KOMP312: A Pilot Study to Create and Phenotype 312 KOMP Knockout Alleles in Mice

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Introduction

The KOMP312 (K312) project was funded by the NHGRI and NCRR to produce and phenotype 312 mutant lines from KOMP targeted stem cell lines over a two-year period; to evaluate LacZ reporter expression patterns in heterozygous (HET) mice from all 312 lines, and to provide additional phenotyping data in homozygous (HOM) mutants from 100 of these lines.

Methods

Targeted stem-cells (B6N), primarily carrying KOMP alleles (either conditional or deleted cell lines produced by the CHORI-Sanger-UC Davis [CSD] Consortium or by Regeneron, Inc.) were injected into blastocysts and ~16 injected blasts were implanted into each pseudo-pregnant recipient. High percentage coat-color male chimeras were bred to C57BL/6NTac females and

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%)

Actual 524 genes in queue:

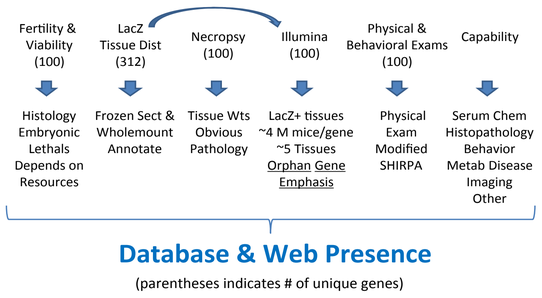
81% (425/524)	21% * (107/524)	12% (48/524)

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

All genes now posted on webpage and publicly available; soliciting public input for gene nominations

appropriate coat-color offspring were genotyped to confirm germ-line transmission. See the International Knockout Mouse Consortium (IKMC) webpage (www.knockoutmouse.org) for a description of the KOMP alleles; and the KOMP Repository webpage (www.komp.org) for a description of the cell culture methods.

K312 Phenotyping Plan

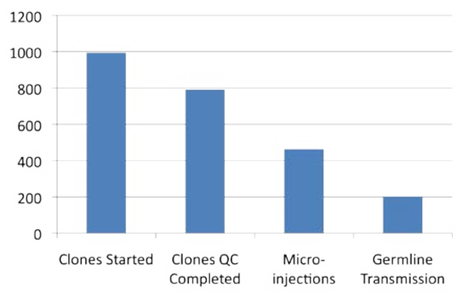


Genes were selected for mouse production based upon an algorithm that emphasized nominations from the scientific community, genes with no known function, and genes implicated in genetic diseases for both mendelian and complex genetic disorders.

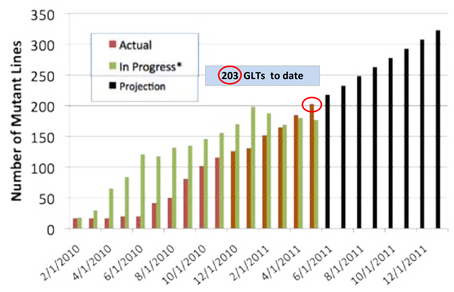
The phenotyping plan includes whole-mount LacZ expression in 1M and 1F HET in ~50 tissues/organs, and frozen Section LacZ staining of 1M and 1F HET in ~ 42 organs and tissues for all 312 mutants. In addition, 100 lines are being bred to homozygosity and 7M and 7F HOMs and approximately 1M and 1F WT littermate will be phenotyped: functional behavioral exam (SHIRPA), physical exam, necropsy with histopathology follow-up of notable findings, serum chemistry in a subset, and compensatory transcriptome analysis.

Production Progress

K312 Total Cumulative Effort



Confirmed Germline



* injected, chimeras breeding or waiting to breed

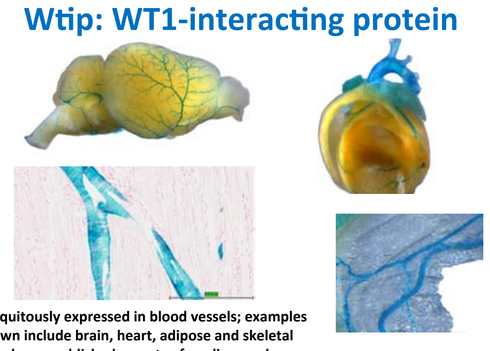
To date, we have started QC on ~ 1000 clones, ~800 clones have finished QC, ~450 clones have been microinjected, and ~ 200 lines have gone germline. Gene selection has met our goals for genes of interest to the scientific community, with a significant fraction having little or no functional information, and a subset selected based upon a genetic and bioinformatic analysis. Our germline transmission rate is on target to meet the goal of 312 lines going germline within two years of starting microinjections.

