MOUSE

- **genus-** *Mus*
- **species-** *Mus musculus*
- **subspecies of** *Mus musculus*
  - *Mus musculus domesticus*
  - *Mus musculus musculus*
  - *Mus musculus castaneus*
  - *Mus musculus bactrianus*

- **species-** *Mus spretus- Western Mediterranean short-tailed mouse*

  *Mus musculus never produces hybrids with Mus spretus in nature*
  *Mus spretus genome is highly polymorphic with Mus musculus.*
  *Mus spretus and Mus musculus can be bred in the laboratory however the F1 males are sterile*

**Inbred strains of mice**

Defined by 20 or more generations of brother/sister matings.

After 20 generations of inbreeding 98.6% of loci are homozygous.

**Today’s classical inbred strains of mice can be considered recombinant inbred strains primarily derived from** *Mus musculus domesticus and Mus musculus musculus*.

**Substrains**

Branches of an inbred strain that have known or probable genetic differences.

1. branches of a strain are separated before the 40th generation of inbreeding.
2. branches of a strain have been maintained separately from other branches for more than 10 generations of inbreeding.
3. genetic differences from other branches are discovered.

**Types of mutations**

**Recessive**

*Loss of function mutation*,

**Dominant**

*Gain of function*

*haplo-insufficient*

*dominant negative*
Types of alleles
null allele
amorph
hypomorph
neomorph

How do we identify new genes in the mouse?
naturally occurring mutations
induced random mutation
random cDNA sequencing
gene identification from genomic sequence.

The mouse genome
20 haploid chromosomes, (19, X, Y)
Diploid DNA content, 2.7X10⁹ bp (about 6.4 pg per nucleus)
8-10% of the genome is repeat sequence

Mapping genes in the mouse
Breeding mice
gestation period-19 days (range is 18-21 days depending on strain)
age at weaning-21 days
sexual maturity-females 4-5 weeks, males-6-8 weeks
birthweight-1 gm
weaning-8-12 gm
adult-30-40 gm

Classical genetic backcross
look for recombination and linkage between two genetic loci-requires the identification of genetic locus by phenotype or by DNA polymorphisms.
ex. dominant white spotting and alfa fetoprotein (AFP),
both located on mouse chromosome 5.
look at coat color and electrophoretic migration of AFP
(or similarly look at an RFLP for the AFP gene)
Inbred strains of mice

inbred strains-defined by 20 or more generations of brother/sister matings

recombinant inbred strains (RI-strains) formed by crossing two inbred strains, followed by 20 or more generations of brother/sister matings

congenic strains-two strains that are genetically identical except at a single locus

RI strains

finite number of strains
infinite amount of DNA
polymorphisms are not too frequent
start with two inbred strains of mice, 20 generations of brother-sister matings, establish 30 RI strains
polymorphisms between the parental progenitor strains become fixed in each RI line.
The pattern of allelic polymorphisms in each RI strain is then matched with that of an unknown locus to determine its map position.

Accurate to within 5 cM.

Example of recent publication:
Cartilage and bone changes during development of post-traumatic osteoarthritis in selected LGXSM recombinant inbred mice.
Hashimoto S, Rai MF, Janiszak KL, Cheverud JM, Sandell LJ.

Congenic Strains

Two strains that are genetically identical except at a single locus.
Interspecific crosses - crosses between laboratory strains and distantly related species (or subspecies) of Mus.
most loci are polymorphic

problems with M. spretus,
small inversions may suppress recombination in some regions.
males are sterile, therefore an F2 intercross is not possible

M. musculus castaneus- more closely related (subspecies),
still have a high degree of polymorphisms
both F1 sexes are fertile.
therefore an intercross is possible
Backcross and intercross panels
large numbers of animals, improved resolution of the genetic map
DNA is limited to that obtained from one mouse, therefore mapping methods that require little DNA are required.

Mapping with anonymous DNA sequences
Simple sequence length polymorphisms (SSLP).
– also called STS (sequence tagged sites)
STRs, short tandem repeats.
– about 100,000 randomly dispersed throughout the genome.
SNPs, single nucleotide polymorphisms.
– occur every 500-1000 base pairs.

(CA)$_n$ repeats
– originally isolated by screening a small insert cDNA or genomic libraries.
- currently isolated electronically

PCR to identify polymorphisms, or direct sequencing of different strains.
di, tri or tetra-nucleotide repeats.
– often polymorphic
– suitable for typing virtually any mouse cross
  (interspecific, intersubspecific or between any inbred strain)

How are different genetic maps integrated?
designated anchor loci (highly polymorphic and robust genetic markers) are mapped on each genetic mapping panel. Anchor loci are spaced 10-20 CM apart. Provides some framework for comparing different genetic maps.
How do we analyze gene function in mice?

