

# More Tails from the Crypt



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# Topics

- Genotyping
- Genetics
- Nomenclature
- Advanced Mating Techniques and ART
- Troubleshooting
- Strain Effects, Health Issues or Phenotype

## Genetics

- **Definitions:**
  - **Wild type:** there are no mutations in the gene of interest on either of the paired chromosomes
  - **Homozygous:** the same mutation is present in both alleles of the gene
  - **Heterozygous:** the mutation is present in one of the two alleles of the gene; the other allele is wild type
  - **Hemizygous:** there is only one copy of the mutant gene; there is no corresponding locus in the paired chromosome
  - **Conditional:** there is a change in the locus that can only be “seen” if certain conditions are met (for example, the DNA is exposed to Cre-recombinase to pop-out DNA between two loxP sites)

## Genetics

- Getting to Homozygosity (1 gene)

A.  $\text{mut}/+$  X  $+/+$



$\text{mut}/+$  (50% of progeny)

B.  $\text{mut}/+$  X  $\text{mut}/+$  (both from Cross A)



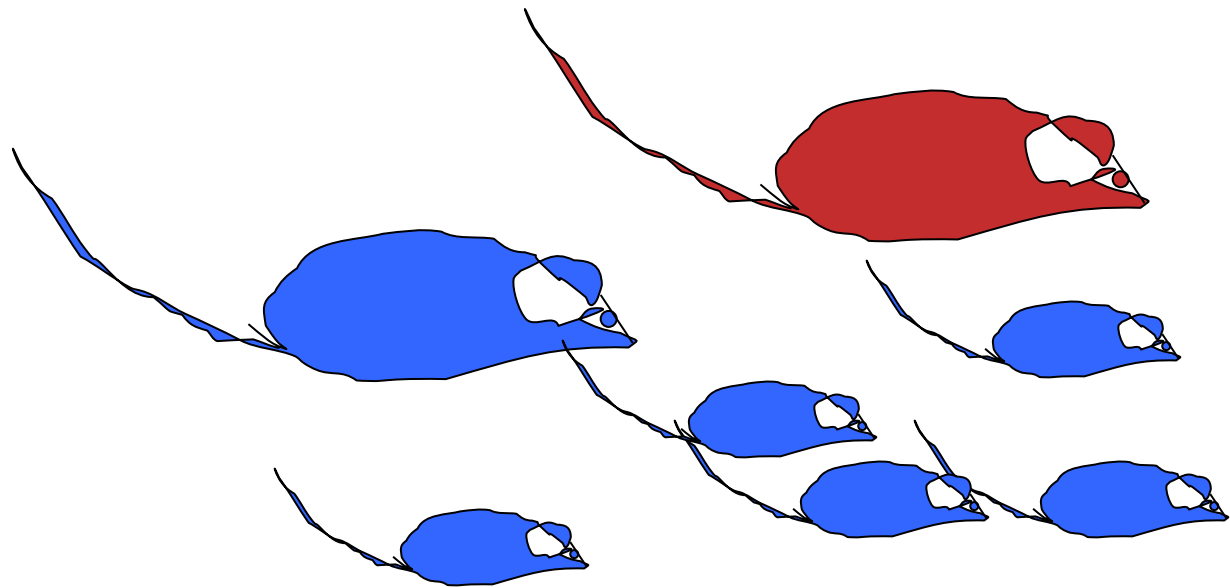
$\text{mut}/\text{mut}$  (25% of progeny)

(mut = any mutation or gene; a knock-out, a knock-in, a point mutation;  
can be dominant or recessive)

## Genetics

Homozygous vs. Heterozygous matings:  
lethality? Recessive? Dominant?

stage? Embryonic lethal, neonatal lethal



## Genetics

- Homozygous lethality

mut/+ X mut/+



mut/mut (25% of progeny expected;  
0% observed )

mut/ + (50% of progeny expected;  
67% observed)

+/+ (25% of progeny expected;  
33% observed)

How many pups need to be tested to verify this?

