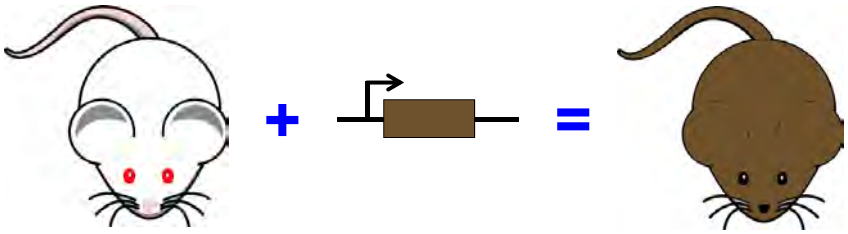
What is a transgenic animal?



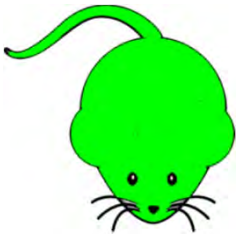
animal + transgene = transgenic

Animal models of albinism

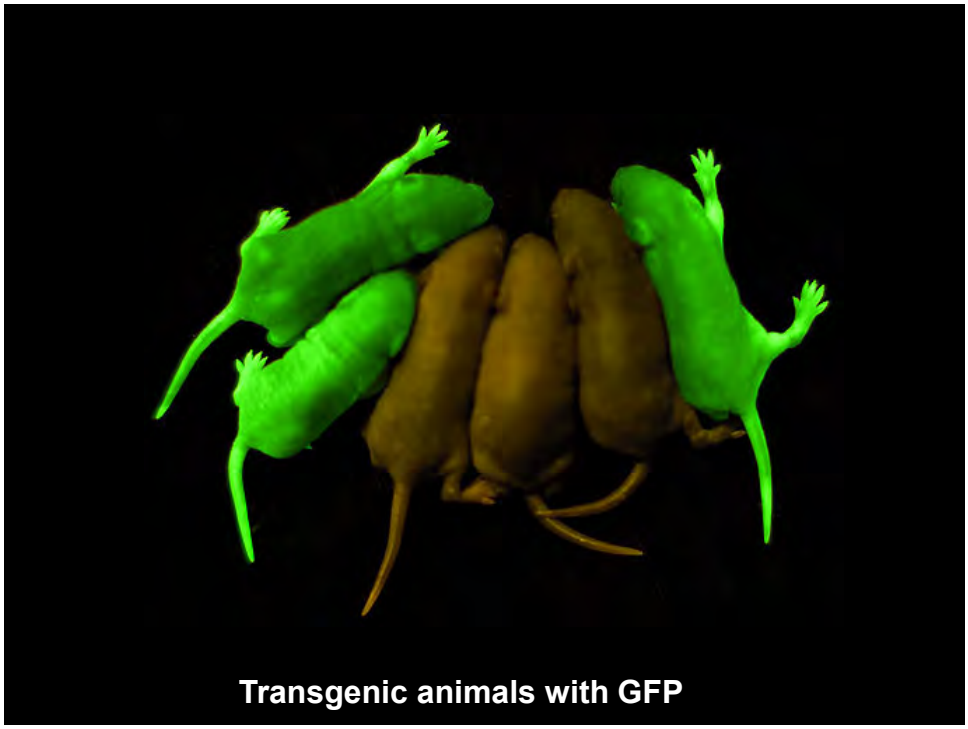
What is a transgenic animal?

+

=

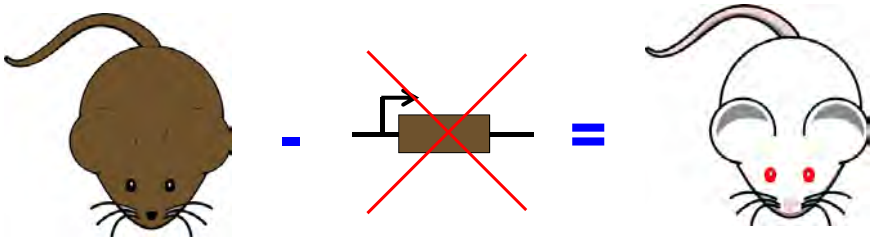


animal transgene transgenic



Transgenic animals with GFP

What is a transgenic animal?



animal - endogenous gene = Mutant Knock-out

Modifying the Mammalian Genome Through Mammalian Embryo Manipulation

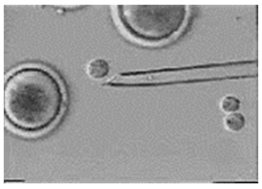
1980 ISTT

Pronuclear microinjection fertilized oocytes

1986 ISTT

Embryonic Stem Cells into blastocysts

1997 ISTT



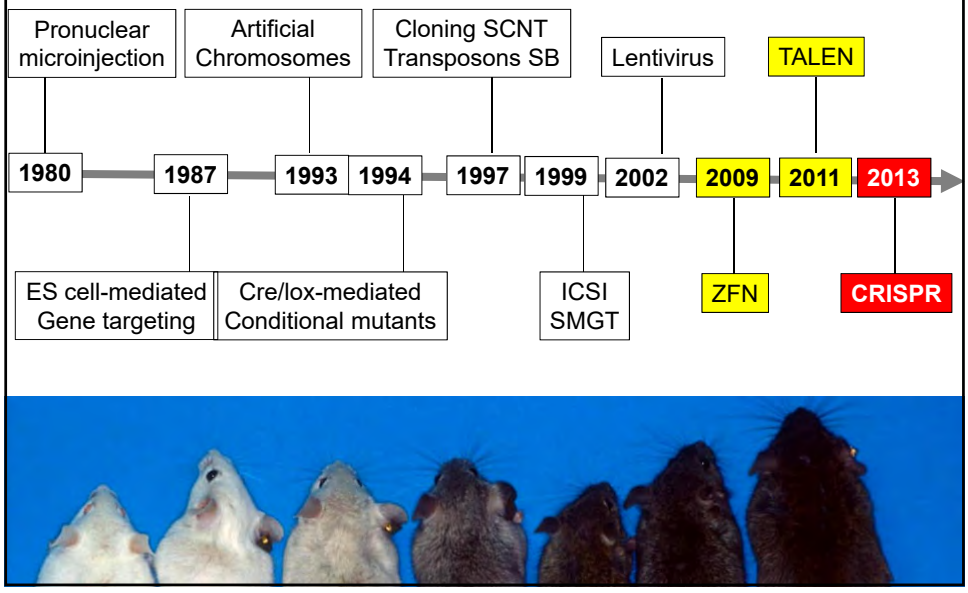
Somatic Cell Nuclear Transfer

1999 ISTT

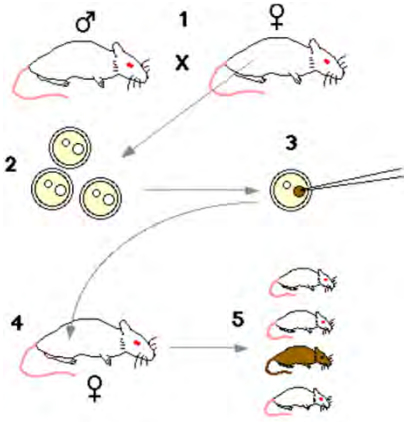
Intracytoplasmic Sperm Injection

Mouse embryos

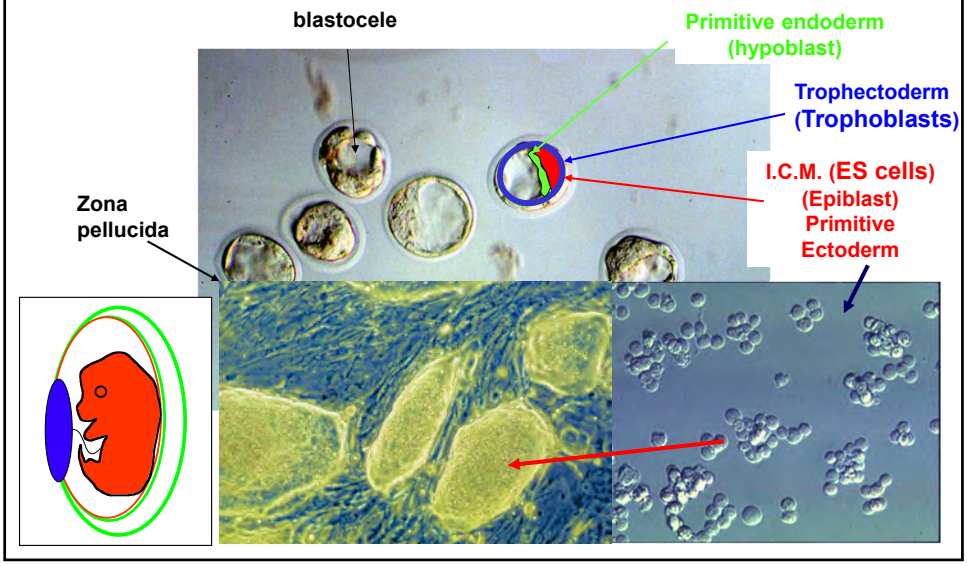
Transgenesis Timeline



Adding a new function: transgenic animals (microinjection)



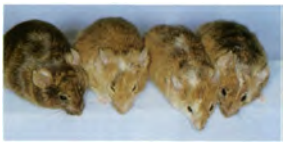
Isolating Embryonic Stem Cells
(ES cells)

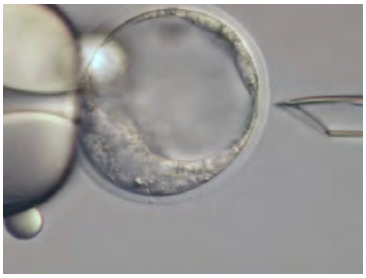


The Nobel Prize in Physiology or Medicine 2007

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

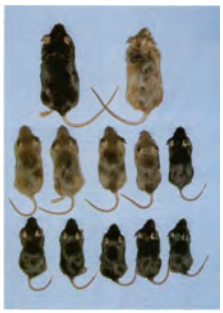
Mario R. Capecchi	Sir Martin J. Evans	Oliver Smithies
1/3 of the prize	1/3 of the prize	1/3 of the prize
USA	United Kingdom	USA
University of Utah Salt Lake City, UT, USA; Howard Hughes Medical Institute	Cardiff University Cardiff, United Kingdom	University of North Carolina at Chapel Hill Chapel Hill, NC, USA
b. 1937 (in Italy)	b. 1941	b. 1925 (in United Kingdom)