Phenotyping Progress

Viability: >99 lines have been set-up with HET x HET breeding; ~33 lines have produced >40 progeny; 4 lines (12%) are embryonic lethal and 2 lines (6%) are subviable defined as fewer than expected HOM mice at weaning.

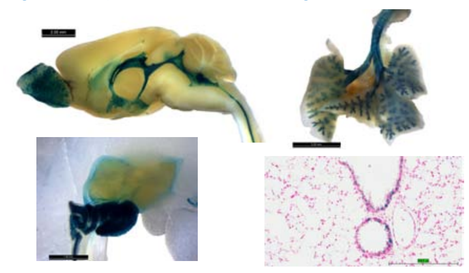
LacZ Reporter: ~122 lines have LacZ data reported on the webpage; ~77% have positive and specific reporter expression; ~24% staining in multiple tissues; 52% express the reporter in the brain; and ~20% reveal unique and previously unknown functional information. Two examples are shown: Wtip and Iqub. For a more detailed discussion of the LacZ findings see Poster #183C (R Pasumarthi et al.)

Wtip: WT1-interacting protein



Ubiquitously expressed in blood vessels; examples shown include brain, heart, adipose and skeletal muscle; no published reports of cardiovascular expression

Iqub: IQ motif and ubiquitin-domain



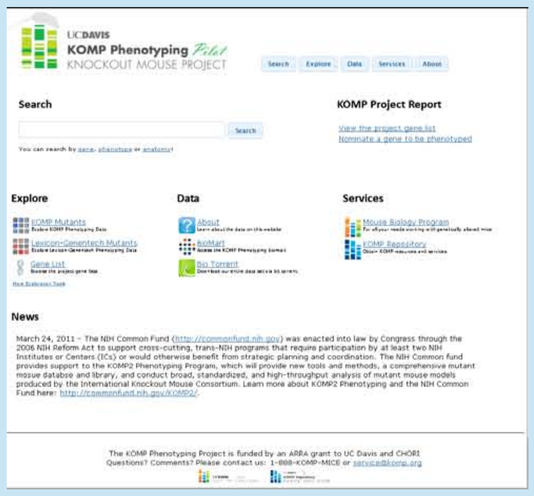
Reporter expressed in epithelial cells of the ventricular ependyma & in the glomerular layer of the olfactory bulb, lung bronchioles, oviduct and sminiferous tubules; no previous reports of gene expression in epithelial tissue

Necropsy, SHIRPA (behavioral tests), Physical Exam & Serum Chemistry: ~ 38 lines have produced >4M and >4F HOM mutants for phenotyping; mutants with behavioral and physical exam phenotypes include 130002K09Rik, 1700108M19Rik, Krt16 and Xkr6; no abberrations have been noted on necropsy or serum chemistry to date. See www.kompphenotype.org for details.

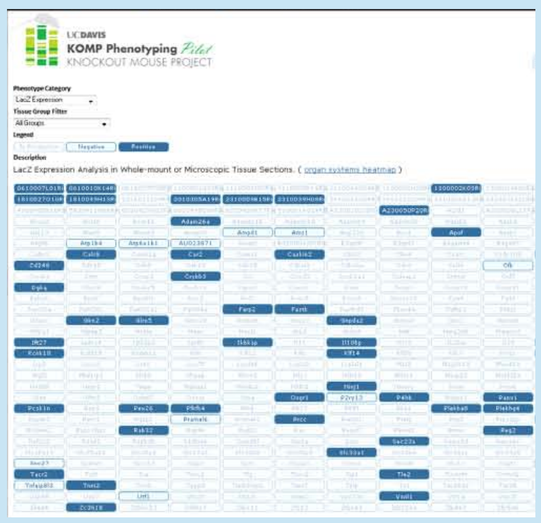
Expression Profiling: A detailed compensatory transcriptome analysis is underway for HOM mutants using Illumina Beadarry technology and RNA-seq. For a more detailed description see Poster 126C (W Chen et al.)

Webpage Progress

The mice or cryopreserved germplasm produced for the K312 project are available for order through the KOMP Repository (www.komp.org) and the data are available for viewing and download at www.kompphenotype .org. Viewing the data on the webpage is enabled for either a gene-focused analysis, or based upon phenotype, using heat maps that allow drilling down to obtain the data for each mutant or for sets of phenotypes.



For a more detailed description of the current webpage and future plans, please see Platform Presentation #31 (E Engelhard et al.).



Significance

The mice produced and characterized by the K312 project, and by the anticipated NIH-Funded KOMP2 project to produce and phenotype 2500 KO mutants over five years as part of the International Mouse Phenotyping Consortium (IMPC), will serve to greatly increase the functional annotation for all of the protein coding genes in the mammalian genome.

Acknowledgements

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