

Compensatory Transcriptome

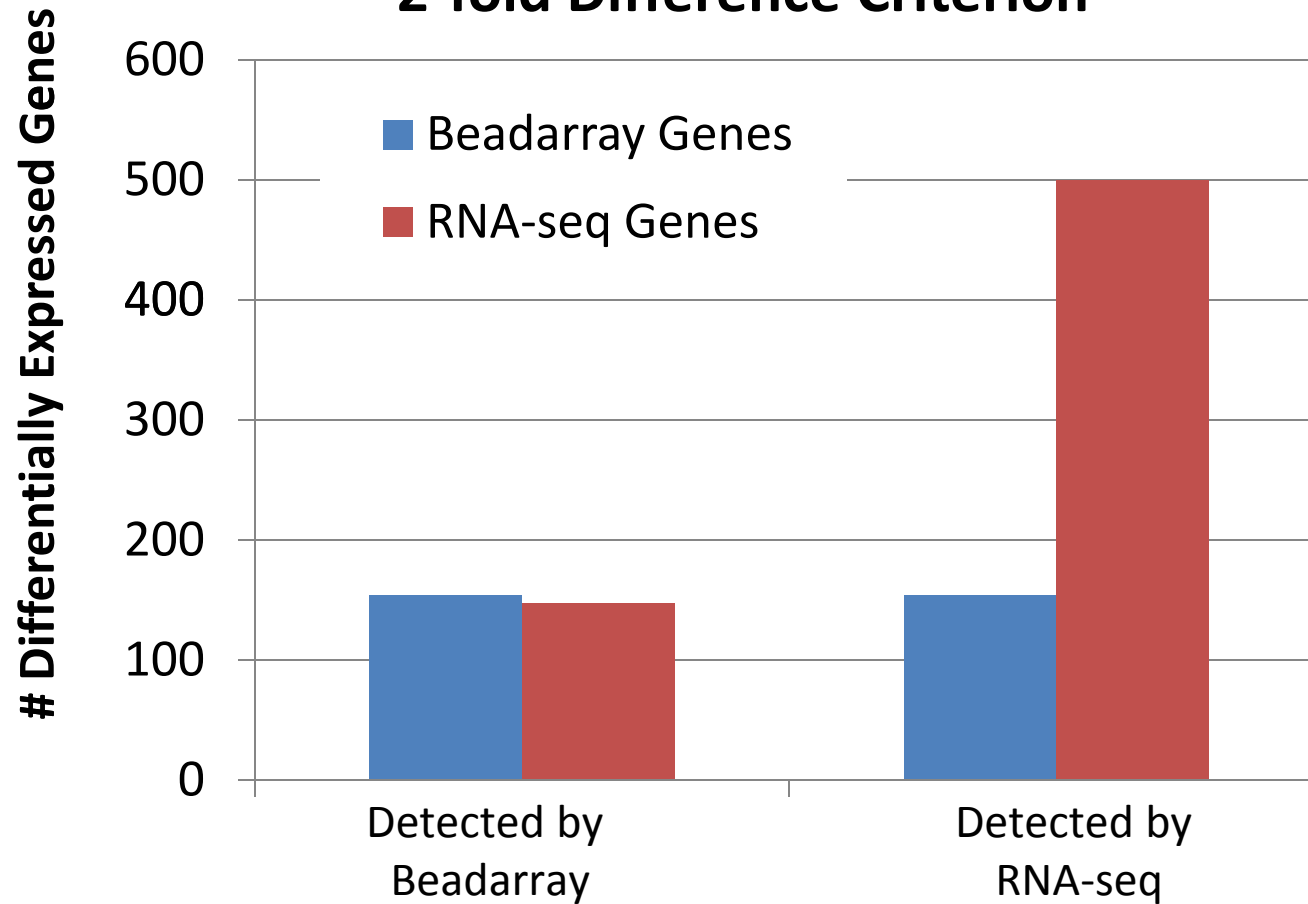
- Technical Approach
- Reducing Cost
- Data Analysis and Interpretation
- Data Presentation to Public
- Strategy – Mutants and Tissues?

