Inbred & Mutant mice
Reverse Genetics

(A) Wild-type

(B) Bmp7 -/-
Inbred mice

- In 1909, developed by Clarence Cook Little
- A line of mice with genetically identical
- Generated by repeated brother-sister crossing more than 20 times...
- Genetic variability is minimized

![Diagram of inbred mice breeding process]

- 50% after first generation
- 75% after second generation
- 88% ... Approaching to 99.99%
Coisogenic & congenic

• **Coisogenic**
  – If a mutation is induced in inbred mice
  – The new line is called by coisogenic

• **Congenic**
  – Changing genetic background
  – i.e mice A with allele X (some phenotype) are crossed with inbred mice B
  – Among F1 mice obtained from A x B cross, mice with X allele crossed again with inbred B
  – By repeating this procedure, you can get congenic mice with X allele on genetic background of B
Coisogenic & Congenic

Wild strain

X

inbred

X

inbred

X

inbred

Congenic mice for Large ear gene!
Coisogenic & Congenic

Injection of Large ear cDNA

Coisogenic mice for Large ear gene!
F1 mice

- Produced by crossing **two inbred mice**
- Many homozygous **mutations** in inbred mice are **to be heterozygous**
- Hybrid vigor...They are clever!
Outbred

- Genetically heterogeneous
- To minimize genetic similarity
Nomenclature of inbred mice

C57BL/6J

- C: Cold Spring Harbor laboratory
- 57: 57\textsuperscript{th} female
- BL: black
- /6: 6\textsuperscript{th} generation
- J: Jackson laboratory
Mutant

**C57BL/6J-a1a\(^{tot}\)**
- C57BL/6J genetic background
- a1a: alpha-1A calcium channel gene
- Tot: tottering phenotype

**129/sv-a1a\(^{null}\)**
- 129/sv genetic background
- a1a: alpha-1A calcium channel gene
- Null: null mutation, Knockout
Genetic Engineering Technologies

- **Transgenesis**
  Adding new genetic material

- **Homologous Recombination**
  Targeting a specific gene using ES cells

- **Random Mutagenesis**
  Altering genetic material via chemicals or irradiation
How to name a mutant allele

• Names based on primary features if the gene product of wild type allele
  • Albino, himalayan, chinchilla → Tyr\textsuperscript{c} Tyr\textsuperscript{c-h} Tyr\textsuperscript{c-ch}
  • Rules for deciding new gene names are made by a nomenclature committee (http://www.informatics.jax.org/mgihome/nomen/index.shtml)
  • Many journals require that new allele names be approved by the nomenclature committee before publication
Laboratory Code

- **J**-The Jackson
- **Mit**-Laboratory Massachusetts Institute of Technology
- **Leh**-Hans Lehrach
- **Kyo**-Kyoto University
- **Ztm**-Central Animal Laboratory Medical School Hannover
- **Unc**-University of Northern California
How to name a gene

• \textit{Glra1}: glycine receptor, alpha 1
• \textit{Hk1-rs1 (ps1)}: hexokinase-1 related sequence 1 (peudogene)
• \textit{Kit}^{W-v}: \textit{Kit} oncogene
  allele name: viable dominant spotting
• \textit{Slc14a2_v1}: solute carrier family 14, member 2, \textit{variant} 1
• \textit{Slc14a2_pr1}: solute carrier family 14, member 2, \textit{promoter} 1
• \textit{D8Mit17}: the 17th locus mapped to mouse Chromosome 8 by M.I.T.
• \textit{Gt(ROSA)26Sor}: the 26th gene "trapped" by the ROSA vector in the laboratory of Phillip Soriano (Sor)
• \textit{Ntn1}^{Gt(pGT1.8TM)629Ska}: the netrin 1 (Ntn1) gene gene-trap allele
How to name transgene ro targeted allele

*Tg(CD8)1Jwg*

A transgene containing the human *CD8* gene, the first transgenic line using this construct described by the lab of *Jon W. Gordon*.

