



The Rich Resources of Mouse Genetics

*Dawn Watkins-Chow, Ph.D.
Genetic Disease Research Branch
NHGRI, NIH*



Outline

- History of Mouse Genetics
- The Mouse Genome
- Reverse Genetics: Engineered Mice
- Forward Genetics: Phenotype Screens
- Public Database Resources
- Strategic Breeding for Diversity

History of the Laboratory Mouse

- 1100 BC “Fancy” mouse breeding of coat color variants in Asia & Europe
- 1900 Retired schoolteacher Abbie Lathrop breeds fancy mice in Granby, MA
- 1909 first inbred strains established
- 1929 The Jackson Laboratory founded
- 1962 nude mouse
- 1980 first transgenic mouse
- 1989 first knockout mouse
- 1990 explosion of engineered mice & beginning of mouse genome project

Mouse Stocks

- Outbred Stocks

- Genetically undefined, bred to maintain heterozygosity
- Cheap & healthy for when genotype does not matter
- Should not be used for breeding experiments

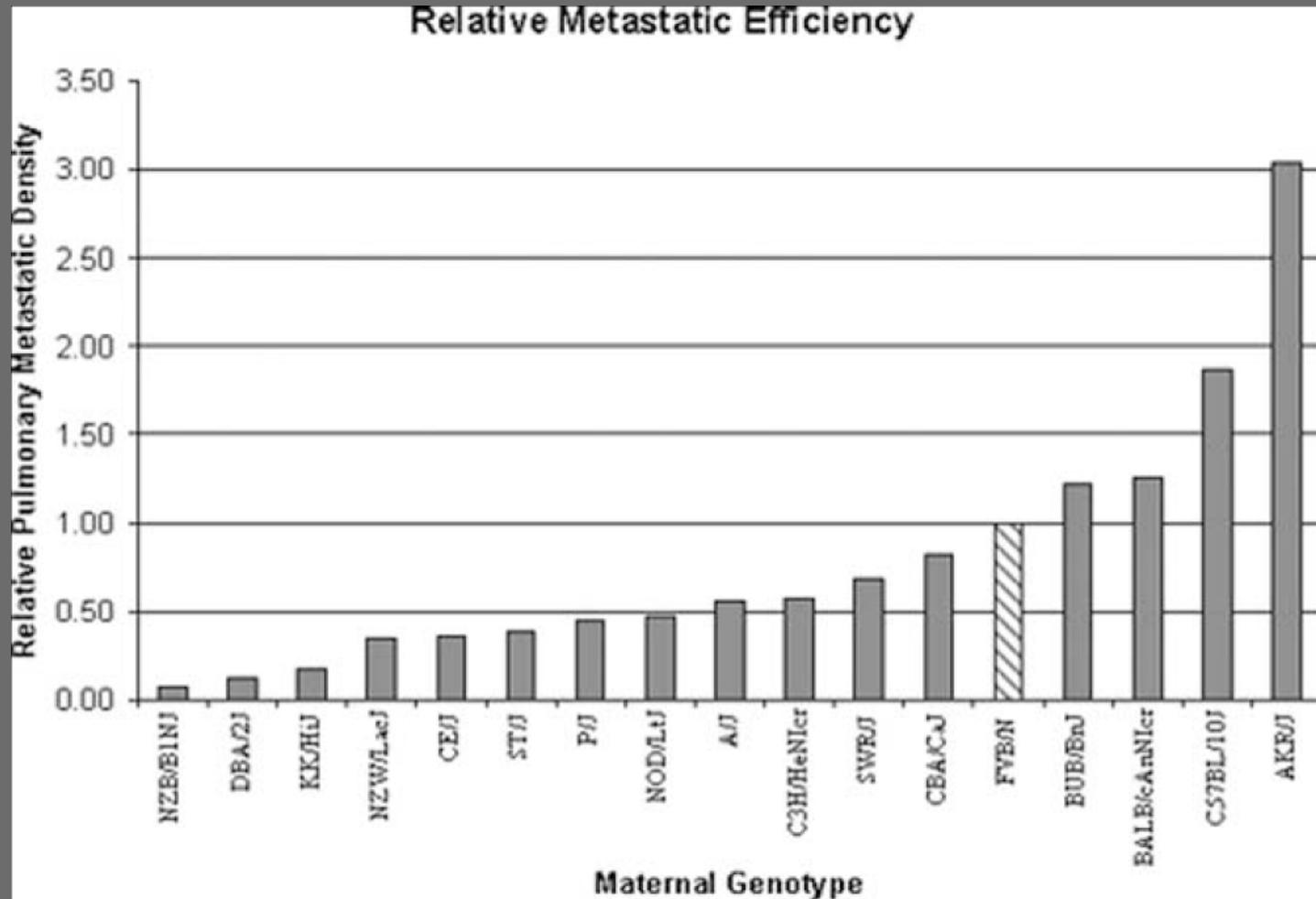
- Inbred Strains

- Bred for homozygosity (>400 strains available)

- Several commercial vendors available

- JAX (jaxmice.jax.org)
- Taconic (www.taconic.com)
- Charles River (www.criver.com)

Effect of Background Strain



T. Lifsted et al. *Int. J. Cancer* (1998) **77**:640-644

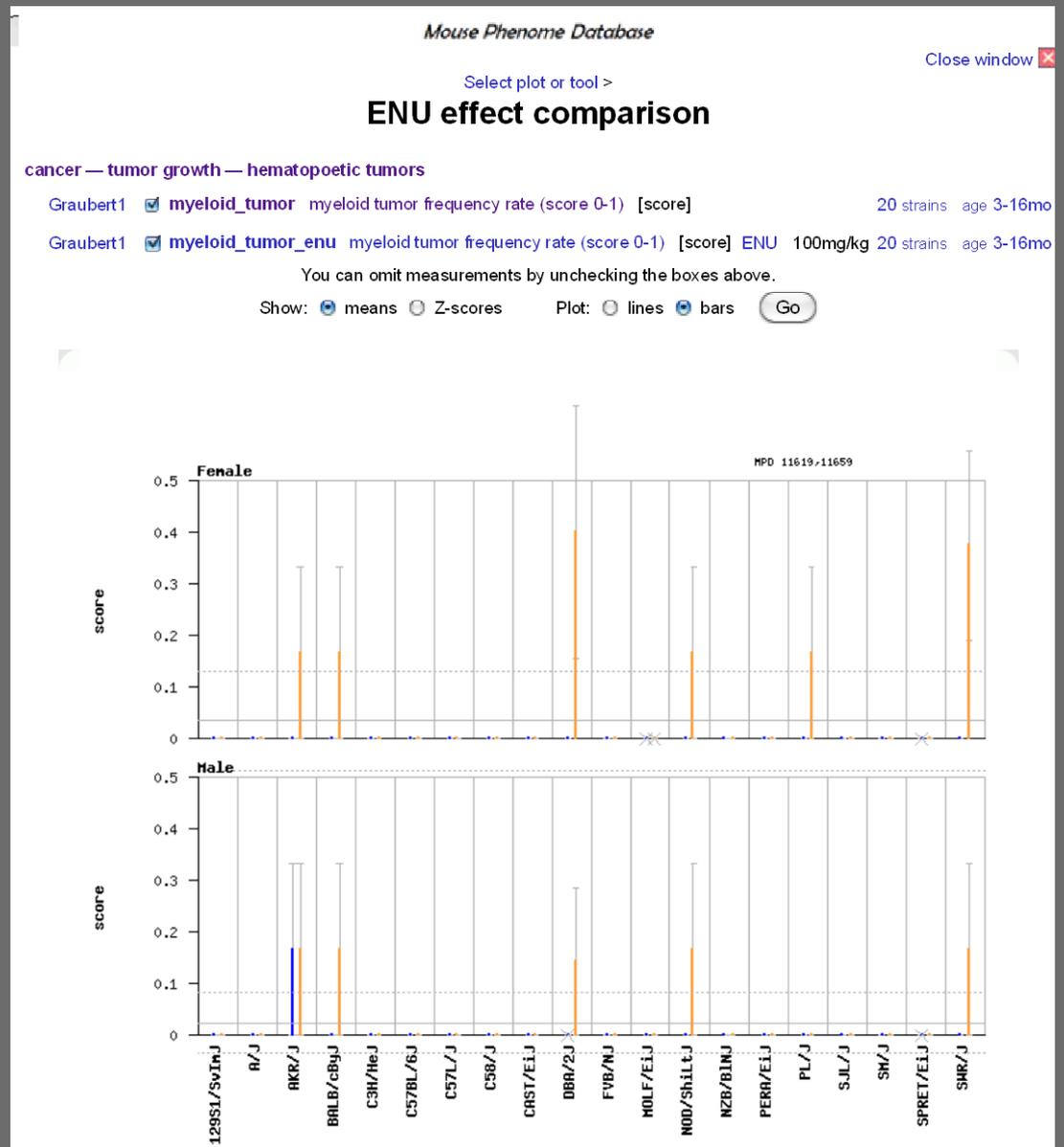
K. Hunter. *Canc Letter* (2003) **200**:97-105

