Belgian Society of Human Genetics

Of Mice and Human Genetics

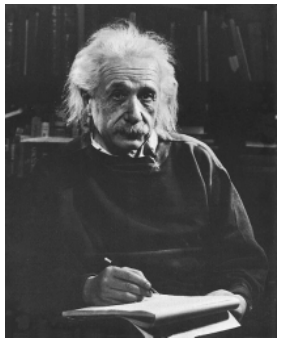
Frank Kooy

Permanent Education Course in Human Genetics

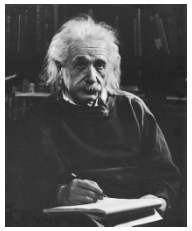
Universiteit Antwerpen

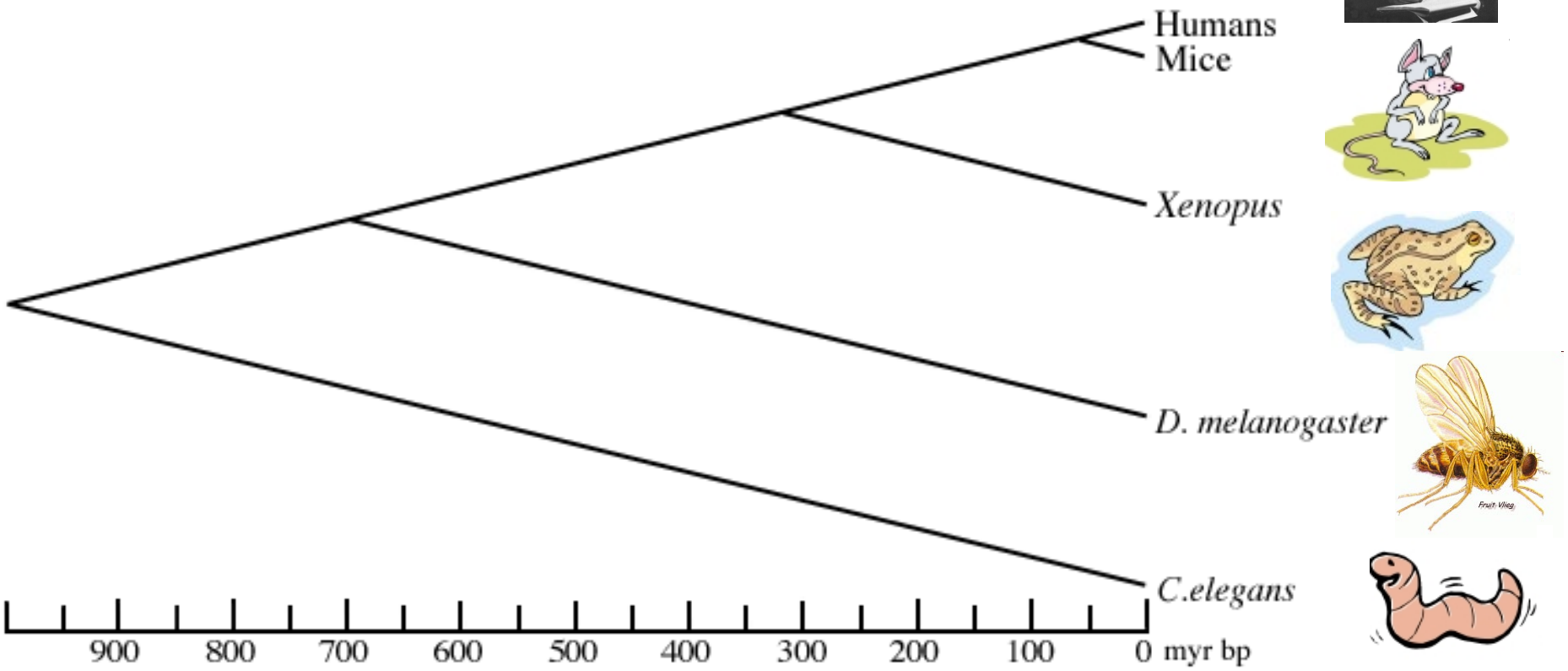
Cognitive Genetics
Centre of Medical Genetics
University of Antwerp

Why study mice



Evolutionary relationships



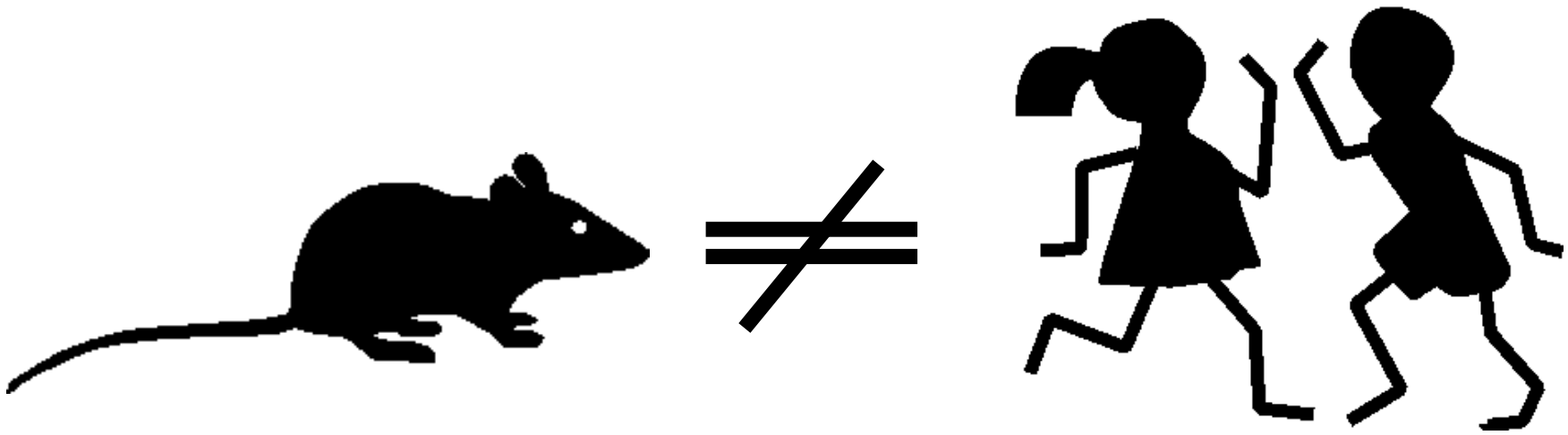


1 Myr = 1 million years

Why study mice ?

- Allow Genetic Manipulation
- Unlimited Numbers can be Bred
- Control of Genetic Background
- Availability of all Organs/Tissues
- Can be used for drug testing

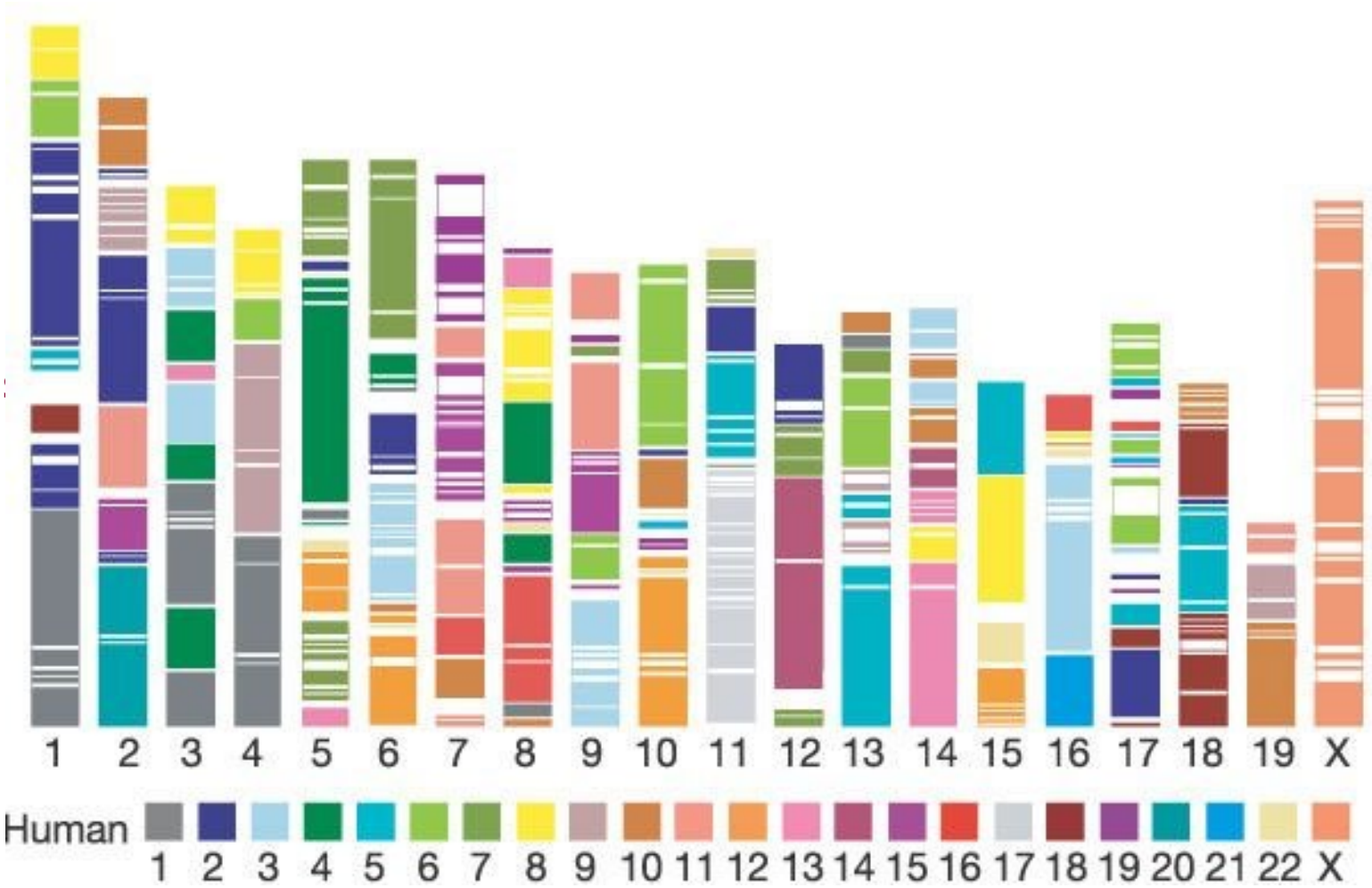
A mouse is not a man



What is in their genome?

Over 90% of the mouse and human genomes can be partitioned into corresponding regions of conserved

Only 1% of mouse genes has no identifiable orthologue in man.



MGSC, Nature 420, 520–562 (2002)

Genetic modification: knockout mouse

- Knockout mouse — Inactivation of coding sequence at defined position in genome

1987-89

The first knockout mouse

Teams led by Martin Evans, Oliver Smithies and Mario Capecchi create the first 'knockout' mice, by selectively disabling a specific target gene in embryonic stem cells. The three receive the Lasker Award in 2001 for this achievement, and the technique goes on to be used to create several thousand knockout mice.

