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Mapping Toxicity Traits using Diversity Outbred Mice

Daniel M. Gatti, Ph.D.

HESI Genomics Genetically Diverse Mouse Models

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The Diversity Outbred (DO) mice are a mapping population

Ideal for mapping complex traits because:

- High genetic diversity,
- Small recombination blocks,
- Balanced allele frequencies.

Outbred nature increases variance for other experiments.

Unique genetic makeup of each mouse means that a specific mouse is not reproducible.

However, the overall results of a mapping experiment <u>are</u> reproducible.

DO mice are derived from 8 inbred founders

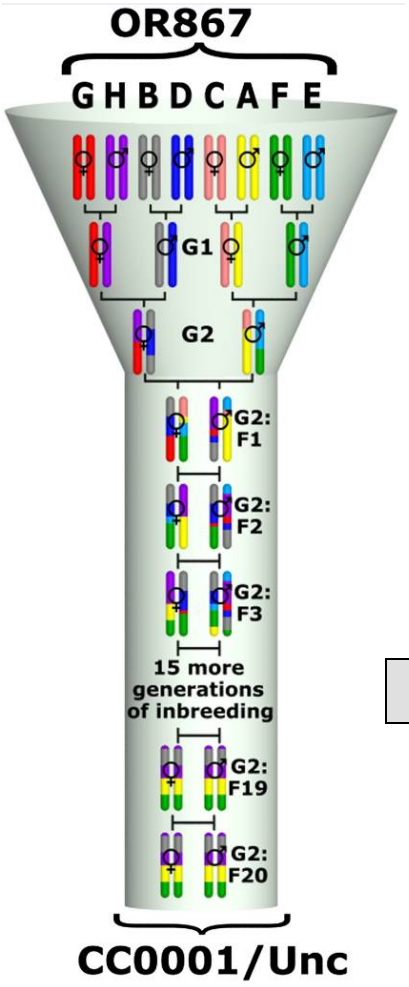
Strain	Letter	Color
A/J	A	
C57BL/6J	B	
129S1/SvImJ	C	
NOD/ShiLtJ	D	
NZO/LtJ	E	
CAST/EiJ	F	
PWK/PhJ	G	
WSB/EiJ	H	

The founder strains contribute high genetic diversity

Number of variants		
SNPs	**Indels**	**Struct. Var.**
38,261,117	5,376,127	228,286

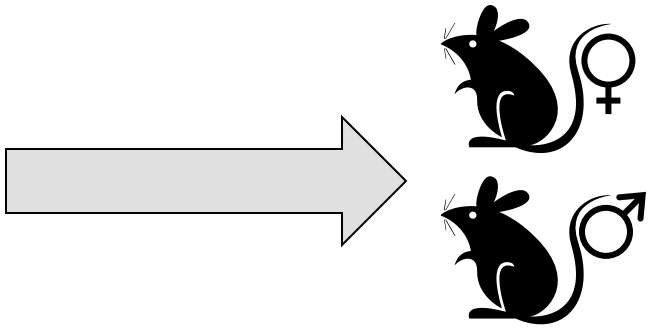
% of protein coding genes with variants in exons or UTRs.		
SNPs	**Indels**	**Struct. Var.**
94%	66%	12%

DO mice were derived from Collaborative Cross funnels

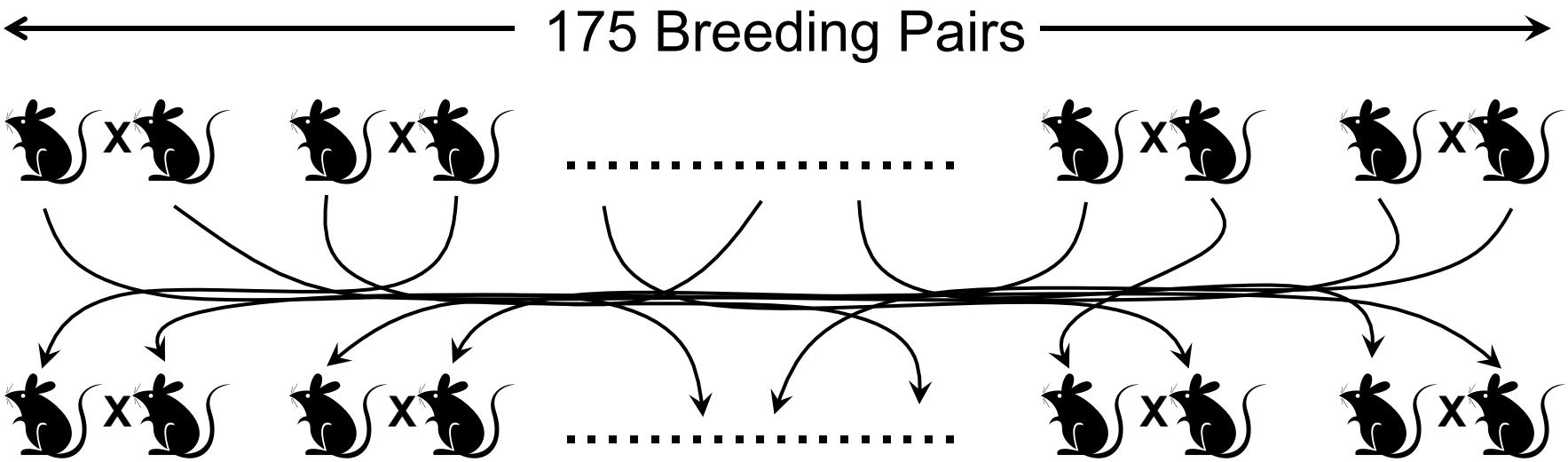


Threadgill & Churchill, 2012

144 pairs of mice from different inbreeding funnels (between G2:F4 & G2:F12)



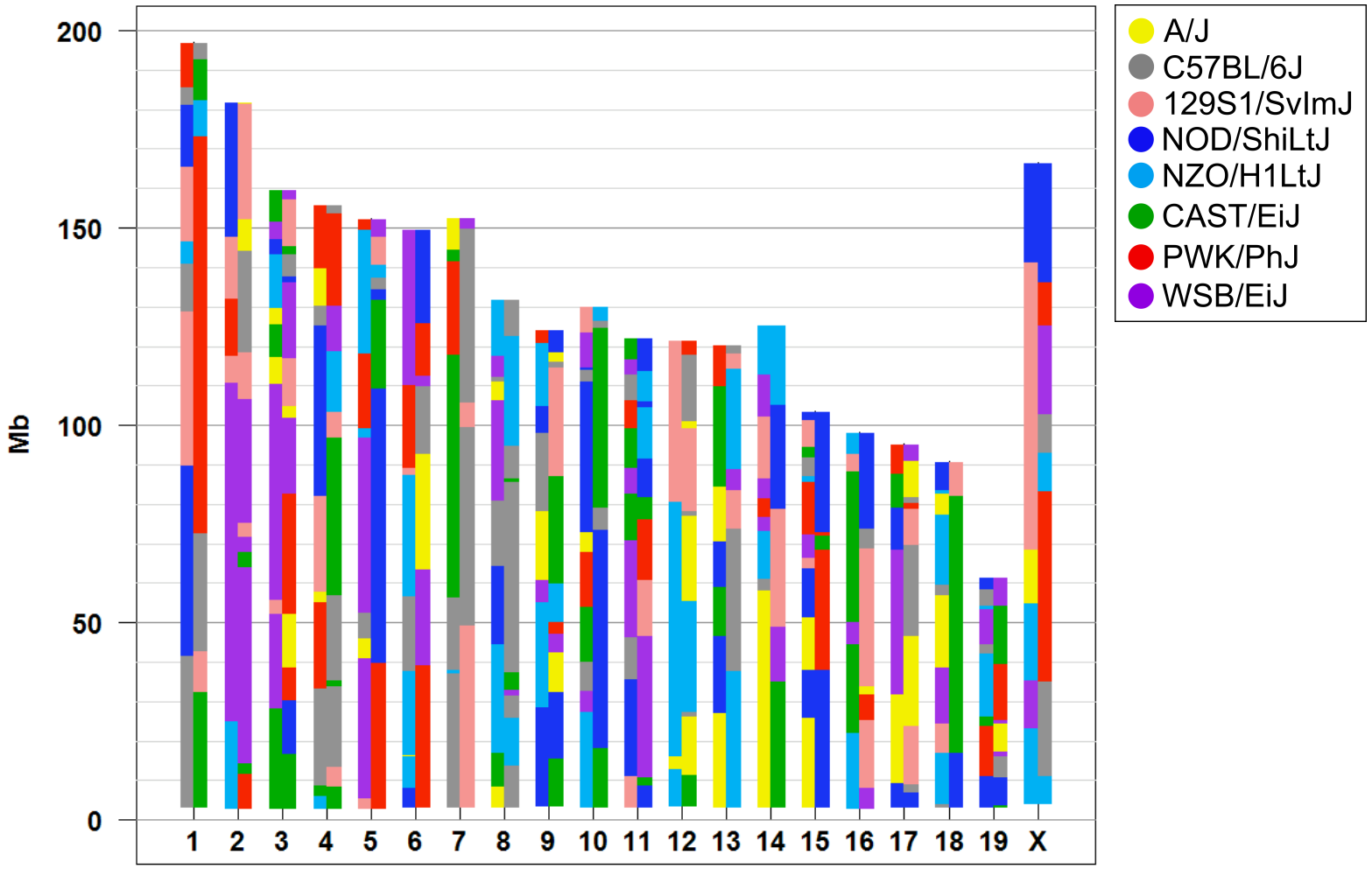
DO Mice are maintained in a random mating scheme



