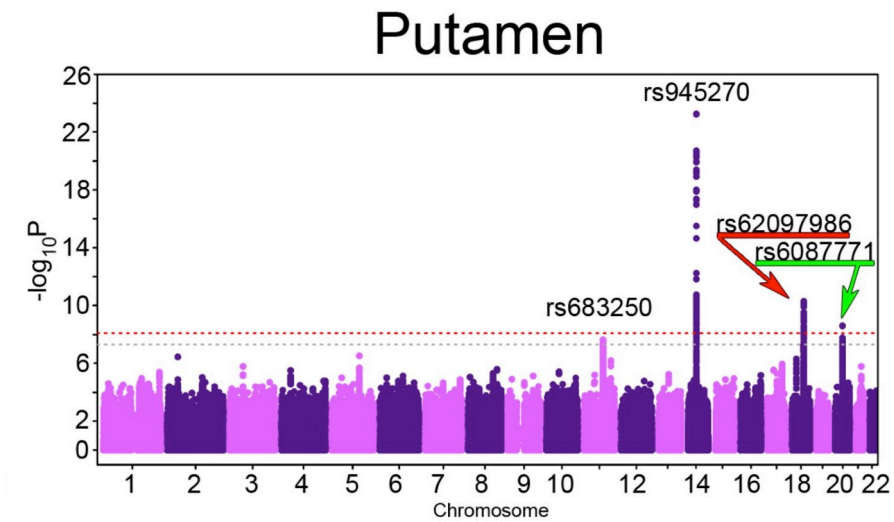
After the association: Functional and Biological Validation of Variants

Jason L. Stein
Geschwind Laboratory /
Imaging Genetics Center
University of California, Los Angeles
(but soon to be at UNC-Chapel Hill)
jasonlouisstein@gmail.com

Organization for Human Brain Mapping
Introduction to Imaging Genetics
Honolulu, HI
June 14, 2015

A hit is just the beginning…



(Hibar et al., 2015)

<u>What you have found</u>

- Variation in a **locus** of the genome which significantly influences your **trait** (brain structure/disease)

<u>What you want to know</u>

- A mechanism by which genetic variation influences brain structure or function and risk for disease
- Causal variant(s)
- Causal gene(s)
- Causal biological pathway(s)
- Causal brain region(s)

But be wary….

APPLICATIONS OF NEXT-GENERATION SEQUENCING

Sequencing studies in human genetics: design and interpretation

David B. Goldstein[1], Andrew Allen[1,2], Jonathan Keebler[1], Elliott H. Margulies[3], Steven Petrou[4,5], Slavé Petrovski[1,6] and Shamil Sunyaev[7]

"Human genomes have a high level of '**narrative potential**' to provide compelling but statistically poorly justified connections between mutations and phenotypes."

"A critical challenge for biologists […] will be avoiding premature hypotheses born of biological plausibility and '**Just So**' stories."

Genome-scale neurogenetics: methodology and meaning

Steven A McCarroll[1,2], Guoping Feng[1,3,4] & Steven E Hyman[1,5]

ORIGINAL ARTICLES

Spurious Genetic Associations

Patrick F. Sullivan

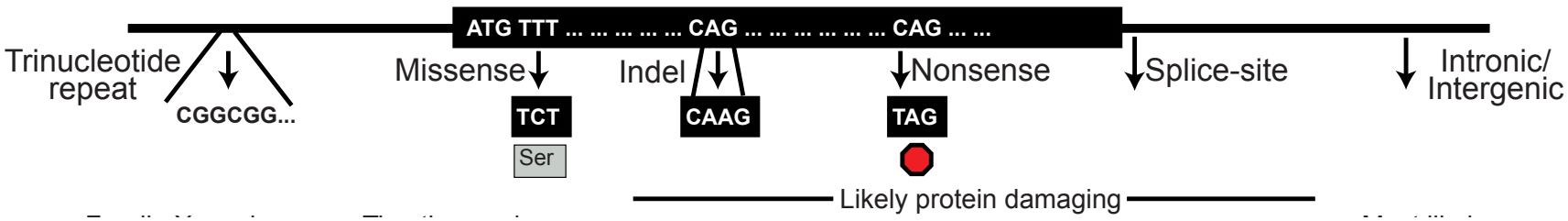
"Findings from single association studies constitute '**tentative knowledge**' and must be interpreted with exceptional caution.

<u>Biological plausibility is not a substitute for statistical significance</u>

Exploring biological mechanisms

- Exploring the genetic locus
- Epigenetics
- Move from locus to gene
- Exploring the expression of the gene
- Enrichment in biological pathways

Genetic hit locations



- 88% of significant GWAS (common variant) loci are often found in intergenic or intronic regions with no clear gene(s) of action (Hindorff et al., *PNAS*, 2009)

- GWAS loci tag very large regions with multiple functional elements including genes
  - For the SCZ GWAS loci: max 800kb (SZ working group, *Nature*, 2014)
  - For the SCZ GWAS loci: mean 171 kb ($r^2 > 0.6$)
  - Mean gene size: 29kb (Gencode v19)

- Rare variant mutations including missense or nonsense mutations have a clear gene of action, but are rare.

UCSC Genome Browser

Human (Homo sapiens) Ge

genome.ucsc.edu/cgi-bin/hgGateway

Genomes | Genome Browser | Tools | Mirrors | Downloads | My Data | Help | About Us

Human (*Homo sapiens*) Genome Browser Gateway

The UCSC Genome Browser was created by the Genome Bioinformatics Group of UC Santa Cruz.
Software Copyright (c) The Regents of the University of California. All rights reserved.

group	genome	assembly	position	search term	
Mammal	Human	Feb. 2009 (GRCh37/hg19)	chr19:45,404,181-45,404,681	enter position, gene symbol or search terms	submit

Click here to reset the browser user interface settings to their defaults.

track search | add custom tracks | track hubs | configure tracks and display

Human Genome Browser – hg19 assembly (sequences)

The February 2009 human reference sequence (GRCh37) was produced by the Genome Reference Consortium. For more information about this assembly, see GRCh37 in the NCBI Assembly database.

Sample position queries

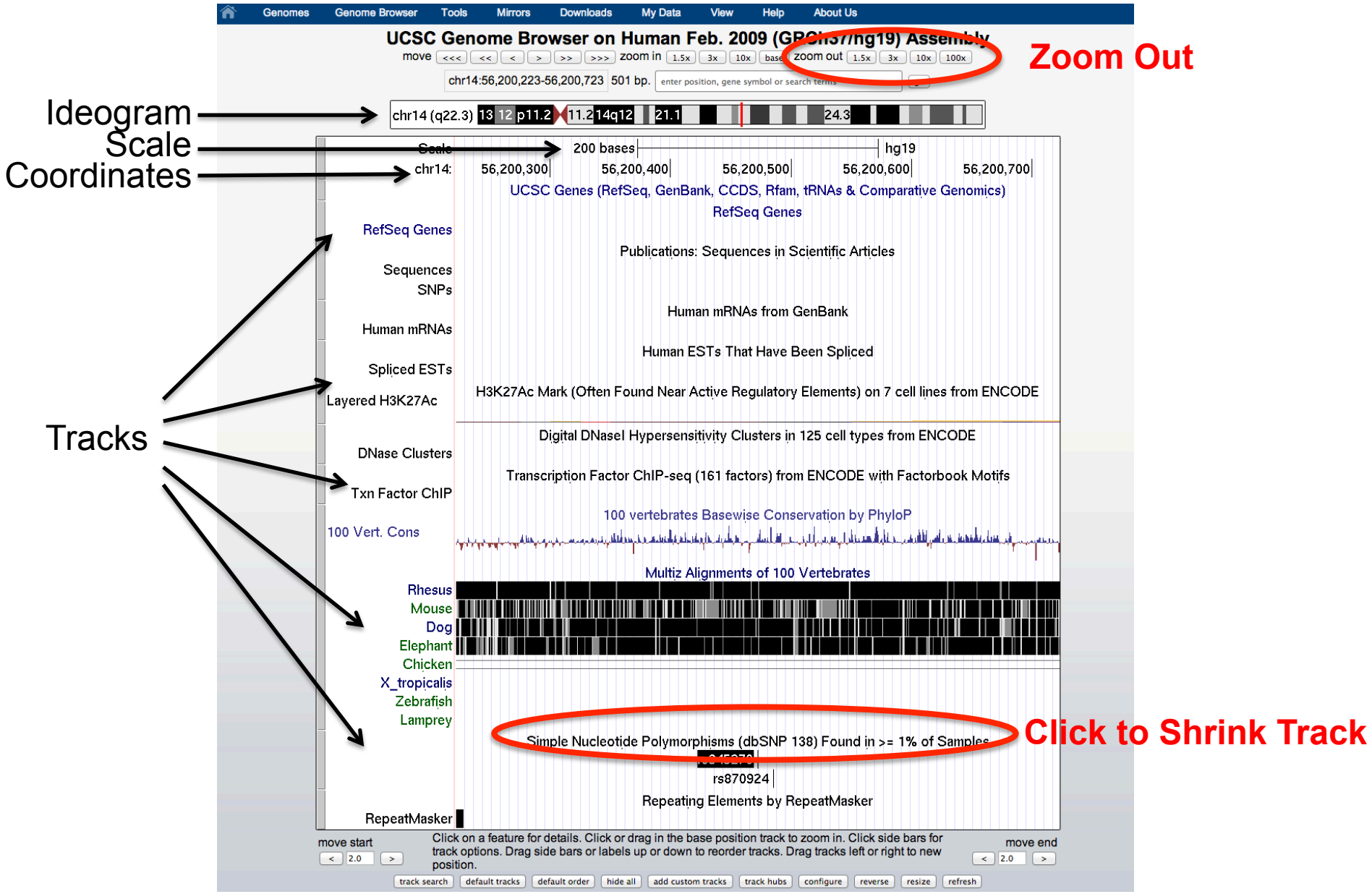
A genome position can be specified by the accession number of a sequenced genomic clone, an mRNA or EST or STS marker, a chromosomal coordinate range, or keywords from the GenBank description of an mRNA. The following list shows examples of valid position queries for the human genome. See the User's Guide for more information.

