

Лисицы и их геном



Why some animals are so nice?

- We still know little about molecular mechanisms influencing animal responses to humans.
- We can easily establish close interactions with some animals but not with others.
- Can animals which are more kin to interact with humans help us to learn about mechanisms of social behavior?



<https://pixabay.com>



photo by Nina Leen



www.dogzombie.com



Courtesy of Ivan Kvasov



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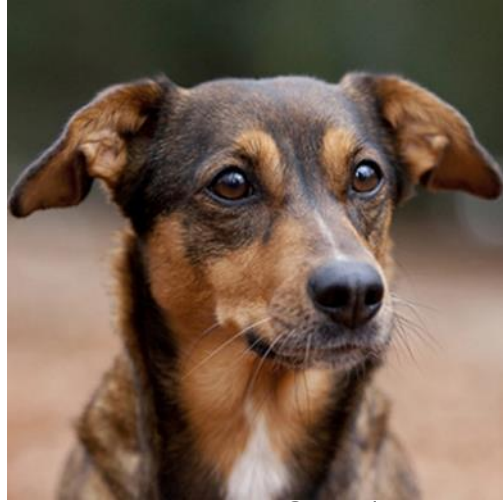


What is domestication?

- Domestication is distinct from mere taming of wild-born animals.
- It is the process whereby an animal is transformed from a life in a wild to a life under some control of humans.
- It involves animal transformations through many generations.



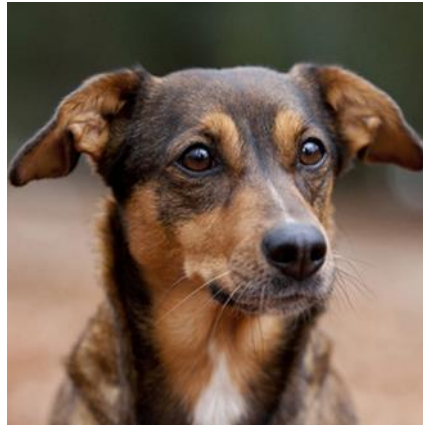
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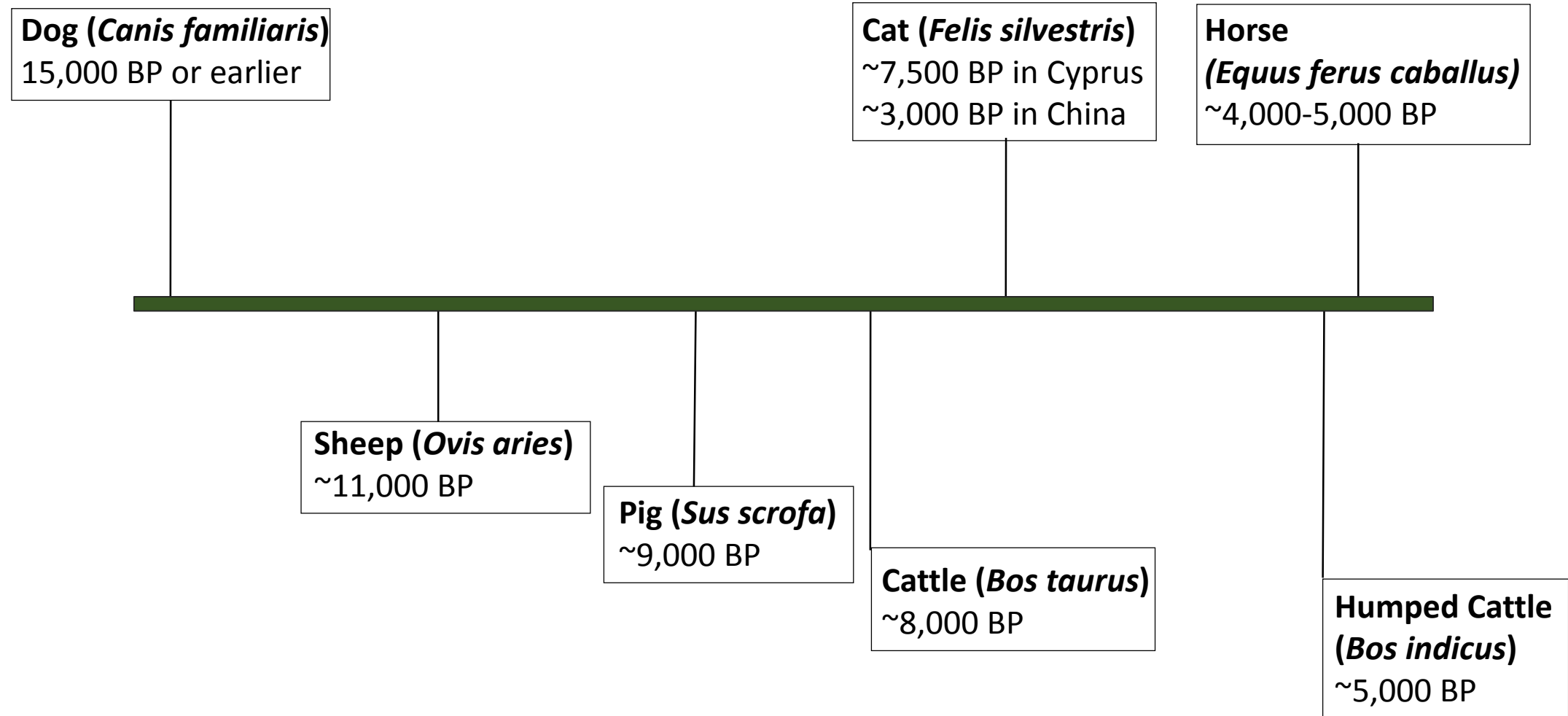


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Time scale of domestication



Physiological and morphological changes



National Geographic, photo by Robert Clark



<https://news.illinois.edu/view/6367/204936>

- “By a domesticate, I mean a species bred in captivity and thereby modified from its wild ancestors in ways making it more useful to humans who control its reproduction and food supply.”

Jared Diamond (Guns, germs, and steel)

Behavioral changes associated with domestication

- Reduced aggressiveness
- Reduced sensitivity to environmental changes
- Increased social tolerance among conspecifics



Courtesy of Darya Shepeleva

Genetics of domestication



- New sequencing technologies allow comparison of genomes and gene expression patterns of domesticated species and their wild counterparts.
- Finding genes and gene networks influencing domesticated behavior will inform us about physiology and neurobiology of animal social behavior.

Red fox (*Vulpes vulpes*) is an experimental model of animal domestication

*The Institute of Cytology and Genetics
of the Russian Academy of Sciences*



1959



Dr. Dmitry Belyaev



Dr. Lyudmila Trut

Selection for tame behavior

?

Red fox (*Vulpes vulpes*) is an experimental model of animal domestication

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1959

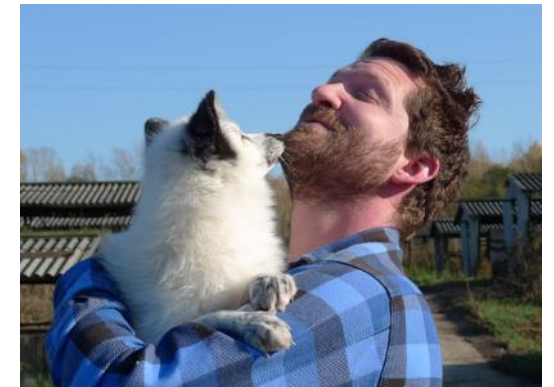
Selection for tame behavior



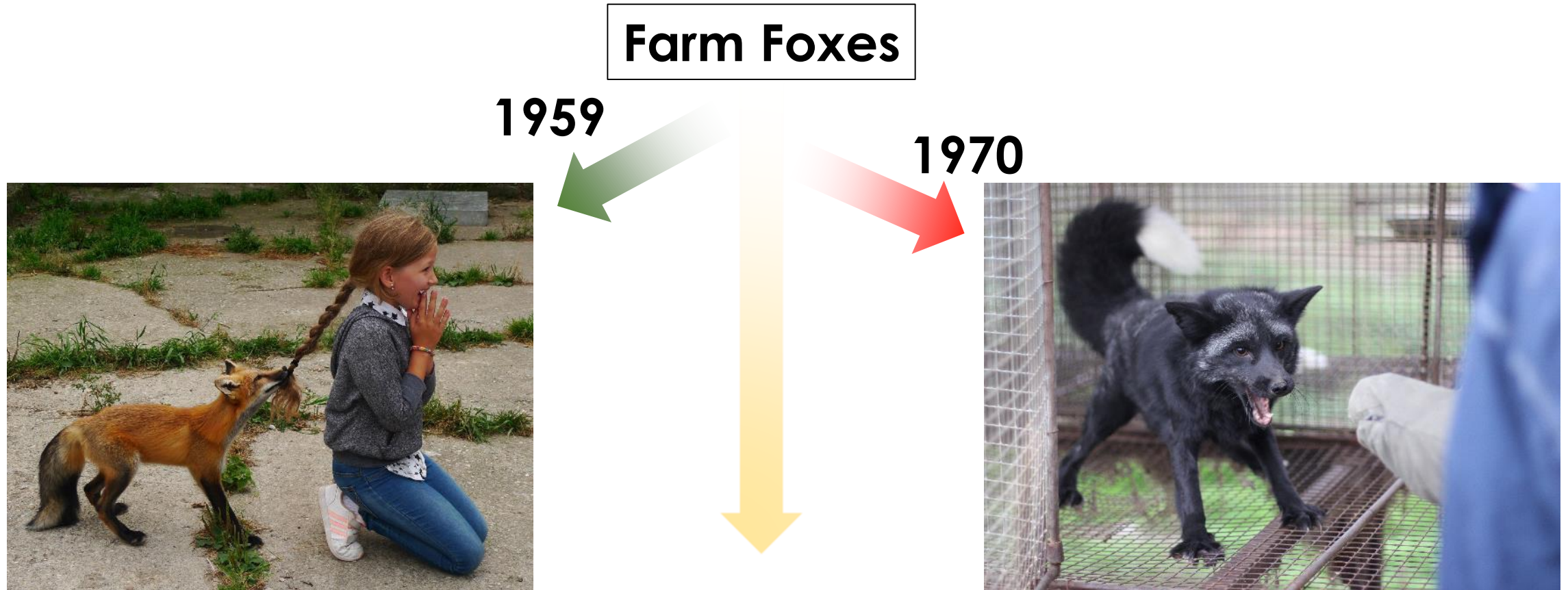
Dr. Dmitry Belyaev



Dr. Lyudmila Trut



Farm-fox experiment





Step A:
STAY

Step B:
OPEN DOOR



Step C:
TOUCH

Step D:
STAY

Advantages of the Fox Model

- Selected solely for behavior
- Genetic inheritance of the tame and aggressive phenotypes was carefully confirmed.
- The populations are outbred and provide an opportunity for high resolution genetic mapping.
- All foxes live under standard farm conditions and their behavior can be tested using standardized methods.