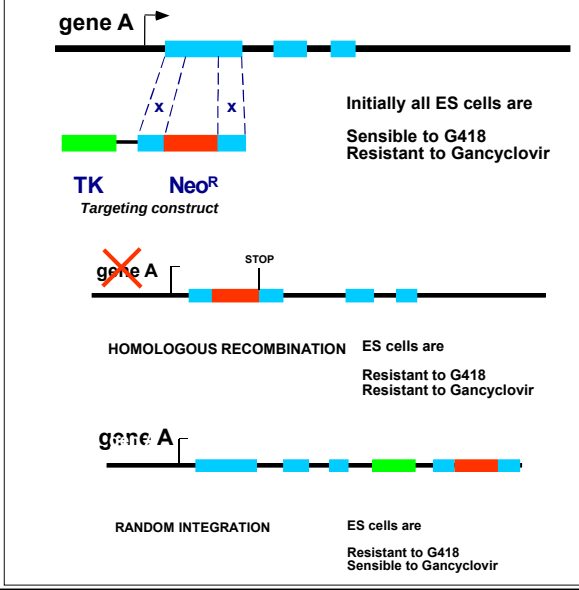


Johannes Wilbertz-ISTT

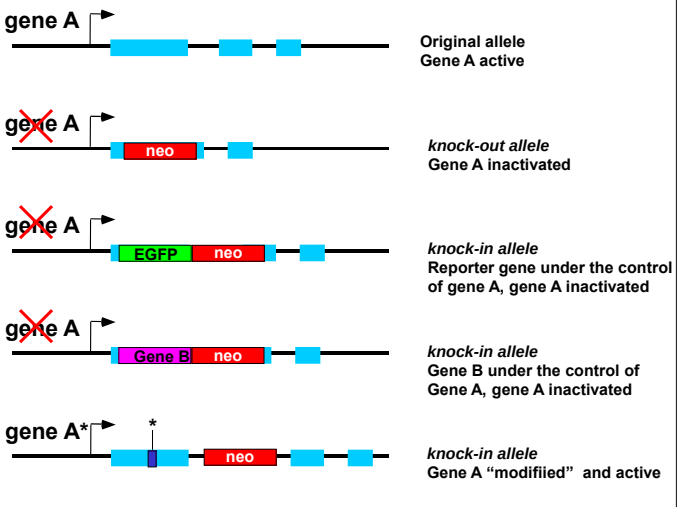
1981 - Isolation of mouse ES cells
1987 - Generation of knockout mice



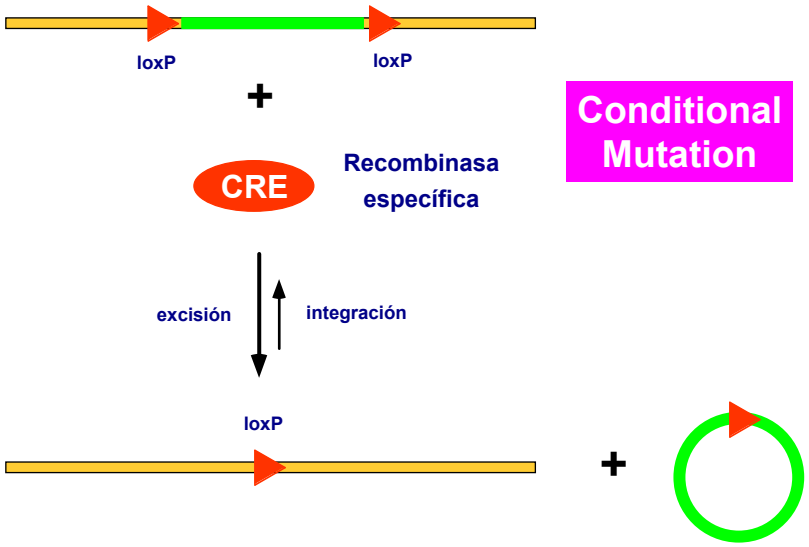
General strategy for gene targeting in ES cells through homologous recombination



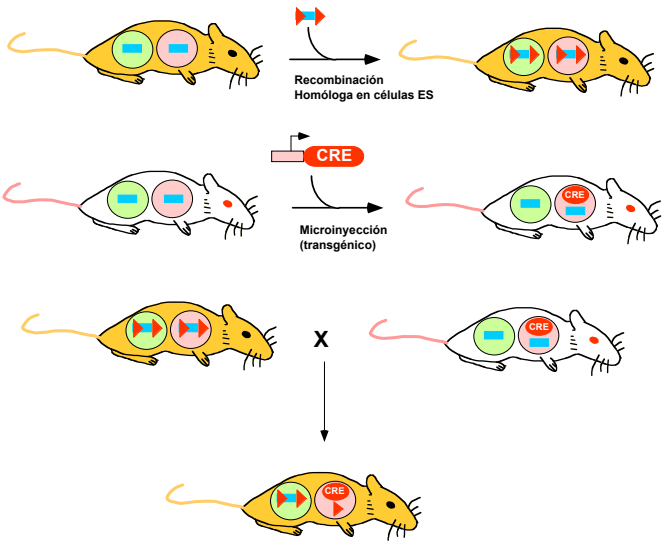
Several strategies to inactivate/alter your favourite gene



El sistema CRE-loxP de recombinación sitio-específica (bacteriofago P1)

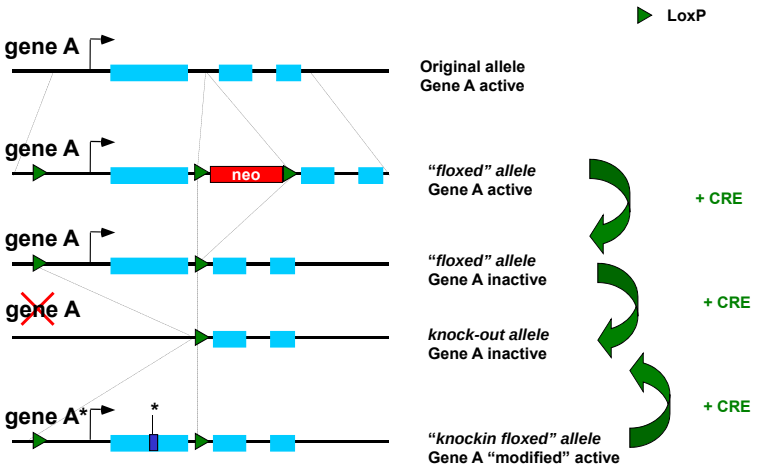


CRE-loxP y la generación de ratones KO condicionales (específicos de tejido)



RATÓN CON LA MUTACIÓN SOLAMENTE EN UN TEJIDO

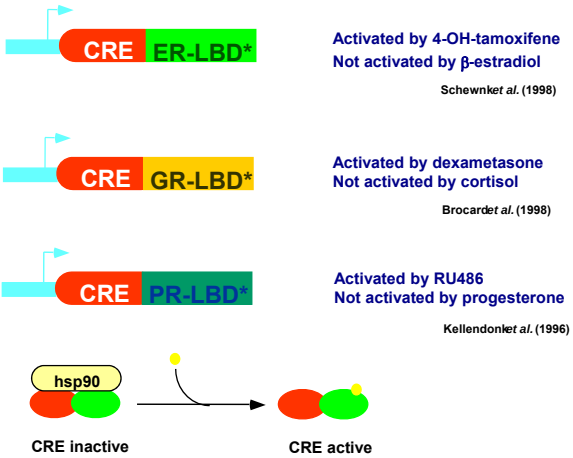
Additional strategies for conditional mutagenesis

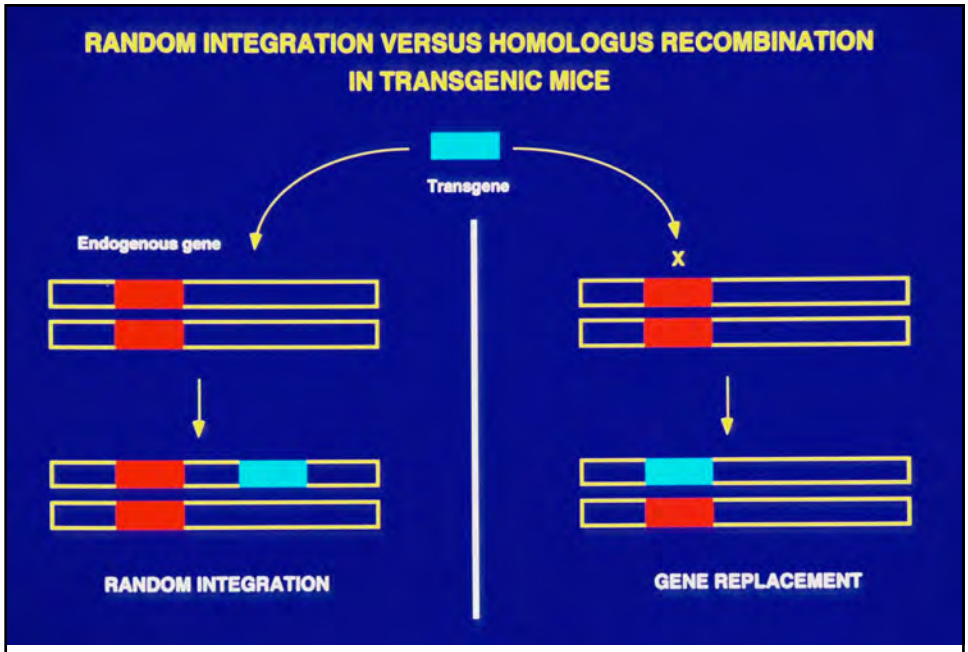


Floxed = "Flanked by LoxP sites"

