

# A Marker Set for Construction of a Genetic Map of the Silver Fox (*Vulpes vulpes*)

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## Abstract

The silver fox, a variant of the red fox (*Vulpes vulpes*), is a close relative of the dog (*Canis familiaris*). Cytogenetic differences and similarities between these species are well understood, but their genomic organizations have not been compared at higher resolution. Differences in their behavior also remain unexplained. Two silver fox strains demonstrating markedly different behavior have been generated at the Institute of Cytology and Genetics of the Russian Academy of Sciences. Foxes selected for tameness are friendly, like domestic dogs, while foxes selected for aggression resist human contact. To refine our understanding of the comparative genomic organization of dogs and foxes, and enable a study of the genetic basis of behavior in these fox strains, we need a meiotic linkage map of the fox. Towards this goal we generated a primary set of fox microsatellite markers. Four hundred canine microsatellites, evenly distributed throughout the canine genome, have been identified that amplify robustly from fox DNA. Polymorphism information content (PIC) values were calculated for a representative subset of these markers and population inbreeding coefficients were determined for tame and aggressive foxes. To begin to identify fox-specific single nucleotide polymorphisms (SNPs) in genes involved in the neurobiology of behavior, fox and dog orthologs of serotonin *5-HT<sub>1A</sub>* and *5-HT<sub>1B</sub>* receptor genes have been cloned. Sequence comparison of these genes from tame and aggressive foxes reveal several SNPs. The close relationship of the fox and dog enables canine genomic tools to be utilized in developing a fox meiotic map and mapping behavioral traits in the fox.

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The red fox (*Vulpes vulpes*) and other fox-like canids last shared a common ancestor with the domestic dog (*Canis familiaris*) and other wolf-like canids about 12–15 million years ago (Wayne et al. 1997; Wayne and Ostrander 1999; Wayne and Vila 2001). This ancient divergence is mirrored in the extensively rearranged karyotype of the fox (34 meta-centric and 0–8 B [micro] chromosomes) compared to the 78 predominantly acrocentric chromosomes of the dog (Breen et al. 1999; Graphodatsky et al. 2001; Yang et al. 1999). In further contrast to the modern dog, which represents the first animal to become fully domesticated, the red fox has, until recently, never been domesticated.

The history of animal domestication is tightly interwoven with the development of human society (Clutton-Brock 1995; Diamond 2002). To be successful, domestication involves behavioral adaptation by animals, enabling them to live in closer proximity to humans than their progenitors

could. Subsequently, or simultaneously, this process is accelerated by artificial selection when breeding becomes controlled by humans and focused on retaining traits regarded as desirable. From molecular data it is evident that the modern dog originated from gray wolves (Vila et al. 1997, 1999). The calculated date of this divergence still depends on the choice of assumptions and analytic methods, but is currently estimated to be as long as 12,000–15,000 years ago (Leonard et al. 2002; Savolainen et al. 2002). Although the archaeological record reveals small canids in association with humans from about the end of this period throughout Eurasia (Clutton-Brock 1995, 1999; Davis and Valla 1978; Olsen 1985), the first unequivocal evidence of the phenotypic diversity characteristic of the modern dog dates from about 5000 years ago in China (Olsen 1985), and 2500–4500 years ago in Babylonian, Assyrian, and Egyptian records (Clutton-Brock 1995, 1999; Zeuner 1963).

Intriguingly, the morphological and physiological changes associated with domestication show similar tendencies among diverse species (Trut 1999). Domesticated animals can be easily distinguished from their wild relatives by skull shape and other skeletal features (Wayne 2001), and even by coat color. The question is, do these changes represent merely human preference for certain morphotypes, or are they consequences of genetic selection for domesticated behavior?

A scientific team from the Institute of Cytology and Genetics of the Russian Academy of Sciences (ICG) in Novosibirsk has shed light on this question by experimentally reconstructing the domestication process in the silver, farm-bred form of the red fox (Belyaev 1969; Trut 1987, 1999). When the ICG studies started in the 1960's, Belyaev and colleagues hypothesized that selection of farm foxes for less fearful and aggressive behavior would lead to development of a domesticated strain of this species. In 40 years of continuous selective breeding, about 50,000 animals were tested for amenability to domestication (Belyaev 1979; Belyaev et al. 1981; Trut 1999). The resultant population of foxes selected for tameness demonstrates emotionally friendly responses to humans from 1 month of age (Trut 1999, 2001; <http://cbsu.tc.cornell.edu/ccgr/behaviour/index.html>). In parallel with this selection for tameness, a second strain of fox was selectively bred for aggressive behavior (Trut 1999). Studies including experimental crossbreeding of domestic and aggressive animals, cross-fostering of newborn pups, and transplantation of embryos have demonstrated the genetic basis of tame and aggressive phenotypes (Trut 1980).

In association with increased amenability for domestication, several de novo traits appeared in the fox population selected for tame behavior without direct selection or inbreeding. In particular, coat color changes such as the appearance of a white spot (*Star* phenotype) on the head (Belyaev et al. 1981), floppy ears, rolled tails, shorter tails, and changes in skull shape mimic the differences between domesticated dogs and wolves (Trut 1999). Significant differences in corticosteroids and several central nervous system (CNS) neurotransmitter levels were also found between animals from the domesticated and control (unselected) populations. In particular, significantly lower density of serotonin 5-HT<sub>1A</sub> receptors and significantly higher levels of serotonin and tryptophan hydroxylase were detected in brains of domesticated strain foxes (Popova et al. 1997; Trut 2001; Trut et al. 1974).

To understand the genetic basis of tame and aggressive behavior in the silver fox, it is first essential to have a genome map of the fox. Fortunately the evolutionary relationship between the dog and fox allows extension of recent progress in canine molecular genetics (Acland et al. 1998, 1999; Breen et al. 2001; Kirkness et al. 2003; Lin et al. 1999; Mellersh et al. 1997; Sidjanin et al. 2002; Yang et al. 2000; Zhang et al. 2002) to the fox genome. Cytogenetically the relationship between the dog and fox genomes is well understood. The remarkable karyotypic differences between fox and dog represent 26 chromosomal fusion events and 4 fission events (Graphodatsky et al. 2000, 2001; Yang et al. 1999, 2000). Cross-

species reciprocal chromosome painting and DAPI banding studies have clearly established the homology of chromosomal segments between domestic dog, red fox, and human (Yang et al. 1999). Thus, in attempting to create a meiotic map of the fox genome, we already know, to a first approximation, how and where the various linkage groups are arrayed, and might be able to use microsatellites from the canine genome map (Breen et al. 2001).