Request:	Genome Browser Response:
chr7	Displays all of chromosome 7
chrUn_gl000212	Displays all of the unplaced contig gl000212
20p13	Displays region for band p13 on chr 20
chr3:1-1000000	Displays first million bases of chr 3, counting from p-arm telomere
chr3:1000000+2000	Displays a region of chr3 that spans 2000 bases, starting with position 1000000

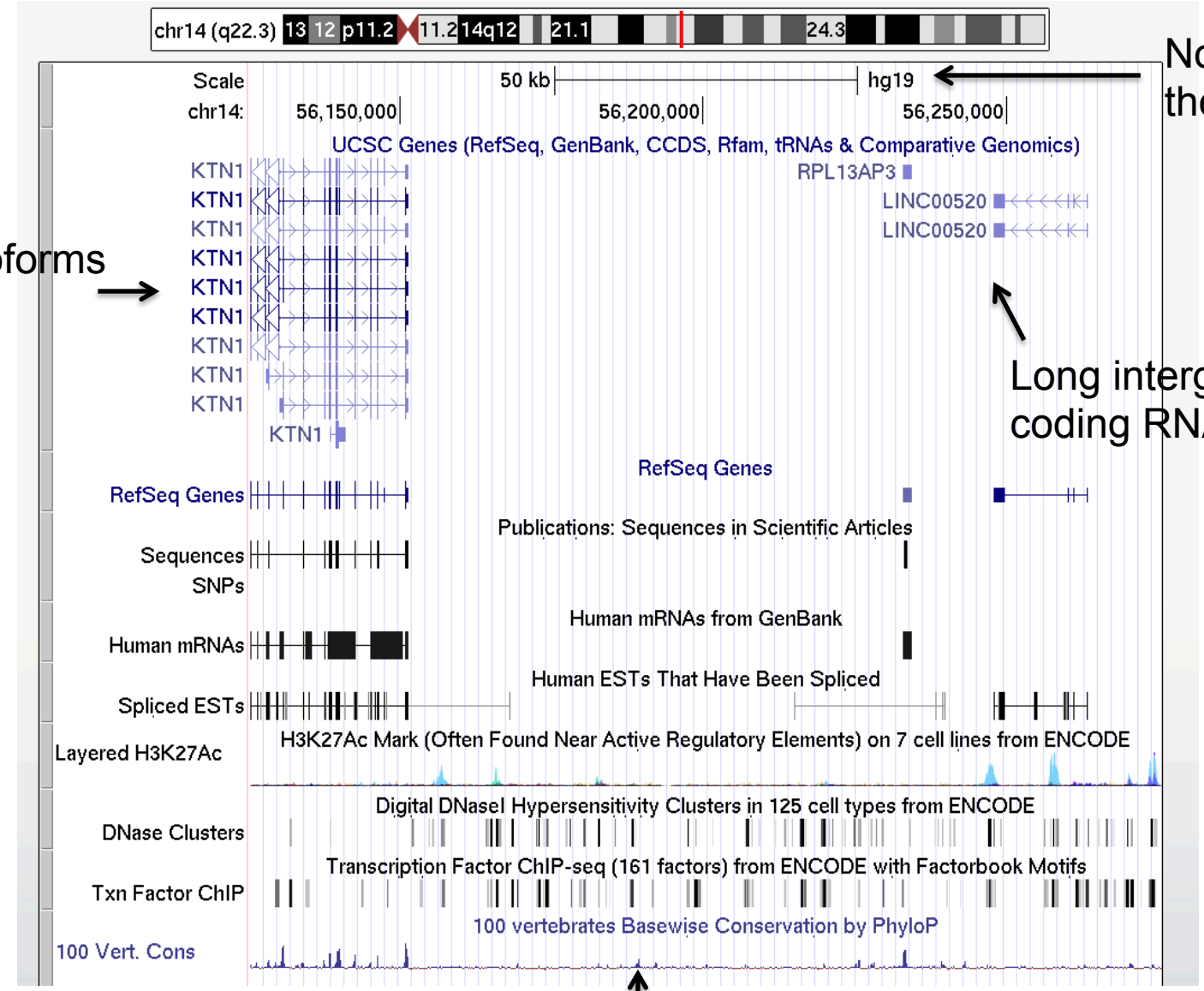
Homo sapiens
(Graphic courtesy of CBSE)

http://genome.ucsc.edu/cgi-bin/hgGateway

Exploring a hit (rs945270)



Exploring a hit (rs945270)



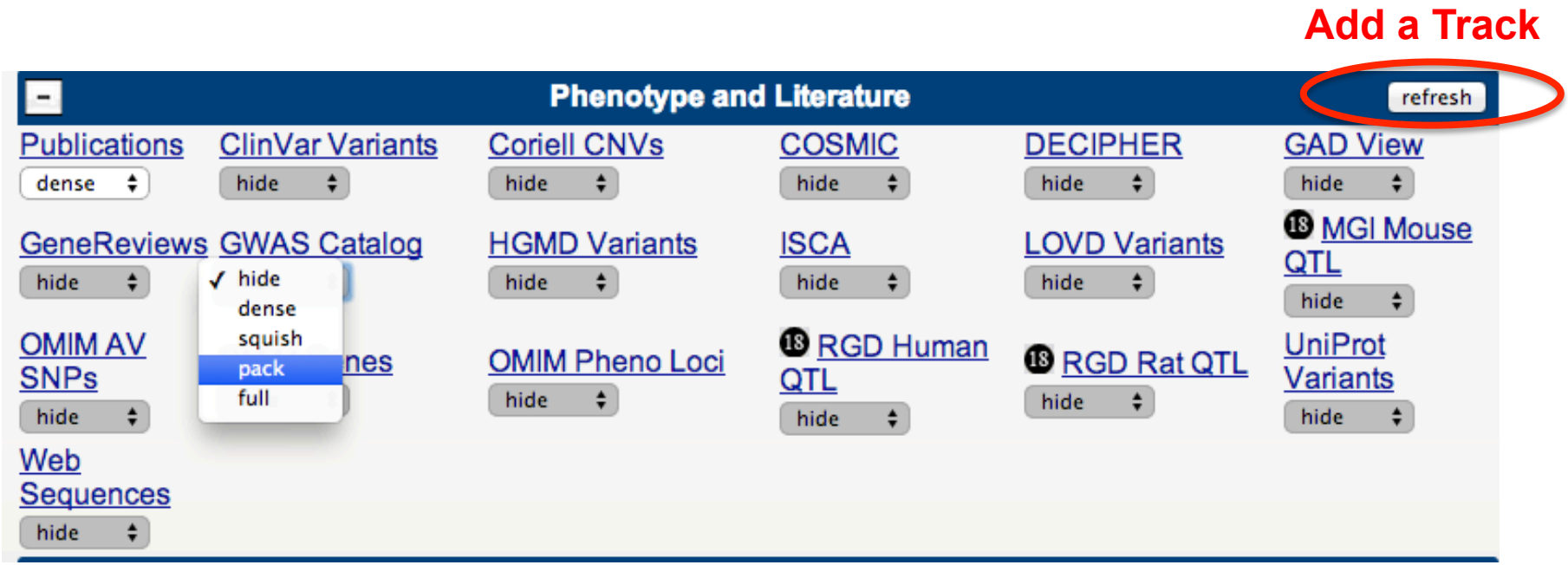
Notice the scale

Multiple isoforms of genes

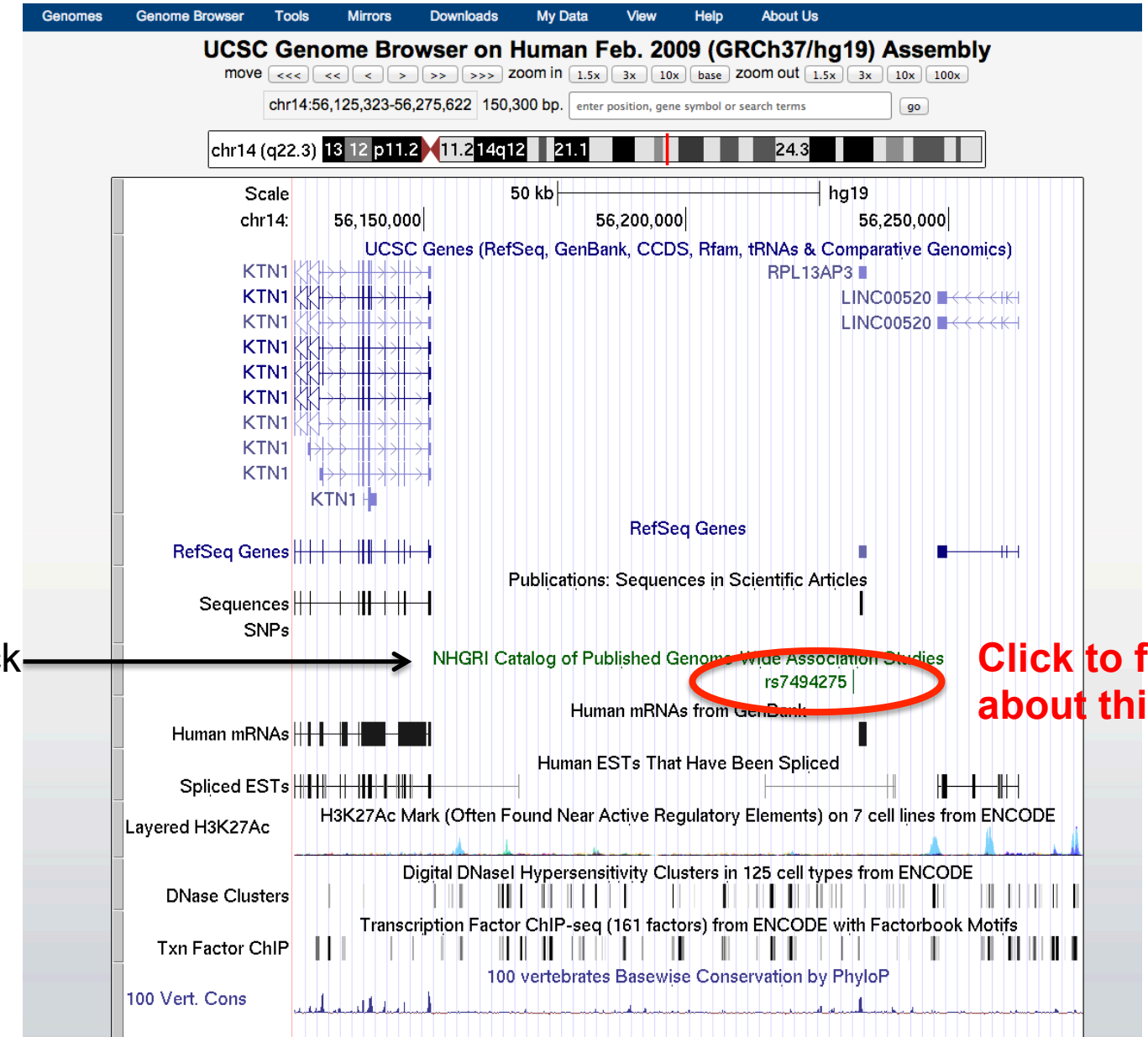
Long intergenic non-coding RNAs

The variant initially entered stays in the center

Exploring a hit (rs945270)



Exploring a hit (rs945270)



New Track

Click to find out about this variant

Exploring a hit (rs945270)

Genomes | Genome Browser | Tools | Mirrors | Downloads | My Data | Help | Abou

NHGRI Catalog of Published Genome-Wide Association Studies (rs7494275)

dbSNP: rs7494275
Position: chr14:56231800-56231800
Band: 14q22.3
Genomic Size: 1
View DNA for this feature (hg19/Human)
Reported region: 14q22.3
Publication: Low SK *et al.* Genome-wide association study of chemotherapeutic agent-induced severe neutropenia/leucopenia for patients in Biobank Japan. *Cancer Sci.* 2013-05-04
Disease or trait: Adverse response to chemotherapy (neutropenia/leucopenia) (all topoisomerase inhibitors)
Initial sample size: 106 Japanese ancestry cases, 187 Japanese ancestry controls
Replication sample size: NA
Reported gene(s): RPL13AP3
Strongest SNP-Risk allele: rs7494275-C
dbSNP build 137 observed alleles for rs7494275: A/C
Risk Allele Frequency: 0.406
p-Value: 9E-6 (Recessive model)
Odds Ratio or beta: 1.73
95% confidence interval: [1.232-2.433]
Platform: Illumina [733,202]
Copy Number Variant (CNV)?: No

View table schema

Go to GWAS Catalog track controls

Data last updated: 2014-05-23

Not super convincing given low sample size and non-genome wide significant P-value.