1. Gene addition (transgenic approach)
   - permits GOF and DN experiments
   - ectopic (spatial or temporal) expression
   - allows gene regulatory elements to be tested
   - allows populations of cells to be marked with a reporter gene

2. Targeted mutations
   - specific genes can be targeted
   - unexpected phenotypes (lethal phenotype may result prior to the spatial and temporal site of interest)
   - *** must be very careful to make a null allele or phenotype may be difficult to interpret.

3. Tissue-specific targeted mutations
   - provides some of the best features of gene targeting and transgenic approaches
   - may be combined with enhancer trap and gene trap experiments.

4. conditional gene targeting
   - a reliable system does not exist yet.

Manipulating the mouse genome

Transgenic mice
- dominant alleles
- dominant negative
- gain of function

Issues with transgenic mice
- tissue-specificity
- temporal-specificity
- level of expression
- chromosomal integration site may effect expression
- insertional mutagenesis

Embryonic stem cells

Teratomas
- tumors composed of various tissues foreign to their site of origin.
- can be formed by transplanting pieces of embryos to extra uterine sites.

Teratocarcinoma
- undifferentiated malignant stem cells, metastasize, lethal
can be made by transplanting day 6-7 mouse embryos under the kidney capsule
resulting tumors can be passaged and cultured to yield embryonic carcinoma cells, a small dense
cell with a well defined nucleus, frequent mitotic figures.

**EC cell lines**

- variety of stages of differentiation
- variable capacity to differentiate
- exponential growth required to prevent differentiation
- often feeder cells are required to prevent differentiation
- differentiation can be induced by aggregation, plate on non-tissue culture plastic, cells inherently
  sticky
- differentiation can be induced by drugs, RA or DMSO.

**ES cells, goal to create a normal pleuripotent cell line**

- isolate stem cells from normal embryo without passing through a tumor stage.
- when reintroduced into the embryo ES cells can generate high grade chimeras.
- essential to grow on feeder cells (STO fibroblasts or MEFs)
- Method to generate ES cells: transfer intact blastocyst into culture, grow to stage of early post
  implantation embryo, dissociate embryonic from extra embryonic portion, continue culture of
  ICM.

**LIF/DIA required to maintain ES cells in an undifferentiated state.**

**Knockout and targeted mice**

- considerations in designing a gene targeting experiment

  - *want null allele*

    - haplo-insufficient
    - recessive
    - difficult to prove that an allele is null
    - may be redundant with other genes

    **targeting may result in partial loss of function (hypomorph)**

    targeting may result in a dominant negative allele

- **knockin mice (gene replacement)**

- **tissue-specific targeting**

**Genomic Editing**

- Zinc finger nucleases (ZFNs)
- TAL effector nucleases (TALENs)
- CRISPR-Cas

General principle is to target a non-specific nuclease (FokI) to a specific DNA sequence
Double stranded break will induce non-homologous end joining, which can disrupt gene function.

Single stranded breaks (nicks) can induce homology-directed repair with a double or single stranded DNA template.

Nonspecific FokI nuclease domain fused to a customizable DNA-binding domain to target a single genomic locus.

FokI nuclease functions as a dimer to cleave double stranded DNA:
- can form unwanted dimers
- off-target mutagenesis is frequent

Obligate heterodimeric FokI nuclease domains (“KK” and “EL”):
- can reduce the formation of unwanted homodimers
- may have improved specificities

Single stranded cuts (nickases) can be promoted by inactivating the catalytic activity of one monomer of a ZFN or TALEN dimer.
### Time line for mouse genetic engineering