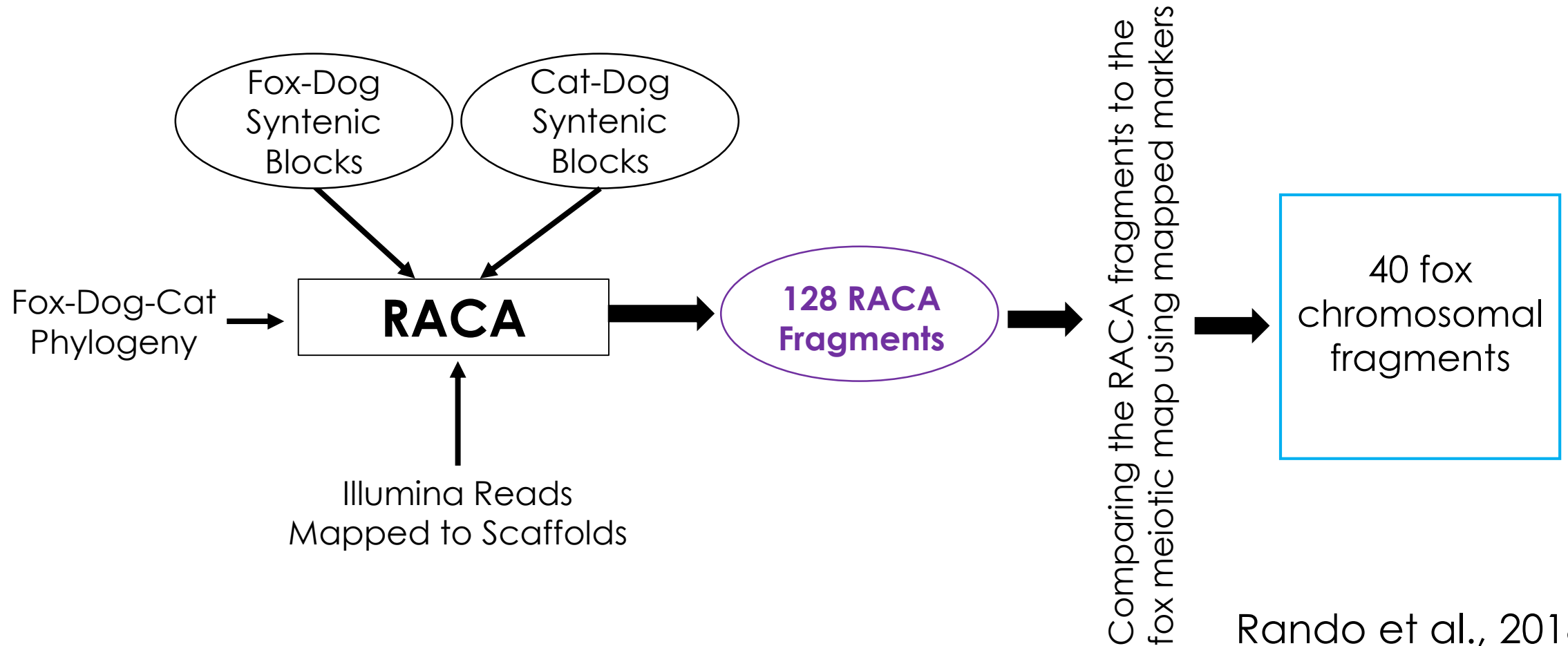
Fox Genome Assembly



- The red fox genome was sequenced at 93X and assembled using SOAPdenovo2
 - Total length: 2,495,544,672 bp
 - Scaffold N50: 11,799,617 bp
 - Number of scaffolds: 676,878
 - 94% of the genome is in top 500 scaffolds ranging from 47.7 Kb to 55.7 Mb

Kukekova et al., Nature E&E, 2018

Construction of Red Fox Chromosomal Fragments

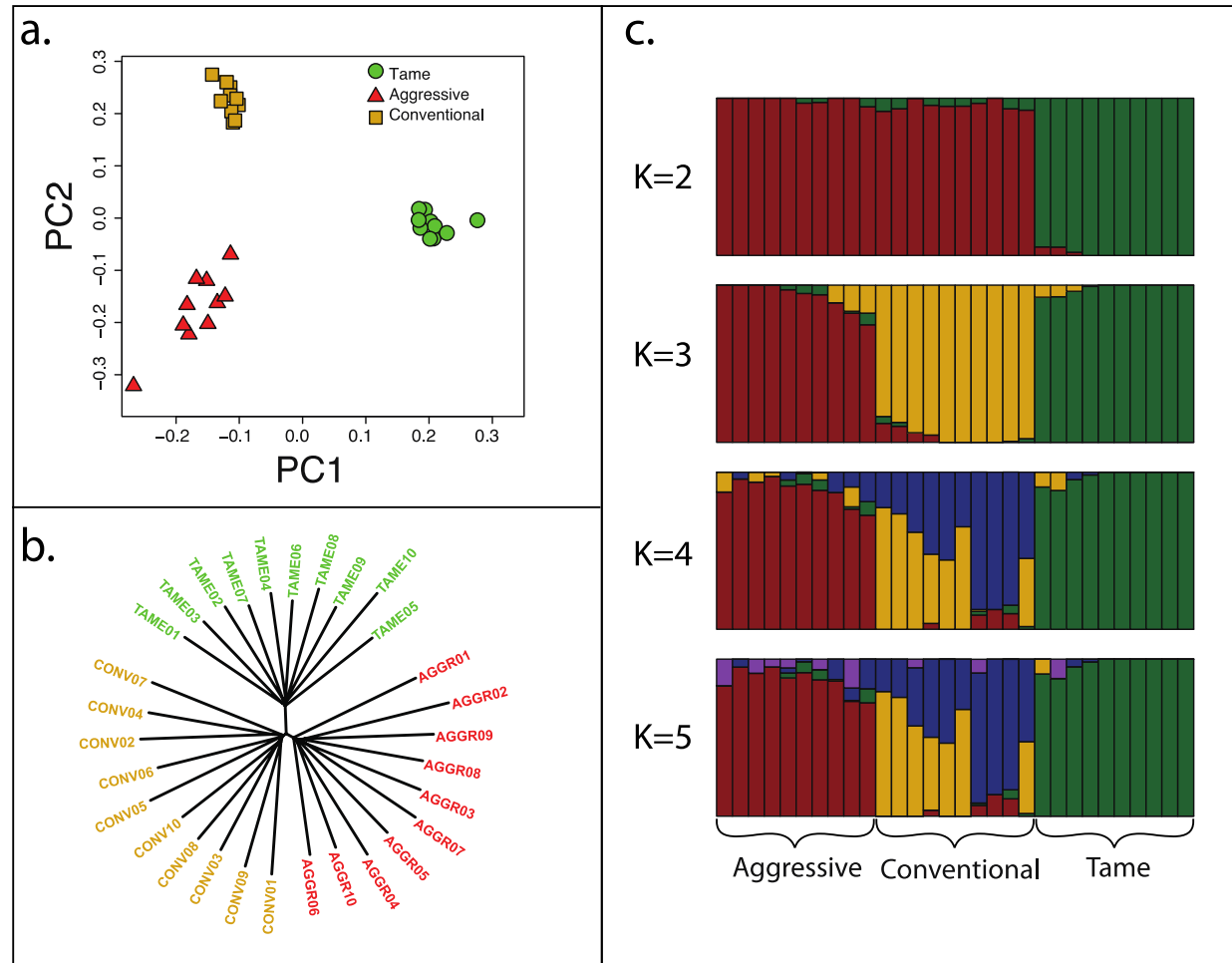


Rando et al., 2018

Comparative analysis of fox populations

- Re-sequenced 10 foxes from each of the three populations maintained at the Institute of Cytology and Genetics in Novosibirsk:
 - Tame
 - Aggressive
 - Conventional
- Identified 8,324,814 fox SNPs

Fox population structure



Search for targets of selection for behavior

- Pooled heterozygosity (H_p) analysis: identification of genomic regions with reduced heterozygosity in each of the three populations.
- F_{ST} analysis: identification of genomic regions with extreme divergence between the populations.

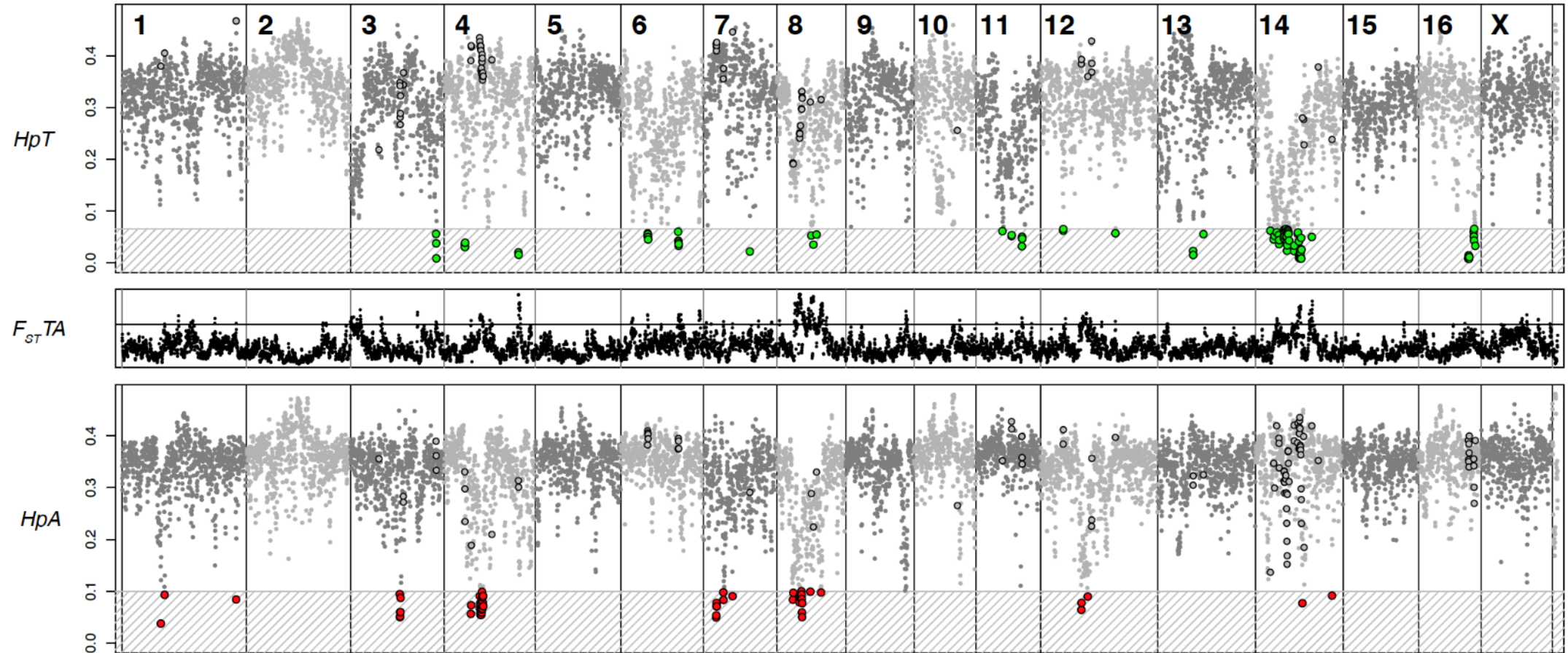
F_{ST} and pooled heterozygosity (H_p)