Uploading a user track

test.bed

```
chr14 56200473 56200473 SIGNIFICANT_HIT
```

move start < 2.0 >

Click on a feature for details. Click or drag in the base position track to zoom in. Click side bars for track options. Drag side bars or labels up or down to reorder tracks. Drag tracks left or right to new position.

move end < 2.0 >

track search | default tracks | default order | hide all | add custom tracks | track hubs | configure | reverse | resize | refresh

collapse all

Use drop-down controls below and press refresh to alter tracks displayed.
Tracks with lots of items will automatically be displayed in more compact modes.

expand all

Paste URLs or data: Or upload: Choose File No file chosen Submit

Clear

chr14 (q22.3) 13 p12 p11.2 11.2 14q12 21.1 24.3 31.3 32.2

Scale 50 kb hg19
chr14: 56,150,000 56,200,000 56,250,000

User Supplied Track
SIGNIFICANT_HIT

UCSC Genes (RefSeq, GenBank, CCDS, Rfam, tRNAs & Comparative Genomics)

KTN1
KTN1
KTN1
KTN1
KTN1
KTN1
KTN1
KTN1
KTN1

RPL13AP3
LINC00520
LINC00520

LocusZoom: Making Prettier Pictures

Make sure not too big a file

LocusZoom

LocusZoom - Plot with Your Data

Plot Your Data

Depending on the size of your data, runs can require 30-60 seconds to generate a plot

Provide Details for Your Data

- **Path to Your File** — Choose File No file chosen
  File will sent to server and used for plotting (Maximum 200MB) [Help]
- **P-Value Column Name** — Default is P.value
- **Marker Column Name** — Default is MarkerName
- **Column Delimiter** — Tab — Default is tab

Set for PLINK data or WikiGWA data

Specify Region to Display

Required: Fill in Only ONE of These Three

SNP	SNP Reference Name	+/-	400 Kb Flanking Size	
Gene	Gene Reference Name	+/-	200 Kb Flanking Size	Optional Index SNP Default=lowest p-value
Region	Chr: None	Mb Starting Chr Position	through	Mb Ending Chr Position; Optional Index SNP Default=lowest p-value

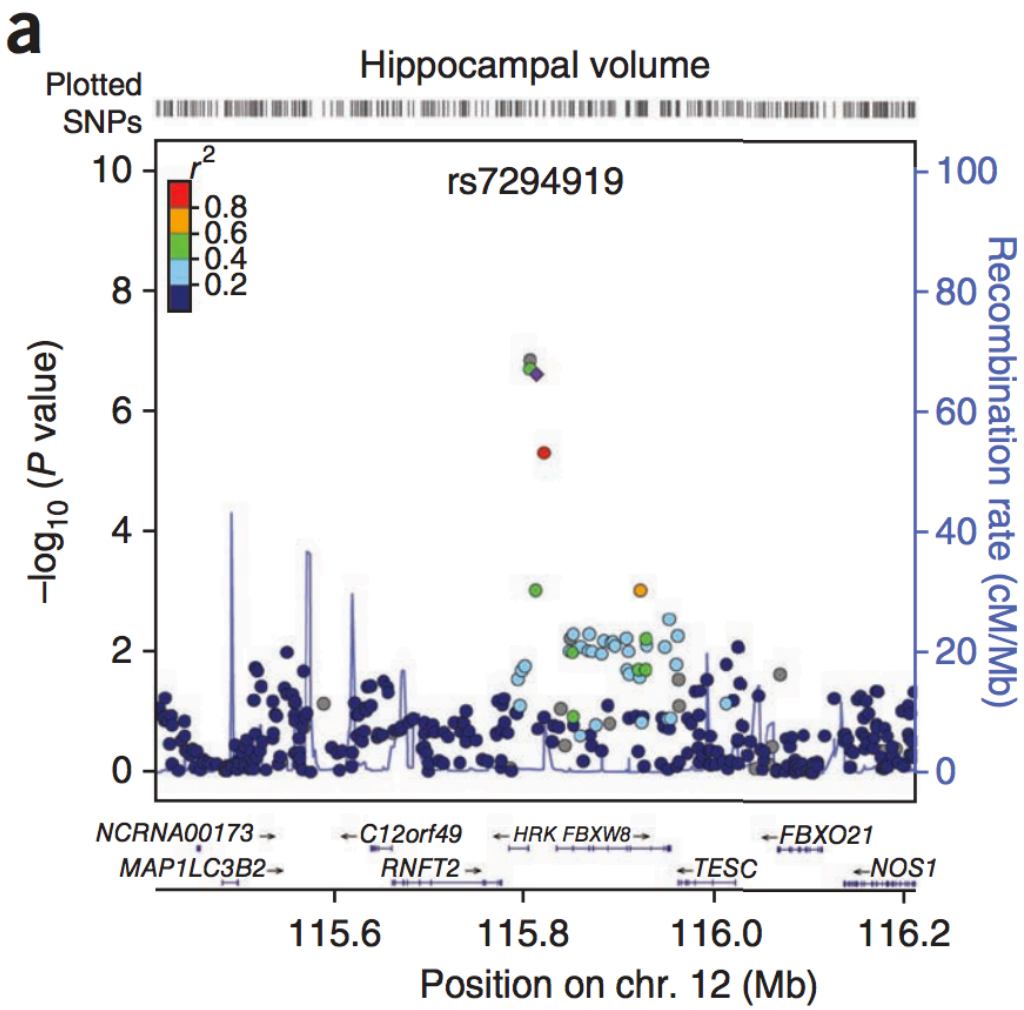
Custom Annotation

Optional: This overrides *Show Annotation* below

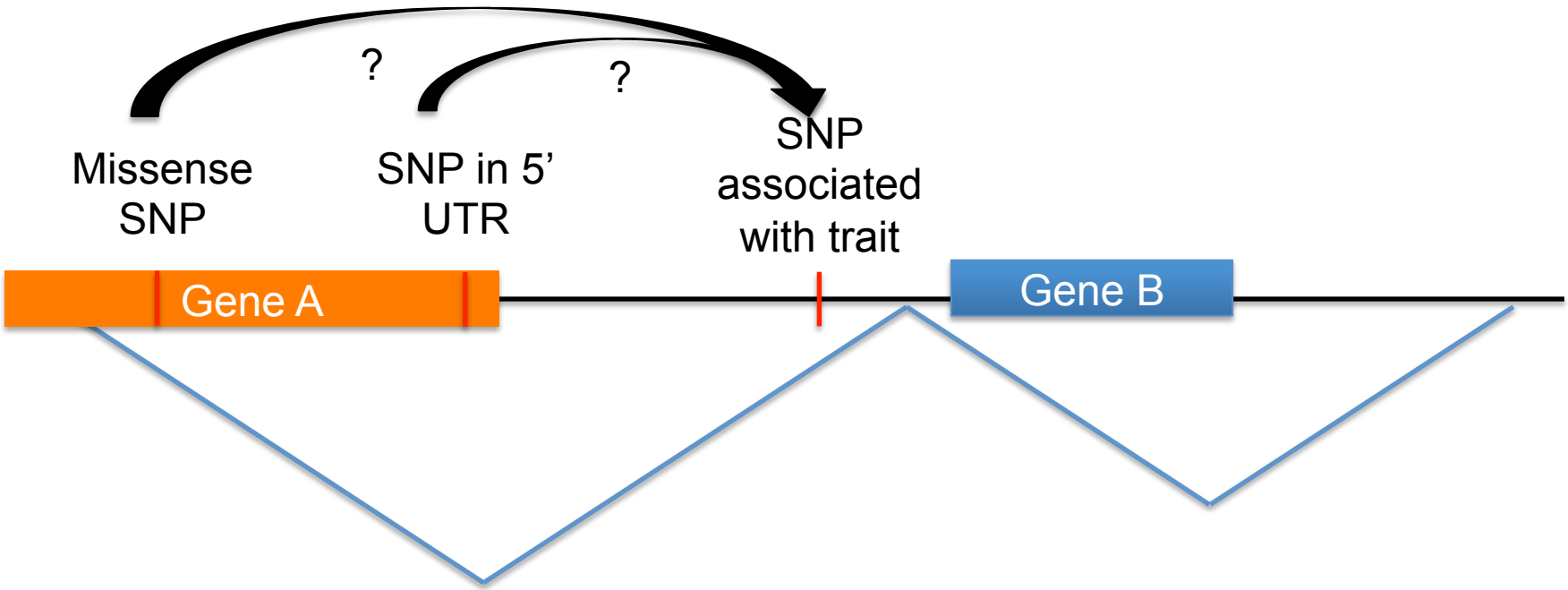
- **Column Name** — Name of annotation column
- **Category Order** — Order of annotation categories

