Key Differences among B6 Substrains and the Research Impact

Technical Information Services
November 3, 2016
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](http://www.jax.org/mouse-search)

- **Mouse Genome Informatics**
  [www.informatics.jax.org](http://www.informatics.jax.org)

- **Mouse Phenome Database**
  [www.jax.org/phenome](http://www.jax.org/phenome)

- **Others, including:**
  - JAX-Clinical Knowledgebase
  - Mouse Tumor Biology Database

www.jax.org/jax-mice-and-services/customer-support/technical-support
Key Differences Among B6 Substrains: Learning Goals

- Know which C57BL/6 substrain you need for your research
- Learn how to use proper nomenclature
- Understand the importance of minimizing genetic drift
How well do you know your B6 mice? Which two are most similar?
How well do you know your B6 mice?
A & B are most similar!

A

B6(Cg)-\textit{Tyr}^{c-2J}/J (000058)

B

C57BL/6J (000664)

C

C57BL/6NJ (005304)

- A & B differ by a single allele (\textit{Tyr}^{c-2J})
- B & C differ in multiple alleles
  - Metabolism
  - Neurobiology
  - Immunology
  - Vision & hearing
  - Behavior
Coat Color Mutations

C57BL/6J-\(A^{w-J}/J\) (000051)
C57BL/6J (000664)
C57BL/6J-\(Lys^{bg-J}/J\) (000629)
B6(Cg)-\(Tyr^{c-2J}/J\) (000058)
C57BL/6J-\(Kit^{W-v}/J\) (000049)
B6J or B6N…

We’ve Got You Covered!

- C57BL/6J (000664)
  - High health status
  - Well characterized
  - Most published

- C57BL/6NJ (005304)
  - Extensive Phenotypic Data
  - Consistent Data Reproducibility
Origins of Inbred Mice

- **Mice are ideal for mammalian genetics**
  - Small and easy to maintain
  - Great reproductive performance
  - Anatomy and physiology similar to humans

- **1900-1918  Abbie Lathrop, Granby, MA**
  - Mouse fancier, raised and sold mice
  - Provided mice to Bussey Institute, Harvard

- **1902 - Dr. William Castle begins using mice, Bussey Institute, Harvard**

- **1909 - C.C. Little begins inbreeding mouse stocks as student of Dr. Castle**
Origins of C57BL Mice

Miss Abbie Lathrop's “pet shop” stock

C.C. Little (1921) mating of female 57

C57BL (BLACK)

C57BR (BROWN)

C57L (LEADEN)
Advantages of Inbred Strains

- Pedigree breeding (brother-sister mating)
  - Inbreds established by 20 generations of brother-sister mating
- Genetic homogeneity
- Statistical reproducibility
Inbred Strain vs. Substrain
Hearing - Avoid Common Research Mistakes

All C57BL/6 substrains ($Cdh23^{ahl}$); consequences of age related hearing loss

- Complication in interpretation of genes influencing diseases, phenotypes & developmental biology of hearing & neurobiology
- Phenotypic analysis of genes implicated in cognitive behavior (fear conditioning in older mice, requires auditory cue)
- Research areas impacted
  - Autism
  - Anxiety & stress disorders
Substrains Develop Quickly

- Colonies separated by 20 or more generations
- Phenotypic or genetic differences are discovered

Labs A & B are 30 generations apart!
Many Substrains of C57BL/6 Exist

Know Your Substrain
Use Proper Nomenclature

- C57BL/6J  
  **Parent strain**

- C57BL/6NJ  
  **Substrain designation**
  NIH (N)
  By (Dr. Baily)

- C57BL/6NCrl  
  **Laboratory maintaining the strain**
  Jackson (J)
  Crl (Charles River Laboratories)

- C57BL/6ByJ

Institute for Laboratory Animal Research (ILAR) Lab Codes
http://dels.nas.edu/global/ilar/Lab-Codes
But Aren’t All B6 Mice the Same?
C57BL/6 substrains are not the same!

