

PhenoGen Informatics

The site for quantitative genetics of the transcriptome
<http://phenogen.ucdenver.edu>

The PhenoGen Group

Initiated and directed by Dr. Boris Tabakoff

Presented by Dr. Laura Saba

Anschutz Medical Campus

University of Colorado Denver

****Please use Firefox for optimal results and to follow along
in the workshop**

Acknowledgements

The PhenoGen Group:

Boris Tabakoff, PhD; Paula L. Hoffman, PhD; Spencer Mahaffey, MA; Laura Saba, PhD; Stephen Flink, PhD; Lauren Vanderlinden, MS; Yinni Yu, MS

Collaborators:

Morton Printz, PhD; Michal Pravenec, PhD

Technical Support:

Adam Chapman; James Huntley; Laura Breen; Donna Moye

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Outline

1. Introduction to PhenoGen Informatics
2. Interactive demo of candidate gene approach
3. Summary of phenotype approach
4. Downloadable data
5. Future directions

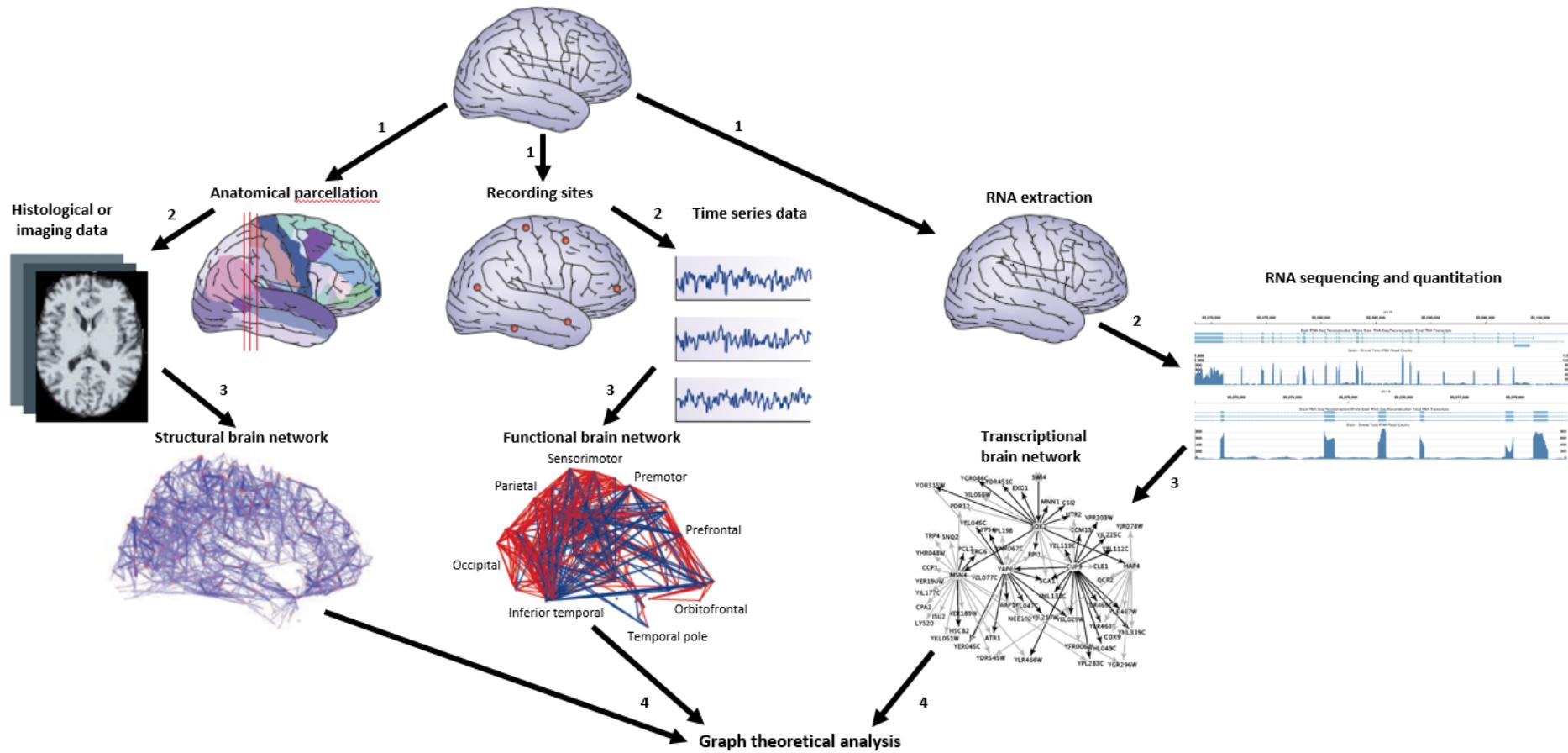
Goal for the PhenoGen Project

Transcriptional Connectome

To generate a new image of organs as networks of interacting elements (transcripts)

- Collect genome sequence and full transcriptome information for organs (brain, liver, heart).
 - We have completed exon array analysis for these three organs
 - RNA-Seq for brain in 30 strains of the HXB/BXH panel completed
 - RNA-Seq for heart and liver of the RI progenitor strains completed

Brain Transcriptional Connectome



Why Networks?

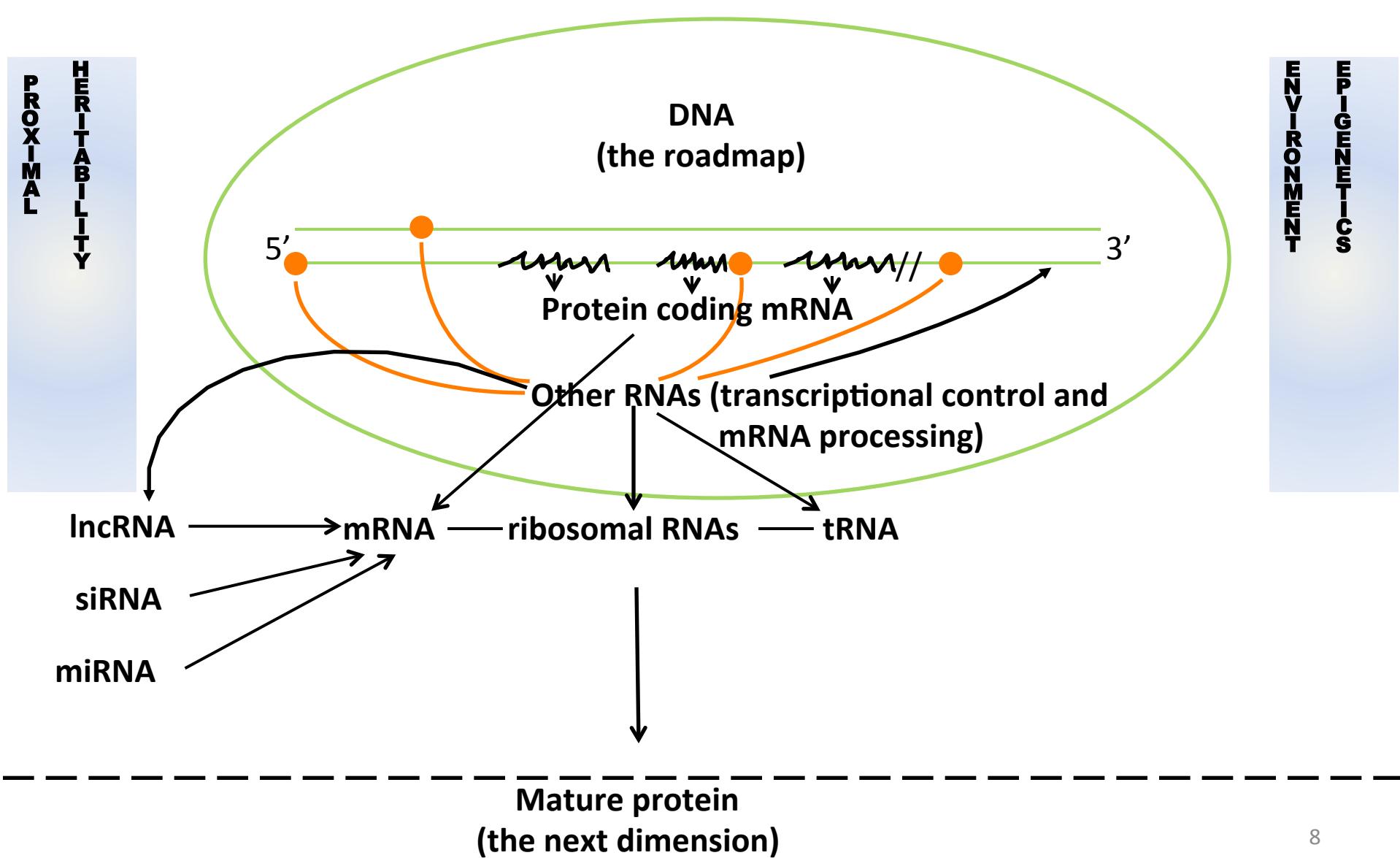
1. The brain is a complex hierarchical network spatio-temporally linked through structure and function.
2. Complex pathologic traits can be conceptualized as systems disorders of failed network regulation.
3. The brains structural and functional systems have features of complex networks that can be described through application of “graph theory” (small world topology).
4. The generation of a “Transcriptional Connectome” representing the Resting State transcriptional networks provides power for understanding predisposition to disease, etiology of organ or behavioral pathology and response to medications or toxins.

Why Study the RNA Dimension

Transcriptome links DNA and complex traits/diseases

- A. RNA is one of the first quantitative links between DNA sequence and phenotype (an endophenotype).
- B. Transcriptome information addresses part of the GWAS Gap: how does an identified DNA polymorphic locus contribute to disease?
- C. First step where DNA sequence and environment interact.
- D. Implementation of graph theory at the transcript level provides insight into genetic/environmental interactions that are the basis for susceptibility to complex diseases.

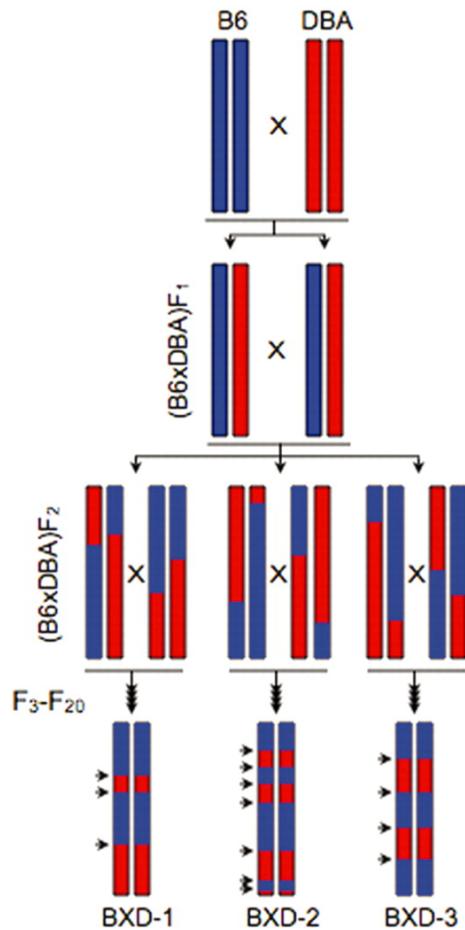
The RNA Dimension (the true intermediate phenotype)



Co-expression as a measure of the “connectome”

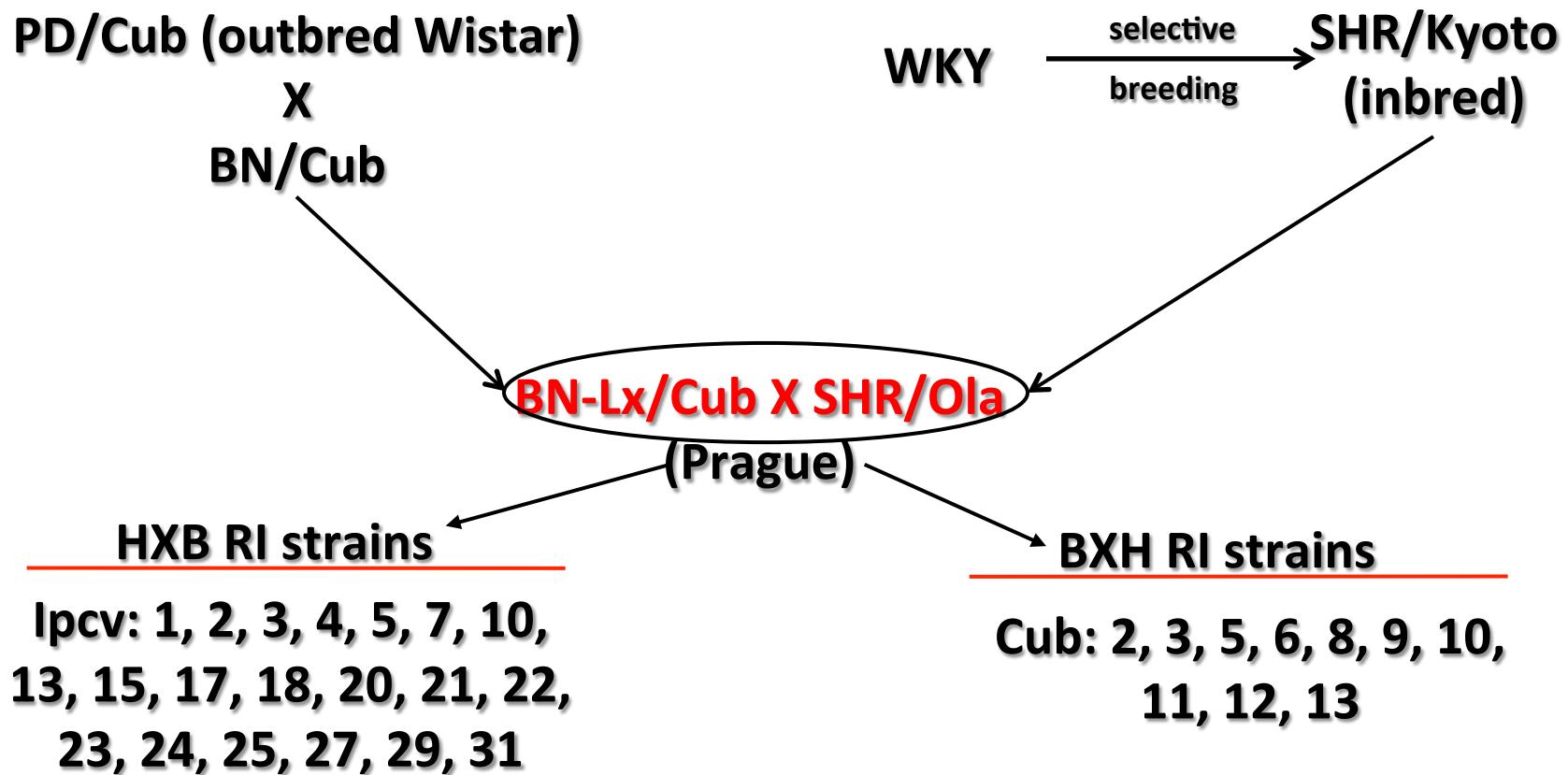
- **Theory** – if the magnitude of RNA expression of two transcripts correlates over multiple “environments” (genomes), then the two transcripts are involved in similar biological processes
- Caveats when multiple environments are multiple genetic backgrounds (false positive correlations)
 - Linkage Disequilibrium
 - Cell-type mixing proportions

Recombinant Inbred Rodent Panel



- Genetic identity is retained over generations
- Cumulative genetic and phenotype data across labs
- Ideal genetic controls for studying interventions/ environmental effects

Origin of HXB/BXH RI Panel



- > 4,500,000 SNPs/indels between progenitors
 - Haplotype map generated

Current Data – RNA-Seq

Strains	Tissue	Sex	Number of Biological Replicates Per Strain	Number of Paired-End Reads (rRNA-depleted total RNA)	Number of Paired-End Reads (polyA+ selected RNA)	Number of Single- End Reads (small RNA)
SHR and BNLx	brain	male	3	645 million	192 million	96 million
SHR and BNLx	brain	female	4	982 million	---	297 million
SHR and BNLx	liver	male	3	583 million	---	342 million
SHR and BNLx	heart	male	4	790 million	---	300 million
30 RI Strains	brain	male	1 to 2	7.3 billion	----	1.9 billion

Current Data - Microarrays

Panel	Platform	Tissue	Number of Biological Replicates
21 HXB/BXH RI Strains	Exon Array	Brain	2 to 4
21 HXB/BXH RI Strains	Exon Array	Liver	2 to 4
21 HXB/BXH RI Strains	Exon Array	Heart	2 to 4
21 HXB/BXH RI Strains	Exon Array	Brown Adipose	2 to 4
60 LXS Mouse RI Strains	Exon Array	Brain	6
30 BXD Mouse RI Strains	3' Array	Brain	4 to 7
26 Inbred Mouse Strains	3' Array	Brain	4 to 6

CANDIDATE GENE EXAMPLE

Transcriptome Information on a Gene of Interest

- Genome View
 - Annotated isoforms
 - DNA variants in parental strains
 - Published QTL
- Transcriptome View
 - Exon array information
 - RNA-Seq results
- Selected Feature
 - Gene details and external links
 - Gene eQTL
 - Weighted gene co-expression network analysis
 - Probe set level data

Genome View

1. Click Genome/
Transcriptome Data
Browser
2. Type in Rat/Mouse
Official Gene Symbol
into “Gene Identifier or
Region” box
3. Select **Rattus Norvegicus**
(rn5) from “Species” box
4. Select **Genome**
(Predefined) from
“Initial View” box
5. Click “Go”

The site for quantitative genetics of the transcriptome.

Welcome to PhenoGen Informatics

The site for quantitative genetics of the transcriptome.

Hover over or click on nodes in the graph below to see the tools/data available on the site.
Green no login required.
Blue sections require a login.

