species - *Mus musculus*

Mouse Molecular Genetics

David Ornitz
History of the laboratory mouse

Eomaia scansoria –
✴ a large mouse-like mammal
✴ the earliest representative of the Eutherian (placental) lineage
✴ gave rise to all modern placental mammals 75-125 million years ago

Genus Mus –
✴ established ~6 million years ago

Mus musculus (the house mouse)
✴ appeared as a distinct species ~8,000 BC
Mus musculus - four main subspecies

Mus musculus domesticus - arose in Western Europe, Mediterranean, Africa and Middle east.

Mus musculus musculus - East Europe, Japan, Russia, Northern China

Mus musculus castaneus - Southeast Asia, Indonesia

Mus musculus bactrianus - Iran, Pakistan, India

Mus spretus
Western Mediterranean short-tailed mouse
The mouse as a genetic model

Ideal mammal for use by early geneticists - small size, relatively large litter size, rapid generation time

The mouse as a tool for genetics began in the early 1900’s
William E. Castle, JBS Haldane and Abbie Lanthrop (Granby mouse farm)
• established a mouse farm for pets, interesting coat colors and behavior
• breeding experiments
• establishment of mutant lines, ancestors of modern inbred strains
Other strains derived by Japanese, Chinese, and European mouse fanciers.
"If you want to get mice with rare color by breeding do not take good dancing mice. They are harder to breed."

Chingan Sodategusa (How to raise rare mice)
Published by Zeniya Choubei in Kyoto in 1787

Early depiction of the mouse, one of the twelve animals of the Chinese zodiac, from a printed scroll, AD 877
Fancy mice become lab mice (~1900)

Ms Abbie Lathorp at Granby farm, 1913

Letter from W. Castle concerning the mice used at Harvard, with mention waltzing mice received from Japan.

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Most of the stocks of mice which we had at the Bussby Institution and with which my wife and I and others of my Students (Snell, Nealon et al.) worked came from Abbie Lathorp of Granby, Mass. None of them came directly from Europe, but we did receive from Japan, a stock of pink-eyed yellow bellied mice, similar to those with which one of Bateson’s pupils worked (Darbishire I think).

Yours in best wishes,

W. E. Castle
Research Associate in Genetics,
Univ. of Calif.
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A sequence-based variation map of 8.27 million SNPs in inbred mouse strains, 2007, Frazer et al., Nature

a, Musculus, castaneus and domesticus subspecies diverged from a common ancestor about one million years ago.
b, Subspecies molossinus is a more recent hybrid between musculus and castaneus.
c, Eighteenth century, mouse fanciers in Japan and China inter-bred the Asian Mus musculus subspecies to produce varieties of mice with different coat colors and behavioral characteristics.
d, Victorian England, ‘fancy’ mice, imported from Asia, were bred with local mice. In the early twentieth century, mating programs using a limited number of founding ‘fancy’ mice were started in the USA, giving rise to many of the modern classical strains.
e, Relative contributions of the Mus musculus subspecies to haplotypes in classical strains.
Fancy Mice have been exhibited in Britain for more than 100 years. The Fancy started in the East End of London in the 1890's. The interest spread to the North of England where it became very popular in the mining communities. The National Mouse Club lays down the standards by which mice are judged. The LSCMRC is working on a library of photographs of all varieties of Fancy Mice and a digital library on the Mouse Fancy.
Backed by two car barons, Edsel Ford (son of Henry) and Roscoe Jackson (head of Hudson Motorcar Company), Clarence Little set up Jackson Laboratory in Bar Harbor, Maine founded in 1929.
M. musculus and M. spretus

✦ Never produce hybrids in nature.
✦ They are biochemically distinct.
✦ Their genomes are highly polymorphic.
✦ They can be bred in the laboratory. (However, the F1 males are sterile)

Extremely useful for genetic mapping studies. However, with the development of highly polymorphic SSLP markers, crosses to “wild” mice are usually no longer necessary.
The Origin of Mouse Genetics

Genetic mapping in the mouse began with Haldane’s report (1915) of linkage between the pink-eye dilution and albino loci.