- Advantages: Specific inactivation of gene of interest
- Disadvantage: technically complicated

The Nobel Prize
in Physiology or Medicine 2007

Generation of a knockout mouse

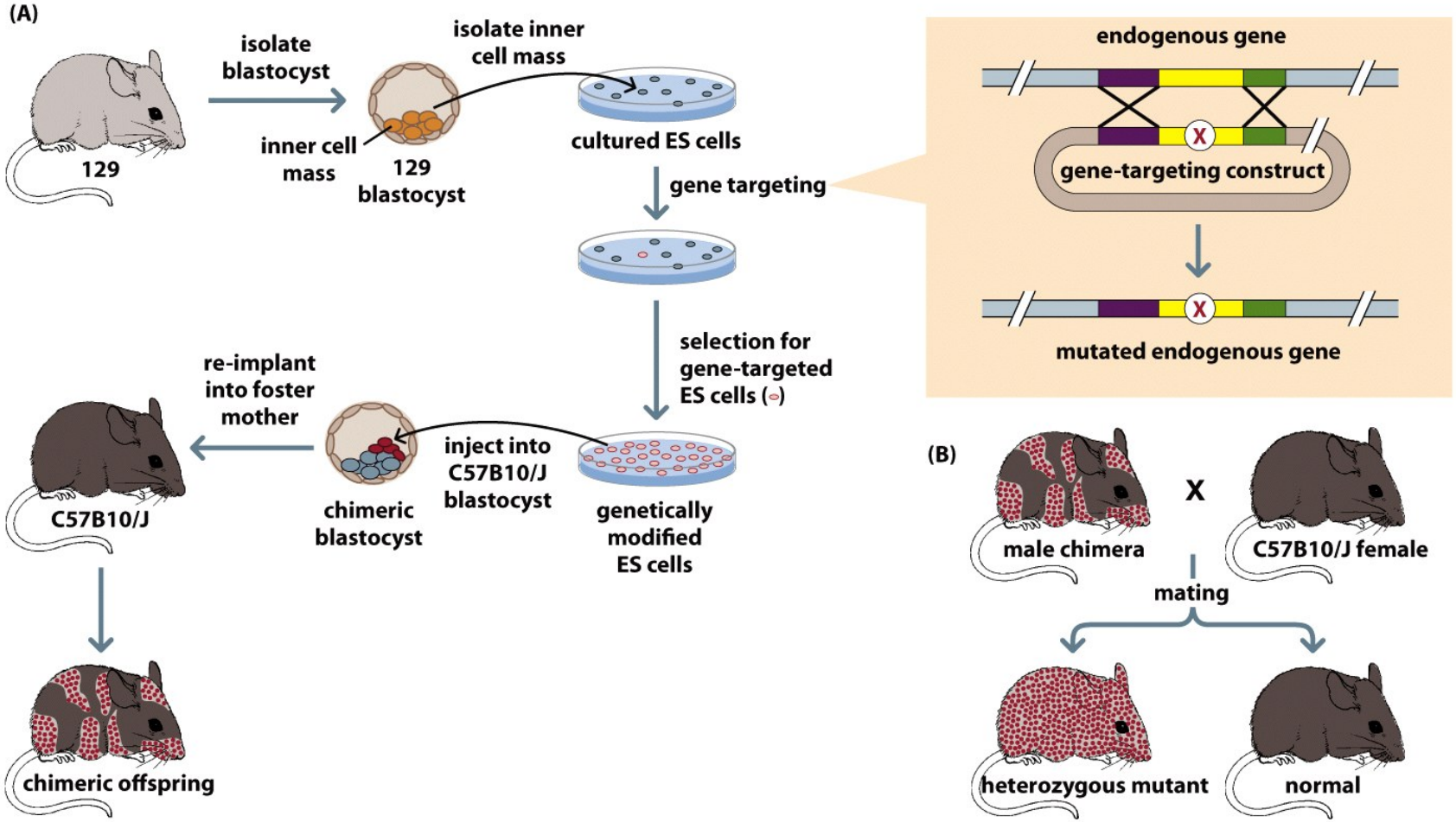


Figure 20.6 Human Molecular Genetics, 4ed. (© Garland Science)

Generation of a knockout mouse

Fragile X mouse

Obese mouse (taconic)

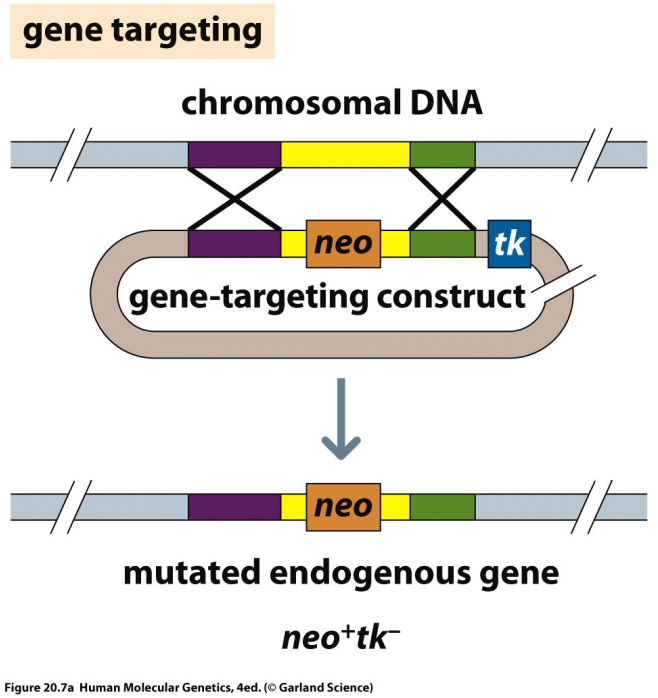


Figure 20.7a Human Molecular Genetics, 4ed. (© Garland Science)

Conditional knockout mouse

Cre-recombinase and flippase are site specific recombinases

Figure 20.10 Human Molecular Genetics, 4ed. (© Garland Science)

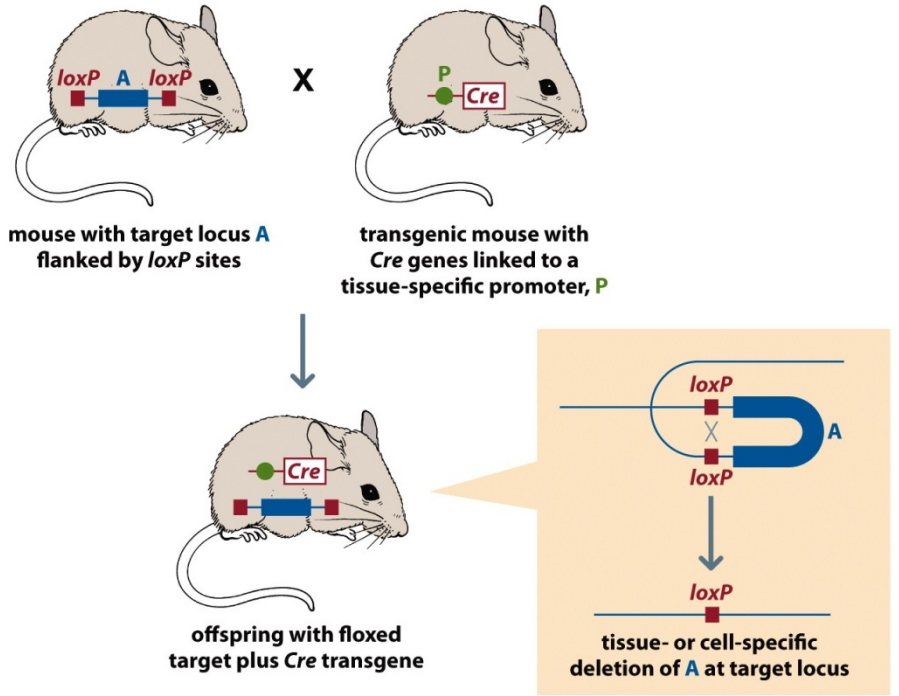
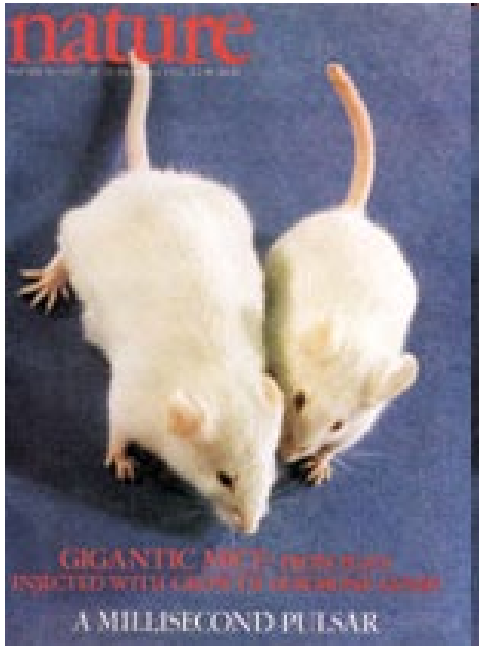


Figure 20.11 Human Molecular Genetics, 4ed. (© Garland Science)

Transgenic mouse

- Transgenesis — Introduction of genetic material at random position in genome



1982

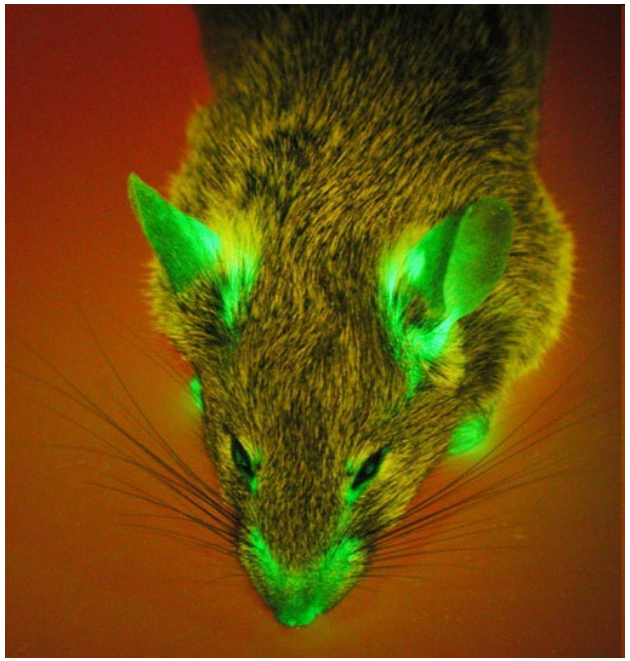
First transgenic mouse

A team led by Richard Palmiter and Ralph Brinster fuse elements of a gene that can be regulated by dietary zinc to a rat growth-hormone gene, and inject it into fertilized mouse embryos. The resulting mice, when fed with extra zinc, grow to be huge, and the technique paves the way for a wave of genetic analysis using transgenic mice.

- cDNA with different promotors
- Large genomic inserts with gene of interest (Cosmids, PAC, BAC, YAC) and natural promotor

- Advantage: relatively simple technology
- Disadvantage: need to analyze several lines

