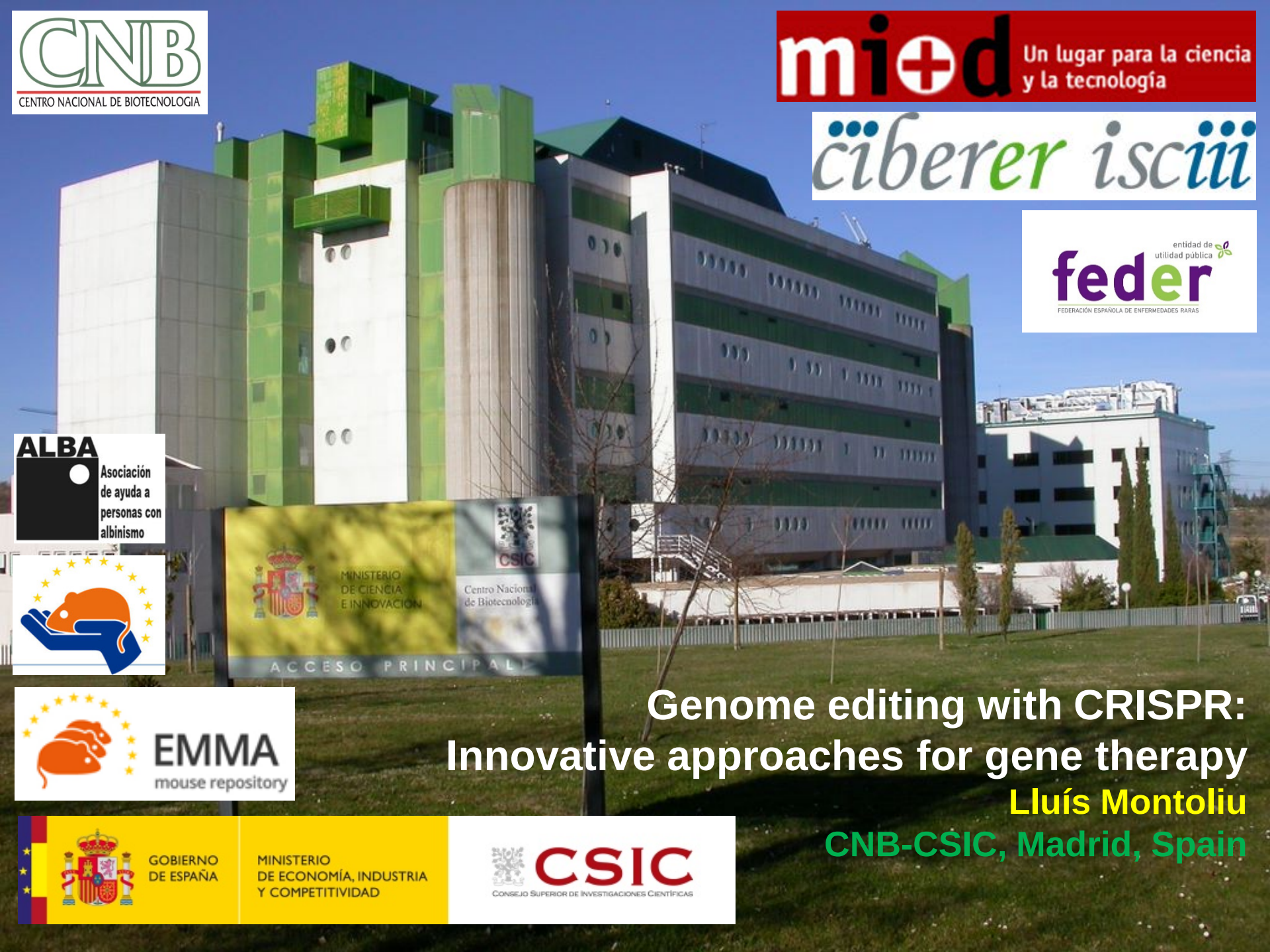

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entidad de utilidad pública
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FEDERACIÓN ESPAÑOLA DE ENFERMEDADES RARAS

ALBA
Asociación de ayuda a personas con albinismo

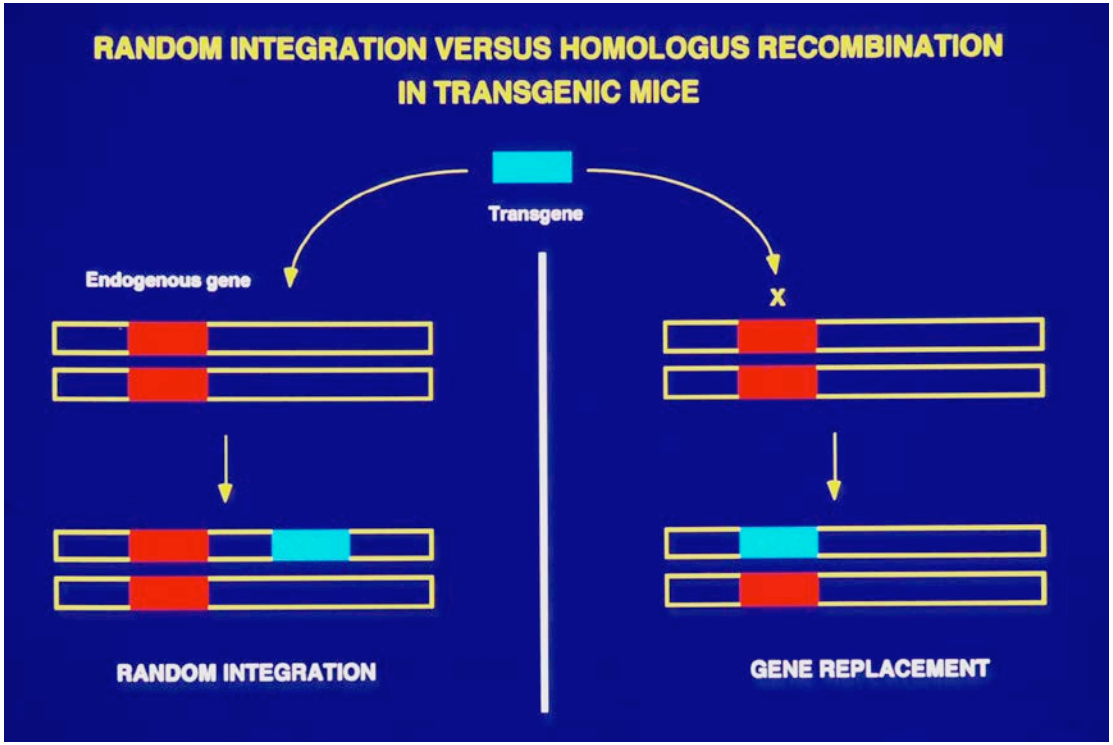
EMMA
mouse repository

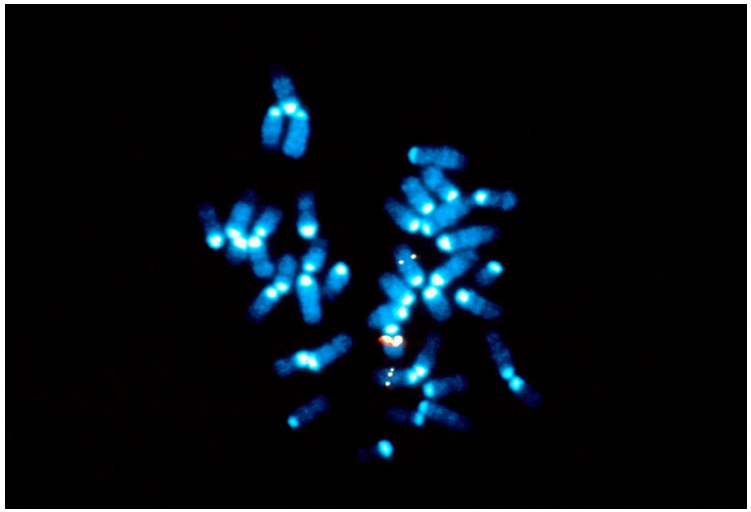
GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA, INDUSTRIA Y COMPETITIVIDAD
CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

Genome editing with CRISPR: Innovative approaches for gene therapy

Lluís Montoliu
CNB-CSIC, Madrid, Spain

The lasting challenge in transgenesis and in gene therapy

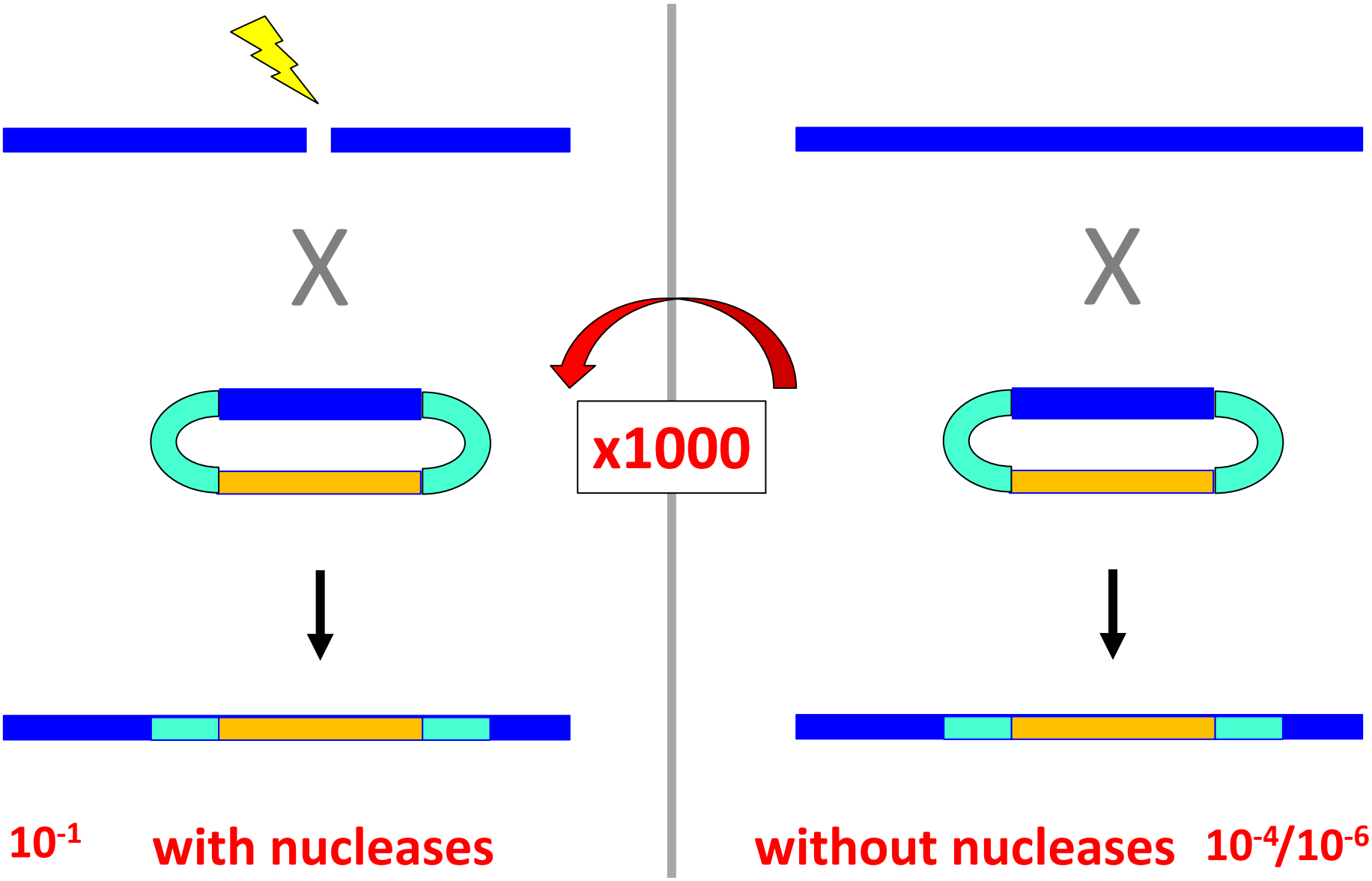




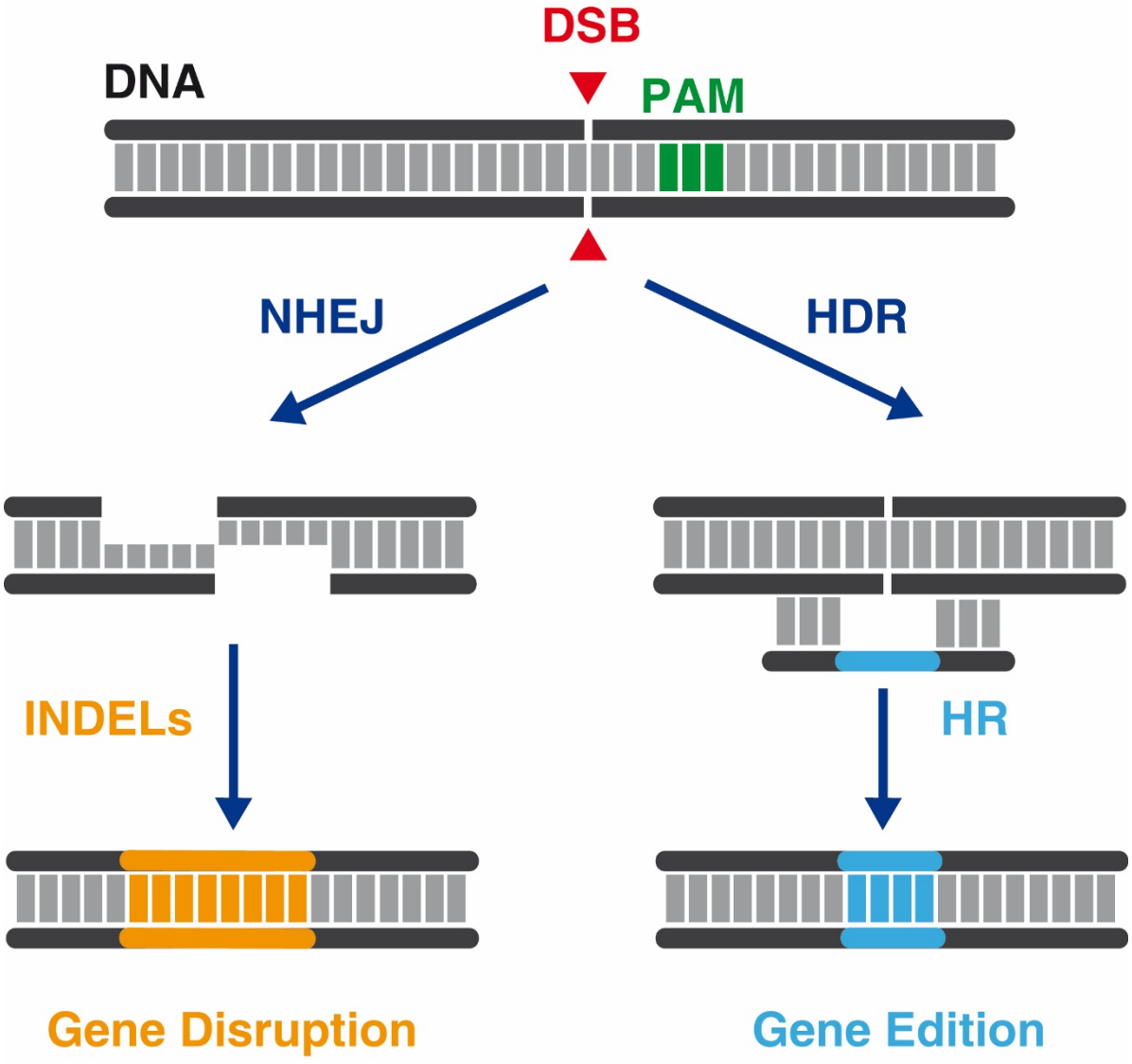
Slides from 1991

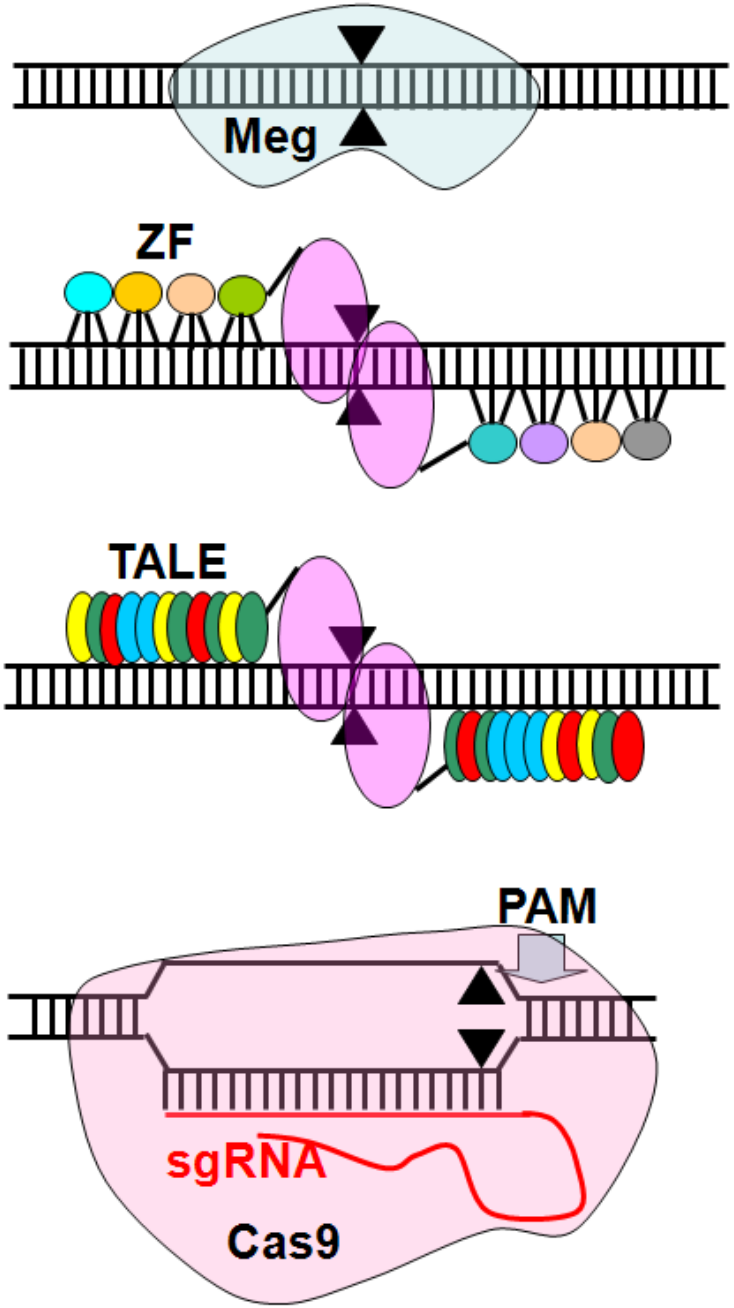
Random versus Targeted genetic modification

Homologous Recombination and Nucleases



Fixing the DSB: NHEJ vs HDR





Meganuclease	20-40 bp/Enzyme
ZFN	3 bp/Finger
TALEN	1 bp/Module
CRISPR-Cas	1 bp/Base

