JAX™ WEBINARS

Welcome Colleagues!

TODAY'S PRESENTATION:

Key Differences among B6 Substrains and the Research Impact

START TIME:

10:00 AM SGT (Singapore)

There will be silence until the webinar begins at the start time.

Organized by:

InVivos
BRINGING LIFE TO YOUR RESEARCH

The Jackson Laboratory

Presented by:

Sarah Edie, PhD
Technical Information Scientist
The Jackson Laboratory

The Jackson Laboratory

January 29, 2021

Key Differences among B6 Substrains and the Research Impact

Development of inbred strains

Resources to find and understand key differences between strains

Accurate reporting of data

Sarah Edie, Ph.D.
Technical Information Scientist

The Jackson Laboratory

JAX MISSION

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

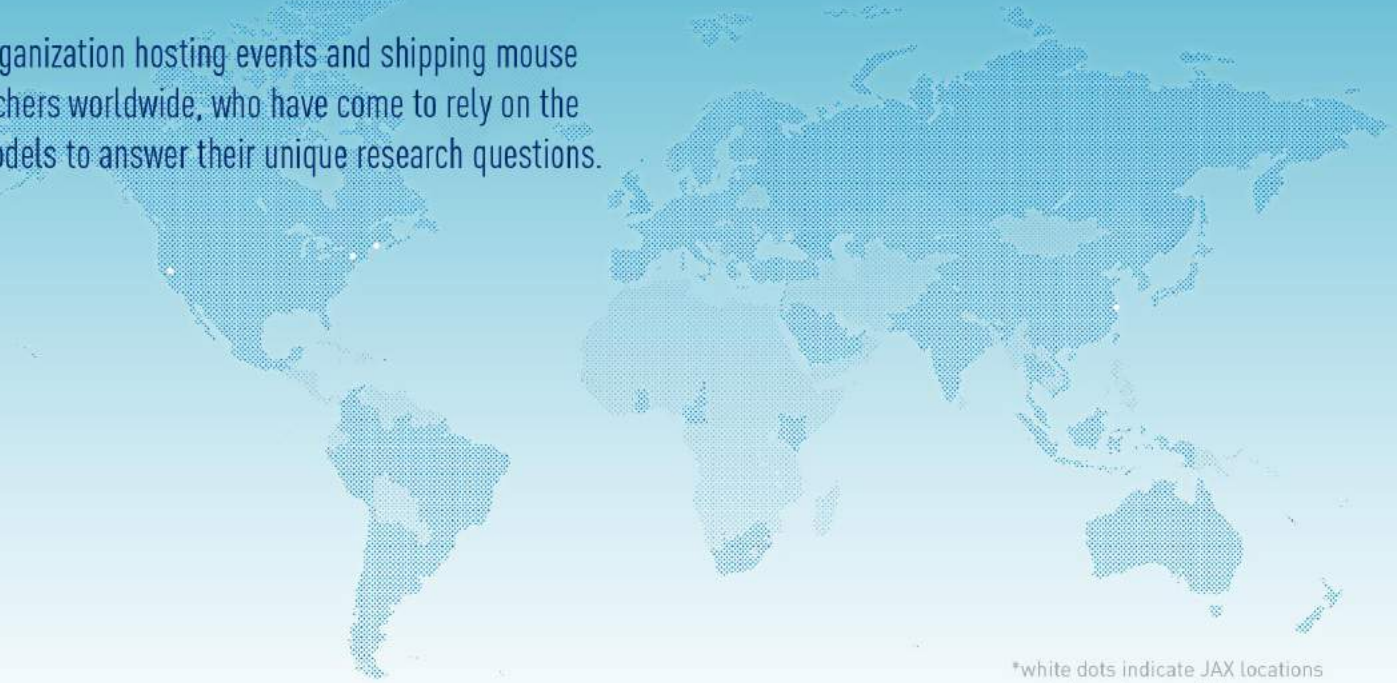
EMPOWERING SCIENTIFIC EXCELLENCE

Our scientific expertise is derived directly from JAX faculty and scientific researchers, who are embarking on ground-breaking research in addition to providing cutting-edge models and powerful preclinical services to researchers worldwide.

Explore the Latest Innovations

GLOBAL EXPERIENCE. GLOBAL INFLUENCE.

JAX is a global organization hosting events and shipping mouse models to researchers worldwide, who have come to rely on the gold-standard models to answer their unique research questions.



*white dots indicate JAX locations and shaded areas of the map are countries JAX has shipped products and services to.

The Story of B6 Substrains

Chapter One

Understanding how substrains arise

Chapter Two

Selecting the appropriate strain for your research

Chapter Three

Accurate reporting through nomenclature

Chapter Four

Reproducibility: Choosing appropriate controls

The Jackson Laboratory

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Where did the first inbred strains come from?

Abbie Lathrop – mouse fancier
1900-1918 Granby, MA

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

Mice are ideal for mammalian genetics

Small and easy to maintain

Great reproductive performance

Anatomy and physiology similar to humans

Where did the first inbred strains come from?

