TACONIC

Survival Guide: Maintaining Genetic Integrity in Breeding Colonies

ICLAS

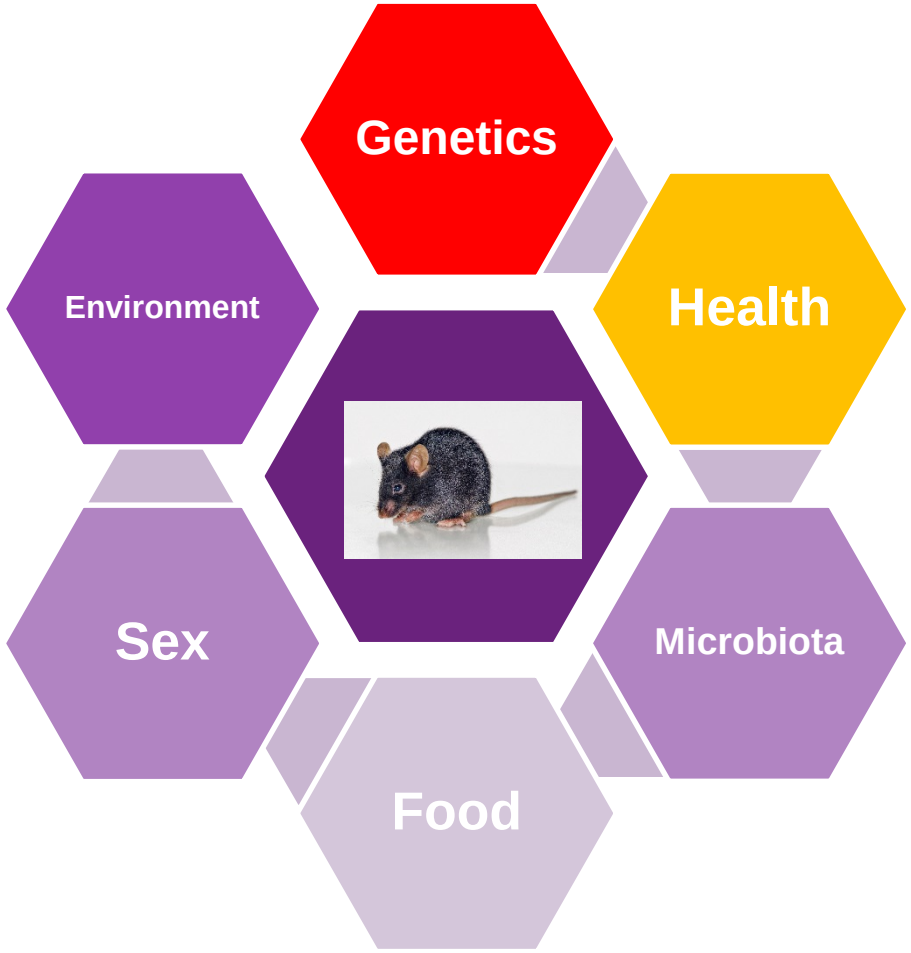
GENETIC SCIENCES & COMPLIANCE

ANA V. PEREZ, Ph.D.

Objectives of this talk

- Importance of Genetic Quality
- Importance of Genetic background
  - Inbreds
    - Development of Congenic Strains
  - Outbreds
    - Maintaining allelic heterozygosity
- Importance of Genetic Monitoring in Genetically Modified Animals
  - Different techniques to generate GEMS
    - Genetic Quality checks- Sequencing
  - Genetic Background Testing
    - SNP Testing, microsatellite markers
    - Establishing an internal genetic monitoring program
    - Considerations of mixed backgrounds
  - Genotyping
    - Test for all alleles
  - Choosing the “Right” Experimental Control

Parameters influencing *In Vivo* Research



Needed Validity and Reproducibility in Science Research

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Using the mouse to model human disease: increasing validity and reproducibility

Monica J. Justice[1,*] and Paraminder Dhillon[2]

Disease Models & Mechanisms (2016) 9, 101-103 doi:10.1242/dmm.024547

Reproducibility in Science

Improving the Standard for Basic and Preclinical Research

C. Glenn Begley, John P.A. Ioannidis

(*Circ Res*. 2015;116:116- 126. DOI: 10.1161/CIRCRESAHA.114.303819.)

The Researchers' View of Scientific Rigor—Survey on the Conduct and Reporting of *In Vivo* Research

Thomas S. Reichlin, Lucile Vogt, Hanno Würbel*

Division of Animal Welfare, Veterinary Public Health Institute, Vetsuisse Faculty, University of Bern, Bern, Switzerland

