

Introduction to the Hybrid Rat Diversity Panel: A renewable rat panel for genetic studies of addiction-related traits

Hao Chen, PhD

Associate Professor

Department of Pharmacology, Addiction Science, and Toxicology

University of Tennessee Health Science Center

Laura Saba, PhD

Associate Professor

Department of Pharmaceutical Sciences

Skaggs School of Pharmacy and Pharmaceutical Sciences

University of Colorado Anschutz Medical Campus

Outline

- Inbred model organisms
- Recombinant inbred panels
- Why rats?
- Hybrid Rat Diversity Panel
- Current resources
- Data integration demo
- Where to now?

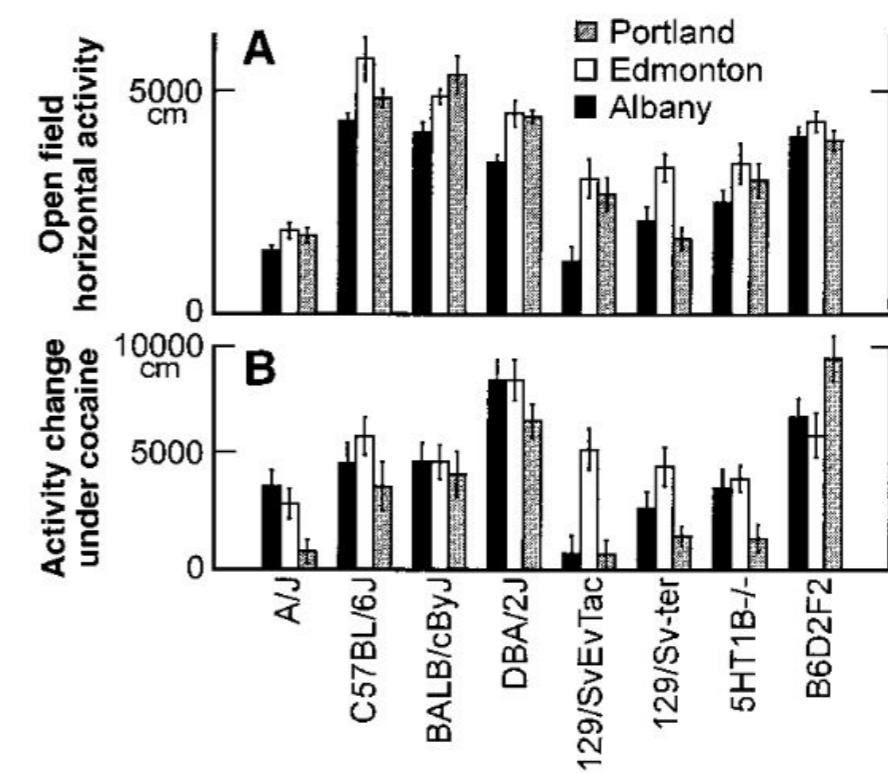
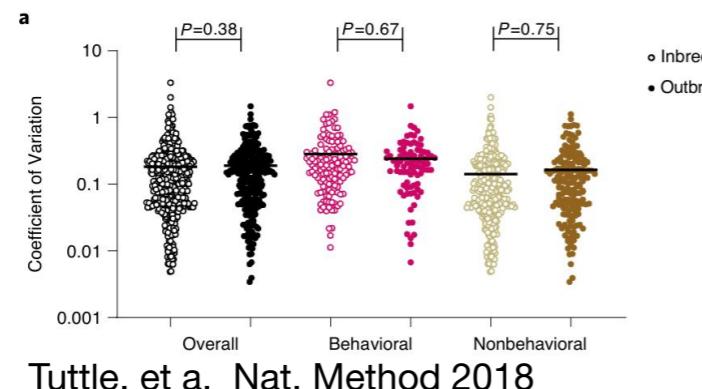
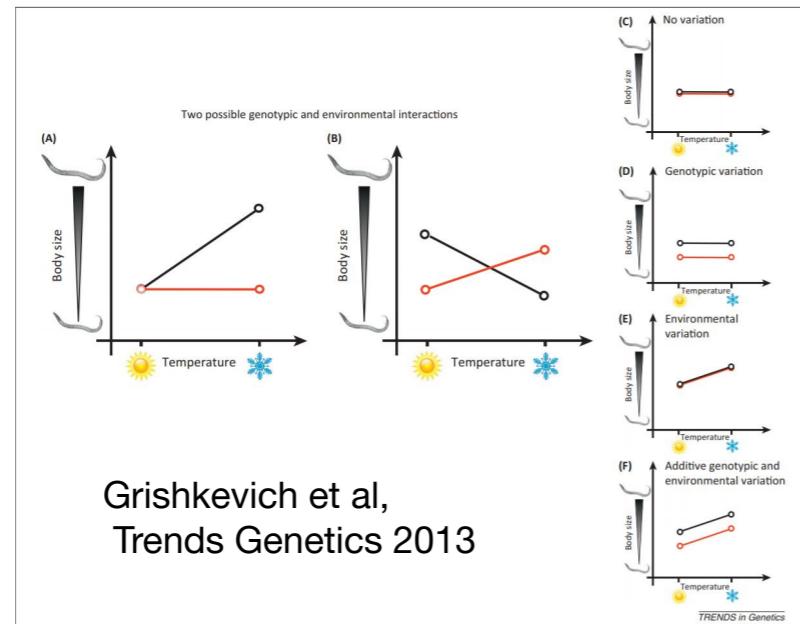
Inbred Model Organisms

Inbred animals

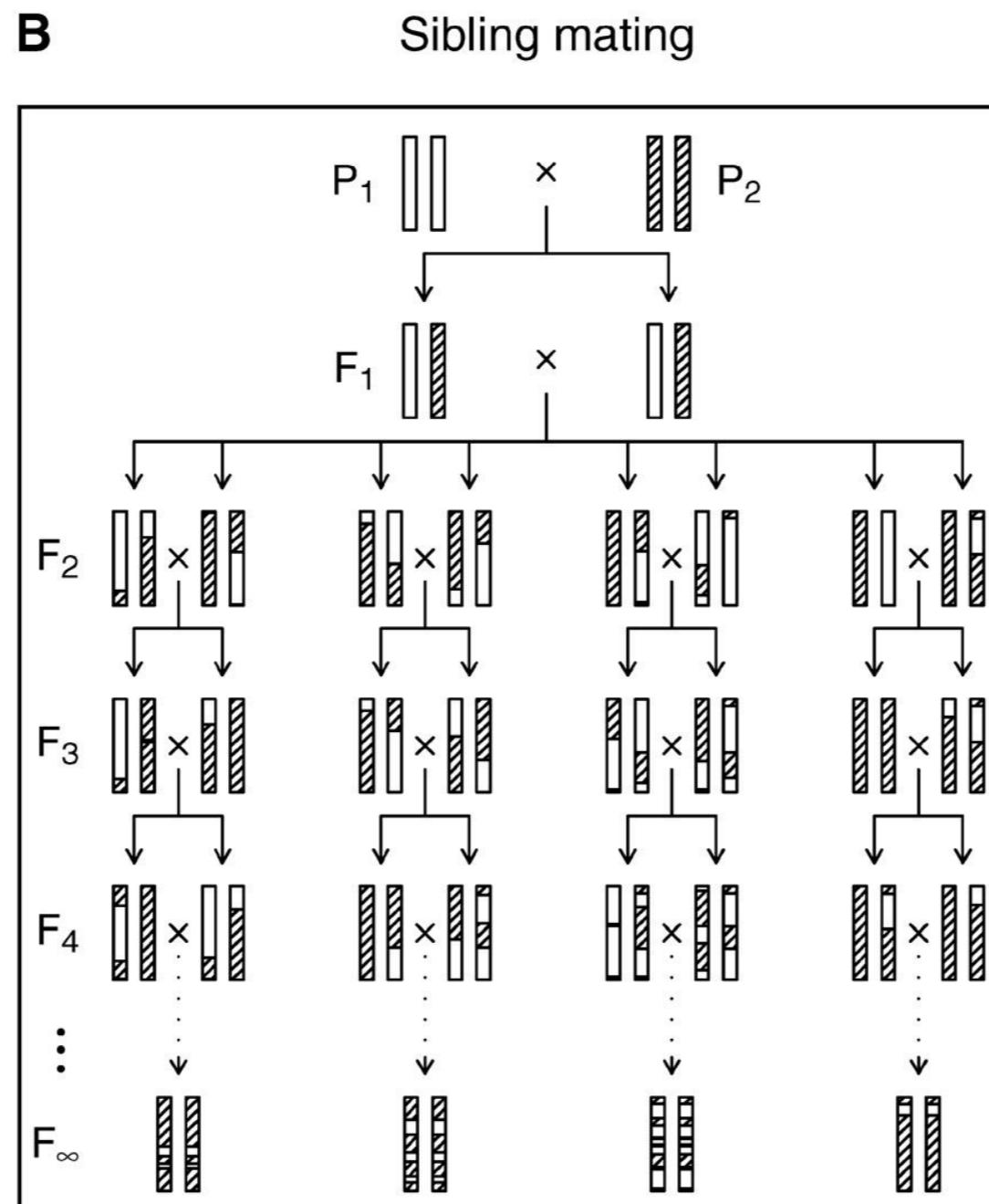
- **Inbreeding** means mating of animals more closely related to each other than the average relationship within the breed (or population)
- The **coefficient of inbreeding** is the probability that two alleles at any locus are identical by descent (Falconer 1981).
- Operationally, inbred strains are produced by full sister x brother mating (known as full-sib) for over **20 generations**.
- Inbred animals are **homozygous** at virtually all genetic loci and also **isogenic** (i.e. all animals of the same strain are genetically identical)
- **Offspring of inbred strains are genetically identical to the parents**

Features of inbred animals

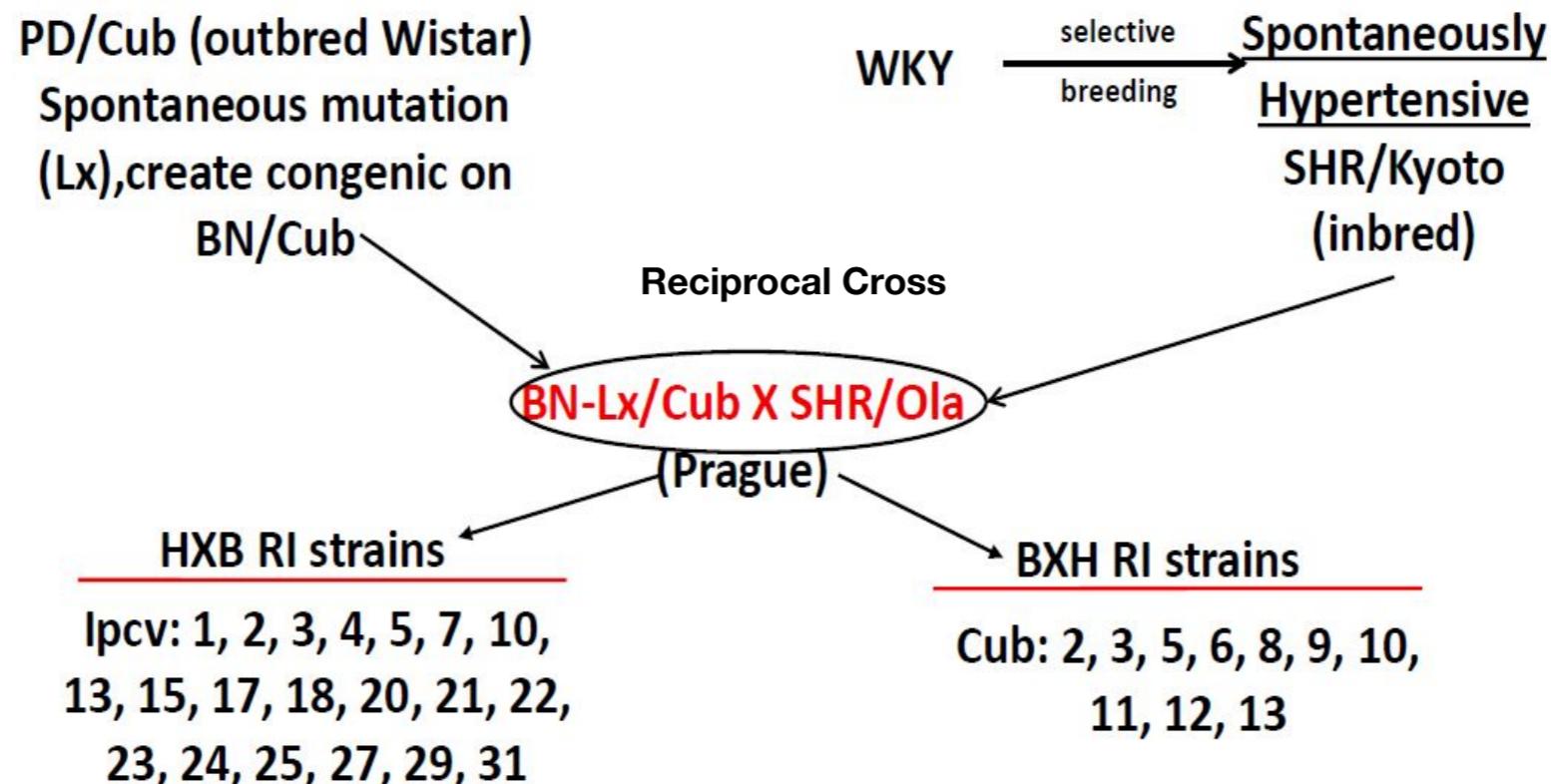
- Phenotypes are stable across generations
 - Accumulation of different phenotype and omics data
 - Studying GXE
- Within strain variation is still expected, even when the environment is well controlled
- Be careful about substrain of the inbred animals (e.g. same strain from different sources)
- Inbred depression