As a first step toward development of a fox genetic map, we have generated a primary set of 400 polymorphic markers for the fox genome based on the canine microsatellite database (Breen et al. 2001; Mellersh et al. 1997). To evaluate the informativeness of canine-derived microsatellites for the fox genome and their potential for constructing a fox meiotic map, we calculated polymorphism information content (PIC) values for 30 markers and estimated inbreeding coefficients of foxes in existing pedigrees. To optimize the map for behavioral studies, we plan to include polymorphic markers corresponding to genes involved in neurotransmitter function. As an initial step, serotonin receptor genes *5-HT<sub>1A</sub>* and *5-HT<sub>1B</sub>* have been selected. In man, abnormalities in serotonin metabolism are implicated in autism, anxiety, depression, obsessive-compulsive disorder, schizophrenia, and many other neuropsychiatric diseases (Cook and Leventhal 1996; Nebigil et al. 2001; Shekhar et al. 2001). In mice, the 5-HT<sub>1A</sub> receptors are implicated in the modulation of exploratory and fear-related behaviors, and aggressiveness in *5-HT<sub>1B</sub>* knockout mice is greater than in wild type (Ase et al. 2000; Miczek et al. 2001; Saudou et al. 1994; Tarantino and Bucan 2000). Serotonin receptor genes *5-HT<sub>1A</sub>* and *5-HT<sub>1B</sub>* have been sequenced from aggressive and domestic foxes and single nucleotide polymorphisms (SNPs) identified for nonparametric linkage and association studies of behavioral trait inheritance in this fox model of domestication.

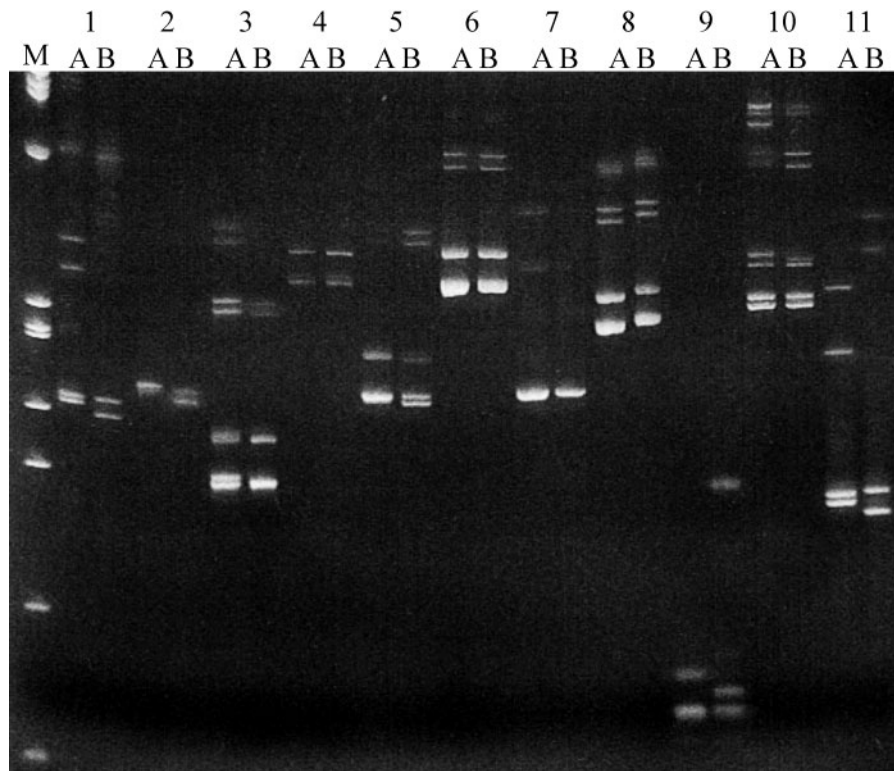
## Materials and Methods

### Fox Colony and DNA Samples

Fox blood samples were collected from tame and aggressive silver foxes maintained at the experimental farm of the ICG of the Russian Academy of Sciences. Canine tissue samples were collected postmortem from animals maintained at the Retinal Disease Studies Facilities (RDSF) in Kennett Square, Pennsylvania. DNA was purified from silver fox blood and dog spleen samples by the phenol-chloroform method (Sambrook et al. 1989).

### PCR Amplification of Canine Microsatellite Markers from Fox DNA

To identify genetic markers that worked robustly on fox DNA, we tested 700 previously published canine microsatellites (Breen et al. 2001; [www-recomgen.univ-rennes1.fr/Dogs/maquette.html](http://www-recomgen.univ-rennes1.fr/Dogs/maquette.html); [www.fhcr.org/science/dog\\_genome/dog.html](http://www.fhcr.org/science/dog_genome/dog.html)) using DNA from two silver foxes and standardized conditions (initial 2 min denaturation at 96°C, followed by 30 cycles: 96°C for 20 s, 58°C for 20 s, 72°C for 20 s, and a final



**Figure 1.** Scoring of fox amplified microsatellites with dog-specific primers. Each marker was amplified on DNA from two unrelated foxes (A, B). A 10  $\mu$ l aliquot of each PCR product (from a total reaction volume 25  $\mu$ l) was electrophoresed in 10% native polyacrylamide gel. (M) *O/HaeIII*; (1) DGN3; (2) REN112I02; (3) REN162B09; (4) REN242N13; (5) AHTK338; (6) REN149E24; (7) FH2274; (8) REN229P15; (9) REN123E09; (10) REN47D17; (11) FH2613. For each marker, amplification robustness was scored, on a five-point scale, as the estimated intensity of the most prominent band. Markers 3, 5, 6, 7, 8, 10, and 11 received a score of 5; markers 1, 2, and 9 received a score of 4; marker 4 received a score of 3.

extension step at 72°C for 5 min). Markers that did not work robustly under these conditions were amplified with the same PCR program but with a  $T_m$  of 55°C or 61°C. PCR products were analyzed in 10% native polyacrylamide gel. Band intensity was scored by eye using a five-point scale. In parallel, a selected set of 12 fox DNA samples was genotyped by Marshfield Laboratories Mammalian Genotyping Service with a standard canine microsatellite set including 241 markers.

#### Estimation of Marker PIC Values and Population Inbreeding Coefficients ( $F_{IS}$ )

Thirty microsatellites, randomly selected from 400 that robustly amplified fox DNA and representing di- and tetra-repeat microsatellites, were tested on fox DNA to evaluate their PIC value in foxes, essentially as previously reported for fluorescently labeled canine markers (Cargill et al. 2002). Each marker was amplified on DNA from 32 colony animals, including 22 domesticated foxes (13 females and 9 males) and 10 aggressive foxes (6 females and 4 males). The forward primers of each pair were labeled with one of three fluorescent dyes: 6Fam, Hex, or Tet (IDT, Coralville, IA;

GIBCO BRL, Gaithersburg, MD) on the 5-end. PCR products with compatible dyes and fragment sizes were combined in multiplex sets and analyzed using the ABI 310 capillary-based genetic analyzer (Applied Biosystems, Foster City, CA). Each multiplex set included a mixture of three or four amplified markers. Collection and analysis of the multiplex sets was performed using the ABI GENESCAN version 3.1 software package (PE Applied Biosystems, Foster City, CA). The PIC value for each marker was calculated in an Excel worksheet.

