# Using the Sequenced Rat Brain Transcriptome to Identify Networks Predisposing Alcohol Consumption

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# Genetics and Alcohol Consumption

- You can't be an alcoholic if you have never tried alcohol, i.e., it is an **etiologic essential**.
- Research shows a strong **genetic influence** on levels of alcohol consumption.
- Voluntary alcohol consumption is a **complex polygenic trait** that manifests through the complex interaction of many biological entities.

http://lets-go-to-the-movies.tumblr.com/tagged/Bridesmaids/page/4

# Goal of This Study

To combine information from rodent models on:

1) DNA sequence
2) brain RNA expression levels
3) variation in voluntary alcohol consumption

to identify **genetic pathways** that predispose to variation in voluntary alcohol consumption.

# Why Pathways and Not Individual Genes?

- Complex polygenic trait ≠ one gene
- Pathways add biological context to unannotated or under annotated genes.
- We hypothesize that different perturbations, i.e., changes in the expression of different genes, of the same genetic network related to voluntary alcohol consumption can produce similar phenotypic outcomes.

# Populations

# Common Genetic Rodent Models For Complex Traits

## Recombinant Inbred Panel

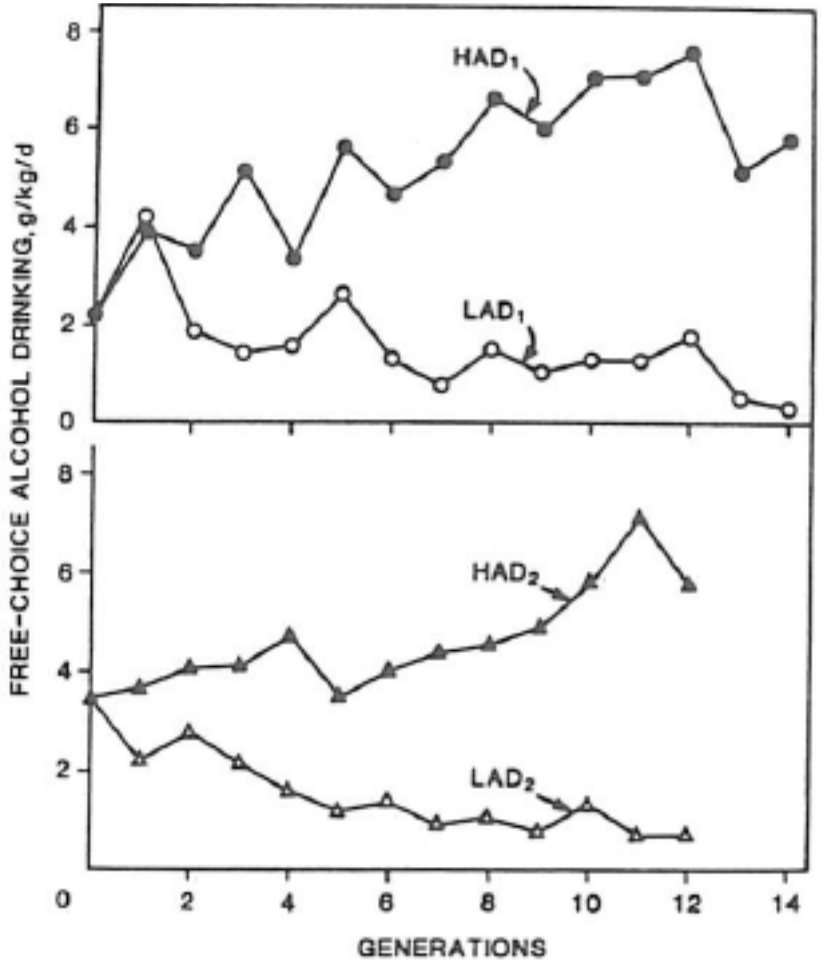
- Definition – a group of inbred strains that were original derived by the crossing of two or more parental strains to produce strains with alternating haplotypes of varying length from the two parental strains
- Similar to a cross-sectional study
  - ***continuous distribution*** of quantitative complex traits
  - different on lots of other traits

## Selected Lines

- Definition – lines of animals that are bred through selection with the intent to "fix" regions of the genome associated with selection trait, while remainder of genome varies randomly
- Similar to a case/control study
  - represents ***extremes*** of the complex trait used for selection