- Window based analyses
 - Window size: 500 Kb
 - Average number of SNPs per window: 1784
- H_p cut off in each population was established by permutations (Qanbari et al., 2012) and simulations
- F_{ST} cut off (>0.458) was established using gene drop simulations

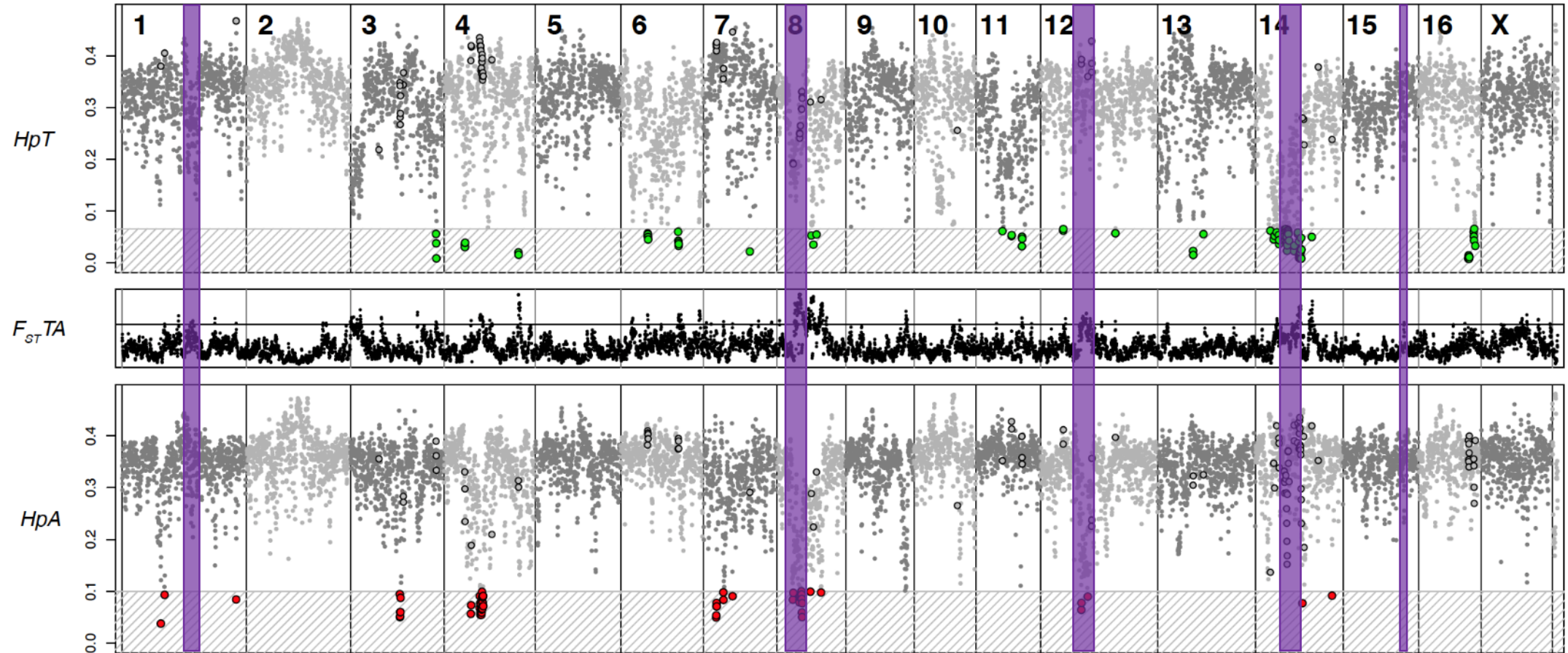
F_{ST} and H_p analyses in tame and aggressive populations

- Number of windows with H_p at $p < 0.0001$
 - Tame (H_{pT}): 96 (combined: 30)
 - Aggressive (H_{pA}): 60 (combined: 19)
- Number of windows with $F_{ST} > 0.458$
 - 276 (combined: 57)

F_{ST} and H_p analyses in tame and aggressive populations



Comparison of behavioral QTL intervals with genomic regions identified in F_{ST} and Hp analyses



Behavioral trait D.PC1

Step A:
STAY

Step B:
OPEN DOOR

Step C:
TOUCH

Step D:
STAY



Behavioral trait D.PC1

PC	Behavioral category	Variance
D.PC1	Avoiding the front part of the cage	50% of behavioral variance at step D
	Aggression	
	Neutral behavior	
	Located or moving to the front part of the cage	
	Exploratory behavior	

Fine mapping

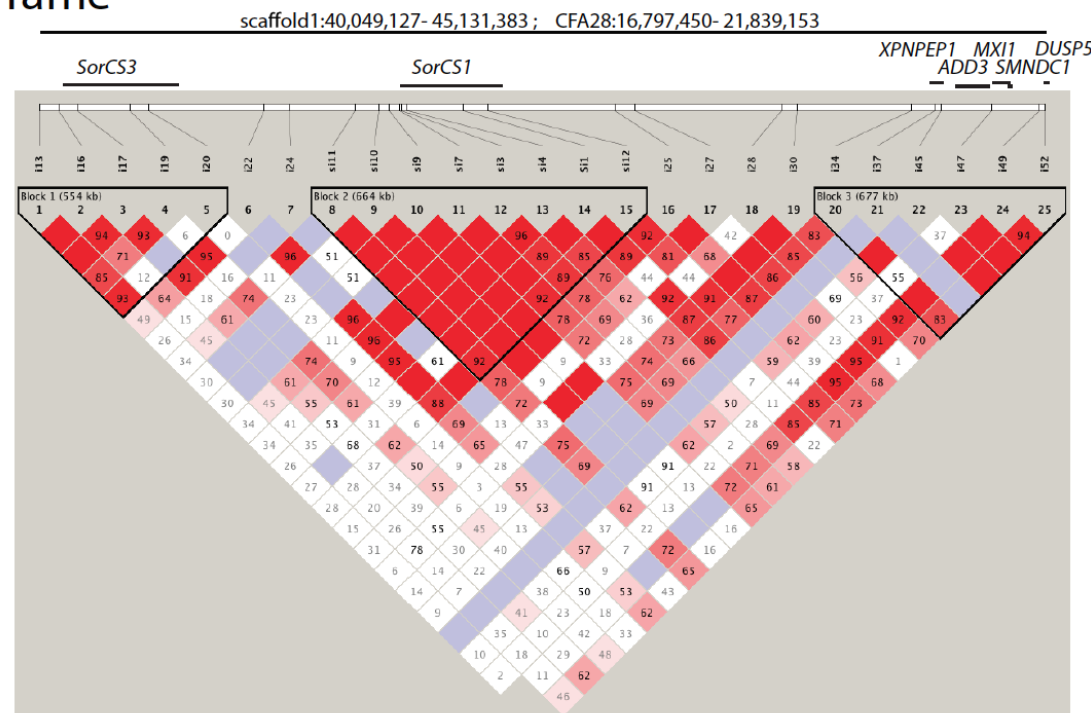
- Fox chromosome 15 contains a single window with $F_{ST} > 0.458$ which is located within quantitative trait loci (QTL) interval for the trait D.PC1.
- The same window has low H_p in both tame (0.20) and aggressive (0.23) populations.



Fine mapping

- Genotyping 25 small insertions/deletions located in a 5 Mb interval in tame (64) and aggressive (70) foxes.