Population heterozygosity was estimated for 25 of the 30 microsatellites used for PIC determination. Markers were amplified from DNA of 22 domestic and 10 aggressive foxes, and analyzed using the ABI 310. The inbreeding coefficient ( $F_{IS}$ ), which measures the reduction of heterozygosity due to nonrandom mating within a subpopulation, was estimated for domestic and aggressive fox populations using GenePop version 3.1 (<http://wbiomed.curtin.edu.au/genepop/>) (Weir and Cockerham 1984) and FSTAT (Goudet 1995). Hardy-Weinberg equilibrium tests were undertaken using option 1 of GenePop. Exact  $P$  values were estimated by the Markov chain method (dememorization: 1000; batches: 100; iterations per batch: 1000).

**Table 1.** 400 microsatellite markers for fox genome mapping

Canine chromosome	Marker identification <sup>a</sup>
CFA1	REN47D17*, C01.673*, FH2294*, FH2016*, FH2598*, FH2309*, FH2109U*, D01505*, C01.424*, AHTK338, REN242N13, REN162B09, REN112I02, DGN3, REN123E09, C01.251, ARGES, REN06N11, C02509, PGKAM, FH2452, FH2634, FH2326, REN229P15, C01.246
CFA2	FH2225*, FH2608*, FH2613*, FH2087U*, REN150M24*, AHT111*, FH3006*, C02.894*, C2.342, FH2274, REN149E24, REN303H07, REN280B15, FH2237, C02.030, REN315H04, FH2062, FH2132
CFA3	FH2131*, C03.895*, CPH19*, PEZ12*, FH2541*, FH2302*, FH2137*, FH2531, REN161A12, FH2316, ZUBECA4, REN157C08, REN260i04, REN216N05, REN73P04, C00802
CFA4	FH2097*, FH2142*, REN298N18*, REN160J02*, AHT103*, FH2412*, FH2534*, FH2457*, PEZ17, REN79O09, REN282C02
CFA5	FH2140*, REN42N13*, REN265H13*, C05.771*, REN192M20*, REN92G21, AHTH248, REN78M01, REN175P10, AHTK315, REN122J03, TAT, C05.377*, CPH18*, CPH14*, ZUBECA6*
CFA6	FH2561*, C06.636*, REN149M14*, REN152F02, FH2576, REN210i14, REN84K01, FH2164*, REN88M24, REN54C11, FH2370
CFA7	C09703*, REN162C04*, REN149P06*, FH2581*, 1B10*, VIASD10*, FH2174*, FH2201*, C07.620, FH2301*, REN200G14, REN99O20, REN286L19, REN69B24, FH2226
CFA8	C08.410*, FH2144*, PEZ11*, AHTH240*, REN288F11*, C08.618*, REN67O13, REN68M10, REN206K11, REN268I01, REN108A18, REN178J05, REN148E17, CMA1,
CFA9	REN198P23*, FH2186*, FH2248, FH2263*, REN278L10*, REN75M10*, REN256F13, REN42F01, REN54L20, REN144L19, G06401, REN126A15, MPO, REN145P07, KRT9
CFA10	FH2293*, FH2422*, ZUBECA1*, FH2537*, C10.16*, C10.602*, C10.781, C10.606, REN171O24, DGN8A, RVC8, REN181G20, REN271K09, DTRCN5, REN73F08, REN04F08
CFA11	AHT137*, REN147O02*, C11.873*, FH2019*, REN207M19, REN181F15, FH2004, REN245N06, REN161D14, REN105L03, C11.868, REN194N17, DTRCN4, REN57H24
CFA12	C05101*, FH2401*, C12.852*, FH2054*, FH2152*, C07003*, C12.406*, REN211B14, PEZ5*, REN170E21, REN213F01, REN258L11, REN153O12, REN194P04, REN89A22, REN208M20, C00104, REN234K01, REN9411, REN213F01
CFA13	REN307K04*, REN65L04*, AHT121*, REN166I13*, REN238L19, REN165L17, REN13N11, DTRCN6, REN154P17, REN107M21, REN66K24, REN227M12, C13.758, FH2348
CFA14	REN169D01*, FH2547*, C14.866*, REN289L09*, FH2258*, PEZ10*, FH2600, REN141P20, FH3019, FH2658
CFA15	REN230G12*, AHT139*, FH2088*, FH2360*, REN06C11*, FH2535*, RVC1*, REN303E22*, REN307J23, REN265J03, REN143N23, REN159B09, CPH4, REN144M10, REN204N22, FH2295, REN123N11
CFA16	FH2175*, C16.436, C16.147*, FH2155, REN96i08, REN275L19, REN54I19, REN210K18
CFA17	PEZ8*, REN50B03*, REN310J13*, FH2321*, REN240A05, CPH10
CFA18	REN183B03*, FH3010*, AHT130*, REN42L13*, REN47J11*, REN186N13, REN50L03, REN248C19, REN266I17, FH2429*, C18.460
CFA19	REN91I114*, FH2380*, FH2279*, PEZ3*, CPH8, REN213G21, REN212E22
CFA20	CPH16*, FH2158*, FH2528*, C20.622*, REN93E07, PEZ19, REN119P03, REN124F16, REN130E03, REN250B12, DGN14, DAG1, FH2365
CFA21	FH2603*, REN285A14*, FH2312*, FH2233*, REN118B15, REN68M20, CCKBR
CFA22	REN262G14*, C22.279*, REN107H05*, REN42F10*, FH2538*, FH2109L*
CFA23	C23.745*, FH2508*, FH2626*, FH2001*, REN46F18*, REN210D03, CPH6
CFA24	AHT125*, FH2168*, FH2010, REN272I16, FH2495, FH2281, FH2261
CFA25	REN166C13*, REN54E19, FH2526*, AHT140*, FH2006*, C25.213*, FH2318*, FH2324*, FH2087L*
CFA26	C26.733*, REN01O23*, N41*, AHTK211*, DTRCN9, REN299M21, REN48E01
CFA27	PEZ6*, REN277O05*, LEI002*, REN208N23*, FH2289*, C27.442*, PEZ16, C27.502, REN65A09, FH2346, SLC6A12, DRPLA, VDR
CFA28	DTRCN14*, REN51i12*, REN309N19*, FH2585*, C28.176*, REN146G17*, REN73i23, C28.434
CFA29	CPH9*, REN45F03*, FH2328*, C29.883, C29.002, FH2364
CFA30	REN245M07*, REN105I08*, REN50N18*, REN248F14*, FH2050*, LEI-1F11*, C30.204
CFA31	REN265M13*, FH2189*, REN43H24*, REN239G04*, REN50I04

**Table 1.** Continued

Canine chromosome	Marker identification <sup>a</sup>
CFA32	AHT127*, CPH2*, REN244E04, D03908, UOR0421
CFA33	FH2507*, REN98D17*, REN291M20*, FH2165*, FH2361*, REN186B12, REN211M13, FH2790
CFA34	REN160M18*, REN53L08*, REN314H10*, FH2377*, REN125M11
CFA35	REN01G01*, REN214H22*, REN94K23*, REN172L08*
CFA36	REN85C13*, FH2611*
CFA37	AHT133*, H10101*, AHT135*
CFA38	AH91*, RENo2C20*, REN86G15*, REN164E17*, REN312K09
CFAX	FH3027*, REN130F03*, F9, FH2584*, FH2916, SSR4, <i>FH2548</i>
Unassigned	<u>FH3367</u> , <u><i>AHTK248</i></u>

\* Marker in Marshfield set.