PLOS ONE | DOI:10.1371/journal.pone.0165999 December 2, 2016

What is Genetic Quality and its Importance

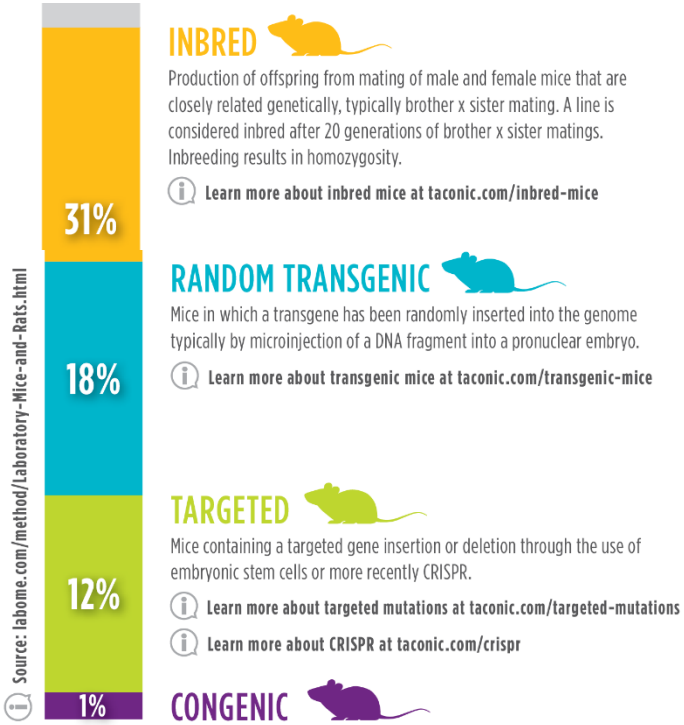
The Preservation of Genetic Purity

Reproducibility

Accuracy

Reliability

Types of Mouse Models



Genetic Characteristics of Inbreds

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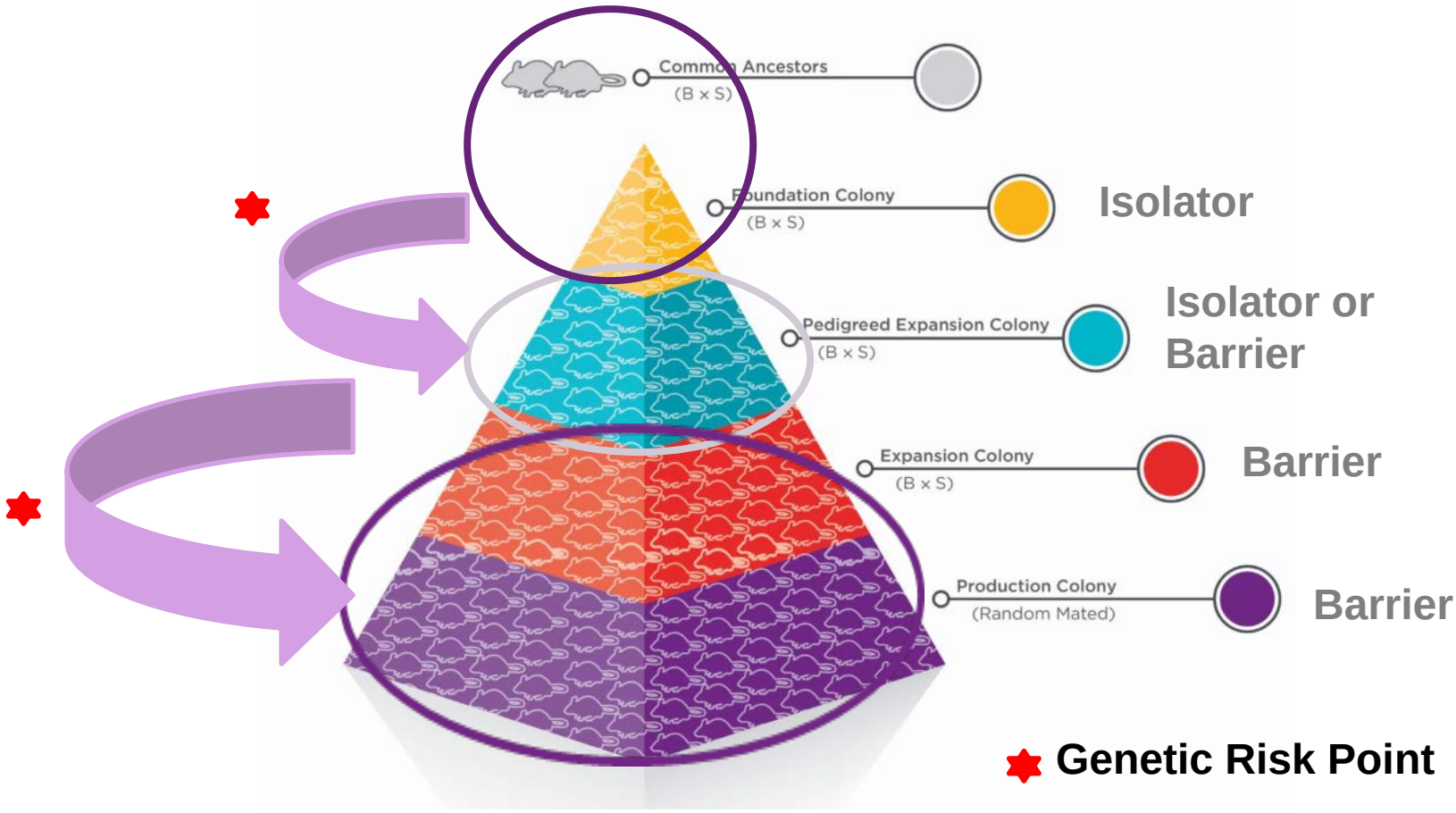
- Used for their homogenous background
- The International Committee on Standardized Nomenclature defines an inbred strain as a colony maintained brother by sister mating for 20 generations
- Inbreds are genetically identical to each other
- Inbreds are homozygous at any locus across their entire genome (HH or hh)

How does Taconic safeguard Genetic Quality of Inbreds

- Genetic refresh from cryopreserved embryos every five years
  - Avoids Genetic Drift
- Reestablish the line from a Common Ancestor pair
- Characterized Genetic Profile
- Global Colony harmonization
  - Different sites are sourced from a single Foundation Colony
- Global Genetic Monitoring Program
  - At Genetic Risk Points

The Inbred Breeding Pyramid



Genetic Characteristics of Outbreds

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- A population of different individuals- Genetic Variability
- Hybrid vigor: long life spans, high disease resistance, early fertility, large and frequent litters, low neonatal mortality, rapid growth, and large size
- Often allows maintenance of mutations that will not be able to maintain in an inbred strain
- Useful in a population studies- toxicity, etc
- Breeding goal is to maximize diversity

How does Taconic Safeguard Genetic Quality of Outbreds

- Genetic refresh from cryopreserved embryos every five to seven years
- Characterized Genetic Profile of stock
- Breeding using Poiley system
  - Number of Groups
  - Number of cages/group
- Global Colony harmonization
  - Outsourced colonies from same stock
- Global Genetic Monitoring Program
  - Monitoring allelic heterozygosity is consistent in all colonies

Different Origins of Genetically Modified Models

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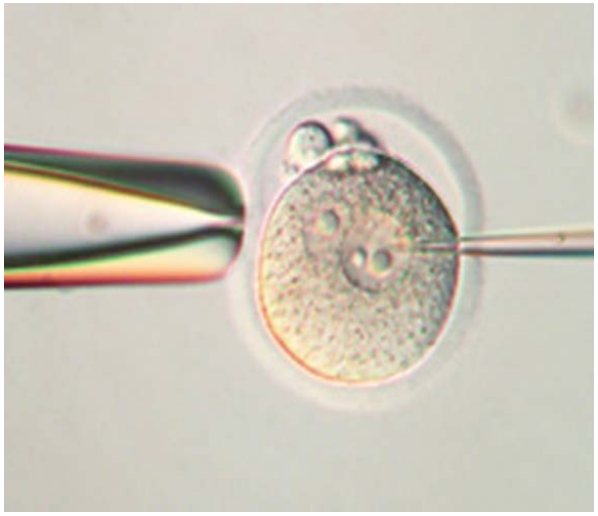
- Pronuclear Injections
- Homologous Recombination
- CRISPR/Cas9 Models

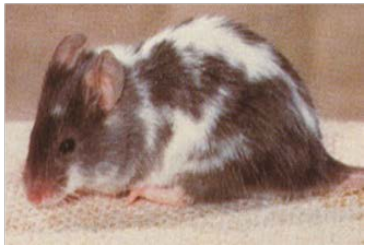
Pronuclear Injections



B6;SJL Tg(APPSWE)2576Kha

- Microinjected DNA inserts anywhere in the genome
- Each Founder is an independent line
- Number of copies of transgene is random
- Make sure that breeding follows Mendelian Segregation
- Find site of insertion
- Determine the stability of the transgene
- Genetic Background

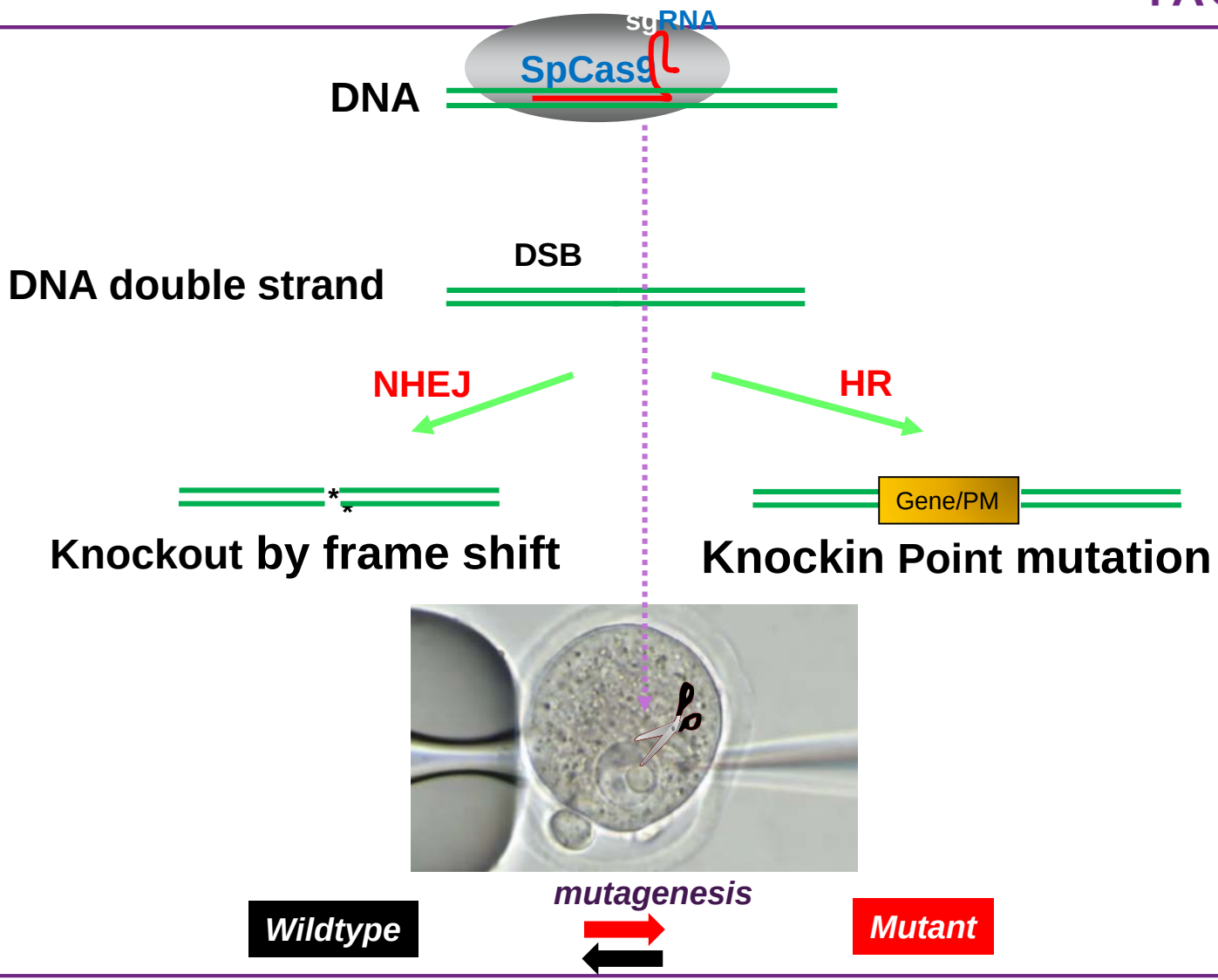
Targeted Mutations by Homologous Recombination



