Genetic Drift: What It Is and Its Impact on Your Research

Technical Information Services

May 11, 2017

The Jackson Laboratory

Leading the search for tomorrow's cures

The Jackson Laboratory's Mission

"To discover precise genomic solutions for disease
and empower the global biomedical community
in the shared quest to improve human health."

Performing Research

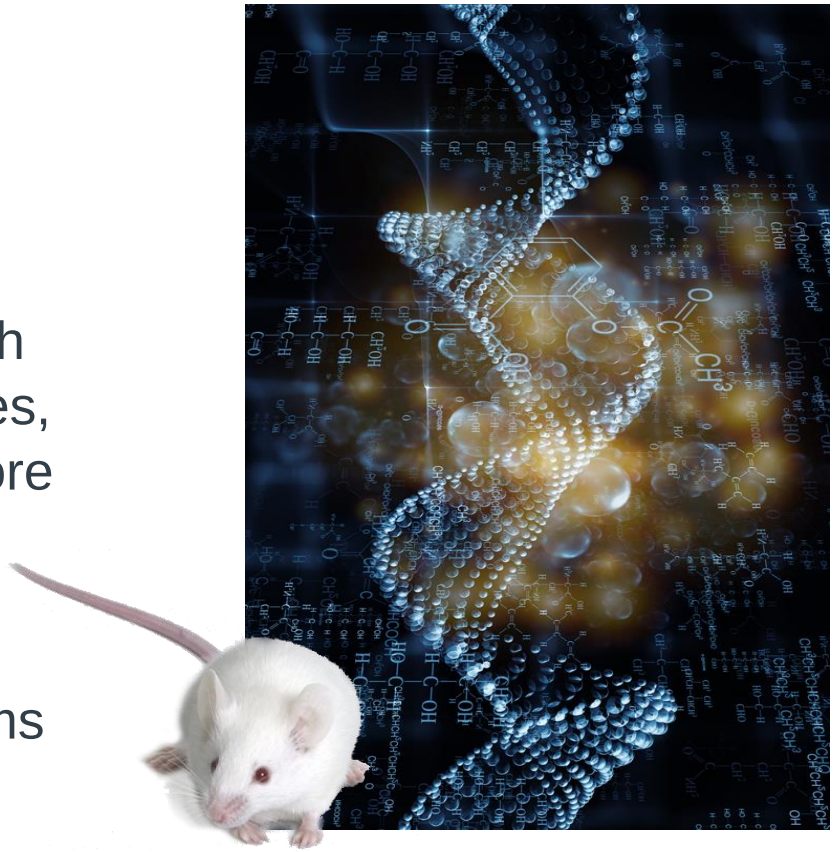
Investigating genetics and biology of human disease

Providing Resources

JAX® Mice Clinical & Research Services, online data resources, technical publications, and more

Educating Scientists

World-class courses, internships, and other programs



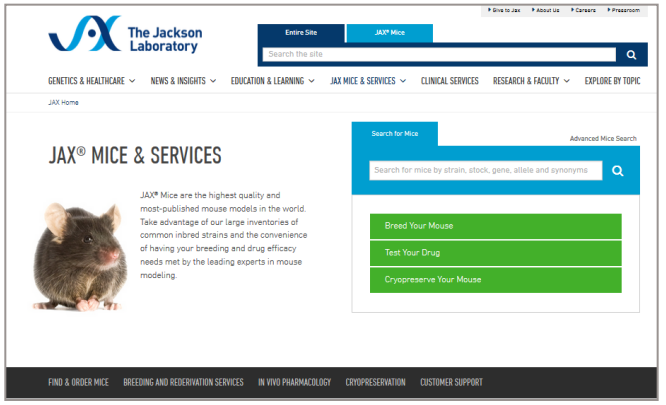
JAX® Mice

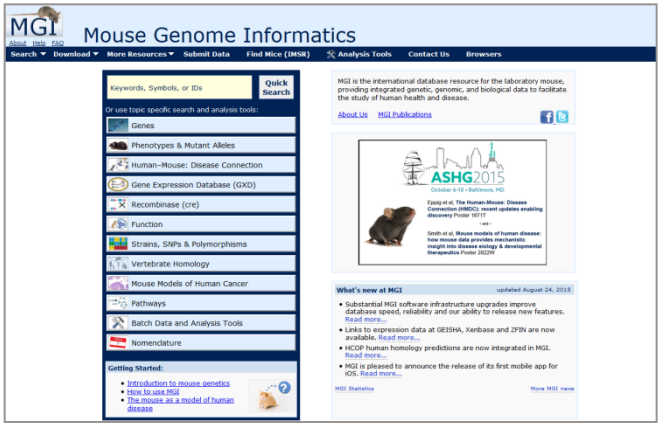
The *Gold Standard* for Biomedical Research

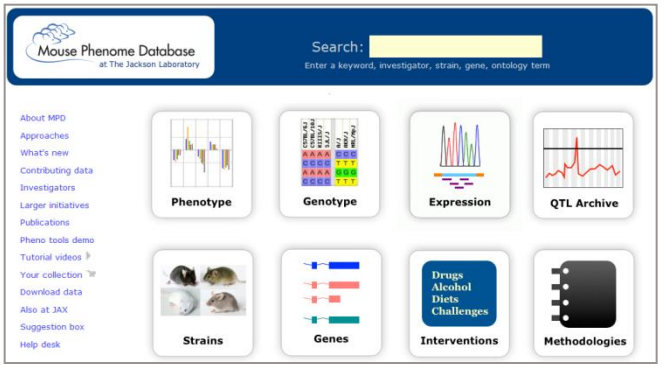
- NIH-funded resource
- >8,000 strains and growing
  - 2.7 million mice shipped annually
- Unsurpassed genetic quality & animal health
- Best characterized & referenced ~100 new pubs/week
- Common inbred strains (C57BL/6J, BALB/cJ, DBA/2J) support development/collection of specialty strains and other valuable community research resources

Online Resources to Expedite Research

- JAX® Mice Database
  www.jax.org/mouse-search
- Mouse Genome Informatics
  www.informatics.jax.org
- Mouse Phenome Database
  www.jax.org/phenome
- Others, including:
  JAX-Clinical Knowledgebase
  Mouse Tumor Biology Database







Today's Learning Goals

- Recognize genetic background of your mouse strain
  - Use proper nomenclature
  - Select appropriate controls
- Implement strategies to reduce genetic drift and increase experimental reproducibility

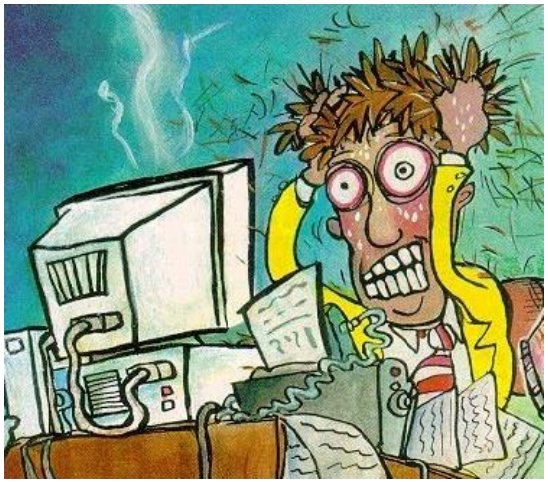
What is your role?