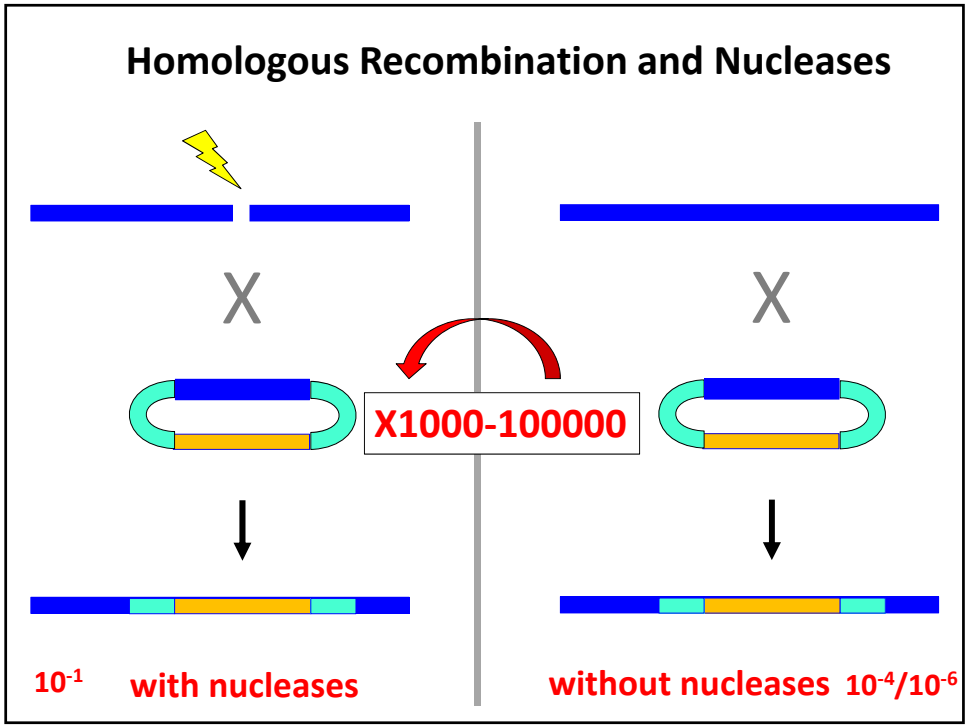
Conditional and inducible mutagenesis

Fusion of LBD from NR with CRE

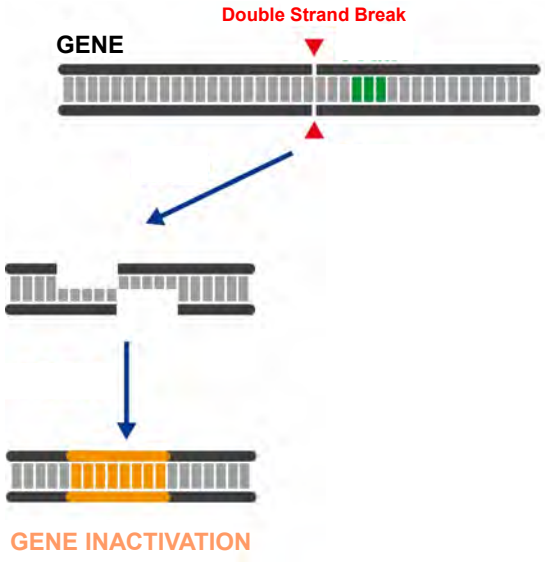


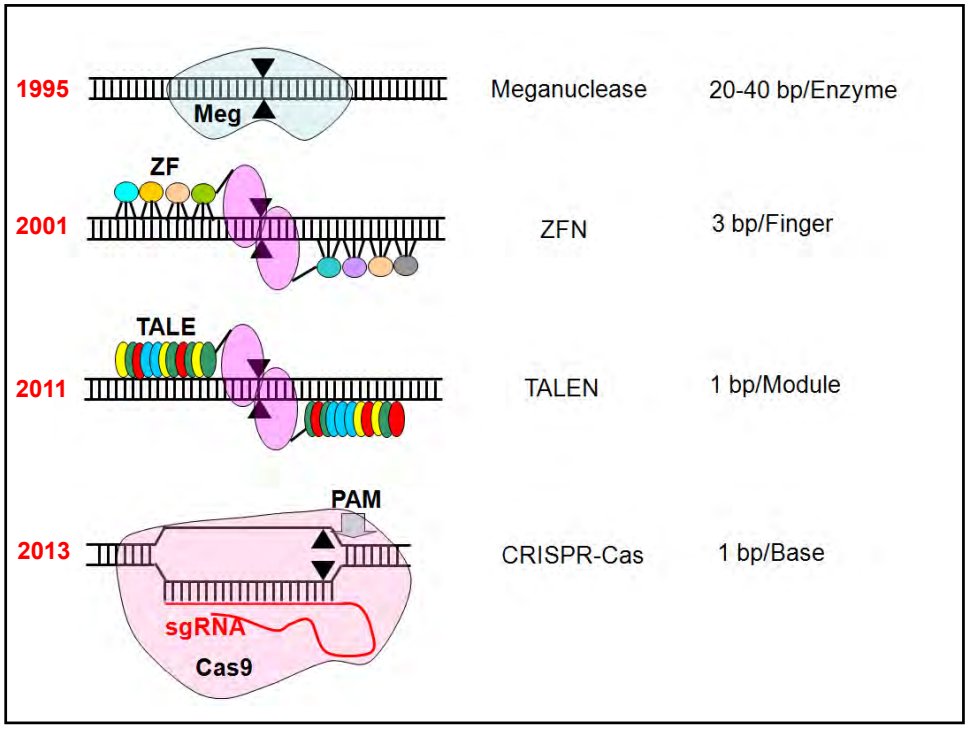


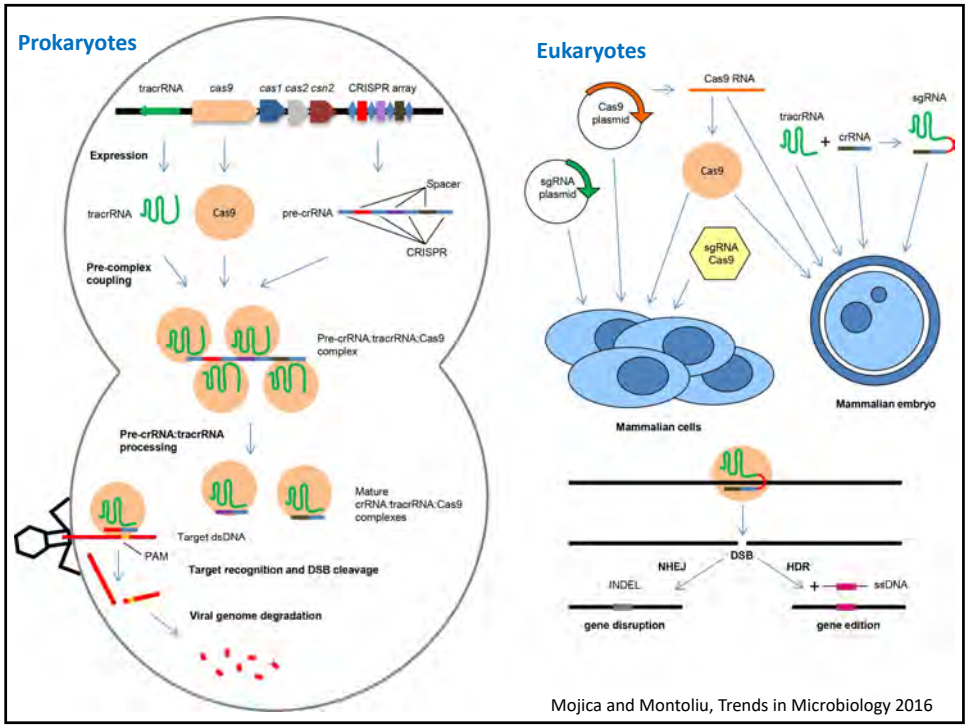
Gene Modification Random or Targeted



How CRISPR-Cas9 tools work?

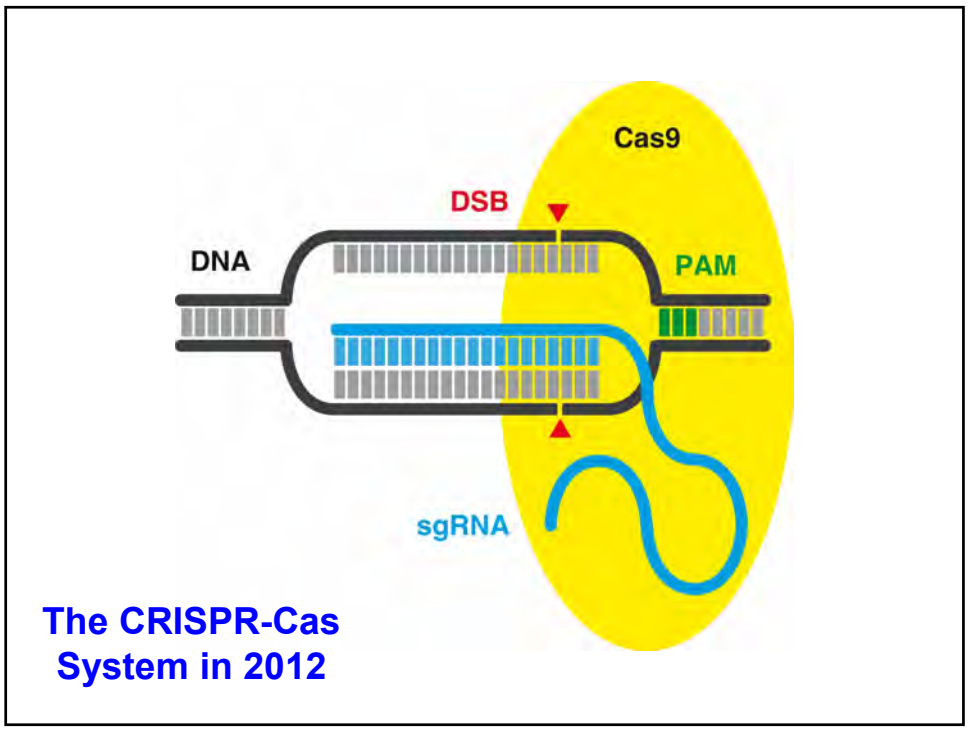




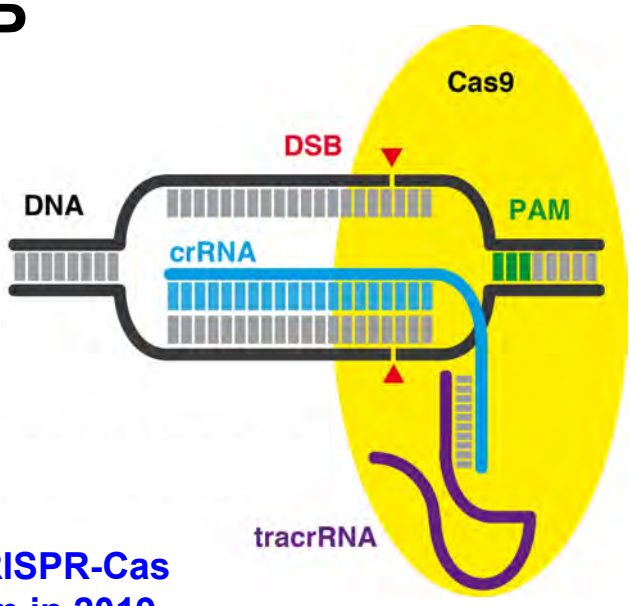


Mojica and Montoliu, Trends in Microbiology 2016

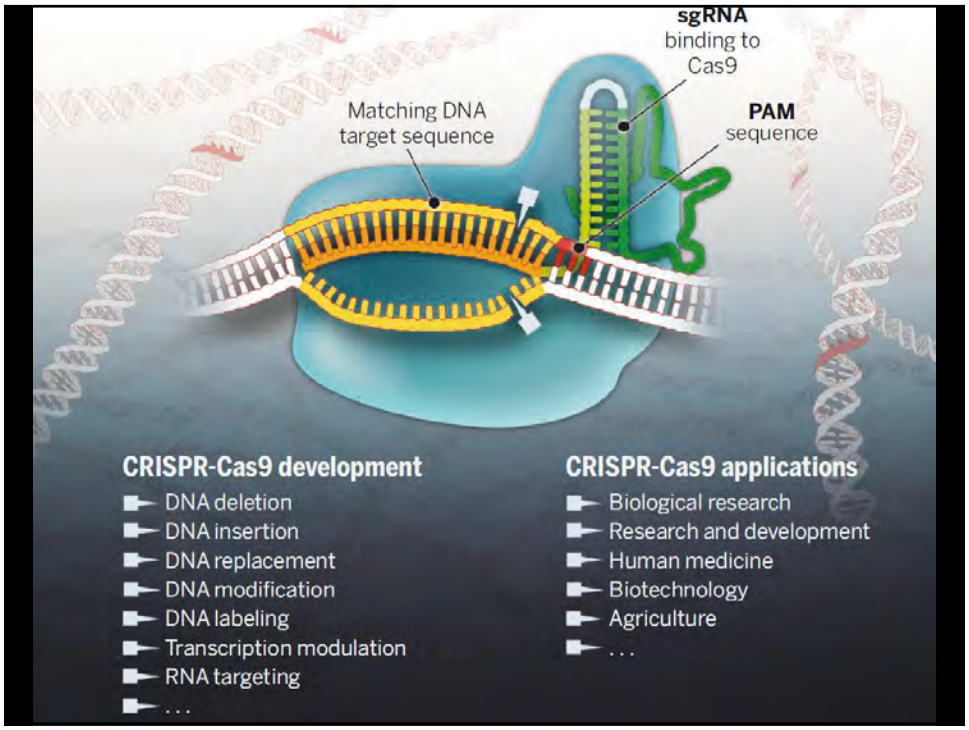

1993
2013
Francisco Mojica
Rodolphe Barrangou
Philippe Horvath
Luciano Marraffini
John van der Oost
Emmanuelle Charpentier
Jennifer Doudna
Virginijus Siksnys
Feng Zhang
George Church
Rudolf Jaenisch
J. Keith Joung


Cas9
DSB
DNA
PAM
sgRNA
The CRISPR-Cas System in 2012

RNP



The CRISPR-Cas System in 2019

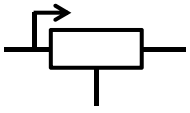


What is a gene-edited animal?