Mouse Phenome Database

- Repository of inbred strain phenotype datasets

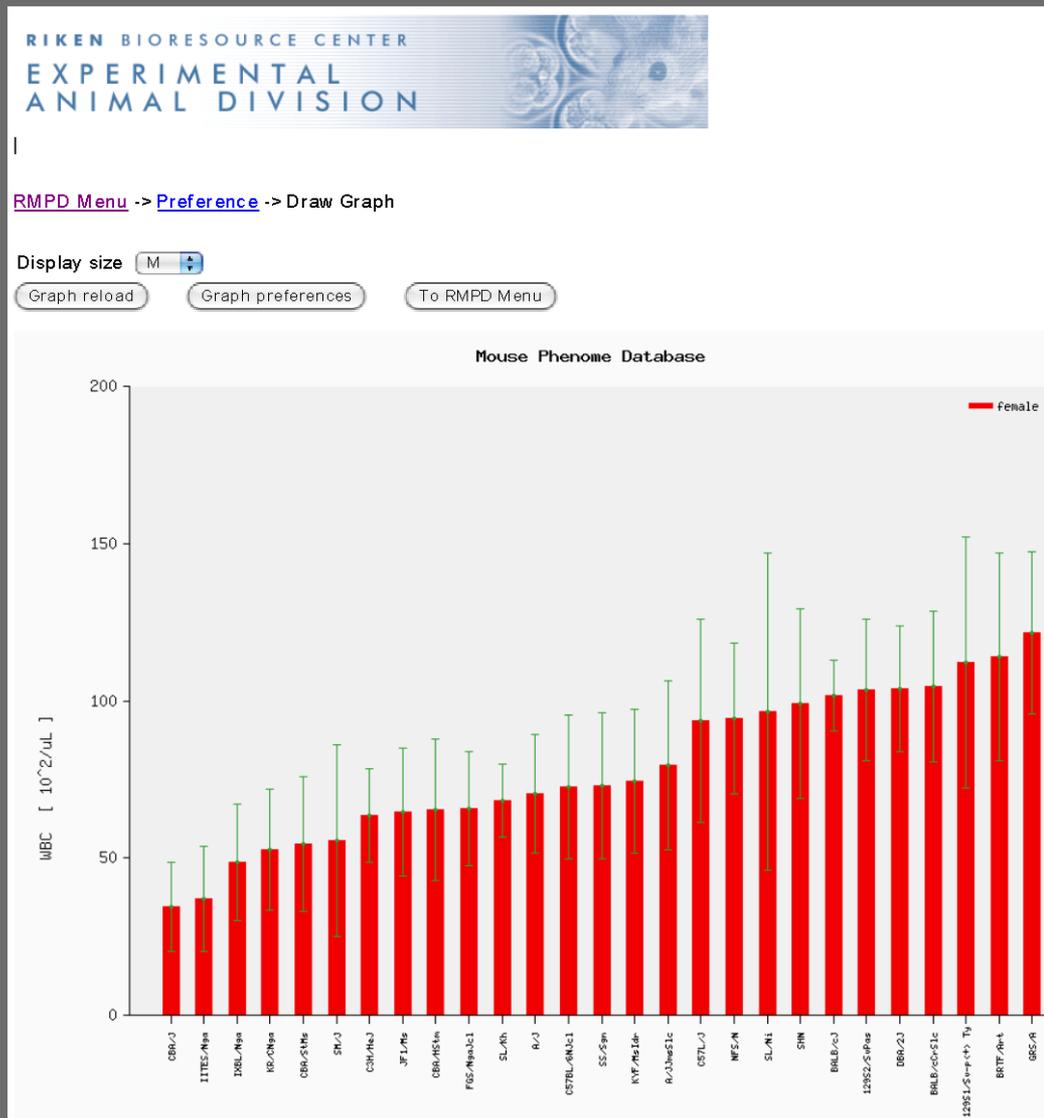
- Example: Strain variation in myeloid tumor frequency

<http://phenome.jax.org>



Riken Phenotype Database

Example: White
Blood cell count



Choice of strains

Why would you choose one strain over another?

