Non-Mendelian Genetics
Epidemiology 217: Lecture 8

Eric Jorgenson, PhD

February 26th, 2019

Epigenetics, Epistasis, and Linking GWAS Findings to Function

~~Non-Mendelian Genetics~~

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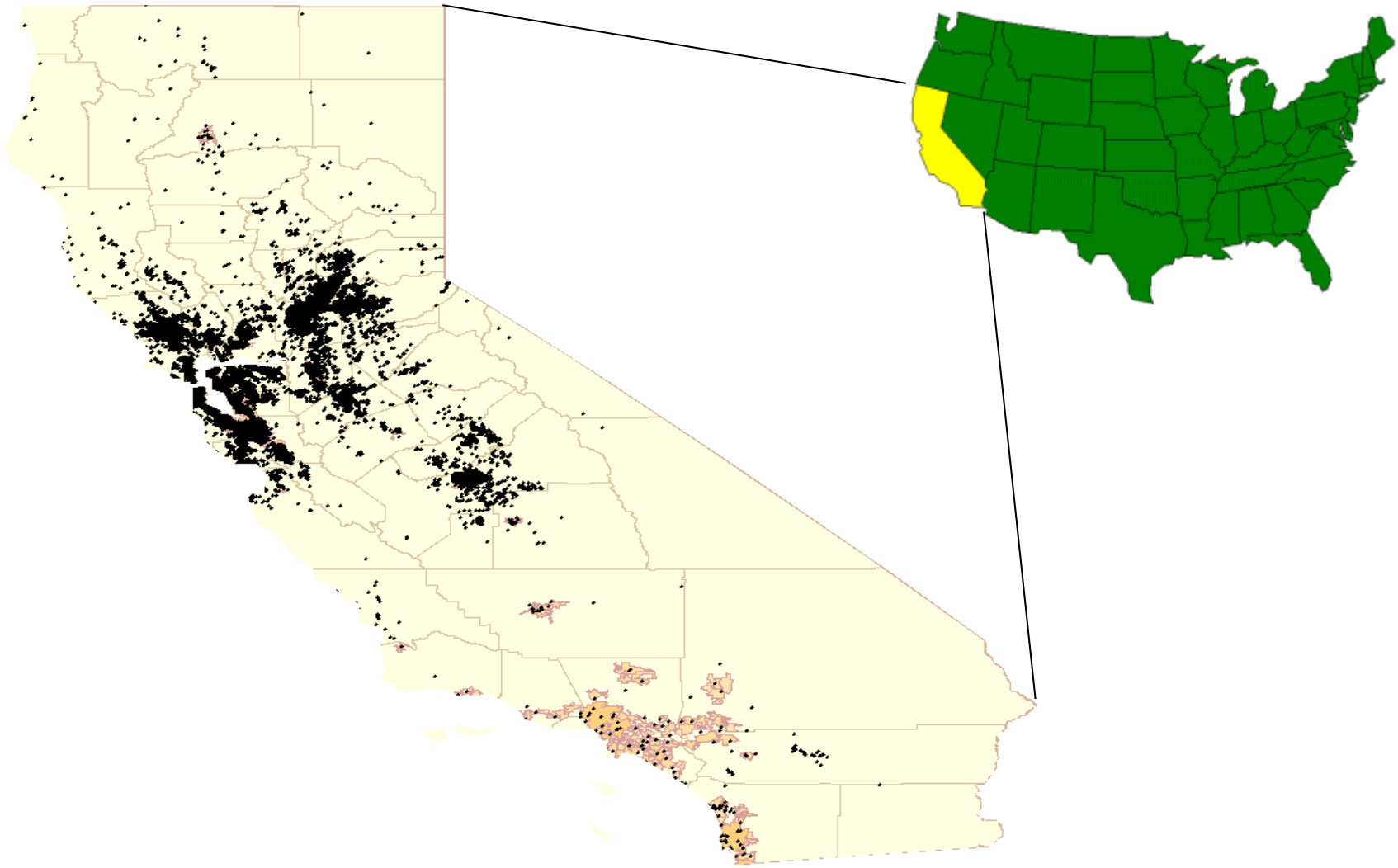
Outline

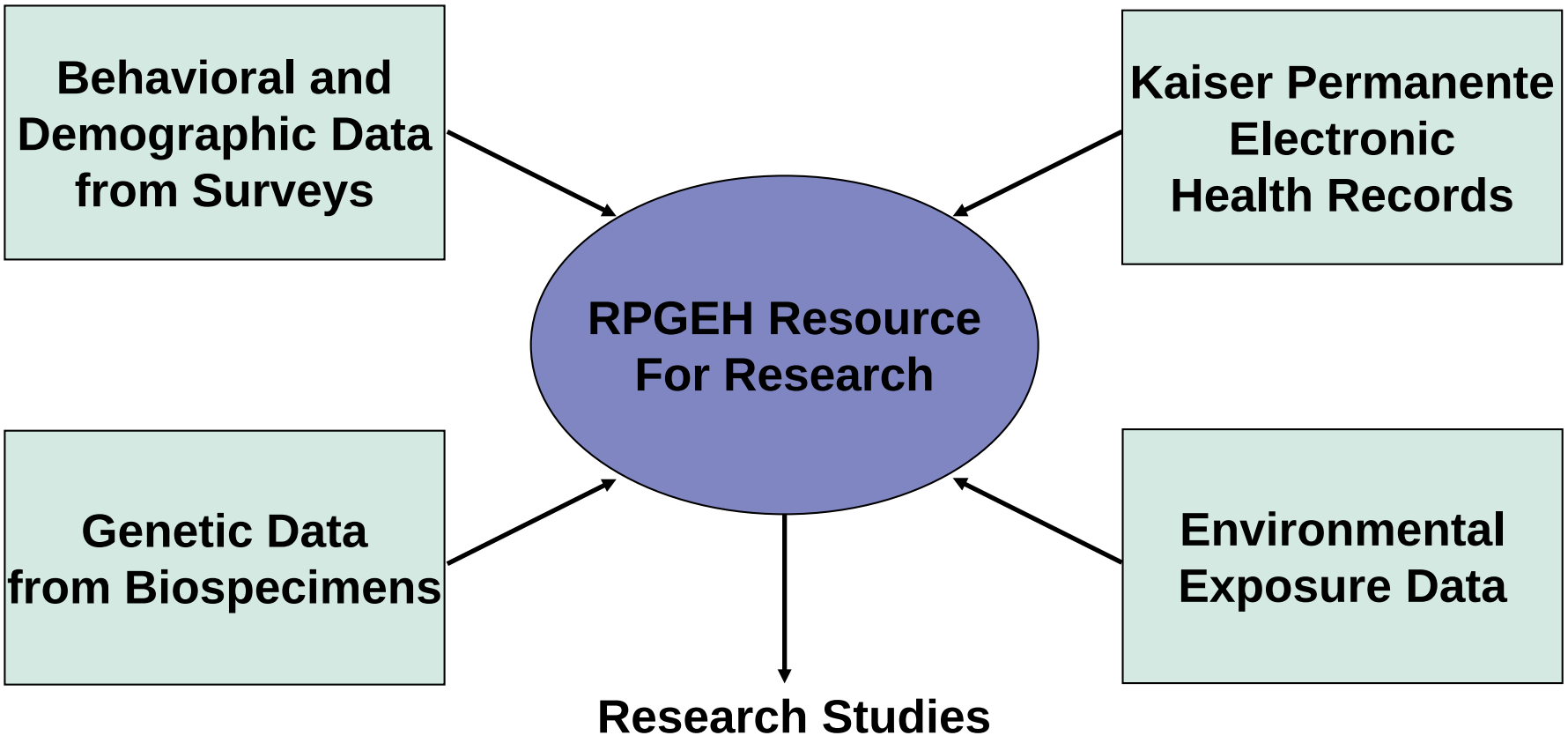
- A GWAS Example: Inguinal Hernia
- Epigenetics: DNA Methylation and Histone Modification
- Epistasis: Cutaneous Squamous Cell Carcinoma

Kaiser Permanente Northern California Members



Research Program on Genes, Environment, and Health

430,000 Adult Members of Kaiser Permanente Northern California Provided Informed Consent



Co-directors: Catherine Schaefer and Neil Risch

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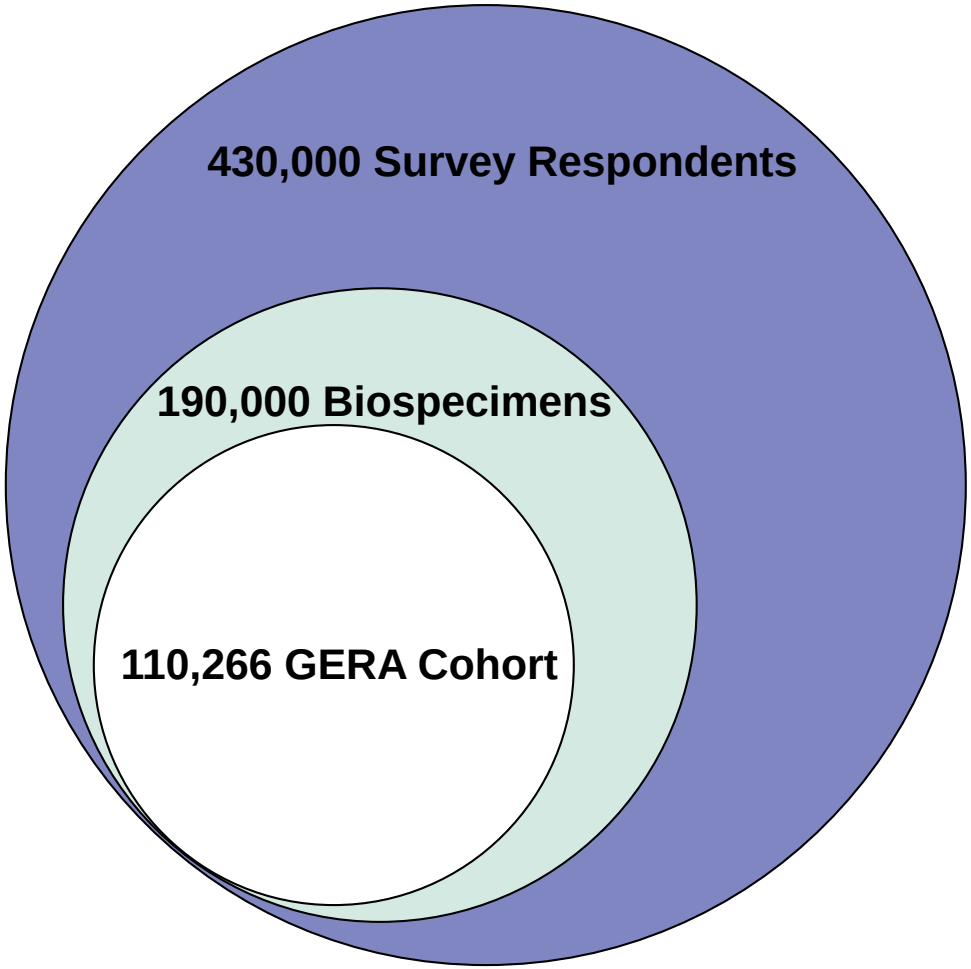
RPGEH Survey Respondents, Biospecimens, and GERA



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GERA Cohort Characteristics

	Number of Subjects	%
Age at Specimen Donation		
18-39	7,025	6.3
40-59	33,344	30.2
60-79	57,856	52.4
80+	12,041	10.9
Sex		
Women	63,883	57.9
Men	46,383	42.1
Race/Ethnicity		
African American	3,765	3.4
Asian	8,512	7.7
Hispanic	7,917	7.2
Non-Hispanic White	89,259	81.0
Total	110,266	100.0

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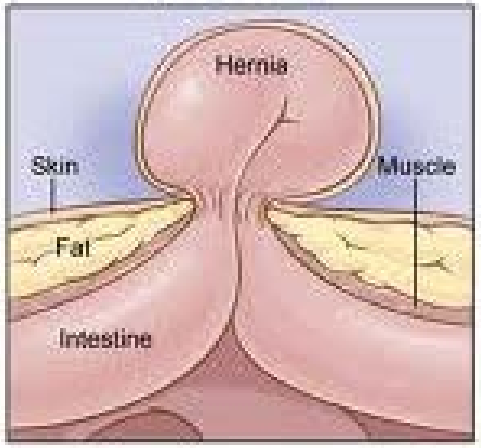
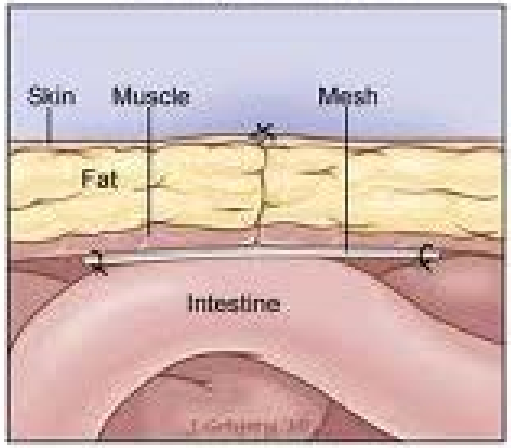
Hernia: A Common Condition

Hernia



Repair Surgery



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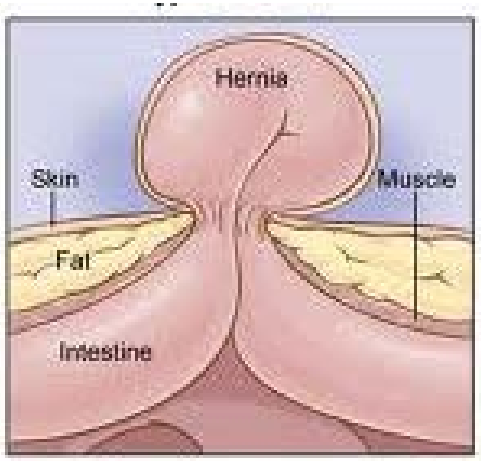
Hernias Are Classified by Anatomical Site

Hernia



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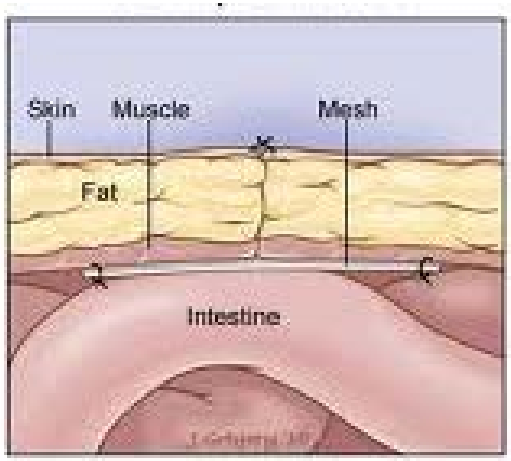