<sup>a</sup> Sequenced markers are underlined; markers which require  $T_m$  55°C are in italics, markers which require 61°C are in italics and underlined.

### Sequencing of *5-HT<sub>1A</sub>* and *5-HT<sub>1B</sub>* Receptor Genes from Fox and Dog

The *5-HT<sub>1A</sub>* and *5-HT<sub>1B</sub>* receptor genes from fox and dog were amplified as a set of overlapping PCR fragments. PCR primers were designed partly from human sequences and partly from dog sequences retrieved from the TIGR canine genomic sequence (Kirkness et al. 2003). Fox and dog PCR products were sequenced and assembled into contigs using Sequencher 4.1. Multiple alignments between *5-HT<sub>1A</sub>* and *5-HT<sub>1B</sub>* receptor gene sequences from different species were performed using MegAlign, part of the DNASTar package. GENESCAN (MIT, Cambridge, MA) was utilized for open reading frame prediction.

## Results

### A Primary Microsatellite Marker Set for the Fox Genome

From 700 canine microsatellite markers tested, 400 (approximately 60%) worked robustly on fox DNA, reliably producing PCR products scored as four or better on a five-point scale (Figure 1 and Table 1). Although patterns of higher molecular weight stutter bands were observed on these gels, similar to those seen with products from canine DNA and arising from secondary structures of PCR products under nondenaturing gel conditions, they did not create problems in assigning marker genotypes. Thirty-five fox-amplified markers were sequenced to determine that the appropriate microsatellite repeats, as expected from canine marker information, were present in the fox PCR products (Table 2); in all cases the sequencing confirmed the expected result. The 400 markers that repeatedly worked well on fox DNA were selected as a primary set of genetic markers for initial mapping of the fox genome. Genotyping results on fox DNA samples analyzed by Marshfield Laboratories Mammalian Genotyping Service (MGS) confirmed the above results, and showed that approximately 55% of the canine marker set used by the MGS worked reliably on fox DNA (Table 1).

### Estimation of PIC Values of Selected Markers and Inbreeding Coefficients for Domestic and Aggressive Fox Populations

A representative multiplex set of fluorescently labeled di- and tetranucleotide microsatellites PCR amplified from fox DNA and analyzed on the ABI 310 is shown in Figure 2. Among the 30 markers tested, the calculated PIC was greater than 0.7 for 6 markers (20%); between 0.5 and 0.7 for 12 (40%); between 0.5 and 0.3 for 9 (30%); and less than 0.3 for 3 markers (10%) (Table 2). The number of fox alleles varied among the microsatellites, with a mean allele number of 5.1 for the 30 analyzed microsatellites. Ninety-two percent of these markers tested in the tame population and 88% of markers tested in the aggressive population were in Hardy-Weinberg equilibrium. Population inbreeding coefficients ( $F_{IS}$ ), calculated from data for 25 polymorphic markers, and for the same animals used for estimation of microsatellite PIC values, yielded mean values of 0.038 for 22 foxes from the tame population and 0.030 for 10 animals from the aggressive population.

### Sequencing of *5-HT<sub>1A</sub>* and *5-HT<sub>1B</sub>* Receptor Genes from Fox and Dog

Both the dog and fox orthologs of the *5-HT<sub>1A</sub>* and *5-HT<sub>1B</sub>* receptor genes were amplified as sets of overlapping fragments using primers designed from human sequence or from fragments of canine *5-HT<sub>A1</sub>* and *5-HT<sub>B1</sub>* gene sequences obtained from the TIGR 1.5X canine genome sequence (Kirkness et al. 2003). All primers used are listed in Table 3; primer positions and fragment sizes are specified relative to the corresponding fox gene sequence; GenBank accessions: AY204569 (*5-HT<sub>1A</sub>*); AY204571 (*5-HT<sub>1B</sub>*). The genomic sequences for the fox *5-HT<sub>1A</sub>* and *5-HT<sub>1B</sub>* receptor genes were obtained from fragments amplified from DNA of three distantly related foxes from the domestic population, and DNA of two animals from the aggressive group, and resolved by alignment of sequence data from cloned fragments. Canine *5-HT<sub>1A</sub>* and *5-HT<sub>1B</sub>* sequences were

**Table 2.** Characteristics of 30 microsatellite markers randomly selected from 400 canine microsatellites that robustly amplified fox DNA

Marker	PIC	FIS domestic	FIS aggressive	Number of animals analyzed	Allele number	Repeat	CFA
DGN3	0.59	0.018	-0.438	31	4	Tetra	1
REN162B09	0.36	0.103	0.067	28	3	Di	1
FH2309	0.39	-0.121	-0.105	29	3	Tetra	1
C01.424	0.36	-0.224	0.517	30	2	Di	1
FH2132	0.43	-0.216	-0.204	30	5	Tetra	2
FH2316	0.77	0.084	-0.085	32	9	Tetra	3
FH2541	0.72	-0.075	0.103	30	7	Tetra	3
FH2531	0.74	-0.029	0.018	31	8	Tetra	3
REN161A12	0.34	0.211	-0.247	31	4	Di	3
FH2097	0.35	-0.074	0.169	32	4	Tetra	4
CPH18	0.51	0.113	0.488	31	4	Di	5
C05.771	0.11			31	4	Di	5
C06.636	0.50	0.439	0.167	31	4	Di	6
FH2174	0.76	0.045	0.038	31	12	Tetra	7
REN69B24	0.06			32	3	Di	7
FH2226	0.73			23	7	Tetra	7
FH2201	0.41	-0.185	0.449	32	4	Tetra	7
REN278L10	0.57	-0.166	-0.292	30	6	Di	9
REN256F13	0.67	0.153	-0.167	31	5	Di	9
REN42F01	0.14	-0.053	0.000	30	3	Di	9
C10.606	0.42	-0.206	-0.532	28	3	Di	10
REN170E21	0.50	0.726	0.641	27	4	Di	12
FH2348	0.52	0.065	-0.302	29	5	Tetra	13
FH2658	0.64	-0.090	-0.067	29	8	Tetra	14
FH3019	0.49	-0.169	-0.151	30	4	Di	14
FH3367	0.55	0.225	0.554	31	7	Unpublished	
DGN14	0.72	0.114	-0.113	31	6	Tetra	20
FH2281	0.57	0.020	0.143	30	7	Tetra	24
FH2289	0.61			28	4	Tetra	27
FH2364	0.42			17	5	Tetra	29

