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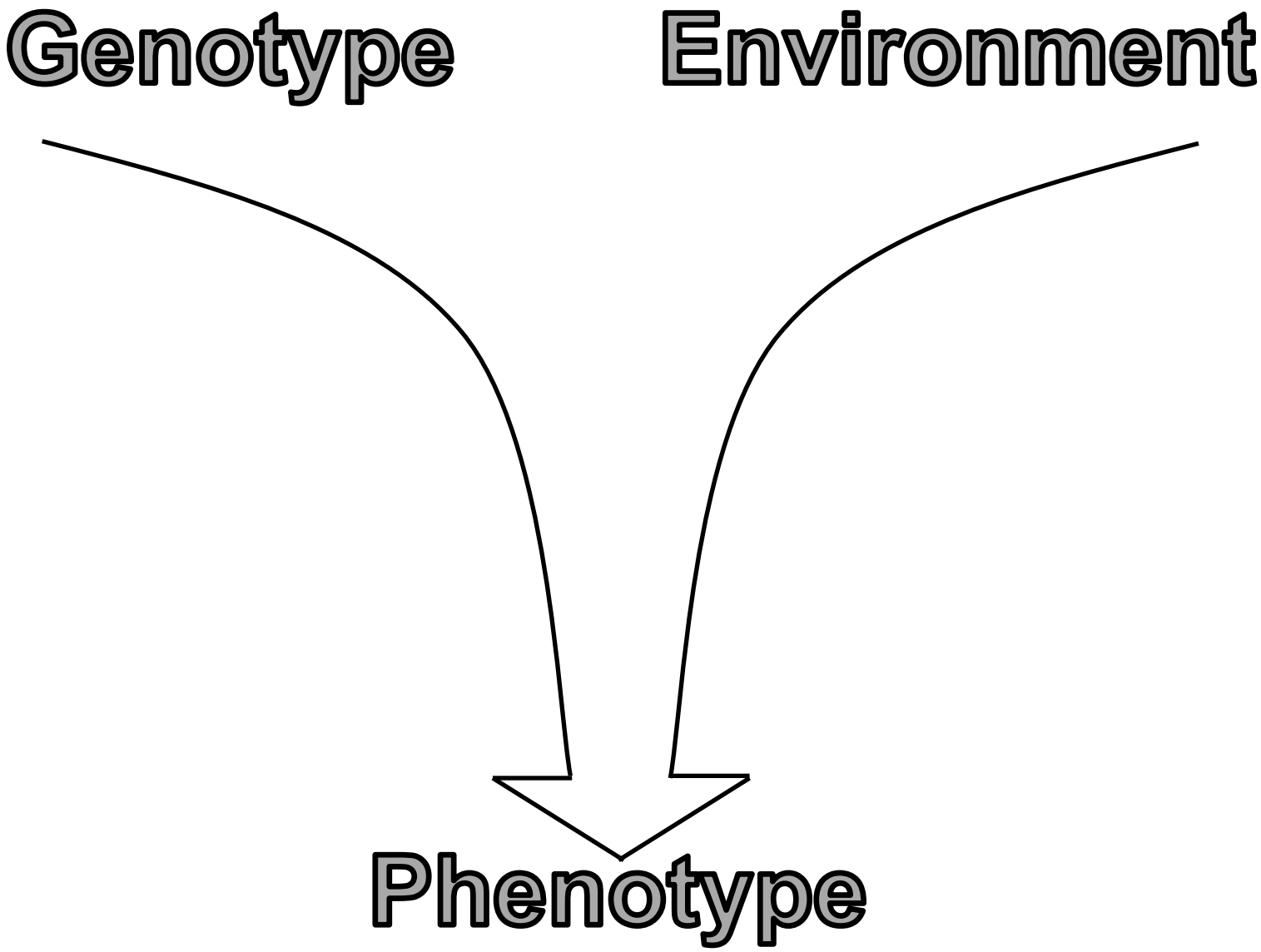
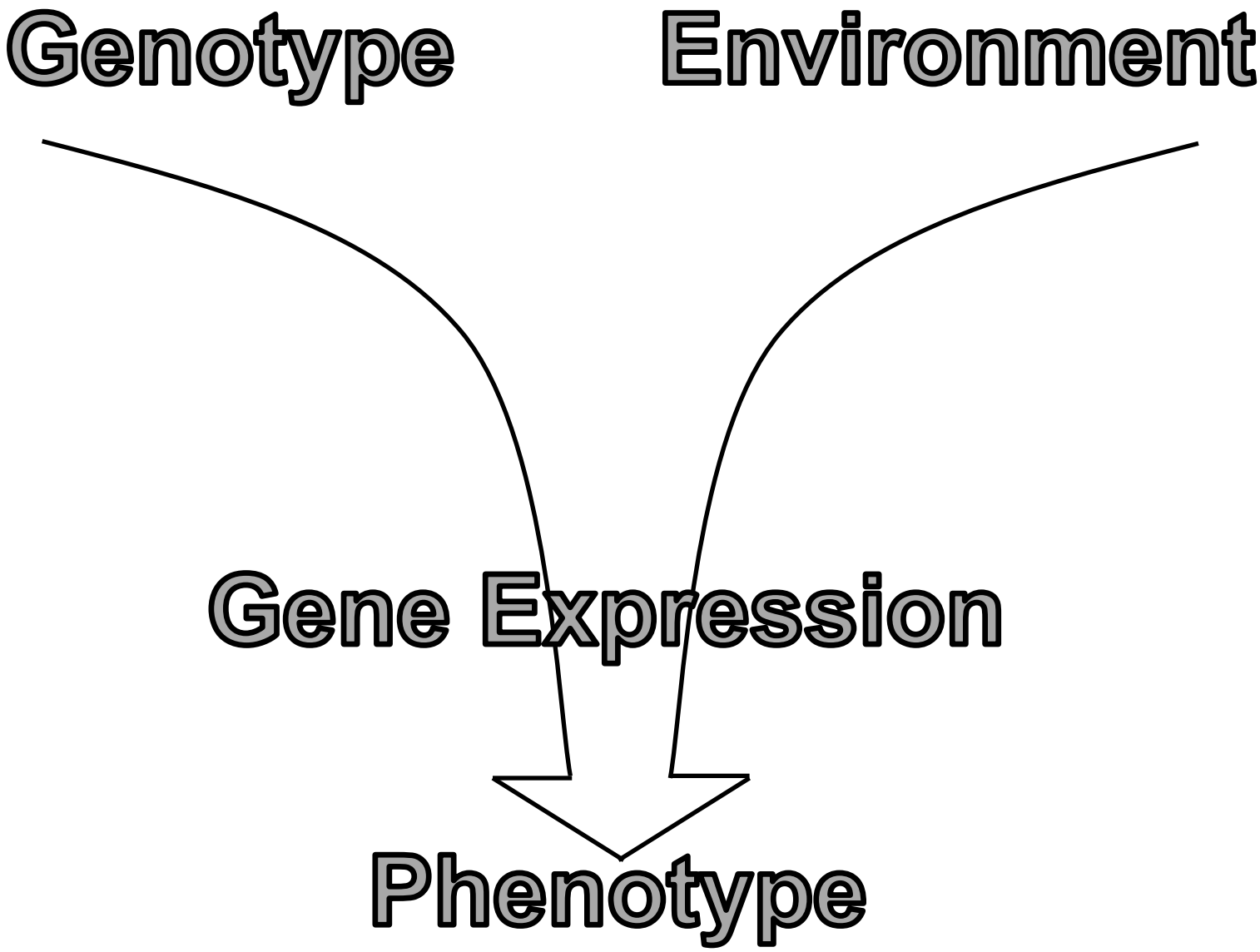
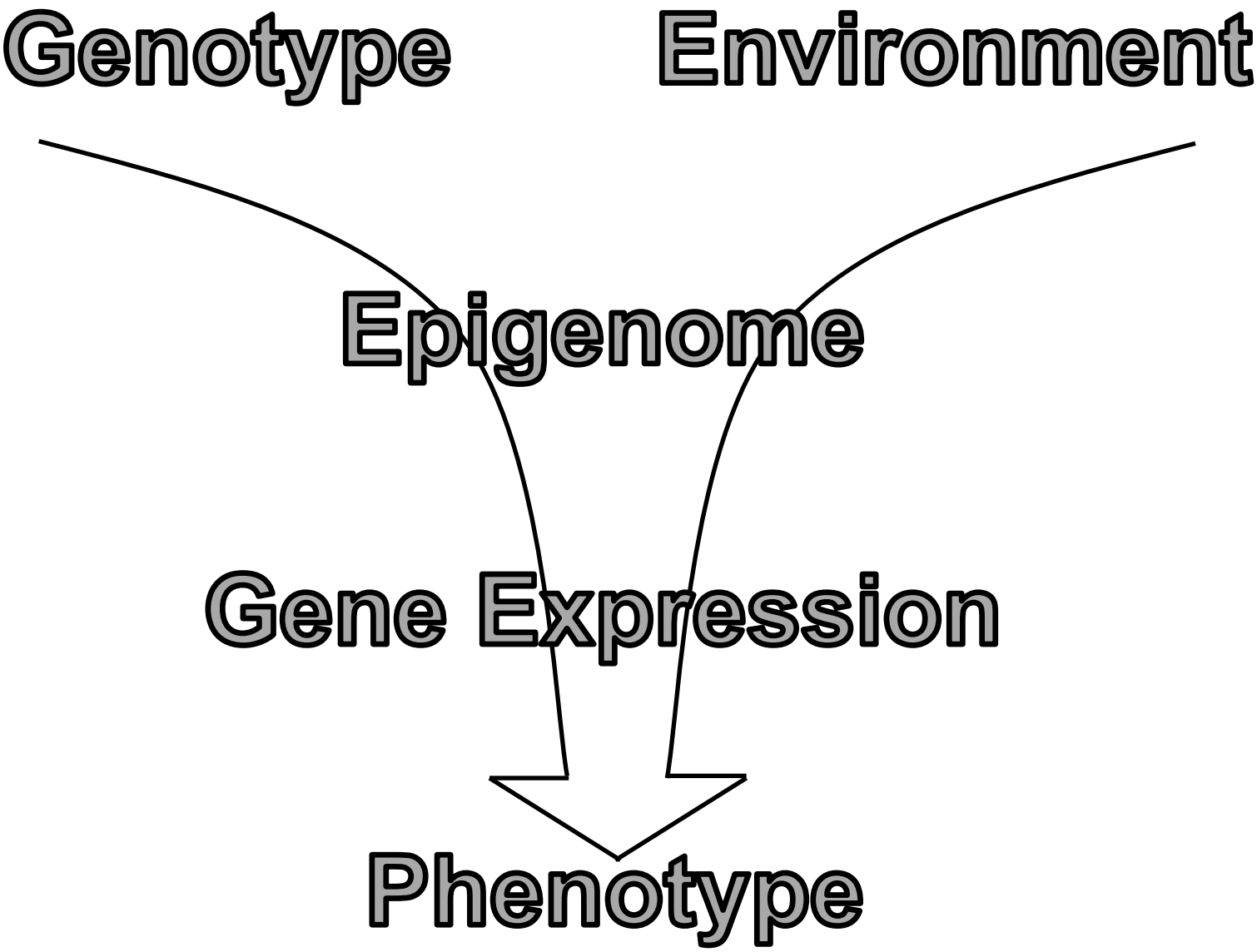
Genetic regulatory variation and environmental response