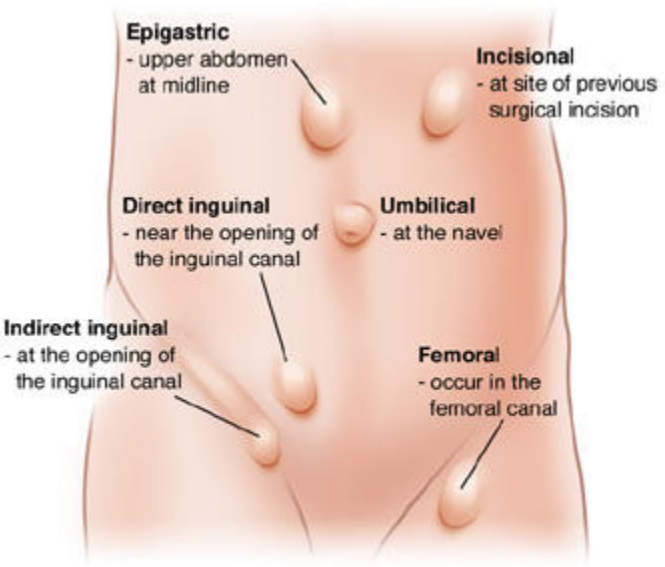

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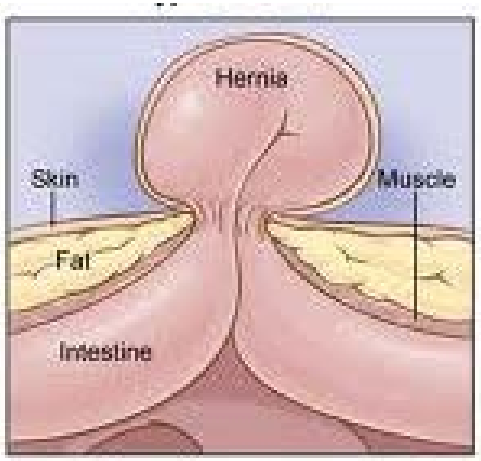
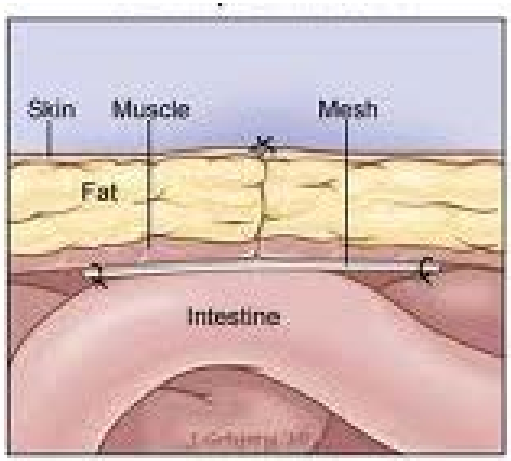
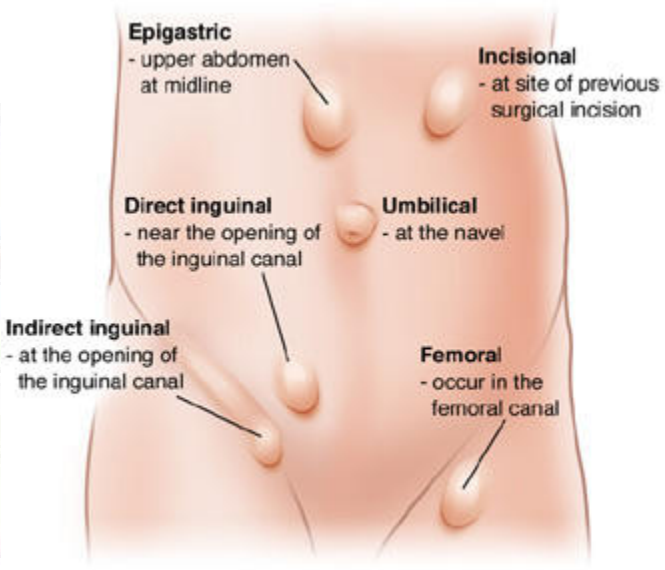
Inguinal Hernias Are Common in GERA

Hernia



Repair Surgery





GERA Cohort	Inguinal Hernia Cases						Controls	
Non-Hispanic whites	All (n=5,295)		Direct (n=2,335)		Indirect (n=2,647)		(n=67,510)	
Men	4,746	89.6%	2,193	93.9%	2,408	91.0%	25,011	37.1%
Women	549	10.4%	142	6.1%	239	9.0%	42,499	63.0%

Jorgenson *et al*. Nature Communications 2015

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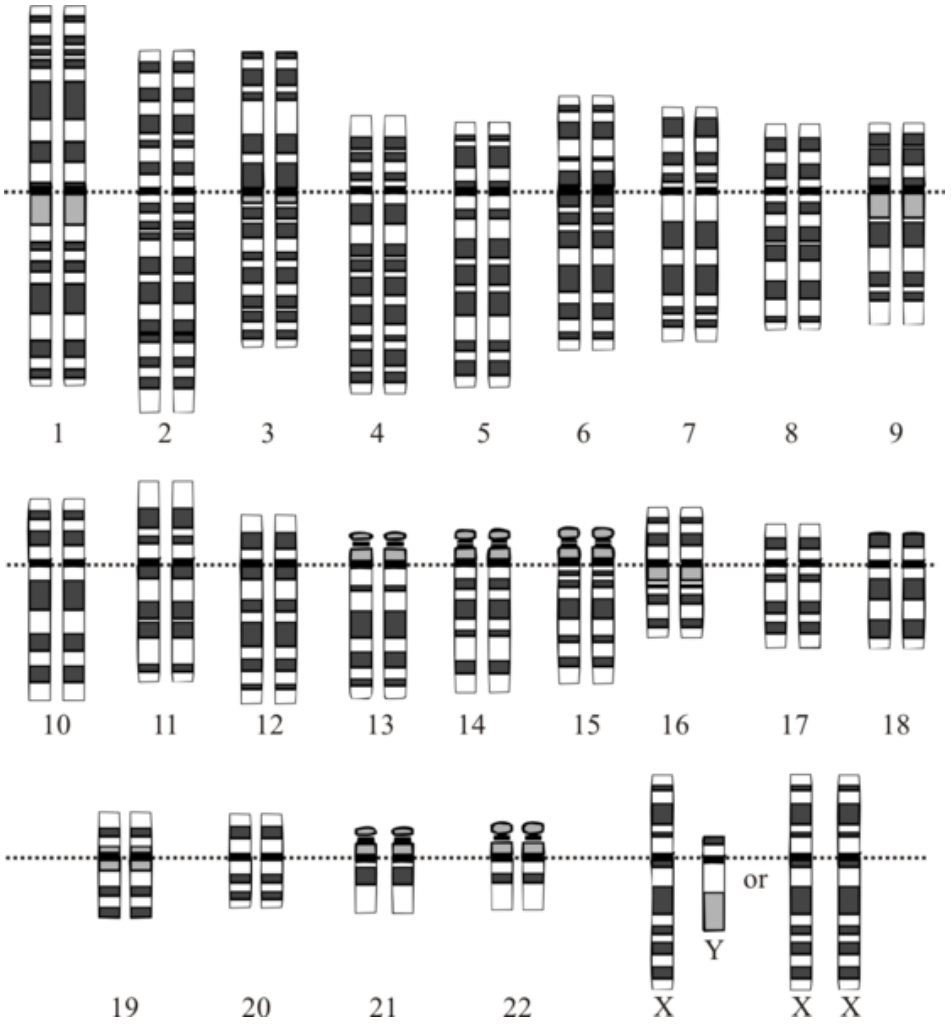
The Human Genome Is 3.2 Billion Basepairs Long



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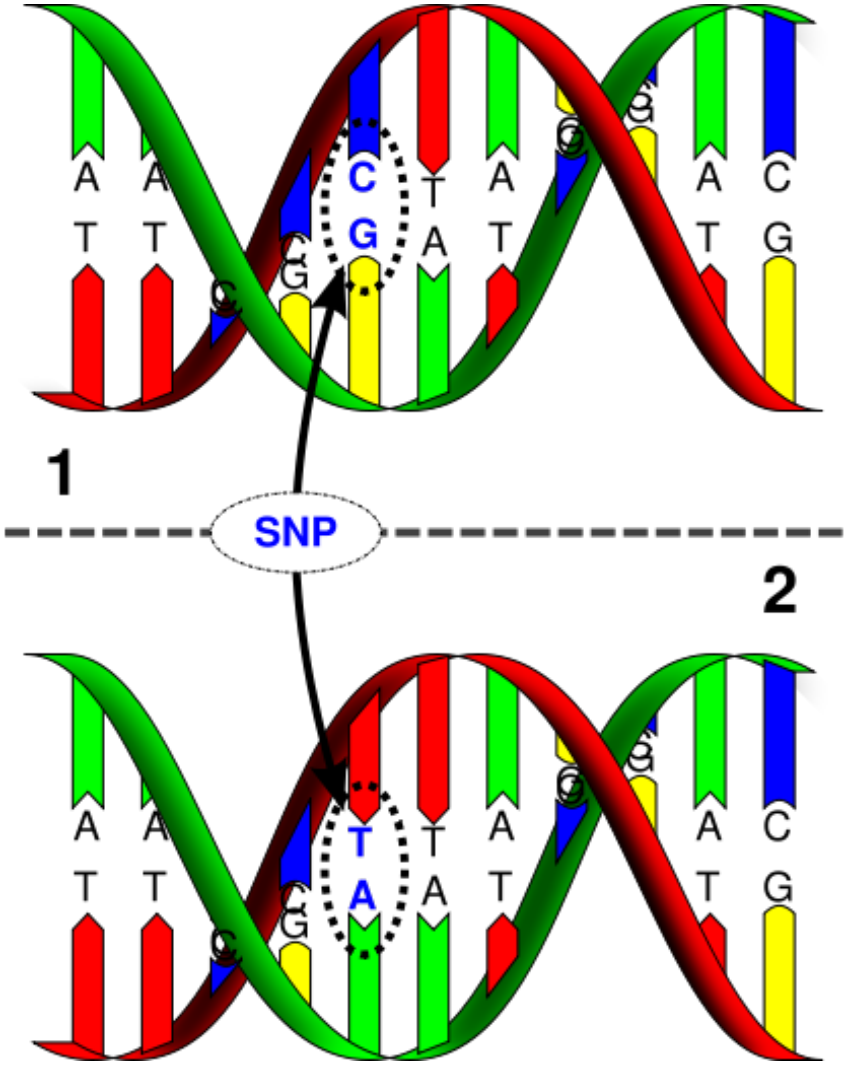
Single Nucleotide Polymorphism (SNP)



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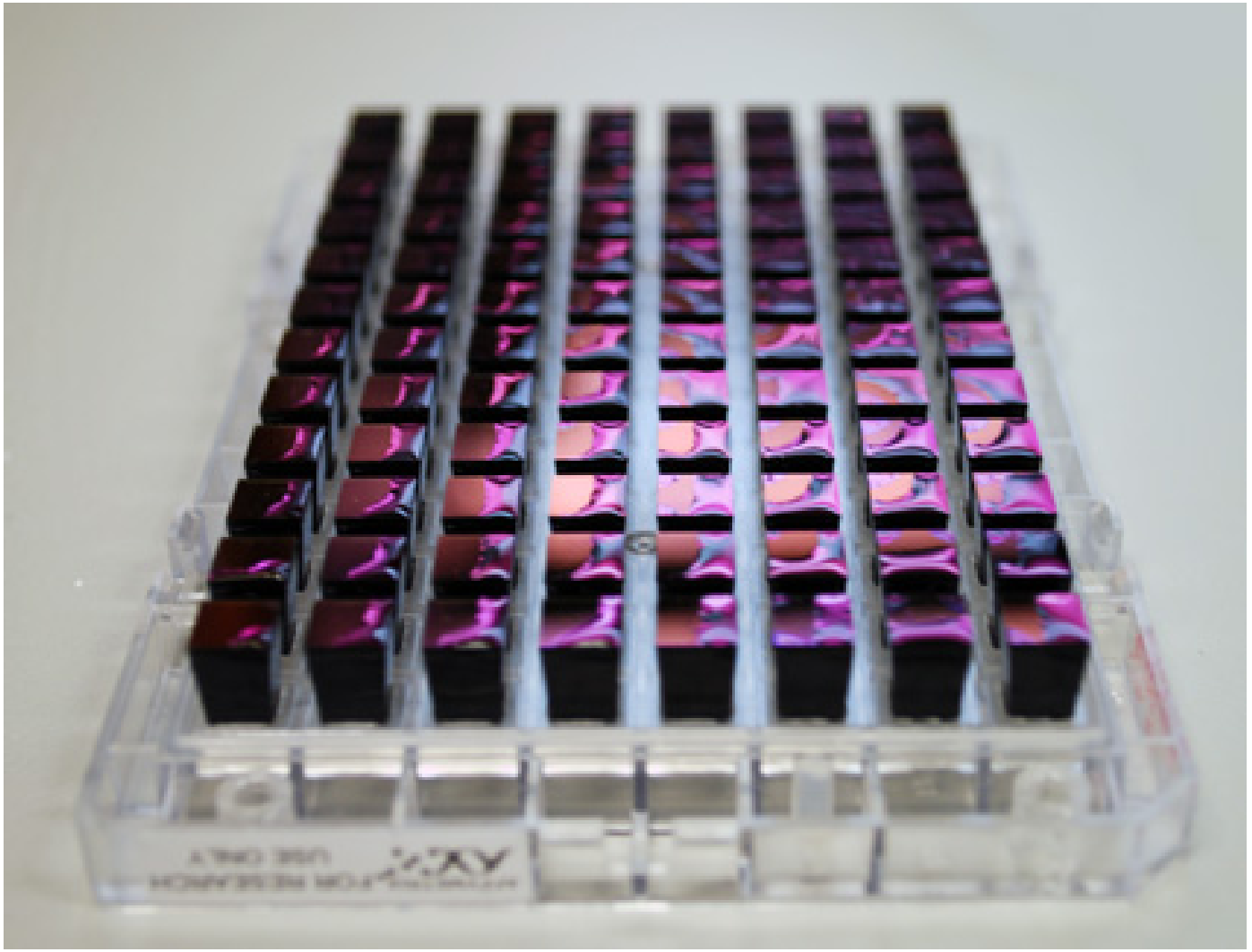
Genotyping Arrays Measure Millions of SNPs in Parallel



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Sample QC Workflow

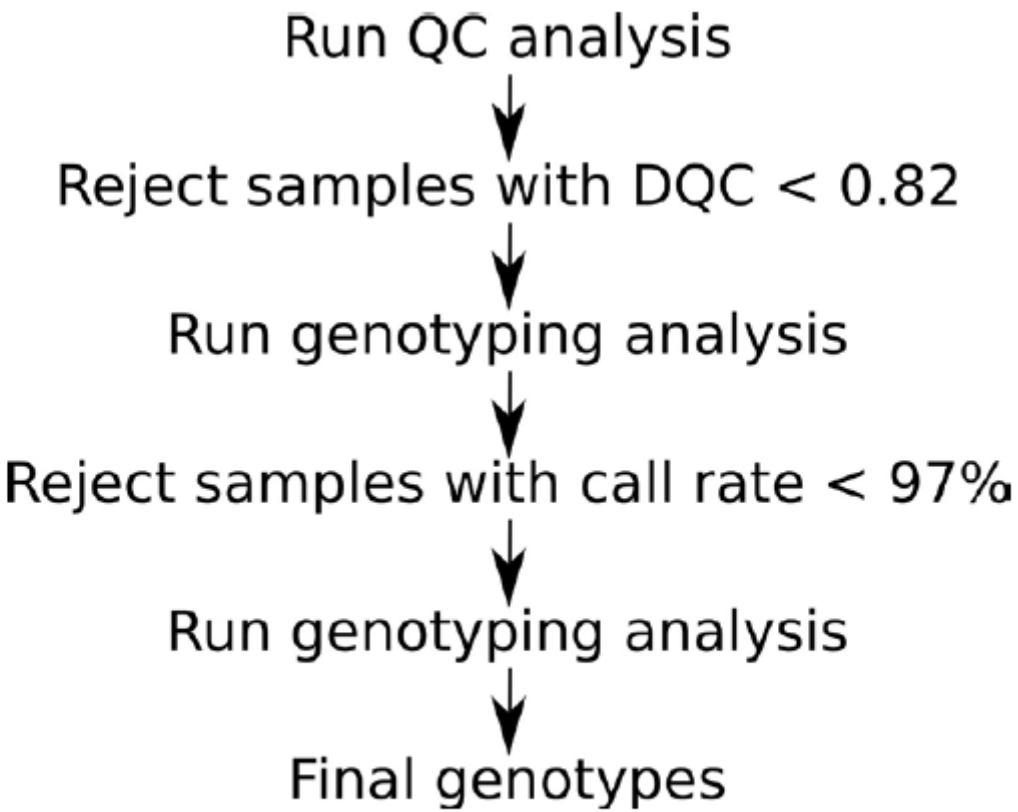

Figure 1 Standard Affymetrix Axiom Genotyping Analysis Workflow.