Recombinant inbred panel



HXB/BXH panel

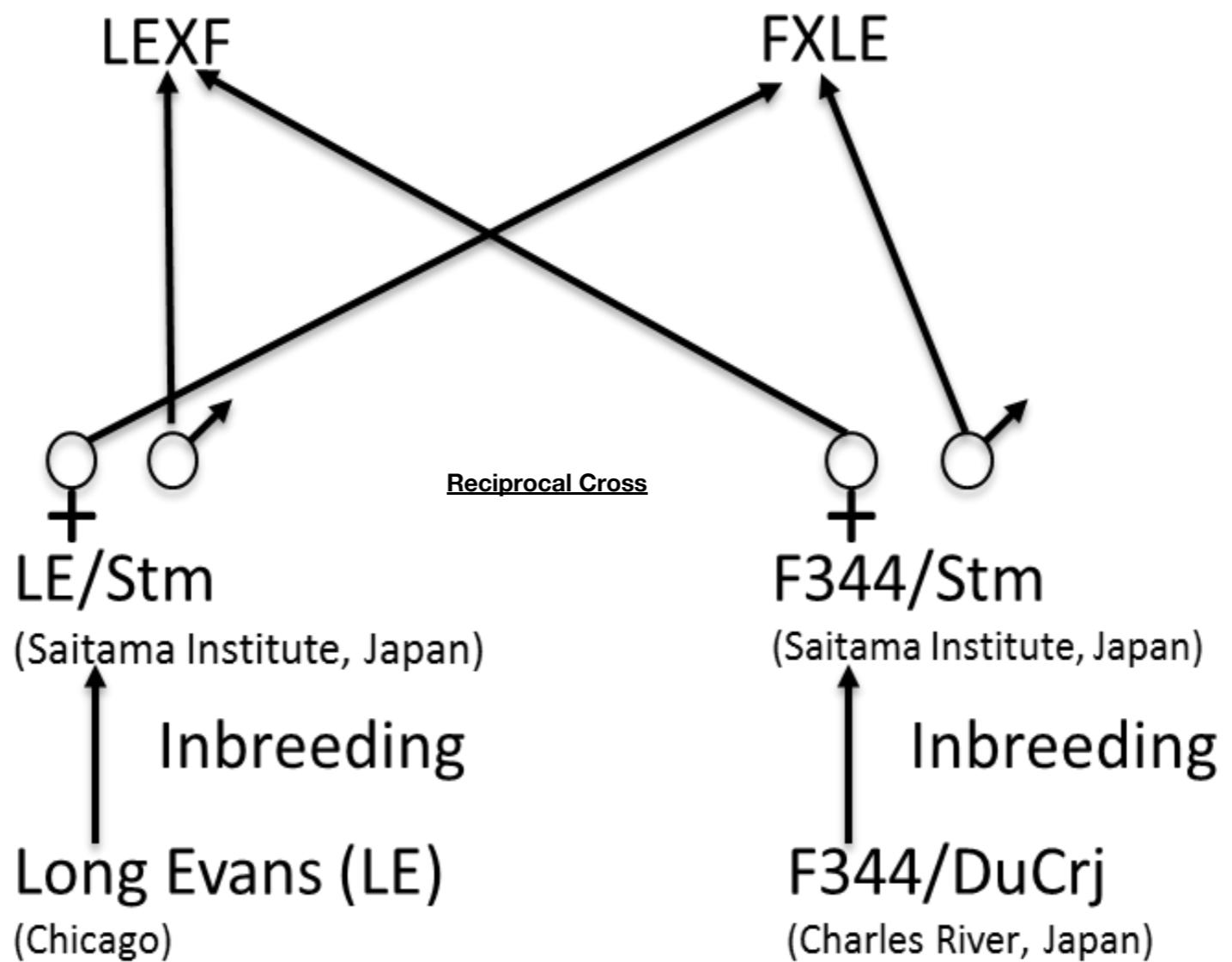


- > 4,500,000 SNPs/indels between progenitors
 - Haplotype map generated

Traits mapped using this panel:

- Cardiovascular traits: arterial pressure, stress-elicited heart rate, and pressor response, and metabolic traits, including insulin resistance, dyslipidemia and glucose handling, and left ventricular hypertrophy.
- Limb development and malformation
- Behavioral traits: startle motor response and habituation, anxiety, elevated plus maze, conditioned taste aversion

LEXF/FXLE panel



Traits mapped using this panel include

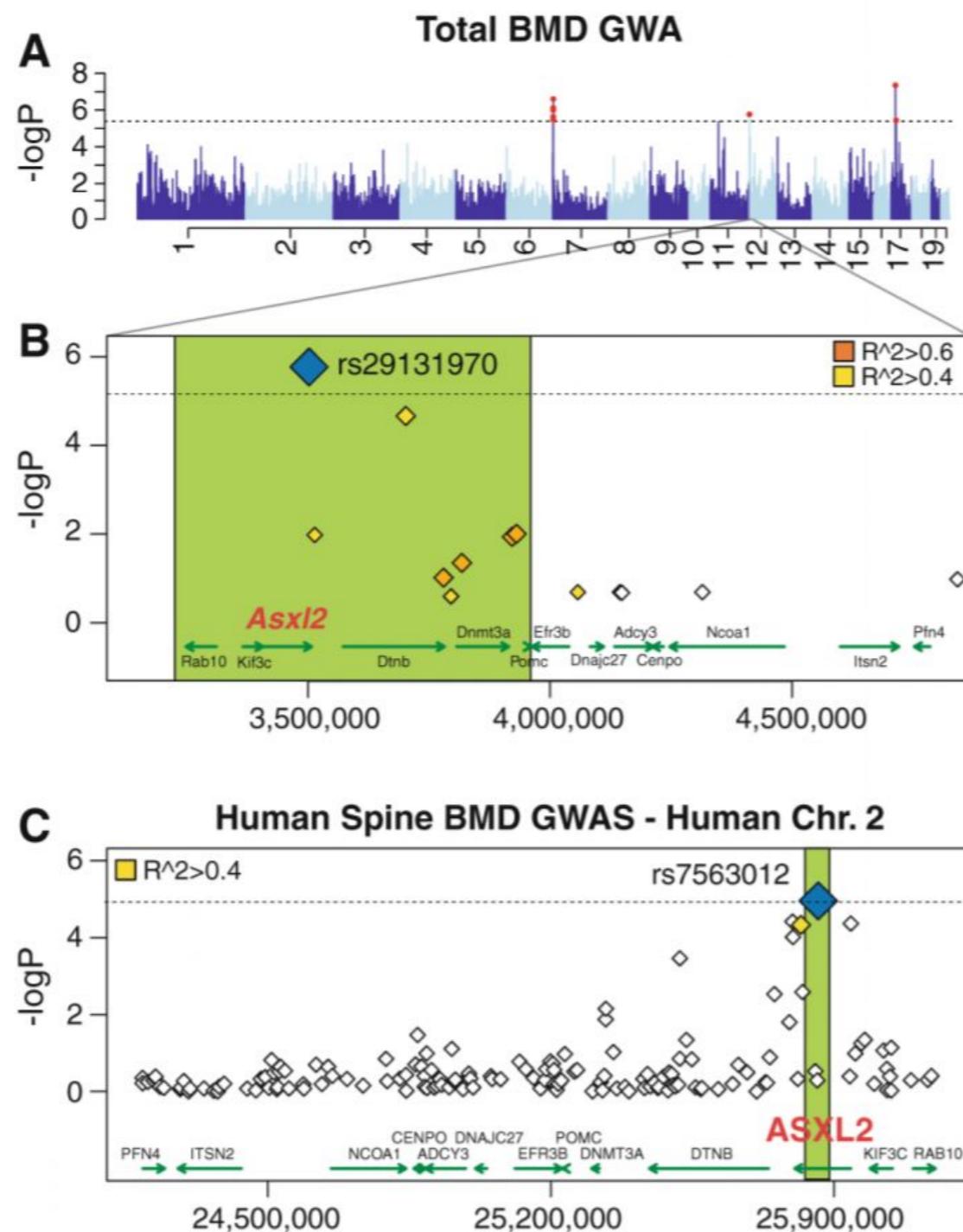
- Physiological: organ weights; blood chemistry
- Behavioral: amygdala kindling
- Cancer: T lymphoma

Hybrid Diversity Panels

Hybrid diversity panel

- **Many inbreds + RI panels**
- Increase resolution of genetic mapping
- Have a renewable resource
- Provide a shared data repository that would allow the integration of data across multiple scales, including genomic, transcriptomic, metabolomic, proteomic, and clinical phenotypes
- Genotyping only need to be done once

GWAS in HMDP for BMD



Advantages of the Rat as a model organism

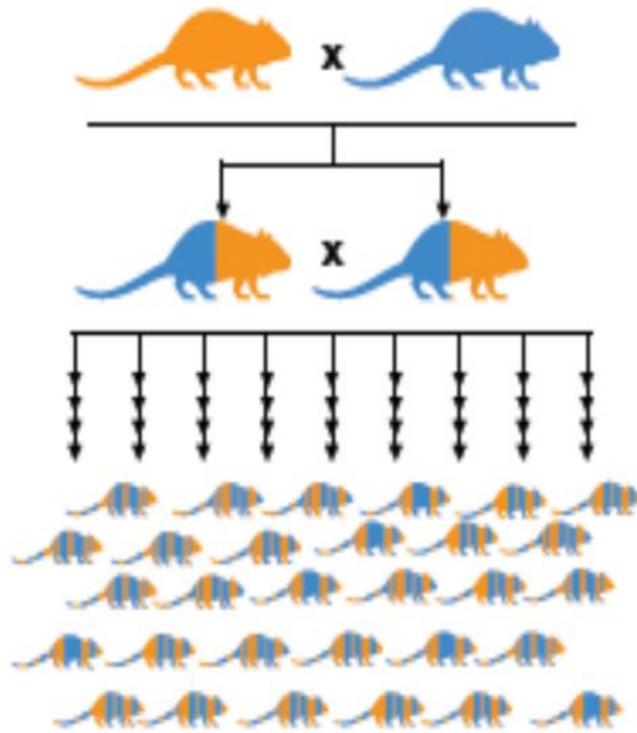
Why Rat? It's Not All or None

- Very rich repertoire of behavioral phenotypes
- Larger than mice for detailed physiological measures
- Disease genes identified in rats shown to play role in human disease
- Amenable to invasive or terminal procedures.
- Reference genome available and newly updated
- Strain specific variants identified
- Allow for further Cross-Species Comparisons
- CRISPR-Cas9 technology for genetic editing available
- Available bioinformatics resources (e.g., RGD, phenogen, genenetwork)
- Cross species validation

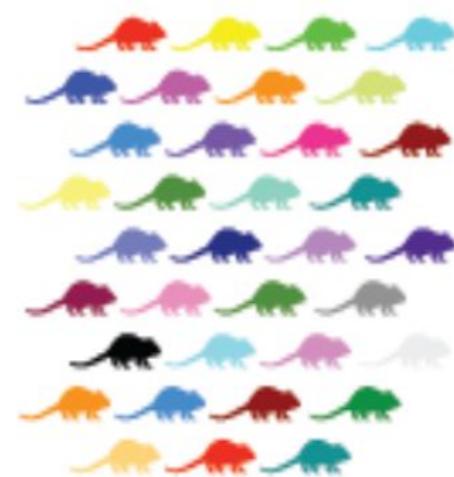
PubMed search	rats	mice
cocaine self-administration [ti] AND x	701	66
heroin self-administration [ti] AND x	211	14
nicotine self-administration [ti] AND x	210	18
methamphetamine self-administration [ti] AND x	128	8
alcohol self-administration [ti] AND x	106	19
morphine self-administration [ti] AND x	85	11
amphetamine self-administration [ti] AND x	67	0
reinstatement [ti] AND self-administration AND x	574	56
withdrawal [ti] AND self-administration AND x	262	37
extinction [ti] AND self-administration AND x	221	24
incubation [ti] AND self-administration AND x	73	4
escalation [ti] AND self-administration AND x	72	8

Hybrid Rat Diversity Panel

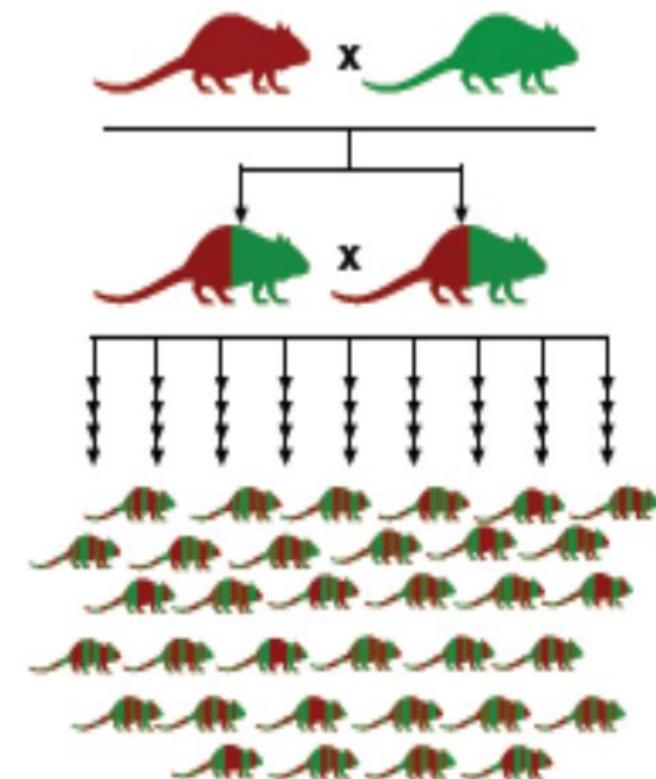
The Hybrid Rat Diversity Panel (HRDP) Developed for Quantitative and Systems Genetic Studies



HXB/BXH Recombinant Inbred Panel (30 strains)



Divergent Classic Inbred Strains (32 strains)



FXLE/LEXF Recombinant Inbred Panel (34 strains)

Professor Michal Pravenec, Prague, Czech Republic

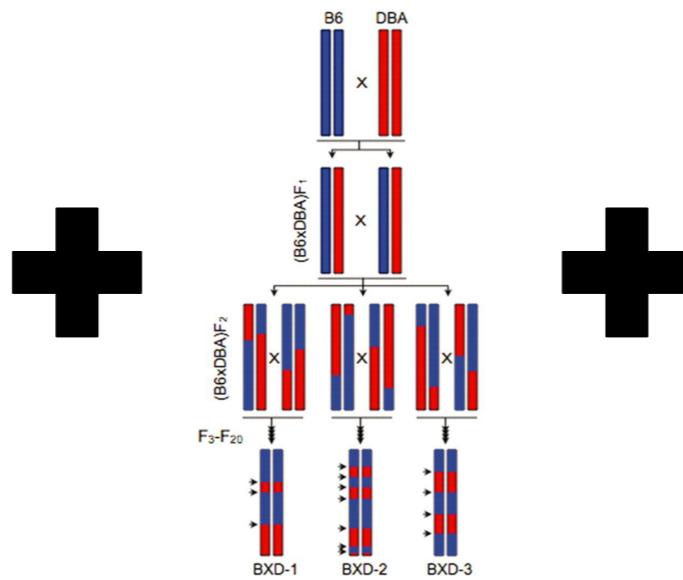
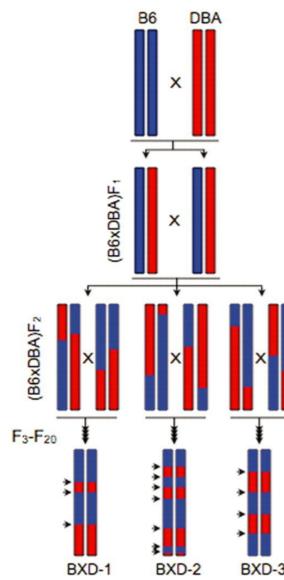
Professor Masahide Asano, NBPR, Kyoto, Japan

All strains being established at the Medical College of Wisconsin, Dr. Melinda Dwinell

Hybrid Rat Diversity Panel (HRDP)

A Renewable Genetically Defined Population for Cumulative Biology

- Susceptibility/predisposition studies
- Mechanistic Biology Studies
- Systems/Network Biology Studies
- Toxicologic Analysis
- Pharmacogenomics/toxicokinetics
- Proof of Concept Studies



**30 HXB/BXH
Recombinant
Inbred Strains**

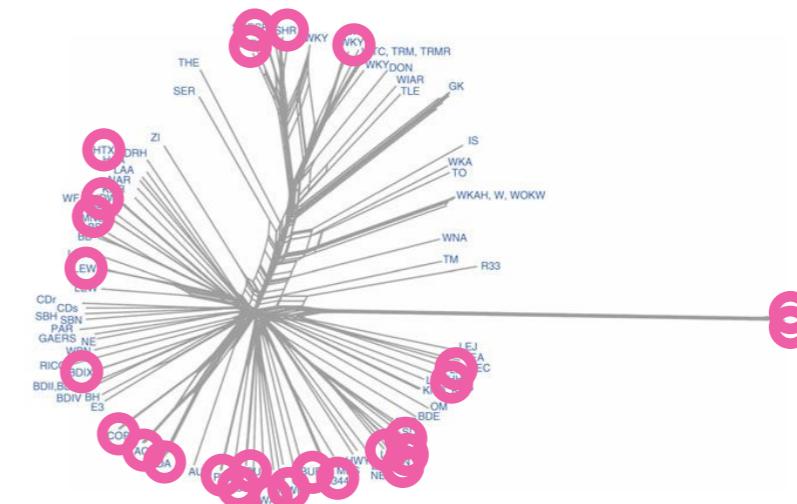
**32 LEXF/FXLE
Recombinant
Inbred Strains**

**34 Classic
Inbred Strains**

Power to detect loci that contribute 10-20% to genetic variance

Power to detect significant genetic correlations as small as 0.28

Power to perform high resolution mapping (median haplotype block size = 225 Kb)



What Do We Want to Capture in the HRDP?