- **Car12**\(^{Tn(sb-T2/GT2/tTA)1.1Dla}\)
  Gene Tn(transposon_class_abbreviation-vector)#Labcode

- **Cftr**\(^{tm1Unc}\)
  The first targeted mutation(tm) of the cystic fibrosis transmembrane regulator (*Cftr*) gene produced at the *University of North Carolina*. 
Explain following words

- Co-isogenic mice
- Congenic mice
- Outbred mice
- F1 mice
- Inbred mice
What is this?

- **C57BL/6Tac- Tbx6 tm 1 Pa**
  - Standard inbred strain designation
  - Holder or supplier
  - Gene symbol for locus
  - Sequential number of mutations at this locus
  - Mode of production: targeted mutation
  - Registered lab code

**C57BL/6J**
**C57BL/6C**
**129SV/Jae**
Factors Affecting Phenotype

- **Transgenesis**
  - Site of integration
  - Number of copies
  - Insertional mutations

- **Targeted Mutagenesis**
  - Incomplete knockout
  - Compensation for loss of gene by other genes

- **Environmental effects**

- **Genetic Background**
Insertional Effects
(positional effects)

B-cell Leukemia/Lymphoma 2  \textit{BCL2}

Promoter: \textit{Igh}, immunoglobulin heavy chain

C57BL/6-TgN(BCL2)\textsubscript{22}Wehi/J (002319) $\rightarrow$ B-cell lineage

C57BL/6-TgN(BCL2)\textsubscript{25}Wehi/J (002320) $\rightarrow$ T-cell lineage

C57BL/6-TgN(BCL2)\textsubscript{36}Wehi/J (002321) $\rightarrow$ B & T-cell lineages
Transgene Copy Number
Amyotrophic Lateral Sclerosis (ALS) Trangenics

B6SJL-TgN(SOD1-G93A)1Gur/J (002726)
- Variant of the human superoxide dismutase-1 gene (glycine to alanine at position 93)
- Limb weakness by 3 - 4 months
- Paralysis due to loss of motor neurons from the spinal cord
- Pathology mimics human ALS

B6SJL-TgN(SOD1-G93A)dl1Gur/J (002300)
- Reduced copy number of transgene
- Delay in symptoms
- Limb weakness by 6-7 months

Environmental Effects

Interleukin 10 targeted mutations ("Knockouts")

◆ Conventional Housing Conditions
Severe inflammatory bowel (colitis)
Breeding problems associated with rectal prolapse

◆ Germ Free or SPF Conditions
No symptoms of colitis
No breeding problems, homozygous breeder pairs
Strains may differ markedly in behavior tests despite standardization of apparatus, protocols, and environmental factors (Crabbe et al., Science 284:1670-1672)

Strains may behave differently if treated with drugs to fight parasite infection (e.g. ivermectin) (Davis et al., Lab Animal Sci 49:2 88-296, 1999)
Open field test

![Bar graph showing activity levels in open field tests for different strains.](image)

- **A**: Open field horizontal activity
  - Bars represent different strains: Portland, Edmonton, Albay

- **B**: Activity change under cocaine
  - Species include: A/J, C57Bl/6J, BALB/cByJ, DBA/2J, 129/SvEVTac, 129/Sv-ter, 5HT1B/−/−, B6D2F2

*Citation: Crabbe et al., Science 284:1670-1672*
Plus maze for anxiety  Alcohol consumption

A

Total arm entries

B

Time in open arms

Ethanol consumed (g/kg)

- Portland
- Edmonton
- Albany

A/J  C57BL/6J  BALB/cByJ  DBA/2J  129/SvE Tac  129/Sv-ter  5HT1B-/-  B6D2F2
Selection of Inbred Strains

- Inbred strains have unique characteristics
- Multifactorial

**DBA/2J**
- Susceptible to audiogenic seizures
- Develop heriditary glaucoma
- Low susceptibility to diet-induced atherosclerosis
- Extreme intolerance to and avoida nce or alcohol & morphine