Transcriptome Technical Approach

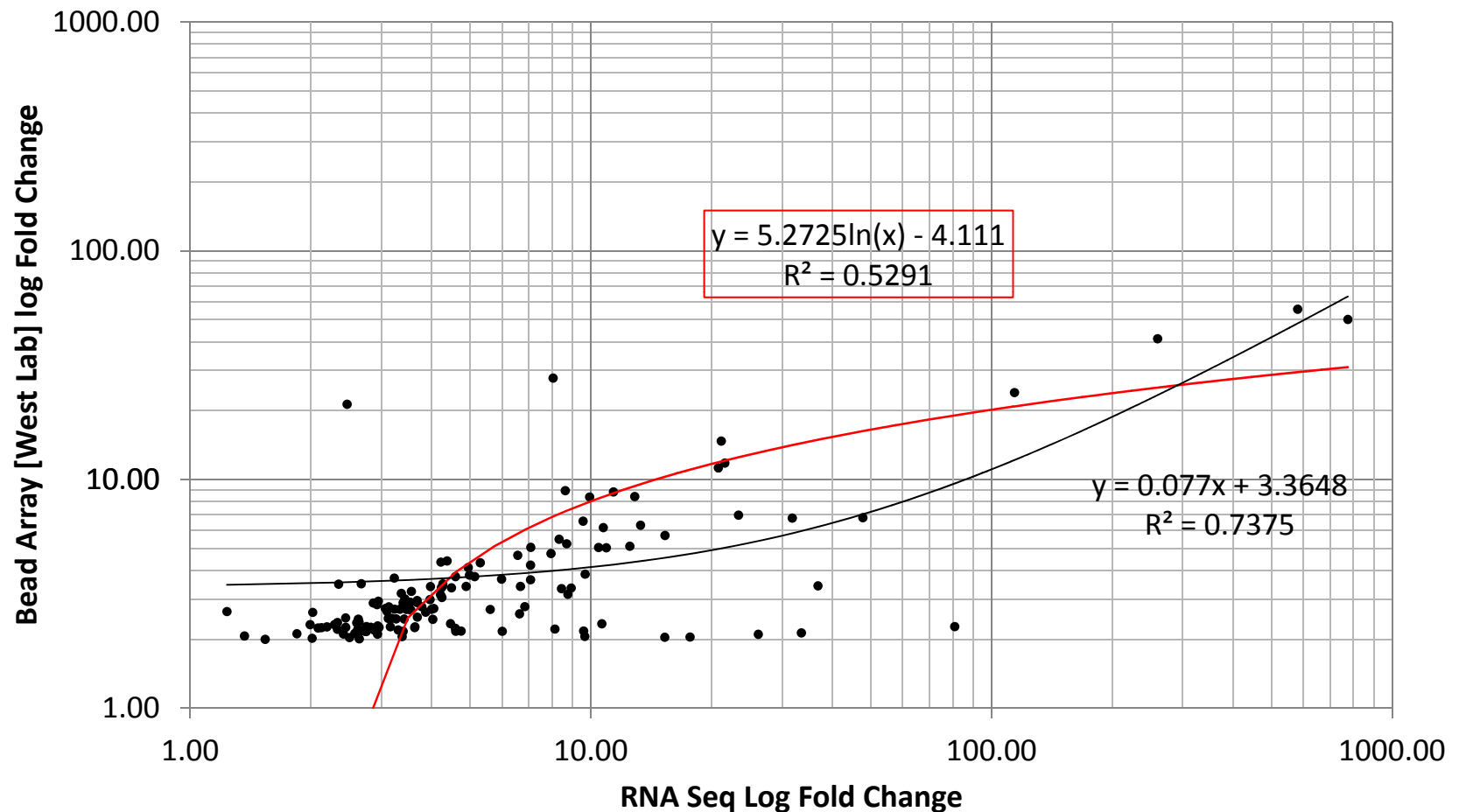
- Array-based Methods- Illumina Bead-Array
 - sensitive to RNA quality
 - detection sensitivity limited
 - specificity dependent upon probe and platform
 - technical variability combined with biological variability requires ~4 replicates per tissue
- RNA-seq - Illumina HiSeq
 - potentially unlimited sensitivity
 - specificity not platform dependent
 - data type and structure will not change with new sequencing platforms or methods