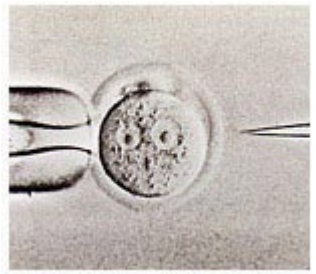
Transgenesis: not limited to mice



Green fluorescent protein (GFP) transgenic

Glowfish

Generation of transgenic mice



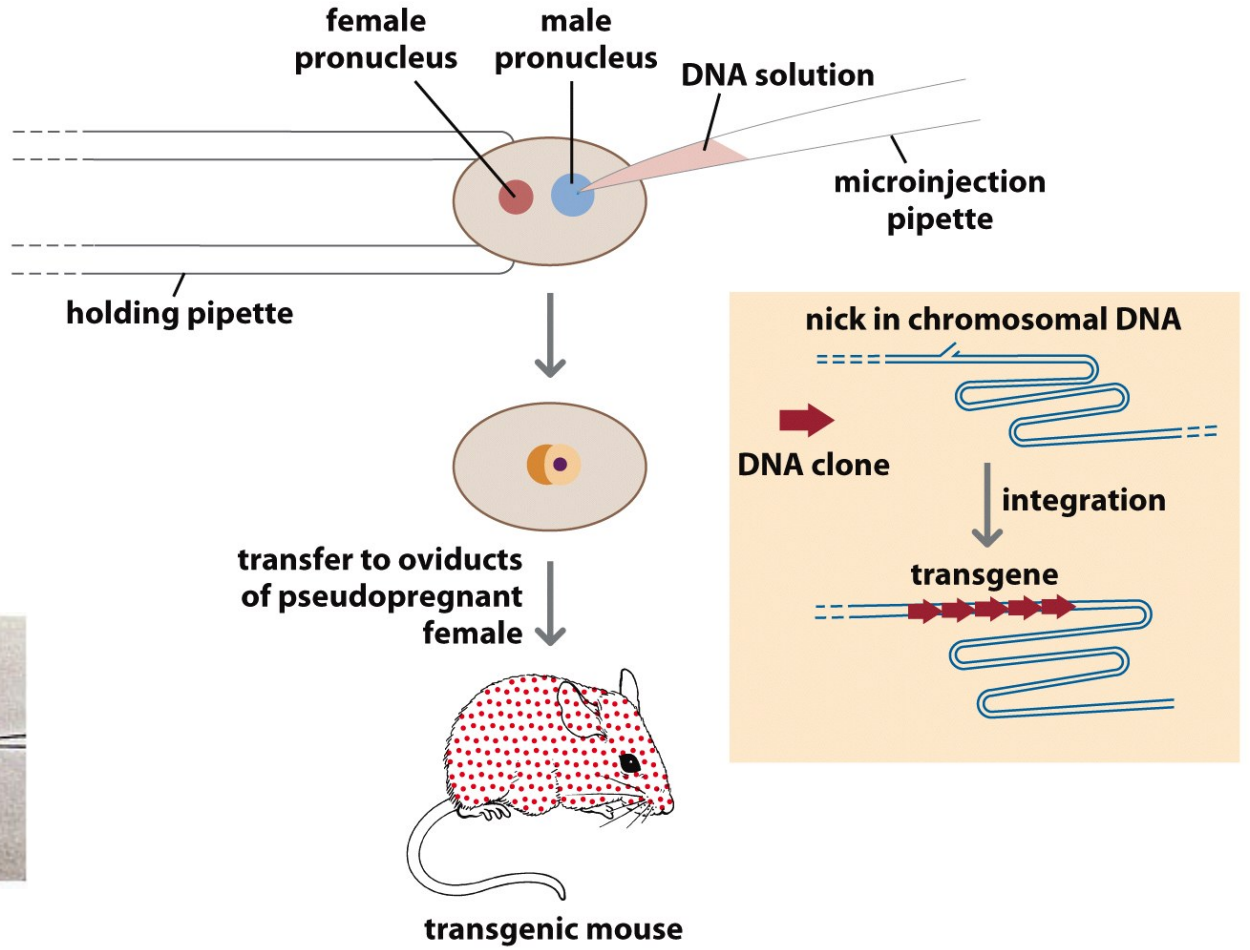


Figure 20.3 Human Molecular Genetics, 4ed. (© Garland Science)

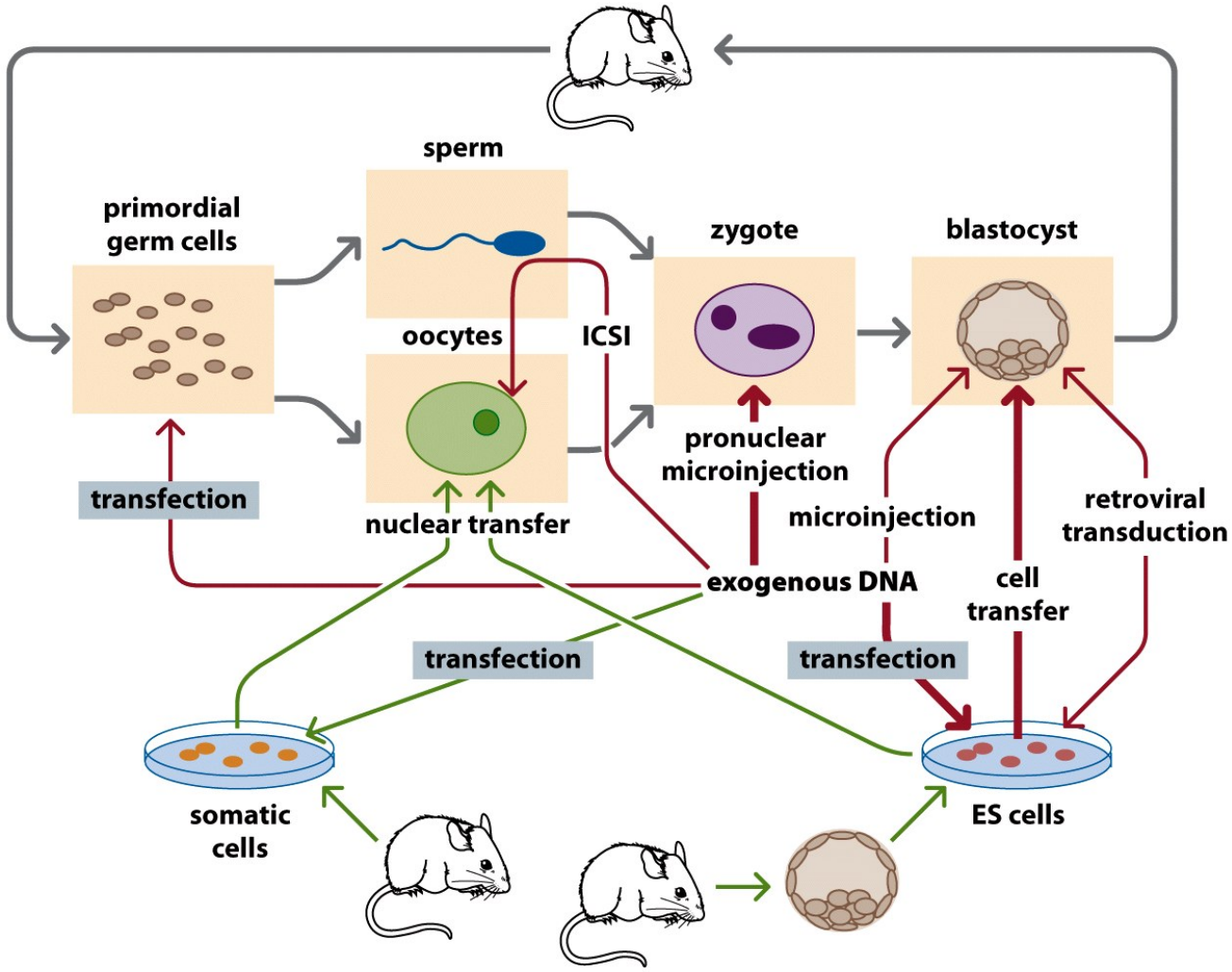


Figure 20.2 Human Molecular Genetics, 4ed. (© Garland Science)

Inducible transgenic mice

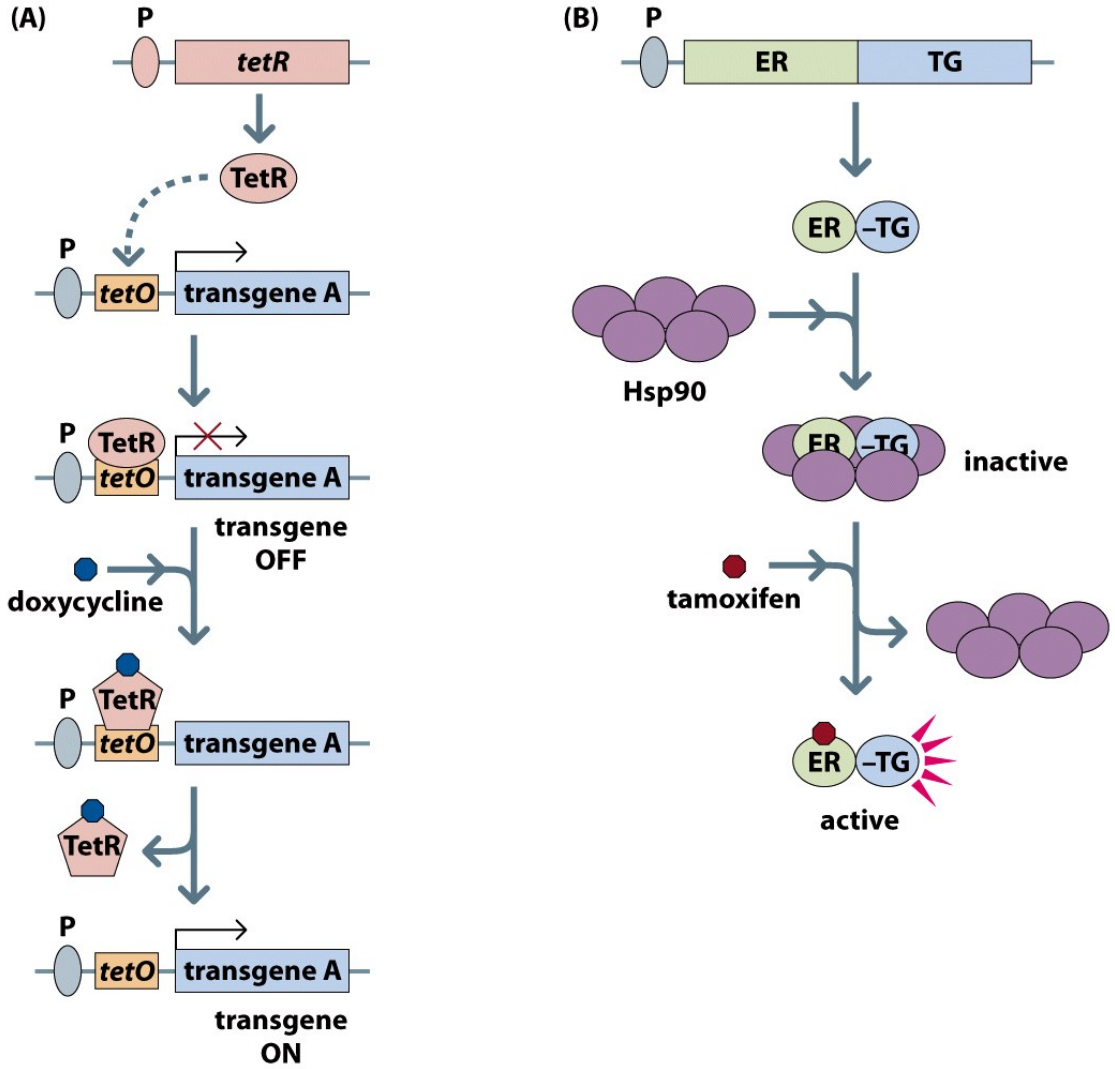
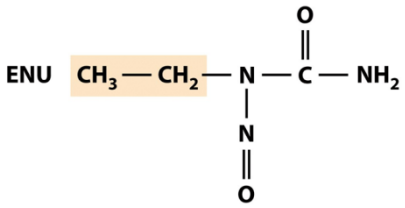


Figure 20.5 Human Molecular Genetics, 4ed. (© Garland Science)

“Getting” a knockout

- Generate one
  - yourselves, in collaboration with specialized University laboratories or commercial
- Seach databases (Jackson) and literature



- Seach chemically induced (ENU) mutant databases

- Seach random mutagenesis databases (genetrap)

Genetrap principle

Figure 2. Schematic of a standard gene trap vector.

