All images of mice from Charles River Laboratories
http://www.criver.com

Mouse Genetics

(GSK Core Course: Experimental Biology - 09/12/2024)

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This **MORNING**

1. **Overview of the mouse model:**
    1. **transgenic mouse models, embryonic stem cells and mouse chimeras.**

2. **Generation and analysis of genetically engineered mouse models (GEMMs):**
    1. Nulls
    2. Hypermorphs
    3. Hypomorphs
    4. Conditionals

3. **Genetic analyses in mice:**
    1. Complementation tests
    2. Pathway analysis (are genes in the same pathway for the specific function)
    3. Autonomous/Non-autonomous functions of a gene (chimera analyses)

4. Genetic screens (forward genetic approaches) **in mice:** dominant and recessive screens

This **AFTERNOON**

Yas Furuta's (Director, Mouse Genetics Core, MSK) lecture

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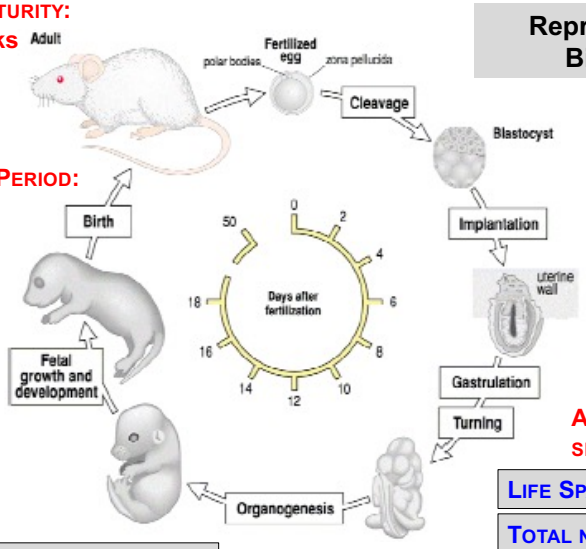
MOUSE – MAMMALIAN MODEL ORGANISM FOR GENETIC ANALYSES

Reproductive Biology

SEXUAL MATURITY: @ 6-7 weeks



GESTATION PERIOD: 19-20 days

AVERAGE LITTER SIZE: 6-8 PUPS

LIFE SPAN: 1.5 -2.5 YEARS

TOTAL NUMBER OF LITTERS PER FEMALE: 4-8

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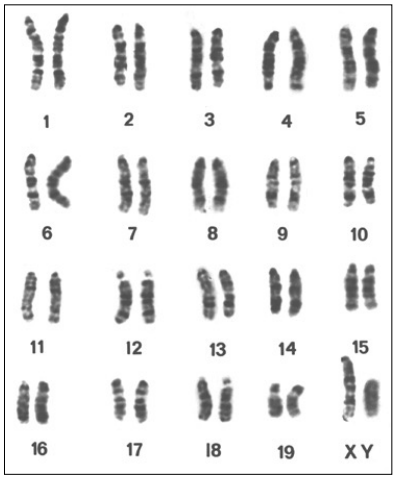
GENERATION TIME: 2-3 MONTHS

3

MOUSE – MODEL ORGANISM FOR GENETIC ANALYSES

Genome



http://www.informatics.jax.org/silver/figures/figure5-1.shtml

Number of chromosomes = 40
19 autosomes; X &Y
All acrocentric, with centromere at one end of the chromosome and the telomere at the other

Haploid DNA content: 3 x 10^9 bp
S. cerevisiae: 1.2 x 10^7 bp
D. melanogaster: 1.65 x 10^8 bp

Number of protein coding genes: 24,000
S. cerevisiae: 5,800
D. melanogaster: 14,000

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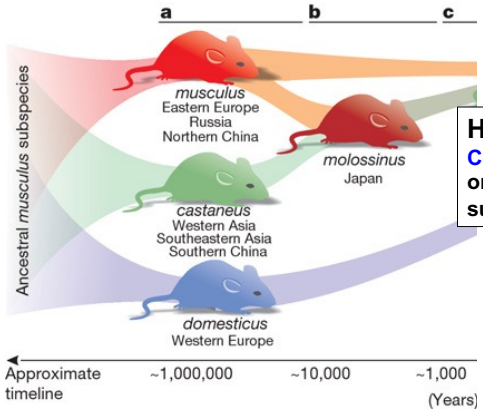
ORIGIN OF CLASSICAL INBRED STRAINS

Laboratory mouse strains derive from subspecies of the *Mus musculus* group: *domesticus*, *musculus*, and *castaneus*



House mice:
Commensal animals that depend on human activity & shelter for survival

A sequence-based variation map of 8.27 million SNPs in inbred mouse strains.
Frazer KA, Eskin E, Kang HM, Bogue MA, Hinds DA, Beilharz EJ, Gupta RV, Montgomery J, Morenzoni MM, Nilsen GB, Pethiyagoda CL, Stuve LL, Johnson FM, Daly MJ, Wade CM, Cox DR.
Nature. 2007 Aug 30;448(7157):1050-3. Epub 2007 Jul 29. PMID: 17660834

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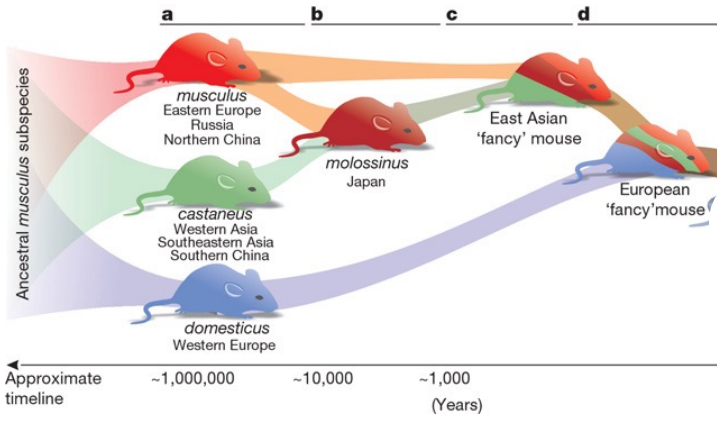
ORIGIN OF CLASSICAL INBRED STRAINS

Fancy mice provided a ready source of genetic variants for testing the applicability of Mendel's laws , rediscovered in 1900 , to mammals.



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http://www.informatics.jax.org/silver/chapters/1-2.shtm

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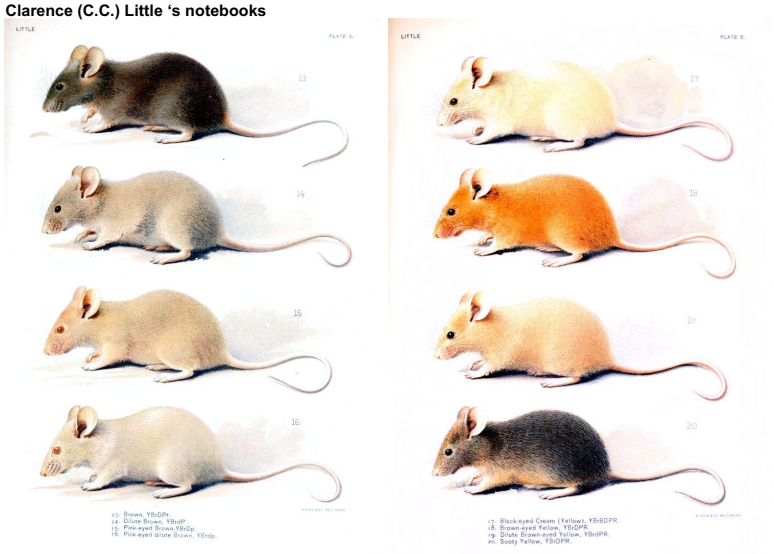
ORIGIN OF CLASSICAL INBRED STRAINS

Clarence (C.C.) Little 's notebooks



Fancy mice served as a conduit for transforming wild house mice into the inbred strains that established the mouse as a model organism

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INBRED STRAINS OF MICE

Strains that have been maintained by brother-sister matings for more than 20 consecutive generations.

At F_{20} any randomly chosen (brother-sister) mating pair will be homozygous for identical alleles in 98.7% of their genom

MATING TYPES AT THE R LOCUS; r = recessive, + = wild type				
Mating Type	Autosomal		X-Linked	Matings between
Incross	♀	♂	♀	♂	
	+/+ x +/+		+/+ x +/Y	Like homozygotes
	r/r x r/r		r/r x r/Y	
Outcross	♀	♂	♀	♂	
	+/+ x r/r		+/+ x r/Y	Unlike homozygotes
	r/r x +/+		r/r x +/Y	
Backcross	♀	♂	♀	♂	
	+/+ x r/+			Homozygote & Heterozygote
	r/+ x +/+			
	r/+ x r/r		r/+ x r/Y	
	r/r x r/+		r/+ x +/Y	
Intercross	♀	♂			
	r/+ x r/+			Heterozygotes

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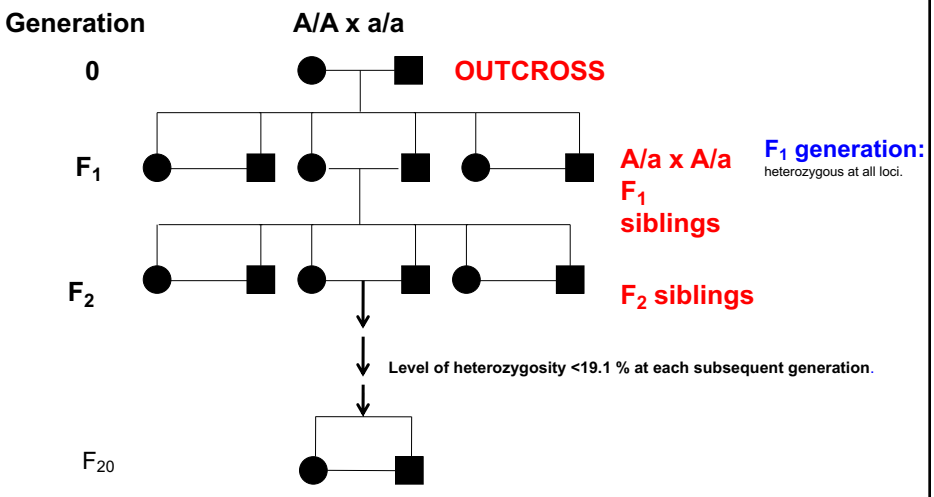
INBRED STRAINS OF MICE

Underlying assumption: the progenitor or first mating pair consists of a male and female that are homozygous for different alleles at all loci.