- They differ genetically
  - Single Nucleotide Polymorphisms (SNPs)
  - Insertions & deletions (Indels)
  - Copy number variations (CNVs)
  - Spontaneous mutations
Genetic Differences Translate into Phenotypic Differences

- Metabolism
- Neurobiology
  - Behavior
  - Vision
  - Hearing
- Immunology
- And more…
Metabolic Differences (DIO)
B6J gains more weight than B6NJ on high fat diet (HFD)

C57BL/6J (000664) vs C57BL/6NJ (005304)

- Mice fed a 60 kcal% high fat diet

Metabolic Differences (DIO)
B6J more impaired than B6NJ on high fat diet (HFD)

C57BL/6J (000664) vs C57BL/6NJ (005304)

Glucose Tolerance Test
- Measures ability of mice to clear glucose from blood
- Both B6J and B6NJ mice have severely impaired glucose tolerance

Metabolic Differences (DIO)
C57BL/6JRj mice are DIO resistant

- B6N mice become obese on high fat diet, B6JRj mice do not
- B6JRj mice have greater food intake on high fat diet

Neurological Differences

C57BL/6JOlaHsd  Mice from Envigo (Harlan), Bicester, UK
Deletion of Snca – no visible phenotype, but…

C57BL/6J
Wild-type Snca

Genomic DNA from The Jackson Laboratory

C57BL/6NCrl
Wild-type Snca

Mice from Charles River, Margate, UK

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

Neurological Differences
Behavior - B6J Prefers Alcohol More Than B6N

C57BL/6J (B6J) consumes more alcohol than C57BL/6N (B6N)

- Increased consumption
- Increased preference
- Notable differences in gene expression

Mulligan, MK et al. 2008. Genes, Brain, and Behavior. 7: 677-689. PMID: 18397380
Neurological Differences

Vision - Substrains Differ In Visual Acuity \((Cr\text{b}1^{rd8})\)

- Localized to Muller cells and photoreceptor (PC) inner segments

- Mutations in CRB1 associated with retinal diseases in man
  - Retinitis pigmentosa
  - Leber congenital amaurosis

- Progressive, spotty retinal degeneration in mice


http://crfb.univ-mrs.fr/Crumbs/section/en/CRB1_function/105
Neurological Differences
Vision - Substrains Differ In Visual Acuity (Crb1<sup>rd8</sup>)

**ALL** C57BL/6N substrains are *Crb1<sup>rd8</sup>/Crb1<sup>rd8</sup>

(Capillary electrophoresis of PCR products)

**C57BL/6J:** *Crb1* wild-type  
(000664)

**C57BL/6NJ:** *Crb1<sup>rd8</sup>/Crb1<sup>rd8</sup>  
(005304)

Neurological Differences
Vision - Avoid Common Research Mistakes

C57BL/6N ($Crb1^{rd8}$); consequences of retinal degeneration

- Complication in interpretation of genes influencing diseases, phenotypes & developmental biology of sight & neurobiology

- Phenotypic analysis of genes implicated in cognitive function (behavioral tests that require visual cues)?
  - Overall retinal function only minimally impaired (@ 10 months)

- Research areas potentially impacted
  - Age-related Macular Degeneration/Retinal Degeneration
  - Neurological/Neurodegenerative disorders (e.g. autism, Down Syndrome, Alzheimer’s)?
  - Diabetic retinopathy?