B6.129P2- *Apoe*$^{tm2(APOE*3)Mae}$

- Precise DNA insertion in the genome
- Maintain each ES clone separately
- Genetic Background
- When breeding mutation to Homozygosity –maintain HET x HET
- Cryopreserve

Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)/Caspase 9

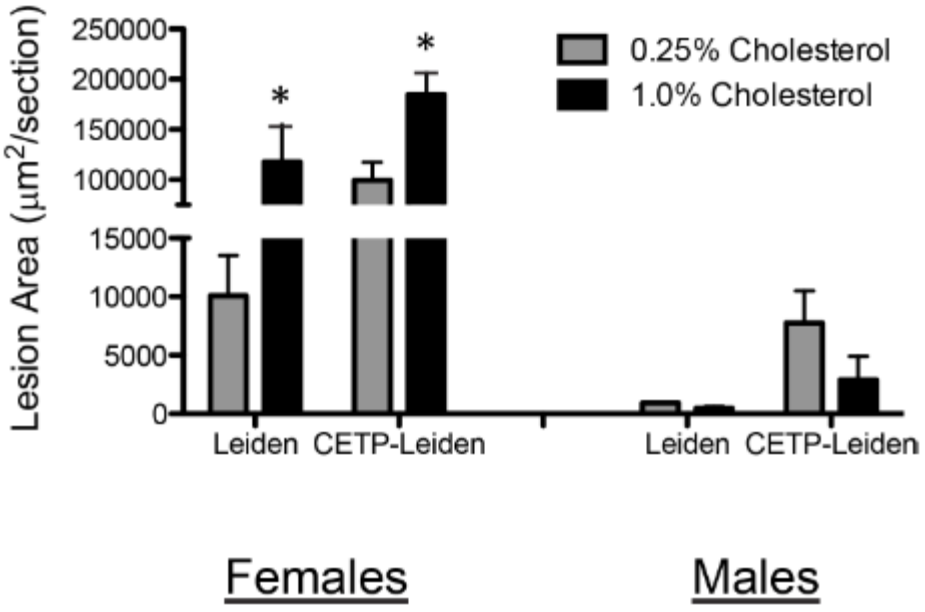


CRISPR/Cas9

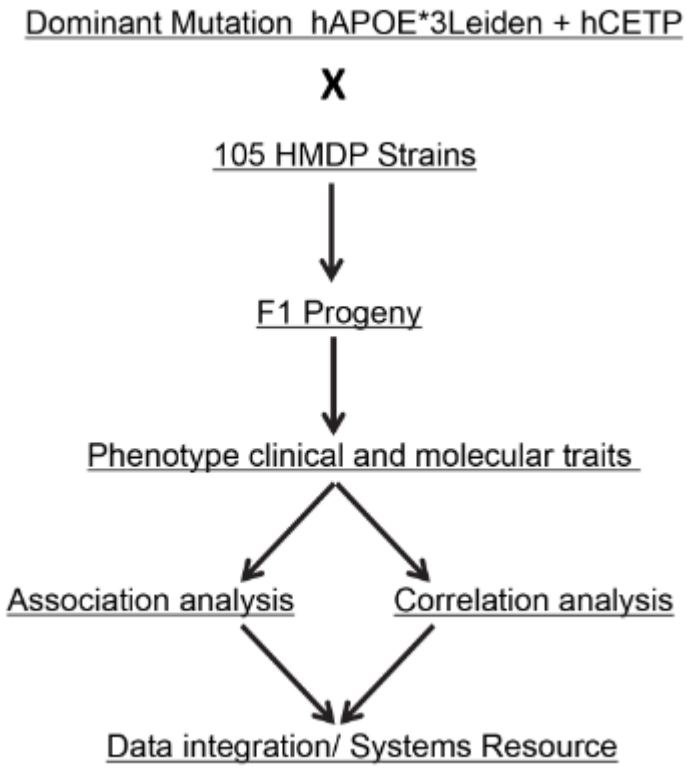
C57BL/6-*Apela*$^{em1.1Hadj}$

- Each Founder (Fo) is an independent line
- Sequencing of founders to verify mutation
- Fo should be mated with inbred strain to verify Mendelian segregation
- N1 generation should be sequenced and verified
- Genetic Background