**C57BL/6J**
- Resistant to audiogenic seizures
- High incidence of microphthalm ia
- High susceptibility to diet-induc ed atherosclerosis
- Preference for alcohol and morp hine
Genetic Background: Definitions

- **Genetic Background**: collection of all genes present in an organism that influences a trait or traits
- **Allelic variance**: sequence differences in genes (inbred strains harbor different alleles)
- **Genetic modifier**: allelic variants at loci (positions) other than the one being genetically modified
How Modifiers Work

- Suppress or enhance the expression of genes involved in physiological or pathological pathways
- Alter DNA transcription rates or mRNA stability
- Epigenetic factors (DNA methylation, position-effects)
- Variation of gene copy number
Understanding Genetic Background Effects

- Effects of genetic modifiers present in the host genome that are unlinked to the modified gene
- Effects of linked genes carried during backcrossing
- Effects of genes present in the host genome which are independent of the gene of interest
Effects of Unlinked Genetic Modifiers

Mom1 = (Pla2g2a)

Chr 4

Chr 18

Apc\textsuperscript{Min}

B6 = \textit{Mom1} null

B6 = \textit{increased} tumor incidence

AKR = \textit{Mom1} wildtype

AKR = \textit{decreased} tumor incidence

\textit{Pla2g2a} = phospholipase A2, group IIA gene
Effects of Linked Genes Carried over during Backcrossing

B6.129P2-Apoel\textsuperscript{tm1Unc/J}
C57BL/6J congenic

N6

129P2
B6

silver

Chr 7

Apoel
p

N10

129P2
B6

black

Chr 7
Effects of Genes Independent of Transgene

retinal degeneration 1 mutation causes blindness by weaning age

$P_{\text{deb}6^{rd1}} \times \text{X} = \text{B6SJLF1/J sighted}$

SJL/J blind  C57BL/6J sighted
Effects of Genes Independent of Transgene

B6SJLF1/J
sighted

B6SJLF1/J
Tg/0
sighted

Pde6b rd1

X

Tg

Tg/0
sighted

Tg/0
blind

+/-
sighted

recombinant

+/-
sighted

recombinant

+/-
sighted

recombinant
Phenotypes Affected by Genetic Background

- Behavior
- Cardiovascular Physiology
- Developmental Defects
- Diabetes
- Immunity and Inflammation
- Metabolism
- Tumor type and incidence
Genetic Background Effects on Type 2 Diabetes

**Diabetes db/db (Lepr\textsuperscript{db})**
- C57BL/6 (B6.Cg-m +/+ Lepr\textsuperscript{db}/J) obesity with transient diabetes
- C57BLKS (BKS.Cg- m +/+ Lepr\textsuperscript{db}/J) obesity with overt diabetes

**Obese ob/ob (Lep\textsuperscript{ob})**
- C57BL/6 (B6.V-Lep\textsuperscript{ob}/J) obesity with transient diabetes
- C57BLKS (BKS.V-Lep\textsuperscript{ob}/J) obesity with overt diabetes
Genetic Background Effects

Interleukin 2 targeted mutations ("Knockouts")

- **B6,129- Il2 \(^{tm1Hor}/J\) original publication**
  - 50% die between 4 and 9 weeks of age
  - Splenomegaly, lymphadenopathy, severe anemia
  - Progressive colitis

- **C57BL/6J-Il2 \(^{tm1Hor}/J\) (002252)**
  - Generalized autoimmune disease (hemolytic anemia)
  - Die by 3 - 6 months of age
  - Progressive colitis dependent on health status

- **C3H/HeJCrIbr-Il2 \(^{tm1Hor}/J\) (002228)**
  - Generalized autoimmune disease (hemolytic anemia)
  - Die by 7 weeks of age

- **BALB/c-Il2 \(^{tm1Hor}/J\) (002229)**
  - Generalized autoimmune disease (hemolytic anemia)
  - Die by 3 - 5 weeks of age

