genes. All of these genes are a part of the MITF transcriptional pathway (Supplemental Figure 1), which is required for proper development, differentiation, and migration of neural-crest derived cells (Figure 1), including melanocytes, mast cells, and osteoclasts along with non-neural crest retinal pigment epithelium cells.

Figure 1. Schematic displaying the migration of neural crest cells and the cell types into which neural crest cells differentiate. (Used with permission from the Kaltschmidt laboratory, University of Bielefeld)



Chapter 1.3: Precursors, Melanocyte Biogenesis, and Developmental History

Pigment cells in all vertebrates, with the exception of pigmented retinal epithelia, are derived early in embryogenesis from the neural crest, from which they migrate as melanocyte precursors (melanoblasts) (Figure 1), ultimately to differentiate into melanocytes and to reside in the skin, hair follicles, inner ear and parts of the eye. The eye is largely pigmented by melanocytes residing in the iris stroma (IMESCH *et al.* 1997). Genetic defects impacting the proliferation, survival, migration or distribution of melanoblasts from the neural crest are readily recognizable in coat hypopigmentation, and thus represent some of the earliest mapped genetic mutations (SILVERS 1979). Research on *White Spotting* loci in mice has been instrumental in understanding the molecular genetics underlying melanocyte biogenesis and migration, identifying many of the genes involved in critical early events in pigmentation, including *Pax3, Mitf, Snai2, Ednrb, Edn3, Sox10* and *Kit* (ATTIE *et al.* 1995; BAYNASH *et al.* 1994; CABLE *et al.* 1994; EPSTEIN *et al.* 1991; HERBARTH *et al.* 1998; HODGKINSON *et al.* 1993; SANCHEZ-MARTIN *et al.* 2002; SOUTHARD-SMITH *et al.* 1998; SYRRIS *et al.* 1999; TACHIBANA *et al.* 1992; TACHIBANA *et al.* 1994).

Chapter 1.4: White Pigmentation Phenotypes in the Cat

The cat displays several distinctive white pigmentation phenotypes that have been under selection by cat fanciers (VELLA *et al.* 1999): (1) Dominant White: uniform white coat, often accompanied by blue irises and

deafness, (2) White Spotting: variable distribution of white areas on the body, and (3) gloving: white pigmentation restricted to the paws. Albinism, the complete absence of pigment, is known to be caused by a locus, (*C*), distinct from *White*. The mutation implicated in albinism has been identified in the tyrosinase (*TYR)* gene, which codes for a critical enzyme in eumelanin synthesis (IMES *et al.* 2006). Albino cats have normal hearing; thus, pigment itself is not critical for the hearing process.

In 1919, Whiting proposed an allelic series at the feline *W* locus controlling white pigmentation in the cat, where *White* is an extreme of piebald and dominant in the allelic series *W (completely white)*> w^m (much spotted) > w^l (little spotted) > *w* (wild type) (Figure 2) (WHITING 1919). *White Spotting* has been reported as linked to the *KIT* locus, and gloving has been reported as exhibiting a mutation in the *KIT* locus (COOPER *et al.* 2006; LYONS, 2010). I report here data implicating two different, but related mutations in feline *KIT* as causative of *Dominant White* and *White Spotting,* respectively.

Figure 2. Pictures of dominant white (*W*), white spotted (w^s), and wildtype domestic cats.

.

Chapter 2: Materials and Methods

Chapter 2.1: Pedigrees and Population Study

White Pedigree

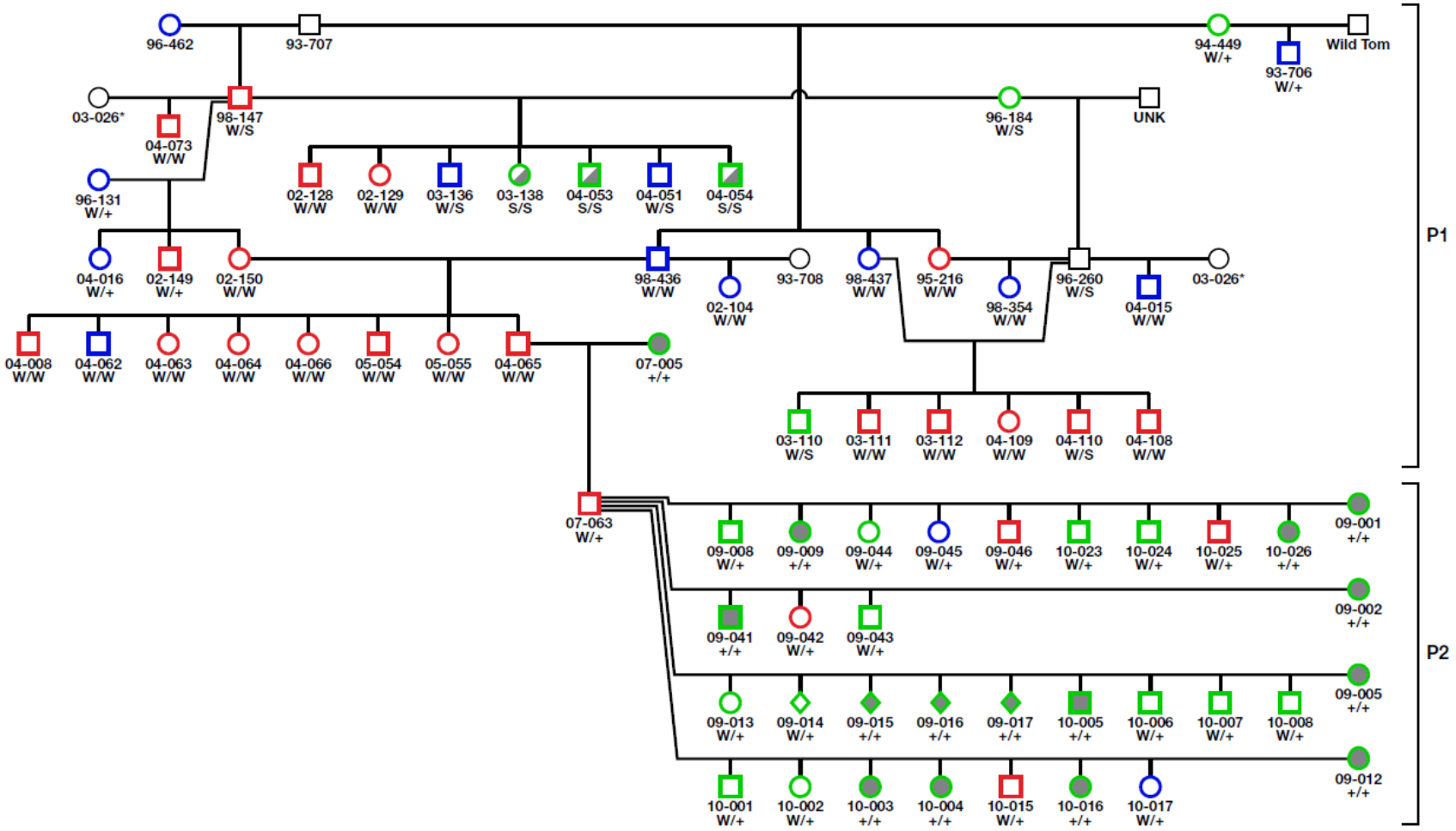
A domestic Deaf White Cat pedigree was maintained for approximately 20 years at The Johns Hopkins University (JHU) in order to research the physical basis of sensorineural deafness in these animals (MORGAN *et al.* 1994; SAADA *et al.* 1996) (RYUGO *et al.* 2003; RYUGO *et al.* 1997; RYUGO *et al.* 1998; SAADA *et al.* 1996) (Pedigree1 in Figure 3). The White Spotting phenotype was additionally observed at low frequency in more recent generations of the pedigree. Archival samples of genomic DNA from this pedigree were utilized in the analysis.

Pedigree Demonstrating Segregation of White

A second pedigree (P2) segregating for *White* and sharing one individual with Pedigree 1, was generated at JHU for mapping of the *W* locus (Figure 3). The progenitor of the pedigree, a white male (07-063), was generated to be heterozygous at *W* by mating a white, deaf male (04-065) with a fully pigmented (no white markings) female (07-005) (Liberty Laboratories) with normal hearing (Figure 3). The heterozygous (*W/+*) male was bred to four fully pigmented females (Liberty Laboratories) to produce 29 offspring, which included ten pigmented and 19 white individuals. A small kindred from a pedigree of cats reported in an earlier study (EIZIRIK *et al.*

2003), was utilized to examine the segregation of *White Spotting*. Genomic DNA from laboratory stocks of the Laboratory of Genomic Diversity was utilized in the study. All animal procedures were conducted in accordance with guidelines established by the NIH and the approval of the Animal Care and Use Committee of The JHU School of Medicine. When necessary, cats were humanely euthanized, as previously described (RYUGO *et al.* 2003) and in accordance with the Institutional Animal Care and Use Committee protocols approved at JHU (# CA10M273).

Figure 3. Depiction of the John's Hopkins University white deaf cat colony. Pedigree 1 (PI) illustrates matings of white to white cats in the JHU archival colony. Pedigree 2 (P2) illustrates pedigree developed to map the *W* locus. White symbols denote individuals with a white coat, gray colored symbols are fully pigmented individuals and half and half symbols (grey/white) are white spotted individuals. Hearing capacity is indicated by color outline of the individual: red outlined are deaf, blue have partial hearing, green have normal hearing, black are unknown. Genotypes are depicted below symbol: *W*, *White*; *S*, *White Spotting*, +, *wild type*.


96-462
93-707
94-449
W/+
Wild Tom
93-706
W/+
03-026*
98-147
W/S
04-073
W/W
96-184
W/S
UNK
96-131
W/+
02-128
W/W
02-129
W/W
03-136
W/S
03-138
S/S
04-053
S/S
04-051
W/S
04-054
S/S
04-016
W/+
02-149
W/+
02-150
W/W
98-436
W/W
02-104
W/W
93-708
98-437
W/W
95-216
W/W
98-354
W/W
96-260
W/S
04-015
W/W
03-026*
04-008
W/W
04-062
W/W
04-063
W/W
04-064
W/W
04-066
W/W
05-054
W/W
05-055
W/W
04-065
W/W
07-005
+/+
03-110
W/S
03-111
W/W
03-112
W/W
04-109
W/W
04-110
W/S
04-108
W/W
P1
07-063
W/+
09-008
W/+
09-009
+/+
09-044
W/+
09-045
W/+
09-046
W/+
10-023
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10-006
W/+
10-007
W/+
10-008
W/+
09-005
+/+
09-012
+/+
10-001
W/+
10-002
W/+
10-003
+/+
10-004
+/+
10-015
W/+
10-016
+/+
10-017
W/+
P2

A Population Genetic Survey of Cat Breeds

Genomic DNA extracted from whole blood or buccal swab samples from a previous study of cat breeds (MENOTTI-RAYMOND *et al.* 2007) was utilized in a population genetic survey of *White* and *White Spotting*. The sample set of 270 individuals included 33 Dominant White cats, 94 cats exhibiting White Spotting (i.e. either exhibiting white paws or bearing white on additional parts of the body) and 143 fully pigmented cats. The sample set represents individuals from 33 cat breeds, including 12 of 21 breeds that allow Dominant White and 16 of 22 breeds that allow White Spotting in their breed standards (Cat Fanciers' Association) (http://www.cfa.org/client/breeds.aspx).

Phenotypes were provided by the owner or from direct observation by Marilyn Menotti-Raymond. All cats were assigned a registry (FCA) number, and phenotypic data were recorded in a database at the LGD to preserve the anonymity of individual cats and their owners.

Chapter 2.2: Molecular Genetic Methodologies

Genomic DNA Extraction

Genomic DNA was extracted from whole blood or tissue using the QIAamp DNA Mini Kit (Qiagen). DNA was quantified using the NanoDrop 1000 spectrophotometer (Thermo Scientific).

Marker development and genotyping

STR selection: Primers were designed for amplification of short

tandem repeat loci (STRs) selected from the domestic cat genome browser (GARField) (http://lgd.abcc.ncifcrf.gov/cgi-bin/gbrowse/cat/) (PONTIUS and O'BRIEN 2007) that were tightly linked to eight candidate genes (Supplementary Table 1), whose orthologs had previously been implicated in a Dominant White phenotype or white-associated deafness.

Table 1. Linkage mapping of the domestic cat *White* locus.

Marker[a]	LOD[b]	θ[b]	Position in Santa Cruz browser (start, Chr.: Mb)[c]
KIT-A	6.32	0	B1: 161.77
KIT-B	6.32	0	B1:161.68
KIT-C	6.02	0	B1:161.64

[a] Markers are shown in genomic order along the domestic cat chromosome B1, on the basis of the most recent genetic linkage and radiation hybrid maps and cat genome assembly

[b] Logarithm of odds (LOD) score and recombination fraction (θ) for linkage between each polymorphic marker and the *WHITE* locus.

[c] Position in the domestic cat whole genome sequence, UCSC browser, Sept. 2011 (ICGSC Felis_catus 6.2/felcat5) Assembly

Amplification and genotyping of simple tandem repeat (STR) loci: PCR amplification was performed with a touchdown PCR protocol as described previously (MENOTTI-RAYMOND *et al.* 2005). PCR products were fluorescently labeled as described (BOUTIN-GANACHE *et al.* 2001). Sample electrophoresis was carried out as described previously (ISHIDA *et al.* 2006). Genotyping was performed using the software package Gene Marker (Soft Genetics, Version 1.85). Inheritance patterns consistent with expectations of Mendelian inheritance were checked as described previously (ISHIDA *et al.* 2006).

Genetic Linkage Analysis for *W*

To identify the *W* locus, single-marker LOD scores were computed using SUPERLINK (FISHELSON and GEIGER 2002; FISHELSON and GEIGER 2004) (http://bioinfo.cs.technion.ac.il/superlink-online/). I modeled *W* as a fully penetrant, AD disease with a disease allele frequency of 0.001. Marker allele frequencies were equal. A logarithm of odds (LOD) score was calculated for each of the markers (Table 1, Table 2).

Table 2. LOD scores for candidate genes (not including *KIT*).

Candidate gene	Marker	Recombination Fraction/LOD Score						
		0.00	0.01	0.05	0.10	0.20	0.30	0.40
EDNRB	EDNRB-1	-Infinity	-1.84	-1.02	-0.64	-0.28	-0.11	-0.03
EDNRB	EDNRB-2	-0.40	-0.25	0.02	0.15	0.18	0.12	0.04
EDNRB	EDNRB-3	-Infinity	-3.24	-1.78	-1.13	-0.51	-0.20	-0.05
SP1	SP1-1	-0.07	-0.06	-0.05	-0.04	-0.02	-0.01	-0.003
SP1	SP1-2	- Infinity	-1.40	-0.72	-0.44	-0.19	-0.08	-0.02
PAX3	PAX-1	- Infinity	-2.51	-1.18	-0.67	-0.25	-0.09	-0.02
PAX3	PAX-2	-0.18	-0.16	-0.11	-0.06	-0.02	-0.004	-0.0002
SNAI2	SNAI2-1	- Infinity	-4.21	-2.16	-1.33	-0.58	-0.23	-0.05
SNAI2	SNAI2-2	- Infinity	-4.21	-2.16	-1.33	-0.58	-0.23	-0.05
SNAI2	SNAI2-3	- Infinity	-5.61	-2.89	-1.77	-0.78	-0.30	-0.07
EDN3	EDN3-1	- Infinity	-4.21	-2.16	-1.33	-0.58	-0.23	-0.05
EDN3	EDN3-2	- Infinity	-1.40	-0.72	-0.44	-0.19	-0.08	-0.02
MITF	MITF-1	- Infinity	-2.59	-1.26	-0.73	-0.29	-0.11	-0.02
MITF	MITF-2	- Infinity	-2.80	-1.44	-0.89	-0.39	-0.15	-0.04
MITF	MITF-3	- Infinity	-2.51	-1.18	-0.67	-0.25	-0.09	-0.02
SOX10	SOX10-1	- Infinity	-1.17	-0.52	-0.28	0.09	-0.03	-0.006
SOX10	SOX10-2	- Infinity	0.20	0.17	0.14	0.08	0.04	0.01

Linkage and association testing for deafness: In this analysis, the two pedigrees in Figure 3 were combined into one, since they share an individual. To test whether the *KIT* FERV1 variation, encoded as a triallelic marker, is genetically linked to deafness, SUPERLINK (FISHELSON and GEIGER 2002; FISHELSON and GEIGER 2004) was used again. Deaf (D) or partially hearing (PH) individuals were coded as affected (2). A range of affection allele frequencies were tested between 0.001 and 0.05. An empirically derived penetrance function of 0.00 0.25 0.75 was varied in the range (0.15, 0.35) and (0.30, 0.80) for the second and third numbers, respectively, to test the robustness of the LOD scores to mis-estimation of the parameter values.