1. Genetic and Phenotypic Variability

- We want the genetic and phenotypic traits to be consistent for long periods allowing for cumulative studies across time.
- Inbred strains fill this criterion – stable genotypes.

2. Effects of Genetic Background.

- Genetic elements or haplotypes on different genetic backgrounds allow for measures of epistasis and other interactions.
- Recombinant Inbred Strains allow for measures of epistasis on stable reproducible backgrounds.

3. High Resolution Association Mapping Capability.

- Generated by proper choice of genomes.
- Proper assortment of inbred strains based on genomic diversity – but have to control for population structure (and those methods established).

4. Useful for Analysis of GXE (Including Drugs, Toxins, etc.).

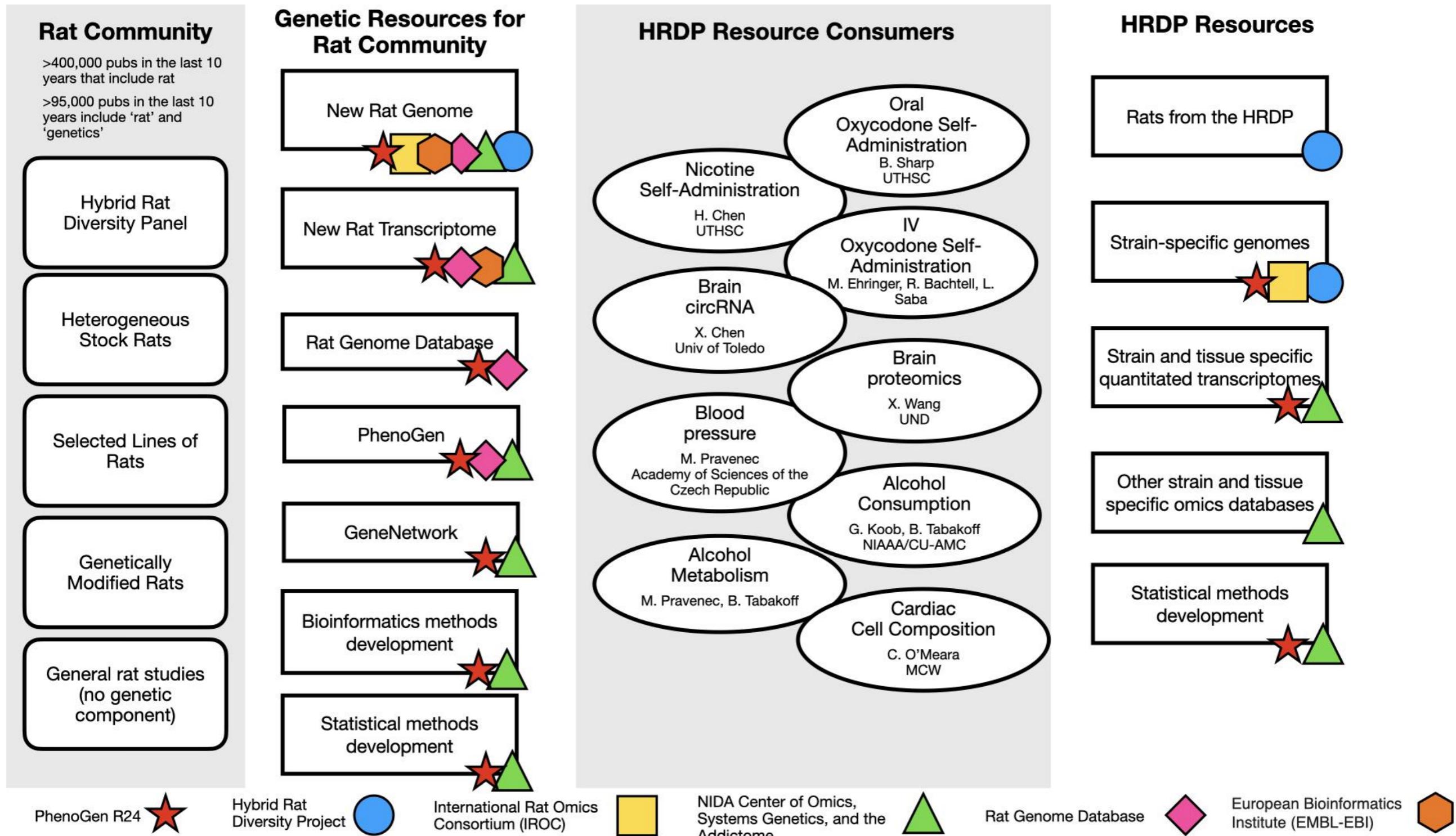
- Should have many sets of genetically identical individuals (Inbreds) exposed to different “environments.”

5. Amenable to Systems Genetics Approaches for Studying Complex Traits.

- accumulation of multiomic data sets

Current Resources for the HRDP

Rat/HRDP Ecosystems



Examples of Current HRDP Resources

- **Live rats and rat tissues** - Melinda Dwinell and colleagues (MCW); https://rgd.mcw.edu/wg/hrdp_panel/
- **Genome sequence information** – Hao Chen (UTHSC); <http://hrdp.opar.io/>
- **Transcriptome information from brain, liver, heart, and kidney** – Boris Tabakoff, Paula Hoffman, and Laura Saba (CU-AMC); <https://phenogen.org/>
- **Phenotype, RNA expression, brain proteomics, and mapping tools** – Rob Williams (UTHSC); <http://genenetwork.org>

Live Rat and Rat Tissues



R24 Resource Grant (R24 OD024617)

Develop the 96 strain Hybrid Rat Diversity Panel to be used to study disease mechanism, environmental impact, and drug development.

Strain	Disease model	Strain	Disease model
ACI/EurMcIw	Renal	MNS/N	Normotension
BDIX/CrCl	Leukemia	MR/N	Alcohol preference, Aging, Anxiety
BN/NHsdMcwi	Asthma, Neoplasms, Reproduction	MWF/Hsd	Renal, Hypertension
BN-Lx/Cub	Polydactyl-luxate, Hypercholesterolemia	PD/Cub	Polydactylos, Metabolic syndrome
BUF/CrCrl	Neoplasms, Autoimmune disorders	PVG/Seac	Immune disorders
COP/CrCrl	Hyperplasia, Exercise	RCS/LavRrrc	Eye disorders
DA/OlaHsd	Cardiovascular, Arthritis, Neoplasms	SBH/Ygl	Hypertension, Renal
F344/NCrl	Aging	SBN/Ygl	Hypertension resistant
F344/Stm	Aging	SHR/OlaPcv	Hypertension, Insulin resistant
FHH/EurMcwi	Hypertension, Renal	SHRSP/A3NCrl	Hypertension, Stroke
GK/CskCrljCrl	Diabetes	SR/JrHsd	Hypertension resistant
HTX/HcjRrrc	Hydrocephalus	SS/JrHsdMcwi	Hypertension, Renal
LE/Stm	Leukemia	WAG/RijCrl	Neoplasms, Eye disorders
LEW/Crl	Neoplasms, Autoimmune disorders	WKY/NCrl	Normotension, Insulin resistant
LH/MavRrrc	Hypertension	HXB/Ipcv RI panel	
LL/MavRrrc	Hypotension	BXH/Ipcv RI panel	
LN/MavRrrc	Normotension	FXLE/Stm RI panel	
LOU/MNCrl	Autoimmune, cancer	LEXF/Stm RI panel	
M520/N	Neoplasms, Renal, Insulin resistance	Leukemia, Lymphoma, Seizures, Reproduction	

Deliverables:

- Rats: live animals, embryos
 - Tissues (organs, blood), genomic DNA
 - Phenotyping
 - Whole genome sequencing (raw & analyzed data)

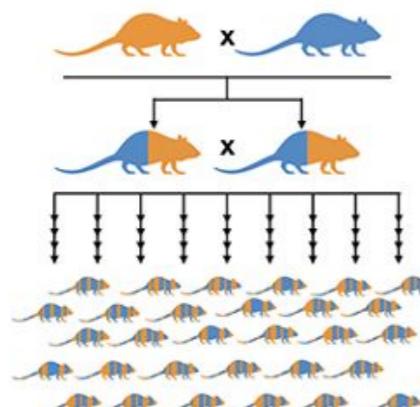


Enter Search Term...

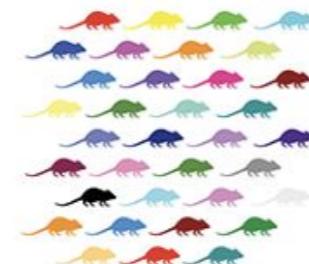
Search RGD

Advanced Search (OLGA)
OntoMate (Literature Search)

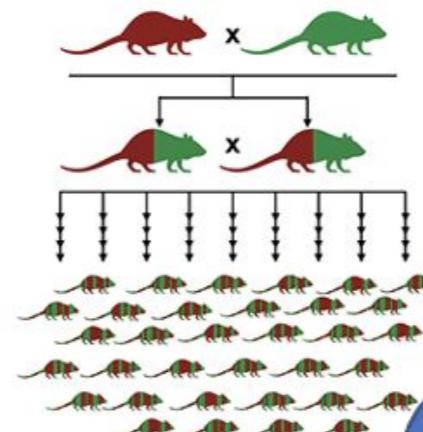
The Hybrid Rat Diversity Panel



HXB/BXH Recombinant Inbred Panel (30 strains)



Divergent Classic Inbred Strains (35 strains)



FXLE/LEXF Recombinant Inbred Panel (34 strains)

The Hybrid Rat Diversity Panel (HRDP) is a panel of 96 inbred rat strains carefully chosen to maximize power to detect specific genetic loci associated with a complex trait and to maximize the genetic diversity among genetically diverse inbred strains of rat and two panels of recombinant inbred rat strains, the FXLE/LEXF (33 strains) from Japan and the HXB/BXH (30 strains) from the Czech Republic. Recombinant inbred strains are derived from two inbred parental rat strains. The two parental strains are crossed to produce F1 pups. F1 pups are subsequently intercrossed to create an F2 generation. These genetically unique F2 generation rats are panelized for at least 20 generations to fix their genomes. The two RI panels used in the HRDP have been well characterized through studies focused on seizures, epilepsy, lymphoma and leukemia, blood pressure regulation, and alcohol consumption.

The HXB/BXH recombinant inbred strains were derived from the Spontaneously Hypertensive Rat (SHR/OlaLpcv) and the normotensive BN-Lx (BN-Lx/Cub), a Brown Norway congenic rat strain with polydactyl-luxury fur. The FXLE/LEXF recombinant inbred strains were derived from the Long Evans strain (LE/Stm) and the Fischer F344 strain (F344/Stm).

The strains included in the HRDP panel are:

Strain	RGD ID	Strain	RGD ID	Strain	RGD ID
ACI/EurMcwi	7364991	HTX/Kyo	1302600	PD/Cub	728161
BDIX/NemOda	2304285	LE/Stm	629485	PVG/Seac	1302722

https://rgd.mcw.edu/wg/hrdp_panel/

HRDP@mcw.edu



Contact us:

- Rats
- Tissues
- WGS data

Currently Available Strains

Classic Inbred Strains

Strain	RGD ID	Colony/Strain status
ACI/EurMcwi	7364991	Live
BN/NHsdMcwi	61498	Live
BN-Lx/Cub	61117	Live
F344/Stm	1302686	Live
FHH/EurMcwi	629509	Live
GK/FarMcwi	10395297	Live
LE/Stm	629485	Live
LH/MavRrrc	60990	Live
M520/N	10024	Live
MWF/Hsd	737906	Live
SHR/OlaPcv	631848	Live
SS/JrHsdMcwi	61499	Live
PVG/Seac	1302722	Live
SR/JrHsd	1582184	Live
WAG/RijCrl	2312498	Live
RCS/LavRrrc	1358258	Live
BDIX/NemOda	2304285	pups born
BUF/Mna	61118	pups born
HTX/Kyo	1302600	MCW has embryos
DA/OlaHsd	734475	Live @ Envigo
F344/NCrl	737926	Live @ CRL
LEW/Crl	737932	Live @ CRL
SHRSP/A3NCrl	2311051	Live @ CRL
WKY/NCrl	1358112	Live @ CRL
LL/MavRrrc	68077	MCW has embryos
LN/MavRrrc	61015	MCW has embryos
LOU/MNCrl	9999143	MCW has embryos
COP/CrCrl	1358153	not available; looking for alternative
MNS/N	2307319	MCW has embryos for MNS (#793)
MR/N	70449	MCW has embryos for MR/Har (#691)
PD/Cub	728161	Will import 2021
SBH/Ygl	631572	Will import 2021
SBN/Ygl	631573	Will import 2021

Recombinant Inbred Strains

Strain	RGD ID	Colony/Strain status
HXB 31	2307081	Live
HXB 10	2307083	Live
HXB 2	2307096	Live
LEXF10A	1302621	Live
FXLE12	1302602	Live
FXLE20	1302633	Live
FXLE15	1302673	Live
LEXF1C	1302666	Live
LEXF6BL2	1302617	Live
BXH 3	2307134	Live
BXH 2	2307121	Live
HXB17	2307085	Live
HXB4	2307077	Live
BXH6	2307136	Live
HXB23	2307093	Live
HXB18	2307082	Live
HXB5	2307099	Founders, but no litters from 3 breeder pairs
LEXF8A	1302653	3 males survived
LEXF1A	1302641	7 pups
FXLE14	1302615	1 pup survived
FXLE17	1302708	5 pups
LEXF2C	1302646	5 pups
LEXF5	1302723	pups born
LEXF10B	1302616	pups born
FXLE13	1302665	pups born
LEXF7B	1302649	
LEXF2B	1302710	pups born
LEXF9	1302618	

Genome Sequence Information

mRatBN7.2

- the new rat reference genome

- Source of genomic DNA
 - a single male Brown Norway rat (BN/NHsdMcwi)
- Sequencing data:
 - Pacific Biosciences CLR long read (80x)
 - 10X Genomics read cloud sequencing (31x)
 - Hi-C (29X)
 - Bionano optical mapping.
- Organization
 - Produced by the Darwin Tree of Life (DToL) project and the Vertebrate Genome Project (VGP) program.
 - Long term maintenance by the Genome Reference Consortium
- Data availability:
 - NCBI: https://www.ncbi.nlm.nih.gov/assembly/GCF_015227675.2
 - ENSEMBL:
https://rapid.ensembl.org/Rattus_norvegicus_GCA_015227675.2/Info/Index
 - UCSC genome browser: pending

Major improvements of mRatBN7.2

- Detailed comparison is on going.
- Much improved contiguity of the genomic sequence
 - From 440 gaps between scaffolds in rn6 to 0 gaps in mRatBN7.2
- Fixed a large inversion in chr6
- Fixed numerous base level sequencing errors -> greatly improves the detection of SNPs/Indels
- Fixed numerous structural errors -> greatly improves the detection of structural variant
- Substantial improvements of annotation accuracy (e.g. genes with frameshifts reduced from 1,476 to 458, further improvements are expected with future maintenance releases)
- Greatly improved mapping of RNA-seq data

Ensembl

e!Ensembl
Rapid Release

BLAST | Tools | Downloads | Help & Docs | Known Bugs | Blog

Rattus norvegicus (BN/NHsdMcwi) - GCA_015227675.2 (mRatBN7.2) ▾

Search Rattus norvegicus

Search... Go
e.g. 1:157231467-157232417

Genome assembly: mRatBN7.2 (GCA_015227675.2)

Download DNA sequence (FASTA)
Display your data in Ensembl

View karyotype
Example region

Statistics

Summary

Assembly mRatBN7.2, INSDC Assembly GCA_015227675.2, Mammal 2020

Base Pairs 2,647,915,728
Golden Path Length 2,647,915,728
Annotation provider Ensembl
Annotation method Full genebuild
Genebuild started Nov 2020
Genebuild released Feb 2021
Genebuild last updated/patched Feb 2021
Database version 103.1

Gene annotation

What can I find? Protein-coding and non-coding genes, splice variants, cDNA and protein sequences, non-coding RNAs.