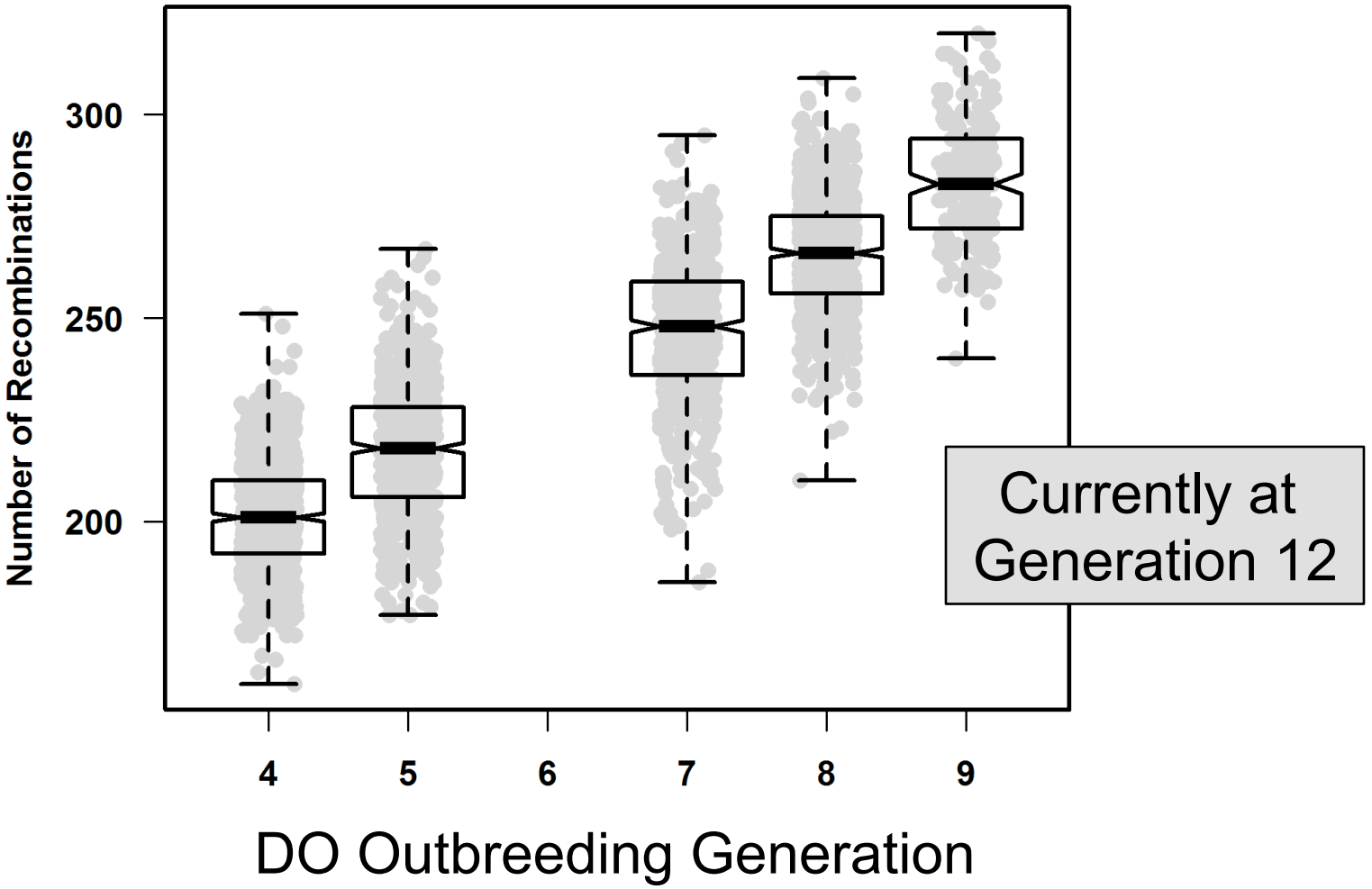
Generations are bred 4 times per year.

Breeding is expanded to meet demand in each generation.

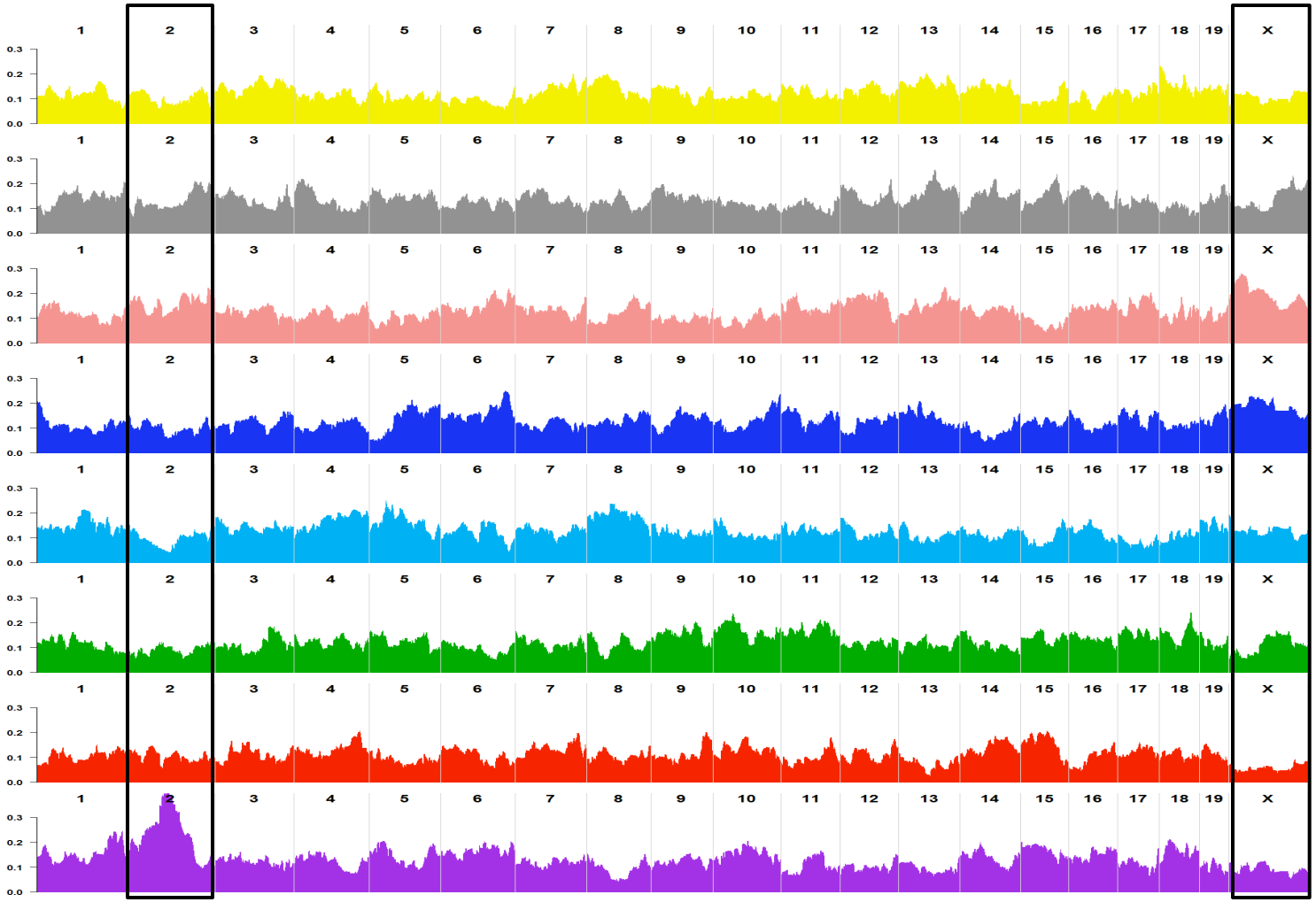
DO genomes are heterozygous mixture of the 8 founders