Kvale *et al*. Genetics 2015

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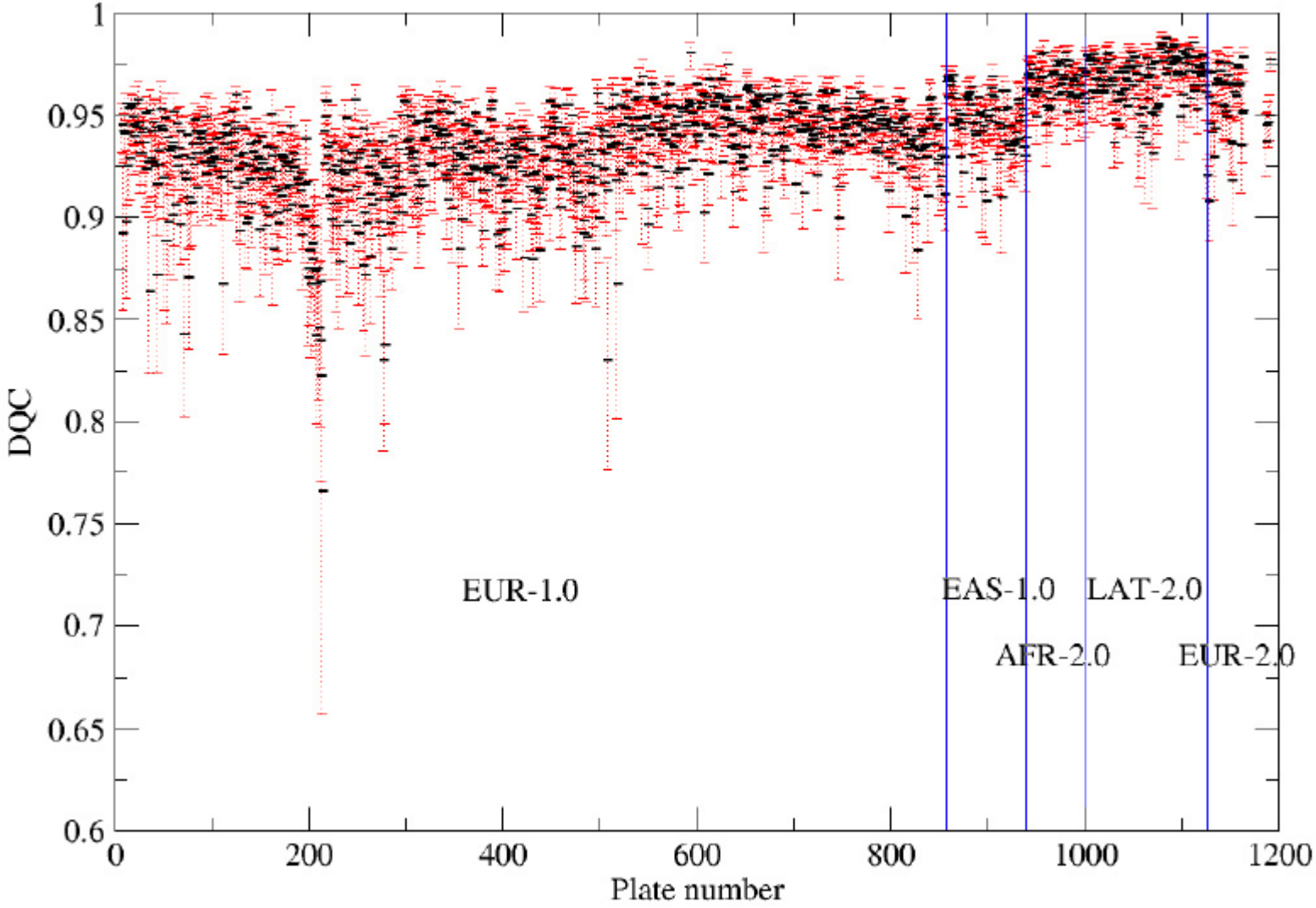
Genotyping Performance Was Tracked in Real Time



Kvale *et al*. Genetics 2015

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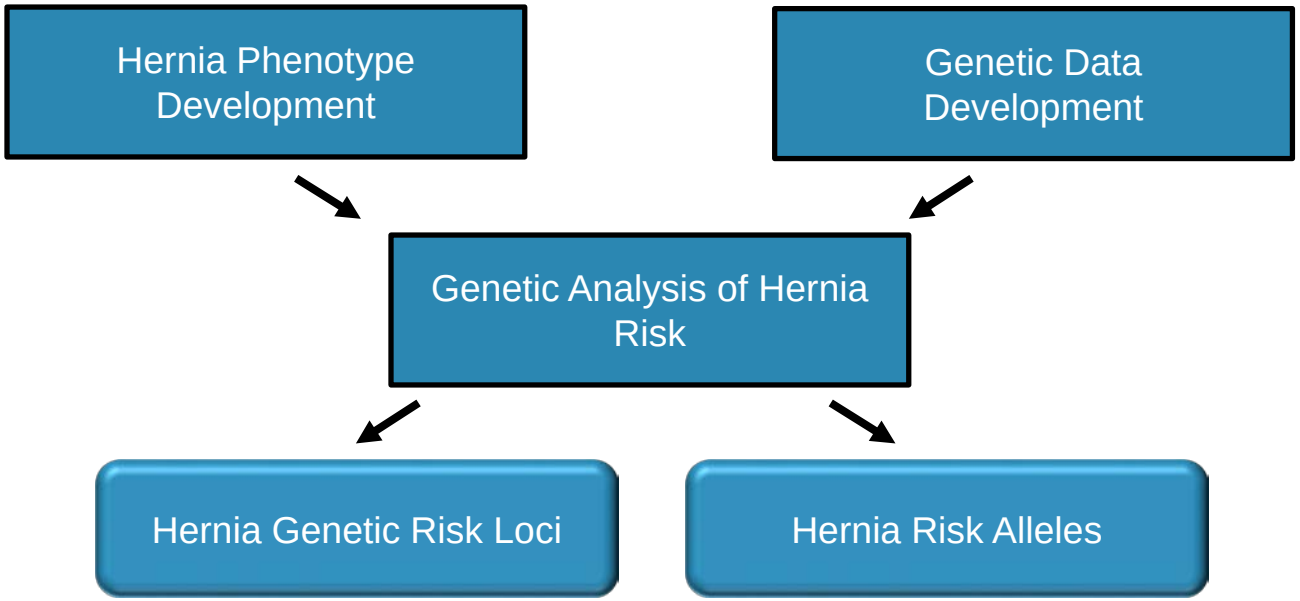
SNP Quality Control

Array	SNPs Assayed	Autosomal	X-linked	Y-Chrom	mtDNA	Passing QC	% of SNPs Passing
EUR	674,518	660,990	13,123	289	116	670,572	99.42%
EAS	712,950	699,324	13,385	158	83	708,373	99.36%
AFR	893,631	867,035	26,264	234	98	878,176	98.27%
LAT	817,810	792,056	25,397	234	123	802,186	98.09%

Kvale *et al*. Genetics 2015

GWAS Can Identify Loci, Not Genes



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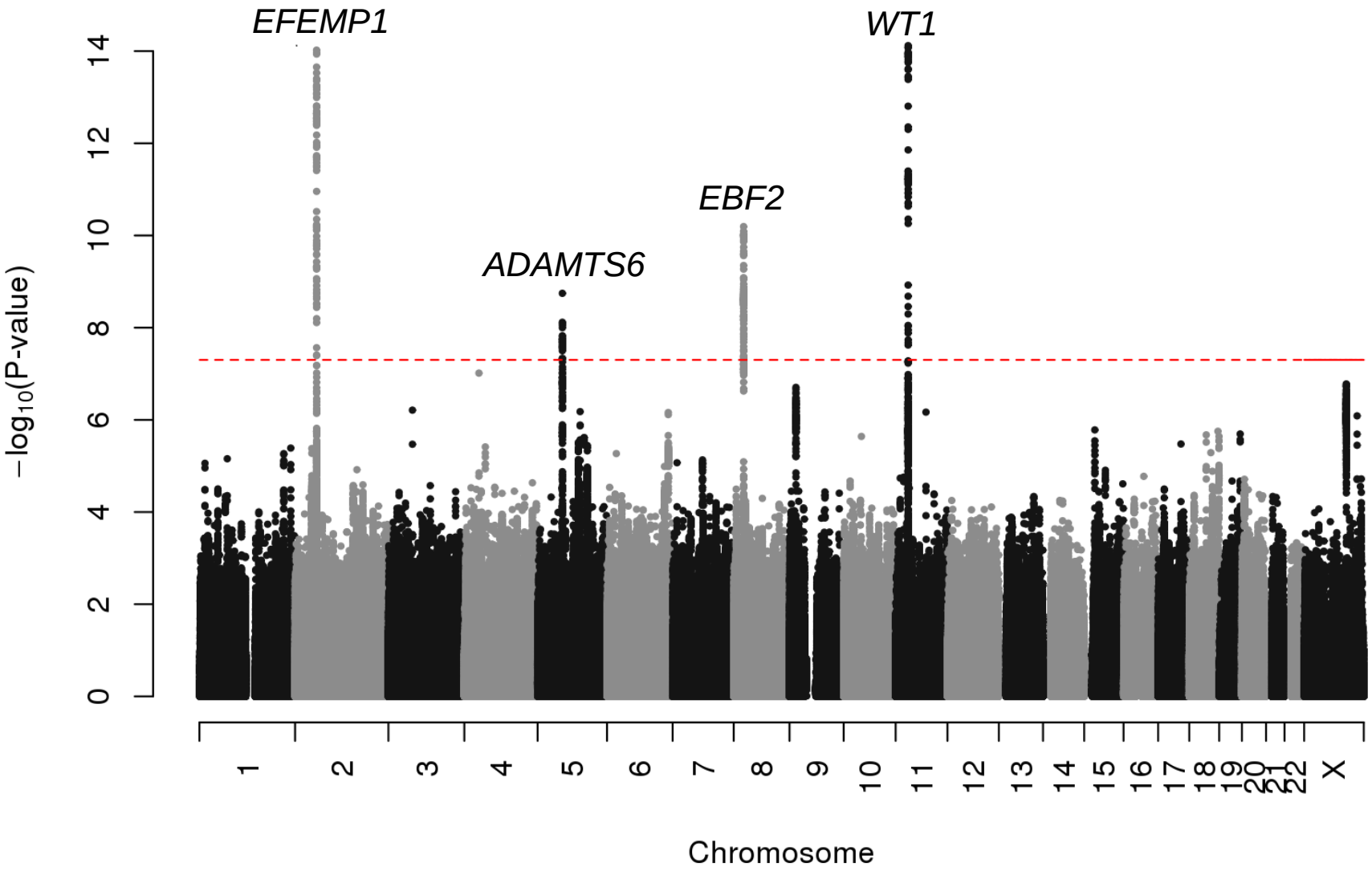
Manhattan Plot of Inguinal Hernia GWAS Results



Jorgenson *et al*. Nature Communications 2015

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Hernia Repair Replication Sample: 23&Me Subjects

“Have you ever had any of the following gastrointestinal surgeries (Hernia repair)?”

23&Me Subjects	Hernia Repair Cases (n=9,701)		Controls (n=82,743)	
Men	6,812	70.2%	41,611	50.2%
<30	246	3.6%	5,147	12.4%
31-45	1,087	16.0%	14,109	33.9%
46-60	1,605	23.6%	10,259	24.7%
>60	3,874	56.9%	12.096	29.1%
Women	1,889	29.8%	41,132	49.8%
<30	63	3.3%	4,252	10.3%
31-45	317	16.8%	11,099	27.0%
46-60	603	31.9%	11,431	27.8%
>60	906	48.0%	14,350	34.9%

Jorgenson *et al*. Nature Communications 2015

Replication of Inguinal Hernia Significant Associations

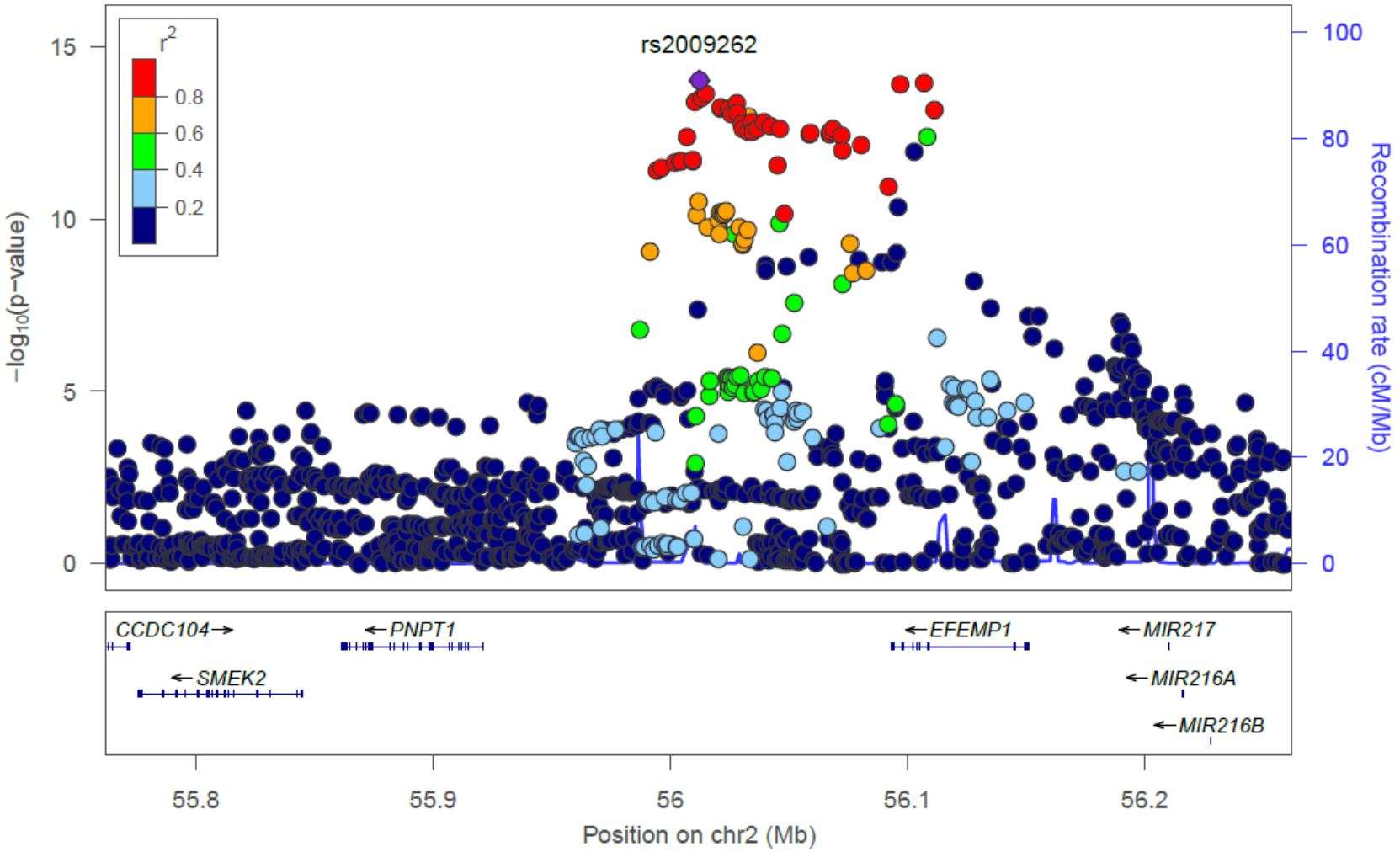
Table 1 | SNP associations reaching genome-wide significance in the combined analysis of discovery and replication cohorts.

