TACONIC BIOSCIENCES

B6 Mice for Research: A Guide to Black 6 Mouse Substrains & Applications

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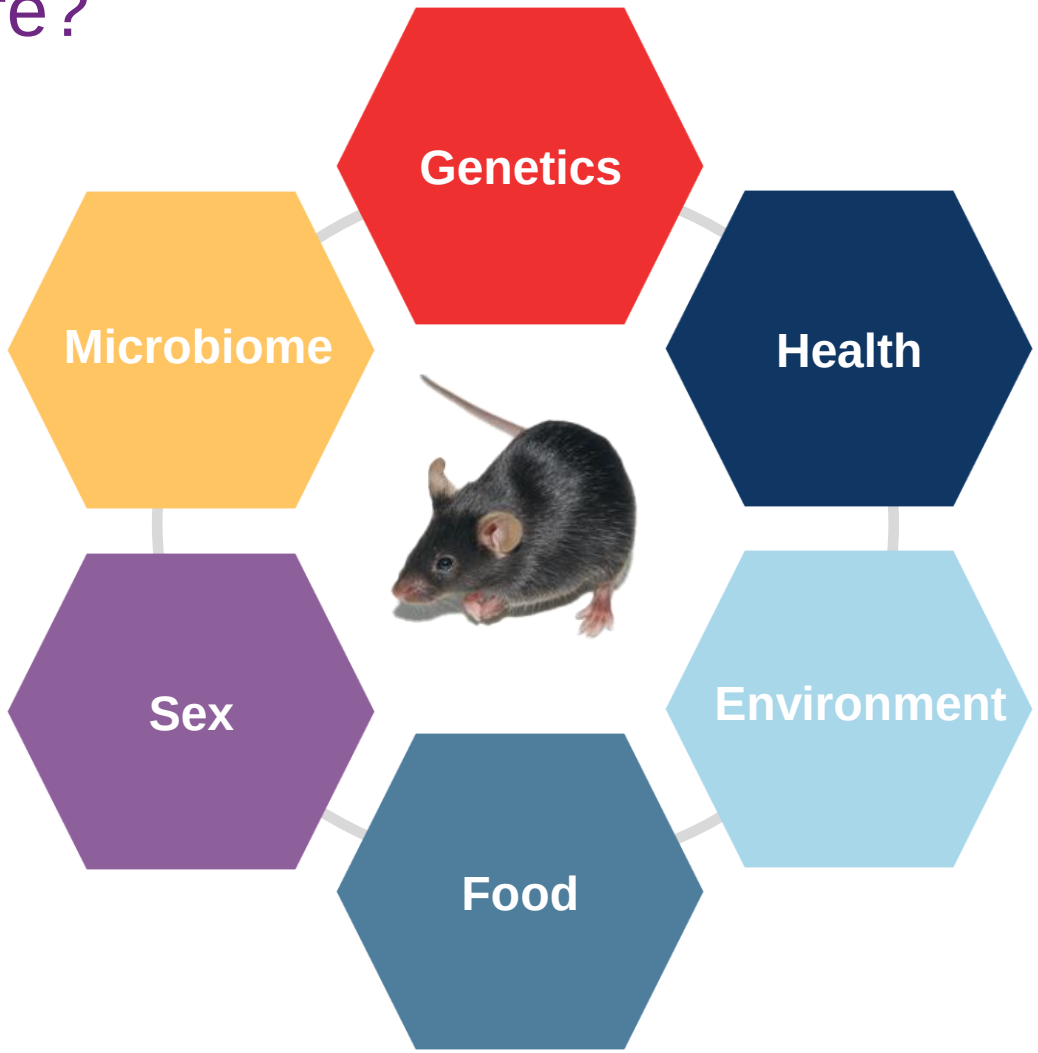
Critical Factors for Mouse Research

Why should you care?

- Model Performance
- Variability
- Reproducibility
- Experimental Validity
- Translational Relevance



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Outline

1. Basic characteristics of C57BL/6 mice
2. Inbred strain considerations
3. Origin of C57BL/6 mice and C57BL/6 substrain diversity
4. C57BL/6 substrain characteristics and implications for study design
5. Implications for genetically engineered mice

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Major Mouse Inbred Strains

• C57BL/6 (B6)

- Preferred in many experimental models
- Metabolism: prone to diet-induced obesity
- Behavior
- Immunology
- Th1-skewed
- Spontaneous tumors

BALB/c

- Preferred in many experimental models
- Metabolism: prone to diet-induced obesity
- Behavior
- Immunology
- Th1-skewed
- Spontaneous tumors

129S6

- Background for many knockouts
- Poor breeder
- Not preferred in many experimental models
- Spontaneous testicular teratomas
- ES cells easily generated

FVB

- Preferred model for generating transgenic mice
- Excellent breeders
- Aggressive males

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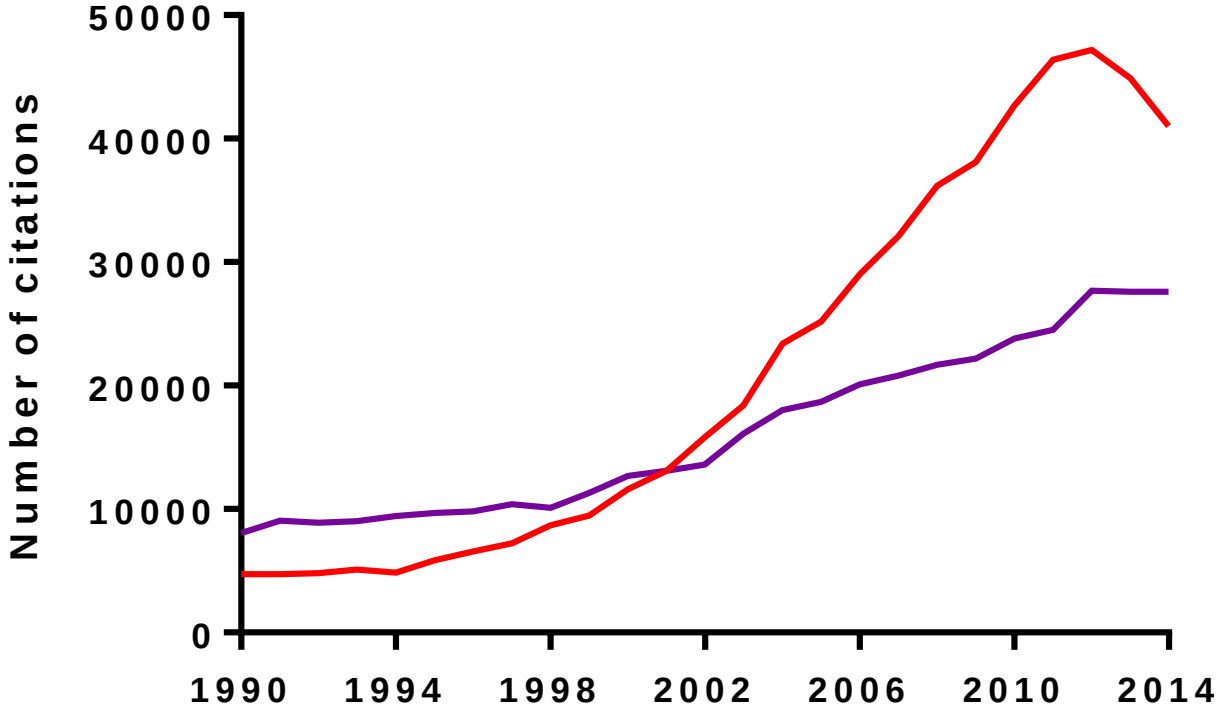
Rise of C57BL/6 in Research



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Common Characteristics of all C57BL/6 Mice

Aggressive males
(not as aggressive as
some strains)

Average parenting behavior
Litter size: 6-8
Breeding lifespan: 10 mo
High fertilization rate for IVF

Well-defined and annotated genome
Large availability of GEMs
on B6 background

Progressive hearing loss
(Cdh23^{ahl})

Prone to weight gain

Preference for alcohol

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Common Health Issues in C57BL/6 Mice

Issue	Frequency*	Preventative/Treatment Options
Aggression	Common (males)	Environmental enrichment Age/Littermates, husbandry controls
Dermatitis	1-26%	Appropriate temperature/humidity Rear toenail clipping Topical ointments
Malocclusion	0.05%	Tooth clipping
Eye Defects (microphthalmia)	2-20%	None
Dystocia	0.05 – 0.8%	Euthanasia
Hydrocephaly	0.05% - 3%	Euthanasia
Tumors Leukemias Lung Mammary	 0-7% 0-3% <1%	None, but useful for research purposes

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What is an inbred mouse?