RNA-seq Sensitivity

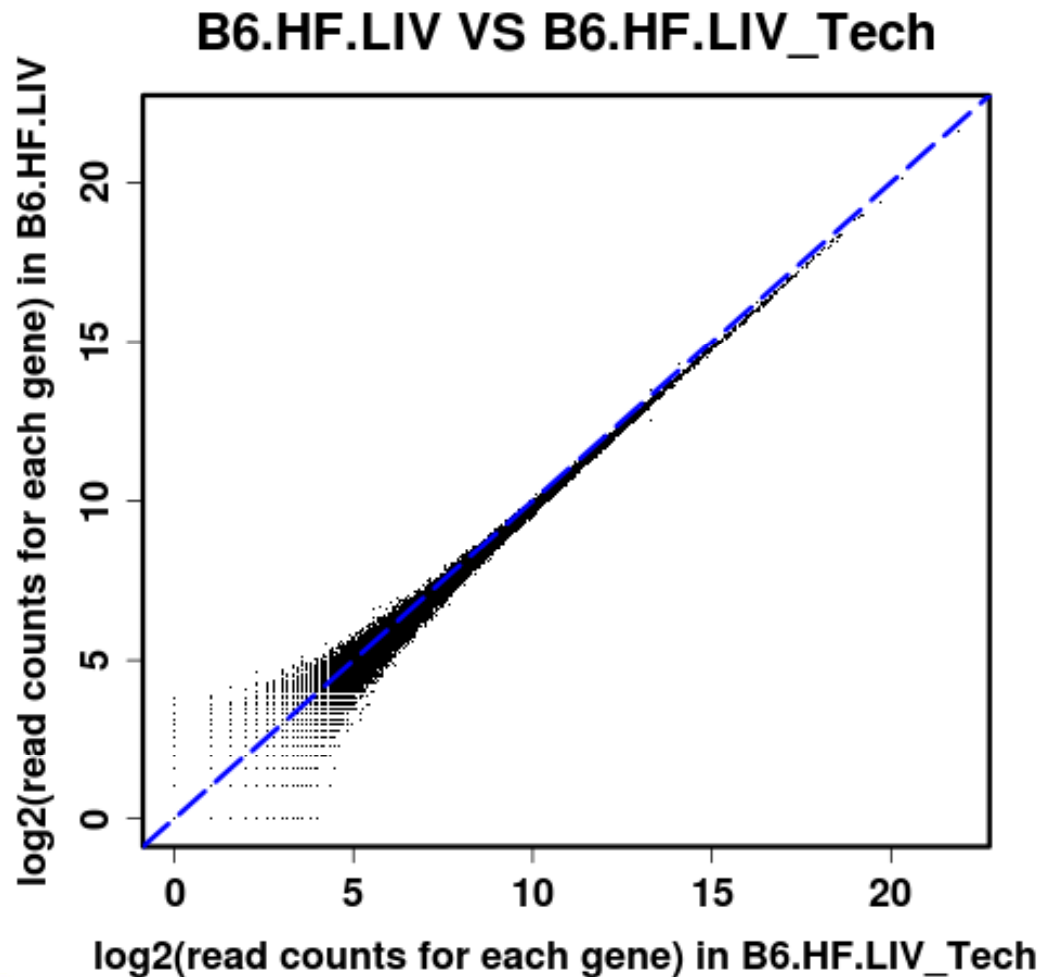
db/db vs. db/+ Liver Expression
2-fold Difference Criterion



Beadarray vs. RNA-seq Fold Change



cDNA Library Prep Variability



Acceptable
variability for
readcounts
above 32 and
minimum 2
fold difference

C · H · O · R · I

Children's Hospital Oakland Research Institute



UC DAVIS

KOMP Phenotyping *Pilot*
KNOCKOUT MOUSE PROJECT

Cost Analysis RNA-seq vs. Beadarray

Reagent, Supply & Assay Costs for RNA-seq vs. Beadarray Analysis (4 mice & 4 tissues)		
Reagent/Supply	Beadarray	RNA-seq
Toral RNA Isolation:		
Rneasy Plus	\$112.00	
Pestles	\$12.68	
QiaShredder	\$23.00	
BioAnalyzer	\$43.66	
RiboGreen	\$0.78	
cDNA Library Preparation:		
TruSeq	n/a	\$348.96
AmPure Beads	n/a	\$107.69
SuperScript II	n/a	\$27.08
BioAnalyzer	n/a	\$10.30
Sample Analysis:		
Sequencing HiSeq	n/a	\$660.00
Illumina Beadarray (n=4)	\$4,400	n/a
TOTAL:	\$4,592.12	\$1,154.03

Additional Cost-Savings

- Fully automating RNA extraction
- Create own kits/reagents for cDNA library production
- Automate cDNA library production
- Anticipate that sequencing costs will continue to drop