Download FASTA, GTF or GFF3 files for genes, cDNAs, ncRNA, proteins
Example gene
Example transcript

Pax6 INS FOXP2 BRCA2 DMD ssh

Coding genes 23,138
Non coding genes 6,503
Small non coding genes 3,582
Long non coding genes 2,524
Misc non coding genes 27
Pseudogenes 918
Gene transcripts 54,992

Other

Genscan gene predictions 57,986

Ensembl Rapid Release - 9 April 2021

NCBI Resources How To

Assembly Assembly Advanced Browse by organism Search Help

Full Report Send to:

mRatBN7.2

Organism name: Rattus norvegicus (Norway rat)
Infraspecific name: Strain: BN/NHsdMcwi
Sex: male
BioSample: SAMN16261960
BioProject: PRJNA662791
Submitter: Wellcome Sanger Institute

See Genome Information for Rattus norvegicus
There are 10 assemblies for this organism
See more

Download Assembly

Access the data

Genome Data Viewer
RefSeq Annotation Report
BLAST the assembly
Run Primer-BLAST
Full sequence report
Statistics report
FTP directory for RefSeq assembly
FTP directory for GenBank assembly
NCBI Datasets NEW

Assembly level: Chromosome
Genome representation: full
RefSeq category: representative genome
GenBank assembly accession: GCA_015227675.2 (latest)
RefSeq assembly accession: GCF_015227675.2 (latest)
Assembly method: FALCON-GRCh38p12 v. 2020
Expected final version: yes
Genome coverage: 92.0x
Sequencing technology: PacBio Sequel; 10X Genomics Chromium; BioNano; Arima Hi-C
Linked assembly: GCA_015244455.1 (alternate pseudohaplotype of diploid)
IDs: 9079111 [UID] 24462838 [GenBank] 24464048 [RefSeq]

Browse data

Download reference genome

Download gene annotation

Global statistics

Total sequence length	2,647,915,728
Total ungapped length	2,626,580,772
Gaps between scaffolds	0
Number of scaffolds	176
Scaffold N50	135,012,528
Scaffold L50	8
Number of contigs	757
Contig N50	29,198,295
Contig L50	27

Assembly Information

Assembly Help
Assembly Basics
NCBI Assembly Data Model

Related Information

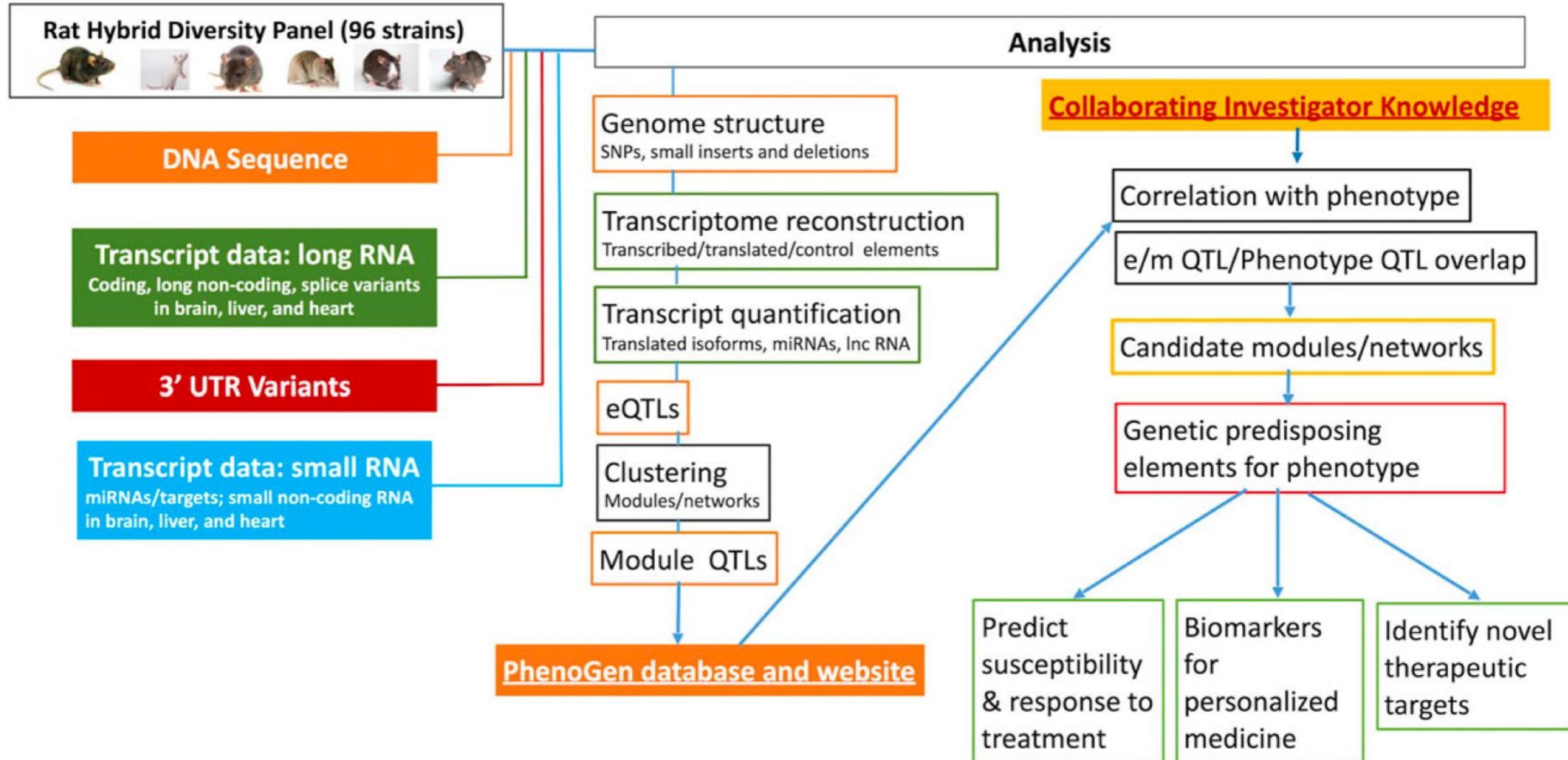
BioProject
BioSample
Genome
Linked assembly from diploid
Nucleotide INSDC
Nucleotide RefSeq
Taxonomy
WGS Master

Genome Sequencing of the HRDP

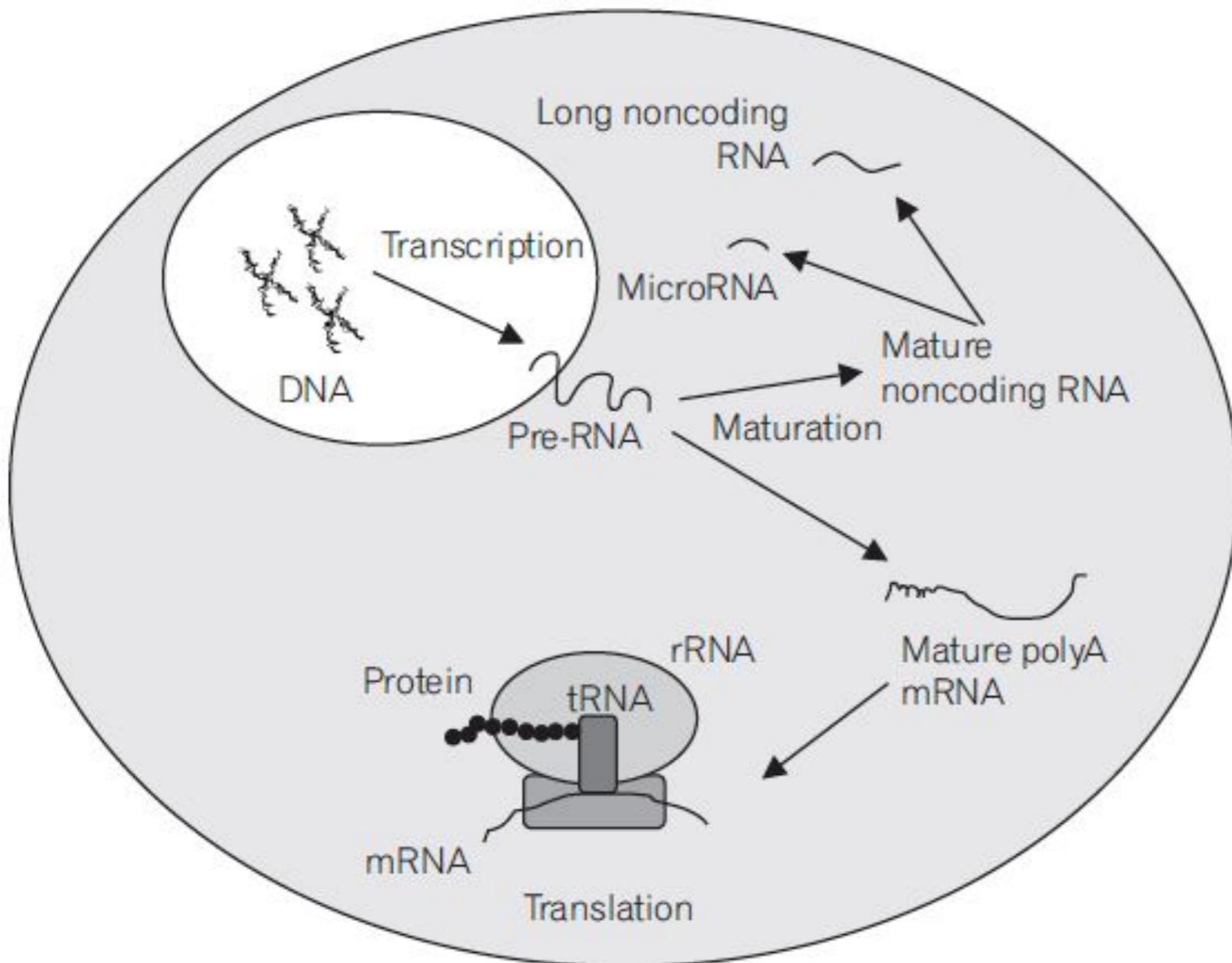
- All strains will be sequenced by two technologies
 - Illumina short reads (Mindy Dwinell, MCW)
 - 48+ strains sequenced and analyzed
 - 10X Genomics linked reads (Hao Chen, UTHSC/Jun Li, UMICH/Clifton Dalgard, USUHS)
 - 80+ strains sequenced, 46 strains analyzed,
- Data will be jointly analyzed and released to
 - RGD
 - genenetwork.org
 - opar.io

Transcriptome Information

The PhenoGen Contribution



Current Types of HRDP RNA Sequencing

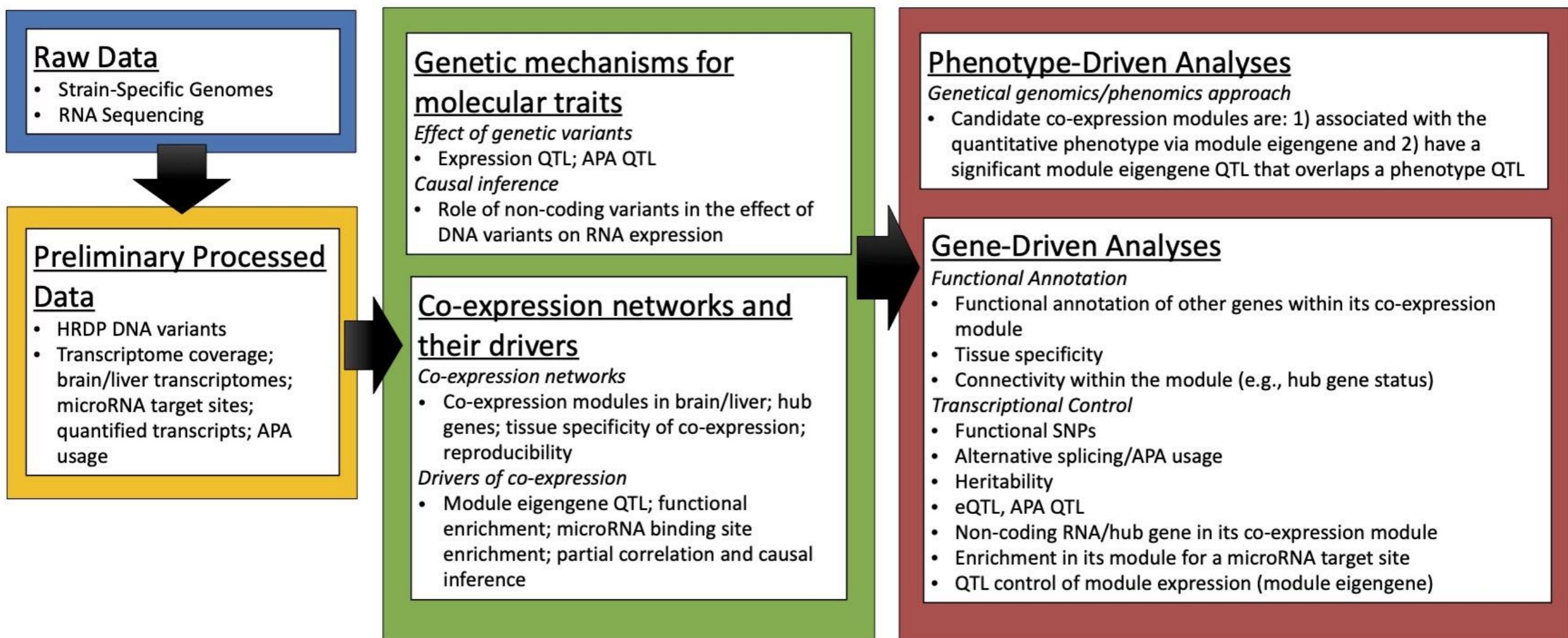


- **Ribosomal RNA-depleted total RNA**
 - >200 nt long
 - Includes both protein coding and non-coding
- **Small RNA**
 - <200 nt long
 - Includes miRNA, snoRNA, and other small RNAs

RNA Sequencing Depth

Number of Strains	Tissue	Sex	Number of Biological Replicates Per Strain	Number of Paired-End Reads (rRNA-deple- ted Total RNA)	Number of Single-End Reads (Small RNA)
			3-4	20.5 billion	6.8 billion
58	Brain	Male	3-4	20.5 billion	6.8 billion
2	Brain	Female across 3 stages of the estrus cycle	3	1 billion	-
58	Liver	Male	3	20.0 billion	7.0 billion
2	Liver	Female across 3 stages of the estrus cycle	3	1 billion	-
32	Heart	Male	1 for RI 5 for inbred	5.5 billion	-
32	Kidney	Male	3	9.8 billion	-

PhenoGen Data



Overview of PhenoGen Website

Major sections are found in the menu along the top.