SNP	Chr.	Position	Gene	Risk allele	Discovery (5,295 cases, 67,510 controls)			Replication (9,701 cases, 82,743 controls)			Combined	
					RAF	OR (95% CI)	*P*-value	RAF	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
rs2009262	2	56,012,214	*EFEMP1*	T	0.78	1.23 (1.17–1.30)	3.66×10^{-15}	0.78	1.10 (1.06–1.15)	3.65×10^{-06}	1.15 (1.11–1.19)	1.45×10^{-17}
rs370763	5	64,355,060	*ADAMTS6*	A	0.65	1.14 (1.09–1.19)	9.70×10^{-09}	0.67	1.06 (1.02–1.09)	3.02×10^{-03}	1.09 (1.06–1.12)	3.73×10^{-9}
rs6991952	8	25,707,412	*EBF2*	G	0.43	1.14 (1.10–1.19)	1.17×10^{-10}	0.43	1.08 (1.05–1.12)	2.04×10^{-06}	1.11 (1.08–1.14)	6.68×10^{-15}
rs3809060	11	32,458,807	*WT1*	G	0.62	1.18 (1.13–1.23)	4.69×10^{-14}	0.63	1.07 (1.03–1.10)	1.69×10^{-04}	1.11 (1.08–1.14)	3.69×10^{-14}

Chr., chromosome; CI, confidence interval; RAF, risk allele frequency; SNP, single-nucleotide polymorphism.

Jorgenson *et al*. Nature Communications 2015

Each Inguinal Hernia Risk Locus Includes Many SNPs



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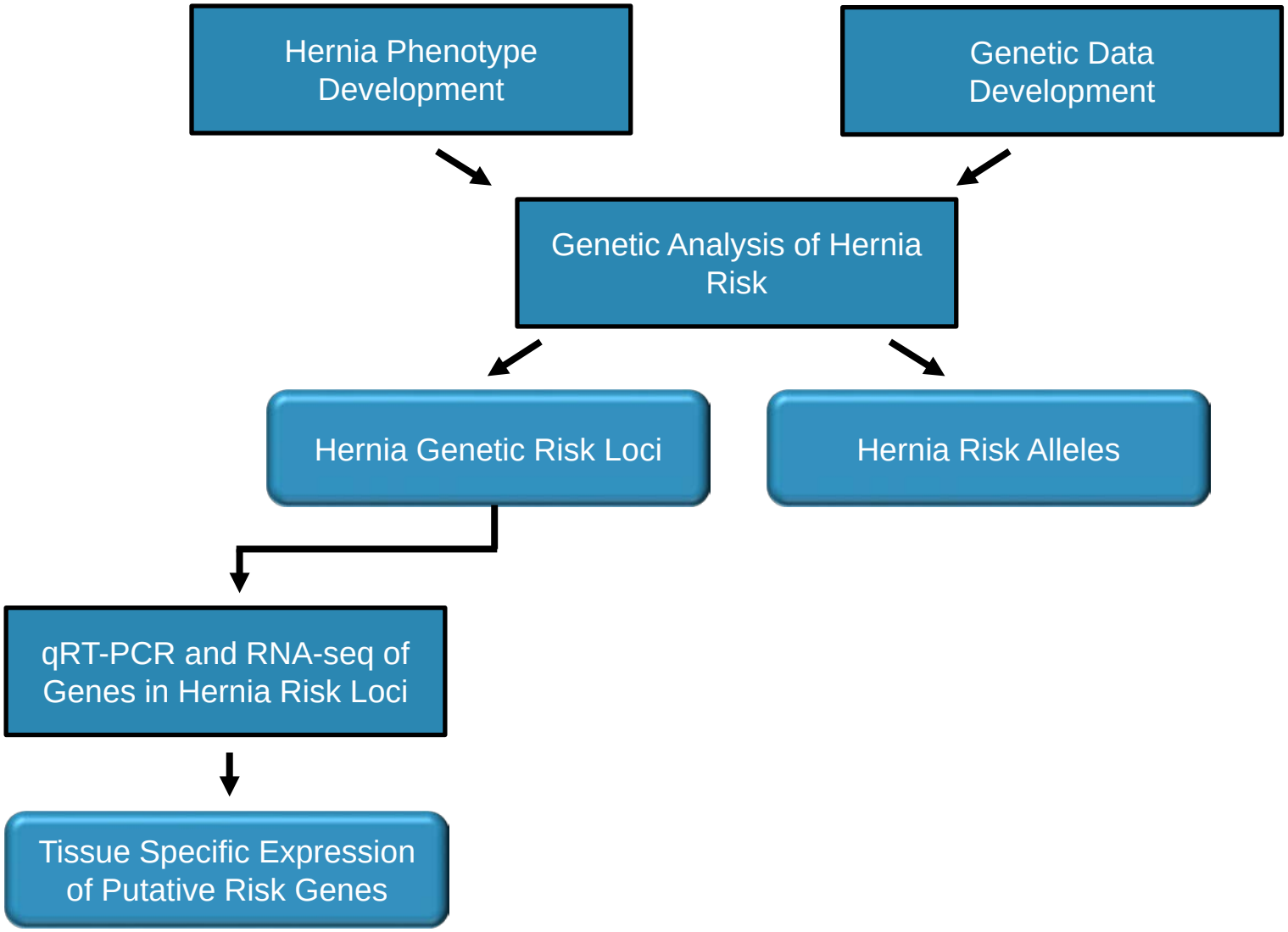
Can We Link Hernia Risk Loci to Specific Genes?



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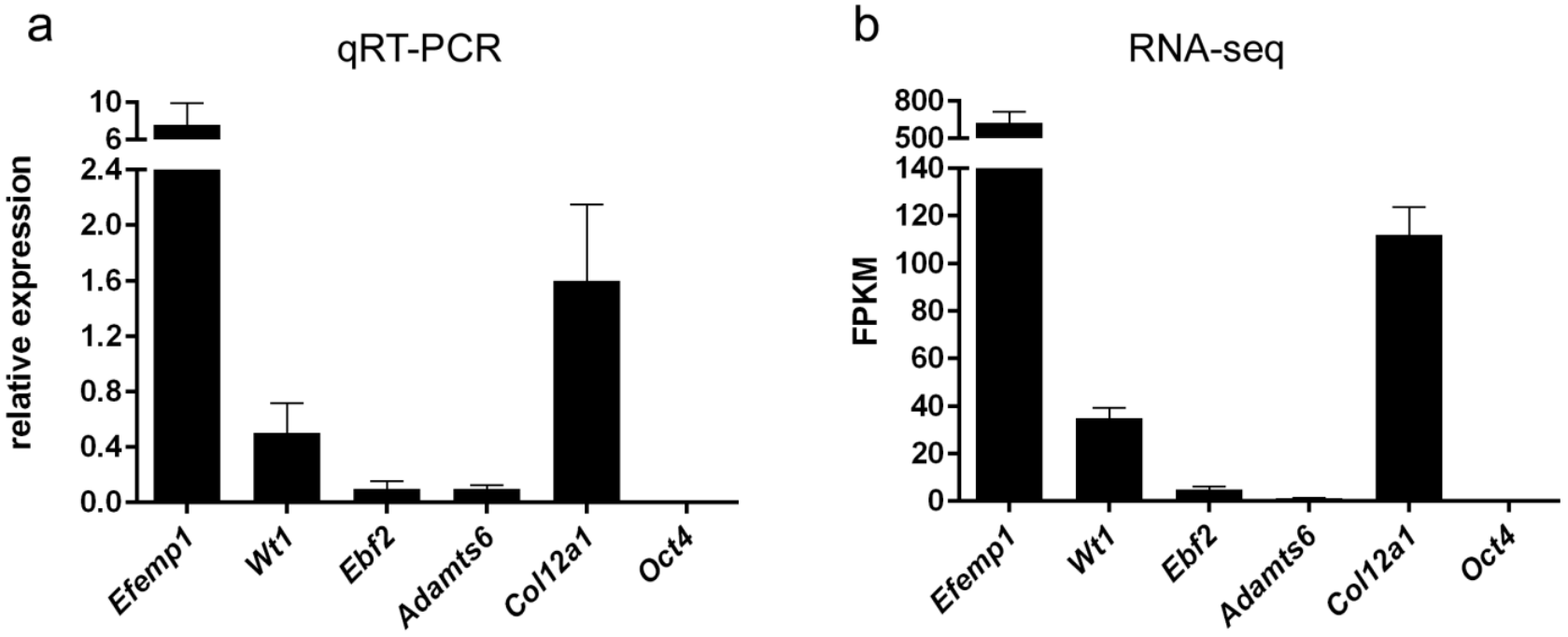
Genes at Inguinal Hernia Risk Loci are Expressed in Mouse Connective Tissue



Jorgenson *et al*. Nature Communications 2015

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What Have We Learned about Inguinal Hernia Genetics?

- 4 Inguinal Hernia Risk Loci
  - Confirmed through replication in an independent cohort
  - Genes in the loci are expressed in equivalent mouse tissue
- Hernia Loci Show Evidence of Pleiotropy
  - *EFEMP1*: Height, Forced Vital Capacity, Multiple Cancers
  - *WT1*: Wilms' Tumor, Tuberculosis
  - *EBF2*: Prostate Cancer (different area in the gene)
  - *ADAMTS6*: Central Corneal Thickness, Osteosarcoma
- Moving Toward Mechanism
  - Collagen homeostasis
  - Matrix metalloproteinases and their inhibitors (*WT1* and *ADAMTS6*)
  - Elastin and chronic obstructive pulmonary disease (*EFEMP1*)

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What Do We Still Need to Learn about Hernia Genetics?

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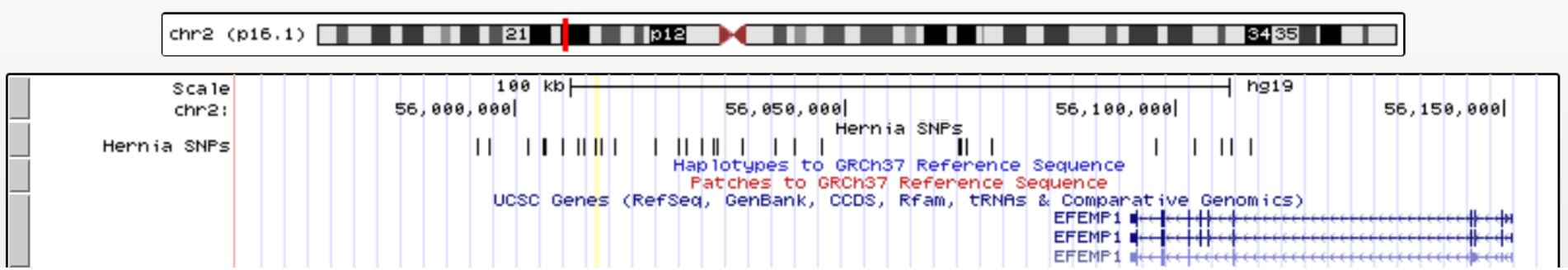
What Do We Still Need to Learn about Hernia Genetics?

- Where are the Regulatory Elements in Hernia Risk Loci?

Genomic Context of Hernia SNPs in the *EFEMP1* Region



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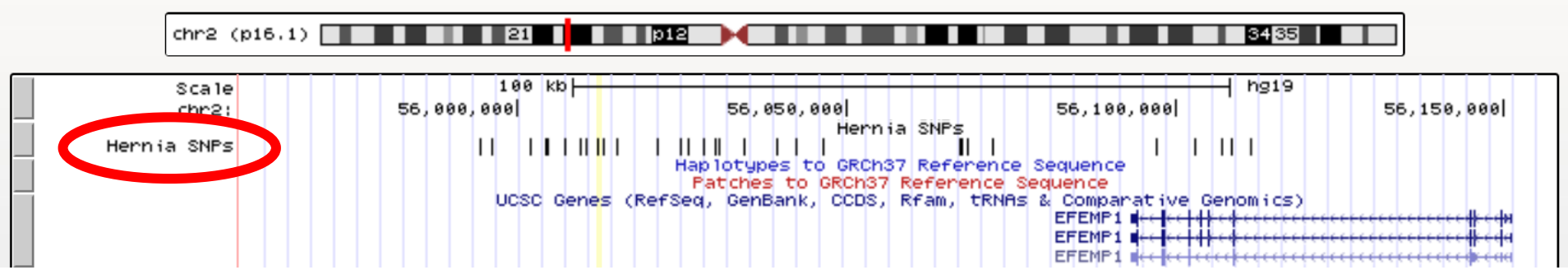

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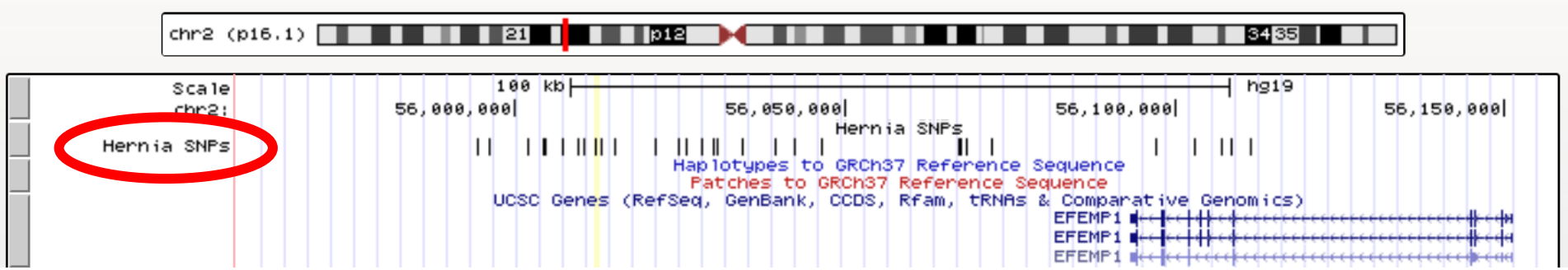

rs2009262

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Genes Are Like Lightbulbs

Genes

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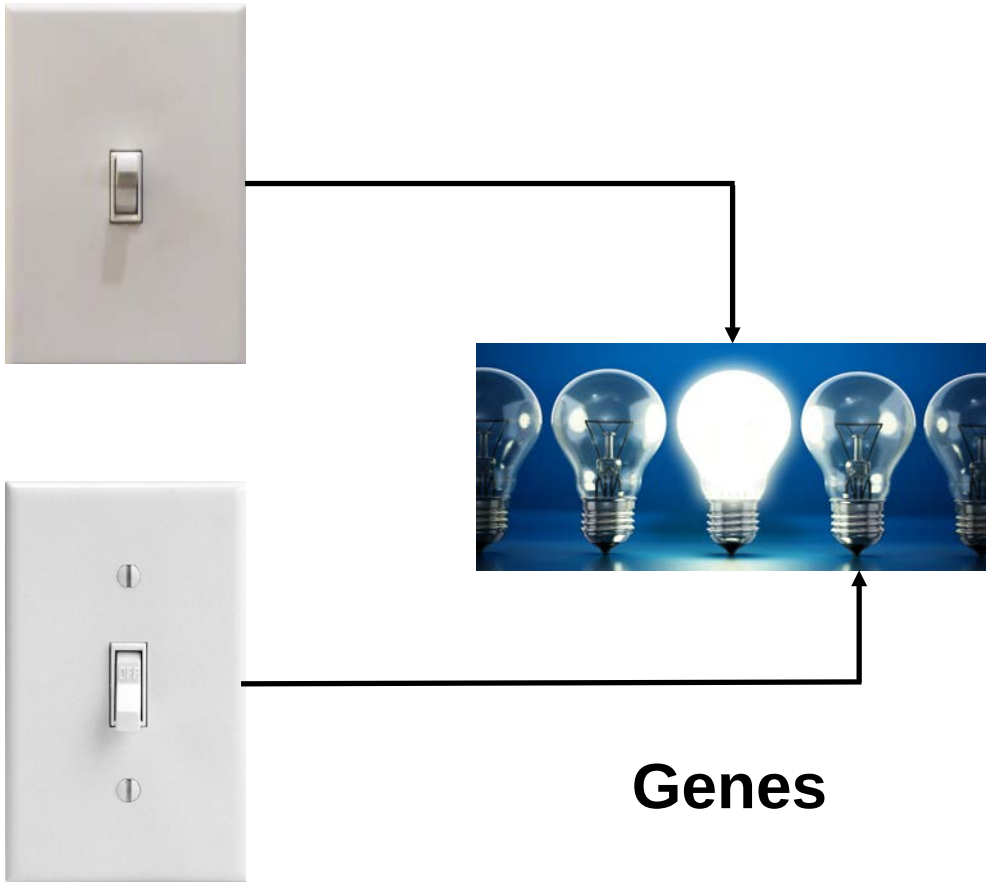