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Inbred Mice 101

- An inbred strain is a genetically homogeneous lineage of mice that carries a unique combination of genes.
- By definition, an inbred strain is created after full sibling mating for 20 generations.
- **NOTE: A specific inbred strain is often treated as genetically-identical individuals, but this is not technically true!**

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Inbred Mice 101

Advantages

- Genetic homogeneity may reduce variation and improve reproducibility
- Inbred strains may have advantageous characteristics
- Simplifies the interpretation of studies with genetically engineered mice
- Breeding schemes to maintain inbred are more practical than outbred

Disadvantages

- Genetic homogeneity may limit translatability
- Inbred strains may have inherent characteristics that limit their applications
- Genetic drift may change an inbred strain over time

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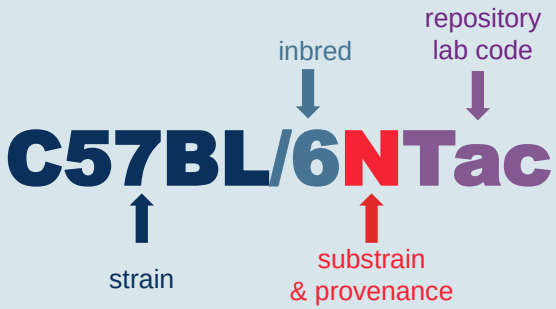
Inbred Mouse Nomenclature

Abbreviation	Full Designation
C57BL/6, B6	
BALB/c	BALB/cAnNTac
129	129S6/SvEvTac

Key Notes

- Abbreviations are ambiguous
- Genetic background explicit in full designation

Lab code registry: https://www.nationalacademies.org/ilar/lab-code-database

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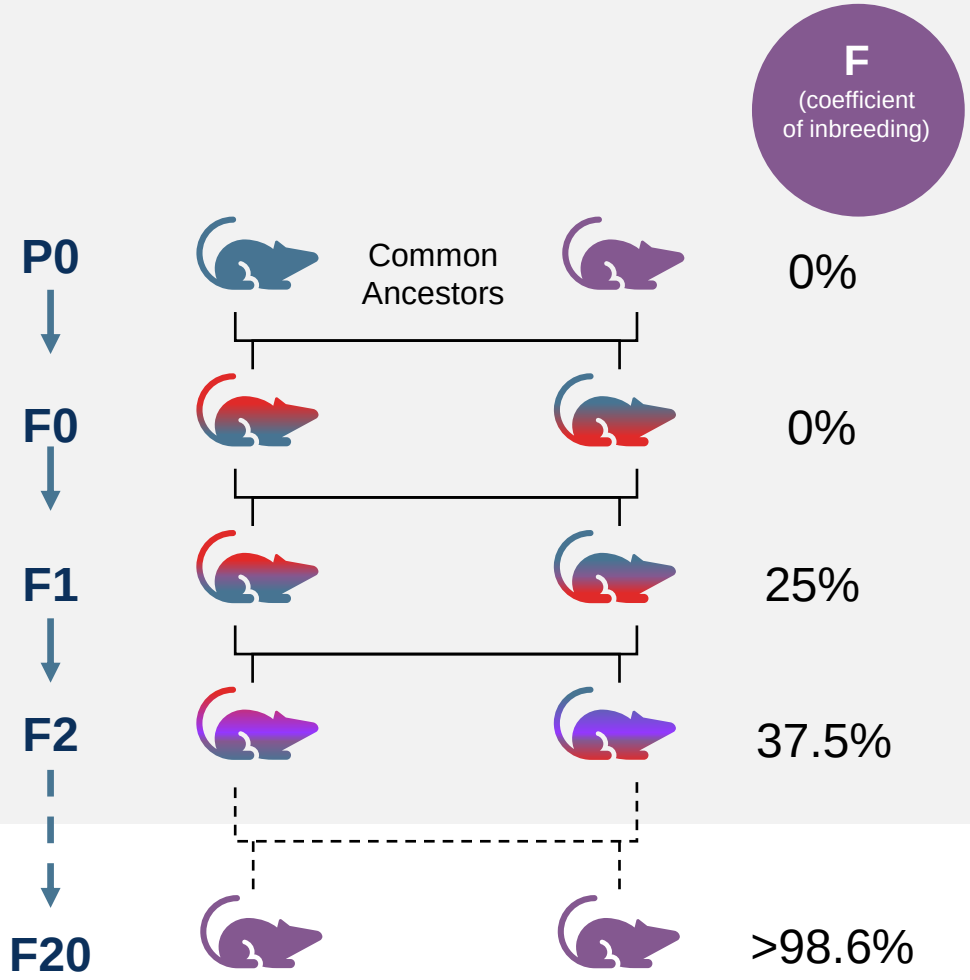
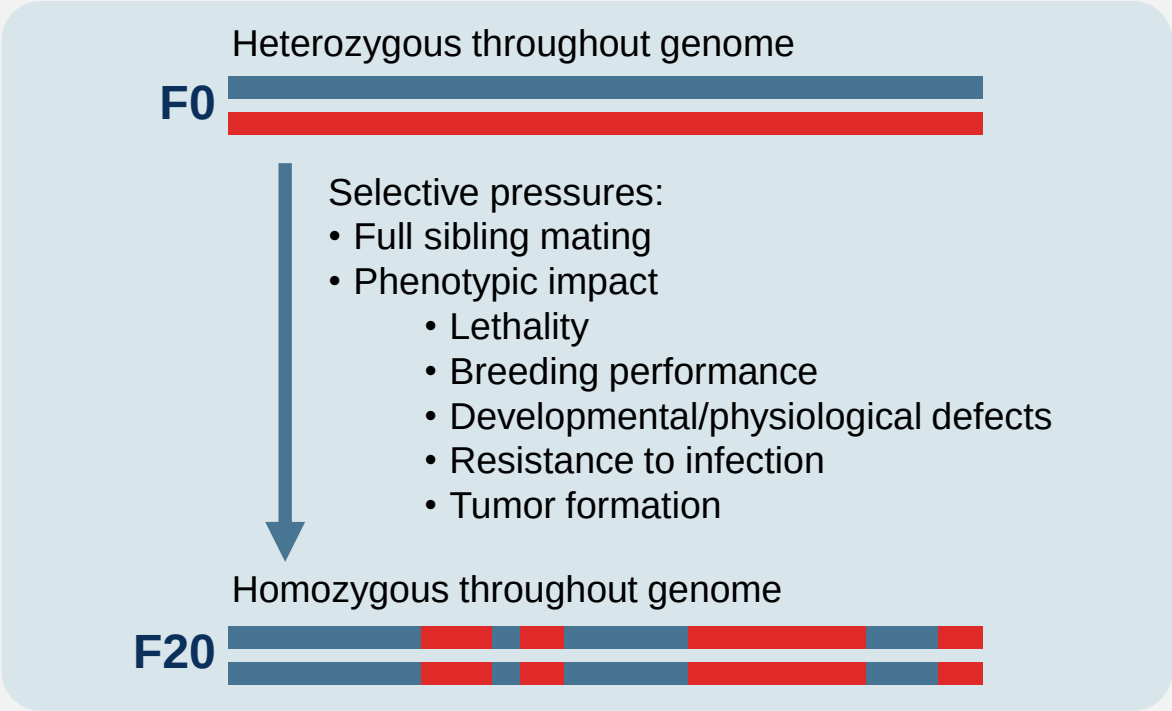
Origin of Inbred Mice



- What occurs at the DNA level during inbreeding?