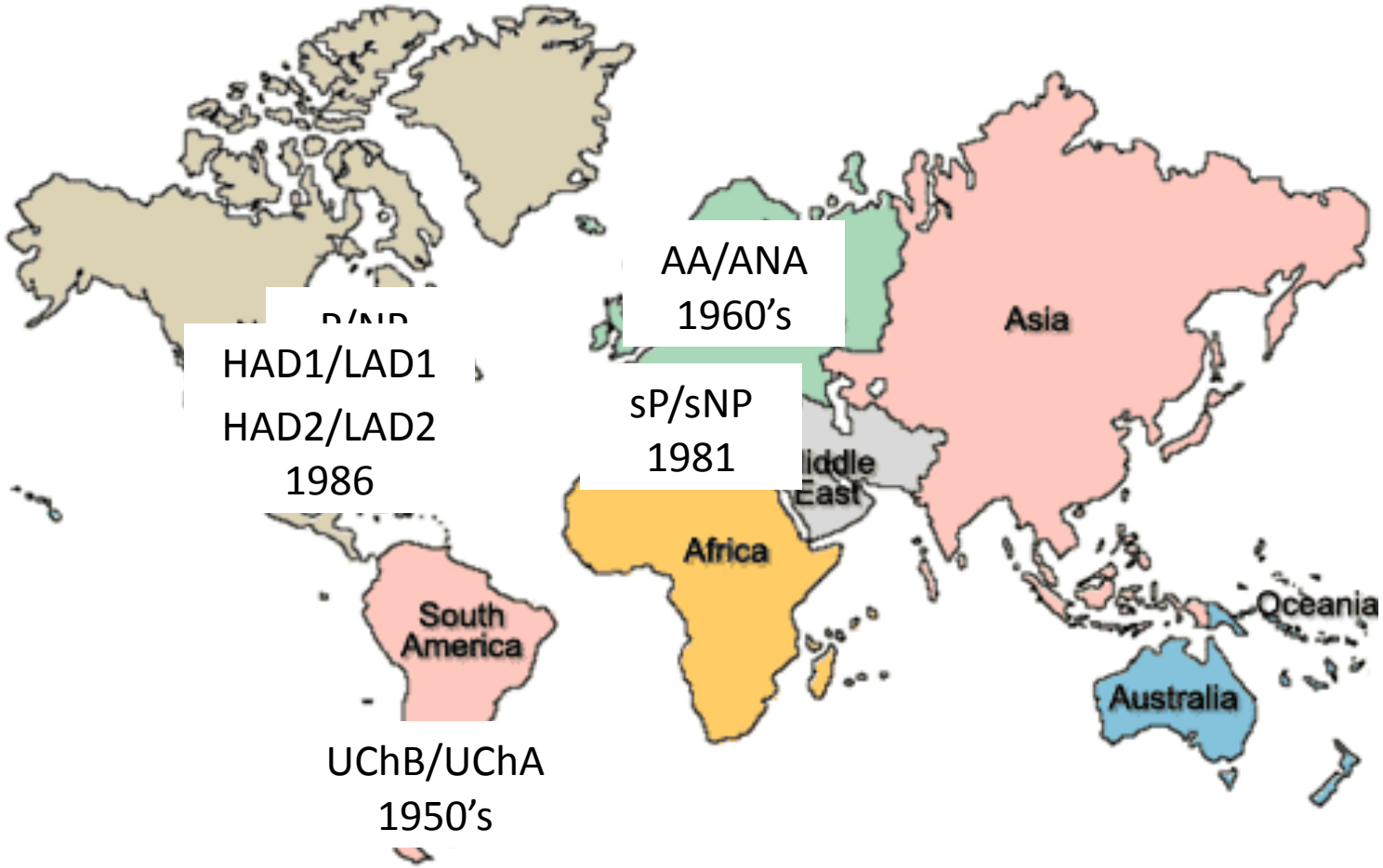
# Selective Breeding

"HERE COMES THE PIMP."

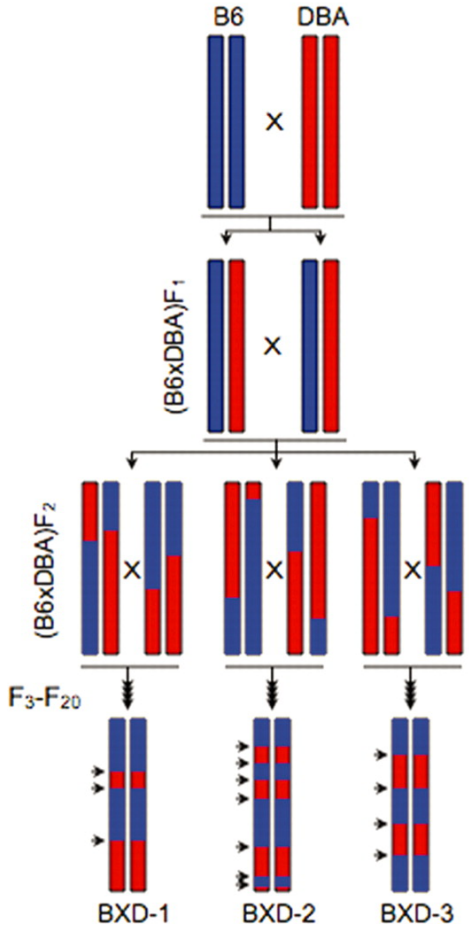



Li T-K, Lumeng L, and Doolittle DP (1993). Selective breeding for alcohol preference and associated responses. Behavior Genetics 23(2):163-170

