

# Using Genotyping to Correlate Pointing, Herding, & Retrieving Behaviors Across Dog Breeds



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## Introduction

In this experiment, we used Sequenom genotyping technologies to look at SNPs (single nucleotide polymorphisms) in regions of the dog genome that correlate with the following behaviors:





used:	
<b>117</b> SNPs	1
x 720 Dogs (49 Breeds & Wolf)	
4.240 Data Points	

## **Project Justification**

### Why dogs?

Breeds strongly selected for behaviors. Dogs in a breed are very similar and frequently homozygous due to inbreeding. •Breeds are diverse. Dog diseases parallel

human diseases and behaviors.

### Why these 117 SNPs?

To focus on regions previously found by a Genome Wide Association (GWA) scan that may be associated with these behaviors.

### Why 49 breeds and 720 individual dogs?

To increase the statistical significance of findings by analyzing all available dog breeds.

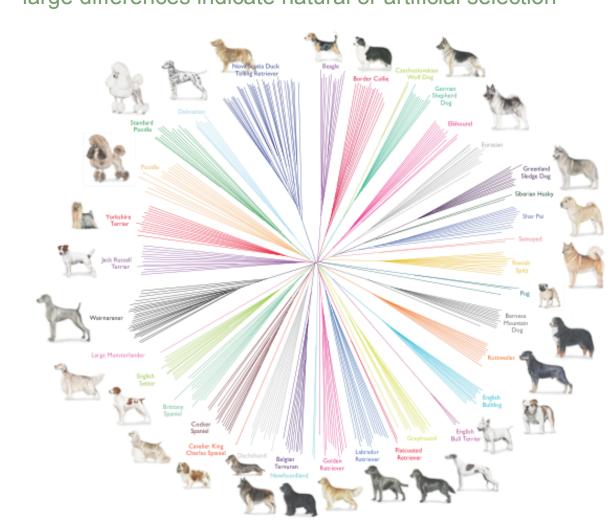


Fig.6 SNP = Single

## **Region Discovery Methods**

Discovery method	Region name	Size (kb)	Associate phenotype
Breed vs breed association	Chr 10 #2	100	Herding
Breed vs breed association	Chr 2 #2	109	Herding
Breed vs breed association	Chr 1	139	Pointing
Breed vs breed association	Chr 15	203	Pointing
Breed vs breed association	Chr X	164	Pointing
XPEHH (long haplotype)	Chr 10 #1	400	N/A
XPEHH (long haplotype)	Chr 12	336	N/A
XPEHH (long haplotype)	Chr 2 #1	1000	N/A
high FST (region)	Chr 24 #1	53	N/A
high FST (region)	Chr 20	100	N/A
high FST (region)	Chr 24 #2	253	N/A
high FST (single SNP)	high_FST_SNP	N/A	N/A

 Breed vs Breed Association: testing for difference between breeds that correlate with phenotypes •XPEHH: testing for long haplotype blocks within a breed, indicates artificial or natural selection •FST: measuring how different breeds are from each other, large differences indicate natural or artificial selection



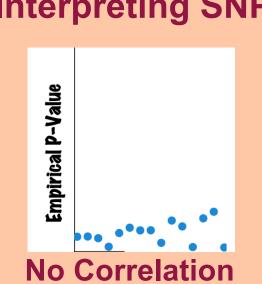
# Introduction to Analysis

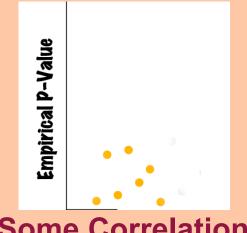
We used PLINK, a whole genome association analysis toolset, to test 3 phenotypes: pointing, herding, & retrieving.

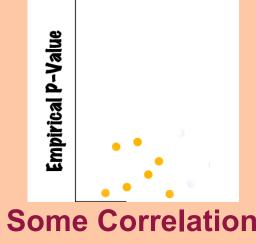
PLINK: calculating a p-value for the phenotypes

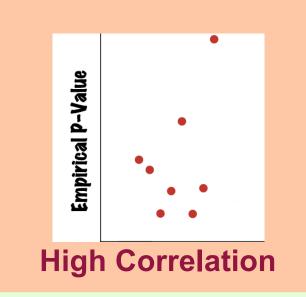
- 1. Measured the difference between affected and unaffected
- 2. Obtained random distribution
- 3. Calculated empirical p-value

Interpreting SNP Correlation Graphs









# Methods

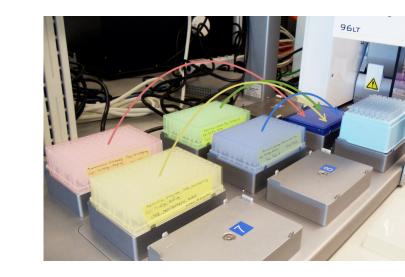
further amplification.

nucleotide.