- to have appropriate controls for study

Many knockouts were originally generated with 129

- to allow comparison across studies

DBA is well characterized in behavioral studies

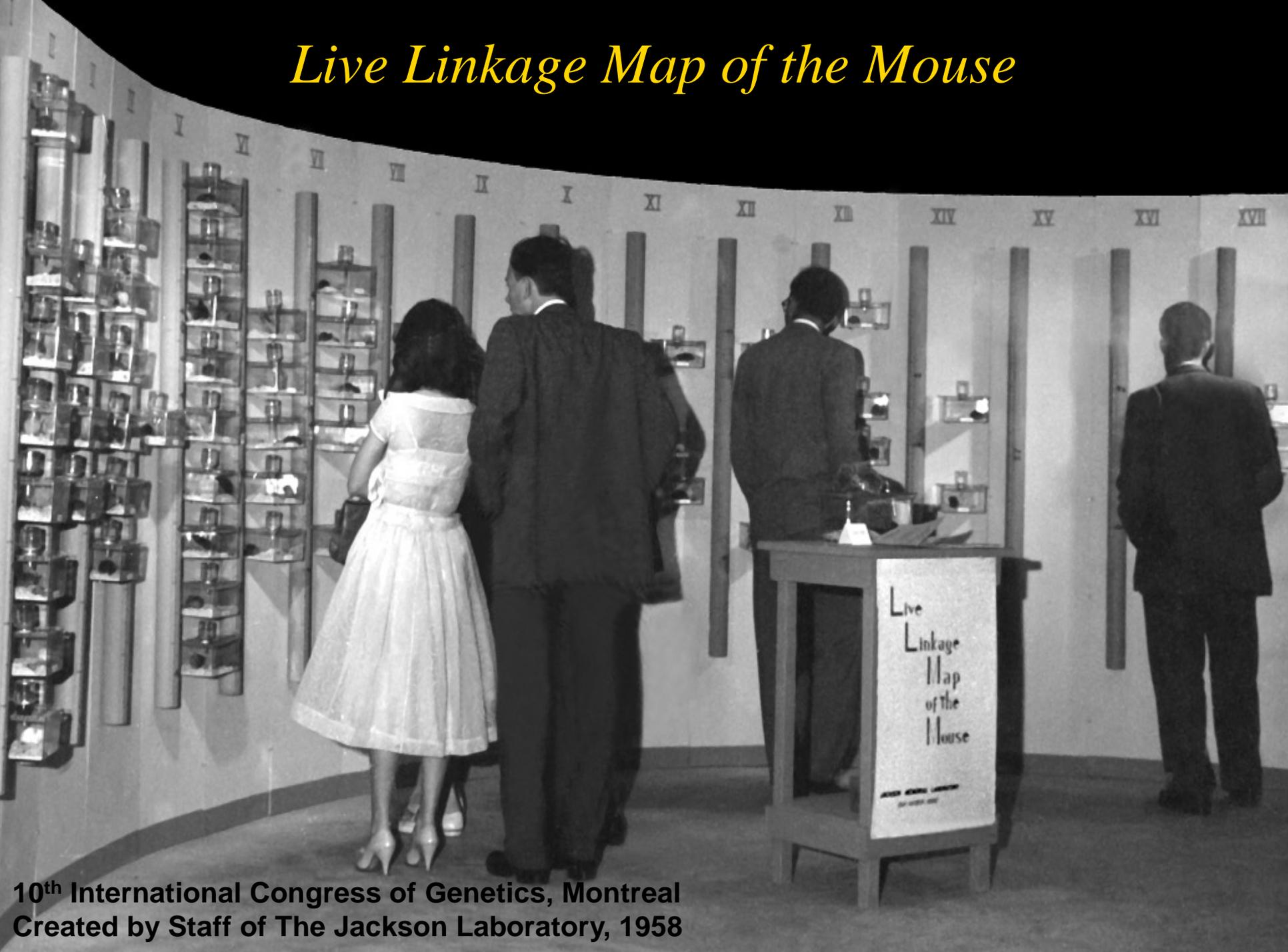
- availability of genome sequence

Current build is based on C57BL/6J

- availability of special mixed strains for follow up

A/J has recombinant inbred strains available

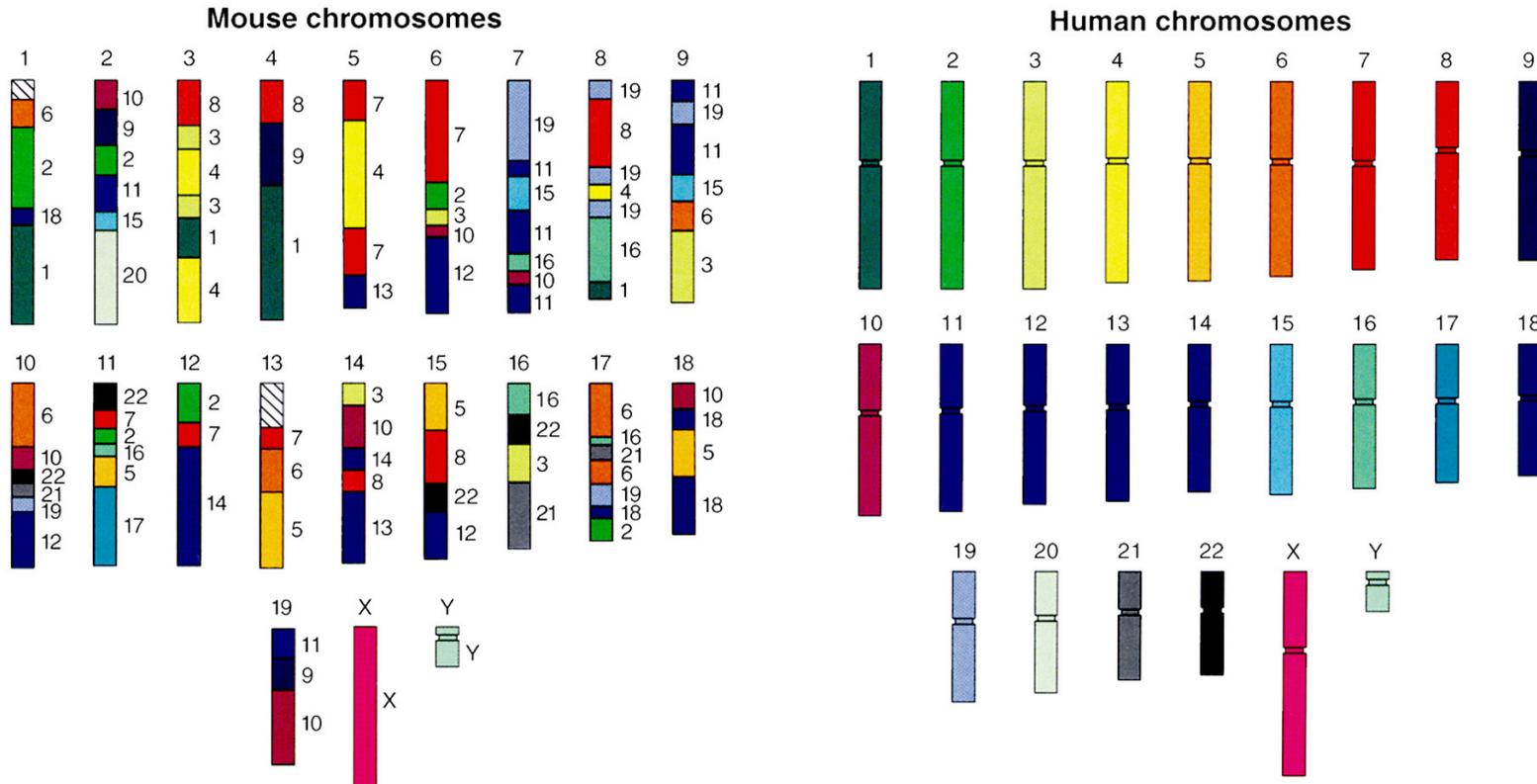
Live Linkage Map of the Mouse



10th International Congress of Genetics, Montreal
Created by Staff of The Jackson Laboratory, 1958

Synteny

Mouse and Human Genetic Similarities



Courtesy Lisa Stubbs
Oak Ridge National Laboratory

The Mouse Genome

- 19 autosomes, XY
- NCBI Build 37
 - 2.66×10^9 bp
 - 124 contigs mostly >5000 kb
 - 28,278 transcripts
- Based on C57BL/6J reference strain
 - Only full clone based assembly

The Mouse Genome

- Additional 17+ strains sequenced
 - Partial clone based assemblies (NCBI)
 - next generation sequencing (Sanger)
- “browsers” are available to help visualize and access the data
 - <http://genome.ucsc.edu>
 - <http://www.ncbi.nlm.nih.gov/mapview>
 - <http://www.ensembl.org>

Polymorphic Variation

- RFLP
- Simple sequence length polymorphism (SSLP) SSRP, STRP, Dinucleotide repeat, microsatellites
- Copy number variation (CNVs)
- Single Nucleotide Polymorphisms
 - Over 10,000,000 SNPs in NCBI dbSNP
 - From 86 strains

Searching for Polymorphic Variation

Example: Strain variation in tumor suppressor *Trp53*

Mouse Genomes Project - SNPs [11:69393861-69403364]

(NOTE: All SNPs, indels and SVs reported have not been experimentally validated)

Gene	Chromosome	Position	Reference	129S1/SvImJ	A/J	BALB/cJ	C3H/HeJ	C57BL/6NJ	CAST/EiJ	DBA/2J	Spretus/EiJ
Trp53	11	69400419	G	-	-	-	-	-	T	-	-
	11	69400874	C	-	-	-	-	-	-	-	T
	11	69400990	C	-	T	-	-	-	-	-	-
	11	69402644	C	-	-	-	-	-	-	-	T
	11	69402663	C	-	-	-	-	-	-	-	T
	11	69403176	T	-	-	-	-	-	-	-	C
	11	69403292	G	-	-	-	-	-	-	-	A
	11	69403304	C	-	-	-	-	-	-	-	T

1

Try:

[New search](#)

[View in Ensembl](#)

Click on SNPs for details

Download:

Formats: [tab](#) or [csv](#).

Legend:

SNP Consequences:

- Splice site
- Non-synonymous coding
- Stop gained
- Stop lost
- Synonymous coding
- + Multiple consequences

Base Calling examples:

- T High confidence SNP
- t Low confidence SNP
- High confidence reference
- Low confidence reference
- Genotype not called

Searching for Polymorphic Variation

- <http://phenome.jax.org> & <http://www.informatics.jax.org>
- 167 SNPs polymorphic between B6 and 129 within 2 kb of Cftr

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Mouse SNPs

Query Results -- Summary

You searched for...

Selected Strain(s): equals **129S1/SvImJ**

Reference Strain: equals **C57BL/6J**

SNPs Returned: equals **different from Reference Strain**

Gene Symbol/Name: equals **Cftr** searching current symbols.

Include SNPs located: **within 2 kb** of genes specified by Gene Symbol/Name

Display Limit: equals **1000**

Allele Display Mode: equals **nucleotide values**

167 matching SNPs displayed

Legend

Results are sorted by chromosome/coordinate value. Chromosome transitions are marked by blank rows.

SNP ID (dbSNP Build 128)	Map Position (NCBI Build 37)	rs orient	Gene : dbSNP Function Class	Assays (ss)	Variation Type	C57BL/6J	129S1/SvImJ	Allele Summary (all strains)
rs3157349 MPD dbSNP MGI SNP Detail	Chr6:18122723	f	Cftr : Intron	2	SNP	A	C	A/C
rs3161305 MPD dbSNP MGI SNP Detail	Chr6:18122761	f	Cftr : Intron	2	SNP	T	A	A/T
rs3157350 MPD dbSNP MGI SNP Detail	Chr6:18122824	f	Cftr : Intron	2	SNP	A	C	A/C

Done

Genotyping Polymorphisms

Genotyping services available

- MDA & MUGA array
 - <http://csbio.unc.edu/CCstatus/index.py?run=Genotype>
- Diversity array
 - <http://jaxservices.jax.org/mdarray/>
- 7,000 – 625,000 polymorphic markers selected for various applications

