K312 Progress: NIH K2 Kickoff

- Gene Selection
- Mouse Production
- Phenotyping Data

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K312 Gene Selection

Selection Criteria for Genes:

- Interest to the scientific community
  (determined by registered interest on www.komp.org)
- Significant percentage with unknown function
- Implicated in human genetic disorders
- No existing available KO mouse models

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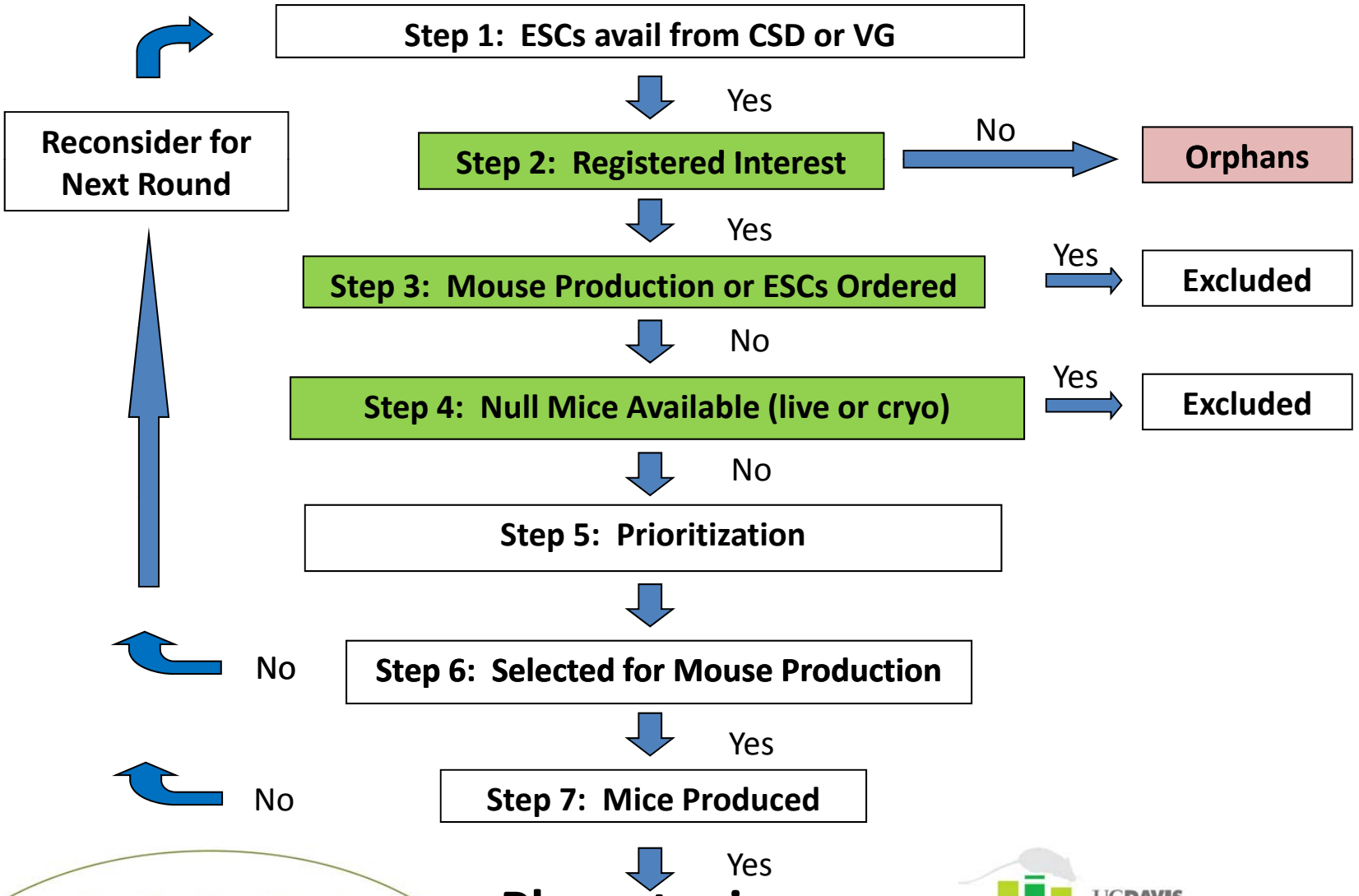
K312 Primary Gene Selection Algorithm



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Paths for K312 Gene Selection

Original Goals for Gene Selection:

Path 1: Prioritized Registered Interest	Path 2: Genes with No Annotation	Path 3: Genetic & Bioinformatic Prioritization
188 genes (60%)	79 genes (25%)	45 genes (15%)

For 357 genes currently active:

79% (284/357)	24% * (85/357)	14% (48/357)

*** Defined as having 3 or fewer GO annotation terms connected to gene ID**

< 2% have existing KO mutants available

All selection goals achieved

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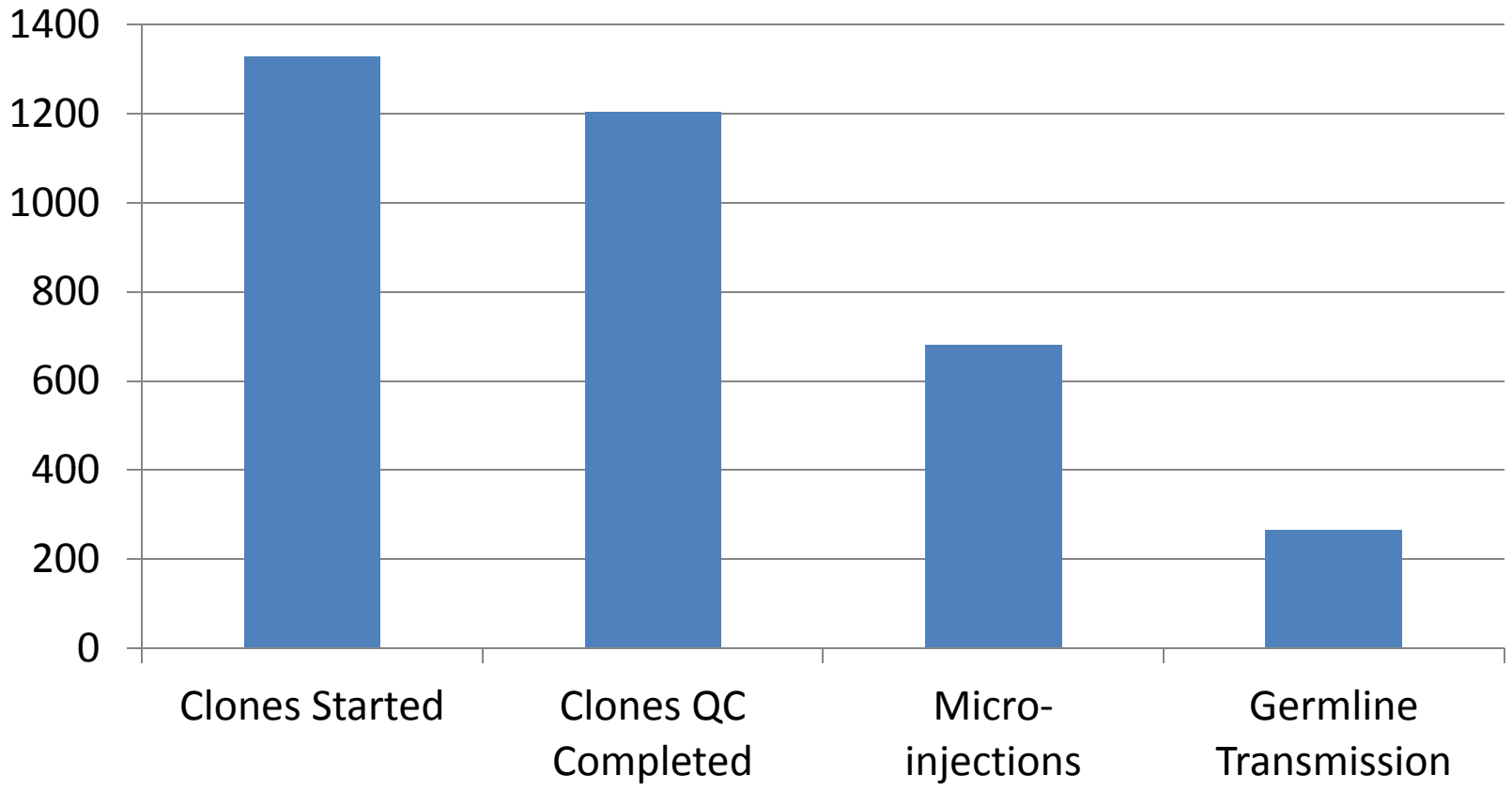
K312 Production: Cumulative Effort