# Origin of Six Pairs of Selected Lines



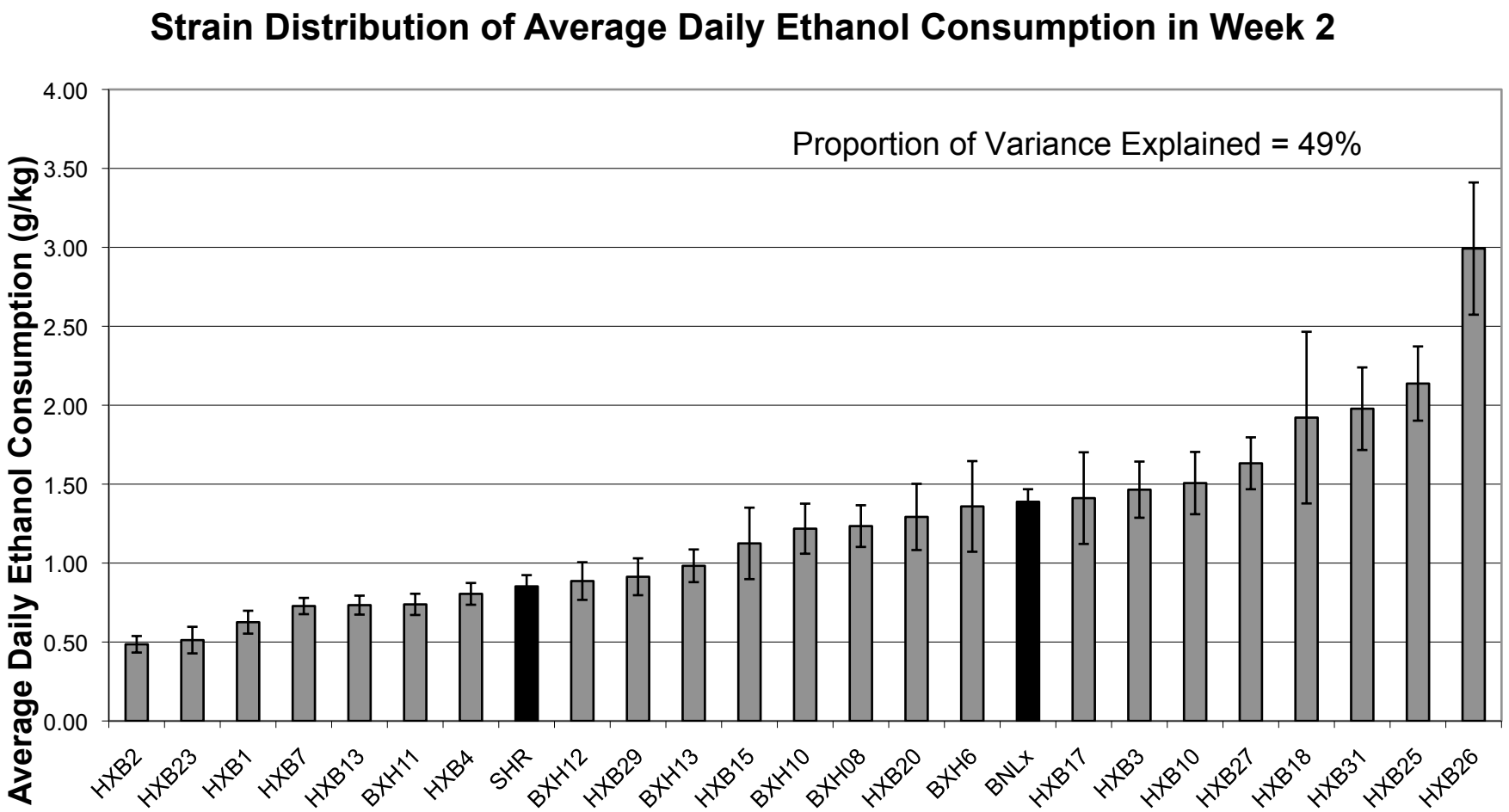
# Recombinant Inbred Rodent Panel




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

**Wu Z K et al. Nucl. Acids Res. 2010;nar.gkp1251**

# Distribution of alcohol consumption in two bottle choice




Copied From Tabakoff B, Saba L et al 2009. BMC Biol. 7:170

# RNA Expression Estimates

# Alcohol Naïve Brain RNA Expression

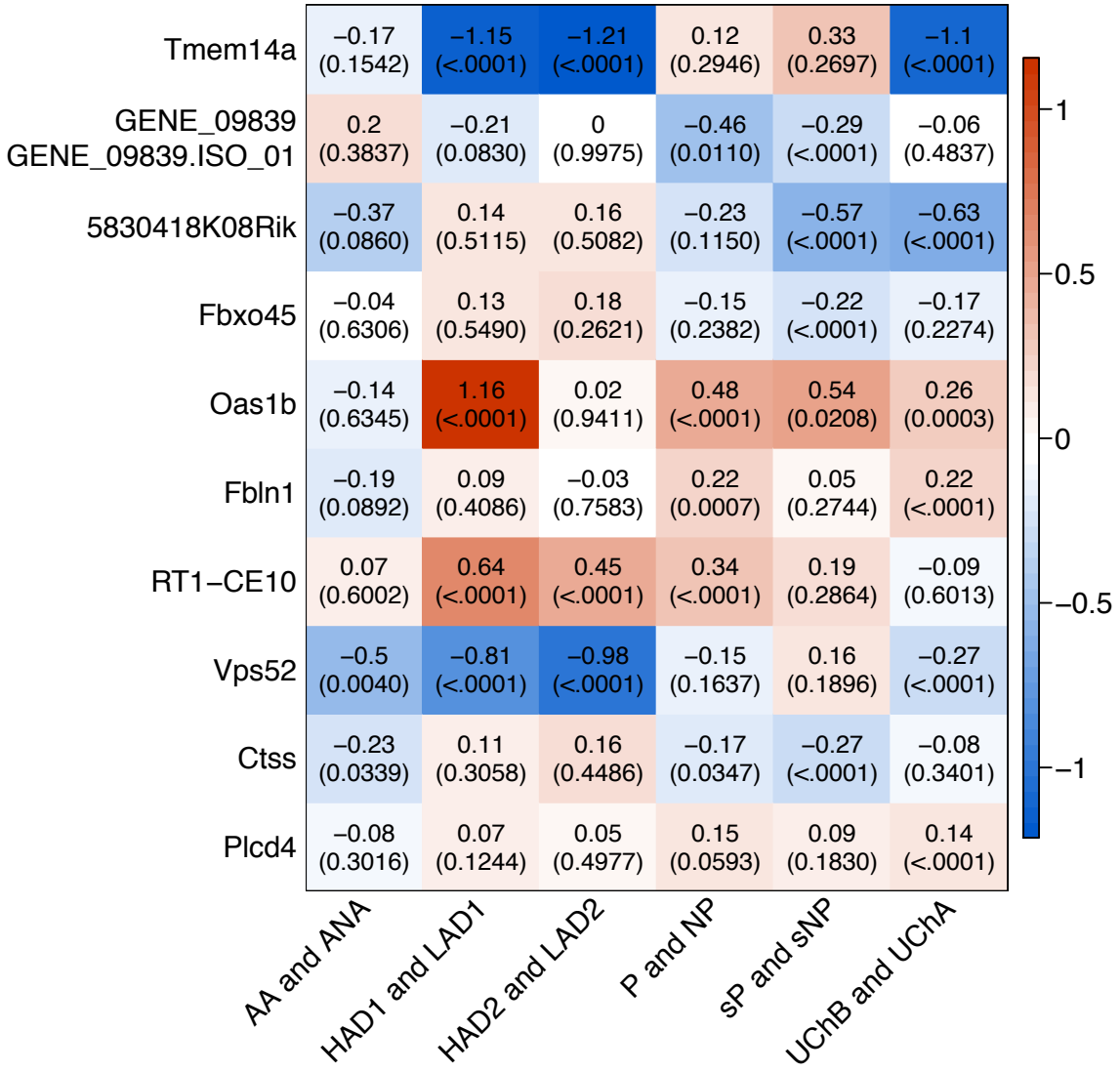
- Selected Lines – 60 Affymetrix Rat Exon 1.0 ST Arrays
  - 6 pairs of selected bred lines
  - 2 lines per pair
  - 5 samples per line
  - 1 array per sample
- RI Panel - 84 Affymetrix Rat Exon 1.0 ST Arrays
  - 21 RI strains
  - 4 samples per strain
  - 1 array per sample

# DNA/RNA-Seq Guided Microarray Analysis

- How good are the probes?
  - DNA-Seq of BN-Lx and SHR inbred strains
- What gene/transcript do the probes represent?
  - RNA-Seq of BN-Lx and SHR inbred strains