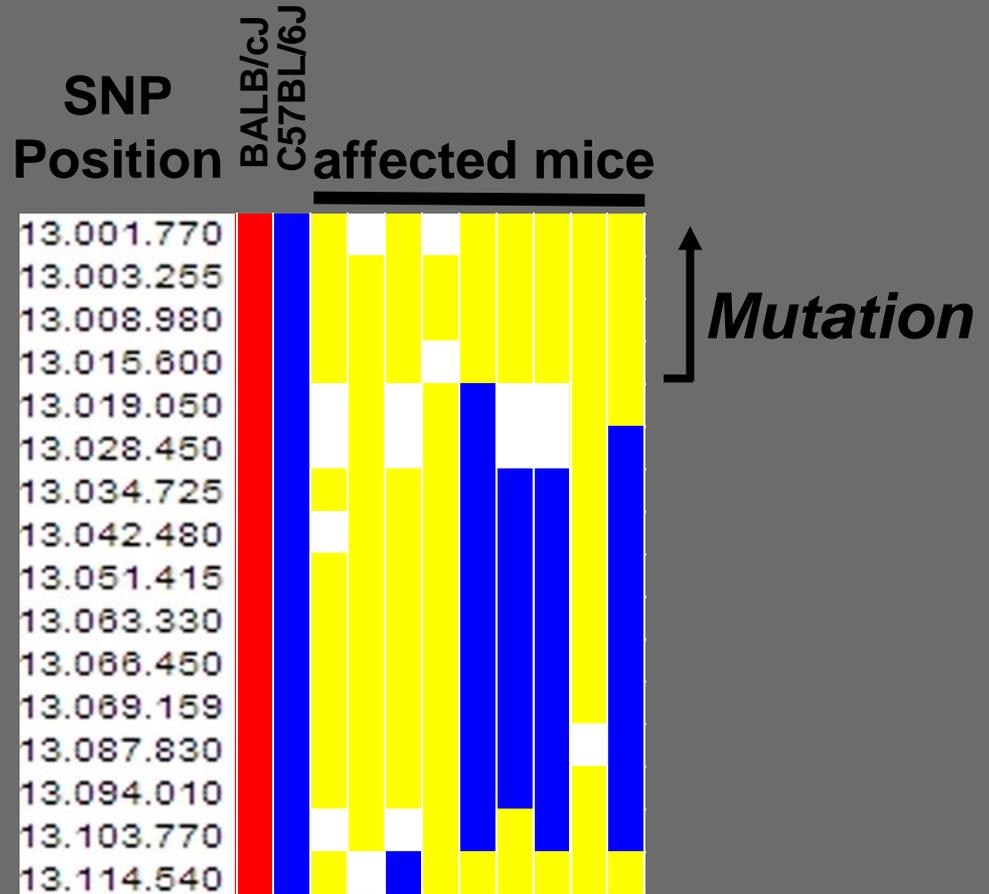
• Genotyping products available

- Illumina GoldenGate products include 2 linkage panels
 - Low density: 200 markers
 - Medium density 1449 SNPs
- For as few as 12 samples

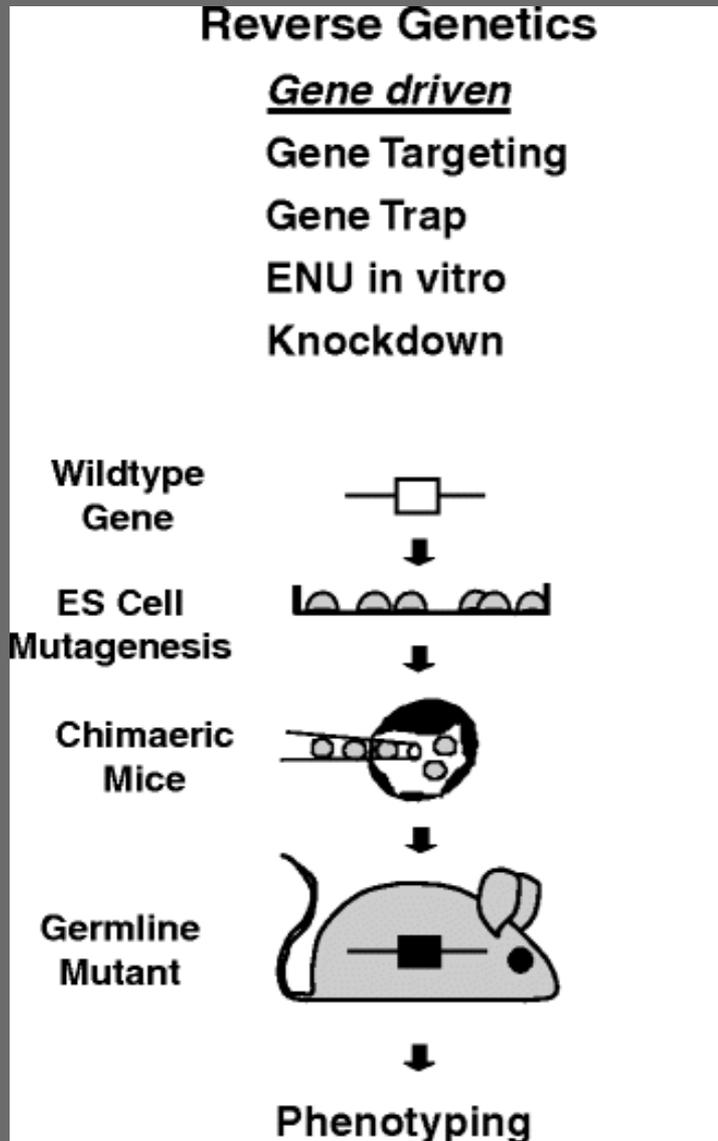
Genotyping Polymorphisms

SNP or SSLP markers polymorphic between the parental mouse strains (C57BL/6J and BALB/cJ) are used to genotype affected mice throughout the genome

- Homozygous BALB/cJ
- Homozygous C57BL/6J
- Heterozygote
- No call



Strategies to identify gene function



Alter a gene and then characterize the resulting phenotype

Examples

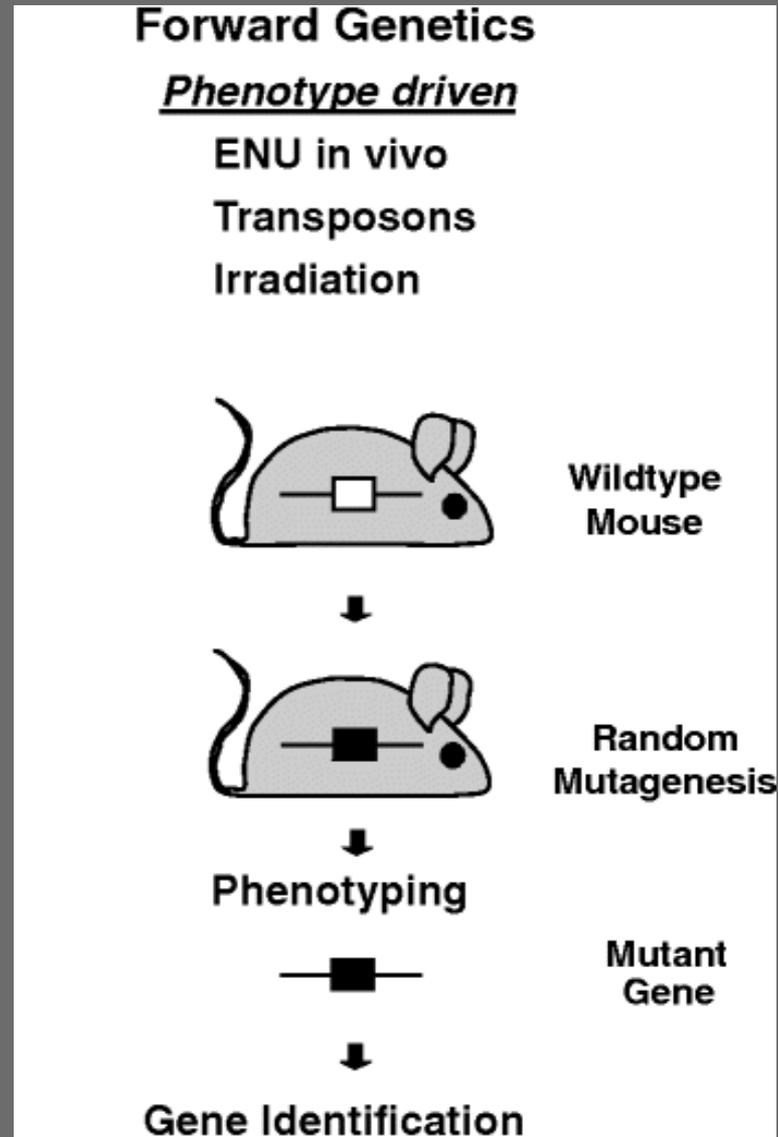
- Create a disease model
- Inactivate candidate tumor suppressor gene
- Overexpress a candidate oncogenes
- Study effects of temporal or spatial gene regulation