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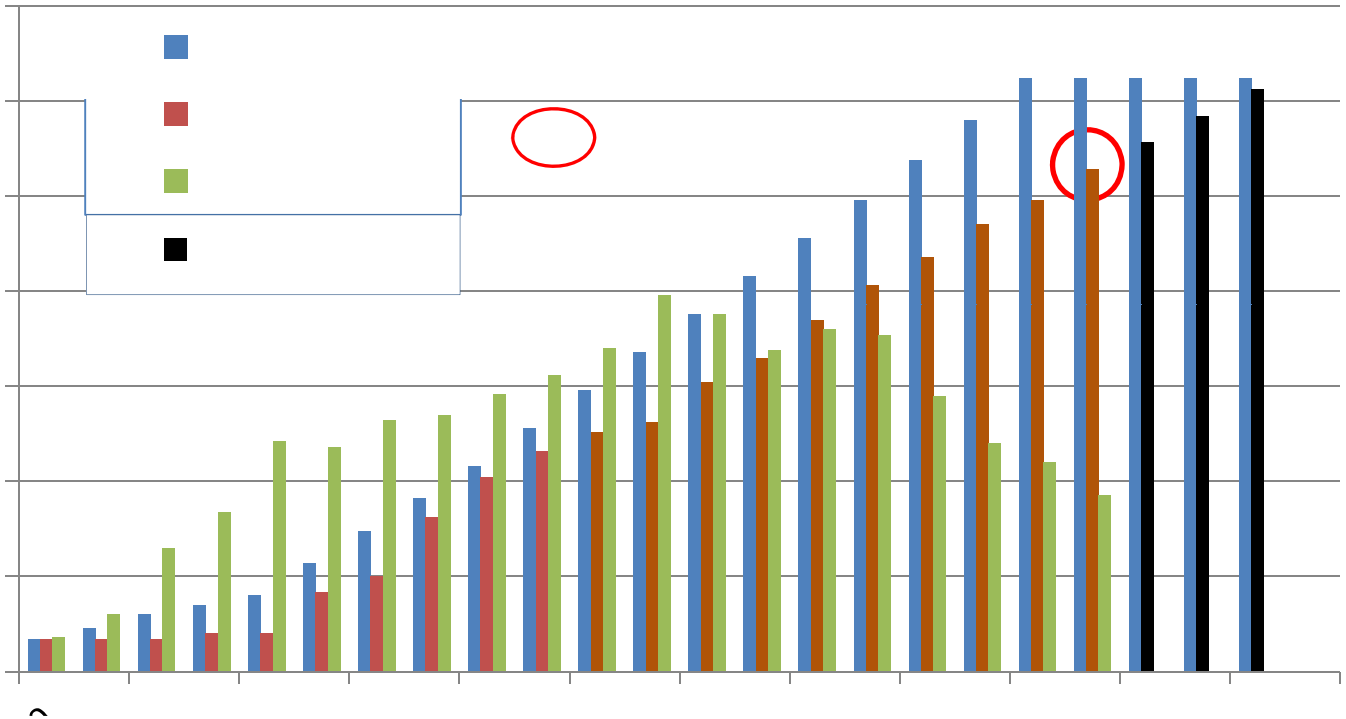

K312 Production Statistics

- 59% of clones pass QC
- 86% of microinjections produce at least one breedable chimeric male
  (>50% coat color chimerism)
- 67% of lines with breedable chimeras go germline

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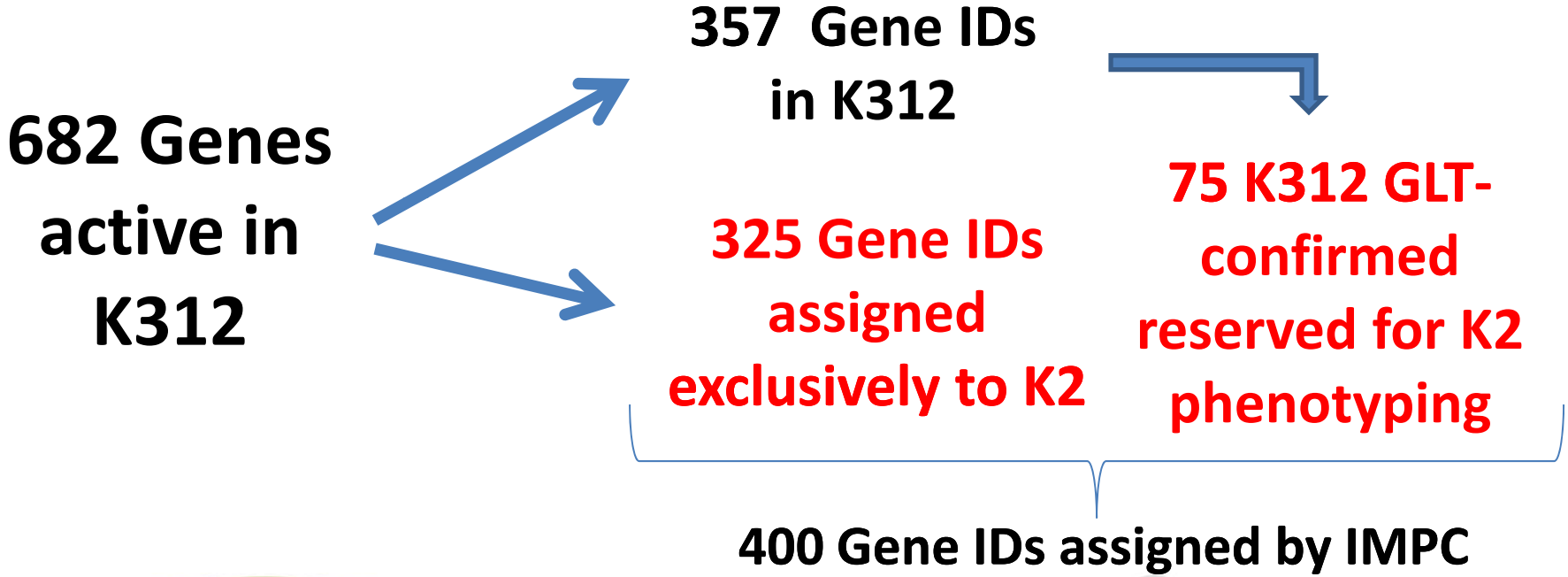
K312 Excess Production will Accelerate K2 Launch

~ 160 injections in pipeline not needed for K312 along with QC on a large number of stemcells



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K312 Production Summary

- 357 genes with clones active in K312 pipeline
  - — 264 GLT-confirmed
  - — ~60-70 additional GLTs in pipeline (chimeras or microinjected)
- 50% of K312 GLT-confirmed have cryo'd material available at www.komp.org

- 34% of K312 now have products ordered

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K312 Phenotyping Plan

HET LacZ Tissue Dist (312)	HOM Viability (100)	HOM Necropsy (100)	HOM** Illumina (100)	HOM Physical & Behavioral Exams (100)	HOM Misc. (100)
Frozen & Wholemount Annotated	Embryo Histology on subset of embryonic lethal	Tissue Wts Obvious Pathology 7M, 7F	LacZ+ tissues ~4 M mice/gene ~5 Tissues Orphan Gene Emphasis	Physical Exam Modified SHIRPA 7M, 7F	Serum Chem Histopathology Behavior Metab Disease Imaging Other 7M, 7F

Database & Web Presence

www.kompphenotype.org

(# of unique genes)