Genetic Background influence on Phenotype of the mutation

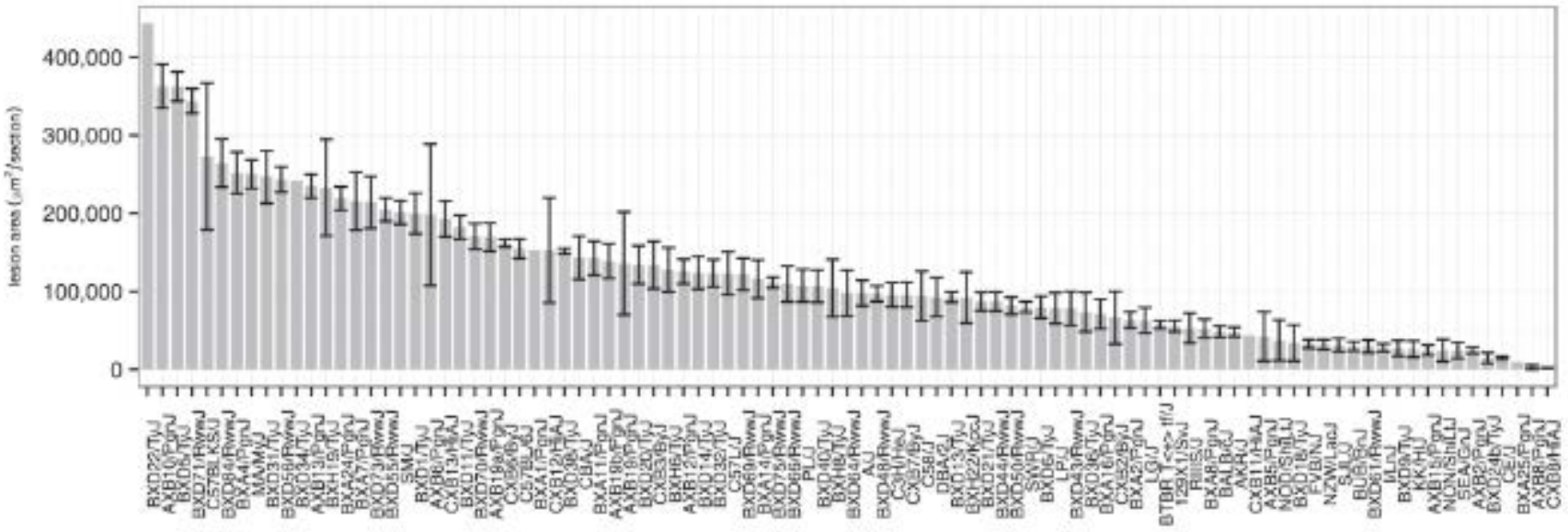


PLOS Genetics | DOI:10.1371/journal.pgen.1005711 December 22, 2015

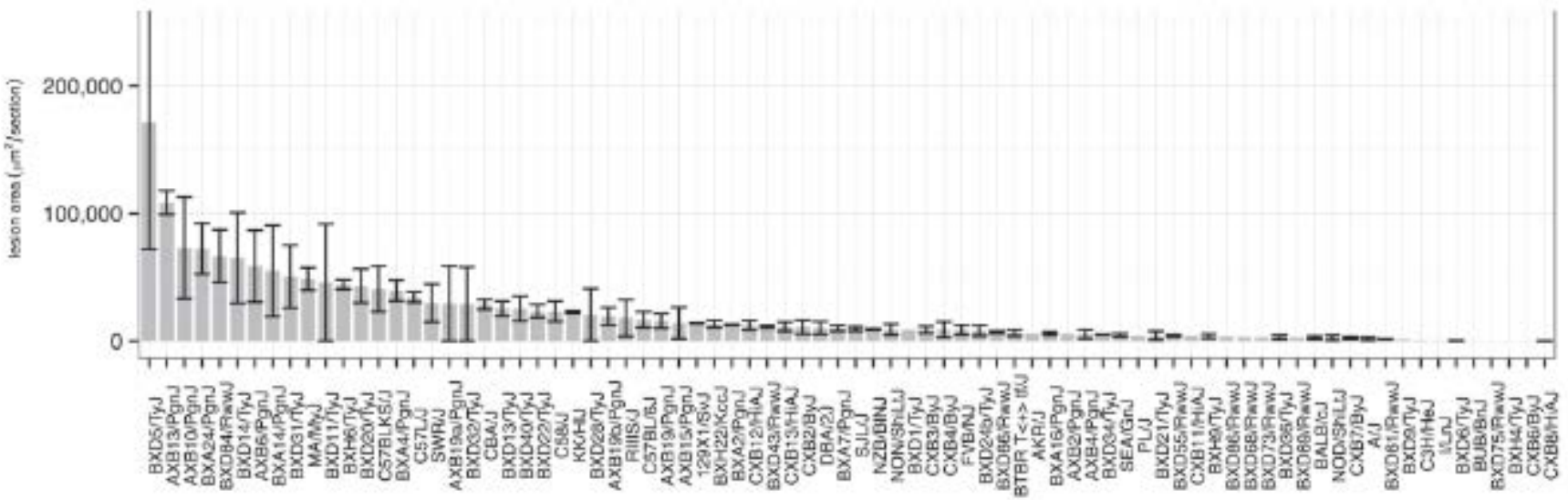


Genetic Background effects on Atherosclerosis Phenotype of Female mice

Artherosclerotic Lesion Size in proximal aorta vs strain



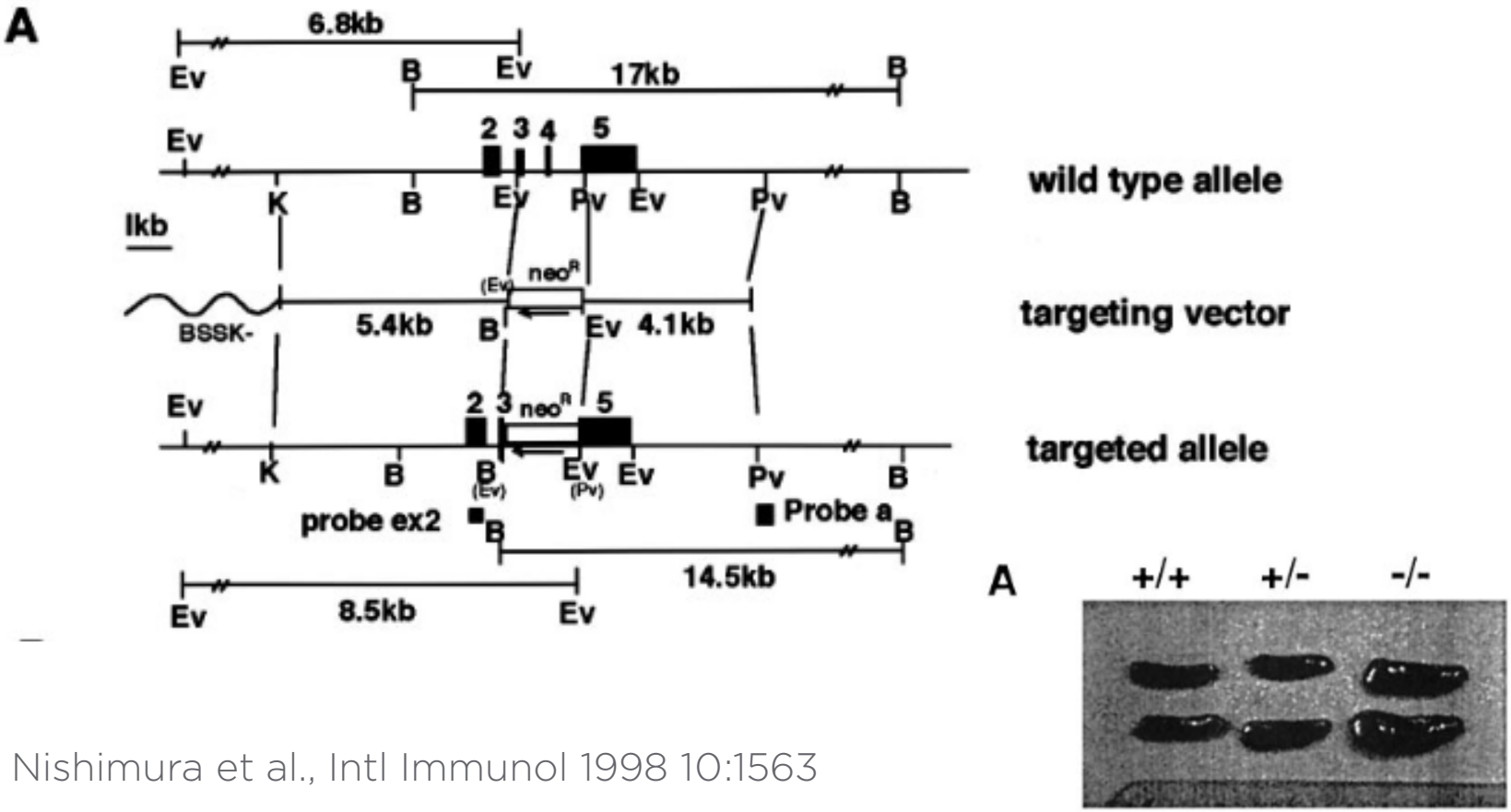
Genetic Background Effects on Atherosclerosis Phenotype of Male mice