David L. Aylor
Population-Based Rodent Resources for Environmental Health Sciences
NIEHS
March 19, 2015


Genotype
Environment
Phenotype


Genotype
Environment
Gene Expression
Phenotype


Genotype
Environment
Epigenome
Gene Expression
Phenotype

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Outline

1. Differential susceptibility to diethylstilbestrol (DES)

1. eQTL meta-analysis in the CC

2. Epigenome profiling in embryonic fibroblasts

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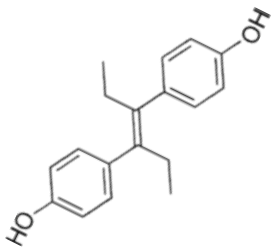
History of diethylstilbestrol (DES)



Prescribed to pregnant women

Efficacy not supported

Formulated

Cervical Clear Cell Adenocarcinoma reported

Pregnancy contraindicated for DES use

1930 1940 1950 1960 1970 1980 1990 2000 2010

FDA bans DES

FDA bans DES in poultry implants

DES used in agriculture

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DES health effects

DES daughters

- Reproductive structural abnormalities
- Increased infertility and pregnancy complications
- Early puberty and menopause
- Breast cancer
- Cervical clear cell adenocarcinoma (CCAC)

DES sons

- Reproductive structural abnormalities
- Decreased sperm count
- Testicular cancer?

DES female mice

- Uterine cancer
- Uterine dysplasia
- Oviduct malformation
- Uterine gene expression changes

DES male mice

- Decreased sperm count
- Testicular gene expression changes

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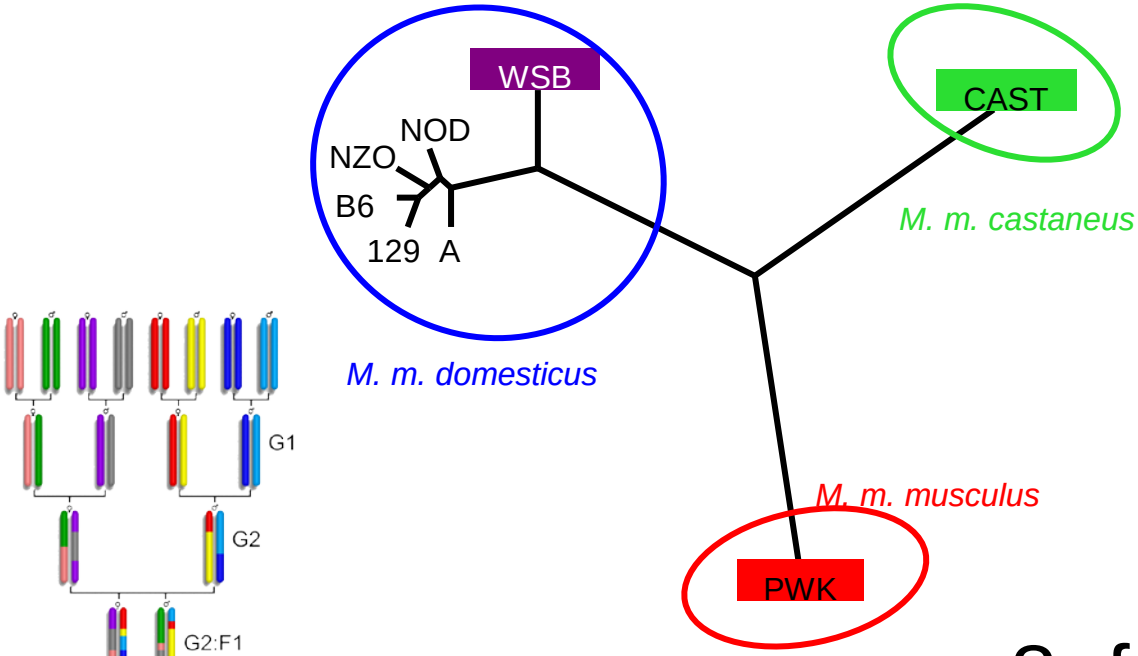
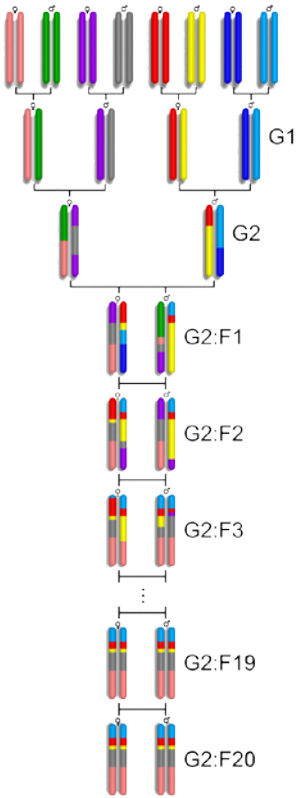
Strain selection





8 founders

50 CC

\+ 2 DBA2 & FVB

60

A/J
C57BL/6J
129S1/SvImJ
NOD/LtJ
NZO/HlLtJ
CAST/EiJ
PWK/PhJ
WSB/EiJ

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DES exposure and phenotyping

- Uterus:
  - RNA expression
  - DNA methylation
  - Cancer Histopathlogy
- Stress?
- Obesity?