This linkage group was eventually assigned to mouse chromosome 7.

This was only 2 years after the first report of genetic linkage in *Drosophila*.

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.

The C57 strain is derived from female #57 from the Granby mouse farm around 1900. Now there are more that 400 inbred strains.

One driving force to establish inbred strains was to study genetics of tumor transplantation and cancer susceptibility.

In 1948 Gorer and Snell described what is now known as the histocompatibility locus.
The mouse as a tool for genetics began in the early 1900’s.
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.
(used to transfer a mutation onto a new genetic background)

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

Example: C57B6 from different vendors are not equivalent

(3) Genetic differences from other branches are discovered.
Types of mutations

Recessive - an allele producing a phenotypic effect only in the absence of the wild-type allele
Most recessive mutations are loss of function mutations,
Most deleterious mutations involve a deficiency of the defective gene. (either amount or activity)

Dominant - an allele producing a phenotypic effect in the presence of a wild-type allele, often involves the production of a novel gene activity. Gain of function mutations are usually dominant.

Haplo - insufficient - reduction in gene activity below a threshold.

Dominant negative - a mutation that blocks the function or activity of the wild-type allele
Types of alleles

**Null** - a mutant with a phenotype that produces no wild-type gene product or activity.

**Amorph** - similar to null but without a detectable phenotype.

**Hypomorph** - a mutant allele producing reduced, but not zero activity. Usually associated with a phenotype.

**Neomorph** - a dominant mutant gene producing a novel phenotypic effect.
How do we identify new genes? new alleles?

**Naturally occurring mutations**

- spontaneous, chemically induced, radiation induced
- allows one to choose a phenotype of interest
- requires gene mapping, identification and cloning
- can lead to the discovery of completely novel genes
- can lead to a mouse model of a human disease
- the gene can be difficult to identify
How do we identify new genes? -cont.

Induced random mutations
random DNA insertion
(by product of transgenic mice, retrovirus insertion, transposon, ES cells)

enhancer trap or gene trap (splice acceptor)

- the type of phenotype cannot be chosen
- provides a sequence tag
- requires gene mapping, identification, and cloning
How do we identify new genes? -cont.

Random cDNA sequencing (ESTs)

- Provides a gene, but not necessarily a function.
- May miss genes that are expressed in very specific spatial and temporal locations.
- May identify gene families.
- A powerful tool in combination with gene targeting.
- May require gene mapping (there are still gaps in the genome sequence).
How do we identify new genes? -cont.

Gene identification from genomic sequence.

✴ Requires genomic sequence and gene identification algorithms.

✴ Not biased by gene expression patterns.

✴ May identify gene families.

✴ A potentially powerful tool in combination with gene targeting.

✴ May identify pseudogenes.

✴ May miss some genes or parts of genes.
The mouse genome

✦ 40 chromosomes (20 haploid chromosomes, 19, X, Y)
✦ Diploid DNA content, $2.7 \times 10^9$ bp (about 6.4 pg per nucleus)
✦ 8-10% of the genome is repeat sequence
✦ Estimated genetic haploid length is 1400CM, 1CM is $1.5 \times 10^6$ bp
✦ Over 90% of the mouse and human genomes can be partitioned into corresponding regions of conserved synteny
✦ Approximately 80% of mouse genes have a single identifiable orthologue in the human genome
✦ Draft sequence was completed in 2002 (Nature, Dec. 2002).
Mouse genome statistics

Genome Length: 2,730,871,774

37,311  Genes (including uncloned mutants)
28,983  Genes with nucleotide sequence data
25,066  Genes with protein sequence data
14,024  Genes with experimentally-based functional annotations
13,233  Genes with gene traps
13,185  Genes with expression assay results
85,345  Mapped genes/markers
Mapping genes

Classical genetic backcross—linkage mapping

Look for recombination and linkage between two genetic loci.

Example: Dominant white spotting and alfa feto protein.