Pdcd1-Programmed cell death 1

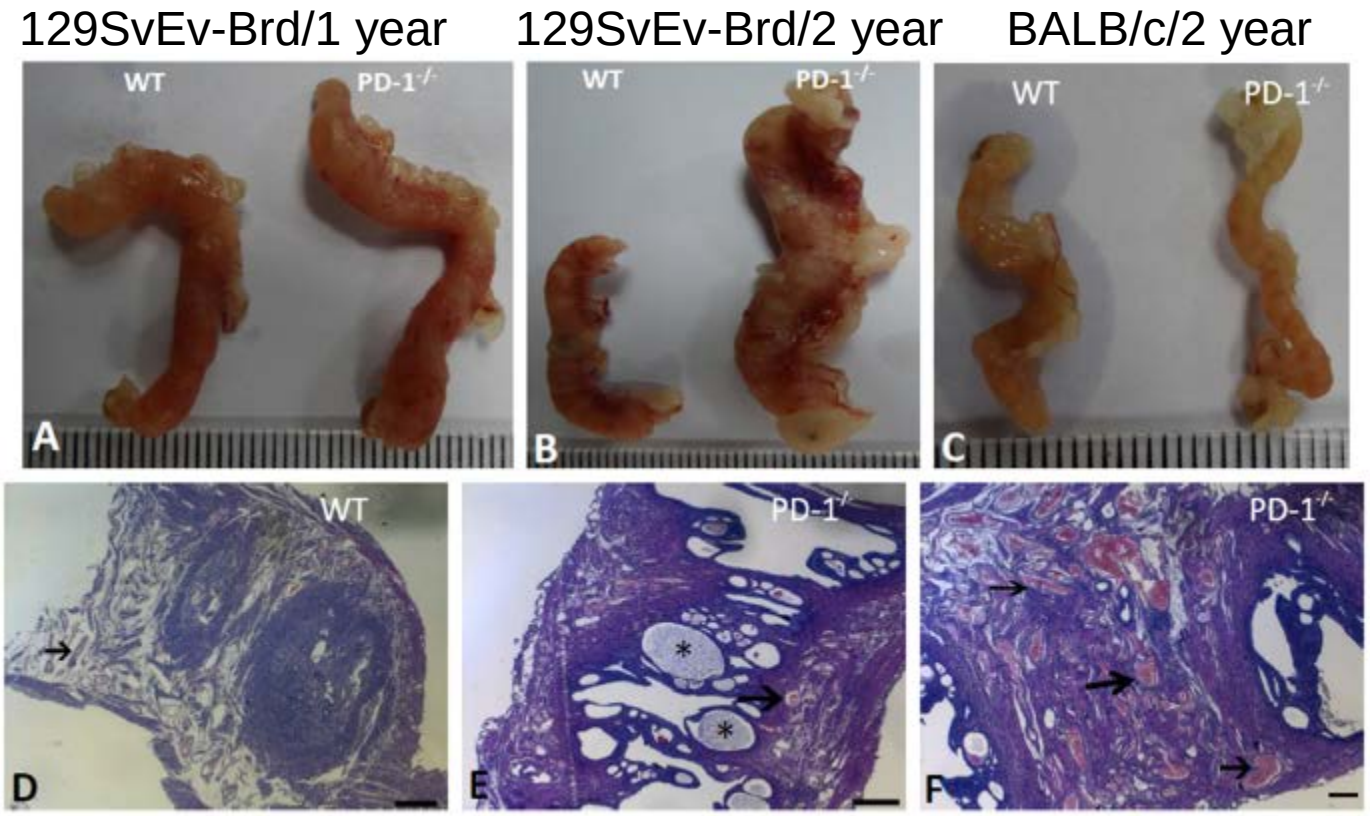
- *Pdcd1* gene, synonym PD-1, is an inhibitory receptor that is expressed in T cells, B cells and myeloid cells and regulates tolerance and autoimmunity
- The phenotype of *Pdcd1* KO varies depending on the genetic background
- In C57BL/6 background, *Pdcd1* deficiency causes lupus-like glomerulonephritis and arthritis
- In BALB/c background, Pdcd1 deficiency causes autoimmune dilated cardiomyopathy and gastritis
- In 129SvEv-Brd, *Pdcd1* deficiency causes endometrial hyperplasia and made them more susceptible to Experimental autoimmune encephalitis
- In NOD background, Pdcd1 deficiency accelerates the onset of Type 1 diabetes
- In MRL background- Fatal myocarditis

Pdcd1 KO in B6;129Sv mixed background- Augmented B cell Response



Nishimura et al., Intl Immunol 1998 10:1563

Pdcd1 KO- Congenic 129SvEv-Brd Endometrial Hyperplasia and BALB/c

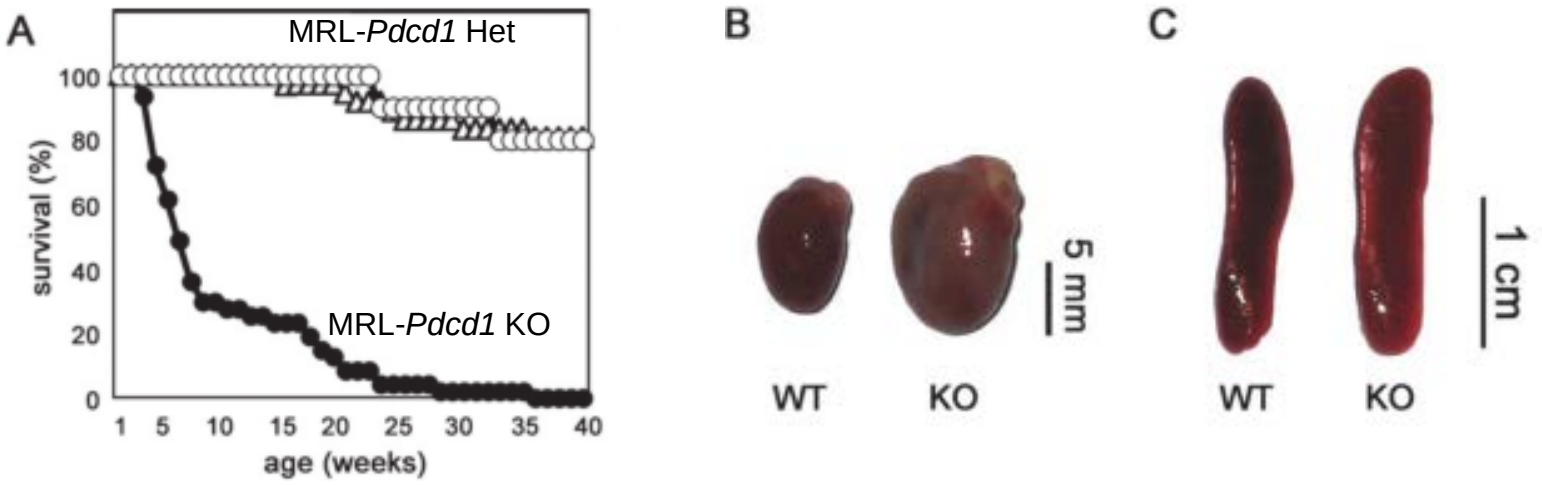


Gross Anatomy and histological Comparison

- Endometrial hyperplasia with Neovascularization and glands

Guo et al., Diagnostic Pathology, 2014 **9**:97

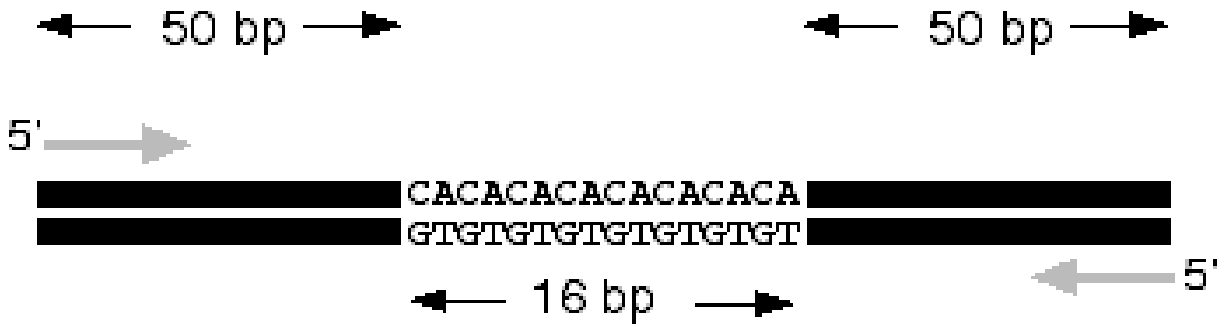
Pdcd1 KO MRL Congenic- Fatal Myocarditis



Wang et al., Intl Immunol 2010 22:443

Genetic Background Testing

- DNA microsatellites
  - Short segments of DNA that have a repeated sequence
  - The length of the sequence repeat varies from species to species and from strain to strain
  - It is somewhat unstable, higher mutation rate
  - Artifact/Technical problems
  - It is difficult to perform in a high-throughput manner



Single Nucleotide Polymorphism or SNP

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- Single Nucleotide Polymorphisms are DNA sequence variations that occur when a single nucleotide (A,T,C,or G) in the genome sequence is altered. For example a SNP might change the DNA sequence

  AAGGCTAA to

  ACGGCTAA

- More Stable than microsatellites

- Scalable for high-throughput, can be tested in the order of thousands

- SNPs allow us to detect strain differences and/or mutations on the different mice we have