♀

PND 1-5 | 8 | 12 | 26 | 52 | 72 wks

- Reproductive morphology
- Sperm count
- Recombination rates
- Testis:
  - RNA expression
  - DNA methylation
  - Histology

♂

Nicole Carns

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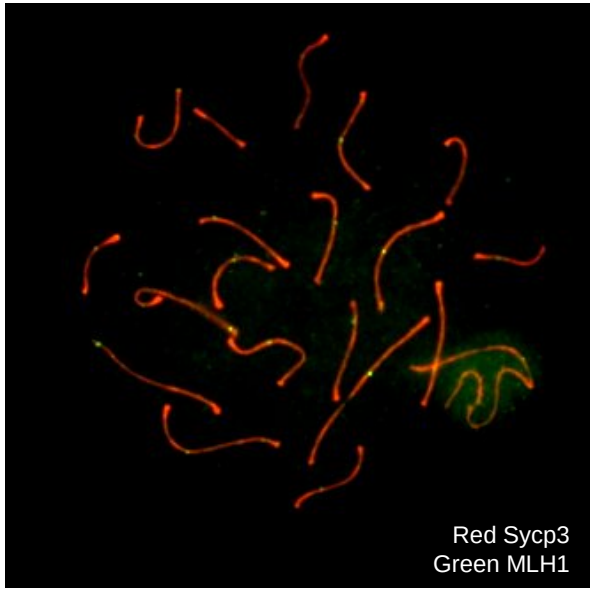
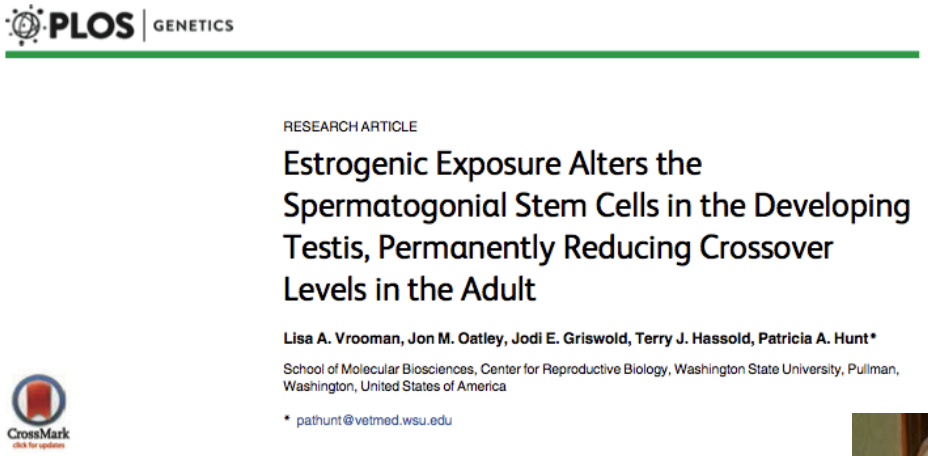
Effects of DES exposure in males

- “Curvy” vas deferens
- Reduced gonadal fat pad
- Recombination rate variation?




PLOS | GENETICS

RESEARCH ARTICLE

Estrogenic Exposure Alters the Spermatogonial Stem Cells in the Developing Testis, Permanently Reducing Crossover Levels in the Adult

Lisa A. Vrooman, Jon M. Oatley, Jodi E. Griswold, Terry J. Hassold, Patricia A. Hunt*

School of Molecular Biosciences, Center for Reproductive Biology, Washington State University, Pullman, Washington, United States of America

* pathunt@vetmed.wsu.edu

Carlee Hemphill

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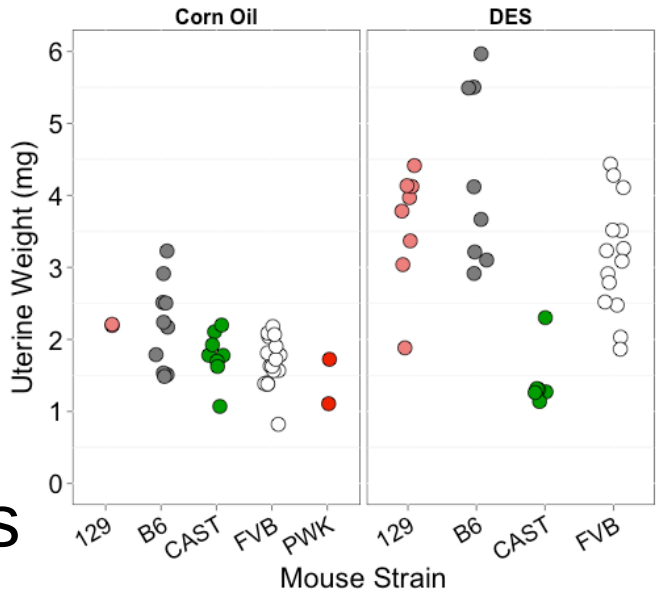
Effects of DES exposure in females

- Anxiety in dams (FVB)
- Vascularized uterus (CAST)
- Increased uterine size
- Gene expression changes



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RNA-sequencing: Acute effects of DES exposure on 5 day old uterus

2	x	2	x	6
TREATMENTS		STRAINS		REPLICATES
DES		B6		
Vehicle		FVB		

- One lane HiSeq 100bp SE reads ~$11x10^6$ reads/sample
- Aligned to B6 and FVB reference genomes
- 11320 genes average expression ≥ 100 reads/sample

Thomas Konneker

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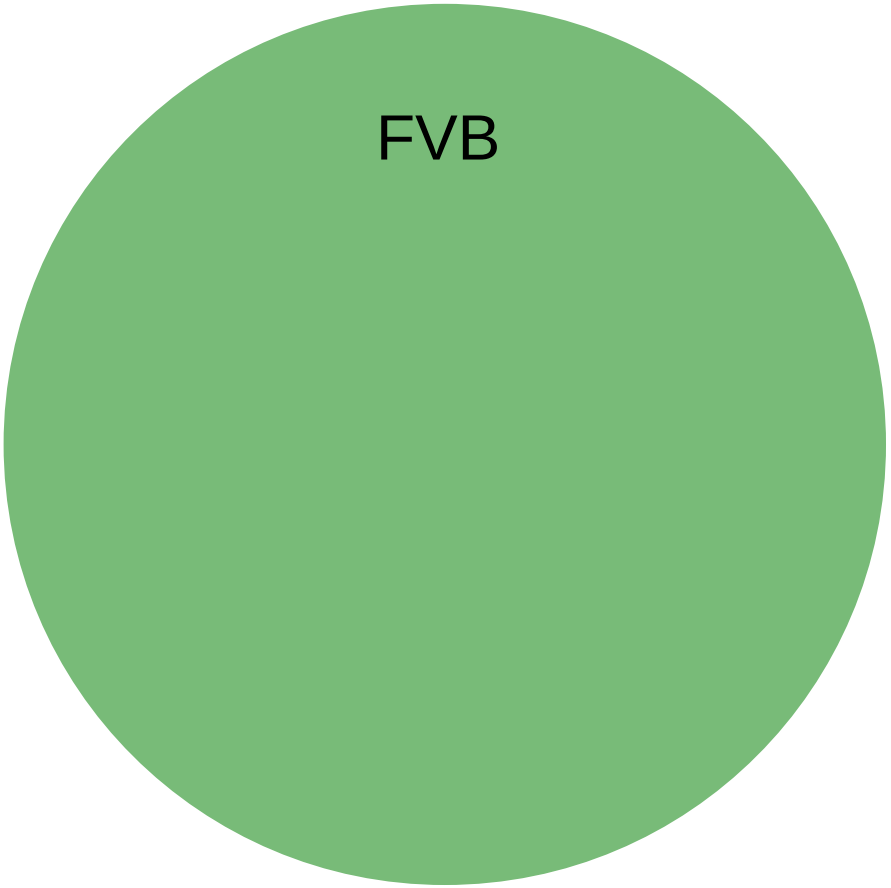
RNA-sequencing – DES v. Vehicle