What do you hope to learn today?

Genetic Drift…Friend or Foe?

Species Diversity

Phenotypic Diversity



Data Diversity

What is Genetic Drift?

- “the constant tendency of genes to evolve even in the absence of selective forces. Genetic drift is fueled by spontaneous neutral mutations that disappear or become fixed in a population at random”
  - Lee Silver, “Mouse Genetics” Oxford University Press, 1995
    www.informatics.jax.org/silverbook/
- Single base changes, deletions, duplications, inversions in the DNA
  - Mistakes in meiosis, DNA repair

Genetic Drift and Colony Size

Small colonies are more vulnerable to fix a mutation

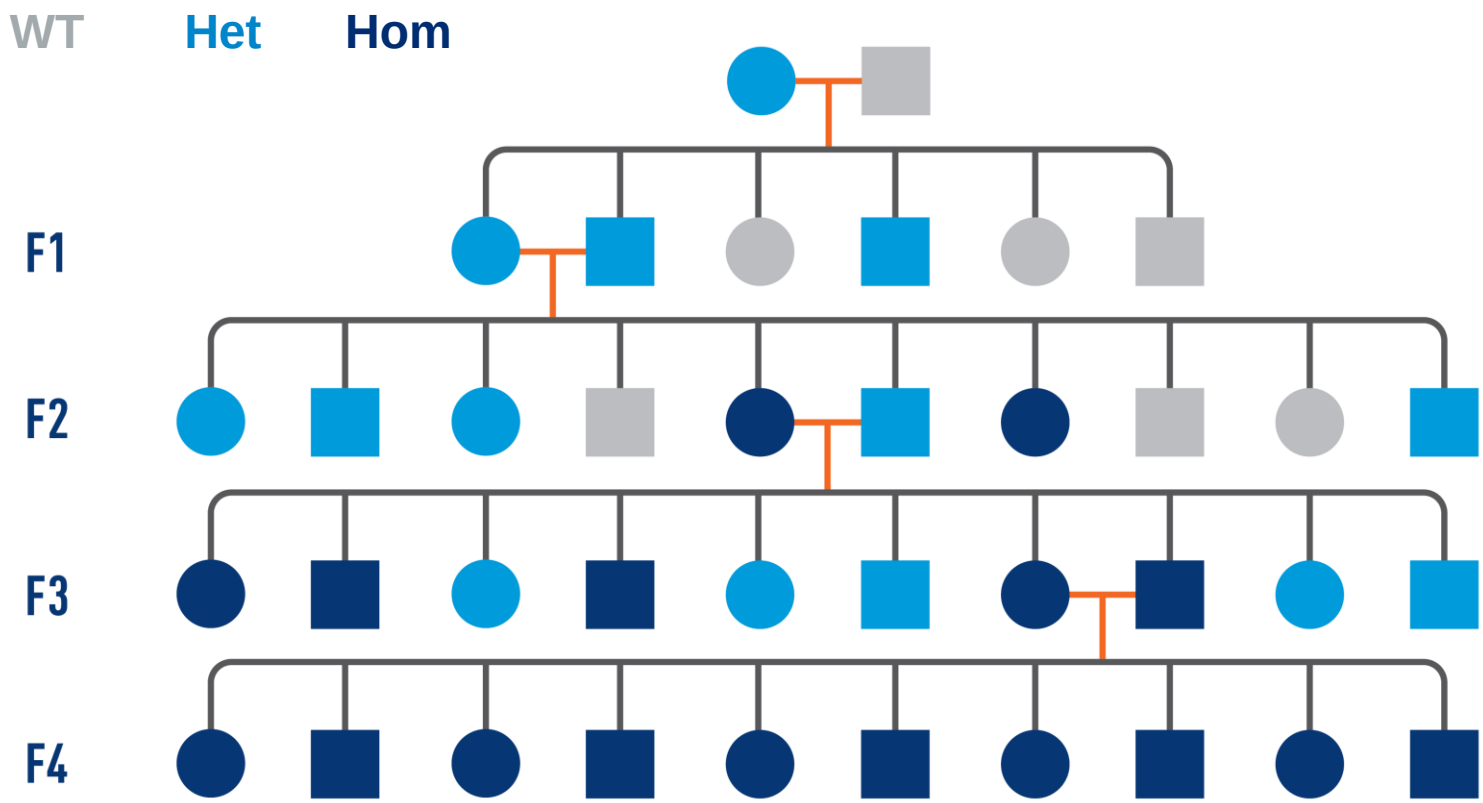
For any given mutation,  = heterozygous mutation

Large Colony

Small Colony

Genetic Drift and Colony Size

Small colonies are more vulnerable to fix a mutation



Visible Genetic Drift

Coat Color Mutations

C57BL/6J-*A*$^{w\text{-}J}$/J
(000051)

B6(Cg)-*Tyr*$^{c\text{-}2J}$/J
(000058)

C57BL/6J
(000664)

C57BL/6J-*Lyst*$^{bg\text{-}J}$/J
(000629)

C57BL/6J-*Kit*$^{W\text{-}v}$/J
(000049)

How Rapidly Do Colonies Drift?

"Visible" mutation example

- Using spontaneous mutation rates in coat color genes,
  - Measured ~1.1 x 10^{-5} mutations/locus/gamete/gen.
- Assuming ~25,000 genes in mice,
  - (1.1 x 10^{-5} mutations/locus/gamete/gen.)*(25,000 loci)
  - 0.275 mutations/gamete/gen.
  - 1 mutations/3.64 gametes/gen.
- ***1 phenotypic mutation arises every 1.8 generations***
  - Likely underrepresents overall mutation rate due to visibility of mutation

Russell LB and Russell WL., 1996. *PNAS* PMID 8917546
Drake JW et al., 1998, *Genetics* PMID 9560386

How Rapidly Do Colonies Drift?

- **Mice have a high rate of spontaneous mutation**
- **Approx. 25% chance that new mutations will become fixed**
- **New mutations in coding sequence become fixed every 6-9 generations**
  - (Assumptions: inbreeding; small breeding population)

How Rapidly Do Colonies Drift?

"Invisible" mutation example

- Using whole genome sequencing of C57BL/6J,
  - Measured 2 samples separated by 69 filial gens.
- Differences found
  - 669 SNPs (~ 10/gen.)
  - 272/669 SNPs were in genetic coding & non-coding regions
  - 7/272 SNPs altered DNA coding sequence or RNA splicing
- ***~1 "impactful" mutation every 10 generations***
  - Likely underrepresents overall mutation rate because the analysis did not include non-SNP mutations (deletions, inversions, CNV changes).

Genetic Drift: Substrain Divergence

Substrains: Branch of an inbred strain known or suspected to be genetically different from the parent colony.

Colonies are considered substrains when. . .

1) Separated from the parent colony for 20+ generations

2) Phenotypic differences with the parent colony are discovered

Nomenclature: Strain name "/" Lab code(s)

e.g. CBA/CaGnLeJ

LAB CODE	ORGANIZATION
Crl	Charles River Laboratories
Hsd	Envigo (formerly Harlan Laboratories)
J	The Jackson Laboratory
N	National Institutes of Health
Rj	Centre D'Elevage R. Janvier
Tac	Taconic Farms, Inc.