**Data/approach to be presented at IMPC kick-off

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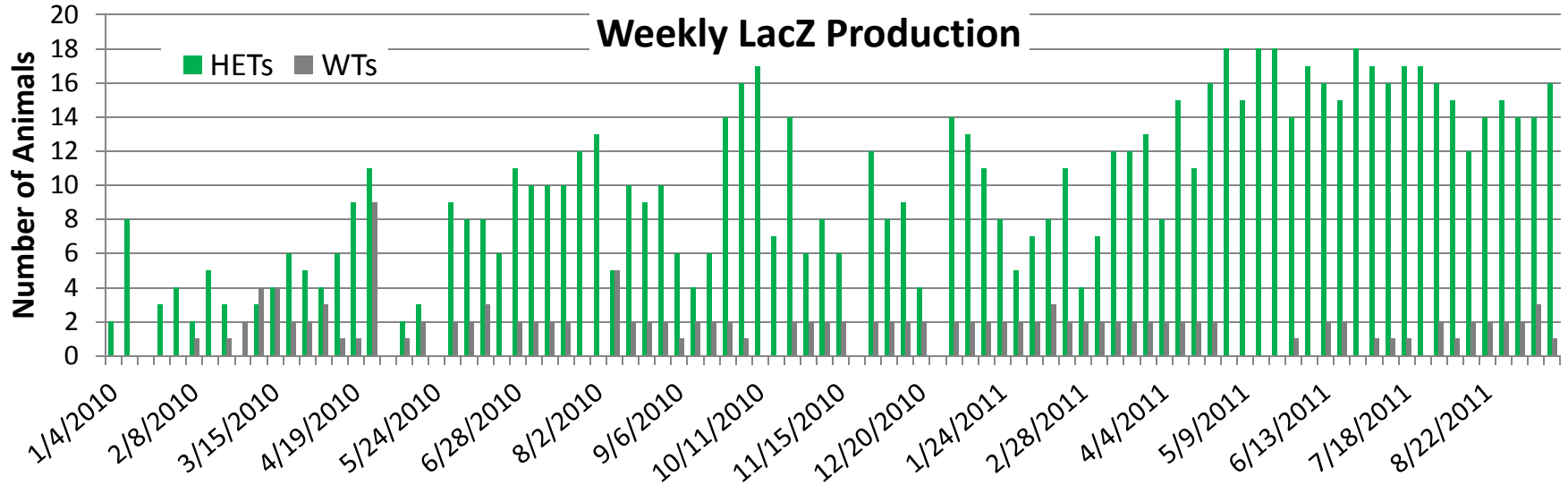
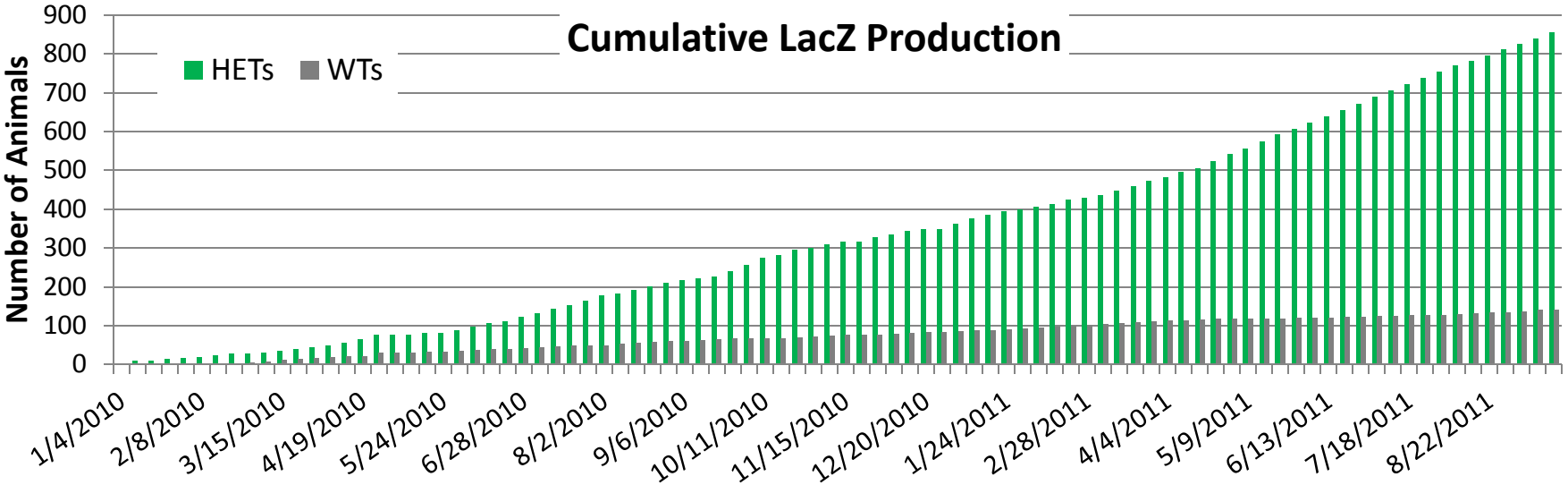
LacZ Production

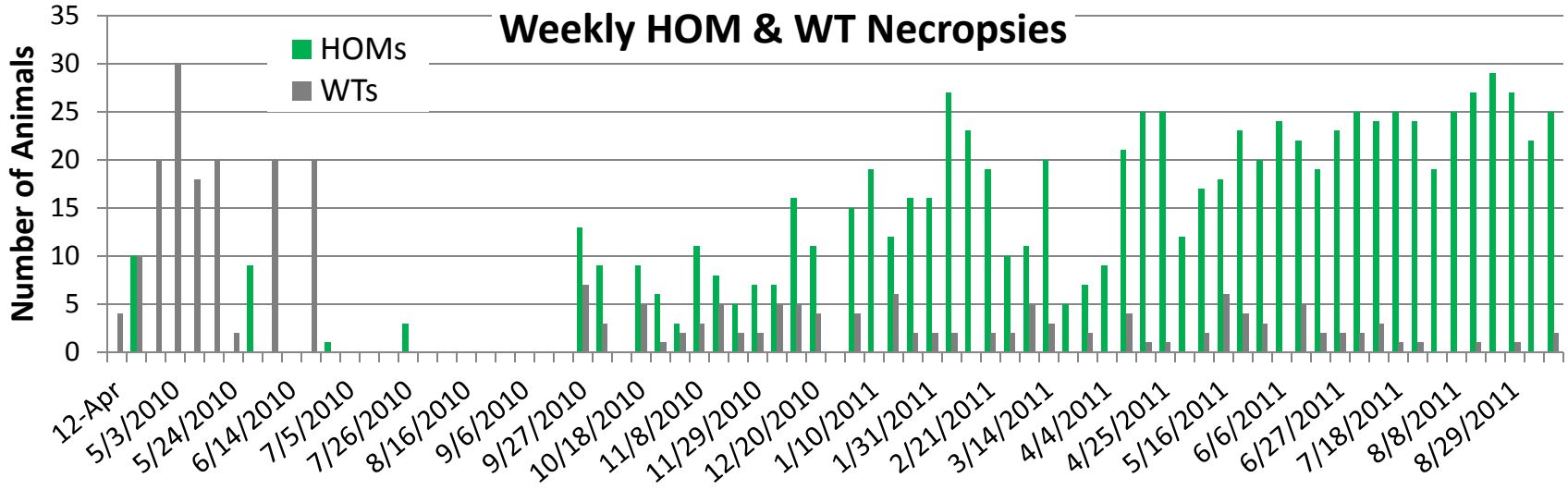
LacZ Analysis Rate significantly above that needed for K2