To test for association of deafness and the *KIT* variant as a marker, Alejandro Schäffer used MQLS (THORNTON and MCPEEK 2007) because it tests for association while controlling for known pedigree relationships. MQLS requires as part of the input pairwise kinship coefficients and inbreeding coefficients. These coefficients were computed with PedHunter (AGARWALA *et al.* 1998) after modifying the kinship and inbreeding programs of PedHunter to produce their output in the format required by MQLS. The MQLS program also requires as input a prevalence (of deafness), which was varied from 0.001 to 0.05 to test robustness of the results. MQLS option 2 was used, which ignores the individuals of unknown phenotype in estimating parameters. Combined linkage and association analysis was done with PSEUDOMARKER (HIEKKALINNA *et al.* 2011) with the empirical

model.

Amplification and Sequencing of *KIT* exons and 5' region of Intron 1

Primers were designed for PCR amplification in intronic regions flanking the 21 exons of *KIT* to include splice junction regions, and in the 5' region of intron 1 using the GARfield cat genome browser (Supplementary Table 2). The exons and the 5' region of intron 1 of *KIT* were amplified using a touchdown procedure and sequenced as described previously (Supplementary Table 3) (ISHIDA *et al.* 2006).

Table 3. White deaf pedigree data. Pedigree 1 and 2 refer to pedigrees P1 and P2 shown in Figure 3.

Sample	Sex	Coat color[a]	Sire	Dam	Hearing status[b]	Hearing threshold[c] (dB SPL)	Hearing threshold (dB SPL) left ear	Iris color[d]	Genotype[e] at *W* locus
Pedigree I									
02-104	F	W	98-436	93-708	PH	95	95		W/W
02-128	M	W	98-147	96-184	D	100	100		W/W
02-129	F	W	98-147	96-184	D	100	100		W/W
02-149	M	W	98-147	96-131	D	100	100		W/+
02-150	F	W	98-147	96-131	D	100	100		W/W
03-026	F	W	96-260	98-437	nd	nd	nd		nd
03-110	M	W	96-260	98-437	H	31	39		W/S
03-111	M	W	96-260	98-437	D	100	100		W/W
03-112	M	W	96-260	98-437	D	100	100		W/W
03-136	M	W	98-147	96-184	PH	50	100		W/S
03-138	F	S	98-147	96-184	H	33	33		S/S
04-008	M	W	98-436	02-150	D	100	100		W/W
04-015	M	W	96-260	03-026	PH	40	100		W/W
04-016	F	W	98-147	96-131	PH	50	50		W/+
04-051	M	W	98-147	96-184	PH	< 50	80		W/S
04-053	M	S	98-147	96-184	H	< 50	< 50		S/S
04-054	M	S	98-147	96-184	H	< 50	< 50		S/S
04-062	M	W	98-436	02-150	PH	50	90		W/W
04-063	F	W	98-436	02-150	D	100	100	BC	W/W
04-064	F	W	98-436	02-150	D	100	100	B	W/W
04-065	M	W	98-436	02-150	D	100	100	B	W/W
04-066	F	W	98-436	02-150	D	100	100		W/W
04-073	M	W	98-147	03-026	D	100	100		W/W
04-108	M	W	96-260	98-437	D	100	100		W/W
04-109	M	W	96-260	98-437	D	100	100		W/W
04-110	M	W	96-260	98-437	D	100	100	C	W/S
05-054	M	W	98-436	02-150	D	100	100		W/W
05-055	F	W	98-436	02-150	D	100	100		W/W
07-005	F	C	0	0	H	< 50	< 50		plus/plus
93-706	M	W	Wild Tom	94-449	PH	95	95		W/+
93-707	M	W	Wild Tom	94-449	nd	nd	nd		nd
93-708	F	W	0	0	nd	nd	nd		nd
94-449	F	W	0	0	H	30	30		W/+
95-216	F	W	93-707	94-449	D	100	100		W/W
96-131	F	W	0	0	PH	95	5		W/+
96-184	F	W	0	0	H	0	40		W/S
96-260	M	W	0	96-184	nd	nd	nd		W/S
96-462	F	W	0	0	PH	10	60		nd
98-147	M	W	93-707	96-462	D	100	100		W/S
98-354	F	W	96-260	95-216	PH	95	95		W/W
98-436	M	W	93-707	94-449	PH	95	95		W/W
98-437	F	W	93-707	94-449	PH	95	95		W/W
Wild Tom	M	W	0	0	nd	nd	nd		nd
Pedigree II									
07-005	F	C	0	0	H	< 50	< 50	C	plus/plus
07-063	M	W	04-065	07-005	D	>95	>95	C	W/+

09-001	F	C	0	0	H	nd	nd	C	plus/plus
09-002	F	C	0	0	H	nd	nd	C	plus/plus
09-005	F	C	0	0	H	nd	nd	C	plus/plus
09-008	M	W	07-063	09-001	H	30.4	37.5	BC	W/+
09-009	F	C	07-063	09-001	H	29.2	29.0	C	plus/plus
09-012	F	C	0	0	H	nd	nd	C	plus/plus
09-013	F	W	07-063	09-005	H	23.7	23.8	C	W/+
09-014	?	W	07-063	09-005	H	35.5	31.8	C	W/+
09-015	?	C	07-063	09-005	H	36.2	29.4	BC	plus/plus
09-016	?	C	07-063	09-005	H	39.4	39.1	C	plus/plus
09-017	?	C	07-063	09-005	H	34.8	31.5	C	plus/plus
09-041	M	C	07-063	09-002	H	36.4	40.4	C	plus/plus
09-042	F	W	07-063	09-002	D	>95	>95	C	W/+
09-043	M	W	07-063	09-002	H	34.6	34.7	C	W/+
09-044	F	W	07-063	09-001	H	40.1	nd	C	W/+
09-045	F	W	07-063	09-001	PH	56.6	52.2	B	W/+
09-046	M	W	07-063	09-001	D	>95	>95	C	W/+
10-001	M	W	07-063	09-012	H	34.8	28.6	C	W/+
10-002	F	W	07-063	09-012	H	38.8	29.3	C	W/+
10-003	F	C	07-063	09-012	H	38.3	29.3	C	plus/plus
10-004	F	C	07-063	09-012	H	38.8	27.5	C	plus/plus
10-005	M	C	07-063	09-005	H	38.5	25.5	C	plus/plus
10-006	M	W	07-063	09-005	H	33.9	34.5	C	W/+
10-007	M	W	07-063	09-005	H	35.3	29.7	C	W/+
10-008	M	W	07-063	09-005	H	36.4	37.3	C	W/+
10-015	M	W	07-063	09-012	D	>95	>95	C	W/+
10-016	F	C	07-063	09-012	H	34.4	33.9	C	plus/plus
10-017	F	W	07-063	09-012	H	44.1	55.2	C	W/+
10-023	M	W	07-063	09-001	H	40.7	45.1	C	W/+
10-024	M	W	07-063	09-001	H	48.8	36.8	C	W/+
10-025	M	W	07-063	09-001	D	>95	>95	C	W/+
10-026	F	C	07-063	09-001	H	37.3	39.1	C	W/+

[a] W, white; C, fully pigmented; S, white spotted

[b] D, Deaf, 100 db; H, normal hearing (< 50 decibels; PH, partial hearing (50-95 decibels)

[c] Hearing threshold; Sound pressure level (SPL) is a logarithmic measure of the effective sound pressure of a sound relative to a reference value. It is measured in decibels (dB) above a standard reference level.

nd:no data

[d] B: blue; C, copper

[e] W, FERV LTR; S, full length FERV; +, wild type; nd: no data as no DNA available

Amplification and Genotyping Assays Developed for FERV1 LTR and Full Length FERV1 Element

FERV1 LTR (*Dominant White* allele) amplification: Primers tagged with M13 tails were designed within genomic regions flanking the FERV1 LTR insertion site in *KIT* intron 1 (TGTAAAACGACGGCCAGTCACCCAGCGCGTTA (7FM13F); CAGGAAACAGCTATGACCCAAATCCTCCTCCTCCACCT (7RM13R)). Fragments were amplified using a TaKaRa LA *Taq* kit (TaKaRa, CloneTech), using GC BufferII following the manufacturer's suggestion. PCR conditions utilized: 94^{o} C for 1 minute followed by 30 cycles of 94^{o} C for 1 minute; 57^{o}C for 2 minutes 30 seconds, followed by an extension at 72^{o}C for 10 minutes. PCR reactions were visualized for presence/absence of products by electrophoresis in a 1% agarose gel and, to verify the presence of the FERV1 LTR insertion, by subsequent DNA sequence analysis of amplification products.

Full length FERV1 (*White Spotting* allele) amplification: (conducted by Victor David) The full length FERV1 insertion causative of White Spotting was amplified using PCR primers designed within genomic regions flanking the FERV1 LTR insertion site in *KIT* intron 1. Primers were M13 tailed and designed to anneal at 65^{o} C:

(KIT_65C_F_M13F):

TGTAAAACGACGGCCAGTATTTTGAGATCTGCAACACCCCTTC;

(KIT_65C_R_M13R):

CAGGAAACAGCTATGACCTCCTCCACCTTCAGACCTAAGTTCC.

PCR conditions were as described above using TaKaRa LA, except that Buffer I and an annealing/extension temperature of 63 º C for 7 minutes were used. Individuals carrying the *White Spotting* allele demonstrated a PCR product band in excess of 7 kbp detected by gel electrophoresis.

Three-primer genotyping assay designed for *White* (FERV1 LTR), *White Spotting* (full length FERV1 element) and wildtype alleles: (conducted by Victor David) A genotyping assay was developed to genotype wild type, *White Dominant* and *White Spotting* alleles in a single PCR reaction. The PCR reaction contained 3 primers, two in genomic regions flanking the FERV1/FERV1 LTR element and a third located within the full length FERV1 element. The primers and expected product sizes are presented in Supplementary Table 8. PCR amplification was performed with TaKaRa LA as described above except that the anneal/extension temperature was 63 C for 2.5 minutes using Buffer I. Products were visualized on a 2% agarose gel.

Identification of the LTR Repeat Type

After identifying and sequencing the LTR in white cats, the cat genome (September 2011 ICGSC Felis_catus 6.2 assembly) (GenBank Assembly ID: GCA_000181335.2) was interrogated for sequences homologous to the LTR using BLAT (KENT 2002) at the UCSC genome browser (http://genome.ucsc.edu/). There were 102 highly homologous sequences with BLAT scores >1000. The top hit was on chromosome D1-

116687546...116694444, which demonstrated 98.4% identity over a span of 6333 bp. RepeatMasker (SMIT *et al.*) identified the repeat element as being part of an endogenous retrovirus (ERV) Class I repeat. The top hit was to ERV1-1_FCa-I. (ANAI *et al.* 2012). A.F.A. Smit, R. Hubley & P. Green, unpublished data. Current Version: open-4.0.0 (RMLib: 20120418 & Dfam: 1.1). A.F.A. Smit, R. Hubley & P. Green, unpublished data. Current Version: open-4.0.0 (RMLib: 20120418 & Dfam: 1.1).

Sequence analysis of full-length FERV1

(conducted by Victor David)

To sequence the > 7kbp product, sequencing primers were designed from the previously published FERV1 sequence (YUHKI *et al.* 2008) (Supplementary Table 4) and sequenced using standard ABI Big Dye sequencing with 99 cycles of amplification using 65F and 65R primers.

RNA Extraction and Generation of cDNA

RNA was extracted from skin cells of white and pigmented cats using the RNAqueous-4 PCR kit (Ambion). Reverse transcriptase PCR (RT-PCR) was performed with the SuperScript III One-Step RT-PCR kit (Invitrogen) to generate amplified cDNA product. RT-PCR products were visualized on 2% agarose gels and sequenced as previously described. The PCR primers used for amplification of the *KIT* cDNA are listed in Supplementary Table 5. Complimentary DNA (cDNA) sequences were aligned in Sequencher version 4.8 (Gene Codes Corp.).

Table 4. Genotype at *White* locus as associated with phenotype.

Table 4

Genotype at White locus as Associated with Phenotype

	Phenotype				
Genotype	Deaf	Partial deafness	Blue iris	Fully pigmented	White
W/W	OR=13.3333 p=1.0000	OR=107.0690 p=1.000	OR=Infinity p=1.0000	OR=0.0495 NT[a]	OR=20.4286 NT
W/+	OR=0.5185 p=1.0000	OR=0.5495 p=1.000	OR=1.5641 NT	OR=0.0428 p=1.0000	OR=30.7288 p=1.0000
W/w^s	OR=0.9545 p=1.000	OR=2.000 p=0.0200	OR=63.000 p=1.0000	OR=0.2129 NT	OR=5.9677 NT
w^s/w^s	OR=0.2536 NT	OR=0.1233 NT	OR=4.846 NT	OR=0.4931 NT	OR=0.0414 NT
+/+	OR=0.0415 p=1.0000	OR=0.0172 p=1.0000	OR=0.4118 p=1.0000	OR=3503.0000 p=1.0000	OR=0.0021 p=1.0000

[a]NT: unable to perform stratified test because data was too sparse

w^s/+ : there were no *w^s/+* individuals in the pedigree

Table 5. Genotype observed with respect to hearing capacity at the *White* locus.

	Phenotype[a]		
Genotype at *W*[o]	Deaf	Partial hearing	Normal hearing
W/W	16	6	0
W/w	6	5	14
w/w	0	0	15
W/w[s]	2	2	2
w[s]/*w*[s]	na[b]	na[b]	3

[a] *W*, *White* allele; *w*, wild type allele; *w*[s] *White Spotting* allele (See Supplementary Table 8 for hearing thesholds of individual animals that were used to assign phenotype.)

[b] No samples observed in pedigree with this phenotype

Chapter 2.3 Collaboration and Statistics

Hearing threshold tests

(conducted by JHU's Ryugo laboratory)

Hearing threshold was determined using standard auditory evoked brainstem response (ABR) techniques in a sound proofed chamber as described previously (RYUGO *et al.* 2003). Each kitten was tested at 30 days and at 30-day intervals to track the animals' hearing statuses over time (RYUGO *et al.* 2003). For 32 pigmented hearing cats and 44 white cats with varying degrees of hearing loss, repeated threshold measures for individual cats varied less than 10 dB from month to month. The final ABR threshold scores just before sacrifice for both ears were reported (Table 3) since this was the endpoint hearing status of the animals. All procedures were conducted in accordance with NIH guidelines and approved by the JHU Animal Care and Use Committee (ACUC) (Protocol # CA10M273).

Case-Control Analysis

A Chi-Square analysis was used to generate P-values for the population genetics section of data. The Ragdoll breed was not included in the statistical analysis of a potential correlation between blue iris and genotype at the W locus as all Ragdolls have blue eyes due to their genotype at the C locus, which results in decreased production of the enzyme tyrosine kinase, critical in the synthesis of pigment (Lyons et al 2005b, Schmidt-Kuntzel et al 2005).

Pathology and Mast Cell Analysis

(conducted by Histoserv, Inc.)

Tissues used in this study were collected after post-mortem perfusion with 4% paraformaldehyde. To compare mast cell number and general histopathological differences between white (n=2) and pigmented cats (n=2), fixed tissues were embedded in paraffin blocks, sectioned, mounted, and stained either with hematoxylin and eosin (H & E) stain for all tissues or toluene blue to visualize mast cells (Histoserv, Inc.).

Chapter 3: Results

Chapter 3.1: History

The mapping and characterization of the feline *White* locus has been complicated by the lack of complete concordance of a white coat with blue irises and deafness (GEIGY *et al.* 2006). Of the three phenotypes, only white coat color exhibits complete penetrance (Figure 3). Thus, I reasoned that mapping *W* using the segregation of white coat color would be a straightforward approach to identify the *W* locus in the domestic cat.

Chapter 3.2: Pedigree Analysis

In order to map *W*, a pedigree segregating for white was generated by mating a male deaf dominant white cat from the John Hopkin's University (JHU) colony, heterozygous at *W* (Materials and Methods, Pedigree 1), with four fully pigmented females, producing 29 progeny, including 19 white and 10 pigmented individuals (Figure 3). *W* exhibited an autosomal, dominant inheritance pattern as had been reported previously (WHITING 1919). Table 3 details phenotypes of the progeny.