Abbie Lathrop – mouse fancier
1900-1918 Granby, MA

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

C.C. Little
Student of Dr. Castle

1909 - begins inbreeding mice

Inbred Mouse Strains

Maintained by sibling (sister x brother) mating for 20 or more consecutive generations (F20+)

Most genetically uniform mouse resource

Best characterized strains

Unique phenotypes

Widely used as models of human disease

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Origins of C57BL/6: Female # 57

Miss Abbie Lathrop's “pet shop” stock

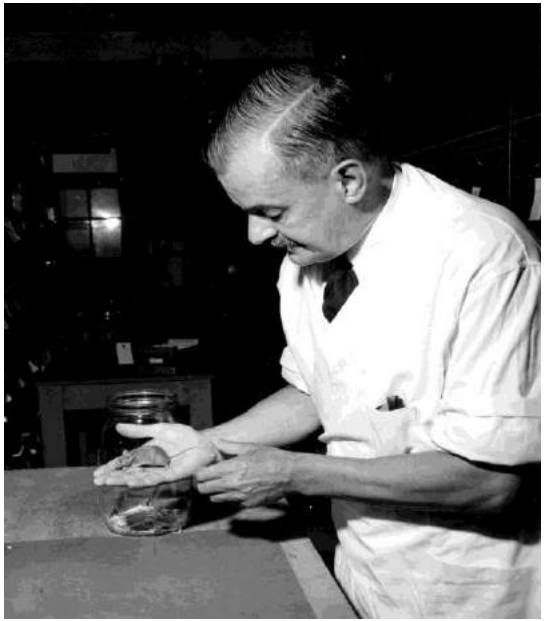
C.C. Little (1921) mating of female 57

C57BL (**BLACK**)

C57BR (**BROWN**)

C57L (**LEADEN**)

Many Substrains of C57BL/6 Exist



C.C. Little

Founded The Jackson Laboratory in 1929

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Effect of Genetic Drift on Substrain Development

- Genetic changes resulting from mistakes in meiosis or DNA repair
- A new mutation becomes fixed every 6-9 generations
- Associated webinar:
  - Genetic Drift: What It Is and How to Minimize Its Impact on Your Research
  - Link to watch webinar

Species Diversity

Strain Diversity

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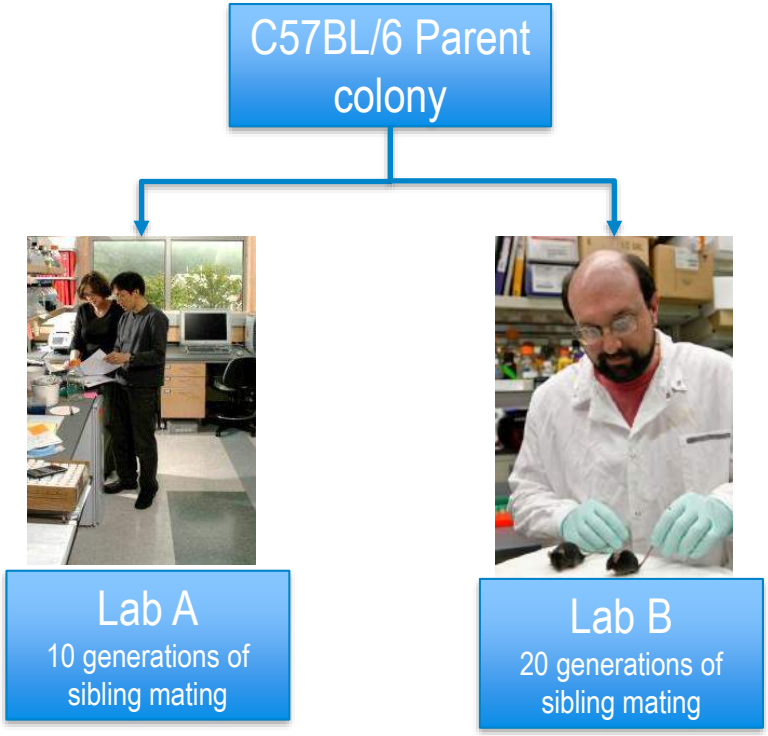
When is a substrain, a substrain?

Colonies separated by 20 or more generations

OR

Phenotypic or genetic differences are discovered



Labs A & B are 30 generations apart!

Genetic Differences between B6J and B6NJ

- Used the data from the 17 Mouse Genomes Project
- 236 validated sequence variants
- 43 Structural Variants (deletions and duplications)
  - 15 overlapping a gene

Table S1. SNP and small indel validation numbers

	No. of filtered variants	No. variants sent for validation*	Failed validation process**	Loci not variant	No. validated***
Coding SNPs	40	40	6	0	34
Non coding SNPs	8484	722	272	304	146
Coding indels	11	11	3	6	2
Non Coding indels	2142	158	82	22	54

Simon, M. M., et al. (2013). *Genome Biology* 14(7): R82. PMID: 23902802

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Genetic Differences Translate to Phenotypic Differences

Genetic or genomic change	Effect	Strain
Dock2 Copy Number Variation	Immune cell changes	C57BL/6NHsd
$Crb1^{rd8}$	Progressive, spotty retinal degeneration	C57BL/6N
Snca deletion	Alpha-synuclein expression	C57BL/6OlaHsd
Nnt	Metabolic	C57BL/6J
$Cdh23^{ahl}$	Age related hearing loss	All C57BL/6 substrains

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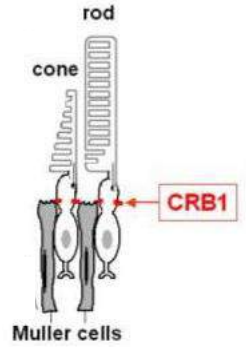
Retinal Degeneration in C57BL/6N Substrains

Crb1 (crumbs-like 1)

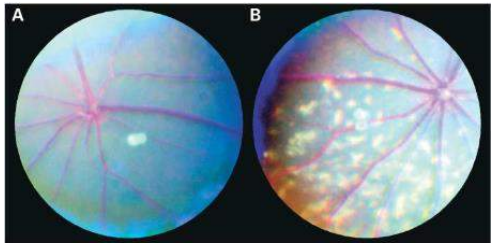
- Localized to Muller cells and photoreceptor (PC) inner segments
- Mutations in CRB1 associated with retinal diseases in humans
  - Retinitis pigmentosa
  - Leber congenital amaurosis