News › Science

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

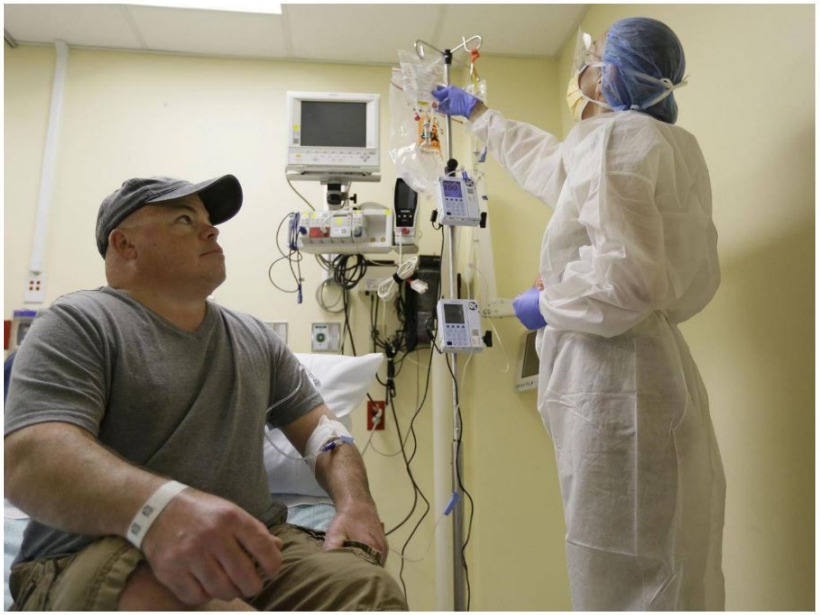
A risky new treatment is being trialled in the US to reverse the effects of an incurable genetic disorder

Josh Gabbatiss | 12 hours ago |

80 shares

Associated Press, 15 Nov 2017

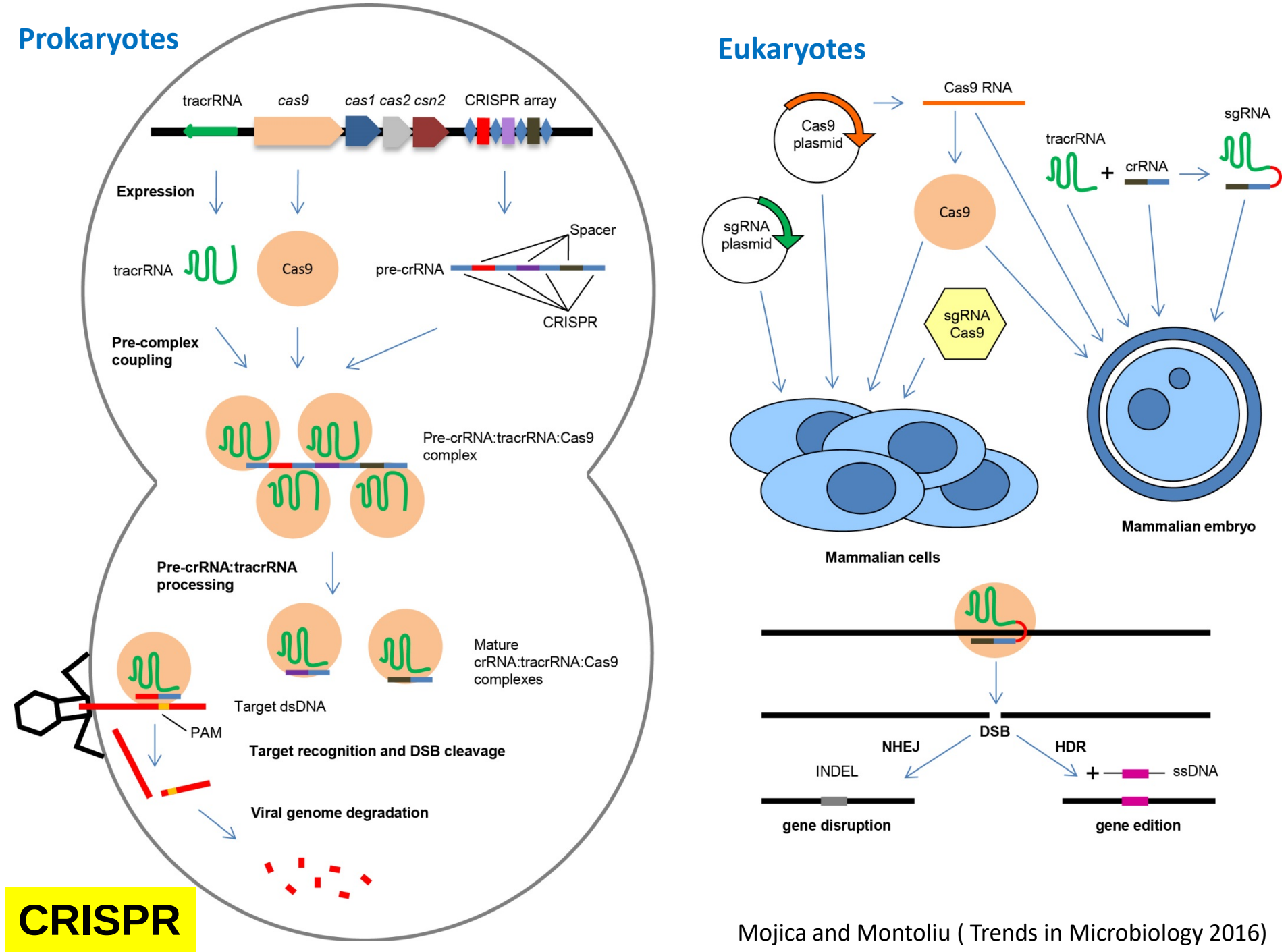
Me gus Click to follow The Independent Online



Brian Madeux, 44, looks up at nurse practitioner Jacqueline Madde while receiving the first human gene editing therapy at the UCSF Benioff Children's Hospital in Oakland, California *ASSOCIATED PRESS*

- 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 last Monday **13 Nov 2017**

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



CRISPR

Mojica and Montoliu (Trends in Microbiology 2016)

Francisco Juan Martínez Mojica

The CRISPR-Cas pioneers

Francisco Mojica
University of Alicante, Spain

Rodolphe Barrangou
North Carolina State Univ, Raleigh, USA

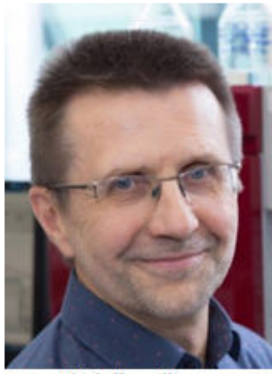
Philippe Horvath
DuPont Nutrition and Health, France

Luciano Marraffini
The Rockefeller Univ, New York, USA

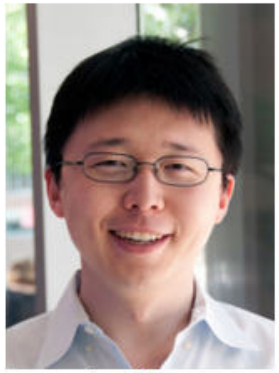
John van der Oost
Wageningen University, The Netherlands

Emmanuelle Charpentier
MPI for Infect. Biol., Berlin, Germany

Jennifer Doudna
Univ California Berkeley, CA, USA



Virginijus Siksnys
Vilnius University, Lithuania



Feng Zhang
BROAD-MIT, Cambridge, MA, USA

George Church
Harvard Med School, Boston, MA, USA

Rudolf Jaenisch
Whitehead Inst, Cambridge, MA, USA

J. Keith Joung
Mass Gen Hosp, Charlestown, MA, USA

The CRISPR-Cas System: targeting nucleases to specific DNA sequences a binary system: Cas9 protein and sgRNA

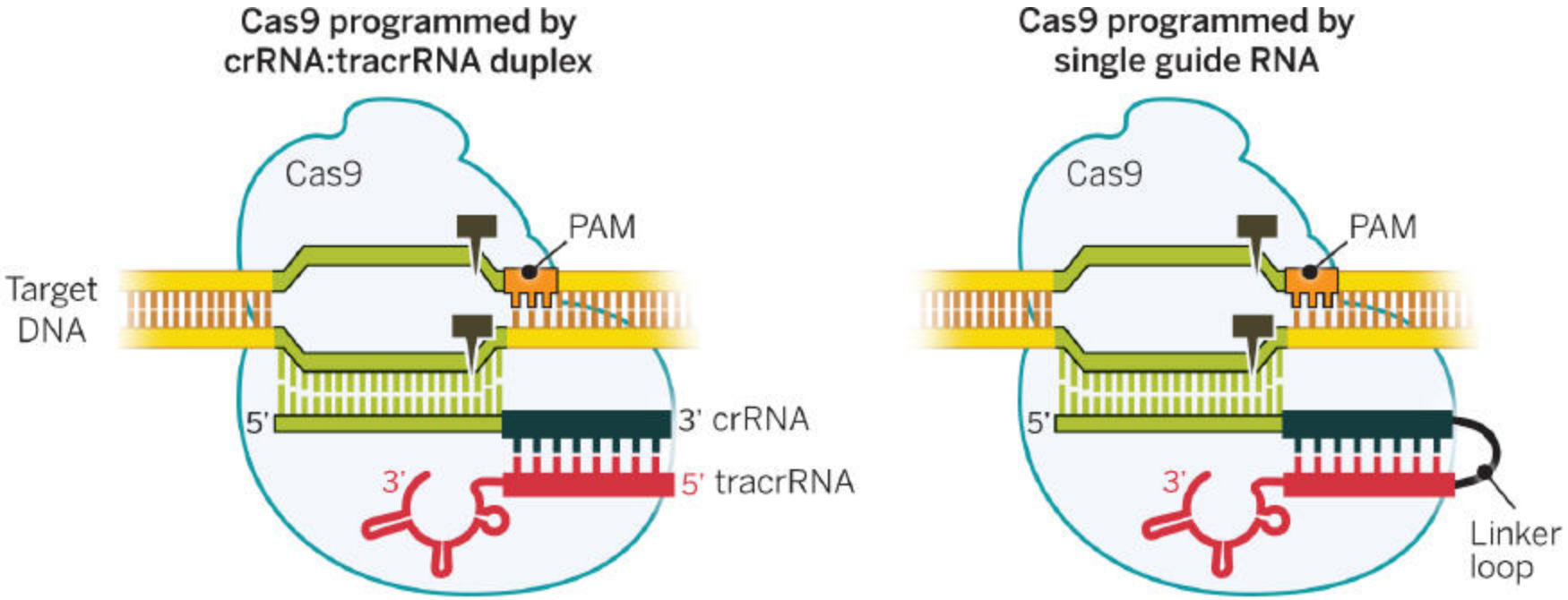
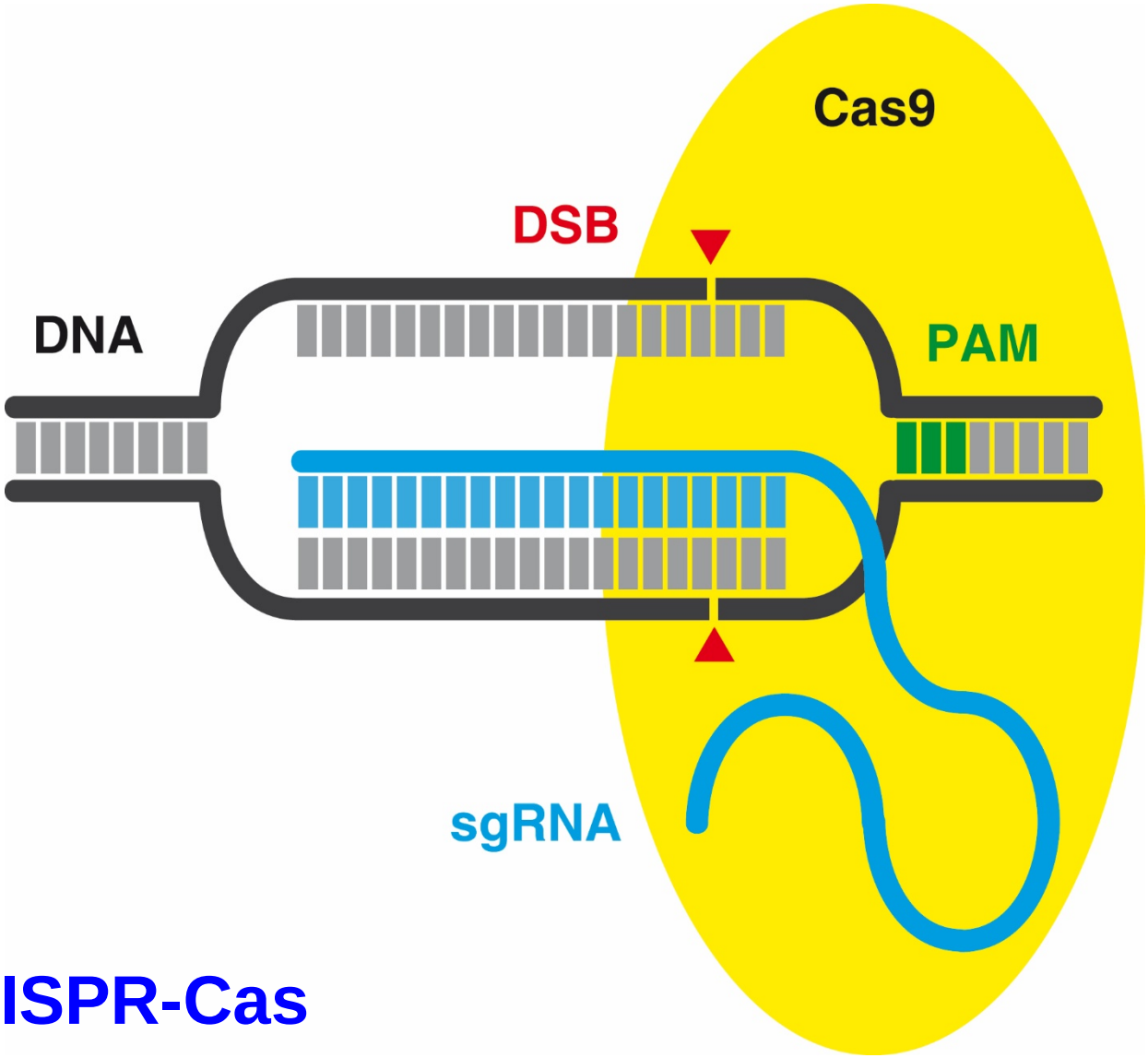


Fig. 3. Evolution and structure of Cas9. The structure of *S. pyogenes* Cas9 in the unliganded and RNA-DNA–bound forms [from (*77*, *81*)].

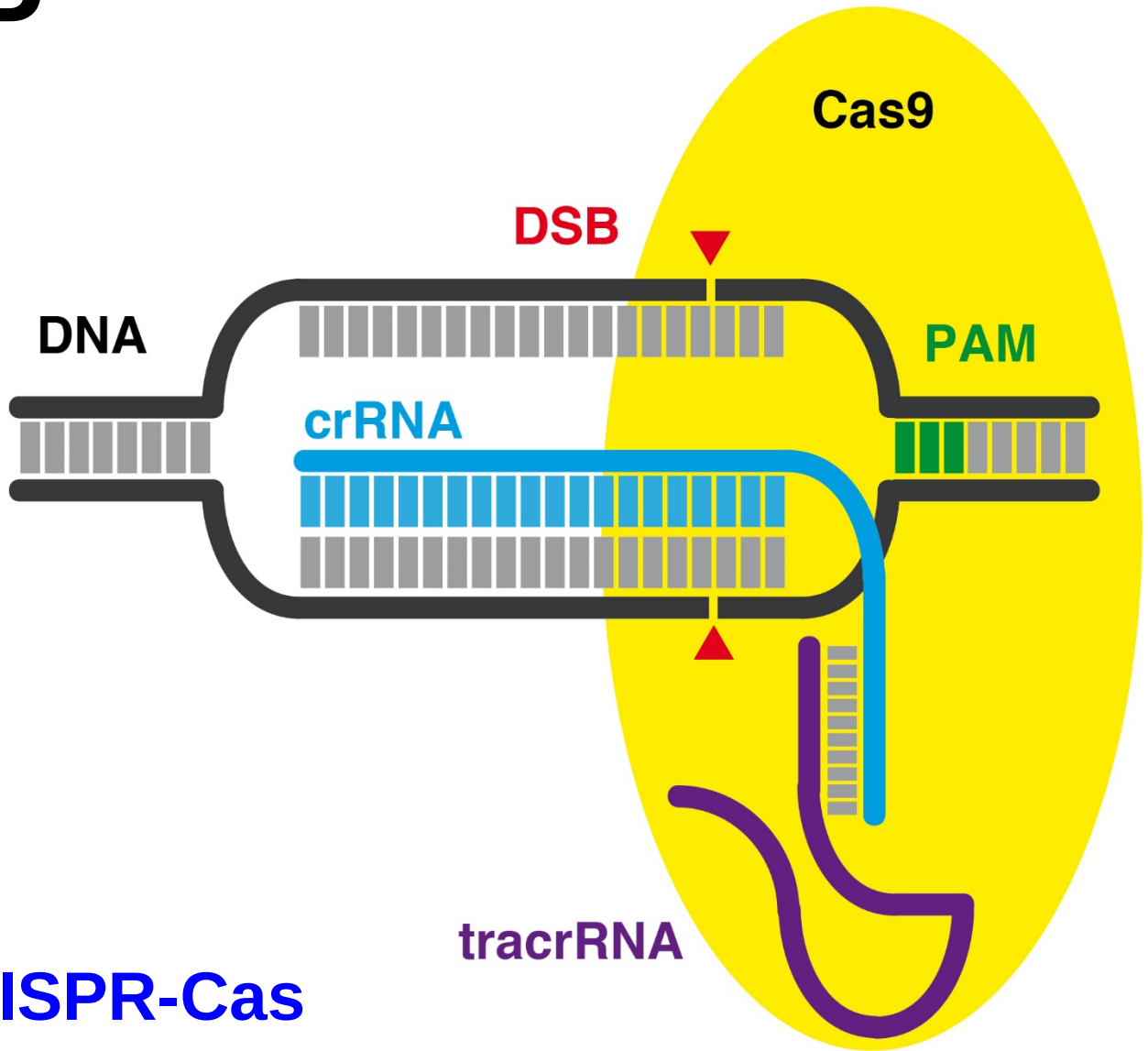
Jennifer Doudna
Emmanuelle Charpentier

Doudna & Charpentier (2014) Science

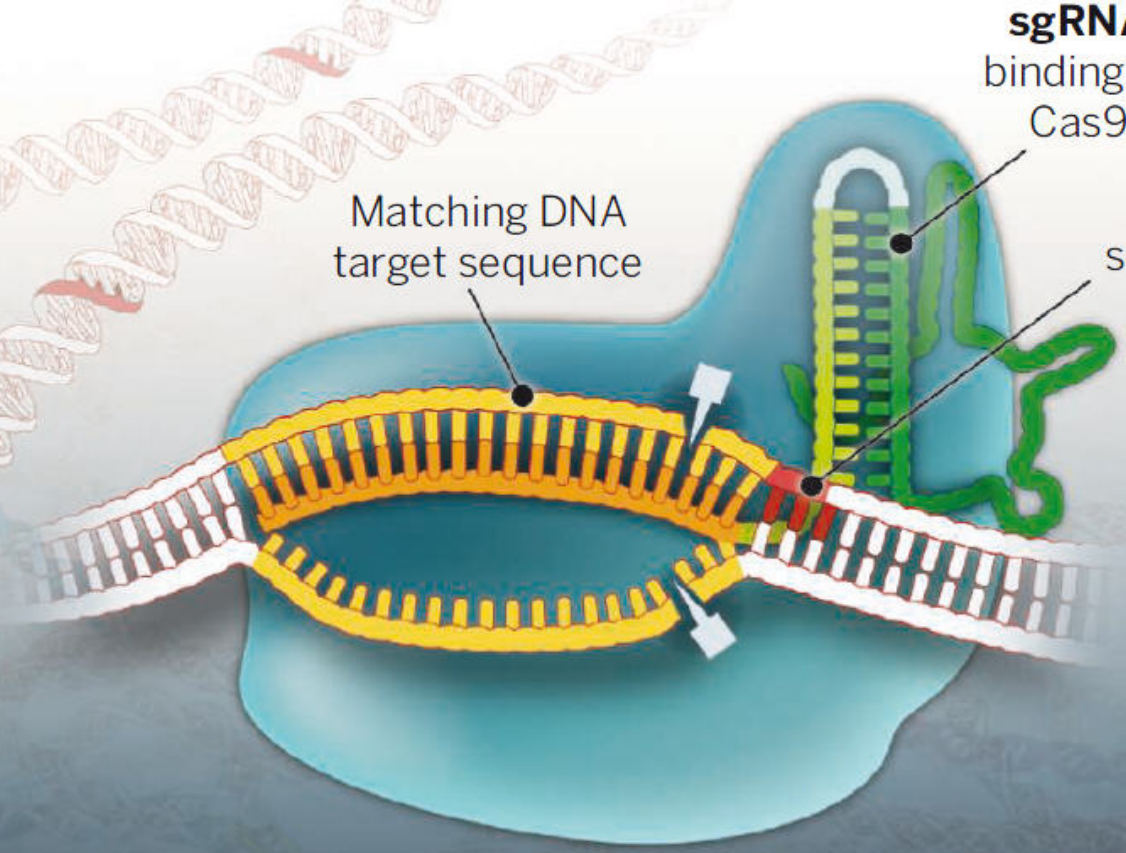


The CRISPR-Cas system

RNP



The CRISPR-Cas system



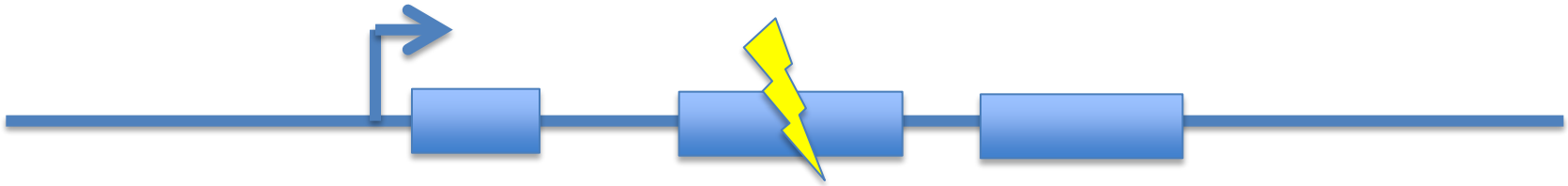
CRISPR-Cas9 development

- DNA deletion
- DNA insertion
- DNA replacement
- DNA modification
- DNA labeling
- Transcription modulation
- RNA targeting
- . . .