9/12/25 All mice in an inbred strain can be traced back to a single progenitor pair.

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INBRED STRAINS OF MICE

Individuals in an inbred strain are essentially identical genetically, i.e. ISOGENIC.

Spontaneous rate for deleterious mutations: ~ 10^{-5}/locus/generation;
each new mutation is either rapidly fixed or lost in further rounds of inbreeding.

Spontaneous mutation rate: 38 x 10^{-9} per nucleotide per generation

C57BL/6J
The most widely used strain and the 1st to have its genome sequenced
(2nd mammalian species to have its genome published !).
a.k.a. Black 6, B6, B6-J, C57 Black.

***a bit of trivia:* in 2013 Black 6 mice were flown into space aboard Bio-M No.1**
& in 2015 Black 6 females were sent to the ISS on SpaceX CR-6 !!

Generation: F226pF230; August 14, 2014
After 226 generations of brother-sister matings, embryos were cryoPreserved and then the strain has undergone another 230 generations of backcrossing.

Estimate of overall mutation rate
0.44 mutations /generation
1 mutation with phenotypic consequences per 2.3 generations.
3-5 years for new mutations to become fixed in a colony maintained by brother-sister matings.

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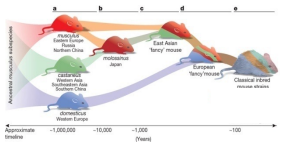

INBRED STARINS WILL DIVERGE: C57BL/6J VS. C57BL/6N

1921: Clarence (C.C.) Little bought female mouse code-numbered 57 from Abbie Lathrop's farm and initiated construction the C57BL inbred strain.

1948: Starting with F24 C57BL mice Jackson Labs established the C57BL/6J inbred strain

1951: Starting with F32 C57BL mice NIH started production of the C57BL/6N inbred strain

2002: genome sequence completed and published in 2002; C57BL/6J

2011: ES cells used to construct the conditional knockout resource for the International Mouse Phenotyping Consortium (IMPC); C57BL/6N

Differences between these two C57BL/6 substrains:
51 coding variants
34 coding SNPs
2 indels
15 structural variants (SVs)

Identified/validated using deep sequencing data and comprehensive detection methods.

A comparative phenotypic and genomic analysis of C57BL/6J and C57BL/6N mouse strains.
Simon MM, Greenaway S, White JK, Fuchs H, Gailus-Durner V, Wells S, Sorg T, Wong K, Bedu E, Cartwright EJ, Dacquin R, Djebali S, Estabel J, Graw J, Ingham NJ, Jackson IJ, Lengeling A, Mandillo S, Marvel J, Meziane H, Preitner F, Puk O, Roux M, Adams DJ, Atkins S, Ayadi A, Becker L, Blake A, Brooker D, Cater H, Champy MF, Combe R, Danecek P, di Fenza A, Gates H, Gerdin AK, Golini E, Hancock JM, Hans W, Hölter SM, Hough T, Jurdic P, Keane TM, Morgan H, Müller W, Neff F, Nicholson G, Pasche B, Roberson LA, Rozman J, Sanderson M, Santos L, Selloum M, Shannon C, Southwell A, Tocchini-Valentini GP, Vancollie VE, Westerberg H, Wurst W, Zi M, Yalcin B, Ramirez-Solis R, Steel KP, Mallon AM, de Angelis MH, Herault Y, Brown SD.
Genome Biol. 2013 Jul 31;14(7):R82. doi: 10.1186/gb-2013-14-7-r82. PMID: 23902802

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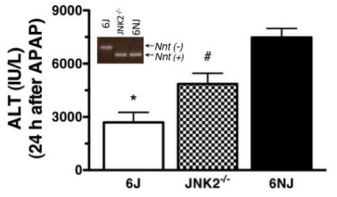
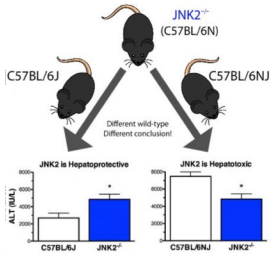
C57BL/6J vs. C57BL/6N





https://www.jax.org/news-and-insights/jax-blog/2016/june/there-is-no-such-thing-as-a-b6-mouse

Testing the effects of a *Mapk9* (*Jnk2*) knockout on acetaminophen-induced liver injury model.

Using C57BL/6J *vs.* C57BL/6N as wild-type controls, the phenotype of the *Mapk9* knockouts fell in between the phenotype for these C57BL/6J and C57BL/6NJ controls.

The researchers found themselves in a situation where they could interpret their data in two opposing ways, depending on which control was used.
If they used C57BL/6J as controls, then the data indicated that MAPK9 was hepatoprotective.
If they used C57BL/6NJ as controls, then MAPK9 seemed to be hepatotoxic.

Mispairing C57BL/6 substrains of genetically engineered mice and wild-type controls can lead to confounding results as it did in studies of JNK2 in acetaminophen and concanavalin A liver injury.
Bourdi M, Davies JS, Pohl LR.
Chem Res Toxicol. 2011 Jun 20;24(6):794-6. doi: 10.1021/tx200143x. Epub 2011 May 24. PMID: 21557537

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Genetic Background Can Modify Mutant Phenotypes

A null allele in the gene encoding the Epidermal Growth Factor Receptor (Egfr), *Egfr*^{m1Cwr}, was backcrossed onto 2 different outbred strains (CF-1 and CD-1), and one inbred strain (129/Sv) with each having a different defect.

- ***Egfr***$^{m1Cwr/m1Cwr}$ **on CF-1 background:**
  peri-implantation death @ E4.5-6.5 with degeneration of the inner cell mass (ICM) of the blastocyst.
- ***Egfr***$^{m1Cwr/m1Cwr}$ **on 129/Sv inbred background:**
  developmental arrest @ mid-gestation (E12.5 - 13.5) with placental defects.
- ***Egfr***$^{m1Cwr/m1Cwr}$ **on CD-1 background:**
  post-natal lethality.
  mutant pups live to 3 weeks and exhibit abnormalities in the skin, kidney, brain, liver, and GI tract.

Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype.
Threadgill DW, Dlugosz AA, Hansen LA, Tennenbaum T, Lichti U, Yee D, LaMantia C, Mourton T, Herrup K, Harris RC, et al.
Science. 1995 Jul 14;269(5221):230-4. PMID: 7618084

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Generating genetically Engineered Mouse Models (GEMMs): Reverse Genetic Approaches

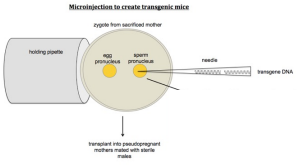
TRANSGENIC MICE

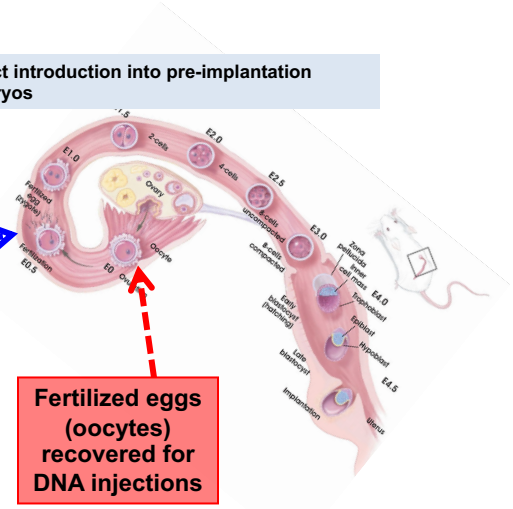
Animals having acquired new genetic information in their **germ line** via manipulation of early the embryo

Pronuclear DNA Injection

Direct introduction into pre-implantation embryos

Injected eggs surgically re-implanted for further development





Fertilized eggs (oocytes) recovered for DNA injections

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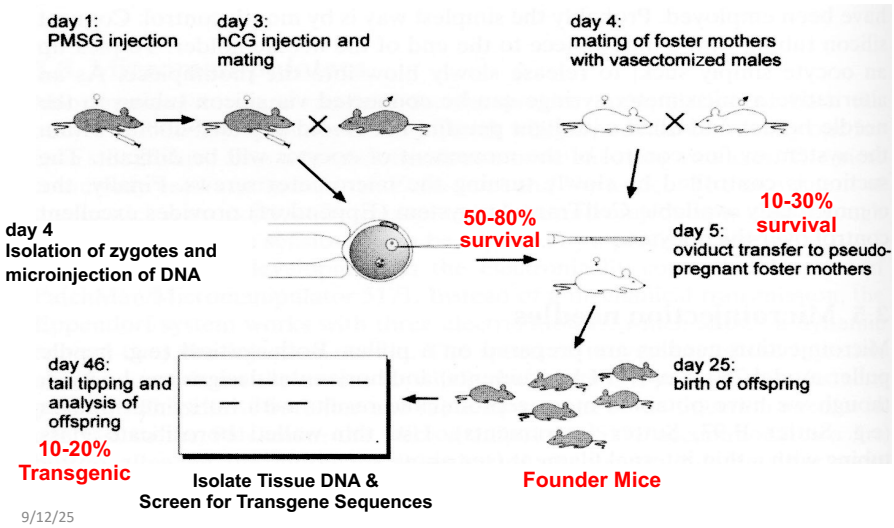
Reverse Genetic Approaches

PRONUCLEAR INJECTION OF DNA INTO FERTILIZED EGGS . . .