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

Crb1^{rd8}

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



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

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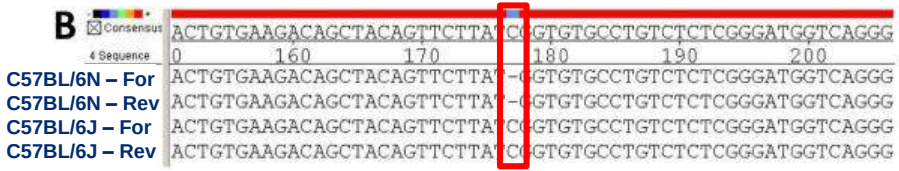
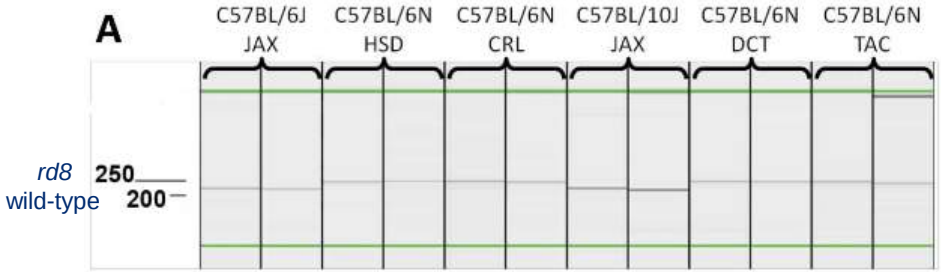
Retinal Degeneration in C57BL/6N Substrains

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

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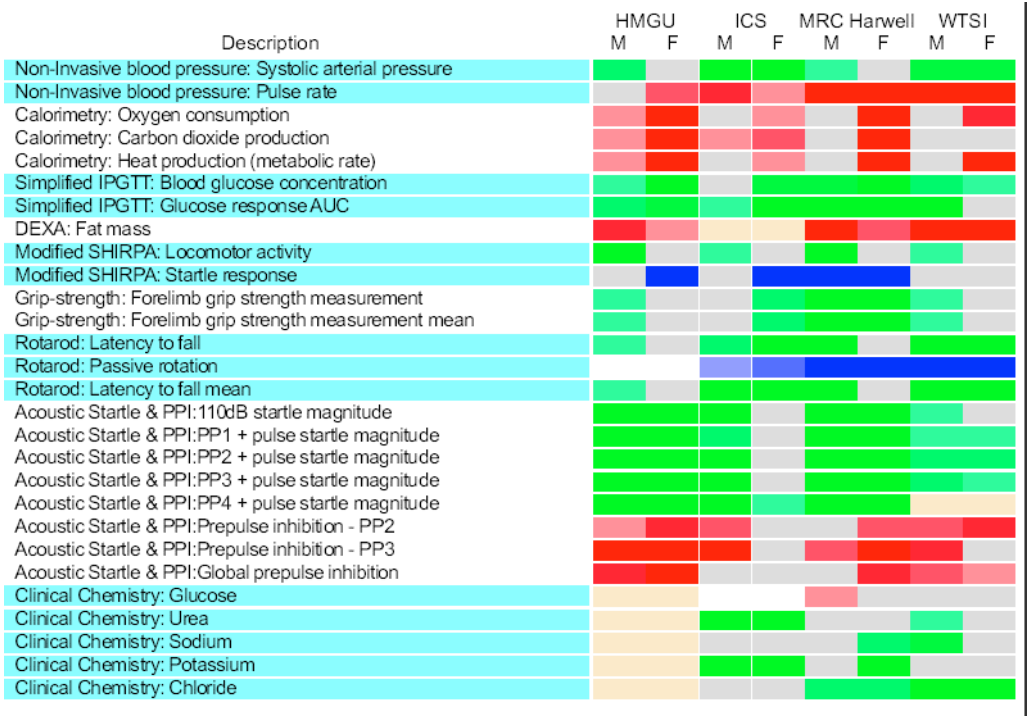
Phenotypic differences between B6J and B6NJ



Simon, M. M., et al. (2013). *Genome Biology* 14(7): R82. PMID: 23902802

IMPReSS:
International Mouse Phenotyping Resource of Standardised Screens

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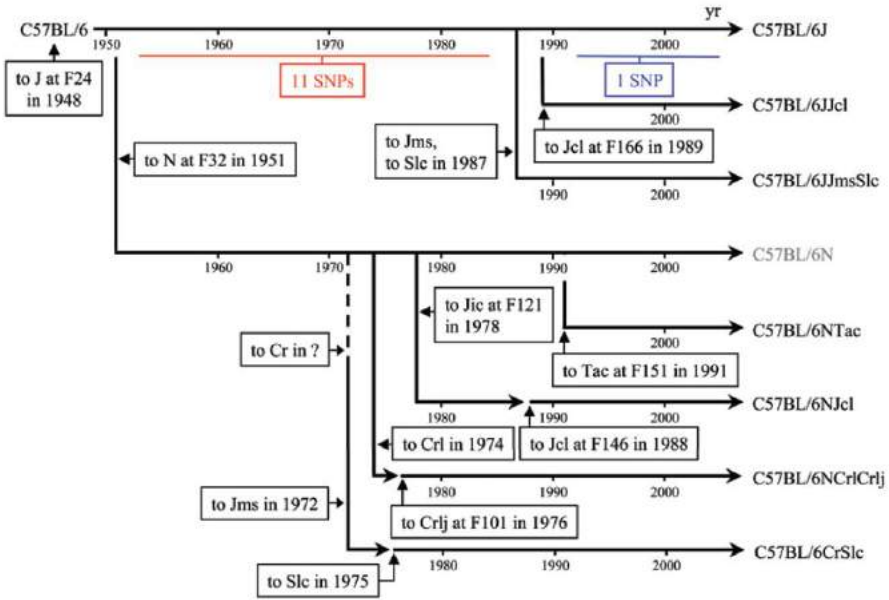
Substrains Continue to Develop Over Time

Genotyped C57BL/6 substrains using ~1500 SNPs



Additional 12 SNPs identified that are different between C57BL/6 “J” strains and C57BL/6 “N” strains