- What occurs at the population level during inbreeding?
- Alleles will tend to become homozygous in all mice at which point they become “fixed”

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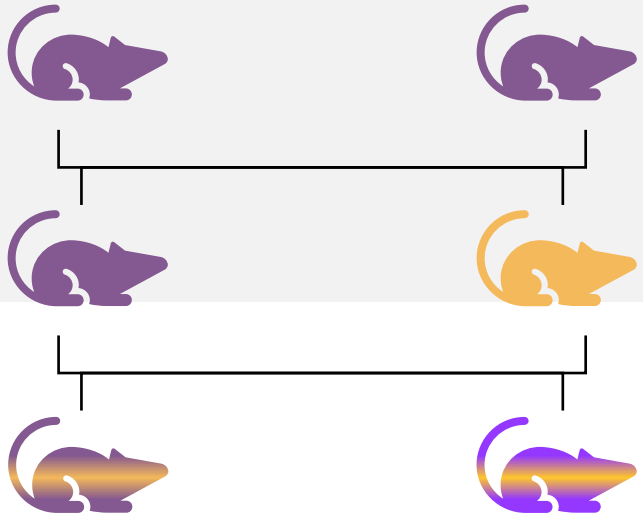
Sources of De Novo Genetic Variation in Inbred Mice

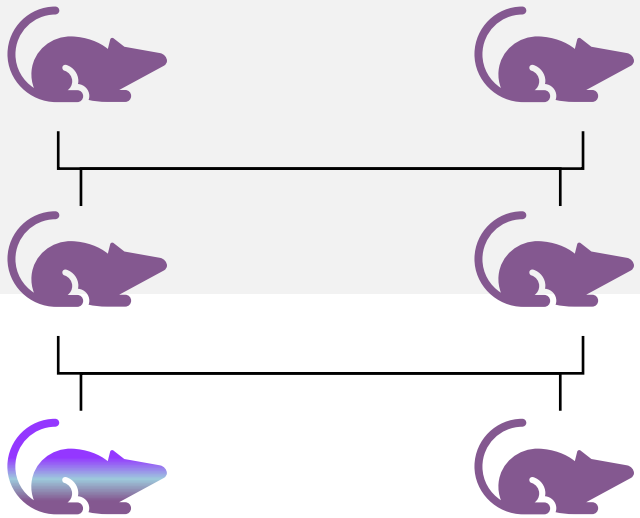
Avoidable

- Contamination of breeding stock
- Crossbreeding to other strains, even if similar
- Retroviral infections

Unavoidable

- Spontaneous substitutions, insertions, deletions, inversions, translocations, copy number variants
- Spontaneous genetic variants can and do occur in both somatic and germ cells but only germ cells variants are passed to subsequent generations





Estimated mutation rate: 7.9×10^{-9} per nucleotide per generation (Heredity (Edinb) 2021;126(1):107-116)

→ Approximately 20/generation given a genome of 2.7×10^{9} bp

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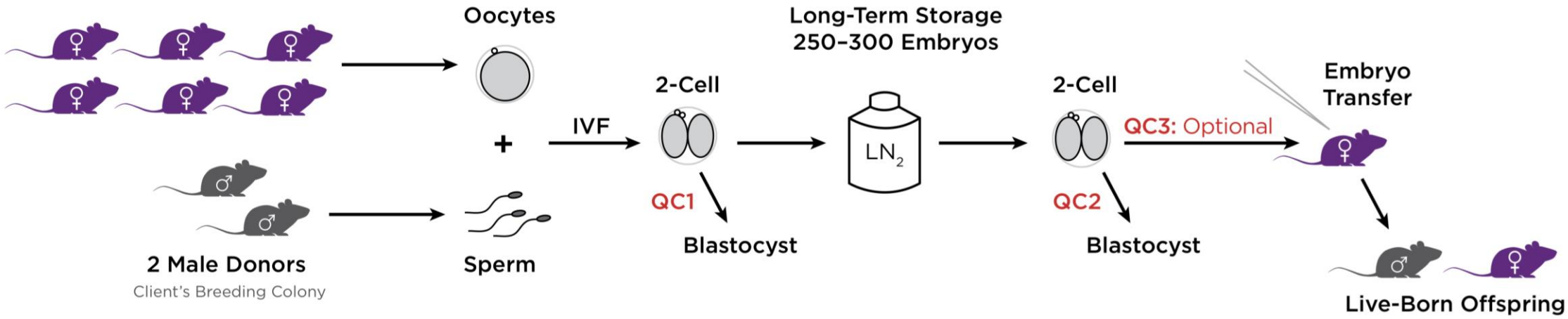
Cryopreservation to Reset the Genetic Clock

- Cryopreserved embryos represent a snapshot in time for a given strain
- Used as a genetic resource to effectively reset the generation number of inbred strains
- May reduce the chances for genetic drift of an inbred strain over time
- This has been a standard practice since 1980's



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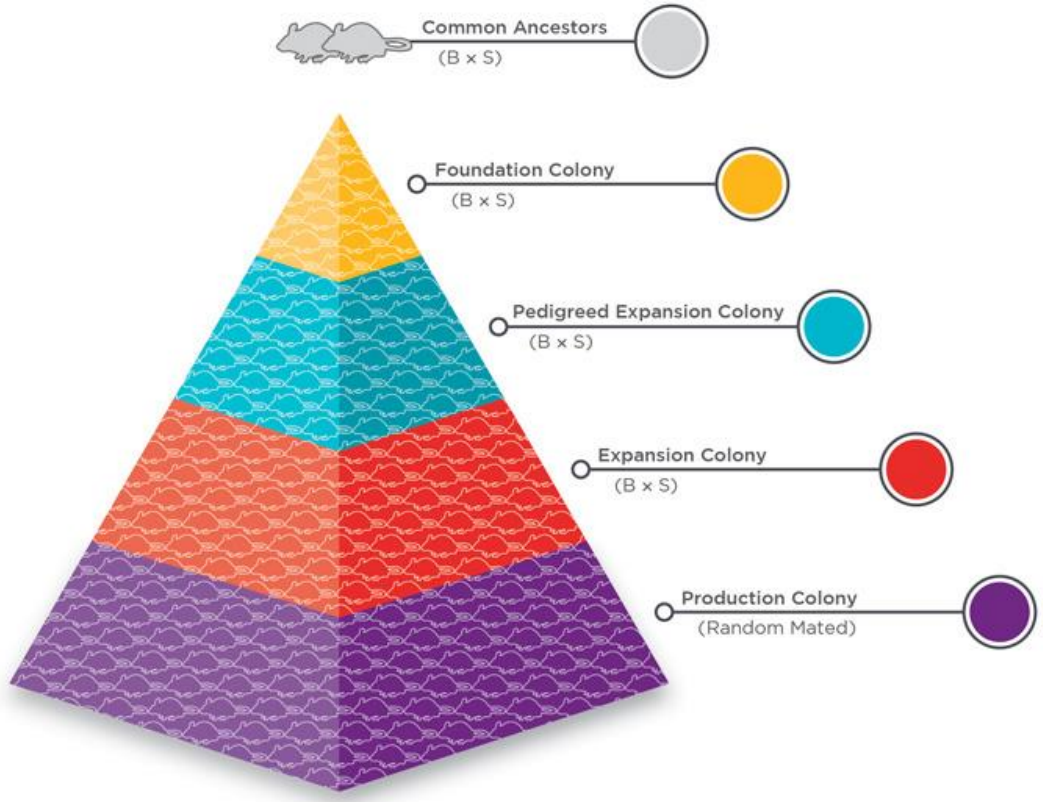
Maintaining Inbred Colonies

- Pedigrees need be carefully tracked and managed to maintain inbred status and produce mice for your studies
- Foundation or nucleus colony:
  - Main function is to propagate the strain
  - Source or common ancestors for all animals of the strain and cryopreserved embryos
  - Should be replenished from cryo on regular basis to avoid genetic drift over time
- Expansion and production colonies
  - Populated from the foundation colony
  - Sized to meet demand and managed to limit genetic distance from foundation



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Testing For Genetic Background

Single Nucleotide Polymorphism (SNP) Panels

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ACCTGCAGATCAGTACAGTCA  C57BL/6NTac
ACCTGCAGAACAGTACAGTCA  BALB/cAnNTac
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SPECIFIC PATTERNS OF BASE PAIR SUBSTITUTIONS ARE CHARACTERISTIC FOR EACH STRAIN

SNP Panel	Application
Mouse GenMon MiniMUGA panel	Mouse genetic background monitoring
Mouse Substrain Panel	Differentiating mouse substrains
Mouse MiniMUGA-Bckgrd panel	Mouse genetic background characterization
Mouse MiniMUGA-SpdCon panel	Mouse genetic speed congenics
Mouse GenMon MiniMUGA panel	Mouse genetic background monitoring

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Inbred Strain Genetic Variation Revealed by SNP Testing

Mouse MiniMUGA panel (8896 SNPs)