Number of recombinations per mouse increases with generation



Founder allele proportions are (mostly) balanced



Steps in a QTL mapping study with DO mice

1. Obtain ~200 – 300 DO mice
   - http://jaxmice.jax.org/strain/009376.html ($50/mouse)
2. Genotype mice
   - MEGA MUGA array run by GeneSeek (Lincoln, NE)
   - http://www.neogen.com/GeneSeek/SNP_Illumina.html
   - ~70,000 SNPs ($100/mouse)
3. Phenotype mice
   - Ideally phenotype pre- and post-dose.
   - Post-dose phenotype has worked well.
4. QTL Mapping: DOQTL package in R

Case Study: Benzene Inhalation

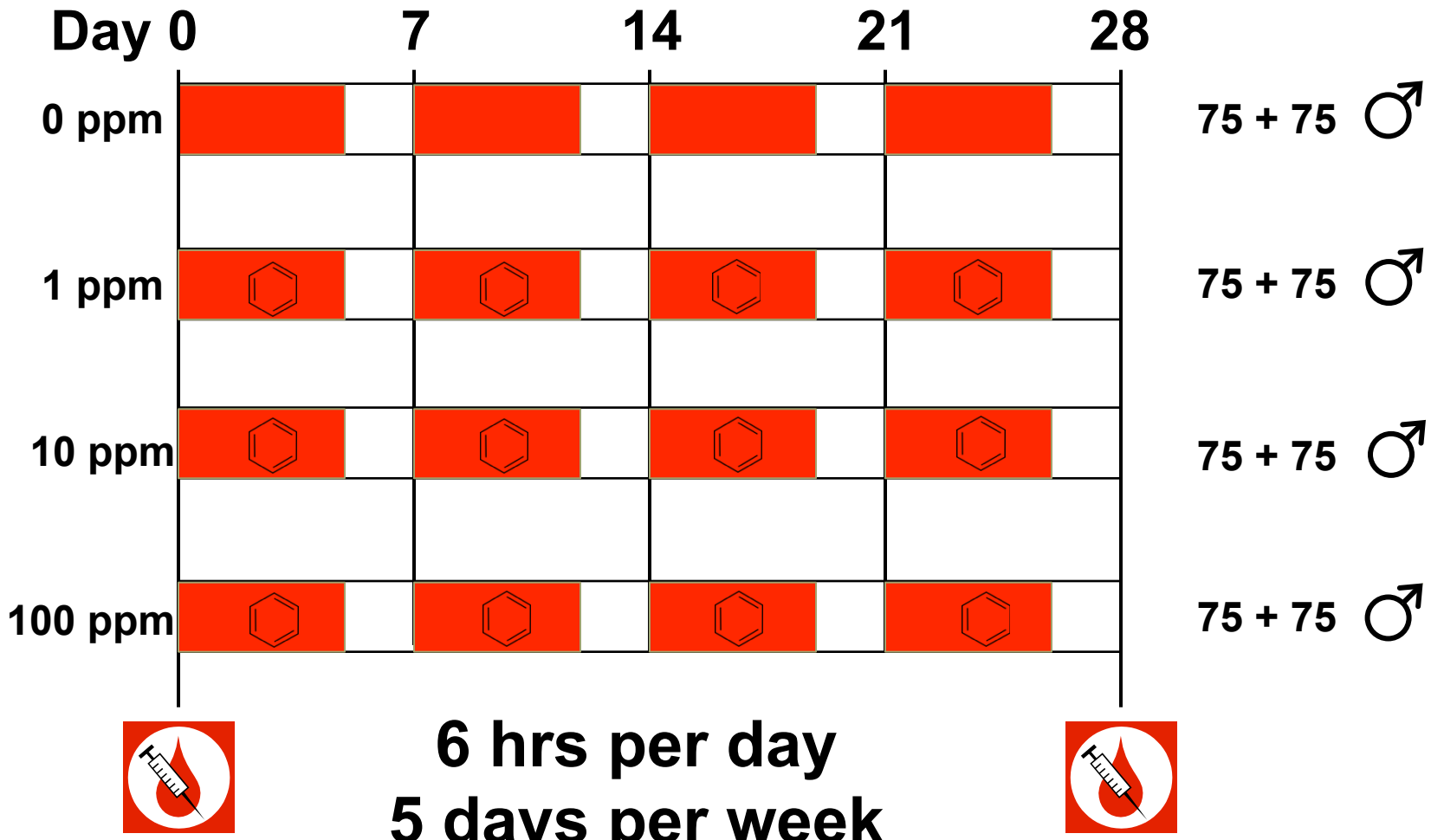
National Institute of Environmental Health Sciences (NIEHS)

Study benzene genotoxicity in reticulocytes.

Search for new genes involved in susceptibility or resistance.

Cyp2e1? Epoxide hydrolase? Phase II enzymes?

Study Design



Benzene Inhalation Endpoints

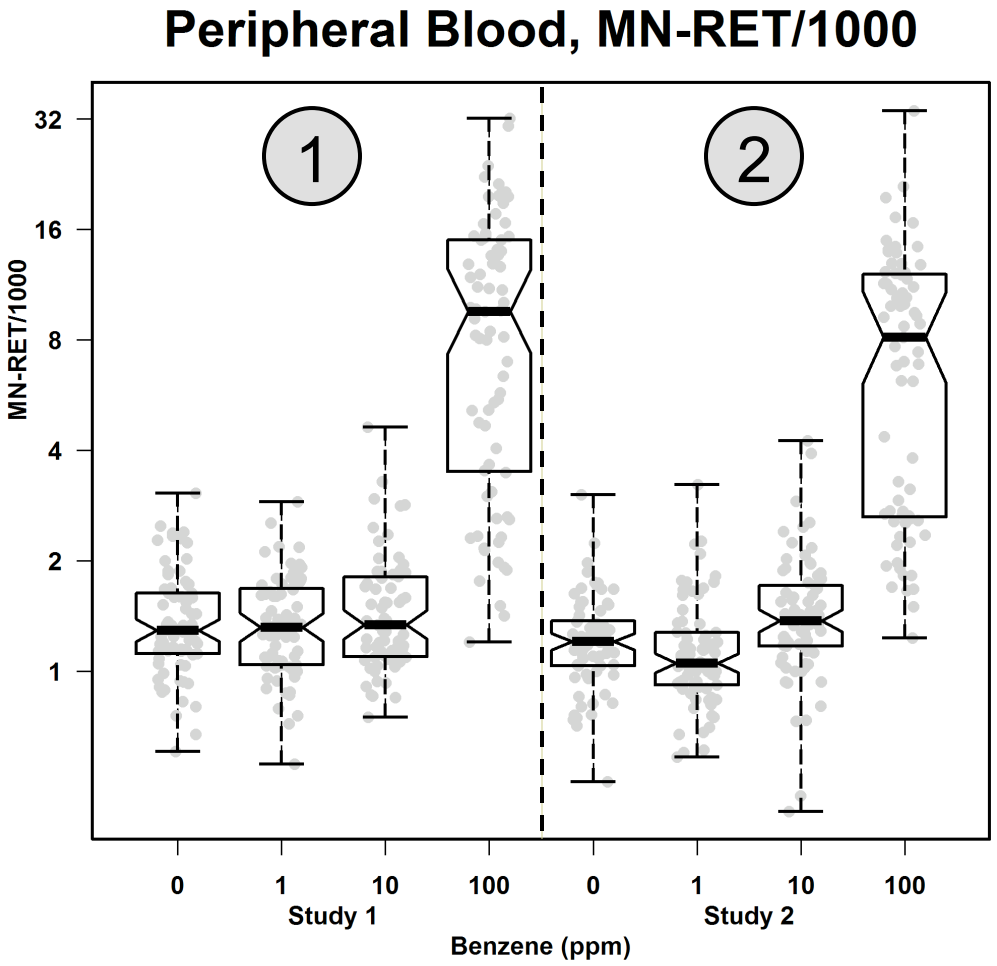
Pre- and post-exposure blood.