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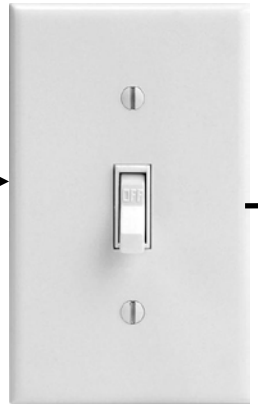

Enhancer

Promoters

Genes

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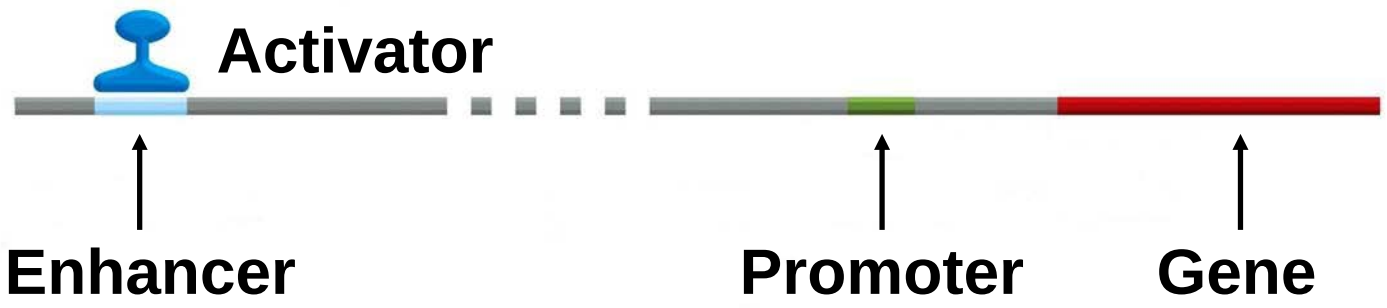
Proteins Bind Regulatory Elements

Proteins Bind Regulatory Elements



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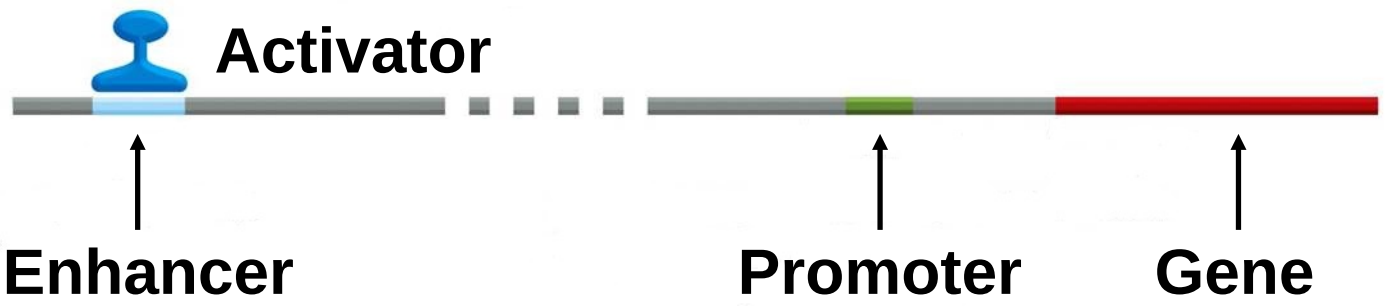
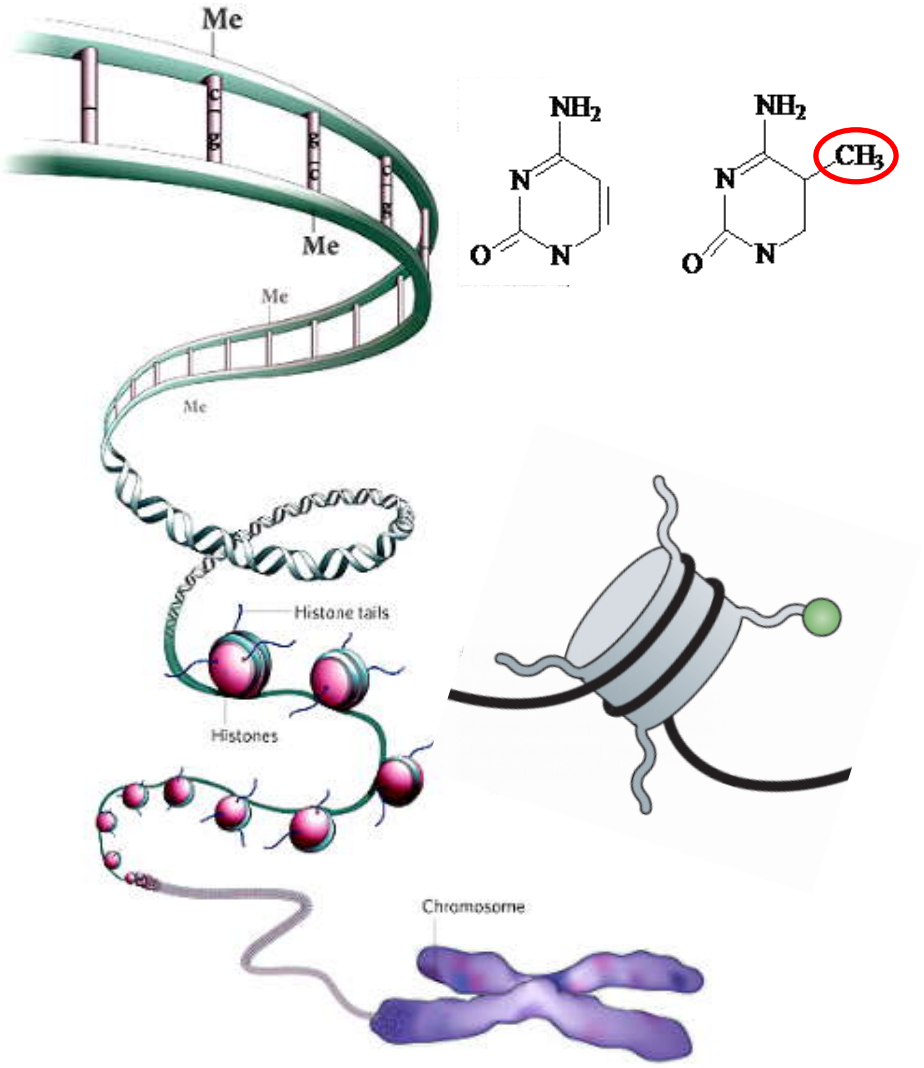


Outline

- A GWAS Example: Inguinal Hernia
- Epigenetics: DNA Methylation and Histone Modification
- Epistasis: Cutaneous Squamous Cell Carcinoma

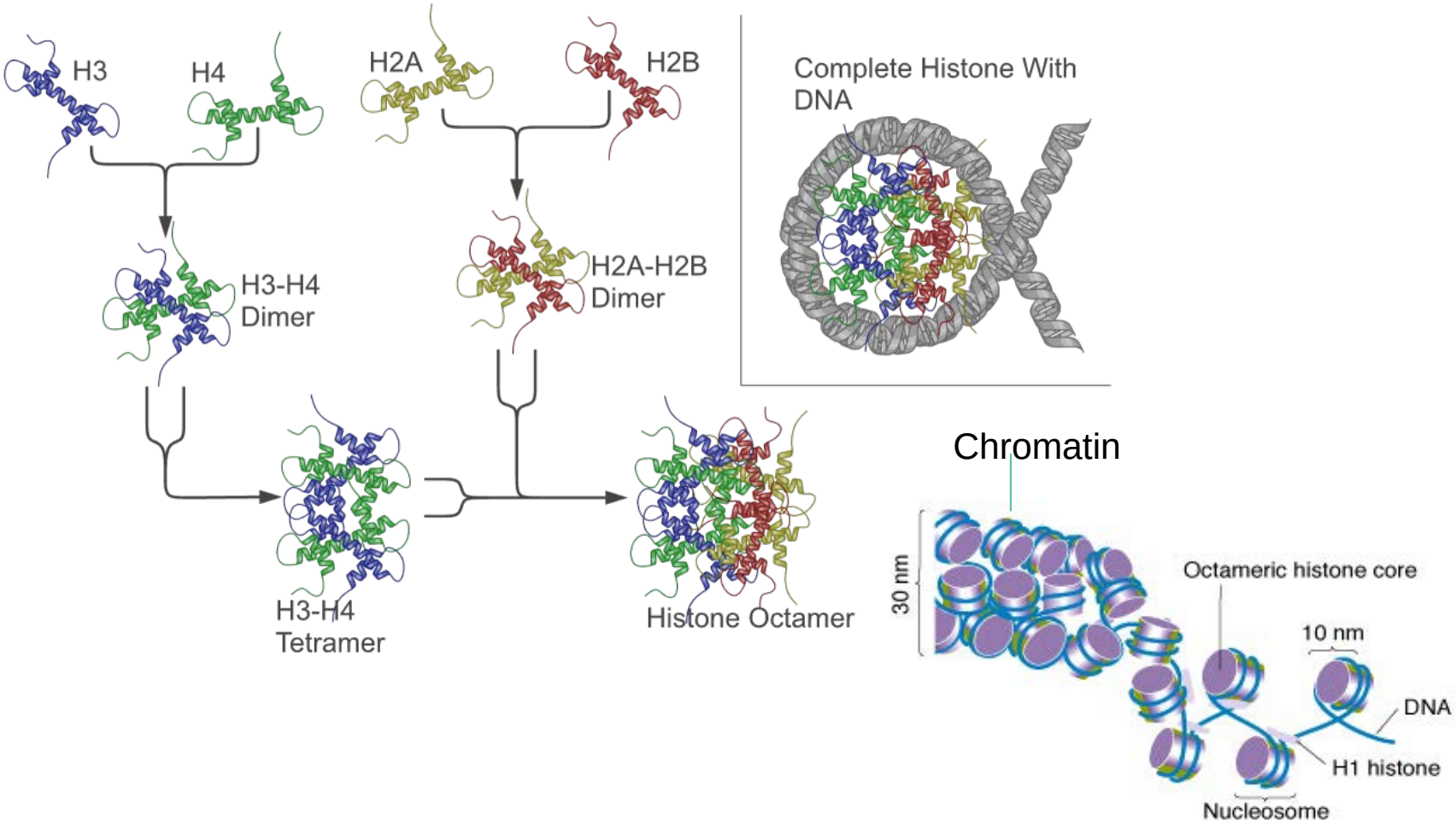
The Two Main Components of the Epigenetic Code



DNA methylation

Histone modification

Chromatin: Histones Provide Structure to DNA



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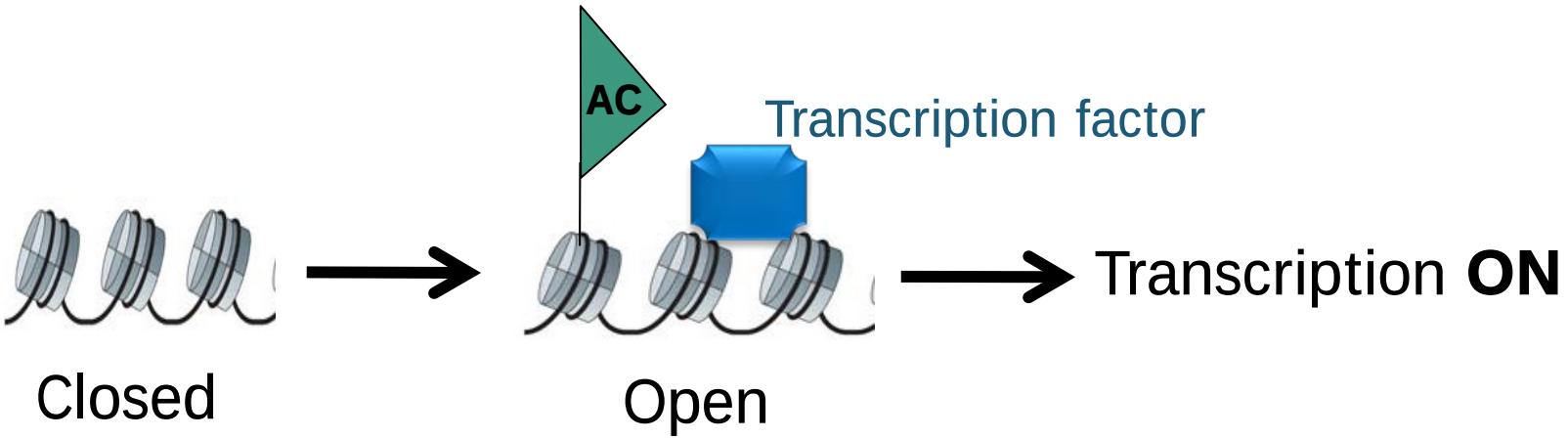
Acetylation Reduces Histone Binding to DNA



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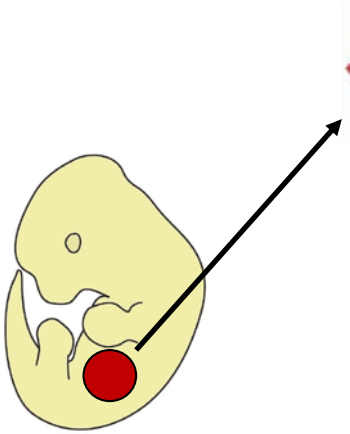
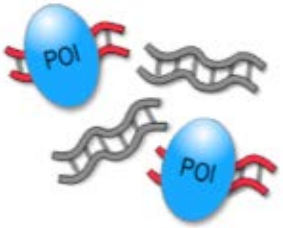
ChIP-seq Can Identify Regulatory Elements

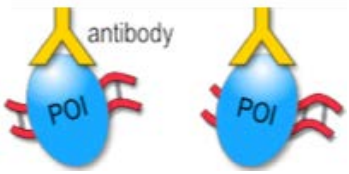


Isolate Tissue

Cross-link DNA and Proteins

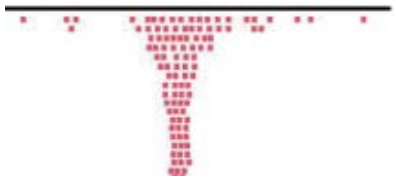


Shear DNA



Immunoprecipitate

Reverse Cross-link



Sequence and Map Reads

Determine Location

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ENCODE has Cataloged Different Types of Marks

https://www.encodeproject.org/data/annotations

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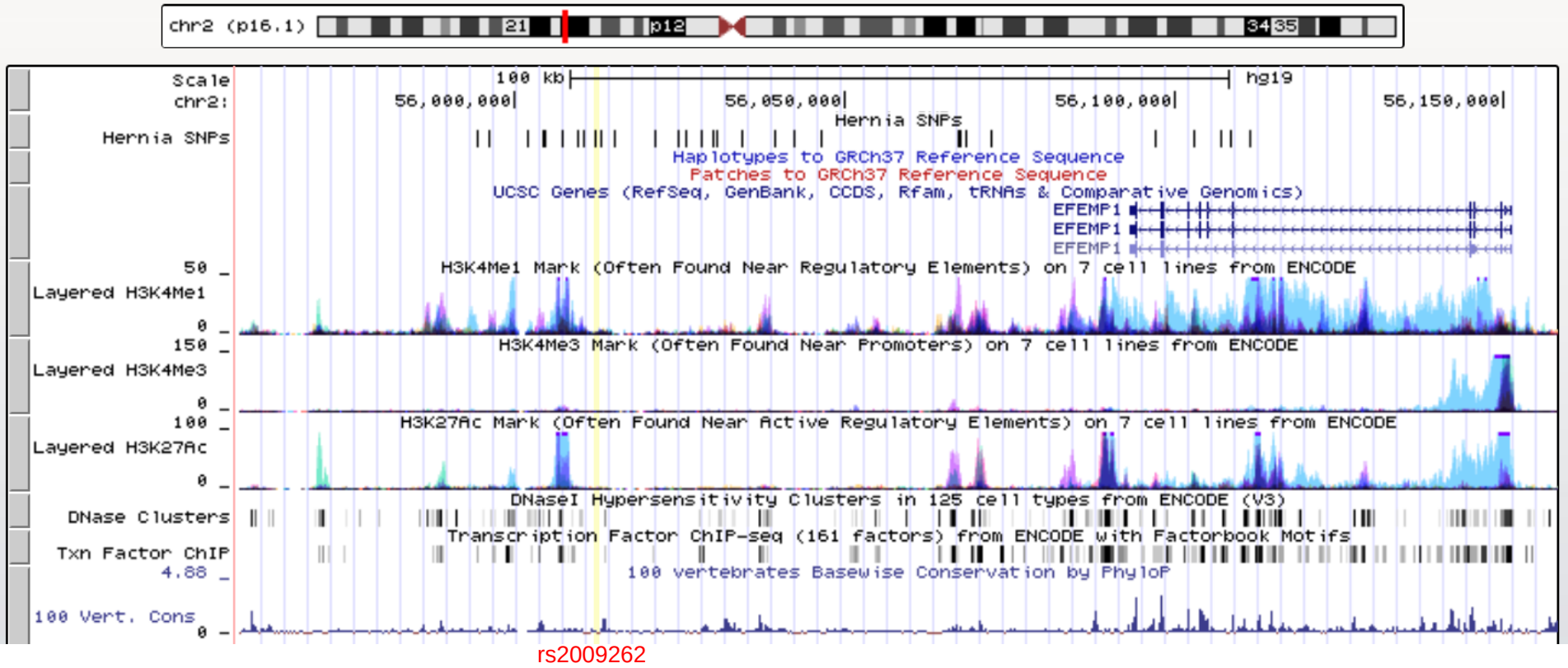
EFEMP1: rs2009262 is not in a Regulatory Element Mark