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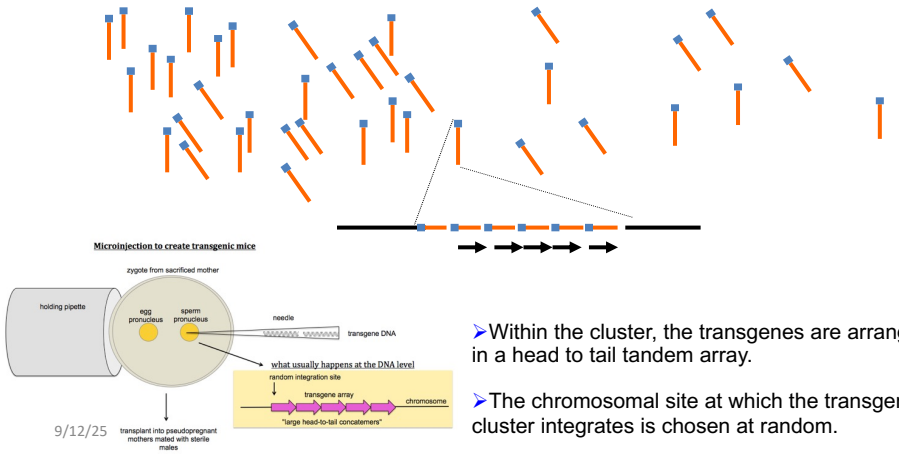
Reverse Genetic Approaches

PROPERTIES OF INTEGRATED TRANSGENES

- Multiple copies of the injected transgene integrate into the genome.
- The multiple copies usually integrate together as a cluster at 1 chromosomal site /founder mouse.



- Within the cluster, the transgenes are arranged in a head to tail tandem array.
- The chromosomal site at which the transgene cluster integrates is chosen at random.

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Reverse Genetic Approaches

Transgene Transmission

- The integrated cluster of transgenes is **transmitted as a single Mendelian trait**.
- **50% transmission from a founder**: all cells contain the integrated transgene, i.e. transgene integration occurred early, possibly before cleavage to 2-cell stage.
- **<50% transmission**: founder is mosaic, i.e. some cells contain the integrated transgene while other cells do not. Transgene likely integrated after the 2-cell stage.
- **>50% transmission**: transgene integrated at more than one chromosomal site.
- The **site of transgene integration**: chosen at random & integrations mediated by mechanisms of illegitimate recombination.
- About **5-10% of transgenic lines carry recessive insertional mutations** caused by the integration of the transgene. These mutations are usually gene disruptions.

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Reverse Genetic Approaches

Transgene Expression

COMMON REQUIREMENT: transgene includes cis-acting transcriptional regulatory elements that function robustly and appropriately at a wide variety of chromosomal locations.

TRANSGENE: cell/tissue–specific regulatory elements direct ectopic expression of heterologous gene & one or more introns

- **cDNA/cDNA-genomic hybrid**
- **Histological/fluorescent reporters (LacZ, GFP, RFP etc)**
- **Modulators of activity of transgene or endogenous reporters/alleles**
- **Enzymes (Cre/FLP recombinases)**
- **Transcriptional regulatory factors (inducers & repressors)**

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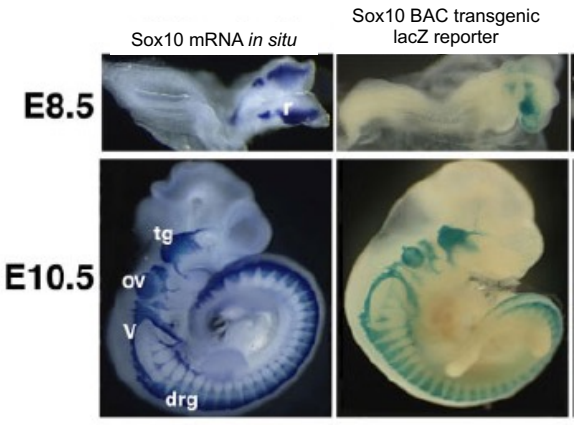
Sox10 Transgene recapitulates *Sox10* expression



Anterior neural folds

Trigeminal ganglion, otic vesicle, vagus & glossopharyngeal nerves & developing spinal nerves

Deal KK et al *Dev Dyn* **235**:1413-1432, 2006

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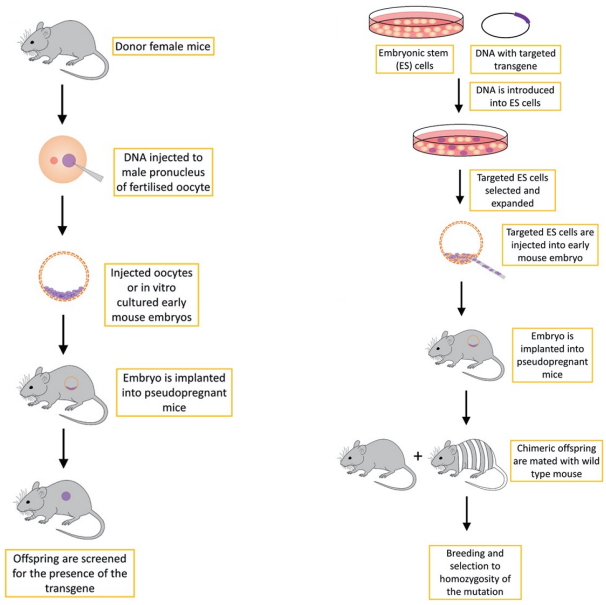
Transgenics Can also be made via ES cells



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Nobelprize.org

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"

Photo: Tim Roberts/PR Newswire, © HHMI

Mario R. Capecchi

1/3 of the prize

USA

University of Utah
Salt Lake City, UT, USA;
Howard Hughes Medical Institute

b. 1937
(in Italy)

Photo: The Press Association Limited

Sir Martin J. Evans

1/3 of the prize

United Kingdom

Cardiff University
Cardiff, United Kingdom

b. 1941

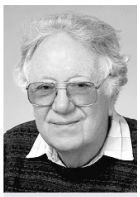

Photo: Scanpix/Dan Sears

Oliver Smithies

1/3 of the prize

USA

University of North Carolina at Chapel Hill
Chapel Hill, NC, USA

b. 1925
(in United Kingdom)

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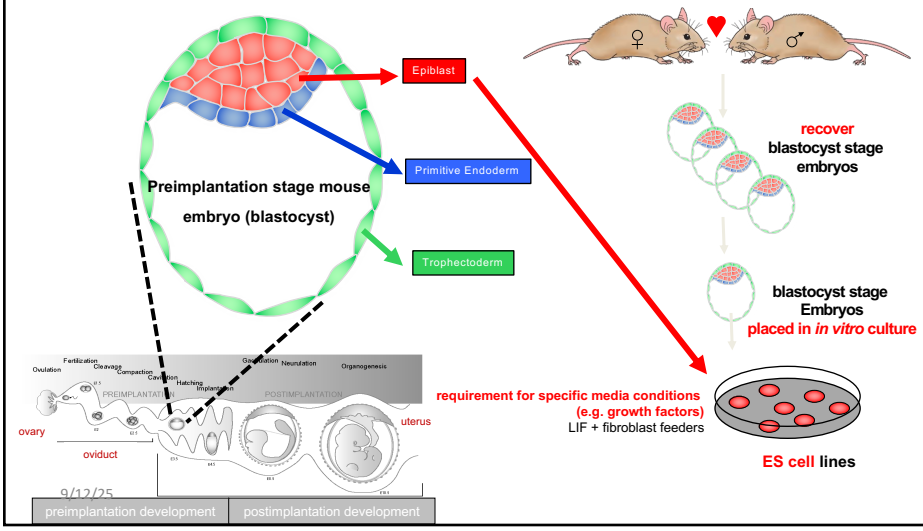
Embryonic stem (ES) cells are pluripotent stem cells derived from early mammalian (and in our case mouse) embryos



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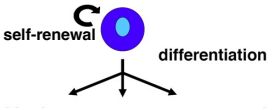
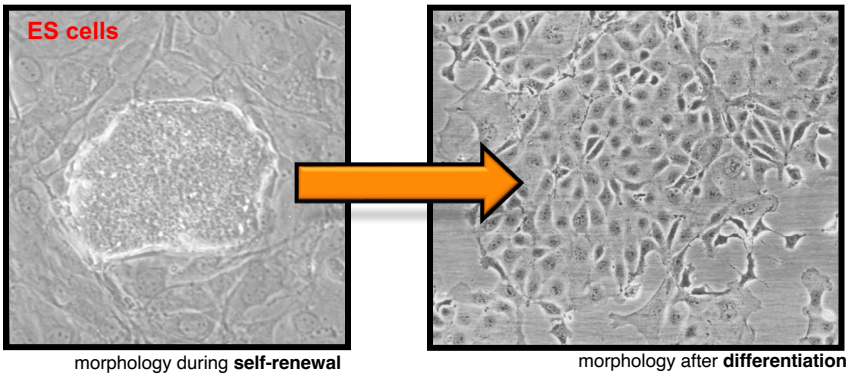
Embryonic Stem (ES) cells (possess 2 signature properties)



without feeders and LIF mouse ES cells differentiate



morphology during **self-renewal**

morphology after **differentiation**

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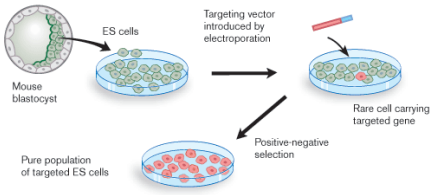
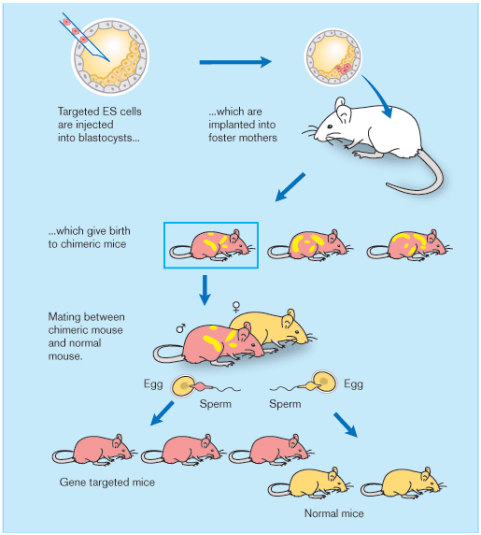
Workflow for genome engineering in ES cells