Make sure you've tested enough progeny to tell.

## Genetics

- 2-gene systems

- A. Obtaining double homozygotes:

Cross single homozygotes to get double heterozygote and then intercross to obtain double homozygotes.

*mut a/mut a*; +/+ X +/+; *mut b/mut b*



*mut a*/+; *mut b*/+ X *mut a*/+; *mut b*/+



*mut a/mut a*; *mut b/mut b* (6.25% of progeny)

## Genetics

- 2-gene systems

- B. Obtaining single homozygote with single heterozygote

A. *mut a/a*; +/+ X +/+; *mut b/+*



*mut a/+*; *mut b/+* (50% of progeny)

B. *mut a/a*; +/+ X *mut a/+*; *mut b/+* (from Cross A)



*mut a/a*; *mut b/+* (25% of progeny)

## Genetics

- 2-gene systems

- B. Obtaining single homozygote with single heterozygote

A. *mut a/a; +/+* X *+/+; Cre/+*



*mut a/+; Cre/+* (50% of progeny)

B. *mut a/a; +/+* X *mut a/+; Cre/+* (from Cross A)



*mut a/a; Cre/+* (25% of progeny)

## Genetics

- 2-gene systems

- C. Obtaining single homozygote with transgenic hemizygote

A. *mut a/a*; 0/0 X *+/+*; *Tg*/0



*mut a/+*; *Tg*/0 (50% of progeny)

B. *mut a/a*; 0/0 X *mut a/+*; *Tg*/0 (from Cross A)



*mut a/a*; *Tg*/0 (25% of progeny)

## Genetics

- 2-gene systems

You need to generate 40 male mice that are homozygous for both p53KO and p73KO. You have both genes as homozygotes in different lines.

- 1) Cross single homozygotes to obtain double heterozygotes (6 mating pairs = 12 mice), then cross the double heterozygotes to obtain double homozygotes
- 2) 6.25% of the progeny will be homozygous for both genes
- 3) 50% of those will be male.

$40 \text{ total} / 0.5 \text{ (males)} / 0.0625 = 1280 \text{ mice to generate!!!!}$

## Genetics

- 2-gene systems

Or, generate 100 progeny

- 1) 6.25% of the progeny will be homozygous for both genes
- 2) 50% of those will be male (3), 50% will; be female (3).
- 3) When you cross these together, all pups will be double homozygotes.

You will still need to generate at least 100 pups, and it will take an additional 8-12 weeks.

- “So many mice, so little time”



# On to Nomenclature

- **Mouse Nomenclature Review**
- International Committee on Standardized Genetic Nomenclature for Mice establishes rules and guidelines
- Mouse Genomic Nomenclature Committee (MGNC) implements the rules
- Institute for Laboratory Animal Research (ILAR) assigns laboratory codes
- Current guidelines: <http://www.informatics.jax.org/nomen/> or send questions to e-mail [nomen@informatics.jax.org](mailto:nomen@informatics.jax.org)
- Mouse Nomenclature Tutorial:  
<http://jaxmice.jax.org/nomenclature.html>

[Nomenclature slides courtesy of Dr. Laura Conour]

# Nomenclature

- Why is this important???

You call JAX and ask for “APOA1 transgenic mice” ...

Do you want this one?

- **B6.129P2-*Apoa1*<sup>tm1Unc</sup>/J**

Or this one?