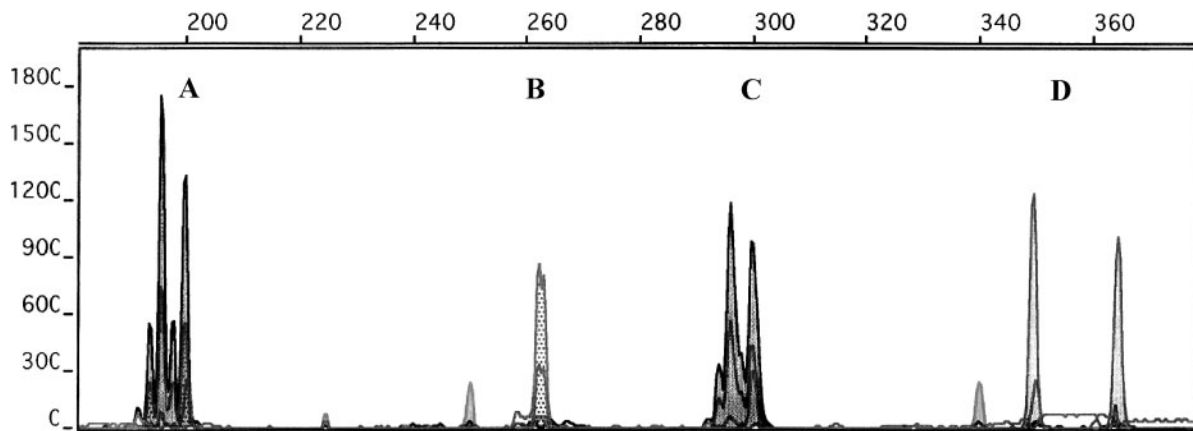
assembled from genomic fragments extracted from the TIGR database, and sequences of *5-HT<sub>1A</sub>* and *5-HT<sub>1B</sub>* receptor genes we amplified from genome DNA of a collie dog. Analysis of fox and dog gene structures predicted only one coding exon for both the *5-HT<sub>1A</sub>* and *5-HT<sub>1B</sub>* receptor genes of the fox and dog.

Multiple sequence alignment of fox, dog, and human sequences for *5-HT<sub>1A</sub>*, including the previously deposited canine *5-HT<sub>1A</sub>* sequence (GenBank accession AY134445; Van Den Berg et al. 2003) revealed 99.5% nucleotide identity between fox and dog sequences, 89.4% between fox and human, and 89.0% between dog and human (Figure 3a). Alignment of fox, dog, and human sequences for *5-HT<sub>1B</sub>* yielded identities of 97.0%, 91.3%, and 91.2%, respectively (Figure 3b).

Sequence comparison among *5-HT<sub>1A</sub>* fragments amplified from different foxes revealed one silent mutation (C/T) in the coding region in position 1184; genotypes C/C and C/T were observed. Two SNPs were identified from comparison of *5-HT<sub>1B</sub>* sequences from tame and aggressive foxes in the noncoding part of the gene. Genotypes C/T and T/T were identified for the SNP located at position 174, and three genotypes (G/T, T/T, and G/G) were observed for the SNP at position 1684.

## Discussion

In developing this set of 400 markers for mapping the fox genome, the opportunity to exploit recent advances in canine and comparative genomics has been advantageous. Each of these markers has been previously mapped meiotically and/or by RH panels on the dog genome (Breen et al. 2001), thus the canine locations are known with confidence. As a starting point in developing this set, a marker set of 172 canine microsatellites previously developed for canine genomewide screens was exploited. For canine application, it is estimated that 42% of the genome is within 5 cM of at least one marker in this set, and 77% of the genome is within 10 cM (Richman et al. 2001). To adapt and optimize the set for application to the fox genome, several markers from this canine set have been replaced by microsatellites located closely nearby, and to improve the resolution and coverage, the overall number of markers was increased to 400. To a first approximation, the comparative location of these markers on fox chromosomes can be estimated by alignment of canine linkage groups with the results of reciprocal painting of dog and fox karyotypes (Yang et al. 1999, 2000). Thus the markers have been deliberately selected to be as uniformly and comprehensively distributed among the 34 fox metacentric chromosomes



**Figure 2.** An example of a multiplex set amplified from DNA of a domestic fox and analyzed with the ABI 310. **(A)** REN162B09: dinucleotide repeat labeled with Hex; allele sizes are 195 and 199 bp; **(B)** FH2174: tetra repeat labeled with Tet; allele size is 262 bp; **(C)** FH3019: dinucleotide repeat labeled with Hex; allele sizes are 296 and 300 bp; **(D)** FH2309: tetra repeat labeled with 6Fam; allele sizes are 250 and 364 bp.

as can be predicted. It remains a possibility that, at finer resolution than is currently seen by reciprocal chromosomal painting, regions of unexpected inconsistency between the dog and fox genomes may become apparent in the process of fox map construction.

Comparative alignment of the fox genome with those of other mammals (dog, human, mouse) will allow rapid prediction of which genes are likely to be in the vicinity of each mapped marker. This latter advantage will accelerate with the recently announced high priority for sequencing the dog genome ([www.nih.gov/news/pr/sep2002/nhgri-12.htm](http://www.nih.gov/news/pr/sep2002/nhgri-12.htm)). A partially assembled 1.5X survey sequence of the dog genome has already been deposited (Kirkness et al. 2003) and installments of an even more comprehensive canine sequenc-

ing project are currently being rapidly and continuously updated in the public domain databases ([www.ncbi.nlm.nih.gov/Traces/trace.cgi?](http://www.ncbi.nlm.nih.gov/Traces/trace.cgi?)).

To test the suitability of the target fox pedigrees for construction of a meiotic map of fox genome using the selected marker set, we estimated the level of heterozygosity in these populations and calculated the PIC value for 30 microsatellites. The average marker PIC value observed in the fox colony was 0.5. Over the multiple generations of selection of these foxes for specific behaviors, a deliberate effort was made to avoid inbreeding (Trut 2001). Analysis of population inbreeding coefficients ( $F_{IS}$ ) confirm that this strategy was successful in maintaining Hardy-Weinberg equilibrium in both tame and aggressive fox populations

**Table 3.** Primers for amplification of fox and canine  $5-HT_{1A}$  and  $5-HT_{1B}$  receptor gene fragments

Primer name	Sequence	Binding site <sup>a</sup>	$T_m$	Fragment size
Primers for $5-HT_{1A}$				
HTR1A-3	GAC GCA TGC ACC ATT AGC AAG G	557–578	58°C	639 bp
HTR1A-4	GGT TAA GCA GAG AGT TGG AGT AGC	1070–1093		
HTR1A-6	GAT GTA GAA AGC GCC AAA GGT GG	600–622	58°C	533 bp
HTR1A-7	GAC GTG ACC TTC AGC TAC CAA GTG	92–115		
HTR1A-10	CTG CCC TTC TGC GAG AGC AG	1106–1125	58°C	261 bp
HTR1A-11	GGA AGT CGC GAG CTG TCC CAG	1344–1364		
HTR1A-12	CAC CAC GCA CGC ATT GCC CAG CA	156–178	58°C	181 bp
HTR1A-18	AGG CAT GGA GGG GCT CAG C	1–19		
Primers for $5-HT_{1B}$				
HTR1B-1	CCA AGG ACT ACA TTT ACC AGG AC	326–348	58°C	756 bp
HTR1B-2	TAC ACA GGA GAT CCG GAT TCG CT	1057–1079		
HTR1B-3	GTG TCG GAA TGC GTG GTG AAC AC	808–830	58°C	573 bp
HTR1B-4	AAC GTA TCA GTT TAT GGA ATG CT	1356–1378		
HTR1B-5	TCA ACT CCC TTA TCA ACC CCA TCA	1301–1324	62°C	695 bp
HTR1B-6	ACT CTT CCA GGT TTC TCT GCA CCA	1970–1993		
HTR1B-18	CGT GGA GTA CAC GGT GTA GAG GAT G	837–861	58°C	863 bp
HTR1B-25	TAG CTA GGC GCT CTG GAA GTG CAG G	1–25		

<sup>a</sup> Binding site refers to position in corresponding fox sequence in GenBank ( $5-HT_{1A}$  receptor gene has accession number AY204569;  $5-HT_{1B}$  receptor gene—AY204571).