Look at coat color (dominant white spotting) and electrophoretic migration (AFP)
(or similarly look at an RFLP for the AFP gene)
Linkage mapping

progenitor strains

Dominant-white spotting (W) locus encodes the c-kit proto-oncogene
Linkage mapping can be used to map any mutation with a phenotype to any polymorphic marker or other mutation with a phenotype.

But this takes a long time and requires breeding.

Mouse tools to facilitate mapping:
• Recombinant Inbred strains
• Interspecific and intersubspecific crosses
Recombinant Inbred (RI) strains

Start with two different inbred strains of mice.  
20 generations of brother-sister matings.  
Establish 30 RI mouse strains.  

Polymorphisms between the parental progenitor strains become fixed in each RI line.  

Each RI strain is unique.  

The map position of novel genetic loci can be determined by matching their pattern of allelic polymorphisms to the strain distribution pattern for the 30 RI strains.  

Limitations in map resolution due to a finite number of strains  

Advantages for large scale mapping - infinite amount of DNA
Mapping with recombinant inbred (RI) strains

progenitor strains
BXD cross
C57B6
DBA

brother-sister mating
G1

brother-sister mating
20 generations
G2

RI line number
G20

\[ \begin{array}{c|c|c|c|c}
\text{RI line number} & 1 & 2 & 3 & 4 \\
\hline
B & B & B & B & B \\
C & C & C & C & C \\
D & D & D & D & D \\
E & E & E & E & E \\
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B=C57BL/6J allele
D=DBA allele

strain distribution pattern
**RI strain distribution pattern**

Example: Map an unknown gene, with RI strain distribution pattern BBDD.

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B=C57BL/6J allele
D=DBA allele

- maps near locus D
BXD strain distribution pattern, mouse chromosome 14

| BXD   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 |
|-------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| D14Pas1 | B | D | B | D | D | B | B | D | B | D | D | B | D | B | B | B | B | B | B | B | D | D | D | D |
| Ms6-5 | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| Nr1    | B | D | B | D | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| Np     | B | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| Tcra   | B | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| Xmv19  | B | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| Ang    | B | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| D14Byu3 | B | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| Rib1   | B | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| D14Byu4 | B | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| Mcpt1  | B | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| Mcpt4  | B | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| Mcpt5  | B | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| Myhca  | B | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| Ctla6  | B | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| D14Mit5 | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| D14Mit6 | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| Raftk  | B | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| D14Byu5 | B | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| Gnrh   | B | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| Es10   | B | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| For5   | B | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| Rbl1   | B | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| Iapls3-15 | B | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| Tst1p98-14 | B | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| D14Mcl | B | D | B | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
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Interspecific and intersubspecific crosses

Crosses between laboratory strains and distantly related species of *Mus*.

**Advantage:** Most sequences are polymorphic.

*M. spretus* (inter-specific):
Small inversions may suppress recombination in some regions. Males are sterile so you cannot do an intercross.

*M. m. castaneus* (inter-sub specific)
More closely related (sub species)

Still has a high degree of polymorphisms. Both F1 sexes are fertile so you can do an intercross.

Resolution is high - large numbers of animals, but DNA is limited to that obtained from one mouse.
Mapping with backcross mapping panels

progenitor strains

C57B6 X *M. spretus*

C57B6  \[\rightarrow\] *M. spretus*

G1  \[\rightarrow\] BSS  \[\rightarrow\] *M. spretus*

BSS polymorphism distribution panel

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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 isolated by screening a small insert DNA library.

PCR to identify polymorphisms.

di, tri or tetra-nucleotide repeats.

★ often polymorphic
★ suitable for typing virtually any mouse cross (interspecific, intersubspecific or between lab substrains)
Ensembl SNP view page
Genotyping with SSLPs

allele

1  GATCACTGTTCGGCACACACACACACACAAATGCTAGCTACCGT
2  GATCACTGTTCGGCACACACACACACACACACACACACACACAAATGCTAGCTACCGT
3  GATCACTGTTCGGCACACACACACACACACACACACACACACACACACACACACAAATGCTAGCTACCGT

[Diagram showing lanes 1, 2, and 3 with alleles 1, 2, and 3 respectively]
Current use of Recombinant Inbred Strains

Cartilage and bone changes during development of post-traumatic osteoarthritis in selected LGXSM recombinant inbred mice.