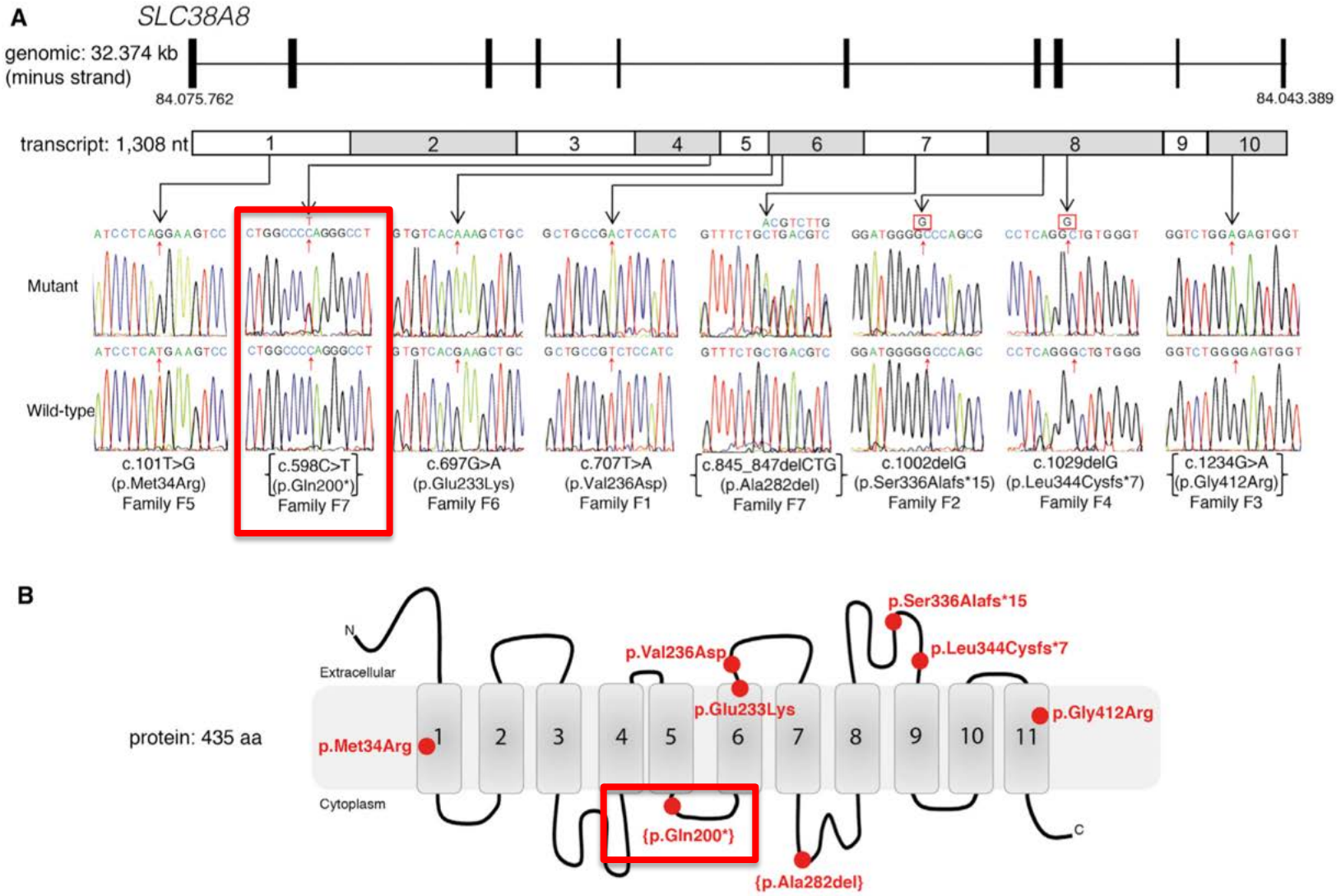
CRISPR-Cas9 applications

- Biological research
- Research and development
- Human medicine
- Biotechnology
- Agriculture
- . . .

Disrupting a gene: KO



- The easiest approach
- almost trivial nowadays
- Many similar alleles will be generated
- Efficient protocol

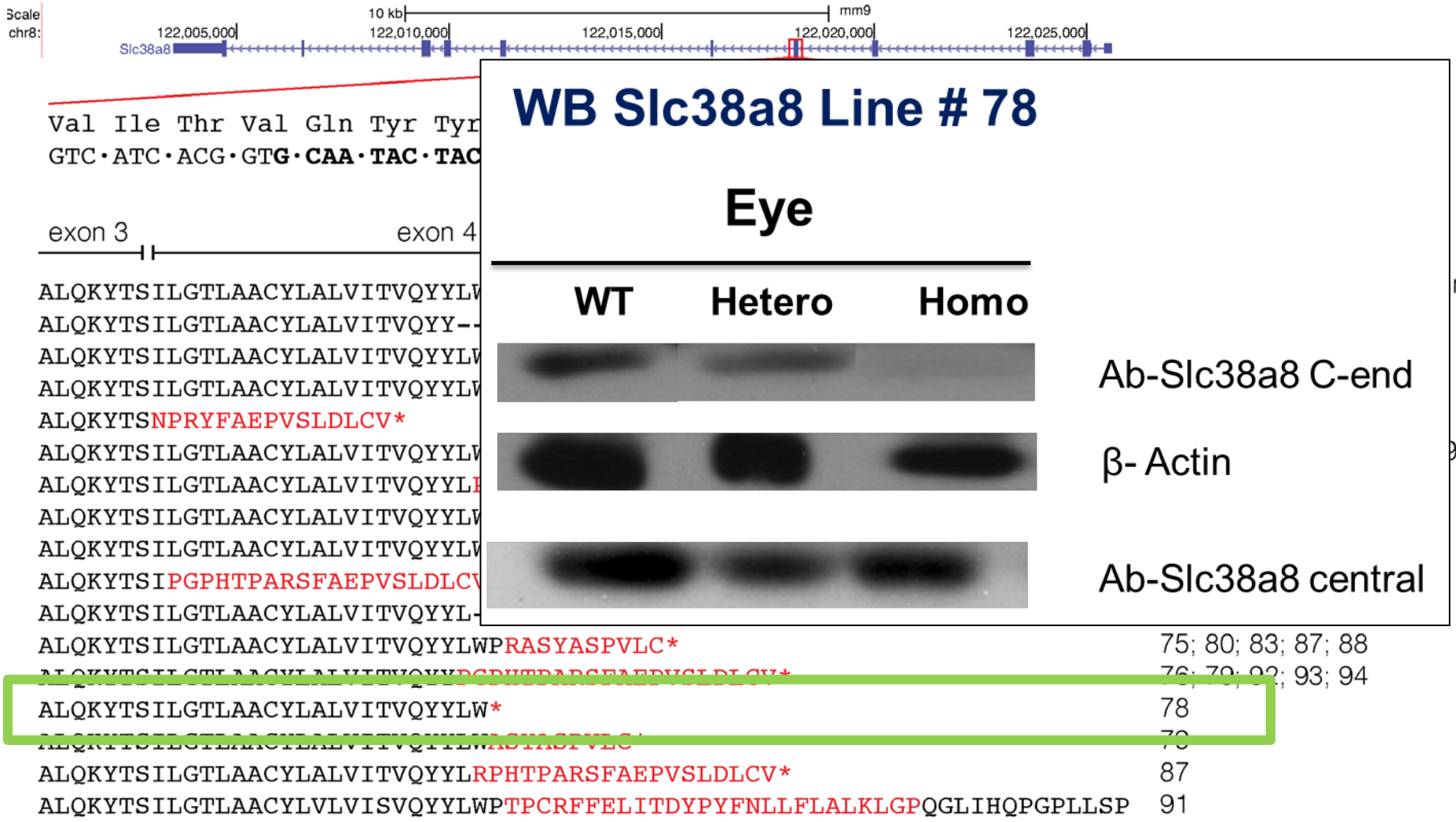


Mouse model of FHONDA

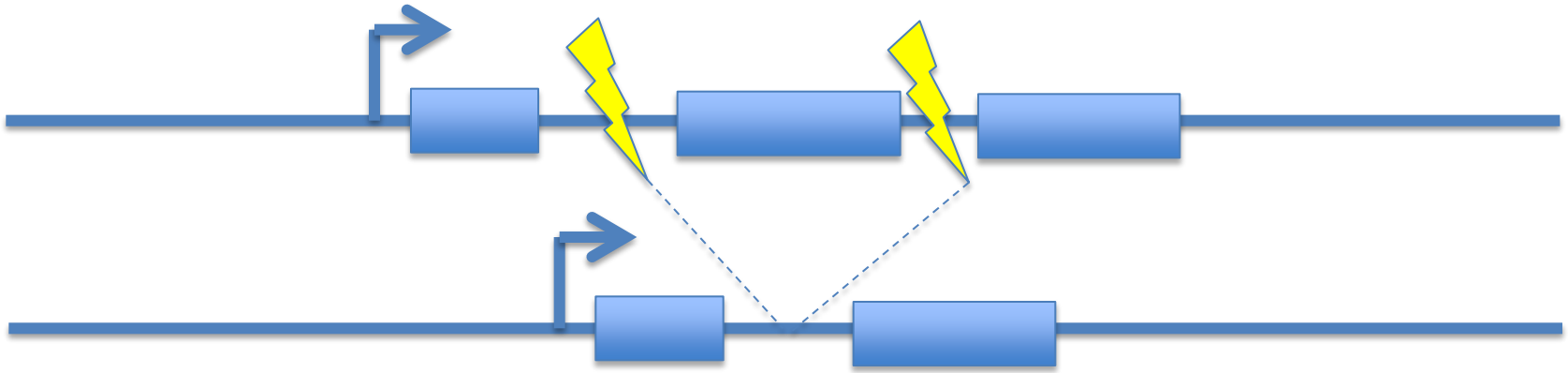
We set to reproduce the Gln200* in mice using CRISPR-Cas9

Mouse model of FHONDA (*Slc38a8*)

Prediction of protein sequence after mutagenesis: 16 different proteins



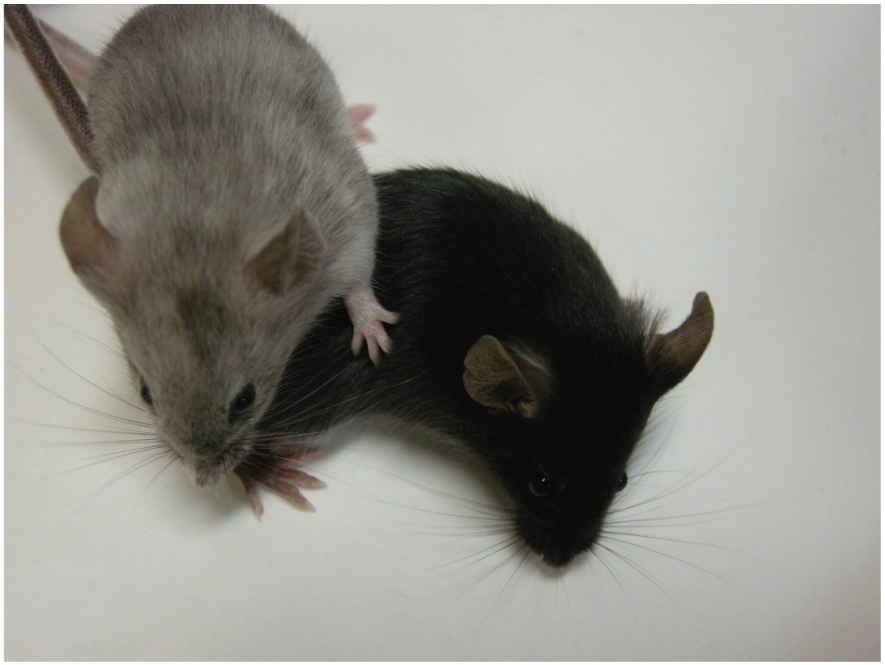
Deletions

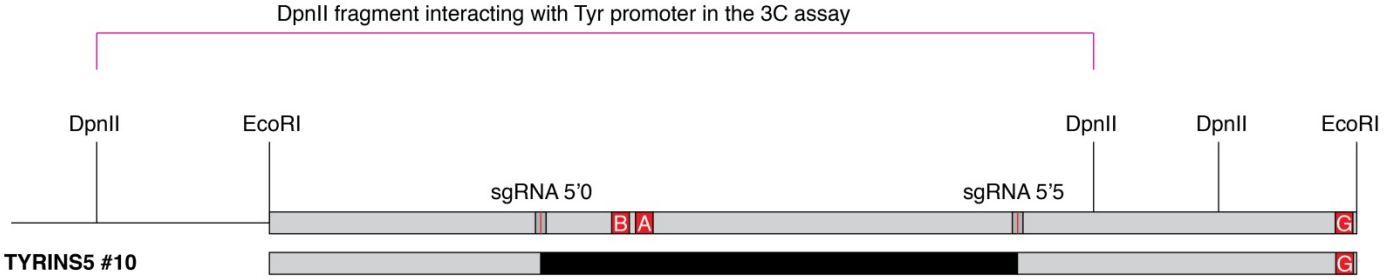


- Relatively straight forward, many alleles will be generated
- INDELs at both targeting sites will be **also** generated
- Consider **inversions** can also be generated
- Efficient protocol

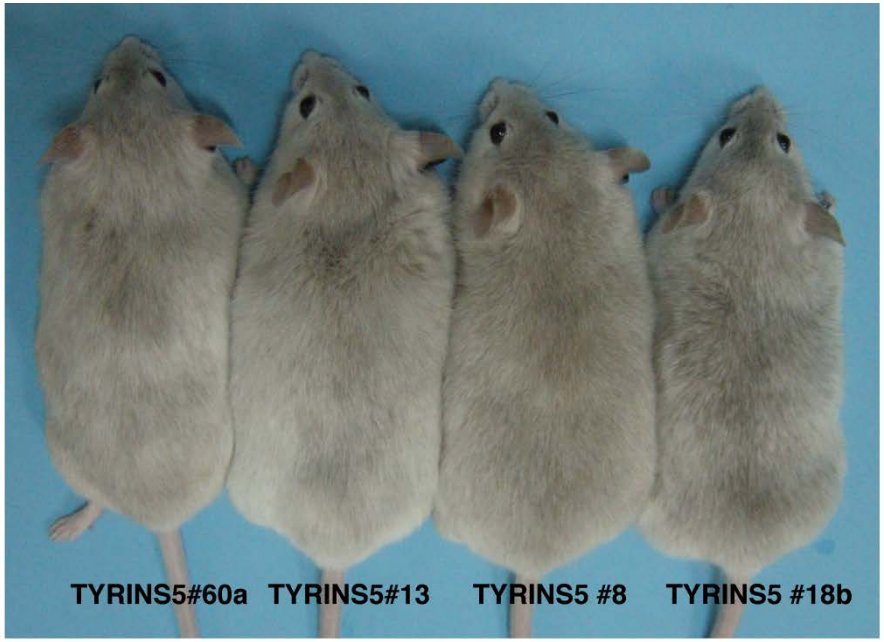
Deleting *Tyr* 5’ boundary with CRISPRs *in vivo*

Founder mosaic mice with clear coat colour pigmentation phenotype carrying BIALLELIC deletion of *Tyr* 5’ boundary

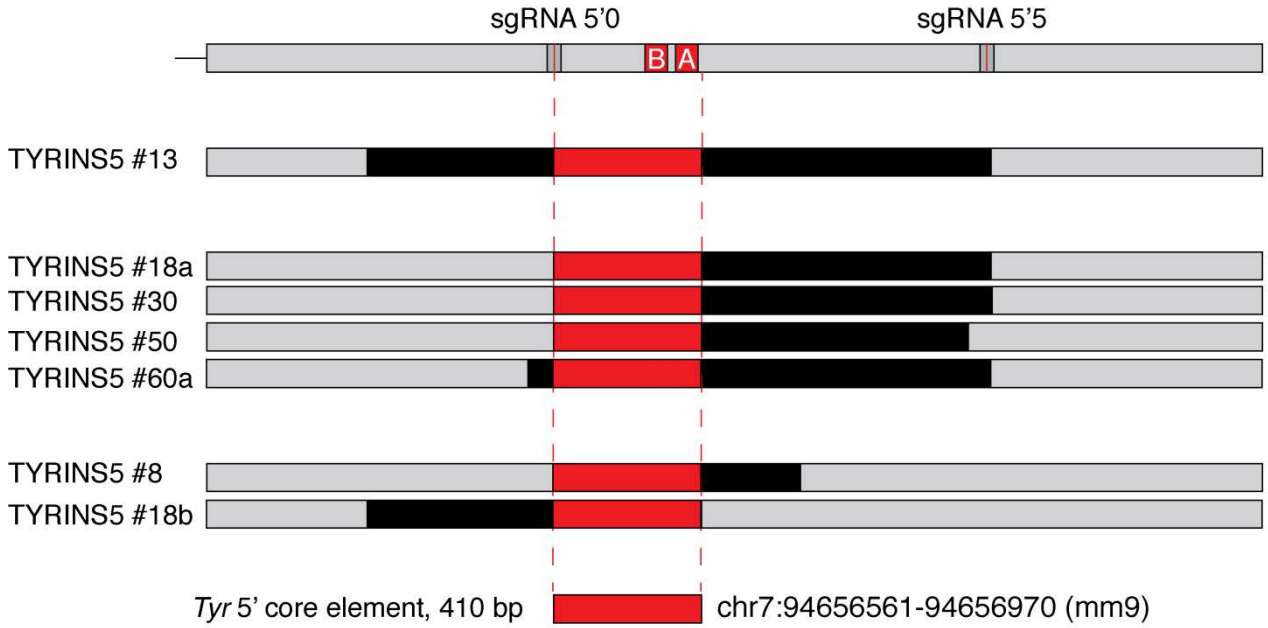





```
reference:    CAAGAATTAAGTGTGACAGTGCAAGATAACAGGAAAATAA......1170bp........CTCTAGGCCAAAATTGCCTAGTTTTATCACTACAAAACTT
TYRINS5#18_1: CAAGAATTAAGTGTGACAGTGCAAGAT-------------......1170bp.....------------TTGCCTAGTTTTATCACTACAAAACTT
TYRINS5#18_1: CAAGAATTAAGTGTGACAGTGCAAGAT-------------......1170bp.....------------TTGCCTAGTTTTATCACTACAAAACTT
```