Taconic Genome Scan SNP Panel

	C57BL/6NTac	C57BL/6J BomTac	C57BL/6J	BALB/c AnNTac	129S6/ SvEvTac	C3H/He NTac	CBA/J	CBSCBG	CB17SC	DBA/1J BomTac	DBA/2 NTac	FVB/ NTac	NOD/ MrkTac	SJL/JCr NTac
C57BL/6NTac	0	135	246	1148	1154	1230	1196	1123	1141	1145	1172	1094	1079	1058
C57BL/6JBomTac	135	0	111	1245	1252	1325	1291	1216	1238	1242	1269	1189	1176	1157
C57BL/6J	246	111	0	1354	1361	1434	1400	1325	1347	1351	1378	1298	1287	1267
BALB/cAnNTac	1148	1245	1354	0	858	618	680	31	9	829	846	812	777	780
129S6/SvEvTac	1154	1252	1369	858	0	829	843	873	861	859	902	779	798	740
C3H/HeNTac	1230	1325	1434	618	829	0	263	621	615	623	616	782	804	804
CBA/J	1196	1291	1400	680	843	263	0	695	685	559	584	767	798	810
CBSCBG	1123	1216	1325	31	873	621	695	0	22	834	851	823	780	797
CB17SC	1141	1238	1347	9	861	615	685	22	0	835	853	815	780	783
DBA/1JBomTac	1145	1242	1351	829	859	623	559	834	835	0	123	817	805	803
DBA/2NTac	1172	1189	1378	846	902	616	584	851	853	123	0	826	796	812
FVB/NTac	1094	1189	1298	812	779	782	767	823	815	817	826	0	622	484
NOD/MrkTac	1079	1176	1287	777	798	804	798	780	780	805	796	622	0	622
SJL/JCrNTac	1058	1157	1267	780	740	804	810	797	783	803	812	584	622	0

2 to 10 Informative markers: Basic Strain Identification

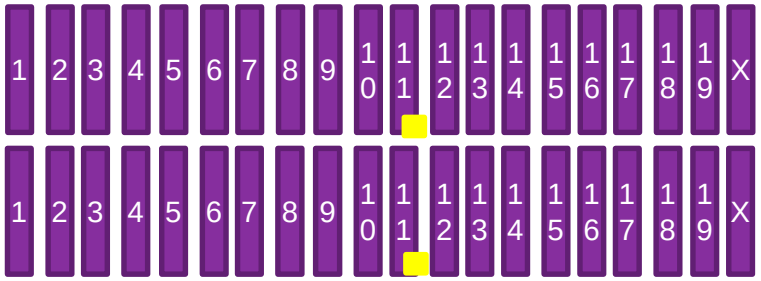
11 to 95 Informative markers: Strain Identification

96 to 400 Informative markers: Accelerated Backcrossing

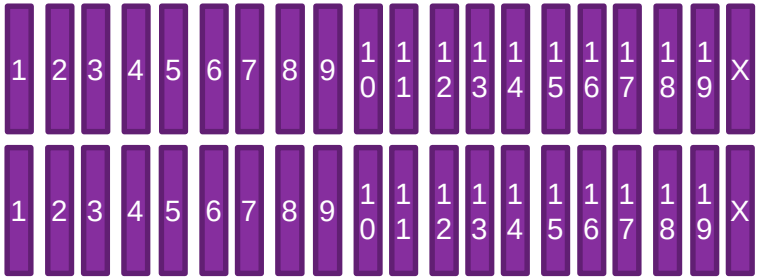
What is a congenic strain?

- In genetics, when referring to inbred strains two individuals that differ in one marker are defined as congenic.

B6 Nude



B6



Why do we need to generate Congenic Strains?

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- If we have a mutation in a mixed background it is important to see the effects of such mutation in a defined background
- Phenotype varies with genetic background so if we need to compare the data generated by our mutant to other models these should be in the same genetic background
- When crossing different mutants verify that they both are in a similar genetic background
- QTL and spontaneous mutants require to be moved from one background to another

Recordkeeping

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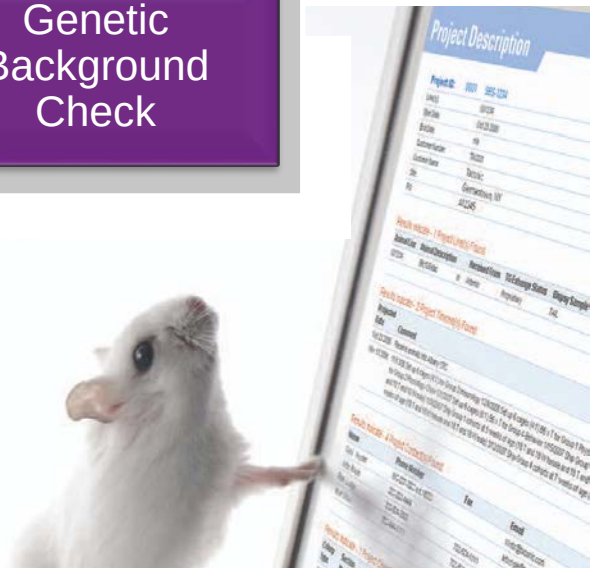
Recordkeeping is critical for accuracy and History of the model

Genetic Background Check

Cryopreservation

Nomenclature

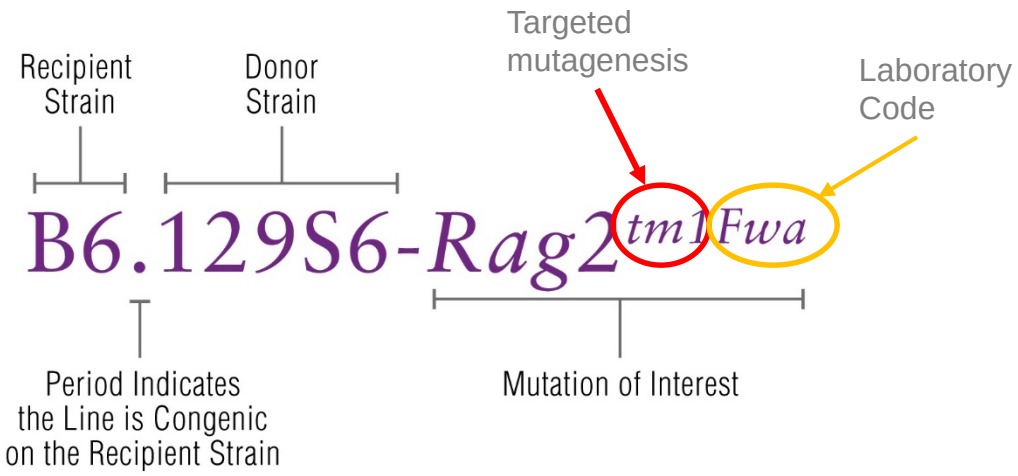


History of Genetically Modified Model Generation

Generation Number

History of Backcrosses

Congenic Nomenclature



Substrain Differences in Inbreds

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J Cell Physiol. 2018 Jan;233(1):371-377. doi: 10.1002/jcp.25895. Epub 2017 Jun 5.

Bone loss in C57BL/6J-OlaHsd mice, a substrain of C57BL/6J carrying mutated alpha-synuclein and multimerin-1 genes.

Liron T[1], Raphael B[1], Hiram-Bab S[1], Bab IA[2], Gabet Y[1].

A Direct Comparison of Metabolic Responses to High-Fat Diet in C57BL/6J and C57BL/6NJ Mice

Diabetes 2016;65:3249–3261 | DOI: 10.2337/db16-0291

Clinical Chemistry Reference Intervals for C57BL/6J, C57BL/6N, and C3HeB/FeJ Mice (*Mus musculus*)

Journal of the American Association for Laboratory Animal Science Vol 55, No 4 July 2016 Pages 375–386

Susceptibility to Plasmodium yoelii preerythrocytic infection in BALB/c substrains is determined at the point of hepatocyte invasion.

Infect Immun. 2015 Jan;83(1):39-47.