**a**

Vv.seq ATGGAGGGGCTCAGCCCGGACAGGGCAACAACACCCCTCGTCCGAGGGGGCCCTTCGGGACGGCGGGCAACGCTACTGGCATCTCCGACGCTGACCTTCA 100  
 Cf.seq ATGGAAGCTGGCTCAGCCCGGACAGGGCAACAACACCCCTCGTCCGAGGGGGCCCTTCGGGACGGCGGGCAACGCTACTGGCATCTCCGACGCTGACCTTCA 100  
 Hs.seq ATGGAAGCTGGCTCAGCCCGGACAGGGCAACAACACCCCTCGTCCGAGGGGGCCCTTCGGGACGGCGGGCAACGCTACTGGCATCTCCGACGCTGACCTTCA 100

Vv.seq GCTACCAAGTGCATCACTCCCTGCTGGGACAGCTCCTATTTCTGGCGGGTGGTGGGCAATGCGTGGTGGGCGCCATCGCCCTGGAGCGCTCCCT 200  
 Cf.seq GCTACCAAGTGCATCACTCCCTGCTGGGACAGCTCCTATTTCTGGCGGGTGGTGGGCAATGCGTGGTGGGCGCCATCGCCCTGGAGCGCTCCCT 200  
 Hs.seq GCTACCAAGTGCATCACTCCCTGCTGGGACAGCTCCTATTTCTGGCGGGTGGTGGGCAATGCGTGGTGGGCGCCATCGCCCTGGAGCGCTCCCT 200

Vv.seq GCAGAAATGTGGCCAAACTATCTCATCGGCTCCGCTGGCTGTGTCACCGACCTCATGGTGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGG 300  
 Cf.seq GCAGAAATGTGGCCAAACTATCTCATCGGCTCCGCTGGCTGTGTCACCGACCTCATGGTGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGG 300  
 Hs.seq GCAGAAATGTGGCCAAACTATCTCATCGGCTCCGCTGGCTGTGTCACCGACCTCATGGTGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGG 300

Vv.seq AAATGGACGCTGGGACAGGTCACCTGTGACCTATTCATTTGCCCTCGACGCTGTGTGCTGCACCTCGTCCATCTGACCTGTGGCCATTGGCGCTGGACA 400  
 Cf.seq AAATGGACGCTGGGACAGGTCACCTGTGACCTATTCATTTGCCCTCGACGCTGTGTGCTGCACCTCGTCCATCTGACCTGTGGCCATTGGCGCTGGACA 400  
 Hs.seq AAATGGACGCTGGGACAGGTCACCTGTGACCTATTCATTTGCCCTCGACGCTGTGTGCTGCACCTCGTCCATCTGACCTGTGGCCATTGGCGCTGGACA 400

Vv.seq GGTACTGGGCGCATCAGGACCCCATCGACTACGTTGAACAAGAGGAGCGCCCGGGCGCCGCTGCGGCTCATCTCGCTCACTTGGCTCATCGGCTTCTCTAT 500  
 Cf.seq GGTACTGGGCGCATCAGGACCCCATCGACTACGTTGAACAAGAGGAGCGCCCGGGCGCCGCTGCGGCTCATCTCGCTCACTTGGCTCATCGGCTTCTCTAT 500  
 Hs.seq GGTACTGGGCGCATCAGGACCCCATCGACTACGTTGAACAAGAGGAGCGCCCGGGCGCCGCTGCGGCTCATCTCGCTCACTTGGCTCATCGGCTTCTCTAT 500

Vv.seq CTCATTTCGGCCCATGCTGGTGTGGGCGACCCCGGAAGACCGCTGGACCCCGGACCGCTGACCAATCAGCAAGGACCAAGGCTACACTATCTACTCCAC 600  
 Cf.seq CTCATTTCGGCCCATGCTGGTGTGGGCGACCCCGGAAGACCGCTGGACCCCGGACCGCTGACCAATCAGCAAGGACCAAGGCTACACTATCTACTCCAC 600  
 Hs.seq CTCATTTCGGCCCATGCTGGTGTGGGCGACCCCGGAAGACCGCTGGACCCCGGACCGCTGACCAATCAGCAAGGACCAAGGCTACACTATCTACTCCAC 600

Vv.seq TTTGGCGCTTTCTATCCCTCGCTGCTCATGCTGGTCTCTACGGGCGCATCTTCCGCGCCGCGCGGCTTCCGCATCCGCAAAACAGTCAAGAAAGGCGG 700  
 Cf.seq TTTGGCGCTTTCTATCCCTCGCTGCTCATGCTGGTCTCTACGGGCGCATCTTCCGCGCCGCGCGGCTTCCGCATCCGCAAAACAGTCAAGAAAGGCGG 700  
 Hs.seq TTTGGCGCTTTCTATCCCTCGCTGCTCATGCTGGTCTCTACGGGCGCATCTTCCGCGCCGCGCGGCTTCCGCATCCGCAAAACAGTCAAGAAAGGCGG 700

Vv.seq AAGAGAAAGGAGCGGACCGCCCGCTCGGCGGCTGGGAGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG 800  
 Cf.seq AAGAGAAAGGAGCGGACCGCCCGCTCGGCGGCTGGGAGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG 800  
 Hs.seq AAGAGAAAGGAGCGGACCGCCCGCTCGGCGGCTGGGAGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG 800

Vv.seq GGGGAGCAAGGCTGGGGGGCTCTGTGCACCAAGCGCGGTTGAGGGGGGGCGGACGACGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG 900  
 Cf.seq GGGGAGCAAGGCTGGGGGGCTCTGTGCACCAAGCGCGGTTGAGGGGGGGCGGACGACGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG 900  
 Hs.seq GGGGAGCAAGGCTGGGGGGCTCTGTGCACCAAGCGCGGTTGAGGGGGGGCGGACGACGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG 900

Vv.seq TCCAAAGAGCACCTGCGGCTGCCAGCGAGGCTGGCGCCATCCCTTGGCGCCCGCCGCTCTTCCGAGAAAGAAATGAGGCGCAACCGCGAGGCGCAAGCGCA 1000  
 Cf.seq TCCAAAGAGCACCTGCGGCTGCCAGCGAGGCTGGCGCCATCCCTTGGCGCCCGCCGCTCTTCCGAGAAAGAAATGAGGCGCAACCGCGAGGCGCAAGCGCA 1000  
 Hs.seq TCCAAAGAGCACCTGCGGCTGCCAGCGAGGCTGGCGCCATCCCTTGGCGCCCGCCGCTCTTCCGAGAAAGAAATGAGGCGCAACCGCGAGGCGCAAGCGCA 1000