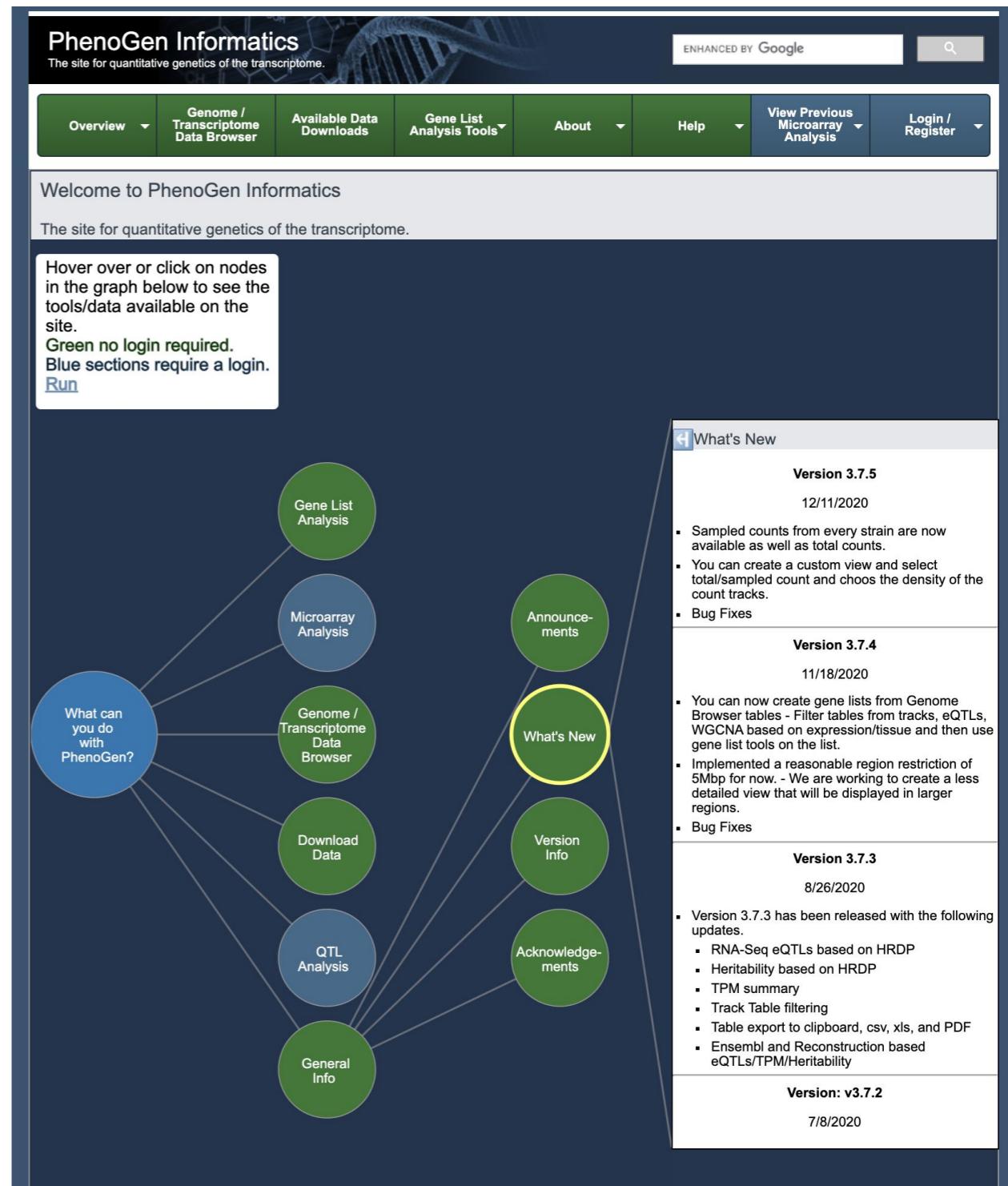
Genome/Transcriptome Data Browser –

Provides a genome browser interface but allows direct access to data/visualizations for features displayed in the browser.

Data Download – Downloads for relevant data displayed on the site and related publications.

Gene List Analysis Tools – provides functions that use a list of genes as a starting point to summarize some aspect of the genes in the list for example miRNA targeting, WGCNA, gene ontology, promoters, etc.

<https://phenogen.org/>



PhenoGen Transcriptome

Data on GeneNetwork

GeneNetwork Intro ▾ Help ▾ Tools ▾ Collections 0 Source Code ▾ Sign in

Genes / Molecules Search All

Select and search

Species: Rat (rn6)

Group: Hybrid Rat Diversity Panel (Includes HXB/BXH) Info

Type: Brain mRNA Info

Dataset: PhenoGen Brain RNA Ensembl rlog (v5 Feb20)

Get Any:

Enter terms, genes, ID numbers in the **Search** field.
Use * or ? wildcards (Cyp*a?, synap*).
Use **quotes** for terms such as "tyrosine kinase".

Combined:

Search Make Default

Affiliates

- [GeneNetwork 1 at UTHSC](#)
- [Genome Browser](#) at UTHSC
- [Systems Genetics](#) at EPFL
- [Bayesian Network Web Server](#) at UTHSC
- [GeneWeaver](#)
- [PhenoGen](#) at University of Colorado
- [WebGestalt](#) at Baylor

News

- Linkage from gene variants to molecular mediators, and to cells, circuits, and behaviors (and confounders)
<https://twitter.com/GeneNetwork2/status/1384182205484703745> ...
<https://twitter.com/GeneNetwork2/status/1384182205484703745>

Posted on Apr 19, 2021

These and other reference populations
genetics analysis to holistically
mechanisms and behavior

Natural

Other HRDP Transcriptome Data on GeneNetwork

GeneNetwork Intro ▾ Help ▾ Tools ▾ Collections 0 Source Code ▾ Sign in

Genes / Molecules Search All

Select and search

Species: Rat (rn6)

Group: Hybrid Rat Diversity Panel (Includes HXB/BXH) ▾

Type: ✓ Traits and Cofactors

Dataset: DNA Markers and SNPs

Get Any: Adipose mRNA
Adrenal Gland mRNA
Brain mRNA
Brain Proteome
Heart mRNA
Hippocampus mRNA
Kidney mRNA
Liver mRNA
Peritoneal Fat mRNA

Affiliates

- GeneNetwork 1 at UTHSC
- Genome Browser at UTHSC
- Systems Genetics at EPFL
- Bayesian Network Web Server at UTHSC
- GeneWeaver
- PhenoGen at University of Colorado
- WebGestalt at Baylor

News

- Linkage from gene variants to molecular mediators, and to cells, circuits, and behaviors (and confounders)
<https://twitter.com/GeneNetwork2/status/1384182205484703745> ...
<https://twitter.com/GeneNetwork2/status/1384182205484703745>

Posted on Apr 19, 2021

These and other reference populations are used for genetic association and genetics analysis to holistically understand the mechanisms and behavior of complex traits.

Natural perturbations Gene-Environment

Proteomic data on GN

Select and search

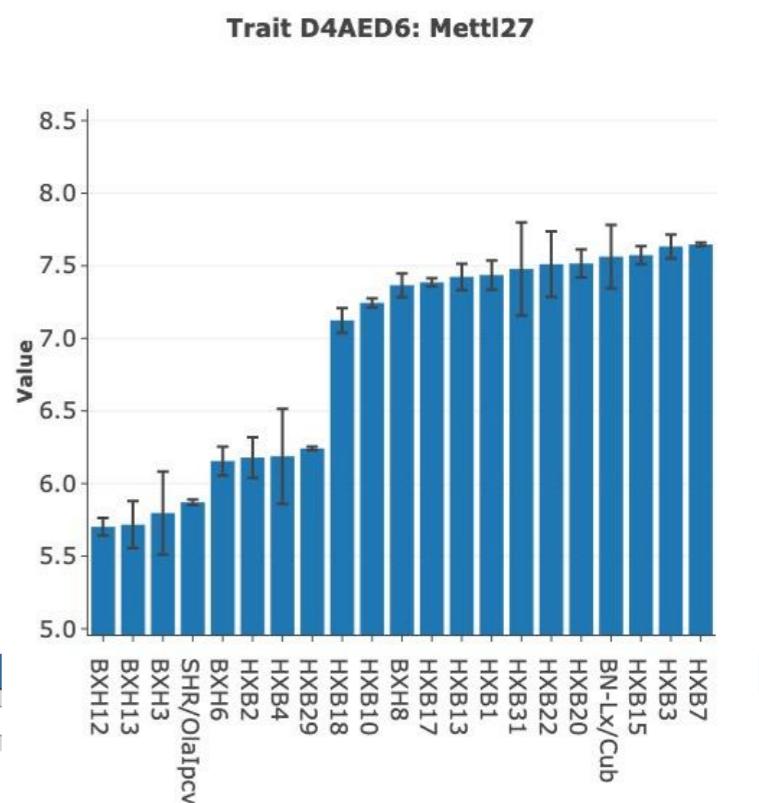
Species: Rat (rn6) ▾

Group: Hybrid Rat Diversity Panel (Includes HXB/BXH) ▾ [Info](#)

Type: Brain Proteome ▾ [Info](#)

Dataset: UND NIDA Brain Proteome (protein-level) log2z+8 (Feb21) ▾

Get Any: ↕



A total of 9,318 records were found.

Use quotes for terms such as "breast cancer".

Correlations Networks WebGestalt GeneWeaver BNW WGCNA CTL Maps MultiMap Comparison Bar Chart

Combined:

Search Make Default

Show/Hide Columns: Symbol Description Location Mean Peak LOD Peak Location Effect Size

Showing 1 to 37 of 9,318 entries

	Index	Record	Symbol	Description	Location	Mean	Peak LOD	Peak Location	Effect Size
<input type="checkbox"/>	4544	D3Z142	Lrsam1	Leucine-rich repeat and sterile alpha motif-containing 1; (test Mendelian in HXB 3@12Mb Rn6)	Chr3: 11.972813	6.954	12.6	Chr3: 11.854192	-0.645
<input type="checkbox"/>	4812	D4AED6	Mettl27	Methyltransferase-like 27	Chr12: 24.767351	6.893	11.4	Chr12: 24.241853	-0.724
<input type="checkbox"/>	8109	E9PTB2	Supt5h	Transcription elongation factor SPT5	Chr1: 85.437780	8.490	10.2	Chr1: 65.742688	0.228
<input type="checkbox"/>	889	Q641Y5	Atg7	Ubiquitin-like modifier-activating enzyme ATG7	Chr4: 146.598413	8.872	9.7	Chr4: 145.012812	-0.119
<input type="checkbox"/>	5476	Q6AY80	Nqo2	Ribosyldihydronicotinamide dehydrogenase [quinone]	Chr17: 32.132347	8.280	9.5	Chr17: 28.265855	-0.857
<input type="checkbox"/>	5102	P18589	Mx2	Interferon-induced GTP-binding protein Mx2	Chr11: 38.035450	7.806	9.2	Chr11: 31.094278	0.627
<input type="checkbox"/>	2987	Q9Z2S9	Flot2	Flotillin-2	Chr10: 65.304902	10.826	8.9	Chr3: 11.854192	0.456
<input type="checkbox"/>	2075	Q64680	Cyp2d4	Cytochrome P450 2D4	Chr7: 123.599266	7.962	8.3	Chr7: 122.316192	0.331
<input type="checkbox"/>	8414	Q5HZA9	Tmem126a	Transmembrane protein 126A	Chr1: 156.283128	7.206	8.2	Chr1: 146.028186	0.313
<input type="checkbox"/>	5101	P18588	Mx1	Interferon-induced GTP-binding protein Mx1	Chr11: 37.891156	7.285	8.0	Chr11: 31.094278	0.696
<input type="checkbox"/>	1113	Q3B8N9	Bph1	Biphenyl hydrolase-like	Chr17: 31.498883	7.436	7.9	Chr17: 28.265855	0.347

Phenotype Information

Substance Use Disorders in HRDP

- SUD-related publications with a subset of the HRDP
 - **Ethanol metabolism** - Alcohol Clin Exp Res. 2018 Jul;42(7):1177-1191.
 - **Voluntary ethanol consumption** - Mamm Genome. 2018 Feb;29(1-2):128-140; FEBS J. 2015 Sep;282(18):3556-78.
- Currently funded grants for SUD research with the HRDP
 - Identification of genes and genetic networks contributing to **opioid use disorder traits** in the Hybrid Rat Diversity Panel (Marissa Ehringer, Ryan Bachtell, and Laura Saba; University of Colorado)
 - System genetics of **menthol and nicotine addiction** (Hao Chen and Rob Williams; University of Tennessee Health Science Center)
 - System genetics of escalating **oral oxycodone intake** (Burt Sharp, Hao Chen, and Rob Williams; University of Tennessee Health Science Center)
 - RGAP: The heritable transcriptome and **alcoholism** (Boris Tabakoff, Paula Hoffman, and Laura Saba; University of Colorado)
 - NIDA Core “Center of Excellence” in Omics, Systems Genetics, and the Addictome (Rob Williams and Laura Saba; University of Tennessee Health Science Center and University of Colorado)

Phenotype Data on GeneNetwork

GeneNetwork Intro ▾ Help ▾ Tools ▾ Collections 0 Source Code ▾ Sign in

Search Results: We searched HXB/BXH Published Phenotypes to find all records in the dataset. A total of 234 records were found.