Workflow generating mice from genetically modified ES cells



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https://www.nobelprize.org/prizes/medicine/2007/advanced-information/

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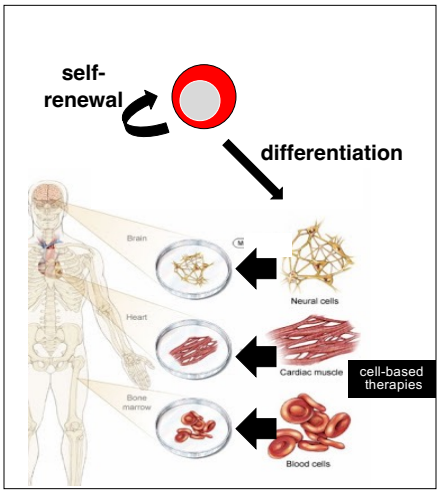
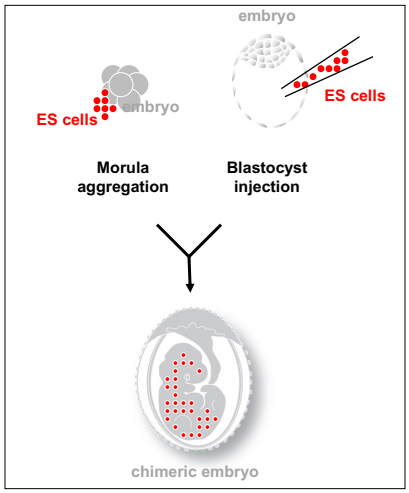
Developmental potential *in vitro*



Developmental potential *in vivo*



- **ES cells can differentiate into a wide variety of cell types in culture (developmental potential *in vitro*)**

- **ES cells can participate in normal development when incorporated into a pre-implantation embryo and produce tumors (called teratomas) containing many differentiated cell types if injected subcutaneously (developmental potential *in vivo*)**

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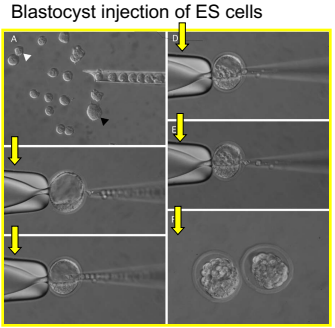
chimeras

- **From the Greek meaning "she-goat"**
- **The Chimera was a mythical fire-breathing creature with the body of a goat, the head of a lion and the tail of a serpent**
- **Upon placement in their normal environment, ES cells will give rise to all cell types in the adult mouse, including germ cells**

Blastocyst injection of ES cells



Adult mouse chimeras

Host embryo and ES cells have different coat colors and their descendants can be distinguished.

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Let's take a break…

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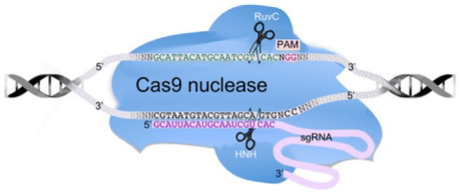
CRISPR-Cas9 as a method to induce more efficient homologous recombination by creating double strand breaks at specific DNA sequences

but creates insertions/deletions (indels) at a higher efficiency…



Double stranded break in DNA is created at site of single stranded RNA (guide RNA - sgRNA) binding to DNA

↓

Non-Homologous End Joining (NHEJ) repair process, or homology-directed repair (HDR)

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Wiles et al., CRISPR-Cas9-mediated genome editing and guide RNA design, Mammalian Genome (2015)

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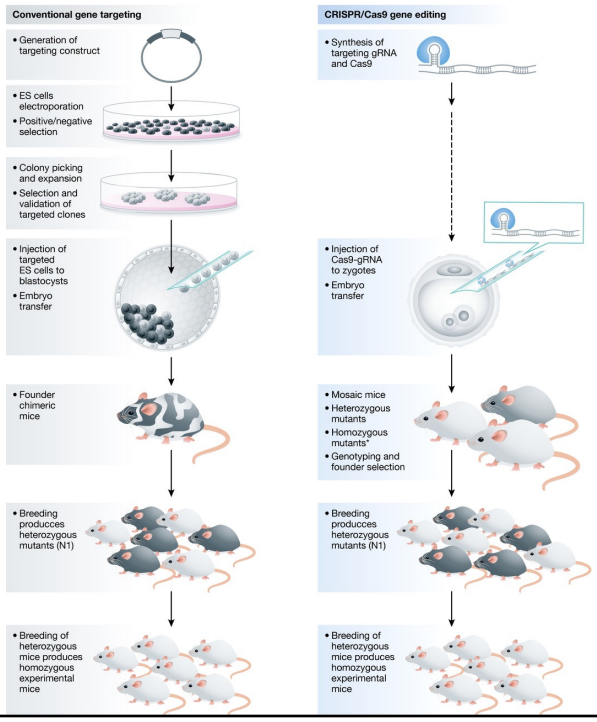

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The percentage cost savings and the timelines using the CRISPR/Cas9 method compared with traditional homologous recombination technology in mouse ES cells

These estimates include project initiation through the N1 stage, at which germline transmission has been achieved and a genetically modified mouse strain has been established. The major difference between the costs and timelines reflects the necessity of performing electroporation, selection, clone picking, and the subsequent screening in mES cells for traditional gene targeting. Downstream phases (injections and subsequent breeding) are similar with respect to the timelines. Note that for conventional targeting for which there are no murine ES cells for the desired background strain, the project timeline will increase by an additional 6–18 months, depending on the extent of backcrossing required to move the mutation to the desired background strain.

	mES cells	CRISPR (in zygotes)				
	conventional gene targeting	indel (small deletion/insertion)	deletion	precise editing (SNP)	conditional KO (LoxP, FRT)	KI (reporter)
% cost savings	baseline	81%	68%	59%	38%	41%
avg. timelines	12 months	6.5 months	7 months	7 months	8 months	8 months

Lui et al., EMBO Rep 2017

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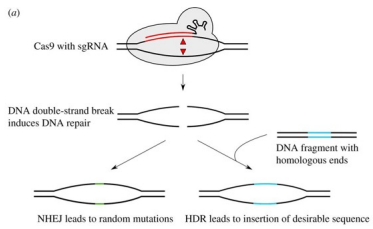
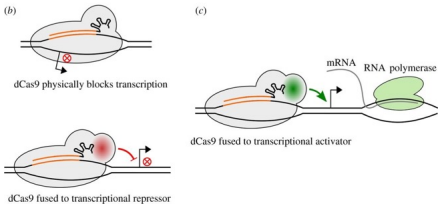
Applications of CRISPR-Cas9 in cells of mice

Indel (insertion/deletion) that can cause a null mutation, hypomorph or make a dominant-negative acting protein



Insertion can be a reporter gene, produce a tagged fusion protein, a point mutation, WT gene copy to repair an endogenous mutation (DNA repair)

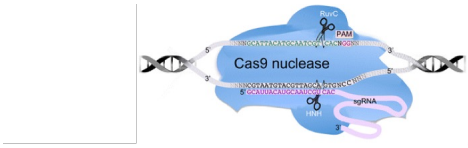
Other: nuclease activity of Cas9 is inactivated and new activities created via fusion proteins

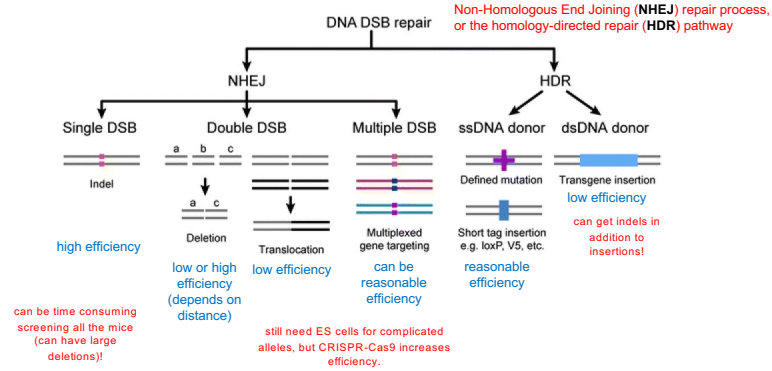


Hille and Charpentier, Philos Trans R Soc Lond B Biol Sci (2016)

32

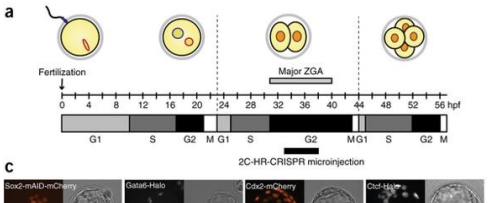
Applications of CRISPR-Cas9 in cells of mice





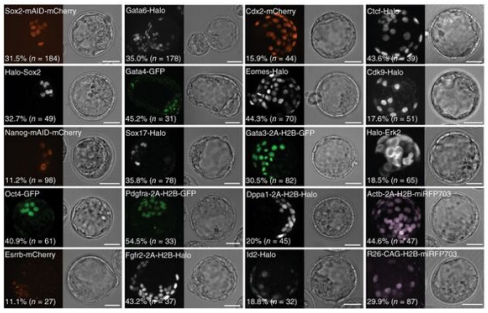
Wiles et al., Mammalian Genome (2015)

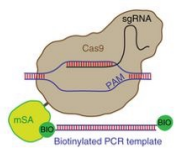
Efficient large-fragment targeting based on HR-mediated CRISPR–Cas9 editing in two-cell-stage mouse embryos (2C-HR-CRISPR)



b

Reporter allele	Zygote	Two-cell
Sox2-mAID-mCherry	0.7% (*n* = 134)	31.5% (*n* = 184)
Gata6-Halo	6.5% (*n* = 138)	35% (*n* = 178)





e

Reporter allele	Cas9-plasmid donor	Cas9-mSA PCR donor	Cas9-mSA BIO-PCR donor
Nanog-mAID-mCherry	11.2% (*n* = 98)	11% (*n* = 36)	43% (*n* = 37)
Oct4-GFP	40.9% (*n* = 61)	53.6% (*n* = 28)	65.6% (*n* = 32)
Gata6-Halo	35% (*n* = 178)	32% (*n* = 25)	95% (*n* = 37)
Sox17-Halo	35.8% (*n* = 78)	28% (*n* = 25)	73.2% (*n* = 41)
Fgfr2-2A-H2B-Halo	43.2% (*n* = 36)	34.6% (*n* = 26)	84.3% (*n* = 32)

- Increase in HR efficiency during long G2 phase and open chromatin structure of the 2-cell embryo.