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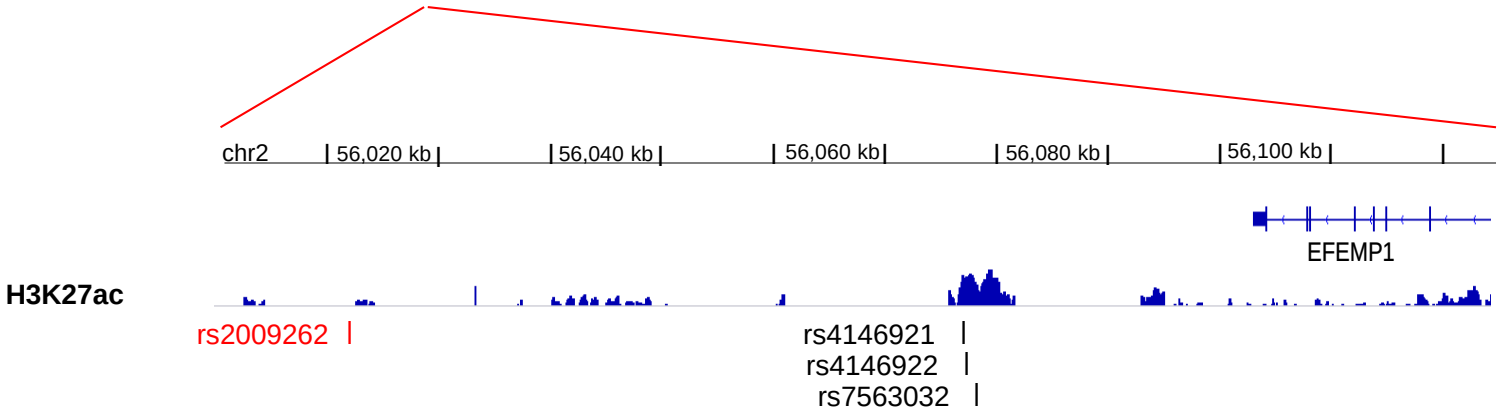
3 SNPs in Connective Tissue Enhancer Marks



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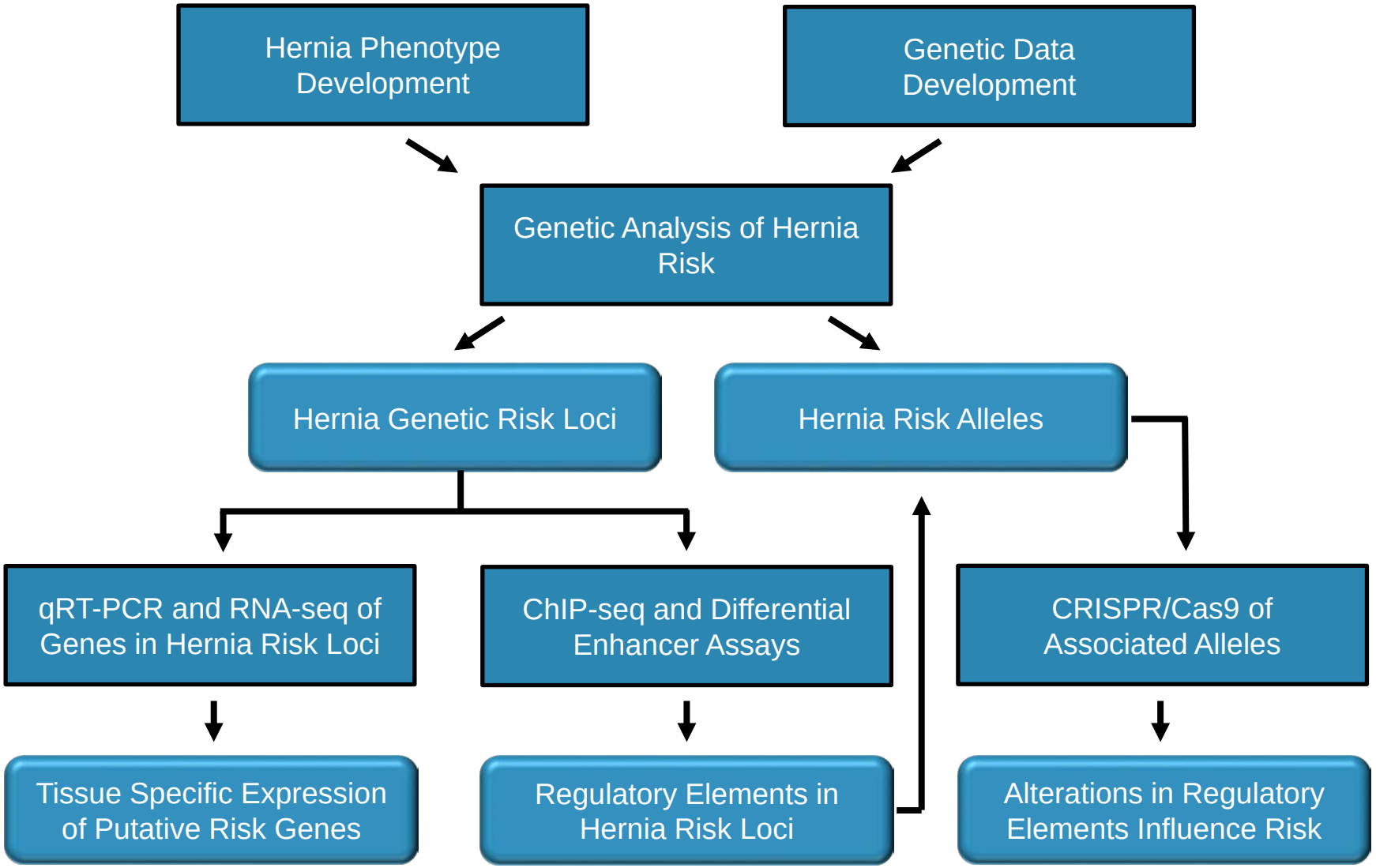
Identifying Causal Variants



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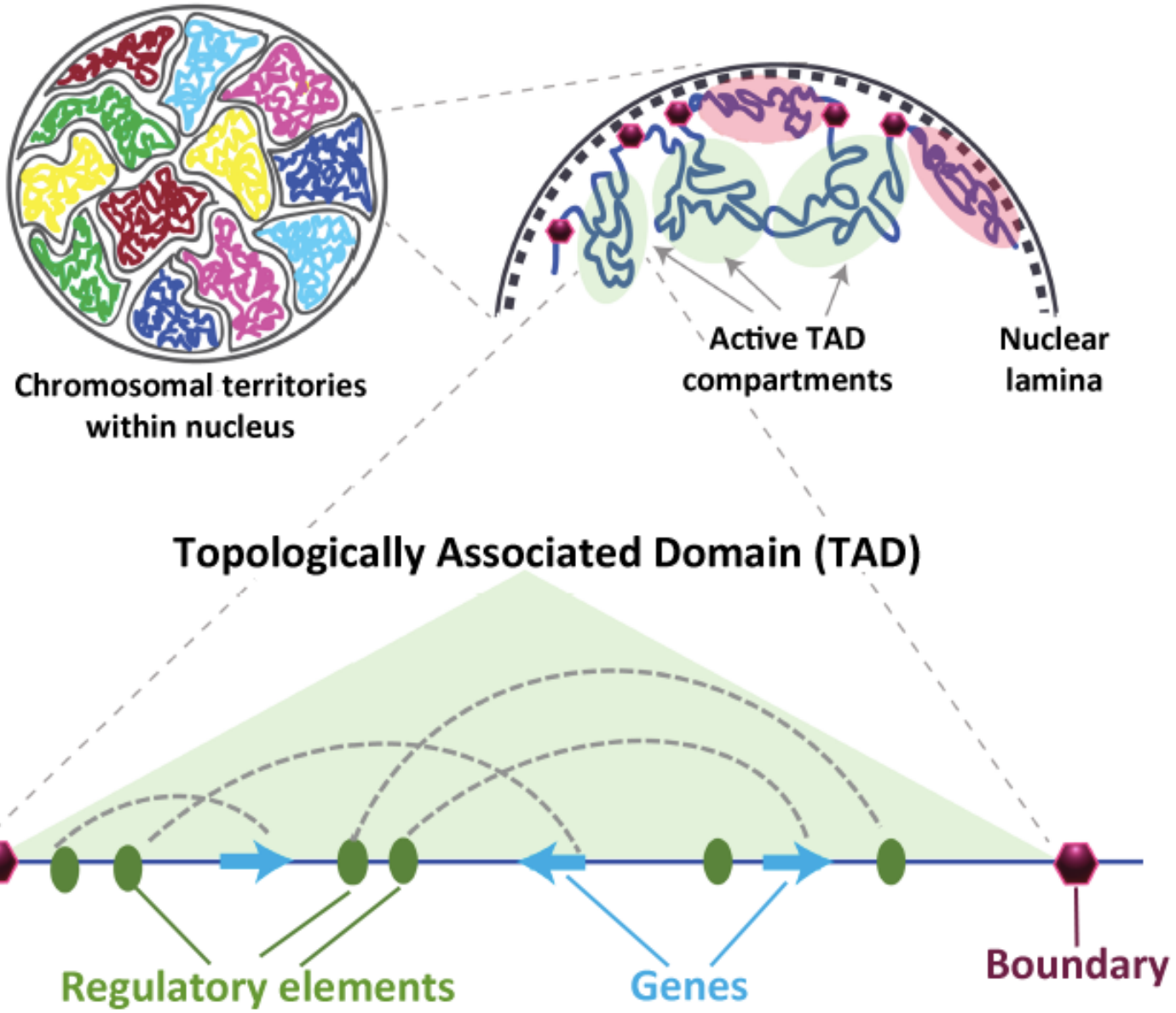
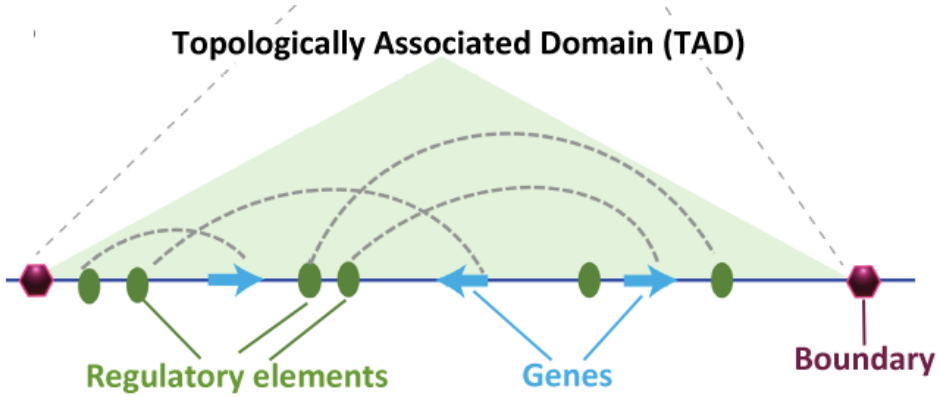
Can We Link Risk SNPs to Specific Genes?

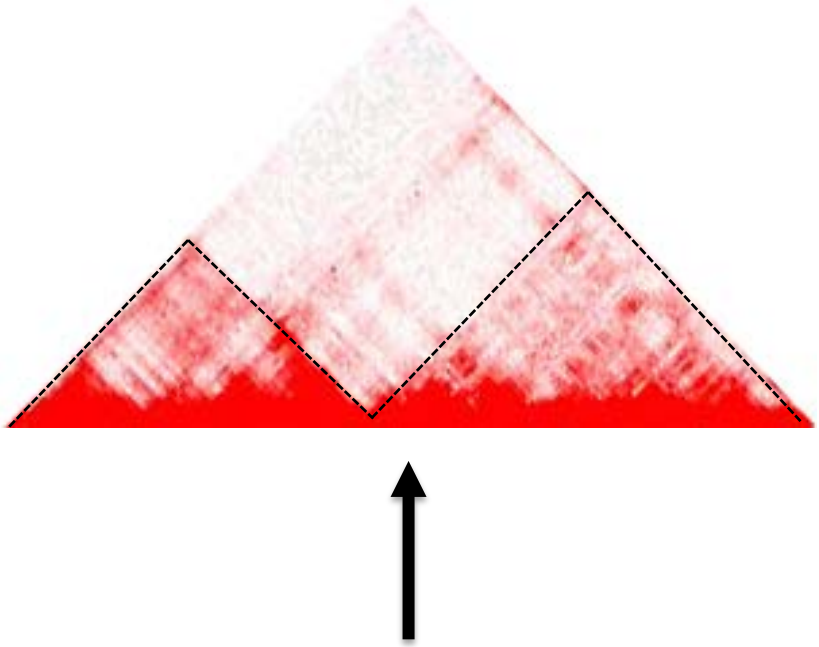
Chromosomes are Organized into Topologically Associating Domains (TADs)