PhenoGen - Genome/Transcriptome Data Browser

PhenoGen Informatics

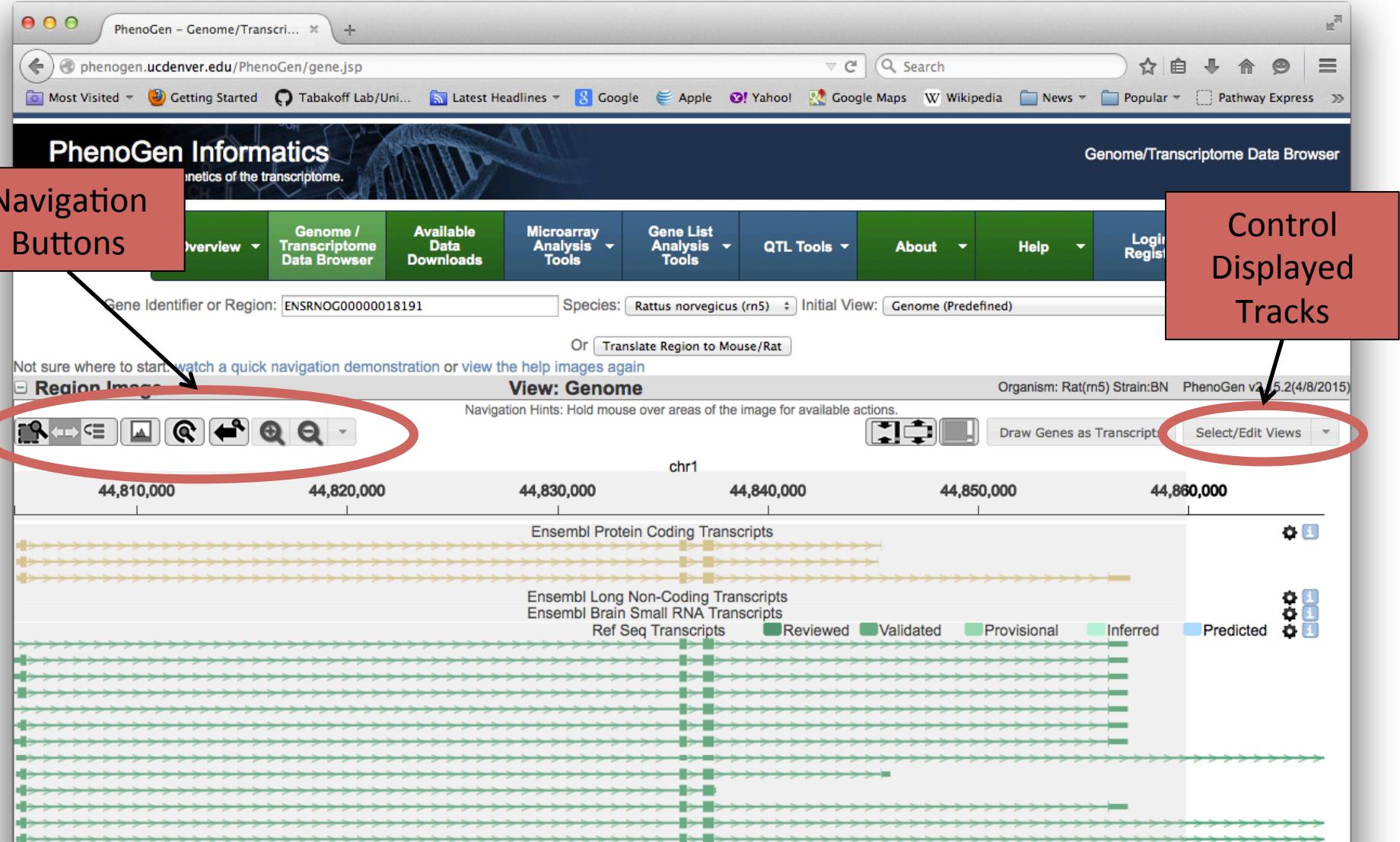
The site for quantitative genetics of the transcriptome.

1. Enter a gene identifier(e.g. gene symbol, probe set ID, Ensembl ID, etc.) in the gene field.
or
Enter a region such as
"chr1:1-50000" which would be Chromosome 1 @ bp 1-50,000.
"chr1:5000+-2000" which would be Chromosome 1 @ bp 3,000-7,000.
"chr1:5000+2000" which would be Chromosome 1 @ bp 5,000-7,000.
or
Click on the Translate Region to Mouse/Rat to find regions on the Mouse/Rat genome that correspond to a region of interest in the Human/Mouse/Rat genome.
2. Choose a species.
3. Click Get Transcription Details.

Gene Identifier or Region: Oprm1 Species: Rattus norvegicus (rn5) Initial View: Genome (Predefined) Go

Or Translate Region to Mouse/Rat

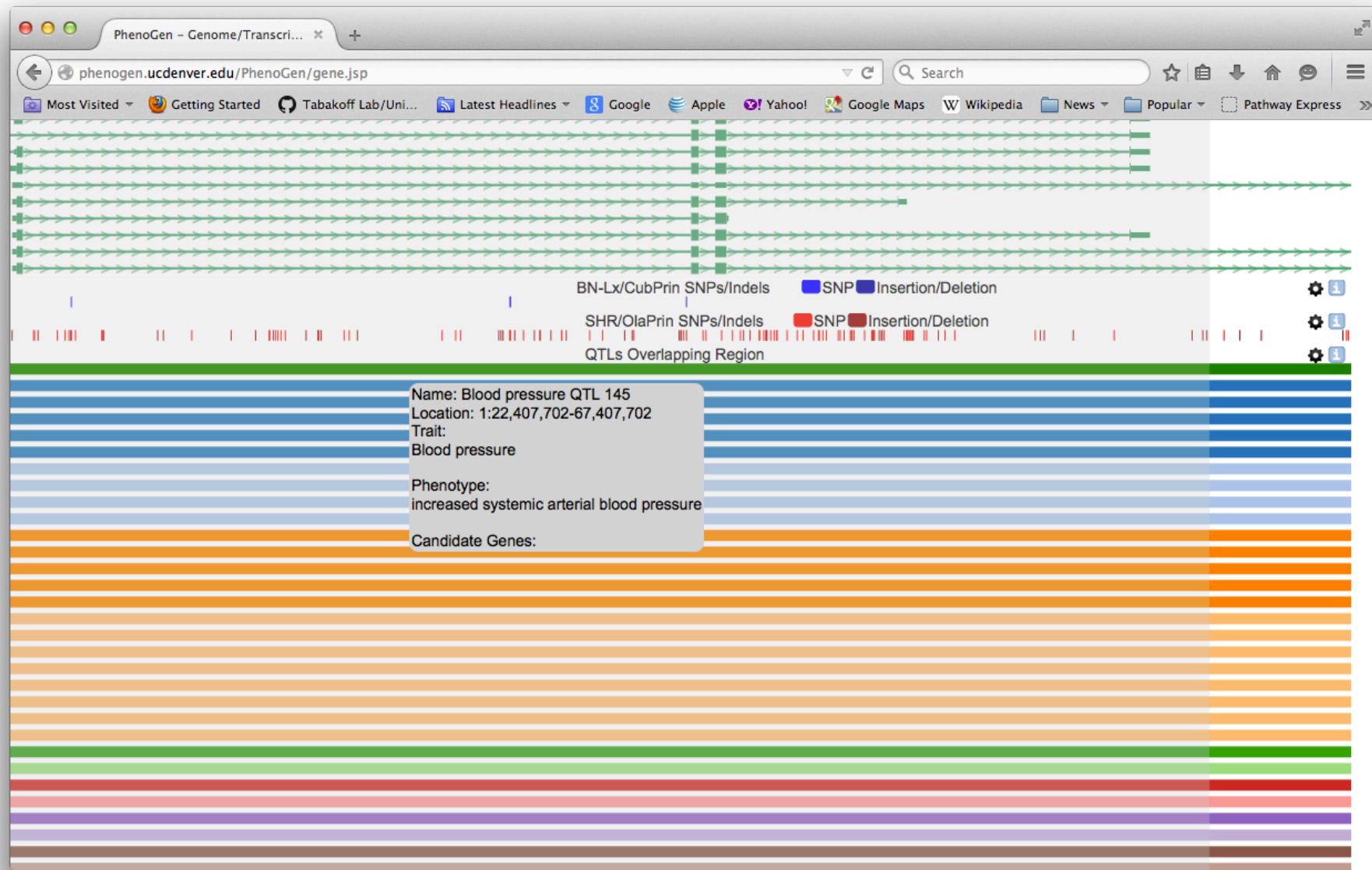
Quick Navigation Demonstration Custom View/Custom Track Demonstration



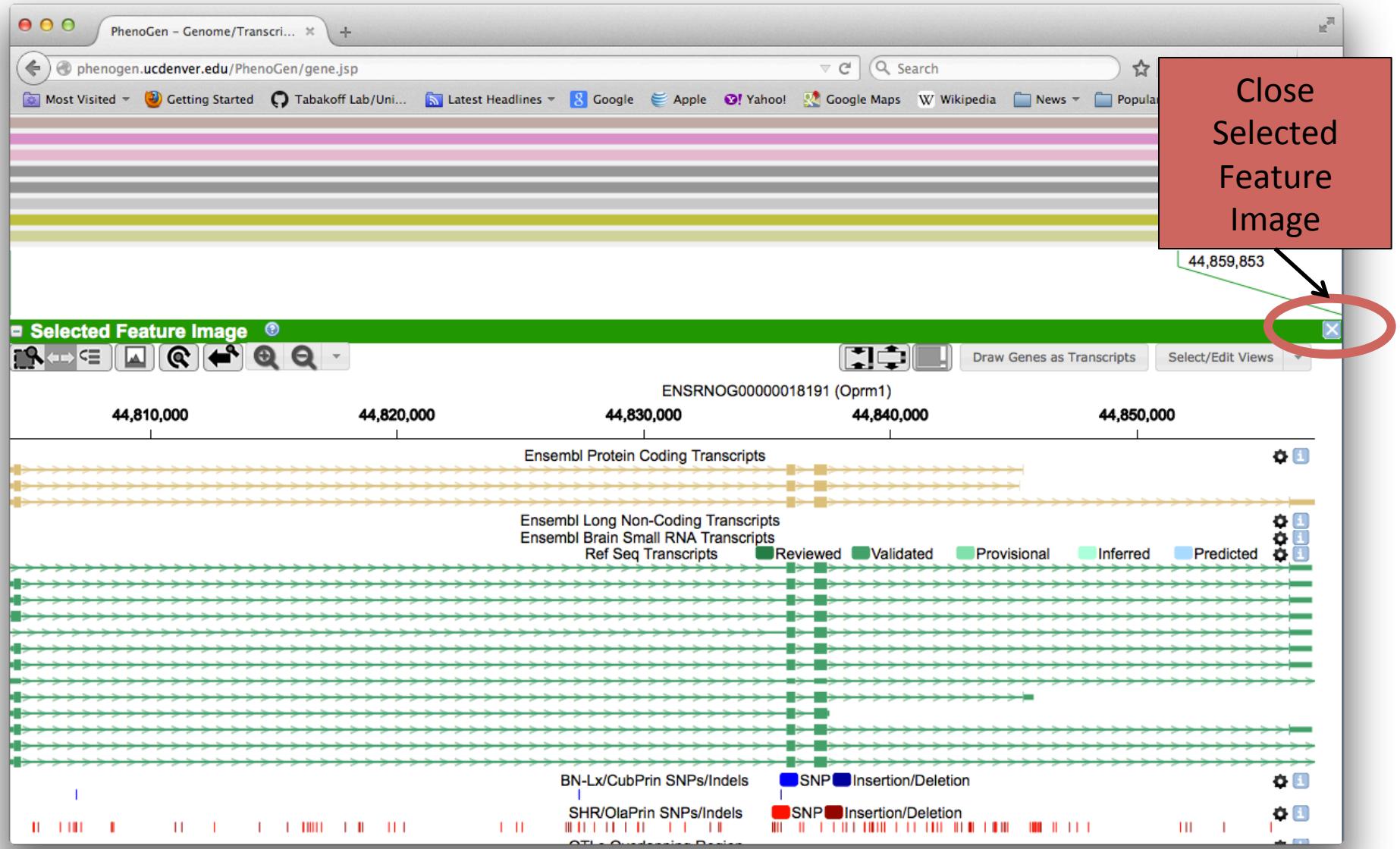
Genetic Variants Between Parental Strains of RI Panel



Published Behavioral/Physiologic QTL



Get Region Summary



Region Summary

PhenoGen - Genome/Transcri... × +

phenogen.ucdenver.edu/PhenoGen/gene.jsp

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Track Details Genes with an eQTL In this region WGCNA

Region Summary

Track List:

- Ensembl Protein Coding Transcripts: 3
- Ensembl Long Non-Coding Transcripts: 0
- Ensembl Brain Small RNA Transcripts: 0
- BN-Lx/CubPrin SNPs/Indels: 4
- Ref Seq Transcripts: 13
- SHR/OlaPrin SNPs/Indels: 132
- QTLs Overlapping Region: 41

Break down of track count*

*Note: Depending on the track settings some features may not be displayed and will not be reflected in the image above.

Features in Selected Track

+ View Columns

RGD ID	QTL Name	Trait	Phenotype	Associated Diseases	References RGD Ref	Candidate Genes	bQTL Region	LOD Score
70225	Blood pressure QTL 58	Blood pressure - direct systolic	increased systemic arterial blood pressure	Hypertension	70067 69692 11082136 68885 11160999		chr1:36,395,668-180,434,202	3.3
631494	Blood pressure QTL 95	Blood pressure - systolic	increased systemic arterial blood pressure	Hypertension		Slc9a3	chr1:23,257,188-50,297,622	40.0

Alternate Region Summary Under Different View

PhenoGen – Genome/Transcri... × +

phenogen.ucdenver.edu/PhenoGen/gene.jsp

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Brain Illumina PolyA+ RNA Read Counts <1 1 5,000+ Read Counts

Brain Illumina Total RNA(rRNA depleted) Read Counts <1 1 5,000+ Read Counts

Track Details Genes with an eQTL in this region WGCNA

Region Summary

Track List:

- Affy Exon 1.0 ST Probe Sets: 38
- Brain RNA-Seq Reconstruction Protein Coding / PolyA+ Transcripts: 0
- Brain RNA-Seq Reconstruction Long Non-Coding / Non-PolyA+ Transcripts: 1

Break down of track count*

Category	Count
Black	14
Green	12
Red	8

*Note: Depending on the track settings some features may not be displayed and will not be reflected in the image above.

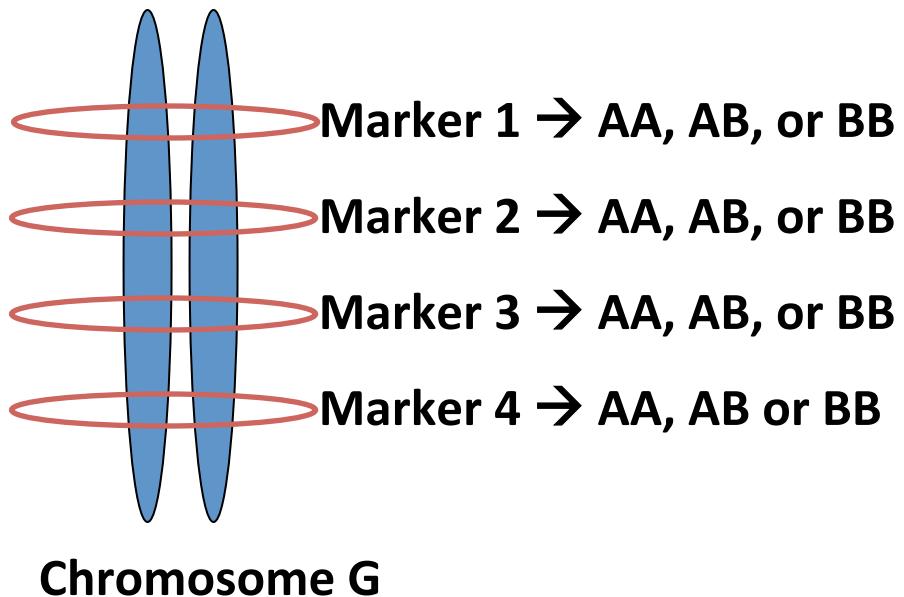
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Source Code (GitHub) Legal Notices Privacy Policy

Follow Follow Follow

Quantitative Trait Loci

- Definition – area of the genome where polymorphisms are associated with a quantitative trait

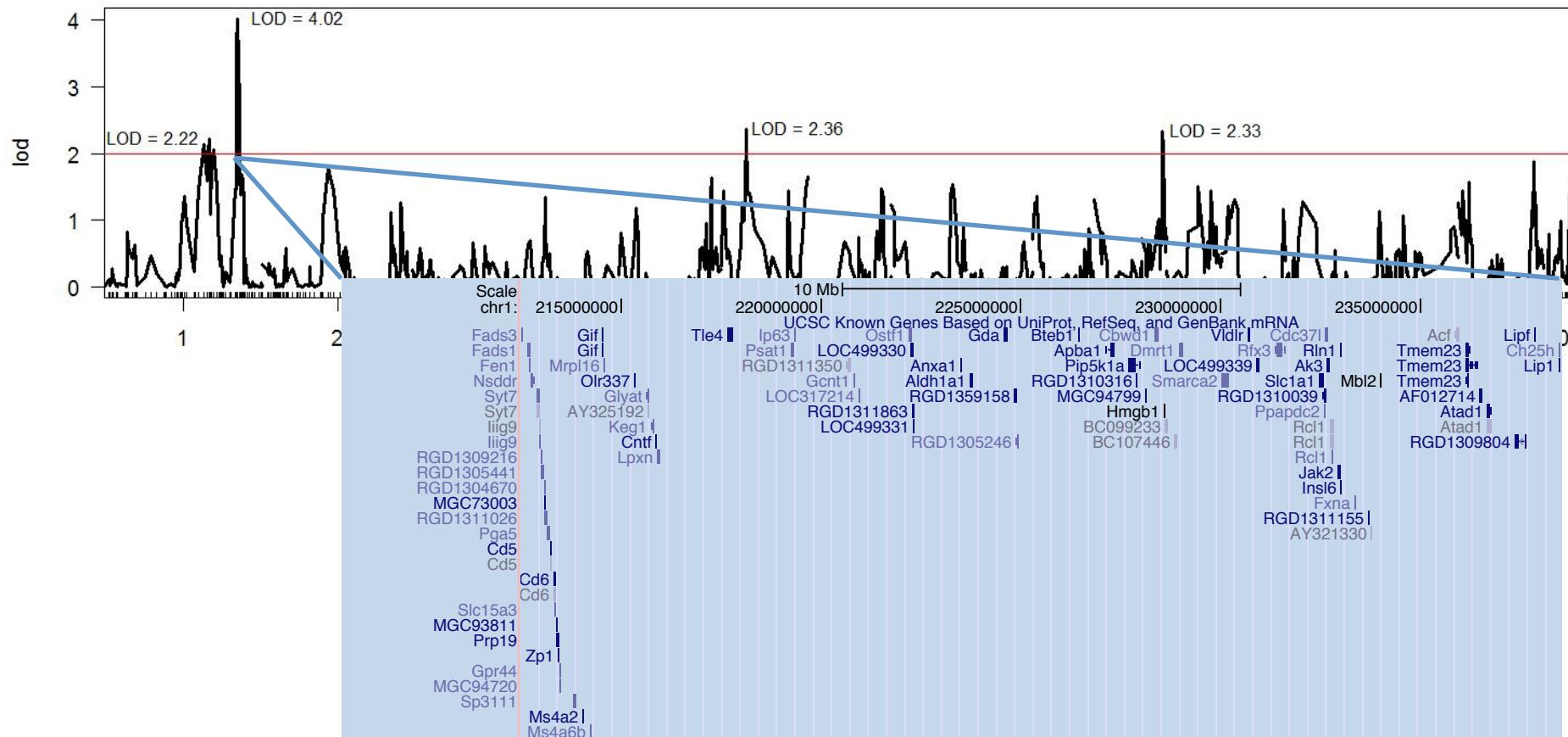


At each marker:

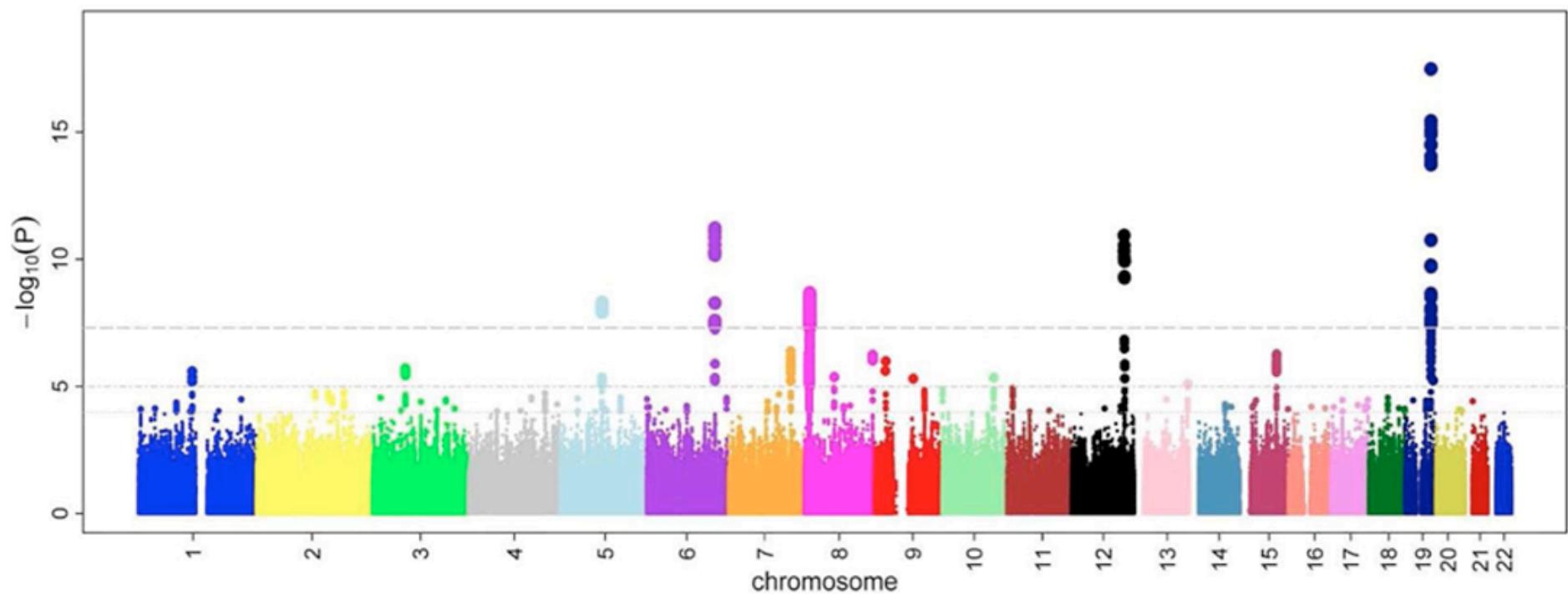
1. Split population into two/three groups based on genotype
2. Compare mean values for the quantitative trait (phenotype) between the groups
3. LARGE Statistically Significant Differences → QTL

Example LOD Plot

Alcohol Consumption in HXB/BXH Rats - Week 2

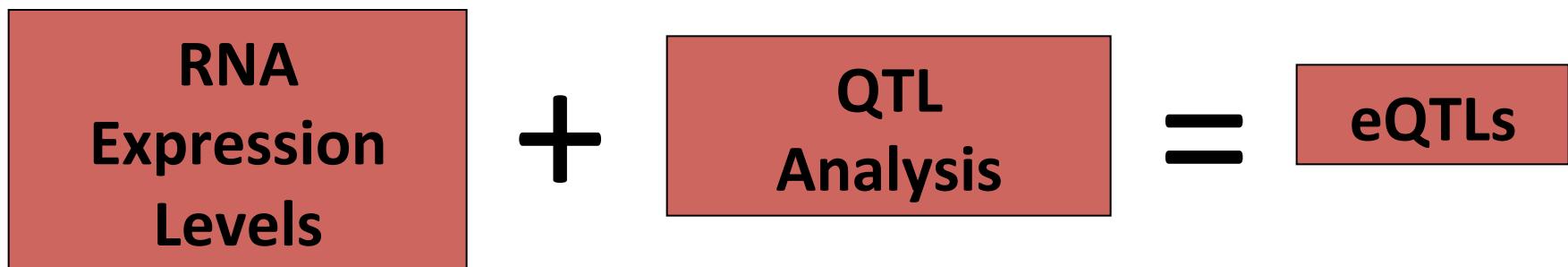


Example Manhattan Plot

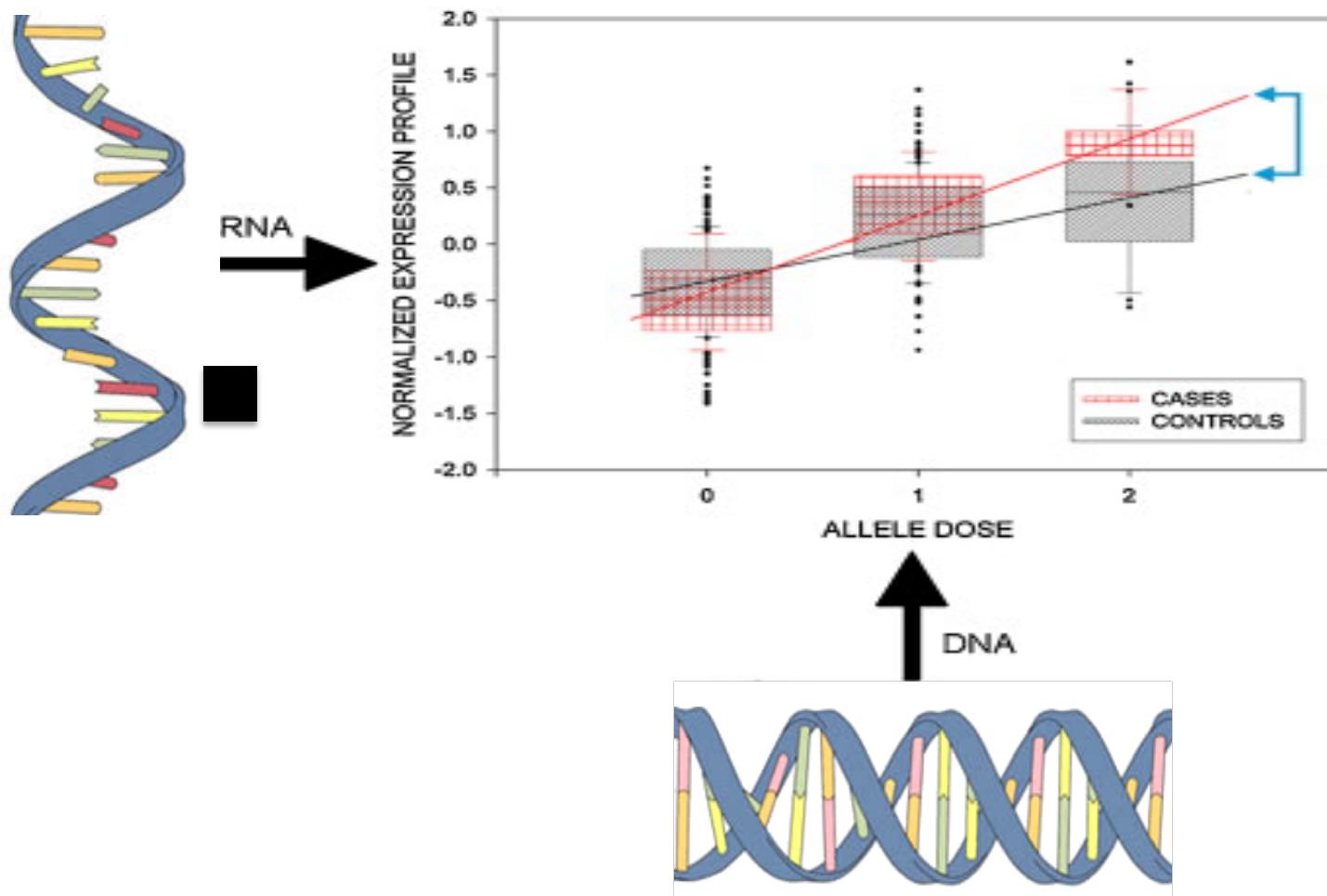


Definition of Genetical Genomics

The study of the genomic location where transcription is controlled by determining expression Quantitative Trait Loci (eQTL).



eQTL Definition



Myers, AJ. The age of the “ome”: Genome, transcriptome and proteome data set collection and analysis. Brain Research Bulletin
Volume 88, Issue 4 2012 294 - 301

Types of eQTL

- *cis*-eQTL (or local eQTL) – the locus controlling transcription is “near” the physical location of the gene in the genome
- *trans*-eQTL (or distal eQTL) – the locus controlling the transcription is NOT “near” the physical location of the gene in the genome

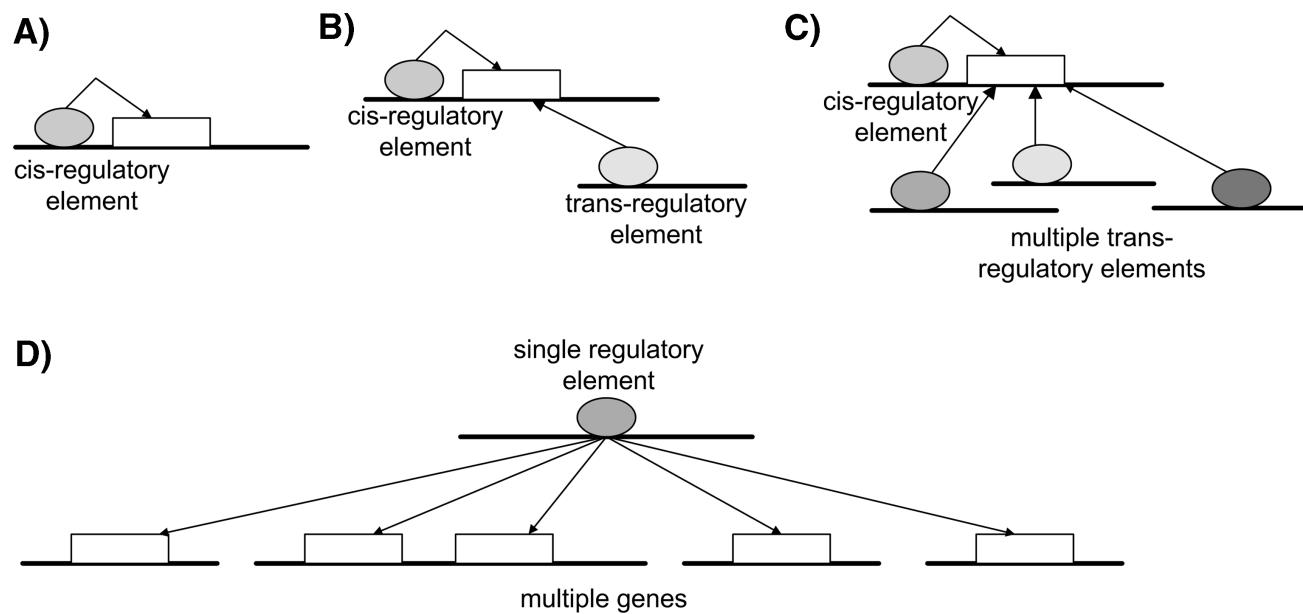
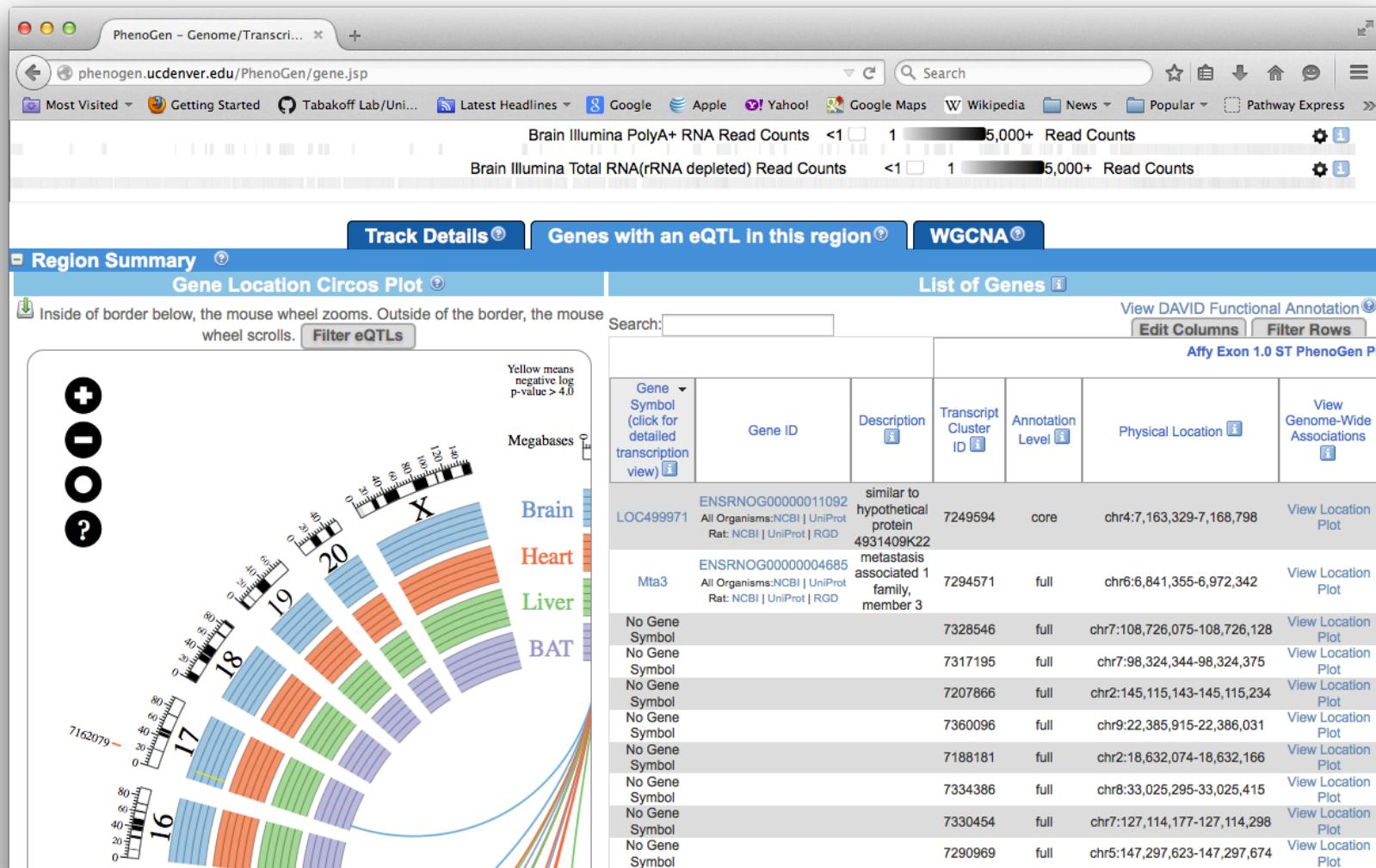


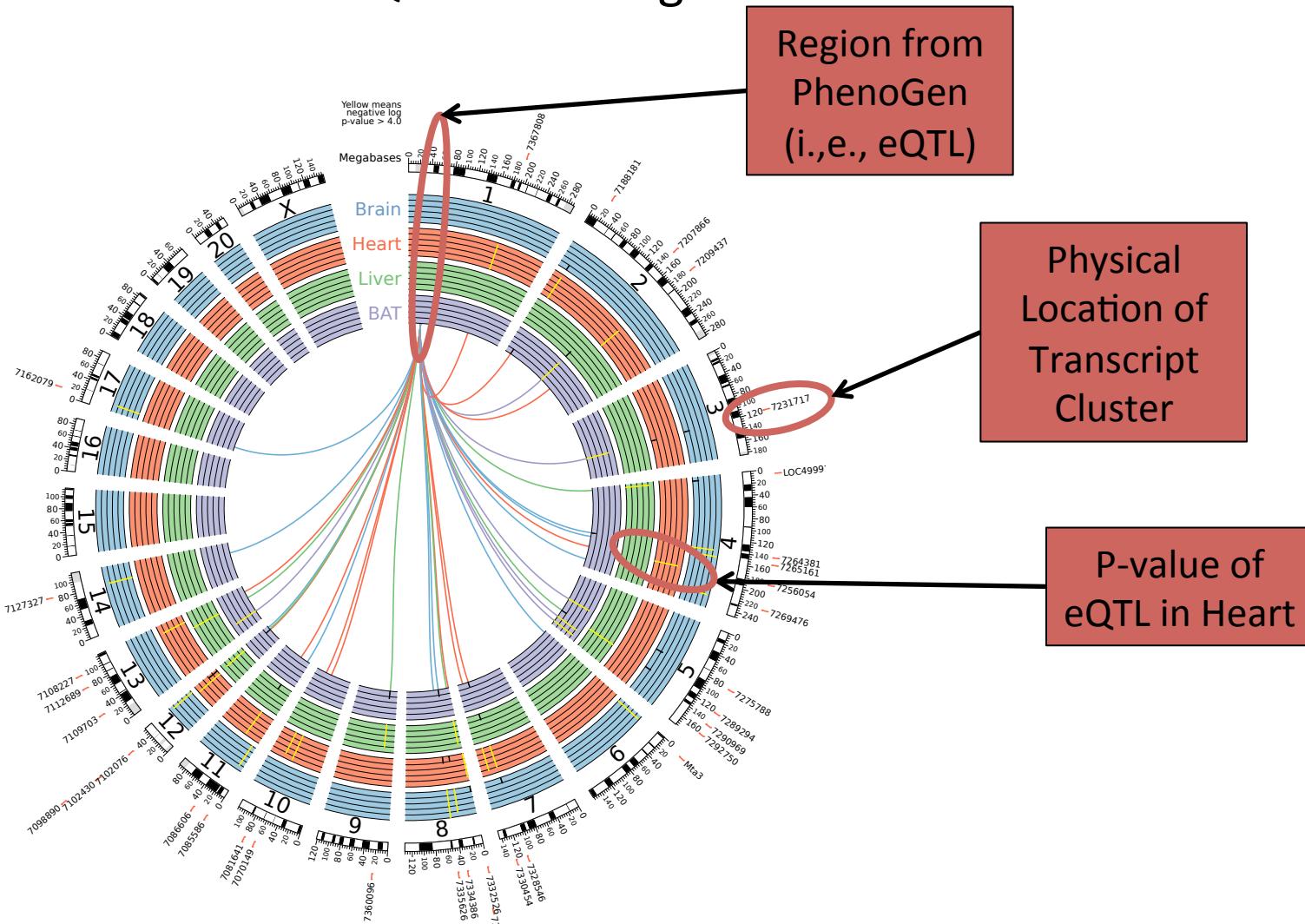
Image copied from Sieberts SK and Schadt EE (2007). [Moving toward a system genetics view of disease](#). Mammalian Genome 18(6): 389-401.