Parent strain

Substrain designations (cumulative)

Lab maintaining strain

Institute for Laboratory Animal Research (ILAR) Lab Codes

C57BL/6 Substrain Divergence

“Invisible” Genetic Drift

Case Study # 1: Sensitivity to infection

1952

C3H/HeJ (000659)

C3H/HeOuJ (000635)

+LPS

+LPS

Endotoxin resistant

***Tlr4*$^{Lps\text{-}d}$ (1958-1965)**

Endotoxin sensitive

***Tlr4* wild-type**

Sultzer BM. 1968. *Nature* PMID 4877918

Watson J et al. 1978. *J Immunol* PMID 202651

Poltarak A et al. 1998. *Blood Cells Mol Dis* PMID 10087992

Poltarak A et al. 1998. *Science* PMID 9851930

“Invisible” Genetic Drift in C57BL/6

Case Study # 2: Alteration of presynaptic protein α-synuclein (*Snca*)

- **C57BL/6J** Genomic DNA from The Jackson Laboratory
  Wild-type *Snca*
- **C57BL/6NCrl** Mice from Charles River, Margate, UK
  Wild-type *Snca*
- **C57BL/6JOlaHsd** Mice from Harlan, Bicester, UK
  Deletion of *Snca* – *No visible phenotype but…*

SNCA protein: implicated in a range of neurodegenerative diseases; primary structural component of Lewy bodies found in Parkinson’s disease brains

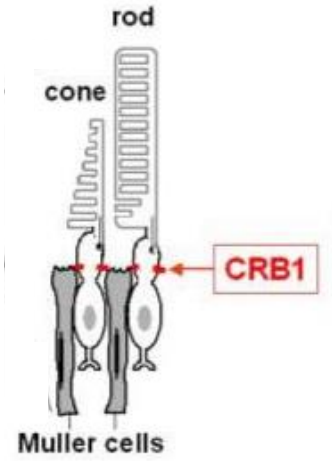
Specht CG and Schoepfer R. 2001. *BMC Neurosci* 2:11. PMID:11591219

“Invisible” Genetic Drift in C57BL/6

Case Study # 3: Retinal degeneration in C57BL/6N substrains

Crb1 (crumbs-like 1)

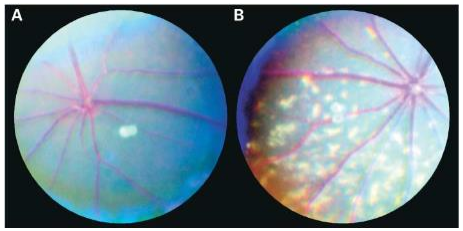
- Localized to Muller cells and photoreceptor (PC) inner segments
- Mutations in CRB1 associated with retinal diseases in man
  - Retinitis pigmentosa
  - Leber congenital amaurosis



http://crfb.univ-mrs.fr/Crumbs/section/en/CRB1_function/105

Crb1^{rd8}

- Single base deletion
- Shorter PC inner & outer segments as early as two weeks
- Progressive, spotty retinal degeneration

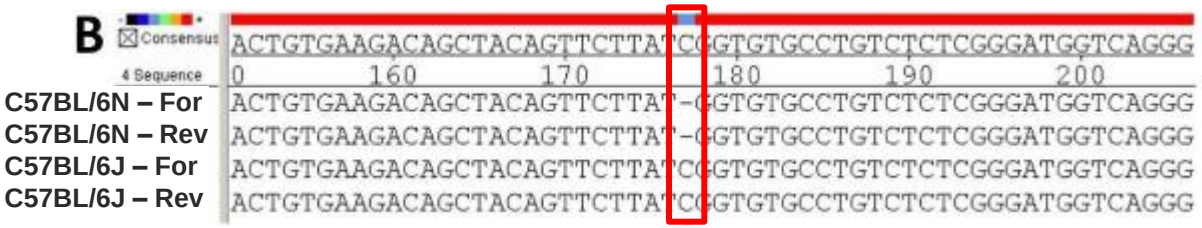


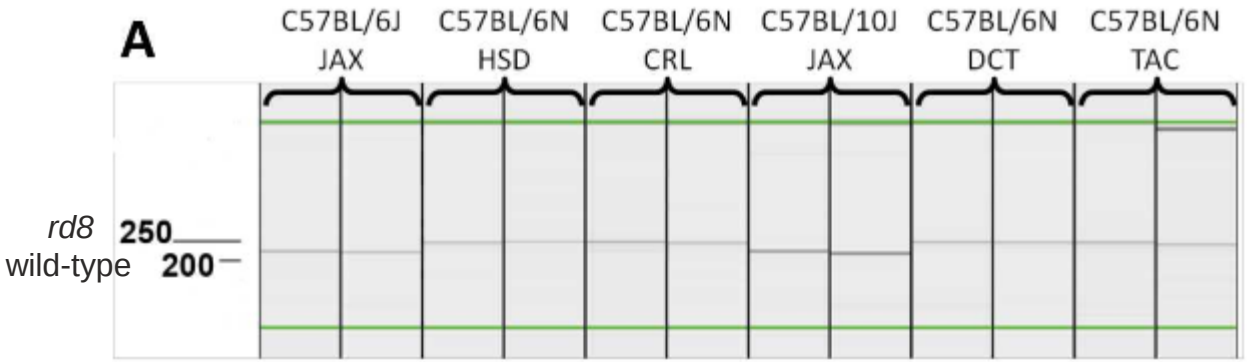
Mehallow AK et al. 2003. *Hum Mol Gen* 12(17):2179-2189. PMID:12915475

“Invisible” Genetic Drift in C57BL/6

Case Study # 3: Retinal degeneration in C57BL/6N substrains

C57BL/6J: *Crb1* wild-type **C57BL/6N:** *Crb1*^{rd8}/*Crb1*^{rd8}





Crb1^{rd8}

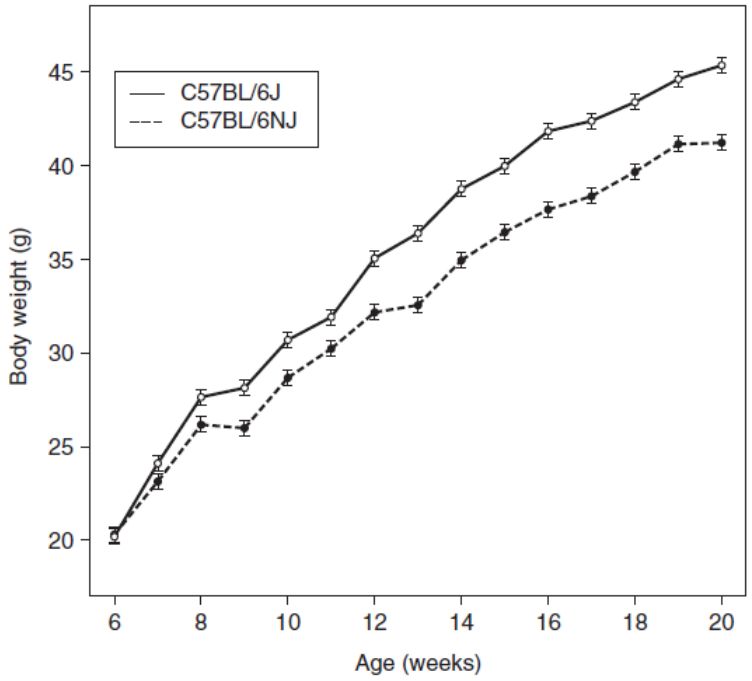
HSD (Harlan)
CRL (Charles River)
DCT (Frederick Nat’l Lab)
TAC (Taconic)