International Knockout Mouse Consortium (IKMC)

Mutate all protein-coding genes in C57BL/6N

- Knockout Mouse Project (KOMP) – USA
- European Conditional Mouse Mutagenesis Project (EUCOMM) – Europe
- North American Conditional Mouse Mutagenesis Project (NorCOMM) – Canada
- Texas A&M Institute for Genomic Medicine (TIGM) - USA

http://www.knockoutmouse.org/
## Reproducible Phenotype Differences

Data consistent from three IKMC phenotyping centers

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<th>Description</th>
<th>HMGU M</th>
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<th>ICS M</th>
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<th>MRC Harwell M</th>
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<td>Non-Invasive blood pressure: Systolic arterial pressure</td>
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<td>Calorimetry: Oxygen consumption</td>
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<td>Calorimetry: Heat production (metabolic rate)</td>
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<td>Simplified IPGTT: Blood glucose concentration</td>
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<td>Simplified IPGTT: Glucose response AUC</td>
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<td>DEXA: Fat mass</td>
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<td>Modified SHIRPA: Locomotor activity</td>
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<td>Modified SHIRPA: Startle response</td>
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<td>Grip-strength: Forelimb grip strength measurement</td>
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<td>Rotorod: Latency to fall</td>
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<td>Rotorod: Passive rotation</td>
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<td>Acoustic Startle &amp; PP1:PP1 + pulse startle magnitude</td>
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<td>Acoustic Startle &amp; PP1:Global prepulse inhibition</td>
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<td>Clinical Chemistry: Glucose</td>
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<td>Clinical Chemistry: Urea</td>
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<td>Clinical Chemistry: Potassium</td>
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**Key**

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<th>N/J</th>
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Immunological Differences
Response to bacteria infection – *Listeria monocytogenes*

- B6J females show greater susceptibility to *Listeria spp.*
- B6N males show significant pro-inflammatory response on day 3

Immunological Differences

B6J mice show greater DTH Response

Delayed Type Hypersensitivity (DTH) Response

- Sensitization and challenge with dinitrofluorobenzene (DNFB)
- B6J males & females show greater inflammatory response

Genetic Analysis

- Identified multiple SNPs & Indels
- Genomic structural variants

Knockout Mouse Phenotyping Project (KOMP²)

Generate and phenotype 2,500 KO mouse strains

- **JAX - The Jackson Laboratory** (Bar Harbor, ME)

- **BaSH Consortium**
  - Baylor College of Medicine, Houston
  - Wellcome Trust Sanger Institute (Hinxton, England)
  - Medical Research Council (MRC) Harwell (Oxfordshire, England)

- **DTCC Consortium**
  - University of California, Davis
  - Toronto Center for Phenogenomics (Canada)
  - Children’s Hospital Oakland Research Institute (Oakland, CA)
  - Charles River Laboratories (Wilmington, MA)
Distribution of Strains at JAX
Both B6J and B6N genetic backgrounds

Knockout, transgenic, spontaneous & induced mutants

- ~1,975 strains on the C57BL/6J background
- ~70 strains on the C57BL/6N background
- ~830 strains on the C57BL/6N background going to be created through KOMP²
- ~200 strains on the C57BL/6N background going to be created through EUCOMM
Considerations for Control Selection

- **Congenic Strains**
  - Littermates (het x het, het x wt, or hemi x wt mating scheme)
    - Wild type or heterozygous for mutant gene or allele
    - Non-carriers of transgene
    - Can also use non-littermate controls from the colony
  - Inbred (hom x hom mating)
    - Match background mutant is on (including substrain)

- **Mixed Background (B6J and B6N)**
  - Littermates
    - Wild type or heterozygous for mutant gene or allele
    - Non-carriers of transgene
    - Can also use non-littermate controls from the colony

* Congenic strains have been crossed more than 10 generations to inbred strain. Acceptable to use inbred as control after N5
Select the Proper C57BL/6 Control
Avoid Common Research Mistakes

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

Select the Proper C57BL/6 Control

Avoid Common Research Mistakes

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

Select the Proper C57BL/6 Control

Avoid Common Research Mistakes

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

Differences Are Reported When Discovered Dock2 copy number variant in C57BL6/NHsd affects immune phenotypes

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

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 Ir35 knockou 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 Cnr2. 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.