Chapter 3.3: Candidate Gene Analysis

A candidate gene approach was utilized to map the *W* locus. Significant linkage to *W* was established with three STRs tightly linked to

the feline *KIT* locus on chromosome B1 (=0, LOD= 6.0 - 6.2) (Table 1). Negative LOD scores were generated for all STRs linked to the seven other candidate genes. For five of these candidate loci, LOD scores of -2 or less were observed, which are considered exclusionary (OTT 1991) (Table 2).

Chapter 3.4: Sequence Analysis of *KIT*

Sequence generated from the 21 exons of *KIT* and splice junction regions displayed no fixed polymorphisms that distinguished between white and non-white individuals. Additionally, sequence of cDNA generated from RNA isolated from skin exhibited no splicing abnormalities (Supplementary Table 5). This was expected because null *KIT* mutations in mice are embryonic lethal (Fleischman 1992).

Chapter 3.5: Analysis of Regulatory Regions

I next examined regions reported to impact regulation of *KIT* in the mouse. Transcriptional regulation of *KIT* is highly complex, can act over large genomic regions, and exhibits tissue specificity (BERROZPE *et al.* 2006; MITHRAPRABHU and LOVELAND 2009; VANDENBARK *et al.* 1996). Cairns et al 2003 identified six DNase I hypersensitive sites (HS) that are “open” allowing for regulation and expression of *KIT* in specific cell types during development. One HS site was found in the *KIT* promoter region immediately 5’ exon 1. This site has been demonstrated in mice to drive green fluorescent protein (GFP) expression in primordial germ cells. Five

more HS sites were identified in the first intron, one of which (HS2) marked an enhancer required to drive GFP expression in hematopoietic cells. The enhancers marked by HS3 to HS6 increased expression to optimal levels. I identified a 623 bp insertion in *KIT* intron 1 interrupting the feline region homologous to the murine *Kit HS2* (Figure 4), which is highly conserved across mammalian species (CAIRNS *et al.* 2003; CERISOLI *et al.* 2009).

The insertion element demonstrated the highest level of identity to a feline endogenous retrovirus 1 (FERV1) family identified in the cat genome, which exhibits similarity to a porcine endogenous retroviral family (PONTIUS *et al.* 2007; YUHKI *et al.* 2008). The inserted fragment was comprised of an incomplete viral sequence including the long terminal repeat (LTR) with a series of seven repeated sequence blocks, 46 bp long (Figure 5). Supplementary Figure 2 presents a sequence alignment of the feline *KIT* wild type intron 1 with the LTR, (henceforth the *W* allele) illustrating insertion breakpoints of the LTR element. (GenBank No. KC893343).

Primers designed in sequence flanking the *W* allele demonstrated that *W* segregated with white in Pedigree 2 (P2 in Figure 3) ($p=0.00014$) and were observed in all white individuals in Pedigree 1 (P1) (Figure 3), with many individuals demonstrating homozygosity for *W* (Table 3).

Alleles for three white spotted siblings in Pedigree 1 (03-138, 04-053, 04-054) (Figure 3) were unable to be amplified for a *W* genotyping assay (using primers 7FM13F and 7RM13R, see methods), demonstrating neither the presence of the *W* allele or the wild type (*w*) allele (Figure 6). Analysis

of short tandem repeat profiles (MENOTTI-RAYMOND *et al.* 1997) confirmed their parentage (data not shown). Since their parents appeared to be homozygous for *W*, these spotted individuals posed contradictions of both phenotypic and genotypic expectations. Ultimately, utilizing long-range PCR methodology, Victor David generated a 7333 bp PCR product from the three white spotted individuals spanning the site of the *W* allele, identified as a full-length 7125 bp feline endogenous retroviral sequence. The sequence exhibited highest identity to the FERV1 element ERV1-1_FCa-I (ANAI *et al.* 2012) on chromosome D1, demonstrating 98.4% identity over a span of 6333 bp. The 5' and 3' ends displayed sequence similarity but not identity to the 800 bp LTR in white individuals (Supplementary Figure 2) (GenBank No. KC893344).

The full-length FERV1 element, henceforth *white spotting* allele, w^s, (Figure 7) demonstrated segregation with the White Spotting phenotype (Figure 3), both in Pedigree 1 and an independent pedigree (Figure 6), as well as exhibiting recessiveness to the *W* allele, and dominance to the wild type allele, *White* (*W*) > *White Spotting* (w^s) > *wild type* (*w*) (Table 4). Interestingly, different degrees of white pigmentation were demonstrated by three progeny (Figure 6) that inherited the identical maternal *White Spotting* allele.

Figure 4. Graphic depiction of feline Chromosome B1 (161.71 Mb-161.62 Mb) (UCSC Genome Browser, Sept. '11 (ICGSC Felis_catus 6.2/felCat5) Assembly. Genomic region of *KIT* intron1 homologous to murine DNase hypersensitive site 2 requisite for high-level expression of *Kit* (Cairns et al 2003). Genomic conservation of the region is demonstrated across six different mammals.

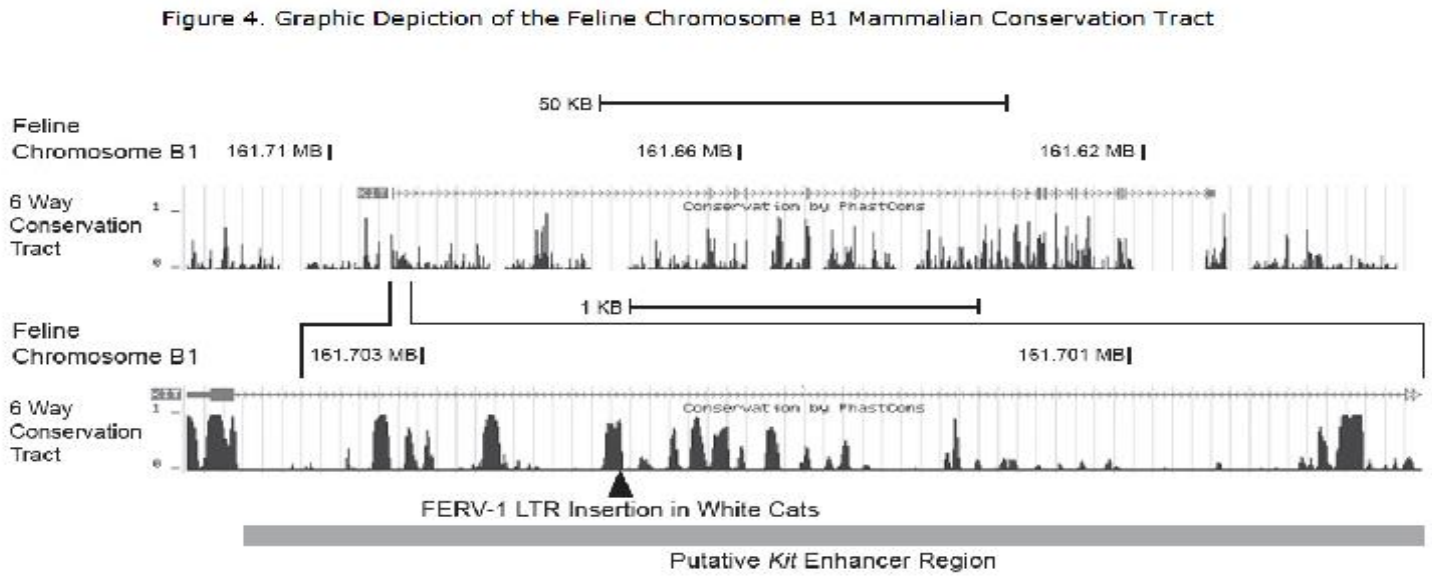


Figure 5. Depiction of *KIT* LTR insertion with repeat sequence and full length FERV-1 sequence.

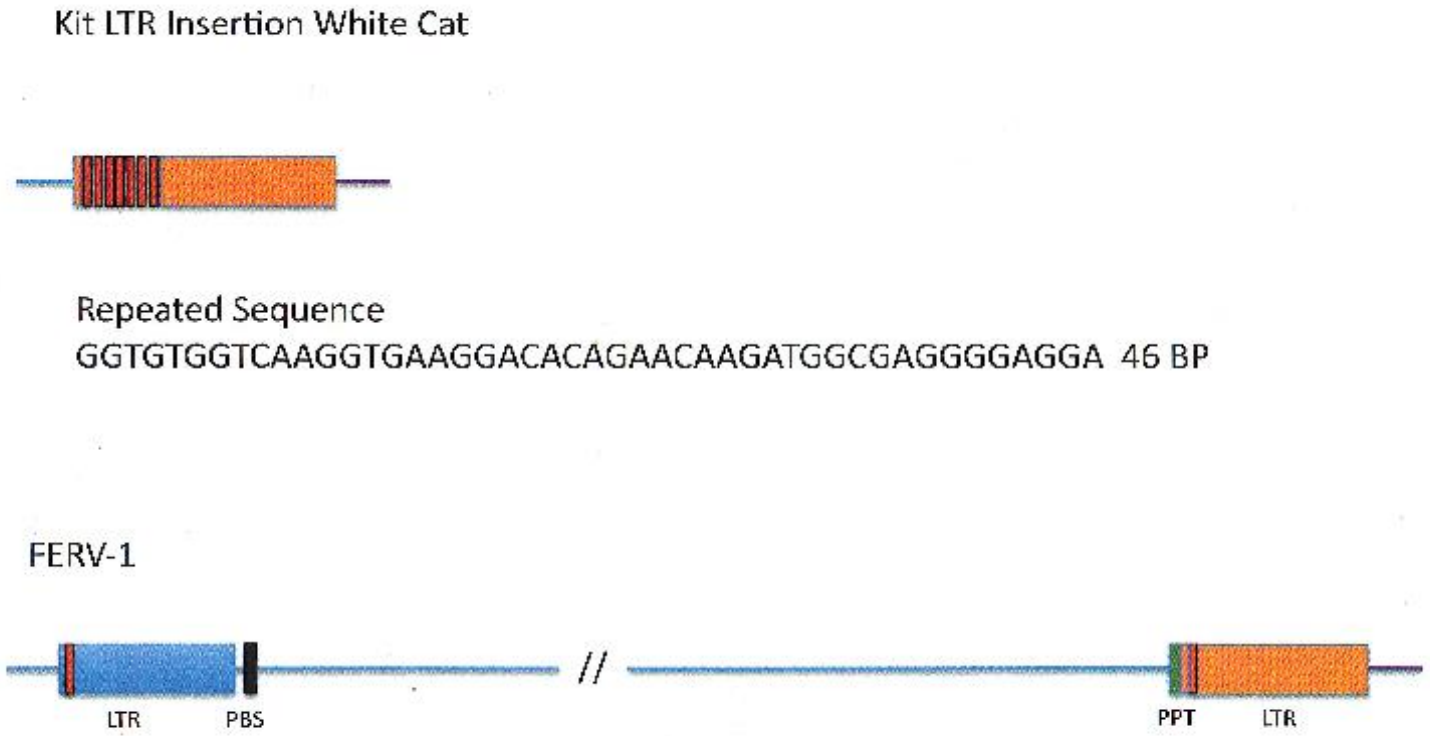


Figure 6. Graphic depiction of a white spotted pedigree. Family of domestic cats segregating *White Spotting* demonstrating difference in degree of White Spotting in individuals inheriting w^S allele identical by descent. Squares = males; circles = females. Filled symbols: White Spotted individuals; open symbol, fully pigmented cat. w^S, indicates genotype of full length FERV element in *KIT*; +, absence of FERV element.

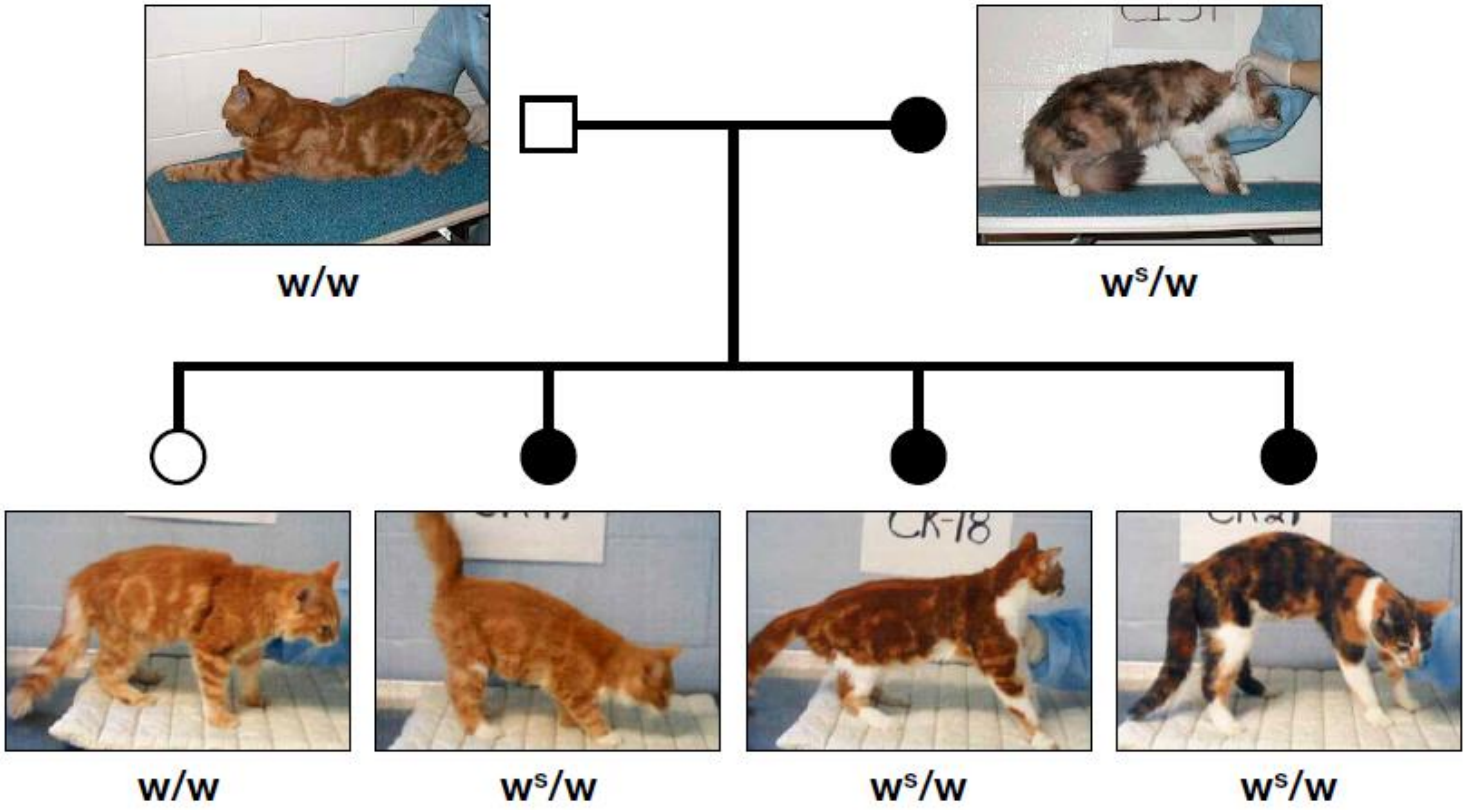
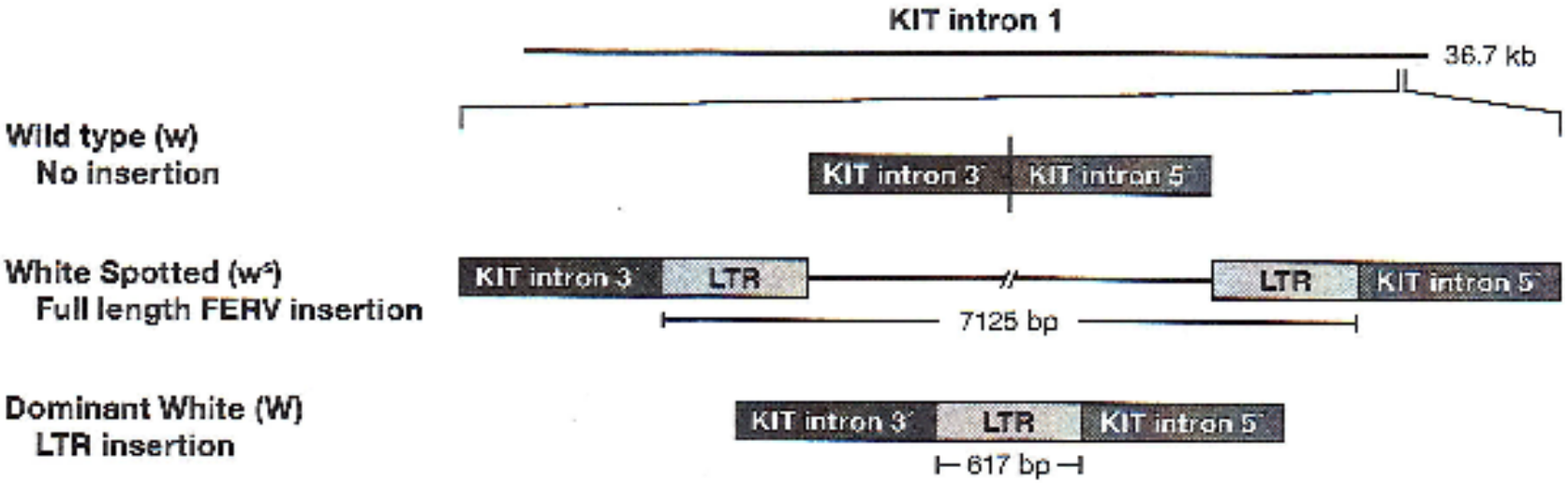


Figure 7. Depiction of *KIT* intron 1 showing *W* and w^s insertions.