Mattapallil, MJ et al. 2012. *Invest Ophthalmol Vis Sci* PMID 22447858

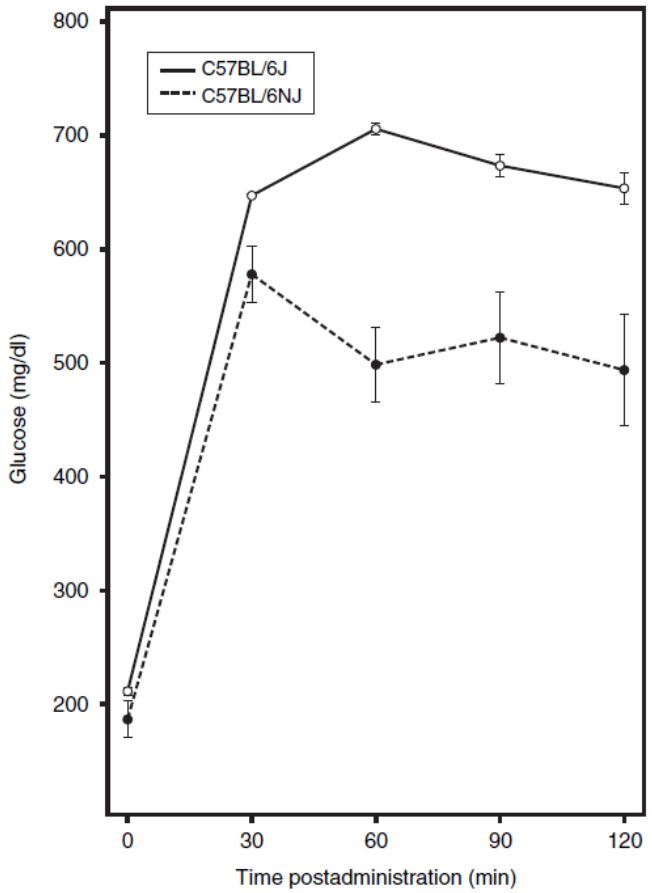
“Invisible” Genetic Drift

Case Study # 4: Response to high-fat diet in C57BL/6

C57BL/6J Outgains C57BL/6NJ



Impaired Glucose Tolerance:
C57BL/6J > C57BL/6NJ



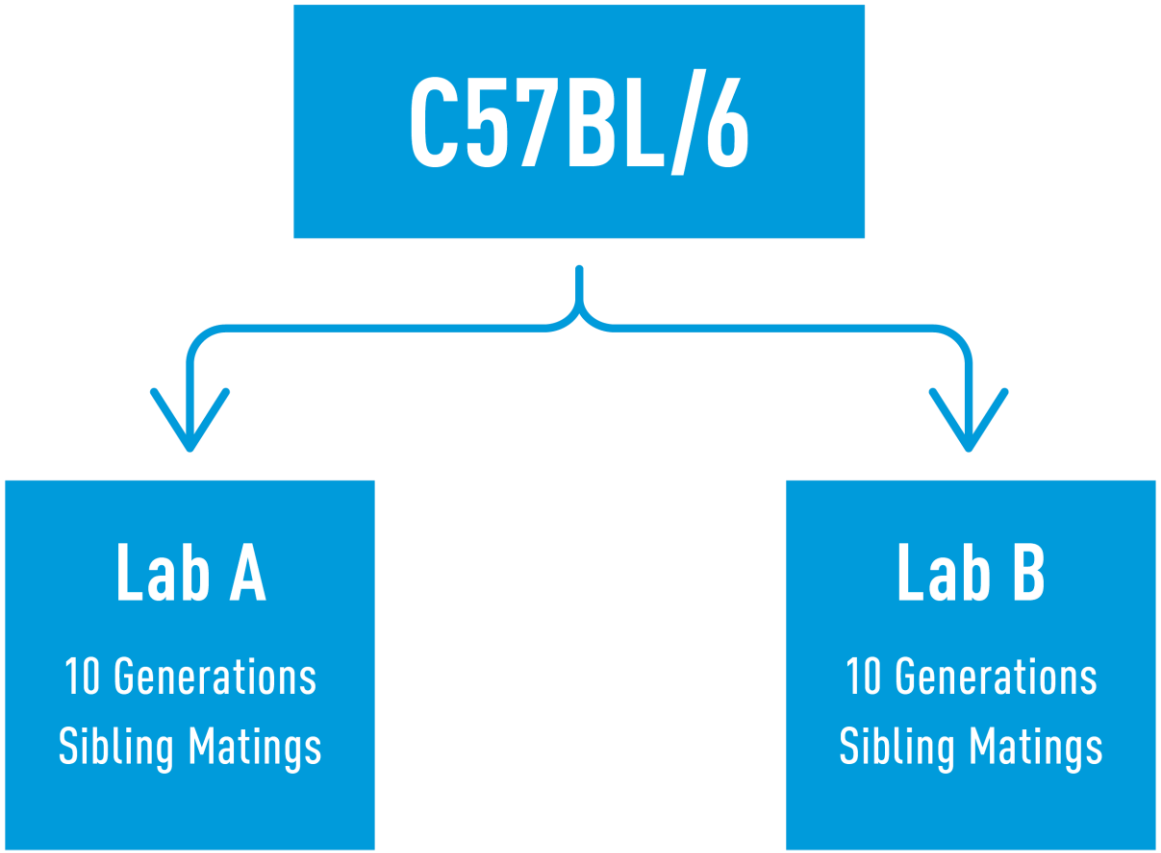
C57BL/6J: *Nnt* loss-of-function

Nicholson A et al. 2010. *Obesity.* 18(10): 1902-5 PMID [20057372](20057372)

The Mice Next Door

- You don’t have enough C57BL/6J mice for your experiment so you got a few from another lab
- The other mice gave really robust responses
- Why do the mice differ in response, even though they are the same strain?

Substrain Development

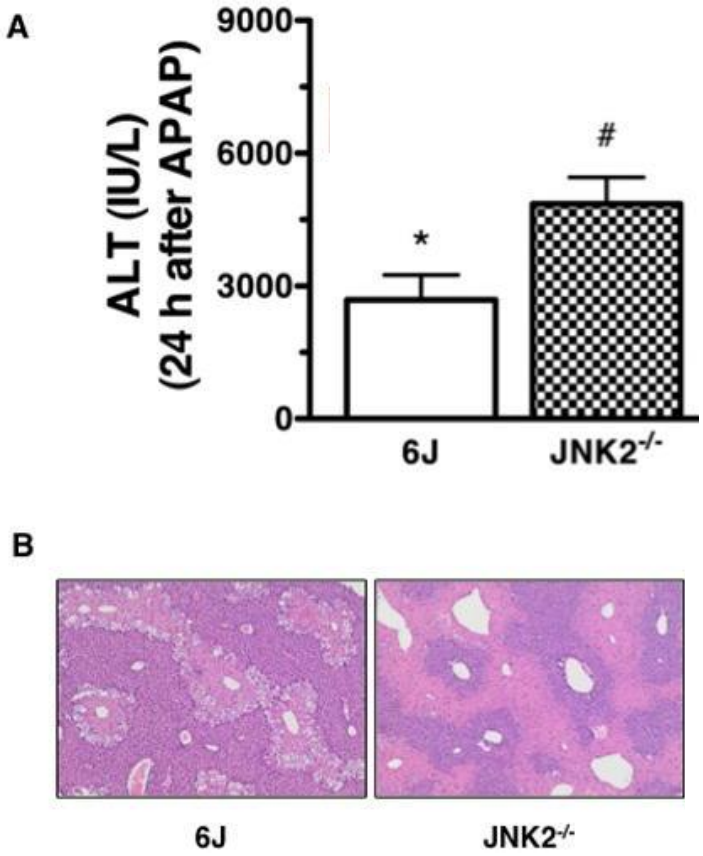


20 generations apart

Selecting Proper Controls

Case Study # 5: C57BL/6 control selection

Influence of Mapk9 (Jnk2) on acetaminophen-induced liver injury (AILI)