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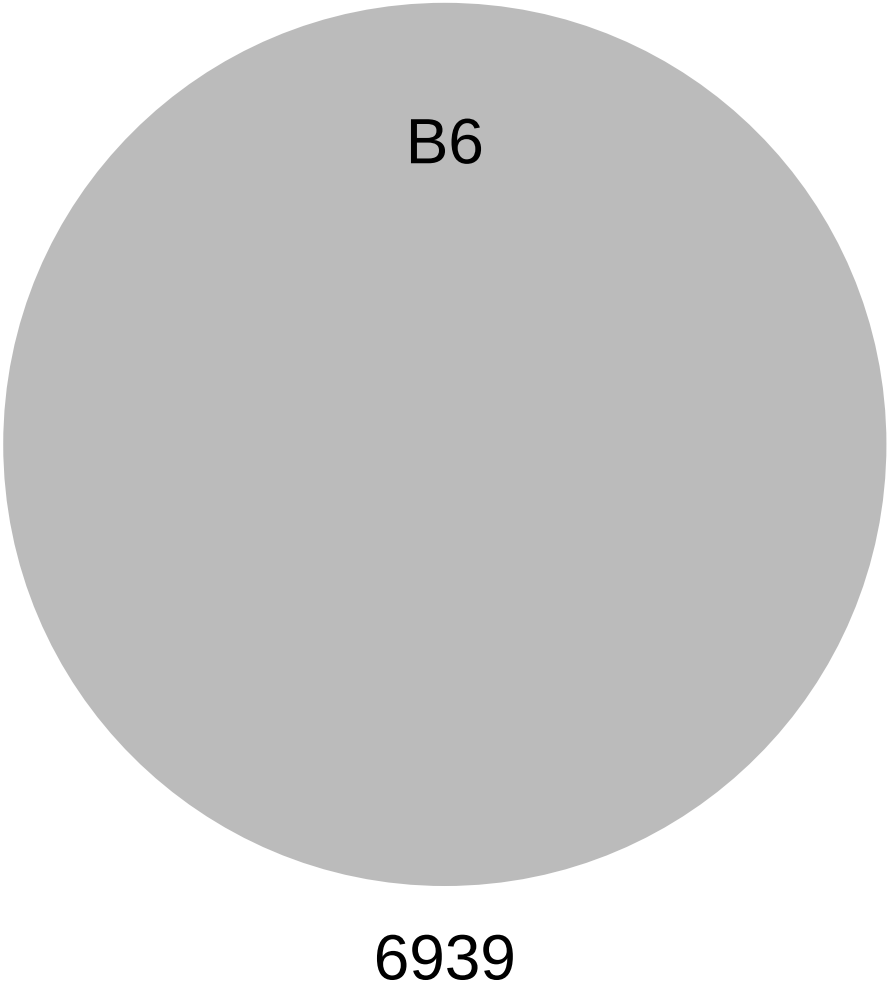
RNA-sequencing – DES v. Vehicle



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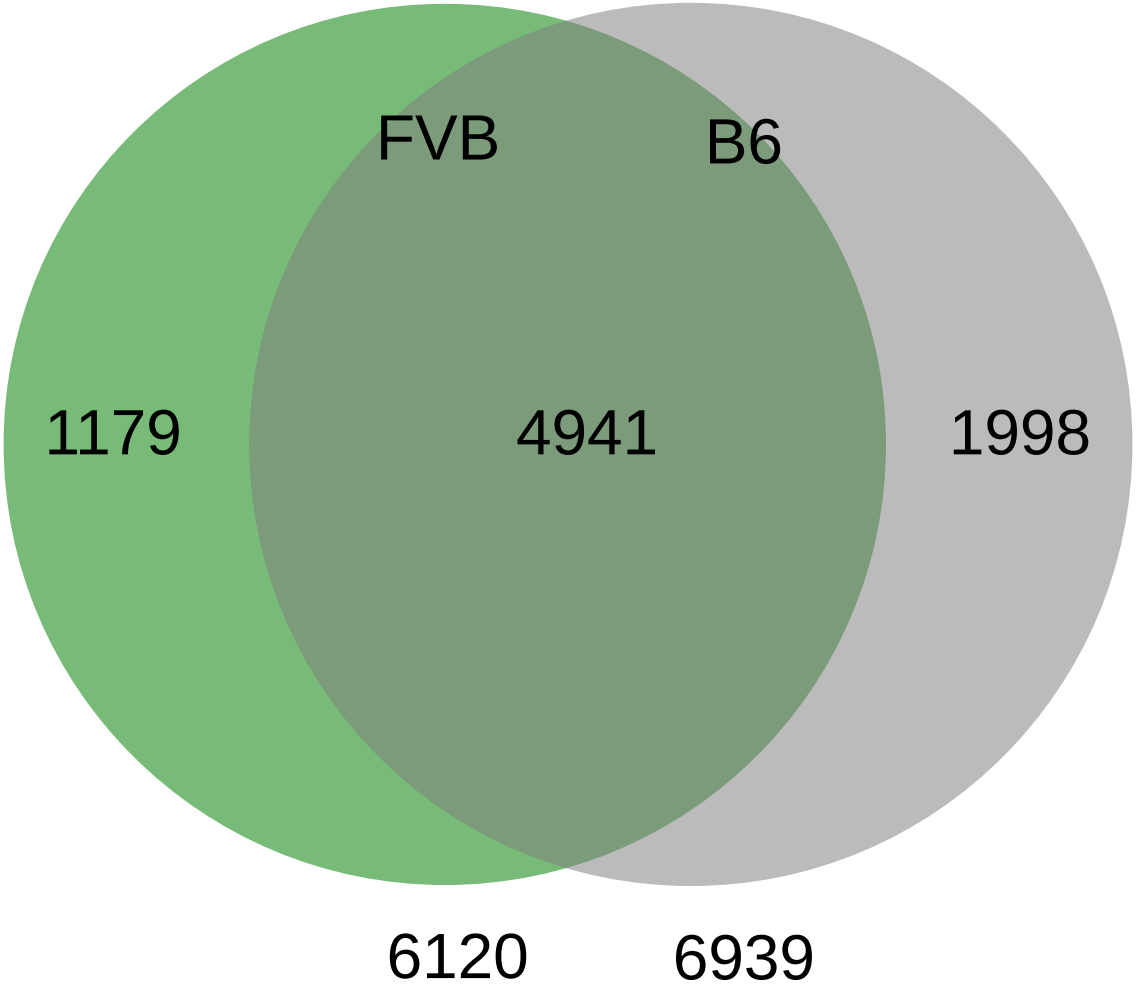
RNA-sequencing – DES v. Vehicle