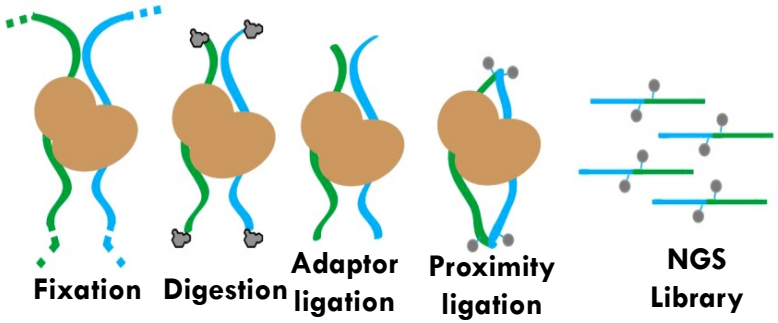
Matharu and Ahituv
PLoS Genetics 2015

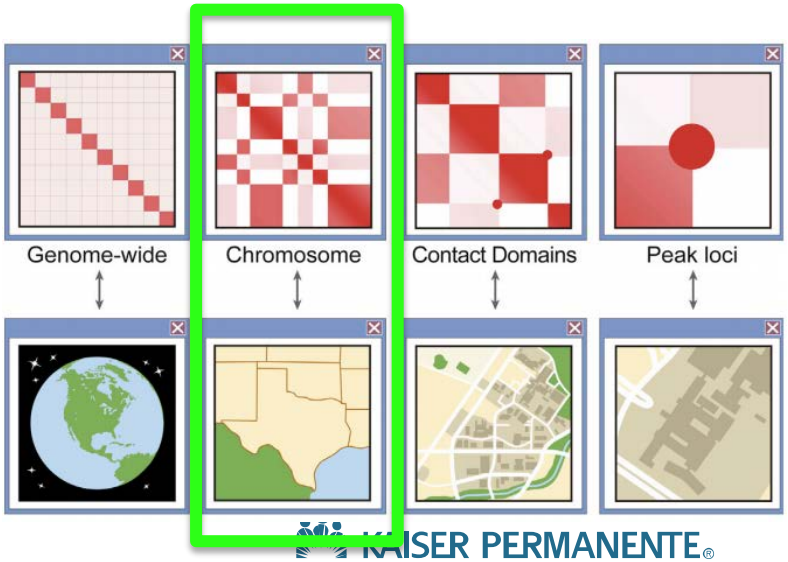
Hi-C contact map





Chromatin conformation capture (Hi-C)





Ramani *et al*. Nature Protocols 2016

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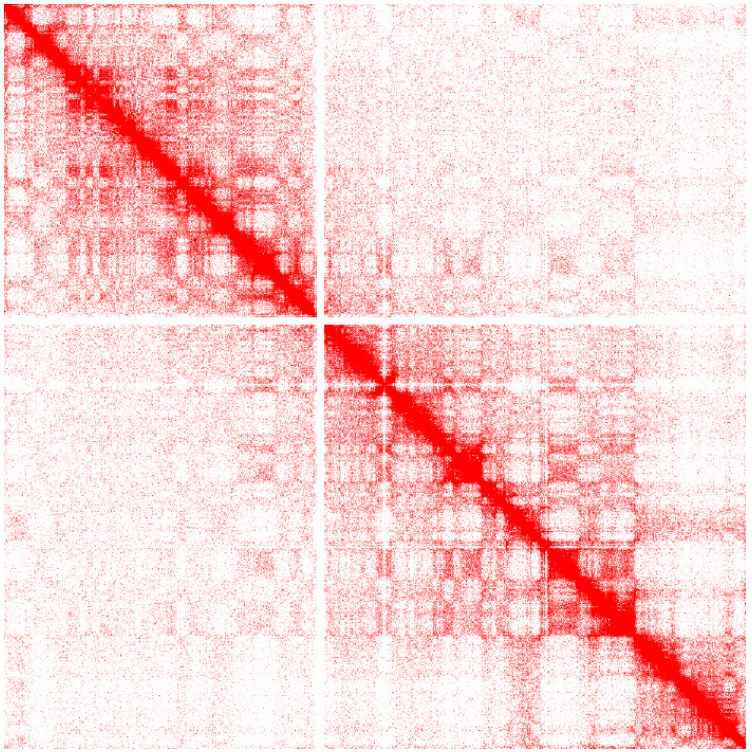
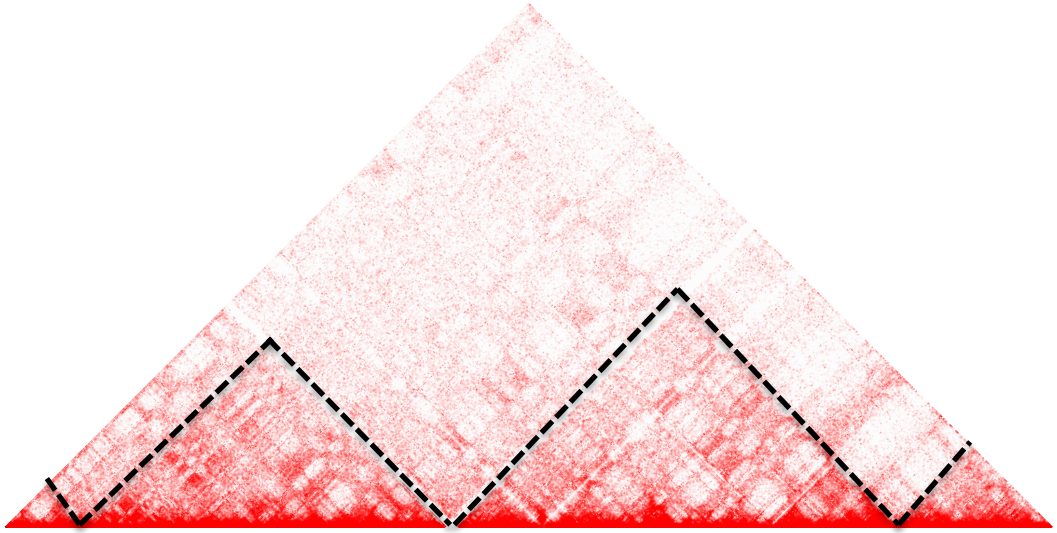
Chromosome Conformation Capture Example



Modified from Lazar *et al*. Genome Research 2018

Chromosome Conformation Capture Example



Modified from Lazar *et al*. Genome Research 2018

TADs around the *EFEMP1* Locus

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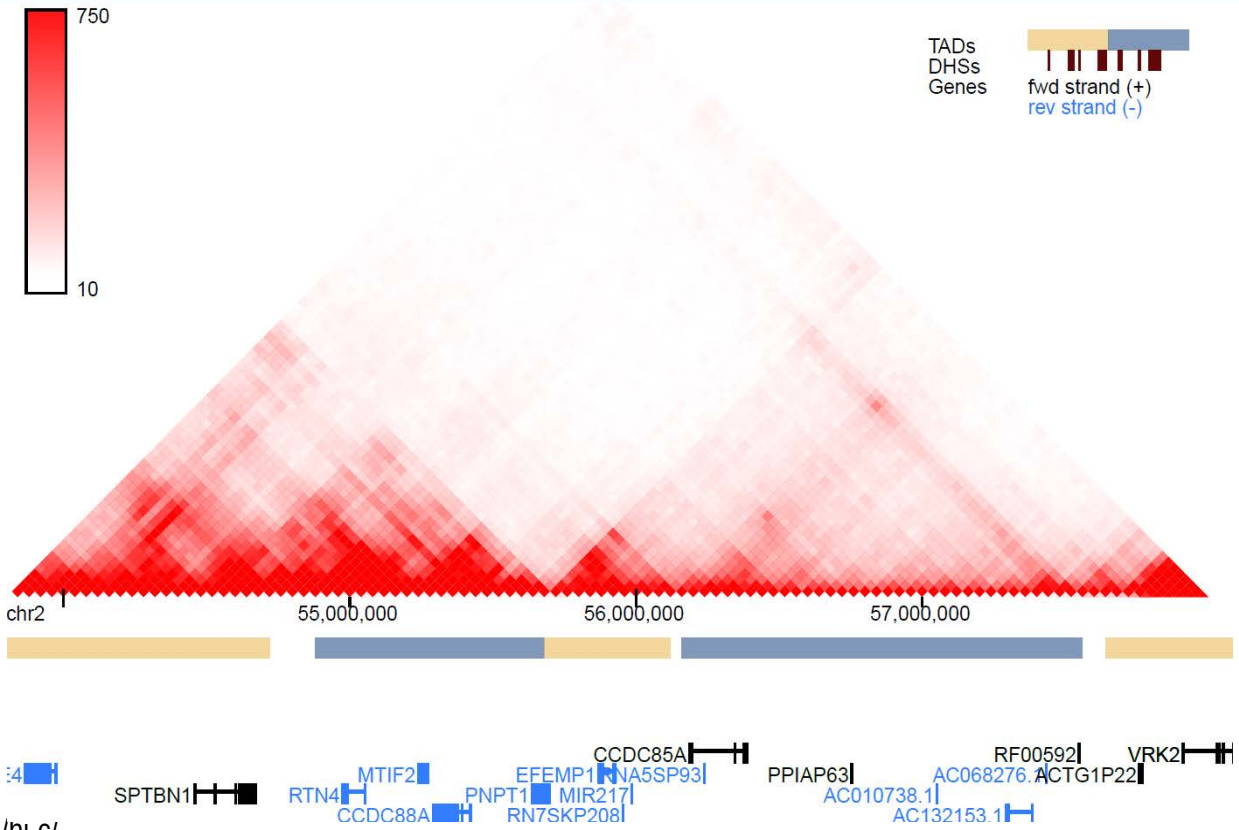
Computational and Functional Genomics/Epigenomics

ENCODE

Intro | Hi-C | Virtual 4C | ChIA-PET & Similar | Capture Hi-C | Compare Hi-C | Inter-chrom | Tutorial | Download



http://promoter.bx.psu.edu/hi-c/

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TADs around the *EFEMP1* Locus

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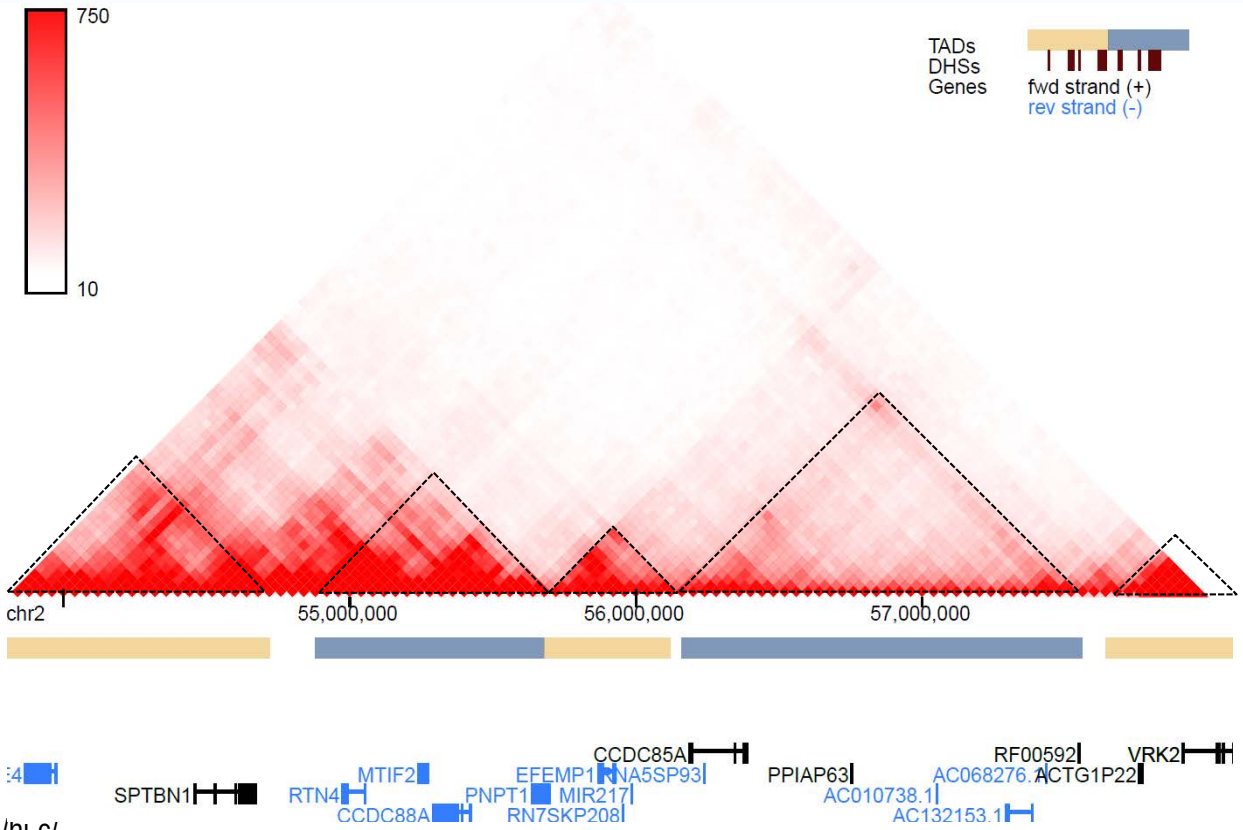

http://promoter.bx.psu.edu/hi-c/

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Virtual 4C Shows Interactions with rs2009262

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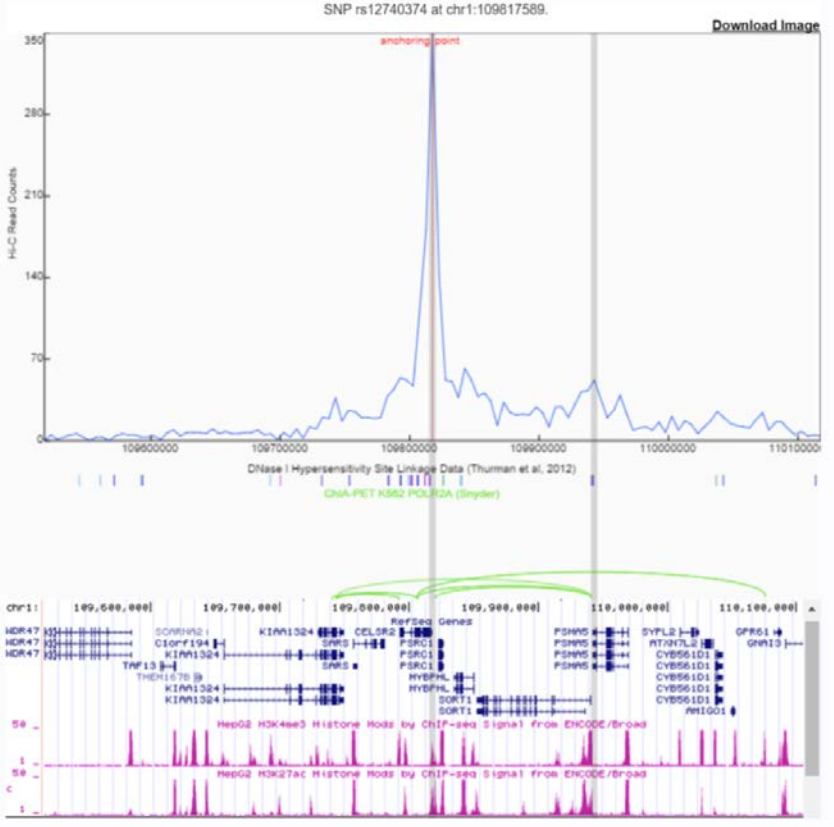

http://promoter.bx.psu.edu/hi-c/

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Does rs2009262 Affect *EFEMP1* Gene Expression?

https://www.gtexportal.org/home

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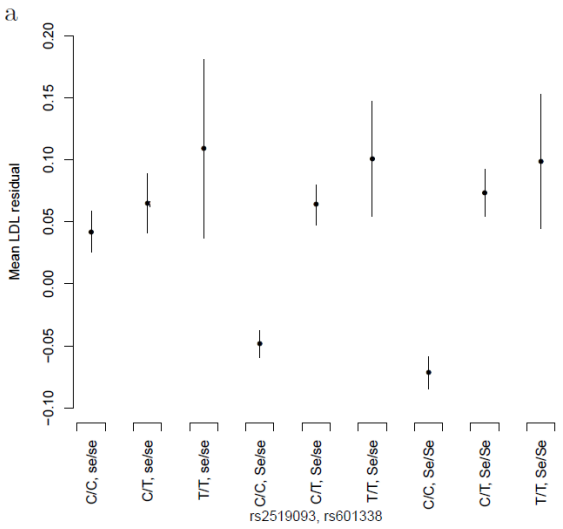
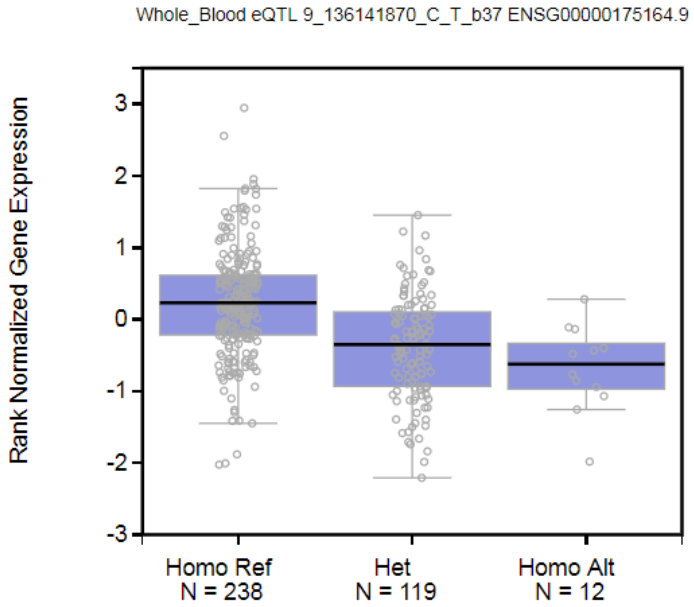
Outline