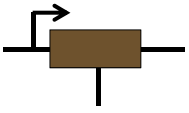
Mutant animal

CRISPR-Cas9

Gene-edited animal



G



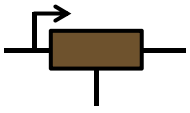
C

What is a gene-edited animal?

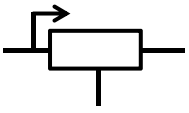
Wild-type animal

CRISPR-Cas9

Gene-edited mutant



C



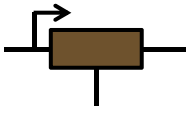
G

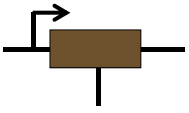
What is a gene-edited animal?

=

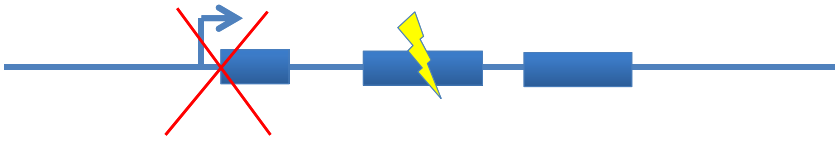
Wild-type animal

Gene-edited animal

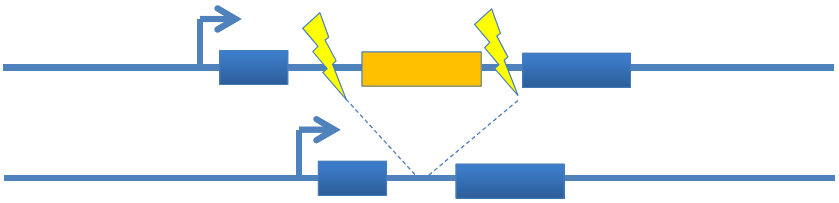




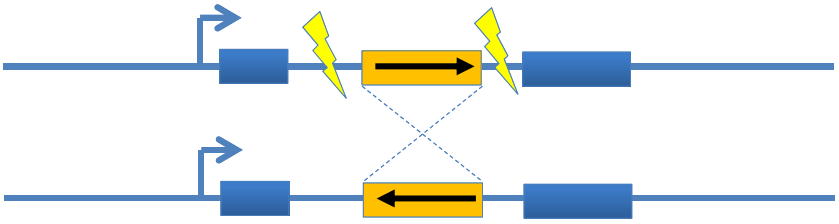
Disrupting a gene: KO



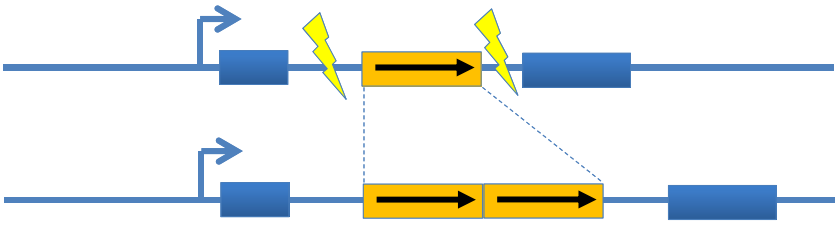
Deletions



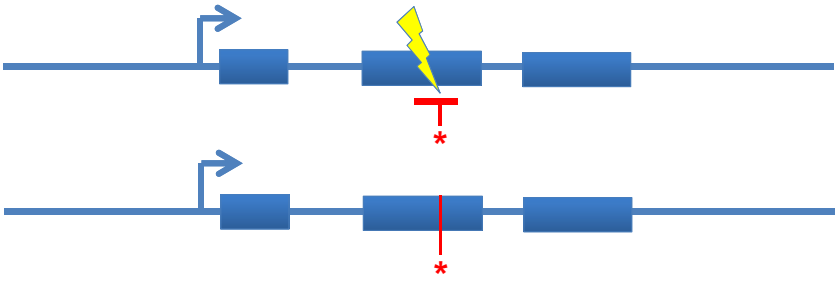
Inversions



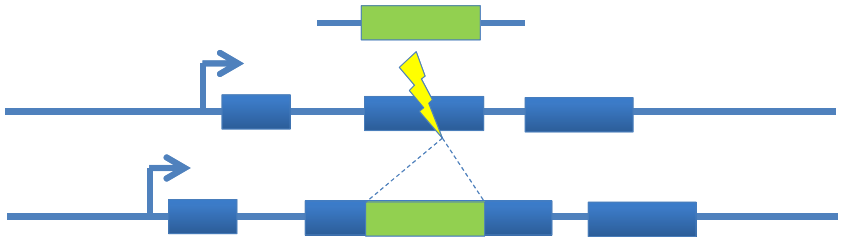
Duplications

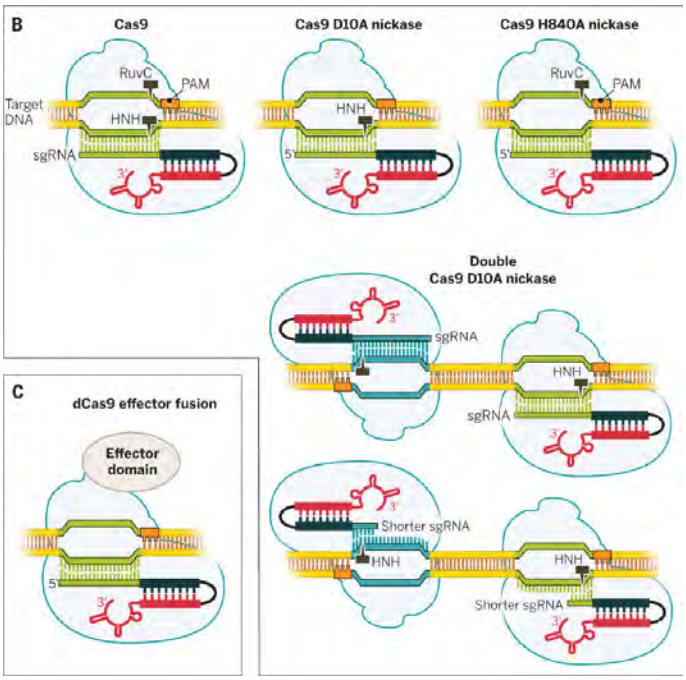


Point mutations



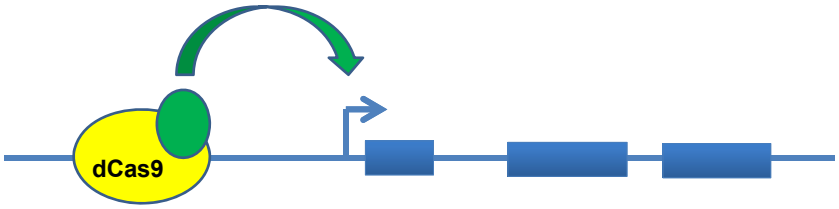
Knock-ins



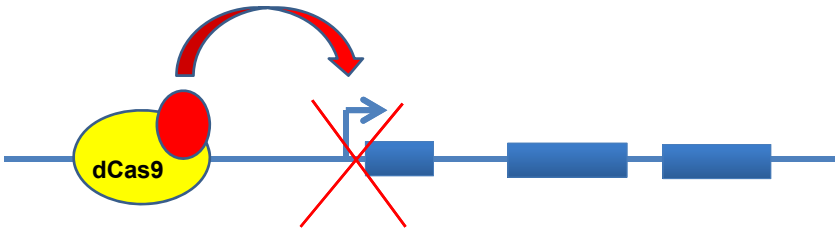


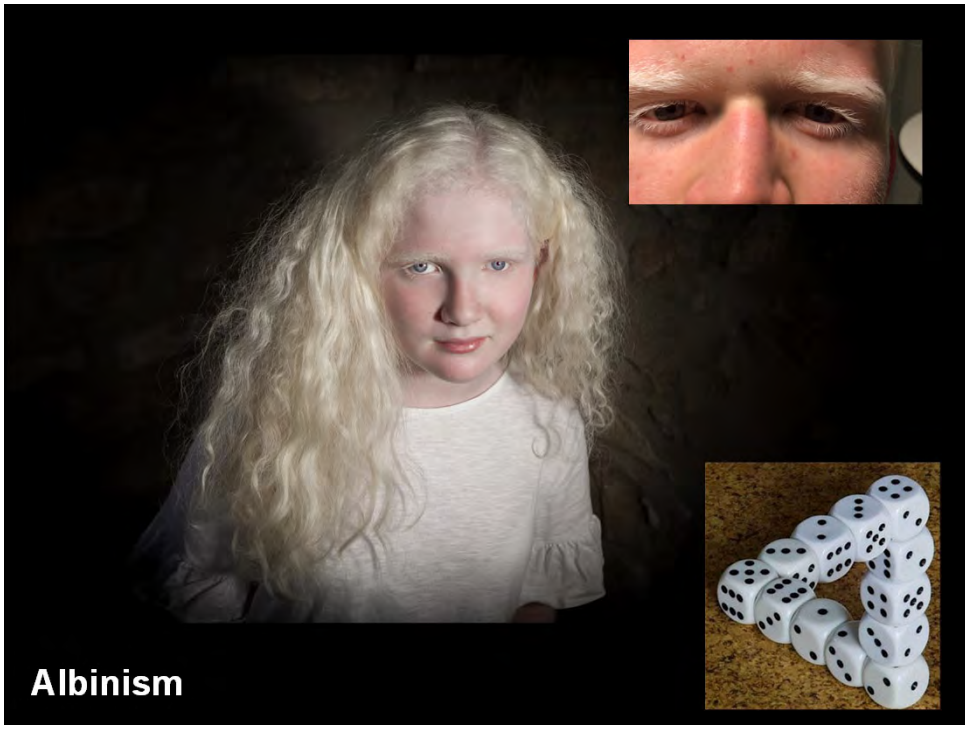
Doudna & Charpentier (2014) Science

Activating a gene



Inactivating a gene

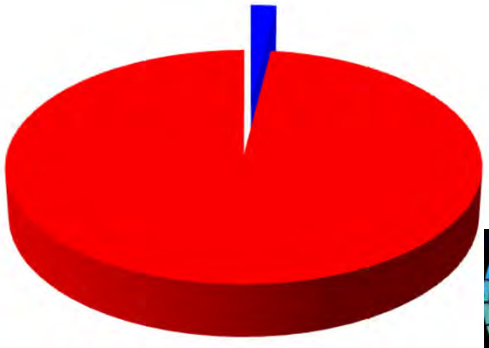




The non-coding genome

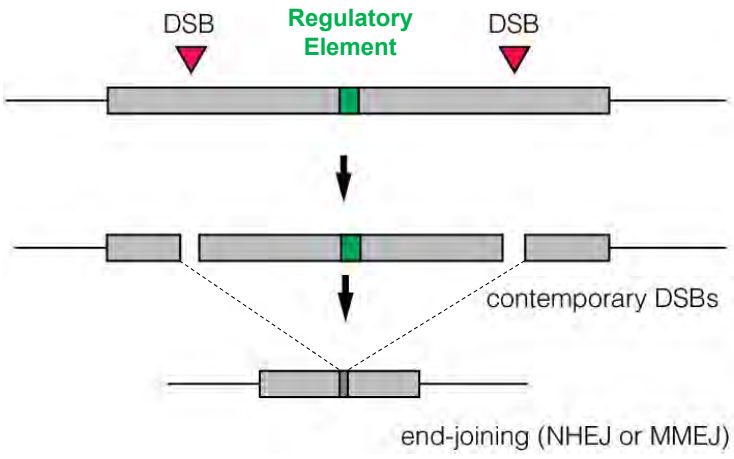
DNA coding sequences represent 2% genome