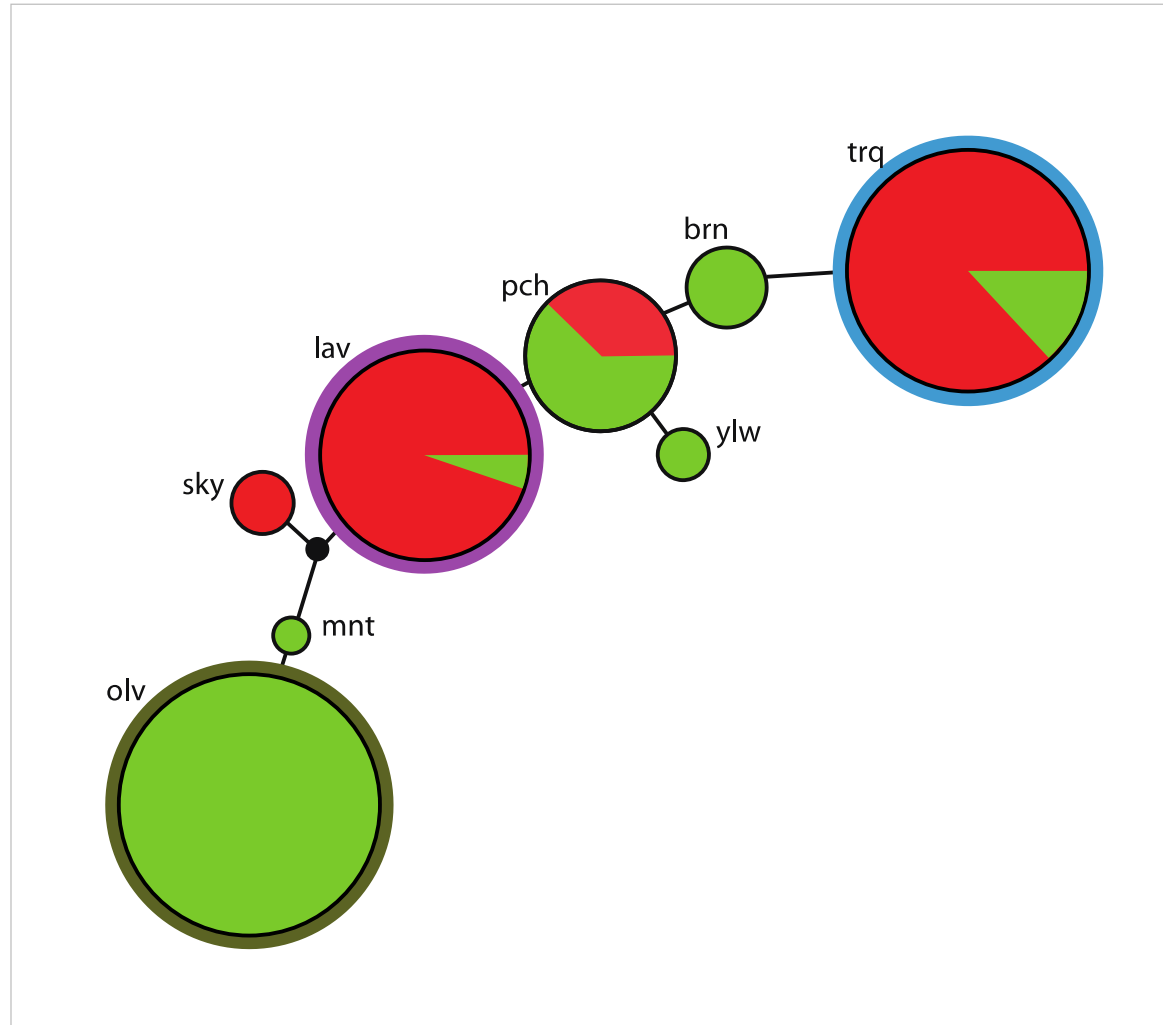
Tame



Central (*SorCS1*) region

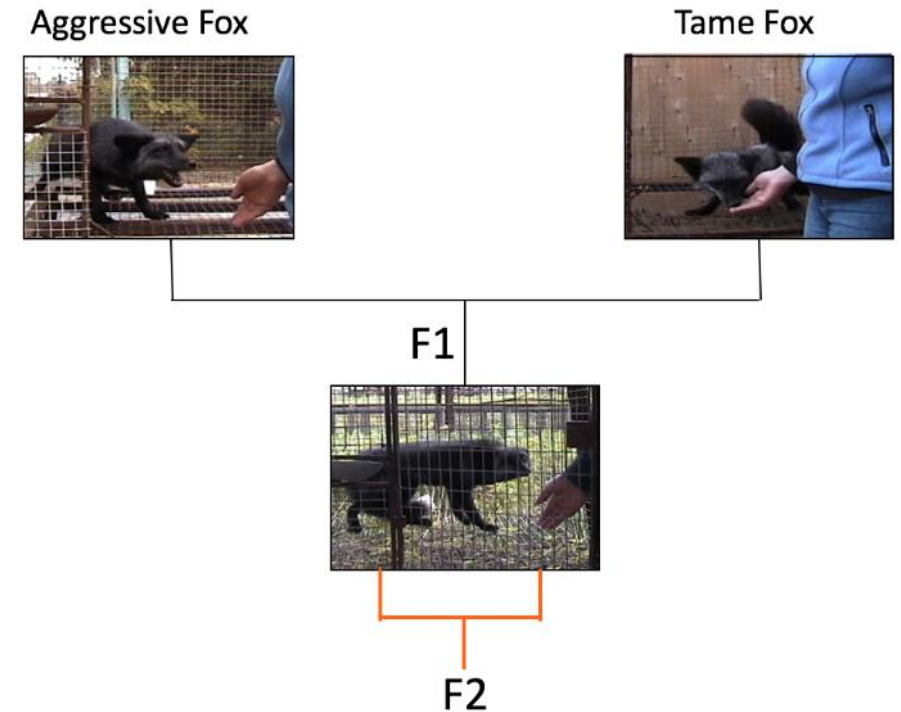
- Haplotype analysis identified three most common haplotypes:
- “Tame (**olv**)” haplotype was observed only in tame population (61%).
- “Aggressive-1 (**trq**)” – 47% in aggressive population and 7% in tame population;
- “Aggressive-2 (**lav**)” – 37% in aggressive population and 2% in tame population.

Distribution of **olv**, **trq**, and **lav** haplotypes in fox populations

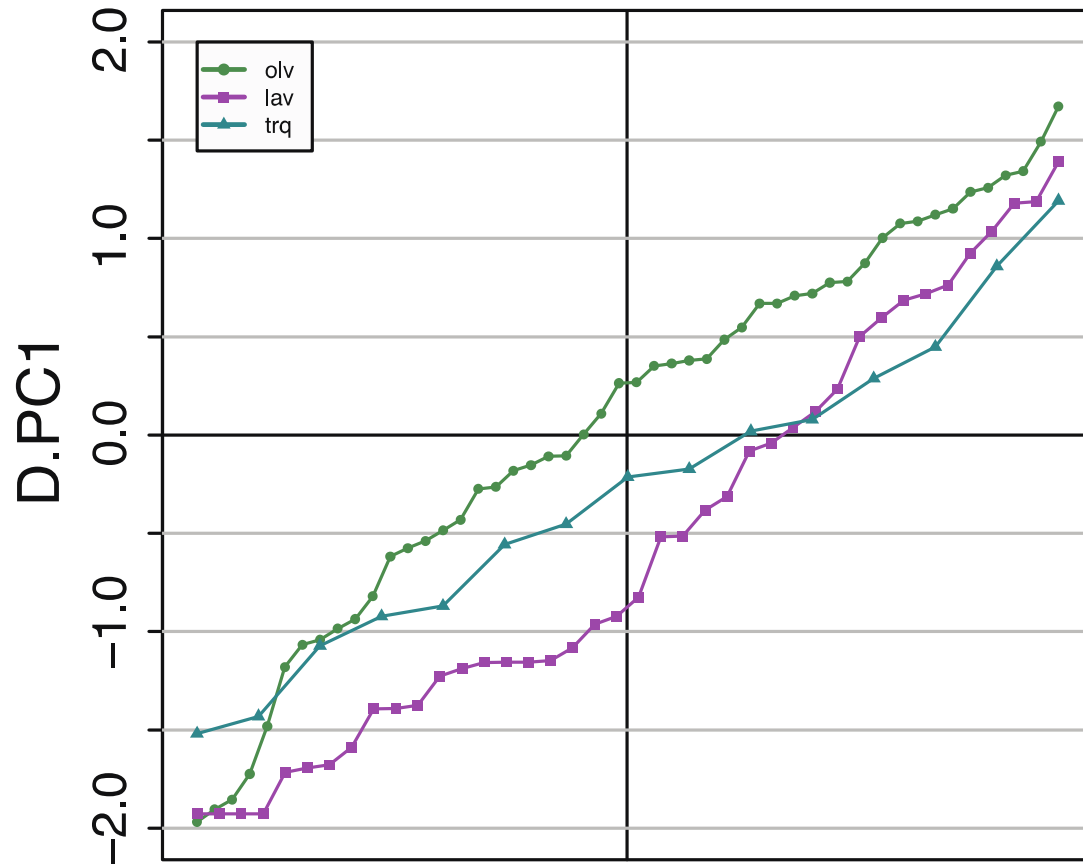


Effect of **olv**, **trq**, and **lav** haplotypes on behavior in F2

- F2 population demonstrates a wide spectrum of behavior
- 25 small insertions/deletions were genotyped in F2 pedigrees previously used for QTL mapping (536 F2 foxes).
- D.PC1 scores were compared for F2's homozygous for **olv**, **trq**, and **lav** haplotypes.



SorCS1 haplotypes and behavior of F2s



- Mean D.PC1 values:
 - Homozygous **olv**: 0.068
 - Homozygous **lav**: -0.546
- Significant differences among three groups:
 - Kruskal-Wallis, $p=0.03$
- A post-hoc Dunn's test with Benjamini-Hochberg correction achieved a $p=0.014$ for the comparison of **olv** and **lav** homozygotes.

SorCS1 is a novel gene for social behavior

- The **olv** haplotype is observed with frequency ~60% in tame population and not found in aggressive and conventional populations.
- The **olv** haplotype influences behavior of F2 foxes in an expected direction.
- *SorCS1* is a strong positional candidate gene for behavioral QTL on fox chromosome 15.

SorCS1

- *SorCS1* (sortilin-related receptor CNS expressed 1) is a member of the Vsp10p-D (vacuolar protein sorting/targeting protein 10-domain) type I transmembrane receptor family, which includes five genes and plays an important role in intracellular trafficking.
- Mutations in *SorCS1* have been found to be associated with late Alzheimer disease, diabetes, and autism.
- *SorCS1* is a key regulator of synaptic receptor trafficking. Neurexins and AMPA glutamate receptor are the major proteins sorted by *SorCS1* (Savas et al., 2015).

Genes in fox regions – Gene Ontology

- 971 genes identified in all significant windows:
- *HpT*: damaged DNA binding, single guanine insertion binding, guanine/thymine mispair binding
- *HpA*: cytokine activity, B cell mediated immunity, carbohydrate binding, receptor binding
- *F_{ST}TA*: natural killer cell activation, B cell mediated immunity, interleukin-1 receptor binding; carbohydrate binding, clathrin-coated vesicle

Genes associated with human behavioral disorders found in fox regions

Genes from four SFARI categories	SFARI category	Bipolar disorder associated genes	Genes known to be involved in mouse aggression
AKAP9	suggestive evidence	BAZ2B	CNGA2
AMPD1	suggestive evidence	CACNA1C	DCT
APH1A	suggestive evidence	CHRNA7	KCNJ3
ATP10A	suggestive evidence	GNG4	NCAM1
CACNA1C	syndromic	GPR50	PAK7
CHRNA7	suggestive evidence	IQGAP2	PRNP
CNTNAP2	syndromic, strong candidate	NCAM1	TACR1
GRIN2B	high confidence	NTF3	
KAT2B	strong candidate	PTPRO	
MAGEL2	syndromic, strong candidate	RASGRF2	
MYO9B	suggestive evidence	RBFOX1	
PIK3R2	syndromic	SCAMP1	
PLCB1	suggestive evidence	ZNF385D	
RBFOX1	suggestive evidence		

Reduced heterozygosity in aggressive foxes in the region syntenic to the Williams-Beuren syndrome in humans

HpT	HpA	HpC	Fox genes
0.266	0.249	0.241	FBXO24 PCOLCE MOSPD3 POP7 EPO EPHB4 SLC12A9 TRIP6 SRRT UFSP1 MUC3A RPL7A TRIM56 VGF MOGAT3 PLOD3 CLDN15 TFR2 GIGYF1 ACHE SERPINE1 AP1S1 NAT16 ZNHIT1 FIS1 ACTL6B
0.282	0.239	0.229	RPL7A TRIM56 VGF MOGAT3 PLOD3 CLDN15 RABL5 SERPINE1 AP1S1 NAT16 ZNHIT1 FIS1 COL26A1
0.322	0.087	0.226	RABL5 MYL10 COL26A1 CUX1
0.289	0.06	0.253	MYL10 CUX1
0.267	0.06	0.295	SH2B2 ORAI2 ALKBH4 LRWD1 POLR2J POLR2J3 UPK3B RASA4 CUX1 DTX2 PRKRIP1
0.281	0.051	0.327	SH2B2 ORAI2 ALKBH4 LRWD1 POLR2J POLR2J3 UPK3B HSPB1 RASA4 ZP3 SRCRB4D YWHAG SRRM3 CUX1 DTX2 STYXL1 PRKRIP1
0.342	0.05	0.342	HSPB1 CCL24 CCL26 ZP3 SRCRB4D YWHAG SRRM3 POR STYXL1 RHBDD2 HIP1
0.348	0.094	0.341	CCL24 CCL26 POR STYXL1 RHBDD2 HIP1 POM121 NSUN5 TRIM50 FKBP6
0.304	0.197	0.33	HIP1 POM121 NSUN5 TRIM50 FKBP6 FZD9 TBL2 BAZ1B BCL7B MLXIPL VPS37D
0.285	0.271	0.317	FZD9 TBL2 DNAJC30 STX1A ABHD11 CLDN3 CLDN4 WBSCR28 BAZ1B BCL7B MLXIPL VPS37D WBSCR22 WBSCR27

What does the fox say?