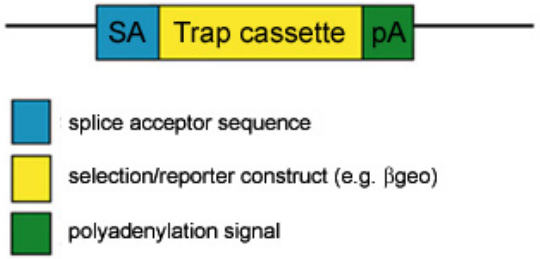
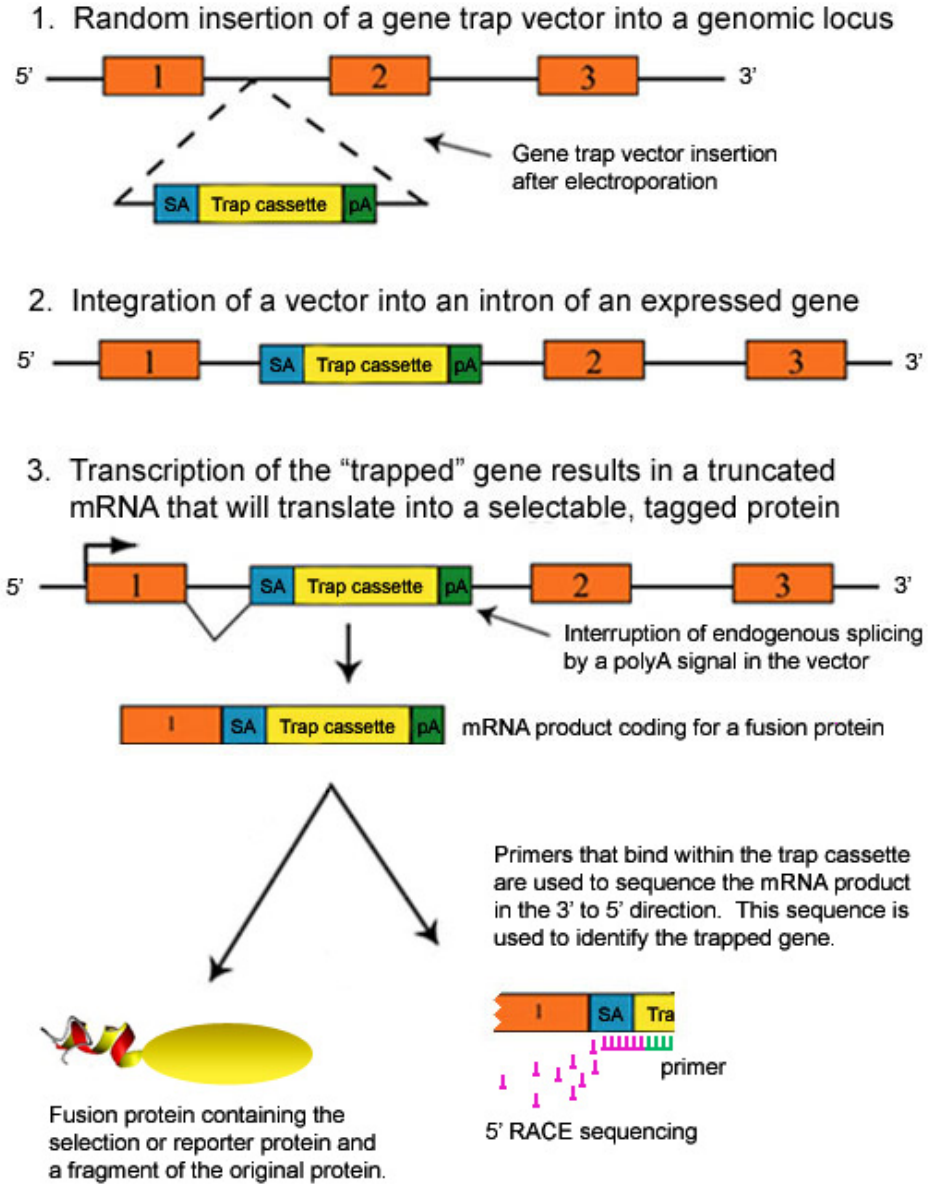


Figure 3. The conventional trapping process.



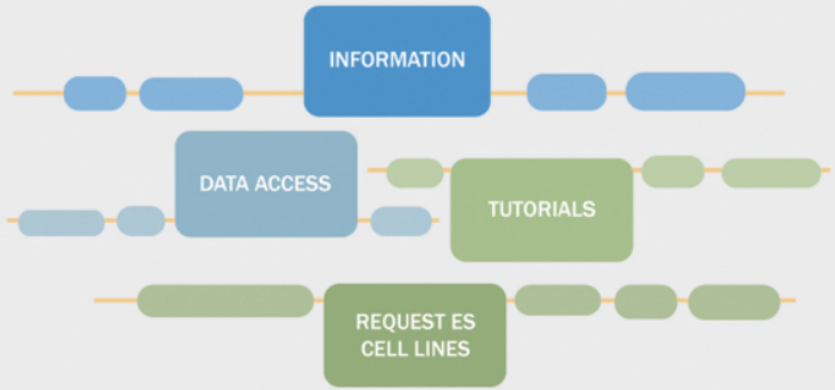
International Gene Trap Consortium

INFORMATION | DATA ACCESS | TUTORIALS | REQUEST ES CELL LINES

About IGTC

Gene trapping is a high-throughput approach that is used to introduce insertional mutations across the genome in mouse embryonic stem (ES) cells. In addition to generating standard loss-of-function alleles, newer gene trap vectors offer a variety of post-insertional modification strategies for the generation of other experimental alleles.

The International Gene Trap Consortium (IGTC) represents all publicly available gene trap cell lines, which are available on a non-collaborative basis for nominal handling fees. Researchers can search and browse the IGTC database for cell lines of interest using accession numbers or IDs, keywords, sequence data, tissue expression profiles and biological pathways.



Statistics and News

› Statistics: Mar 20, 2020

IGTC cell lines in database:121703 cell lines
Pipeline Status:116765 (95.94%) processed cell lines
Last dbGSS deposit:2014-02-19
Ensembl version:54, NCBI m37, IGTC cell line coverage 12393 (39.33%) genes
Entrez version:NCBI m37, IGTC cell line coverage 16377 (25.88%) genes
More statistics...

› The International Gene Trap Consortium is moving to a new home in the Mouse Biology Program at UC Davis

Oct 14, 2010

The International Gene Trap Consortium (IGTC) web portal, www.genetrap.org, is moving to a new home at the Mouse Biology Program (MBP) at the University of California, Davis. The move became effective October 1, 2010 and was designed to enhance IGTC's longevity of services by providing a seamless transition of support to MBP's scientific and informatics staff and in-house web services infrastructure.

› Genomic Sequence Alignment Images

Jul 29, 2010

Individual cell line and gene pages have long included an image depicting how cDNA cell lines align to the trapped gene. As most IGTC cell lines are genomic traps, we are introducing new genomic alignment images. Now both cDNA and genomic traps are aligned against the associated gene transcripts and viewable in a scrollable Scalable Vector Graphics (SVG) image.

› IGTC Running on New Hardware

May 27, 2010

The IGTC web site is now running on a Red Hat Enterprise Linux server cluster.

All news and events

CONTACT US

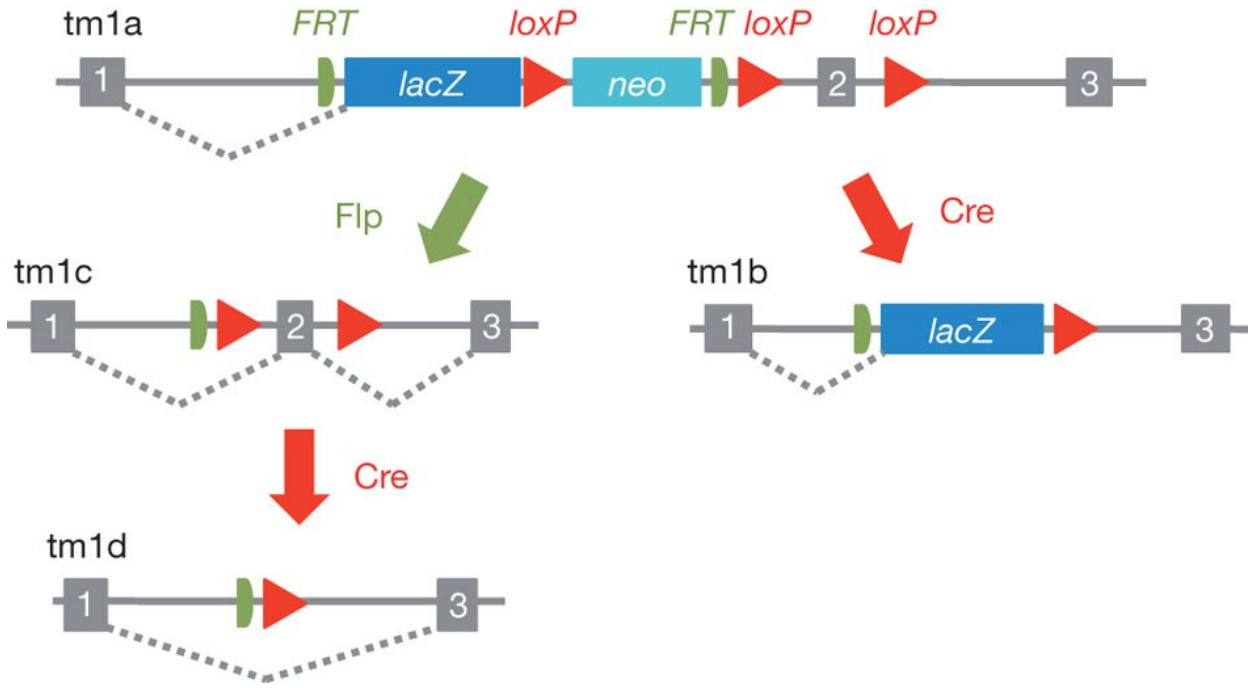
ARTICLE

doi:10.1038/nature10163

A conditional knockout resource for the genome-wide study of mouse gene function

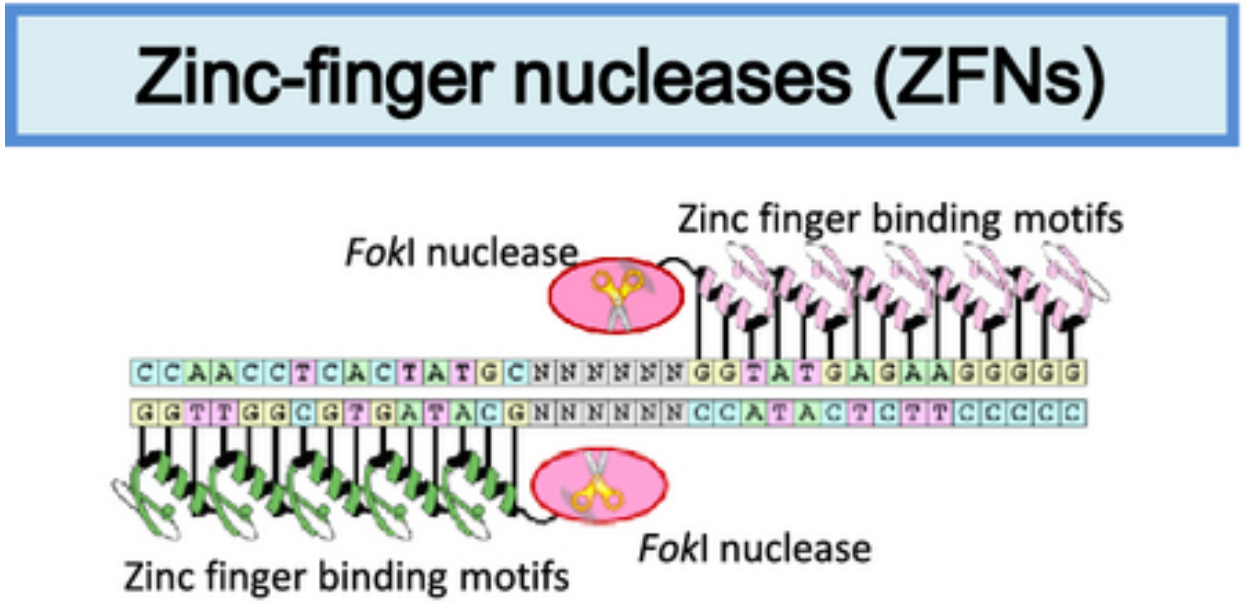
William C. Skarnes[1], Barry Rosen[1], Anthony P. West[1], Manousos Koutsourakis[1], Wendy Bushell[1], Vivek Iyer[1], Alejandro O. Mujica[1]†, Mark Thomas[1], Jennifer Harrow[1], Tony Cox[1], David Jackson[1], Jessica Severin[1]†, Patrick Biggs[1]†, Jun Fu[2], Michael Nefedov[3], Pieter J. de Jong[3], A. Francis Stewart[2] & Allan Bradley[1]

16 JUNE 2011 | VOL 474 | NATURE | 337

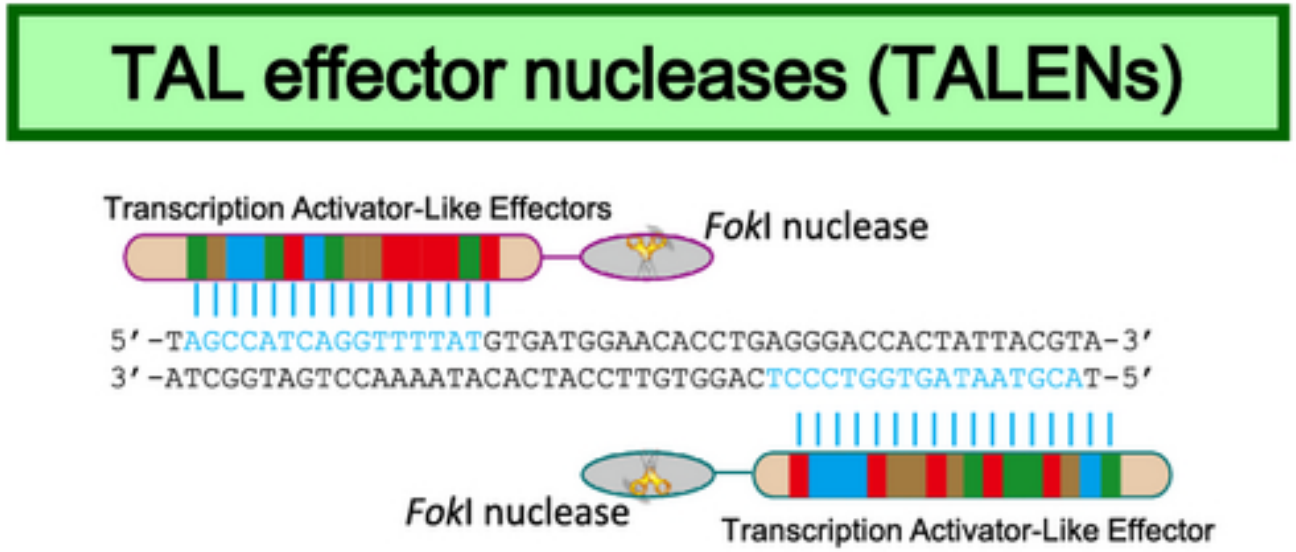


The 'knockout-first' allele (tm1a) contains an IRES:*lacZ* trapping cassette and a floxed promoter-driven *neo* cassette inserted into the intron of a gene, disrupting gene function. Flp converts the 'knockout-first' allele to a conditional allele (tm1c), restoring gene activity. Cre deletes the promoter-driven selection cassette and floxed exon of the tm1a allele to generate a *lacZ*-tagged allele (tm1b) or deletes the floxed exon of the tm1c allele to generate a frameshift mutation (tm1d), triggering nonsense mediated decay of the deleted transcript.