Phenotype Data on RGD



Home ▾ Data ▾ Analysis & Visualization ▾ Diseases ▾ Phenotypes & Models ▾ Pathways
Community ▾

Enter Search Term... Advanced Search (OLGA)
OntoMate (Literature Search) [f](#) [t](#) [in](#) [y](#)

Phenotype Data

Cardiovascular

[Blood Pressure](#)
[Heart Rate](#)
[Myocardial Ischemia](#)

Blood Chemistry

[Glucose Level](#)
[Cholesterol Level](#)

Renal

[Protein Excretion](#)
[Urine Electrolytes](#)
[Plasma Renin](#)
[Plasma Creatinine](#)

Morphological

[Body Weight](#)
[Organ Weights](#)

Pulmonary

[Ventilation](#)
[Pulmonary Vascular Resistance](#)
[Airway reactivity](#)

Vascular

[Vasodilation](#)
[Vasoconstriction](#)

Exercise

[Blood Pressure](#)
[PaCO₂](#)

Behavior

[Anxiety](#)
[Cage Center Time](#)
[Cage Margin Time](#)
[Locomotor Activity](#)



Data Integration Demo

Data Integration Demo

1. Initial Genetical Genomics/Phenomics study of alcohol consumption
2. Extending results to other tissues/phenotypes
3. Validating of network components
4. Layering of other omics data

Genetical Genomics/Phenomics Approach

Goal of this analysis:

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.

Candidate Modules from Genetical Genomics/Phenomics Approach

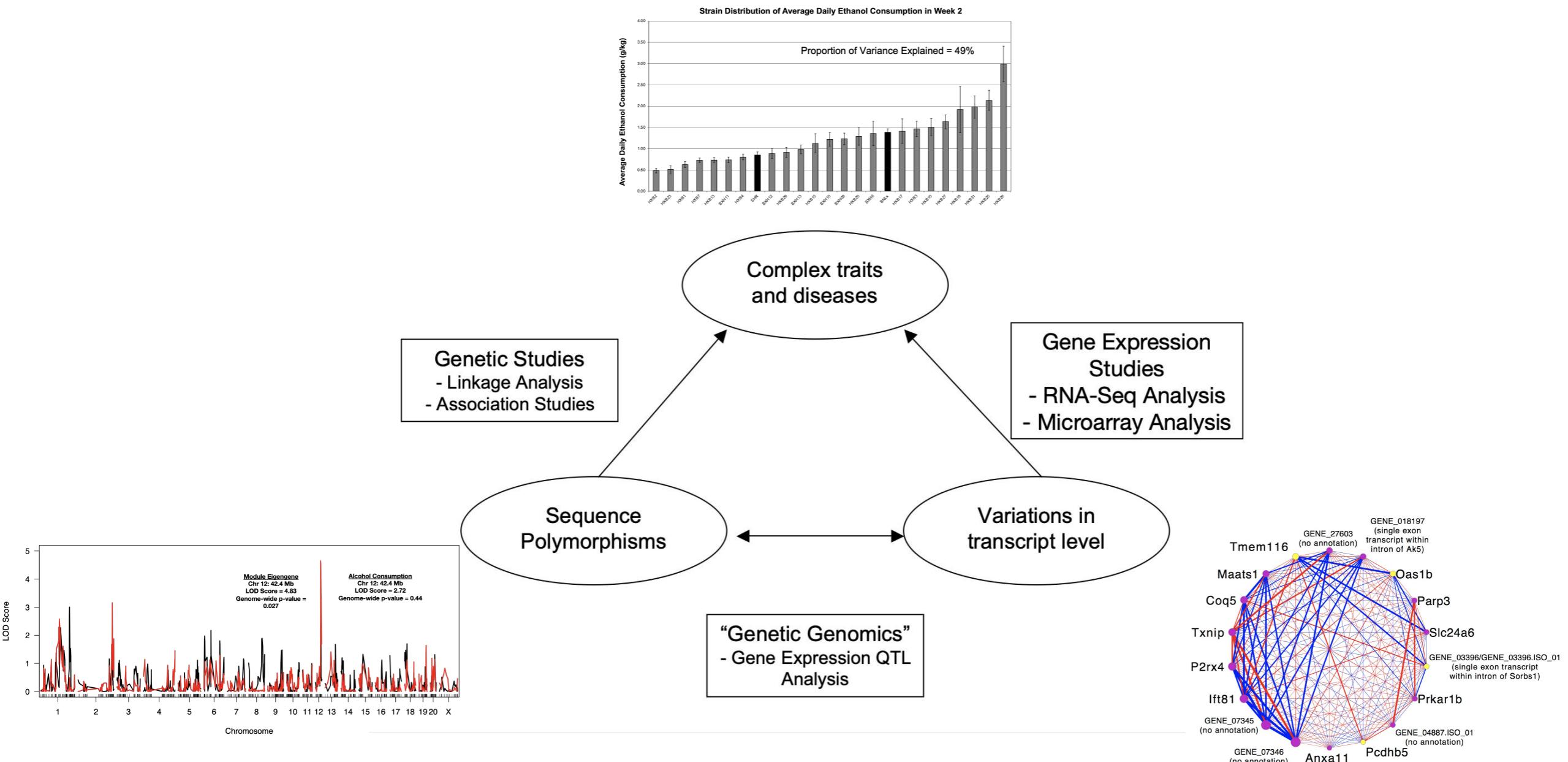
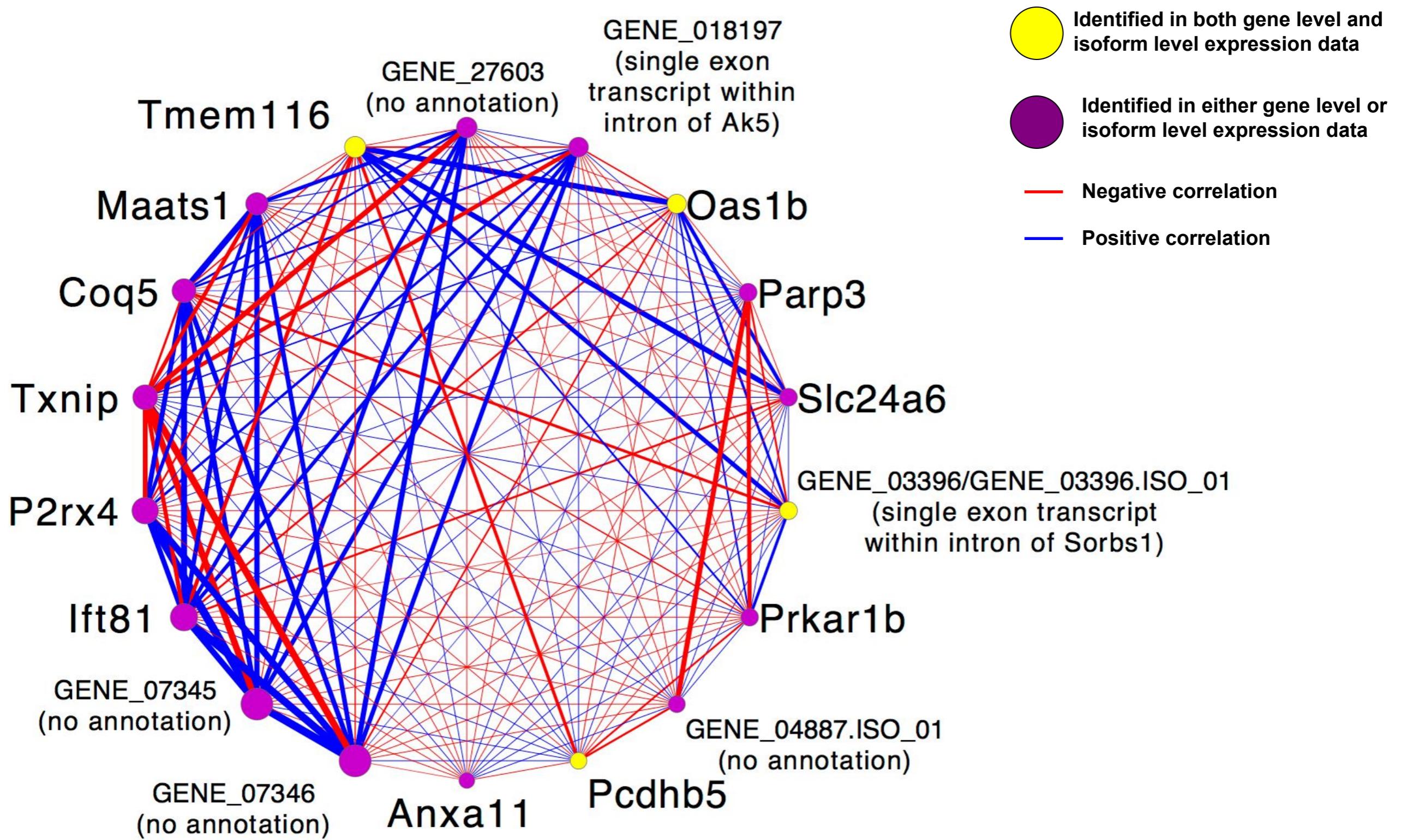


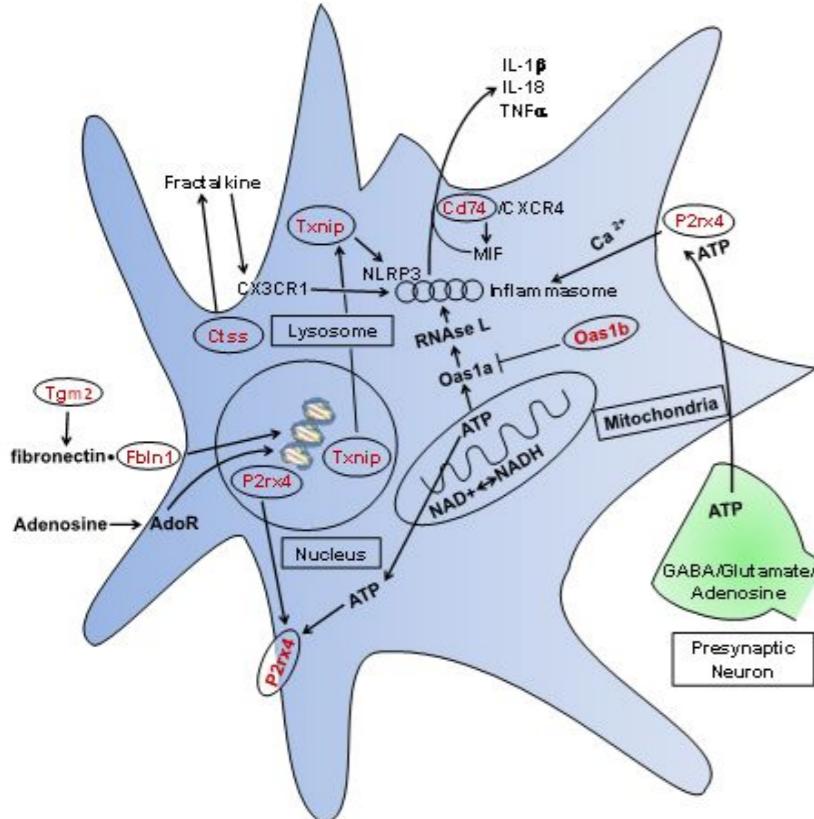
Image copied from Saba et al, *The Marriage of Phenomics and Genetical Genomics: A Systems Approach to Complex Trait Analysis*. In *Systems Biology in Psychiatric Research: From High-Throughput Data to Mathematical Modeling*, edited by Tetter F, Winterer G, Gebicke-Haerter PG, and Mendoza E. Wiley-VCH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

Combined Candidate Module

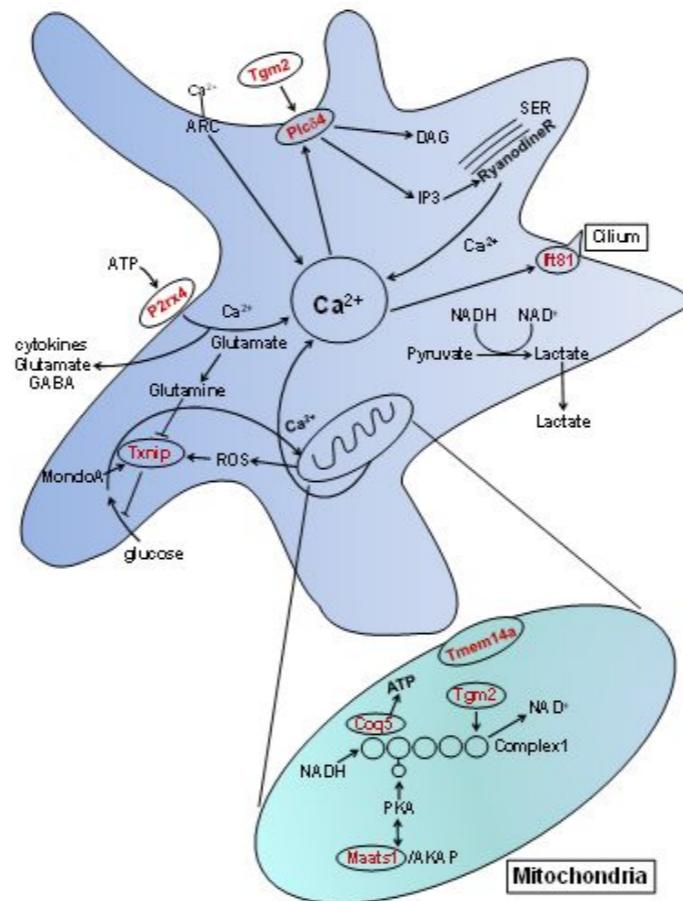


Saba et al (2015). The sequenced rat brain transcriptome, its use in identifying networks predisposing alcohol consumption.
FEBS J. 2015 Sep;282(18):3556-78.