<table>
<thead>
<tr>
<th>Event</th>
<th>Year(s)</th>
<th>Authors/Institutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Development of chimeras between embryos with different genotypes</td>
<td>1960s</td>
<td>Tarkowski, Mintz, Gardner</td>
</tr>
<tr>
<td>Transgenic mice first derived by infecting embryos with retroviruses</td>
<td>1974, 1976</td>
<td>Jaenisch and Mintz</td>
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<tr>
<td>First DNA injection into mouse eggs</td>
<td>1980</td>
<td>Gordon,</td>
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<td>First embryonic stem cells developed</td>
<td>1981</td>
<td>Martin, Evans, Kaufman</td>
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<td>Germline contribution of ES cells</td>
<td>1984</td>
<td>Bradley</td>
</tr>
<tr>
<td>First genetic modification of an ES cell (HPRT gene)</td>
<td>1987</td>
<td>Hooper et al.,</td>
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<td>Improved vectors for homologous recombination</td>
<td>1987</td>
<td>Thomas and Capecchi.</td>
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<td>Phenotypic consequences of targeted genes</td>
<td>1990+</td>
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<tr>
<td>Conditional gene targeting-cre/lox</td>
<td>1992/1993</td>
<td>Marth, Rajewsky</td>
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<tr>
<td>Conditional gene targeting-flip/FRT</td>
<td>1996</td>
<td>Dymecki</td>
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<td>Multiple conditional alleles, cre, flip</td>
<td>1998</td>
<td>Martin</td>
</tr>
<tr>
<td>Somatic cloning of mice</td>
<td>1998</td>
<td>Wakayama et al</td>
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<tr>
<td>Lentiviral vectors for transgenesis</td>
<td>2002</td>
<td>Lois, Baltimore</td>
</tr>
<tr>
<td>RNAi in transgenic mice</td>
<td>2002</td>
<td>Conklin, Rosenquist</td>
</tr>
<tr>
<td>Sleeping Beauty Transposons</td>
<td>2005</td>
<td>Copeland and Jenkins</td>
</tr>
<tr>
<td>Conditional Mouse Knockout Project</td>
<td>2006-2010</td>
<td>EUCOMM, KOMP</td>
</tr>
<tr>
<td>Genomic editing-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc fingers, TALENs, CRISPR-Cas</td>
<td></td>
<td>Meyer, Tesson, Mali, Jinek</td>
</tr>
</tbody>
</table>
Selected references

Mouse evolution, genetics, development
(1-4)

Embryonic Stem Cells
(5-10)

Cloning
(11-16)

Conditional Gene targeting
(17-23)

Reporter alleles, lineage tracing
(24-28)

Lentiviral vectors, gene silencing
(29-32)

Large scale gene targeting
(33-41)

Genomic editing
(42-49)

lentivirus-based system to functionally silence genes in primary mammalian cells, stem cells and transgenic mice by RNA interference, Nat. Genet. 33(3), 401-406.


43. Tesson, L., Usal, C., Menoret, S., Leung, E., Niles, B. J., Remy, S., Santiago, Y., Vincent, A.
 generated by embryo microinjection of TALENs, Nat. Biotechnol. 29(8), 695-696,
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45. Mali, P., Yang, L., Esvelt, K. M., Aach, J., Guell, M., DiCarlo, J. E., Norville, J. E., and
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Cas system, Nat. Biotechnol.,
Mouse Molecular Genetics, homework problems.

A. You have recently cloned a cDNA that you think may be responsible for a mouse mutant phenotype that maps to chromosome 14. You decide to map this cDNA using RI mapping panels. After checking Southern blots of DNA from C57/B6, AKR and DBA mice you note a RFLP between C57/B6 and DBA using the enzyme TaqI. Attached is a Southern blot experiment in which BXD RI DNA is digested with the enzyme TaqI and probed with your cDNA. Alleles B and D are marked, as are the numbers (names) of the individual RI lines.

Mouse RI lines screened: 1 2 5 6 8 9 11 12 13 14 15 16 18 19 20 21 22 23 24 25 27 28 29 30 31 32

Does your gene map to chromosome 14? If so, where?

You will need to determine the strain distribution pattern from the Southern blot and match it to the appropriate RI data set, which you can find in the Jackson laboratory database. The purpose of this problem is to demonstrate RI mapping and to explore the databases at the Jackson laboratory. Below are two ways to find the RI mapping data.

http://www.jax.org/
go to Search JAX® Mice database
search for BXD strains
click on one of the BXD stains
under “description” click on “Mouse Genome Informatics Recombinant Inbred Strain Distribution Patterns”
copy the BXD dataset and paste into Excel as text.

Go to Mouse Genome Informatics (http://www.informatics.jax.org/)
Under more resources, click on “contributed data sets”
Click on: 8. RI Strain Distribution Patterns
copy the BXD dataset and paste into Excel as text.

B. You created a null allele of the gene shown below. Homozygous embryos were never recovered beyond embryonic day 7, however, you are interested in the function of this gene in adult pancreatic islet cells. Design an experiment that will address your goals. (Draw a diagram showing maps of your DNA vectors and appropriate mating experiments. Don’t write more than a short paragraph).

C. Design a strategy to introduce point mutation ‘A’ into the hypothetical gene shown below, into the germline of mice. (Draw a diagram showing your strategy and appropriate matings. Don’t write more than a very short paragraph).
B, BamHI
S, Scal
X, XhoI
A, point mutation