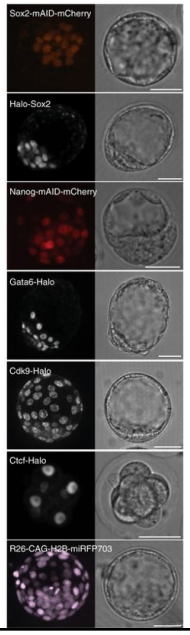
Efficient generation of targeted large insertions by microinjection into two-cell-stage mouse embryos.
Gu B, Posfai E, Rossant J.
Nat Biotechnol. 2018 Aug;36(7):632-637. doi: 10.1038/nbt.4166. Epub 2018 Jun 11. PMID: 29889212

Efficient large-fragment targeting based on HR-mediated CRISPR–Cas9 editing in two-cell-stage mouse embryos (2C-HR-CRISPR)

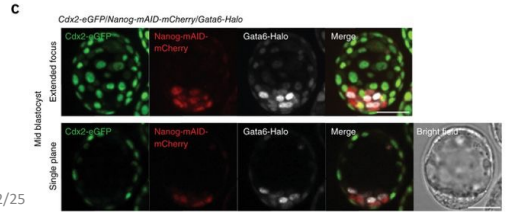
a

Reporter allele	Method	Embryos transferred	Pups born	Positive founders	Founder tested	Germline transmission
Sox2-mAID-mCherry	Cas9 plasmid	150	32	3 (9.3%)	#1	14% (3/21)
					#2	6% (4/60)
Halo-Sox2	Cas9 plasmid	75	15	7 (46%)	#1	46% (13/28)
Nanog-mAID-mCherry	Cas9-mSA BIO-PCR	75	25	2 (8%)	#1	40% (10/25)
					#2	53% (15/28)
Gata6-Halo	Cas9 plasmid	150	8	3 (37.5%)	#1	45% (9/20)
					#2	48% (13/27)
Gata6-Halo	Cas9-mSA BIO-PCR	60	3	3 (100%)	#1	100% (23/23)
					#2	100% (25/25)
					#3	34% (8/23)
Cdk9-Halo	Cas9 plasmid	75	39	3 (7.6%)	#1	24% (7/29)
					#2	14% (4/27)
Ctcf-Halo-mAID	Cas9-mSA BIO-PCR	75	31	15 (48%)	#1	52% (13/25)
					#2	47% (11/23)
R26-CAG-H2B-miRFP703	Cas9 plasmid	75	22	2 (9.1%)	#1	39% (9/23)
					#2	42% (9/21)

b



c



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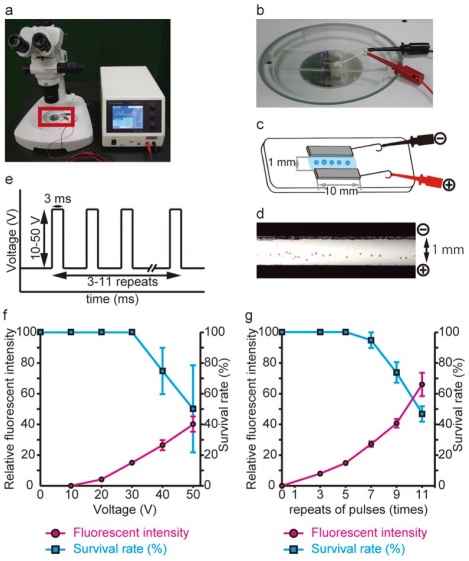
CRISPR-Cas9-mediated gene targeting by electroporation of mouse zygotes

on average 10 embryos electroporated per indel/mutant recovered (~5X more efficient than injection methods) and down from 20% (injection) to 10% of experiments need to be repeated



Electroporation enables the efficient mRNA delivery into the mouse zygotes and facilitates CRISPR/Cas9-based genome editing.
Hashimoto M, Takemoto T.
Sci Rep. 2015 Jun 11;5:11315. doi: 10.1038/srep11315. Erratum in: Sci Rep. 2015;5:12658.
PMID: 26066060

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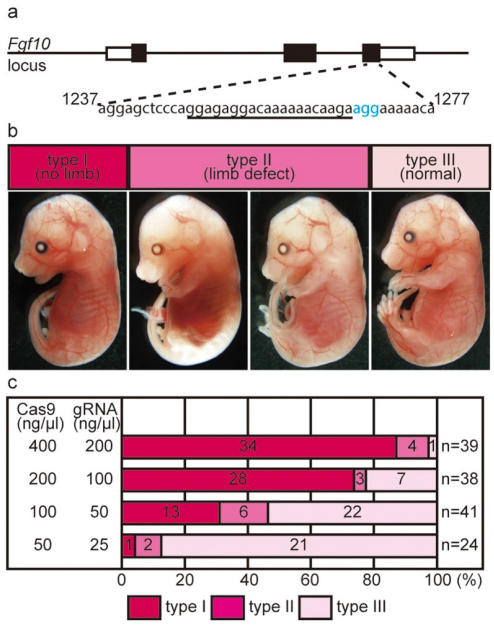
Generation of mosaics



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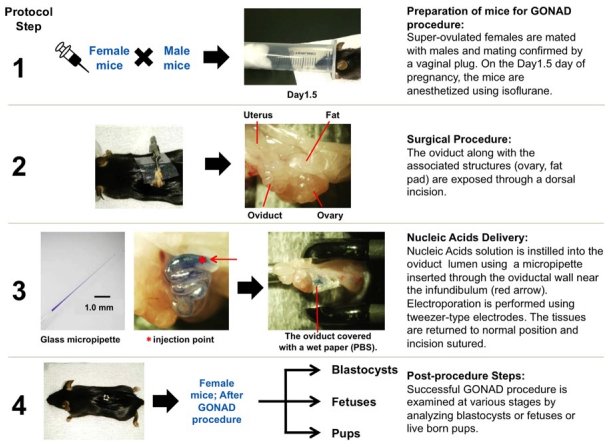
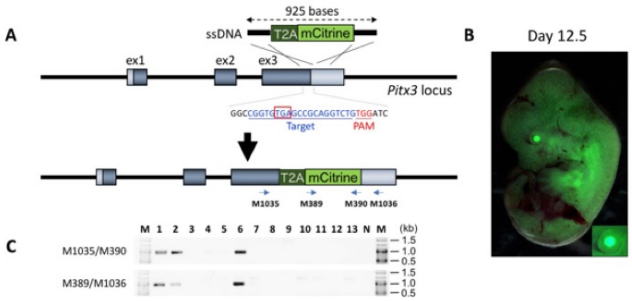
GONAD (Genome-editing via Oviductal Nucleic Acids Delivery)



GONAD: Genome-editing via Oviductal Nucleic Acids Delivery system: a novel microinjection independent genome engineering method in mice.
Takahashi G, Gurumurthy CB, Wada K, Miura H, Sato M, Ohtsuka M.
Sci Rep. 2015 Jun 22;5:11406. doi: 10.1038/srep11406. PMID: 26096991

38

i-GONAD insertion of reporter into *Pitx3* gene



E

No. mice treated	Concentration of ssDNA	G0 fetuses/newborns obtained	No. G0 mice with modified allele	No. G0 mice with knock-in allele
2	2.2 μg/μl	21	11 (52%)	2 (10%)
1	1.4 μg/μl	13	10 (77%)	3 (23%)
total 3	-	34	21 (62%)	5 (15%)

Fig. 5. Generation of reporter knock-in mice using the GONAD method. **A.** A schematic diagram showing insertion of "T2A-mCitrine" cassette into *Pitx3* locus. The target sequence and the genotyping primer sets are shown. A 925 bases-long ssDNA synthesized by *iv*TRT method was used as the donor DNA. **B.** mCitrine fluorescence in fetus collected at E12.5. The eye of the fetus is enlarged as an inset. **C.** Example of genotyping analysis of knock-in G0 fetuses. Expected fragment sizes: M1035/M390 = 948-bp, M389/M1036 = 956-bp. N: negative control, M: size marker. **D.** A representative sequencing chromatogram showing 5′ and 3′ junctional regions of the inserted cassette. The junctional sequences showing insertion derived from G0-#1 in **C** is shown. Red arrows indicate junctions between the arms and the genomic sequences. **E.** Genome editing efficiency of the *Pitx3* locus by the GONAD method.

i-GONAD: a robust method for in situ germline genome engineering using CRISPR nucleases.
Ohtsuka M, Sato M, Miura H, Takabayashi S, Matsuyama M, Koyano T, Arifin N, Nakamura S, Wada K, Gurumurthy CB.
Genome Biol. 2018 Feb 26;19(1):25. doi: 10.1186/s13059-018-1400-x. PMID: 29482575

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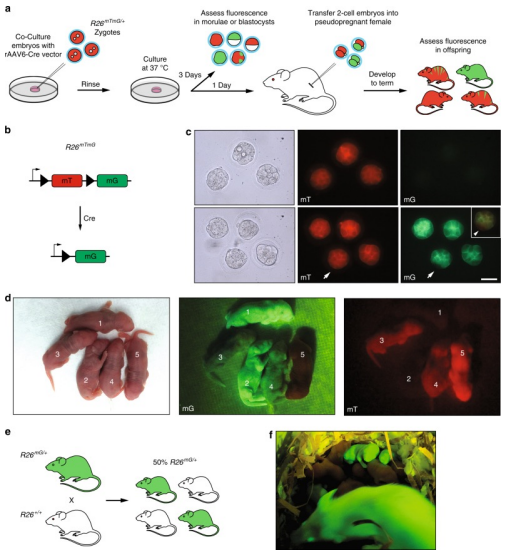
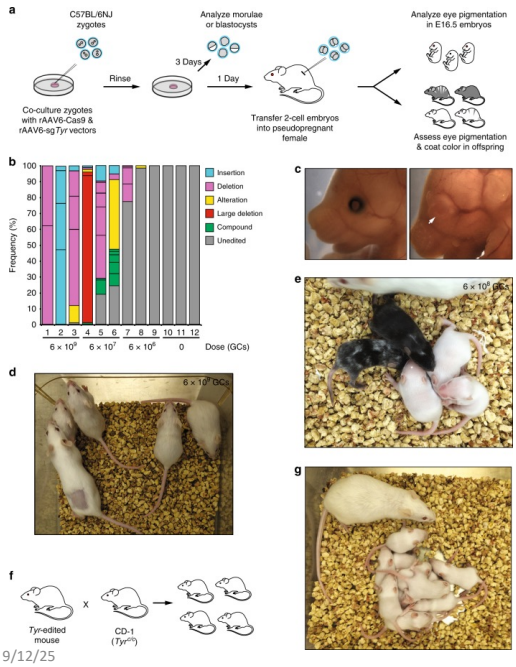
Genome Editing with Ease: Dunkin Transgenics