https://statgen.sph.umich.edu/locuszoom/genform.php?type=yourdata

LocusZoom: Making Prettier Pictures

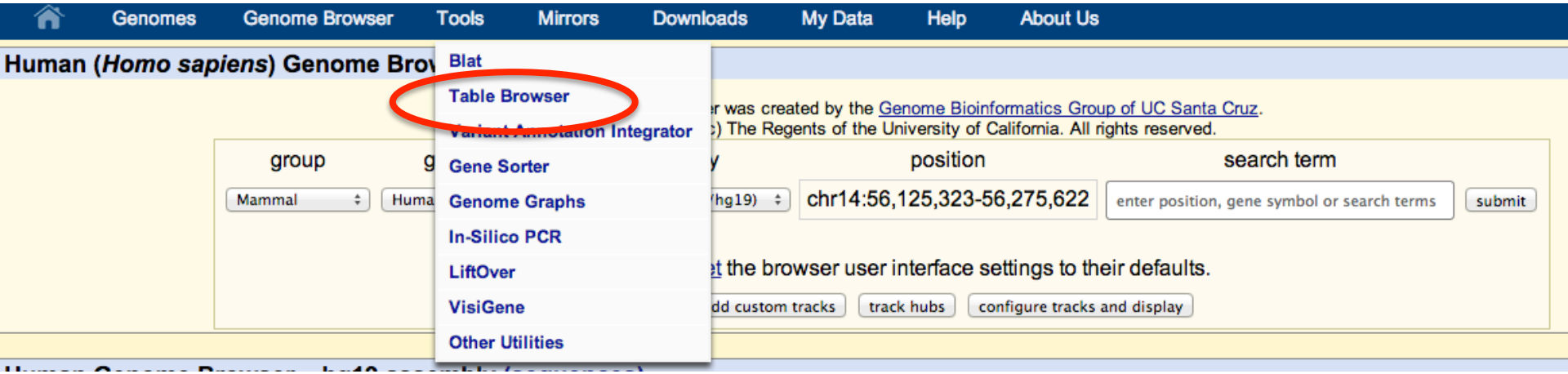


Trying to find possible gene function

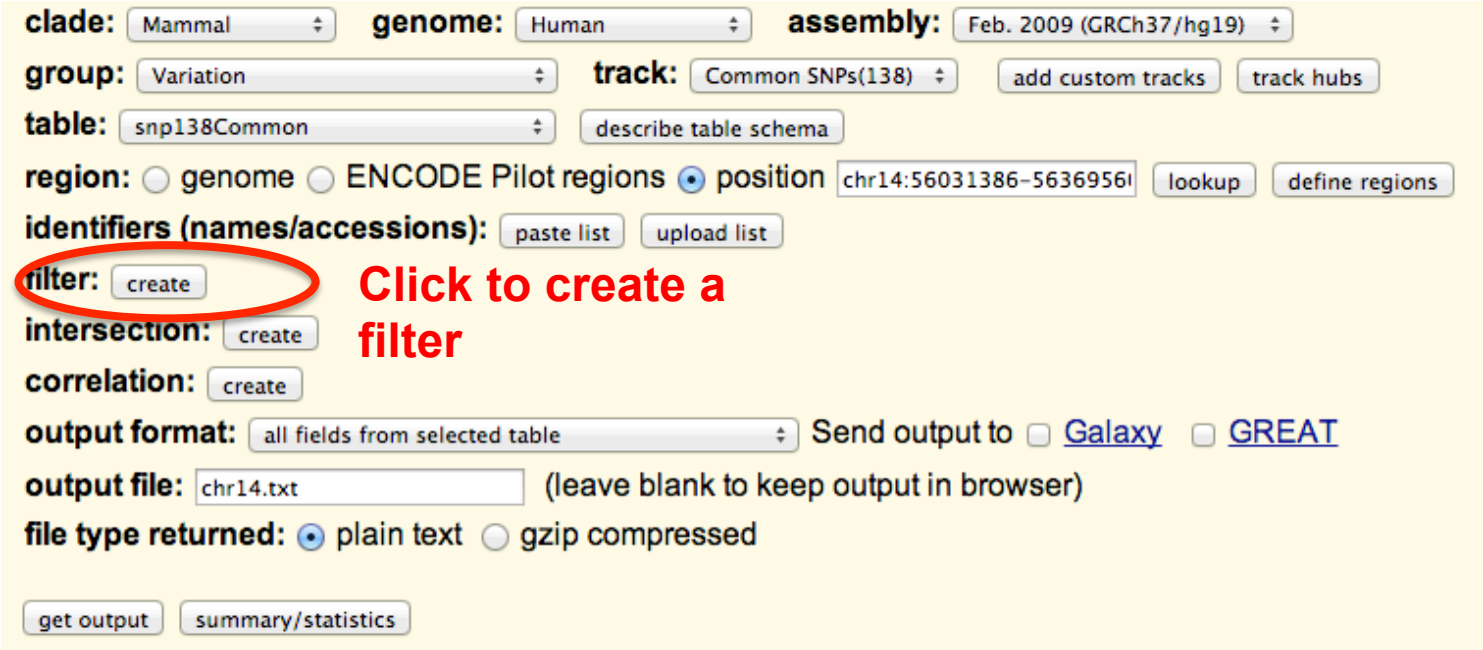


We found a genetic variant, but is it in LD with anything of known functionality?

Finding Variants of Known Functionality



Go to the Table Browser in UCSC Genome Browser



Finding Variants of Known Functionality

Select interpretable functional Variants

func | does | include
- [ ] *
- [ ] unknown
- [x] coding-synon
- [ ] intron
- [ ] near-gene-3
- [ ] near-gene-5
- [ ] ncRNA
- [x] nonsense
- [x] missense
- [x] stop-loss
- [x] frameshift
- [ ] cds-indel
- [x] untranslated-3
- [x] untranslated-5
- [x] splice-3
- [x] splice-5

submit | cancel

Click to create a filter

clade: Mammal | genome: Human | assembly: Feb. 2009 (GRCh37/hg19)

group: Variation | track: Common SNPs(138) | add custom tracks | track hubs

table: snp138Common | describe table schema

region: ○ genome ○ ENCODE Pilot regions ◉ position chr14:56,031,386-56,369,! | lookup | define regions

identifiers (names/accessions): paste list | upload list

filter: edit | clear

intersection: create

correlation: create

output format: all fields from selected table | Send output to ☐ Galaxy ☐ GREAT

output file: chr14.txt (leave blank to keep output in browser)

file type returned: ◉ plain text ○ gzip compressed

get output | summary/statistics

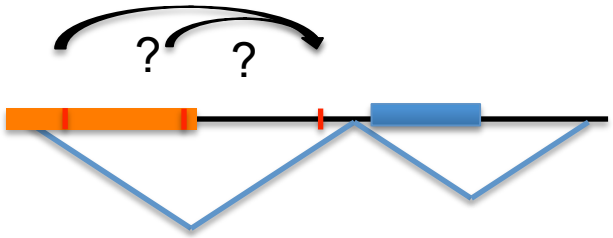
Get output spreadsheet

Finding Variants of Known Functionality

#filter: (FIND_IN_SET('splice-5', snp138Common.func)>0 OR FIND_IN_SET('splice-3', snp138Common.func)>0 OR FIND_IN_SET('untranslat

#bin	chrom	chromStart	chromEnd	name	refNCBI	refUCSC	observed	func
1012	chr14	56068483	56068484	rs116289145	C	C	C/T	intron,ncRNA,untranslated-5
1012	chr14	56068520	56068521	rs10083303	T	T	C/T	intron,ncRNA,untranslated-5
1012	chr14	56078738	56078739	rs1138345	T	T	G/T	ncRNA,untranslated-5
1012	chr14	56079038	56079039	rs34879854	A	A	A/T	coding-synon,ncRNA
1012	chr14	56094725	56094726	rs17128636	C	C	A/C	coding-synon,ncRNA
1012	chr14	56096685	56096686	rs74053638	A	A	A/C	coding-synon,ncRNA
1012	chr14	56096730	56096731	rs2274075	A	A	A/G	coding-synon,ncRNA
1013	chr14	56146356	56146357	rs11546	G	G	A/G	coding-synon,ncRNA

Variants of known function



Are variants of known function in LD with our top hit?