Chapter 3.6 Association to Deafness

Deafness was determined to be genetically linked to the triallelic *KIT W* variant. For the initial penetrance function and a disease allele frequency of 0.01 the LOD score is +2.67. Varying the model parameter values (see Materials and Methods) caused the LOD score to vary in the range [+2.42, +2.83]. Because linkage was tested to only one marker, these LOD scores are significant at $p < 0.0038$ for the lowest score of +2.42 and $p < 0.0015$ for the highest score of +2.83 (OTT 1991). The correction for genome-wide multiple testing implicit in the typically used LOD score thresholds of +3.0 or +3.3 is not applicable in this usage of genetic linkage analysis. Deafness is statistically associated with the genotype of the *KIT* variant in the combined pedigree 1 and 2. MQLS estimated p-values in the range [0.007, 0.010], varying with the input prevalence of deafness and with the method of p-value estimation. For the combined hypothesis of linkage and association, PSEUDOMARKER reported a p-value of 0.000023. Work in MQLS and PSEUDOMARKER was done by Alejandro Schäffer.

There appears to be an influence of homo- or heterozygosity at *W* relative to hearing capacity. In Pedigree 1, all *W/W* homozygotes (*n*=22) demonstrated some degree of hearing impairment: 73% were deaf and 27% demonstrated partial hearing (Table 3) (Table 5). In contrast, individuals that were heterozygous (*W/w)* (*n*= 24) were much more likely to display some hearing capacity: 58% demonstrated normal hearing, 16.7% partial hearing and 20.8% were deaf (Table 3). All wild type individuals demonstrated

normal hearing. In individuals exhibiting the *White Spotting* allele, though sample sizes are small, w^s/w^s homozygotes (*n*=3) demonstrated normal hearing, W/w^s heterozygotes (*n*=6) were equally divided (33%) into hearing, deaf, or hearing impaired (Table 3). There were no w^s/w individuals examined. (Table 5)

Chapter 3.7: Population Survey

Victor David and I examined the correlation of the w^s and W alleles, respectively, with coat color in a population genetic survey of cats of registered breed (n= 270), including 33 Dominant White cats, 94 white spotted individuals and 143 fully pigmented cats (MENOTTI-RAYMOND *et al.* 2007) (Table 6) (Supplementary Table 6). All Dominant White individuals demonstrated the presence of the *W* allele, with 6 individuals demonstrating homozygosity for *W* ($p < 0.0001$). With the exception of one individual, all individuals demonstrating White Spotting exhibited the w^s allele ($p<0.0001$). All but three of the fully pigmented individuals exhibited absence of both the *W* or w^s allele (Supplementary Table 6) (Table 6) ($p<0.0001$). Two of these individuals were from a near-hairless breed (Sphynx) where lack of pigmentation can be difficult to phenotype, often appearing pink instead of white (Solveig Pfluger, personal communication). I had no phenotypic information for hearing status in the population sample, except that one W/W homozygous individual was reported as both blue eyed and deaf.

Table 6. Summary of genotypes at the *White* Locus in a population survey of 30 cat breeds.

	Genotype at the *White* locus[a]				
	W/W	*W/w*	w^s/w^s	w^s/w	*w/w*
Coat Color Phenotype					
Dominant White	6	27	0	0	0
White Spotting	0	0	40	53	0
Fully pigmented	0	0	2	1	140
Total individuals	6	27	42	54	140

[a] *W*, *White* allele; w^s, *White Spotting* allele; *w*, wild type allele

Chapter 3.8: Histopathology

In humans, an activating point mutation of *KIT* is causative of a heterogeneous disorder, mastocytosis, that exhibits proliferation and accumulation of mast cells in the skin, bone marrow, and internal organs such as the liver, spleen and lymph nodes (for review, (ORFAO *et al.* 2007). A survey for mast cell profiles in tissues of white (n=2) and pigmented cats (n=2) revealed no substantive differences in mast cell distribution (Supplementary Table 7).

Chapter 4: Discussion

Chapter 4.1: KIT

KIT encodes the mast/stem cell growth factor tyrosine kinase receptor. Extensive research has demonstrated the critical role of *KIT* and its ligand, *KITLG* in the development, differentiation, migration and proliferation of stem cells (hematopoietic, cardiac and neural stem cells of the eye), primordial germ cells and several types of early committed progenitors, including melanoblasts (AOKI *et al.* 2005; CABLE *et al.* 1995; CAIRNS *et al.* 2003; FLEISCHMAN 1993).

In mice, homozygous null mutations in *Kit* are embryonic lethal; milder mutations may cause anemia, infertility, and albinism (FLEISCHMAN 1992). The heterozygous *W* mouse phenotype is similar to the human piebald trait, also caused by a *KIT* mutation, which is characterized by a congenital white hair forelock and ventral and extremity depigmentation (FLEISCHMAN *et al.* 1991). Activating mutations in *Kit* are associated with cancer and mastocytosis while deactivating mutations are seen in piebaldism and deafness. Mutations in coding or regulation of *KIT* have been characterized in additional species as causative of defects in pigmentation and hearing (HAASE *et al.* 2007; RUAN *et al.* 2005; SPRITZ and BEIGHTON 1998).

Cable et al. have demonstrated that mutations in *Kit* do not prevent

early melanoblast migration or differentiation, but severely affect melanoblast survival during embryonic development in white spotting mouse mutants (CABLE *et al.* 1995). It follows that this is the reason calico cats have large blotches of color when compared with tortoiseshells cats which have smaller dappled orange and black coats. Calico cats are tortoiseshells cats that have the *White Spotting* allele, fewer melanocytes have survived and populate the skin. This leads to larger patches of color.

Cairns et al. described murine cell type-specific DNase I hypersensitive sites that delineated *Kit* regulatory regions in primordial germ cells, hematopoietic stem cells, and melanoblasts (CAIRNS *et al.* 2003). Genomic regions defined by the hypersensitive sites, once engineered into transgenic constructs driving green fluorescent protein (GFP) expression, demonstrated expression of GFP *in vitro* and *in vivo* through development of hematopoietic and germ cell lineages (CERISOLI *et al.* 2009). The *W* and w^s alleles map within the 3.5 Kb DNase 1-hypersensitive site 2 (HS2) fragment, required for high level expression of *Kit* (CAIRNS *et al.* 2003). This genomic region is evolutionarily conserved across a range of mammals (Figure 4), suggesting that it is under selective constraint. I suggest that disruption of this regulatory region in the cat impacts melanocyte survival and/or migration.

Chapter 4.2: ERV sequences

Similar to other mammalian species, cats carry endogenous retroviral (ERV) genomic sequences descended from ancestral infections and integrations into the germ line. Approximately 4% of the assembled feline genome consists of sequence segments that are retroviral-like with the FERV1 family comprising approximately 1.05% of the genome (PONTIUS *et al.* 2007). The FERV1 integration site in *KIT* is unusual relative to the pattern of ERV insertions in the human genome, which are generally found in intergenic regions and rarely within an intron or in close proximity of a gene (MEDSTRAND *et al.* 2002). I would envision the integration of the full-length retroelement, the *White Spotting* allele (w^s), followed at some point by recombination between the two LTRs of the integrated provirus, generating a single *LTR,* the *W* allele*.* Fixed solo LTRs are found for many classes of endogenous retroviruses and outnumber their full-length ancestral progenitors (JERN and COFFIN 2008)*.*

Retroviral insertions can be powerful agents for phenotypic change, and are reported to impact a host of genetic mechanisms which can impact phenotype, including gene expression, splicing and premature polyadenylation of adjacent genes (for review see (JERN and COFFIN 2008) (BOEKE and STOYE 1997; ROSENBERG and JOLICOEUR 1997). Why the full length retroviral element (w^s) results in a less extreme phenotype (White Spotting) than the solo LTR (*W*) is open for speculation. It has been previously reported in *D.melanogaster* that excision of all but a solo LTR

insertion can result in reversion to a near wildtype phenotype (Carbonare and Gehring 1985). In the full-length element, a large imperfect 4908 bp open reading frame persists that corresponds to the Gag-Pol precursor protein of feline ERV DC-8 of the ERV1-1 family (ANAI *et al.* 2012). However, presence of 3 stop codons precludes potential translation of a complete Gag-Pol polyprotein.

Other retroviral insertion events have been reported to impact pigmentation, (CLARK *et al.* 2006; JENKINS *et al.* 1981) and there is report of a retroviral insertion that can effect transcriptional regulation of several unlinked loci (NATSOULIS *et al.* 1991), but to our knowledge this is the first report of the evolution of a retroviral element in mammals impacting multiple mutant phenotypes (*W* and w^s) controlled by a single gene.

Chapter 4.3: Melanocytes and Hearing

My laboratory initially embarked on this project to explore the genetic basis underlying deafness in white, deaf cats. Deaf white cats are lacking melanocytes in the inner ear (BILLINGHAM and SILVERS 1960). In contrast, albino cats are not deaf, have a normal distribution of melanocytes but lack the enzyme tyrosinase and so are incapable of producing melanin pigment (IMES *et al.* 2006). Therefore, pigment is not necessary for hearing. Only recently has the molecular mechanism correlating the absence of melanocytes in the cochlea and deafness been elucidated. Early in embryogenesis melanoblasts migrate from the neural crest on either a

dorsal-lateral path to populate the skin or on a dorsal-ventral route to populate regions of the eye and the inner ear. In the cochlea, melanocytes become abundant in a vascularized epithelium that lines the lateral wall of the cochlea, known as the stria vascularis (SV) (Figure 8). The SV plays a critical role in secreting high levels of K+ into the endolymph, the extracellular fluid of the inner ear that surrounds the mechanosensory hair cells (Figure 9). This secretion establishes an endocochlear potential (EP) that is critical for depolarization and auditory nerve electrical signal transduction (Marcus et al. 2002). As the only cell type in the SV to express the KCNJ10 (Kir4.1) potassium channel protein, melanocytes directly facilitate K+ transport (Figure 9) (Marcus et al. 2002). Knockouts of the *Kcnj10* gene in mice eliminate the EP and reduce endolymph potassium concentration, with resultant deafness (Marcus et al. 2002).

White cats with blue eyes represent the classic model of feline deafness. The inner ears of such cats exhibit degeneration of the cochlea and saccule, termed cochlea-saccule degeneration (MAIR 1973). The cochleae of white kittens do not appear different from those of normal pigmented kittens at birth, with inner and outer hair cells intact in both groups. Within the first postnatal week, the cochleae of white kittens manifest degenerative changes, characterized by a pronounced atrophy of the stria vascularis and incipient collapse of Reissner's membrane (BAKER *et al.* 2010; MAIR 1973). By the start of the second postnatal week the tectorial membrane; the sensory receptors have been obliterated (Figure

10). Perhaps the most economical interpretation of the available evidence is that these latter events are secondary to some primary event involving the *KIT* mutation, missing melanocytes, and reduced EP.

Figure 8. Graphic of the inner ear showing the stria vascularis.

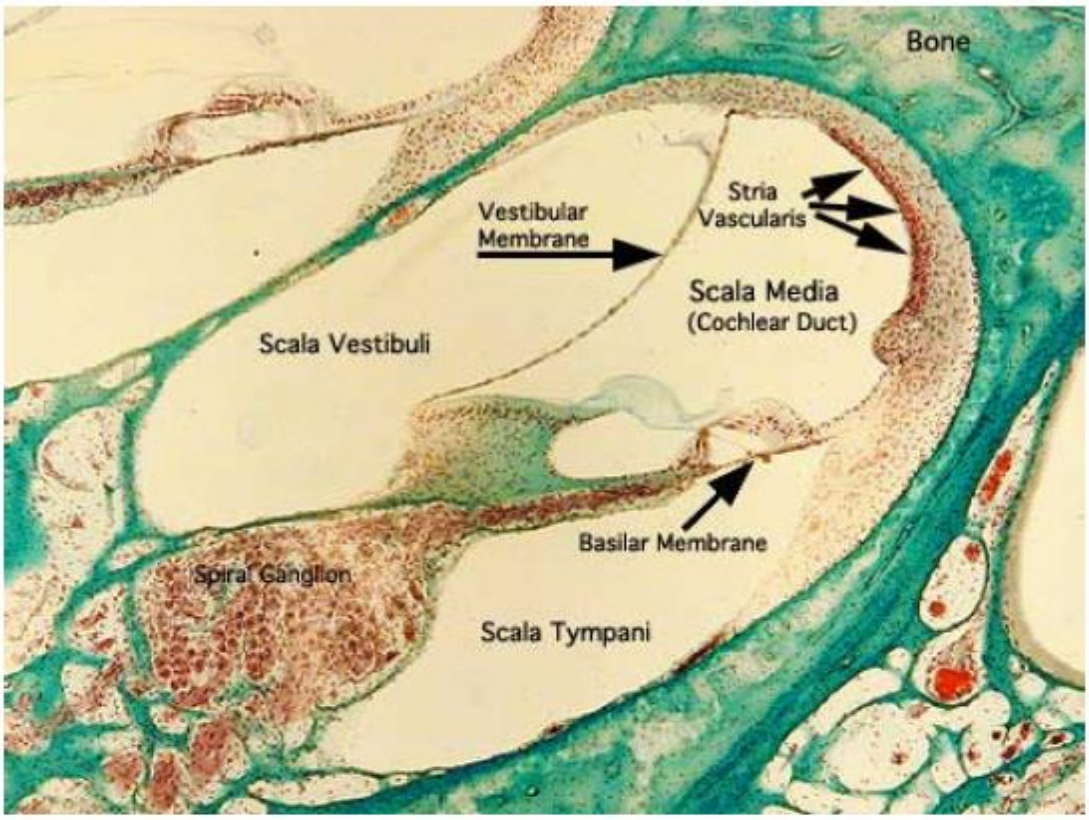


Figure 9. Graphic illustration demonstrating endocochlear potential. Intermediate cells are melanocytes which express KCNJ10. (Marcus et al 2002)