- delivery of CRISPR-Cas9 system components into embryos, as well as embryo handling, remain challenging
- nucleic acid delivery methods such as microinjection and electroporation can be replaced by transduction of pre-implantation embryos with recombinant adeno-associated viruses (rAAVs)
- many AAV serotypes can successfully transduce embryos, but serotype 6 proved the most efficient

Streamlined ex vivo and in vivo genome editing in mouse embryos using recombinant adeno-associated viruses.
Yoon Y, Wang D, Tai PWL, Riley J, Gao G, Rivera-Pérez JA. Nat Commun. 2018 Jan 29;9(1):412.

40



Targeting *tyrosinase* gene (involved in eye pigmentation and coat color), gives chimeras with up to 100% efficiency.

Most of the animals in the experiment developing into albinos

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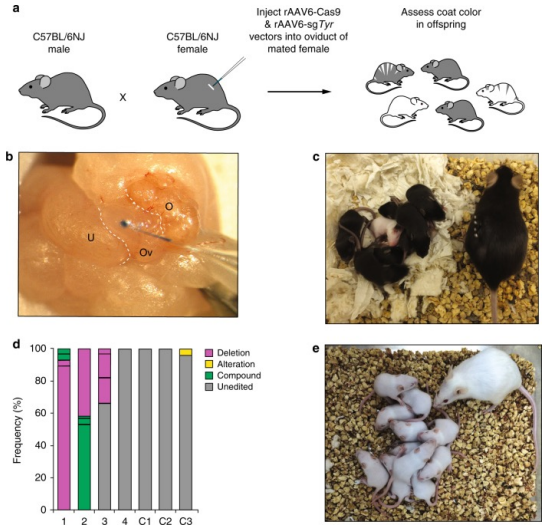
Further simplified genome-editing process in mice

introducing rAAVs into oviducts of pregnant females: embryos can be transduced *in situ*



- Approach makes genome editing possible within pregnant animals.
- Avoids the need for embryo handling outside of the female reproductive tract.

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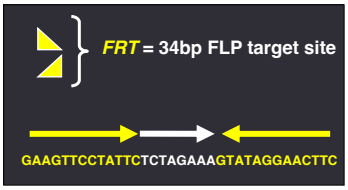
More tools to use:

Site-specific recombinases (SSRs) can be used in the mouse

Cre (causes recombination)
38 KDa protein
from bacteriophage P1
resolves DNA dimers into monomers

Flp (pronounced 'flip')
43 KDa protein
encoded by yeast 2μ plasmid



9/12/25

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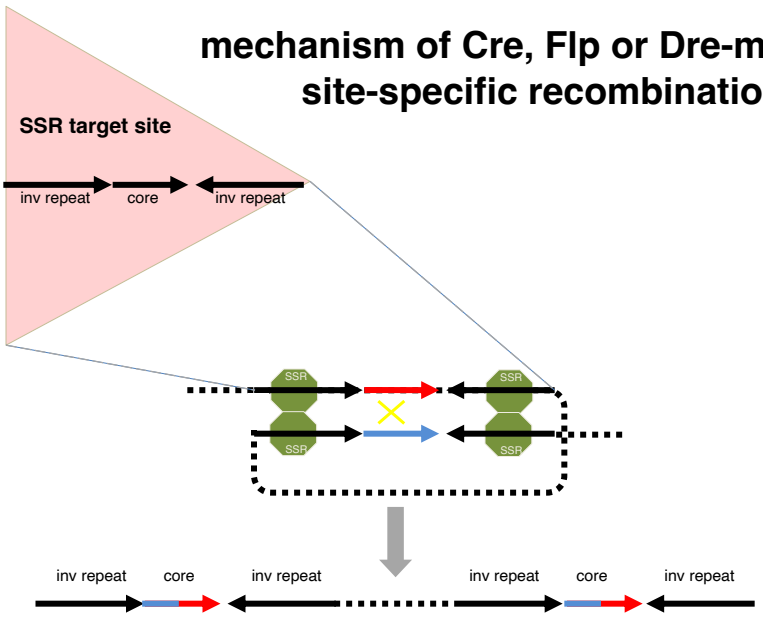
mechanism of Cre, Flp or Dre-mediated site-specific recombination (SSR)



9/12/25

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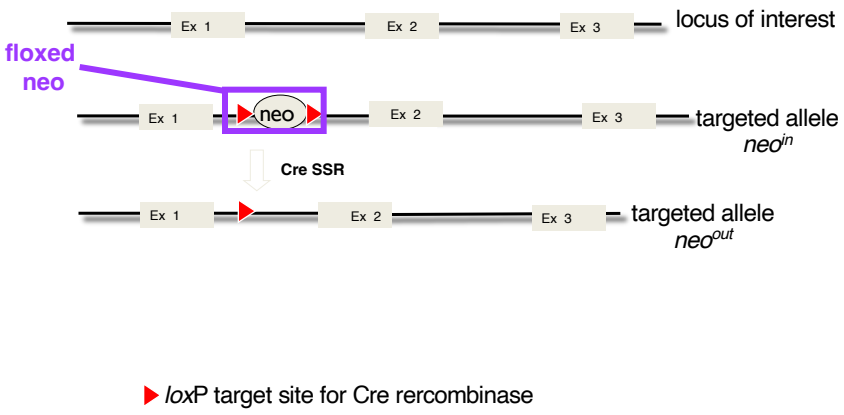
Cre mediated excision of a drug selection cassette (in ES cells or mice)



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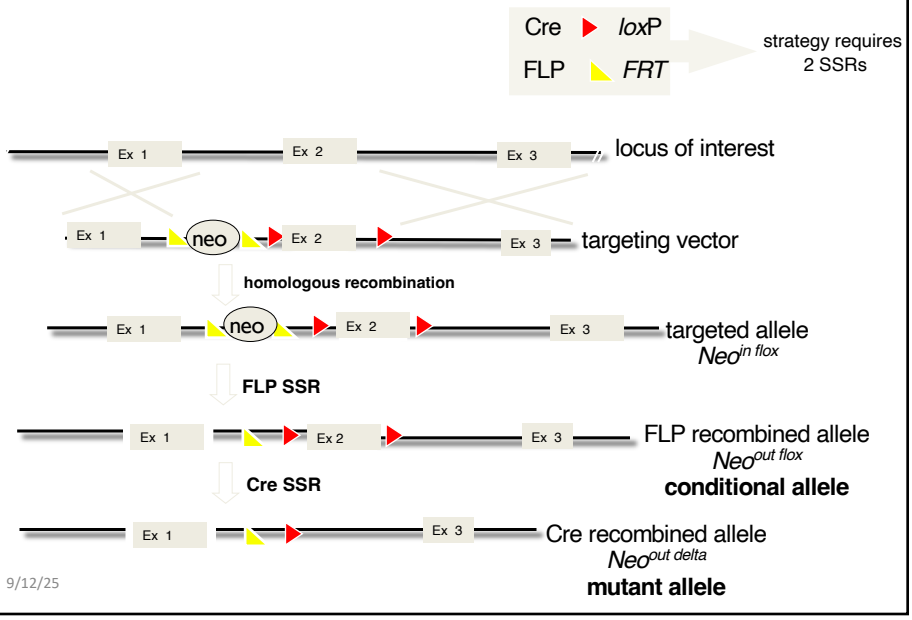
Using homologous and site-specific recombination to produce a conditional allele



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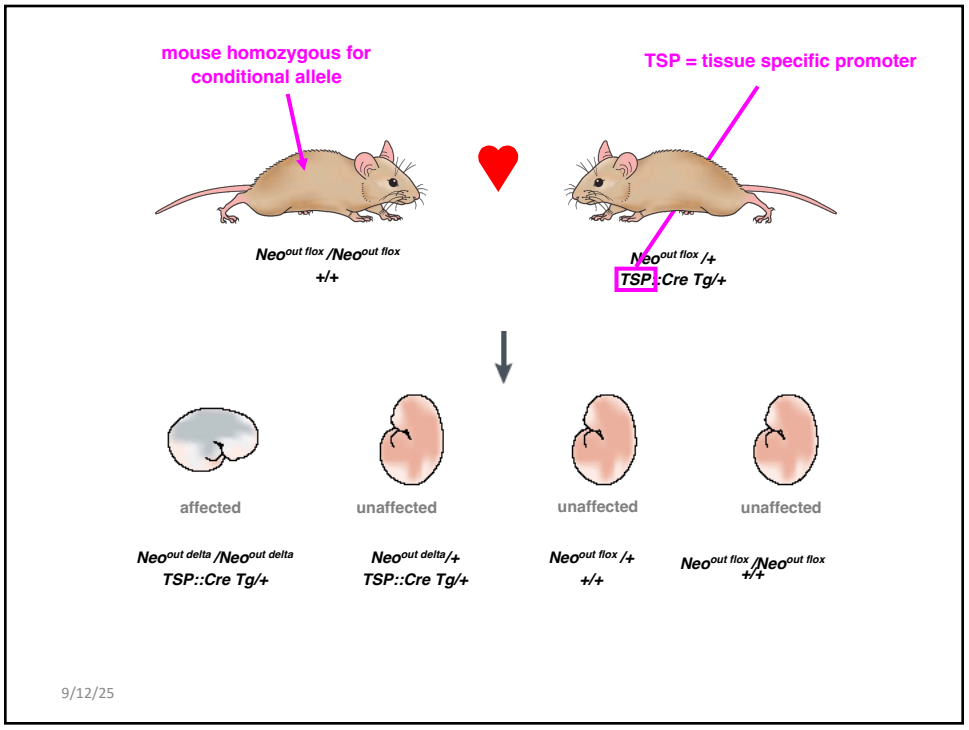
mouse homozygous for conditional allele
TSP = tissue specific promoter
Neo^out flox /Neo^out flox +/+
Neo^out flox /+ TSP::Cre Tg/+
affected
unaffected
unaffected
unaffected
Neo^out delta /Neo^out delta TSP::Cre Tg/+
Neo^out delta/+ TSP::Cre Tg/+
Neo^out flox /+ +/+
Neo^out flox /Neo^out flox +/+
9/12/25