- **C57BL/6-Tg(*APOA1*)1Rub/J**

[Nomenclature slides courtesy of Dr. Laura Conour]

# Nomenclature

- Advantage
  - Unique identifier, conveys technology, and conveys line and producer
- Disadvantage
  - Long, sometimes incomplete historic information, and can change frequently
- User importance
  - Scientists –publications
  - Purchasing agent/facility manager –ordering information
  - Animal care techs –pedigree and record keeping

# Nomenclature

## Outbred Lines

- **Outbred Nomenclature**

Contain breeder code, producer stock designation, and origin of stock

- Example:

**CrI:CD-1(ICR)**

**Hsd:ICR(CD-1)**

**Tac:SW**

# Nomenclature

## Inbred Strains

- **Inbred Nomenclature**

- Named based on coat color

- C57BL (nonagouti -black); C57BR (brown); C57L (brown leaden -grey)

- Named based on origin

- SJL (Swiss, Jim Lambert); NZB (New Zealand Black)

- Named based on phenotype

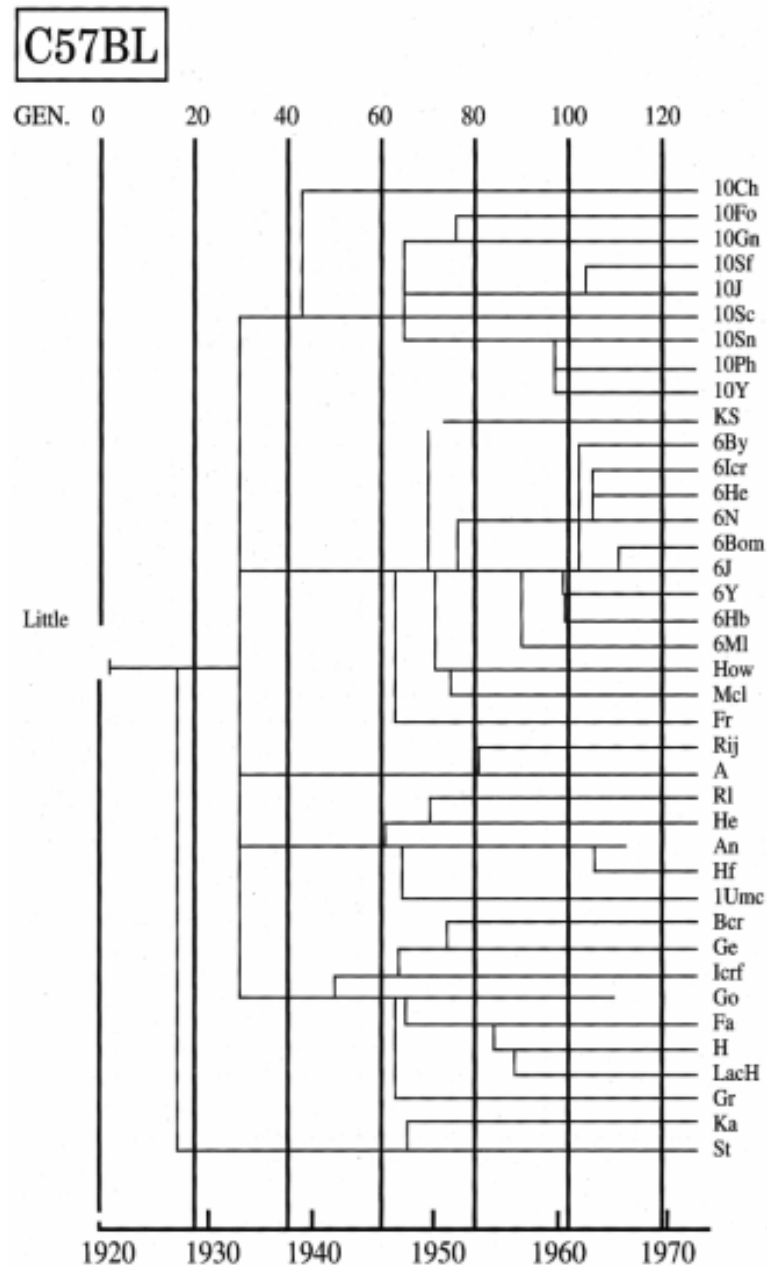
- NOD (Nonobese Diabetic); NU (nude)