Genes with an eQTL in this region



Circos Plot

Genes within an eQTL in this region



Transcriptome View

The screenshot shows the PhenoGen - Genome/Transcriptome Data Browser interface. The main window displays a genome browser view for chromosome chr1, showing Ensembl Protein Coding Transcripts and Ensembl Lnc Non-Coding Transcripts. The navigation bar includes links for Overview, Genome / Transcriptome Data Browser, Available Data Downloads, Microarray Analysis Tools, Gene List Analysis Tools, QTL Tools, About, Help, and Login/Register. A search bar at the top has the gene identifier ENSRNOG00000018191, species Rattus norvegicus (rn5), and initial view set to Genome (Predefined). A 'Select/Edit Views' button is highlighted with a red oval.

Not sure where to start: watch a quick navigation demonstration or view the help images again

Region Image **View: Genome** Organism: Rat(rn5) Strain:BN PhenoGen v2.15.2(4/8/2015)

Navigation Hints: Hold mouse over areas of the image for available actions.

chr1

44,810,000 44,820,000 44,830,000

Ensembl Protein Coding Transcripts
Ensembl Lnc Non-Coding Transcripts

Select/Edit Views

Sign in to see views/tracks not created on this computer.

Click on a view to select it and view preview/details.

View types: All Views

Select a view below (click apply to display the view and return to the browser):

- Transcriptome (Predefined) (6 tracks)
- General Transcriptome (Predefined) (141 tracks)
- Liver RNA-Seq (Predefined) (6 tracks) (Rat Only)
- Brain RNA-Seq (Predefined) (8 tracks)
- Liver Microarray/RNA-Seq (Predefined) (7 tracks) (Rat Only)
- Brain Microarray/RNA-Seq (Predefined) (7 tracks)
- Heart RNA-Seq (Predefined) (6 tracks) (Rat Only)

Apply View

Description/Preview **View/Edit Track List**

Provides general Transcriptome Data including RNA-Seq transcriptomes, Affymetrix probesets, and RNA-Seq read count depth tracks.

Preview

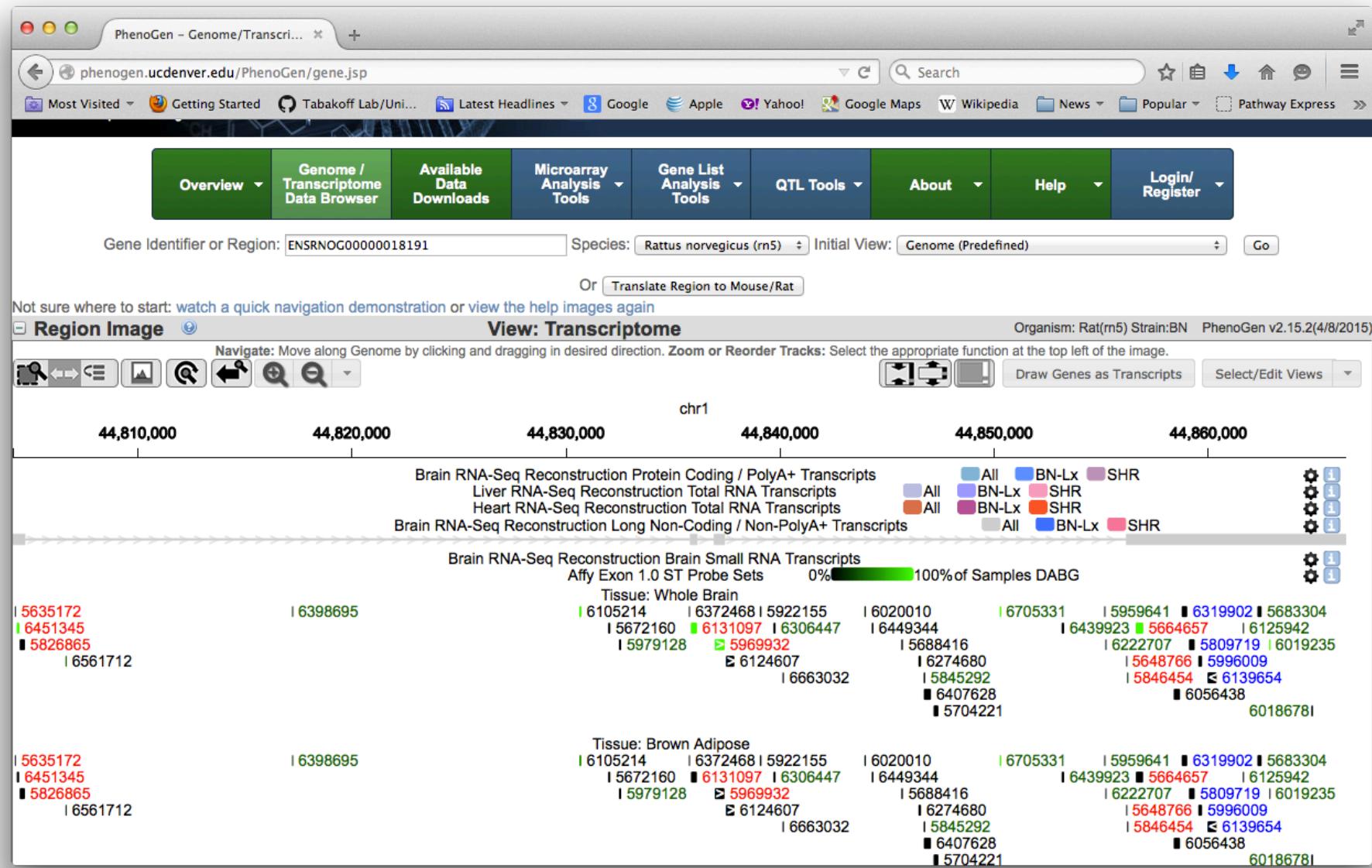
chr1

44,820,000 44,840,000 44,860,000

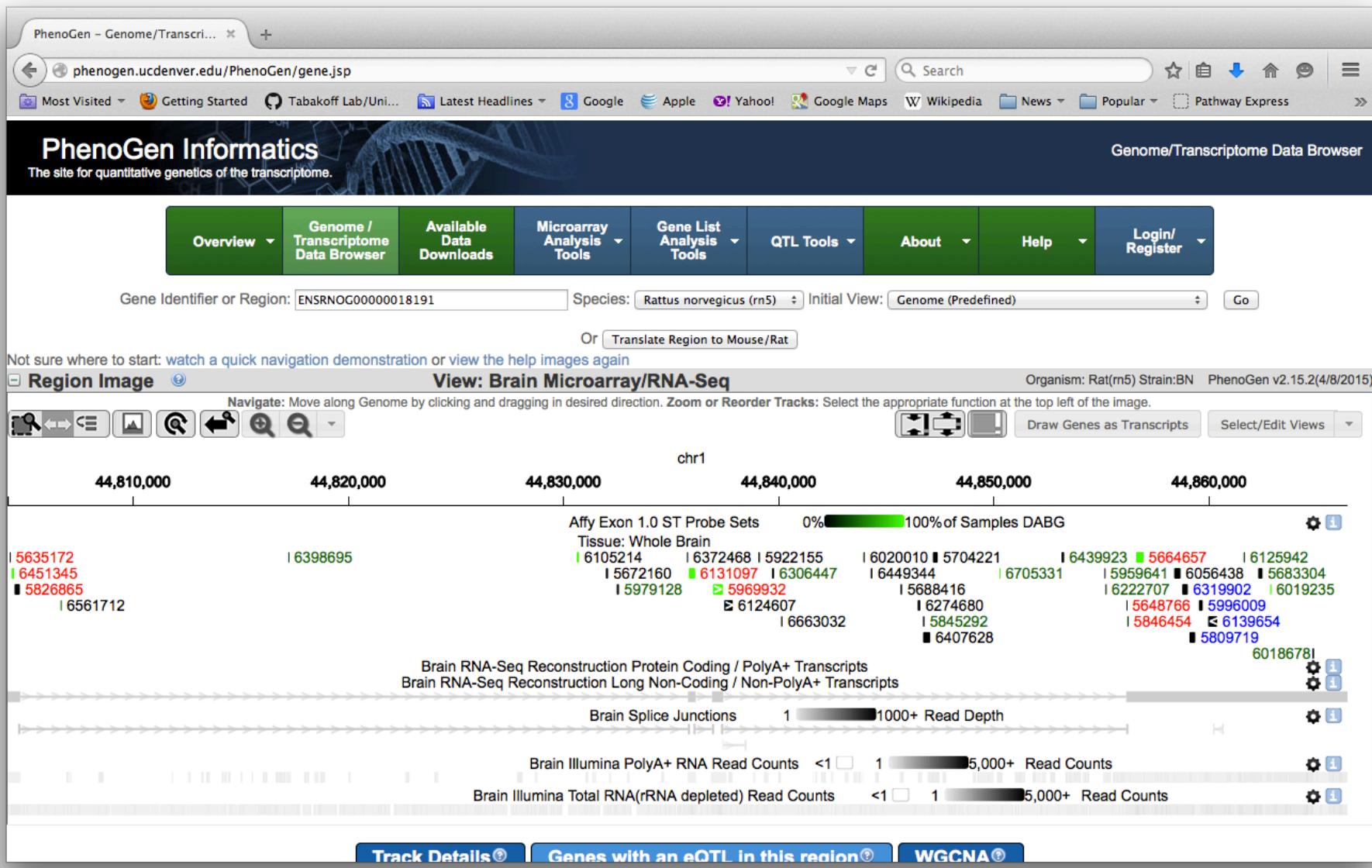
Brain RNA-Seq Reconstruction Protein Coding / PolyA+ Transcripts

1. Click “Select/Edit Views” box.
2. Select “Transcriptome (Predefined) (6 tracks)” in Select a view below box
3. Click “Apply View” button

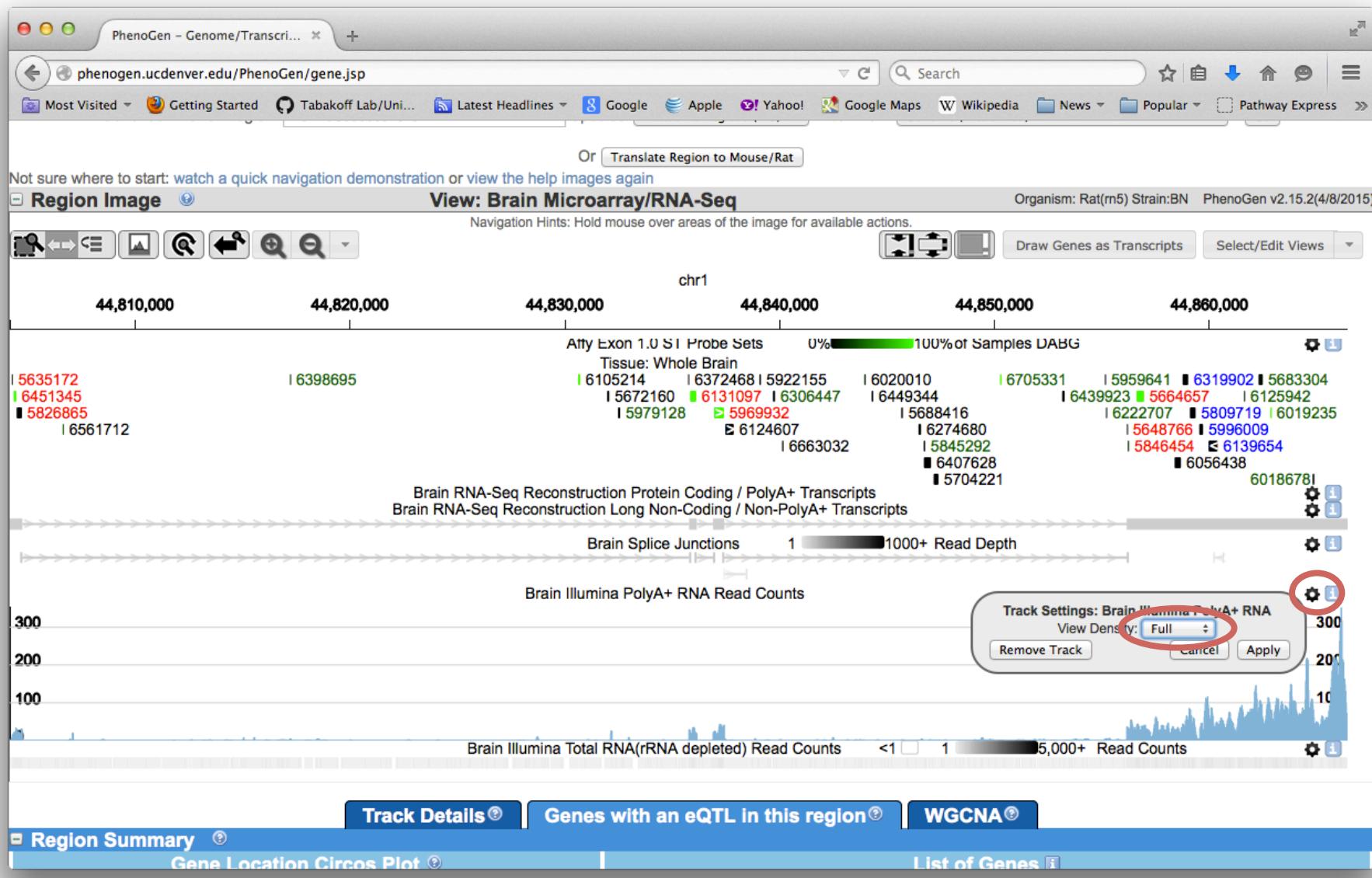
Oprm1 – Only in Brain Reconstruction



Brain Microarray/RNA-Seq View



Expand RNA-Seq Read Counts



Add Ensembl Track

The screenshot shows the PhenoGen web application interface. At the top, there is a navigation bar with links for 'Data Browser', 'Downloads', 'Tools', and 'Register'. Below the navigation bar, the URL is phenogen.ucdenver.edu/PhenoGen/gene.jsp. The main search area has fields for 'Gene Identifier or Region' (ENSRNOG00000018191), 'Species' (Rattus norvegicus (rn5)), and 'Initial View' (Genome (Predefined)). A 'Go' button is also present.

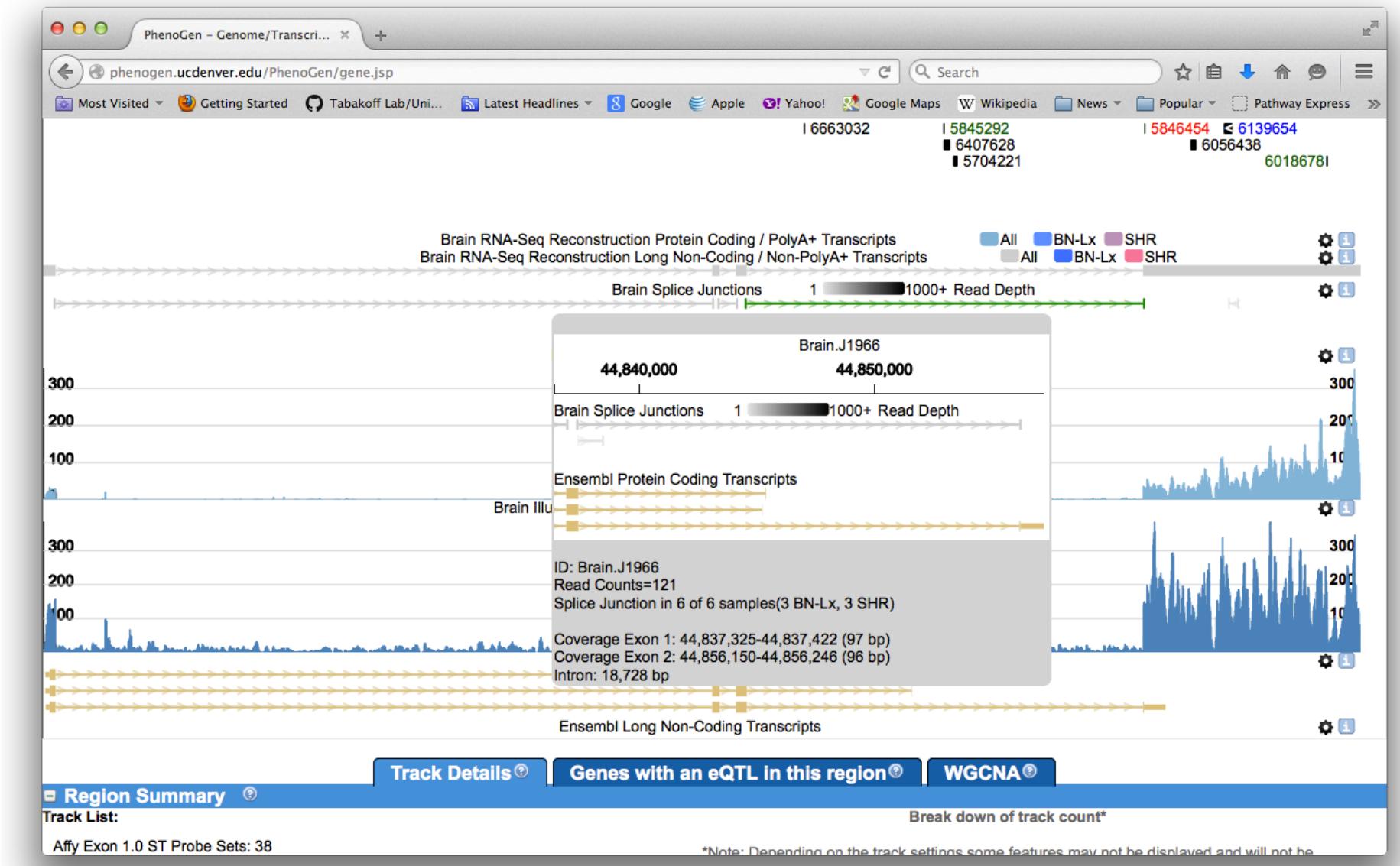
Below the search area, a message says 'Not sure where to start? watch a quick navigation demonstration or view the help images again'. The title of the page is 'View: Brain Microarray/RNA-Seq (Modified)'. The organism is listed as Rat(rn5) Strain:BN and the version is PhenoGen v.2.15.2(4/8/2015).