Finding Variants of Known Functionality

Query SNPs

Input SNPs: Text Entry Example

One snp per line: rs945270

Search Options

SNP data set: 1000 Genomes Pilot 1

r^2 threshold: 0.8

Population panel: CEU

Distance limit: 500

Output Options

Download to: File

☑ Include each query snp as a proxy for itself

☐ Suppress warning messages in output

http://www.broadinstitute.org/mpg/snap/ldsearch.php

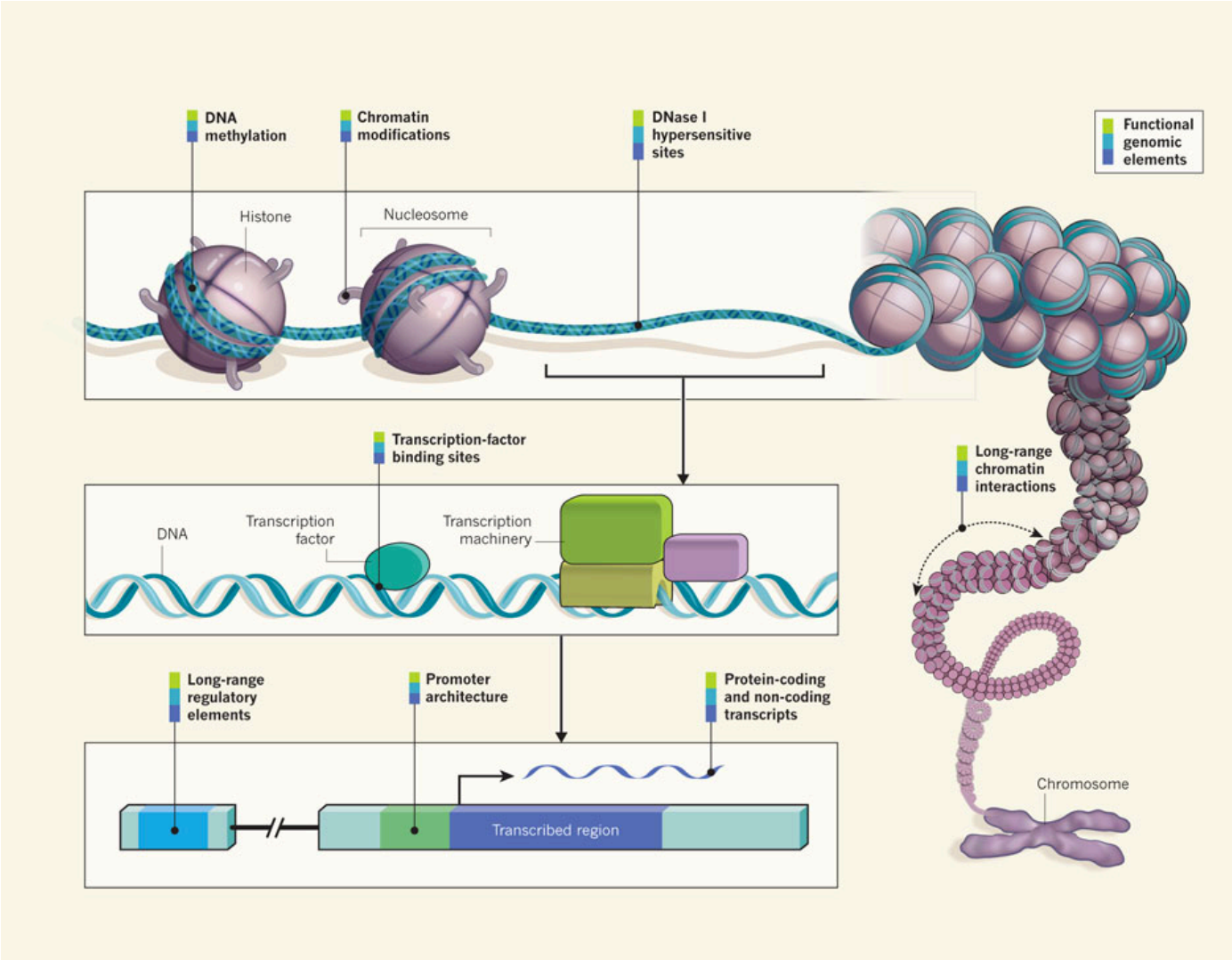
Finding Variants of Known Functionality

SNP	Proxy	Distance	RSquared	DPrime	Arrays	Chromosome	Coordinate_HG18
rs945270	rs945270	0	1	1	None	chr14	55270226
rs945270	rs8017172	1425	1	1	I2,I5,I6,I6	chr14	55268801
rs945270	rs1959089	2636	0.875	1	None	chr14	55267590
rs945270	rs1953350	3199	0.875	1	None	chr14	55267027
rs945270	rs1953351	3248	0.875	1	None	chr14	55266978
rs945270	rs1953352	3314	0.875	1	I3,I5,I6,I6	chr14	55266912
rs945270	rs2342589	3405	0.875	1	None	chr14	55266821
rs945270	rs2342588	3434	0.875	1	None	chr14	55266792
rs945270	rs868202	4711	0.875	1	None	chr14	55265515
rs945270	rs10129414	7201	0.875	1	None	chr14	55263025
rs945270	rs8012377	9060	0.875	1	AN,A5,A6	chr14	55261166
rs945270	rs10145631	11143	0.875	1	A6,OQ	chr14	55259083
rs945270	rs8014725	13520	0.875	1	None	chr14	55256706
rs945270	rs7157327	8023	0.84	0.964	None	chr14	55262203
rs945270	rs8021018	22965	0.807	0.929	I2,I5,I6,I6	chr14	55247261

Are any of the proxy SNPs in the list of functional SNPs?... Nope.

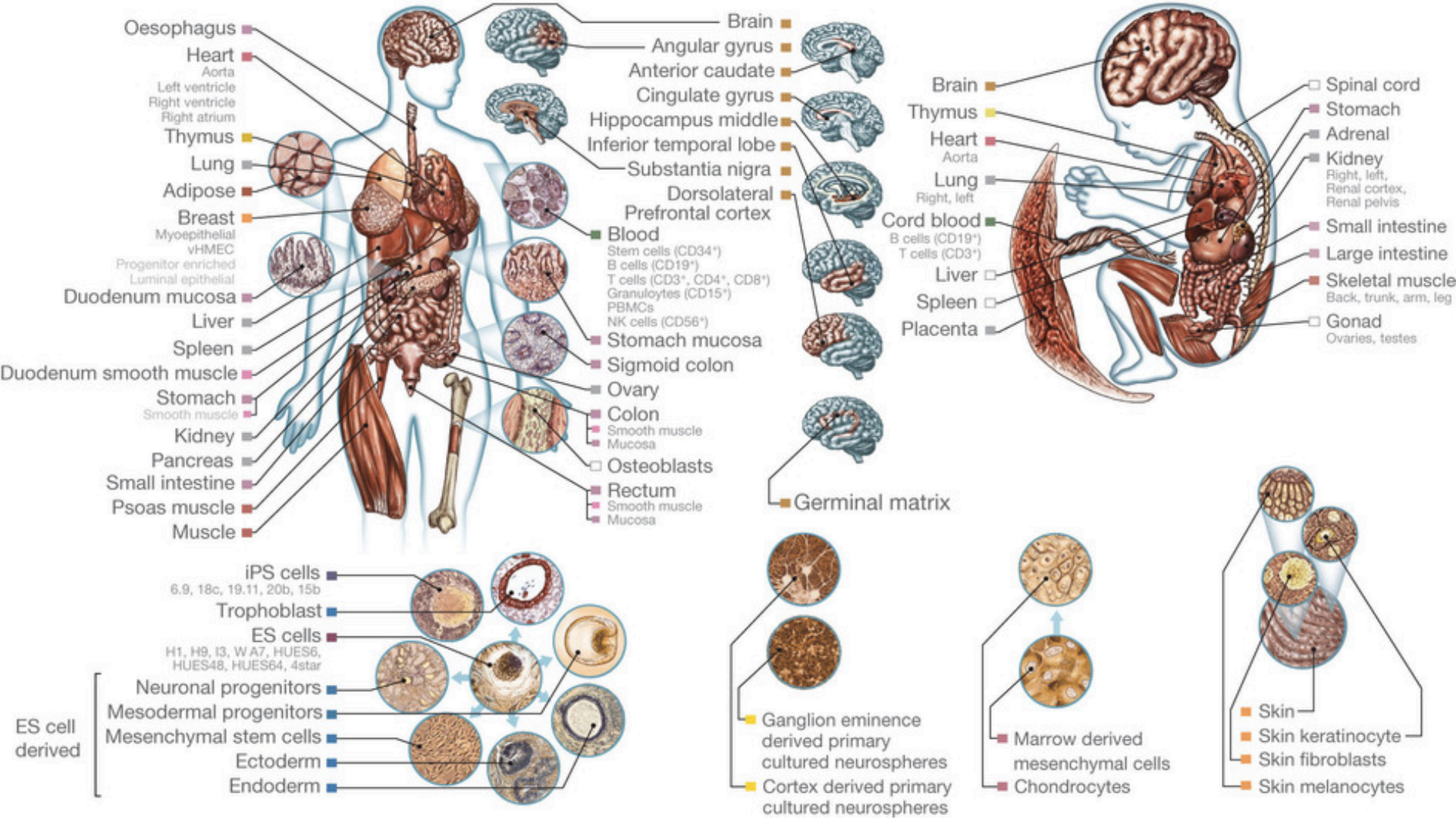
Our hit cannot be explained by known functional variants

Epigenetics



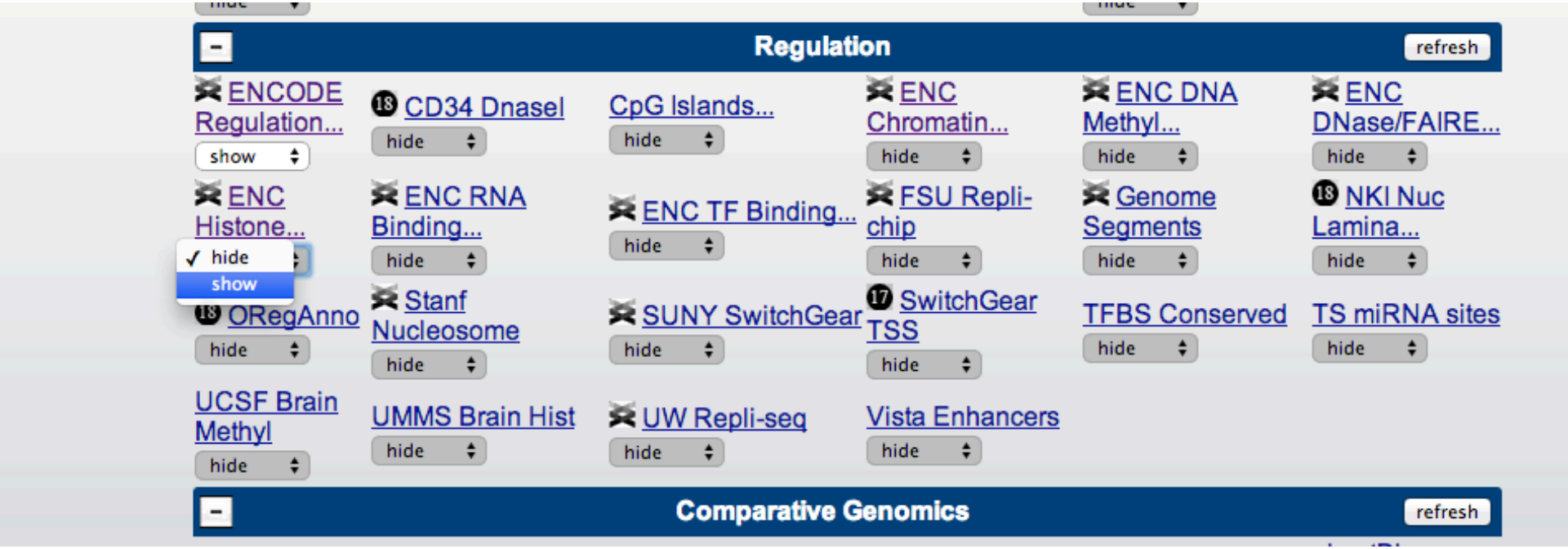
(Ecker et al., 2012)

Epigenetics Roadmap



(Roadmap Epigenetics Consortium et al., 2015)