	C57BL/6NTac	C57BL/6JBomTac	C57BL/6J	129S6/SvEvTac	BALB/cAnNTac	BALB/cJ	BALB/cJBomTac	CBA/J	CB17SC	CBSCBG	C3H/HeNTac	DBA/1JBomTac	DBA/2NTac	FVB/NTac	NOD/MrkTac	SJL/JCrNTac
C57BL/6NTac	X	71	171	2659	2652	2748	2685	2660	2630	2618	2811	2619	2696	2526	2512	2419
C57BL/6JBomTac	71	X	178	2676	2672	2768	2705	2678	2650	2637	2829	2636	2713	2544	2533	2437
C57BL/6J	171	178	X	2769	2769	2865	2802	2777	2747	2734	2928	2735	2812	2644	2634	2536
129S6/SvEvTac	2659	2676	2769	X	2767	2798	2735	2433	2762	2756	2578	2453	2500	2417	2429	2375
BALB/cAnNTac	2652	2672	2769	2767	X	208	148	2010	21	26	1934	2471	2450	2373	2251	2207
BALB/cJ	2748	2768	2865	2798	208	X	150	2071	214	215	1995	2530	2509	2447	2357	2344
BALB/cJBomTac	2685	2705	2802	2735	148	150	X	2007	154	155	1932	2468	2446	2386	2294	2282
CBA/J	2660	2678	2777	2433	2010	2071	2007	X	2009	2007	808	1659	1670	2258	2213	2271
CB17SC	2630	2650	2747	2762	21	214	154	2009	X	5	1927	2464	2443	2370	2244	2199
CBSCBG	2618	2637	2734	2756	26	215	155	2007	5	X	1925	2456	2435	2364	2234	2194
C3H/HeNTac	2811	2829	2928	2578	1934	1995	1932	808	1927	1925	X	1949	1886	2405	2369	2351
DBA/1JBomTac	2619	2636	2735	2453	2471	2530	2468	1659	2464	2456	1949	X	503	2446	2370	2420
DBA/2NTac	2696	2713	2812	2500	2450	2509	2446	1670	2443	2435	1886	503	X	2509	2339	2412
FVB/NTac	2526	2544	2644	2417	2373	2447	2386	2258	2370	2364	2405	2446	2509	X	1786	1392
NOD/MrkTac	2512	2533	2634	2429	2251	2357	2294	2213	2244	2234	2369	2370	2339	1786	X	1848
SJL/JCrNTac	2419	2437	2536	2375	2207	2344	2282	2271	2199	2194	2351	2420	2412	1392	1848	X

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Inbred Strain Genetic Variation Revealed by SNP Testing

Mouse MiniMUGA panel (8896 SNPs)

	C57BL/6NTac	C57BL/6JBomTac	C57BL/6J	129S6/SvEvTac	BALB/cAnNTac	BALB/cJ	BALB/cJBomTac	CBA/J	CB17SC	CBSCBG	C3H/HeNTac	DBA/1JBomTac	DBA/2NTac	FVB/NTac	NOD/MrkTac	SJL/JCrNTac
C57BL/6NTac	X	71	171	2659	2652	2748	2685	2660	2630	2618	2811	2619	2696	2526	2512	2419
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BALB/cAnNTac	2652	2672	2769	2767	X	208	148	2010	21	26	1934	2471	2450	2373	2251	2207
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NOD/MrkTac	2512	2533	2634	2429	2251	2357	2294	2213	2244	2234	2369	2370	2339	1786	X	1848
SJL/JCrNTac	2419	2437	2536	2375	2207	2344	2282	2271	2199	2194	2351	2420	2412	1392	1848	X

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Origin of C57BL/6 Mice & C57BL/6 Substrain Diversity

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C57BL/6 Origins

- Originally derived from Abbie Lathrop's mice in Granby, MA (early 1900's)
- C.C. Little used stock from Lathrop to establish the C57BL/6 line between 1921 and 1937
- All C57BL/6 and C57BL/10 mice are direct descendants of ♀C57 and ♂C52 from Lathrop's stock
- The strains derive their name from ♀C57 and coat color

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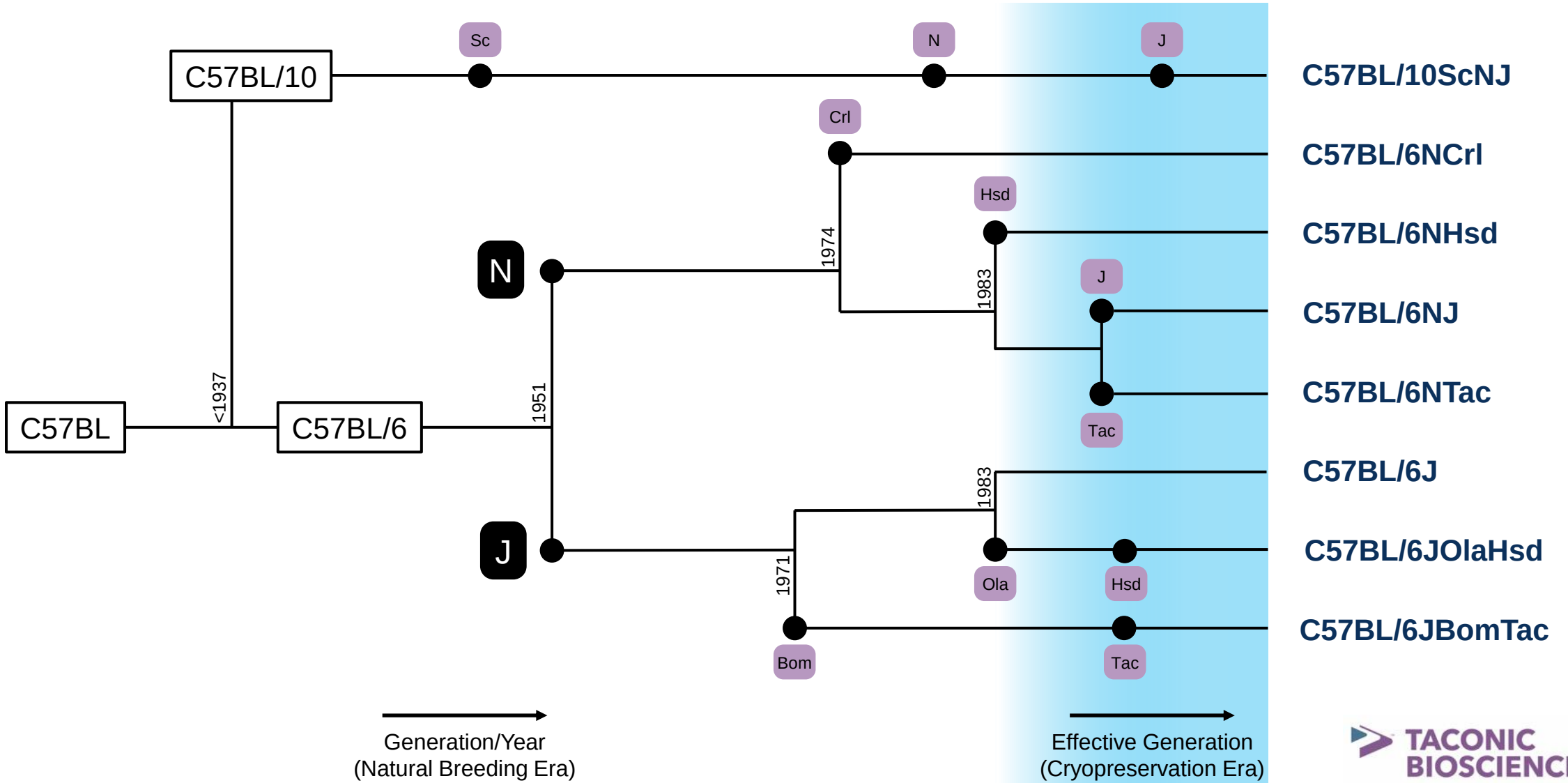
The C57BL/6 Family Tree



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Testing for C57BL/6 Substrains via SNP Analysis