The main content area is divided into two panels:

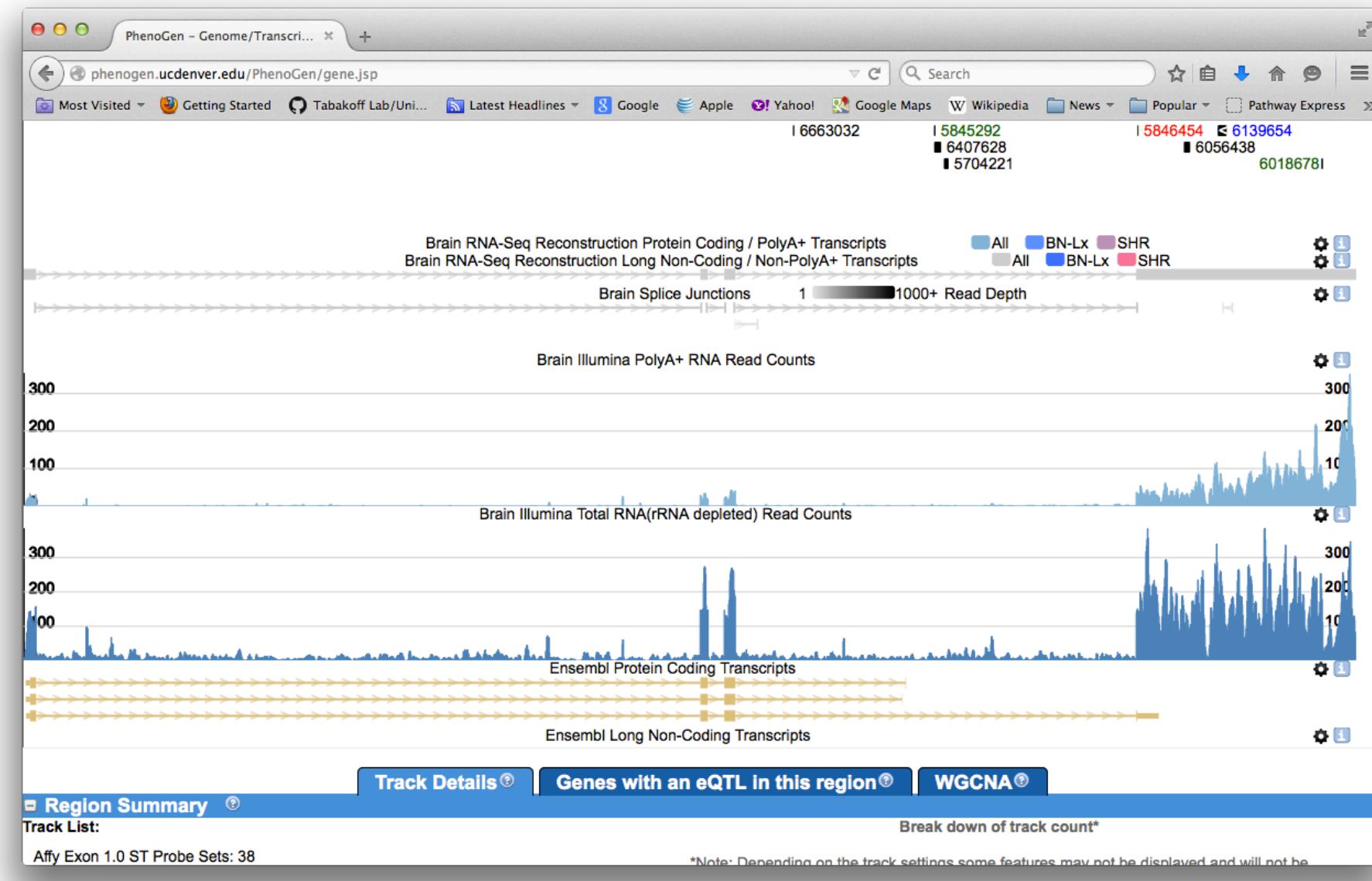
- Left Panel: Select a Track to add to Brain Microarray/RNA-Seq**
 - A warning message: 'Sign in to see tracks not saved to this computer. (Any tracks created will only be saved locally)'.
 - An 'Add Track' button with a plus sign icon.
 - A table titled 'Available Tracks' with columns: 'Ensembl Protein Coding Genes', 'Genome', 'Annotation', and 'Public'. It lists tracks like 'Ensembl Long Non-Coding Genes', 'Ensembl Small RNA Genes', 'Ref Seq Genes', 'SHR SNPs small Insertion / Deletions', etc.
- Right Panel: Select/Edit Views**
 - A warning message: 'Sign in to see views/tracks not created on this computer.'
 - An 'Apply View' button.
 - A list of 'Select a view below (click apply to display the view and return to the browser)':
 - Genome (Predefined) (8 tracks)
 - Transcriptome (Predefined) (6 tracks)
 - Genome/Transcriptome (Predefined) (14 tracks)
 - Liver RNA-Seq (Predefined) (6 tracks) (Rat Only)
 - Brain RNA-Seq (Predefined) (8 tracks)
 - Liver Microarray/RNA-Seq (Predefined) (7 tracks) (Rat Only)
 - Brain Microarray/RNA-Seq (Predefined) (7 tracks)
 - Heart RNA-Seq (Predefined) (6 tracks) (Rat Only)
 - A 'Description/Preview' button with a green plus sign icon, which is circled in red.
 - A 'View/Edit Track List' button.
 - A message: 'Click to add a track to the current view.'
 - A table titled 'View/Edit Track List' showing five tracks:

Order	Track Name	Organism	Edit
1	Reference Genomic Sequence	Rat	Up/Down, Gear, X
2	Affymetrix Exon 1.0ST Probes	Rat only	Up/Down, Gear, X
3	Brain Transcriptome Protein Coding Genes	Rat only	Up/Down, Gear, X
4	Brain Transcriptome Long Non-Coding Genes	Rat only	Up/Down, Gear, X
5	Brain Splice Junction Support	Rat only	Up/Down, Gear, X

Junction Reads



Alternative 3' UTR



Selected Feature Summary

PhenoGen – Genome/Transcri... +

phenogen.ucdenver.edu/PhenoGen/gene.jsp

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Gene Details Gene eQTLs Probe Set Level Data WGCNA

Selected Feature Summary

Gene Symbol: Oprm1
Location: chr1: 44,804,261-44,857,206
Strand: +
Description: opioid receptor, mu 1
Links: ENSRNOG00000018191
All Organisms:NCBI | UniProt
Rat: NCBI | UniProt | RGD

Exonic Variants:
Common: 0 (SNPs) / 0(Insertions/Deletions)
BN-Lx/CubPrin: 0 (SNPs) / 0(Insertions/Deletions)
SHR/OlaPrin: 4 (SNPs) / 7 (Insertions/Deletions)
SHR/NCrlPrin: 4 (SNPs) / 7 (Insertions/Deletions)
F344: 4 (SNPs) / 6 (Insertions/Deletions)

Transcripts:
ENSRNOT00000024682
ENSRNOT00000045144
ENSRNOT00000051837
Brain_T18812 - Transcript Match: ENSRNOT00000051837 2 Perfect Exon Matches, 3' Extended, 5' Extended,

Affy Probe Set Data: Overlapping Probe Set Count:23

Probe sets detected above background*:

Tissue	Number of probe sets detected above background* in more than 1% of samples (out of 23 probe sets for this gene)	Avg % of samples DABG*	Range
Brain	12	57 %	2.78 - 100 %
Heart	3	20 %	1.9 - 55.24 %
Liver	2	84 %	72.64 - 96.23 %
Brown Adipose	5	18 %	2.08 - 56.25 %

Probe Set Heritability:

Tissue	Number of probe sets with a heritability greater than 0.33 (out of 23 probe sets for this gene)	Avg Herit	Range
Brain	5	0.36	0.33 - 0.4
Heart	9	0.39	0.33 - 0.47
Liver	11	0.38	0.34 - 0.44
Brown Adipose	6	0.41	0.34 - 0.5

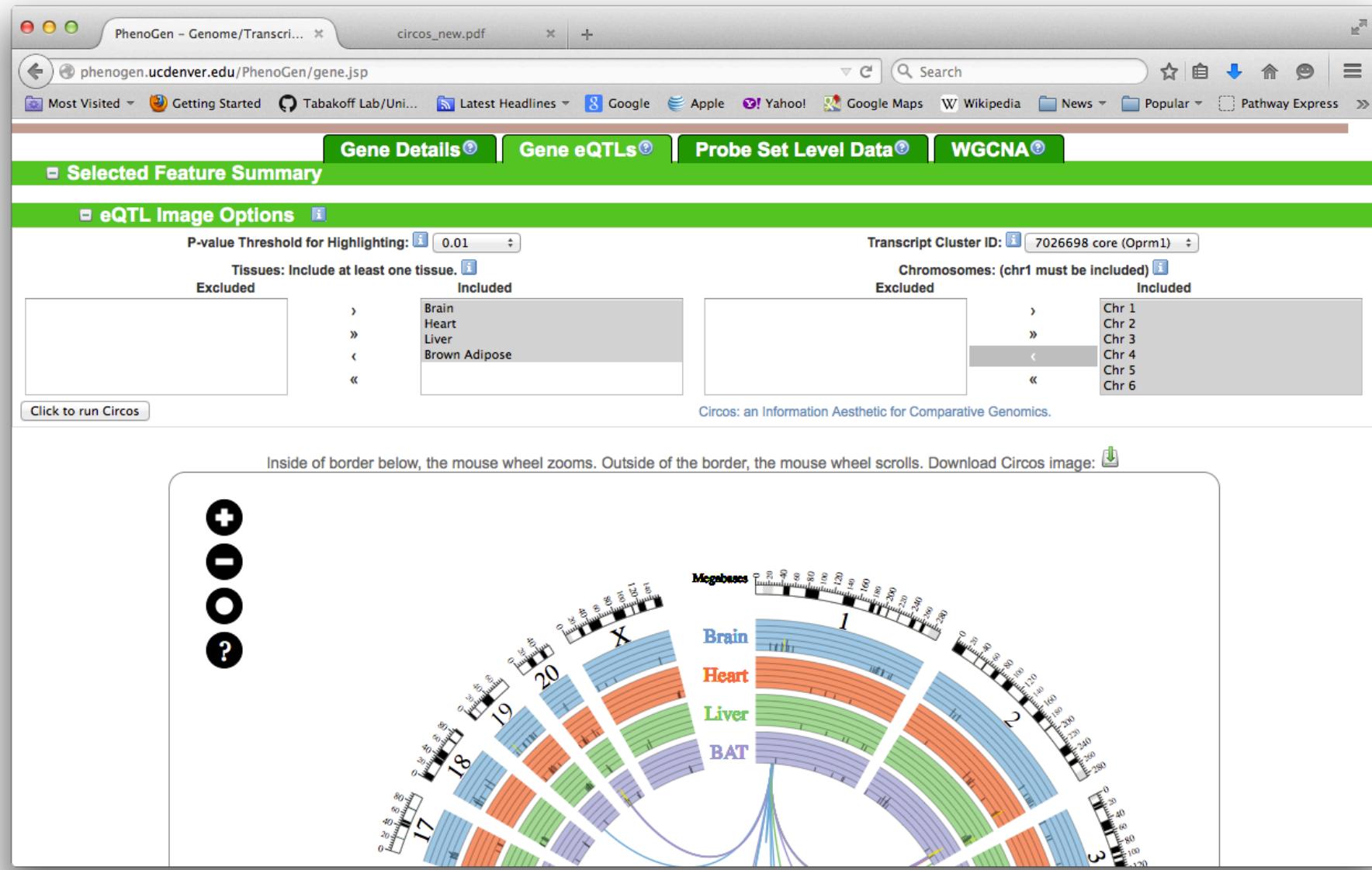
*DABG is based on Affymetrix software that assigns a P-value to the probe sets detection above background. Using a comparison of RNA-Seq data probe sets that overlap a high confidence exon in the transcriptome are not detected above background roughly 5% of the time. Increasing the P-value cutoff of 0.0001 can reduce this but only at the expense of greatly elevated false positives.

eQTLs Affymetrix Transcript Cluster(Confidence Level): 7026698 (core)

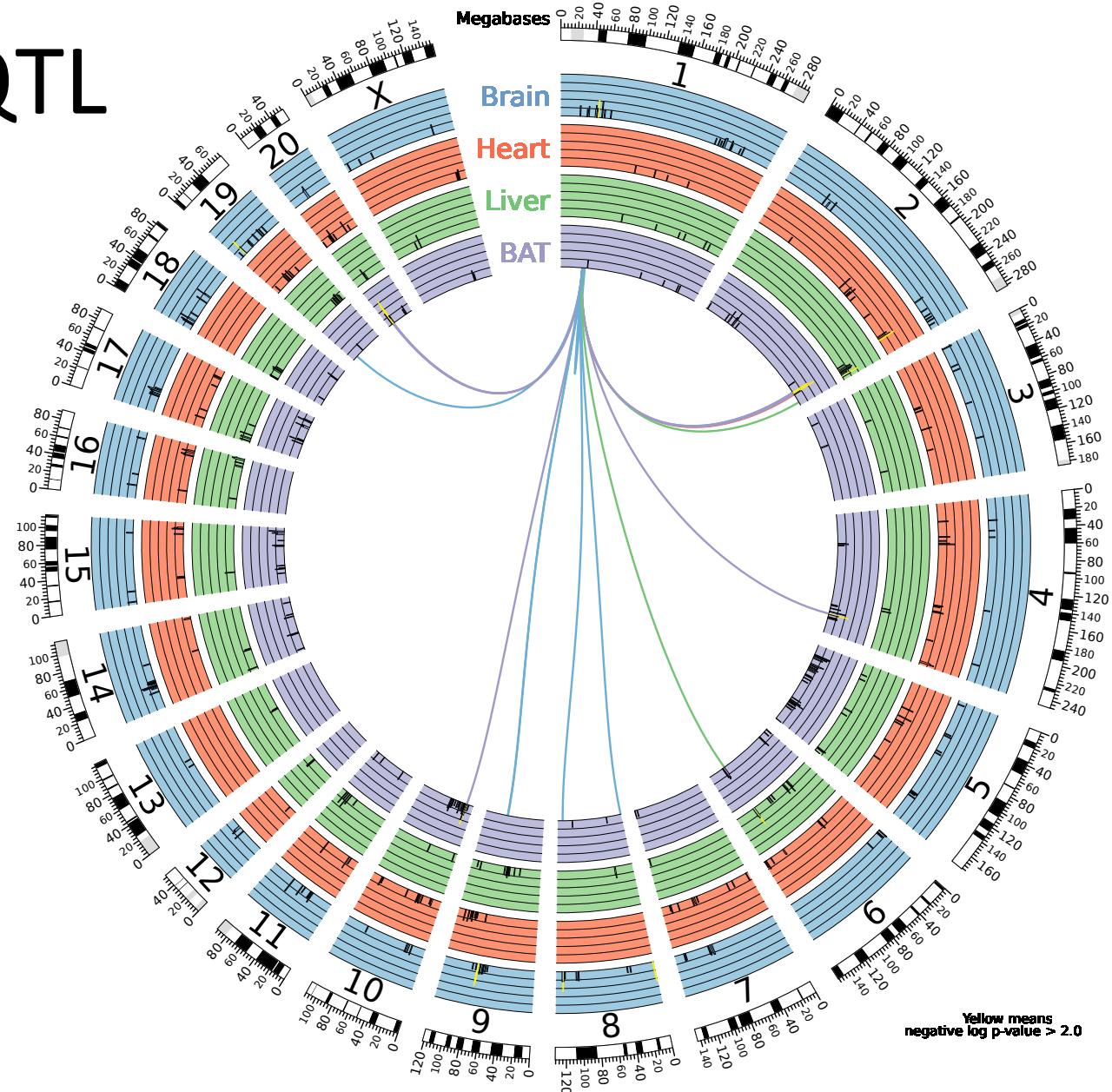
Tissue	Number of eQTLs	Minimum P-value EQTL	
		P-value	Location
Whole Brain	6	0.0024	chr9:76,343,169
Heart	1	0.0096	chr2:259,028,982
Liver	2	0.0052	chr6:119,885,523
Brown Adipose	8	0.0006	chr20:12,052,918

Click the Gene eQTLs Tab above to view a Circos Plot of the eQTLs listed above.