Strategies to identify gene function

Begin with a phenotype and
identify the causative gene

Example Uses

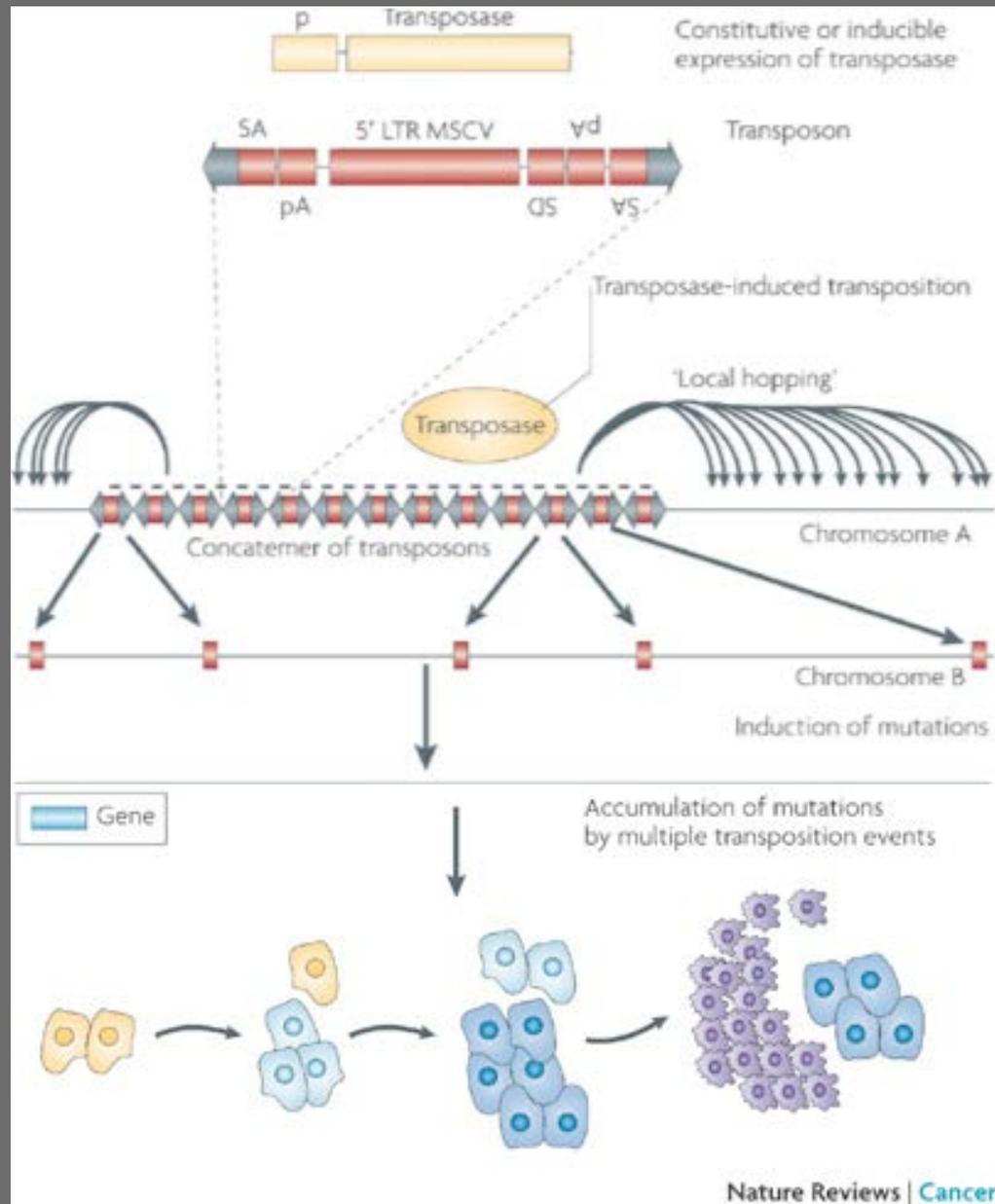
- Creating disease model
- Identifying disease modifiers



Forward Genetics

- *Retroviral Insertional Mutagenesis*
 - *MMTV, MuLV*
- *Transposon Mediated Mutagenesis*
- *ENU Mutagenesis*

Transposon Mediated Mutagenesis



Kool & Berns
Nature Reviews Cancer
(2009) 9:389-399.

Transposon Mediated Mutagenesis

- Sleeping beauty

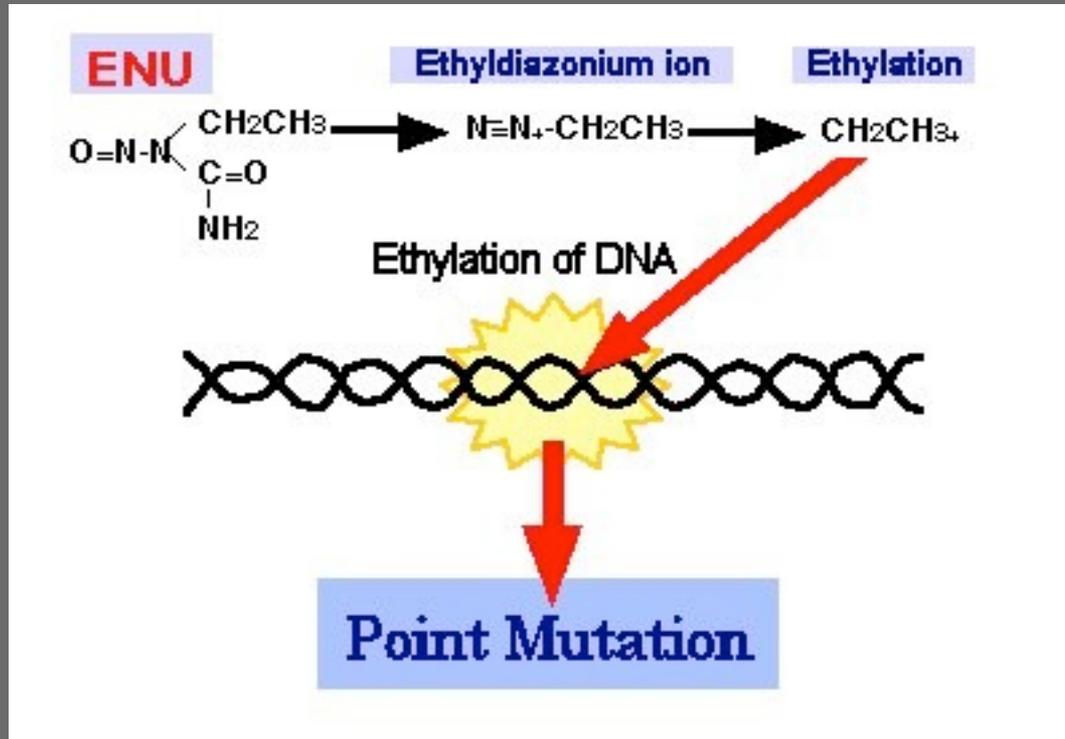
Keng et al. (2005) *Nature Methods* **2**: 763-769

- piggyBac

Ding et al. (2005) *Cell* **122**: 473-483

ENU Mutagenesis

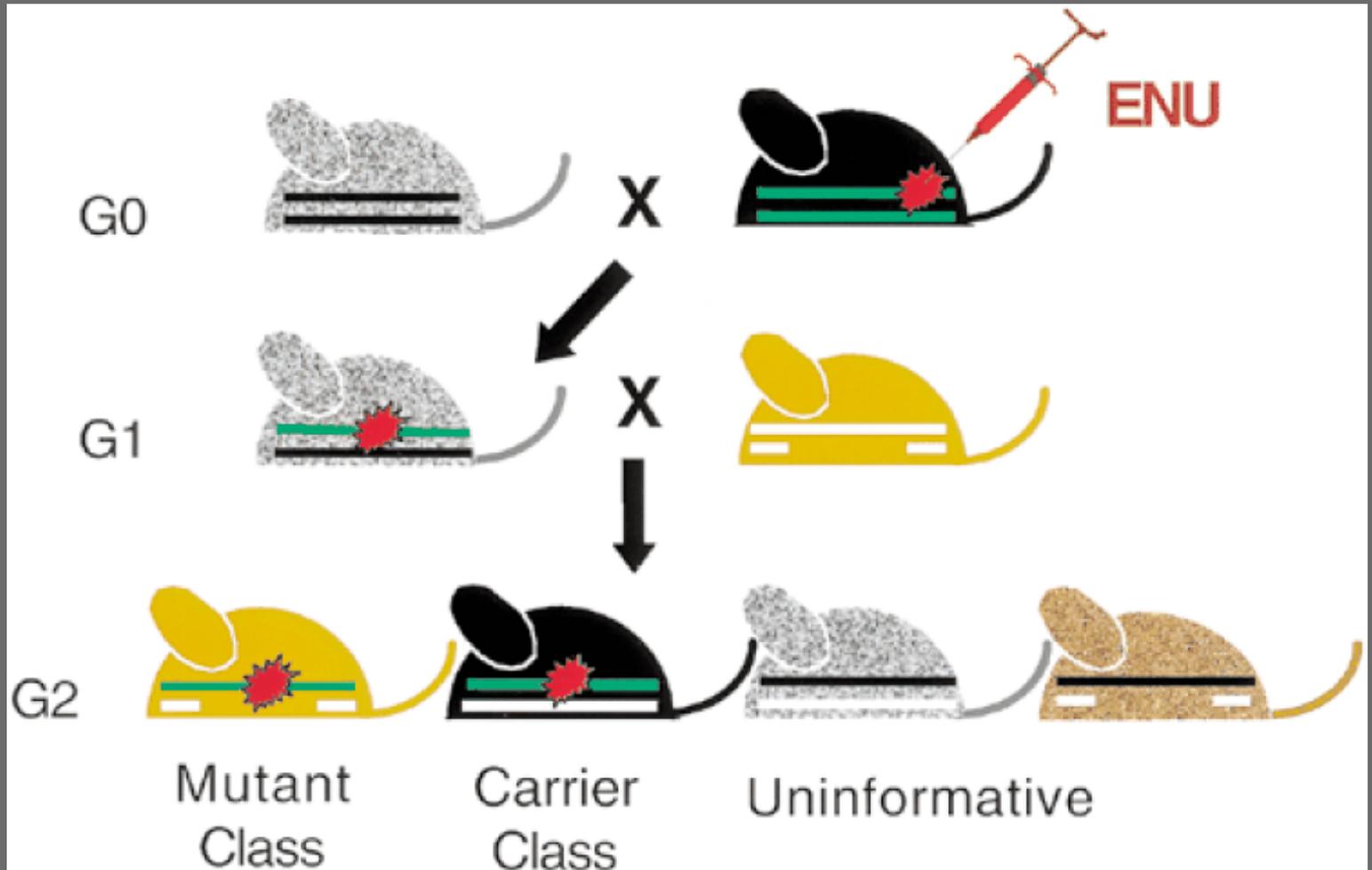
- ENU mutagenesis (ethylnitrosourea)