DNA non-coding sequences represent 98% genome



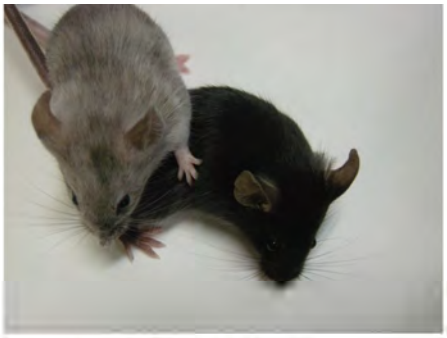
DNA non-coding sequences contain mainly:
DNA repetitive elements, mobile elements and
DNA regulatory elements

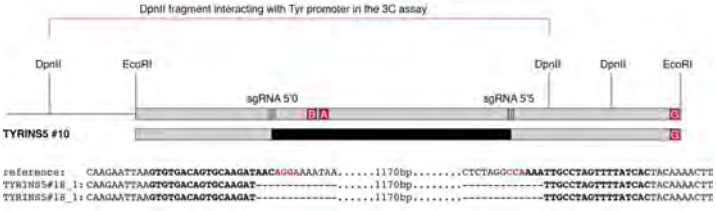
Using CRISPR-Cas9 genome editing to target *Tyr* regulatory elements



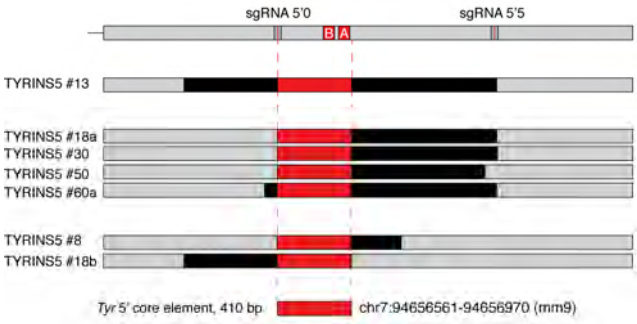
CRISPR-Cas9 genome editing

Deleting *Tyr* regulatory elements with CRISPRs *in vivo*





Comparing different *Tyr* 5' Boundary targeted alleles with similar phenotypes reveals the location of the functionally relevant endogenous regulatory DNA sequences

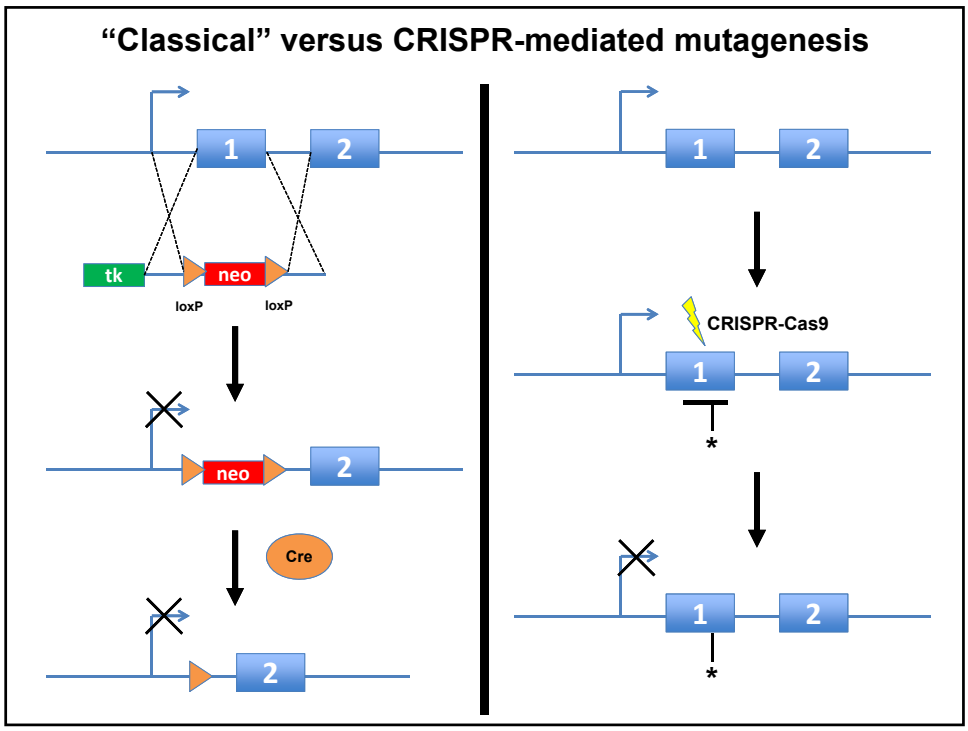


Genetic analysis now possible in mice!

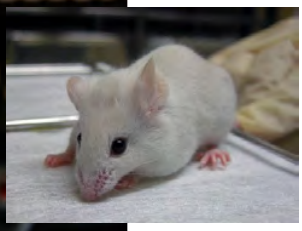
Seruggia et al. 2015 NAR

CRISPR

"Avatar" mice


"Classical" versus CRISPR-mediated mutagenesis
1
2
tk
neo
loxP
loxP
neo
2
Cre
2
1
2
CRISPR-Cas9
1
2
*
1
2
*


OCA4 (SLC45A2) c.986delC
Patty

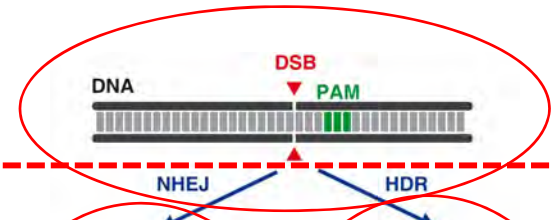


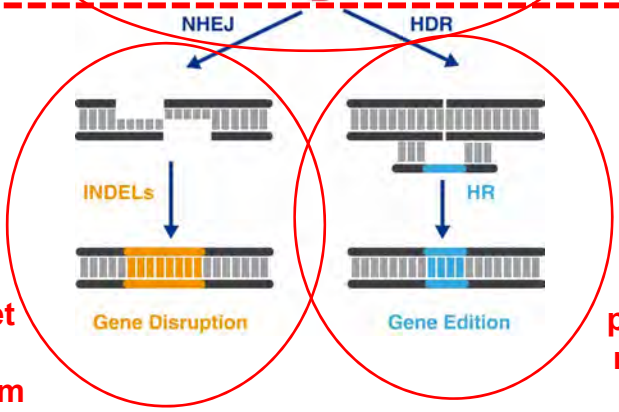
Treatments

Limitations of CRISPR-Cas9 technology

Off-target effects

Related to CRISPR





Unrelated to CRISPR

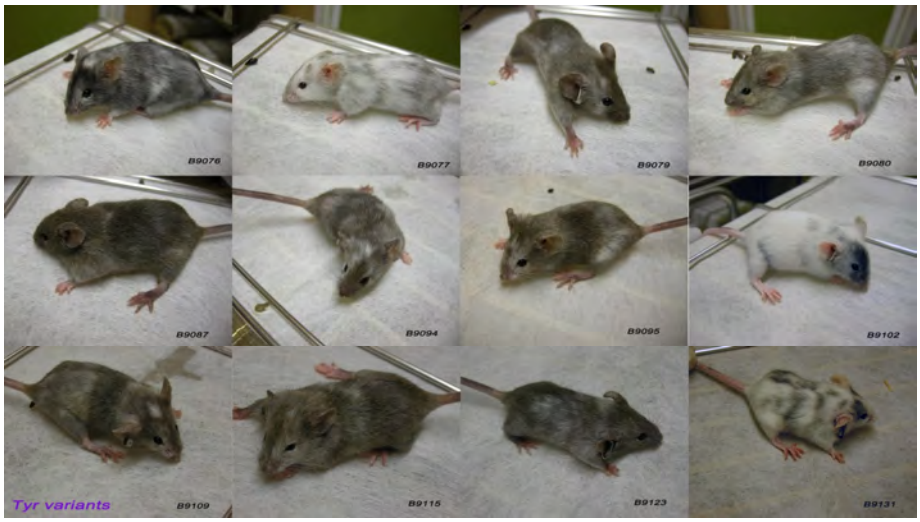
On-target effects Mosaicism

HDR is not the preferred repairing pathway

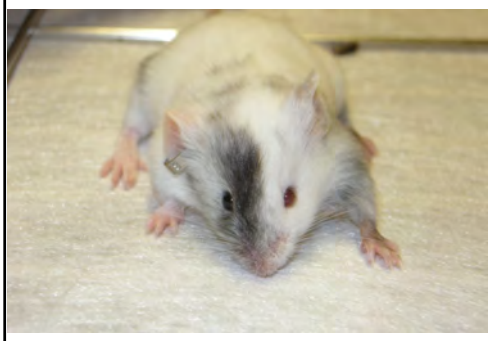
Improved design of RNA guides for optimized CRISPR experiments

Oliveros *et al.* Nucleic Acids Res. 2016

CRISPR gene-edited founder mice are mosaic



On-targets: the real problem



- Founder animals are nearly always complex mosaic
- Many different alleles can be present
- Not all of them might transmit through germline