The genome architecture of the collaborative cross mouse genetic reference population.
Collaborative Cross (CC)

Multiparental recombinant inbred panel derived from eight laboratory mouse inbred strains

Genome architecture analyzed for 350 genetically independent CC lines

Custom genotyping array with 7500 SNPs
The mouse *tilted* mutation

- *tilted* (*tlt*) is a spontaneous recessive mutation in a C57B6/J background
- The *tlt/tlt* mutant specifically lacks otoconia and consequently does not sense spatial orientation relative to the force of gravity
- The *tlt* defect is highly penetrant
- Exhibits no degeneration of the sensory epithelium
- Has no apparent abnormal phenotype in other organ systems (Non-syndromic)
- The *tlt* mutation maps to the proximal region of mouse Chr5
Phenotyping the tilted mouse
Anatomy and Histology of the Vestibular System

Semi-circular Canals (angular acceleration)

Cochlea (Hearing)

Saccule & Utricle (linear acceleration + gravity)

Otoconia

Gelatin layer

Supporting cell

Hair cell Type I

Hair cell Type II
Histopathology of the *tlt* mouse
The *mergulhador* (diver) mice

- *mergulhador (mlh)* is a recessive mutation
- The *mlh/mlh* mutant displays non-swimming behavior
- *mlh/mlh* mice lack otoconia but show no other gross malformations of the inner ear or of any other organ system
- *mlh* maps to the proximal region of mouse Chr 5

Are *tilted* and *mlh* two mutant alleles of the same gene?
Complementation test:

\( tlt/tlt \times mlh/mlh \)

Result: \( tlt/mlh \) mice do not swim normally

Conclusion: \( tlt \) and \( mlh \) are allelic
The tilted locus was originally mapped to mouse chromosome 5 by establishing linkage to Hm and Kit.
Mouse Chr 5

Hammertoe locus

c-Kit proto-oncogene

Lane, (1986, 1987)
Positional Cloning Strategy

**Genetic Mapping**
- MIT Markers (non-coding)
- Genes (coding)
- F2 intercross C57BL/6J- \textit{tilt} x \textit{Mus castaneous} (500 mice)
- T3 Mouse-Hamster Radiation Hybrid panel (100 cell lines)
- Synteny with human

**Physical Mapping**

**Candidate Gene**
Strategy for mapping the tlt locus (T)

parental strains
B6 X M. castaneus
(TT) (tt)

C57B6
TT

M. castaneus
tt

G1

Tt

C57B6
TT

Tt

Save all TT progeny (can’t swim)
Haplotype analysis - intersubspecific cross

F2 (C57BL6/J-\textit{tlt} x \textit{M. m. castaneus}) x (C57BL6/J-\textit{tlt} x \textit{M. m. castaneus})

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<th>Genotype</th>
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\textit{tlt}

Total: 487 456 1 2 1 2 1* 3 1 8 12
Mouse Chr 5

Hm

Kit

$tlt$ (40 cM)

$tlt$ (1.1 cM)

$Dmo3$

$D5Mit353$
Genetic Map
Synteny between Human Chr 4 and Mouse Chr 5

Human Chr 4

Mouse Chr 5

4p1

4p16.3-4p15.1

tlt
Human-Mouse Comparative Mapping of the tlt Locus
Positional Cloning Strategy

- **Genetic Mapping**
- **Physical Mapping**
  - Chromosome Walking (BAC clones)
  - Genomic sequence
- **Candidate Gene**
Positional Cloning Strategy

- Genetic Mapping ✓
- Physical Mapping ✓
- Candidate Gene

[*]
Preliminary Expression Study

1. Extract RNA from mouse otocyst (E16.5 and P0 stages)
2. Determine the gene expression of 4 candidate genes (RT-PCR assay)
Novel gene (26795 bp)