# SEQUENOM® Genotyping

## **1** DNA Preparation

We dispensed individual dog DNA samples 4 times onto different 384-well plates. Eight 384well plates were used.



4 Shrimp Alkaline Phosphatase (SAP)

We added SAP to amplified DNA. The enzyme

disassociated two phosphate groups from the

ends of DNA strands and free nucleotides to stop

**5** Single Base Extension (SBE)

We added the remaining four oligo pools

onto plates. A single nucleotide was added

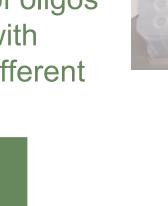
to the DNA, complimenting the SNP's

Reverse SBE primer

Reverse SBE primer

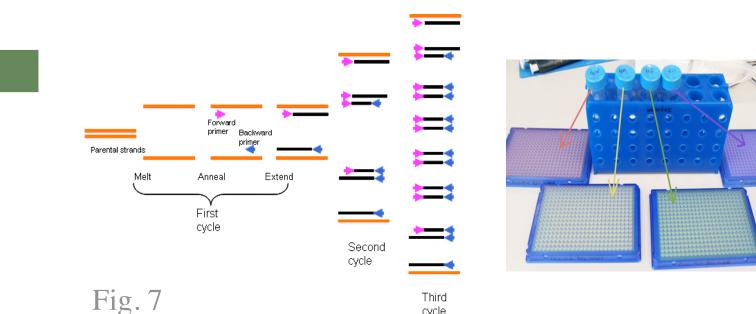
# 2 Oligo Pooling

We customized and ordered oligonucleotide primers (oligos) to attach near targeted SNPs. Then we made 4 pools of oligos for PCR and 4 for SBE with each pool attaching to different sites on the DNA.



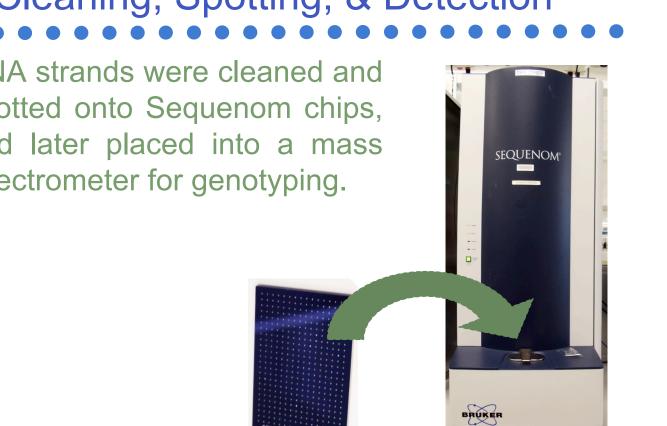
## 3 Polymerase Chain Reaction (PCR)

Each plate received one of the four oligo pools for PCR. Oligo primers in pools attached to sites near the desired SNPs. DNA amplification started at the oligos.