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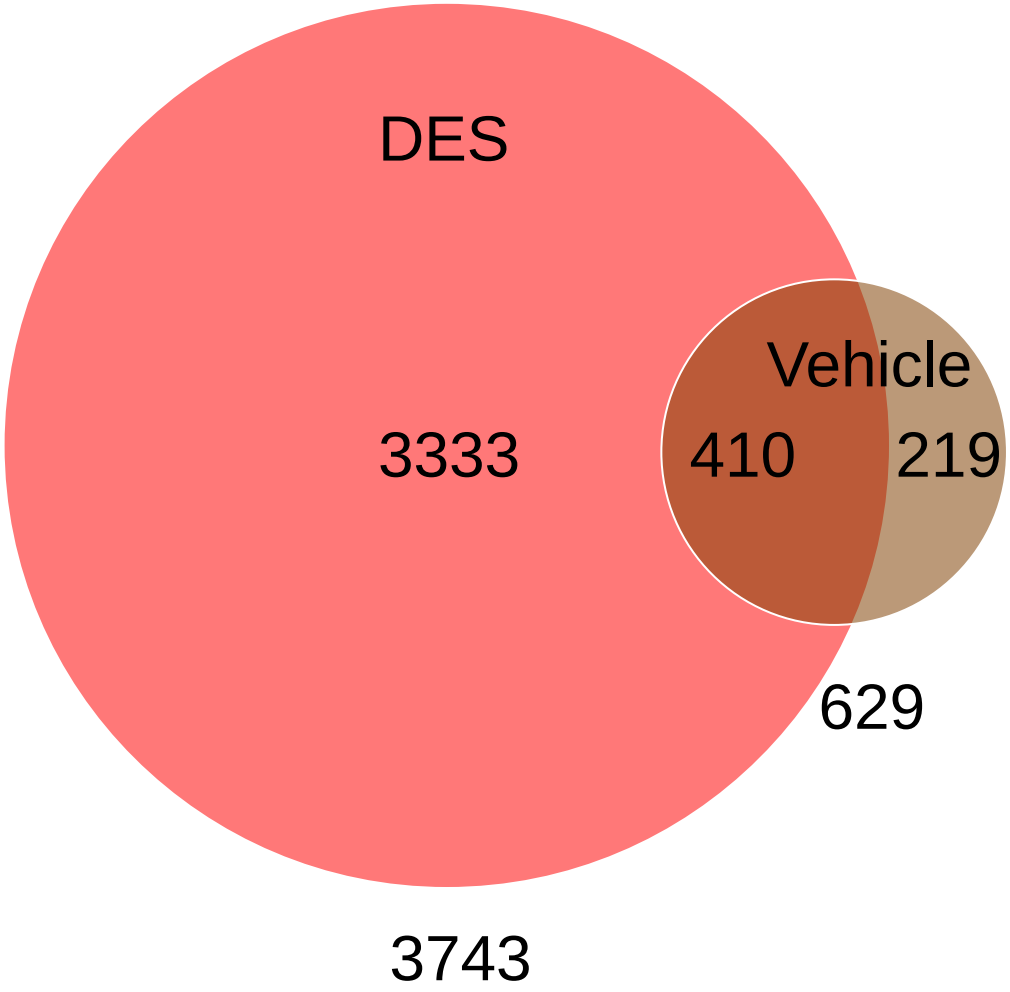
RNA-sequencing – FVB v. B6



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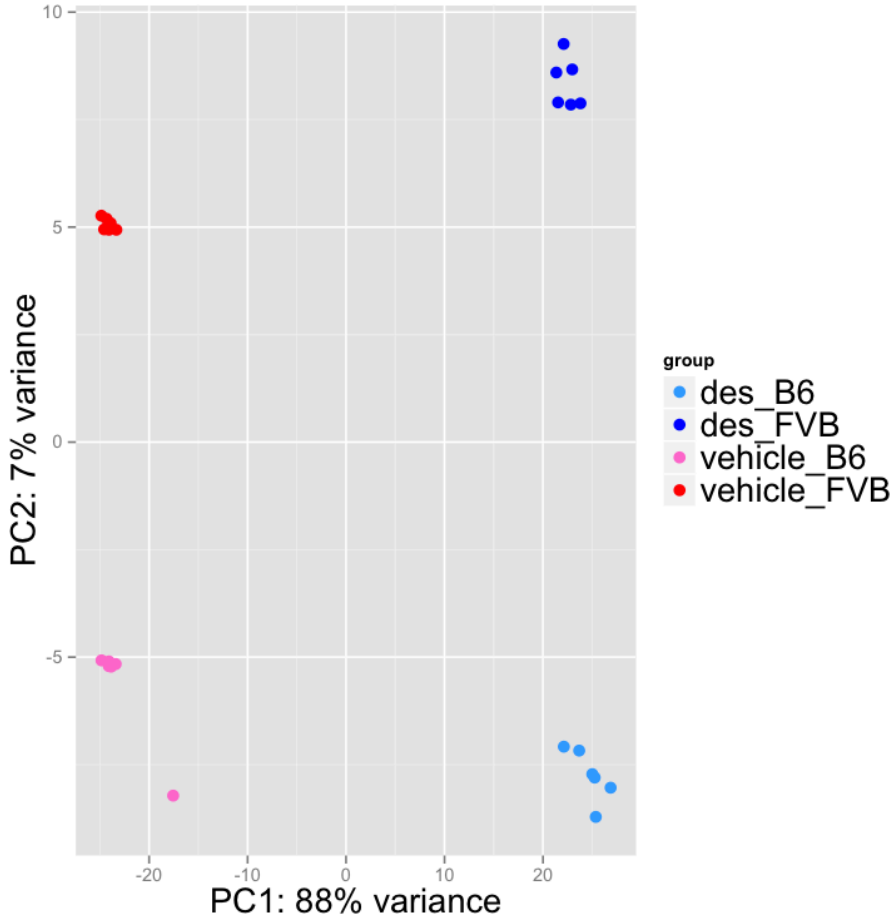
GxE in the DES-exposed transcriptome



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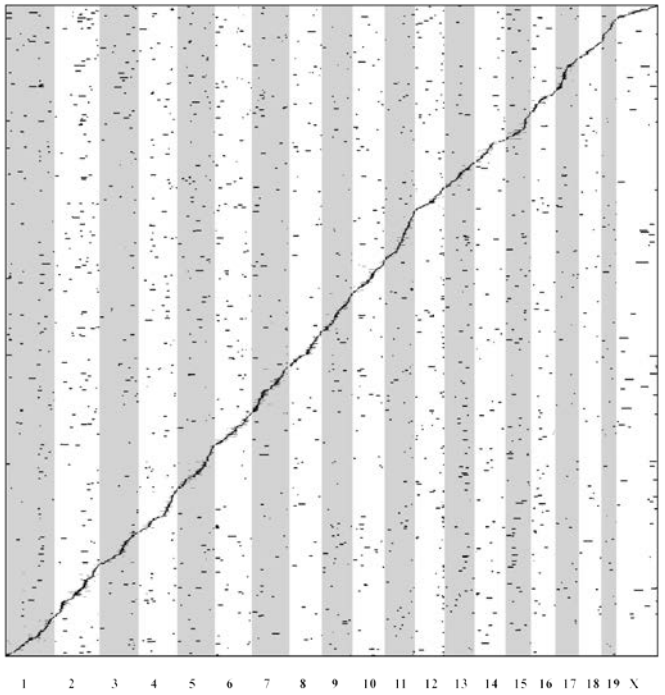
CC gene expression QTL

Study	Year	Local eQTL	Distant eQTL
pre-CC liver	2011	4042	1881
pre-CC lung (asthma)	2013	7331	2523