RNAseq Technical Methods Issues

- Multiplexing libraries (n=4 per sequencing lane)
- Technical assay variability_ minimal
- Variability due to cDNA library construction – minimal
- Pooling of biological replicates (n=4-5 replicates)
 - normalized RNA concentration
 - cDNA library generated from pool
 - need to monitor as part of QC
- WT control
 - not pooled
 - repeated at intervals

RNAseq Computational Pipeline

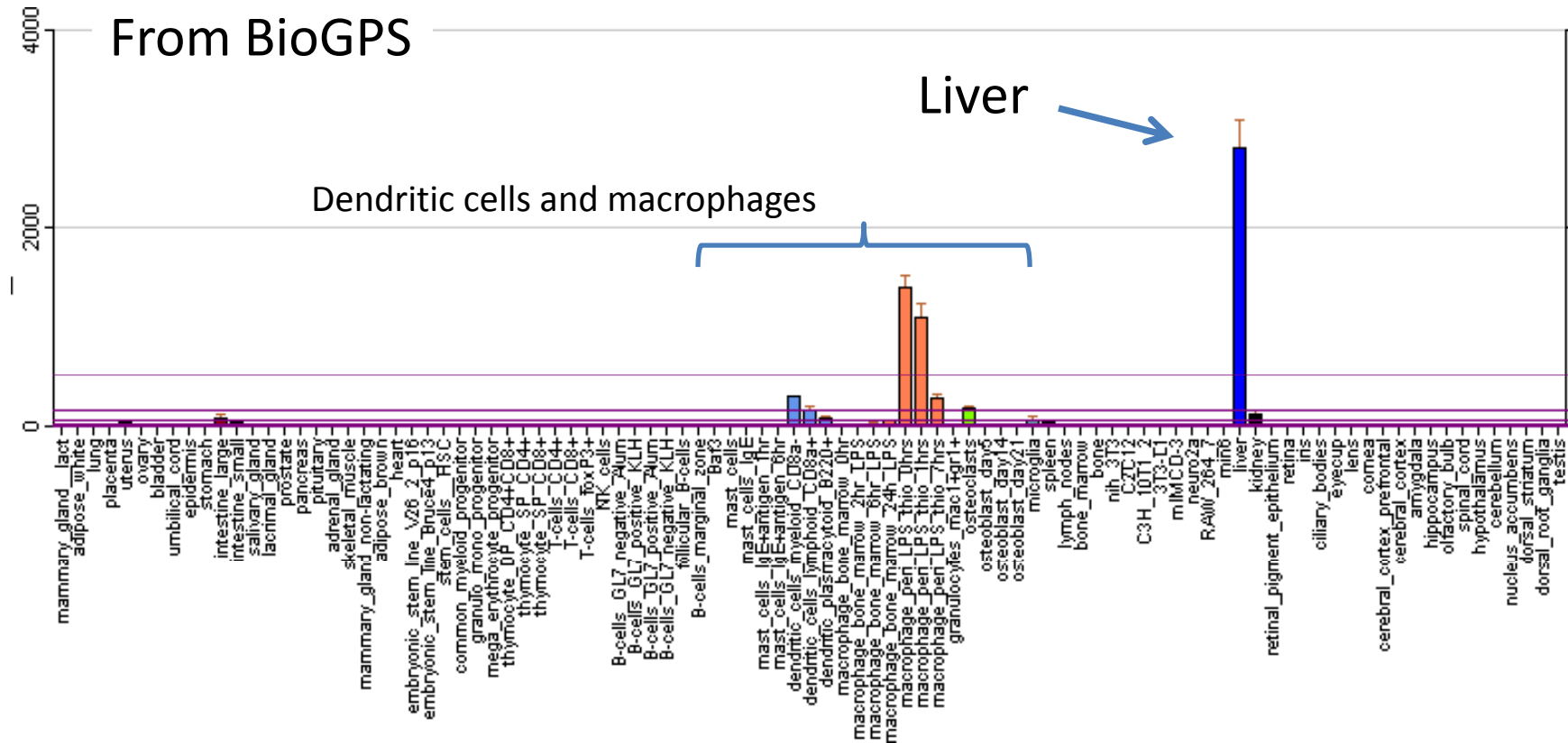
Automated pipeline modules

- data download and archiving
- alignments against transcriptome and genome
- statistical analysis of digital counts
- automation of QC needed