One 8-cell embryo = 16 possible alleles

Multiple alleles present in CRISPR founder gene-edited mice


```
Reference:  AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACAGCACGGTAGGGTTGATTTCAGGAAATGTAA
B9040.1     AACATTGGAGGAGCTGC ACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAG----------GTAGGGTTGATTTCAGGAAATGTAA
B9040.2     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACAGGTAGGTAGG-TTGATTTCAGGAAATGTAA
B9040.3     AACACTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAG----------GTAGGGTTGATTTCAGGAAATGTAA
B9040.4     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACAGC-----AGGGTTGATTTCAGGAAATGTAA
B9040.5     CAAGCTCCTGCCCCACTTTCAAAGCTGTACTGAACTGCAGTTTCTTCTCCACCCAGATTCCTGCAAGACCTTGCACCGGGG----------(437bp)----------
B9040.6     ----------(561bp)----------
B9041.1     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACAGCTGTTACCTAGGGTTGATTTCAGGAAATG
B9041.2     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAAC--------AGGGTTGATTTCAGGAAATGTAA
B9041.3     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACA----GGTAGGGTTGATTTCAGGAAGTGTAA
B9041.4     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACG----GGTAGGGTTGATTTCAGGAAATGTAA
B9041.5     AACATTGGAGGAGCTGCCACTGCTATTTGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACA----GGTAGGGTTGATTTCAGGAAATGTAA
B9042.1     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACAGCACGGTAGGGTTGATTTCAGGAAATGTAA
B9042.2     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAAT--------------GGTAGGGTTGATTTCAGGAAATGTAA
B9042.3     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAA-TGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACAGCACGGTAGGGTTGATTTCAGGAAATGTAA
B9042.4     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAAT--------------GGTAGGGTTGATTTCAGGAAGTGTAA
B9043.1     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAAT--------------GGTAGGGTTGATTTCAGGAAGTGTAA
B9043.2     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACAGCACACTGGTAGGGTTGATTTCAGGAAGTG
B9043.3     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTCCCAATCAGGAGTTGAGAAAAAT--------------GGTAGGGTTGATTTCAGGAAGTGTAA
B9043.4     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGATAGACATGTCGAGGA------(134bp)----------
B9044.1     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAAT--------------GGTAGGGTTGATTTCAGGAAATGTAA
B9044.2     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCGATCAGGAGTTGAGAAAAATGGTAGGTAAC--------AGGGTTGATTTCAGGAAATGTAA
B9045.1     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTTGTTTAT----------CAAGGTAGGGTTGATTTCAGGAAATGTAA
B9045.2     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAAT----------GGTTGATTTCAGGAAATGTAA
B9045.3     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTTGTTTAT----------CAAGGTAGGGTTGATTTCGGAAATGTAA
B9046.1     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACAGCACCTAGGTAGGGTTGATTTCAGGAAATG
B9046.2     AACATTG----------(87bp)----------TAGGGTTGATTTCAGGAAATGTAA
B9046.3     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACAGCAATGGTAGGGTTGATTTCAGGAAATGTA
B9046.4     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACAGCACCTAGGTAGGGTTGATTACAGGAAATG
B9060.1     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACA----GGTAGGGTTGATTTCAGGAAATGTAA
B9060.2     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAGATGGTAGGTAACA----GGTAGGGTTGATTTCAGGAAATGTAA
B9060.2     GACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACA----GGTAGGGTTGATTTCAGGAAATGTAA