75-87% of eQTL are local



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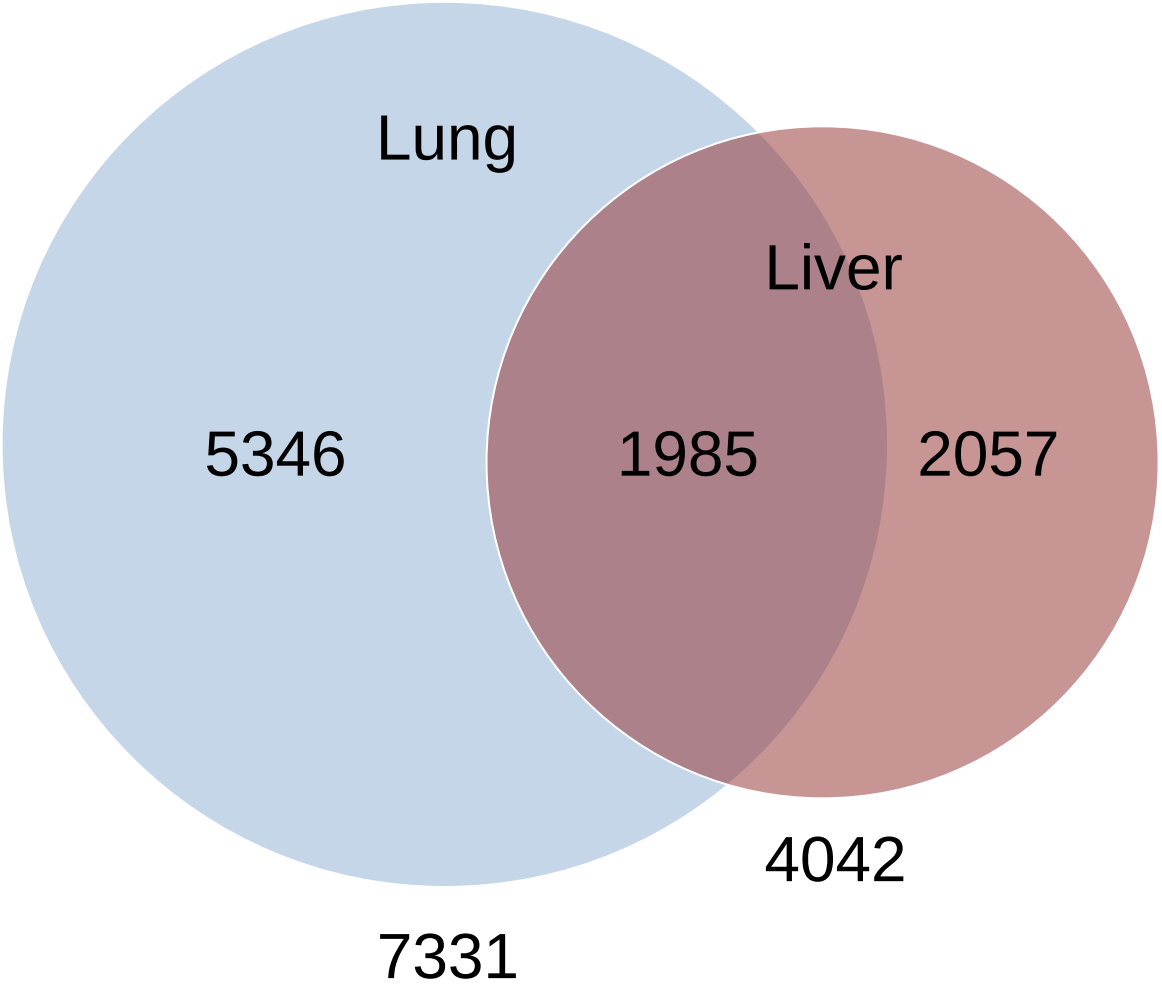
Local eQTL shared in liver and lung



Sam Widmayer

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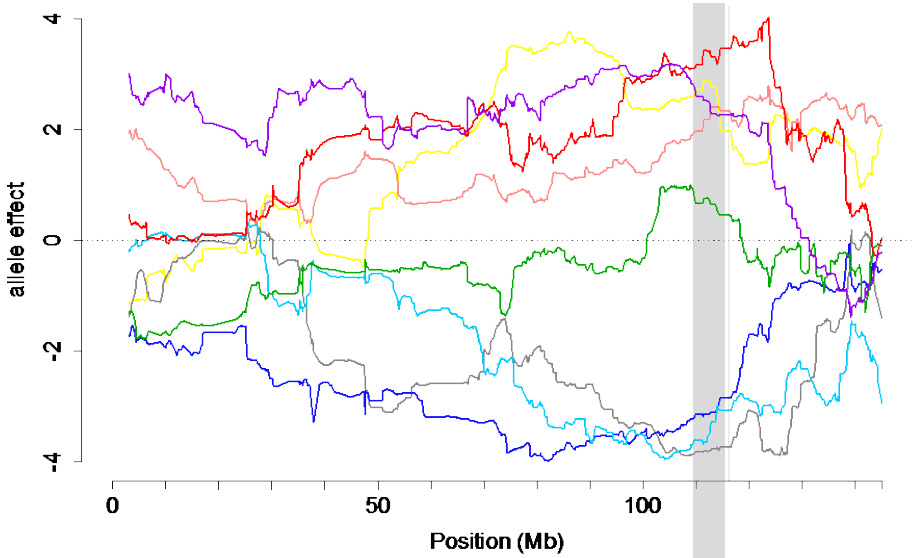
Allele effect patterns



<u>254 possible patterns</u>

2^8 – 2 null patterns

	A	B6	129S1	NOD	NZO	CAST	PWK	WSB
High	1	1	1	1	1	1	1	1
Low	0	0	0	0	0	0	0	0
	1	0	1	0	0	1	1	1

A/J
C57BL/6J
129S1/SvImJ
NOD/LtJ
NZO/HlLtJ
CAST/EiJ
PWK/PhJ
WSB/EiJ

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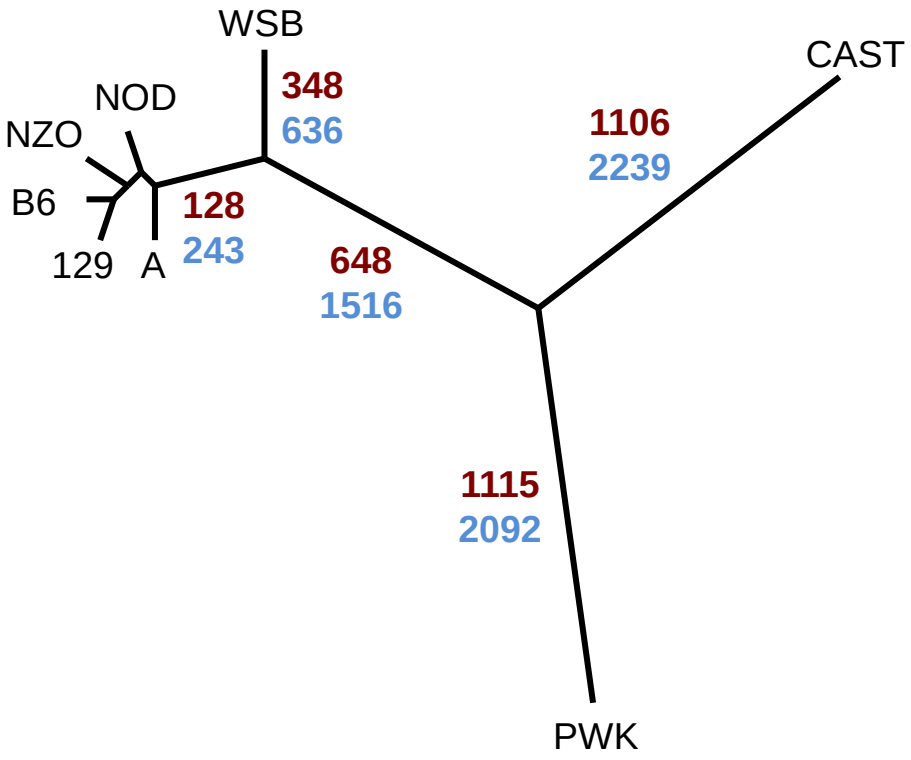
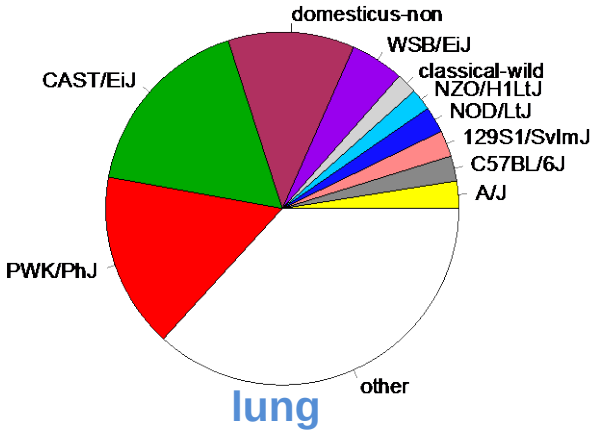
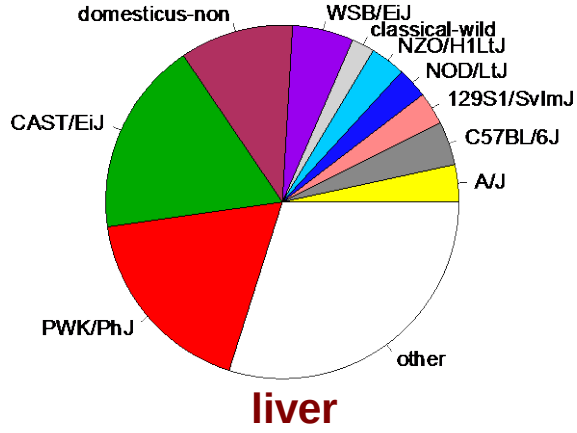
Allele effects reflect strain relationships