Data Analysis & Presentation

- Gene lists of +/-2X changes
- Pathway Analysis
 - KEGG
 - Ingenuity
 - Seralogix
- More detailed information available as secondary queries
 - novel/alternative transcripts
 - Mapping reads back to genome
 - Comparison between tissues and among mutants

Tissue Expression of 1300002K09Rik



Histochemical staining in liver from HET 1300002K09Rik KO mice using a LacZ reporter driven by the native gene promoter; only the liver stained specifically for LacZ in this mutant



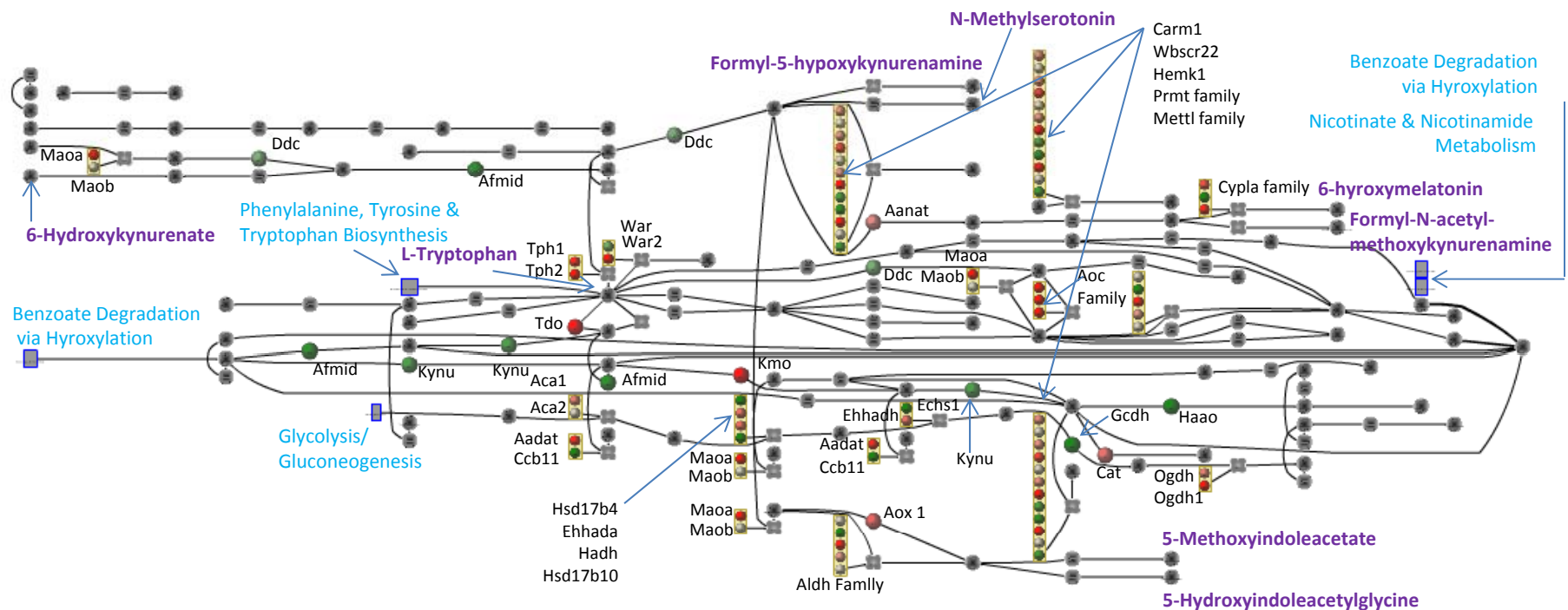
Top Genes Affected in 130002K09Rik Liver

Top gene	Fold up	Top gene	Fold down
GCLC	6.19	BHMT	-3.12
HSPE1	5.50	PECI	-2.83
HSPA5	5.42	GADD45G	-2.74
GSTA1	5.30	PAIP2	-2.74
ID2	4.82	SLC38A4	-2.73
KMO	4.04	SPON2	-2.62
ALDH1A1	3.91	PECR	-2.38
TDO2	3.66	FGF21	-2.37
MT-ND5	3.55	GSTA2	-2.29
NAMPT	3.44	MT-CO2	-2.25
color: hit 1 pathway; color: hit 3 or more pathways			