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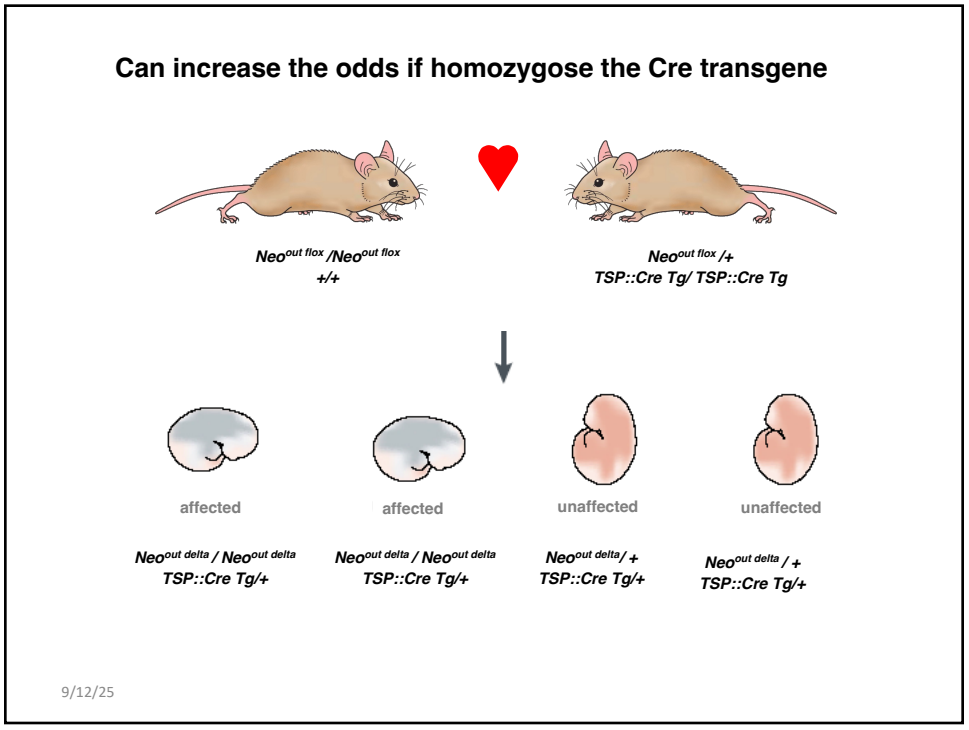
Can increase the odds if homozygose the Cre transgene
Neo^out flox /Neo^out flox +/+
Neo^out flox /+ TSP::Cre Tg/ TSP::Cre Tg
affected
affected
unaffected
unaffected
Neo^out delta / Neo^out delta TSP::Cre Tg/+
Neo^out delta / Neo^out delta TSP::Cre Tg/+
Neo^out delta/ + TSP::Cre Tg/+
Neo^out delta / + TSP::Cre Tg/+
9/12/25

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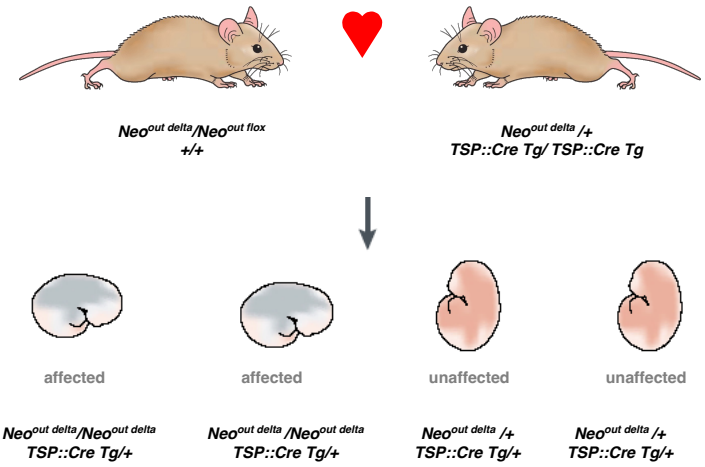
But Cre is not always efficient!!

Inefficient SSR -> mosaicism. So sometimes best to use null allele over conditional!



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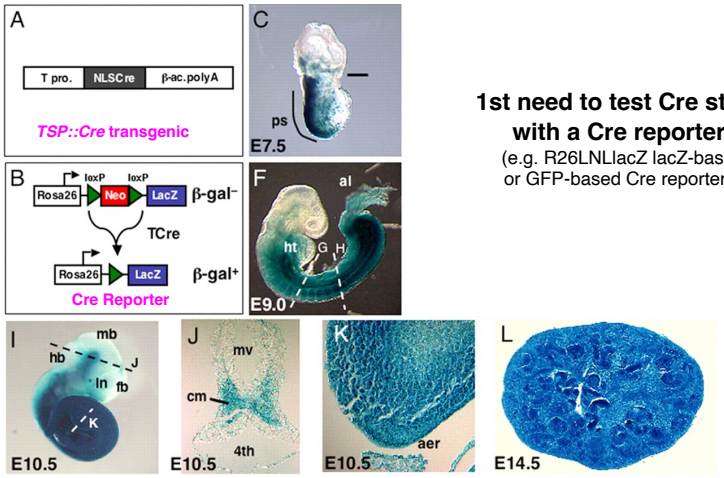
Tissue-specific Cre-mediated gene inactivation

–

T-Cre **functions (i.e. recombines) only in the embryonic mesoderm**



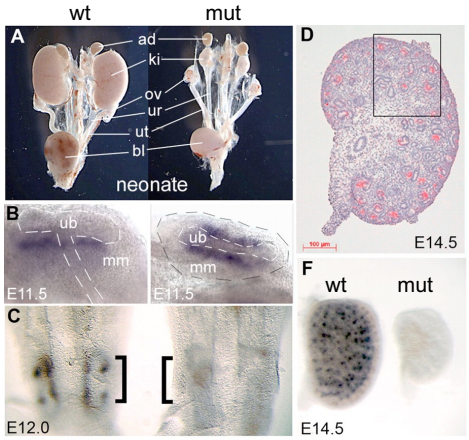
1st need to test Cre strain with a Cre reporter!

(e.g. R26LNLacZ lacZ-based or GFP-based Cre reporters)

Perantoni *et al.*, 2006

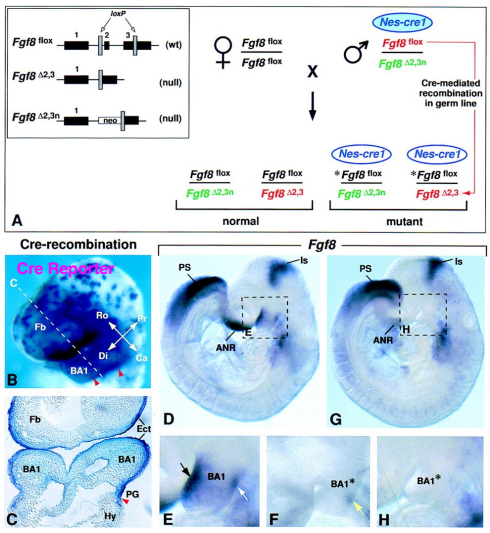
50

Cre-mediated gene inactivation
–
Fgf8 ablation in mesoderm reveals requirement in kidney formation



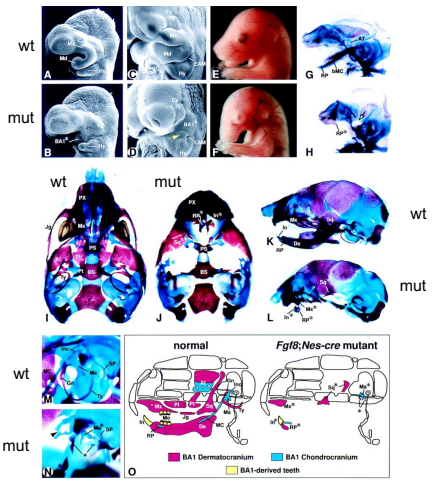
Perantoni *et al.*, 2006

Cre-mediated gene inactivation
-
use of *Nestin-Cre* removes *Fgf8* in first branchial arch cells



Trumpp *et al.*, 1999

Cre-mediated gene inactivation
-
***Fgf8* ablation in first branchial arches (BAs) reveals requirement in craniofacial development**



Trumpp *et al*., 1999

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This **MORNING**

1. **Overview of the mouse model**
   1. **transgenic mouse models, embryonic stem cells and mouse chimeras.**
2. **Generation and analysis of genetically engineered mouse models (GEMMs):**
   1. Nulls
   2. Hypermorphs
   3. Hypomorphs
   4. Conditionals
3. **Genetic analyses in mice:**
   1. Complementation tests
   2. Pathway analysis (are genes in the same pathway for the specific function)
   3. Autonomous/Non-autonomous functions of a gene (chimera analyses)