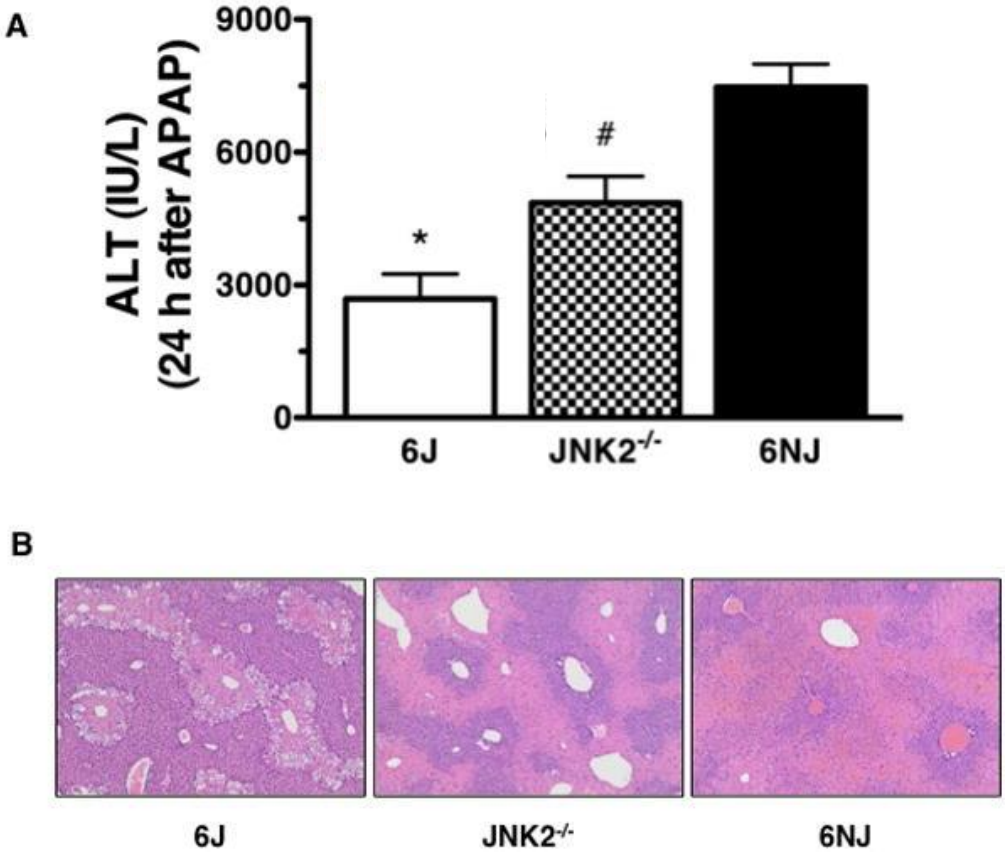


Bourdi M et al. 2011. *Chem Res Toxicol* PMID [21557537](21557537)

Selecting Proper Controls

Experimental conclusions may be in opposition

Effects of Mapk9 (Jnk2) on acetaminophen-induced liver injury (AILI)

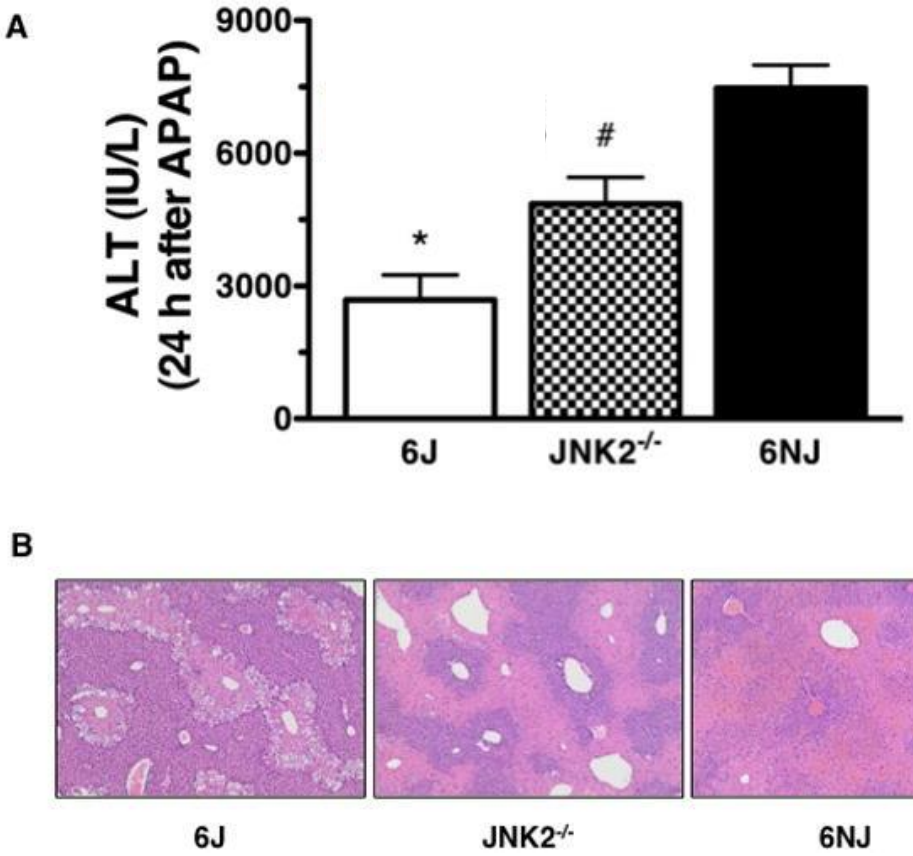


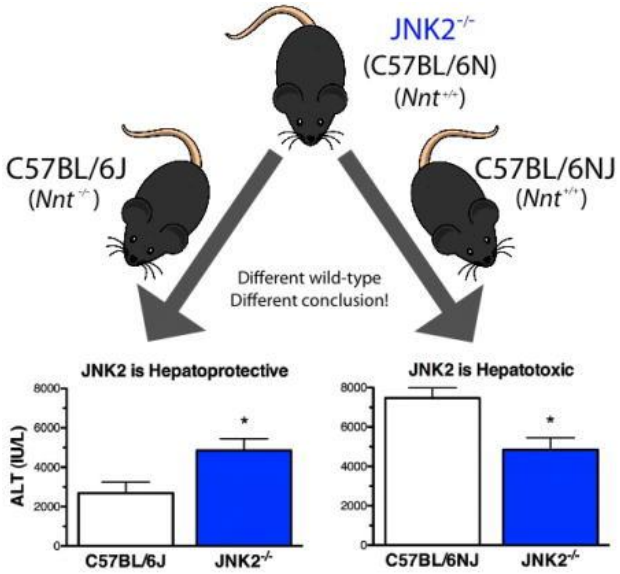
Bourdi M et al. 2011. *Chem Res Toxicol* PMID 21557537