- Even a relatively simple behavioral pattern can be comprised by several behaviors with relatively independent genetic inheritance.
- Application of a combination of approaches (population and pedigree analyses) is powerful for finding genes influencing behavior.
- Genetics/genomics of domesticated behavior can provide a novel insight into molecular mechanisms involved in regulation of social behaviors.

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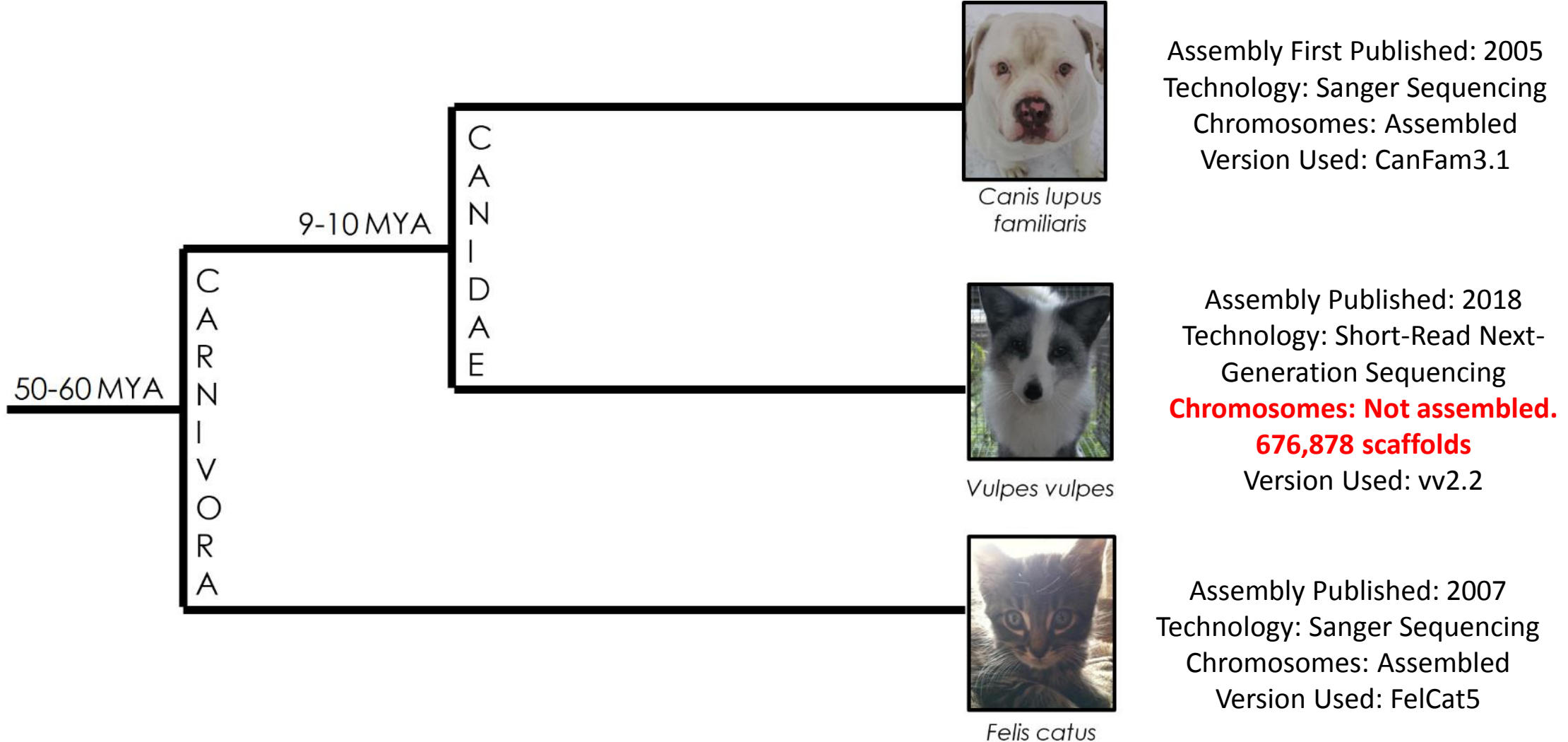
- **Support**

NIH GM120782
ICiG

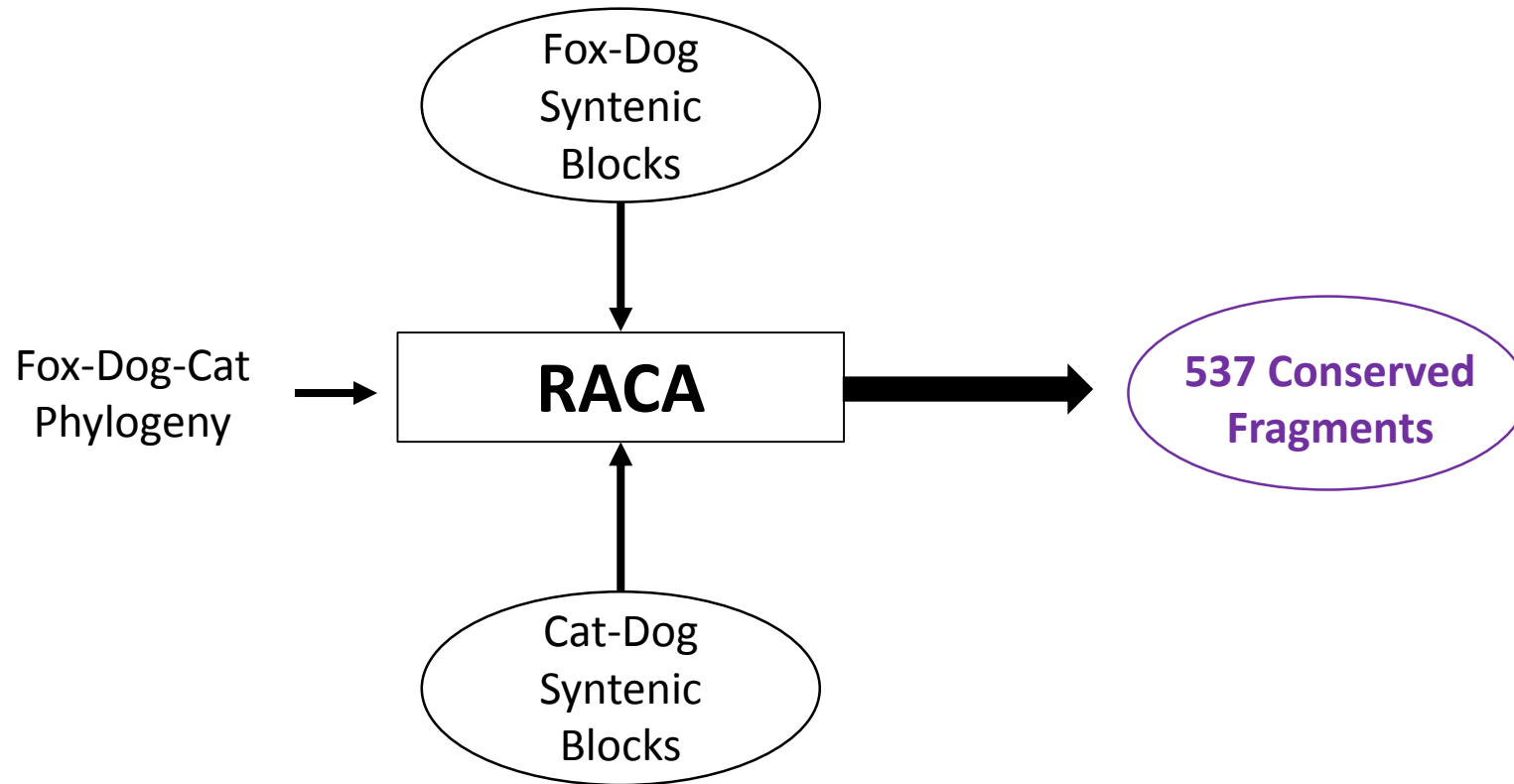
Russian Fund for
Basic Research
UIUC Research Board



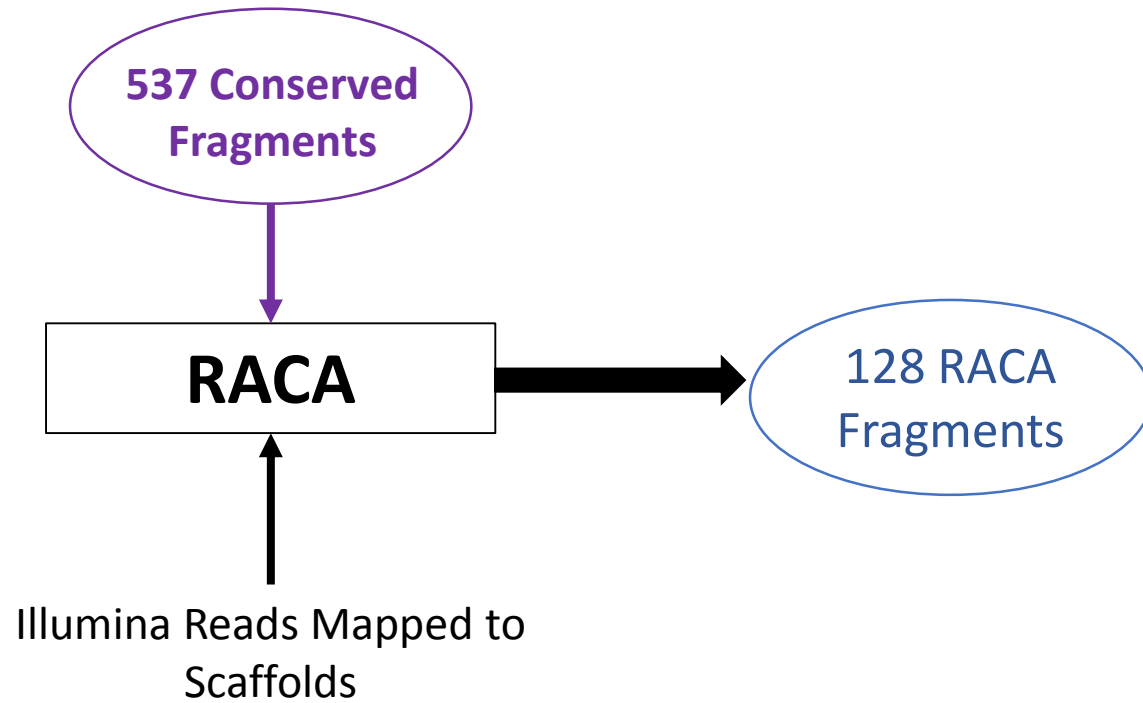
Felid & Canid Genome Assemblies



Reference-Assisted Chromosome Assembly (RACA) looks for conservation among species