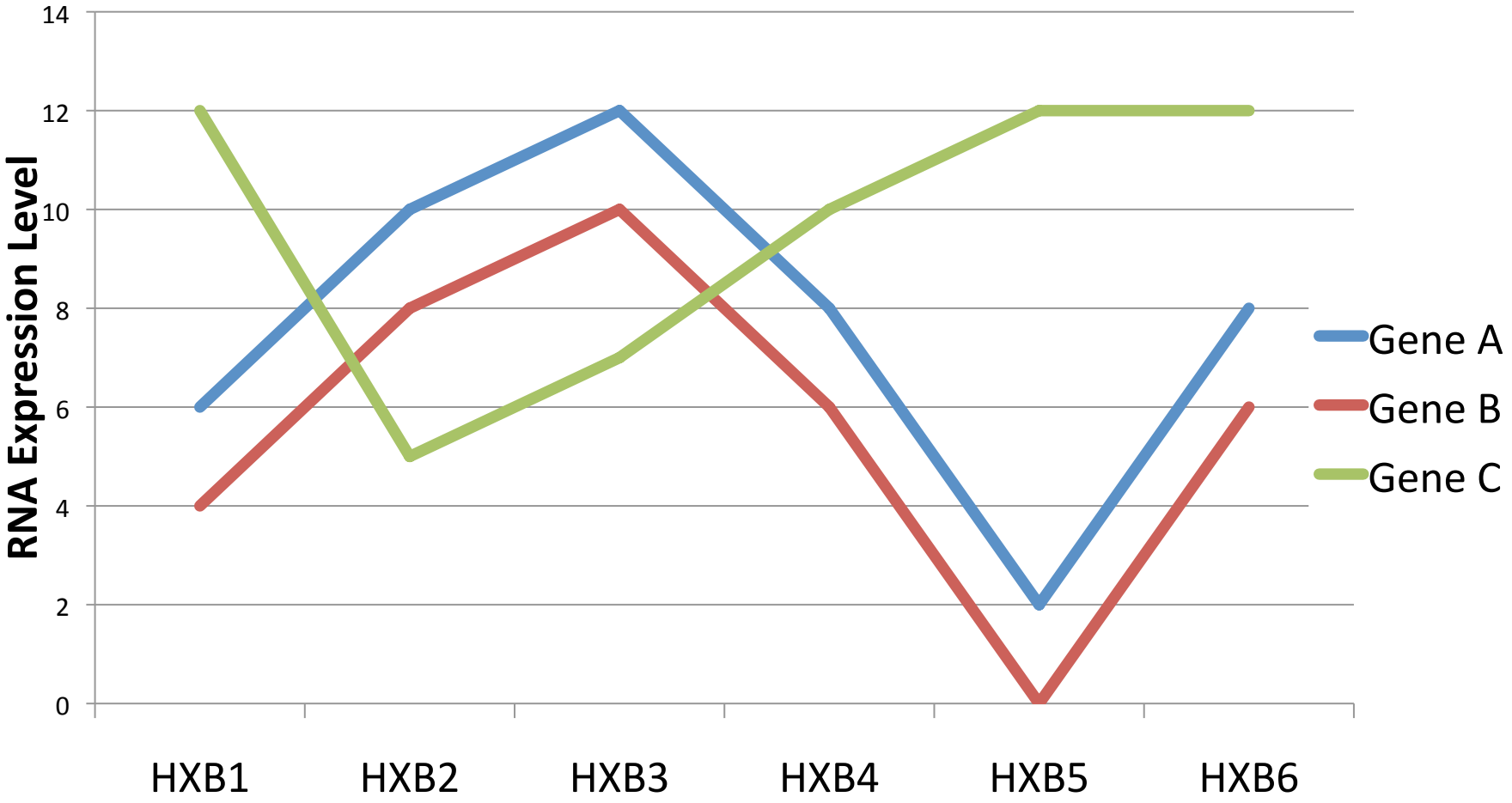
# Selected Lines

# Genes/Isoforms differentially expressed between high alcohol consuming and low alcohol consuming selected lines of rats



# Recombinant Inbred Panel – HXB/BXH

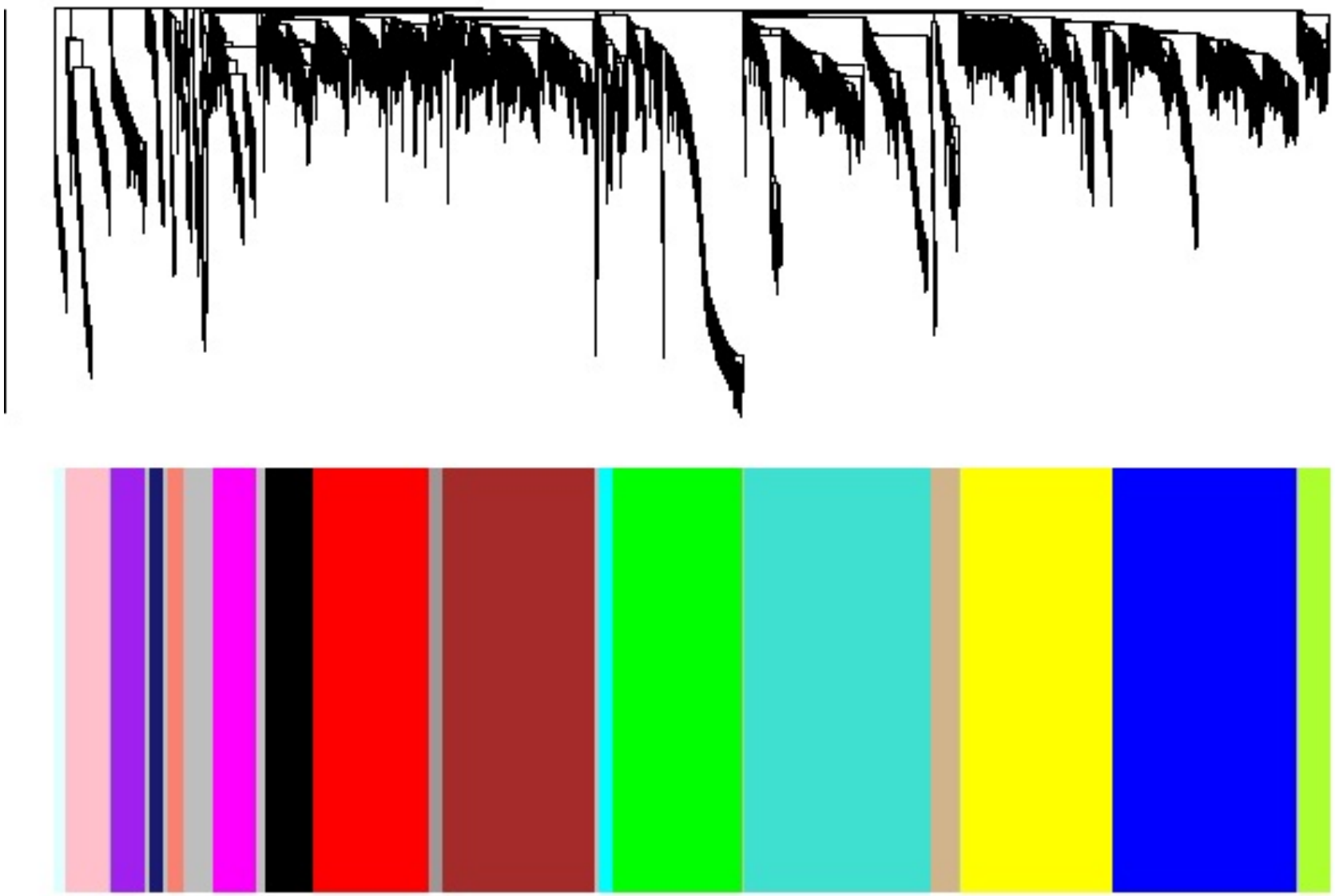
# How are transcripts related, one to another?