- A GWAS Example: Inguinal Hernia
- Epigenetics: DNA Methylation and Histone Modification
- Epistasis: Cutaneous Squamous Cell Carcinoma

Epistasis: LDL and a *FUT2* x *ABO* Interaction

- rs492602 (*FUT2*) and rs2519093 (*ABO*) are hits in overall GWAS
- Interaction $P_{LDL} = 8.1 \times 10^{-10}$
- *FUT2* secretor status and greater ABO expression lower LDL





Hoffmann *et al*. Nature Genetics 2018

https://gtexportal.org/home/snp/rs2519093

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Cutaneous Squamous Cell Carcinoma (cSCC)

- 4.3 Million cases per year of non-melanoma skin cancer
- $4.8 Billion public health costs
- 4,000 Deaths per year

Asgari *et al*. JID 2016

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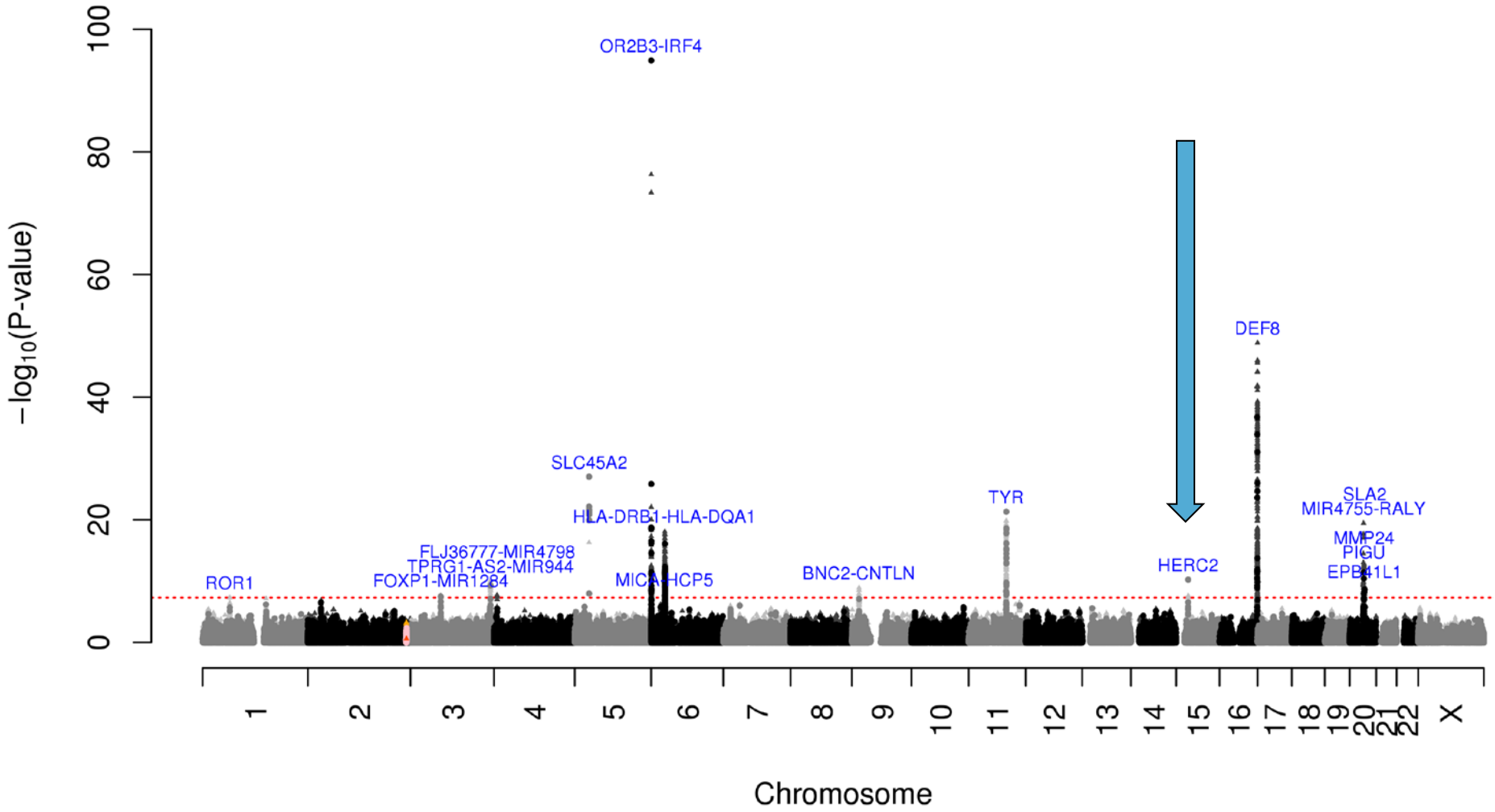
GWAS of cSCC in GERA



Asgari *et al*. JID 2016

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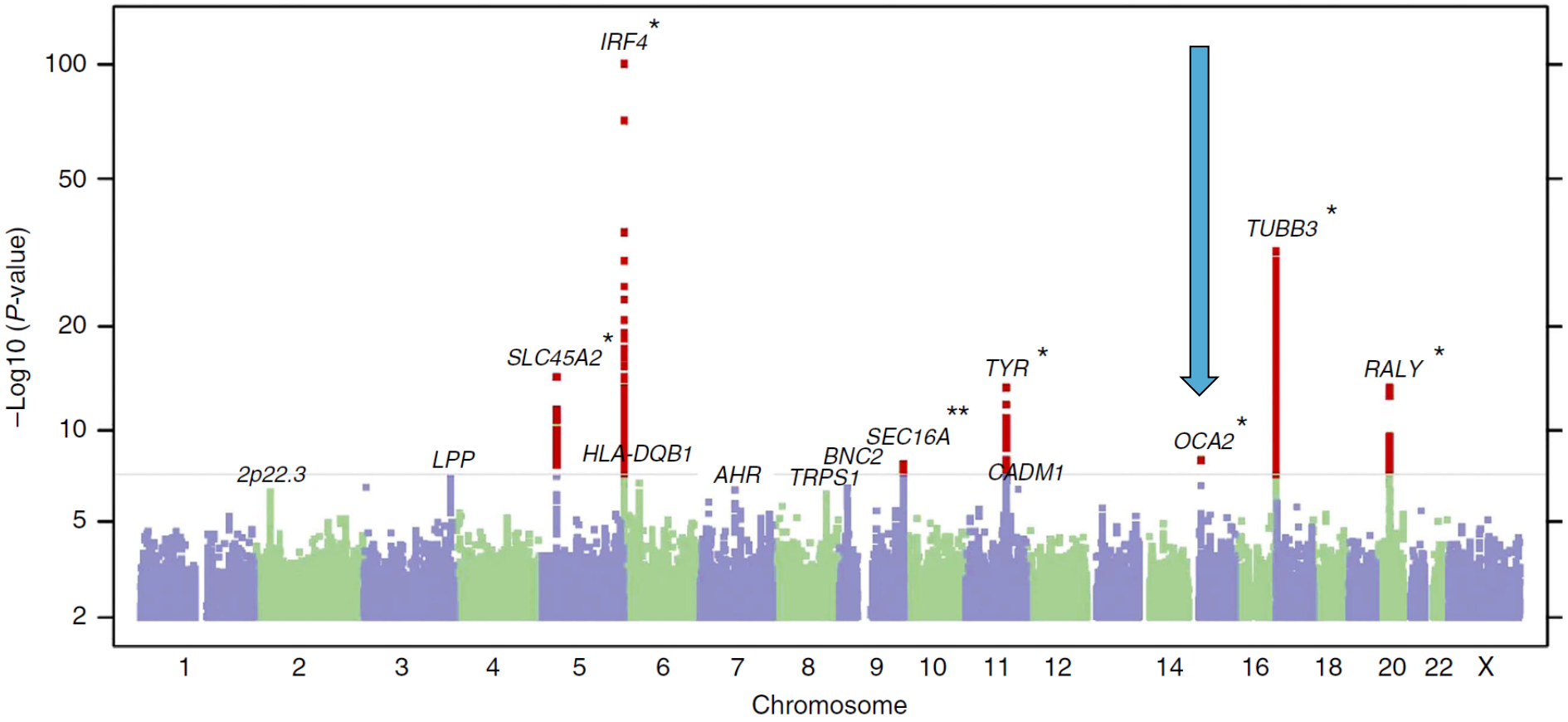
GWAS of cSCC in 23&Me



Chahal *et al*. Nature Communications 2016

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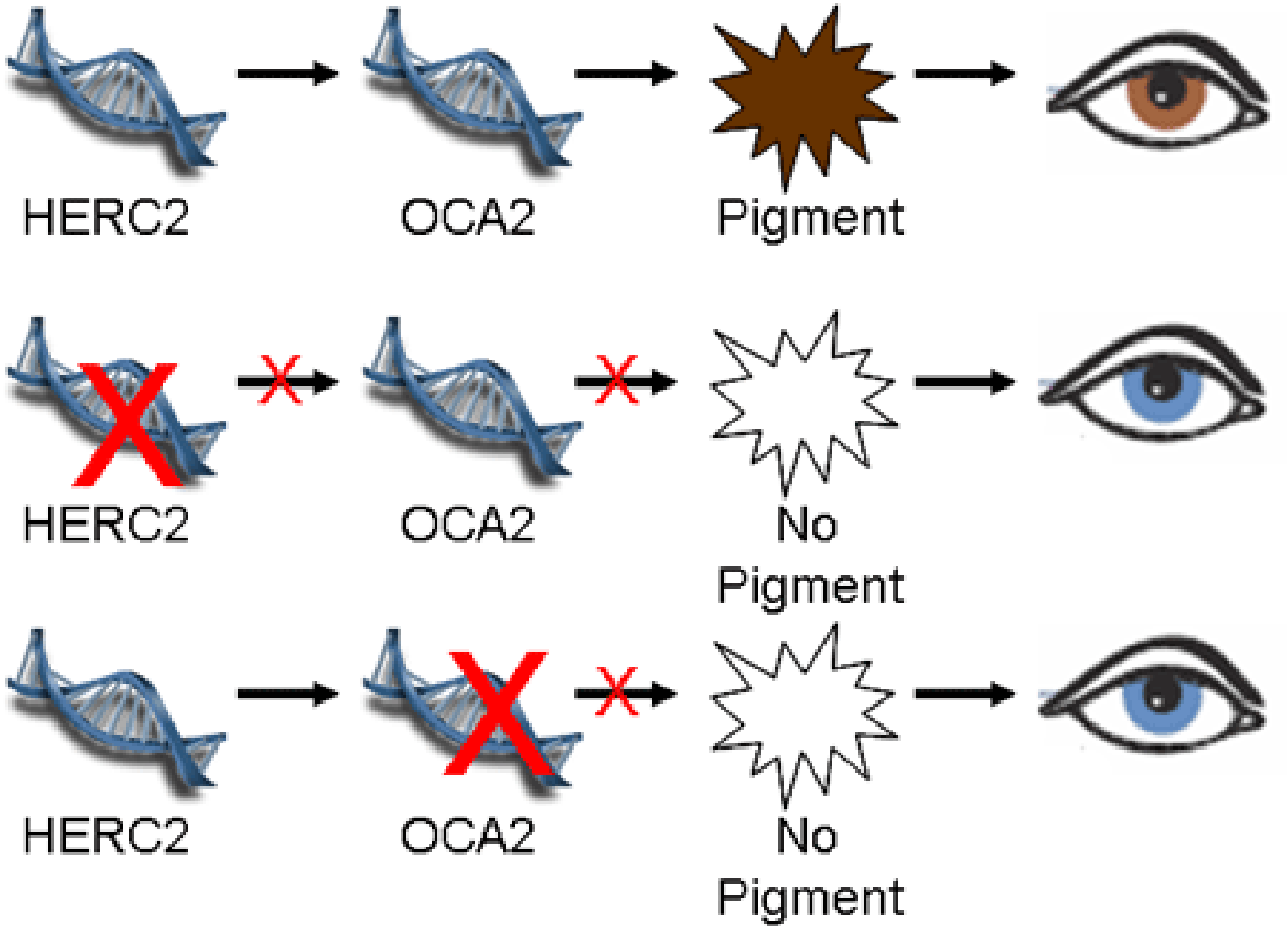
Epistasis and Eye Color



https://genetics.thetech.org/how-blue-eyed-parents-can-have-brown-eyed-children

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cSCC Risk by *HERC2* and *OCA2* Genotypes

		rs12916300 (*HERC2*)		
	OR (CI)	CC	CT	TT
rs1800407 (*OCA2*)	CC	1.00 (ref)	1.45 (1.26, 1.66)	1.91 (1.67, 2.18)
	CT	1.25 (0.99, 1.57)	2.13 (1.83, 2.48)	1.84 (1.55, 2.19)
	TT	2.26 (1.40, 3.65)	1.76 (1.12, 2.76)	1.85 (0.83, 4.11)

Subjects with Blue Eyes have Similar cSCC Risk

		rs12916300 (*HERC2*)		
	OR (CI)	CC	CT	TT
rs1800407 (*OCA2*)	CC	1.00 (ref)	1.45 (1.26, 1.66)	1.91 (1.67, 2.18)
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	TT	2.26 (1.40, 3.65)	1.76 (1.12, 2.76)	1.85 (0.83, 4.11)

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Interaction Coding Does Not Reflect Blue Eye Phenotype

Allele Coding		0	1	2
	OR (CI)	CC	CT	TT
0	CC	0	0	0
1	CT	0	1	2
2	TT	0	2	4

Interaction p > 0.05

Interaction Coding Does Not Reflect Blue Eye Phenotype

Allele Coding		0	1	2
	OR (CI)	CC	CT	TT
0	CC	1.00 (ref)	1.45 (1.26, 1.66)	1.91 (1.67, 2.18)
1	CT	1.25 (0.99, 1.57)	2.13 (1.83, 2.48)	1.84 (1.55, 2.19)
2	TT	2.26 (1.40, 3.65)	1.76 (1.12, 2.76)	1.85 (0.83, 4.11)

Interaction p > 0.05

Paper Discussion

PNAS

Genetic variation in the *SIM1* locus is associated with erectile dysfunction

Eric Jorgenson[a,1], Navneet Matharu[b,c], Melody R. Palmer[d], Jie Yin[a], Jun Shan[a], Thomas J. Hoffmann[c,e], Khanh K. Thai[a], Xujia Zhou[b,c], James M. Hotaling[f], Gail P. Jarvik[d], Nadav Ahituv[b,c], Hunter Wessells[g], and Stephen K. Van Den Eeden[a,1]

[a]Division of Research, Kaiser Permanente Northern California, Oakland, CA 94612; [b]Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, CA 94158; [c]Institute for Human Genetics, University of California, San Francisco, CA 94158; [d]Division of Medical Genetics, University of Washington School of Medicine, Seattle, WA 98195; [e]Department of Epidemiology and Biostatistics, University of California, San Francisco, CA 94158; [f]Department of Surgery (Urology), University of Utah School of Medicine, Salt Lake City, UT 84132; and [g]Deparment of Urology, University of Washington School of Medicine, Seattle, WA 98195

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