B9060.3     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACAGCT-------GTTGATTTCAGGAAATGTAA
B9060.4     AACATCGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACA----GGTAGGGTTGATTTCAGGAAATGTAA
B9064.1     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACAGCAAAATGGTAGGTAGGGTTGATTTCAGG
B9064.2     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTA----------GGTAGGGTTGATTTCAGGAAATGTAA
B9064.3     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAGTGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACAGCAAAATGGTAGGTAGGGTTGATTTCAGG
B9064.4     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACAGC-----AGGGTTGATTTCAGGAAATGTAA
B9064.5     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCA----------GGTAGGGTTGATTTCAGGAAATGTAA
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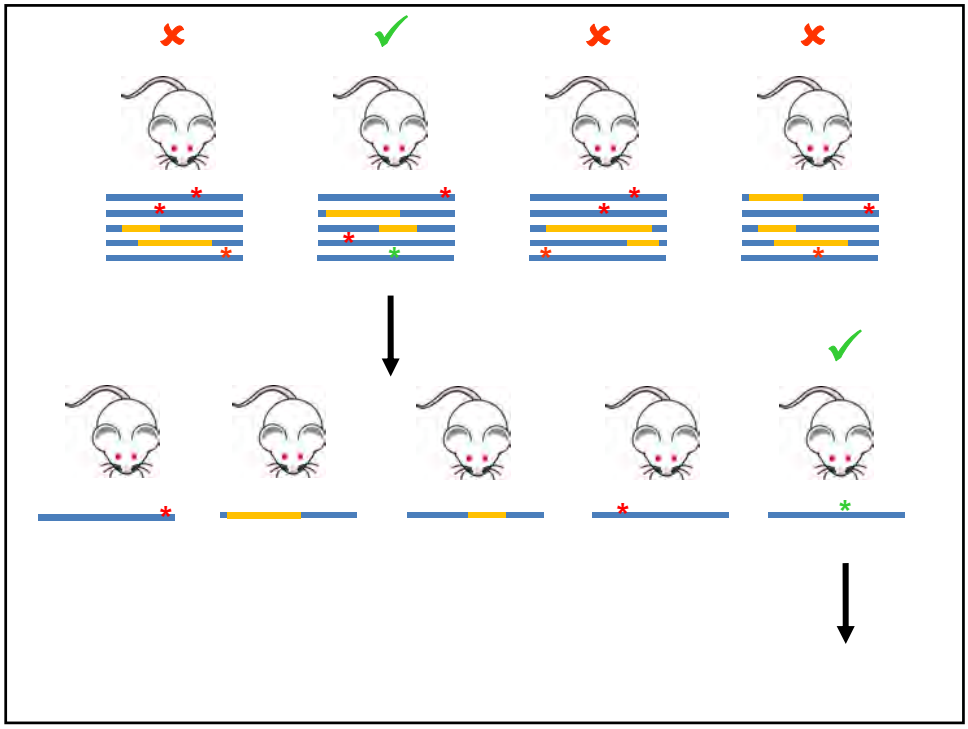
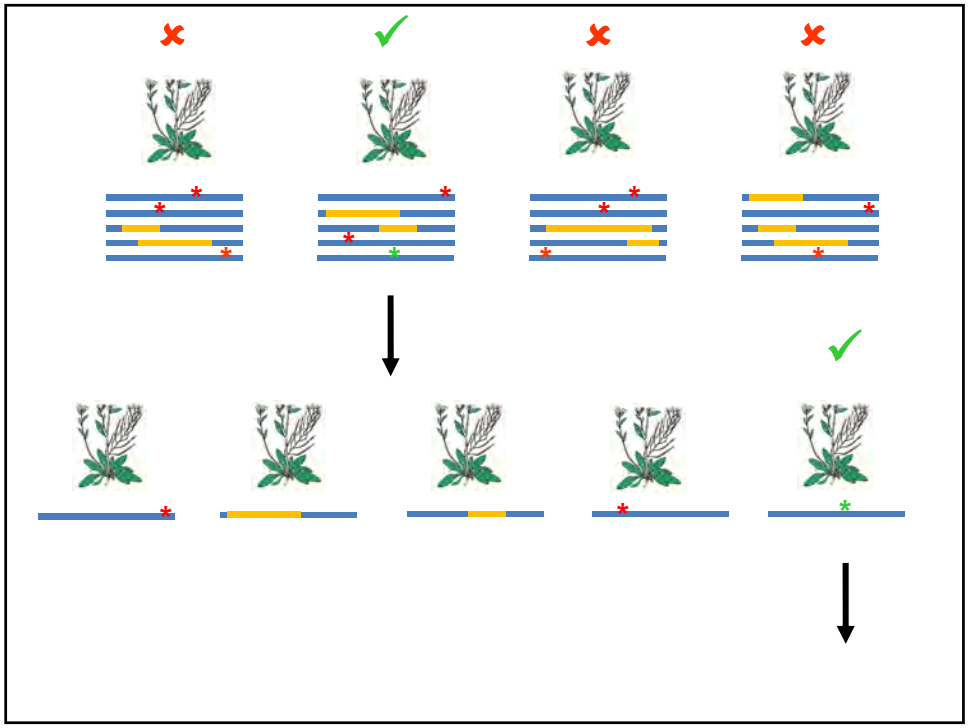
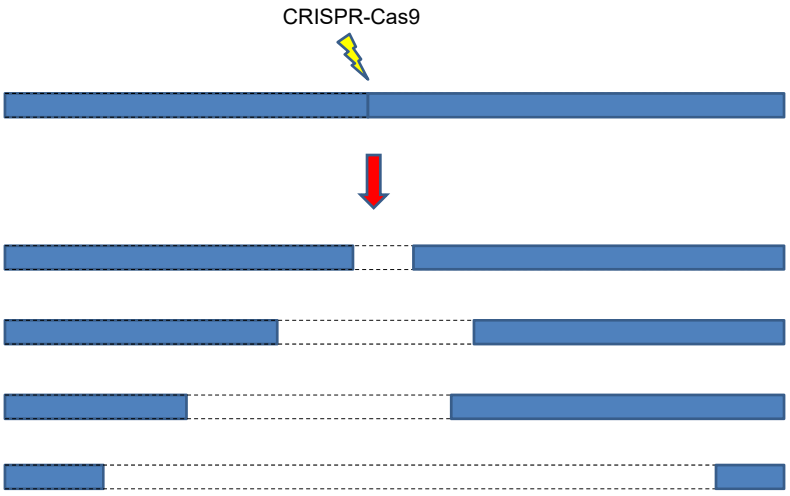




Large deletions after DSB induced by CRISPR-Cas9



Kosicki et al. 2018 Nature Biotech.
Adikusuma et al. 2018 Nature Comm.

CRISPR-Cas is the future

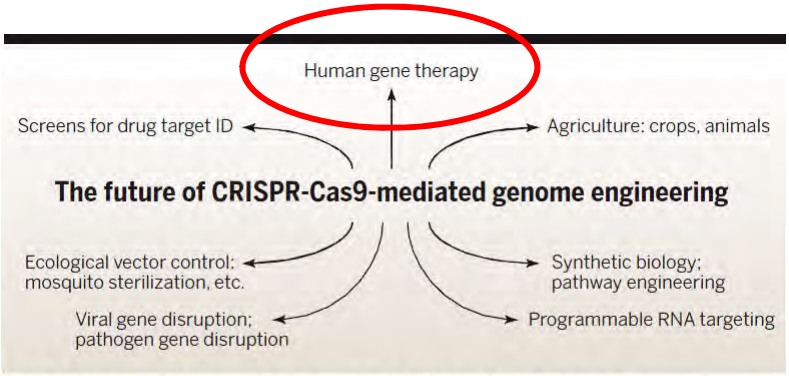
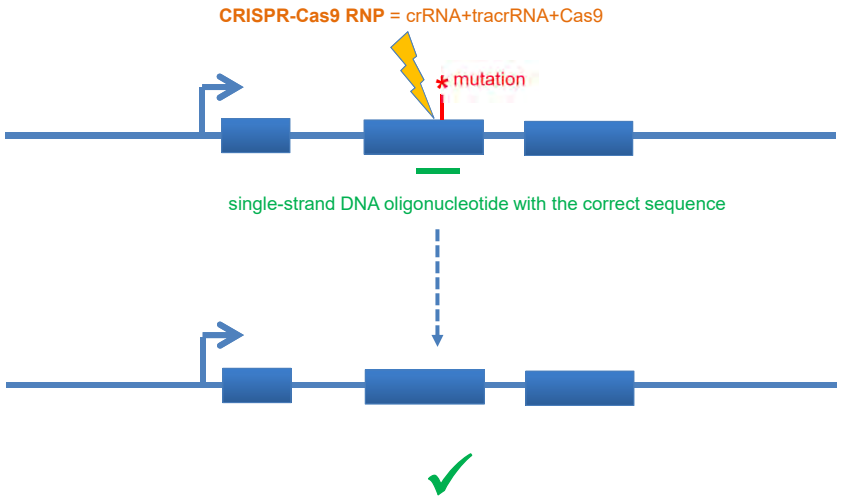


Fig. 6. Future applications in biomedicine and biotechnology. Potential developments include establishment of screens for target identification, human gene therapy by gene repair and gene disruption, gene disruption of viral sequences, and programmable RNA targeting.

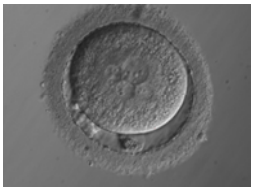
Doudna & Charpentier (2014) *Science*

Gene Therapy with CRISPR



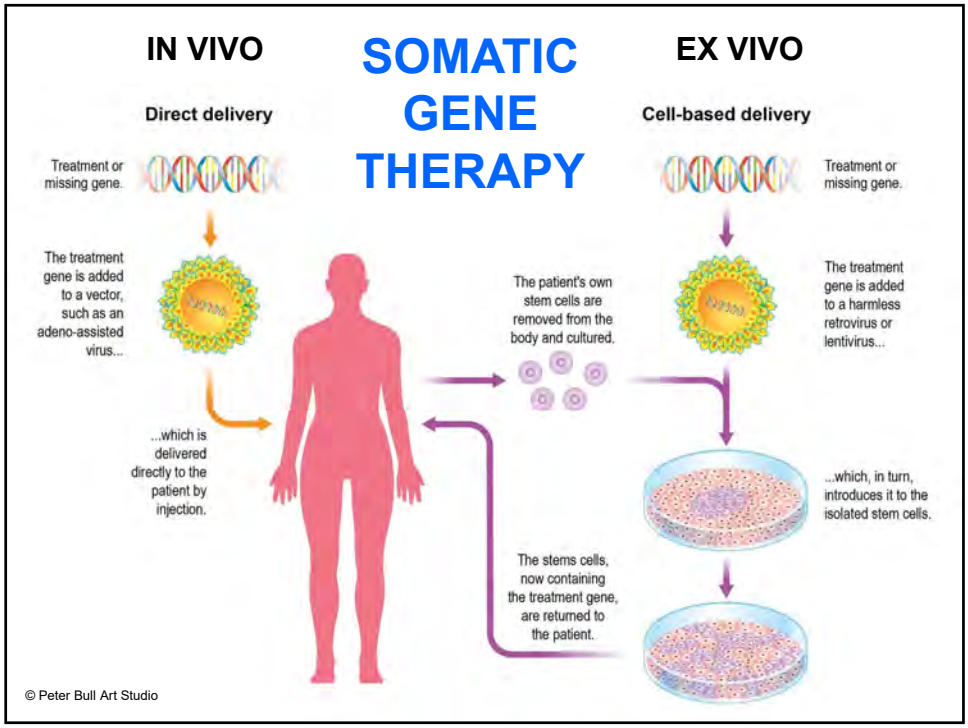
CRISPR and human embryos

- >3 studies from China using 3n/2n embryos
- Many **different alleles** are produced
- Most edited embryos are **mosaic**
- Anticipate potential **off-target** effects
- Need for **careful risk/benefit** analysis
- Consider alternative technologies (**PGD**: preimplantation genetic diagnosis)
- Need to be **cautious** before applying
- Poses Ethics dilemas (art 13 and art. 18, Asturias Convention, 1997)

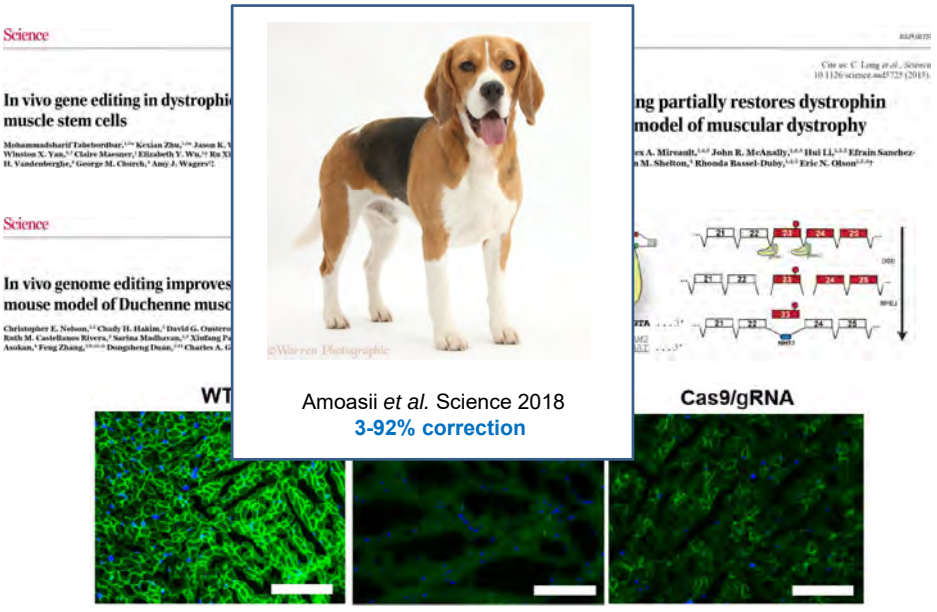


Lunes, 26 Noviembre 2018

He Jiankui (Shenzhen, China)



CRISPR-Cas9 and *in vivo* somatic gene therapy



Amoasii *et al.* Science 2018
3-92% correction

Increasing number of animal models of rare monogenic diseases corrected via CRISPR

Preclinical animal models

- Duchenne muscular distrophy (DMD)
- Ornithine transcarbamylase (OTC) deficiency
- Hereditary tyrosinemia I (FAH deficiency)
- Congenital cataract (CRYGC)
- Chronic granulomatous disease (CGD)
- Retinitis pigmentosa (RP)
- Leber congenital amaurosis (LCA)
- Hungtinton Disease (HD)
- …
- Also **many iPS cells models** correcting gene mutations via CRISPR strategies

News › Science

Scientists make first attempt to permanently change a person's DNA to cure a disease

A risky new treatment is being trialled in the US to reverse the effects of an incurable genetic disorder

Josh Gabbatiss | 12 hours ago

80 shares

Associated Press, 15 Nov 2017

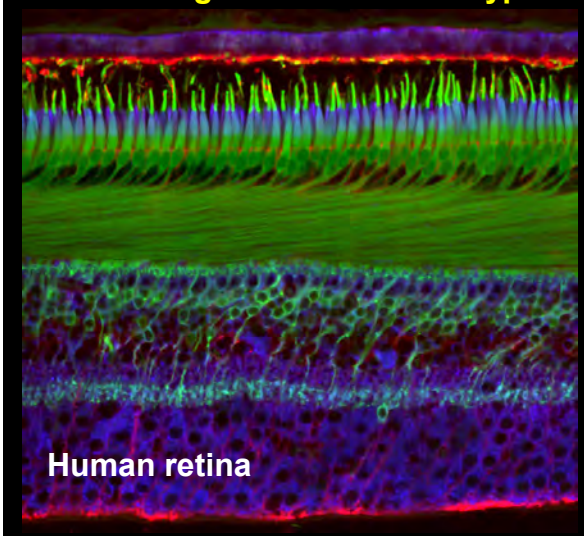
IN VIVO

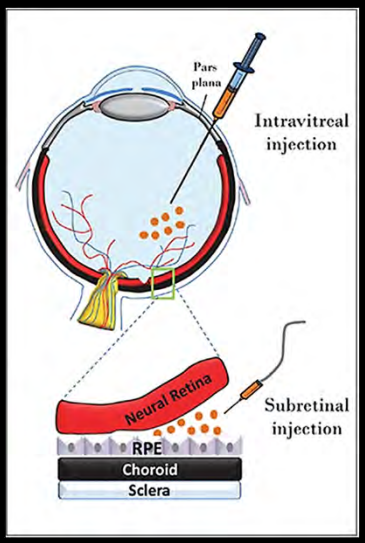
- UCSF Benioff Children's Hospital in Oakland, California
- IV injection of viral particles with ZFNs
- Approved by NIH
- Sangamo
- Hunter's syndrome (I2S gene) Mucopolysaccharidosis II (MPS II)
- Lysosomal storage disease
- Injected on **13 Nov 2017**
- **No therapeutic effect seen**
- **No toxicity detected**

First genome editing (driven by ZFN) somatic gene therapy in a patient IN VIVO