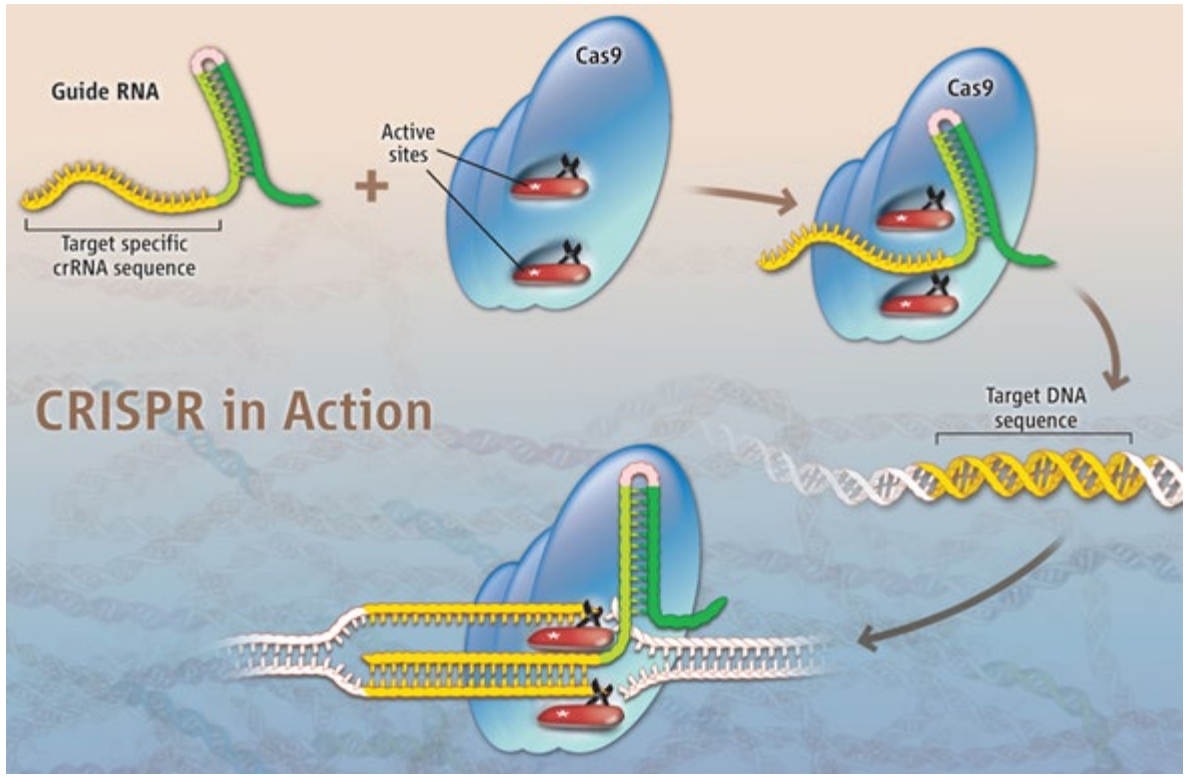
Novel methods:
Zinc-finger nucleases



Novel methods:
Transcripion activator-like effectors



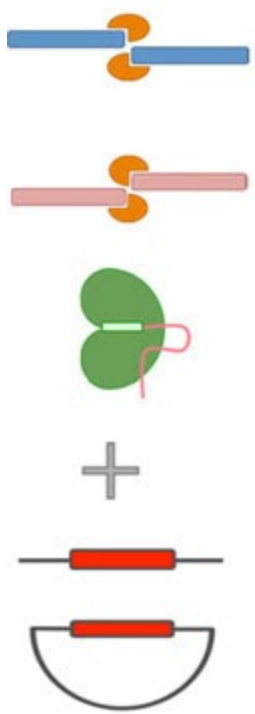
Novel methods: Clustered, regularly interspaced, short palindromic repeats-associated endonuclease (CRISPR/Cas)

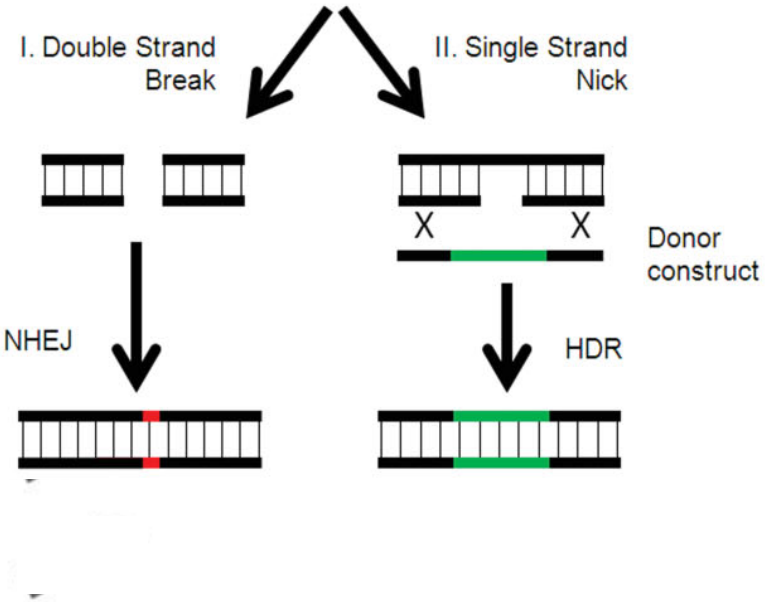


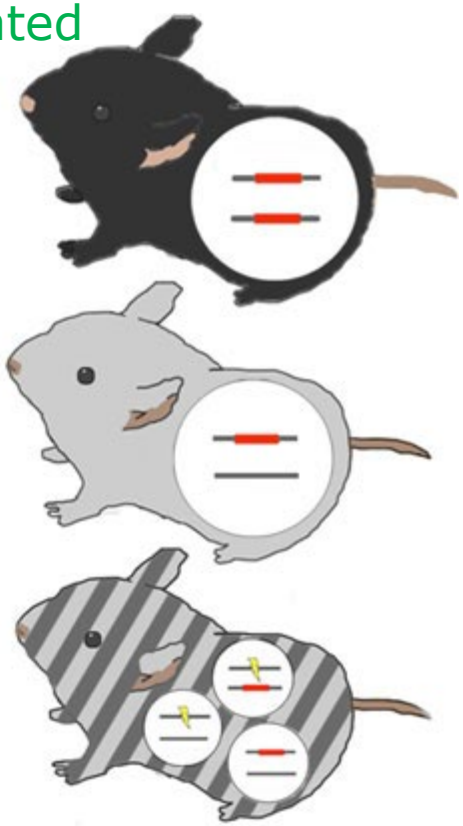
Transcription activator-like effector nucleases (TALENs),

Zinc-finger nucleases (ZFNs)

Clustered, regularly interspaced, short palindromic repeats-associated endonuclease (CRISPR/Cas)







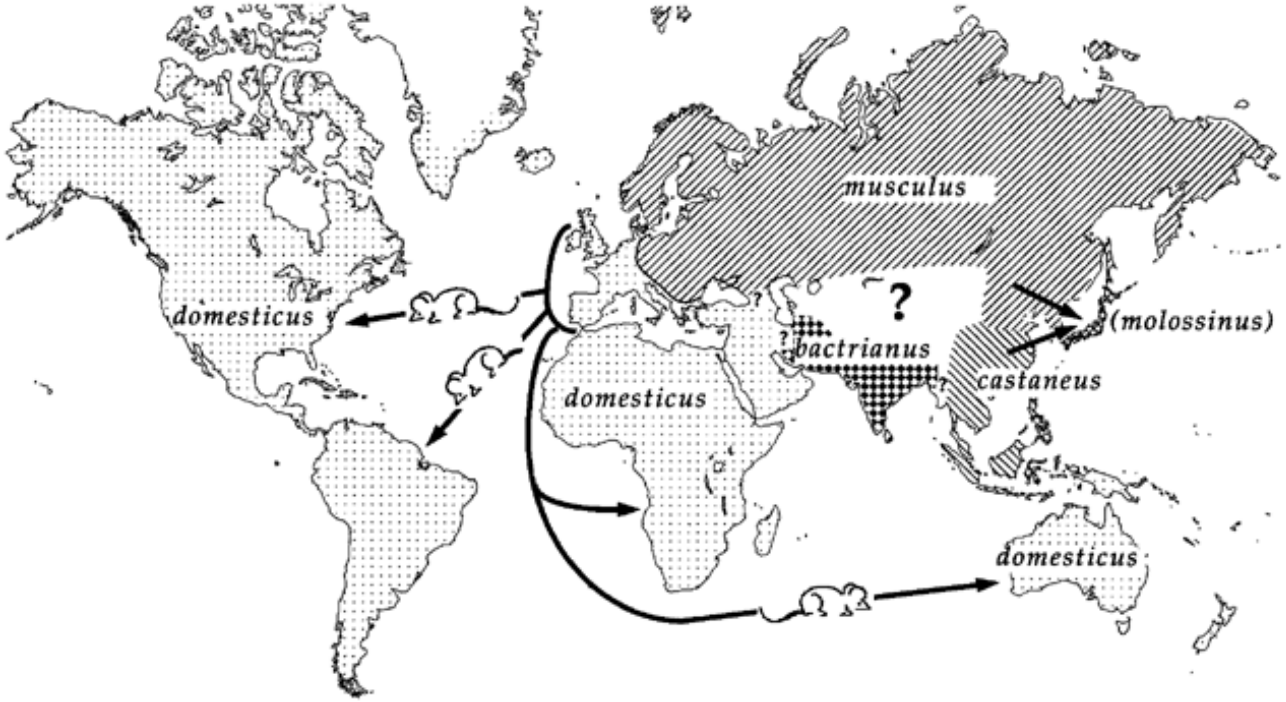
Nonhomologous end-joining (NHEJ)