Selecting Proper Controls

Experimental conclusions may be in opposition

Effects of Mapk9 (Jnk2) on acetaminophen-induced liver injury (AILI)



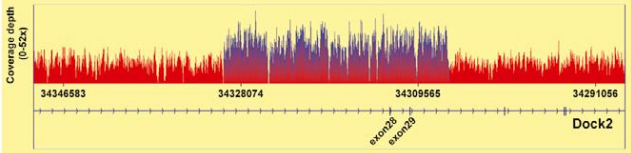


Bourdi M et al. 2011. *Chem Res Toxicol* PMID 21557537

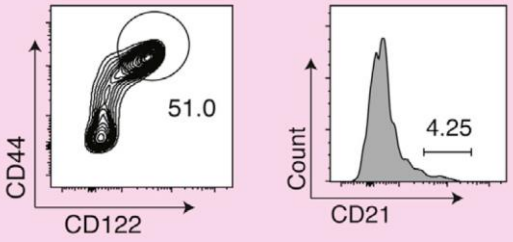
A Recent High-Profile Example:

Case Study # 6: Copy number variant confounds results

Dock2 copy number variant (duplication of exons 28 and 29) in a commercial C57BL/6 strain



Multiple hematopoietic phenotypes unrelated to the targeted genes



Increased CD8 memory T cells

Loss of marginal zone B cells

Mahajan et al., 2016, Cell Reports *15*, 1–9
May 31, 2016 © 2016 The Author(s)
http://dx.doi.org/10.1016/j.celrep.2016.04.080

Cell Reports
Report

OPEN ACCESS
CellPress

Striking Immune Phenotypes in Gene-Targeted Mice Are Driven by a Copy-Number Variant Originating from a Commercially Available C57BL/6 Strain

Vinay S. Mahajan,[1,3] Ezana Demissie,[1,3] Hamid Mattoo,[1] Vinay Viswanadham,[1] Ajit Varki,[2] Robert Morris,[1] and Shiv Pillai[1,*]
[1]Ragon Institute of MGH, MIT and Harvard, 400 Technology Square, Cambridge, MA 02139, USA
[2]Departments of Medicine and Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA 92093, USA
[3]Co-first author
*Correspondence: pillai@helix.mgh.harvard.edu
http://dx.doi.org/10.1016/j.celrep.2016.04.080

SUMMARY

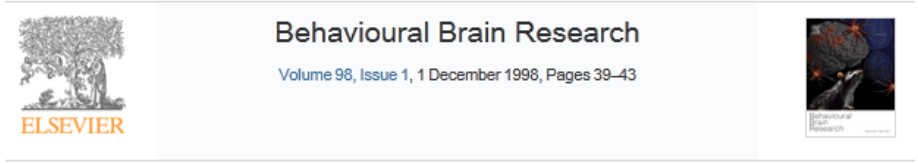
We describe a homozygous copy-number variant that disrupts the function of Dock2 in a commercially available C57BL/6 mouse strain that is widely used for backcrossing. This *Dock2* allele was presumed to have spontaneously arisen in a colony of *Irf5* knockout mice. We discovered that this allele has actually been inadvertently backcrossed into multiple mutant mouse lines, including two engineered to be deficient in Siae and Cmah. This particular commercially obtained subline of C57BL/6 mice also exhibits several striking immune phenotypes that have been previously described in the context of *Dock2* deficiency. Inadvertent backcrossing of a number of gene-targeted mice into this background has complicated the interpretation of several immunological studies. In light of these findings, published studies involving immune or hematopoietic phenotypes in which these C57BL/6 mice have been used as controls, as experimental animals, or for backcrossing will need to be reinterpreted.

signaling (Cariappa et al., 2009). Given that these mice generate altered forms of sialic acid that are not recognized by key regulatory Siglecs expressed on B cells (such as CD22/Siglec-2 and Siglec-G), the defects in B cell development observed in these mice were presumed to arise from perturbations in Siglec function (Cariappa et al., 2009; Pillai et al., 2009). In addition, the observed phenotypes were largely compatible with previous studies of Siglec function (Mahajan and Pillai, 2016; Pillai et al., 2009). Both *Siae*$^{\Delta ex2/\Delta ex2}$ and *Cmah* knockout mice had been backcrossed into a specific commercially obtained C57BL/6 background for ten generations (Cariappa et al., 2009; Hedlund et al., 2007). We found that Siae-deficient mice unexpectedly lost their aberrant B cell development phenotype upon backcrossing for 13 additional generations into the C57BL/6J (Jackson Laboratory) background. We created an independent knockout line of Siae-deficient mice in the C57BL/6N background, and these mice exhibited no defects in B cell development.

Given these discrepant results, we re-examined the genetic basis of aberrant B cell development in *Siae*$^{\Delta ex2/\Delta ex2}$ mice using genetic crosses, SNP arrays, and whole-genome sequencing. These studies revealed that the defects in B cell development were not linked to *Siae*, which is present on chromosome 9 (chr9), but instead to a gene encoding a guanine nucleotide exchange factor, *Dock2*, on chromosome 11 (chr11).

Mahajan, V et al. 2016. *Cell Reports* PMID 27210752

More Published Examples



ELSEVIER

Behavioural Brain Research

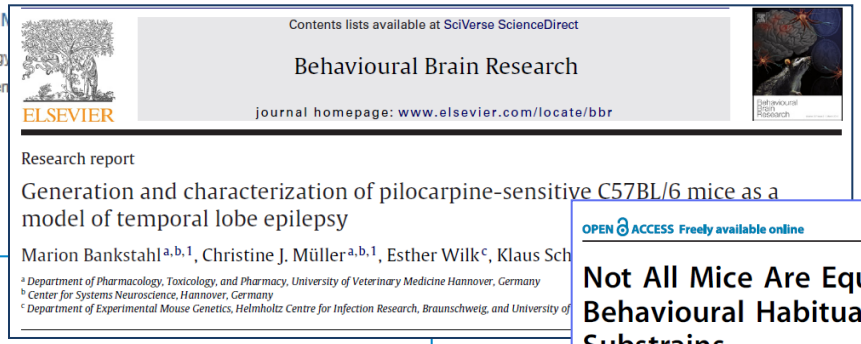
Volume 98, Issue 1, 1 December 1998, Pages 39–43

Research report

Further phenotypical characterisation of two substrains of C57BL/6J inbred mice differing by a spontaneous single-gene mutation

Frans Sluyter[a], Charlotte C.M

[a] Department of Psychoneuropharmacology
[b] Génétique, Neurogénétique et Comporten
Orléans, Cedex 2, France



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Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