Mekada et al. Exp. Anim.: 58(2), 2009 PMID: 19448337

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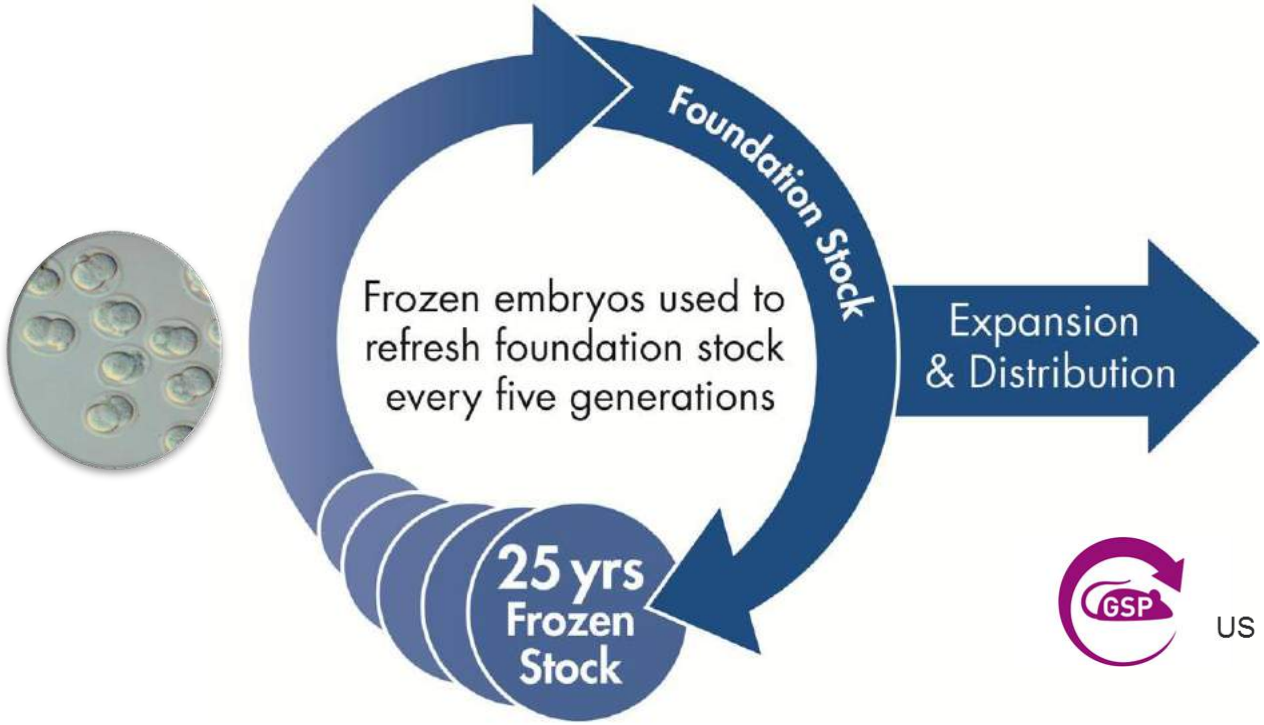
The Jackson Laboratory's Genetic Stability Program (GSP)



GSP

US patents 7592501, 8110721

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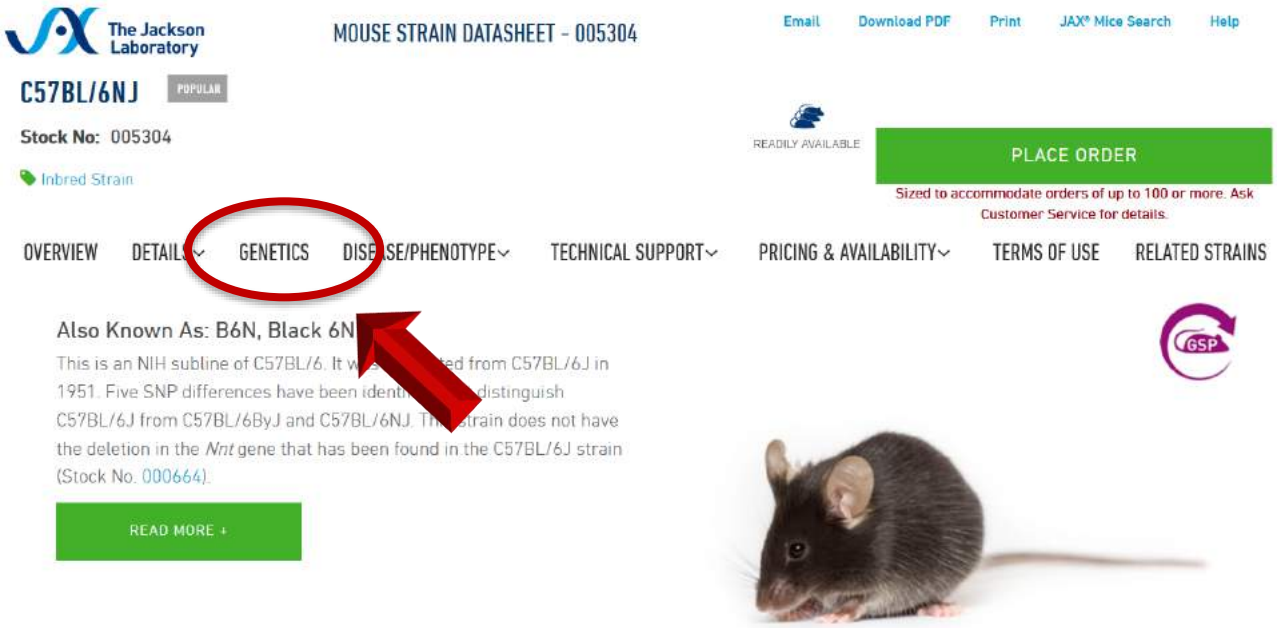
JAX Strain Datasheets