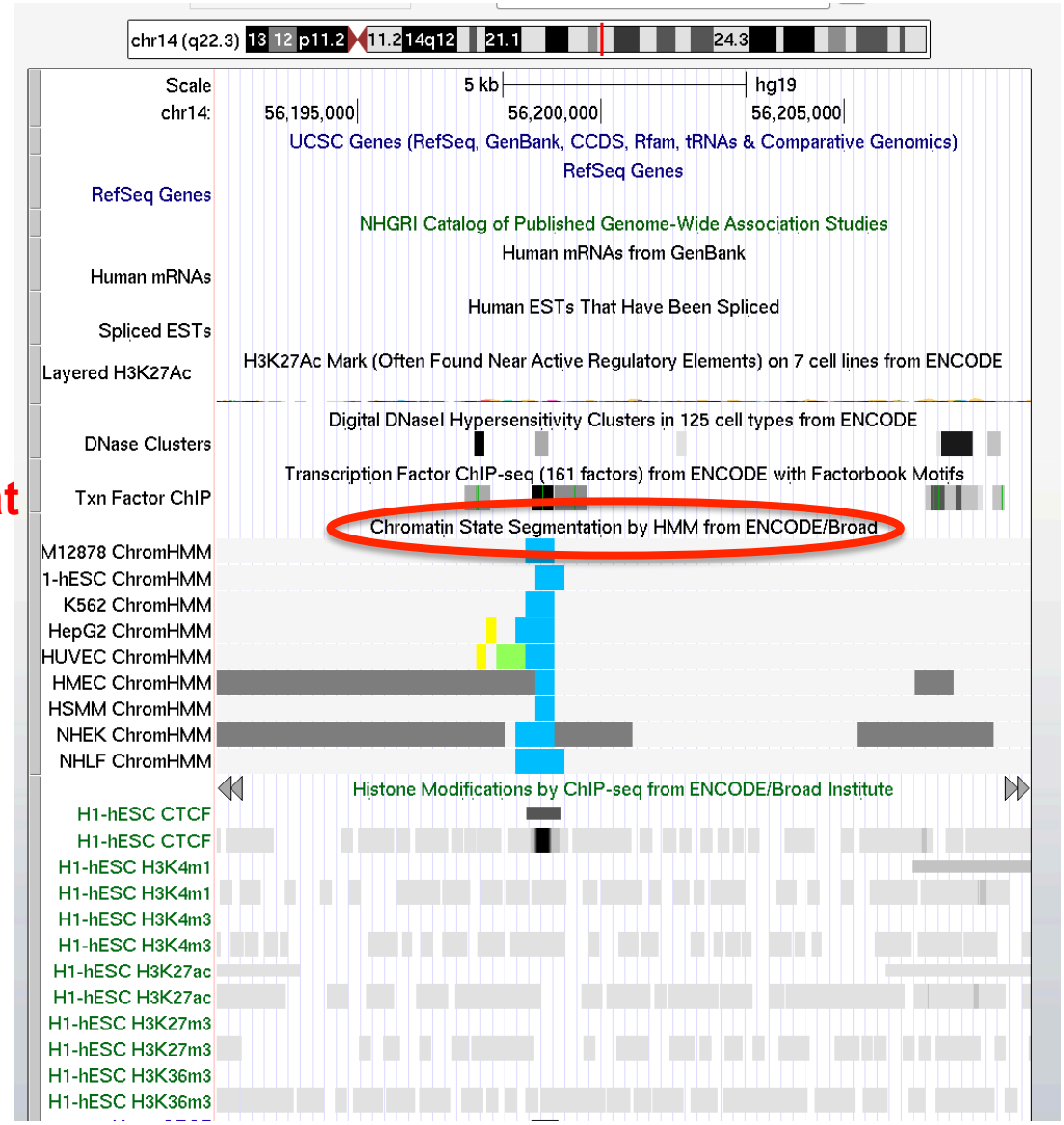
ENCODE in UCSC Genome Browser



Add some tracks that may help us explore function

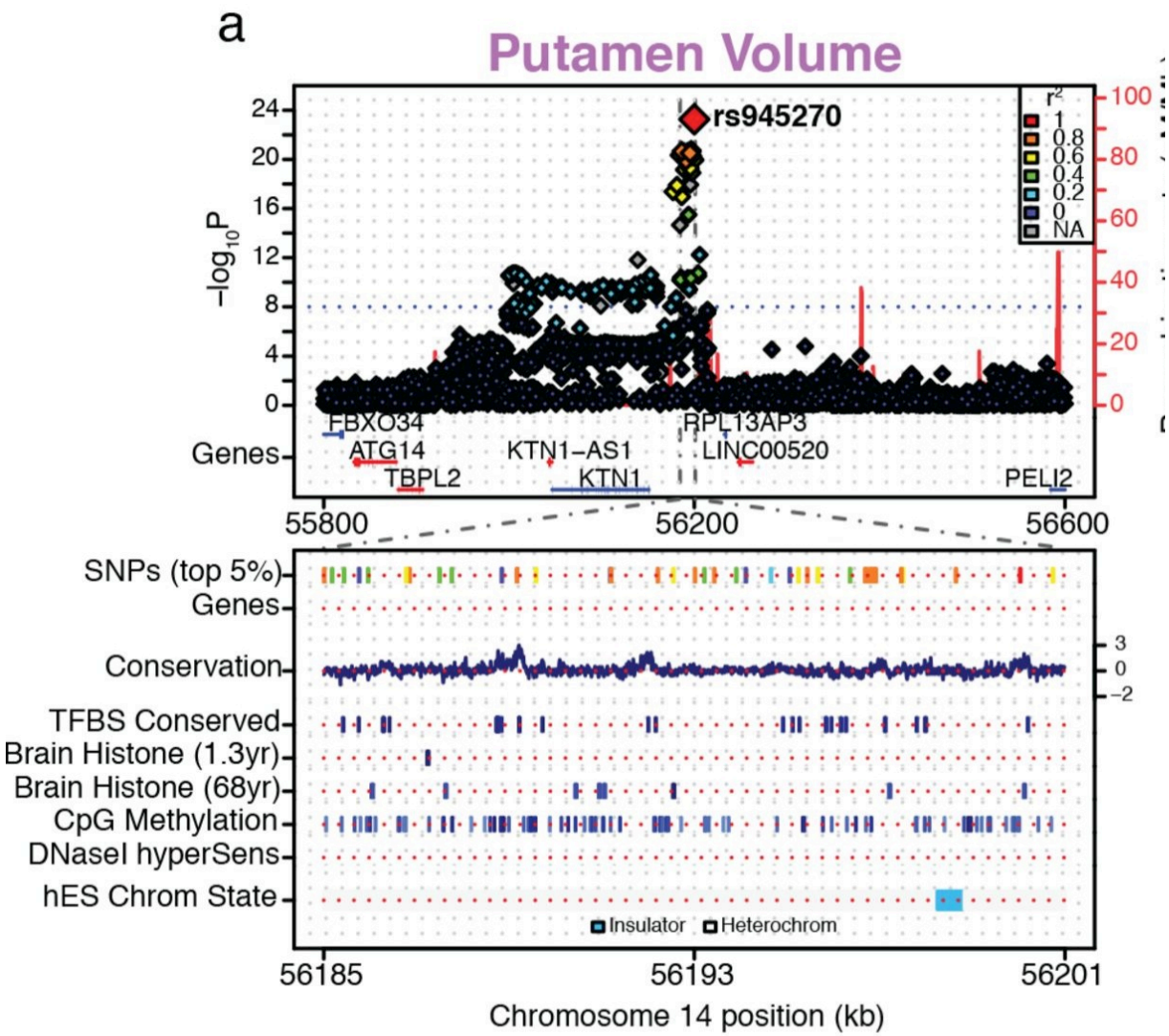
Epigenetics & ENCODE

Click to see what the colors mean



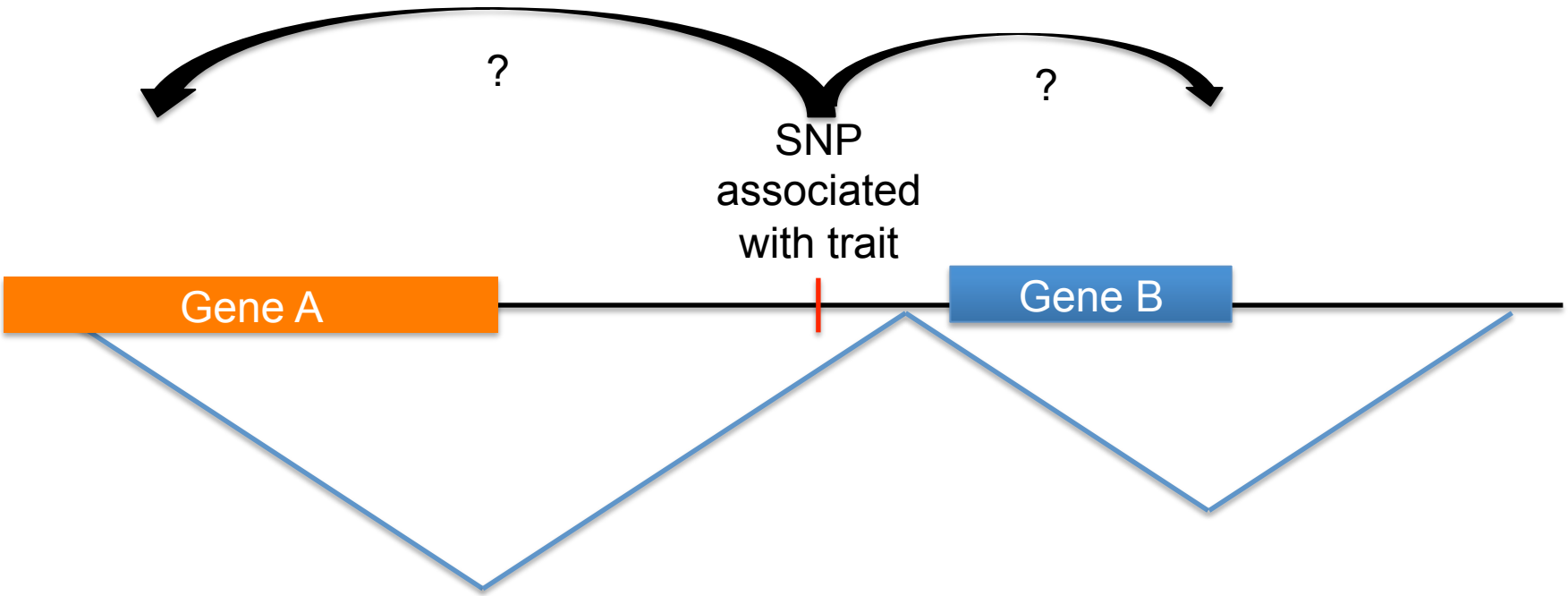
Appears to be a CTCF binding site (insulator) very close to the locus!

LocusTrack: Other ways to visualize



http://gump.qimr.edu.au/general/gabrieC/LocusTrack/index.html

expression QTLs (eQTLs)

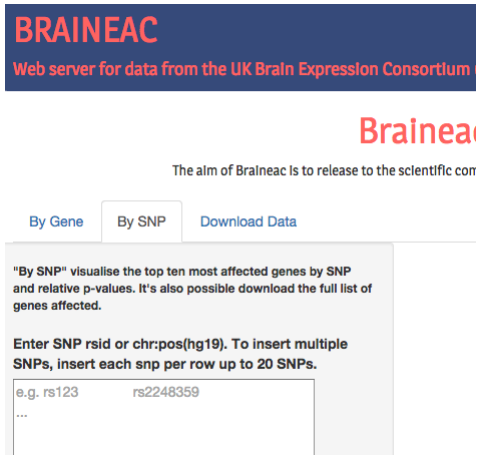


Do the alleles at the risk SNP affect the expression of any nearby genes?