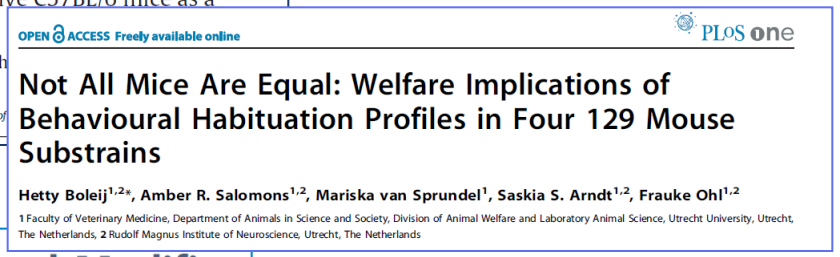
journal homepage: www.elsevier.com/locate/bbr

Research report

Generation and characterization of pilocarpine-sensitive C57BL/6 mice as a model of temporal lobe epilepsy

Marion Bankstahl[a,b,1], Christine J. Müller[a,b,1], Esther Wilk[c], Klaus Sch

[a] Department of Pharmacology, Toxicology, and Pharmacy, University of Veterinary Medicine Hannover, Germany
[b] Center for Systems Neuroscience, Hannover, Germany
[c] Department of Experimental Mouse Genetics, Helmholtz Centre for Infection Research, Braunschweig, and University of



OPEN ACCESS Freely available online

PLoS one

Not All Mice Are Equal: Welfare Implications of Behavioural Habituation Profiles in Four 129 Mouse Substrains

Hetty Boleij[1,2]*, Amber R. Salomons[1,2], Mariska van Sprundel[1], Saskia S. Arndt[1,2], Frauke Ohl[1,2]

1 Faculty of Veterinary Medicine, Department of Animals in Science and Society, Division of Animal Welfare and Laboratory Animal Science, Utrecht University, Utrecht, The Netherlands, 2 Rudolf Magnus Institute of Neuroscience, Utrecht, The Netherlands

—Original—

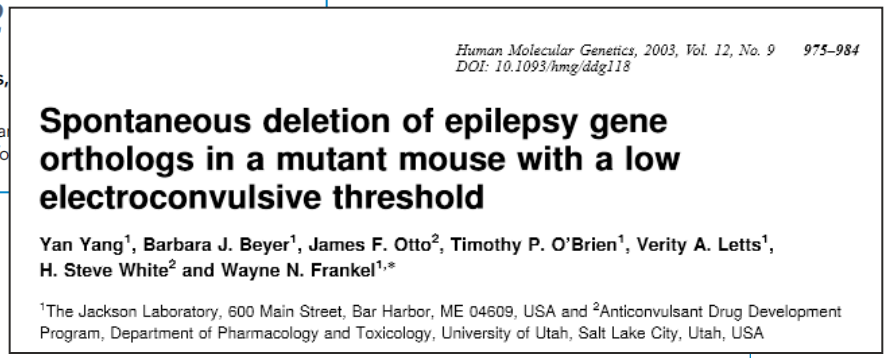
Genetic Differences among C57BL/6 Substrains

Kazuyuki MEKA
Hatsumi NAKAT

RIKEN Bio

The C57BL/6J Mouse Strain Background Modifies the Effect of a Mutation in *Bcl2l2*

Stefanie J. Navarro, Tuyen Trinh, Charlotte A. Lucas, Andrea J. Ross,
and Grant R. MacGregor[1]
Department of Developmental and Cell Biology, School of Biological Sciences, a
Mitochondrial Medicine and Genetics, University of California Irvine, Irvine, Califo



Human Molecular Genetics, 2003, Vol. 12, No. 9 975–984
DOI: 10.1093/hmg/ddg118

Spontaneous deletion of epilepsy gene orthologs in a mutant mouse with a low electroconvulsive threshold

Yan Yang[1], Barbara J. Beyer[1], James F. Otto[2], Timothy P. O'Brien[1], Verity A. Letts[1], H. Steve White[2] and Wayne N. Frankel[1,*]

[1]The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609, USA and [2]Anticonvulsant Drug Development Program, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, Utah, USA

C57BL/6 Publications

SEARCH TERM	PUBMED ENTRIES
C57BL/6	37122
C57BL/6ByJ	112
C57BL/6J	16390
C57BL/6JOlaHsd	53
C57BL/6JBomTac	11
C57BL/6JRj	7
C57BL/6N	1182
C57BL/6NCrl	71
C57BL/6NJ	11
C57BL/6NHsd	41
C57BL/6NTac	78

Complete nomenclature benefits everyone!

Based on May 1, 2017 PubMed citations search (without limits)

Which Genetic Controls to Use?

- I want to study the effect of GeneX on blood pressure. My GeneX KO has been bred hom x hom for 10 consecutive generations.
- Which wildtype/genetic controls should I use?
  - A. Controls? I don't need controls!
  - B. C57BL/6 mice
  - C. Outbred mice
  - D. A suitable inbred, F1, or F2 hybrid strain
  - E. A littermate
  - F. Answer not listed

Genetic Drift and Substrains

- **Spontaneous mutations can be overt or hidden**
  - only apparent by physiological assay
- **Substrains can vary significantly genetically and phenotypically**
- **Know what substrain backgrounds your strains are, and use the proper control**

How to Detect Genetic Drift

- Comparing whole genome sequence data
  - Single Nucleotide Polymorphism (SNP) scans won't do it
- Look for phenotypic differences

image

Minimizing Genetic Drift

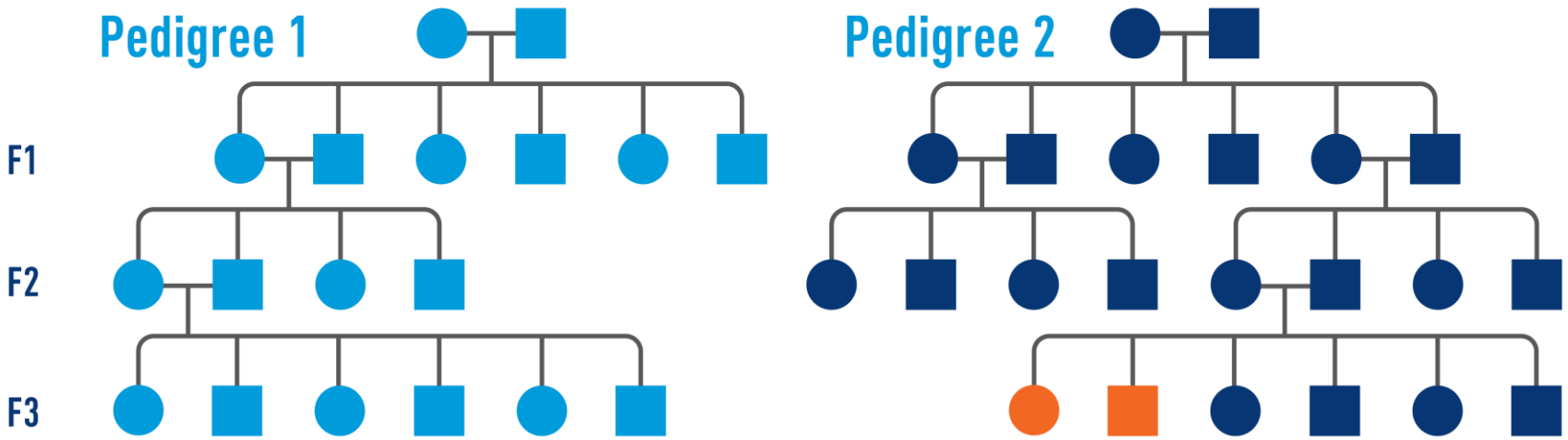
**Genetic change can't be stopped,
but it can be slowed down!**

- Maintain pedigrees and detailed colony records

Maintaining a Pedigreed Colony

Single Established Colony - any strain type

sister-brother mating only!