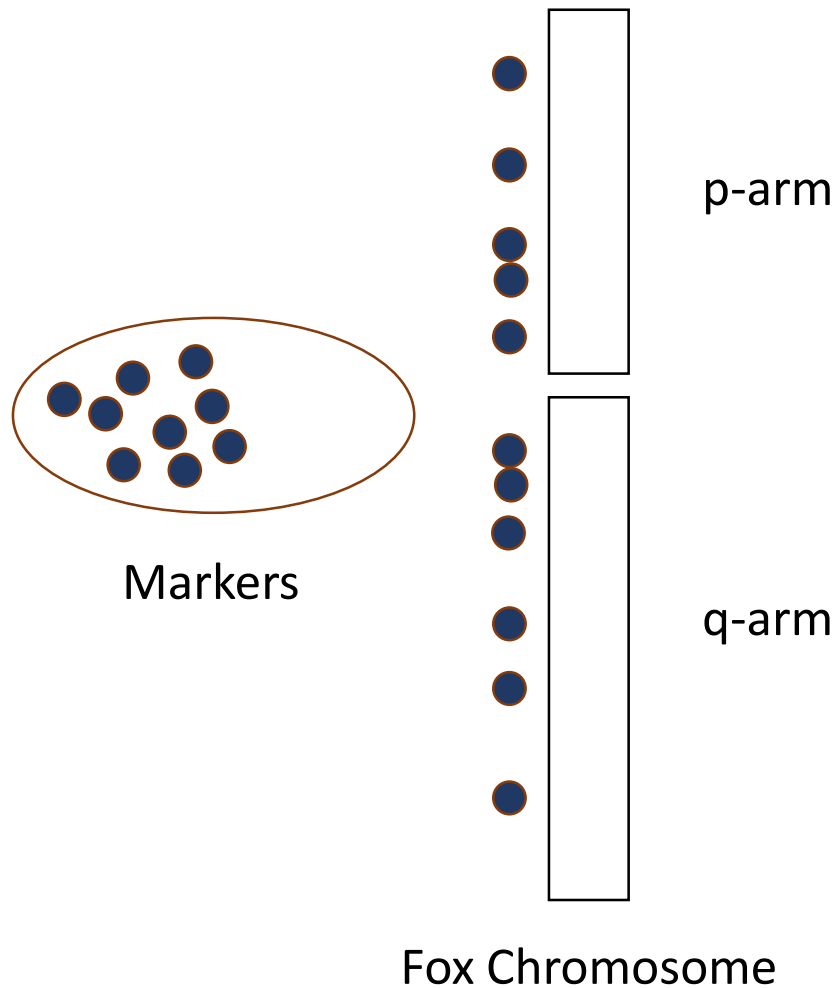


RACA also integrates information from sequencing libraries



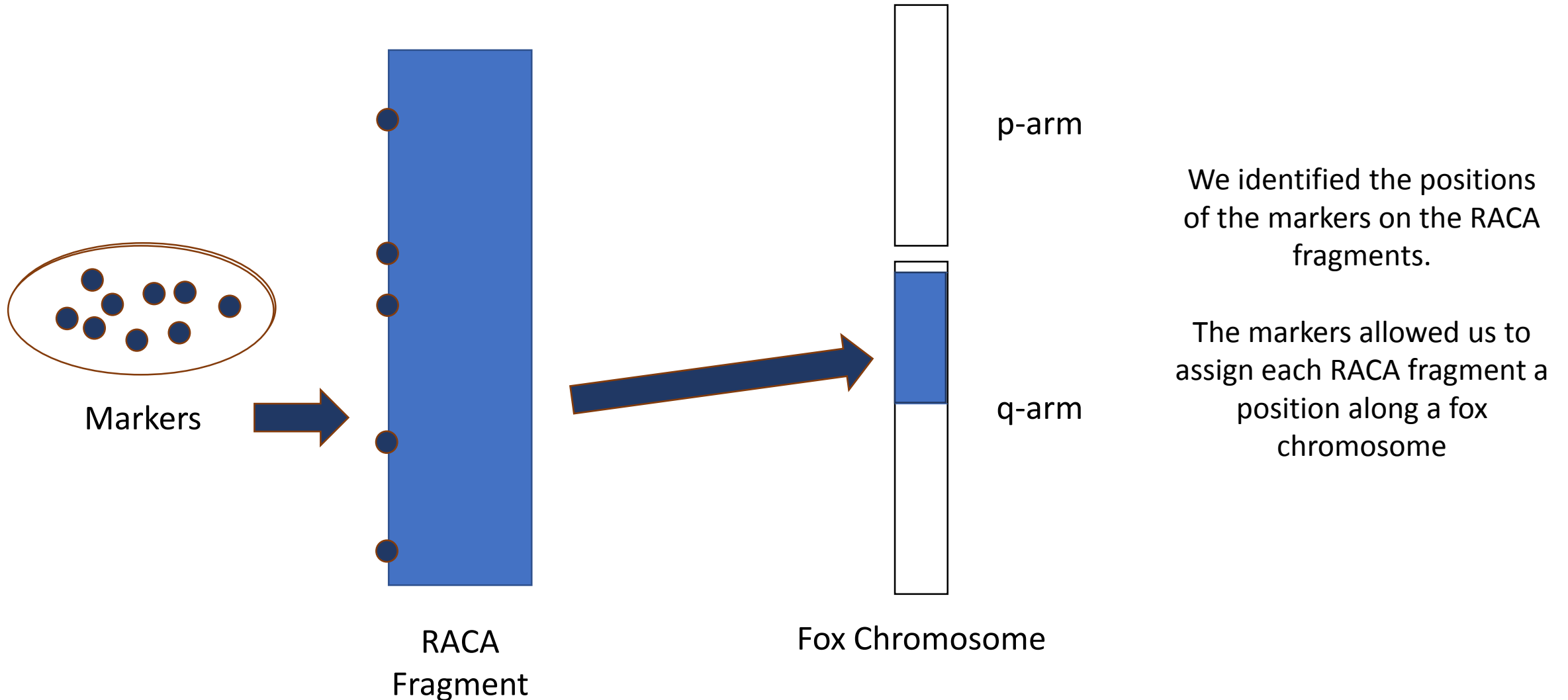
Each RACA fragment has a known position in the dog chromosomes and fox scaffolds

Comparing the RACA fragments to the fox meiotic map

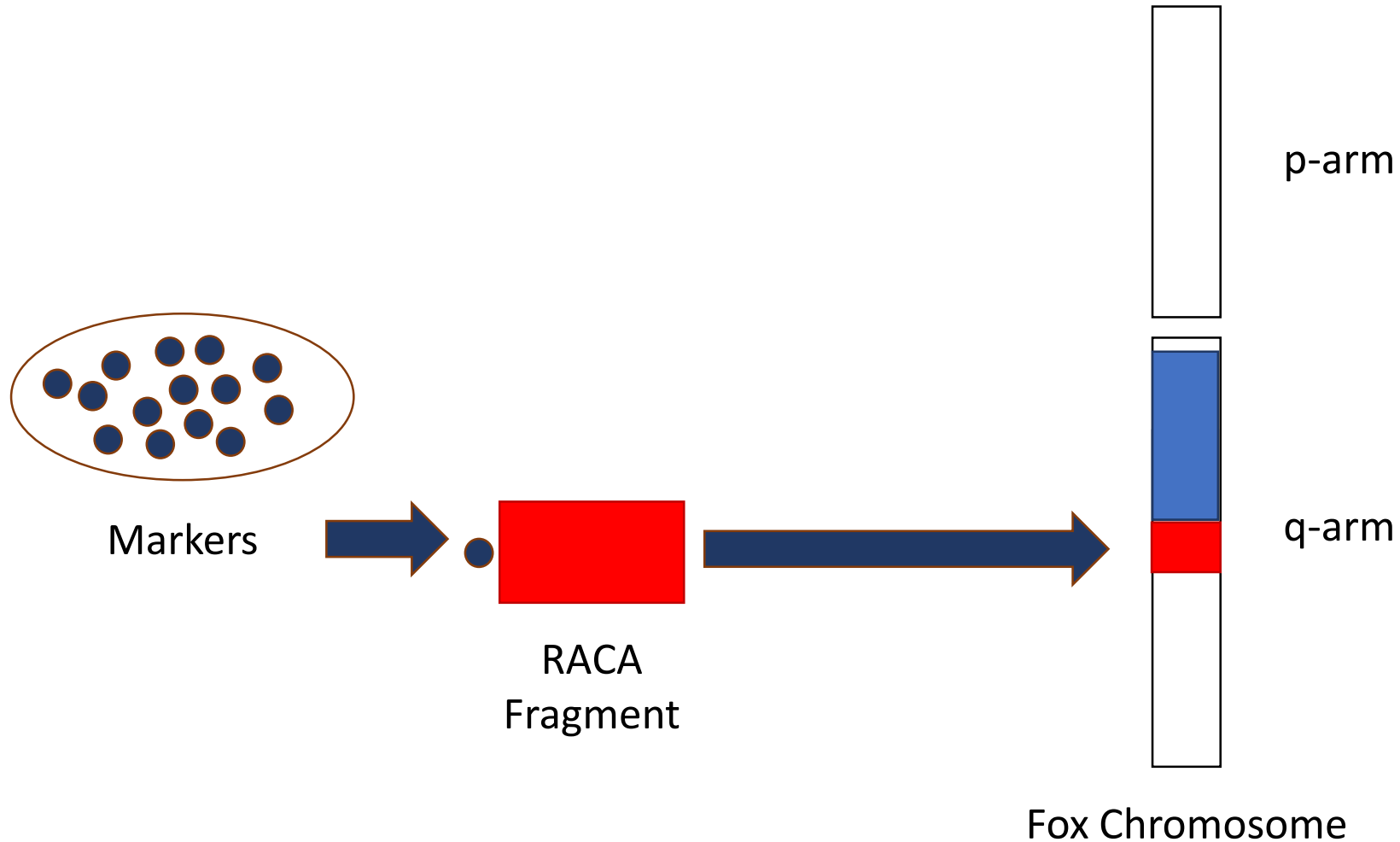


The microsatellite markers used in the fox meiotic linkage Map have known positions on the fox chromosomes.