A way to move from variant to gene.

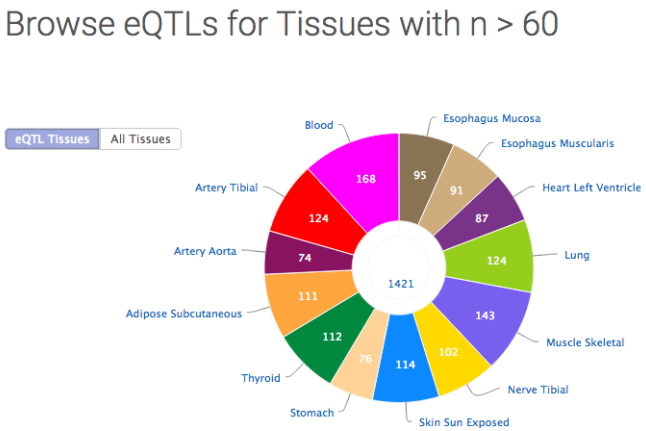
eQTL databases

BRAINEAC



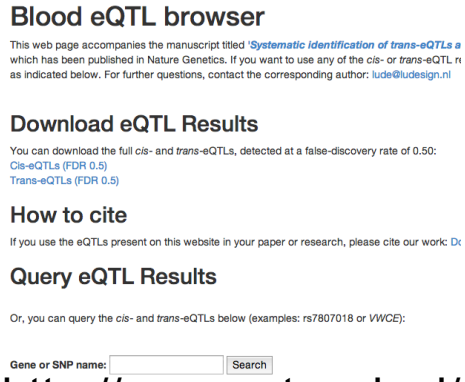
http://www.braineac.org/

GTEx



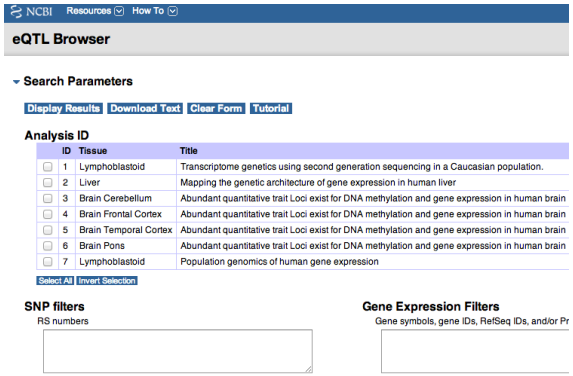
http://www.gtexportal.org/home/
(Brain on its way)

Blood eQTL browser



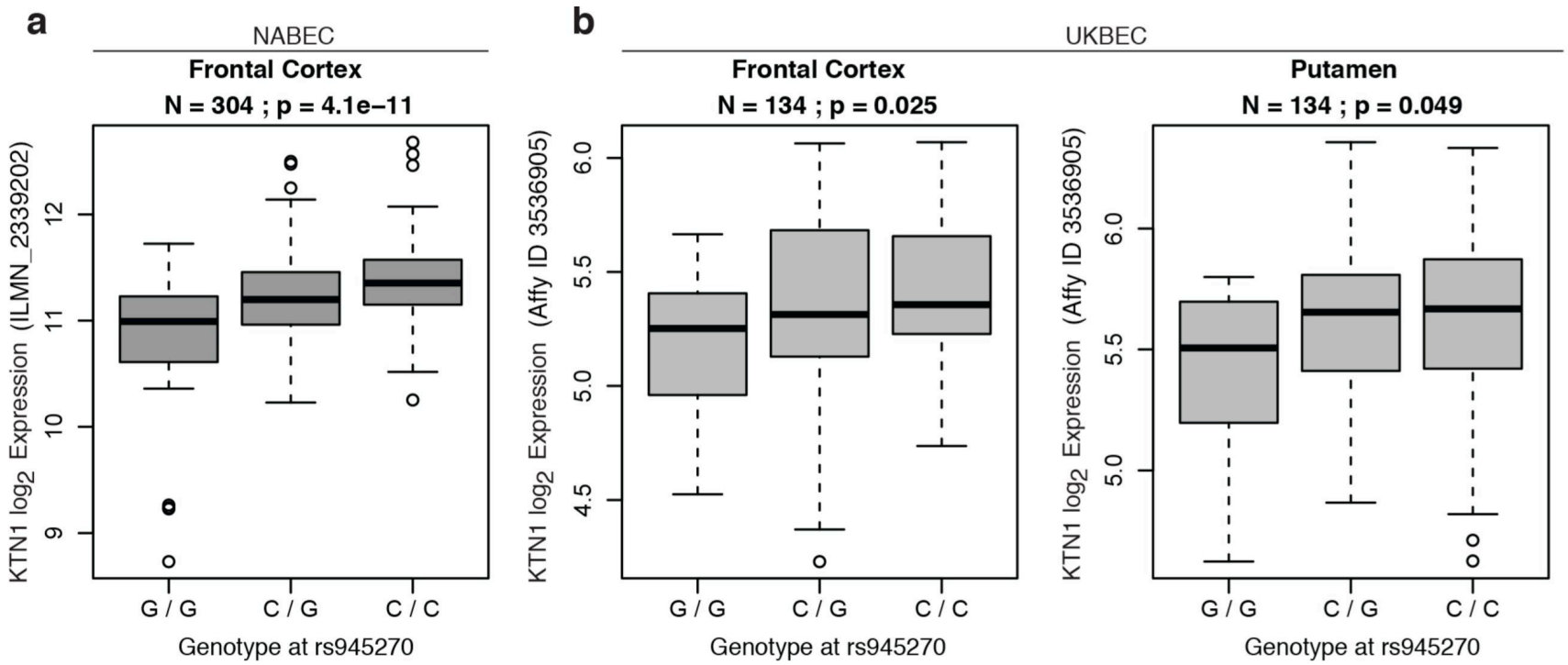
http://genenetwork.nl/bloodeqtlbrowser/

NCBI eQTL Browser



http://www.ncbi.nlm.nih.gov/projects/gap/eqtl/index.cgi

eQTL phone a friend



This SNP affects a gene (replicated in brain),
we now have a gene!

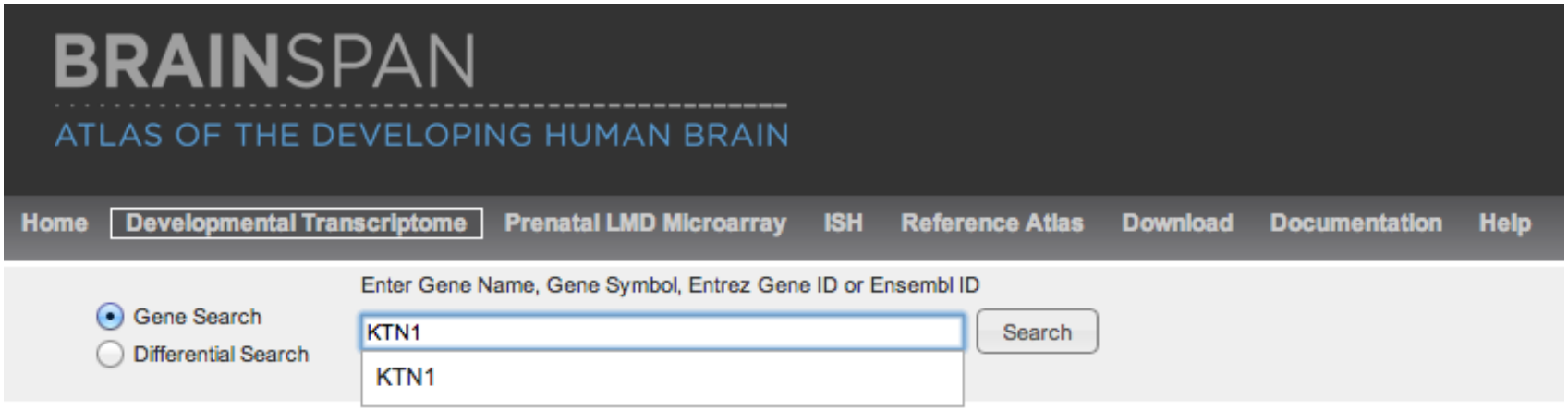
When and Where is Gene Expressed?

- 86-95% of genes are expressed in the brain at some point during the lifespan and 90% of those were differentially regulated across region or time (Kang et al., 2011; Miller et al., 2014).

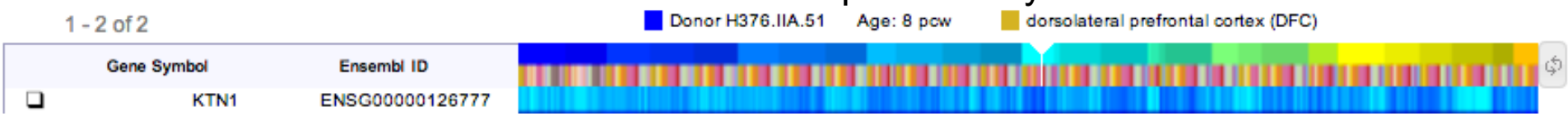
- Expression of a gene in brain does little to implicate it as causal.

- However, finding the time period or region of gene expression may lead us to cell type hypotheses.

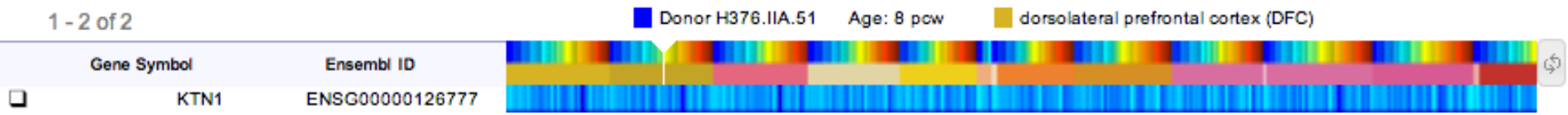
When and Where is Gene Expressed?