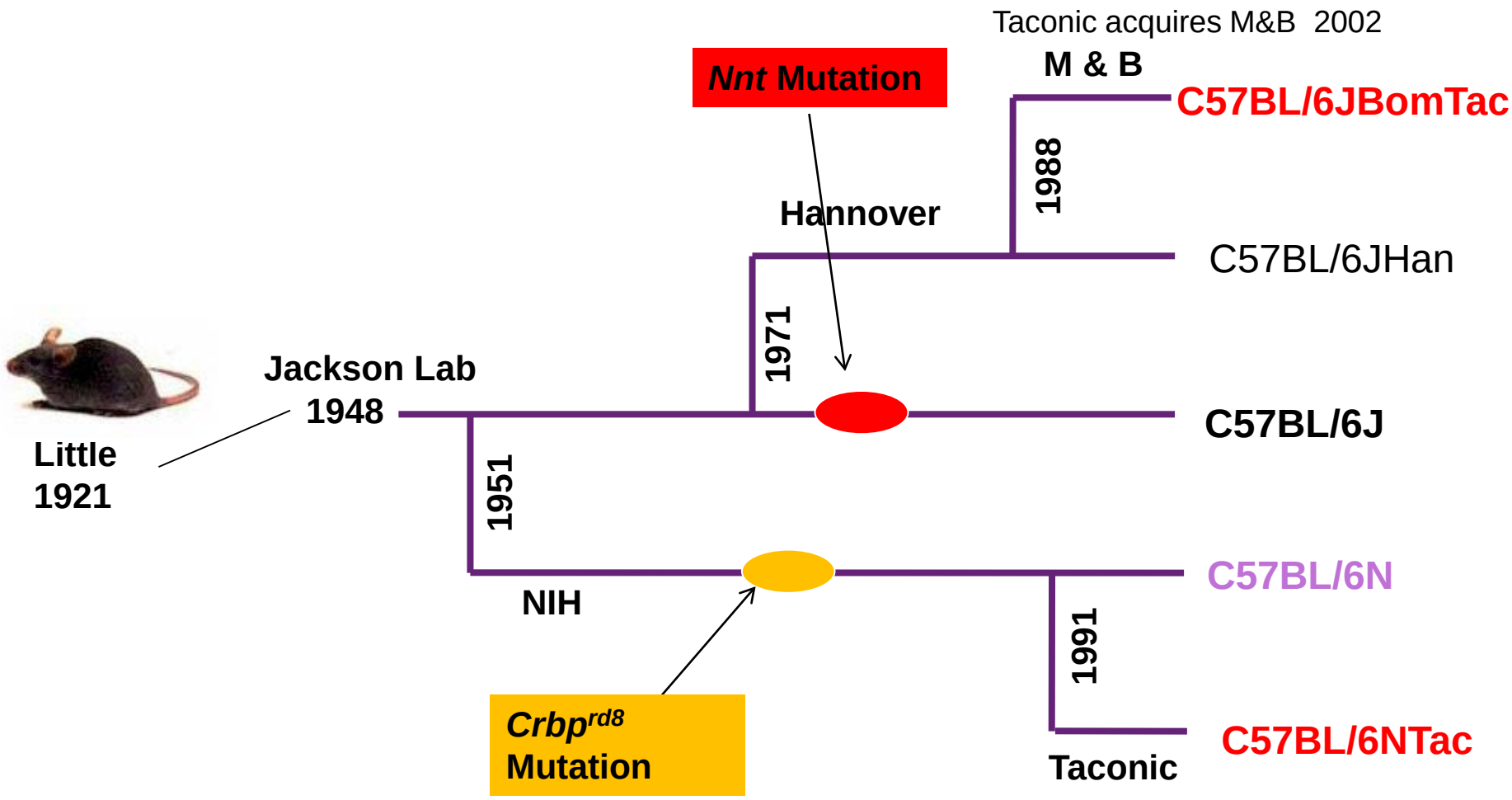
Behavioral Differences among C57BL/6 Substrains: Implications for Transgenic and Knockout Studies

J Neurogenet. 2008 ; 22(4): 315–331.

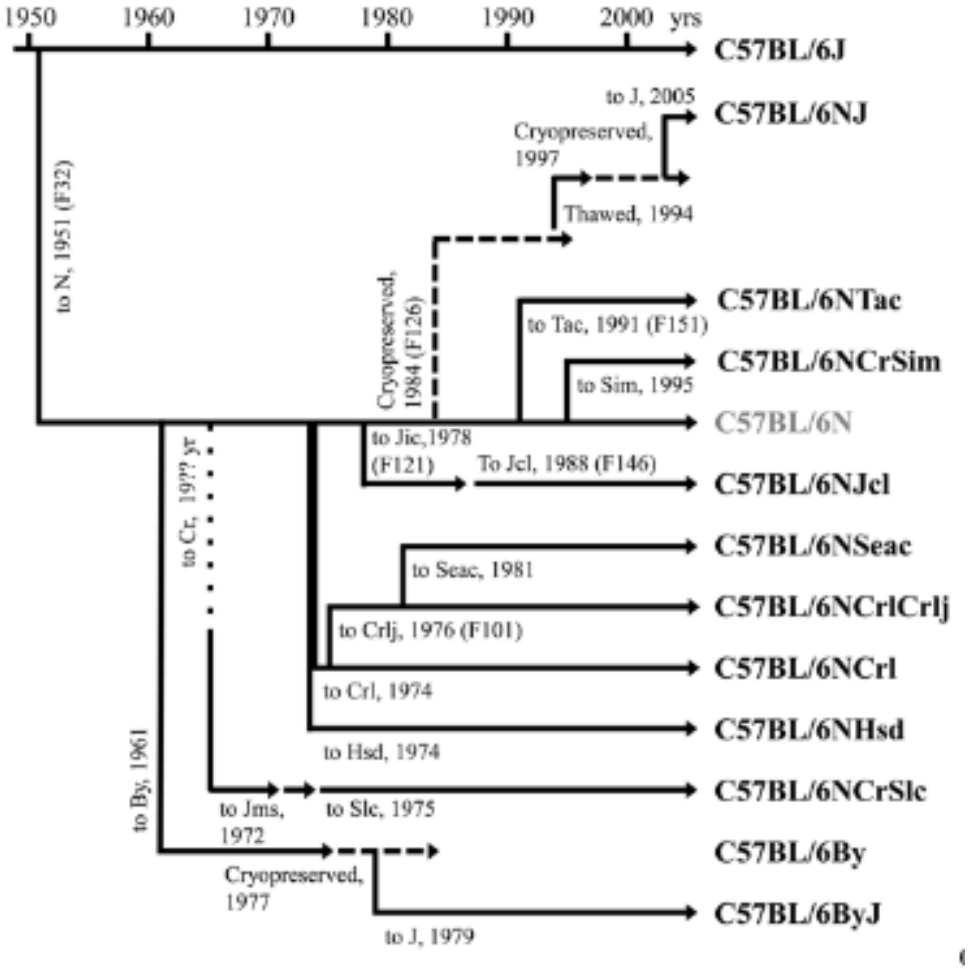
Segregation of a spontaneous Klrd1 (CD94) mutation in DBA/2 mouse substrains.

G3 (Bethesda). 2014 Dec 17;5(2):235-9.

Origin of Taconic's C57BL/6 substrains



Genealogy of C57BL/6N Substrains



Development of SNP markers for C57BL/6N-derived mouse inbred strains. Exp Anim. 2015;64(1):91-100.

Genetic Variants in C57BL/6 Mice

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- *Nnt*$^{C57BL/6J}$- Nicotinamide nucleotide transhydrogenase, associated with impair glucose homeostasis and reduced insulin secretion. Reported as ~17 Kbp deletion
- *Cyfip2*^{M1N} –single base pair substitution resulting in an aminoacid change from Phe to Ser. Abnormal response to cocaine and methamphetamine.
- *Snca*- Synuclein alpha and , associated with resistance to the effects of MPTP (1-methyl-4 phenyl -1,2,3,6-tetrahydropyridine, used as prototypical toxin) on dopamine levels, neurodevelopmental abnormalities. Reported as 365 Kbp deletion
- *Crb1*^{rd8}- single base pair mutation in *Crb1* (Crumbs homolog 1) gene which causes a form of mild retinal degeneration
- *Nlrp12*$^{C57BL/6J}$- single base pair mutation in rod-like receptor pyrin domain containing 12 resulting in an aminoacid change from Arg to Lys, impaired immune response
- Y chromosome partial deletion, deletion of 40 Mbp of the Y chromosome, causes sperm abnormalities and increased Female to Male ratio