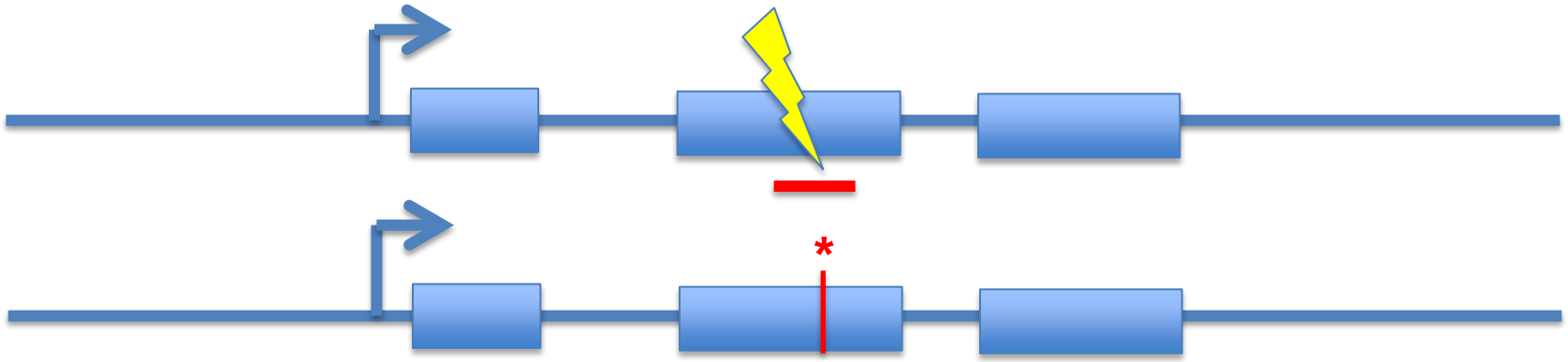
Comparing different *Tyr* 5' Boundary targeted alleles with similar phenotypes reveals the location of the functionally relevant endogenous regulatory DNA sequences



Genetic analysis now possible in mice!

Seruggia et al. 2015 NAR

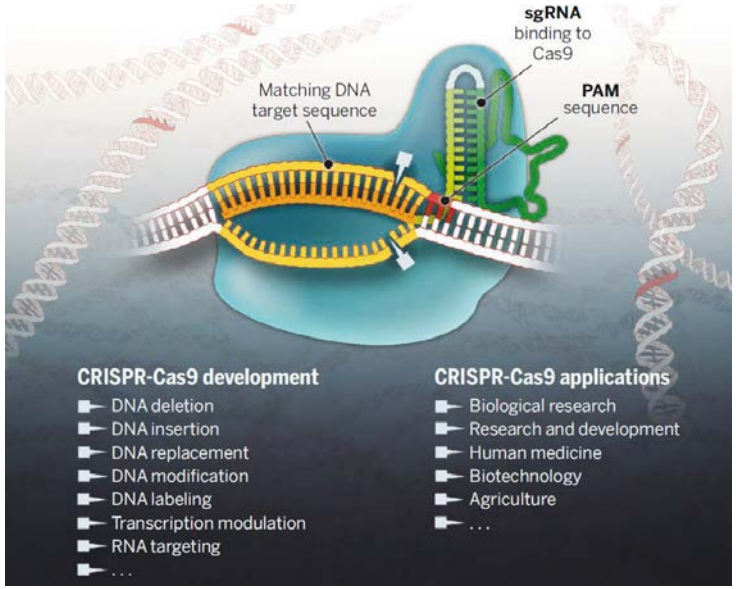
Point mutations



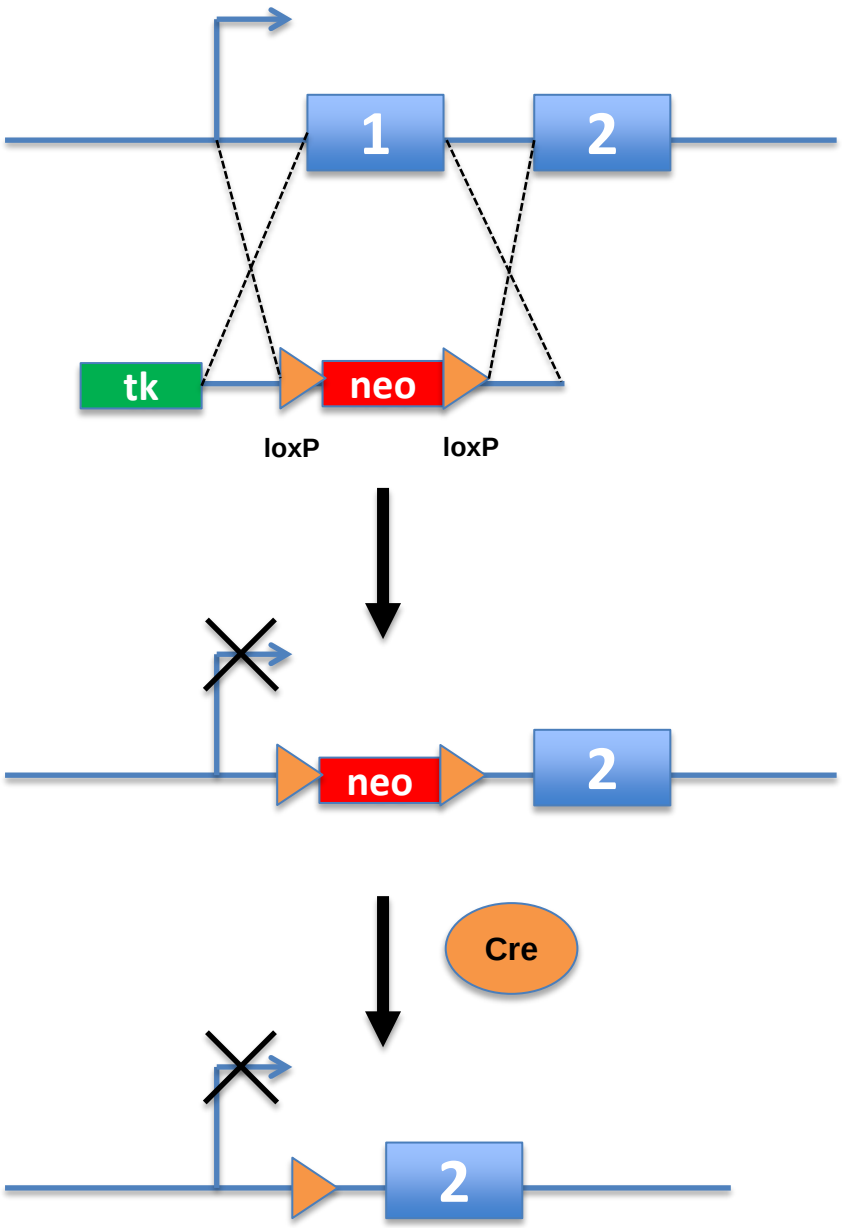
- Relatively straight forward, but can be challenging, many alleles will be generated
- INDELs will mostly be **also** generated
- Relatively doable protocol (however, if expected mutation not found, the number of embryos/animals to be used rapidly increases)
- Optimally: must have screening protocols in place (silent mutations/RFLPs/sequencing…)

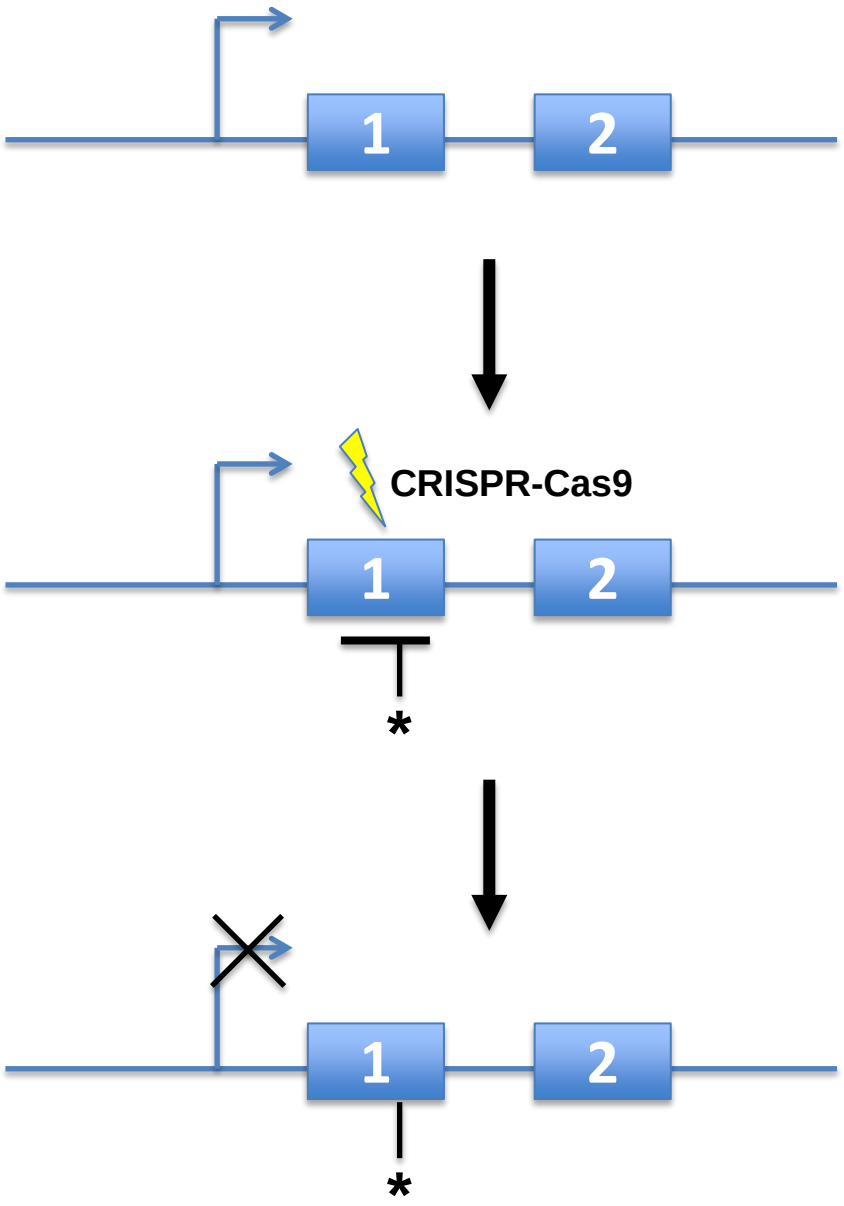
AVATAR CRISPR mice

- Easier approach to reproduce human mutations in animal models



“Classical” versus CRISPR-mediated mutagenesis





New specific mouse models of OCA4

Patients with oculocutaneous albinism type 4 (OCA4)

Mutation: c.986delC (*SLC45A2*) homozygous

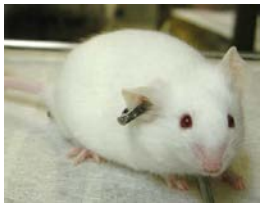
Celia de Lara

Gene edited Oca4 (*Slc45a2*) mice

OCA4 avatar mouse model

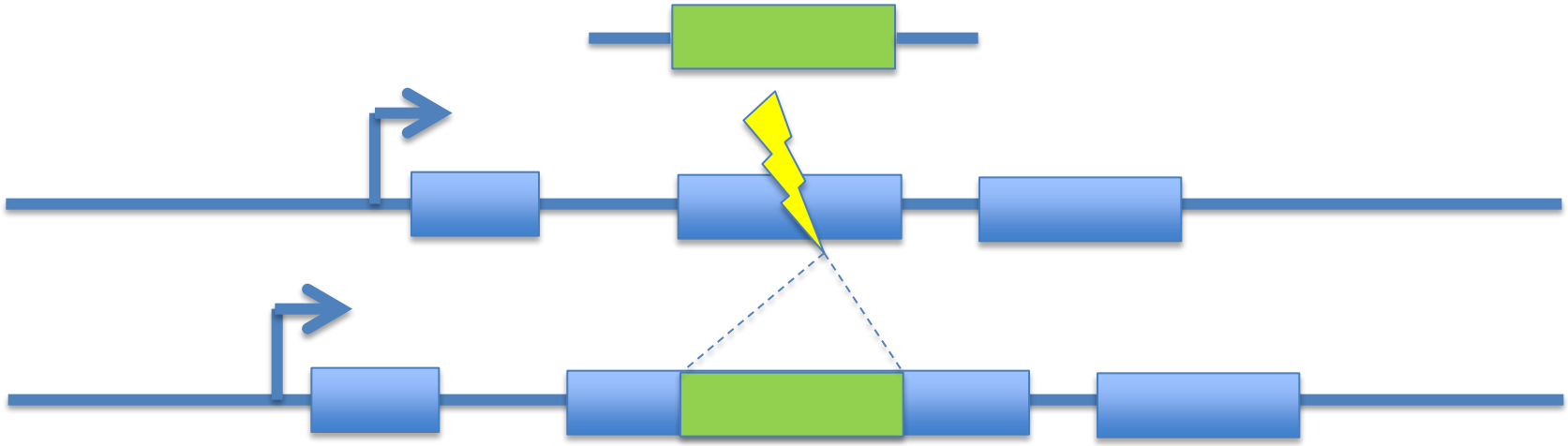
Mutation: c.986delC (*SLC45A2*)

```
Wt:   GTAAACATGCCTTCCCATTATCGCTGCCTTTGCGTCAGCCACCTGATTGGATGGACTGCCTTCCTGTCAAACATG
Mu:   GTAAACATGCCTTCCCATTATCGCTGCCTTTGCGTCAGCCACCTGATCGGATGGA-TGCCTTCCTGTCAAACATG
63:   GTAAACATGCCTTCCCATTATCGCTGCCTTTGCGTCAGCCACCTGATTGGATGGACTGCCTTCCTGTCAAACATG
      GTAAACATGCCTTCCCATTATCGCTGCCTTTGCGTCAGCCACCTGATTGGATGGACTGCCTTCCTGTCAAACATG
64:   GTAAACATGCCTTCCCATTATCGCTGCCTT--------------------------------CCTGTCAAACATG
      GTAAACATGCCTTCCCATTATCGCTGCCTT--------------------------------CCTGTCAAACATG
65:   GTAAACATGCCTTCCCATTATCGCTGCCTTTGCGTCAGCCACCTGATTGGATGGACTGCCTTCCTGTCAAACATG
      GTAAACATGCCTTCCCATTATCGCTGCCTTTGCGTCAAACA----------TGGATGGACTGCCTTCCTGTCAAACATG
      GTAAACATGCCTTCCCACTATCGCTGCCTTTGCGTCAGCCACCTGATTGGATGGACTGCCTTCCTGTCAAACATG
66:   GTAAACATGCCTTCCCATTATCGCTGCCTTTGCGTCAGCCACCTGATTGGATGGACTGCCTTCCTGTCAAACATG
      GTAAACATGCCTTCCCATTATCGCTGCCTTTGCGTCAGCCACCTGATTGGATGGACTGCCTTCCTGTCAAACATG
67:   GTAAACATGCCTTCCCATTATCGCTGCCTTTGCGTCAGCCAG---GATTGGATGGACTGCCTTCCTGTCAAACATG
      GTAACCATGCCTTCCCATTATCGCTGCCTTTGCGTCAGCCAG---GATTGGATGGACTGCCTTCCTGTCAAACATG
68:   GTAAACATGCCTTCCCATTATCGCTGCCCTTGCGTCAGCCAC-TGATTGGATGGACTGCCTTCCTGTCAAACATG
      GTAAACATGCCTTCCCATTATCGCTGCCTTTGCGTCAGCCACCTGATTGGATGGACTGCCTTCCTGTCAAACATG
      GTAAACATGCCTTCCCATTATCGCTGCCTTTGCGTCAGCCAC-TGATTGGATGGACTGCCTTCCTGTCAAACATG
      GTAAACATGCCTTCCCATTATCGCTGCCTTTGCGTCAGCCACCTGATCGGATGGA-TGCCTTCCTGTCAAACATG
69:   GTAAACATGCCTTCCCATTATCGCTGCCTTTGCGTCAGCCACCTTGATTGGATGGACTGCCTTCCTGTCAAACATG
      GTAAACATGCCTTCCCATTATCGCTGCCTTTGCGTCAGCCACCTGATTGGATGGACTGCCTTCCTGTCAAACATG
      GTAAACATGCCTTCCCATTATCGCTGCCTTTGCGTCA---------TTGGATGGACTGCCTTCCTGTCAAACATG
70:   GTAAACATGCCTTCCCATTATCGCTGCCTTTGCGTCAGCCACCTGATTGGATGGACTGCCTTCCTGTCAAACATG
      GTAAACATGCCTTCCCATTATCGCTGCCTTTGCGTCAGCCACCTTGATTGGATGGACTGCCTTCCTGTCAAACATG
71:   GTAAACATGCCTTCCCATTATCGCTGCCTTTG-----------------GATGGACTGCCTTCCTGTCAAACATG
      GTAAACATGCCTTCCCATTATCGCTGCCTTTG-----------------GATGGACTGCCTTCCTGTCAAACATG
R1:   GTAAACATGCCTTCCCATTATCGCTGCCTTTGCGTCAAACATGCTCTTCCCATTATCGCTGCC---------TTGGATGGACTGCCTTCCTGTCAAACATG
```