Expression by time →

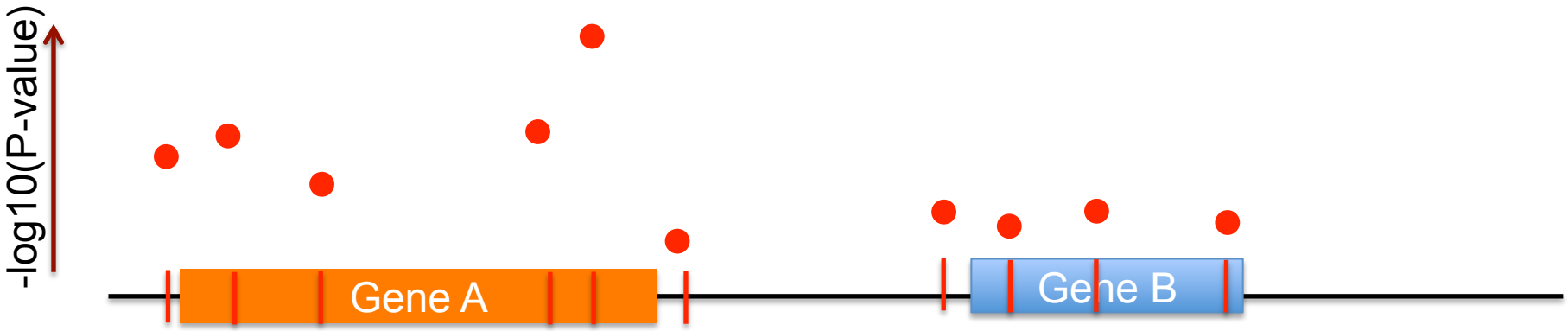


Expression by region →



http://brainspan.org/rnaseq/search/index.html

Gene-based tests



Combines SNP associations across genes to form a gene based p-value

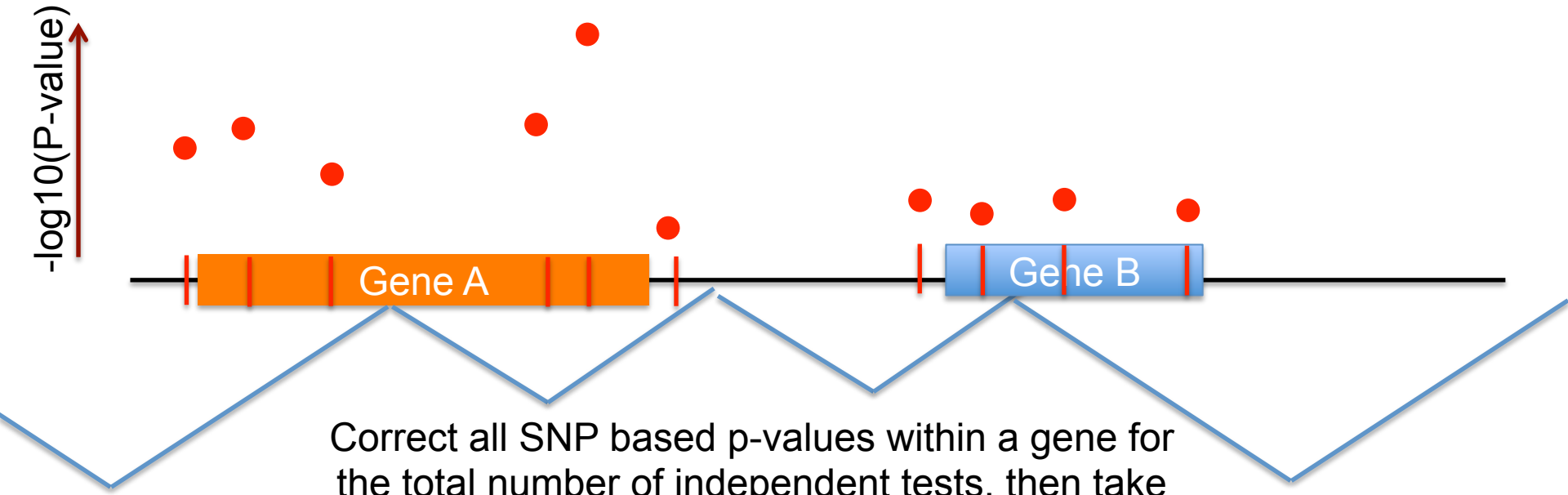
<u>Advantages</u>

- Greater interpretability
- Fewer multiple comparisons
- Can feed into pathway based approaches

<u>Disadvantages</u>

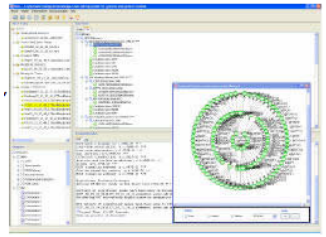
- Ignores intergenic variation (most of genome)
- Generally ignores direction of association
- Ignores that a variant within the intron of one gene may be affecting a totally different gene

Tools for Gene Based Analyses



Correct all SNP based p-values within a gene for the total number of independent tests, then take the minimum p-value.

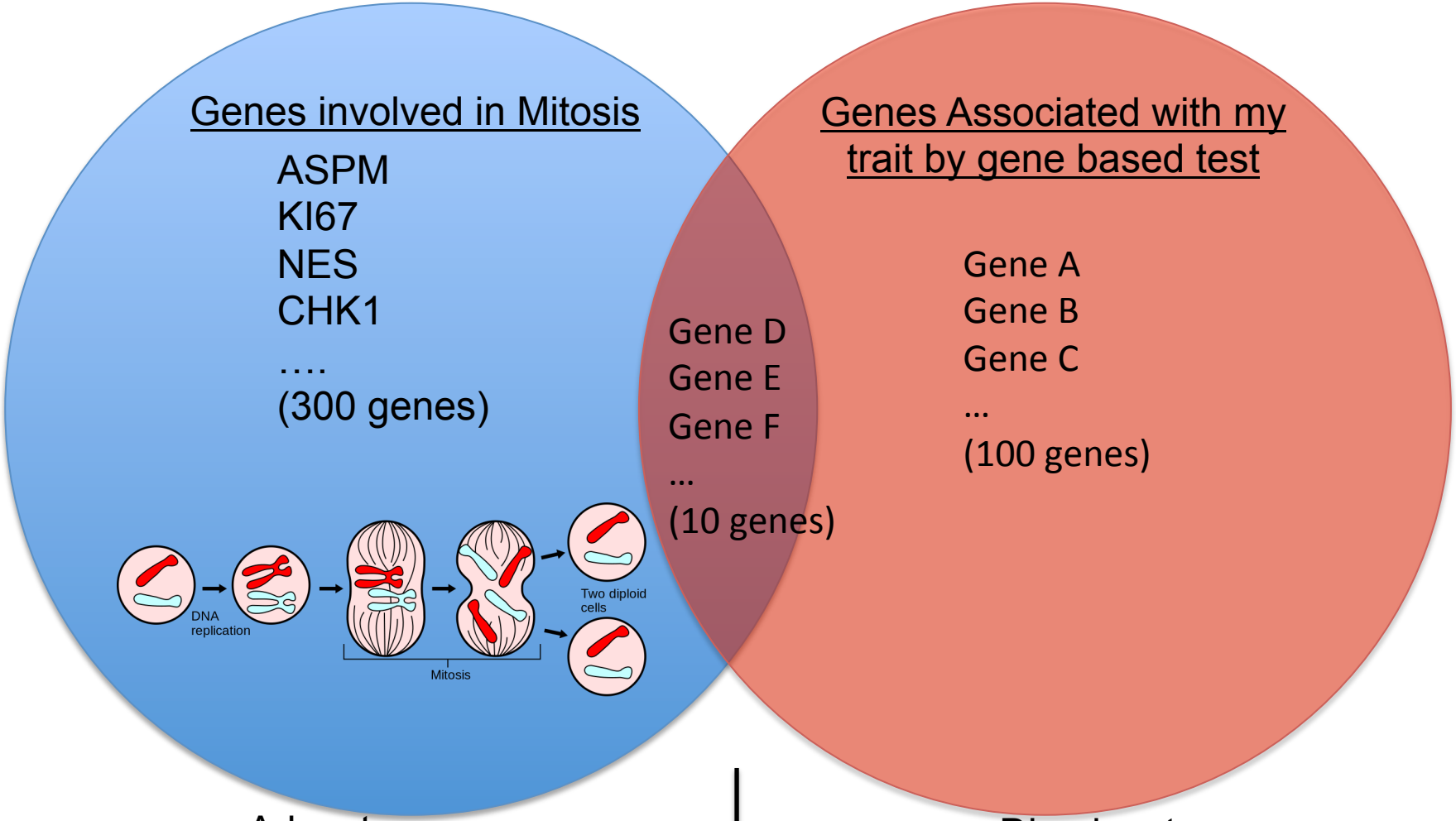
See GATES algorithm implemented in KGG toolbox (Li et al., 2011)



http://statgenpro.psychiatry.hku.hk/limx/kgg/index.html

Pathway Analysis

Look for enrichment of your associated genes in known pathways



Advantages

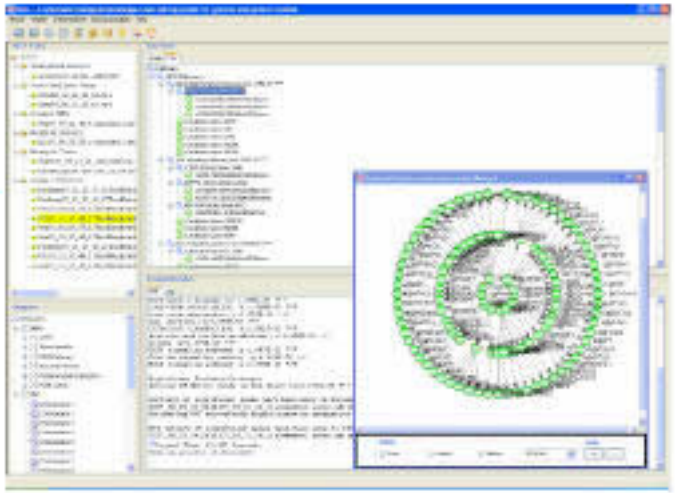
- Amazing interpretability – exactly what you're looking for

Disadvantages

- Uses gene based tests
- Pathway gene lists are generally not well known

Tools for Pathway Based Analyses

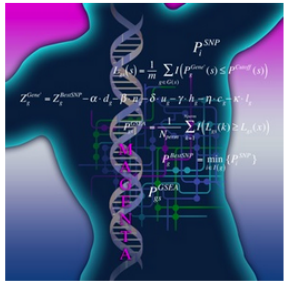
Knowledge-Based Mining System for Genome-Wide Genetic Studies (KGG)



http://statgenpro.psychiatry.hku.hk/limx/kgg/index.html

MAGENTA

MAGENTA: Meta-Analysis Gene-set Enrichment of variaNT Associations



http://www.broadinstitute.org/mpg/magenta/

Conclusions

- Identifying the genetic locus is a causal foothold into understanding novel biological mechanisms.
- There are many databases and tools that will allow you to form hypotheses about the biological mechanisms.
- It’s easy to make a story! Let the evidence guide you.

Acknowledgements

Derrek Hibar, IGC
Sarah Medland, QIMR
Miguel Renteria, QIMR