Vv.seq AGATGGCCCTGGCCCGGAGAGGAAACAGGTTGAAGACCGTGGGACATCATGAGGCGAGCTTTCATCTGCTGGCTGGCTTCTTCACTCGTGGCGCTGG 1100  
 Cf.seq AGATGGCCCTGGCCCGGAGAGGAAACAGGTTGAAGACCGTGGGACATCATGAGGCGAGCTTTCATCTGCTGGCTGGCTTCTTCACTCGTGGCGCTGG 1100  
 Hs.seq AGATGGCCCTGGCCCGGAGAGGAAACAGGTTGAAGACCGTGGGACATCATGAGGCGAGCTTTCATCTGCTGGCTGGCTTCTTCACTCGTGGCGCTGG 1100

Vv.seq CTTGCCCTTCTGGGAGAGCAGCTGCCACATGCCACCTTGTGGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG 1200  
 Cf.seq CTTGCCCTTCTGGGAGAGCAGCTGCCACATGCCACCTTGTGGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG 1200  
 Hs.seq CTTGCCCTTCTGGGAGAGCAGCTGCCACATGCCACCTTGTGGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG 1200

Vv.seq GCGTACTTCAACAAGGACTTCCAGAACCGCTTTAAAGAAAGATCGTCAAGGTTGCAAGTTCTGCCCGCGGAGCGG 1270  
 Cf.seq GCGTACTTCAACAAGGACTTCCAGAACCGCTTTAAAGAAAGATCGTCAAGGTTGCAAGTTCTGCCCGCGGAGCGG 1270  
 Hs.seq GCGTACTTCAACAAGGACTTCCAGAACCGCTTTAAAGAAAGATCGTCAAGGTTGCAAGTTCTGCCCGCGGAGCGG 1270

**b**

Vv.seq ATGGAAAGAGCGCCGGGCTCTCGGTGGGCGCCCGCCCGCGGCGGCGGCTCCAGACCGGGGGGCGCTCCAGCGCAACCTGTCTTGGGCTCCG --- CACAACCTGCA 97  
 Cf.seq ATGGAAAGAGCGCCGGGCTCTCGGTGGGCGCCCGCCCGCGGCGGCTCCAGACCGGGGGGCGCTCCAGCGCAACCTGTCTTGGGCTCCG --- CACAACCTGCA 97  
 Hs.seq ATGGAAAGAGCGCGGGCTCTCGGTGGGCGCCCGCCCGCGGCGGCTCCAGACCGGGGGGCGCTCCAGCGCAACCTGTCTTGGGCTCCG --- CACAACCTGCA 100

Vv.seq GCGCCGAGGGGCTACATCTACCGGACTCCATCGCGCTGGCCCTGGAAAGTGTCTCTGCGCTATTCTGCTGGCACTCTCTACCTTGGCCACCAACGCTCTCCCA 197  
 Cf.seq GCGCCGAGGGGCTACATCTACCGGACTCCATCGCGCTGGCCCTGGAAAGTGTCTCTGCGCTATTCTGCTGGCACTCTCTACCTTGGCCACCAACGCTCTCCCA 197  
 Hs.seq GCGCCGAGGGGCTACATCTACCGGACTCCATCGCGCTGGCCCTGGAAAGTGTCTCTGCGCTATTCTGCTGGCACTCTCTACCTTGGCCACCAACGCTCTCCCA 200

Vv.seq GCGCTTTGTGTGATCGCCACAGGTTGACCGGACCCCGGAAAGCTGTGCACACCCCGGCAACTACCTGATTTGGCTTCCCTGGCGGCTACCGGACCTGCTGCTCA 297  
 Cf.seq GCGCTTTGTGTGATCGCCACAGGTTGACCGGACCCCGGAAAGCTGTGCACACCCCGGCAACTACCTGATTTGGCTTCCCTGGCGGCTACCGGACCTGCTGCTCA 297  
 Hs.seq GCGCTTTGTGTGATCGCCACAGGTTGACCGGACCCCGGAAAGCTGTGCACACCCCGGCAACTACCTGATTTGGCTTCCCTGGCGGCTACCGGACCTGCTGCTCA 300

Vv.seq CTGGTGTATGCCCATCAGCACCATGTAACCGGTCACCGGCGGCTGGACGCTGGGCGAGGTTGGTCTGGCACTTGTGGCTGTGCTGGGACATCACTTGTGCA 397  
 Cf.seq CTGGTGTATGCCCATCAGCACCATGTAACCGGTCACCGGCGGCTGGACGCTGGGCGAGGTTGGTCTGGCACTTGTGGCTGTGCTGGGACATCACTTGTGCA 397  
 Hs.seq CTGGTGTATGCCCATCAGCACCATGTAACCGGTCACCGGCGGCTGGACGCTGGGCGAGGTTGGTCTGGCACTTGTGGCTGTGCTGGGACATCACTTGTGCA 400

Vv.seq GCGCCTTCCATCTCTGACCTCTGCGCTCATGCGCCTGGACCGCTACTGGGCGCATCAGGACCGCGCTGGAGTACTCCGCAAAAGGACTCCCAAGAGGGCGCG 497  
 Cf.seq GCGCCTTCCATCTCTGACCTCTGCGCTCATGCGCCTGGACCGCTACTGGGCGCATCAGGACCGCGCTGGAGTACTCCGCAAAAGGACTCCCAAGAGGGCGCG 497  
 Hs.seq GCGCCTTCCATCTCTGACCTCTGCGCTCATGCGCCTGGACCGCTACTGGGCGCATCAGGACCGCGCTGGAGTACTCCGCAAAAGGACTCCCAAGAGGGCGCG 500

Vv.seq GGTGATGATCGCGGCTCTGCTGGGCTTCTTCCATCTCTGCTGCGGCTGGGCGGCTTCTTCTGGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG 597  
 Cf.seq GGTGATGATCGCGGCTCTGCTGGGCTTCTTCCATCTCTGCTGCGGCTGGGCGGCTTCTTCTGGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG 597  
 Hs.seq GGTGATGATCGCGGCTCTGCTGGGCTTCTTCCATCTCTGCTGCGGCTGGGCGGCTTCTTCTGGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG 600

Vv.seq GTGAACACCGACCAATCTCTACACCGTGTACTCCAGGGTGGGCGGCTTCTTACTTCCCGACCTTGTCTTCCATCGCCCTCTACGGGCGGCTCTACGTTG 697  
 Cf.seq GTGAACACCGACCAATCTCTACACCGTGTACTCCAGGGTGGGCGGCTTCTTACTTCCCGACCTTGTCTTCCATCGCCCTCTACGGGCGGCTCTACGTTG 697  
 Hs.seq GTGAACACCGACCAATCTCTACACCGTGTACTCCAGGGTGGGCGGCTTCTTACTTCCCGACCTTGTCTTCCATCGCCCTCTACGGGCGGCTCTACGTTG 700