Correcting a point mutation in CEP290 gene with NHEJ CRISPR

Leber's congenital amaurosis type 10





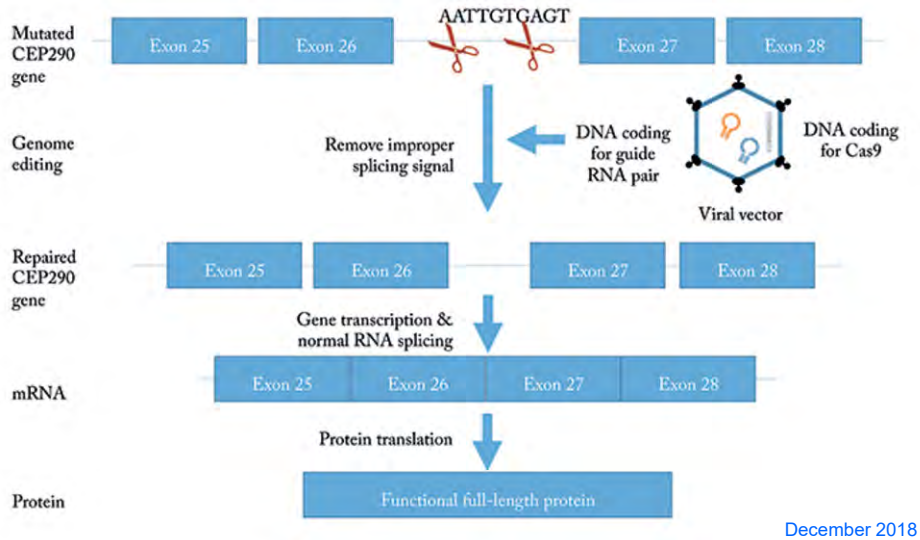
editas MEDICINE | Allergan

In vivo

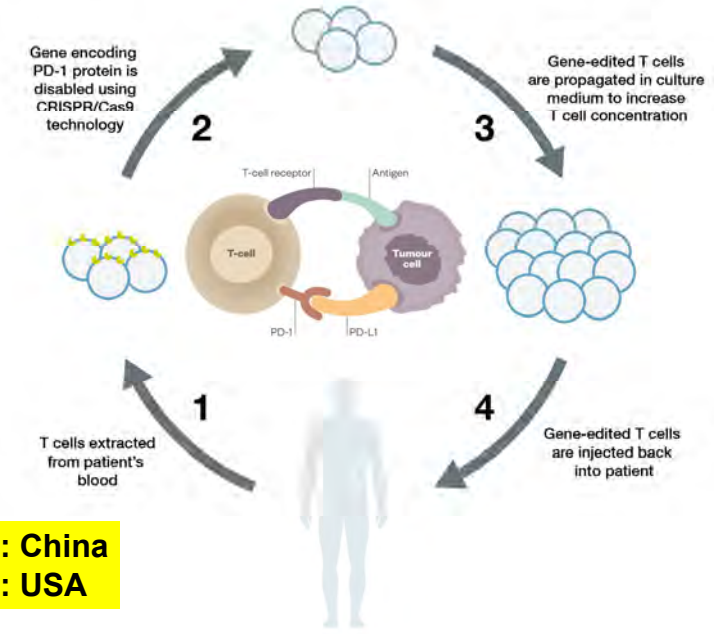
December 2018

Correcting a point mutation in CEP290 gene with NHEJ CRISPR

Leber's congenital amaurosis type 10



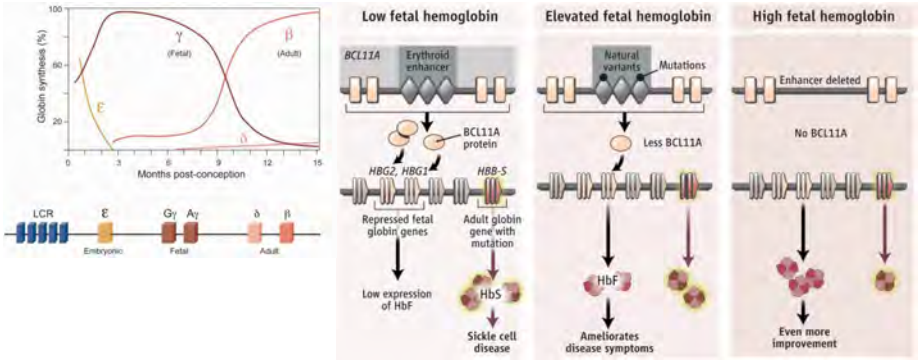
December 2018



2016: China
2019: USA

Ex-vivo immunotherapy: targeting PD-1 gene with CRISPR tools

Ex-vivo CRISPR targeting of *BCL11A* enhancer for Sickle cell anemia and beta-thalassemia



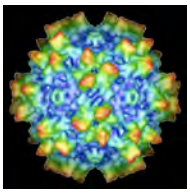
Bauer Lab (Dana Farber-Boston Children's)

CRISPR THERAPEUTICS

VERTEX

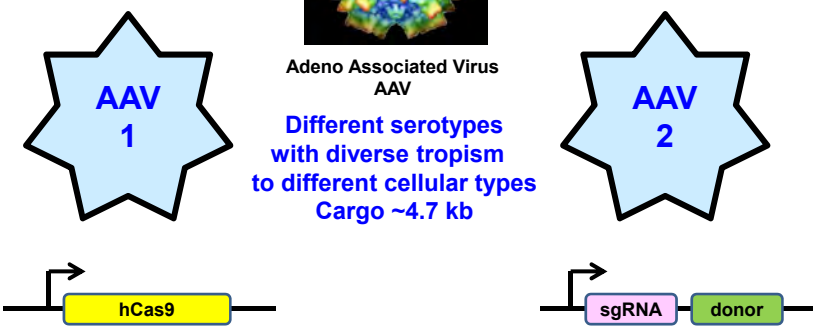
First ex-vivo CRISPR therapy approved in Europe

CRISPR tools and somatic gene therapy of human rare diseases

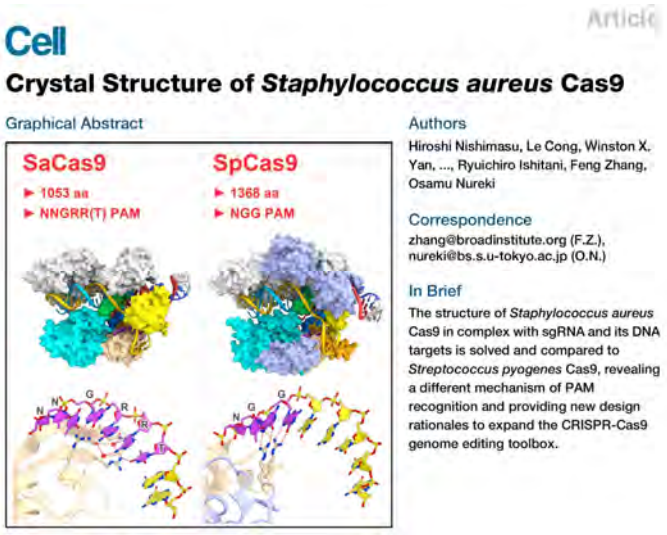


Adeno Associated Virus
AAV

Different serotypes with diverse tropism to different cellular types
Cargo ~4.7 kb



Smaller Cas9 variants found (useful for gene therapy approaches)



Cell

Article

Crystal Structure of *Staphylococcus aureus* Cas9

Graphical Abstract

SaCas9
- 1053 aa
- NNGRR(T) PAM

SpCas9
- 1368 aa
- NGG PAM

Authors

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Correspondence

zhang@broadinstitute.org (F.Z.), nureki@bs.s.u-tokyo.ac.jp (O.N.)

In Brief

The structure of *Staphylococcus aureus* Cas9 in complex with sgRNA and its DNA targets is solved and compared to *Streptococcus pyogenes* Cas9, revealing a different mechanism of PAM recognition and providing new design rationales to expand the CRISPR-Cas9 genome editing toolbox.

Nishimasu et al. 2015 (Cell)

Cas9
Streptococcus pyogenes
Staphylococcus aureus

Cas9: Bang Wong, Broad Institute of Harvard and MIT, Cambridge, MA

bioRxiv beta
THE PREPRINT SERVER FOR BIOLOGY

5 Jan 2018 – **28 Jan 2019** ***Nature Med.***

nature medicine

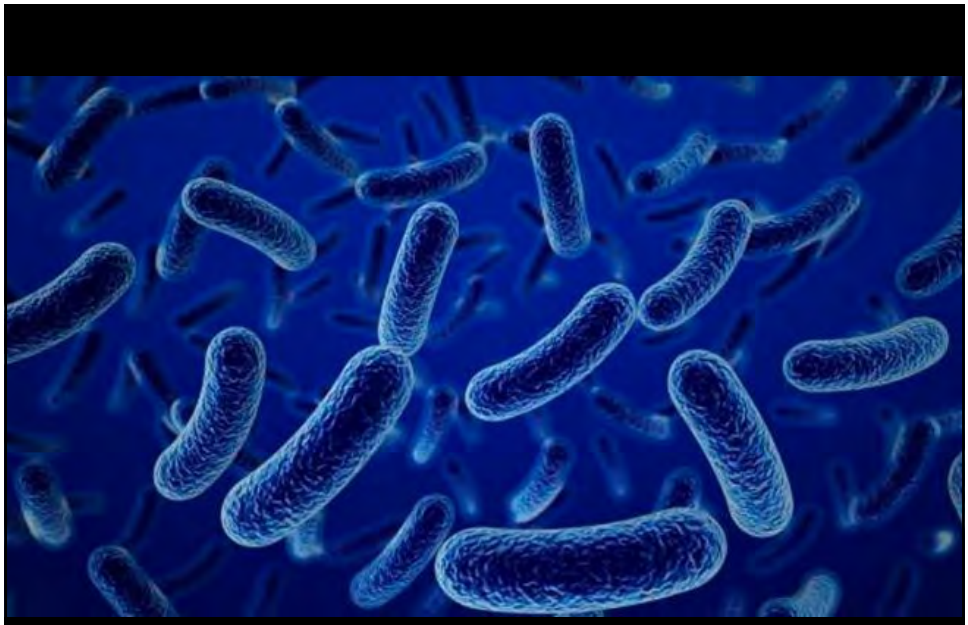
New Results

Identification of Pre-Existing Adaptive Immunity to Cas9 Proteins in Humans

Carsten Trevor Charlesworth, Priyanka S Deshpande, Daniel P Dever, Beruh Dejene, Natalia Gomez-Ospina, Sruthi Mantri, Mara Pavel-Dinu, Joab Camarena, Kenneth I Weinberg, Matthew H Porteus

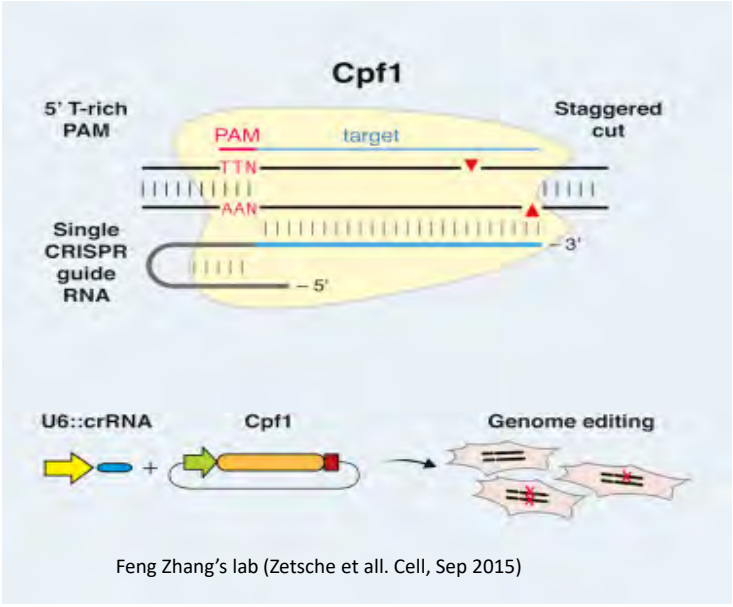
doi: https://doi.org/10.1101/243345

- Cas9 antibodies found in human serum
- Anti-Cas9 T lymphocites found in human blood
- 79% individuals have antibodies against SaCas9
- 65% individuals have antibodies against SpCas9
- 46% individuals have anti-Cas9 T cells
- Immunosupression or alternative Cas proteins



CRISPR systems from other bacteria…

Alternative Cas-like proteins

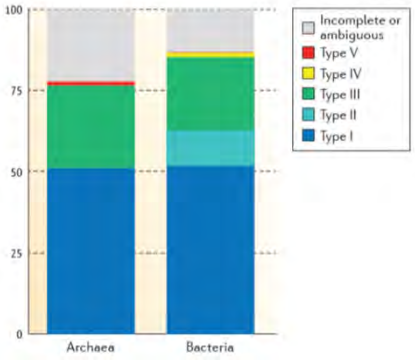


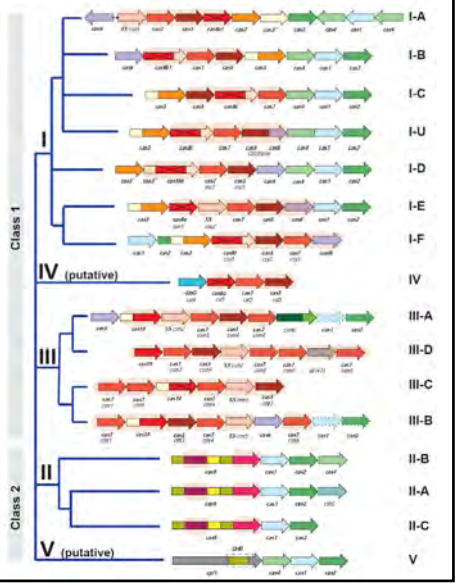
Feng Zhang's lab (Zetsche et all. Cell, Sep 2015)

Cas12a *Francisella tularensis*

Diversity of CRISPR-Cas systems

An updated evolutionary classification of CRISPR–Cas systems
Makarova *et al.* **Nature Rev. Microbiol. 2015**

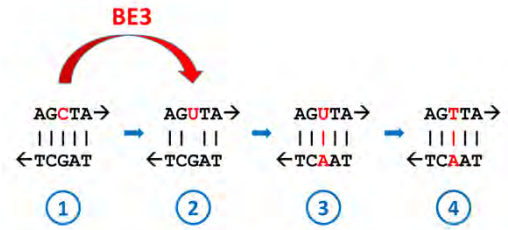


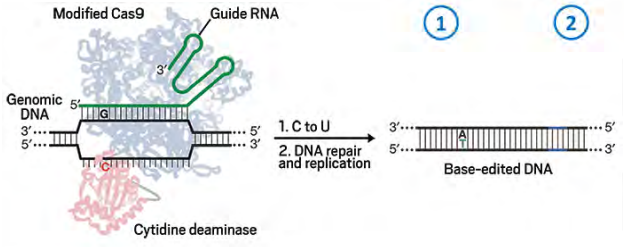


David Liu Lab

CRISPR-derived BASE EDITORS (BE3)

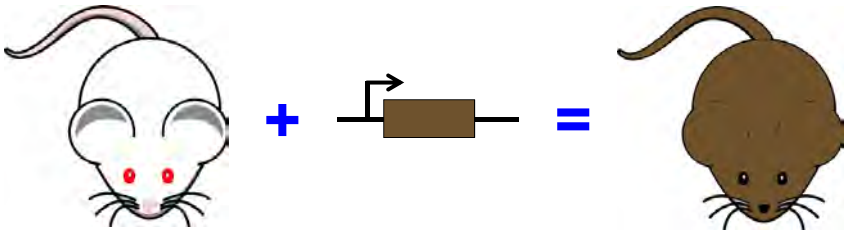
C → U → T
A → I → G





Kim et al. Nat Biotech. 2017

What is a transgenic animal?



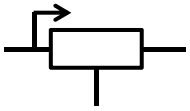
animal + transgene = transgenic

This is a transgenic animal

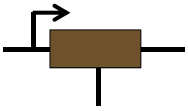
This is NOT a transgenic animal

Mutant animal

Gene-edited animal



CRISPR-Cas9



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