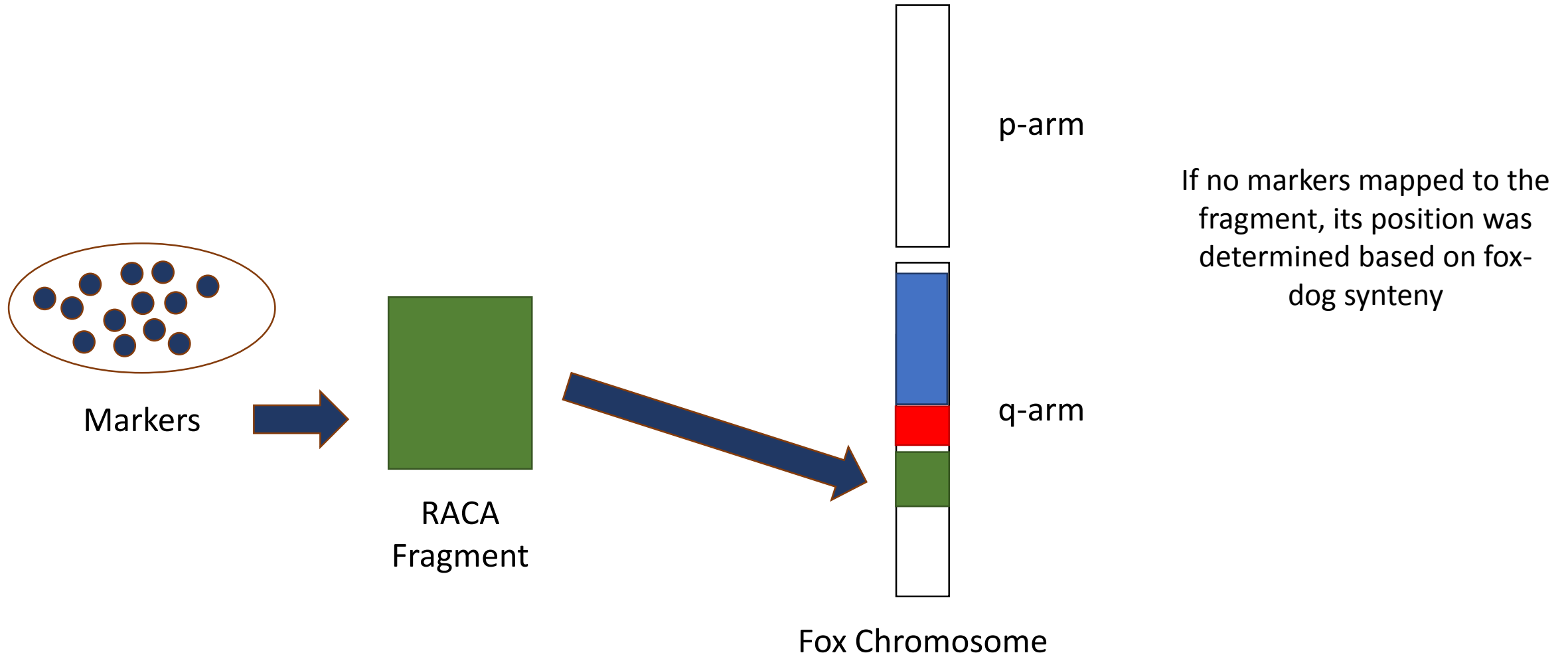
Comparing the RACA fragments to the fox meiotic map



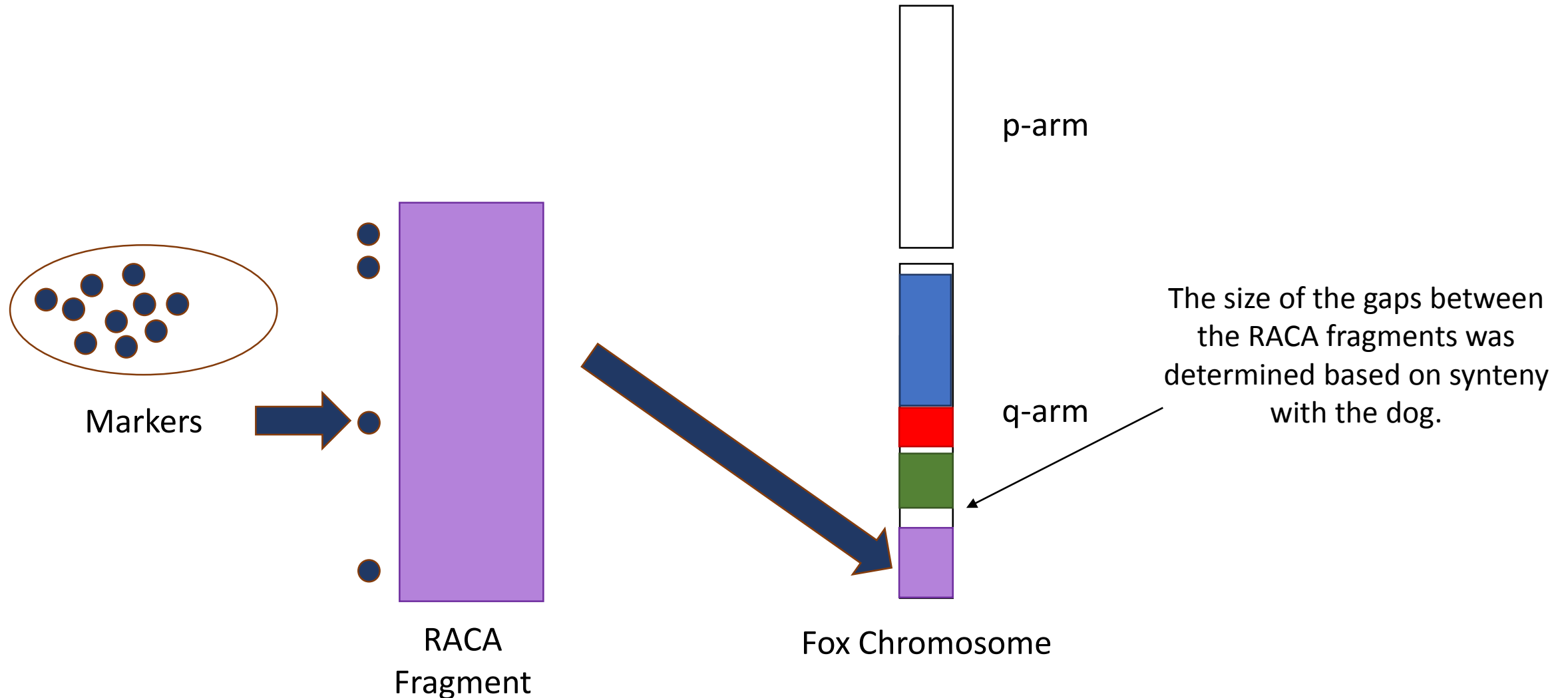
Comparing the RACA fragments to the fox meiotic map



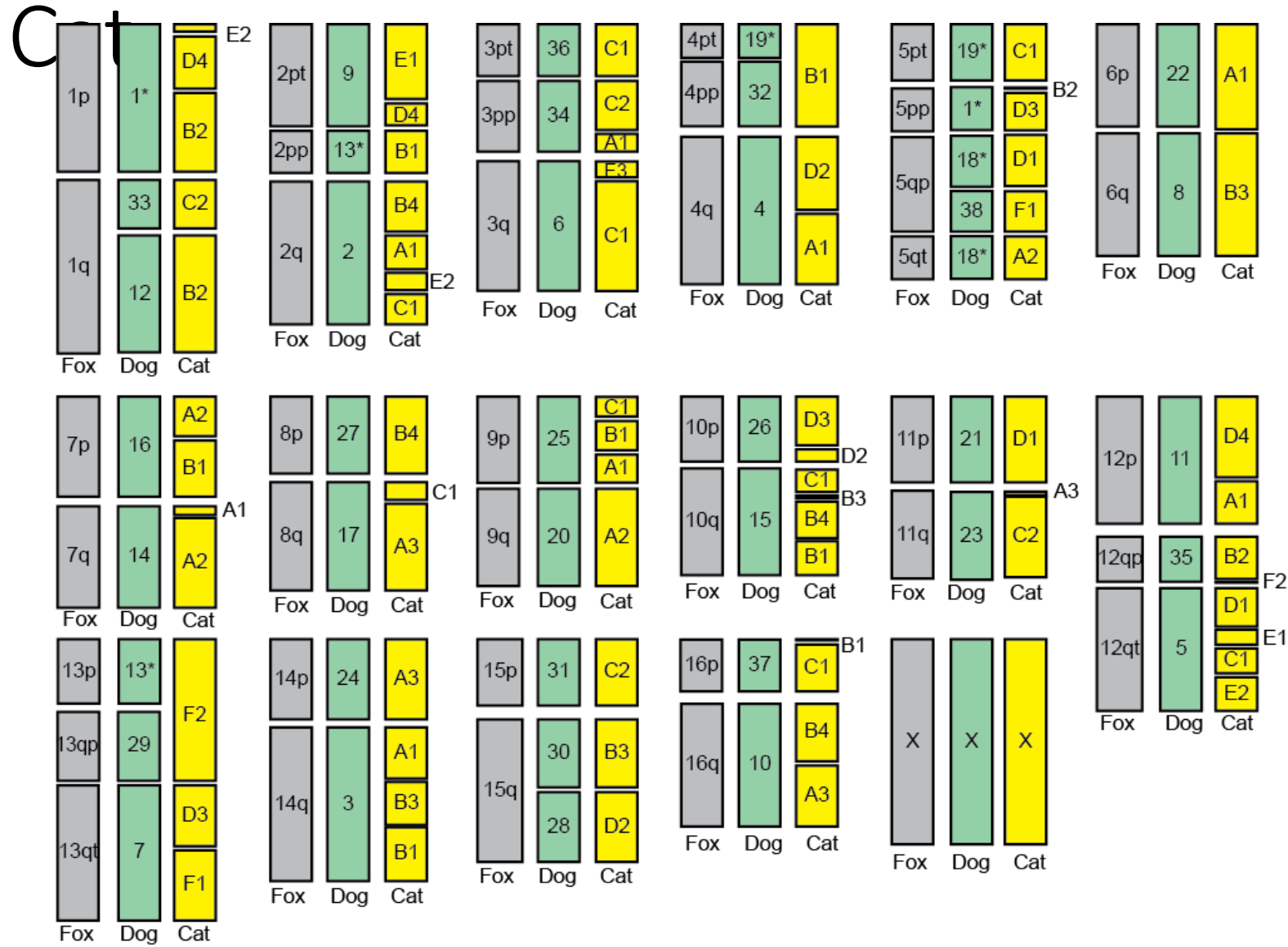
Comparing the RACA fragments to the fox meiotic map