MiniMUGA - C57BL/6 Substrains													
	C57BL/6ByJ	C57BL/6J	C57BL/6JBomTac	C57BL/6JEiJ	C57BL/6JJicTac	C57BL/6JOlaHsd	C57BL/6JRj	B6N-Tyr<c-Brd>/BrdCrCrl	C57BL/6NCrl	C57BL/6NHsd	C57BL/6NJ	C57BL/6NRj	C57BL/6NTac
C57BL/6ByJ	X	143	43	30	142	54	172	20	29	5	52	23	28
C57BL/6J	143	X	178	113	11	183	0	155	172	148	195	166	171
C57BL/6JBomTac	43	178	X	65	177	89	207	61	72	48	95	66	71
C57BL/6JEiJ	30	113	65	X	112	70	142	47	59	35	82	53	58
C57BL/6JJicTac	142	11	177	112	X	182	30	154	171	147	194	165	170
C57BL/6JOlaHsd	54	183	89	70	182	X	212	72	83	59	106	77	82
C57BL/6JRj	172	0	207	142	30	212	X	184	201	177	224	195	200
B6N-Tyr<c-Brd>/BrdCrCrl	20	155	61	47	154	72	184	X	3	23	70	31	46
C57BL/6NCrl	29	172	72	59	171	83	201	3	X	26	73	33	49
C57BL/6NHsd	5	148	48	35	147	59	177	23	26	X	47	19	23
C57BL/6NJ	52	195	95	82	194	106	224	70	73	47	X	52	50
C57BL/6NRj	23	166	66	53	165	77	195	31	33	19	52	X	5
C57BL/6NTac	28	171	71	58	170	82	200	46	49	23	50	5	X

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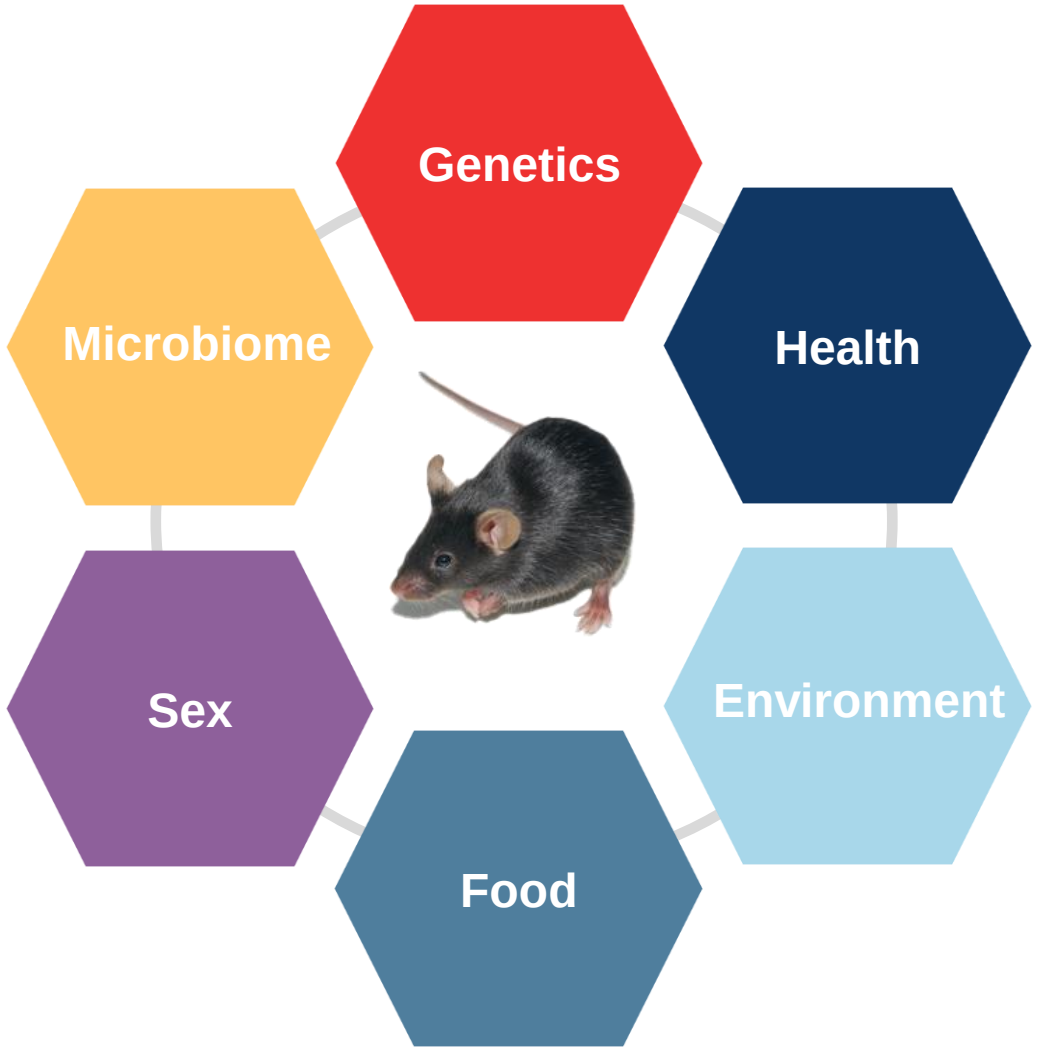
A Caveat About Comparing Different C57BL/6 Substrains

- Genetics isn’t everything
- Association **vs.** causation
- Substrains reported in literature are not always specified or accurate
- Mice bred without proper genetic control may not reflect the entire substrain



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Summary of Fixed Genetic Variants in C57BL/6 Substrains with Demonstrated Phenotypic Impact

	C57BL/6NTac	C57BL/6NHsd	C57BL/6NCrl	C57BL/6J	C57BL/6JBomTac	C57BL/6JOlaHsd	Phenotypic Impact (selected references)
Nnt $^{C57BL/6J}$	No	No	No	Yes	No	Yes	Impaired insulin secretion, redox abnormalities (1, 2)
Nlrp12 $^{C57BL/6J}$	No	No	No	Yes	Yes	Yes	Impaired innate immune response (3, 4)
Snca deletion	No	No	No	No	No	Yes	Neurodevelopmental abnormalities (5)
Y chromosome (Del(Y)1Tac)	No	No	No	No	Yes	No	Sperm abnormalities and increased female:male ratio at birth (6)
Crb1 ^{rd8}	Yes	Yes	Yes	No	No	No	Mild retinal degeneration (7-10)
Cyfip2 ^{M1N}	Yes	Yes	Yes	No	No	No	Impaired response to amphetamines (11)

References:

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9. Invest Ophthalmol Vis Sci. 2012 Jun 1;53(7):3764-5.
10. Invest Ophthalmol Vis Sci. 2012 May 17;53(6):2921-7.
11. Science. 2013 Dec 20;342(6165):1508-12.

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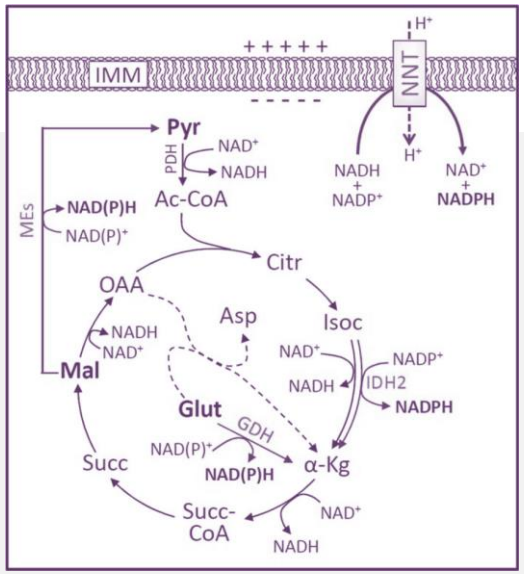
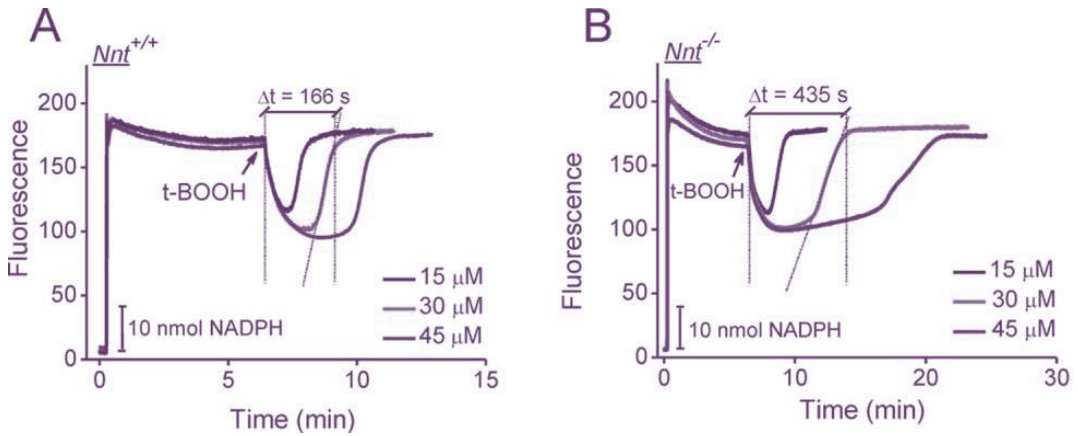
C57BL/6J Genetic Variants–*Nnt*