Commercial C57BL/6 Genetic Variants

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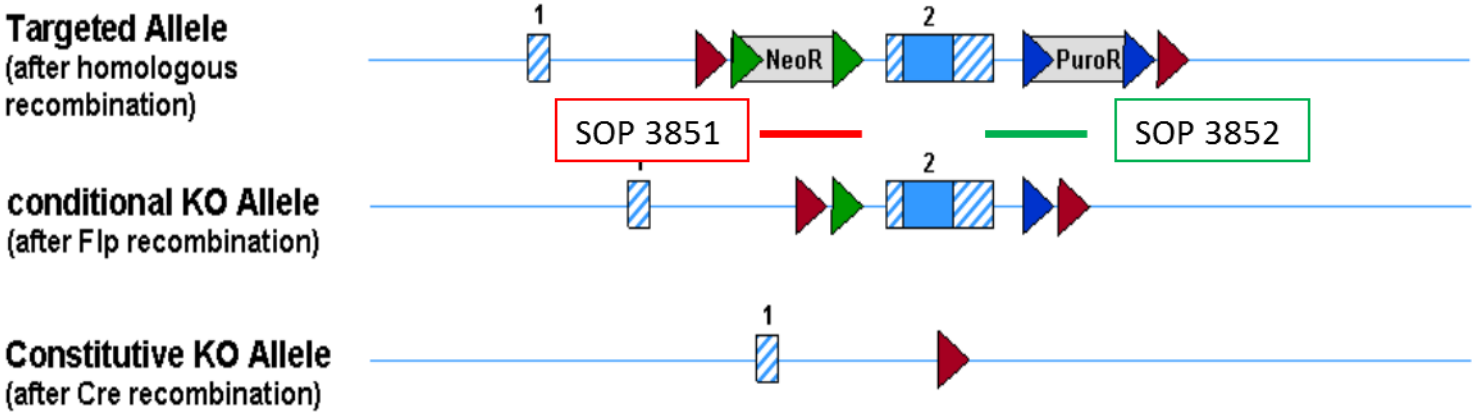
Genetic Variant	C57BL/6NTac	C57BL/6NHsd	C57BL/6NCrl	C57BL/6J	C57BL/6JBomTac	C57BL/6JOlaHsd
Nnt $^{C57BL/6J}$	No	No	No	Yes	No	Yes
Snca deletion	No	No	No	No	No	Yes
Nlrp12 $^{C57BL/6J}$	No	No	No	Yes	Yes	Yes
Cyfip2 ^{M1N}	Yes	Yes	Yes	No	No	No
Y chromosome partial deletion	No	No	No	No	Yes	No
Crb1 ^{rd8}	Yes	Yes	Yes	No	No	No
Dock2 ^{m1Hsd}	No	Yes	No	No	No	No

Taconic Biosciences
Envigo
Charles River
Jackson Labs

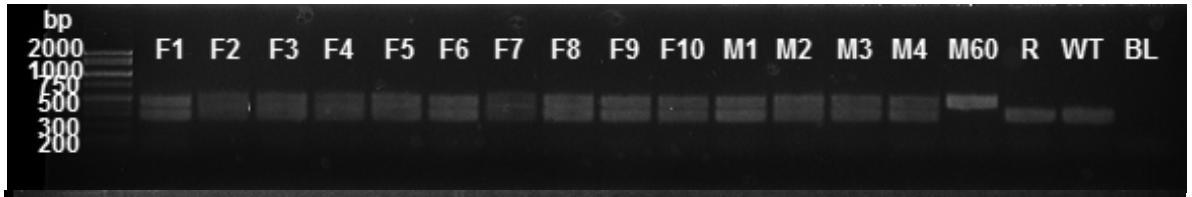
Consider Genetic Background

- Potential genetic drift of the background
- Potential phenotype variation
- Non-reproducible or inconsistent results
- Unable to interpret the data due to presence of unwanted alleles

Testing for all potential alleles in your genetically modified ~~mouse/rat~~

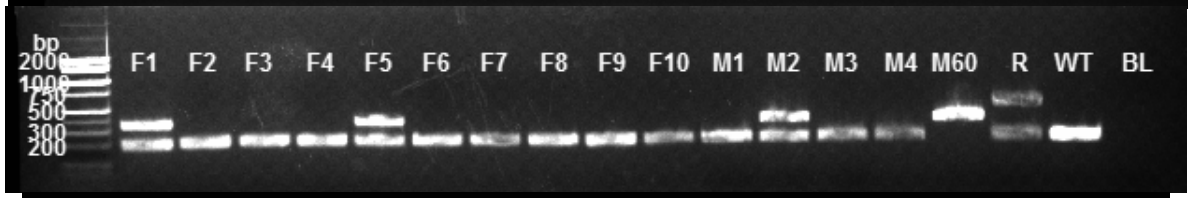


SOP 3851



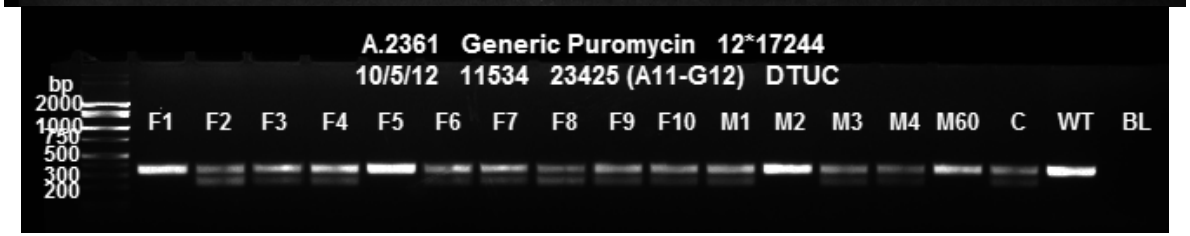
Top band amplification when deleted

SOP 3852



Top band amplification when deleted

PURO



Top band amplification when puro present

Choosing the right controls

Table 2. Comparison of eye phenotypes in control *Le-Cre*$^{Tg/-}$; *Pax6*$^{+/+}$, experimental *Le-Cre*$^{Tg/-}$; *Pax6*$^{fl/+}$ and different *Pax6* heterozygous mice.

Genotype	*Le-Cre*$^{Tg/-}$; *Pax6*$^{+/+}$ (from stage 1 crosses)	*Le-Cre*$^{Tg/-}$; *Pax6*$^{fl/+}$ (from stage 1 crosses)	*Pax6*$^{+/Sey\text{-}Neu}$	*Pax6*$^{Sey/+}$	*Pax6*$^{Leca4/+}$
Genetic background	~78% CBA, ~5% FVB & ~17% CD1	~78% CBA, ~5% FVB & ~17% CD1	75% CBA & 25% C57BL* or 100% CBA**	CD1 (outbred)	mixed (unspecified)
Reference	present study	present study	[19,22,41]	[47]	[39]
Phenotypes					
1. Eye size (mass or diameter)	normal (Fig. 3)	some are small (Fig. 3)	small	small	very small
2. Corneal epithelial layers	normal (Figs. 4-6)	reduced (Figs. 4-6)	reduced	reduced	reduced
3. Keratin 12 immunostaining in cornea	positive staining (Figs. 5T,X)	absent (Figs. 5U,Y)	reduced staining	ND	ND
4. Keratin 19 immunostaining in	limbus not cornea	limbus & patchy in cornea	limbus & cornea**	ND	ND
cornea	(Figs. 5K,P)	(Figs. 5L,M,Q)			
5. Goblet cells in corneal epithelium	absent (Fig. 4K)	present (Fig. 4L)	present	ND	absent
6. Blood vessels in cornea	none seen	none seen	present in some	present	present very early
7. Lens structure	normal (Figs. 2C,D)	cataracts and abnormal (Figs. 2E,F)	cataracts	cataracts	cataracts and vacuolated.
8. Lens-corneal plug in corneal epithelium (persistent lens stalk)	absent (Figs. 2,4,5)	lens-corneal plug in some corneas (Fig. 5M)	lens-corneal plug present	lens-corneal plug in some corneas	absent
9. Kerato-lenticular adhesions or strands	absent	absent	some adhesions	strands	adhesions

Citation: Dorà NJ, Collinson JM, Hill RE, West JD (2014) Hemizygous *Le-Cre* Transgenic Mice Have Severe Eye Abnormalities on Some Genetic Backgrounds in the Absence of *LoxP* Sites. PLoS ONE 9(10): e109193. doi:10.1371/journal.pone.0109193

Critical Genetic Quality Checks

- Consider Potential genetic drift when working with mixed background
- Potential phenotype variation in different background strains
- Non-reproducible or inconsistent results perhaps due to additional genetic modifiers and therefore unable to interpret data
- Have in mind sub-strain differences these also may change the phenotype
- When crossing different models consider the final genetic background of the desired model
- Give careful thought in using the right control
- Always verify all alleles present in the model

QUESTIONS