Table xxx. Top Pathways Affected in 130002K09Rik Liver

1300002K09Rik-LIVER	IPA (p-value)	Seralogix	KEGG pathway#	Category
Glycolysis/Gluconeogenesis	6.60E-07	5.05	mmu00010	Carbohydrate Metabolism
Citrate Cycle	1.01E-05	3.14	mmu00020	Carbohydrate Metabolism
Inositol Metabolism	1.39E-05	-3.81	mmu00031	Carbohydrate Metabolism
Glutathione Metabolism	7.65E-05	-3.92	mmu00480	Metab of Other Amino Acids
Fatty Acid Metabolism	8.60E-05	-3.46	mmu00071	Lipid Metabolism
Bile Acid Biosynthesis	1.09E-04	-3.22	mmu00120	Lipid Metabolism
Tryptophan Metabolism	1.39E-04	3.48	mmu00380	Amino Acid Metabolism
Propanoate Metabolism	1.64E-04	3.68	mmu00640	Carbohydrate Metabolism
Pyruvate Metabolism	2.12E-04	3.49	mmu00720	Carbohydrate Metabolism
Valine, Leucine and Isoleucine Degradation	2.31E-04	-3.21	mmu00280	Amino Acid Metabolism
Metabolism of Xenobiotics by P450	6.00E-04	-4.14	mmu00980	Xenobiotics Biodegrad & Metab
Retinol Metabolism	1.14E-03	3.43	mmu00830	Metab of Cofactors and Vitamins
Lysine Degradation	1.42E-02	4.09	mmu00310	Amino Acid Metabolism
Glycine, Serine and Threonine Metabolism	1.37E-01	-3.42	mmu00260	Amino Acid Metabolism
Nicotinate & Nicotinamide Metabolism	1.49E-01	4.20	mmu00760	Metab of Cofactors and Vitamins
Tyrosine Metabolism	2.95E-01	3.04	mmu00350	Amino Acid Metabolism
Mat Onset Diab Young (MODY) Signaling	5.08E-01	3.30	mmu04950	Metabolic Disorders
Taurine and Hypotaurine Metabolism	6.76E-01	4.30	mmu00430	Metab of Other Amino Acids
color: hit by 1 of top 10 genes	color: hit by 2 of top 10 genes		color: hit by 3 + of the top 10 genes	