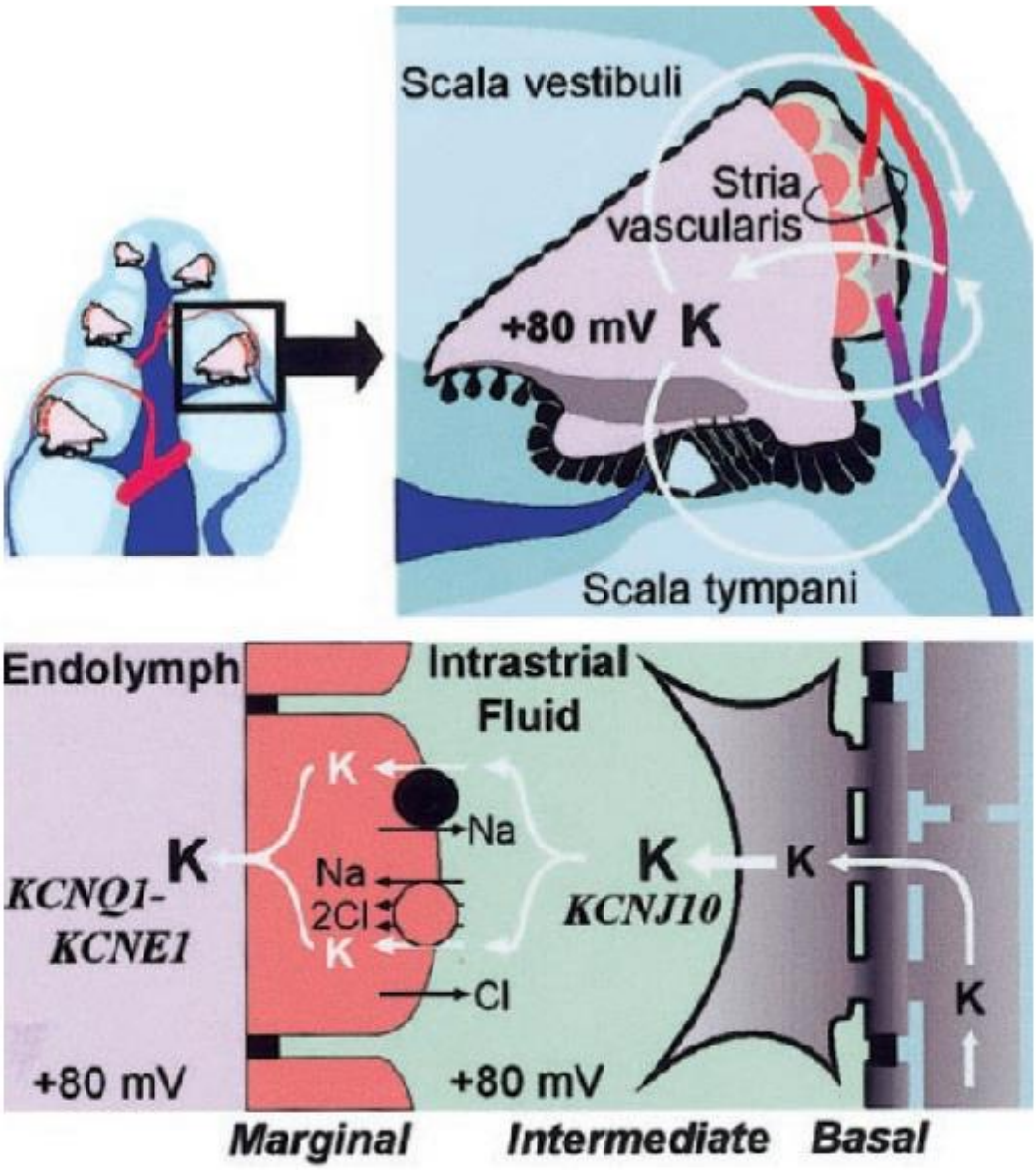
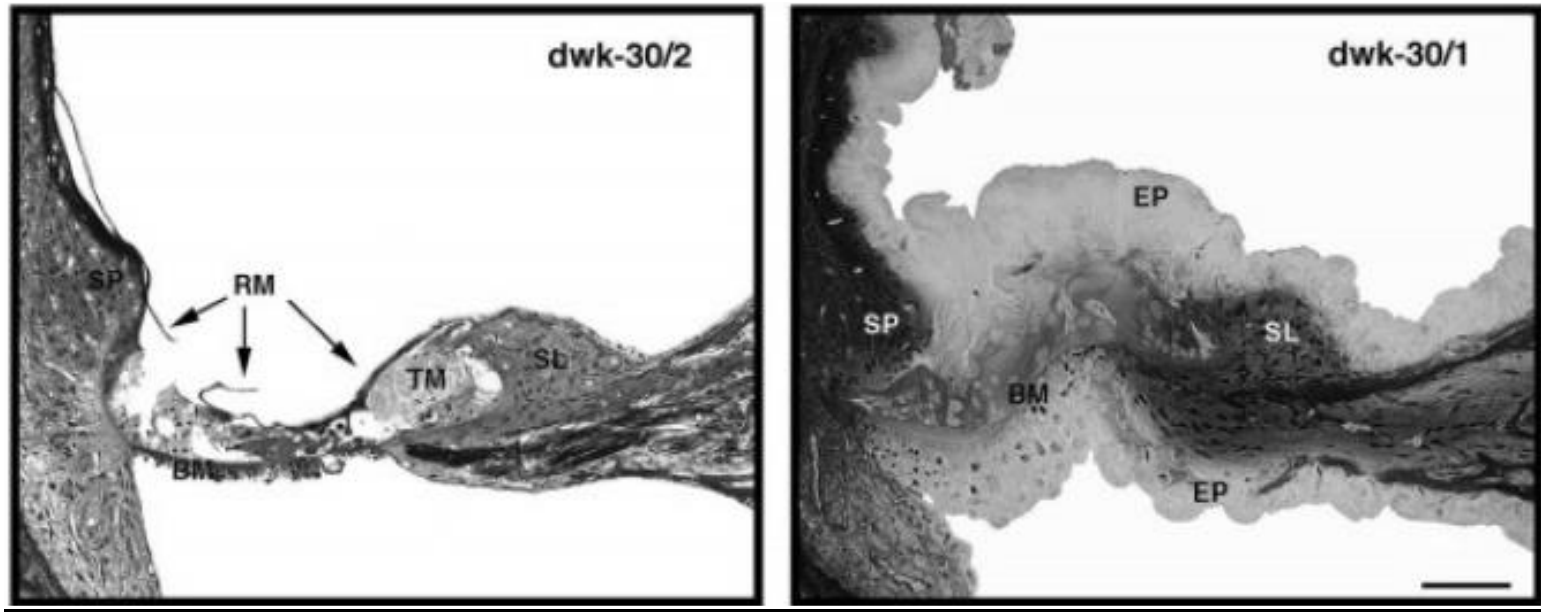


Figure 10. Pathology of the deaf white cat cochlear duct. (Ryugo et al 2003)



Chapter 4.4: Two Melanocyte Lineages

We observed that homozygous (*W/W*) individuals were more likely to be deaf (Table 5) than heterozygotes. Aoki et al. report that melanocytes that migrate to the inner ear are less sensitive to Kit signaling than the melanocyte lineage that migrates to pigment hair and skin (AOKI *et al.* 2009). I have demonstrated relative to the *W* allele, that genotype has a strong impact on phenotype, with homozygous (*W*/*W*) individuals more likely to be deaf and have blue eyes (Table 4). A report in the literature provides compelling evidence addressing the reduced incidence of deafness in W/+ individuals. Aoki et al. report that melanocytes derive from two distinct lineages with different sensitivity to Kit signaling (Aoki et al. 2009). "Classical" murine melanocytes that migrate from the neural crest along a dorsal-lateral route to pigment skin and hair are highly Kit-sensitive. However, non-cutaneous melanocytes, which travel a dorsal-ventral route to the inner ear and the eye, are more effectively stimulated by endothelin 3 (Edn3) or hepatocyte growth factor (HGF), than by Kit (Aoki et al. 2009). Extensive loss of Kit signaling does however lead to a complete loss of non-cutaneous melanocytes (Aoki et al., 2009). I propose that suppression or availability of Kit may be less severe in heterozygous individuals, allowing for modest survival and migration of non-cutaneous melanocytes to the inner ear and iris of W/+ individuals. While this may explain some of the perceived lack of penetrance for deafness at the *W* locus, I have not observed a complete correlation between genotype for the FERV1 insertion and phenotype,

suggestive of additional genetic modifying factors (i.e. modulating tissue or temporal specific expression of EDN3 or HGF).

We propose that suppression or availability of *KIT* may be less severe in heterozygous individuals, allowing for modest survival and migration of non-cutaneous melanocytes to the inner ear and iris of (*W/w^s, W/w*) individuals. While this may explain some of the perceived lack of penetrance for deafness at the *W* locus, I have not observed a complete correlation between genotype for the FERV1 insertion and phenotype.

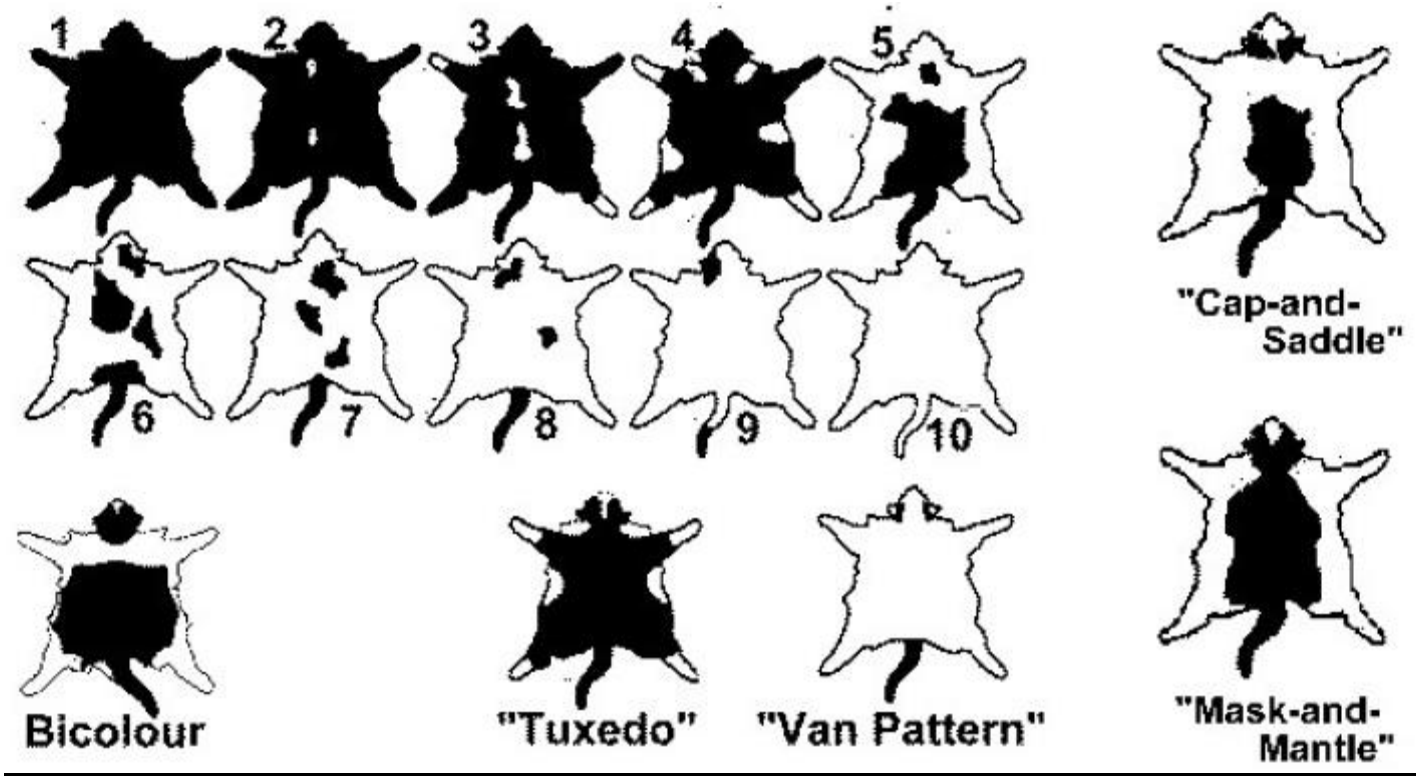
Chapter 4.5: Epigenetics

Epigenetic regulation may impact hearing capacity and iris color in Deaf White Cats. Yin et al. reported cloning white cats by somatic cell nuclear transfer from fibroblasts of a male Turkish Angora Deaf White Cat (YIN *et al.* 2007). Whereas, the donor cat was deaf, the five white cloned individuals were not deaf and had blue irises (YIN *et al.* 2007).

White Spotting in the cat is observed as a *continuum* of white pigmentation from low grade (face/paws/legs/white stomach) to medium grade spotting covering 40-60 percent of the body to high grade spotting (van pattern) where most of the body is white, other than the head and tail (VELLA *et al.* 1999) (Figure 11). Homozygosity vs heterozygosity for the *w^s* allele appears to have an influence on the degree of white pigmentation. In our population survey, of two cat breeds known to demonstrate a high degree of white spotting (Turkish Van, Japanese Bobtail) (FOGLE 2001), 13

of 16 individuals demonstrated (w^s/w^s) genotypes (Supplementary Table 6). Homozygous (w^s/w^s) individuals for whom I have pictures are generally predominantly white (data not shown). As observed with the *W* allele, other genetic modifiers appear to influence melanoblast survival and migration as observed by the different degrees of white pigmentation in siblings that inherited the identical w^s allele (Figure 6). None of the individuals of the Birman cat breed, which all exhibit white pigmentation of the paws demonstrated the w^s allele supporting a recent report of Lyons et al. of an independent mutation possibly in *KIT* causative of Birman gloving (LYONS ET AL. 2010).

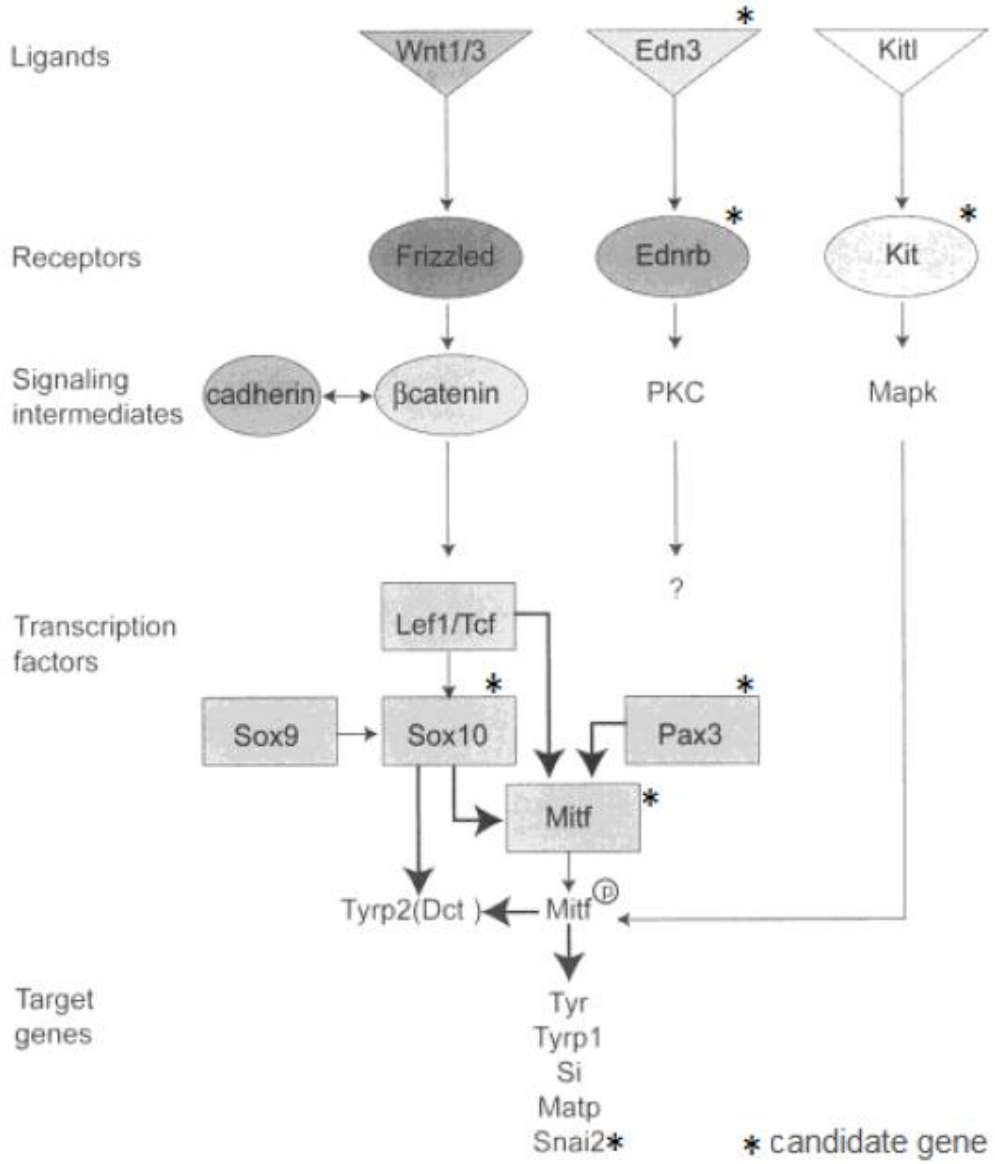
Figure 11. Graphic depicting degrees of white spotting, and some common names for specific patterns.