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<th>Exon</th>
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<th>Splice acceptor</th>
<th>Intron</th>
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</table>
 Mutation screening test (sequencing)

1. Draft sequence 4 mouse BACs
2. Determine the gene structure of 4 candidate genes (total of 37 exons)
3. Sequence exons and splice site junctions in \( tlt \) mutant
4. Compare with WT sequence

![Diagram of genetic loci with annotations for \( tlt \) and candidate genes.]
RP23-426E16: full sequence and annotation

**Lyar**

**Otopetrin (Otop)**

**Drd5**

**Glut9**
Mutation Screening of the tlt mutant

C57B6/J +/+  

T C G C  
A G C G

C57B6/J tlt/tlt

T C G A  
A G C T

TaqI

T C G A
A G C T

+/-  tlt/tlt  +/-

-391bp  -230bp  -161bp

GSITLFAFITVVLGCLKVAYFIGFSECLSAATEGVFPVTHAVHTLLQ
GSITLFAFITVVLGCLKVEYFIGFSECLSAATEGVFPVTHAVHTLLQ
Mutation Screening of the \textit{mlh} mutant

\begin{itemize}
\item \textbf{Balb/c}^{+/+} \\
\hspace{1cm} \text{[Image of DNA sequences]}
\item \textbf{Balb/c}^{mlh/mlh} \\
\hspace{1cm} \text{[Image of DNA sequences]}
\end{itemize}

\begin{itemize}
\item \textbf{Mscl} \\
\hspace{1cm} \text{[Image of DNA sequences]}
\end{itemize}

\begin{itemize}
\item \textbf{+/+} \hspace{1cm} \text{[Image of DNA bands]}
\item \textbf{mlh/mlh} \hspace{1cm} \text{[Image of DNA bands]}
\end{itemize}

\begin{itemize}
\item \textbf{542bp} \hspace{1cm} \text{[Image of DNA bands]}
\item \textbf{481bp} \hspace{1cm} \text{[Image of DNA bands]}
\end{itemize}

\begin{itemize}
\item \textbf{DLLVATGSGSWLLSWGSLAIACACAETRPPYTWNLPYSVLVIVEKYVQNI}F
\item \textbf{DLLVATGSGSWLLSWGSIQAIACACAETRPPYTWNLPYSVLVIVEKYVQNI}F
\end{itemize}
# Time line for mouse genetic engineering

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<th>Contributors</th>
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<td>Development of chimeras between embryos with different genotypes</td>
<td>1960s</td>
<td>Tarkowski, Mintz, Gardner</td>
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<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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<td>First DNA injection into mouse eggs</td>
<td>1980</td>
<td>Gordon, Brinster, Constantini, Lacy, Wagner</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>
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<td>First genetic modification of an ES cell (HPRT gene)</td>
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<td>Smithies</td>
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<td>Improved vectors for homologous recombination</td>
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<td>Thomas and Capecchi.</td>
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<td>Phenotypic consequences of targeted genes</td>
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<td>Conditional gene targeting-cre/lox</td>
<td>1992/1993</td>
<td>Marth, Rajewsky</td>
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<td>Conditional gene targeting-flip/FRT</td>
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<td>Multiple conditional alleles, cre, flip</td>
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<td>Wakayama et al</td>
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<td>Lentiviral vectors for transgenesis</td>
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<td>RNAi in mice</td>
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<td>Conklin, Rosenquist</td>
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<td>Jenkins, Copeland</td>
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<td>Conditional Mouse Knockout Project</td>
<td>2006 -</td>
<td>EUCOMM, KOMP</td>
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<tr>
<td>Genomic editing</td>
<td>2010 -</td>
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The Nobel Prize in Physiology or Medicine
2007

"for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells"

Mario R. Capecchi  Sir Martin J. Evans  Oliver Smithies
How do we analyze gene function in mice?

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

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

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.

Conditional gene targeting
- An effective method to circumvent embryonic lethality.