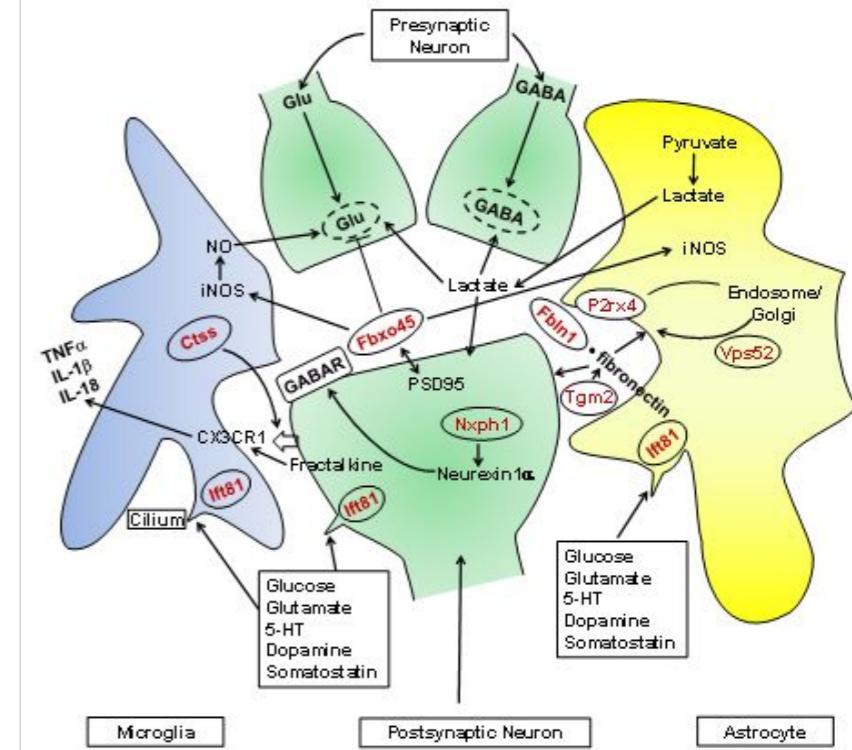
Biological Context from Module



Inflammation/Immune Response



Energy/Ca $^{2+}$
Homeostasis/Redox



Glial/Neuronal
Communication

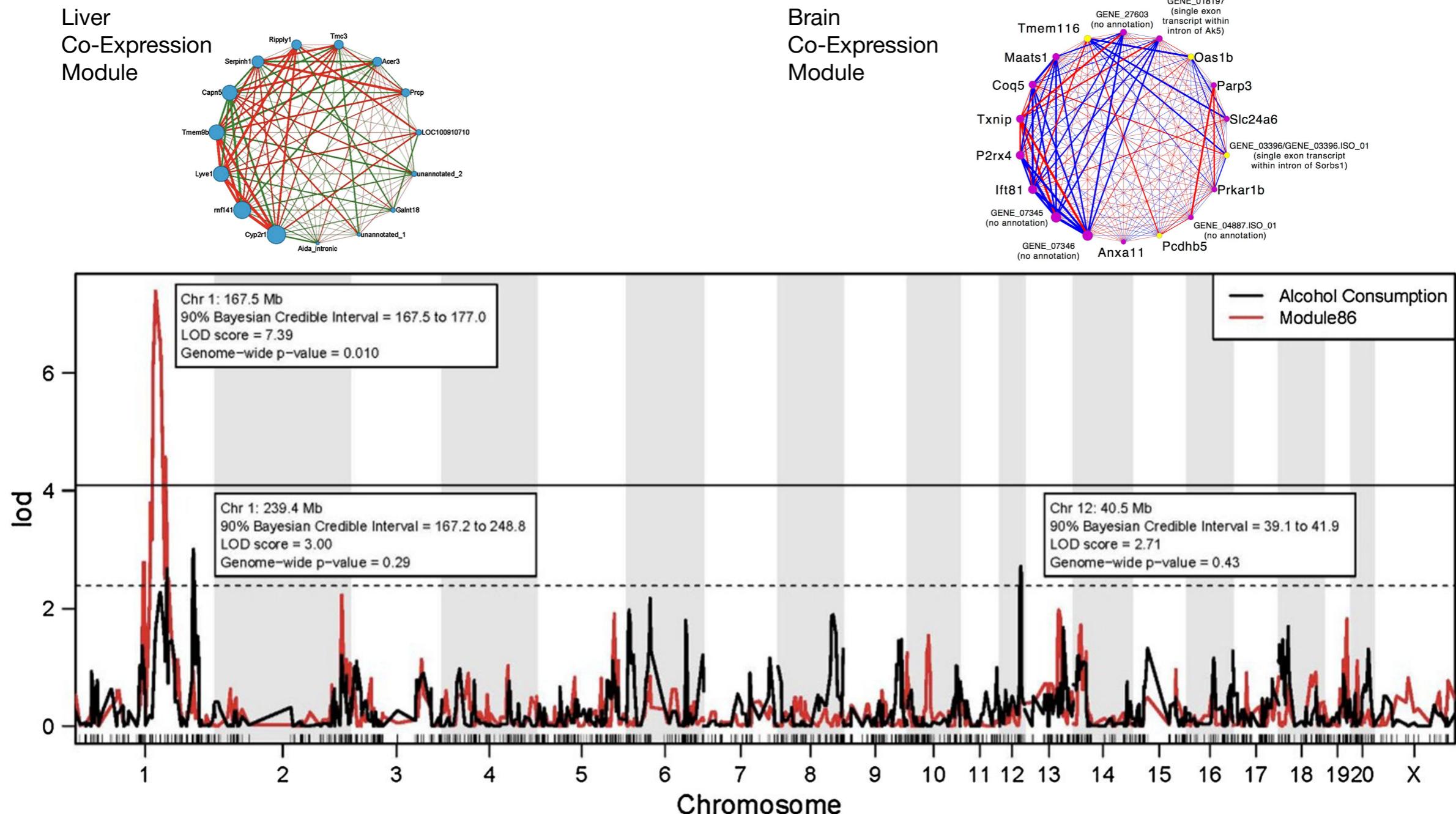
Saba et al (2015). The sequenced rat brain transcriptome, its use in identifying networks predisposing alcohol consumption. FEBS J. 2015 Sep;282(18):3556-78.

Data Integration Demo

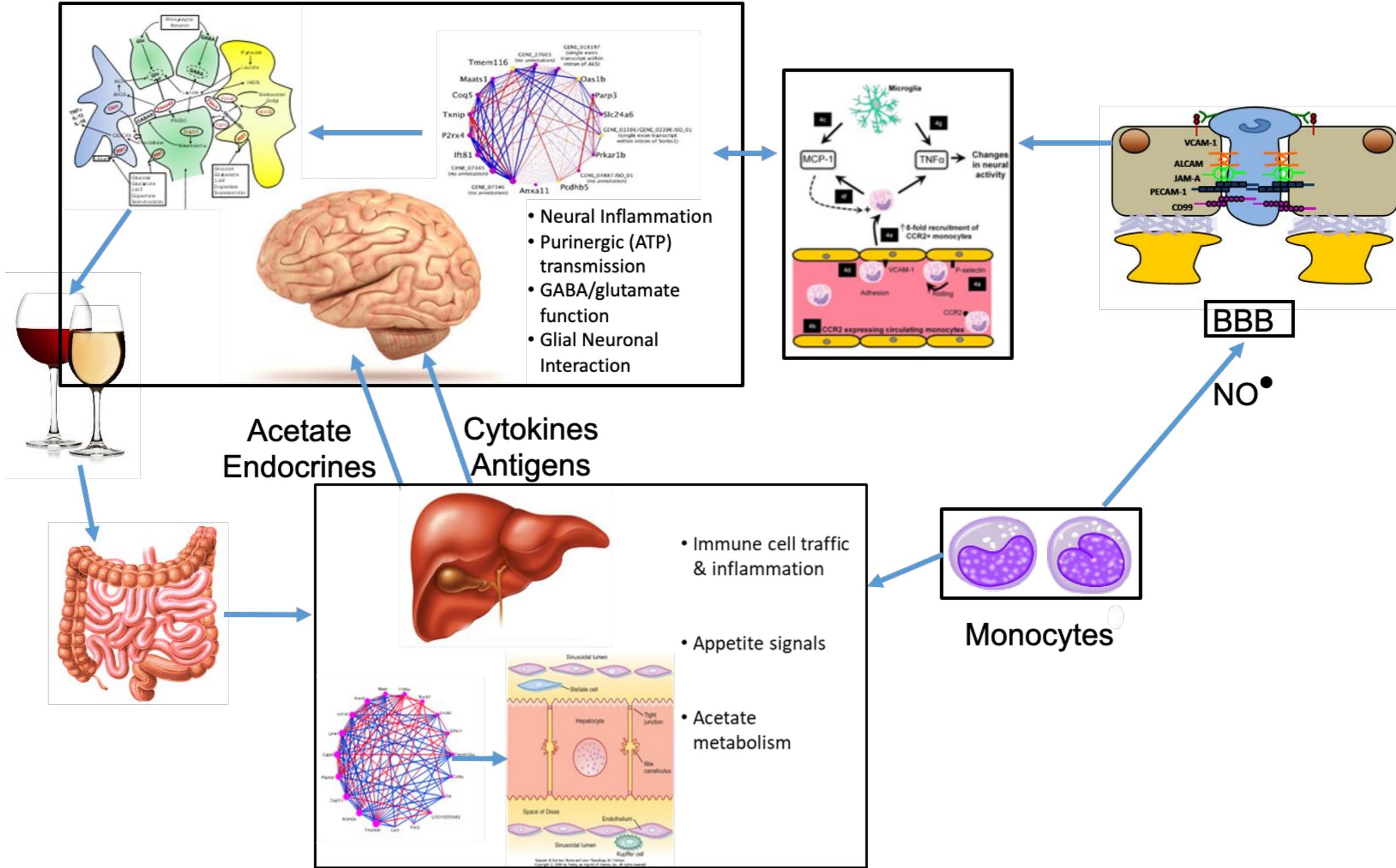
1. Initial Genetical Genomics/Phenomics
study of alcohol consumption
2. Extending results to other
tissues/phenotypes
3. Validating of network components
4. Layering of other omics data