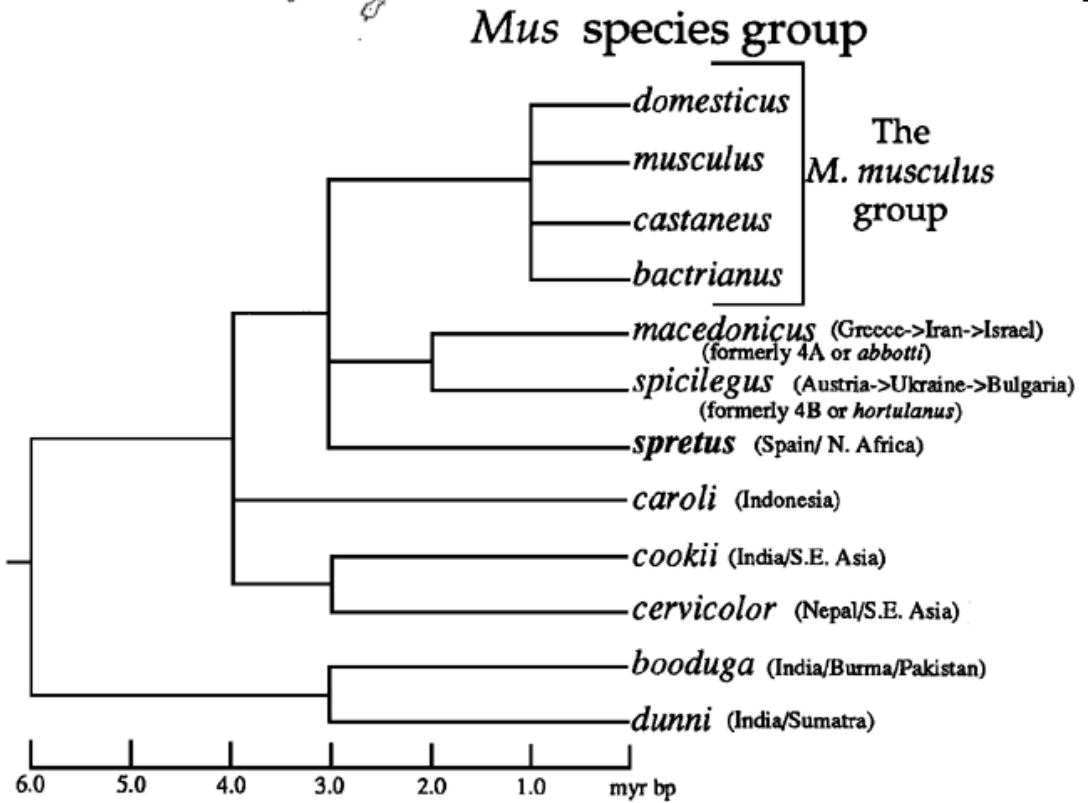
Double-strand break (DSB) and subsequent homology-directed repair (HDR)

What mice are we using ?
Fancy mice become lab mice

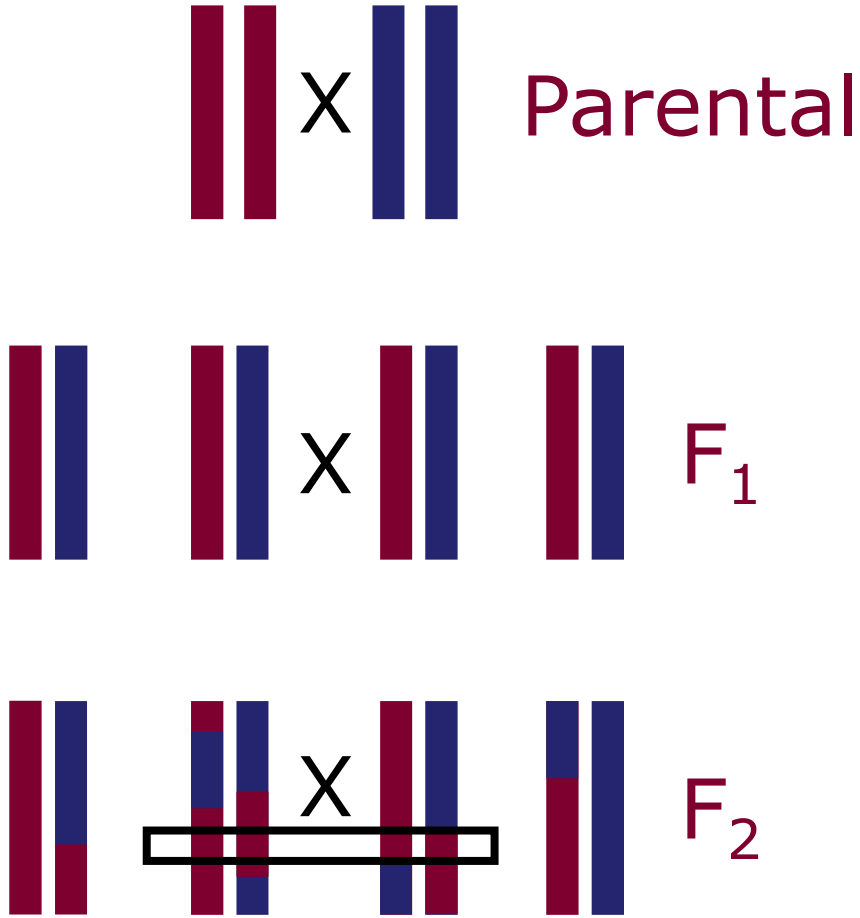
William Ernest Castle
(Harvard)

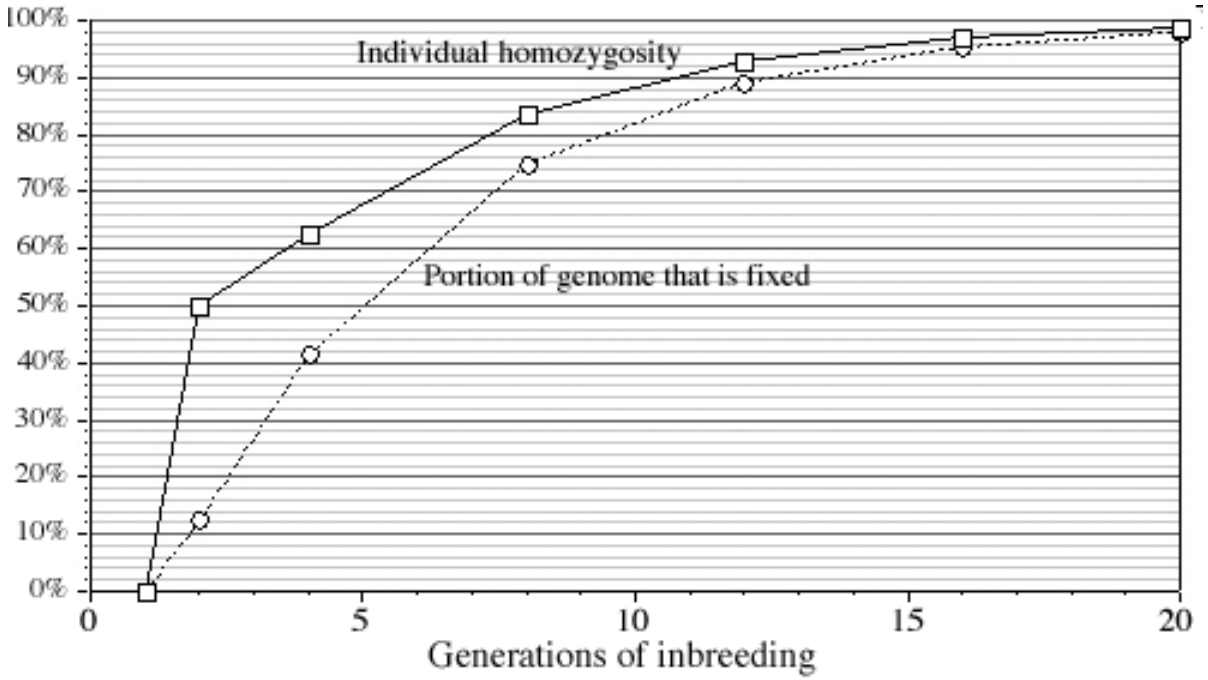
Abbie Lathrop, a retired schoolteacher,
breeds fancy mice (Granby, MA)


musculus
?
domesticus
bactrianus
(molossinus)
castaneus
domesticus
domesticus


Mus species group
domesticus
musculus
castaneus
bactrianus
The M. musculus group
macedonicus (Greece->Iran->Israel)
(formerly 4A or abbotti)
spicilegus (Austria->Ukraine->Bulgaria)
(formerly 4B or hortulanus)
spretus (Spain/ N. Africa)
caroli (Indonesia)
cookii (India/S.E. Asia)
cervicolor (Nepal/S.E. Asia)
booduga (India/Burma/Pakistan)
dunni (India/Sumatra)
6.0
5.0
4.0
3.0
2.0
1.0
myr bp

Inbred strains: 20 generations of brother x sister matings





First inbred strain: DBA

bred by Clarence Cook Little (Harvard)

- At F_{20} generations: 98.7% of loci homozygous

Commonly used inbred strains

Strain	Use	Breeding performance	Remarks
129	Construction ES cell lines	Poor	Poor cognition
Balb/c	Hybridoma generation	Poor	Albino
C57BL/6	Reference strain	Good	Old age hearing impaiment
C3H	Common in genetic studies	Poor/Good	Large animals
DBA/2	For F1 and other inbreds	Poor	
FVB	Production of transgenic mice	Excellent	Blind

What's in a mouse name?

- C57BL/6J — C57BL substrain 6, Jackson colony
- C57BL/10J — C57BL substrain 10, Jackson colony
- C57BL/6NCrl — C57BL/6, NIH colony, distributed by Charles River Laboratories
- FVB/NCrl — FVB, NIH colony, distributed by Charles River Laboratories
- FVBS/Ant — sighted FVB, Antwerp colony
- F_1: C57BL/6 female x DBA/2 male is named B6D2F1

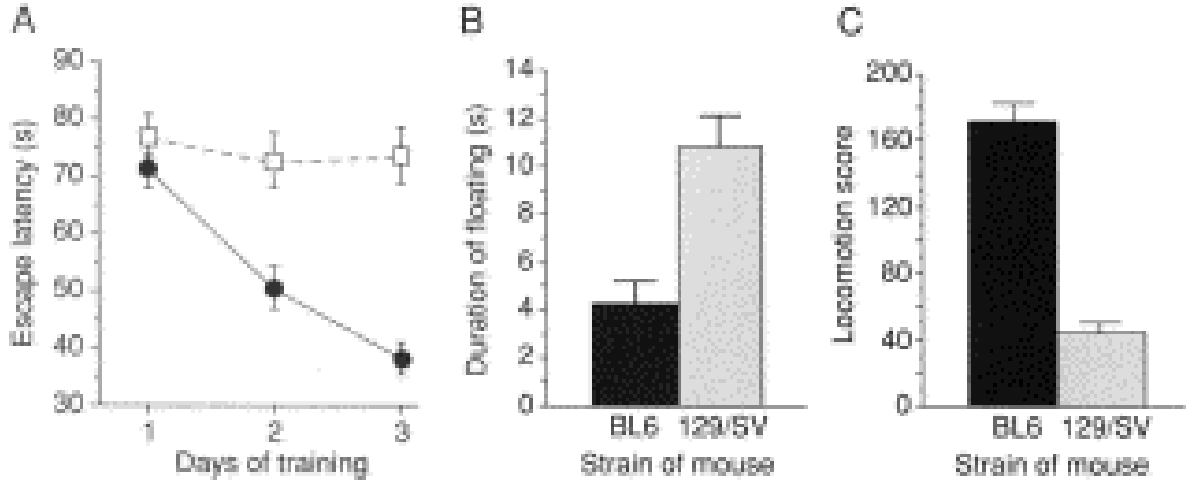
Lab codes registered with Institute for Laboratory Animal Resources, USA national acedemy of Sciences, Washington DC

Gene-targeting studies of mammalian behavior: is it the mutation or the background genotype?