Nicotinamide Nucleotide Transhydrogenase (NNT)

- NNT is an inner mitochondrial membrane protein
- Regulates mitochondrial redox potential by reducing $NADP^+$ to NADPH while oxidizing NADH
- $Nnt^{C57BL/6J}$ –deletion of exons 7 to 11 leads to absence of mature protein
- Absence of *Nnt* linked to dysfunctional:
  - Responses to hypoxia/ischemia
  - Carbohydrate metabolism
  - Insulin secretion
  - Cardiovascular function
  - Immune function





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Nnt Deletion Impairs Response to Infection

Nicotinamide Nucleotide Transhydrogenase (NNT)

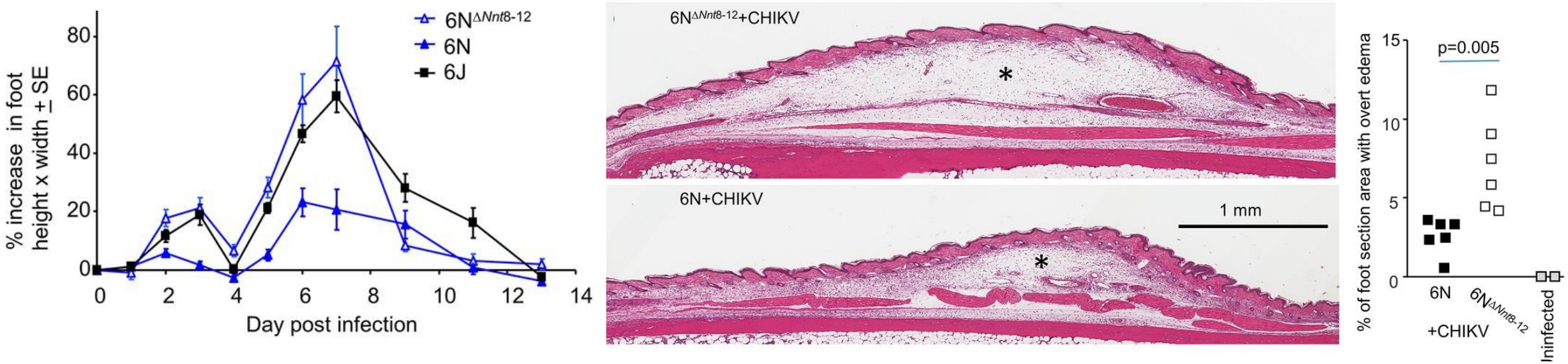

Figure from: Rawle DJ, et al. Widespread discrepancy in Nnt genotypes and genetic backgrounds complicates granzyme A and other knockout mouse studies. Elife. 2022 Feb 4;11:e70207. doi: 10.7554/eLife.70207. Licensed under CC BY 4.0 (www.creativecommons.org/licenses/by/4.0/).

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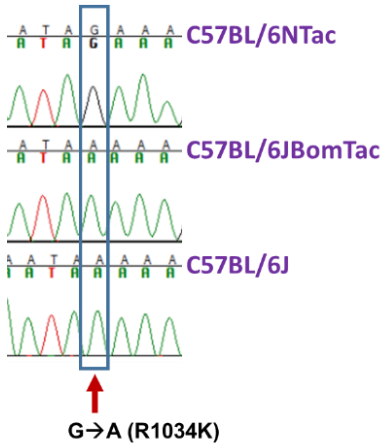
C57BL/6N Genetic Variants–*Crb1*^{rd8}

Crb1 (crumbs family member 1, photoreceptor morphogenesis associated)

- Cytoplasmic innate immune sensor regulates inflammasomes, immune cell trafficking and cytokine production
- *Nlrp12*$^{C57BL/6J}$ – G→A substitution in all C57BL/6J mice leading to Arg→Lys in ligand binding LRR domain
- Associated with impaired chemokine production, immune cell infiltration and pathogen clearance (Nat Commun. 2016 Oct 25;7:13180)
- Effect of mutation phenocopied in *Nlrp12*$^{-/-}$ mice



C57BL/6N	K	L	R	V	L	W	L	F	G	M	D	L	N	R	K	T	H	R	R	M	A	A
129P2/OlaHsd	K	L	R	V	L	W	L	F	G	M	D	L	N	R	K	T	H	R	R	M	A	A
129S1/SvImJ	K	L	R	V	L	W	L	F	G	M	D	L	N	R	K	T	H	R	R	M	A	A
129S5SvEvBrd	K	L	R	V	L	W	L	F	G	M	D	L	N	R	K	T	H	R	R	M	A	A
A/J	K	L	R	V	L	W	L	F	G	M	D	L	N	R	K	T	H	R	R	M	A	A
AKR/J	K	L	R	V	L	W	L	F	G	M	D	L	N	R	K	T	H	R	R	M	A	A
BALB/cJ	K	L	R	V	L	W	L	F	G	M	D	L	N	R	K	T	H	R	R	M	A	A
C3H/HeJ	K	L	R	V	L	W	L	F	G	M	D	L	N	R	K	T	H	R	R	M	A	A
CBA/J	K	L	R	V	L	W	L	F	G	M	D	L	N	R	K	T	H	R	R	M	A	A
DBA/2J	K	L	R	V	L	W	L	F	G	M	D	L	N	R	K	T	H	R	R	M	A	A
FVB/NJ	K	L	R	V	L	W	L	F	G	M	D	L	N	R	K	T	H	R	R	M	A	A
LP/J	K	L	R	V	L	W	L	F	G	M	D	L	N	R	K	T	H	R	R	M	A	A
NOD/ShiLtJ	K	L	R	V	L	W	L	F	G	M	D	L	N	R	K	T	H	R	R	M	A	A
NZO/HlLtJ	K	L	R	V	L	W	L	F	G	M	D	L	N	R	K	T	H	R	R	M	A	A
PWK/PhJ	K	L	R	V	L	W	L	F	G	M	D	L	N	R	K	T	H	R	R	M	A	A
C57BL/6J	K	L	R	V	L	W	L	F	G	M	D	L	N	K	K	T	H	R	R	M	A	A
														1034								

Source: Taconic internal data

C57BL/6J Genetic Variants – *Nlrp12*

NLRP12 (Nod-like receptor pyrin domain containing 12)

- Mutations in human *CRB1* cause early-onset retinal degeneration and complete loss of vision
- *Crb1*^{rd8} (retinal degeneration 8)–recessive truncation mutation expressed in all C57BL/6N substrains
- Impact of mouse rd8 mutation:
  - Responses to hypoxia/ischemia
  - Carbohydrate metabolism
  - Insulin secretion
  - Cardiovascular function
  - Immune function

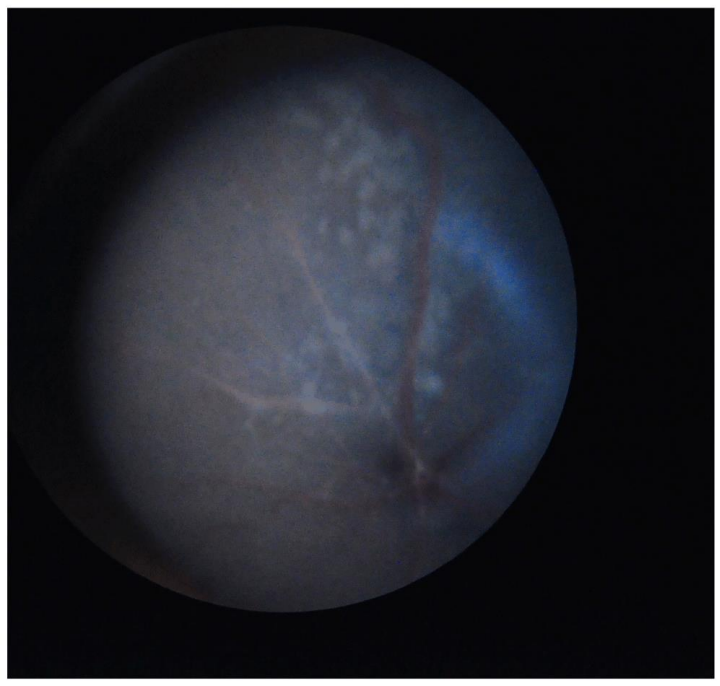


Photo from: Concas D, et al. A scoring system for the evaluation of the mutated Crb1/rd8-derived retinal lesions in C57BL/6N mice. F1000Res. 2017 Mar 31;6:404. doi: 10.12688/f1000research.11252.1. Licensed under CC BY 4.0 (www.creativecommons.org/licenses/by/4.0/).