4. Genetic screens (forward genetic approaches) in mice: dominant and recessive screens

This **AFTERNOON**

5. The International Mouse Phenotyping Consortium (IMPC)

1. unbiased comprehensive catalog of mammalian gene function

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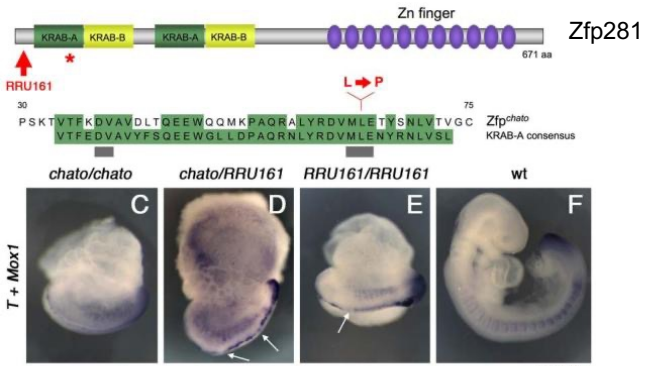
Genetic analysis in mice: complementation tests



Garcia *et al.*, Development 2008

Chato = recessive mutant identified in ENU screen (Leu-to-Pro point mutation in Zfp281)

RRU161 = null allele of *Zfp281*

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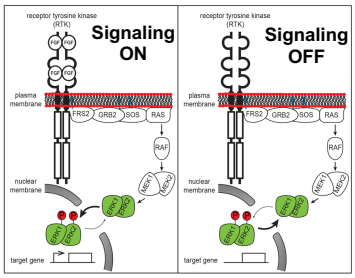
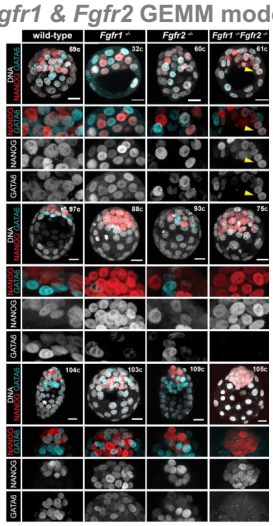
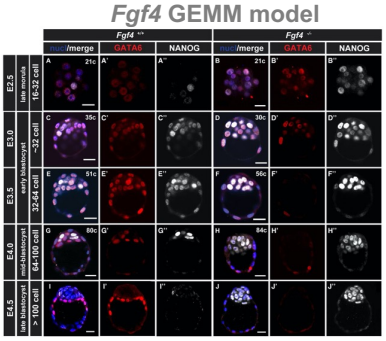
Pathway analysis: *are genes in the same pathway required for function*?



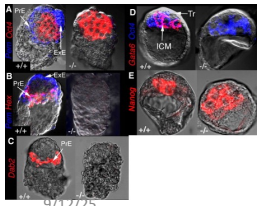
Fgfr1 & Fgfr2 GEMM mode



Fgf4 GEMM model



Grb2 GEMM model



Chazaud *et al.*, Developmental Cell 2006

Kang *et al.*, Development 2013

Kang *et al.*, Developmental Cell 2017

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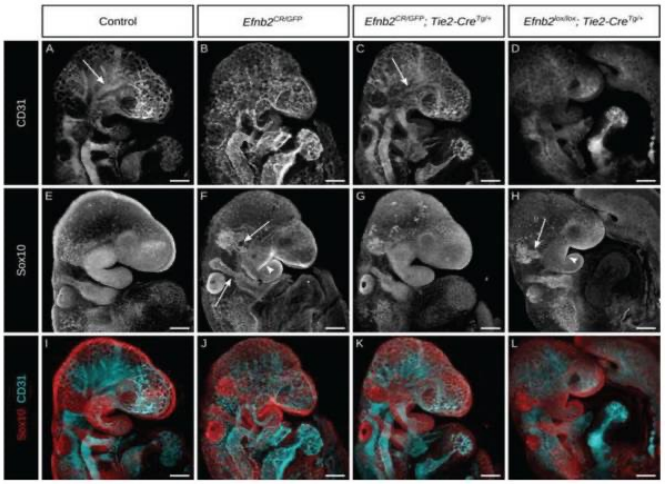
Cell autonomous *vs.* Cell non-autonomous functions of a gene



Neural crest defects in ephrin-B2 mutant mice are non-autonomous and originate from defects in the vasculature

Lewis *et al*., Developmental Biology 2015

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FORWARD GENETIC APPROACHES

CHEMICAL MUTAGENESIS FOR GENOME-WIDE SCREENS

N-ethyl-N-nitrosourea (ENU):
powerful alkylating agent that transfers its ethyl group to nucleophilic nitrogen or oxygen sites in DNA; if unrepaired, the mis-pairing caused by the ENU-induced DNA adducts will result in mutations during DNA replication in spermatogonial stem cells.

ENU-induced mutations
single base-pair substitutions: A-T to T-A transversions (44%) or A-T to G-C transitions (38%)

Missense mutations (46%); splicing (26%); nonsense (10%)

Frequency of ENU-induced mutations
Sequence based studies: 1 mutation every 1-to-2.7 Mb →1,000 mutations per male gamete; with ~20 with the potential to generate a phenotype

Enable unbiased genome-wide screens that yield in discovery of novel gene functions

Random mutagenesis of the mouse genome: a strategy for discovering gene function and the molecular basis of disease.
Nguyen N, Judd LM, Kalantzis A, Whittle B, Giraud AS, van Driel IR.
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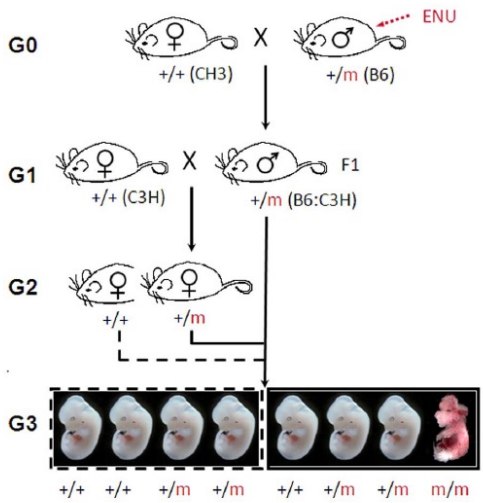
FORWARD GENETIC APPROACHES

ENU MUTAGENESIS FOR RECESSIVE EMBRYONIC MUTATIONS



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Essential genes

Genes that are critical to the survival of an organism

- Survive in a dish
- Survive to birth/weaning
- Survive until a reasonable life expectancy as an adult

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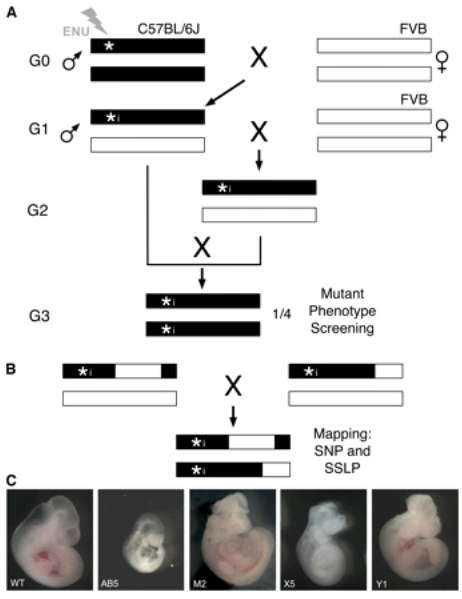

Multiplex Chromosomal Exome Sequencing Accelerates Identification of ENU-Induced Mutations in the Mouse
Miao Sun, Kajari Mondal, Viren Patel, Vanessa L. Horner, Alyssa B. Long, David J. Cutler, Tamara Caspary, Michael E. Zwick and B. J. Andrews
G3: GENES, GENOMES, GENETICS *January 1, 2012 vol. 2 no. 1 143-150; https://doi.org/10.1534/g3.111.001669*

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The Nobel Prize in Physiology or Medicine 2017
Jeffrey C. Hall, Michael Rosbash, Michael W. Young

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The Nobel Prize in Physiology or Medicine 2017


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Michael W. Young
Prize share: 1/3

The Nobel Prize in Physiology or Medicine 2017 was awarded jointly to Jeffrey C. Hall, Michael Rosbash and Michael W. Young *"for their discoveries of molecular mechanisms controlling the circadian rhythm"*.

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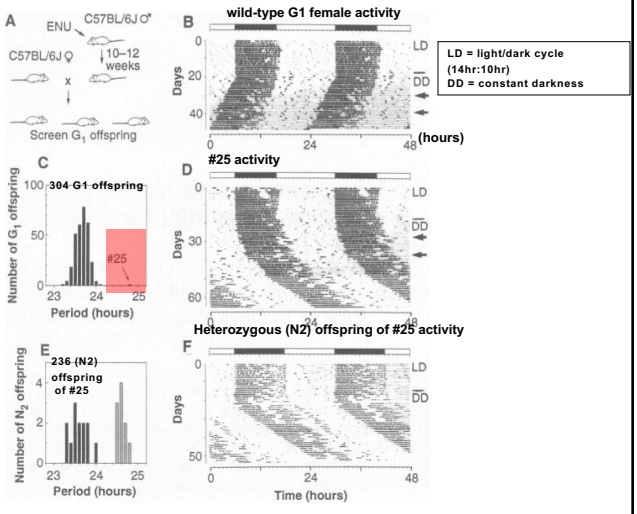
FORWARD GENETIC APPROACHES

ENU MUTAGENESIS FOR DOMINANT EMBRYONIC MUTATIONS

Led to the discovery of the mouse and human clock genes & description of conserved circadian clock mechanism in animals

http://www.utsouthwestern.edu/labs/takahashi-joseph/research/forward-genetics-screen.html

screen details



Clock is a **semidominant** mutation, that lengthens circadian period and abolishes persistence of rhythmicity

An allele "A" is said to be semidominant with respect to the allele "a"
if the A/A homozygote has a mutant phenotype, A/a heterozygote has a less severe phenotype, while a/a homozygote is wild-type.
(Definition from Mouse Genome Informatics (MGI))

Mutagenesis and mapping of a mouse gene, Clock, essential for circadian behavior.
Vitaterna MH, King DP, Chang AM, Kornhauser JM, Lowrey PL, McDonald JD, Dove WF, Pinto LH, Turek FW, Takahashi JS.
Science. 1994 Apr 29;264(5159):719-25. PMID: 8171325

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