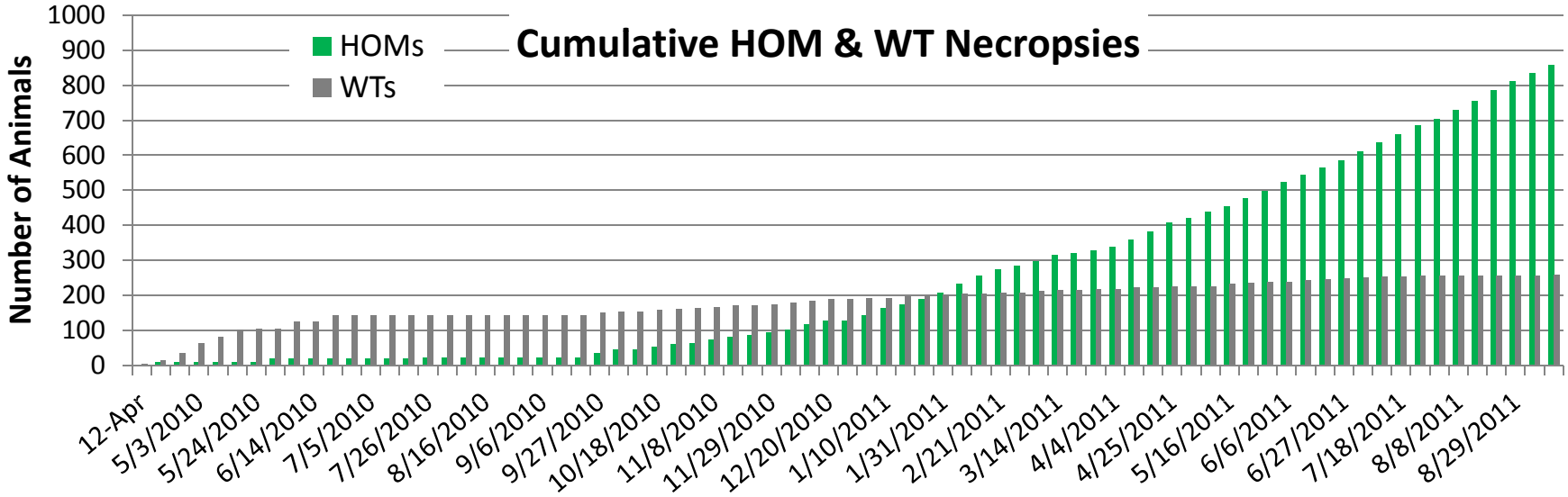




HOM Phenotyping

HOM analysis rate ~71% of that needed for K2

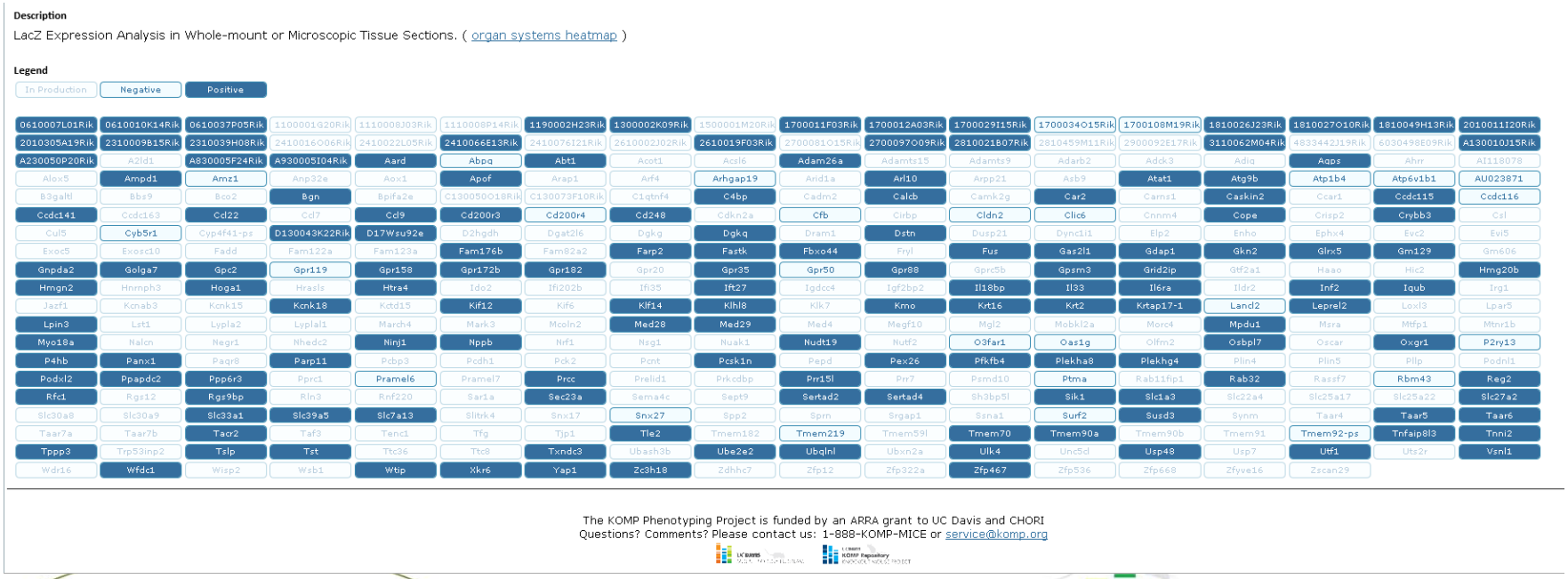




K312 HET LacZ Staining

Procedure:

- 50 +/- 2 days of age
- 1M & 1F Wholemount; 1M & 1F Frozen-Section*
- Substructure Annotation
- Detailed Brain Annotation*



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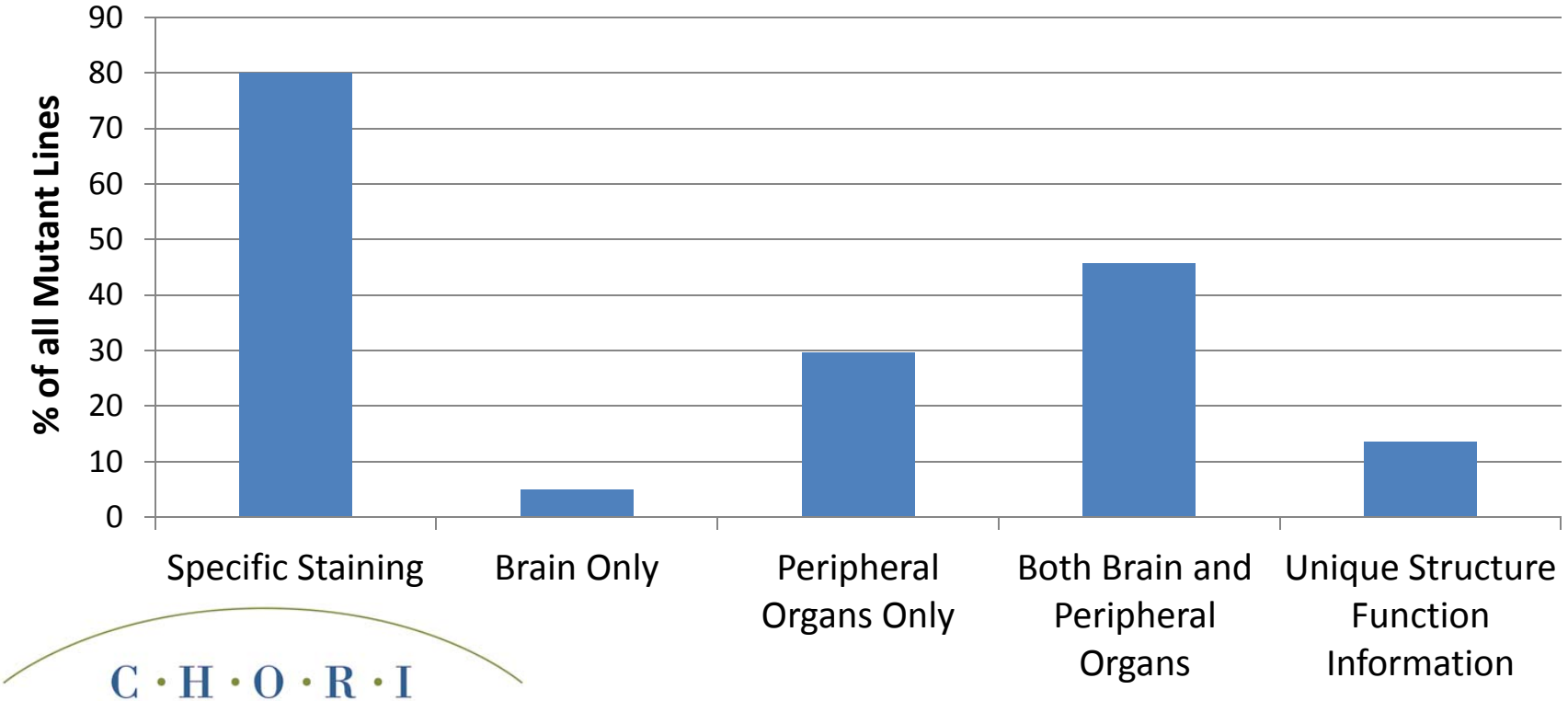
K312 HET LacZ Staining

- Provides unique structure-function information for 15-20% of genes
- Complements existing gene expression profile data
- Reveals unique CNS patterns of expression