Chapter 4.7: Evolution of *W* and w^s

The *KIT* insertion event is likely of relatively recent origin. The cat was domesticated from the Near Eastern wildcat, *Felis sylvestris lybica*, less than ten thousand years ago (DRISCOLL *et al.* 2007). Similar to other species that have experienced domestication, multiple coat color phenotypes rapidly arose in the cat (DROGEMULLER *et al.* 2007; EIZIRIK *et al.* 2010; EIZIRIK *et al.* 2003; ISHIDA *et al.* 2006; KEHLER *et al.* 2007; LYONS *et al.* 2005a; LYONS *et al.* 2005b; MENOTTI-RAYMOND *et al.* 2009; SCHMIDT-KÜNTZEL *et al.* 2005), likely as the consequence of selection by man of desirable phenotypes (CIESLAK *et al. 2011*). A white cat, or white spotted cat (females can be calico), would likely have been a prized possession. Our population genetic data suggest that single mutational events are causative of the Dominant White and White Spotting phenotypes, demonstrating the remarkable impact on phenotype at *W* by retroviral insertion and evolution. This evolution likely happened via site specific recombination between the 5' and 3' LTRs on the same strand or double recombination in a w^s/w^s individual of the retroviral LTR, excluding a large portion of the original insertion. An allelic series of mutations in *KIT* has also been observed in the pig for several hypopigmentation phenotypes (GIUFFRA *et al.* 1999; JOHANSSON *et al.* 2005; PIELBERG *et al.* 2002) and is proposed in the horse (HAASE *et al.* 2007). This investigation provides molecular genetic data largely supporting Whiting's proposal of an allelic series for the feline *W* locus; *White* (*W*) > *White Spotting* (w^s) > wild type (*w*) (WHITING 1919).

Supplementary Figure 1. This is a schematic of the *MITF* Transcriptional Pathway, showing ligands, receptors, signal intermediates, transcription factors, and target genes. Candidate genes are denoted with an asterisk. The only candidate gene not shown is *SP1*. (From Melanocytes to Melanoma)



Supplementary Figure 2. Clustal alignment of *Felis catus KIT* intron 1 including sequences from a wild type (fully pigmented) individual, White individual and White Spotted individual characterizing the retrotransposition of 7125 bp of a feline endogenous retrovirus (White Spotted) or 617 bp of a solo LTR (White) into *KIT*. The breakpoint of the FERV retrotransposition is on Chromosome B1 between positions 16702321 and 16702320 on Assembly NCBI genome/78 (Felis catus) September 2011

```
Normal Cat            ATTTTGAGATCTGCAACACCCCTTCCCACGTGATAGCTACACTACTTAAGGGCCGCCTGG
White Cat             ATTTTGAGATCTGCAACACCCCTTCCCACGTGATAGCTACACTACTTAAGGGCCGCCTGG
White Spotted Cat     ATTTTGAGATCTGCAACACCCCTTCCCACGTGATAGCTACACTACTTAAGGGCCGCCTGG
                      ************************************************************

Normal Cat            GGCGGGGGTGGGAGATGGAGTGGAACTTT--------------------------------
White Cat             GGCGGGGGTGGGAGATGGAGTGGAACTTT--------------------------------
White Spotted Cat     GGCGGGGGTGGGAGATGGAGTGGAACTTTTGTTATGCCCAAAATTCGTGATCCCCAAAGA
                      ****************************

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     CCACCAGGGAGCCGAGTCCGATGCAAAAGCAAAGAGCCTTTATTCGAGCTAGCTCGAGCT


Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     CAATCCCCTACCTGCACCGACGCAGCGGTGAGATACCAGGGAAAGAGCACGAGTTTCAAA


Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     AAGGACAAAGGTTTTATTGGGGCCTGGGGGCAGTTGGTGAGGTAATGGCTGTGGCCTCAG


Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     CTGATTGGCTGGGGAGGGGTCCTGGGGAAGGGTCTGGCAGGTGAGGGAGGGTTTACTCAA


Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     GGGGAGGAGGTGTGGTCAAGGTGAAGGACACAGAACAAGATGGCGAGGGGAGGAGGTGTG


Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     GTCAAGGTGAAGGACACAGAACAAGATGGCGAGGGGAGGAGGTGTGGTCAAGGTGAAGGA


Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     CACAGAACAAGATGGCGAGGGGAGGAGGTGTGGTCAAGGTGAAGGACACAGAACAAGATG


Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     GCGAGGGGAGGAGGTGTGGTCAAGGTGAAGGACACAGAACAAGATGGCGAGGGGAGGAGG


Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     TGTGGTCAAGGTGAAGGACACAGAACAAGATGGCGAGGGGAGGAGGTGTGGTCAAGGTGA


Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     AGGTCACAGAACAAGATGGCGACGGCTGGCGTAGGCCCGCCCTTTCATTCCCCCCTTGTC
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Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     ATGTAGCTTACGGACCCAATCATGGGACCGGCTGCATTTATGGTGACAAGGAGAACAGAG

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     TCTGGAGGTTTACGCAAAGTTCTGGGAACCAAGAGTCCCTGGGGCGGCTCTGGGAGGTCT

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     GATTAAGTATTGTCCCCGAGCTGGTGTCTATGATCTGCCAGGTGATGTTTTGGGGGCGTG

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     GGGACTCCCGGCAGCATGAGCAATGACAAGCAGAGTTAACAGAGTTACCAATATTAGGTA

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     GGTCGAATGCGCTGTAGCTTGAGCTTGAGCGGGTTGTGTCGGTCCCGACTGATGGCCCAT

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     CGCGTGACGAAGTCCTTCCGGATCGAGGAGGGGTCCGCTGGCTGAGCGTGGGTGTGATGG

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     ACCCAGGTCGCGATGCCGTCTACCTTGAGAGCGGTGGGGGTTGTCAACACCACGATGTAG

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     GGTCCCTTCCAGCGCGGCTCGAGAGTCTCTCGGTGGTGCCTCTTGACGTAGACCCAGTCT

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     CCCGGCCTGTACTGATGAGGTGTCGGGATCGGGCCAGCCTCGTAGATGGCACGGAGGCGC

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     GGCCAAATGTCCTCGTGCGCCCTCTGGAGCCCGCTCAAGGAAAGAAAAAGTTCTTGATCT

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     TTAAACTCAGCAATAAGTTCAGCTCGAAGGCTGGGAATAACAGGGGGTGGCCTGCCAAAC

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     ATGATTTCGTAGGGAGTAAAACCCAGAGTGTAAGGAGTGTTTCTAACCCGGTAAAGGGCG

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
```

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White Spotted Cat      TACGGTAGGAGAGTCACCCAGTCCCCGCCAGTCTCCATGGTTAATTTGGTAAGGGTCTCT

Normal Cat             ------------------------------------------------------------
White Cat              ------------------------------------------------------------
White Spotted Cat      TTTAGGGTTCTATTCATTCTTTCTACCTGTCCTGAGCTCTGGGGCCTATAAGCACAATGT

Normal Cat             ------------------------------------------------------------
White Cat              ------------------------------------------------------------
White Spotted Cat      AATTTCCAGTTTGCCCCCACCGCCTTGGCTACTGCCTGTGTTACCTGCGAGATAAAAGCT

Normal Cat             ------------------------------------------------------------
White Cat              ------------------------------------------------------------
White Spotted Cat      GGTCCATTGTCTGATCCTACCAGGGCAGGAAAACCATACCTGGGTAAGATGTCTTCTAGT

Normal Cat             ------------------------------------------------------------
White Cat              ------------------------------------------------------------
White Spotted Cat      AGCTTCTTAGCCACCGTCTGAGCCGTTTCATGCTTGGTTGGGTATGCCTCCACCCAGCCA

Normal Cat             ------------------------------------------------------------
White Cat              ------------------------------------------------------------
White Spotted Cat      GAGAAGGTGTCTGTAAATACTAAAAGATATTTAAAACCATACTTTCCTGGTTTGACTTCA

Normal Cat             ------------------------------------------------------------
White Cat              ------------------------------------------------------------
White Spotted Cat      GTGAAGTCGACTTCCCATTGGGCTCCCGGTCTGGTGCCTCTGAGCCTGGTTCCTTTTTCA

Normal Cat             ------------------------------------------------------------
White Cat              ------------------------------------------------------------
White Spotted Cat      TTTGATGTGGCCCTCGCGTTGGTGAGTTGGCAGGTCTTGCAGGCAGATACAACTTGCTCT

Normal Cat             ------------------------------------------------------------
White Cat              ------------------------------------------------------------
White Spotted Cat      ATTTTGGTGTCCTGTTGGTGAATCTTAATTCCGGCATGTCGGATTAAGTCTTTTAATTTT

Normal Cat             ------------------------------------------------------------
White Cat              ------------------------------------------------------------
White Spotted Cat      CGGGCCCCCATGTGAGTAGACCGATGCATGTGCTCTAATATTGAGACTCCGAGCTGGTCT

Normal Cat             ------------------------------------------------------------
White Cat              ------------------------------------------------------------
White Spotted Cat      GGCAACACGAGCTCCTTGTTAGGTGTATACCACCATCCCTTTATCTCCTGGGCCATGGGG

Normal Cat             ------------------------------------------------------------
White Cat              ------------------------------------------------------------
White Spotted Cat      AGTTTCTTGATCCGCTGTAATTCCTCCTGGGAGTACTTGGGCTGGTCTGGTAAAACTGGG

Normal Cat             ------------------------------------------------------------
White Cat              ------------------------------------------------------------
White Spotted Cat      TCTCCTGGGTCTGGTAGTTGTATGGTCATGGTGGGGACTGGAGTAAGGGCTACTGCCTTG

Normal Cat             ------------------------------------------------------------
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White Cat             ------------------------------------------------------------
White Spotted Cat     GCTGCCTGGTCAGCCTTTCGATTACCTCTAGCTACTGGGTTACCAGCTTTTTGGTGCCCT

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     TGGCAGTGGATAATGGCTAGCTTGGCAGGAAGCCATAAGGCCGTAAGCAGGTTAAGTATC

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     TCCTGCTTATTTTTTATAGTCCGTCCTTCTGCCGTCAGTAACCCCCTCTCCTGATAAATT

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     GCCCCATGAATATGAGCTGTGGCAAATGCATAACGGCTGTCTGTGTAGATGTTGAGCCGT

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     TTTCCAGCTCCCAGCATCAGCGCCTTGGTGAGGGCTATGAGCTCTGCTCGCTGGGCTGAC

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     GTTCCGGAGGGTAGAGCCTCCGCCCATACGGTGTTGGTTTCGGTGACCACCGCTGCCCCC

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     GCATACCTGTGTCCGCCTCGCACAAAGCTGCTGCCATCAGTGAACCAAGTAGCCTCAGCA

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     TCGGGGAGGGGCCGGTCGGTCAGGTCCGTCCGGAATCCATGTACTTGTTCCAGGATTTCC

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     ACACAGTCATGTAATGGAGCACCTAGGTCAGGGTCGGGCAGCAGGGTTGCAGGATTGAGG

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     GCTACACTGGGGTGGAACCGCACTCGTGGAGGGTTGAGTAGGAGGCTCTGGTAATGAGTC

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     ATACGCGTATTGCTCATCCATCTATCTGGAGGCTGTTTCAGGACCCCTTCAATGGCGTGT

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     GGGGTCGTGATCCAGATCTCCTGTCCTAGGGTCAGTTTGTCTGCATCCTTGACTAGGAGT

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     GCTGTCGCTGCAATAATTCTTAGGCATGGCGGCCAGCCGGCAGCCACTGGGTCTAGTTTC
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Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     GCCAAGGAGCTCAAGAGAGTATACGGGTTGGGAACAGTTGGGTGTATGTCCATGACCCTC

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
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Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     GGCAGTAGGGGGGTGTTCCAGGCAGACTGGCAAGGAACTAGTACCCCTAGGCTTCGTAGT

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     CTCCGGATGTGTGGCTGGATCCCCTTCCAGGCCTCCTGAGACATGGGGTATTGTTTGATC

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     CTTACCGGACTCTCTCCTGGCTTGAGCTCTACCAGGACTGGGGTCCTATGAGCGGCTAGT

Normal Cat            ------------------------------------------------------------
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White Spotted Cat     CCCATCCCCCCTGTCTCTGCCCAAACCGAGGGGAATTCTTGTAACCATCTGTCTATATTA

Normal Cat            ------------------------------------------------------------
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Normal Cat            ------------------------------------------------------------
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Normal Cat            ------------------------------------------------------------
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White Spotted Cat     TGTGGGGTGCGGAGGACCGAATGTTGTGCTCCGGTGTCGACAAGGAAGTCAATAGGGGTC

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White Spotted Cat     CCCTCCACTTTAAGAGTTACCCTGGGTTCGGGGAGAGGGTCCGAACCCTGACTCCCCTAA
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Normal Cat            ------------------------------------------------------------
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Normal Cat            ------------------------------------------------------------
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White Spotted Cat     TTTTCTCCTAGTCTATCTATTTTCTGTAATTTTCTCCTAATGTCTACGGCCGATTGGTTT

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White Spotted Cat     ACAAAGGCCATGATAACAGCTGCCTTGCTTTCCGGAGCCTCTGGATCCATGGGGGTGTAG

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White Spotted Cat     GTACGGAATGCCTCCATGATCCGTTCTAAAAAGGCAGCTGGAGATTCATCTTTTCCCTGT

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White Spotted Cat     TGTACATTTCCTACCTTGGCCAAATTGGTTGGCTTTCTAGCAGCCATTCGGAGACCCCCC

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White Spotted Cat     ATTAGAGTCTGGCGGTAGACCCGGAGCCTCTCCTTACCTTCTGCCGTGTTGAAATCCCAC

Normal Cat            ------------------------------------------------------------
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White Spotted Cat     TGGGGCCGAGTTAAGGGGAAGGAGGCATCTATCTGAGCTTGGTTGGTGGTGGGATTCCCG

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
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White Spotted Cat      TCTGCGCCCGGAACTAGTTTTCGGGCCTCATTGAGGATTCTTTCTCTTTCTTCAGTCGTG

Normal Cat             ------------------------------------------------------------
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White Spotted Cat      AACAGGACCTGCAAAAGCTGCTGGCAATCGTCCCACGTGGGCTGATGGGTAAAAAGAACA

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White Spotted Cat      ATAGGGATTTTGGCTCCGGAGGAAGCAGGGTTAGGAAGGGCTTAAGCCAAGAGGGTGGGT

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White Spotted Cat      CTTCTACAAGGTCCTGCCAAGTGATAATGTAAGGGAGCTGATCAAGATGGCCCGTCTTAG

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White Spotted Cat      GCTGAGAGATGATACTCCTGACTCGGTGGATGGTAGGGAGGTCGAAGGTCCCCTCTGGTG

Normal Cat             ------------------------------------------------------------
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White Spotted Cat      GCCATCCGACATTGAAAGTTGGCCACTCGCTAGAACAAAAAAACTGCAACCGACCCTTTC

Normal Cat             ------------------------------------------------------------
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White Cat             ------------------------------------------------------------
White Spotted Cat     GGACTTCCACACTGAGGTTGTTAGCTCTTCCCCTCACATCCTTAAAGTGATCAATCATAA

Normal Cat            ------------------------------------------------------------
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White Spotted Cat     TACTTAGAGGAGTAGTCTGAGTCTGTCCCATAATGTCCGTCCAGTAAGTCCACAGAGCAA

Normal Cat            ------------------------------------------------------------
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White Spotted Cat     AACAGAGAAACACAAAAACAGACAAACAGAGGGCCCCTAGAAAGTCTTCCAACTCCATGG

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White Spotted Cat     AAGCAAAACGGAAAGCTAGCTTTTGAGGGGATTCCATGTCCCTCCAAAACCGATGAGGGG

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     ATTCCACGTCCCTCCAAAGACGACGGCCTCACGCCGACCAGCGGGAGCGACCCGCCTCGT

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     CTCAGACCTTTGAGGGGATTCCACGTCCCTCCAGAAGGGAGAATCGGAACGTCTTCCGAG

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     ACTCCCGGCCCGTGGTCCTCCAGTGCGTCCACCTAGACCGCGTCGGGCACTACCAGAATT

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     CCAGAAATGAGCTCACACAGAAAAGACAGAACAAACAGACACTACCGTGGCCAGTCAGGC

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------TGTTATGC
White Spotted Cat     TCTCCGGGTCGGGGGTCCCTCGGGGTCTTGGGGATCCCGGACGAGCCCCCAATGTTATGC

Normal Cat            ------------------------------------------------------------
White Cat             CCAAAATTCGTGATCCCCAAAGACCACCAGGGAGCCGAGTCCGATGCAAAAGCAAAGAGC
White Spotted Cat     CCAAAATTCGTGATCCCCAAAGACCACCAGGGAGCCGAGTCCGATGCAAAAGCAAAGAGC

Normal Cat            ------------------------------------------------------------
White Cat             CTTTATTCGAGCTAGCTCGAGCTCAATCCCCTACCTGCACCGACGCAGCGGTGAGATACC
White Spotted Cat     CTTTATTCGAGCTAGCTCGAGCTCAATCCCCTACCTGCACCGACGCAGCGGTGAGATACC

Normal Cat            ------------------------------------------------------------
White Cat             AGGGAAAGAGCACGAGTTTCAAAAAGGACAAAGGTTTTATTGGGGCCTGGGGGCAGTTGG
White Spotted Cat     AGGGAAAGAGCACGAGTTTCAAAAAGGACAAAGGTTTTATTGGGGCCTGGGGGCAGTTGG