## 6 Cleaning, Spotting, & Detection

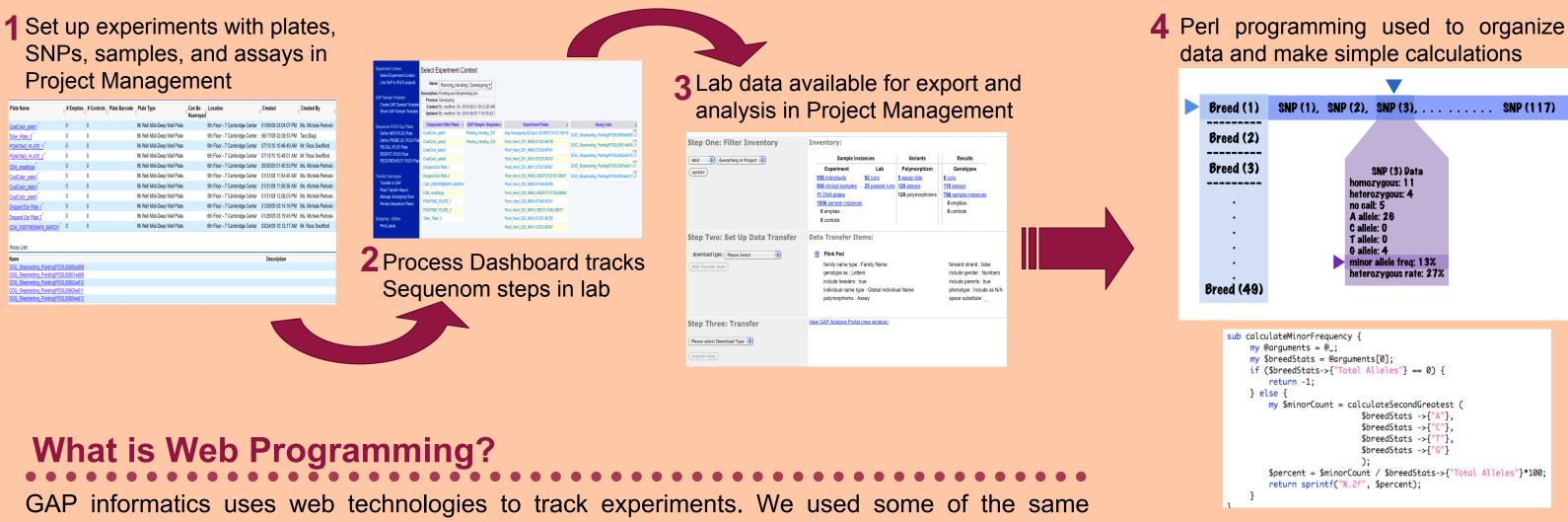
DNA strands were cleaned and spotted onto Sequenom chips, and later placed into a mass spectrometer for genotyping.



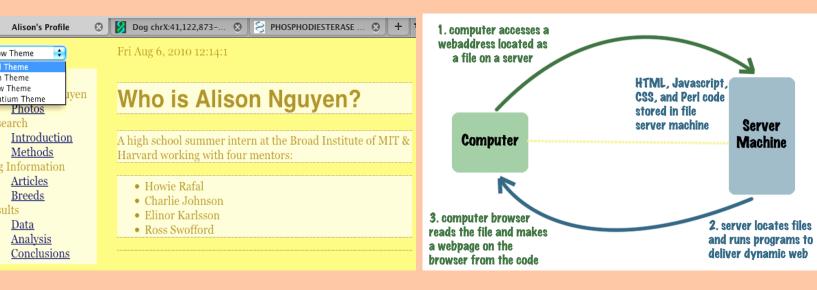


# Informatics & Analysis Workflow

The Genetic Analysis Platform (GAP) programmed a web interface that facilitates and tracks projects done within the platform.



technologies to create a simple page that changed its appearance by user selection.