JAX Strain Datasheets

JAX Strain Datasheets

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C57BL/6NJ POPULAR

Stock No: **005304** | B6N

Genetics

- *Crb1*^{rd8}
- *Cyfip2*^{M1N}
- *Nlrp12*$^{C57BL/6N}$
- *Cox7a2l*^{s}

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C57BL/6J POPULAR

Stock No: **000664** | B6

Genetics

- *Ahr*$^{b-1}$
- *Cdh23*^{ahl}
- *P2rx7*$^{rs48804829-T}$
- *Gluchos1*$^{C57BL/6J}$
- *Gluchos2*$^{C57BL/6J}$
- *Gluchos3*$^{C57BL/6J}$
- *Nnt*$^{C57BL/6J}$ 

JAX Strain Datasheets

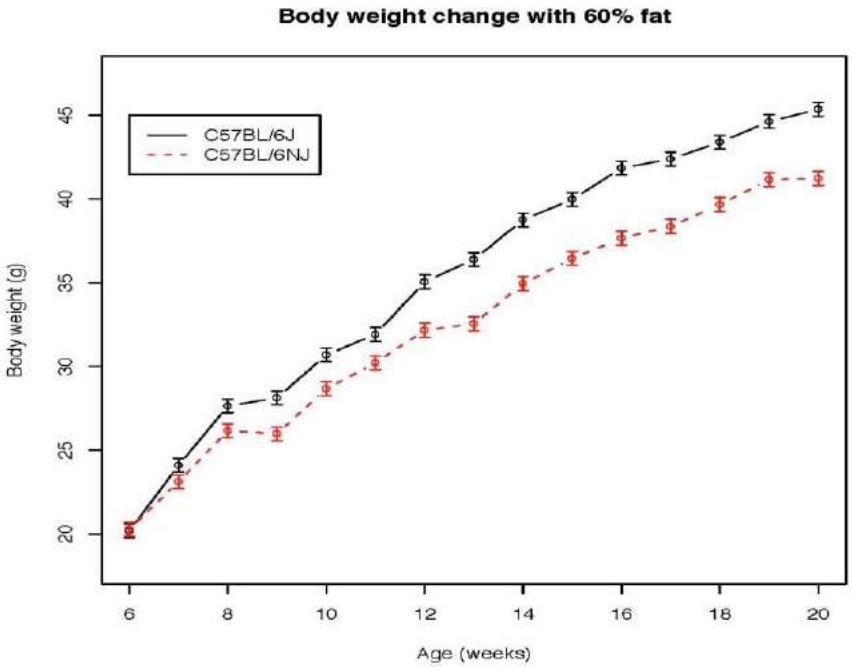
Nnt$^{C57BL/6J}$

Allele Symbol: *Nnt*$^{C57BL/6J}$ MGI↗

Allele Name	C57BL/6J
Allele Type	Spontaneous (Null/Knockout)
Allele Synonym(s)	Ant1^{-}
Gene Symbol and Name	*Nnt* MGI↗ , nicotinamide nucleotide transhydrogenase
Gene Synonym(s)	
Strain of Origin	C57BL/6J
Chromosome	13
Molecular Note	This allele contains a stretch of 17,814 bp missing between exons 6 and 12. RT-PCR demonstrated cDNA corresponding to exons 7-11 was absent. Mature protein was not detected in these mutants.

Metabolic Differences between B6J and B6N

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



- Mice fed a 60 kcal% high fat diet
- B6J gains more weight than B6NJ on high fat diet (HFD)

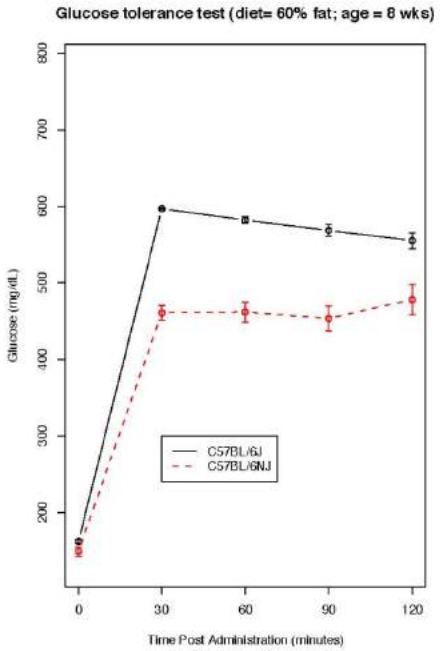
Nicholson, A et al. 2010. *Obesity* 18(10): 1902-1905. PMID: 20057372

Metabolic Differences between B6J and B6N

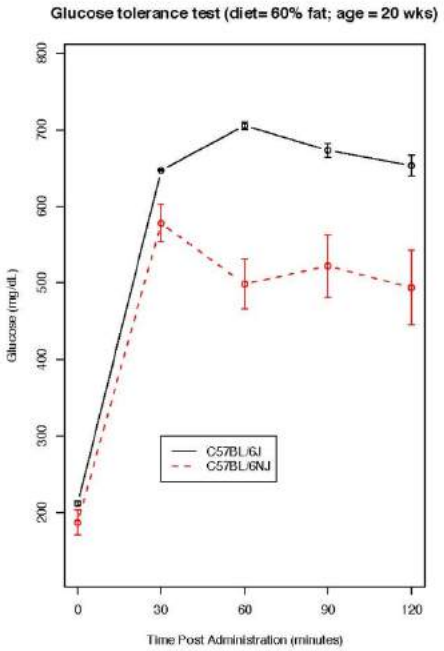
Glucose Tolerance Test

- Measures ability of mice to clear glucose from blood
- Both B6J and B6NJ mice have severely impaired glucose tolerance
- B6J more impaired than B6NJ on high fat diet (HFD)

2 wks on HFD



14 wks on HFD



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What resources can you use?