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
Background Strain Information: Questions You May Want to Ask

- What strain was used to develop this stock?
  - What oocyte donor?
  - What ES cell line?
- What strains have been introduced through breeding?
  - Cre/FLP
  - Reporters
  - Other mutations
- What is the current breeding scheme?
- What is the current generation?
- Has it been cryopreserved?
  - At what generation?
  - Has the strain been backcrossed to an inbred strain?
- Has the genetic background been verified?
A 32 SNP (single nucleotide polymorphism) panel analysis, with 27 markers covering all 19 chromosomes and the X chromosome, as well as 5 markers that distinguish between the C57BL/6J and C57BL/6N substrains, was performed on the rederived living colony at The Jackson Laboratory Repository. While the 27 markers throughout the genome suggested a C57BL/6 genetic background, all 5 markers that determine C57BL/6J from C57BL/6N were found to be segregating. These data suggest the mice sent to The Jackson Laboratory Repository were on a C57BL/6N genetic background.
Genetic Stability Program (GSP)
Diminish cumulative drift, stabilize phenotype

Frozen embryos used to refresh foundation stock every five generations

C57BL/6J (000664)

C57BL/6NJ (005304)

US patents 7592501, 8110721

www.jax.org/jaxmice/genetichealth/stability
Resources Supporting B6 Mice
The best characterized & most published strain

- Mouse Phenome Database (MPD) [www.phenome.jax.org](http://www.phenome.jax.org)
  - Over 2700 measurements for [C57BL/6J](http://www.phenome.jax.org) (000664)

- Whole genome sequence data - Sanger Institute
  - [Mouse Genomes Project](http://www.mousegenomes.org)

- Preconditioned mice
  - [Streptozotocin (STZ) induced diabetes](http://www.streptozotocin.com)
  - [Diet induced obesity (DIO)](http://www.dietinducedobesity.com)

- Inventoried aged mice
  - [Custom aging services](http://www.customaging.com)

- High Health Status at no extra charge!
Choose Wisely….Background Matters
Which strain would you choose?

Mouse Phenome Data (MPD): Total cholesterol @ 10 weeks – 43 strain survey

Access MPD at www.jax.org/phenome
Ensuring Data Validity & Reproducibility

Consider your rodent, your most important reagent

• Choose wisely – know thy mouse
• Use proper nomenclature
• Minimize genetic drift
• Educate and establish a QC culture

Good science results in reduced animal use
Summary

- Multiple genetically and phenotypically unique substrains have developed over time (and continue to do so)

- Knowing and understanding the B6 substrain you are working with is key to proper selection of controls and data interpretation

- Comparison of phenotypes between B6 substrains may allow identification of unique modifier alleles

- At JAX, genetic drift is diminished in C57BL/6J & C57BL/6NJ by GSP to stabilize phenotype over time
JAX® Mice & Services: Leading Experts in Mouse Modeling

- Common inbred and specialty JAX® Mice
- Study-ready, aged C57BL/6J Mice (25-78 wks)
- Basic and complex mouse breeding, speed congenics, and rederivation
- Mouse genome scanning
- Cryopreservation and recovery
- Humanized and PDX preclinical models and compound evaluation
Upcoming JAX Webinars™

Subscribe to the monthly webinar announcements email list: https://subscribe.jax.org/

- **Cre-lox Basics: Generating Knockout Mice**
  - Nov. 10, 2016, 1:00 PM ET USA

- **Efficient Mouse Colony Management**
  - Nov. 17, 2016, 1:00 PM ET USA

- **Comparing Immunodeficient Mice for Cancer, Immunity and Transplant Research**
  - Nov. 30, 2016, 6:30 AM ET USA

- **Predictive Cancer Models Using Patient-derived Xenograft Mice**
  - Dec. 1, 2016, 1:00 PM ET USA

- **Advanced Cre-lox: Generating Reporters, Inducible Mice, and Disease Models**
  - Dec. 8, 2016, 1:00 PM ET USA

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Thank you!

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