# Weighted Gene Co-Expression Network Analysis
# Why Not Just Use Correlation?

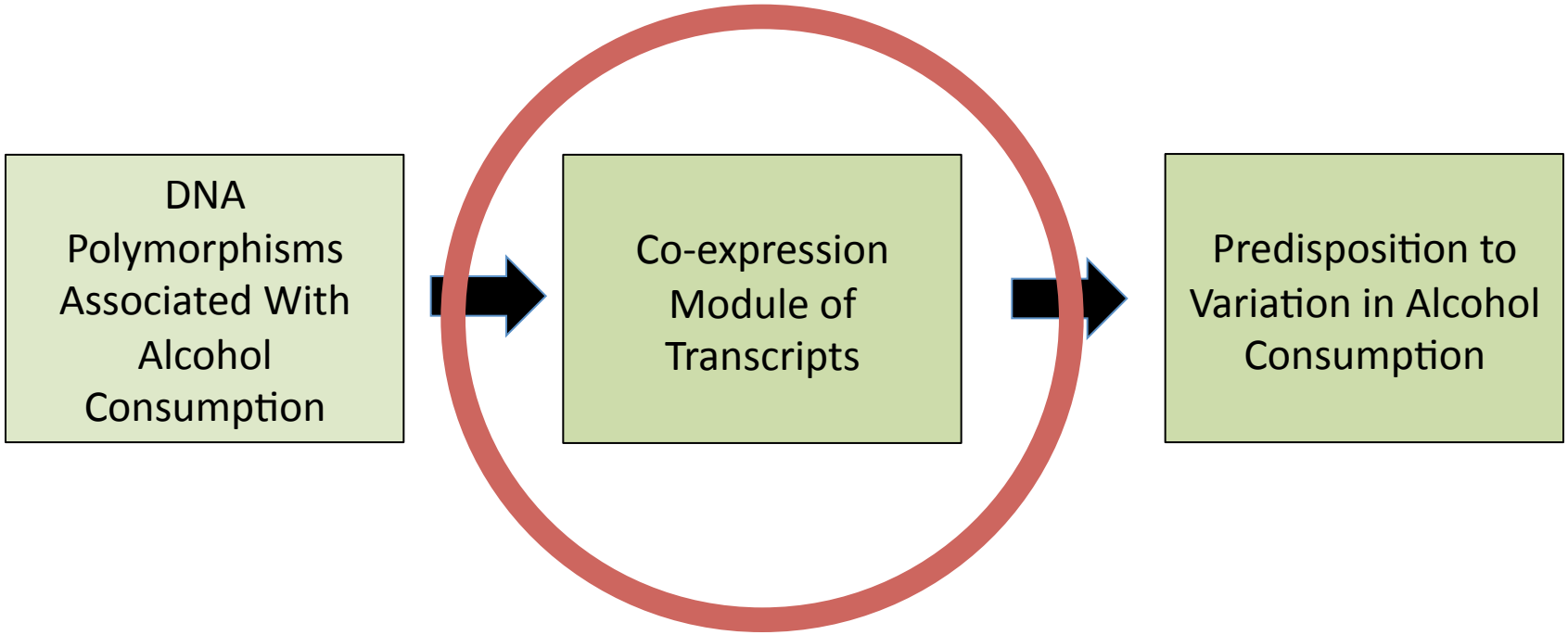
1. Simple correlation does not give connectivity.
2. How are we measuring co-expression?
   - Scale-Free Network
     - Network has few highly connected genes rather then each gene have similar connectivity
     - **Biologically motivated**, fewer highly connected genes means that a system is more robust 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