Gene eQTL



Gene eQTL



WEIGHTED GENE CO-EXPRESSION NETWORK ANALYSIS (WGCNA)

**What are we missing by
considering each candidate gene
individually?**

- Define relationships among genes
- Infer biological function from other co-expressed genes
- Define the context in which the gene exerts its effect
- Find multiple therapeutic targets within the same pathway

**What do we gain by building
networks and identifying
modules?**

- No gene product acts independently in the cell
- Information about biological function in cell
- Information on causes/consequences of differential expression

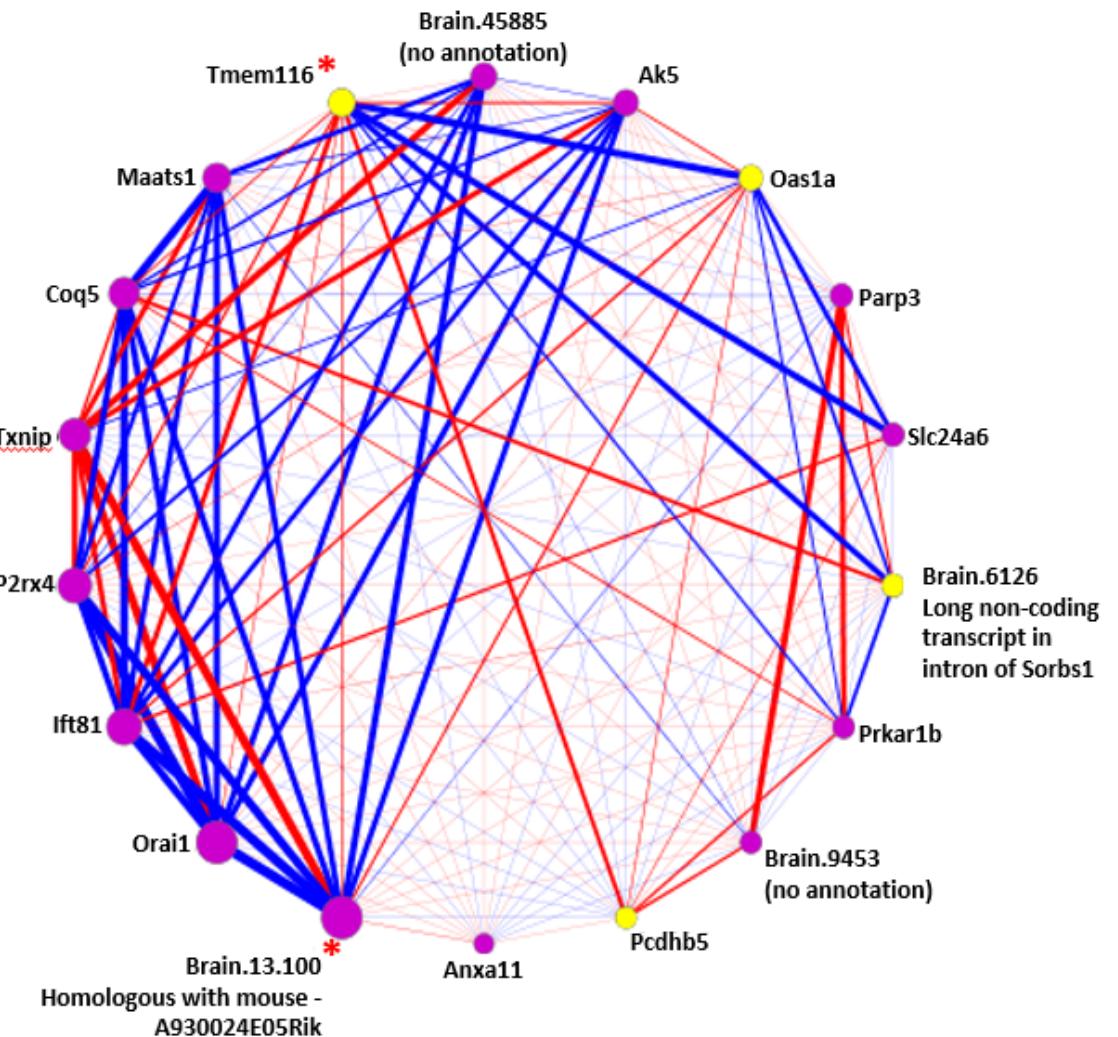
Weighted Gene Co-Expression Network Analysis

Why Not Just Use Correlation?

1. Simple correlation does not give connectivity.
2. How are we measuring co-expression?
 - Scale-Free Network
 - Network has few highly connected genes rather than each gene have similar connectivity
 - **Biologically motivated**, fewer highly connected genes means that a system is more robust to failure of any one gene
3. How do we get a **robust** measure of connectivity for identifying modules?
 - Topological Overlap Measure
 - Includes a measure of how many “friends” two genes have in common
 - Protects against spurious correlations among genes

Example Co-Expression Module

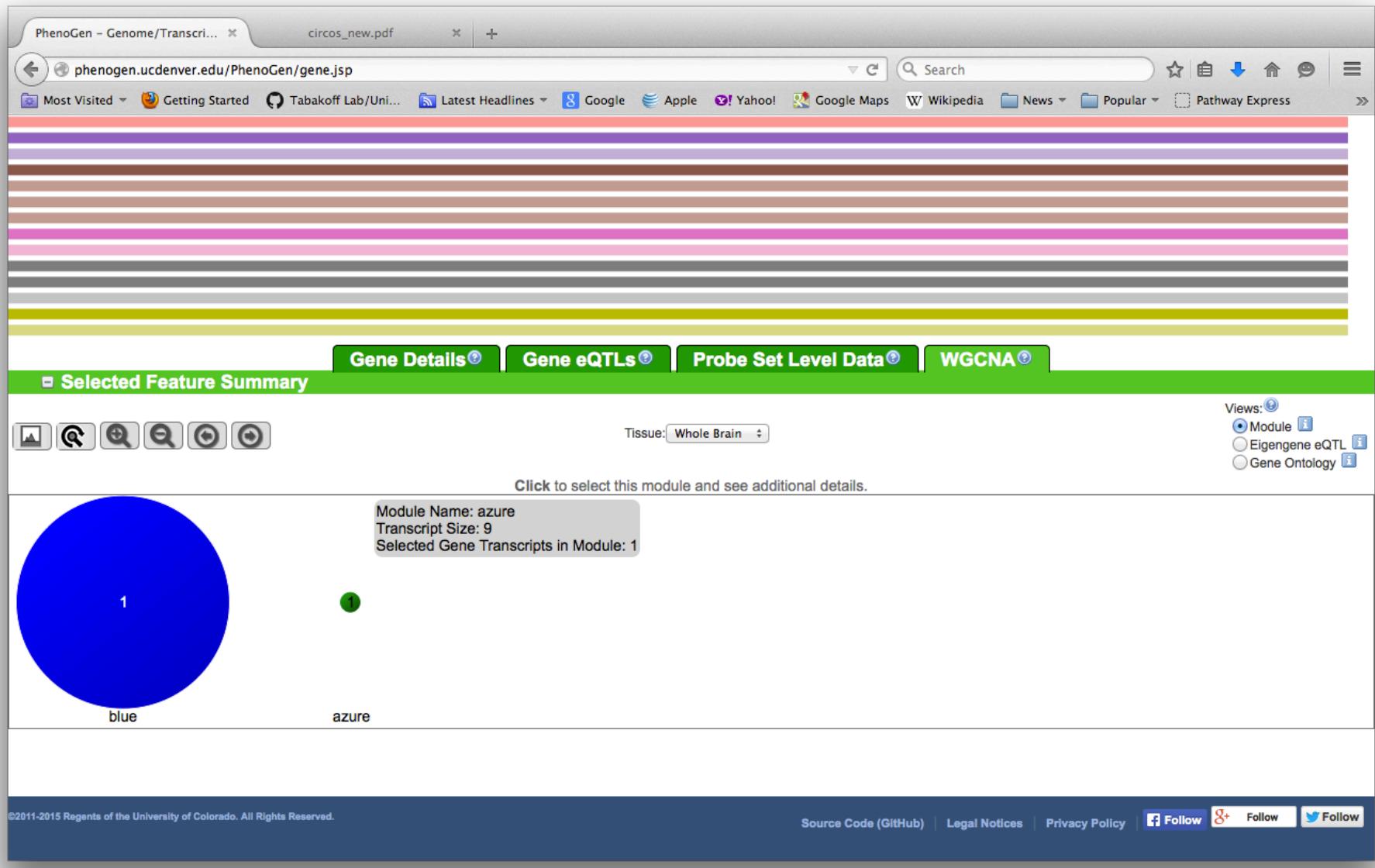
- Edge thickness is weighted based on magnitude of correlation between nodes.
- Blue edges represent a positive correlation between nodes.
- Red edges represent a negative correlation between nodes.
- Node size is weighted based on connectivity within module.



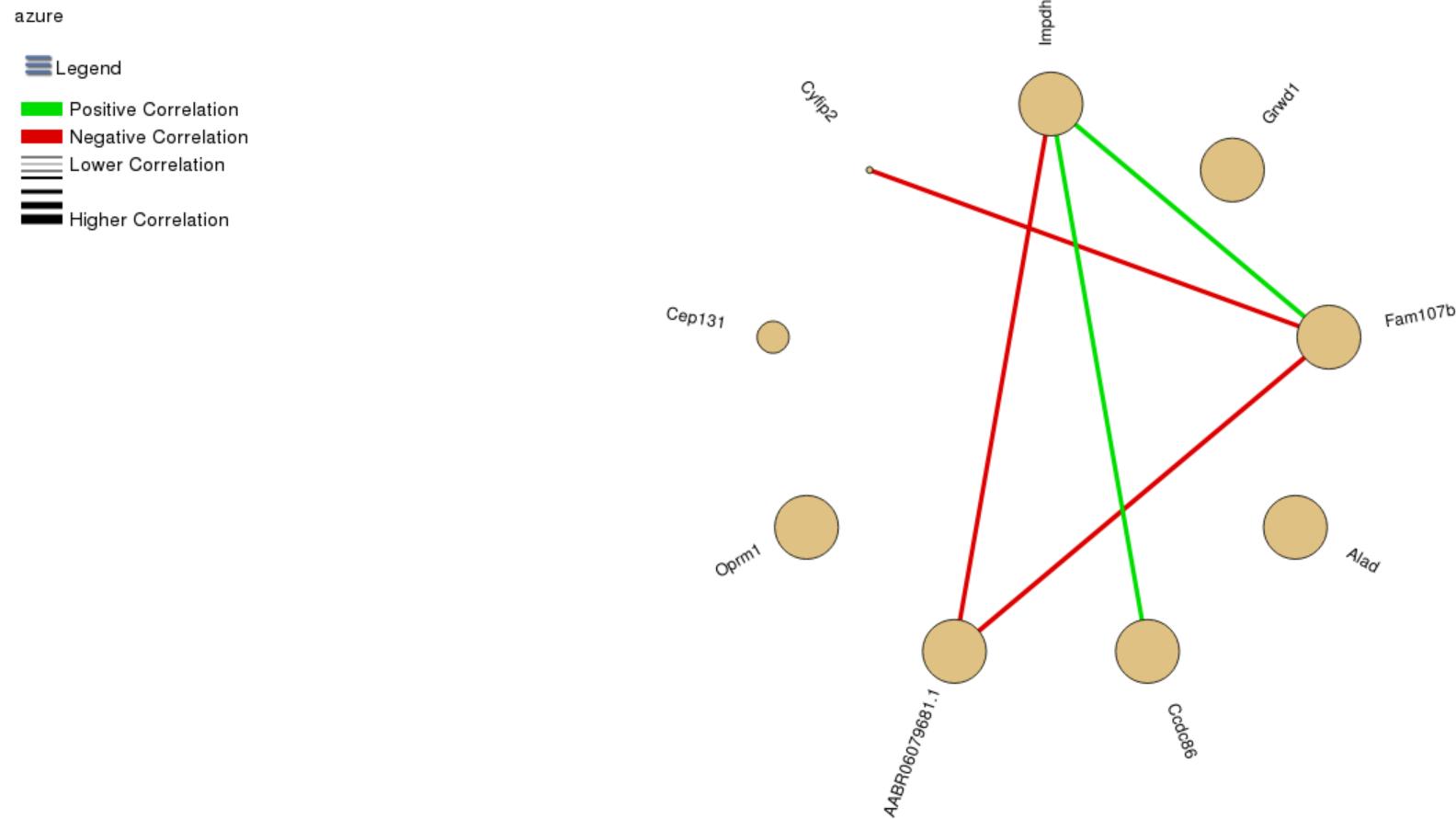
Summary of Expression Pattern Within Co-Expression Module

- Eigengene
 - First Principal Component
 - Maximize the amount of variance in expression captured by a single value per strain/sample
- Hub Gene
 - Most ‘connected’ gene within a module
 - May have biological implications