# Genetic Background Effects

**Innate immunity of “scid” mice**

<table>
<thead>
<tr>
<th></th>
<th>NOD/LtSz–Prkdc\textsuperscript{scid/J}</th>
<th>C.B-17/Sz–Prkdc\textsuperscript{scid/J}</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK cell activity</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Complement activity</td>
<td>Absent</td>
<td>High</td>
</tr>
<tr>
<td>Macrophage development</td>
<td>Impaired</td>
<td>Normal</td>
</tr>
<tr>
<td>Antigen presenting cell function</td>
<td>Impaired</td>
<td>Normal</td>
</tr>
</tbody>
</table>
Options for Controlling Genetic Background Effects

- **Isogenicity**
  1) Creating a strain that is coisogenic to it’s control strain
  Generation of transgenics on defined genetic background *(e.g. C57BL/6J, FVB/NJ)*
  2) The 129 strain
  3) Other options *(e.g. C57BL/6 derived ES cell lines)*

- **Congenicity**
  Creating congeneric strains that differ from control strain in only small region

- **Use F1 strain**
Recommendations
Banbury Conference Guidelines

- Published reports must include a detailed description of genetic background of the mice studied that is sufficient enough to allow replication of the study.

- The genetic background chosen for the studies should not be so complex as to preclude replication.

- Use of common or standardized genetic background would facilitate comparison of experimental results among laboratories → Use of F1 (B6x129).

Background Problem in using F2 mice

B6 129 (chimera germ-line)

\[ P \]

\[ B6 \] \[ 129 \] (chimera germ-line)

\[ F1 \] \[ X \]

\[ +/+ \] \[ +/- \] \[ +/- \]

\[ F2 \]

\[ +/+ \] \[ +/- \] \[ -/- \]
Breeding Strategies to minimize background effects

B6 129

N1

B6 X

N2

B6 X

N5,6,7…. 129 cosiogenic

B6 congenic

+/- +/+- -/-
Make a group and
Make a mouse model!
Title: Mouse model for drowsiness

Why?
Drowsiness is a transition state between consciousness and unconsciousness. By using this model, we could examine the **switching mechanism of consciousness**

How?
We easily feel sleepiness **in the lecture** after eating a lot of food containing fat as a lunch so will try to feed **spam** to mice **in the open field**
Title: Mouse model for drowsiness
Mouse model for playfulness?
Response to novelty: Movable toy experiment

Habituation, 1-hour \rightarrow \text{Place novel objects} \rightarrow \text{Return}

Recording with a cage-top CCD camera
Active engagements in the mutants: pushing, towing, biting
Active engagement with novel objects by the mutants

Object contacts

- **1st contact latency**
- **Contact duration**

Response patterns

- Total
- Approaching
- Digging
- Pushing, Towing, & biting

Object dislocation

Distance dislocated (cm)

* : p < 0.05
Response to novel objects in home cage

+/-

0  5  10  15  20 min

-/-
Difference anxiety levels in inbred mice
Response to novel objects in inbred mice

Figure 2: Pattern analysis of active manipulations in DBA/2J and BALB/cJ mice. (a) Three examples of object dislocation are illustrated by videotracking traces. (b) Temporal changes of object dislocation are displayed at 5-min intervals. (c) Total summation of object dislocation for 20 min. (d) Wild gnawing of objects by BALB/cJ mice. The time spent in object exploration (e) and object gnawing expressed as the 'stripping score' (f) are presented as a function of repeated presentations of similar objects for 5 h at 1-h intervals. Mean (±SEM) is depicted for each group. *P<0.05 by post hoc test. The number of mice used in the test: C3H, n=8 (five males and three females); DBA/ 2, n=8 (five males and three females); BALB/c, n=10 (five males and five females); C57BL/6J, n=10 (five males and five females); 129/Sv, n=10 (five males and five females).
Response to novel objects in inbred mice

C57BL/6J  BALB/C
Make a group and
Make a mouse model!