https://phenome.jax.org/

Mouse Phenome Database

https://phenome.jax.org/

C57BL/6ByJ	JAX:001139
C57BL/6CrSlcRbrc	Rbrc
C57BL/6J	JAX:000664
C57BL/6JArc	Arc
C57BL/6JBomTac	Tac 764
C57BL/6JCrl	(none)
C57BL/6JEiJ	JAX:000924
C57BL/6JJclRbrc	Rbrc
C57BL/6JJmsSlcRbrc	Rbrc
C57BL/6JOlaHsd	Hsd
C57BL/6JRbrc	Rbrc
C57BL/6JRccHsd	Hsd
C57BL/6N	(none)
C57BL/6NCrl	Crl c57bl-6n-mouse
C57BL/6NCrlCrljRbrc	Rbrc
C57BL/6NHsd	Hsd
C57BL/6NJ	JAX:005304
C57BL/6NJclRbrc	Rbrc
C57BL/6NNIH	(none)
C57BL/6NTac	Tac 760

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Mouse Phenome Database

Mouse Phenome Database
at The Jackson Laboratory

https://phenome.jax.org/

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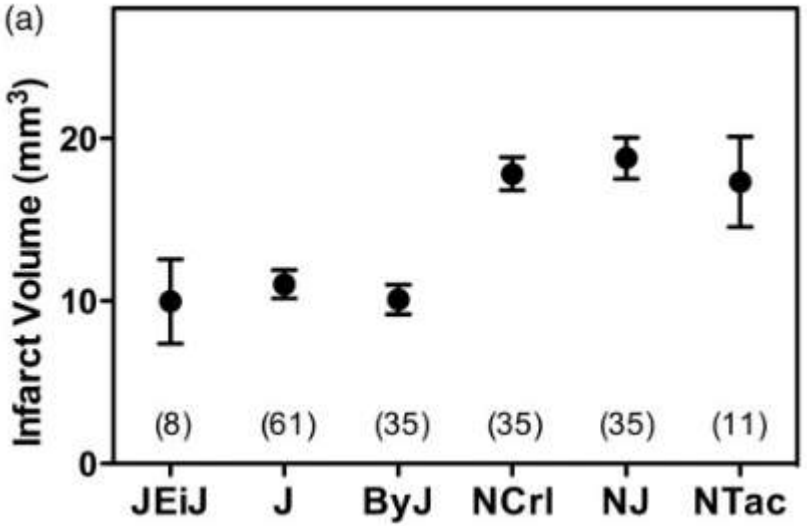
Literature Searches

Modeling Stroke vulnerability in C57BL/6 substrains

- 3 “J” substrains:
  - smaller and fewer infarcts following stroke induction
  - Showed sex skewing – females had smaller infarct sizes compared to males
- 3 “N” substrains
  - Larger infarct sizes
  - No sex skewing



Zhao, A, Mulligan MK, and Nowak T 2019. *JCBFM 39*(3): 426-438. PMID: **29260927**

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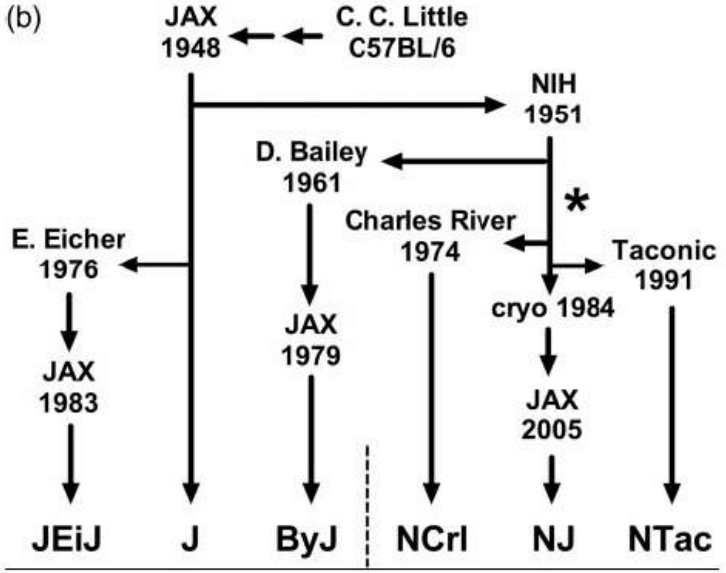

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Know *Your* Substrain: Use Proper Nomenclature

C57BL/6J

Parent strain

C57BL/6NJ

Substrain designation

NIH (N)

By (Dr. Bailey)

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

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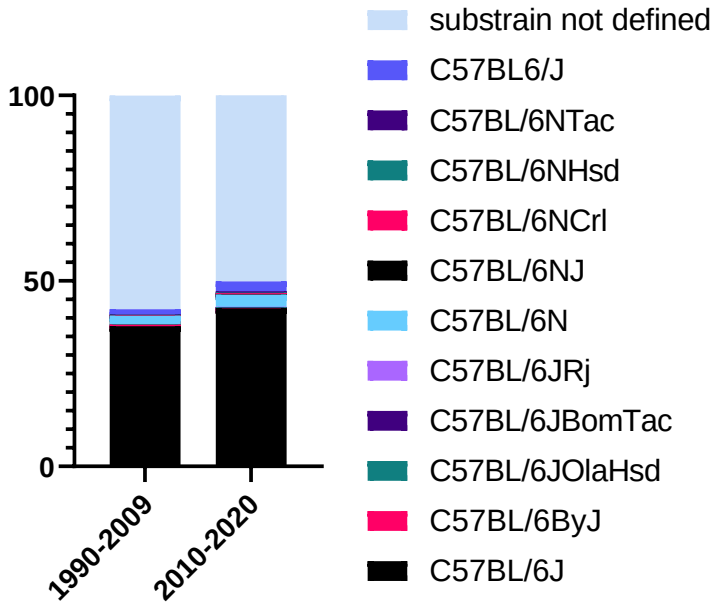
C57BL/6 Publications

Pubmed Search Term	1990-2009	2010-2020
C57BL6	18655	25,546
C57BL/6J	7043	10,893
C57BL/6ByJ	88	21
C57BL/6JOlaHsd	26	43
C57BL/6JBomTac	2	11
C57BL/6JRj	1	28
C57BL/6N	426	834
C57BL/6NJ	0	24
C57BL/6NCrl	16	87
C57BL/6NHsd	18	28
C57BL/6NTac	20	92
C57BL6/J	267	671

Complete & correct nomenclature benefits everyone!