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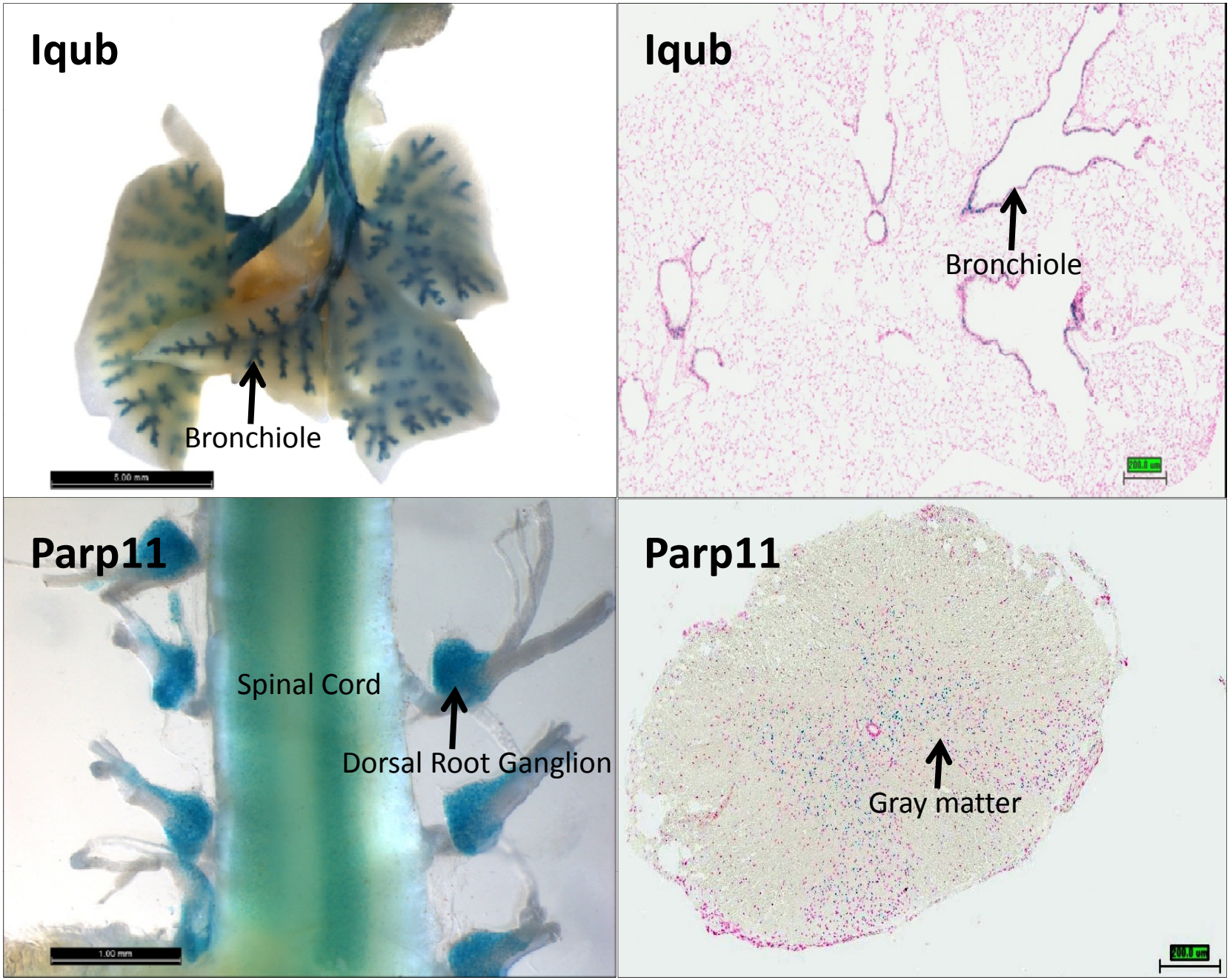

Iqub
Bronchiole
5.00 mm
Iqub
Bronchiole
200.0 um
Parp11
Spinal Cord
Dorsal Root Ganglion
1.00 mm
Parp11
Gray matter
200.0 um

Taar6

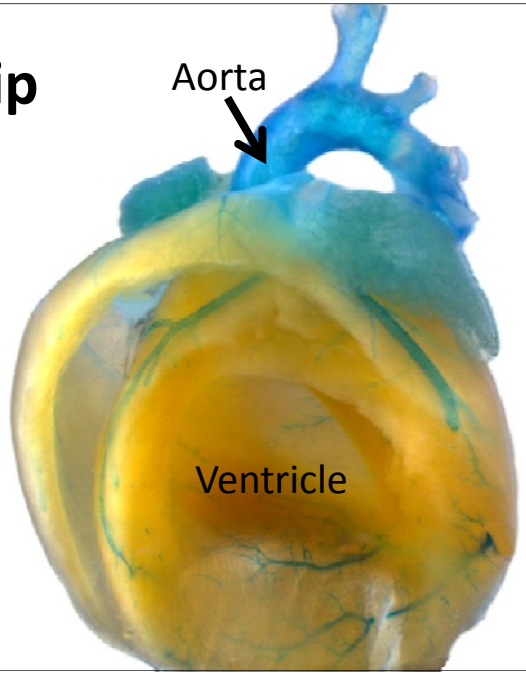
Olfactory bulb

Taar6

Olfactory nerve layer

Wtip

Aorta



Ventricle

Wtip

Blood vessel

Ventricle

Kcnk18 LacZ Annotation

Structure Name	Intensity
Posterior hypothalamic nucleus	−
Medial	
Dorsomedial hypothalamic nucleus	−
Medial preoptic region	++
Suprachiasmatic nucleus	++
Ventromedial hypothalamic nucleus	−
Periventricular	
Arcuate nucleus	−
Paraventricular hypothalamic nucleus	Not in image
Mammillary Body	
Medial mammillary nucleus	−
Dorsal tuberomammillary nucleus	−
Pituitary gland	Not in image
Other	
blood vessel	Not in image
Midbrain	
Cerebral aqueduct	−
Midbrain periaqueductal grey	−

Detailed Annotation of Brain LacZ staining

- More than 100 structures annotated
- Uses standard anatomy ontology
- Improves understanding of structure function relationships
- Facilitates comparison with other brain atlases (Allan Brain Atlas, GenSat)

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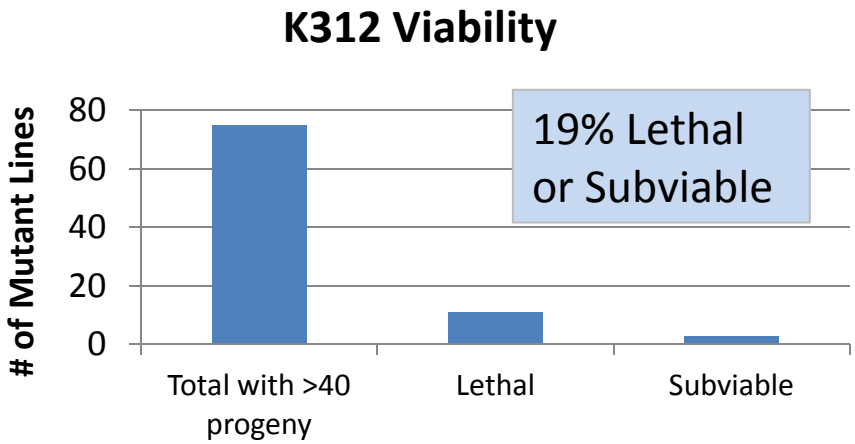
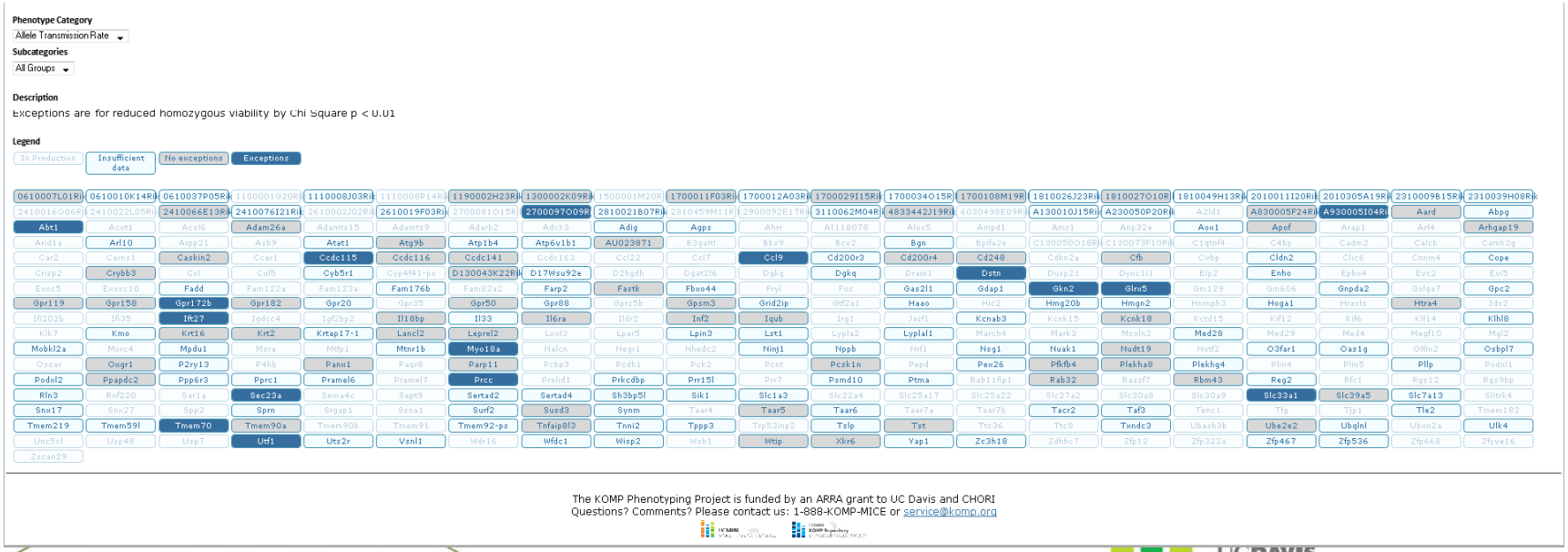
K312 HOM Viability

Procedure:

- HET x HET crosses
- Minimum of 40 progeny genotyped
- Chi-square for significance (P< 0.01)





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K312 HOM Viability

Prcc

Name: papillary renal cell carcinoma (translocation-associated)

Resources: MGI:2137738 NCBI:94315 OMIM, PubMed, Gene Cloud, Additional resources at KOMP