Comparing the RACA fragments to the fox meiotic map



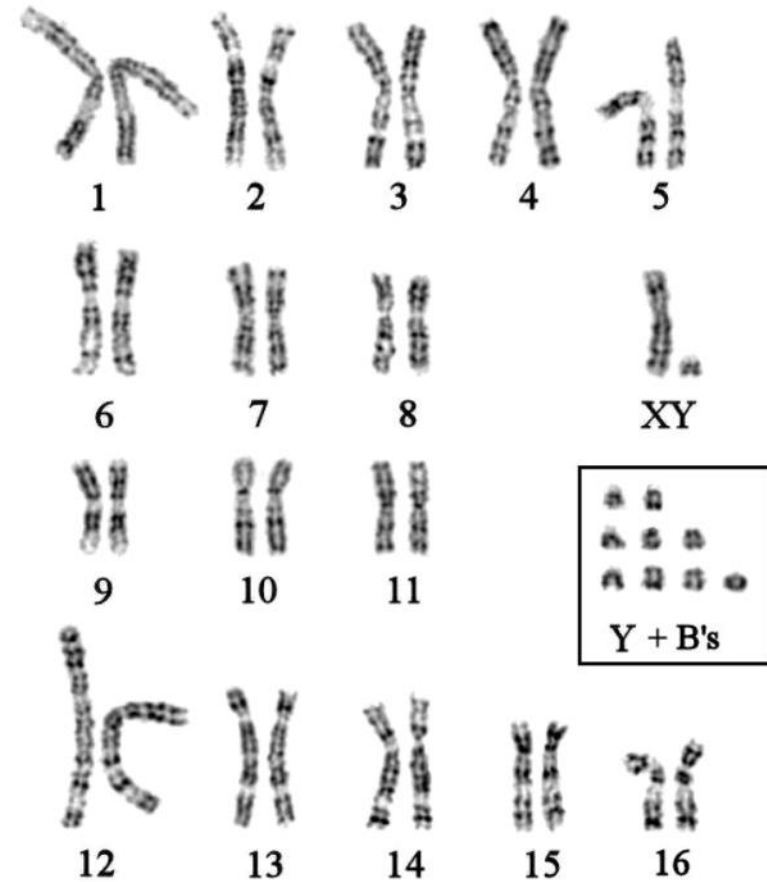
Fox Chromosomes Aligned to Dog and Cat



The end result is 40 fox chromosomal fragments with corresponding syntenic positions in dog and cat

Fox chromosomal fragment assembly

- 40 chromosomal fragments
- Deeply anchored to the dog genome...



Deep sequencing data

- 41 tame and 42 aggressive foxes were sequenced with >15x coverage per individual.
- Reads aligned against the fox genome with Bowtie2.
- SNVs called with GATK 4.
- 15,907,200 SNVs remained after filtering.
- One missense mutation identified in *SorCS1* gene.

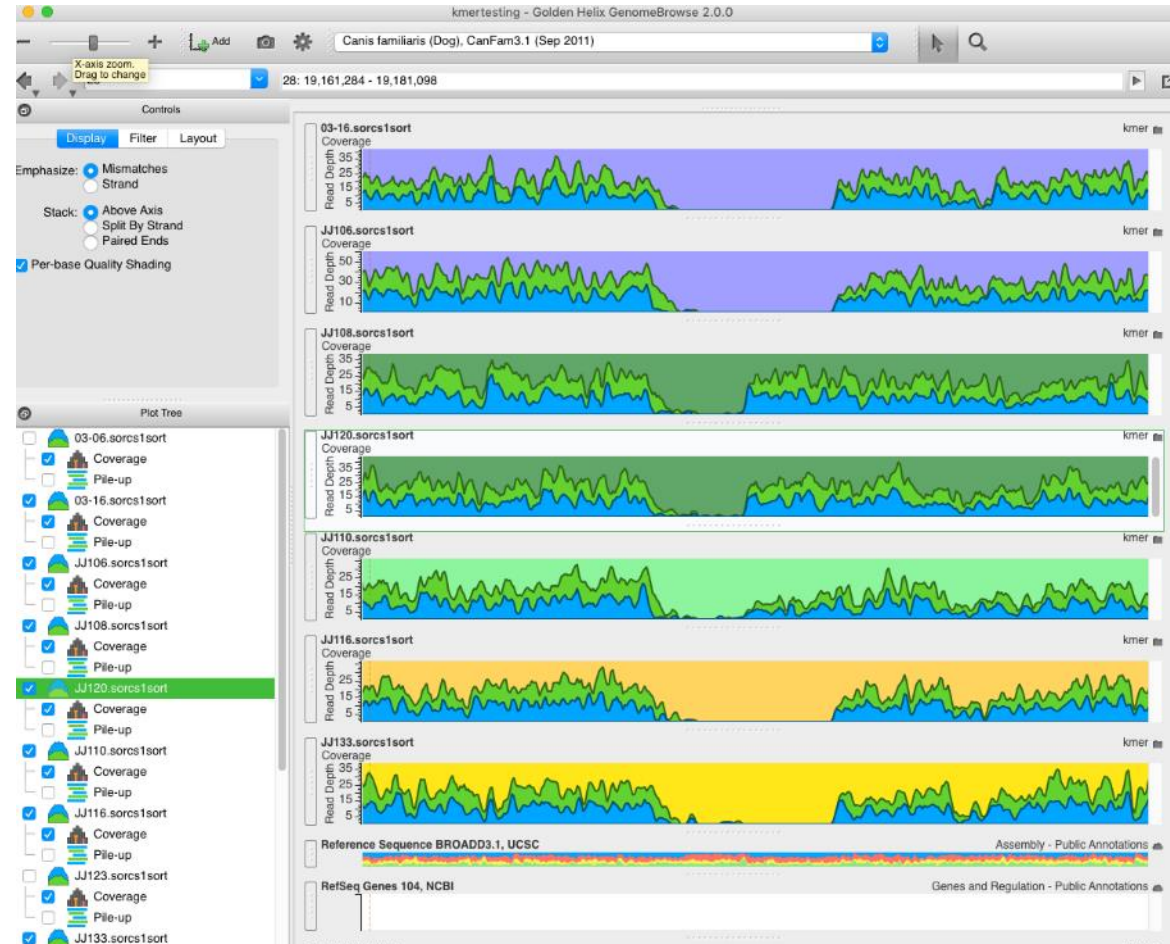
Association mapping of tame and aggressive foxes using k-mers *(following Rahman et al. eLife 2018;7:e32920)*

- 2,470,983,266 k-mers (21 bp long) were identified in tame and aggressive reads using Jellyfish.
- 9.23% of the k-mers were enriched in tame and 8.05% of the k-mers were enriched in aggressive samples.
- K-mers enriched in tame and aggressive samples were assembled separately using ABySS.
- K-mer assemblies were mapped against the dog genome using Bowtie2.

Tame enriched k-mers upstream *SorCS1*

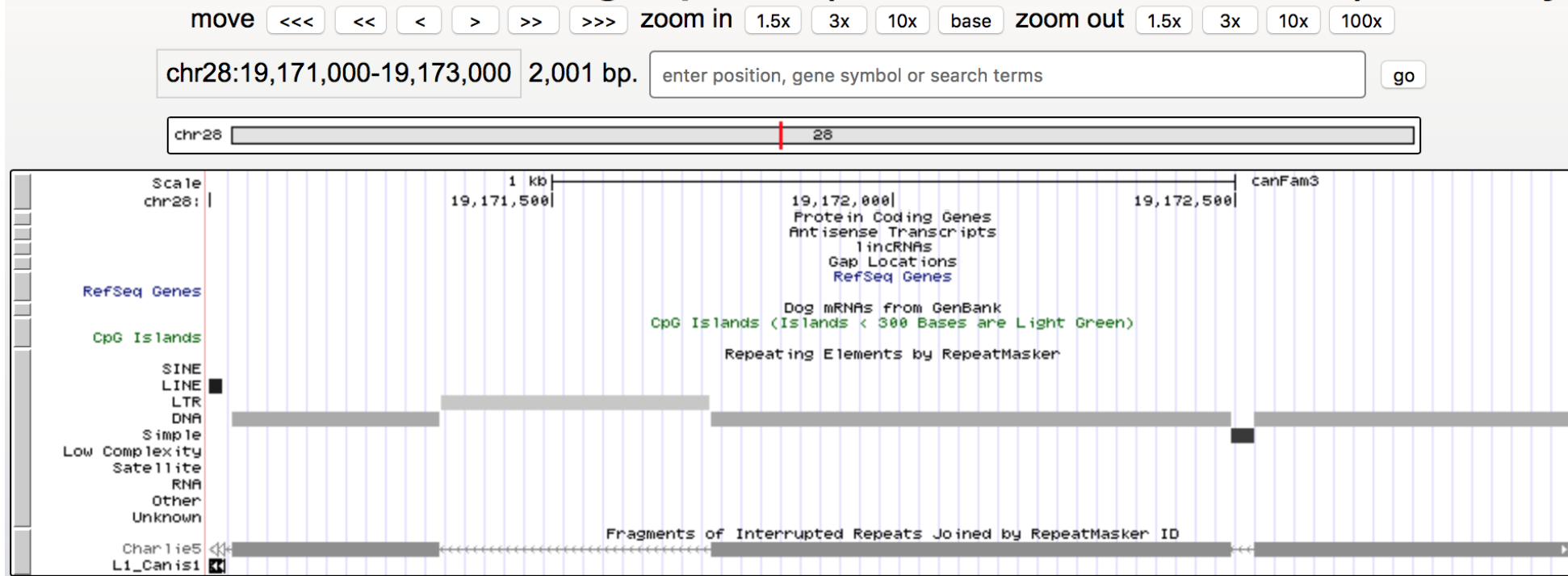
- A region with multiple tame-enriched k-mers located approximately 55 Kb upstream from the *SorCS1* gene in the dog genome was identified.
- The region is ~2kb in length (~CFA28: 19,171,000-19,173,000) and present only in tame foxes carrying **olv** haplotype.

Olv haplotype upstream *SorCS1* is different



Region upstream *SorCS1* identified by k-mer mapping

UCSC Genome Browser on Dog Sep. 2011 (Broad CanFam3.1/canFam3) Assembly



~2 Kb insertion/deletion upstream of *SorCS1* gene differentiates **olv** from other fox haplotypes in this region.