Bone marrow at necropsy.

MN-RET: micronucleated reticulocytes

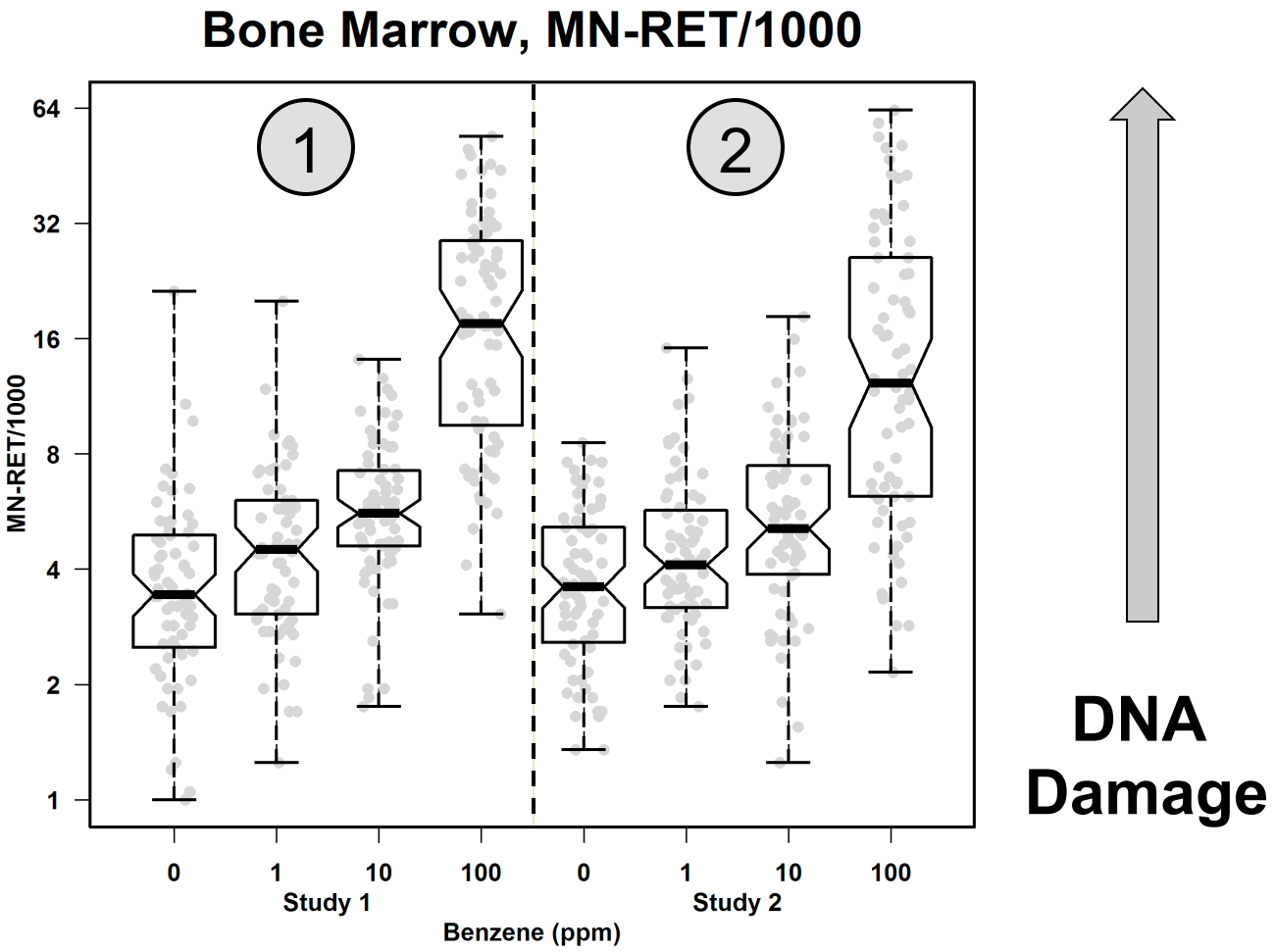
indicator of DNA damage.

Micronucleated Reticulocytes in Blood



DNA Damage

Micronucleated Reticulocytes in Bone Marrow



QTL mapping model estimates founder effects

Map with 150 samples from 100 ppm dose.

$$y_{ij} = \sum_{j=1}^{8} \beta_j g_{ij} + \lambda + \varepsilon$$

y_{ij} = phenotype for sample *i* at SNP *j*.

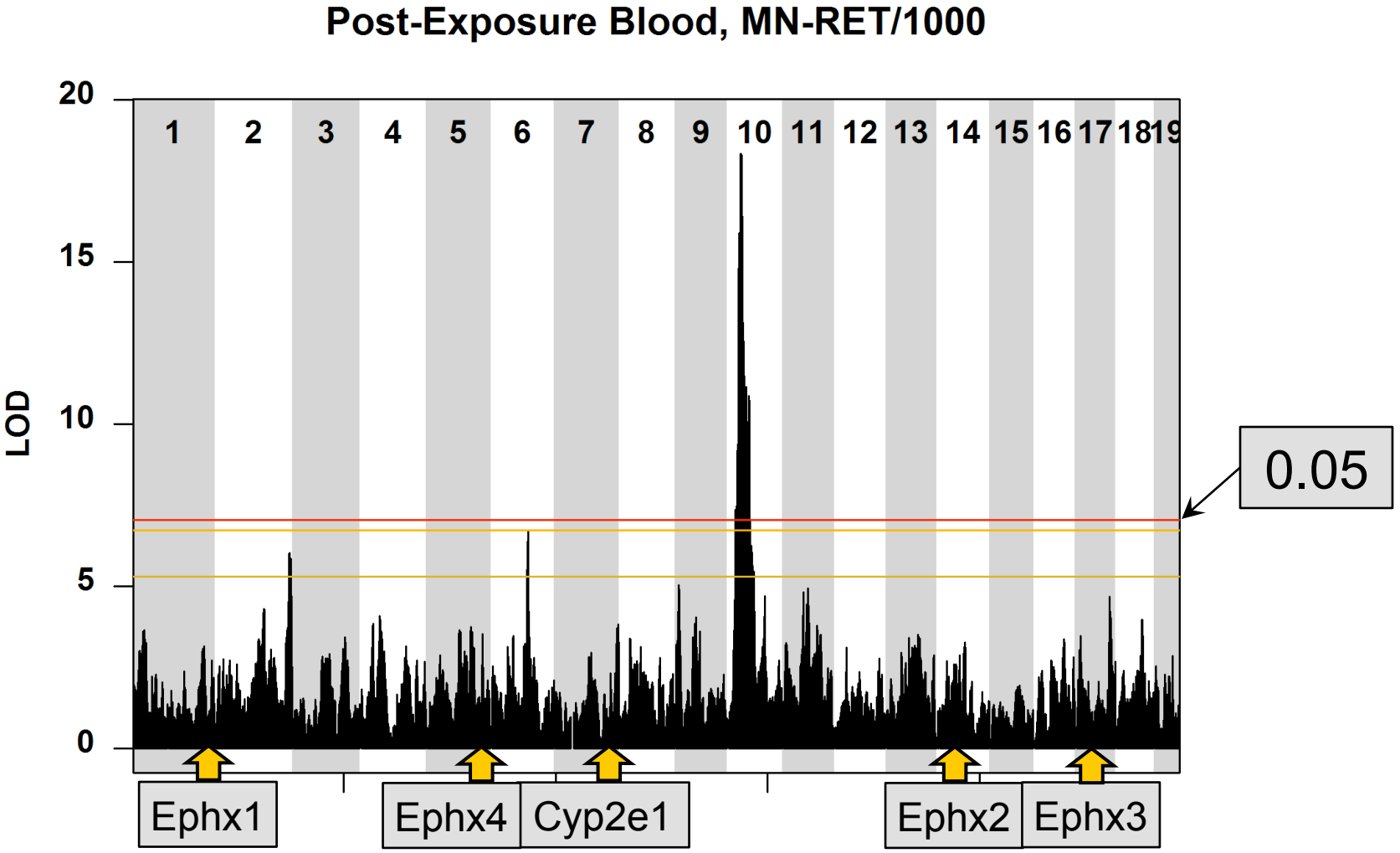
β_j = founder allele effect at SNP *j*.

g_{ij} = genotype for sample *i* at SNP *j*.