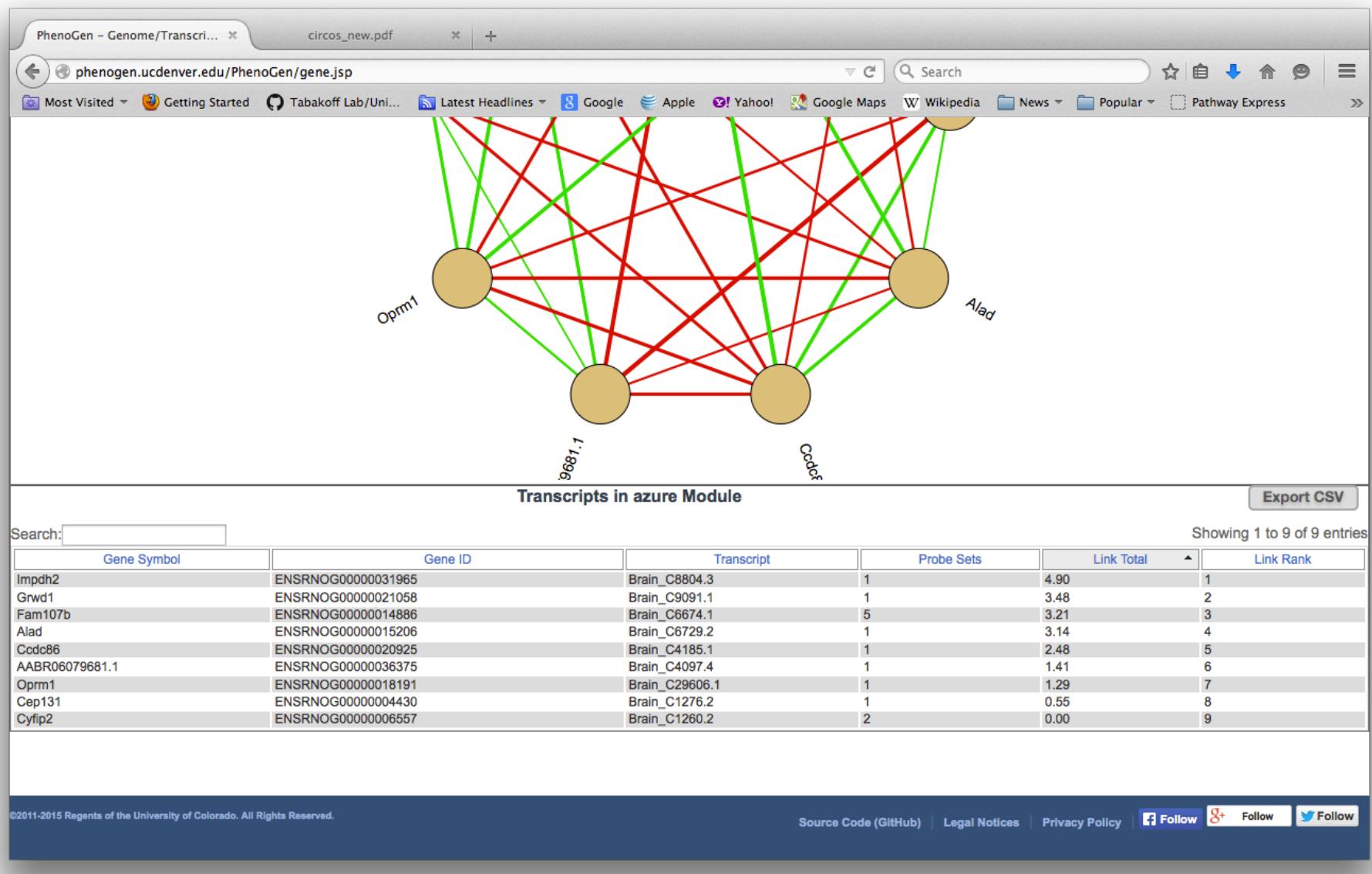
WGCNA



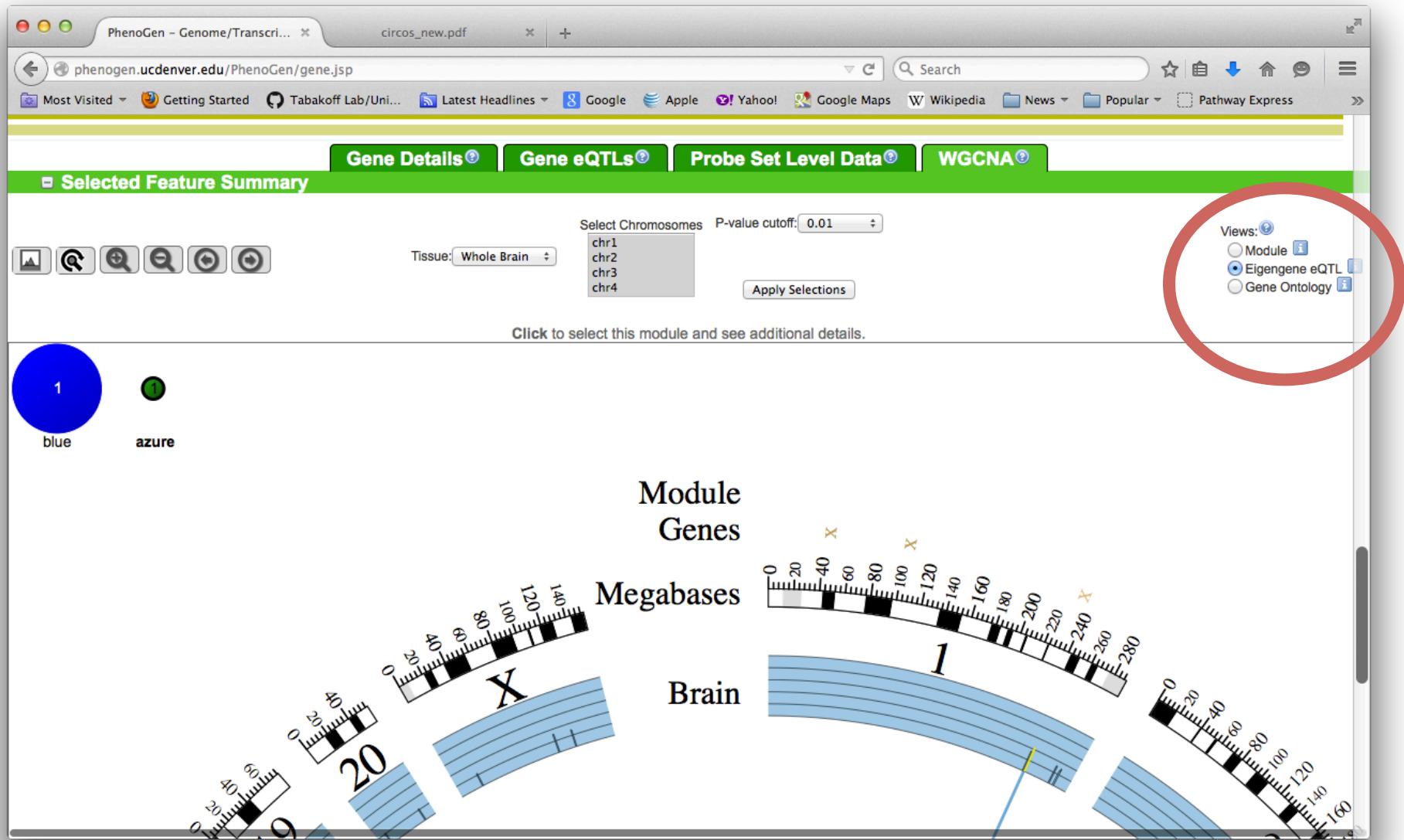
Azure Module



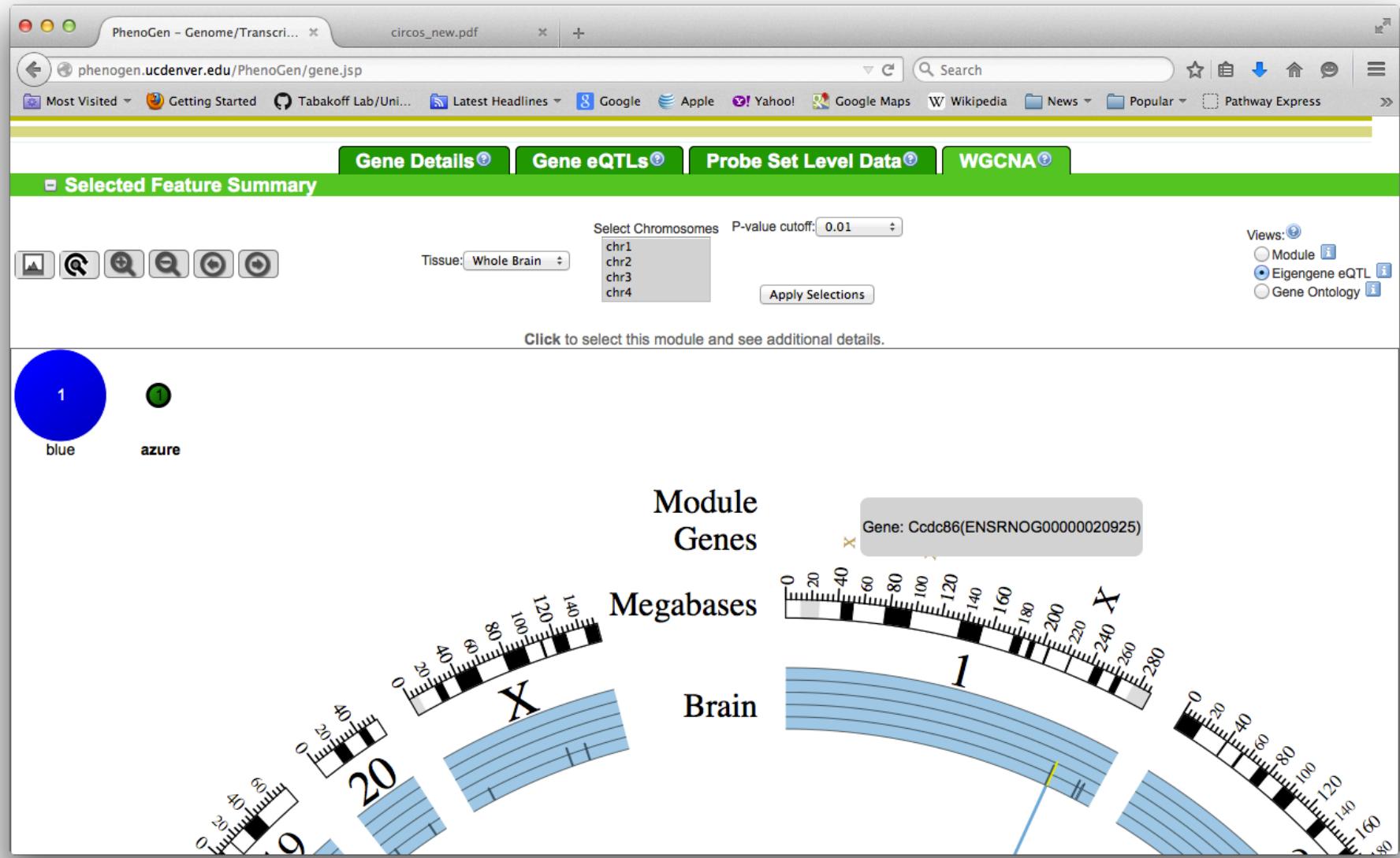
Azure Module Table



Eigengene eQTL

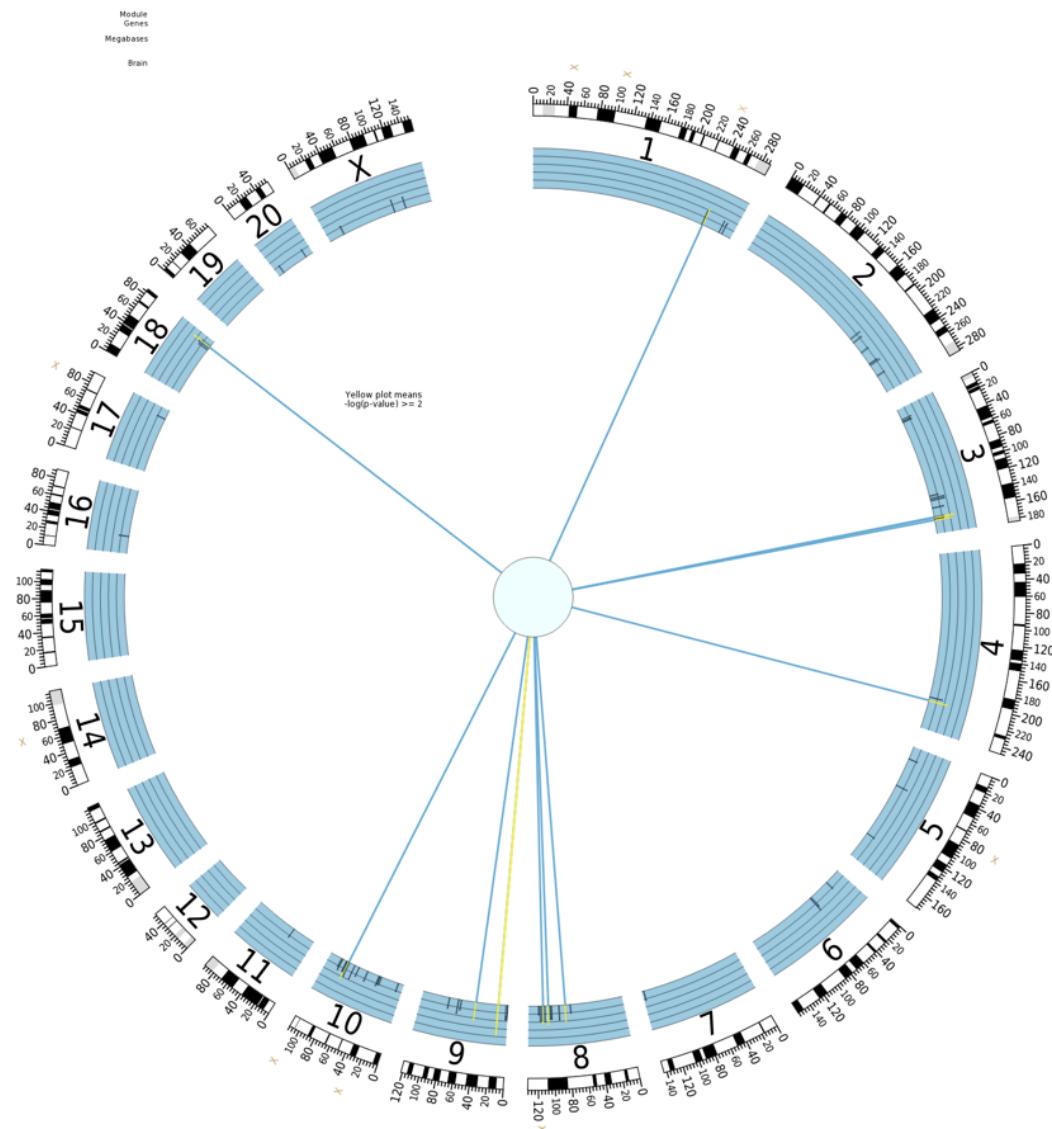


Physical Location of Genes with Module

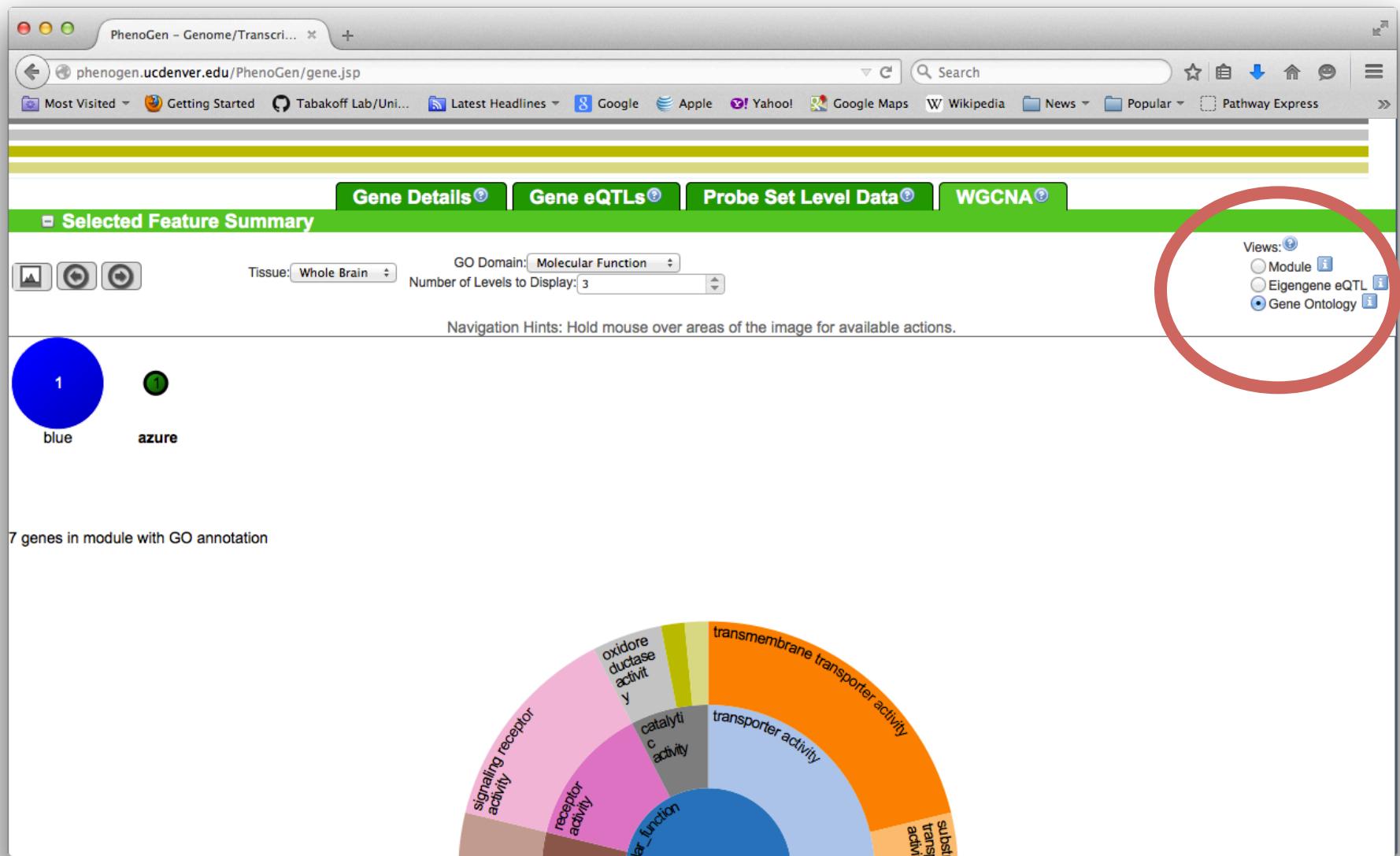


Circos

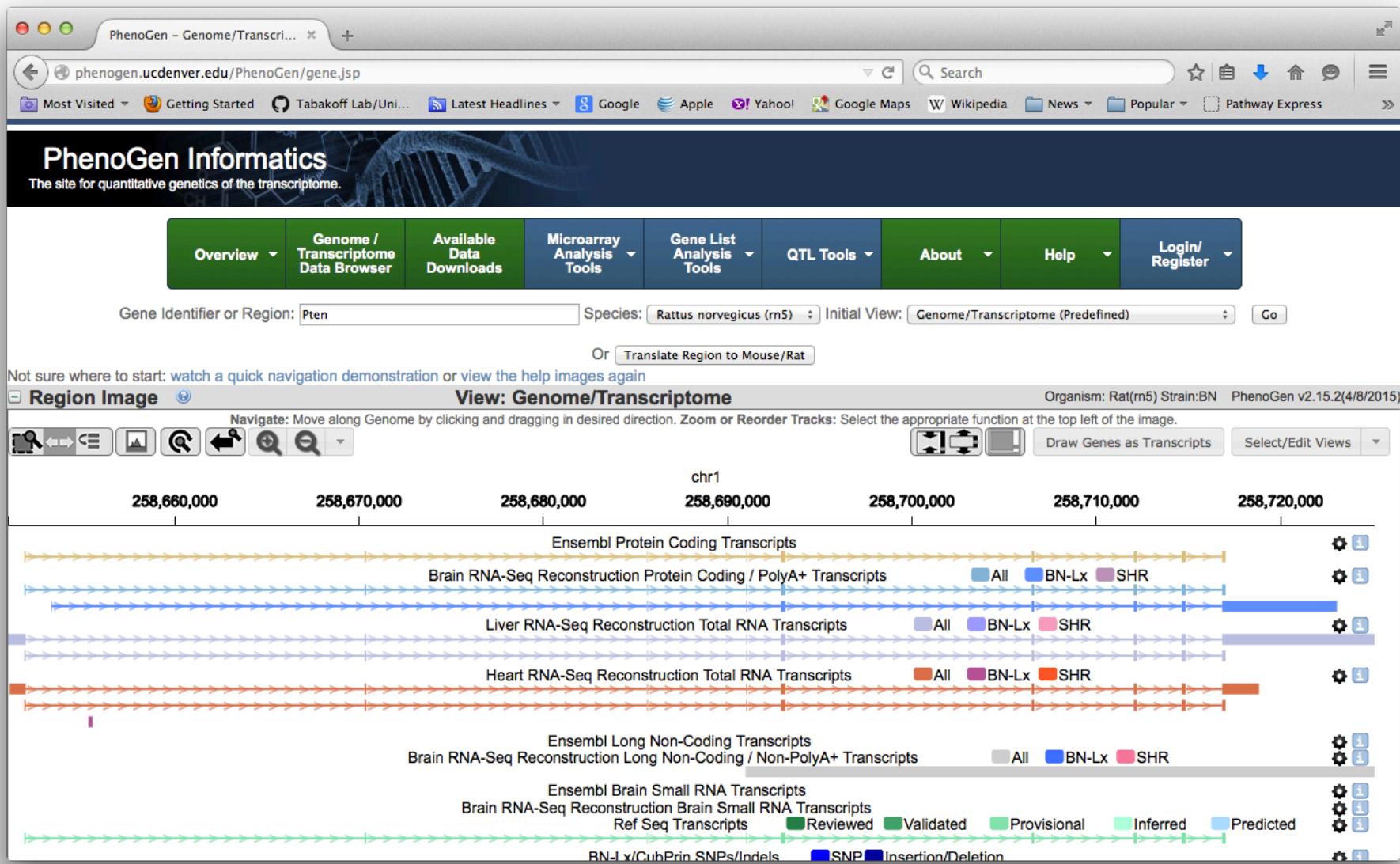
Eigengene eQTL



Gene Ontology



RNA Expression Across Tissues



Probe Set Level Data

Probe Set Level Data needs to collect and parse lots of data.

Click on the tab, and wait a minute before pushing the View Affy Probe Set Details button

PhenoGen - Genome/Transcri... +

phenogen.ucdenver.edu/PhenoGen/gene.jsp

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Gene Details Gene eQTLs Probe Set Level Data WGCNA

Selected Feature Summary

This feature requires Java which will open in a separate window, when you click the button below. Java will be automatically detected and directions will be displayed on the next page if there are any issues to correct before proceeding.