Based on September 2020 PubMed citations search (without limits)

Reproducibility through Accurate Reporting

ARRIVE

The ARRIVE guidelines
Animal Research: Reporting *In Vivo* Experiments

8a	Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight.
8b	Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.

https://arriveguidelines.org/arrive-guidelines/experimental-animals

NIH National Institutes of Health
Turning Discovery Into Health

RIGOR AND REPRODUCIBILITY

animals: report source, species, strain, sex, age, husbandry, inbred and strain characteristics of transgenic animals

https://www.nih.gov/research-training/rigor-reproducibility

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Reproducibility through Accurate Reporting

ARRIVE

The ARRIVE guidelines
Animal Research: Reporting *In Vivo* Experiments

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RIGOR AND REPRODUCIBILITY

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Cell Press STAR★Methods

providing the available and detailed information related to the species, strain and backcrossing status, developmental stage, weight, genotype, health/immune status, drug or test naive, previous procedures, housing, and husbandry.

https://www.cell.com/star-authors-guide

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Considerations for Control Selection

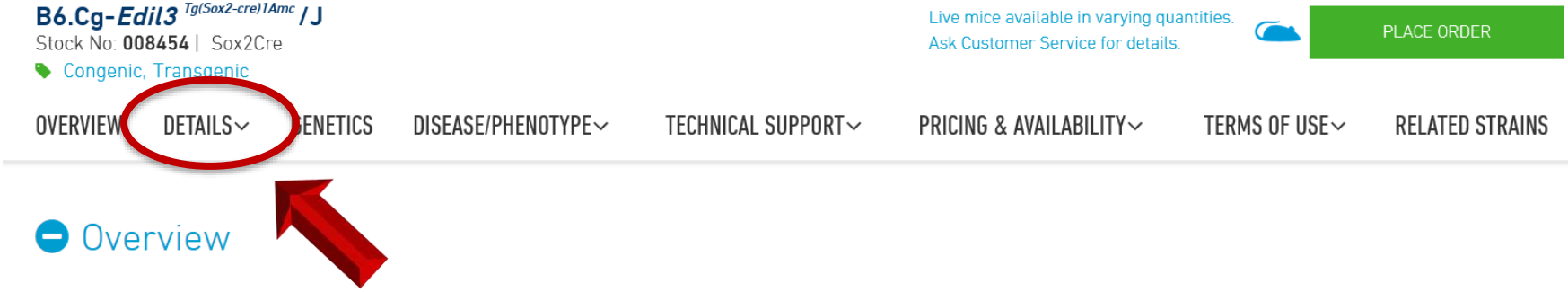
- **Congenic Strains***
  - Littermates (het x het, het x wt, or hemi x wt mating scheme)
    - Wild type or heterozygous for mutant gene or allele
    - Non-carriers of transgene
    - Can also use non-littermate controls from the colony
  - Inbred (hom x hom mating)
    - Match background mutant is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

What Resources Can You Use?

Review Strain Development



What Resources Can You Use?

Review Strain Development

Development

The Sox2Cre transgene was designed with 12.5 kb of upstream regulatory sequence from the mouse *Sox2* locus (SRY-box containing gene 2), a chicken β-actin intron, a Cre recombinase gene, and a rabbit β-globin poly(A) sequence. This transgene was introduced into B6CBAF1 donor eggs. The resulting founder animals were initially crossed to C57BL/6 mice, and then crossed to outbred Swiss Webster mice. The mice were then backcrossed to C57BL/6 for 11 generations (see SNP notes below), and then sent to The Jackson Laboratory Repository.

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, 1 of 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.

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Background Strain Information