Vv.seq AGCGCCGCTCCCGGCTATTTGTAACAGGACCGCCCAACAGGACCGGCAAGCGGCTGACCGGAGCCAGCTGATAACCGACTCCCGGGGTTCAACGCTCTCGGT 797  
 Cf.seq AGCGCCGCTCCCGGCTATTTGTAACAGGACCGCCCAACAGGACCGGCAAGCGGCTGACCGGAGCCAGCTGATAACCGACTCCCGGGGTTCAACGCTCTCGGT 797  
 Hs.seq AGCGCCGCTCCCGGCTATTTGTAACAGGACCGCCCAACAGGACCGGCAAGCGGCTGACCGGAGCCAGCTGATAACCGACTCCCGGGGTTCAACGCTCTCGGT 800

Vv.seq CACCTCCGTTGAACTCGGGGCTCCCGACGCTGCCAGCGGAAATCGGGGTTCCCGGCTGACGTTGAAACCAAGTCAAAGTGGGCTCTCGGACCGCTGCTGGA 897  
 Cf.seq CACCTCCGTTGAACTCGGGGCTCCCGACGCTGCCAGCGGAAATCGGGGTTCCCGGCTGACGTTGAAACCAAGTCAAAGTGGGCTCTCGGACCGCTGCTGGA 897  
 Hs.seq CACCTCCGTTGAACTCGGGGCTCCCGACGCTGCCAGCGGAAATCGGGGTTCCCGGCTGACGTTGAAACCAAGTCAAAGTGGGCTCTCGGACCGCTGCTGGA 900

Vv.seq AAGAAAGAACTCATGGCCGCTAGGGAGCGCAAAAGCACCAAGACCTTGGGATCATTTTGGGAGCCTTTATCGTGTGGTGGGCTGGGCTTCTTCACTCATCT 997  
 Cf.seq AAGAAAGAACTCATGGCCGCTAGGGAGCGCAAAAGCACCAAGACCTTGGGATCATTTTGGGAGCCTTTATCGTGTGGTGGGCTGGGCTTCTTCACTCATCT 997  
 Hs.seq AAGAAAGAACTCATGGCCGCTAGGGAGCGCAAAAGCACCAAGACCTTGGGATCATTTTGGGAGCCTTTATCGTGTGGTGGGCTGGGCTTCTTCACTCATCT 1000

Vv.seq CCTGGTGTATGCTATTTGCAAGGATGCTGCTGGTTCACCTGGCCATTTTGAATTTCTTCACTGGGCTGGGCTTCTTCACTTCACTCCCTTATCAACCCCAT 1097  
 Cf.seq CCTGGTGTATGCTATTTGCAAGGATGCTGCTGGTTCACCTGGCCATTTTGAATTTCTTCACTGGGCTGGGCTTCTTCACTTCACTCCCTTATCAACCCCAT 1097  
 Hs.seq CCTGGTGTATGCTATTTGCAAGGATGCTGCTGGTTCACCTGGCCATTTTGAATTTCTTCACTGGGCTGGGCTTCTTCACTTCACTCCCTTATCAACCCCAT 1100

Vv.seq CATCTATACCATGCTCAATGAGGACTTCAAAACAGGCTTCCATAAACTGATACCGCTTTAAAGTGGCGCAAGGTTGA 1171  
 Cf.seq CATCTATACCATGCTCAATGAGGACTTCAAAACAGGCTTCCATAAACTGATACCGCTTTAAAGTGGCGCAAGGTTGA 1171  
 Hs.seq CATCTATACCATGCTCAATGAGGACTTCAAAACAGGCTTCCATAAACTGATACCGCTTTAAAGTGGCGCAAGGTTGA 1174

**Figure 3. (A)** Alignment of coding regions of fox (Vv), dog (Cf), and human (Hs) *5-HT<sub>1A</sub>* receptor gene. **(B)** Alignment of coding regions of fox (Vv), dog (Cf), and human (Hs) *5-HT<sub>1B</sub>* receptor gene.

( $F_{IS}$ , tame = 0.038; aggressive = 0.030; overall = 0.036). The  $F_{IS}$  95% confidence interval (-0.040, 0.124) was estimated by the bootstrapping algorithm of FSTAT. Significant positive  $F_{IS}$  values were observed for markers REN170E21 (0.641), FH3367 (0.554), C01.424 (0.517), FH2201 (0.449), and

CPH18 (0.488) in the aggressive population, and for markers REN170E21 (0.726) and C06.636 (0.439) in tame population. As these high  $F_{IS}$  values were observed for only a few loci, it is likely that these result from small sample sizes rather than inbreeding. Alternatively, they might represent the result of

selection for specific genome regions; this will be tested by sampling more individuals and genotyping additional closely located markers.

A genomewide screen of fox families informative for both the behavioral phenotypes and the associated morphological traits that arose de novo in the domestic fox population can now be undertaken. Such a study would allow valuable insights into the molecular genetics of tame and aggressive behaviors. Because the populations of tame and aggressive foxes were created relatively quickly by selection focused only on specific behavioral traits, there are likely to be relatively few major genetic loci influencing the development of these phenotypes. It is known that a single gene mutation can cause some types of abnormal behavior. For example, mutation in the monoamine oxidase gene A (*MAOA*), involved in the metabolism of serotonin, dopamine, and noradrenalin, has been identified in an X-linked impulsive aggressive behavioral disorder in humans (Brunner et al. 1993).

To use the fox meiotic linkage map optimally, we will also need sequence data for identified regions of interest. Availability of comparative genomic sequence from the dog and other mammals is expected to be highly exploitable in such a situation. To test this hypothesis we cloned and sequenced the dog and fox orthologs of two genes, *5-HT<sub>1A</sub>* and *5-HT<sub>1B</sub>*, which are involved in serotonin metabolism. As anticipated, cloning and sequencing of the fox *5-HT<sub>1A</sub>* and *5-HT<sub>1B</sub>* receptor genes demonstrated very strong sequence conservation (Figure 3). Similar conservation was seen in comparative alignments of orthologous fox and dog gene sequences available from GenBank (NCBI, NIH). For example, comparison of the coding regions of the *MCR1* (X90844, AF064455) and growth hormone genes (E07594, AF69071) using MegAlign demonstrated 99.1% and 92.8% similarity, respectively. These data demonstrate the high identity between coding regions of fox and dog genes and let us predict a broad potential for using canine sequences to clone fox genes.

The close evolutionary relationship between fox and dog predicts and is confirmed by the analyses of gene sequences and microsatellites reported here. This allows the application of modern tools developed for canine molecular genetics to genetic studies of the silver fox. Development of a fox genetic map and the mapping of behavioral and morphological phenotypes segregating in selected fox populations will provide insight into the mechanisms underlying canid domestication and will help to identify candidate genes potentially involved in human disorders of behavior and social interaction. Higher-resolution comparisons of the fox and dog genomes than has previously been possible will also yield insights into the chromosomal and genomic reorganizations associated with the divergence of the wolf-like and fox-like members of the *Canidae*.

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