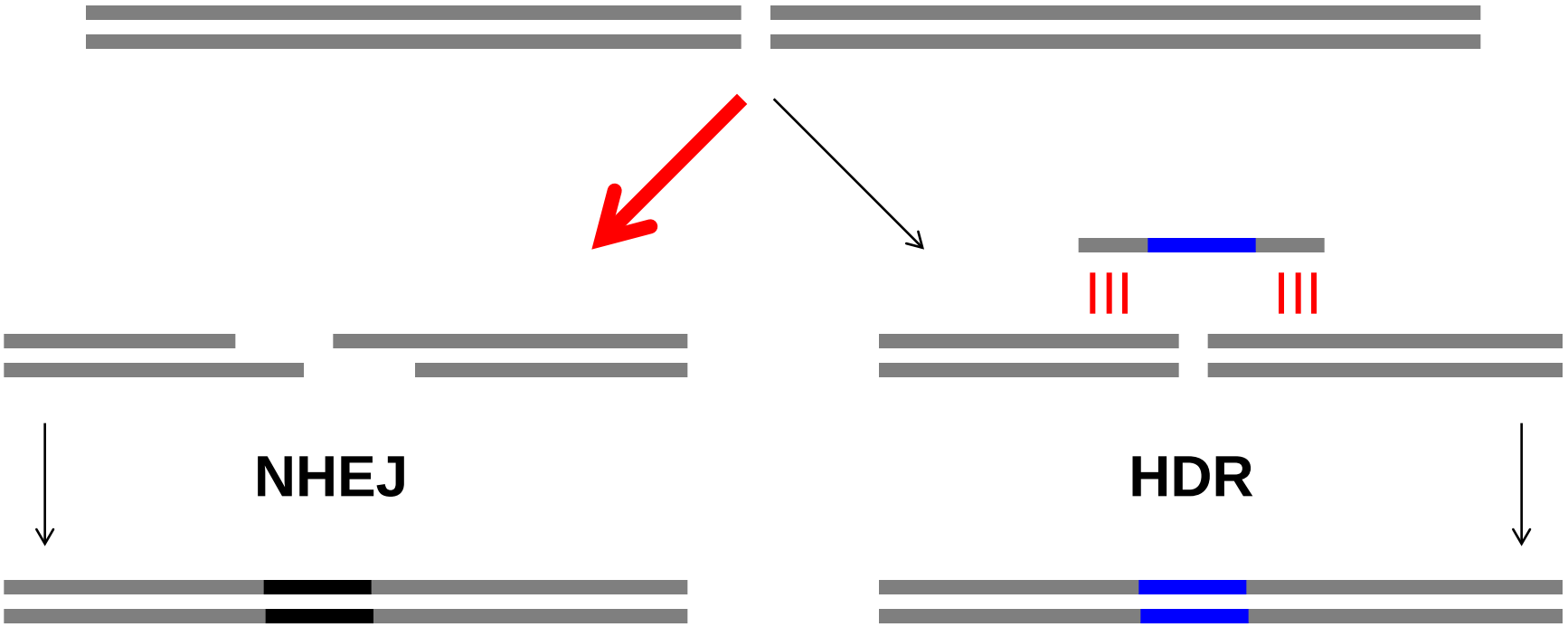
Celia de Lara

Knock-ins



- Most challenging approach, many protocols, not really optimized
- Useful for small (loxP) and larger (EGFP) insertions
- INDELs will mostly be **also** generated
- Relatively in-efficient approach
- Must have screening protocols in place (PCR/RFLPs/sequencing…)

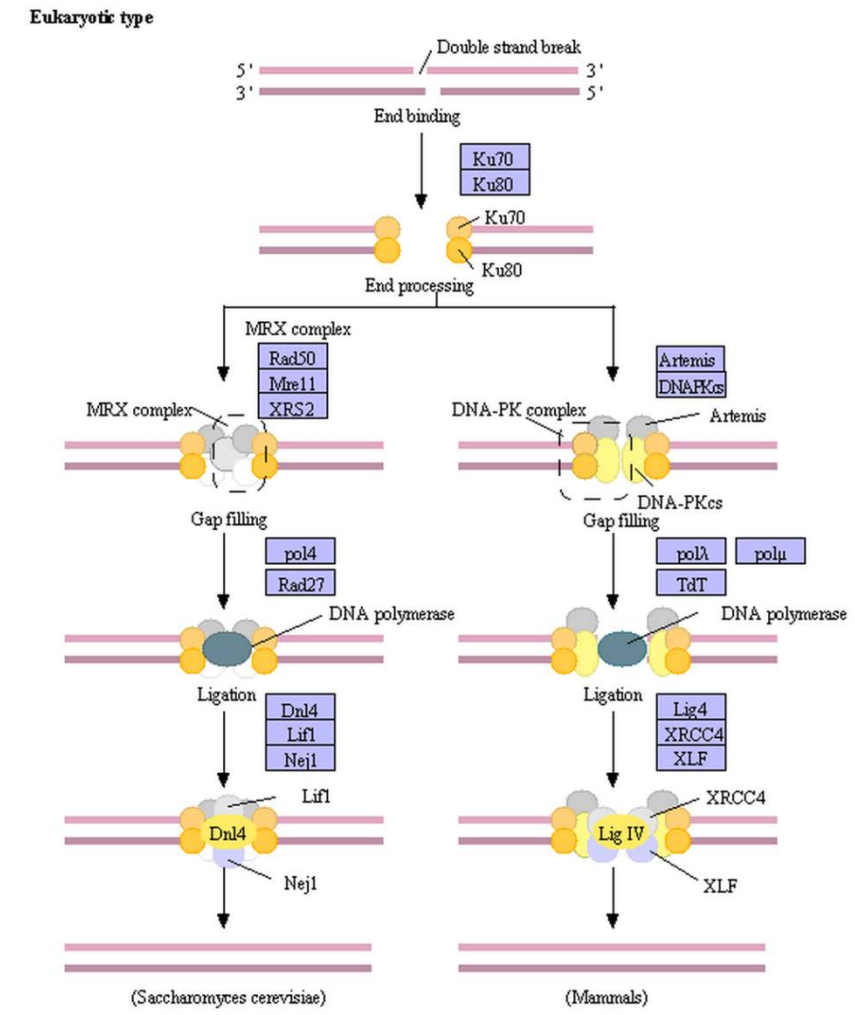
Improving Gene Edition



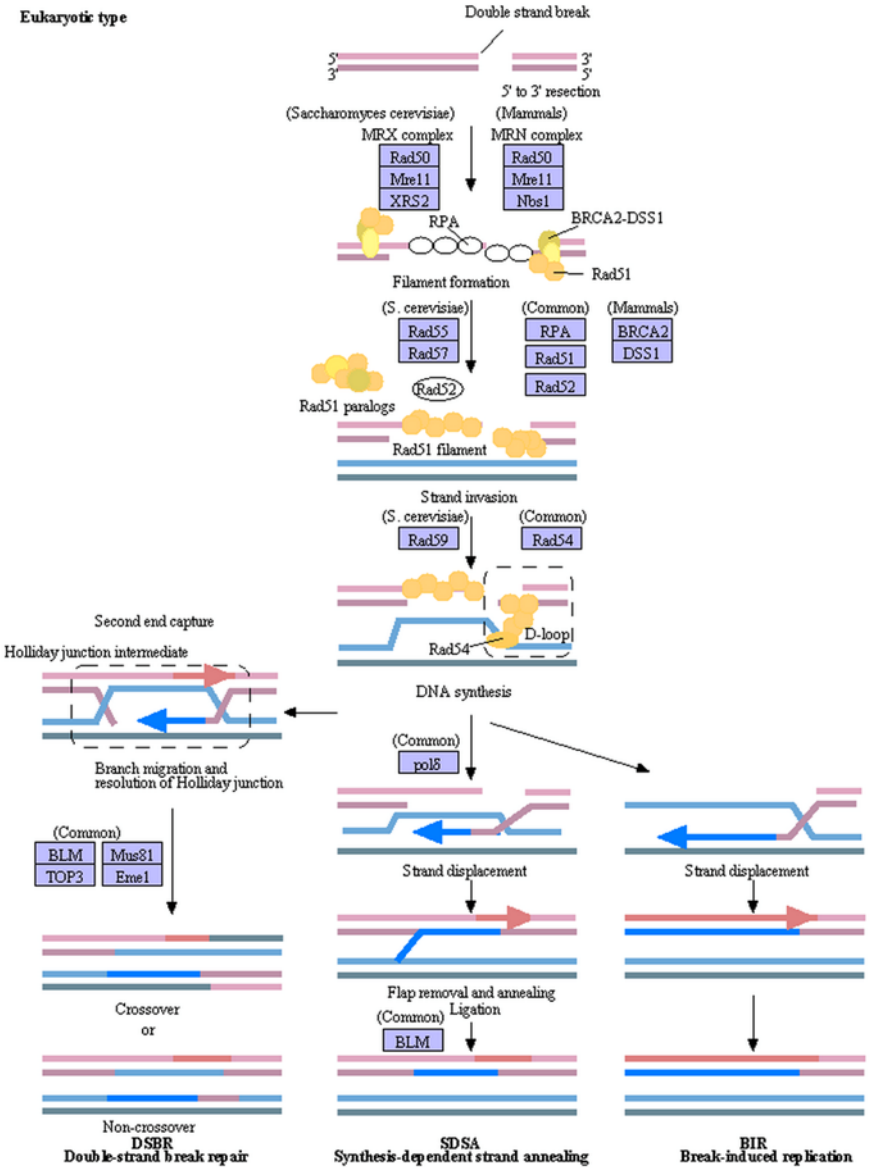
INDELs – Gene disruption

Gene repair / edit

NHEJ



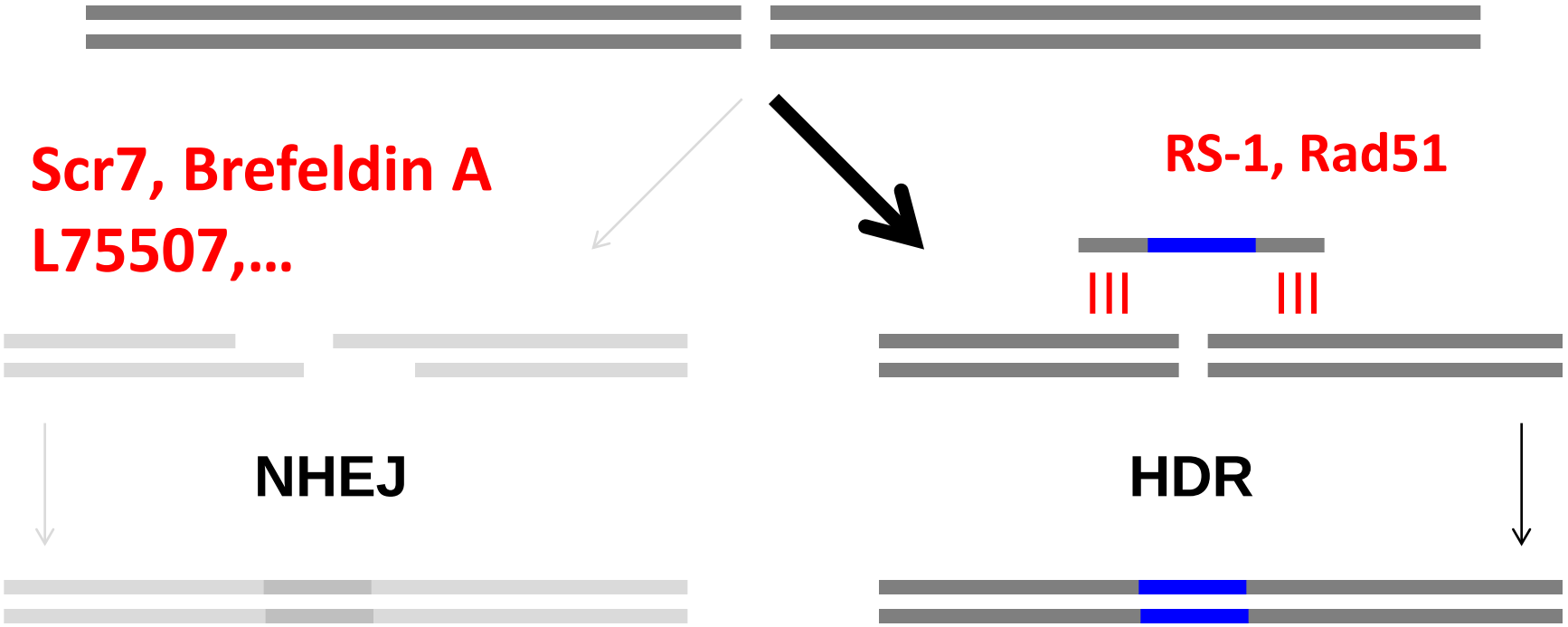
HDR



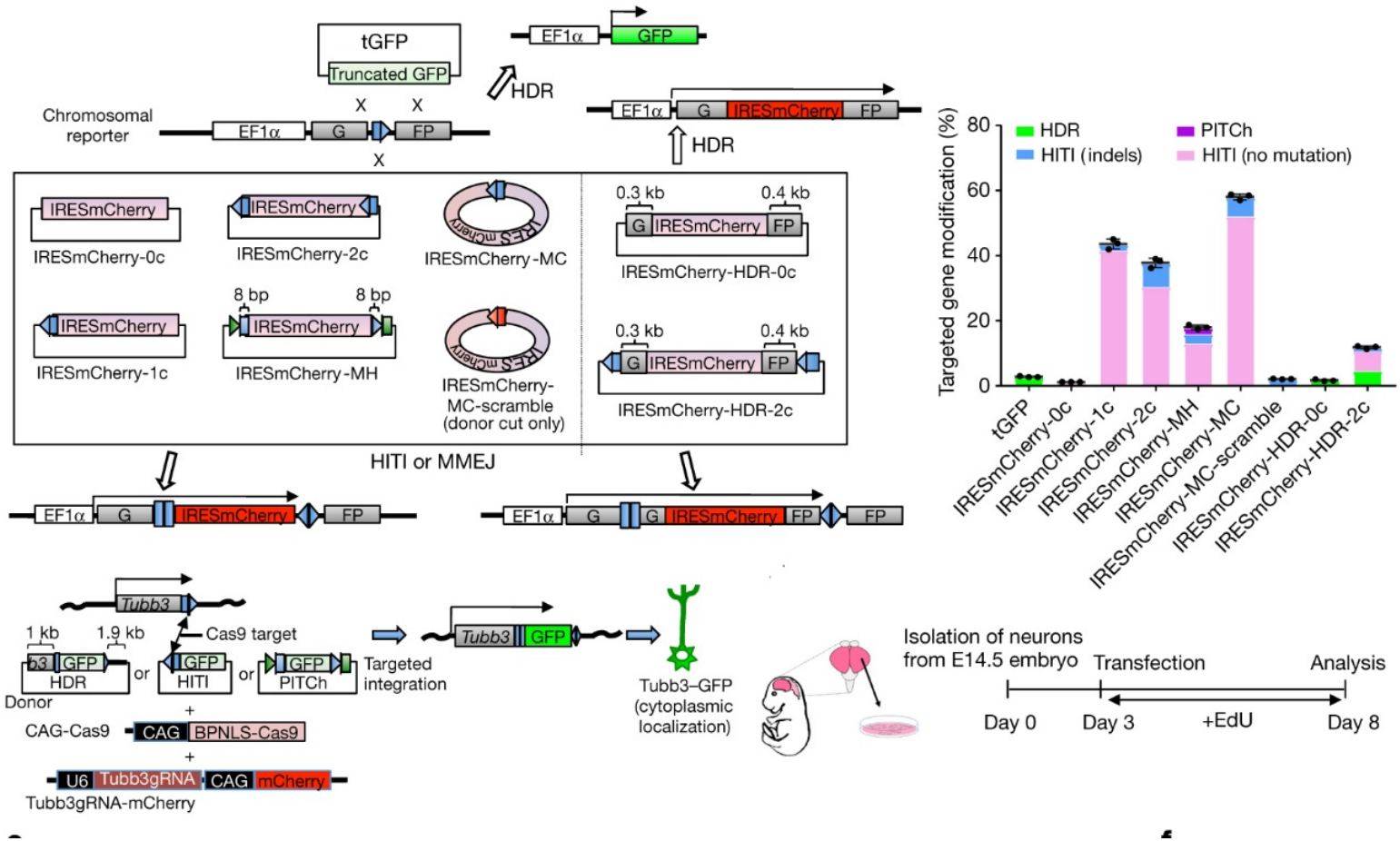
http://www.genome.jp/kegg-bin/show_pathway?ko03450

Using NHEJ inhibitors to boost HDR

Yu et al. Cell Stem Cell Feb 2015; Maruyama et al. Nat Biotech March 2015; Chu et al. Nat Biotech March 2015; Song et al. Nat. Comm. 2016