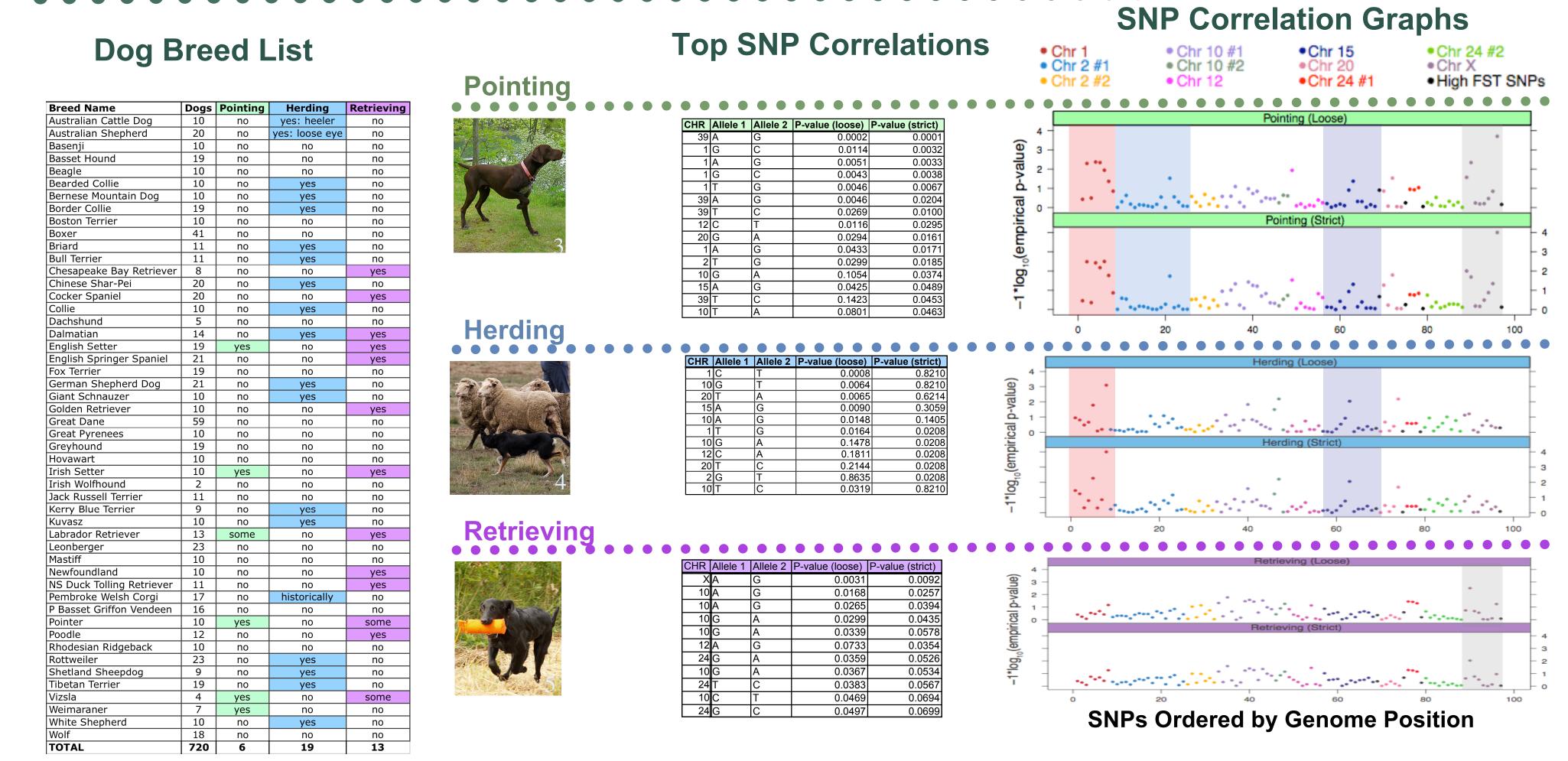




## 5 Data sent to 2) 2009 Shaun Purcell, GNU General Public License, v For documentation, citation & bug-report instructi http://pngu.mgh.harvard.edu/purcell/plink/ Web-based version check ( --noweb to skip ) Connecting to web... OK, v1.07 is current Writing this text to log file [ plink.log ] Analysis started: Thu Aug 5 09:04:40 2010

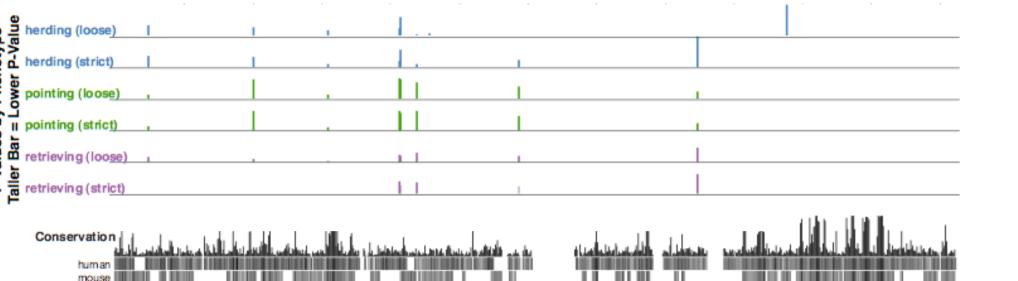
Before frequency and genotyping pruning, there are 0 SNPs

# Results & Conclusions



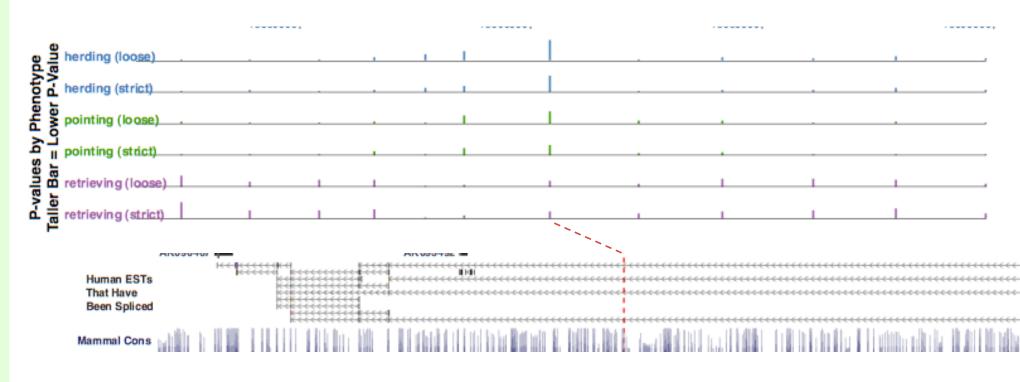
## SNPs in a Genome Browser (by Chromosome)