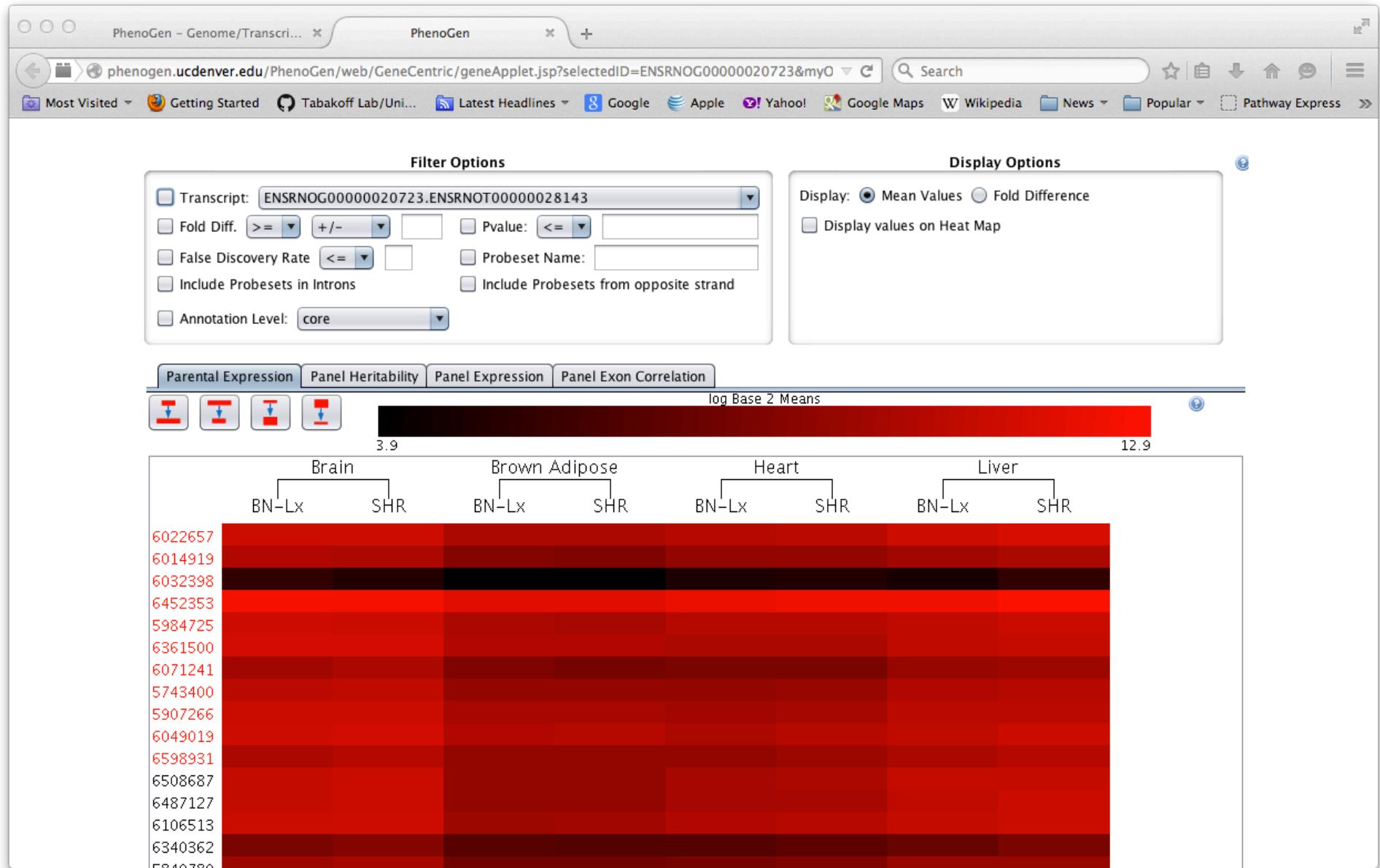
View Affy Probe Set Details

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Source Code (GitHub) Legal Notices Privacy Policy

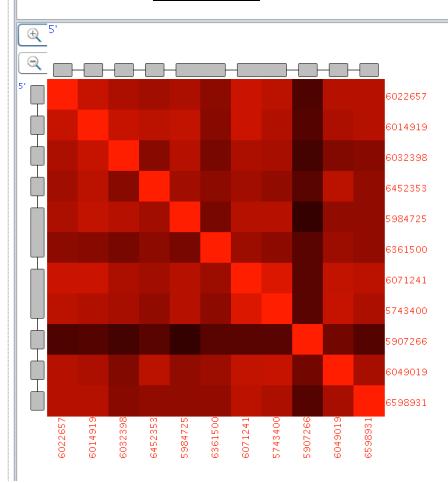
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Differences Between Parental Strains Across Tissues

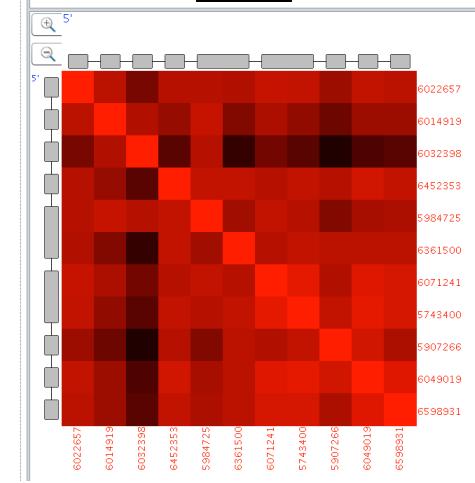


Panel Exon Correlation In Different Tissues

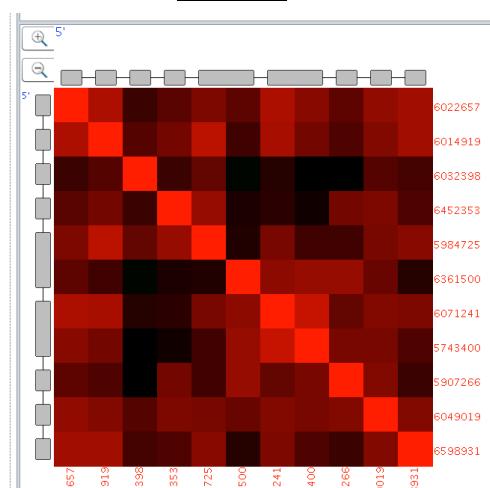
Brain



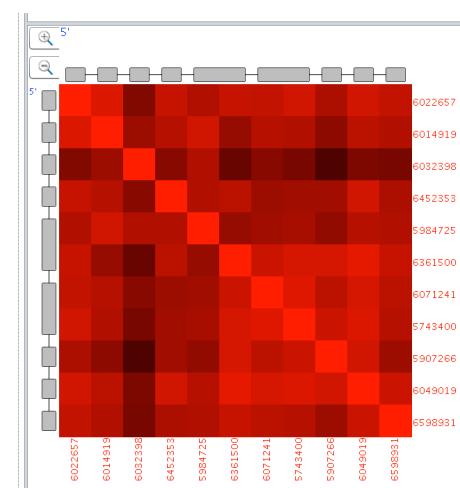
Liver



Heart



Brown Adipose



FROM PHENOTYPE TO CANDIDATE GENES

Genetical Genomics/Phenomics Approach

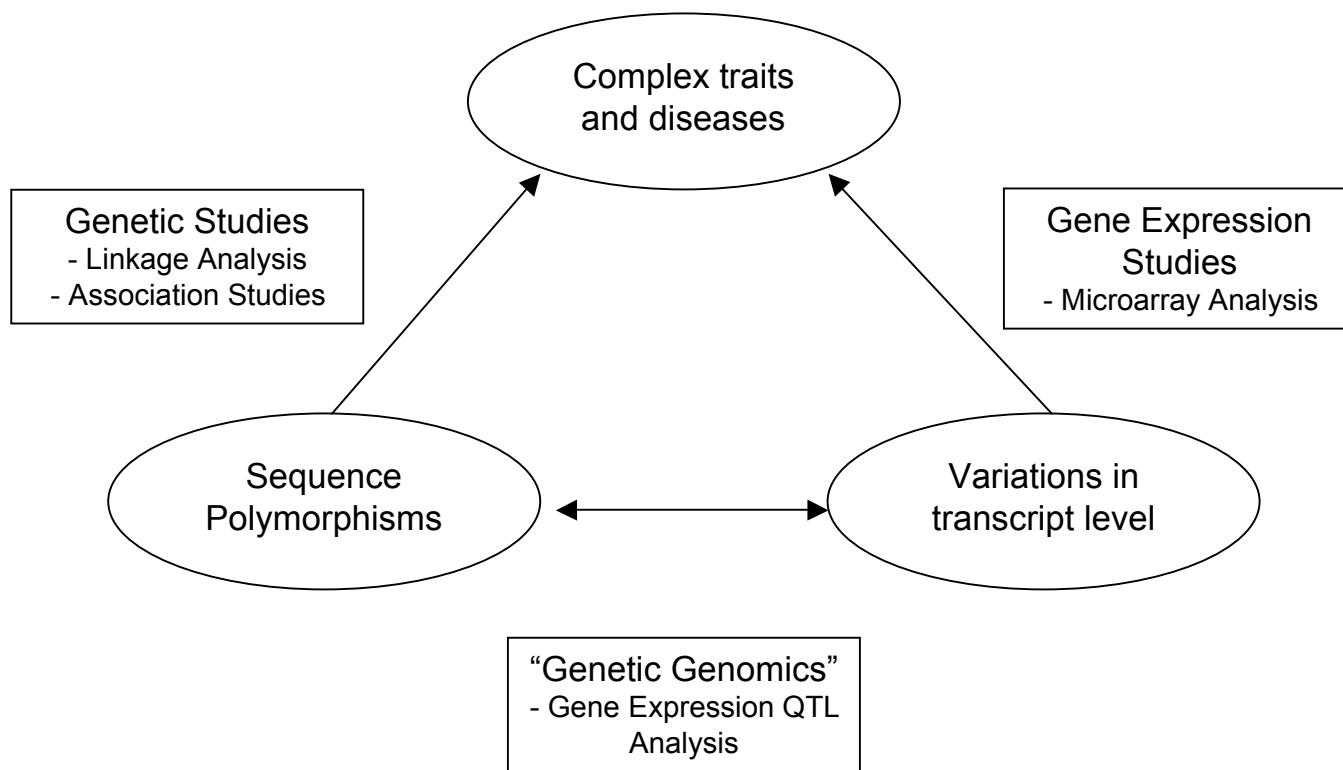


Image copied from “The Marriage of Phenomics and Genetical Genomics: A Systems Approach to Complex Trait Analysis” in Systems Biology in Psychiatric Research: From High-Throughput Data to Mathematical Modeling, edited by Tetter F, Winterer G, Gebicke-Haerter PG, and Mendoza E. Wiley-VCH 2010.

Data

- What you provide
 - Quantitative phenotype data from one of the following panels:
 - BXD RI mice
 - LXS RI mice
 - HXB/BXH RI rats
- What we provide
 - RNA expression levels
 - SNP information

Genetic Correlation

RNA Expression Levels Correlated with Phenotype

The screenshot shows a web browser window for the PhenoGen Informatics platform. The URL is phenogen.ucdenver.edu/PhenoGen/web/datasets/correlation.jsp?datasetID=707&datasetVersion=4&analysisType=correlation. The page title is "PhenoGen Informatics" with the subtitle "The site for quantitative genetics of the transcriptome".

The main content area displays the message "You are Analyzing: Public ILSXISS RI Mice v4". Below this, a "Steps to run a correlation analysis:" diagram shows a sequence of seven steps: Choose Dataset → Choose Dataset Version → Choose Type of Analysis → Choose Phenotype Data → Filter Probe(s) → Run Statistical Test → Correct for Multiple Testing → Save Gene List. To the right of the steps are two buttons: "Dataset Version Details" and "Create New Phenotype".

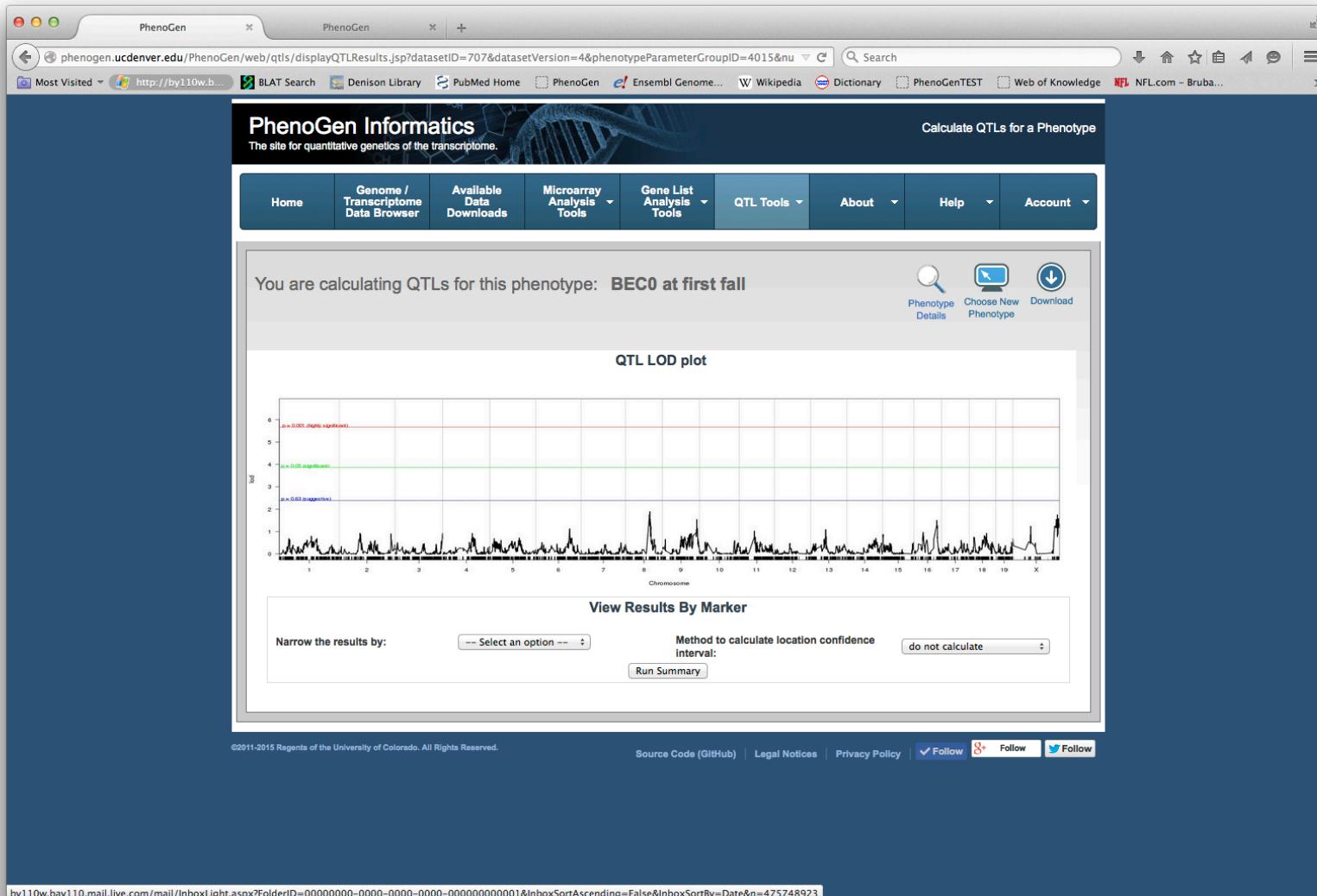
Below the steps, a message says "Click on the phenotype data you would like to use, or enter new phenotype data." A table titled "Phenotype Values (Matching 5 or more strains)" lists four entries:

Phenotype Name	Description	Details	Delete	Download
BEC0 at first fall	from GeneNetwork	View	X	Download
Longevity	longevity in days for male mice from MPD	View	X	Download
LORR from GeneNetwork	loss of righting reflex	View	X	Download
ME1130	eigengene for Mecp2 module	View	X	Download

At the bottom of the page, there is a footer with links: "©2011-2015 Regents of the University of Colorado. All Rights Reserved.", "Source Code (GitHub)", "Legal Notices", "Privacy Policy", "Follow" (with links to Google+ and Twitter), and "Follow" (with a link to the PhenoGen GitHub page).

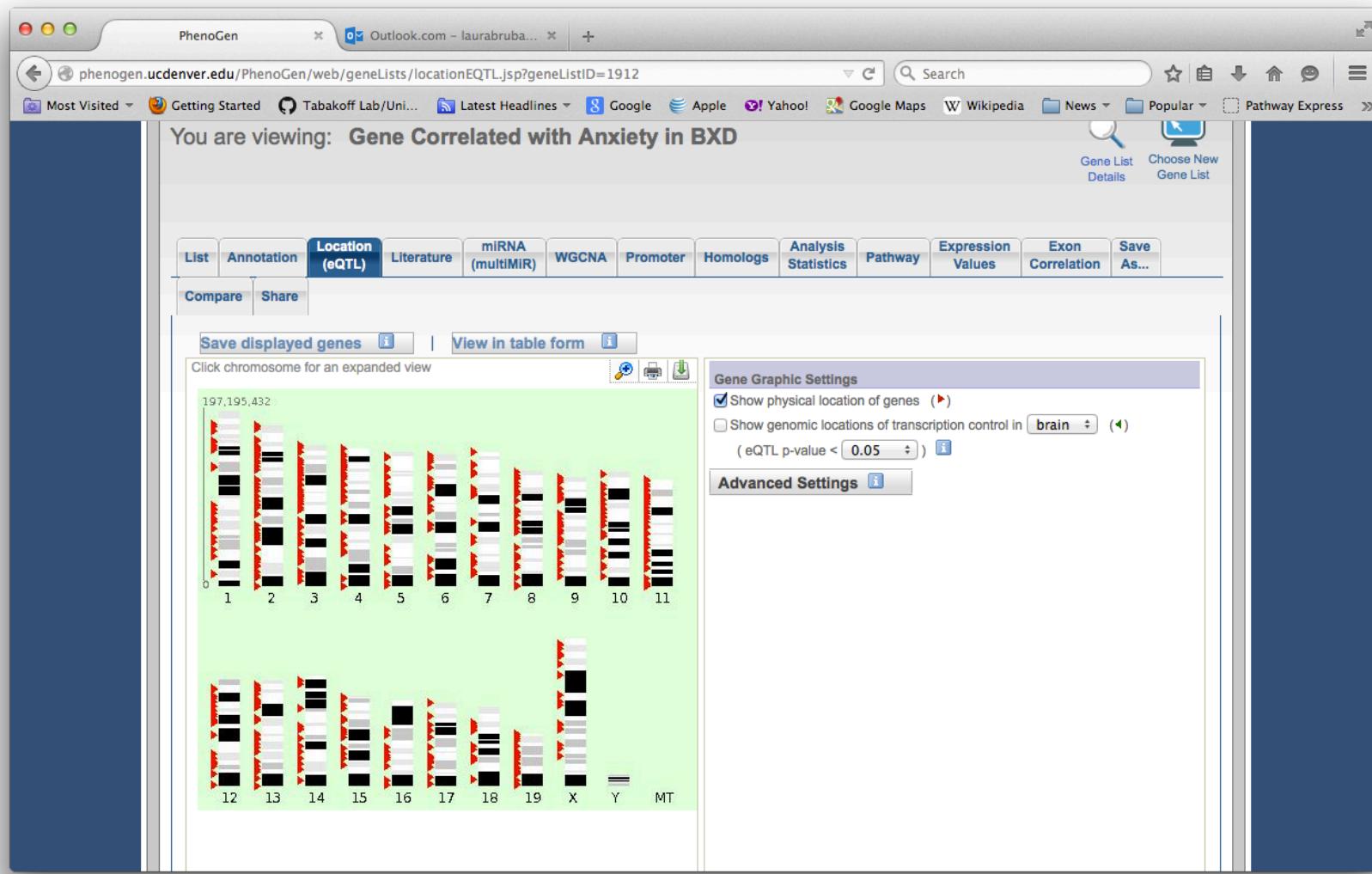
Phenotypic QTL

DNA Variants Associated with Phenotype



Expression QTL

DNA Variants Associated with RNA Expression Levels



DOWNLOADS

Downloadable Files

- Microarray Expression Data
 - Raw
 - Processed
 - eQTL/heritability
- Genotype Data
- RNA-Seq Data
 - Raw
 - Processed
- Code
 - Github

FUTURE DIRECTIONS

Future Data Plans

Strains	Tissue	Sex	Number of Biological Replicates Per Strain	Number of Paired-End Reads (rRNA-depleted total RNA)	Number of Single- End Reads (small RNA)
30 Classic Inbred Rat Strains	brain	male	4	18 trillion	5.5 billion
30 RI Rat Strains	brain	male	4	18 trillion	5.5 billion
30 Classic Inbred Rat Strains	liver	male	4	18 trillion	5.5 billion
30 RI Rat Strains	liver	male	4	18 trillion	5.5 billion

Functions to Add in Future

- multiMiR (miRNA/mRNA pairs) for rat
 - Currently implemented for mouse
- WGCNA for uploaded data sets
- Causal inference in co-expression modules
- Google drive integration