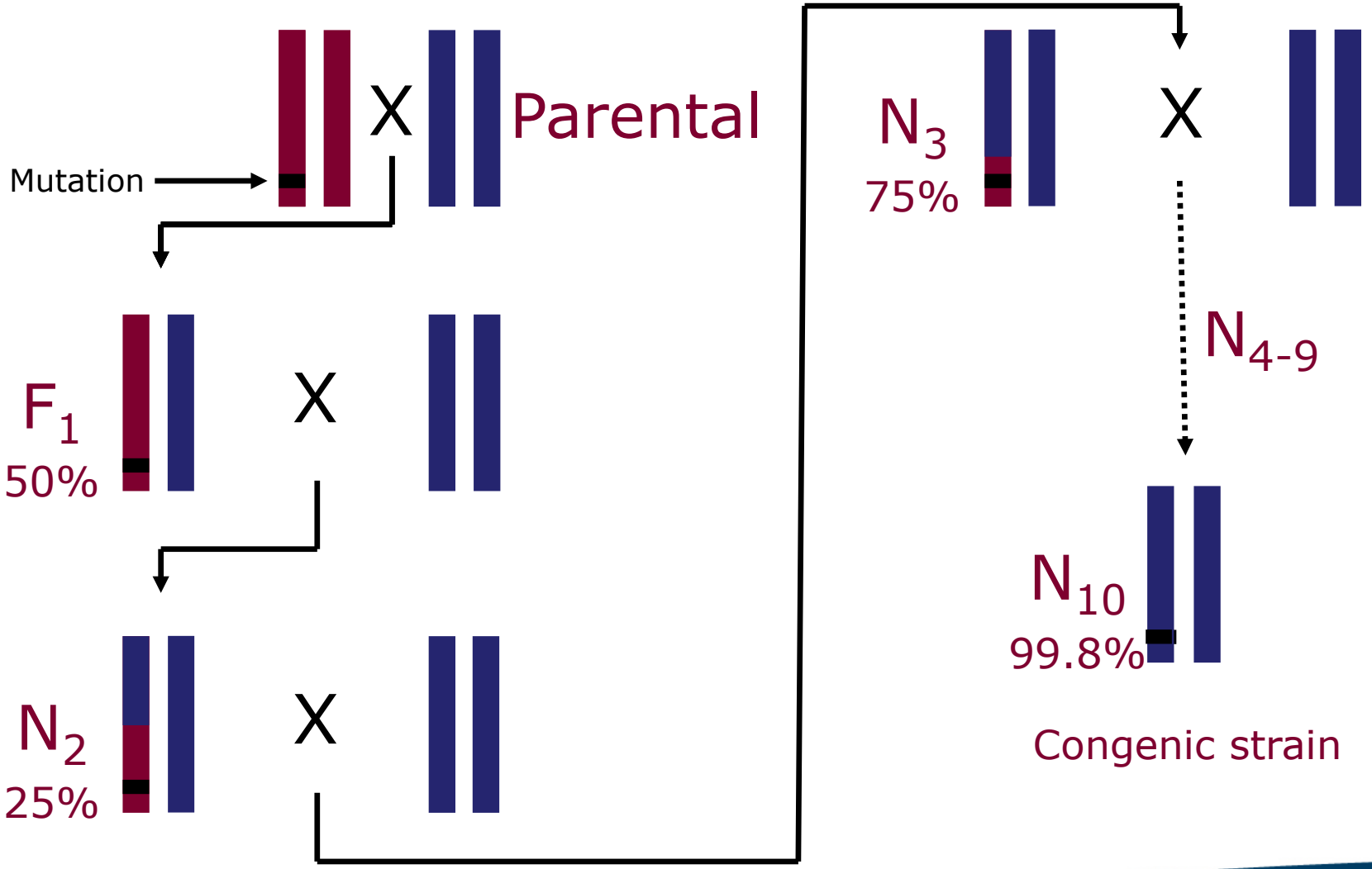
Robert Gerlai

Gene targeting to create null mutations in mice is a powerful new tool in biology which will allow the molecular dissection of complex phenotypes such as mammalian brain function, and learning and memory. However, the attempt to interpret the phenotypical changes which arise in null-mutant mice is subject to several caveats. For example, the ability to disrupt a single gene in a targeted manner might lead one to overlook the effects of other genes. Ignoring the genetic background might lead to misinterpretation of results: the present article will demonstrate that the phenotypical abnormalities attributed to the null mutation in several molecular-neurobiological studies could simply result from the effects of background genes.

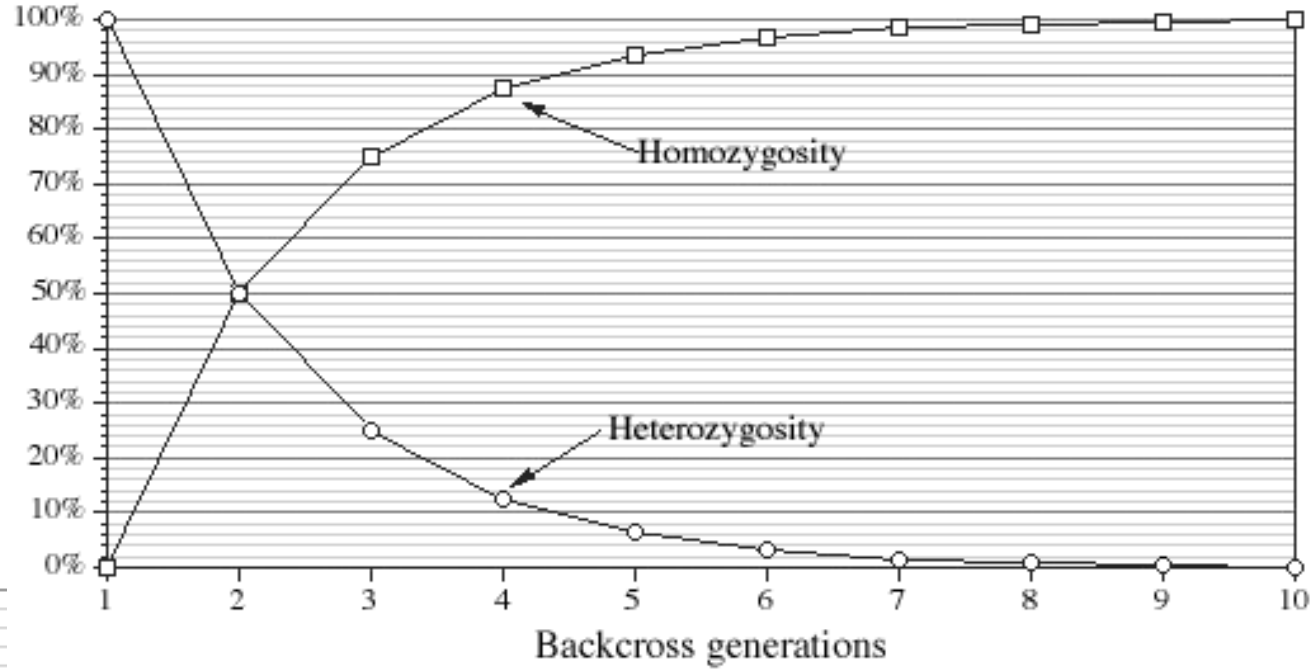
Trends Neurosci. (1996) 19, 177–181

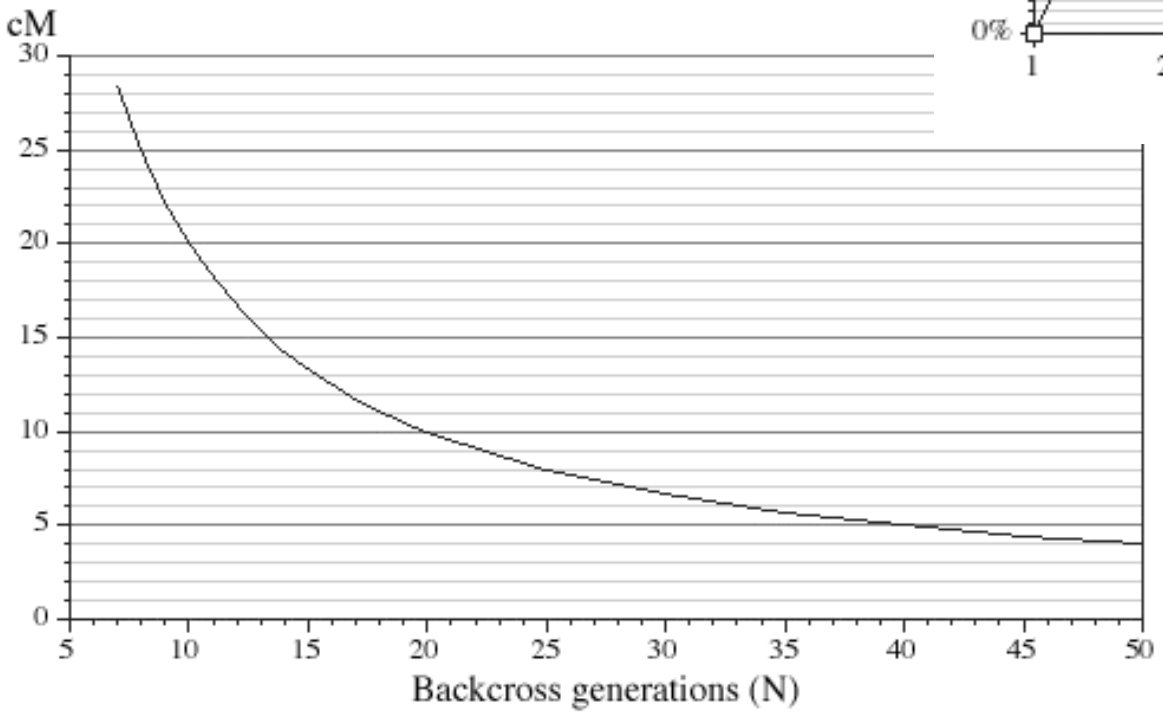


Creation of a congenic strain: 10 repeated backcross matings



At generation N_{10}: 98.8% of loci homozygous.....





..... but not at the
the chromosomal segment
flanking the mutation

Genetic nomenclature

- Gene name: *Fmr1* – *Italics* and lower case
- Alelles - mutant: *Fmr1*
  - wild type: + or $Fmr1^{+}$
- Congenics: abrreviated strain symbols recipient.donor

  B6.129P2 for mutant created in 129P2 backcrossed to C57BL/6 (; if not fully congenic)
- Knockout: congenic name followed by - gene symbol

  B6.129P2-$Fmr1^{tm1Cgr}$/J

1. Tm1 targeted mutation 1
2. Cgr (lab code) created by Ben Oostra, Erasmus University, Rotterdam
3. /J bred at Jackson

Genes, Brain and Behavior (2007)

FVB.129P2-Pde6b^{+} Tyr$^{c\text{-}ch}$/Ant, a sighted variant of the FVB/N mouse strain suitable for behavioral analysis

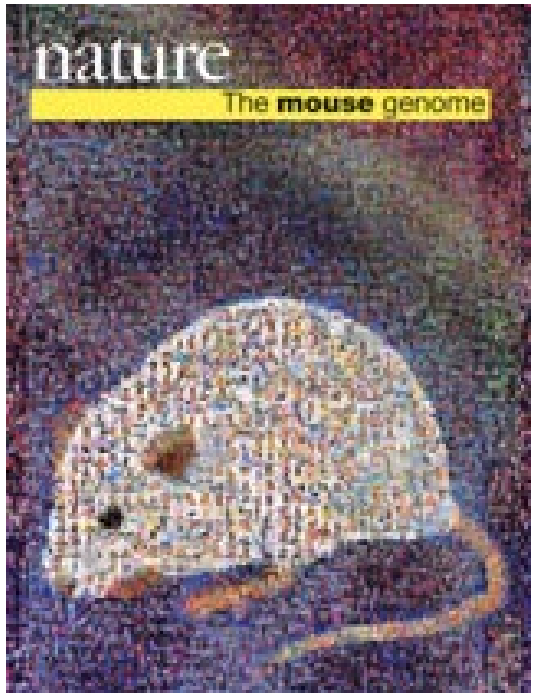
V. Errijgers[†], D. Van Dam[‡], I. Gantois[†], C. J. Van Ginneken[§], A. W. Grossman[¶], R. D'Hooge[††], P. P. De Deyn[‡,‡‡] and R. F. Kooy[†,*]

[†]Department of Medical Genetics, [‡]Laboratory of Neurochemistry and Behavior, Institute Born-Bunge, Department of Biomedical Sciences and [§]Department of Veterinary Medicine, University of Antwerp, Antwerp, Belgium[¶]Neuroscience Graduate Program, Beckman Institute, University of Illinois at Urbana – Champaign, IL, USA, [††]Department of Biological Psychology, University of Leuven, Leuven, and [‡‡]Memory Clinic, Department of Neurology, Middelheim General Hospital, ZNA, Antwerp, Belgium
**Corresponding author: R. F. Kooy, Department of Medical Genetics, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium. E-mail: frank.kooy@ua.ac.be*

Mice of the FVB/N strain are severely visual impaired as a result of tyrosinase gene defects, leading to a deficiency of the key enzyme for melanin synthesis in skin and eye and of cyclic guanosine monophosphate phosphodiesterase gene defects, which results in albinism (Tyr$^{c/c}$) and retinal degeneration (Pde6b$^{rd1/rd1}$), respectively. Nevertheless, FVB/N mice are commonly used for the generation of transgenic animals because of their large, strong pronuclei and high breeding performance. However, due to visual impairment of the FVB/N animals, the resulting transgenic animals cannot be used in tests that depend on vision, including tests of cognitive behavior. Therefore, we have bred a sighted version of the FVB/N strain by an outcross between FVB/N and 129P2/OlaHsd, followed by repeated backcrosses to FVB/N mice while selecting against albinism and homozygosity of the retinal degeneration mutation. After 11 generations of backcrossing, sighted animals were intercrossed to generate the congenic FVB.129P2-Pde6b^{+} Tyr$^{c\text{-}ch}$/Ant strain, which is pigmented (Tyr$^{c\text{-}ch/c\text{-}ch}$) and devoid of the genetic predisposition to retinal degeneration. The accurate visual abilities of the FVB.129P2-Pde6b^{+} Tyr$^{c\text{-}ch}$/Ant mice, for which we propose the name FVBS/Ant, demonstrated a clear visual evoked potential in the presence of normal eye histology and improved performance in the Morris water maze test.