Extending to Liver

Expression

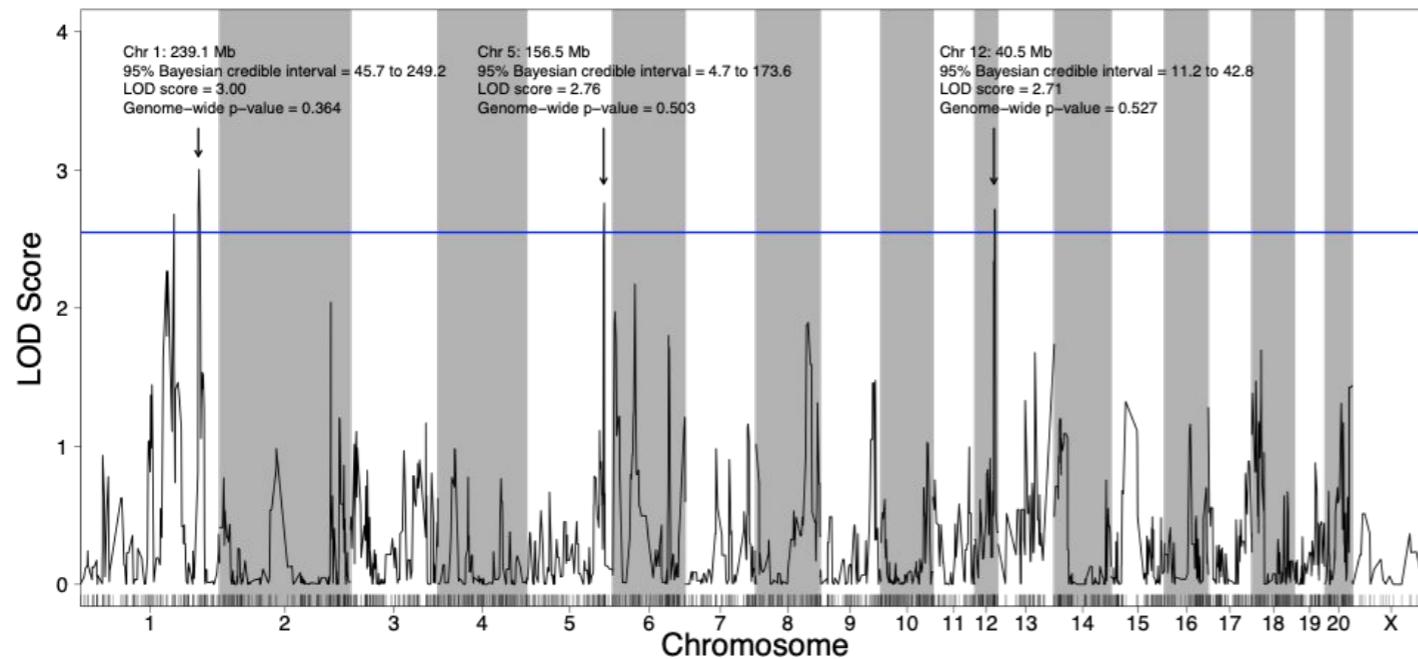


Alcohol and the Gut-Liver- Brain Axis

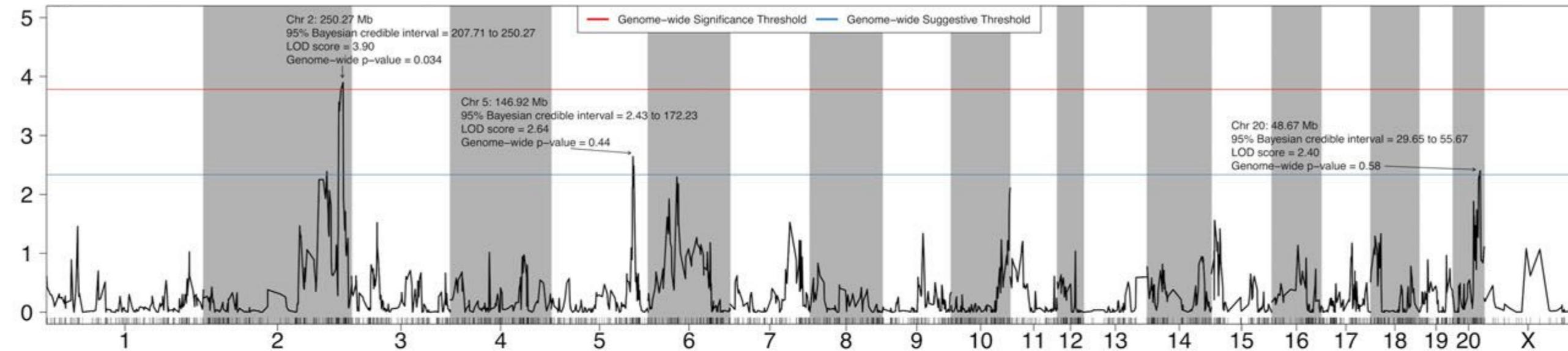


QTL overlap among alcohol phenotypes

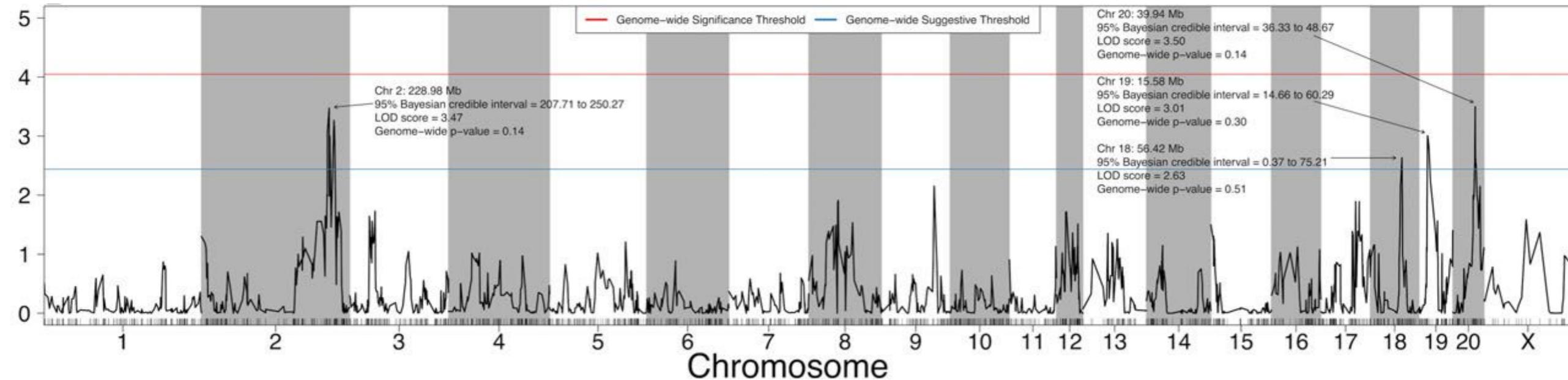
Alcohol Consumption



Alcohol Clearance



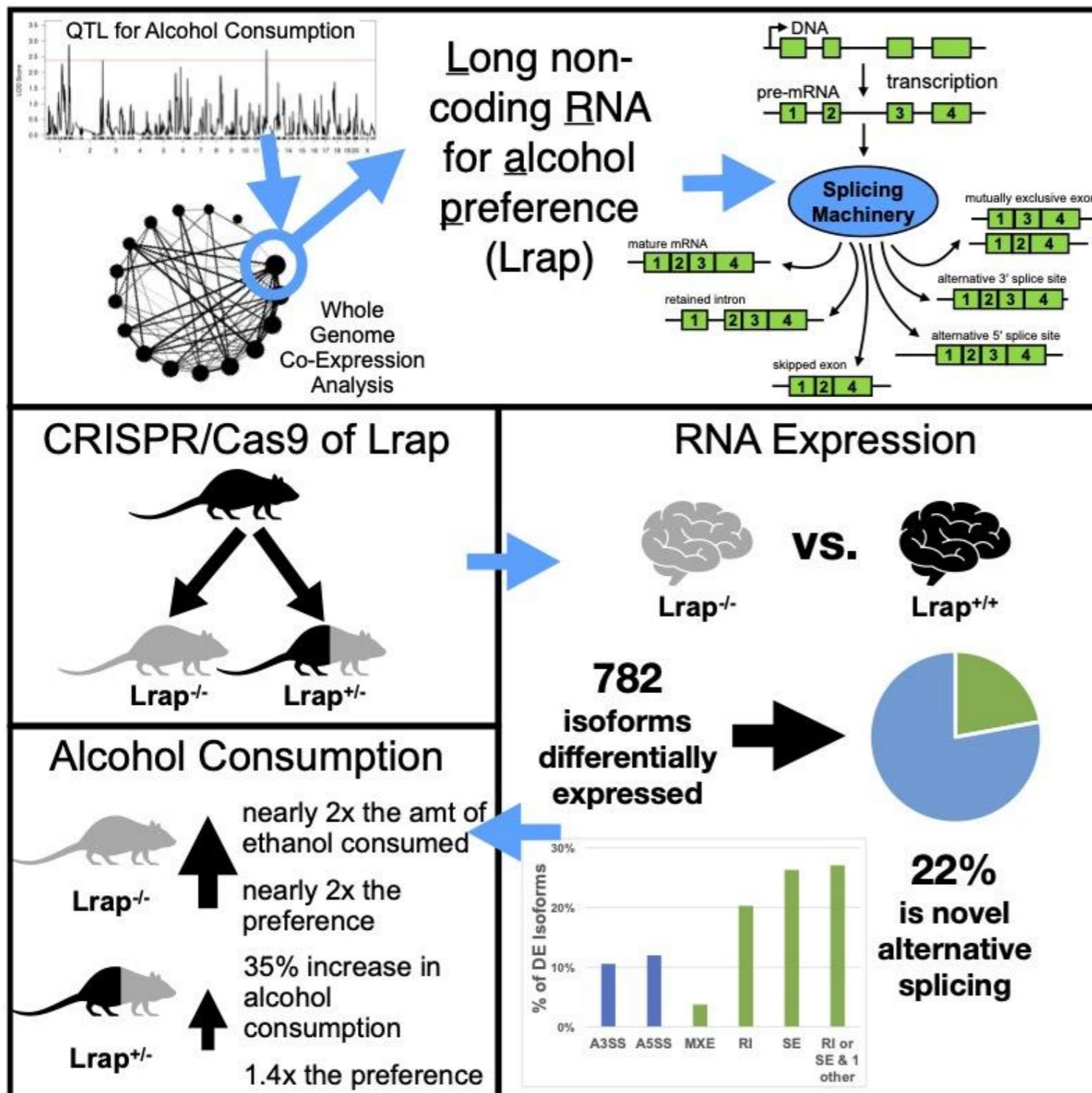
Acetate AUC



Data Integration Demo

1. Initial Genetical Genomics/Phenomics
study of alcohol consumption
2. Extending results to other
tissues/phenotypes
3. Validating of network components
4. Layering of other omics data

Validation of a Network Component



❖ We previously used the systems genetic approach to identify a brain gene co-expression module associated with the predisposition of rats in the HRDP to consume alcohol. The hub gene of the co-expression module ("Lrap") is an unannotated transcript with a structure resembling a long non-coding RNA (lncRNA).

❖ In this study, we disrupted the hub gene (CRISPR/Cas9) and validated its role in alcohol consumption. In addition, RNASeq analysis of the genetically modified rats demonstrated changes in expression of over 700 other brain transcripts and changes in splicing.

❖ The transcriptional effects are consistent with the identification of Lrap as a lncRNA.

Data Integration Demo

1. Initial Genetical Genomics/Phenomics
study of alcohol consumption
2. Extending results to other
tissues/phenotypes
3. Validating of network components
4. Layering of other omics data

Proteomics Data on GeneNetwork

Select and search

Species: Rat (rn6) ▼

Launchpad

Group: Hybrid Rat Diversity Panel (Includes HXB/BXH) ▼ Info

Type: Brain Proteome ▼ Info

Dataset: UND NIDA Brain Proteome (protein-level) log2z+8 (Feb21) ▼

Get Any: *

Enter terms, genes, ID numbers in the **Search** field.
Use * or ? wildcards (Cyp*a?, synap*).
Use quotes for terms such as "tyrosine kinase".

Combined:

Search Make Default

Search Results: We searched UND NIDA Brain Proteome (protein-level) log2z+8 (Feb21) to find all records in the dataset. A total of 9318 records were found.

Proteomics Correlation

With Alcohol Consumption

Correlation Table

Trait: 10190

Values of record 10190 in the [HXB/BXH Published Phenotypes](#) dataset were compared to all records in the [UND NIDA Brain Proteome \(protein-level\) log2z+8 \(Feb21\)](#) dataset. The top 500 correlations ranked by the Genetic Correlation (Pearson's r) are displayed. You can resort this list by clicking the headers. Select the Record ID to open the trait data and analysis page.

[Correlations](#) [Networks](#) [WebGestalt](#) [GeneWeaver](#) [BNW](#) [WGCNA](#) [CTL Maps](#) [MultiMap](#) [Comparison Bar Chart](#)

[Select All](#) [Invert](#) [Add](#) [Search Table For ...](#) [Select Top ...](#) [Deselect](#) [Reset Columns](#)

[Download](#) [More Options...](#)

Show/Hide Columns:

[Symbol](#) [Description](#) [Location](#) [Mean](#) [Peak LOD](#) [Peak Location](#) [Effect Size](#)

Showing 1 to 49 of 500 entries

	Index	Record	Symbol	Description	Location	Mean	Sample r	N	Sample p(r)	Lit r	Tissue r	Tissue p(r)	Peak LOD	Peak Location	Effect Size
<input checked="" type="checkbox"/>	1	Q62839	Golga2	Golgin subfamily A member 2	Chr3: 11.317183	8.693	-0.664	19	1.934e-03	--	--	--	2.3	Chr7: 129.026739	0.019
<input checked="" type="checkbox"/>	2	Q64680	Cyp2d4	Cytochrome P450 2D4	Chr7: 123.599266	7.962	-0.631	19	3.746e-03	--	--	--	8.3	Chr7: 122.316192	0.331
<input checked="" type="checkbox"/>	3	P12938	Cyp2d3	Cytochrome P450 2D3	Chr7: 123.625590	6.487	-0.607	19	5.858e-03	--	--	--	7.1	Chr7: 122.316192	0.342
<input checked="" type="checkbox"/>	4	F1LPV8	Suclg2	Succinate-CoA ligase [GDP-forming] subunit beta, mitochondrial	Chr4: 127.552101	9.053	-0.570	19	1.079e-02	--	--	--	1.9	Chr18: 12.985992	-0.046
<input checked="" type="checkbox"/>	5	P10633	Cyp2d1	Cytochrome P450 2D1	Chr7: 123.625590	6.234	-0.549	19	1.486e-02	--	--	--	4.6	Chr7: 122.316192	0.335
<input checked="" type="checkbox"/>	6	P17988	Sult1a1	Sulfotransferase 1A1	Chr1: 198.100586	7.065	-0.539	19	1.727e-02	--	--	--	2.6	Chr19: 41.590495	0.210
<input checked="" type="checkbox"/>	7	F1LPS6	Ifit1	Interferon-induced protein with tetratricopeptide repeats 1	Chr1: 252.944103	5.920	-0.525	19	2.097e-02	--	--	--	2.4	Chr3: 32.972945	-0.314
<input checked="" type="checkbox"/>	8	P17164	Fuca1	Tissue alpha-L-fucosidase	Chr5: 154.269118	8.011	-0.524	19	2.119e-02	--	--	--	1.8	Chr11: 69.315012	-0.069
<input checked="" type="checkbox"/>	9	B5DEJ9	Sbf2	LOC691036 protein	Chr1: 174.948913	8.774	-0.522	19	2.193e-02	--	--	--	2.4	Chr19: 41.590495	0.053
<input checked="" type="checkbox"/>	10	P02680	Fgg	Fibrinogen gamma chain	Chr2: 181.987217	9.009	-0.512	19	2.514e-02	--	--	--	2.8	Chr8: 110.061256	-0.156
<input checked="" type="checkbox"/>	11	Q5EBC0	Itih4	Inter alpha-trypsin inhibitor, heavy chain 4	Chr16: 6.970342	9.307	-0.510	19	2.570e-02	--	--	--	2.1	Chr19: 57.380159	0.126



WEB-based GEne SeT AnaLysis Toolkit

Translating gene lists into biological insights...

Gene Set	Description	Size	Expect	Ratio	P Value	FDR
GO:0006805	xenobiotic metabolic process	70	0.12576	31.807	0.0000067862	0.078258
GO:0042738	exogenous drug catabolic process	30	0.053896	55.663	0.000020037	0.11553
GO:0072378	blood coagulation, fibrin clot formation	6	0.010779	185.54	0.000046196	0.13283
GO:0019369	arachidonic acid metabolic process	40	0.071862	41.747	0.000048188	0.13283
GO:0044281	small molecule metabolic process	1355	2.4343	4.1079	0.000057593	0.13283
GO:0034116	positive regulation of heterotypic cell-cell adhesion	8	0.014372	139.16	0.000086044	0.16538
GO:0071466	cellular response to xenobiotic stimulus	141	0.25331	15.791	0.00010723	0.17666
GO:0009410	response to xenobiotic stimulus	296	0.53178	9.4024	0.00015525	0.19525
GO:0042730	fibrinolysis	11	0.019762	101.20	0.00016846	0.19525
GO:0019752	carboxylic acid metabolic process	702	1.2612	5.5504	0.00016931	0.19525

20 per page ▾

1

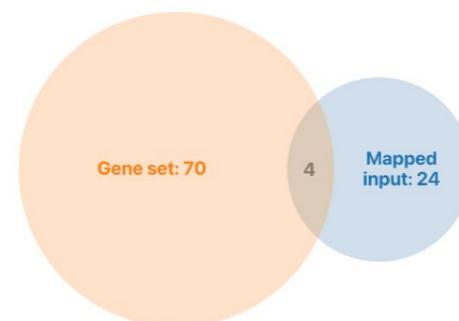
Select an enriched gene set...

GO:0006805: xenobiotic metabolic process

Gene set: GO:0006805 xenobiotic metabolic process

FDR
0.078258 P Value
0.0000067862

Gene Set Size
70 Expected Value
0.12576 Overlap
4 Enrichment Ratio
31.807



User ID	Gene Symbol	Gene Name	Entrez Gene ID
171522	Cyp2d4	cytochrome P450, family 2, subfamily d, polypeptide 4	171522
24303	Cyp2d3	cytochrome P450, family 2, subfamily d, polypeptide 3	24303
266684	Cyp2d1	cytochrome P450, family 2, subfamily d, polypeptide 1	266684
83783	Sult1a1	sulfotransferase family 1A member 1	83783

Conclusions

Summary

The HRDP is an useful tool of studying complex substance use related traits because we can:

- parse genetic variation from non-genetic variation
- map quantitative traits to the genome with both precision and accuracy
- accumulate data on across experiments and laboratories to enable systems genetics approaches, trans-omics studies and eventually PheWAS
- study gene by environment/exposure effects by capitalizing on the renewable genetic backgrounds

Where can we go from here?

Diallele cross - studying F1 crosses of inbred strains (4560 possible F1 combinations from 96 strain), e.g.,

- Chen H, Hiler KA, Tolley EA, Matta SG, Sharp BM. Genetic factors control nicotine self-administration in isogenic adolescent rat strains. PLoS One. 2012;7(8):e44234.
- Neuner SM, Heuer SE, Huentelman MJ, O'Connell KMS, Kaczorowski CC. Harnessing Genetic Complexity to Enhance Translatability of Alzheimer's Disease Mouse Models: A Path toward Precision Medicine. *Neuron*. 2019 Feb 6;101(3):399-411.e5.
- Han W, Wang T, Chen H. Social learning promotes nicotine self-administration by facilitating the extinction of conditioned aversion in isogenic strains of rats. *Sci Rep*. 2017 Aug 14;7(1):8052.

Single molecule RNA sequencing - enumerate splice variants and alternative transcription start and stop sites; role of genetics and differences in transcript structure.

Other omics - metabolomics; chromatin structure; single cell RNA-Seq and ATAC-Seq

Gene by environment/drug interaction - tease out predisposition from response in behavioral and molecular phenotypes

Gene by mutation (interaction) - penetrance of a disease related variant based on different genetic backgrounds.

- Neuner SM, Heuer SE, Huentelman MJ, O'Connell KMS, Kaczorowski CC. Harnessing Genetic Complexity to Enhance Translatability of Alzheimer's Disease Mouse Models: A Path toward Precision Medicine. *Neuron*. 2019 Feb 6;101(3):399-411.e5.

Differential co-expression - networks activated or disrupted based on exposure to a drug

Acknowledgements

Grant sponsors:

- NIDA Core “Center of Excellence” in Omics, Systems Genetics, and the Addictome - NIH (NIDA) P30 DA044223 (MPI - Rob Williams; Laura Saba; Saunak Sen)
- RGAP: The heritable transcriptome and alcoholism - NIH (NIAAA) R24 AA013162 (MPI - Boris Tabakoff; Paula Hoffman; Laura Saba)
- Identification of genes/genetic networks contributing to opioid use disorder traits in the Hybrid Rat Diversity Panel - NIH (NIDA) U01 DA051937 (MPI - Marissa Ehringer; Ryan Bachtell; Laura Saba)
- System genetics of escalating oxycodone intake - NIH (NIDA) U01 DA053672 (MPI - Burt Sharp; Hao Chen; Rob Williams)
- System genetics of menthol and nicotine addiction - NIH (NIDA) U01 DA047638 (MPI - Hao Chen; Rob Williams, co-I: Saunak Sen)

Community:

- Hybrid Rat Diversity Project (PI - Melinda Dwinell)
- Rat Genome Database (PI - Anne Kwitek)

Scientists:

- **Colorado** - Boris Tabakoff; Paula Hoffman; Spencer Mahaffey; Jennifer Mahaffey; Lauren Vanderlinden; Harry Smith; Ryan Lusk; Cheyret Wood; Sam Rosean; Angela Yoder
- **Memphis** - Rob Williams; Saunak Sen; Tristan de Jong; Hakan Gunturkun; Tengfei Wang; Angel Garcia Martinez