- Alkylating agent
- Mutations introduced into spermatogonial stem cells

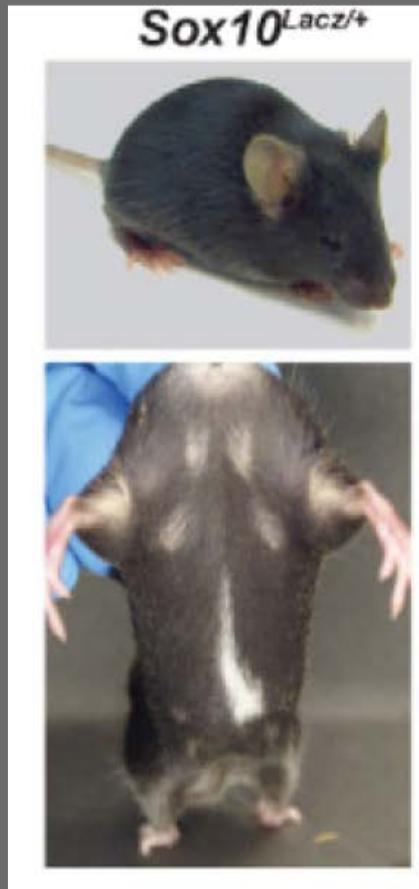
ENU Mutagenesis

Region specific screens



ENU Mutagenesis

Sensitized/Modifier screens



Large Scale Efforts



International Knockout Mouse Consortium

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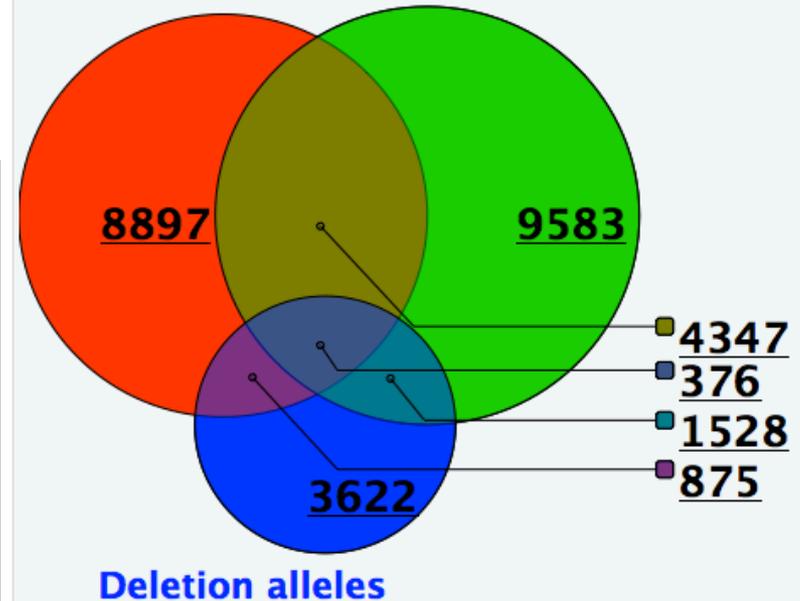
Welcome to the IKMC



The International Knockout Mouse Consortium (IKMC) aims to mutate all protein-coding genes in the mouse using gene trapping and gene targeting in C57BL/6 ES cells. [Read more...](#)

Bar Chart Venn

Conditional alleles Gene Trap alleles



Number of genes for which IKMC ES cells with a given allele type are available. Overlaps indicate the number of genes for which 2 or 3 types of alleles have been generated.

Large Scale Efforts



International Knockout Mouse Consortium

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Enter gene symbols, gene IDs or genome location

Search

e.g., Adam19, Pax, ENSMUSG00000020681,
Chr13:22210730-22311689

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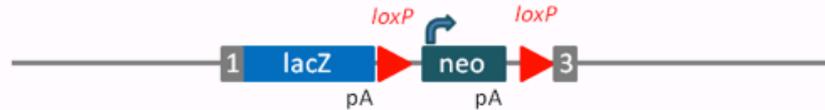
About...

- IKMC
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 - IKMC alleles
- I-DCC
- KOMP
 - EUCOMM
 - NorCOMM
 - TIGM

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View all IKMC allele types

Allele type:

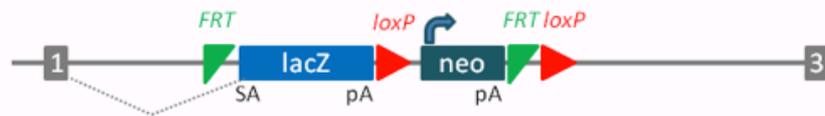
Deletion (lacZ replacement)



Pipeline:

KOMP-REGN

Deletion (lacZ-tagged)



KOMP-CSD

Deletion (lacZ-tagged, conditional-ready)



NorCOMM

ENU Mutagenesis Large Scale Screens

Riken

broad phenotyping

MRC Harwell

broad phenotyping

The Jackson Laboratory

Neurological, reproductive, heart, lung, blood

Baylor College of Medicine

Chromosome 11

Finding the Mice you Need

IMSR Summary										
Query Summary										
State equals live mouse										
Gene/Allele Symbol/Name contains RAS searching Current/Synonyms										
Display Limit equals 10000										
281 matching items displayed										
+ Name carries approved nomenclature. - Name does not carry approved nomenclature. ? Name has not been reviewed for nomenclature.										
N	Strain/Stock Designation	Holder Site	State	Strain Type(s)	Strain/Stock Synonyms	Chr	Allele Symbol	Allele Name	Gene Name	Mutation Type(s)
+ 129-A p tm1(cre)Nagy _J	JAX	live	coisogenic strain			4	A p tm1(cre)Nagy	targeted mutation 1, Andras Nagy	alkaline phosphatase, liver/bone/kidney	recombinase(cre/flip)
						10	Cdh23 ^{ahl}	age related hearing loss 1	cadherin 23 (otocadherin)	
+ 129P1 Re J	JAX	live	inbred strain			18	Pojl ^d	polymerase iota deficient	polymerase (DNA directed), iota	spontaneous mutation
						8	Disc1 ^{del}	deletion	disrupted in schizophrenia 1	spontaneous mutation
+ 129P3 J	JAX	live	inbred strain, segregating inbred	129/J		10	Cdh23 ^{ahl}	age related hearing loss 1	cadherin 23 (otocadherin)	
						5	Rmcf ^f	MCF resistant	resistance to MCF virus	spontaneous mutation
						8	Disc1 ^{del}	deletion	disrupted in schizophrenia 1	spontaneous mutation
						18	Pojl ^d	polymerase iota deficient	polymerase (DNA directed), iota	spontaneous mutation
+ 129S-Parp1tm1Zqw _J	JAX	live	mutant strain	129S-Adprt1tm1Zqw _J , 129S-Aprt1tm1Zqw, 129S-Aprt1tmZqw		1	Parp1tm1Zqw	targeted mutation 1, Zhao-Qi Wang	poly (ADP-ribose) polymerase family, member 1	targeted mutation
+ 129S-Top2b ^{tm2Jcw} _J	JAX	live	coisogenic strain			14	Top2b ^{tm2Jcw}	targeted mutation 2, James C Wang	topoisomerase (DNA) II beta	targeted mutation
+ 129S6.CB(B6)-Del(1)1Brk Gpi1 ^b /Gpi1 ^f /BrkMdf _J	JAX	live	congenic strain, mutant strain	129S6.CBA(B6)-Del(1)1Brk Gpi1 ^b /Gpi1 ^c /BrkMdf _J		1	Del(1)1Brk	deletion, Chr 1, Jane Barker 1	deletion, Chr 1, Jane Barker 1	chromosomal aberration
						7	Gpi1 ^b	b variant	glucose phosphate isomerase 1	

Sharing the Mice you Have



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Submissions

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Start Submission

Instructions

Overview

Special Arrangements

Donor MTA

MMRRC Submission Application

Strain Submission Terms and Conditions

As the Donating Investigator, you are asked to agree to and meet the following terms and conditions for any strain submitted to the MMRRC:

1. I confirm that my mouse strain was created and maintained under an approved Institutional Animal Care and Utilization Committee (IACUC) protocol.
2. If this strain is accepted, I agree, in a timely fashion, to:
 - a. provide a detailed genotyping protocol;
 - b. complete and submit the standard MMRRC Donor Material Transfer Agreement (see [sample Donor MTA](#)) for my strain, which details the formal agreement between me, my institution and the MMRRC for preservation and redistribution of my strain;
 - c. provide a recent and institutionally certified health status report in advance of shipping this mouse strain to the assigned facility;
 - d. provide this strain, **within six months**, in a single shipment of live mice or cryopreserved materials in the form and quantities that I have agreed to provide to the MMRRC facility (see [Transfer Methods](#) for additional information);
 - e. take responsibility for the cost of preparation and shipment of my mouse strain to the assigned facility.
3. If I transfer my strain via cryopreserved embryos or germplasm, I accept the risk that it may not be possible to resuscitate my strain. I understand that the MMRRC will make a reasonable effort at resuscitation. I will not hold the MMRRC responsible for the loss of my strain.
4. I accept that the MMRRC will maintain my strain as appropriate for the overall benefit of the entire strain repository resource, and that my strain may be maintained as a cryopreserved-only archive if demand is inadequate to justify maintenance of a breeding colony.
5. I acknowledge that the MMRRC is not liable and will not reimburse for expenses incurred in the event that availability of this strain is further restricted subsequent to its acceptance due to ownership or distribution rights of third parties.

Please indicate that you have read, accept and can meet the above terms and conditions.

I Accept

Database Resources



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Mouse Genome Informatics

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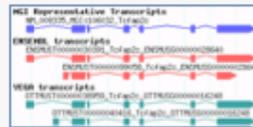
Keywords, Symbols, or IDs

[Quick Search](#)

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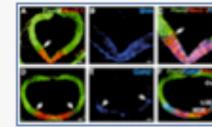
Genes



Phenotypes



Expression



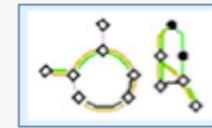
Recombinases (cre)



Function



Pathways



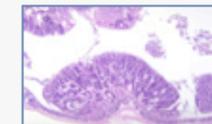
Strains / SNPs

Variation Type	DMP/J		PVE/NJ		Allele Summary (all strains)
	ROF/EJ	ROF/EJ	ROF/EJ	ROF/EJ	
SNP	G	G	A	A	A/G
SNP	C	C	T	T	C/T

Orthology



Tumors



Done

Standard Crosses

- Incross: mating between individuals of an inbred strain.

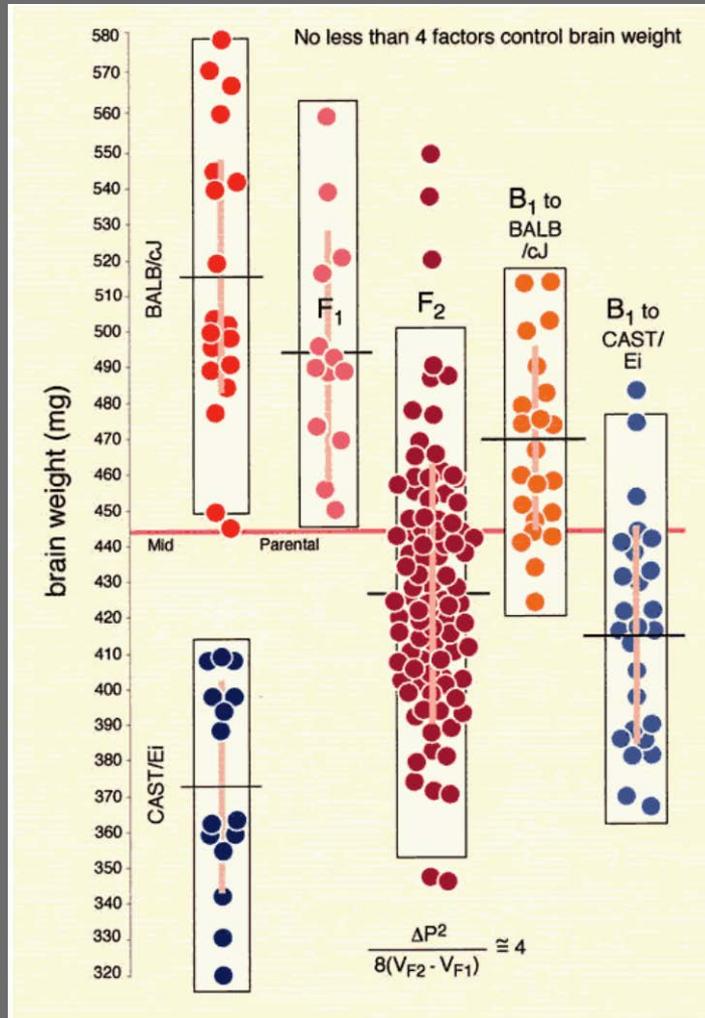
Used for inbred strain maintenance.

- Outcross: mating between 2 unrelated strains. To generate F1s.

- Backcross: mating between a heterozygous F1 and one of the parent strains. Used in linkage analysis.

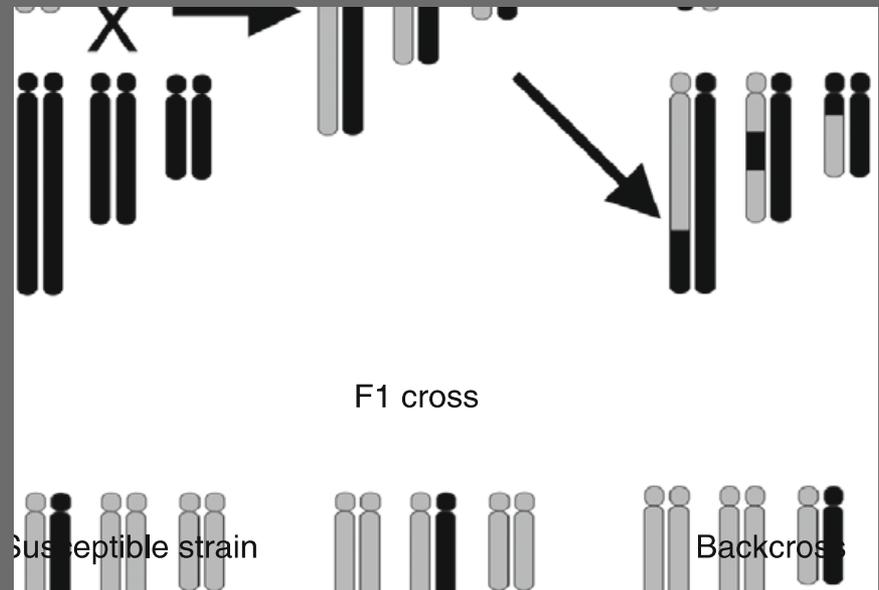
- Intercross: mating between two identically heterozygous individuals. Used in linkage analysis.