Tryptophan Metabolism in 130002K09Rik Liver

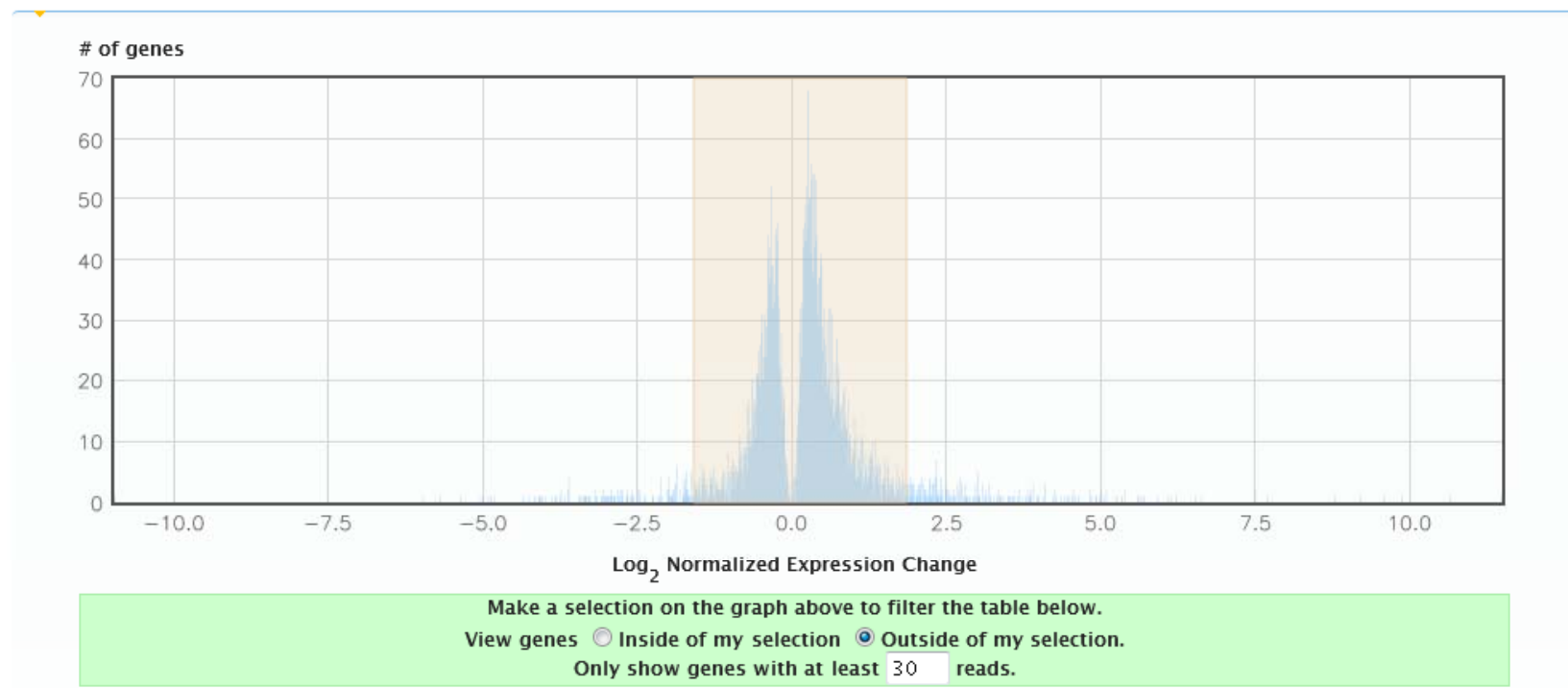


- Gene node icon. Gradient color from light to bright red indicates increasing up modulation.
- Gradient color from light green to bright green indicates increasing down modulation.
- Grey color indicates a gene with no change from control.

Data Presentation

RNASeq db/db db/+ expression data for tissue: LIV

[Return to tissue list](#)



Data Presentation

Copy  Excel  PDF  Print 

Show entries

Search:

Gene Symbol	db/+ Average Reads	db/db Average Reads	Log2 Expression Change	Expression Change	p value	z score
Sult2a2	0	1341	11.49	2,876.69	0.00E+0	28.67
Hsd3b5	6358	3	-10.95	-1,975.90	0.00E+0	-63.08
Sult2a1	0	756	10.66	1,621.76	0.00E+0	23.05
Cyp2b9	15	13209	9.88	944.52	0.00E+0	102.23
Sult1e1	1	721	9.59	773.34	0.00E+0	24.38
Sult2a5	0	274	9.20	587.78	0.00E+0	15.43
Sult2a4	0	208	8.80	446.20	0.00E+0	13.78
Cyp2b13	23	4445	7.70	207.29	0.00E+0	67.47
Cidea	3	510	7.51	182.34	0.00E+0	23.03
Krt20	0	85	7.51	182.34	0.00E+0	9.40
Sprr1a	5	461	6.63	98.89	0.00E+0	22.52
Sult3a1	0	43	6.53	92.24	0.00E+0	6.89
Gm12629	0	35	6.23	75.08	0.00E+0	6.25
Slco1a1	16451	246	-5.96	-62.35	0.00E+0	-131.11
Sult2a3	1	66	6.15	70.79	0.00E+0	8.59
Pla2g4f	3	193	6.11	69.00	0.00E+0	14.69
Fmo3	6	341	5.93	60.96	0.00E+0	19.55
Gpr110	277	5	-5.69	-51.65	0.00E+0	-17.02
Ly6d	41	1998	5.71	52.27	0.00E+0	47.34
Hao2	7	333	5.67	51.02	0.00E+0	19.33
Cyp2b23	4	182	5.61	48.80	0.00E+0	14.28
Moxd1	44	1	-5.36	-41.02	0.00E+0	-6.77
Gprc5b	16	632	5.40	42.37	0.00E+0	26.57
A4gnt	9	354	5.40	42.19	0.00E+0	19.09
A1bg	10	358	5.26	38.40	0.00E+0	19.96

Showing 1 to 25 of 334 entries (filtered from 8,290 total entries)