Products: vector, mice, ESC, sperm

LacZ Summary | Lacz Expression | Viability | Blood Chem | Cardiology | Growth/Gross/Histology | Metabolism | Neurology & Behavior | Physical

Allele Transmission Rates (Heatmap)

Male Parent (Het)	Female Parent (Het)	Number of pups produced						
		WT Male	HET Male	HOM Male	WT Female	HET Female	HOM Female	Total
BL1495-77	BL1495-71	1	2	0	0	2	0	5
BL1495-77	BL1495-72	0	1	0	0	3	0	4
BL1495-78	BL1495-74	0	4	0	2	2	0	8
BL1495-81	BL1495-80	0	0	0	1	0	0	1
BL1495-109	BL1495-108	3	5	0	0	9	0	17
BL1495-98	BL1495-96	1	1	0	0	4	0	6
	Total:	5	13	0 *Reduced HOM viability	3	20	0 *Reduced HOM viability	41

Genotype Frequencies

	Male	Chi Square (p)	Female	Chi Square (p)	Combined	Chi Square (p)
Actual Genotype Ratio (Wt:Het:Hom)	1.1 : 2.9 : 0	6.33 (0.05)	0.5 : 3.5 : 0	7.07 (0.04)	0.8 : 3.2 : 0	18.37 (<0.01)
Expected Genotype Ratio (Wt:Het:Hom)	1:2:1		1:2:1		1:2:1	

The KOMP Phenotyping Project is funded by an ARRA grant to UC Davis and CHORI
Questions? Comments? Please contact us: 1-888-KOMP-MICE or service@komp.org

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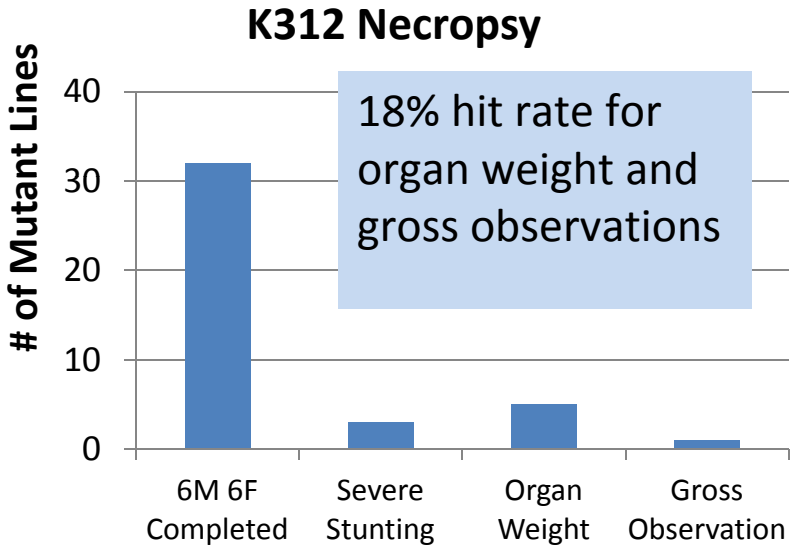
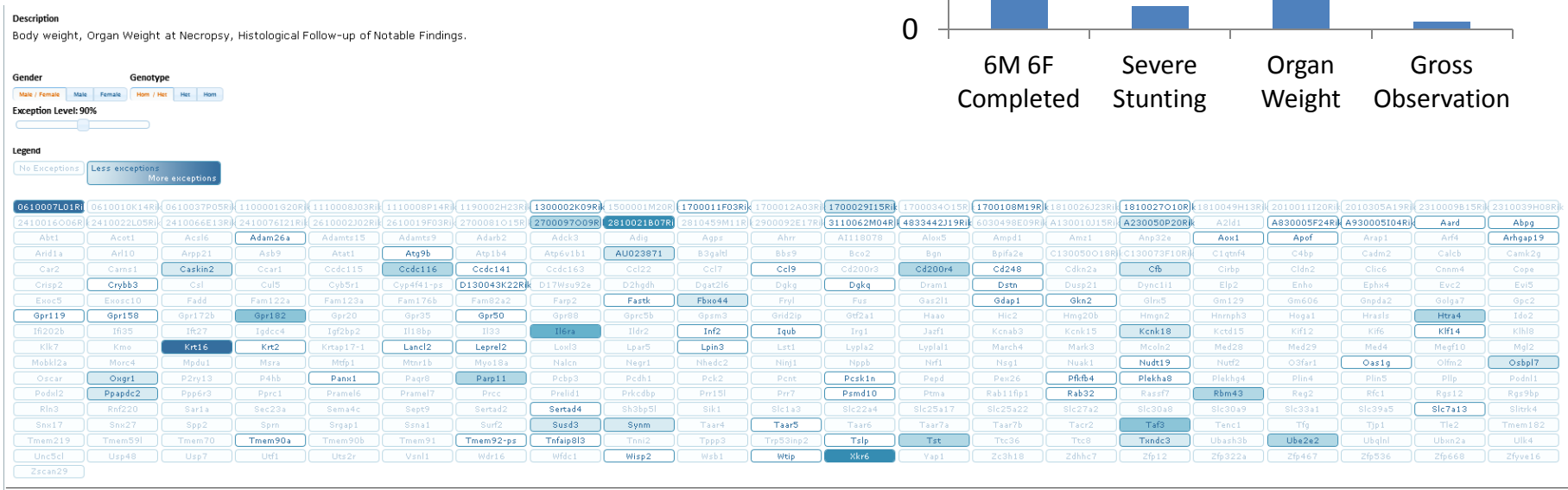
K312 Necropsy

Procedure:

- 50 +/- 2 days of age
- Detailed physical exam
- 10 organs weighed and frozen (7M & 7F)
- Up to 5 organs RNA-later (7M)
- Comprehensive formalin fix (3M & 3F)





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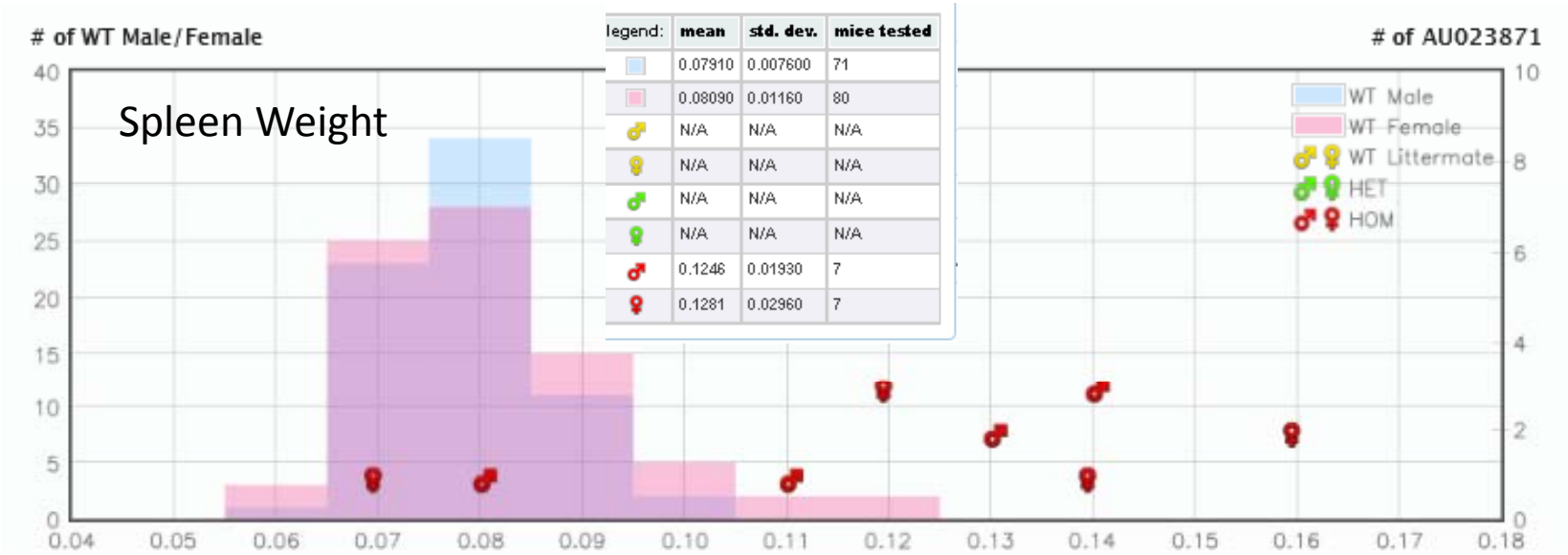
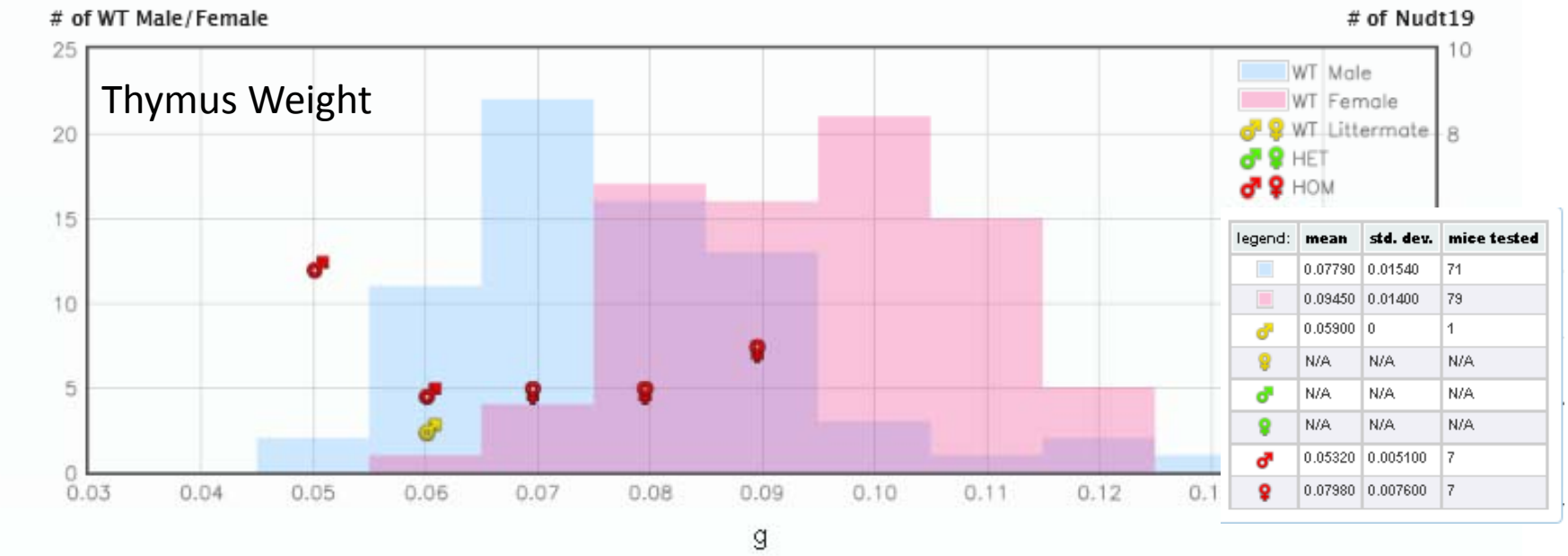
K312 Necropsy: Organ Weights