A/J
C57BL/6J
129S1/SvImJ
NOD/LtJ
NZO/HlLtJ
CAST/EiJ
PWK/PhJ
WSB/EiJ

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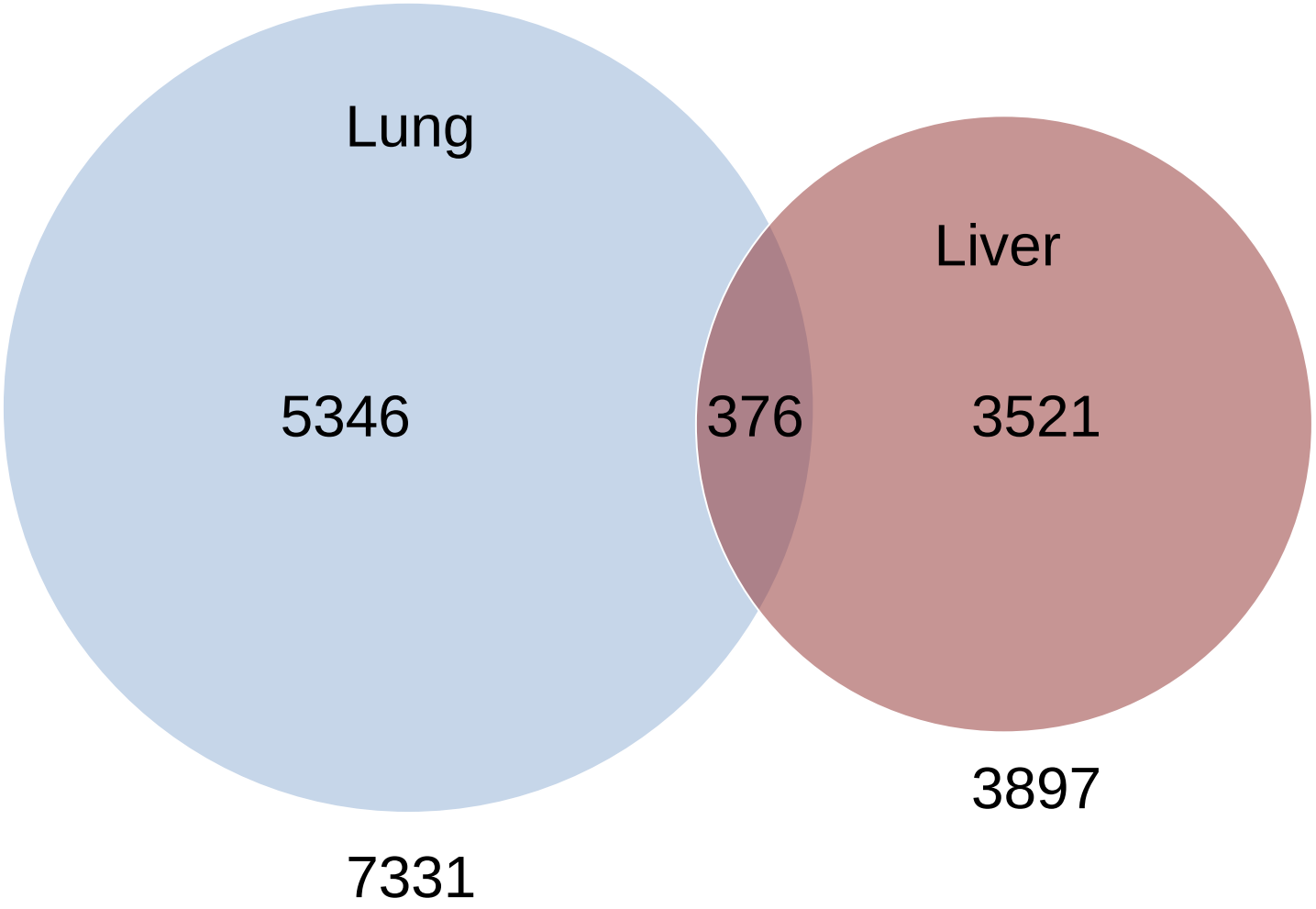
Shared eQTL driven by same alleles



State of eQTL knowledge – 2015

- Abundant
- Local and *cis*-acting
- Allele effects agree between CC, DO, and founder strains
- Proportional to genetic distance
- Shared across tissues

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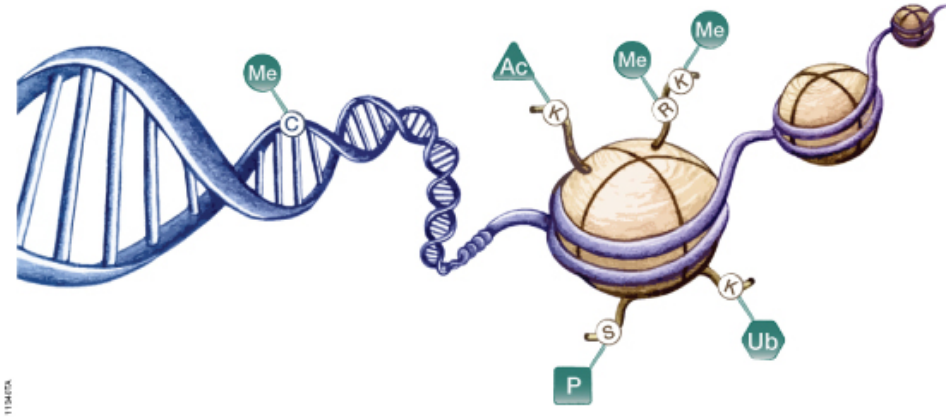
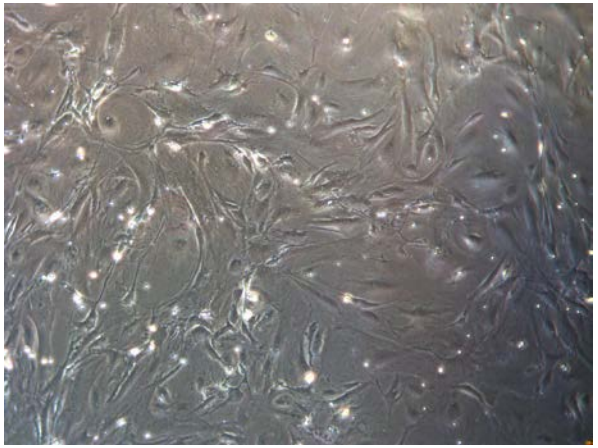
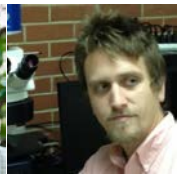
Open chromatin profiling of embyronic fibroblasts





- Embryonic fibroblasts (MEFs) from 8 inbred strains
- Two biological replicates per strain
- 124K-141K peaks/strain
- Average peak size is 169bp
- 249,534 DNaseI hypersensitive sites