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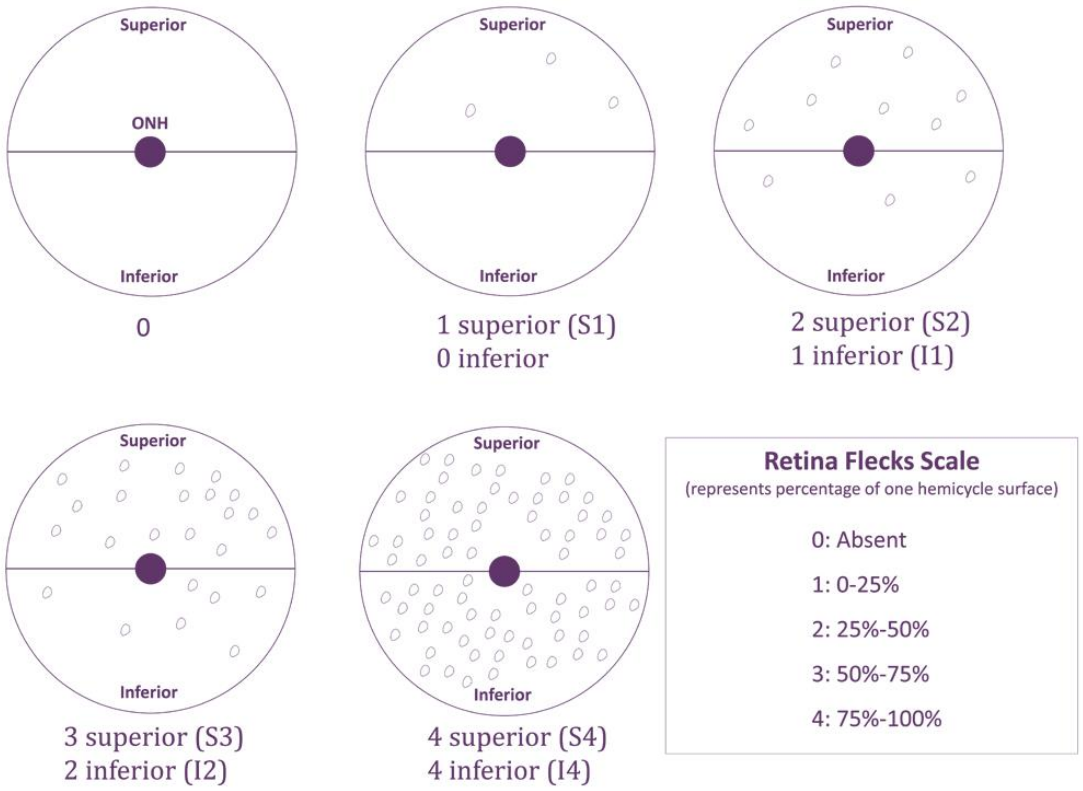
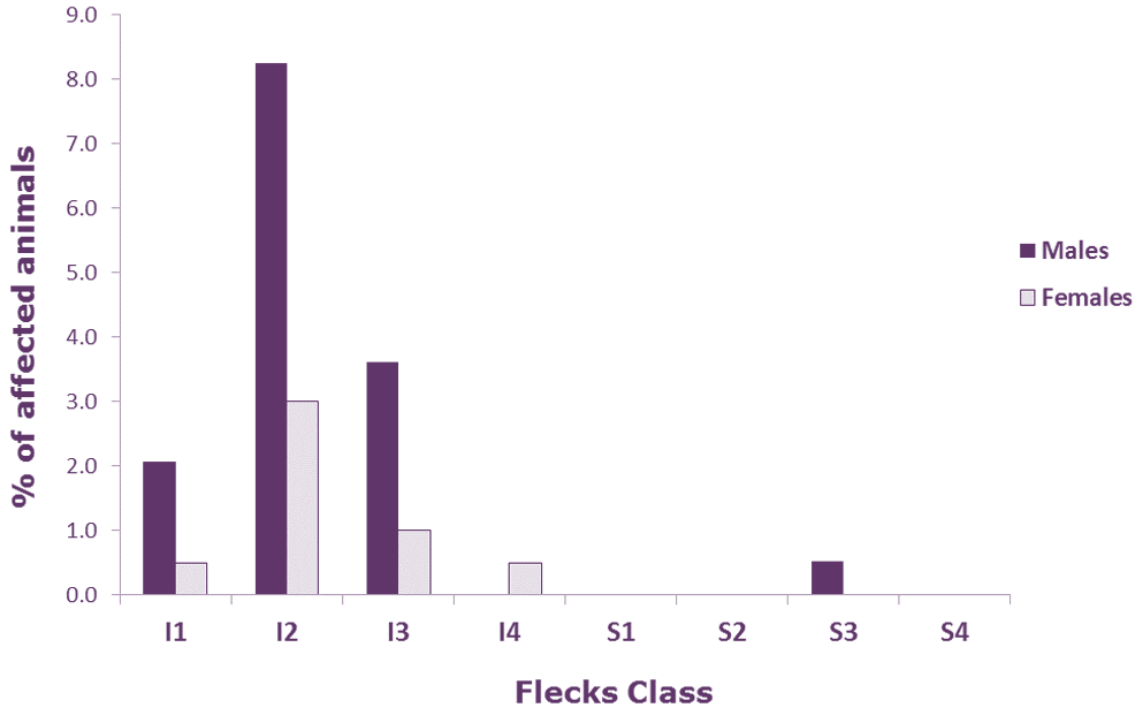
Retinal Phenotype in C57BL/6NTac Mice





Figures from: Concas D, et al. A scoring system for the evaluation of the mutated Crb1/rd8-derived retinal lesions in C57BL/6N mice. F1000Res. 2017 Mar 31;6:404. doi: 10.12688/f1000research.11252.1. Licensed under CC BY 4.0 (www.creativecommons.org/licenses/by/4.0/).

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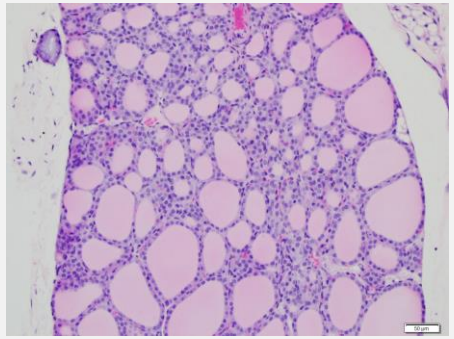
C57BL/6NTac Genetic Variant—*Tg*$^{tdys\text{-}Tac}$

Tg–Thyroglobulin

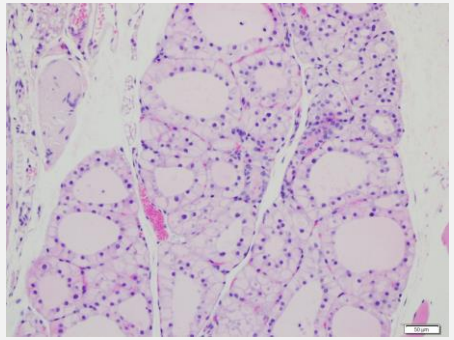
- Thyroglobulin is the most abundant thyroid protein and is the precursus to thyroid hormones, T3 and T4
- Tg$^{tdys\text{-}Tac}$ (Thyroid Dysplasia—Taconic) is a LINE-1 insertion in intron 25 of the Tg gene, which causes skipping of exon 26, resulting in omission of 64 amino acids from the TG protein product



WT-–normal

Impact of the *LINE-1*

- Congenital thyroid follicular epithelial cell dysplasia in HET and HOM animals that progresses to hyperplasia and thyroid adenoma in HOM animals at 9 months of age
- Mutant TG is instable and accumulates in the ER of the thyroid follicular epithelial cells
- Thyroid hormone levels are affected in HET and HOM animals at 12 months of age, but not at earlier ages
- No body weight or other physiological abnormalities associated with the LINE-1 insertion
- The LINE-1 was removed from the B6NTac colonies



HOM-–dysplasia

Bailey, Smits et al., under review

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C57BL/6 Substrain Characteristics and Implications for Study Design

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Immunology of C57BL/6 Substrains

- No known differences in cell surface markers or MHC alleles between C57BL/6 substrains
- Transplants (cell or tissue) between C57BL/6 substrains are typically not an issue
- Genetic considerations for C57BL/6J substrains:
  - *Nlrp12*$^{C57BL/6J}$ mutation:
    - Impaired innate immune function in C57BL/6J substrains may be related to altered ligand binding
    - Impaired chemokine production, neutrophil chemotaxis and pathogen clearance
    - Effect of mutation phenocopied in *Nlrp12*$^{-/-}$ mice
  - *Nnt*$^{C57BL/6J}$ mutation:
    - Increased macrophage ROS production and *S. pneumoniae* clearance (FASEB J. 2012 Aug;26(8):3550-62.)
    - Altered cytokine responses to viral infection (Elife. 2022 Feb 4;11:e70207.)