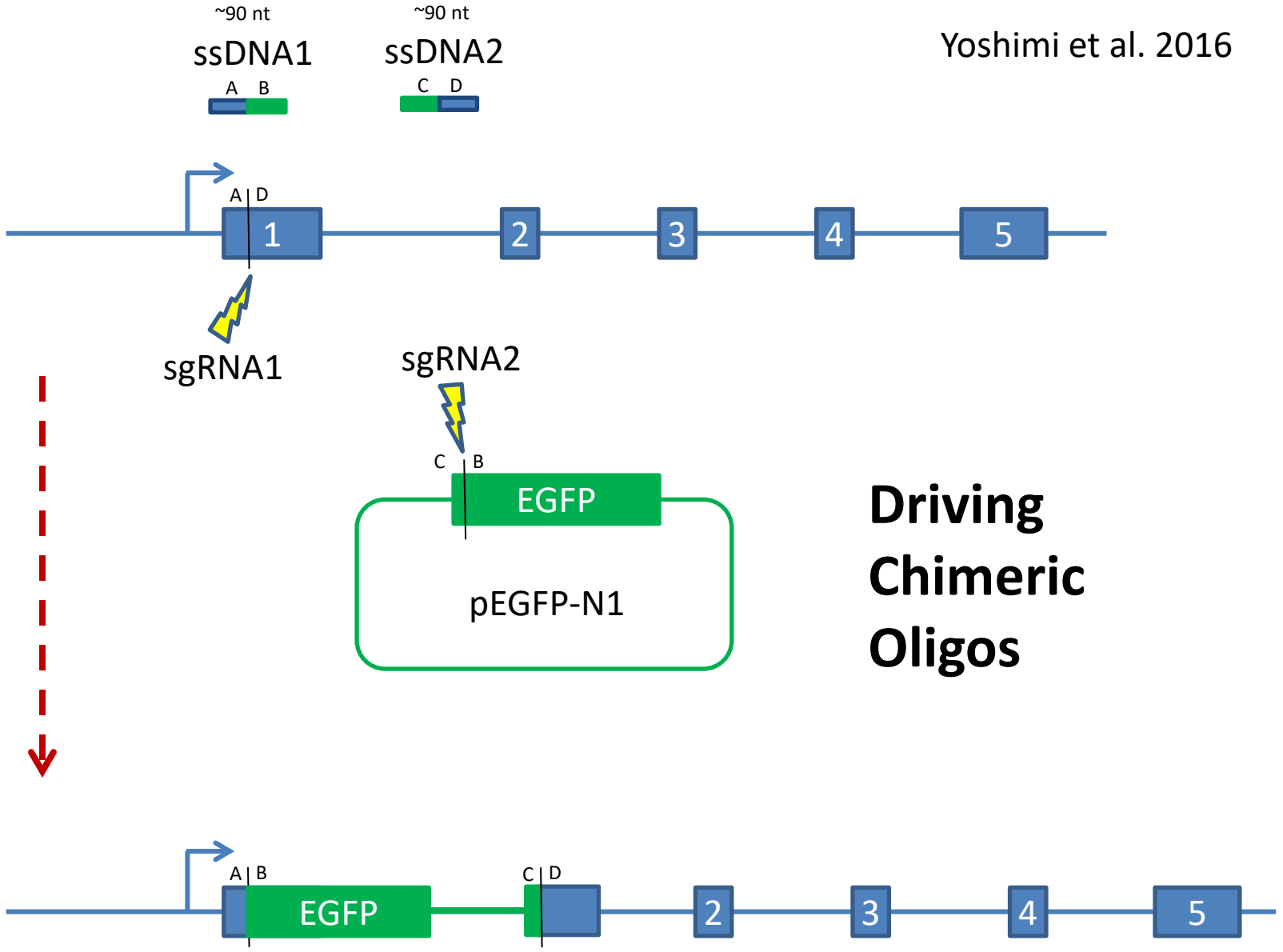


HITI: Homology-independent targeted integration

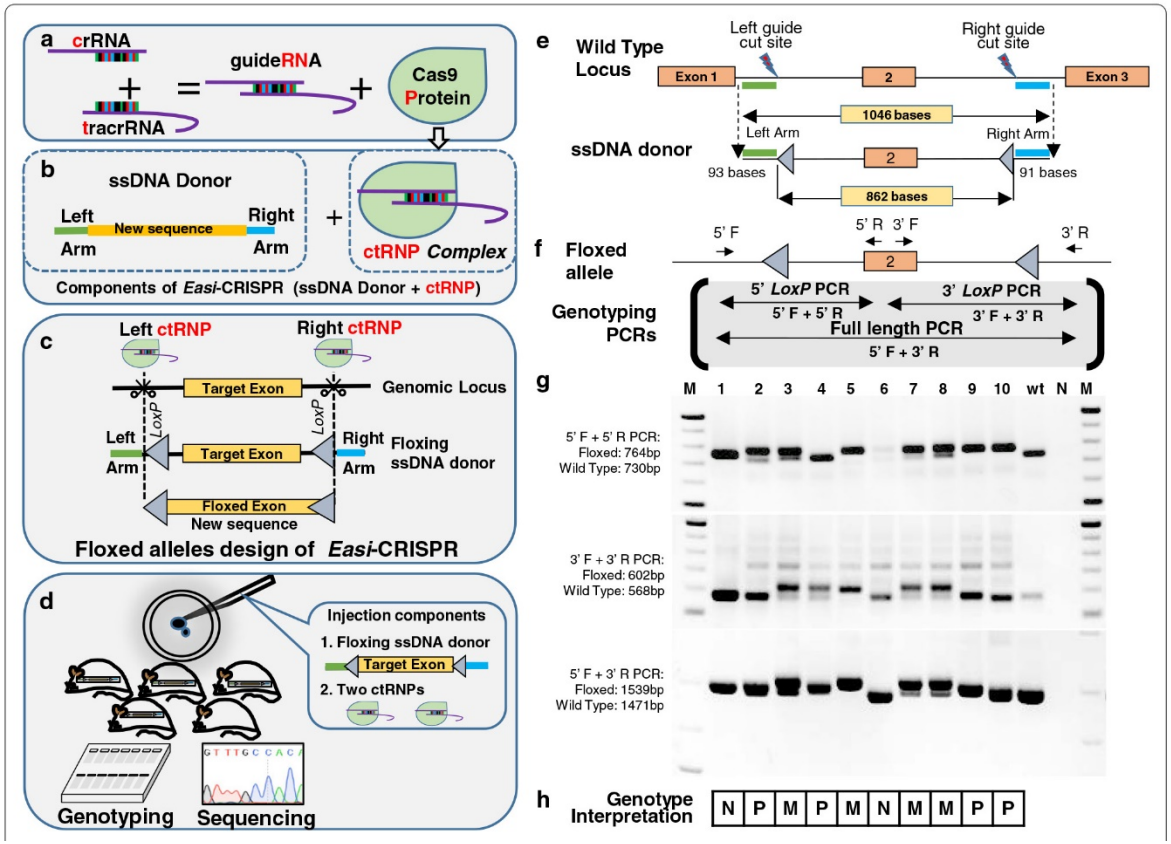


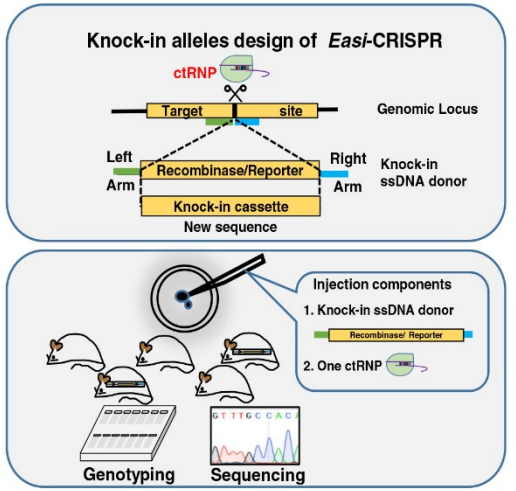
Suzuki et al. 2016 Nature

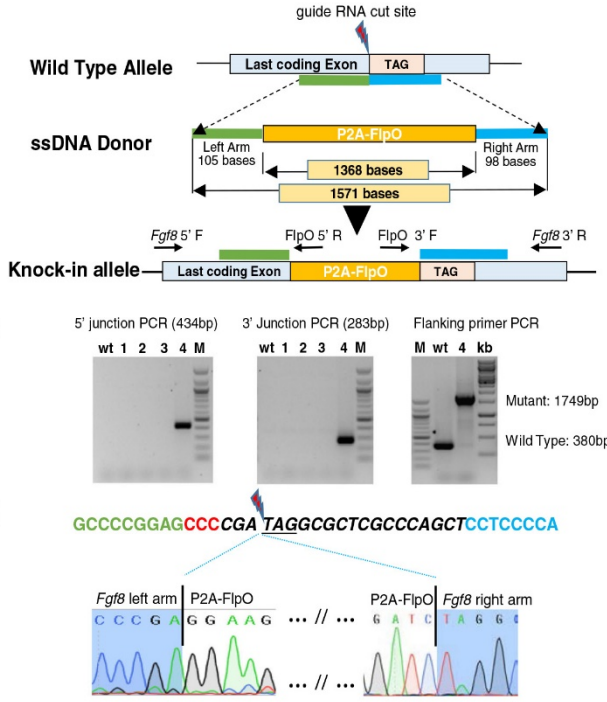
Alternative strategies for knock-in experiments: 2H2OP



Easi-CRISPR: improved knock-in approach







Combining RNPs + long ssDNA donor template

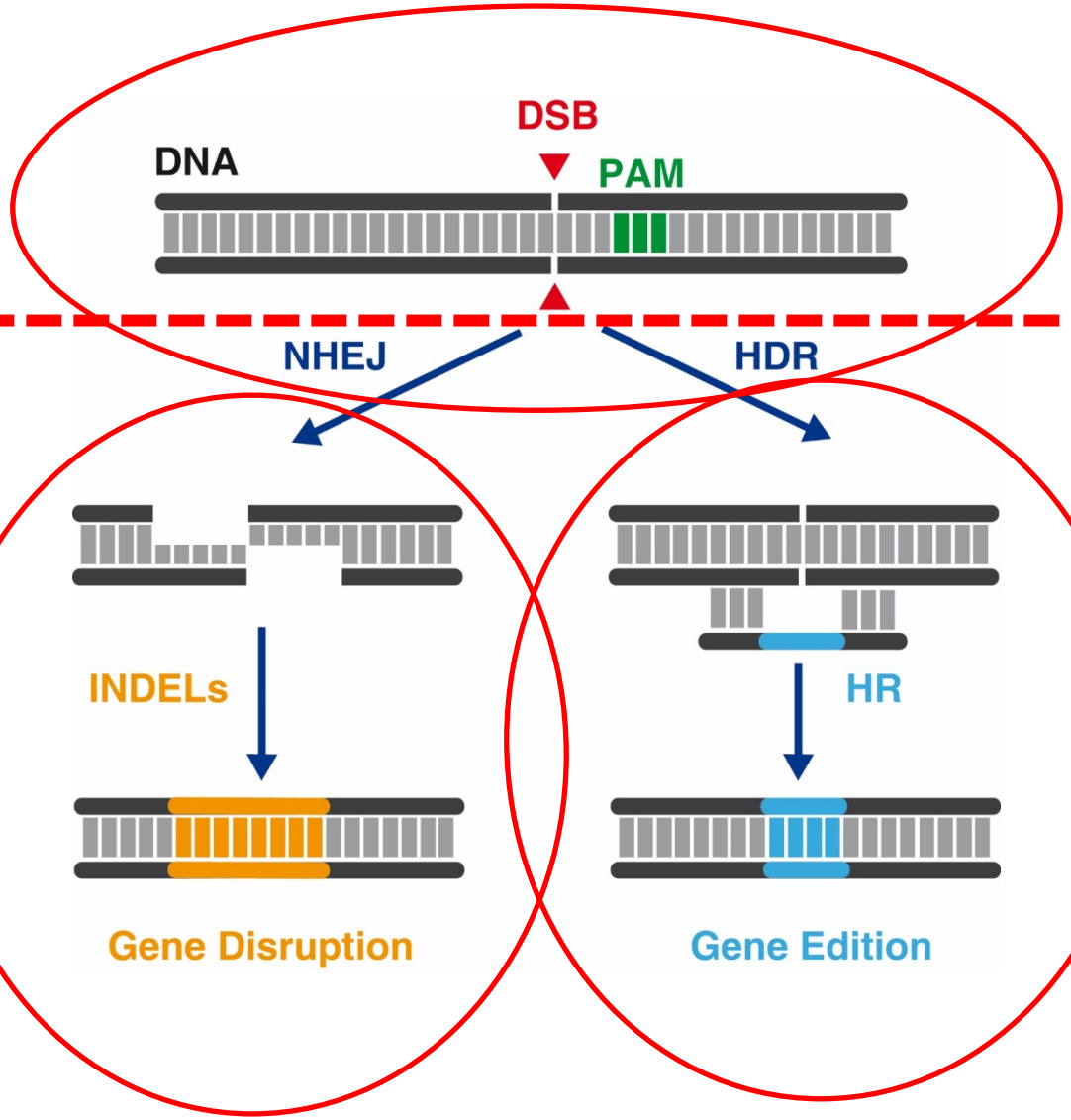
RNP = crRNA + tracrRNA + Cas9 protein

Quadros et al. 2017 Genome Biol.

Current limitations of CRISPR

Off-target effects

Related to CRISPR



Unrelated to CRISPR

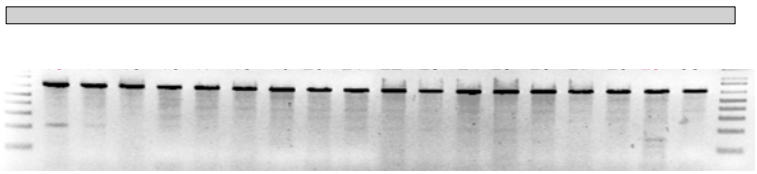
On-target effects
Mosaicism

HDR is not the preferred repairing pathway

Improved design of RNA guides for optimized CRISPR experiments

Oliveros *et al.* Nucleic Acids Res. 2016

What about off-targets?



Mostly observed in vitro

We have not found off-target sites with altered sequences in genome-edited mice

E

p

F

Off-target mutations are rare in Cas9-modified mice

Vivek Iyer, Bin Shen, Wensheng Zhang, Alex Hodgkins, Thomas Keane, Xingxu Huang & William C Skarnes

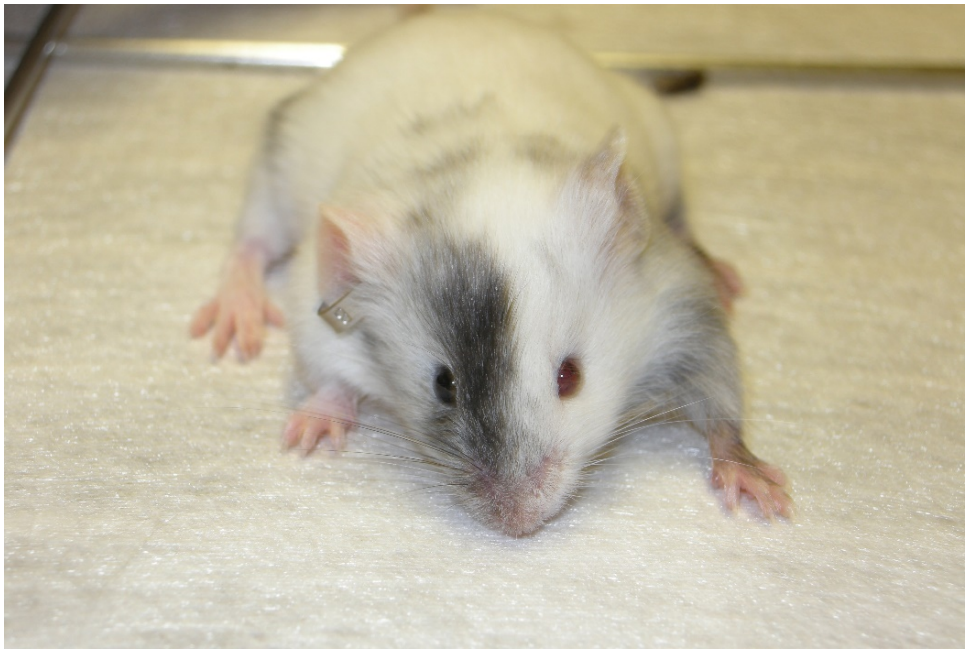
Affiliations | Corresponding authors

Nature Methods **12**, 479 (2015) | doi:10.1038/nmeth.3408
Published online 28 May 2015

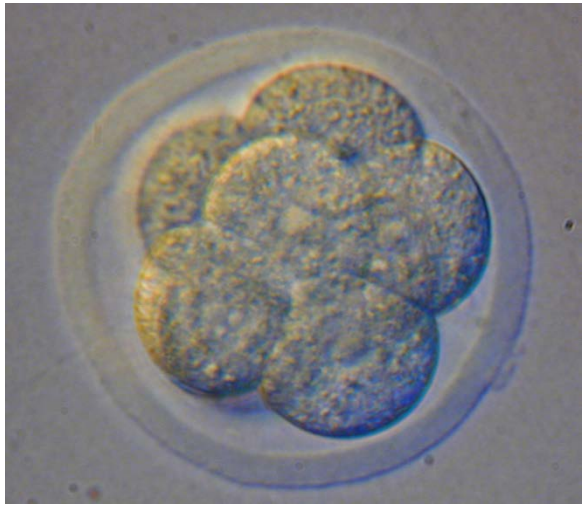
Confirmed by NGS

Seruggia et al. 2015 NAR

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
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B9040.3     AACACTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAG----------GTAGGGTTGATTTCAGGAAATGTAA
B9040.4     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACAGC-----AGGGTTGATTTCAGGAAATGTAA
B9040.5     CAAGCTCCTGCCCCACTTTCAAAGCTGTACTGAACTGCAGTTTCTTCTCCACCCAGATTCCTGCAAGACCTTGCACCGGGG-----------(437bp)----------------
B9040.6     ----------------------------------------(561bp)-----------------------------------------------------------------
B9041.1     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACAGCTGTTACCTAGGGTTGATTTCAGGAAATG
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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
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B9043.3     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTCCCAATCAGGAGTTGAGAAAAAT----------GGTAGGGTTGATTTCAGGAAGTGTAA
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B9045.2     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAAT----------GGTTGATTTCAGGAAATGTAA
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B9046.4     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACAGCACCTAGGTAGGGTTGATTACAGGAAATG
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B9064.4     AACATTGGAGGAGCTGCCACTGCTATTGGGGACCCACCAAATGTTATCATTGTTTCCAATCAGGAGTTGAGAAAAATGGTAGGTAACAGC-----AGGGTTGATTTCAGGAAATGTAA
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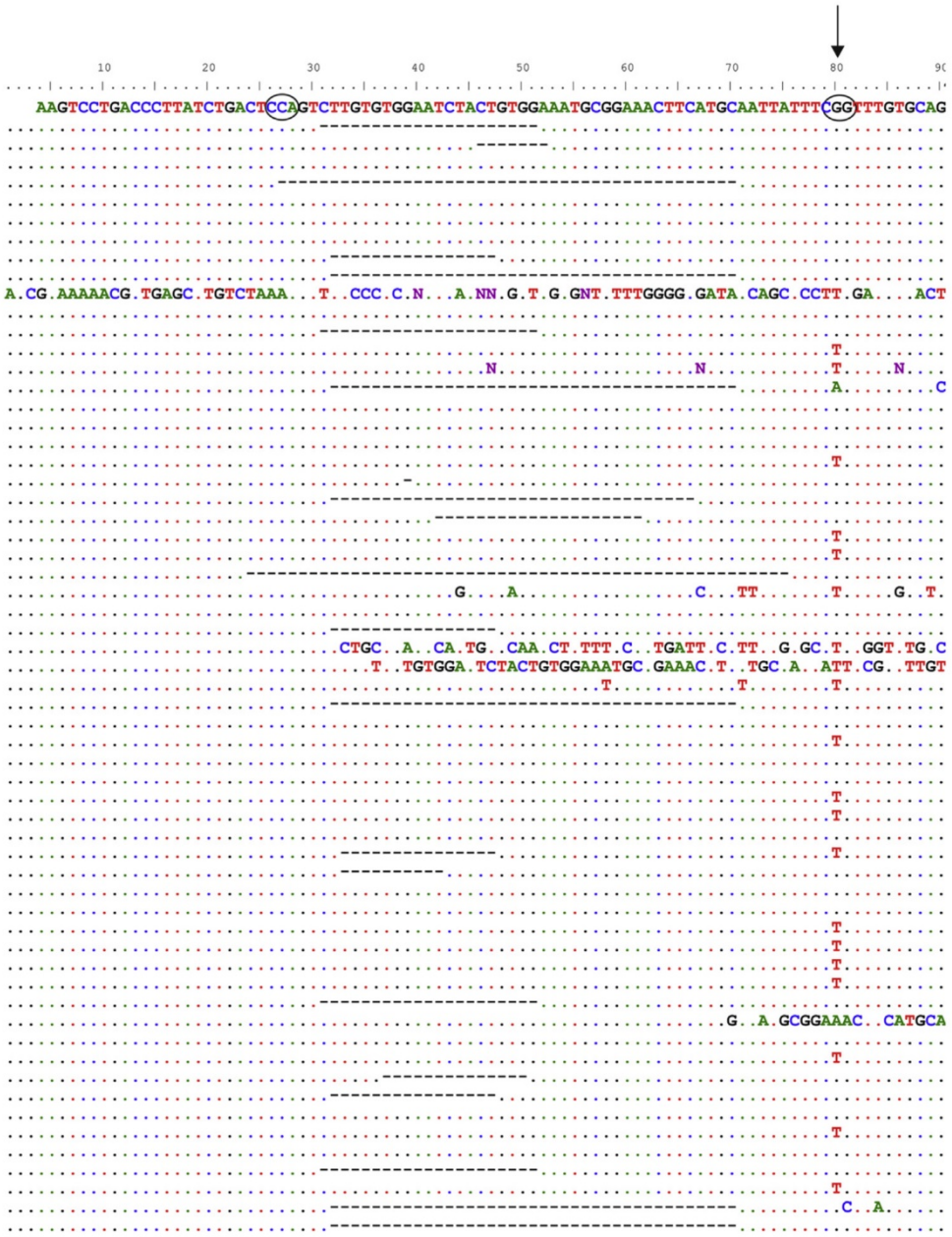


CRISPR-mediated gene therapy of a human rare disease: chronic granulomatous disease (CGD) in human iPS cells

Challenge: multiple alleles are generated

Flyn et al. (2015) Experimental Hematology

CRISPR-Cas is the future

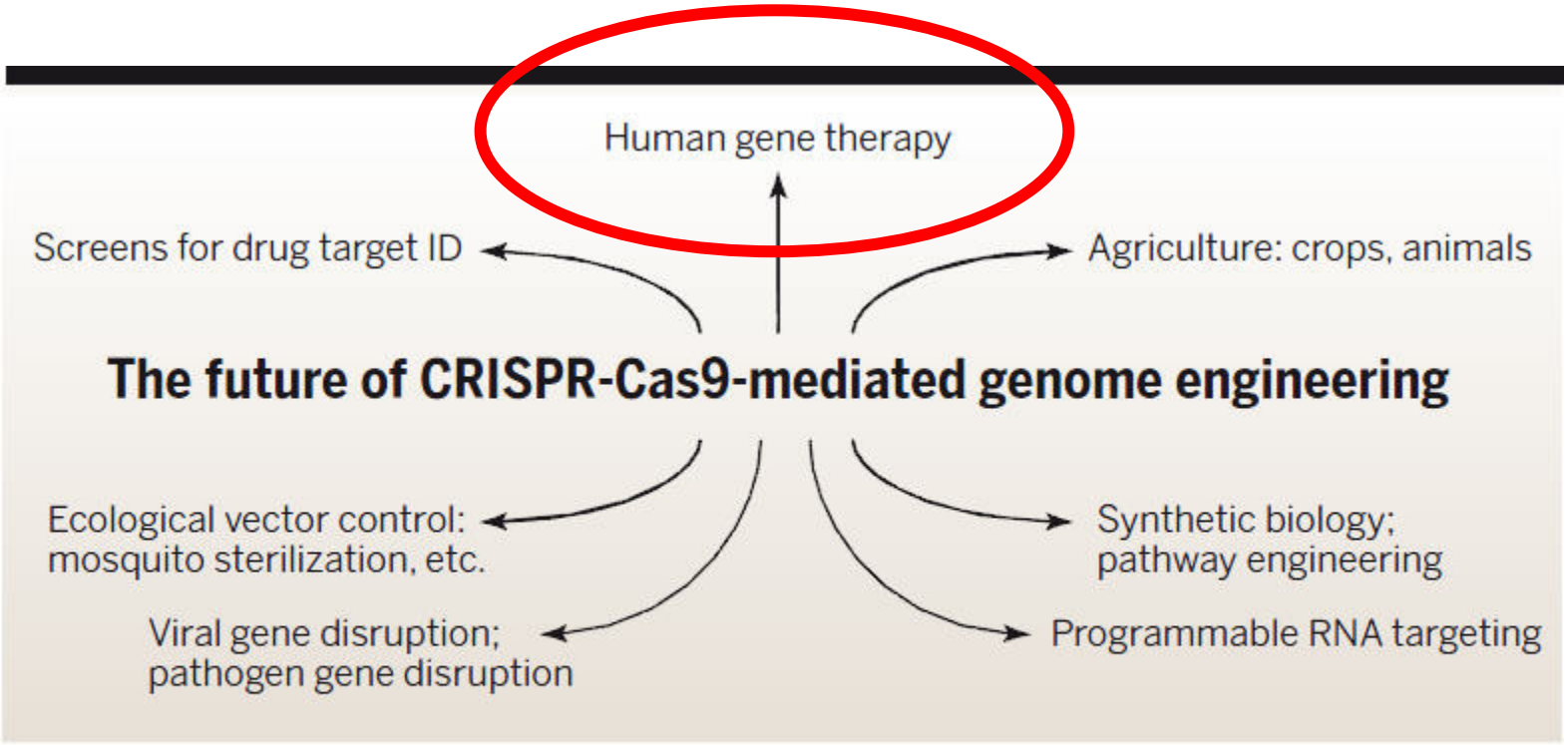


Fig. 6. Future applications in biomedicine and biotechnology. Potential developments include establishment of screens for target identification, human gene therapy by gene repair and gene disruption, gene disruption of viral sequences, and programmable RNA targeting.