Standard Crosses & QTLs

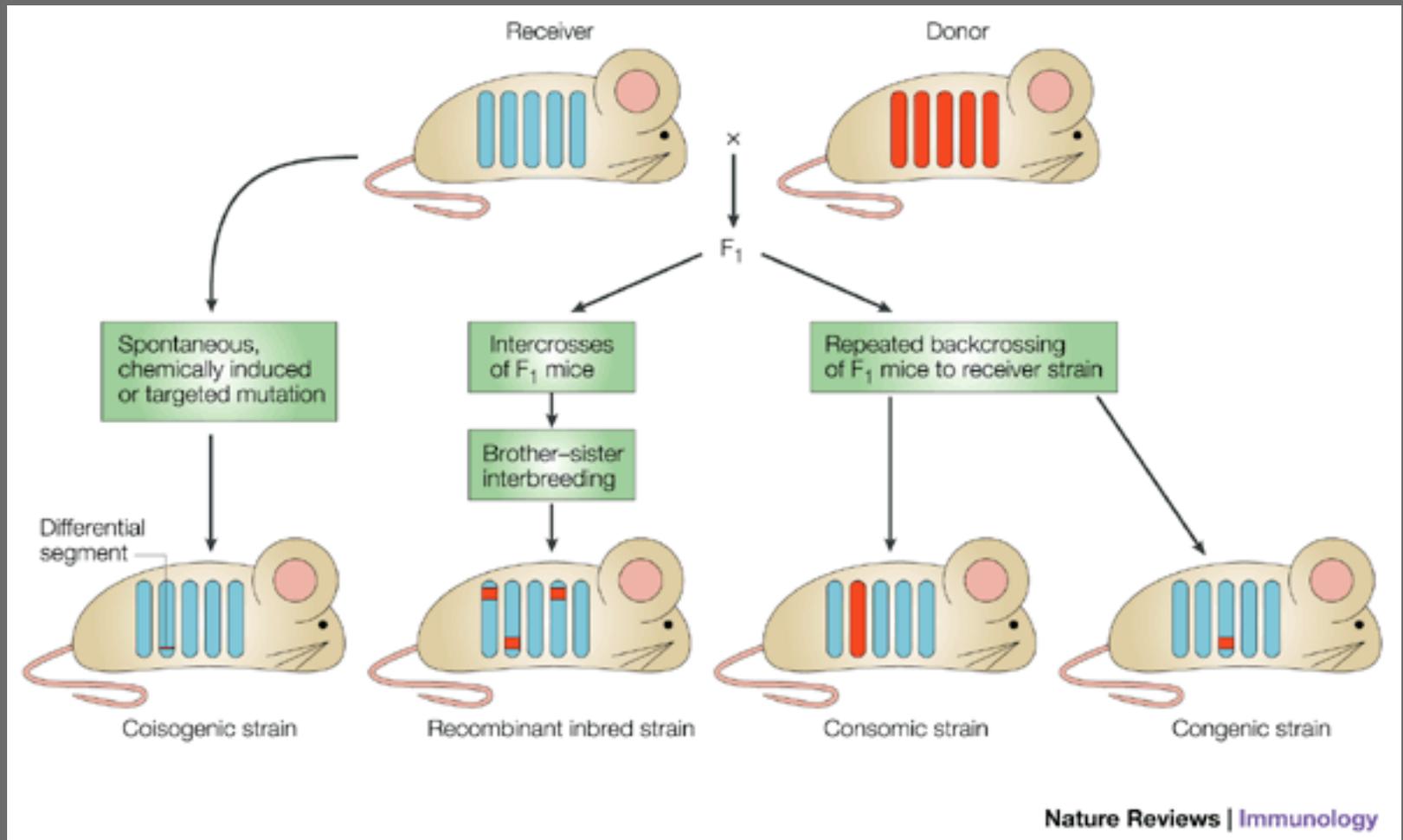


Example

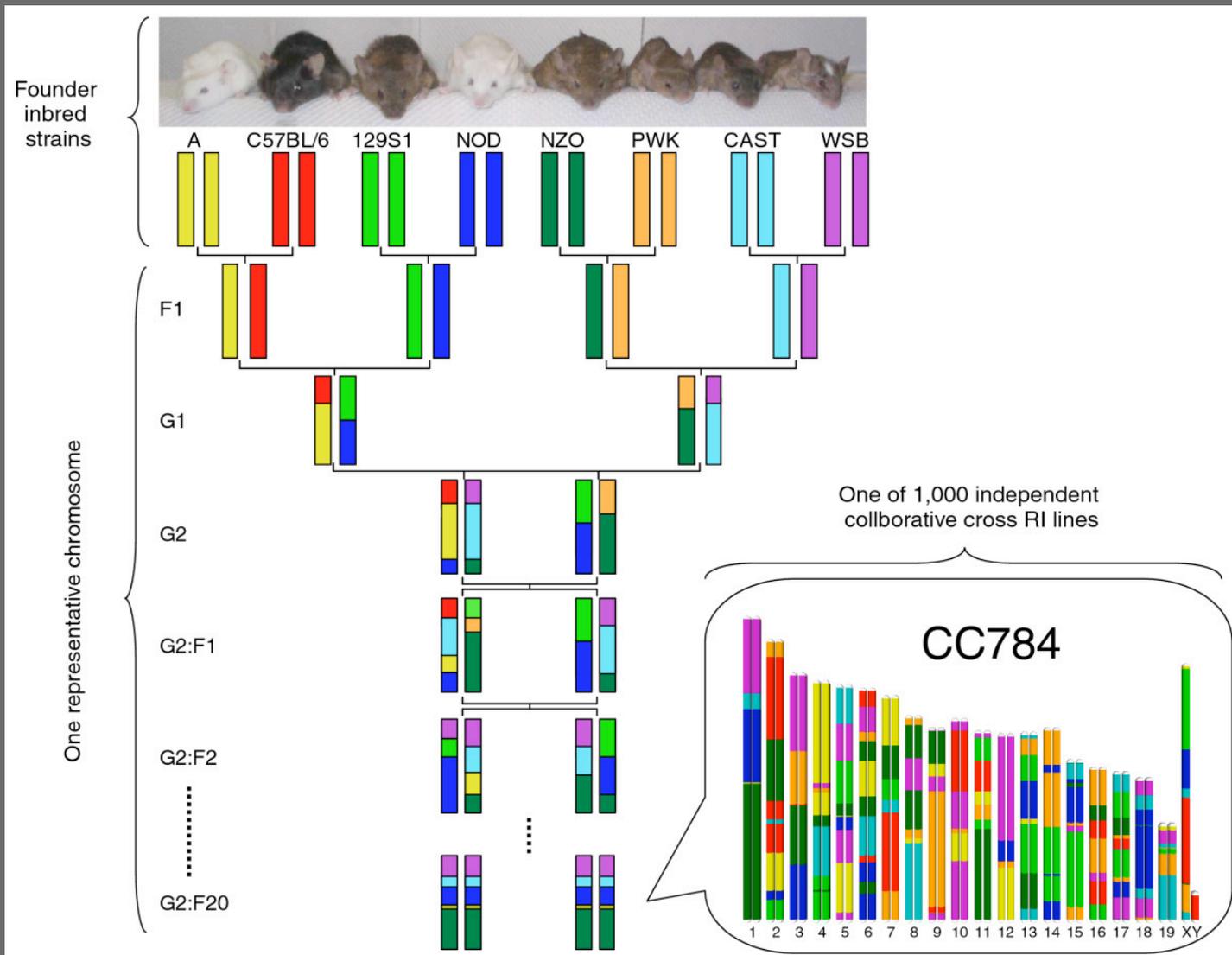
A combination of intercross and backcrosses were used to estimate the number of loci affecting brain weight.



Designer Crosses

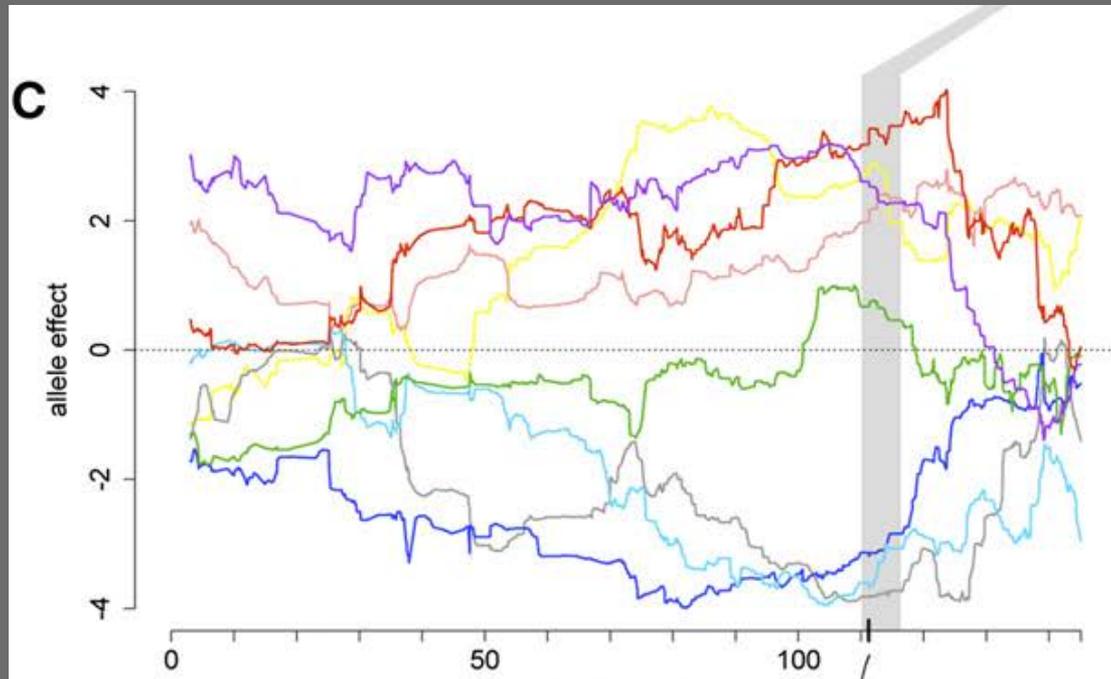


Collaborative Cross



Collaborative Cross

Example: Use of the collaborative cross to identify modifiers of hematological parameters.



Reference Material

- *Mouse Genetics, Concepts and Applications* (1995) Silver, L.M. Oxford University Press, New York, NY
- *Genetic Variants and Strains of the Laboratory Mouse*, 3rd Edition (1996) Edited by M. F. Lyon, S. Rastan and S.D.M. Brown. Oxford University Press, New York, NY
- **Online Books:**
http://www.informatics.jax.org/resources.shtml#res_books
- **Online Resources:**
<http://www.informatics.jax.org/resources.shtml>