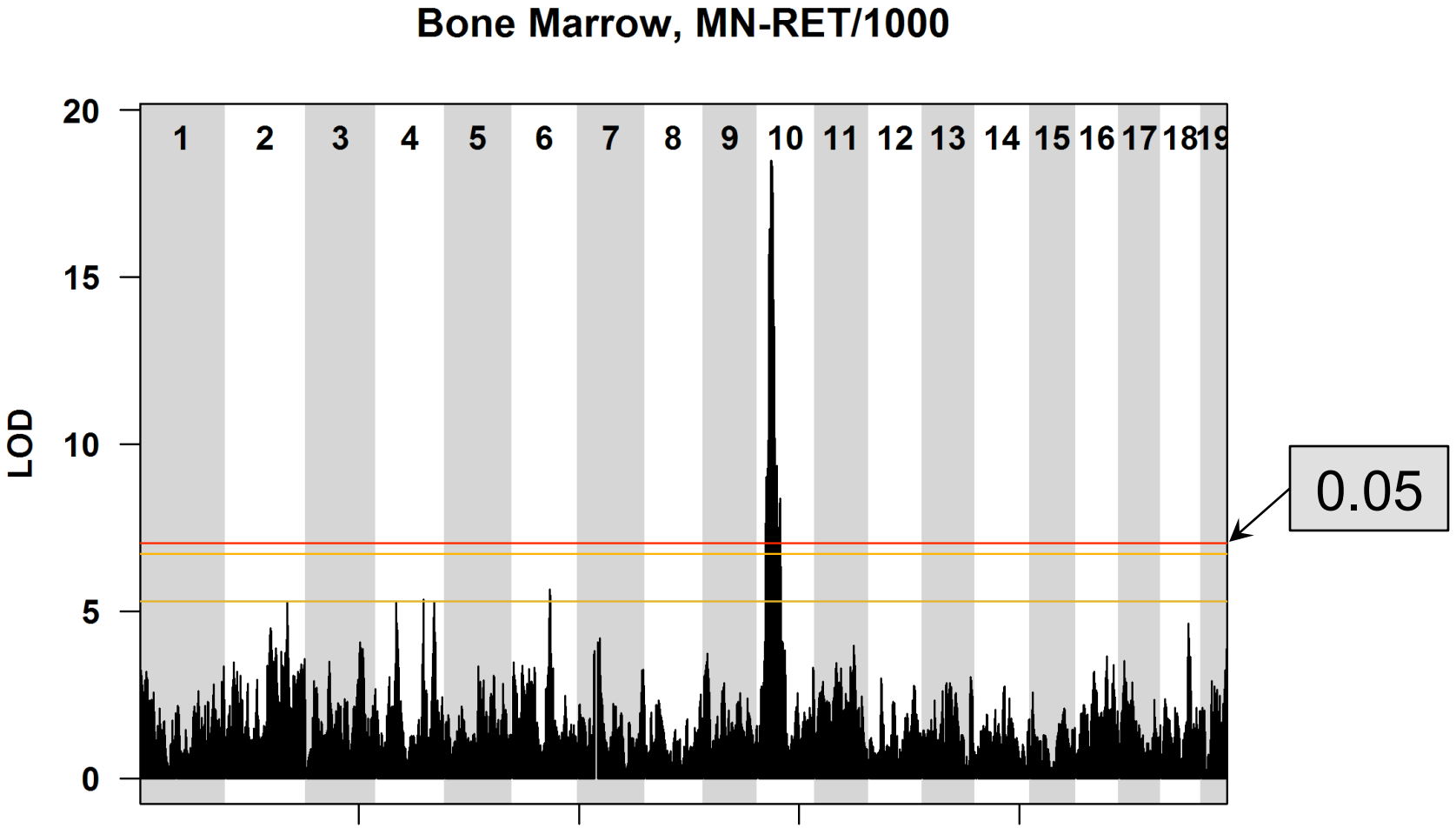
λ = correction for population structure.