Mutations become fixed more rapidly in sister-brother pedigrees

- More easily identified/more easily removed

Minimizing Genetic Drift

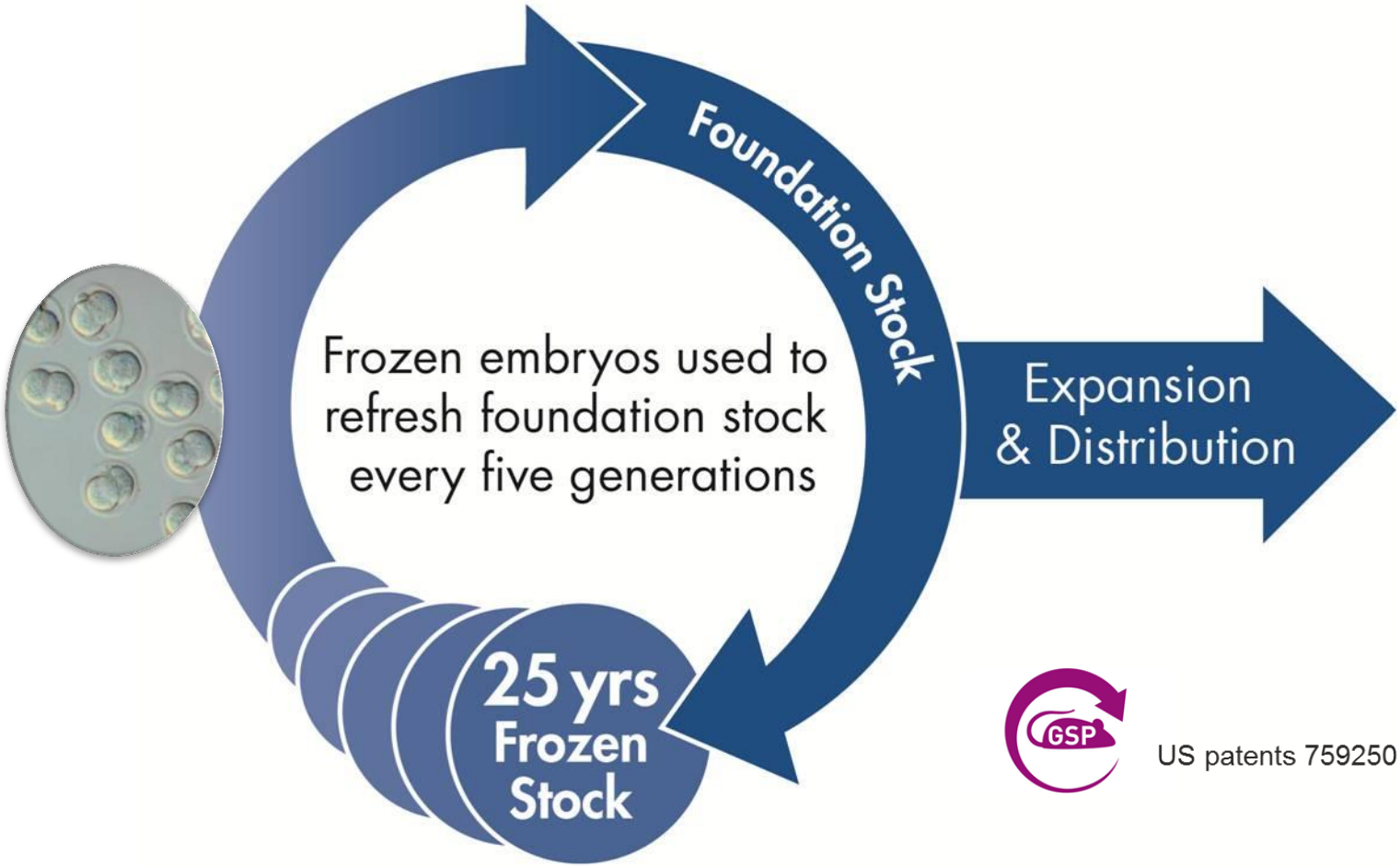
Genetic change can't be stopped, but it can be slowed down!

- Maintain pedigrees lines and detailed colony records
- Watch for phenotypic changes in mutants and controls
- Refresh breeders frequently (~10 generations)
- Avoid selection pressure
- Verify genetic background with genome scanning
- Cryopreserve unique strains

True or False?

- Large production colonies that breed mice professionally do not experience genetic drift.

The Jackson Laboratory's Genetic Stability Program (GSP)



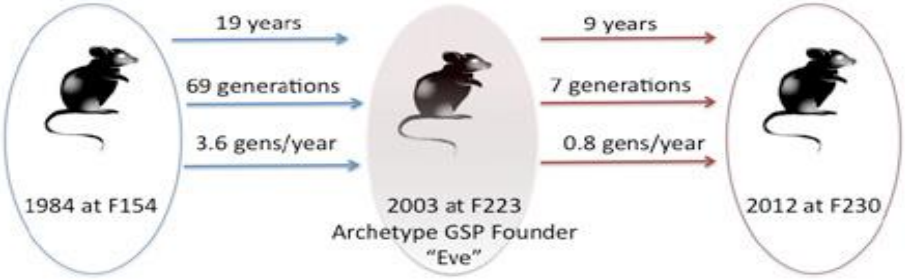
US patents 7592501, 8110721

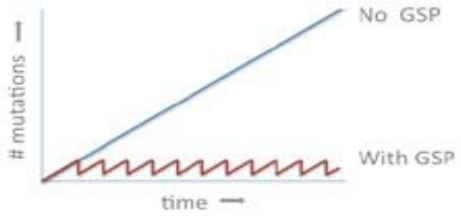
Genetic Stability Program Works!

Whole genome sequencing on C57BL/6J genomic DNA

- 1984 @ F154 (pre GSP) } 69 gen.
- 2003 @ F223 (GSP "Eve") } 7 gen.
- 2012 @ F230 (post-GSP)

Evaluated high quality single nucleotide variants (SNPs)





- Constant mutation rate in pre- & post-GSP period
- Mutations accumulate more slowly post-GSP

US patents 7592501, 8110721

Summary

- Spontaneous mutations occur frequently and can be overt or hidden
  - Implement breeding and colony maintenance strategies that minimize genetic drift
- Substrains can vary significantly genetically and phenotypically
  - Know the substrain that you use, and use the proper control

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- Mouse Genome Scanning
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  - May 18, 2017, 1:00 pm (ET); 5:00 pm (GMT)
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  - May 23, 2017, 6:30 am (ET); 10:30 am (GMT); 12:30 pm (CEST); 4:00 pm (IST)
- Modeling HIV, Ebola and Other Infectious Diseases in Mice
  - Jun. 1, 2017, 2017, 1:00 pm (ET); 5:00 pm (GMT)
- CRISPR/Cas: Moving from Founder Mice to Phenotyping
  - Jun. 13, 2017, 9:00 am (ET); 1:00 PM (GMT); 3:00 pm (CEST)
- Predictive Cancer Models Using Patient-derived Xenograft Mice
  - Jun. 22, 2017, 1:00 pm (ET); 5:00 pm (GMT)

Thank You!

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