First Previous 1 2 3 4 5 Next Last

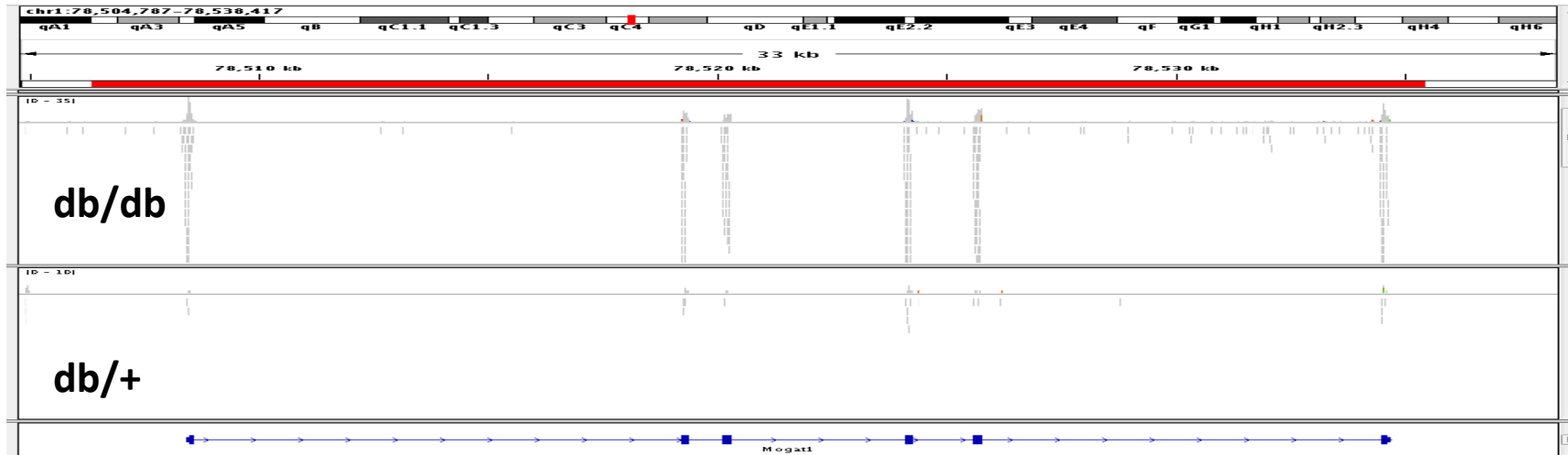
Transcriptome Strategy

- Which Mutants?
 - Selective?
 - Unannotated genes?
 - Complement physiological phenotypes?
- Which Tissues?
 - Survey major organ systems?
 - Selective tissues based on other information?
 - Microdissection of substructures

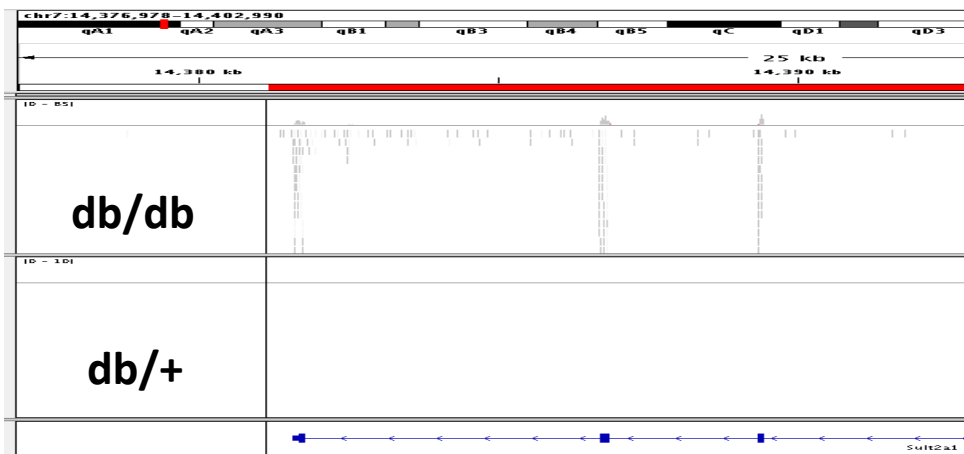
Acknowledgements

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 - William Chen
- CHORI
 - Pieter De Jong
 - Michael Adkisson

RNA-seq Pile-ups for db/db vs. db/+ Liver Differentially Expressed Genes



Mogat1: monoacylglycerol O-acyltransferase 1; Beadarray: 3.3 Fold; RNA-seq 8.4 Fold



Sult2a1: sulfotransferase family 2A, dehydroepiandrosterone (DHEA)-preferring, member 1; Beadarray: Not detected; RNA-seq 200 Fold

Entire family of Sults (Sult2a1, Sult2a3, Sult2a4, Sult2a5, Sult2a7, Sult3a1, Sult5a1) all increased 8-200 Fold in db/db