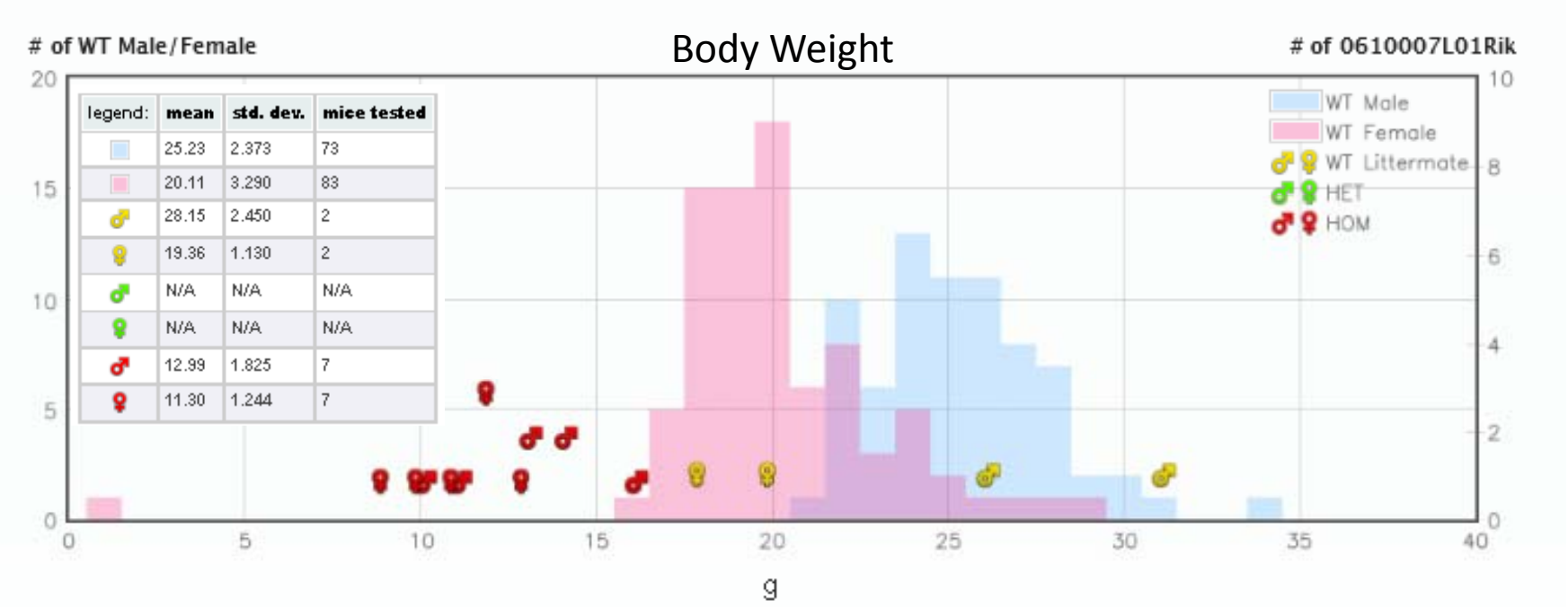
legend:	mean	std. dev.	mice tested
	0.07910	0.007600	71
	0.08090	0.01160	80
	N/A	N/A	N/A
	N/A	N/A	N/A
	N/A	N/A	N/A
	N/A	N/A	N/A
	0.1246	0.01930	7
	0.1281	0.02960	7



legend:	mean	std. dev.	mice tested
	0.07790	0.01540	71
	0.09450	0.01400	79
	0.05900	0	1
	N/A	N/A	N/A
	N/A	N/A	N/A
	N/A	N/A	N/A
	0.05320	0.005100	7
	0.07980	0.007600	7

g

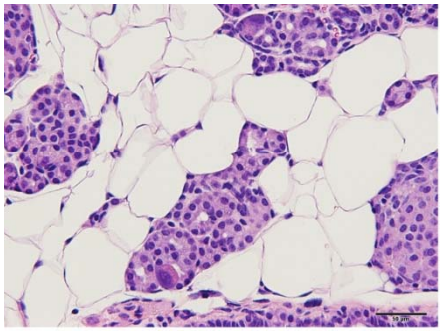
K312 Necropsy: Histopathology



Clinical Features 0610007LO1Rik:

- Stunted
- Little adipose tissue
- Distended cecum and colon
- Atrophied pancreas

Thymus has a decreased cortical:medullary distinction and medulla is slightly decreased in size. Pancreas is small with marked fatty infiltration. The pancreas consists predominantly of ducts and islets with only few individual acinar cells which are enlarged, some are binucleated, and distended with zymogen granules..