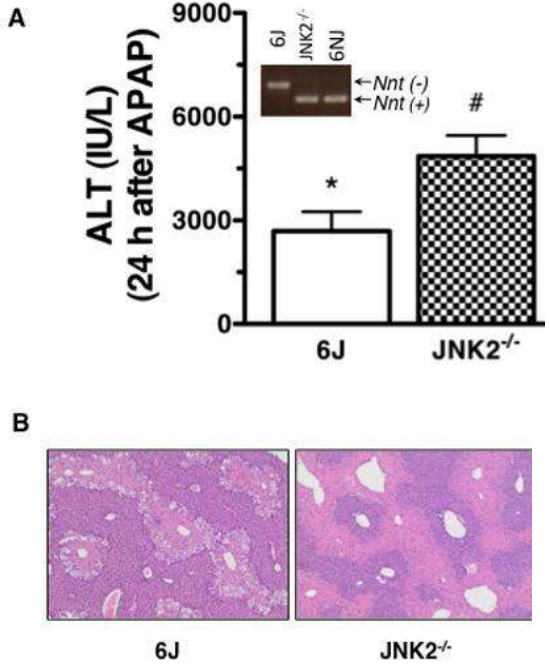
Questions You May Want to Ask

- **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?**

Select the Proper C57BL/6 Control

Avoid Common Research Mistakes

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



Bourdi M et al. 2011. *Chem Res Toxicol* 24: 794-6. PMID:21557537

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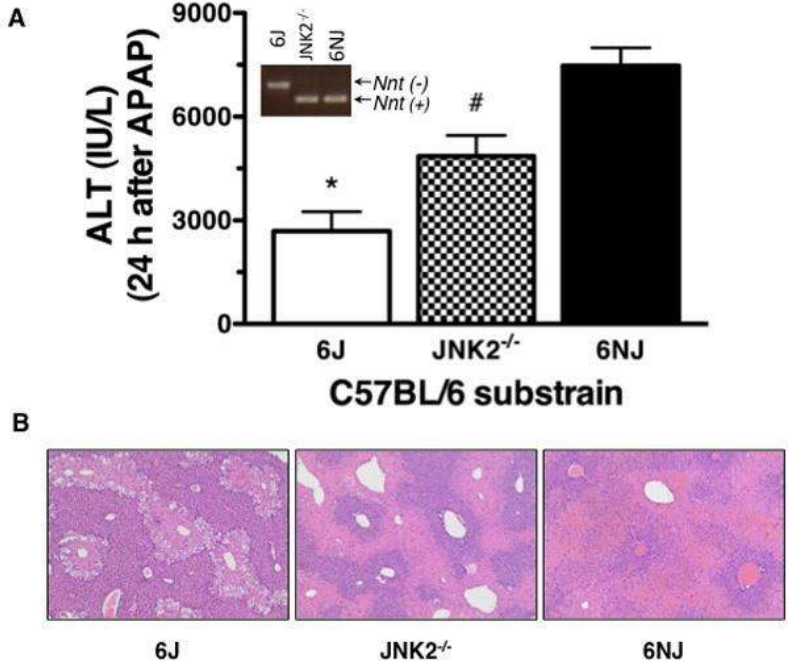

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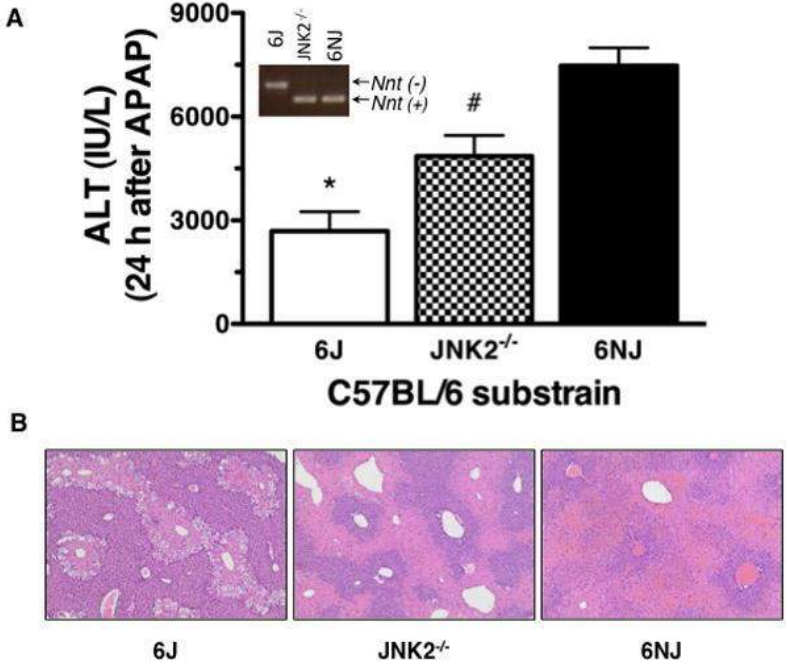

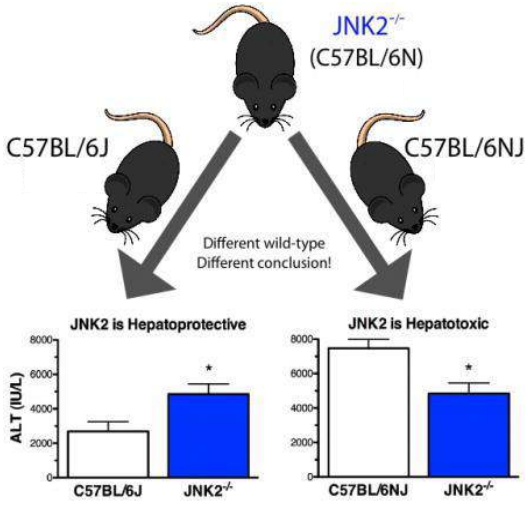

Bourdi M et al. 2011. *Chem Res Toxicol* 24: 794-6. PMID:21557537

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Ensuring Data Validity & Reproducibility

Consider your rodent, your most important reagent

- Choose wisely – “Know thy mouse!”
- Use proper nomenclature and controls
- Minimize genetic drift
- Educate and establish a QC culture

Good science results in reduced animal use

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Upcoming JAX™ Webinars

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

- Efficacy and Safety of Immunomodulatory Therapeutics - Induced Cytokine Release Syndrome
  - Feb 11, 2021, 1:00 PM USA Eastern Time (New York)
- Neuromuscular Platforms for Drug Discovery
  - Feb 18, 2021, 1:00 PM USA Eastern Time (New York)

MiceTech Talks: 15 minute chat sessions with JAX Technical Information Scientists on mouse-based research topics. Join us on YouTube or LinkedIn. Watch past episodes.

The Jackson Laboratory

THANK YOU FOR THE ADVENTURE

At JAX, we enjoy the journey as much as reaching the destination, and we're so happy you joined us.

Authorized JAX™ Mice Distributor in Singapore:

InVivos

Website: www.invivos.com.sg

Tel: +65 6643 8600

Email: enquiries@invivos.com.sg

JAX Mice Technical Support:
micetech@jax.org

JAX B6 substrains at INVIVOS

C57BL/6J	C57BL/6NJ (New**)
JAX stock#000664	JAX stock#005304
Deletion in Nnt gene	No deletion in Nnt gene
	Similar to B6N of Taconic

** Promotional discount of 10% till 31 Mar 2021 for C57BL/6NJ mice

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