(a) FVB/N (b) F_1 (c) FVBS/Ant

The mouse genome & sequence



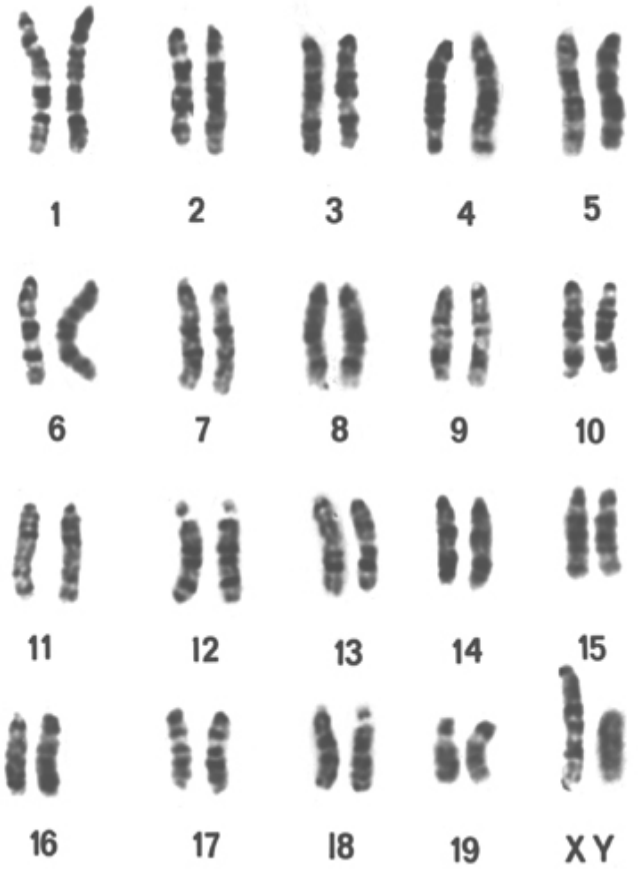
2002 Dec
The mouse genome

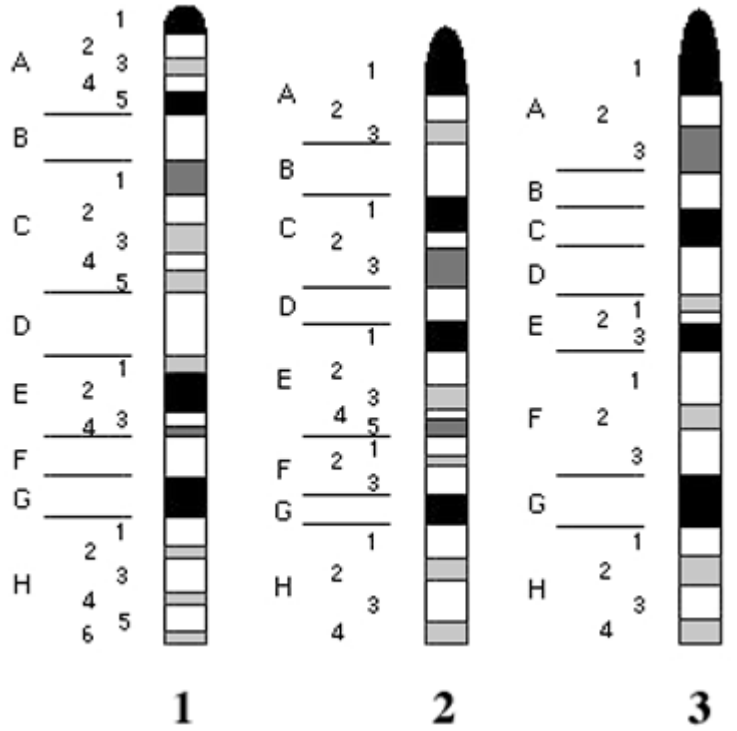
The Mouse Genome Sequencing Consortium publishes the culmination of international efforts - a high-quality draft sequence and analysis of the genome of the C57BL/6J mouse strain. The estimated size is 2.5 Gb, smaller than the human genome, with less than 30,000 genes. About 40% of the human and mouse genomes can be directly aligned with each other, and about 80% of human genes have one corresponding gene in the mouse genome. Accompanying papers detail various other aspects of the genetic make-up of the mouse, and through the efforts of the RIKEN Genome Science Laboratory in Japan, many essential resources are made freely available.

- Genome size: ~2.600 Mb
- Genetic length: ~1450 cM
- Less than 30.000 genes
- Only 1% has no identifiable homologue
- Large set of non-coding RNA

Gap

Mouse karyotype



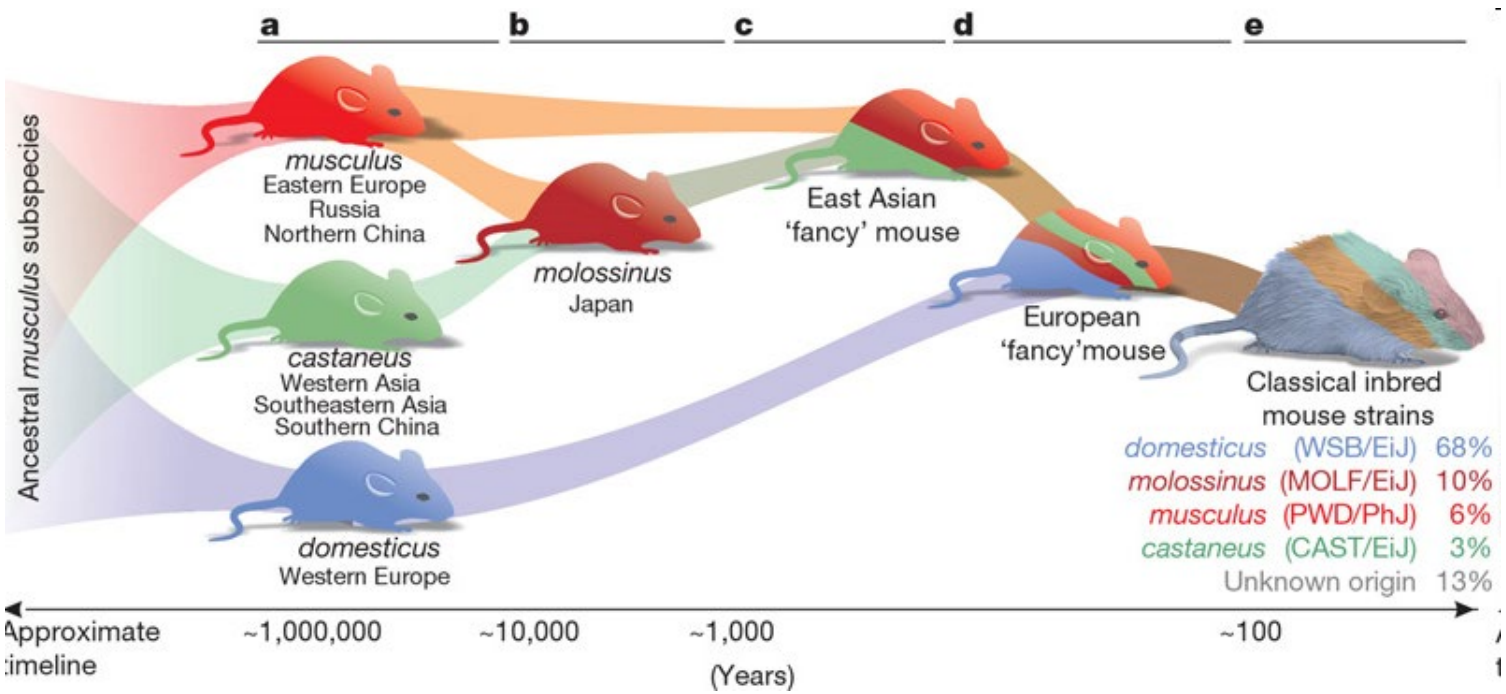


Mus musculus karyotype

Acrocentric chromosomes,
19 pairs, XY

Laboratory mouse genome

- 1 SNP per 250-300 bp between classical inbred strains
- Mouse Genome consisting of haplotype blocks
- Regions of high and low SNP density exist



Information

The Jackson Laboratory
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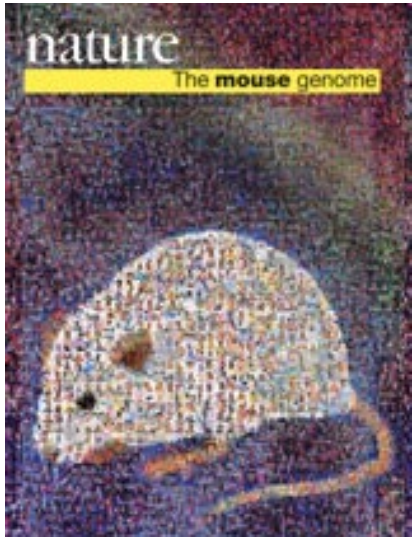
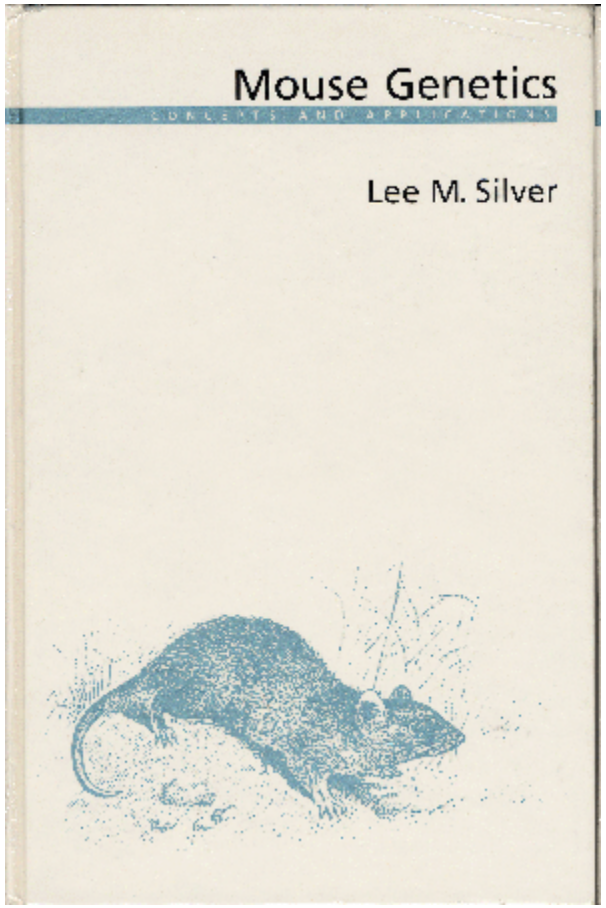
http://www.jax.org/index.html



http://www.nature.com/
nature/mousegenome/



http://www.informatics.jax.org/
silverbook/index.shtml