Doudna & Charpentier (2014) *Science*

CRISPR-Cas9 and *in vivo* somatic gene therapy

Science REPORTS

Cite as: M. Tabebordbar *et al.*, *Science* 10.1126/science.aad5177 (2015).

In vivo gene editing in dystrophic mouse muscle and muscle stem cells

Mohammadsharif Tabebordbar,[1,2*] Kexian Zhu,[1,3*] Jason K. W. Cheng,[1] Wei Leong Chew,[2,4] Jeffrey J. Widrick,[5] Winston X. Yan,[6,7] Claire Maesner,[1] Elizabeth Y. Wu,[1†] Ru Xiao,[8] F. Ann Ran,[6,7] Le Cong,[6,7] Feng Zhang,[6,7] Luk H. Vandenberghe,[8] George M. Church,[4] Amy J. Wagers[1‡]

Science REPORTS

Cite as: C. Long *et al.*, *Science* 10.1126/science.aad5725 (2015).

Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy

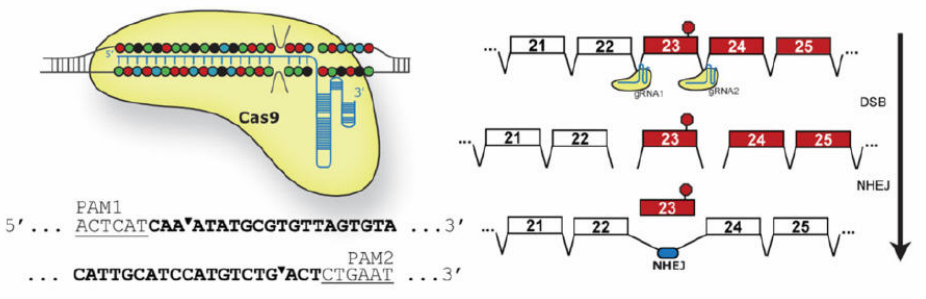
Chengzu Long,[1,2,3*] Leonela Amoasii,[1,2,3*] Alex A. Mireault,[1,2,3] John R. McAnally,[1,2,3] Hui Li,[1,2,3] Efrain Sanchez-Ortiz,[1,2,3] Samadrita Bhattacharyya,[1,2,3] John M. Shelton,[4] Rhonda Bassel-Duby,[1,2,3] Eric N. Olson[1,2,3†]

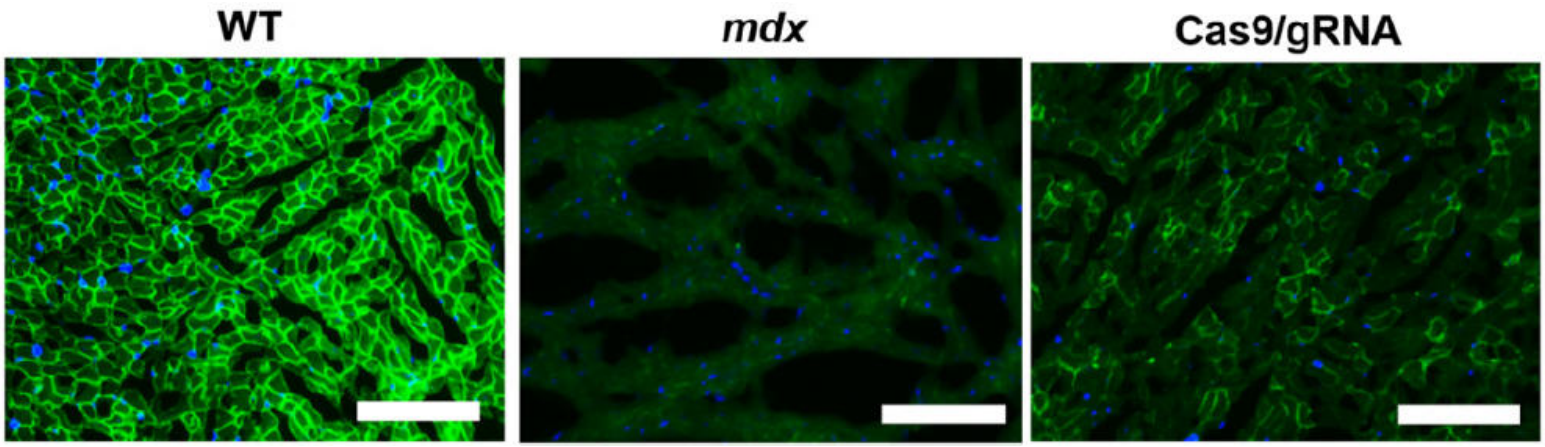
Science REPORTS

Cite as: C. E. Nelson *et al.*, *Science* 10.1126/science.aad5143 (2015).

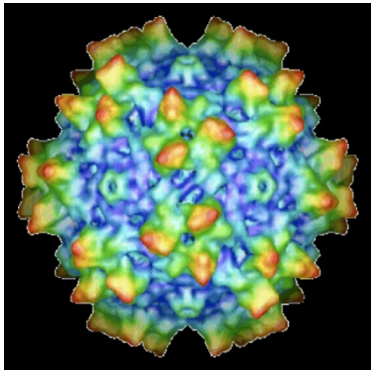
In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy

Christopher E. Nelson,[1,2] Chady H. Hakim,[3] David G. Ousterout,[1,2] Pratiksha I. Thakore,[1,2] Eirik A. Moreb,[1,2] Ruth M. Castellanos Rivera,[4] Sarina Madhavan,[1,2] Xiufang Pan,[3] F. Ann Ran,[5,6] Winston X. Yan,[5,7,8] Aravind Asokan,[4] Feng Zhang,[5,9,10,11] Dongsheng Duan,[3,12] Charles A. Gersbach[1,2,13*]



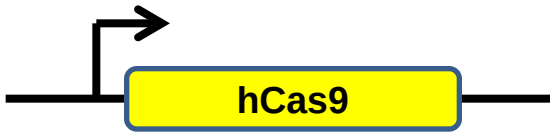


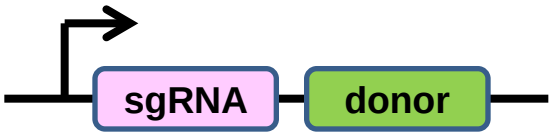
CRISPR tools and somatic gene therapy of human rare diseases



Adeno Associated Virus
AAV

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





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

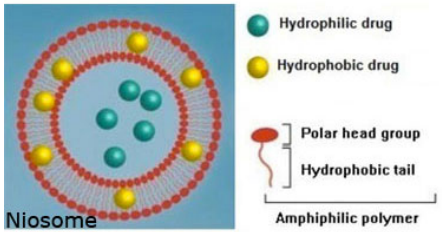
Preclinical animal models

- 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

Challenges for CRISPR-mediated gene therapy in patients

- Immune response against Cas9
- Immune response against AAV
- Finding the right AAV serotype (preferential)
- Non-viral delivery systems (nanoparticles, liposomes) → the future

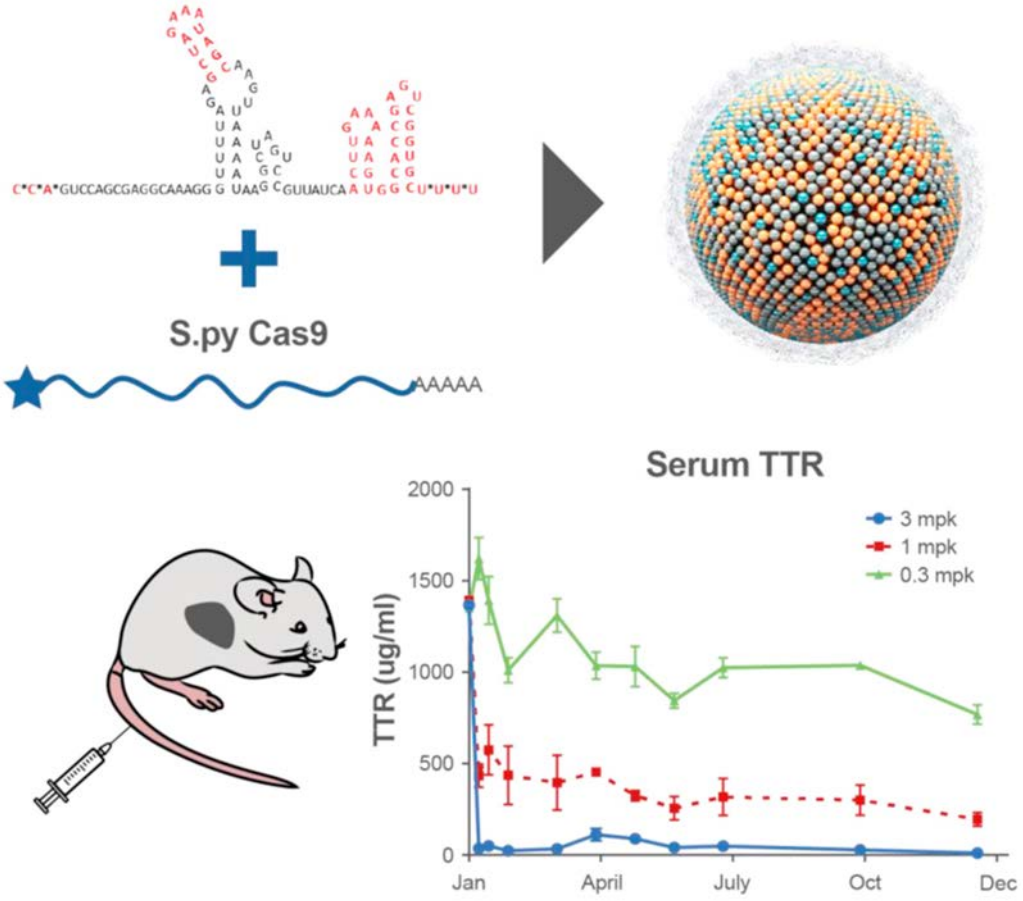
ciber-bbn



NanoCRISPRalbino
Niosomes
J.L. Pedraz (UPV/EHU)

- Reaching a significant number of target cells
- On-target allelic multiplicity (indetermination)
- Off-targets
- HDR (dividing cells) versus NHEJ (quiescent cells/neurons) →HITI (homology-independent targeted integration)

Single Administration of CRISPR/Cas9 Lipid Nanoparticles achieves Robust and Persistent In Vivo Genome Editing



Finn et al. Cell Reports 2018 22, 2227-2235DOI: (10.1016/j.celrep.2018.02.014)