R packages: DOQTL and QTLRel

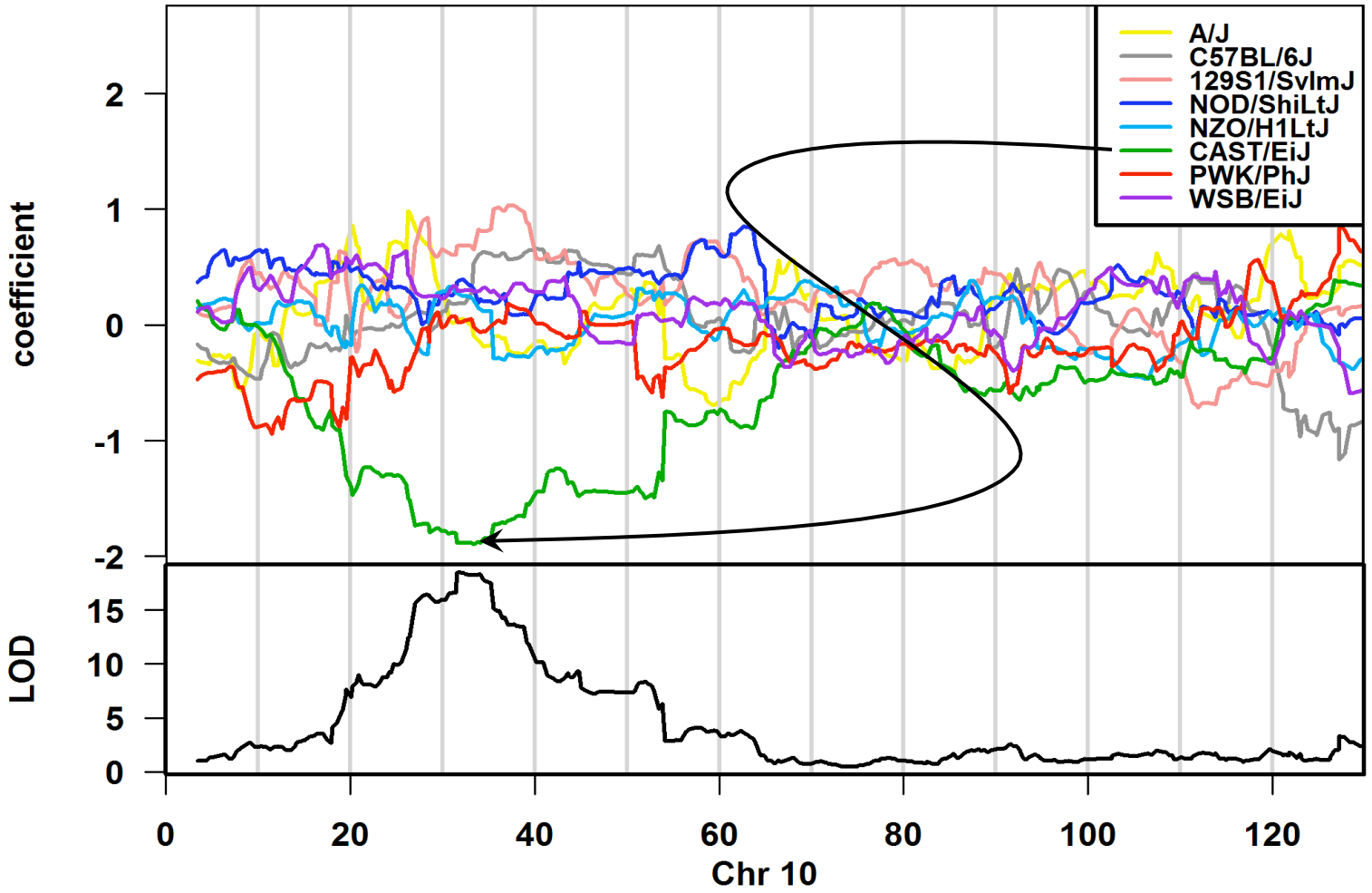
Blood MN-RET has a QTL on Chr 10



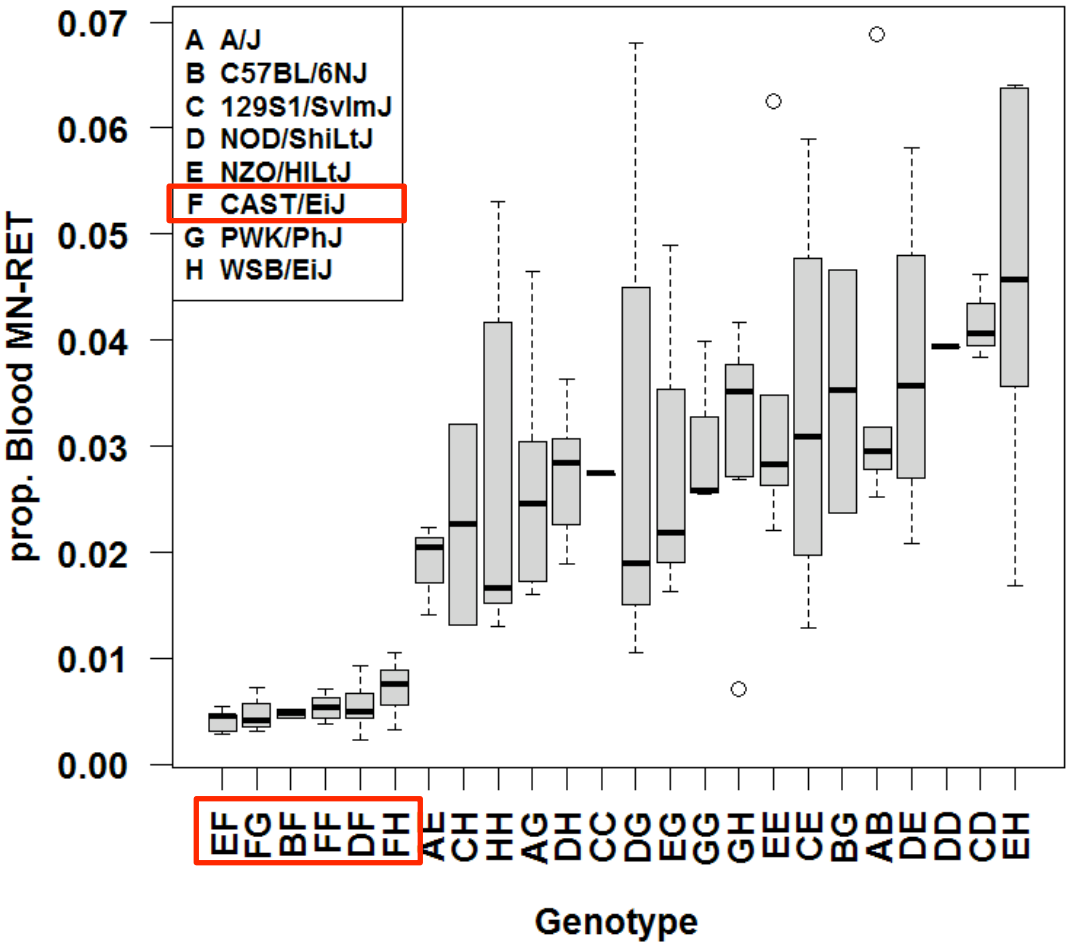
Bone Marrow MN-RET has a QTL on Chr 10



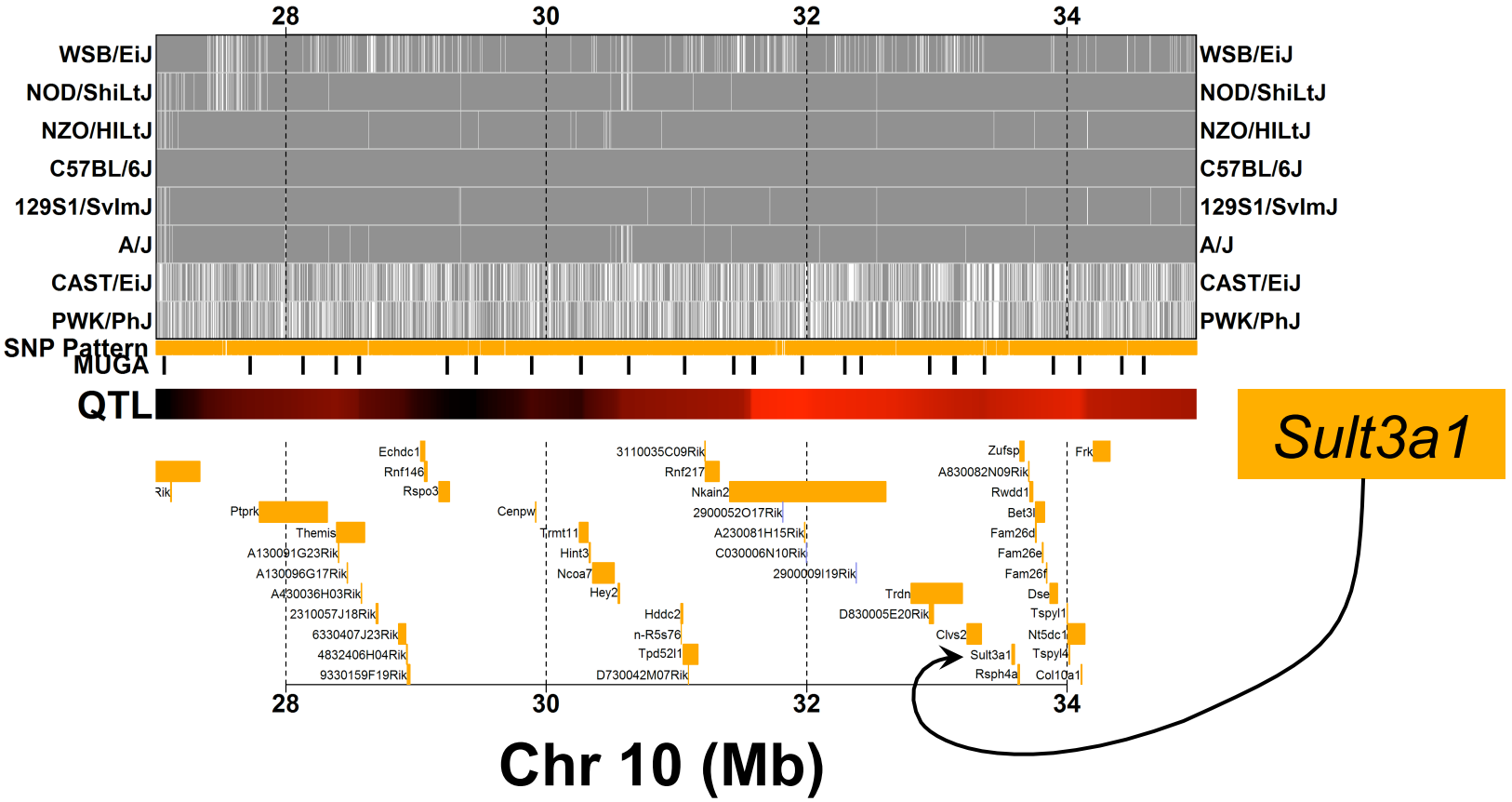
CAST/EiJ allele is resistant



CAST/EiJ allele is dominant



Candidate genes in the QTL interval

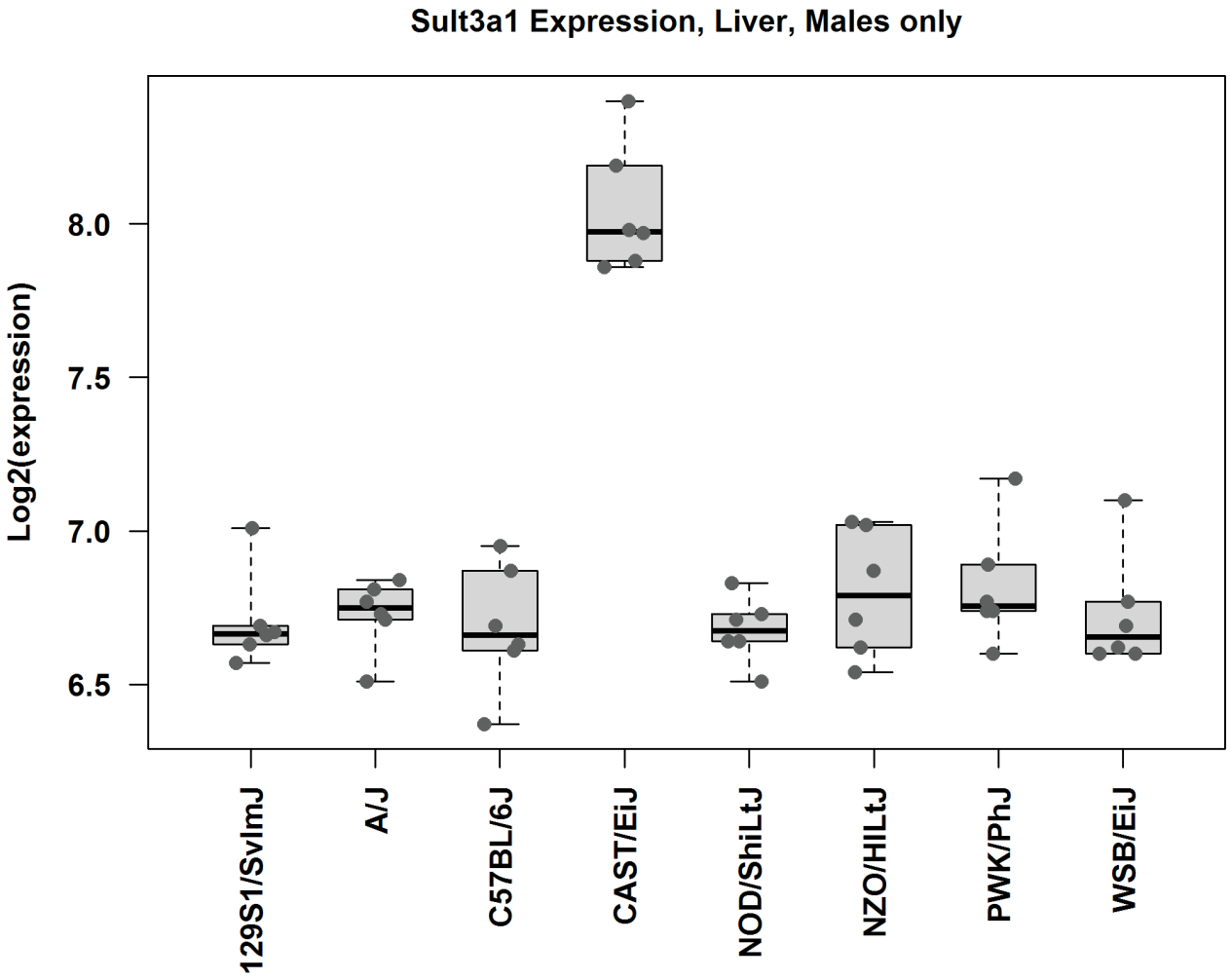