Greg Crawford



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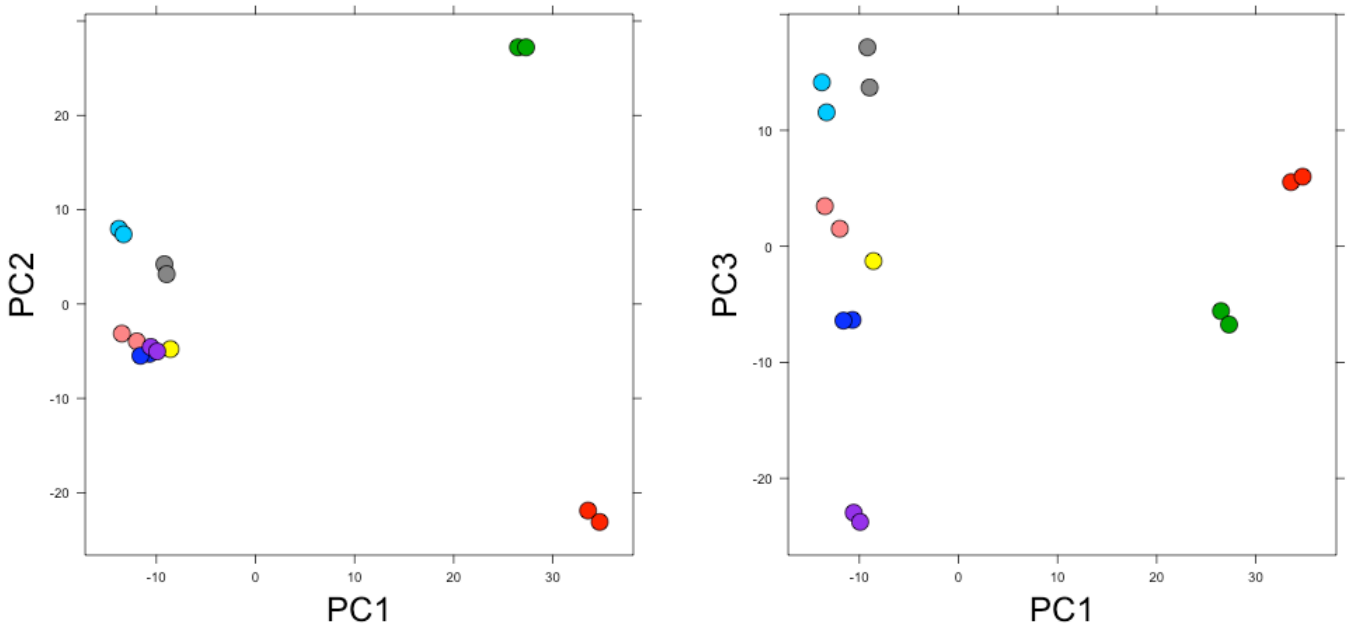
Open chromatin differs by strain



- 51% of DHS peaks vary between strains
- PCA shows genetic diversity is proportional to functional diversity

A/J
C57BL/6J
129S1/SvImJ
NOD/LtJ
NZO/HlLtJ
CAST/EiJ
PWK/PhJ
WSB/EiJ

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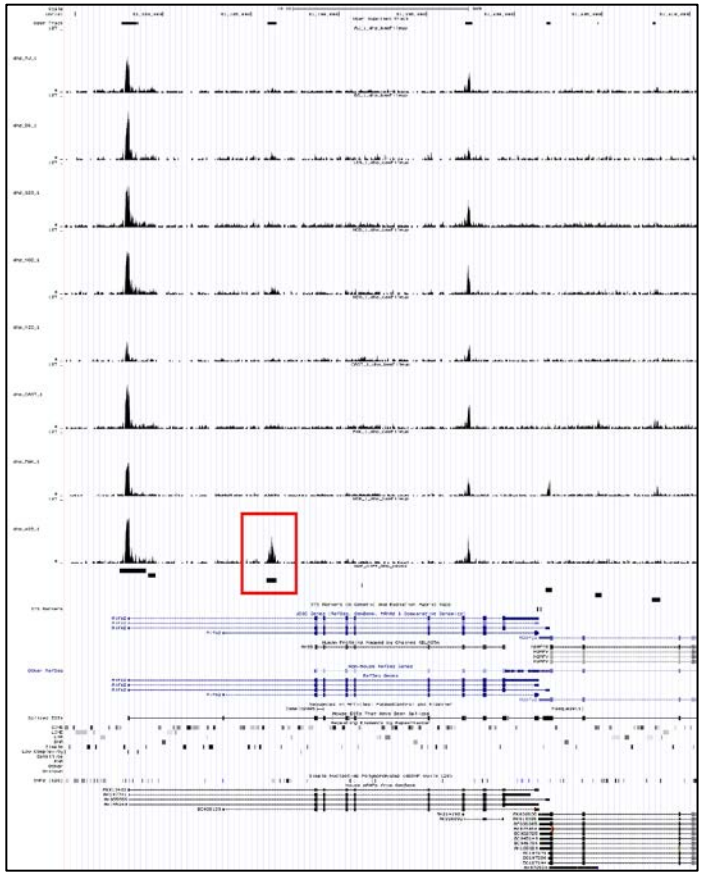
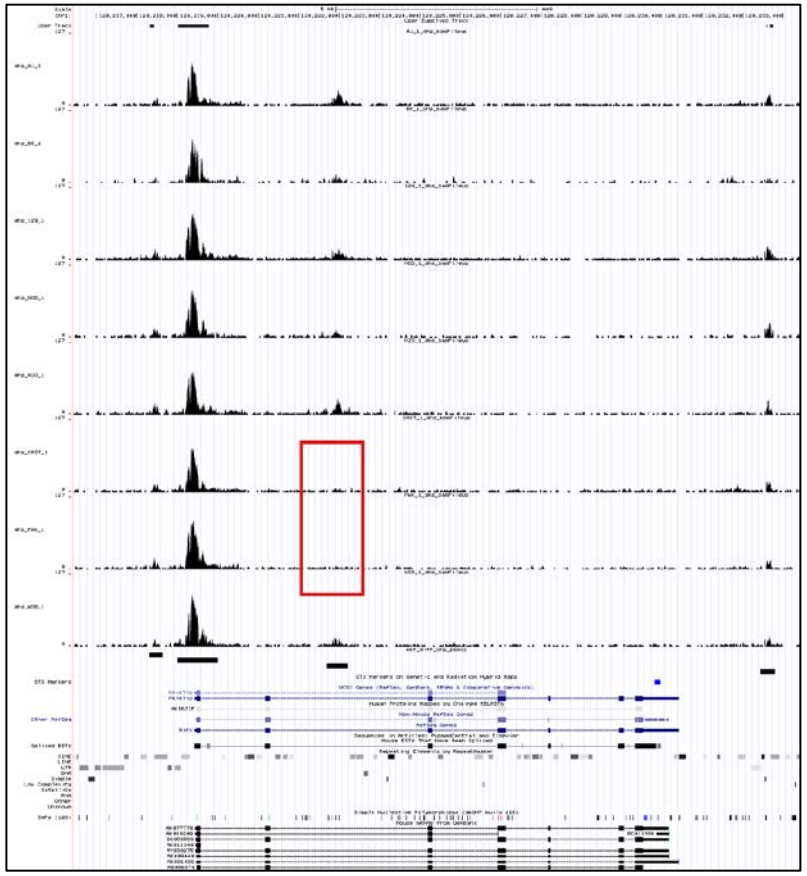
Genetic control of gene regulation



Aifm2



Mki67ip

A/J
C57BL/6J
129S1/SvImJ
NOD/LtJ
NZO/HlLtJ
CAST/EiJ
PWK/PhJ
WSB/EiJ

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The vast GxE landscape

Genetic Variants	4×10^7
Genes	2.2×10^5
Cell types	2×10^2
Tox21 compounds	1×10^5
	1.6×10^{20}

Multiple approaches

- Experimental and computational
- In vivo and ex vivo
- Single compound, many compounds, no compound

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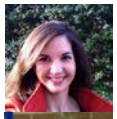
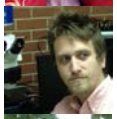
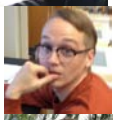
Thank you

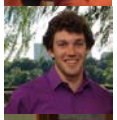
Aylor Lab


Nicole Carns


Thomas Konneker


Tiffany Garbutt


Kevin Gillespie


Sam Widmayer


Carlee Hemphill

Duke
Greg Crawford

UNC
Samir Kelada

NCSU
Dahlia Nielson

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