# Co-expression Modules



# Candidate Transcriptional Pathways

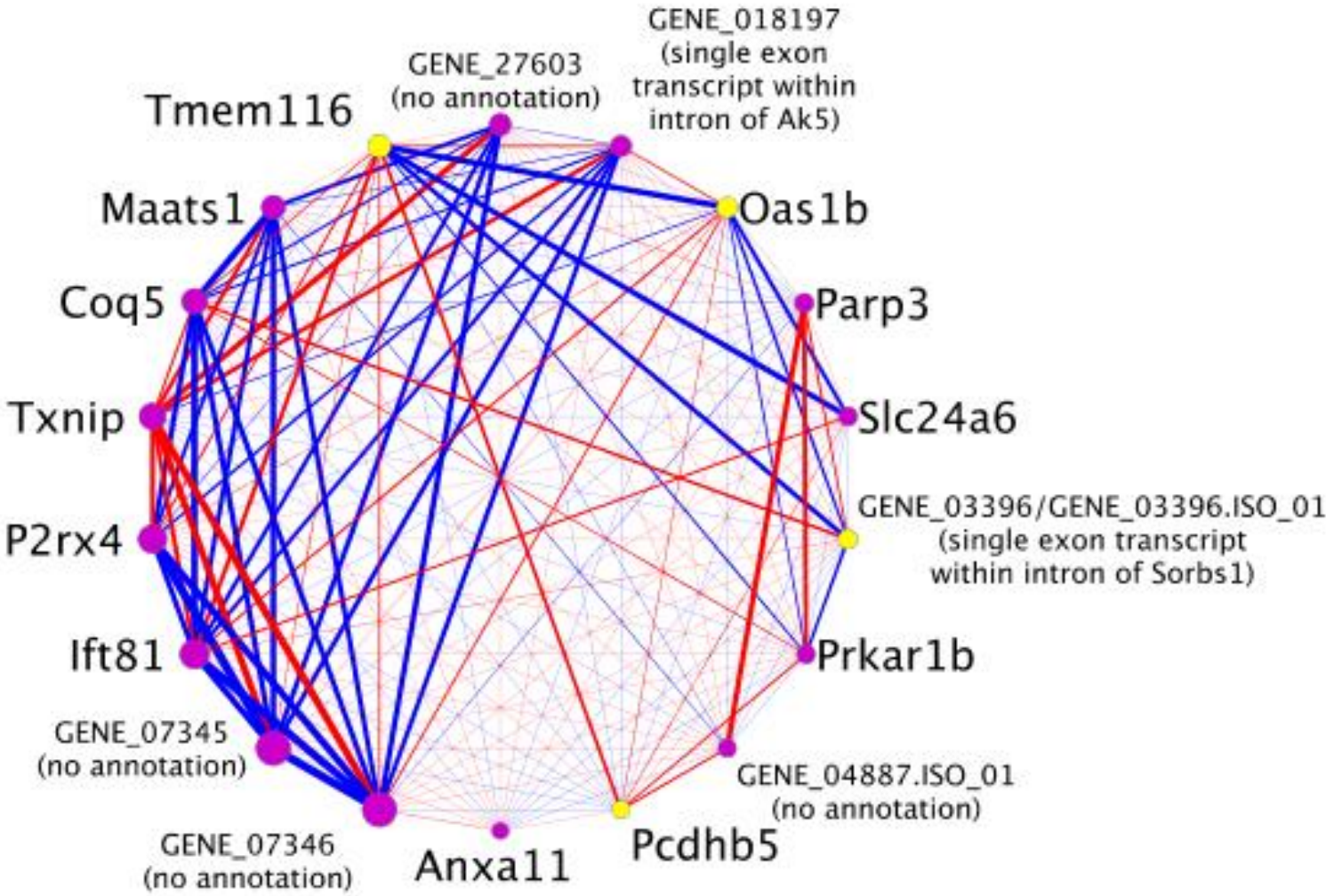
# Transcriptional Pathway



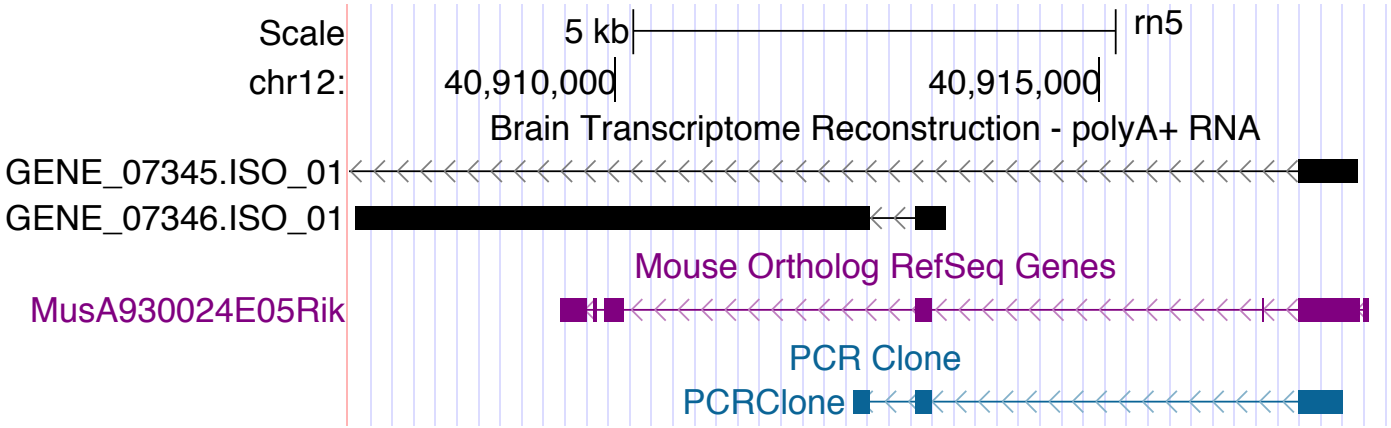
# Candidate Modules

1. Significant eQTL for the co-expression module within a QTL for alcohol consumption **(DNA → RNA)**
2. Module expression correlated with alcohol consumption and/or genes within the coexpression module tend to be differential expressed in the selected lines **(RNA → phenotype)**