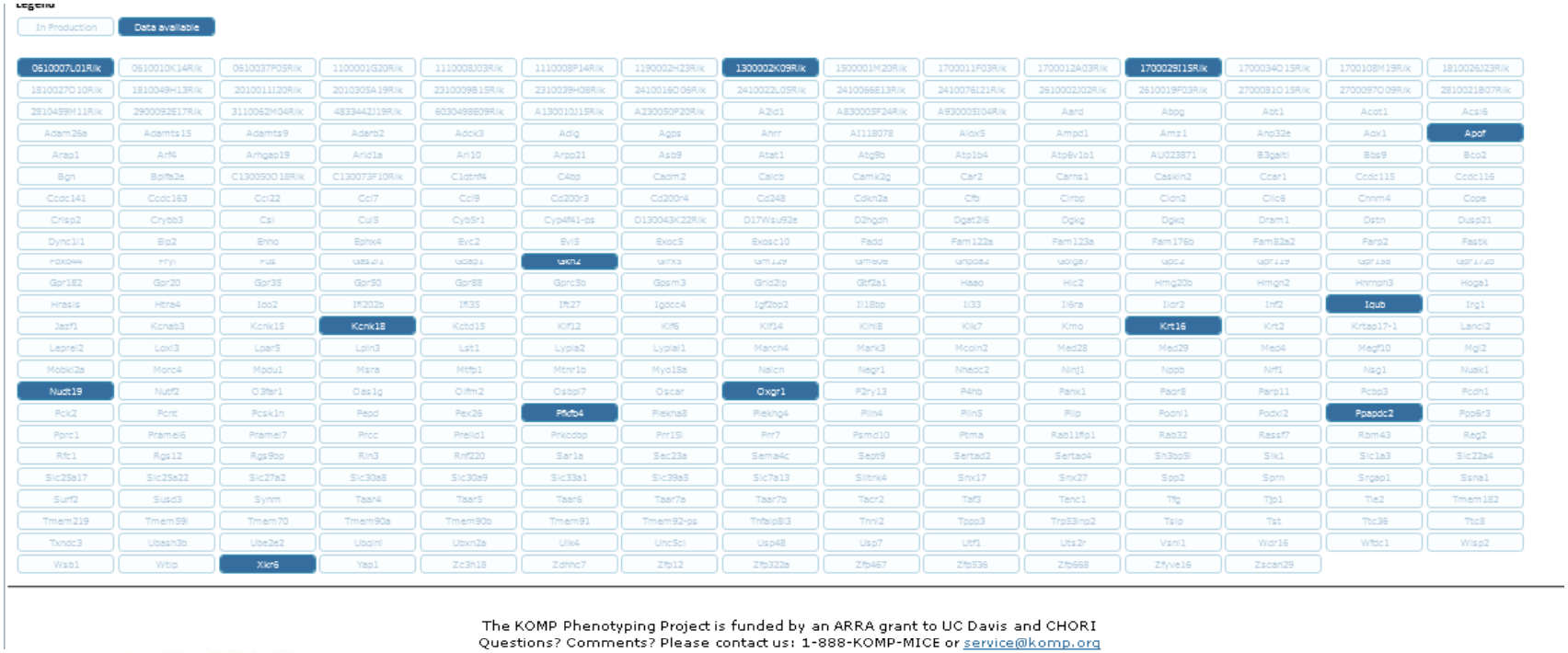


BL1425-211 Pancreas1.tif

K312 Serum Chemistry

Procedure:

- Non-fasted blood collected at necropsy by heart stick
- 22 analytes evaluated to date in 13 mutants
- Selective mutant analysis: for K312 will not be all mutants
- Several notable findings



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K312 SHIRPA and Physical Exam

Procedure:

- Modified SHIRPA followed per EUMODIC – 30 behaviors
- Supplemented with Functional Observation Battery Tests
- Physical exam records 32 physical features

Results:

- Low hit-rate (~3%)
- Complicated pattern of behavior in wild-types
- One clearly affected mutant with altered SHIRPA tests has mutiple traits affected

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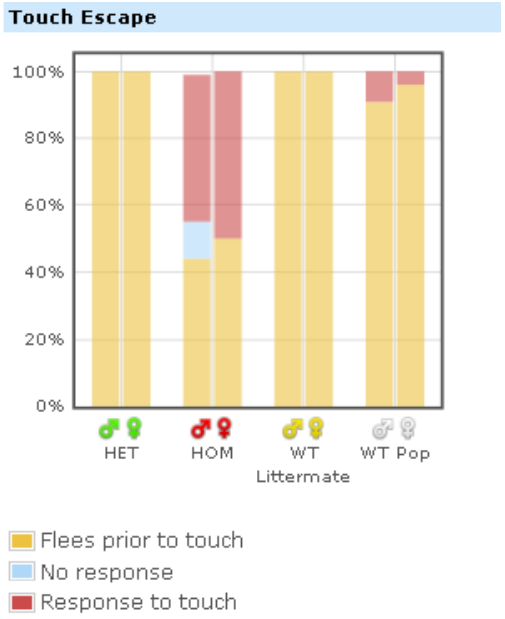
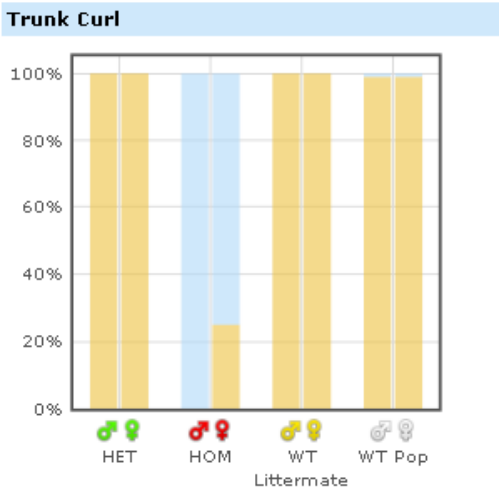
Xkr6 Cluster of Behavioral Traits

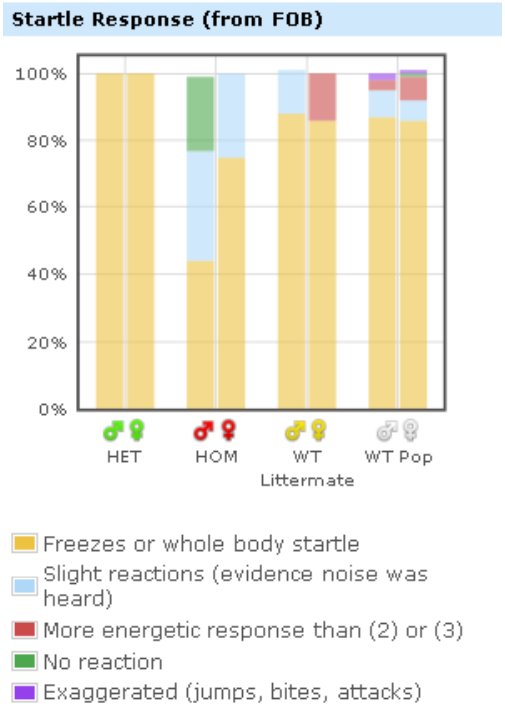
Xkr6 Mutant Features:

- LacZ staining only in brain
- Moderate stunting of growth
- Normal gait, posture, movement & reflexes in SHIRPA exam

Total of 3 Het M (♂) were tested
Total of 3 Het F (♀) were tested
Total of 9 Hom M (♂) were tested
Total of 4 Hom F (♀) were tested
Total of 8 Wt littermates M (♂) were tested
Total of 7 Wt littermates F (♀) were tested
Total of 134 Pop Wt M (♂) were tested
Total of 141 Pop Wt F (♀) were tested







Geotaxis and strength FOB tests also affected (data not shown)

Webpage Functionality

www.kompphenotype.org

Search | Explore | Data | Services | About

Search

Search

You can search by gene, phenotype or anatomy!

KOMP Project Report

View the project gene list
Nominate a gene to be phenotyped

Explore

KOMP Mutants
Explore KOMP Phenotyping Data

Lexicon-Genentech Mutants
Explore Lexicon-Genentech Phenotyping Data

Gene List
Browse the project gene lists

More Exploration Tools

Data

About
Learn about the data on this website

BioMart
Access the KOMP Phenotyping biomart

Bio Torrent
Download our entire data set via bit torrent.

Services

Mouse Biology Program
For all your needs working with genetically altered mice

KOMP Repository
Obtain KOMP resources and services

News

Jun 3, 2011 - Mouse Molecular Genetics (20-23 September 2011)
For the past 24 years, the Mouse Molecular Genetics meeting has been a leading forum for researchers who apply the methods of genetics and genomics to fundamental problems in mammalian biology, including from stem cell biology, early development, and models of human disease. In particular, the meeting showcases the latest technical developments in genetics and engineering of the mouse genome, and this year will feature a session devoted to imaging. The Mouse Molecular Genetics meeting assembles leaders in the field to present unpublished research findings, encourages junior investigators to participate in oral and poster presentations, and provides a stimulating environment for the exchange of ideas and information. More Information / Registration

March 24, 2011 - The NIH Common Fund (http://commonfund.nih.gov) was enacted into law by Congress through the 2006 NIH Reform Act to support cross-cutting, trans-NIH programs that require participation by at least two NIH Institutes or Centers (ICs) or would otherwise benefit from strategic planning and coordination. The NIH Common fund provides support to the KOMP2 Phenotyping Program, which will provide new tools and methods, a comprehensive mutant mosue databse and library, and conduct broad, standardized, and high-throughput analysis of mutant mouse models produced by the International Knockout Mouse Consortium. Learn more about KOMP2 Phenotyping and the NIH Common Fund here: http://commonfund.nih.gov/KOMP2/.

K312 Current Status

- 264 GLT-confirmed lines finished with mice or cryo-preserved material available for ~ 130 lines
- K312 excess-production will feed K2 effort
- ~200 lines with HET LacZ data
- HOM phenotyping
  - ~ 60 lines with HOM data on webpage
  - HOM lethal/subviable rate ~19%
  - Necropsy HOM phenotypes in ~18% of completed lines (6M & 6F)
  - SHIRPA behavioral phenotypes in HOMs for ~3%
  - Serum chemistry analysis underway
  - Expression profiling data to be presented at IMPC session
- Webpage up and showing all data live!

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