## Substrains

- **Substrain Formation**
  - Branch separated before 40 generations of inbreeding
    - Residual heterozygosity still likely
    - Ex: C57BL/6J vs C57BL/10J
  - Separated for 20+ generations from the “parent” strain
    - Genetic mutation and drift
    - Ex: BALB/cNCrI vs. BALB/cNCr
  - Detection of a previously unknown genetic difference
    - Ex: C57BL/6J vs C57BL/6N or 129S6 vs 129S7

# Nomenclature

## C57BL genealogy



# Nomenclature

## Inbred Strains

- **Inbred Nomenclature**
- Inbred strains named with numbers and capital letters
  - C57BL, C3H
- Substrains identified by parent strain followed by slash (/)
  - C57BL/6, C57BL/10
  - C3H/He, C3H/HeSn
- First letter of lab code designation capitalized, rest lowercase
  - C57BL/6J, C57BL/6NCrl

# Nomenclature

## Hybrid Strains

- **Hybrid Nomenclature**
- Use standard strain abbreviations
- Female parent first, male parent second
- Example:
- C57**B**L/6J x **D**BA/2J = **B**6**D**2F1/J
- B6D2F1/J x B6D2F1/J = B6D2**F**2/J

# Nomenclature

## Genes

- **Mouse Gene Nomenclature**
- Typically 3-5 characters, but <10 characters
- Begin with a capital letter followed by all lowercase letters
- Recessive mutations begin with a lowercase letter
- Gene and allele designations are **italicized in published articles**
- Superscript gene alleles
  - *Lepr<sup>db</sup>*, *Foxn1<sup>nu</sup>*

# Nomenclature

## Transgenic Lines

Genetic background-Tg(YYYYYY)#####Zzz

- Tg: Denotes transgene inserted
- (YYYYY): The official gene symbol of the inserted DNA (promoter designations encouraged). Can use punctuation to separate sequences
- #####: Laboratory assigned number (1-99,999, founder line number)
- Zzz: Laboratory code

# Nomenclature

## Transgenic Lines

- C57BL/6J-Tg(*APOA1*)1*Rub*
- *JAX B6 mouse*
- *transgenic*
- *containing the human APOA1 gene*
- *and the 1st germline transmission (founder)*
- *from the lab of E.M. Rubin*

## Nomenclature

# Transgenic Lines

- **C57BL/6-Tg(*ACTB-EGFP*)1Osb/J**

- Background Strain: C57BL/6
- Transgene: Tg
- Promoter in Tg segment: ACTB (chicken  $\beta$  actin)
- Expressed transgene: EGFP (enhanced GFP)
- Line number: 1
- Lab code: Osb(Osaka University)
- Maintained by: The Jackson Laboratory

# Nomenclature

## Targeted Mutations (tm)

Recipient;<sup>.</sup>donor background-**XXXX****tm****###****Zzz**

<b>;</b>	Denotes <5 backcrosses
<b>.</b>	Denotes 5+ backcrosses
<b>XXXX:</b>	<i>Gene name</i>
<b>tm:</b>	<i>Denotes targeted mutation</i>
<b>#####:</b>	Laboratory assigned number (1-99,999, founder line number)
<b>Zzz:</b>	Laboratory code

[Nomenclature slides courtesy of Dr. Laura Conour]

# Nomenclature

## Targeted Mutations (tm)

Example:

**B6**; **129P2**-*Apoa1* *tm1* *Unc*

Recipient strain:	C57BL/6
Semicolon (;):	< 5 backcrosses to B6
Donor strain(ES cell origin):	129P2/OlaHsd
Targeted gene:	<i>Apoa1</i>
Targeted mutation:	<i>tm</i> , <i>founder line 1</i>
Lab Code:	<i>Unc</i> ( <i>University of North Carolina</i> )

# Now, back to the mouse



Let's get together: Mating techniques