Normal Cat            ------------------------------------------------------------
White Cat             TGAGGTAATGGCTGTGGCCTCAGCTGATTGGCTGGGGAGGGGTCCTGGGGAAGGGTCTGG
White Spotted Cat     TGAGGTAATGGCTGTGGCCTCAGCTGATTGGCTGGGGAGGGGTCCTGGGGAAGGGTCTGG
```

```
Normal Cat              ------------------------------------------------------------
White Cat               CAGGTGAGGGAGGGTTTACTCAAGGGGAGGAGGTGTGGTCAAGGTGAAGGACACAGAACA
White Spotted Cat       CAGGTGAGGGAGGGTTTACTCAAGGGGAGGAGGTGTGGTCAAGGTGAAGGACACAGAACA

Normal Cat              ------------------------------------------------------------
White Cat               AGATGGCGAGGGGAGGAGGTGTGGTCAAGGTGAAGGACACAGAACAAGATGGCGAGGGGA
White Spotted Cat       AGATGGCGAGGGGAGGAGGTGTGGTCAAGGTGAAGGACACAGAACAAGATGGCGAGGGGA

Normal Cat              ------------------------------------------------------------
White Cat               GGAGGTGTGGTCAAGGTGAAGGACACAGAACAAGATGGCGAGGGGAGGAGGTGTGGTCAA
White Spotted Cat       GGAGGTGTGGTCAAGGTGAAGGACACAGAACAAGATGGCGAGGGGAGGAGGTGTGGTCAA

Normal Cat              ------------------------------------------------------------
White Cat               GGTGAAGGACACAGAACAAGATGGCGAGGGGAGGAGGTGTGGTCAAGGTGAAGGACACAG
White Spotted Cat       GGTGAAGGACACAGAACAAGATGGCGAGGGGAGGAGGTGTGGTCAAGGTGAAGGACACAG

Normal Cat              ------------------------------------------------------------
White Cat               AACAAGATGGCGAGGGGAGGAGGTGTGGTCAAGGTGAAGGACACAGAACAAGATGGCGAG
White Spotted Cat       AACAAGATGGCGAGGGGAGGAGGTGTGGTCAAGGTGAAGGACACAGAACAAGATGGCGAG

Normal Cat              ------------------------------------------------------------
White Cat               GGGAGGAGGTGTGGTCAAGGTGAAGGTCACAGAACAAGATGGCGACGGCTGGCGTAGGCC
White Spotted Cat       GGGAGGAGGTGTGGTCAAGGTGAAGGTCACAGAACAAGATGGCGACGGCTGGCGTAGGCC

Normal Cat              --------------CTGCAAAATCTTACTTGGATCCTAAAGCTGTAGTGAAAATCCGGTT
White Cat               CGCCCTTTCACTTTCTGCAAAATCTTACTTGGATCCTAAAGCTGTAGTGAAAATCCGGTT
White Spotted Cat       CGCCCTTTCACTTTCTGCAAAATCTTACTTGGATCCTAAAGCTGTAGTGAAAATCCGGTT
                                ********************************************

Normal Cat              TTATCTGCGCGGGAACTTAGGTCTGAAGGTGGAGGA
White Cat               TTATCTGCGCGGGAACTTAGGTCTGAAGGTGGAGGA
White Spotted Cat       TTATCTGCGCGGGAACTTAGGTCTGAAGGTGGAGGA
                        ************************************
```

CLUSTAL O(1.1.0) multiple sequence alignment

```
Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     TTAGACAGGTAAGCCACTGGACGGTTCCAGGGGCCTAAGGCTTGAGTTAGAACTCCTTTT