### **Chromosome 1**



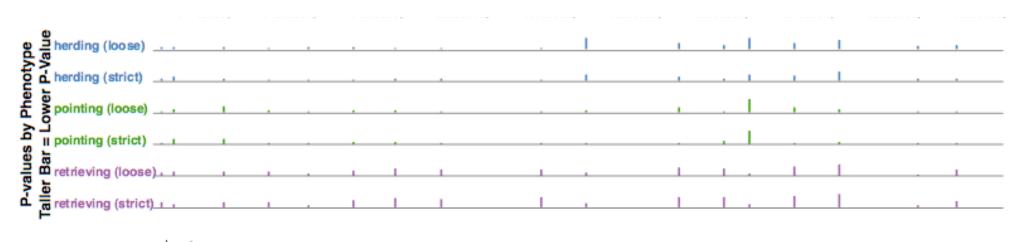
 Herding SNP upstream of human gene with function poorly known Pointing SNP in human gene linked to schizophrenia

### **Chromosome 15**



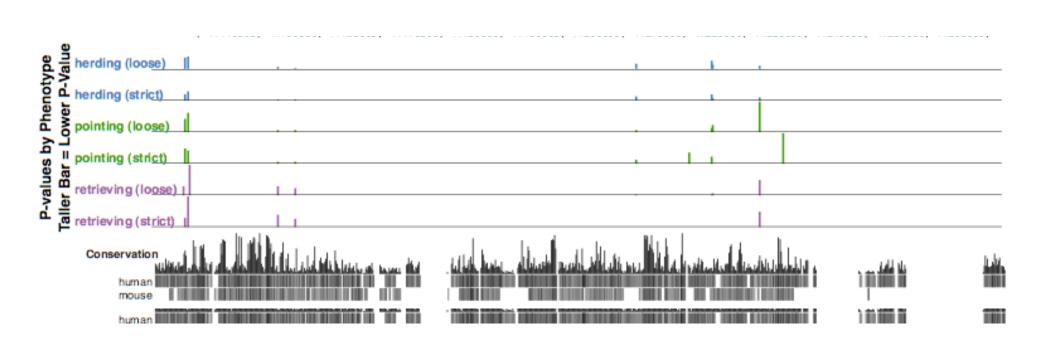
• SNPs in region with no known genes but some functional annotations, including ESTs isolated from brain cells

## **Chromosome 2**



• Pointing SNPs in human chromatin remodeling gene expressed in brain.

### Chromosome X (39)



- Pointing SNP in human gene for androgen receptor
- Retrieving SNP in human oncogene, unknown brain function
- Peaks flank synaptic signaling (epilepsy) gene

# Significance

Dog Chromosome	Dog Phenotype	Potential Human Disease
Chr 1	herding	unknown
Chr 1	pointing	schizophrenia
Chr 2	pointing	unknown
Chr 15	herding	unknown
Chr 15	pointing	unknown
Chr X (39)	pointing	unknown
Chr X (39)	retrieving	unknown

For thousands of years, humans have shaped dog behaviors through artificial selection. Identifying the genes correlated with these behaviors and using crossspecies genomic analysis reveal that they are also involved in human psychiatric diseases and disorders, giving us insight into the functions of these genes.

### References •••••••

1 http://www.flickr.com/photos/56761195@N00/4774916833/ http://www.dogbreedsaz.com/wp-content/uploads/2010/06/West\_20Highland\_20White\_20Terrier.jpg http://www.flickr.com/photos/smilla4/3615313293/ 4 http://www.flickr.com/photos/ppym1/426585244/ 5 http://www.flickr.com/photos/35136177@N02/4845155288/ 6 http://fightcolorectalcancer.org/images/posts/2008/10/snp.png 7 http://www.obgynacademy.com/basicsciences/fetology/genetics/images/pcr.png

### **Acknowledgments**

8 http://www.biomedcentral.com/1471-2105/9/253/figure/F2?highres=y

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