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Syngeneic Tumor Studies

C57BL/6-derived tumor

C57BL/6 recipient

C57BL/6-derived Tumor Cell Lines
(word size represents relative number of tumors developed)

- B6 substrains share histocompatibility and will accept tumor grafts from other B6 substrains
- The recipient substrain may have unique immune responses to implanted tumors or the tumor microenvironment

Tumor Cell Line	Reported Strain	Strain Determined by SNP
B6-206	C57BL/6J	C57BL/6J
B16-F1	C57BL/6J	C57BL/6N
B16-F10	C57BL/6N	C57BL/6N
MC38	C57BL/6N	C57BL/6N
BPC4	C57BL/6N	C57BL/6N

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Metabolic Considerations for C57BL/6 Mice

- Preferred mouse model for metabolism studies
- C57BL/6 mice susceptible to:
  - High fat diet-induced obesity, insulin-resistance and atherosclerosis
  - Streptozotocin (STZ)-induced diabetes
  - Atherogenic diet-induced atherosclerosis
  - Diet-induced NAFLD/NASH
  - Wealth of C57BL/6-background genetic models

Taconic DIO C57BL/6NTac

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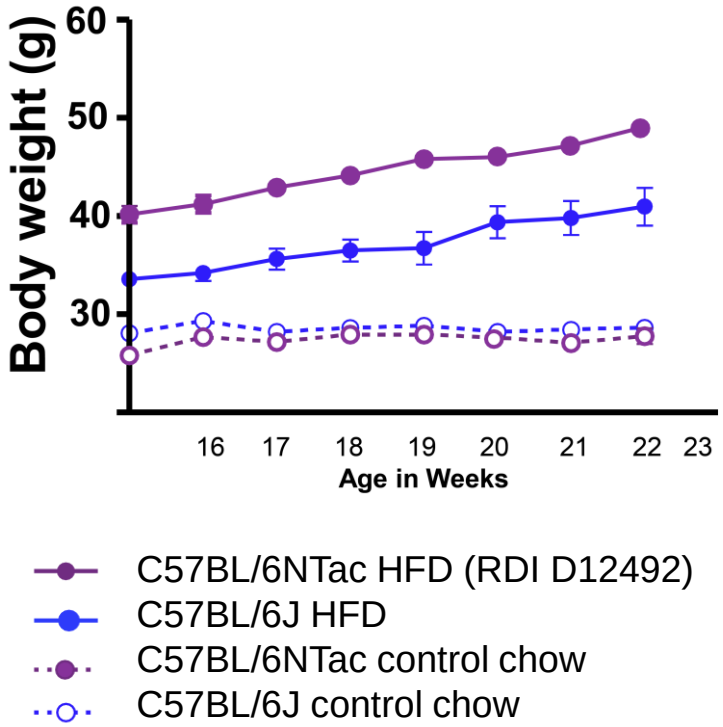
Metabolic Differences Between C57BL/6 Substrains

- C57BL/6N substrains may be preferred for metabolic studies:
  - Accelerated weight gain on high fat diet vs C57BL/6J
  - Weight gain and fat accumulation can be enhanced in C57BL/6JRj by crossing to C57BL/6NTac (Horm Metab Res. 2020 Dec;52(12):877-881.)
  - Enhanced first and second phase insulin secretion but similar incretin effect (Mol Metab. 2014 Dec; 3(9): 848–854)
  - Enhanced glucose stimulated insulin secretion (GSIS) from isolated islets (J Endocrinol. 2017 Apr;233(1):53)



Source: Internal Taconic data

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Considerations for Genetically Engineered Mice on C57BL/6 Background

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Generating GEMs with C57BL/6 ES Cells

- GEMs are now routinely created on a multitude of genetic backgrounds due to the ease of using CRISPR
- The majority of ES cell projects will produce mice with a C57BL/6N background unless crossed with other strains
  - C57BL/6N-derived ES cells are predominant and used exclusively by the International Mouse Phenotyping Consortium (IMPC)
  - C57BL/6J-derived ES cells are challenging to produce and use for unknown reasons

IMPC Release 19.1 (July 6, 2023):

- Number of phenotyped genes: 8,483
- Number of phenotyped mutant lines: 9,135
- Number of phenotype calls: 102,926

ES Cell Line	Background
JM8	C57BL/6NTac
JM8.F6	C57BL/6NTac
JM8.N4	C57BL/6NTac
JM8A3	C57BL/6NTac
JM8A3.N1	C57BL/6NTac
JM8A1.N3	C57BL/6NTac
C2	C57BL/6NTac
D4	C57BL/6NTac
WB6d	C57BL/6NTac
VGB6	C57BL/6NTac
iTL IC1	C57BL/6NTac
WB6a	C57BL/6NTac
WB6b	C57BL/6NTac

A chimera demonstrating presence of C57BL/6NTac-derived ES cells

Source: https://www.mousephenotype.org

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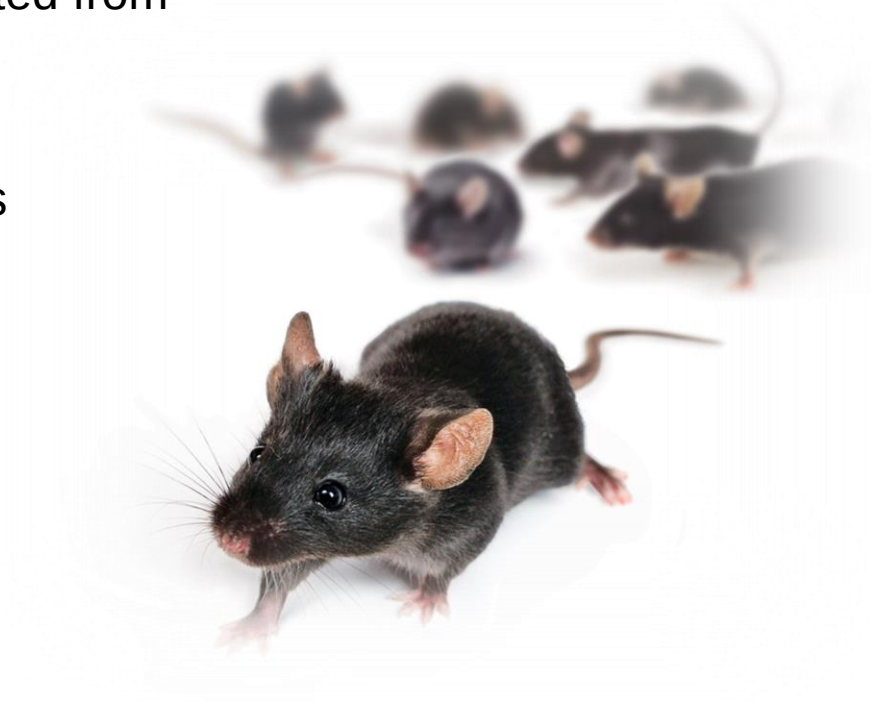
Avoiding Genetic Heterogeneity in C57BL/6-based GEMs

- Consider strain and substrain suitability during model generation
- Confirm genetic background (including C57BL/6 substrain) for all novel lines, even if created from known ES cell sources, via SNP panel
- Use caution when crossing different lines or choosing controls
- Avoid genetic drift by cryopreservation or backcrossing to defined inbred strains



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Questions?

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