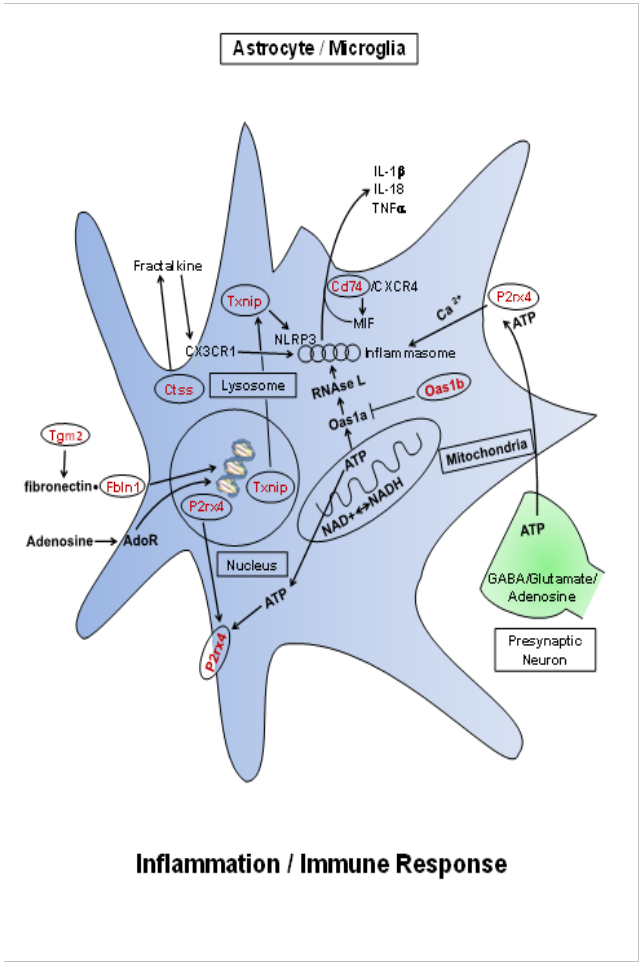
# Transcriptional Pathway Associated With Alcohol Consumption

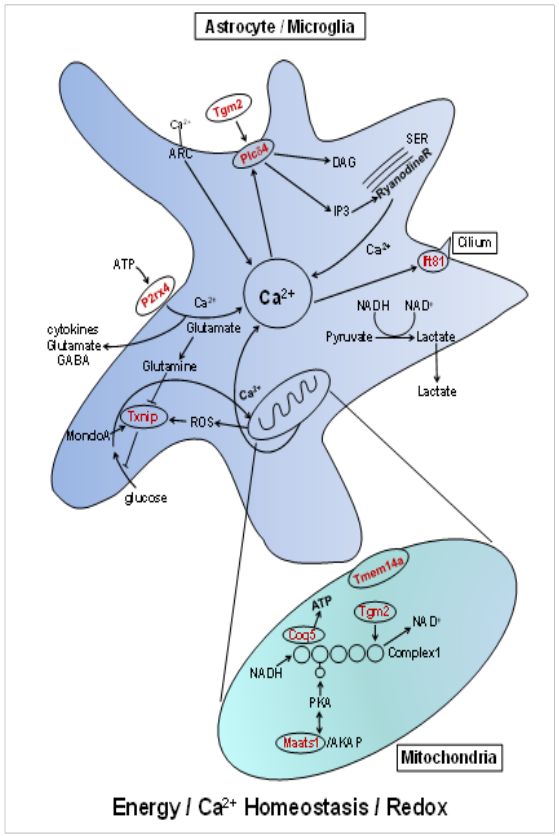


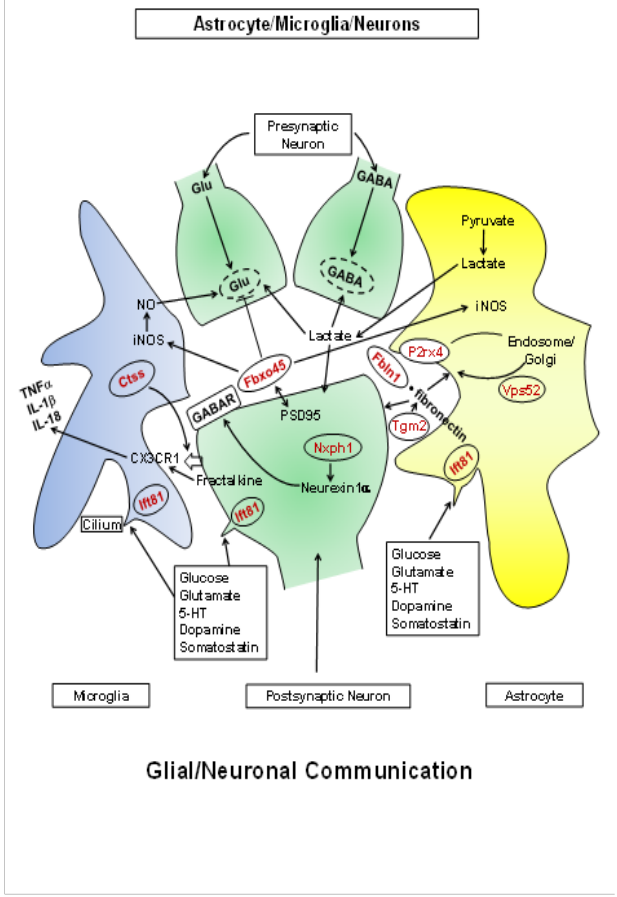
# RNA-Seq and Unannotated Genes



# Biological Context from Pathway



# Future Statistical Directions

- Identification and quantification for several large **RNA-Seq data** sets we are wadding through (http://phenogen.ucdenver.edu)
- Genetic **causal inference**, i.e., can we determine if the relationship between two 'correlated' transcripts and/or phenotypes is causal, reactive, or, completely independent
- **Predictive** network analysis when merging multiple 'omics' data sets in a single model

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