SNPs from: http://www.sanger.ac.uk/cgi-bin/modelorgs/mousegenomes/snps.pl

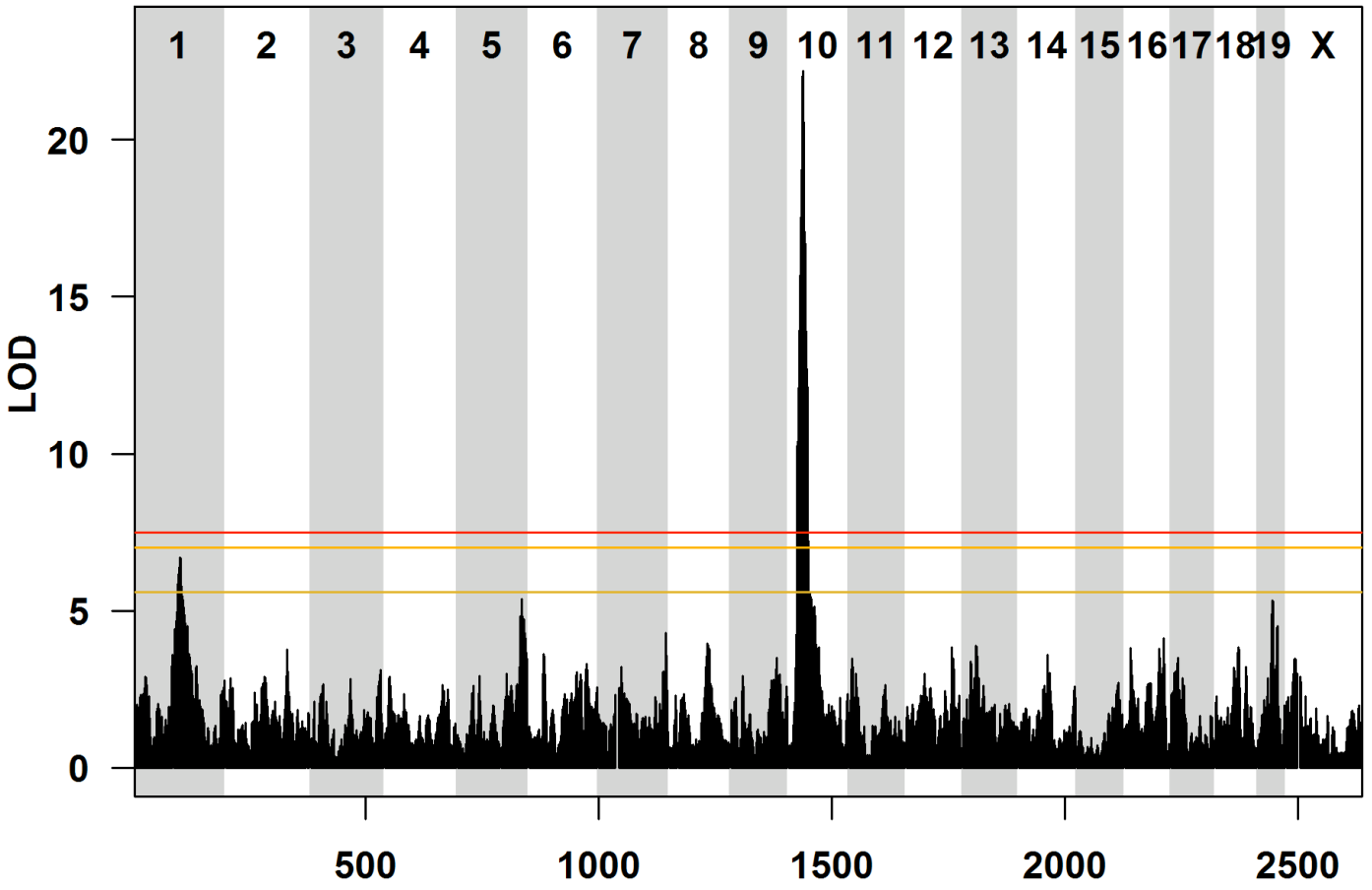
Keane TM, Goodstadt L, Danecek P, *et al.* (2011), Mouse genomic variation and its effect on phenotypes and gene regulation, *Nature*, 477(7364):289-294.

Yalcin B, Wong K, Agam A, *et al.* (2011), Sequence-based characterization of structural variation in the mouse genome, *Nature*, 477(7364):326-329.