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     GCTATTCCCTTATGCTCGTCTACAAAGAGGTGGAAGGGCTTCGTAATGTCCGGTAGGCCC

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     AGGGCTGGGGCACTTAGGAGGGCCCTTTTTAACTGATTAAAGGCAGTTTCTTCTTTTTCC

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     AGCCCATTTTAAATGTTTTCCCCTCTTTTGGTAGCTTCATATAGGGGGCCTGGCGATCTC

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     AGCAAAAACCTGGAACCCCAGAGGCGGGCAGTAGCCGGCTGATCCTAGGAATTCCCCTCA

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     CTTCCCCTTCGGGAGGTGGGAGTAGGGATCTTTAGGACAGTTTCTTTTCTGGCTTCTGAT

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     AACCGCCGCTGTCCGCCCTCCAGGATATATCCCAGGTAACTTACCCTCTCCCTGCATATC

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     TGAGCCTTCTTCGCAGATACGCGGTATCCTAAGGTCCCCAGGGTAGCCAGCAGGTCCTGG

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     GTCCCTCGCTCACAGTCTTTGGCAGTGTCGGCAGCAATCAGGATGTCATCTACATACTGT

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     AGAAGGGTGAGGCCAGGGTGCTCCCTTCTGTACTCACCCAGGTCCTCGTGTAGCGCCTCG

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     TCAAAGATGGTGGGTGAATTTTTGAATCCCTGAGGTAGCCGTGTCCAGGTGAGTTGTCCA

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     CTGTAGCCCTCCTCCGGATCATGCCACTCGAAGGCGAACAAGGGTTGGCTCTGGGGTGCC

Normal Cat            ------------------------------------------------------------
White Cat             ------------------------------------------------------------
White Spotted Cat     AGCGGCAGACTGAAGAAGGCGTCCTTTAAATCTAGTACAGTATACCAGACCCTGGAGGGC
```

Supplemental Table1. Primers used to amplify STRs linked to candidate genes.

Gene	Primer Name	Primer Sequence (with M13 or PIGtail in caps)
SOX10	SOX10A_F_M13F	TGTAAAACGACGGCCAGTGcagagggctcaggagacta
	SOX10A_R_PIG	GTGTCTTcccccacacatgtatgcttt
	SOX10B_F_M13F	TGTAAAACGACGGCCAGTaccccaagggagtcttgtct
	SOX10B_R_PIG	GTGTCTTttgtctggctggttgtgtgt
	SOX10C_F_M13F	TGTAAAACGACGGCCAGTcaggtcccattccaagtcat
	SOX10C_R_PIG	GTGTCTTgtcatgatctcacggtgtcg
PAX3	PAX3A_F_M13F	TGTAAAACGACGGCCAGTtgtgtgaactgcagggattt
	PAX3A_R_PIG	GTGTCTTtggtgatttttctccccatt
	PAX3B_F_M13F	TGTAAAACGACGGCCAGTccagccttctgcatttccta
	PAX3B_R_PIG	GTGTCTTcaaagtagacagaaggcaagga
	PAX3C_F_M13F	TGTAAAACGACGGCCAGTctcccctccccaaactctat
	PAX3C_R_PIG	GTGTCTTctggttctcccttgtccaaa
KIT	KITA_F_M13F	TGTAAAACGACGGCCAGTcattgggctctatgctgaca
	KITA_R_PIG	GTGTCTTtctgagcaggaagtttatgaatga
	KITB_F_M13F	TGTAAAACGACGGCCAGTcgtttgctttgacttccaat
	KITB_R_PIG	GTGTCTTcactcatgcagcagaggaaa
	KITC_F_M13F	TGTAAAACGACGGCCAGTcgtgtagggctctctgctg
	KITC_R_PIG	GTGTCTTaaatcaaacgtgggttttgc
EDNRB	EDNRBA_F_M13F	TGTAAAACGACGGCCAGTaaaaagcccaaacaaattttca
	EDNRBA_R_PIG	GTGTCTTggaaaaggcagtcatccaaa
	EDNRBB_F_M13F	TGTAAAACGACGGCCAGTagcctgctttggattctgtg
	EDNRBB_R_PIG	GTGTCTTaatgcatttagaacctcagca
	EDNRBC_F_M13F	TGTAAAACGACGGCCAGTttgaggtcacattgtcaaaaca
	EDNRBC_R_PIG	GTGTCTTccactggacacttccaggat
EDN3	EDN3A_F_M13F	TGTAAAACGACGGCCAGTcgcccataggtactgcattt
	EDN3A_R_PIG	GTGTCTTccccactcatgctctttctc
	EDN3B_F_M13F	TGTAAAACGACGGCCAGTacctccacatcctgctgttc
	EDN3B_R_PIG	GTGTCTTccccactcatgctctttctc
	EDN3C_F_M13F	TGTAAAACGACGGCCAGTtgaccttgcacagacacagg
	EDN3C_R_PIG	GTGTCTTctgcttcggattctgcatct
SNAI2	SNAI2A_F_M13F	TGTAAAACGACGGCCAGTatttcgtgctcttgcagctt
	SNAI2A_R_PIG	GTGTCTTatgaggaatctggctgctgt
	SNAI2B_F_M13F	TGTAAAACGACGGCCAGTctctggggatgtgggttaaa
	SNAI2B_R_PIG	GTGTCTTcctgggaacacacaggaaat
	SNAI2C_F_M13F	TGTAAAACGACGGCCAGTtgtgagattgaccctgcatc
	SNAI2C_R_PIG	GTGTCTTgtcagtggggagagctgtgt
SP1	SP1A_F_M13F	TGTAAAACGACGGCCAGTgcccattccaaagaatctga
	SP1A_R_PIG	GTGTCTTgtcttcgtgtcaggctcctc

Supplemental Table 2. Primers designed to amplify *KIT* Exons.

Primer[a]	Exons amplified	Forward Primer	Reverse Primer
KIT_EX1	1	GAGCAGGAACGTGGAACG	CCACCTCTGCCGACGAAC
KIT_EX2	2	ATGCTTTATTTCGCCAAGGA	CATGAAAGAAAGCCACACGTT
KIT_EX3	3	CAAAAATGTTTTCAACCATTCAA	CGTGTACCAATCAACATCAACA
KIT_EX4	4	TGGCAAGTGAAAATGGCATA	GAGAAAAACAAAGGGAACAAGC
KIT_EX5	5	TTTATCTAGCTAGGAAAGATCCTGAA	TTTCACTACTGTCGGTAATTTATACG
KIT_EX6	6	TCCCTGTTCTATTTTGTTAT	ACATCTGATCCTCAGCGTAA
KIT_EX7	7	CAGGCCCTTCACAAGTGATT	CCAACACGAGCCACAACTTA
KIT_EX8	8	GGTGAGGTTTTCCAGCAGTC	GTCCTTCCCTTACGCATGTC
KIT_EX9	9	TTTCTGGAGTAAATCGGGTTG	GCAGGCAGAGCCTAAACATC
EX10_11	10,11	GGCTGTAAAATGGGAGATGG	GCACCCAAAGAGGTTACACG
EX12_13	12,13	ACCACCACGTGCTCTCTTCT	TTTGATCATTTGAAAGATAATAAAAGG
KIT_EX14	14	TCTCATCTCTCTTTATTTAACCTTCTC	ACCCTTATGACCCCTCGAAC
KIT_EX15	15	CCCCTTTTTCCCATTTTGTT	TGGGGAACCAGTCACTATGG
KIT_EX16	16	TGGTATCCCTGTTGTCACCAT	GTTGGCGTGGGAGTGCTT
KIT_EX17	17	CGTTGCACGTAGTTTTCATTC	TGAGACTAACATCCTTCATTGGA
KIT_EX18_19	18,19	AACTTGGCCGAATCTGTTGT	GGGGAAGCACTATCTGAAGG
KIT_EX20	20	GGGTGAGAGAAAAATGGCTTT	TAAAGGTCTTCACCCCCAGA
KIT_EX21	21	GGTGTAGGGACTGGCATGTT	GAACCAAAAGAAGAGGGATCG

Supplemental Table 3. Primers designed to amplify DNase sensitive regions in the *KIT* 5' region and intron 1.

Primer Name	Sequence
KitReg_F1[a]	CTTGTGCCTACCAAGGTGCT
KitReg_R1	TGGGGAAGAGAGCCTAGTGA
KitReg_F2	GGGCTTAGCACAACGATTCT
KitReg_R2	GGAACAAAATAATGCGTGTATCC
KitReg_F3	GTGAAAGCCCTAGCGAACTG
KitReg_R3	CATGTAGGGCTCTGTGCTGA
KitReg_F4	GGAGAGAGAGAATCCCAAGC
KitReg_R4	CTCTGGAGGACCTCACCTTG
KitReg_F5	TCTGCTTCTTTCCCACCAAT
KitReg_R5	CGGAGGCTGAAAAGCAAG
KitReg_F6	GTCCAGACAGGTTGGGAGAG
KitReg_R6	GGCATGGGATTTACAAAAGC
KitReg_F7	CACCCAGCGCGTTATCTC
KitReg_R7	CAAATCCTCCTCCTCCACCT

[a]Primer sets 1-4 are 5' of the KIT gene, set 5 flanks exon 1 and sets 5-7 are in the 5' region of intron 1.

Supplemental Table 4. Primers designed to sequence the *White Spotted* allele.

Primer	Sequence
FERV1_1f_M13F	CAACCCGGAGAGCCTGACTG
FERV1_1r_M13F	CAGGGTAGAGGGGGTGCTGA
FERV1_2f_M13F	TCTTGCTTTGCAGGGGACAA
FERV1_2r_M13F	CCTGGTTGGTGGTGGGATTC
FERV1_3f_M13F	TGACTGAAGAAAGAGAAAGAATCCTCA
FERV1_3r_M13F	TTCCTCGGGGAAGTCAGCAG
FERV1_4f_M13F	CGAGACCTGGCCAGAATACTGCT
FERV1_4r_M13F	CCTTGCCATTGGTGACCTGA
FERV1_5f_M13F	TGACCAAGATTGGAGCTCAG
FERV1_5r_M13F	CGAACAAGGGTTGGCTCTGG
FERV1_6f_M13F	CCCTCCAAGGTCTGGTATACTG
FERV1_6r_M13F	GGCACTCAGGAGGGCCTTTT
FERV1_7f_M13F	ACCAAAGAGGGGAAAACATT
FERV1_7r_M13F	GCCTCCACCCATACGGTGTC
FERV1_8f_M13F	GCAGCAGCTTTGTGCGAGAC
FERV1_8r_M13F	CCAGGGCAGGAAAACCATACC
FERV1_9f_M13F	CTGGCTGGGTGGAGGCATAC
FERV1_9r_M13F	ACGAACGTGGGTGTGATGGA
FERV1_10f_M13F	CATCGTGGTGCTGACAACC
FERV1_11f_M13F	TCCCATGATTGGGTCCGTAA

All sequences had the M13Forward sequence tag appended for future sequencing of cDNA.

Supplemental Table 5. Primers designed to amplify *KIT* cDNA.

Primer Name[a]	Sequence
kitcDNA1_F	GAGCAGGAACGTGGAACG
kitcDNA1_R	GATTGTGATGCCAGCCTTG
kitcDNA2_F	GTGCGAGGGGAAGCCTCT
kitcDNA2_R	GTGCTCAGGCTTGGGATATG
kitcDNA3_F	TCGTGAATGATGGCGAGAA
kitcDNA3_R	AGAAGTCTTGCCCACATTGTT
kitcDNA4_F	GCCGTCTGGAAAACTAGTGG
kitcDNA4_R	TTCATGTGATTGCCGAGGTA
kitcDNA5_F	CATTTGACAGAACGGGAAGC
kitcDNA5_R	TCATTCTTGATGTCTCTGGCTA
kitcDNA6_F	TTCACAGAGACTTGGCTGCT
kitcDNA6_R	TCTACCCTGGAACAGGATGC
7_F_M13F[a]	CACCCAGCGCGTTA
7_R_M13R[a]	CAAATCCTCCTCCTCCACCT
8_F_M13F	CCCACGTGATAGCTACACTACTT

[a] Forward primers are tagged with M13-Forward sequence (TGTAAAACGACGGCCAGT) for sequencing of PCR product
Reverse primers are tagged with M13-Reverse sequence (CAGGAAACAGCTATGACC)
for sequencing of PCR product

Supplemental Table 6. Data from population genetic survey of breed cats.

Sample no.	Coat Color[a]	Breed[b]	Genotype[c]	Iris color[d]
2751	Spotting	AMW	S/+	
3066	Spotting	AMW	S/+	
3073	Spotting	AMW	S/+	
3078	Spotting	AMW	S/+	
5086	Spotting	AMW	S/+	
2521	Spotting	ANG	S/+	
2523	Spotting	ANG	S/+	
2657	Spotting	ANG	S/+	
1341	wild type	BEN	S/+	C
2577	Spotting	BOB	S/+	
3017	Spotting	BOB	S/+	
3041	Spotting	BOB	S/+	
2111	Spotting	CRE	S/+	
2113	Spotting	CRE	S/+	
2116	Spotting	CRE	S/+	
2118	Spotting	CRE	S/+	
2564	Spotting	CRE	S/+	
2595	Spotting	CRE	S/+	
2767	Spotting	CRE	S/+	
2768	Spotting	CRE	S/+	
2770	Spotting	CRE	S/+	
2771	Spotting	CRE	S/+	
2772	Spotting	CRE	S/+	
1904	Spotting	ESH	S/+	
2488	Spotting	EXO	S/+	
2727	Spotting	EXO	S/+	
2736	Spotting	EXO	S/+	
2242	Spotting	MAX	S/+	
2613	Spotting	MAX	S/+	
2491	Spotting	MCC	S/+	
2845	Spotting	MCC	S/+	
253	Spotting	MUN	S/+	
2199	Spotting	PER	S/+	
2042	Spotting	RAG	S/+	
2043	Spotting	RAG	S/+	
2207	Spotting	RAG	S/+	B
2208	Spotting	RAG	S/+	B
2285	Spotting	RAG	S/+	B
2479	Spotting	RAG	S/+	B
2493	Spotting	RAG	S/+	B

354	Spotting	SFO	S/+	
2799	Spotting	SFO	S/+	
2832	Spotting	SFO	S/+	
2833	Spotting	SFO	S/+	
2844	Spotting	SFO	S/+	
2892	Spotting	SFO	S/+	
1419	Spotting	SPH	S/+	C
2496	Spotting	SPH	S/+	
2788	Spotting	SPH	S/+	C
2794	Spotting	SPH	S/+	
2906	Spotting	SPH	S/+	C
2993	Spotting	SPH	S/+	C
2994	Spotting	SPH	S/+	C
3047	Spotting	SRE	S/+	
2664	Spotting	AMW	S/S	
2519	Spotting	ASH	S/S	
2609	Spotting	BOB	S/S	C
2610	Spotting	BOB	S/S	C
2673	Spotting	BOB	S/S	
2731	Spotting	BOB	S/S	
2826	Spotting	BOB	S/S	
3018	Spotting	BOB	S/S	
3026	Spotting	BOB	S/S	
3029	Spotting	BOB	S/S	
3042	Spotting	BOB	S/S	
3062	Spotting	BOB	S/S	
3063	Spotting	BOB	S/S	
2851	Spotting	BSH	S/S	
2109	Spotting	CRE	S/S	
2117	Spotting	CRE	S/S	
2769	Spotting	CRE	S/S	
2726	Spotting	EXO	S/S	
2520	Spotting	MAX	S/S	
1911	Spotting	MCC	S/S	C
2847	Spotting	MCC	S/S	
2598	Spotting	NFC	S/S	
2198	Spotting	PER	S/S	
2589	Spotting	PER	S/S	
2480	Spotting	RAG	S/S	B
2536	Spotting	SFO	S/S	
2575	Spotting	SFO	S/S	

2649	Spotting	SFO	S/S	
2650	Spotting	SFO	S/S	
2797	Spotting	SFO	S/S	C
2830	Spotting	SFO	S/S	
2896	Spotting	SFO	S/S	
2898	Spotting	SFO	S/S	
2571	Spotting	SPH	S/S	
2573	Spotting	SPH	S/S	
2787	Spotting	SPH	S/S	
2789	Spotting	SPH	S/S	C
2907	Spotting	SPH	S/S	
2793	wild type	SPX	S/S	
2908	wild type	SPX	S/S	C
2517	Spotting	VAN	S/S	
2923	Spotting	VAN	S/S	
2524	*W*hite Dominan	ANG	W/+	
2561	*W*hite Dominan	ANG	W/+	C
2703	*W*hite Dominan	ANG	W/+	
2593	*W*hite Dominan	BSH	W/+	C
2100	*W*hite Dominan	CRE	W/+	C
2106	*W*hite Dominan	CRE	W/+	B
967	*W*hite Dominan	DRE	W/+	C
1277	*W*hite Dominan	DRE	W/+	C
1278	*W*hite Dominan	DRE	W/+	ODD-EYED
1280	*W*hite Dominan	DRE	W/+	C
1281	*W*hite Dominan	DRE	W/+	C
1412	*W*hite Dominan	DRE	W/+	C
2035	*W*hite Dominan	DRE	W/+	C
2280	*W*hite Dominan	DSH	W/+	C
fsx479	*W*hite Dominan	FSX	W/+	
2240	*W*hite Dominan	MAX	W/+	
2818	*W*hite Dominan	MAX	W/+	C
2608	*W*hite Dominan	MCC	W/+	
2132	*W*hite Dominan	NFC	W/+	
2215	*W*hite Dominan	NFC	W/+	C
2058	*W*hite Dominan	OSH	W/+	C
2921	*W*hite Dominan	OSH	W/+	
2927	*W*hite Dominan	OSH	W/+	B
2091	*W*hite Dominan	PER	W/+	C
2167	*W*hite Dominan	PER	W/+	
2894	*W*hite Dominan	SFO	W/+	

2863	White Dominan	SRE	W/+	C
2563	White Dominan	ANG	W/W	C
2702	White Dominan	ANG	W/W	
2105	White Dominan	CRE	W/W	B
961	White Dominan	DRE	W/W	C
2059	White Dominan	OSH	W/W	B
356	White Dominan	SFO	W/W	B
1902	wild type	ABY	plus/plus	C
2498	wild type	ABY	plus/plus	C
2545	wild type	ABY	plus/plus	C
2947	wild type	ABY	plus/plus	C
4848	wild type	ABY	plus/plus	C
2391	wild type	ACU	plus/plus	
3056	wild type	ACU	plus/plus	
3057	wild type	ACU	plus/plus	
2750	wild type	AWH	plus/plus	
2752	wild type	AWH	plus/plus	
5087	wild type	AWH	plus/plus	
2387	wild type	ASH	plus/plus	
230	wild type	BEN	plus/plus	C
294	wild type	BEN	plus/plus	C
670	wild type	BEN	plus/plus	C
953	wild type	BEN	plus/plus	C
1347	wild type	BEN	plus/plus	C
1423	wild type	BEN	plus/plus	C
1424	wild type	BEN	plus/plus	C
1597	wild type	BEN	plus/plus	C
1599	wild type	BEN	plus/plus	C
1618	wild type	BEN	plus/plus	C
1619	wild type	BEN	plus/plus	C
1635	wild type	BEN	plus/plus	C
2379	wild type	BEN	plus/plus	C
2380	wild type	BEN	plus/plus	C
2381	wild type	BEN	plus/plus	C
2474	wild type	BEN	plus/plus	C
409	wild type	BOM	plus/plus	C
475	wild type	BOM	plus/plus	C
2412	wild type	BOM	plus/plus	C
2413	wild type	BOM	plus/plus	C
2795	wild type	BOM	plus/plus	C
302	wild type	BUR	plus/plus	C

314	wild type	BUR	plus/plus	C
317	wild type	BUR	plus/plus	C
360	wild type	BUR	plus/plus	C
2475	wild type	BUR	plus/plus	C
2098	wild type	CHA	plus/plus	C
2471	wild type	CHA	plus/plus	C
2472	wild type	CHA	plus/plus	C
2773	wild type	CHA	plus/plus	C
2775	wild type	CHA	plus/plus	C
2777	wild type	CHA	plus/plus	C
2110	wild type	CRE	plus/plus	
2112	wild type	CRE	plus/plus	
2114	wild type	CRE	plus/plus	
2115	wild type	CRE	plus/plus	
2602	wild type	CRE	plus/plus	
2604	wild type	CRE	plus/plus	
2033	wild type	DRE	plus/plus	C
1919	wild type	EXO	plus/plus	
1920	wild type	EXO	plus/plus	
1921	wild type	EXO	plus/plus	
1922	wild type	EXO	plus/plus	
1934	wild type	EXO	plus/plus	
1938	wild type	EXO	plus/plus	
1956	wild type	EXO	plus/plus	
1957	wild type	EXO	plus/plus	
1958	wild type	EXO	plus/plus	
1959	wild type	EXO	plus/plus	
2486	wild type	EXO	plus/plus	
2579	wild type	EXO	plus/plus	
2874	wild type	EXO	plus/plus	
2875	wild type	EXO	plus/plus	
2876	wild type	EXO	plus/plus	
737	wild type	HAV	plus/plus	C
756	wild type	HAV	plus/plus	C
765	wild type	HAV	plus/plus	C
2549	wild type	KOR	plus/plus	C
2550	wild type	KOR	plus/plus	C
523	wild type	MAU	plus/plus	C
524	wild type	MAU	plus/plus	C
1671	wild type	MAU	plus/plus	C
1672	wild type	MAU	plus/plus	C

1673	wild type	MAU	plus/plus	C
1674	wild type	MAU	plus/plus	C
1675	wild type	MAU	plus/plus	C
1676	wild type	MAU	plus/plus	C
1677	wild type	MAU	plus/plus	C
1684	wild type	MAU	plus/plus	C
1686	wild type	MAU	plus/plus	C
2340	wild type	MAU	plus/plus	C
2503	wild type	MAU	plus/plus	C
2655	wild type	MAU	plus/plus	C
2900	wild type	MAU	plus/plus	C
2904	wild type	MAU	plus/plus	C
2243	wild type	MAX	plus/plus	
2615	wild type	MAX	plus/plus	
2812	wild type	MAX	plus/plus	
2813	wild type	MAX	plus/plus	
2815	wild type	MAX	plus/plus	
2816	wild type	MAX	plus/plus	
2817	wild type	MAX	plus/plus	
2819	wild type	MAX	plus/plus	
2872	wild type	MAX	plus/plus	
2477	Spotting	MCC	plus/plus	
252	wild type	MUN	plus/plus	
4506	wild type	MUN	plus/plus	
4507	wild type	MUN	plus/plus	
2247	wild type	OCI	plus/plus	C
2248	wild type	OCI	plus/plus	C
2249	wild type	OCI	plus/plus	C
2250	wild type	OCI	plus/plus	C
2386	wild type	OCI	plus/plus	C
2400	wild type	OCI	plus/plus	C
2583	wild type	OCI	plus/plus	C
2605	wild type	OCI	plus/plus	
2606	wild type	OCI	plus/plus	
2607	wild type	OCI	plus/plus	C
2704	wild type	OCI	plus/plus	C
2837	wild type	OCI	plus/plus	
3008	wild type	OCI	plus/plus	C
3038	wild type	OCI	plus/plus	
4686	wild type	OCI	plus/plus	C
4687	wild type	OCI	plus/plus	C

1204	wild type	PER	plus/plus	
1205	wild type	PER	plus/plus	
2061	wild type	PER	plus/plus	C
2064	wild type	PER	plus/plus	C
2165	wild type	PER	plus/plus	
2166	wild type	PER	plus/plus	
2275	wild type	PER	plus/plus	
2278	wild type	PER	plus/plus	C
2862	wild type	PER	plus/plus	
648	wild type	RUS	plus/plus	C
758	wild type	RUS	plus/plus	C
759	wild type	RUS	plus/plus	C
1094	wild type	RUS	plus/plus	C
2347	wild type	SFO	plus/plus	
2348	wild type	SFO	plus/plus	
2532	wild type	SFO	plus/plus	
2576	wild type	SFO	plus/plus	
2893	wild type	SFO	plus/plus	
2897	wild type	SFO	plus/plus	
2781	wild type	SPX	plus/plus	
2782	wild type	SPX	plus/plus	
2790	wild type	SPX	plus/plus	
2791	wild type	SPX	plus/plus	
2864	wild type	SRE	plus/plus	C
2865	wild type	SRE	plus/plus	

[a]:Spotting: White Spotting; wild type: completely pigmented cat (no white fur)
[b:] ABY, Abyssinian; ACU, American Curl; ANG, Turkish Angora;
ASH, Americn Shorthair; AWH, American Wirehair; BEN, Bengal; BOB, Bobtail;
BOM, Bombay;BSH, British Shorthair; CHA, Chartreux; CRE, Cornish Rex;
DRE, Devon Rex; DSH, domestic shorthair (not a cat breed);
ESH, European shorthair (not a breed);EXO, Exotic; FSX, Felis sylvestris/domestic cat
hybrid;HAV, Havana; KOR, Korat; MAU, Egyptian Mau; MAX, Manx; MCC, Maine Coon Cat;
MUN, Munchkin; NFC, Norwegian Forest Cat; OCI, Ocicat; OSH, Oriental Shorthair;
PER, Persian; RAG, Ragdoll;RUS, Russian Blue; SFO, Scottish Fold; SPH, Sphynx;
SRE, Selkirk Rex; VAN, Turkish Van
[a]: *W* , FERV LTR allele; S, full length FERV allele; +, wild-type allele
[d] B, blue iris; C, pigmented iris

Supplemental Table 7. Mast cell pathology inquiry report.

Sample				Brain	Eye		Skin		Colon		Small intestine		Bone		Bone m
	Coat color	Sex	DOB	Histopath	Histopath	Toluidine	Histopath	Toluidine	Histopath	Toluidine	Histopath	Toluidine	Histopath	Toluidine	Histopath
8138	pigmented	F	4/7/2009	None	None	Neg	mixed mononuclear cell infiltrate	occasional mast cells scattered throughout dermis	Infiltrate, mixed mononuclear	few mast cells in submucosa	N/E	N/E	N/E	N/E	None
8139	White, deaf	F	8/19/2010	None	None	Neg	None	occasional mast cells scattered throughout dermis; one section with cartilage- more mast cells, but in normal limits	None	Neg	N/E	N/E	None	Neg	None
8140	pigmented	F	11/8/2008	None	None	few mast cells in retrobulbar connective tissue	None	occasional mast cells scattered throughout dermis; one section with cartilage- more mast cells, but in normal limits	Infiltrate, mixed mononuclear	few mast cells in submucosa	Infiltrate, mixed mononuclear	few mast cells in submucosa	None	Neg	None
8141	White, deaf	F	7/18/2004	None	None	Neg	None	5 sections, negative for mast cells	Infiltrate, mixed mononuclear	few mast cells in submucosa	Infiltrate, mixed mononuclear	few mast cells in submucosa	None	Neg	fewer bone trabeculae present compared to other submitted bones

DOB: date of birth
Histopath: Results of histopathology
Toluidine: Results of toluidine staining, sensitive for mast cells
None: no pathology reported
Neg: negative for mast cells
N/E: not examined

Pathology report: Mast cells were most prominent in sections of skin with cartilage that were interpreted to be from the ear.
The distribution and numbers of mast cells appeared to be consistent in each cat, and therefore were considered to be within normal limits.
In other tissues mast cells were seen in locations including submucosa or lamina propria(small intestine, colon, soft palate), peribronchia and perivascular (lung), peritoneal fat (from sections of pancreas), and retrobulbar connective tissue (eye). Mast cells were not detected in the sections of bone, bone marrow, lymph node, or brain by morphology or Toluidine blue stain.

narrow	Liver	Spleen	Pancreas		Kidney	Lymph node		Adrenal		Skeletal muscl	Heart	Lung	
Toluidine	Histopath	Histopath	Histopath	Toluidine	Histopath	Histopath	Toluidine	Histopath	Toluidine	Histopath	Histopath	Histopath	Toluidine
Negative	None	None	N/E	N/E	N/E	N/E	N/E	N/E	N/E	N/E	N/E	N/E	N/E
Neg	None	None	N/E	N/E	N/E	N/E	N/E	N/E	N/E	N/E	N/E	N/E	N/E
Neg	N/E	None	None	Neg	None	None	Neg	None	Neg	None	Histopath	None	Occasional mast cells in peribronchial and perivascular locations
Neg	None	None	None	occasional in surrounding peritoneal fat	Infarcts, bilateral	None	Neg	N/E	N/E	None	None	None	Occasional mast cells in peribronchial and perivascular locations

Supplemental Table 8. Primers and product sizes for *White / White Spotting* genotyping assay.

Primers:

1. FERV internal_65C_M13F: TGTAAAACGACGGCCAGTGTCTTGGGGATCACGGACGA

2. KIT_65C_F_M13F: TGTAAAACGACGGCCAGTATTTTGAGATCTGCAACACCCCTTC

3. KIT_65C_R_M13F: CAGGAAACAGCTATGACCTCCTCCACCTTCAGACCTAAGTTCC

Expected Product Sizes:

Primer Sets	PCR Amplicon Size		
	Wildtype	White Spotting	Dominant White
65F and 65R	207 bp	7333 bp*	829 bp
Ferv Int., 65R	No product	769 bp	No product

* This product fails to amplify under PCR conditions used for 3 primer assay because the extension time of 2.5 minutes is too short.

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