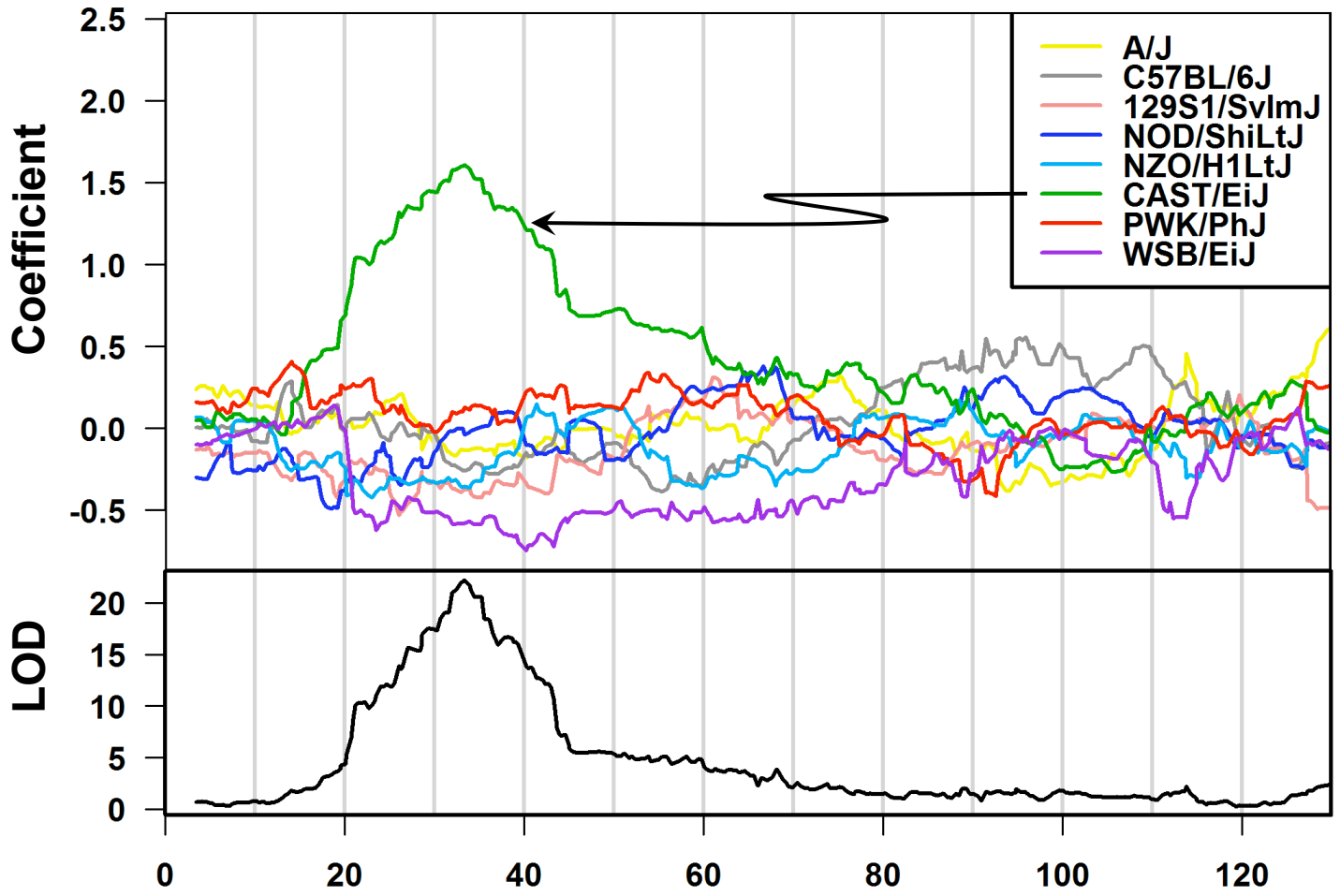
Sult3a1 has high expression in liver



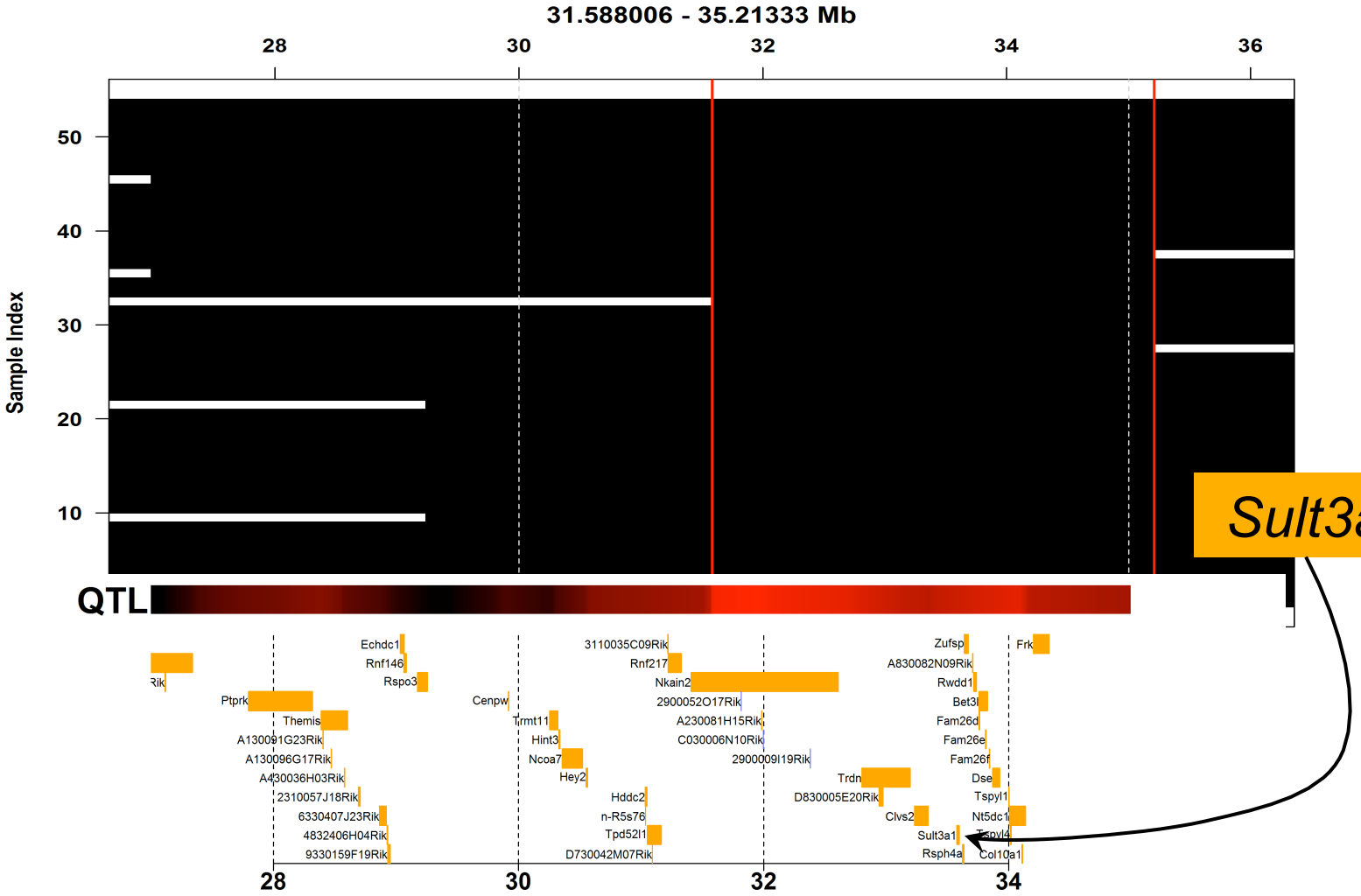
Sult3a1 expression in liver has a co-located QTL on Chr 10



Sult3a1 founder effect pattern is the same as MN-RET



CAST/EiJ haplotype blocks can be used to narrow the QTL



Future work

No direct ortholog of *Sult3a1* in humans, but its active catalytic site accepts phenol, napthylamine, and paranitrophenol.

Investigation of the homology of the *Sult3a1* binding site and substrate affinity with phenol human SULT*s* in progress.

Sulfate conjugates of benzene are observed in urine.

Differential mRNA expression liver and bone marrow in progress.

Collaborative Cross mice and targeted knockouts will aid reproduction of phenotype and results.

Summary

Diversity Outbred mice are derived from 8 inbred founders.

DO mice have high genetic diversity, fine recombination block structure and balanced allele frequencies, making them ideal for mapping.

DO mice can be used to find genes underlying toxicity.

Data from the founders can help inform results.

CC mice may be useful for validation and follow up.

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