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

Cas9
Streptococcus pyogenes
Staphylococcus aureus

- 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

Cold Spring Harbor Laboratory

bioRxiv beta
THE PREPRINT SERVER FOR BIOLOGY

5 Jan 2018

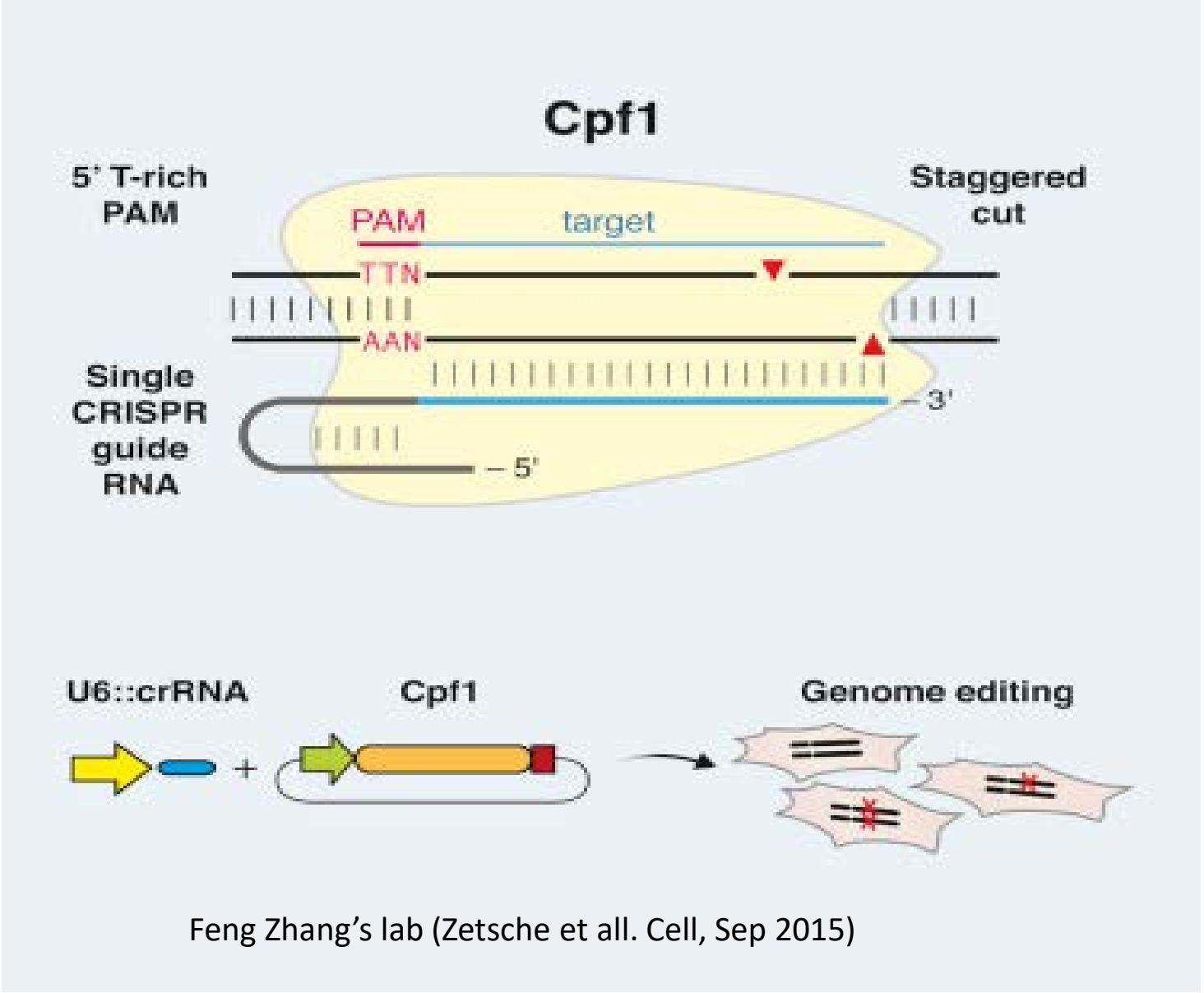
New Results

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

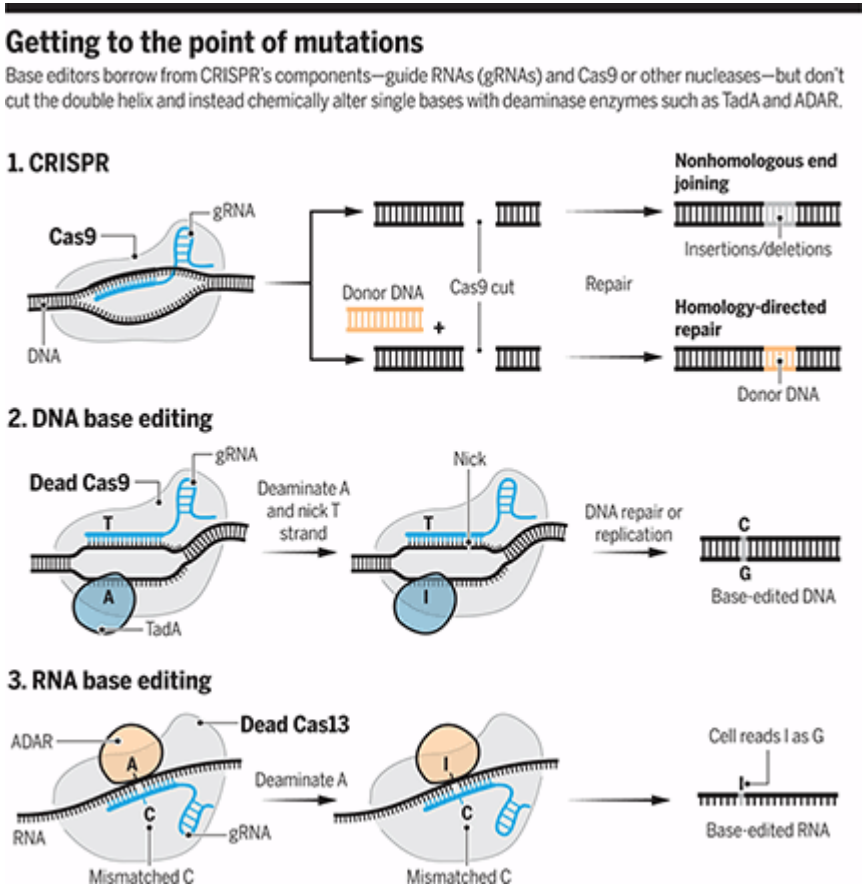
Alternative Cas-like proteins



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

Cas12a

Francisella tularensis



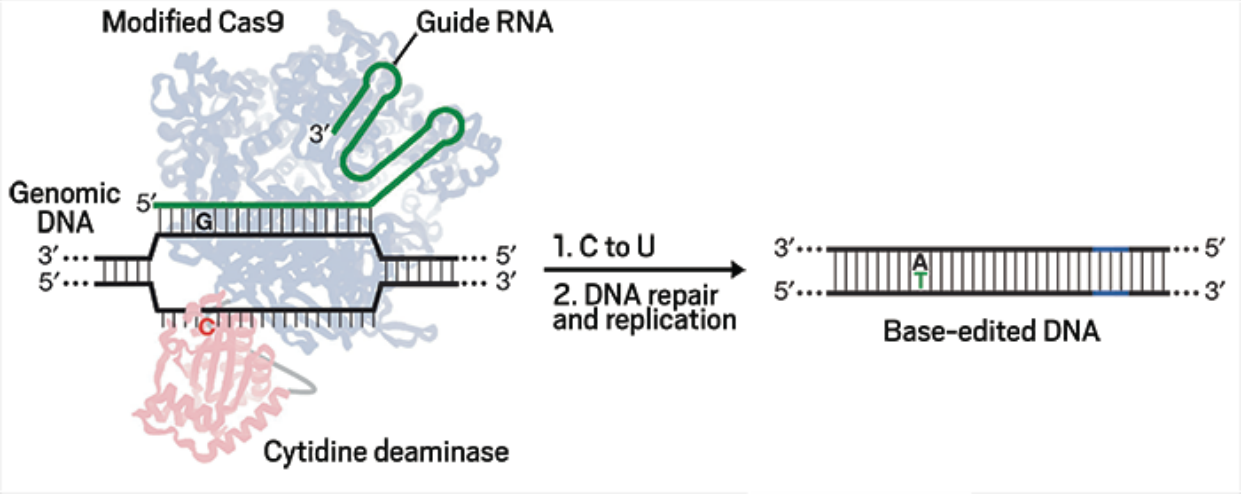
C → U → T

A → I → G

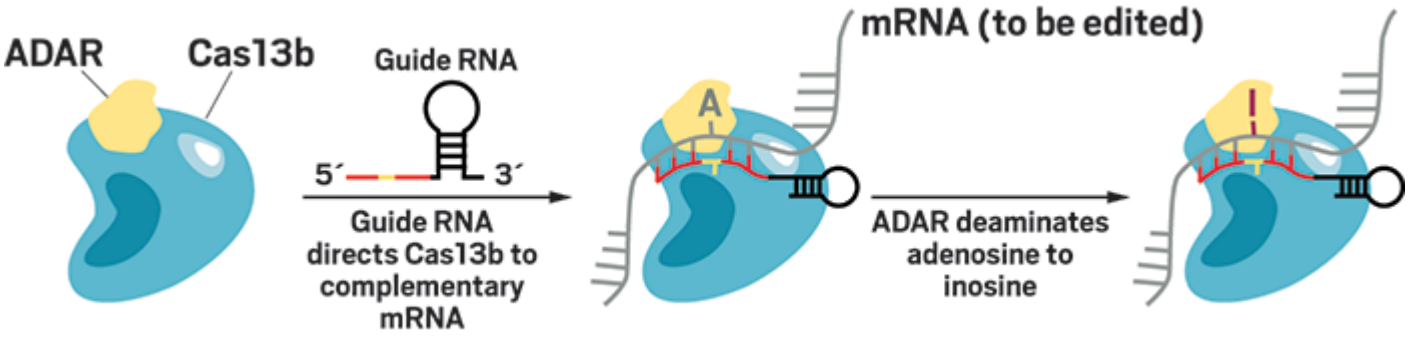
Kim et al. Nat Biotech. 2017

David Liu Lab

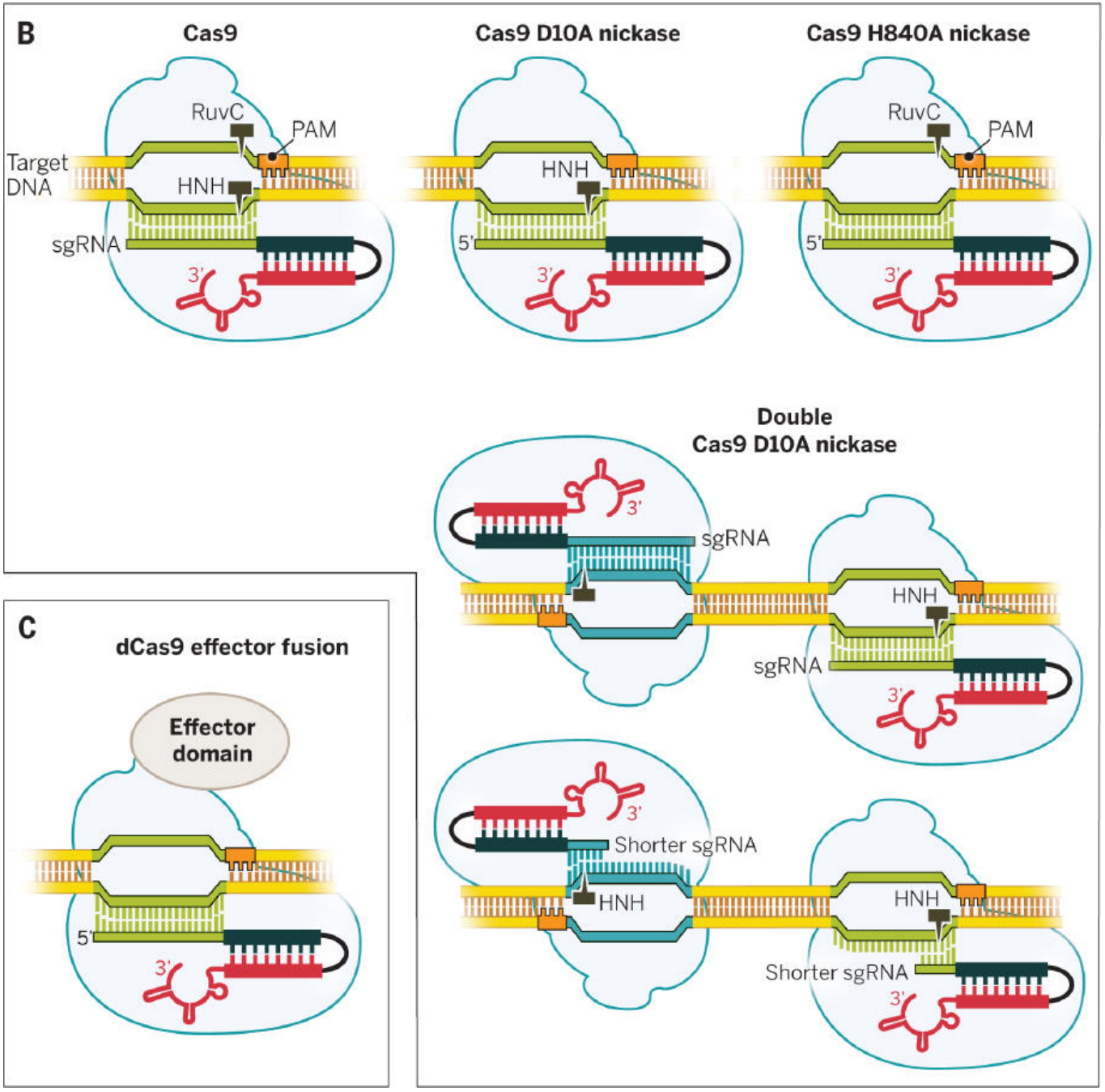
CRISPR-derived BASE EDITORS



CRISPR 2.0 (editing RNA)



Feng Zhang's Lab Nature 2017

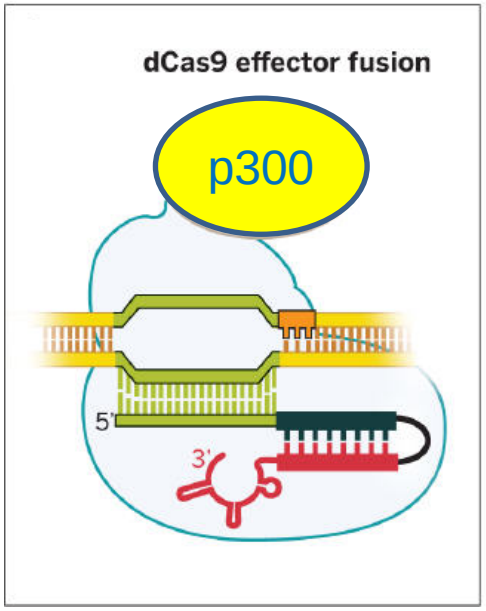


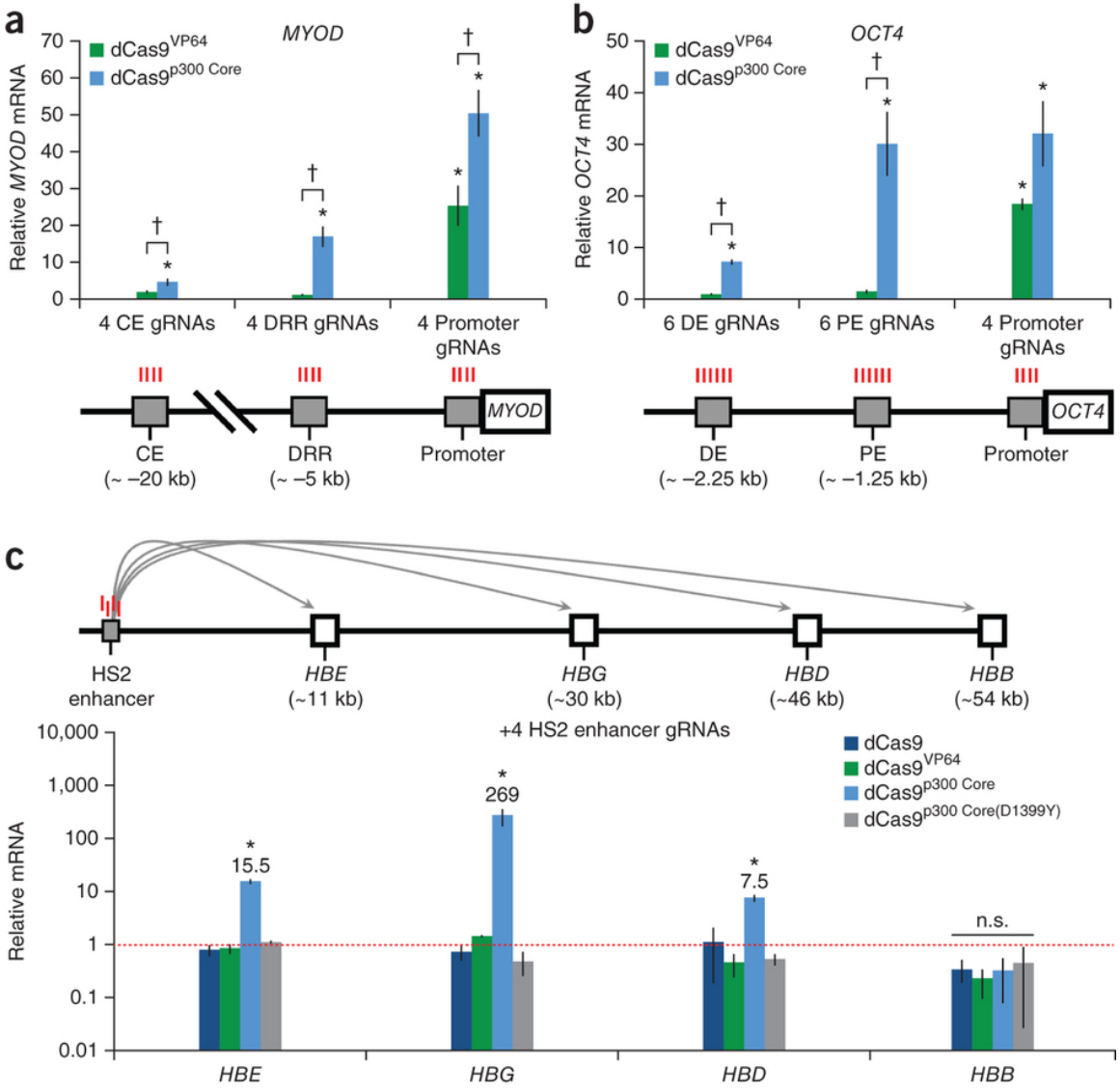
Doudna & Charpentier (2014) Science

Targeting acetylation of Histone H3 Lysine 27 in the genome

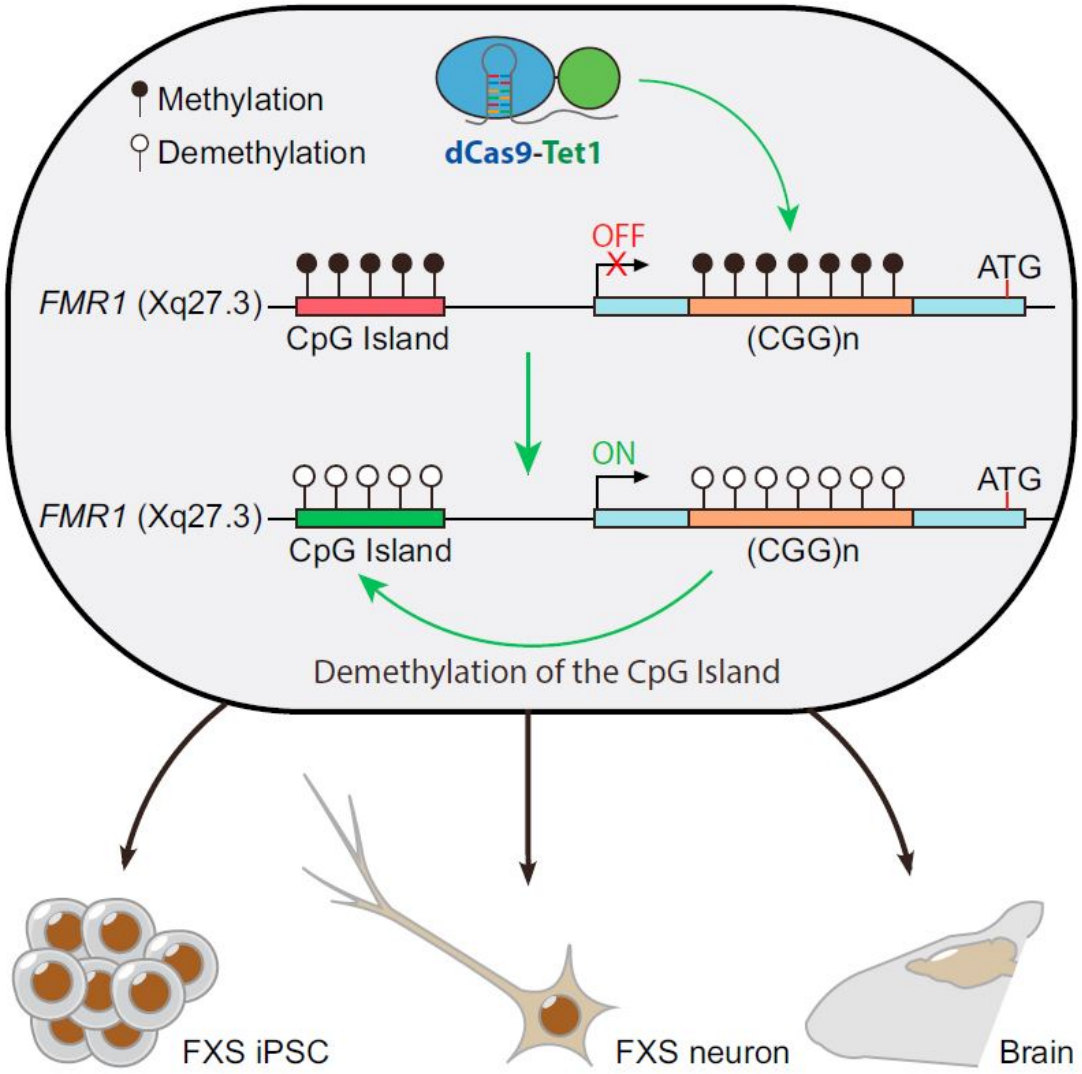
Activating gene expression by CRISPR-dCas9/p300

Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers
Hilton *et al.*
Nature Biotechnology 33, 510–517 (2015)

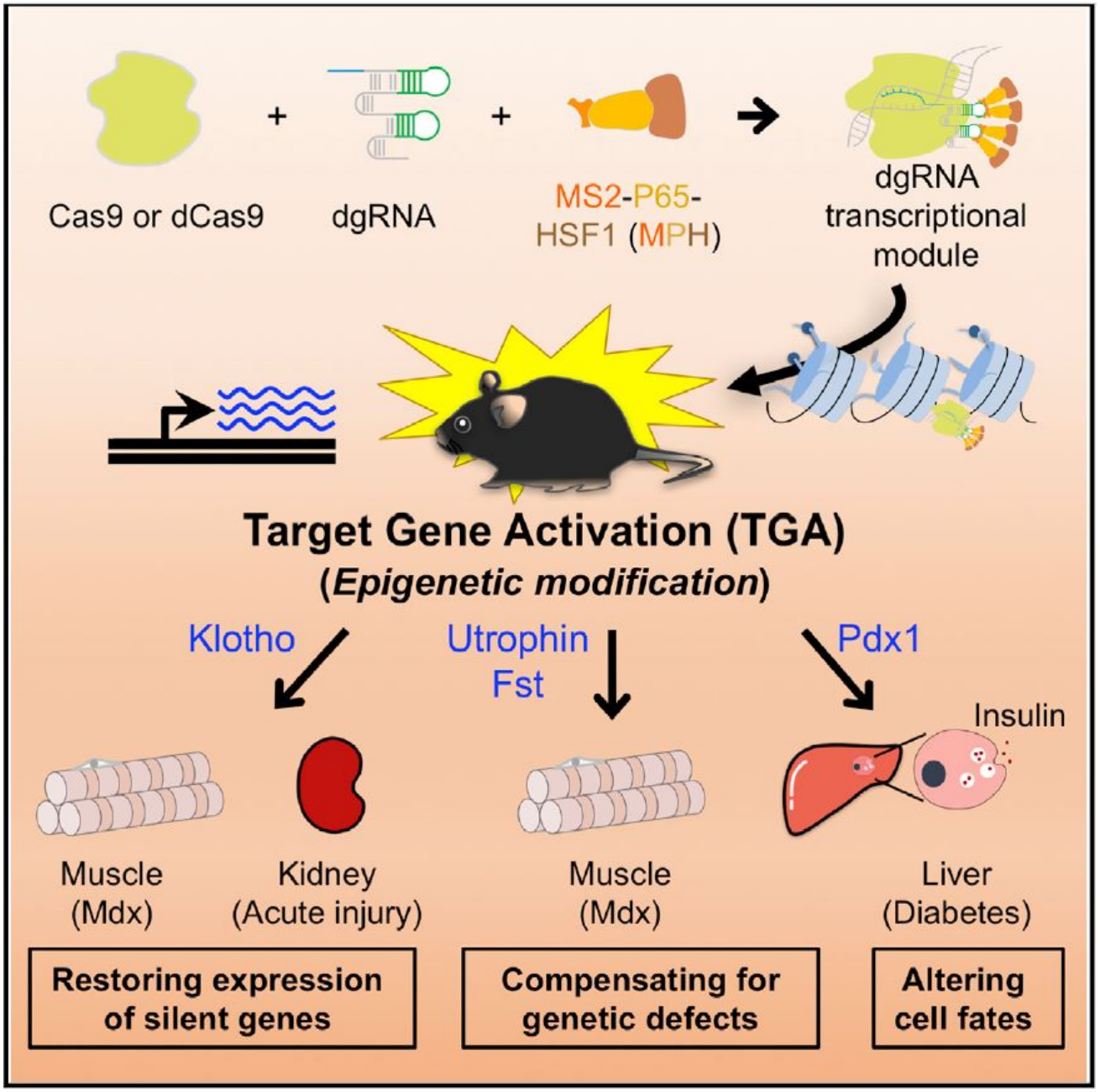




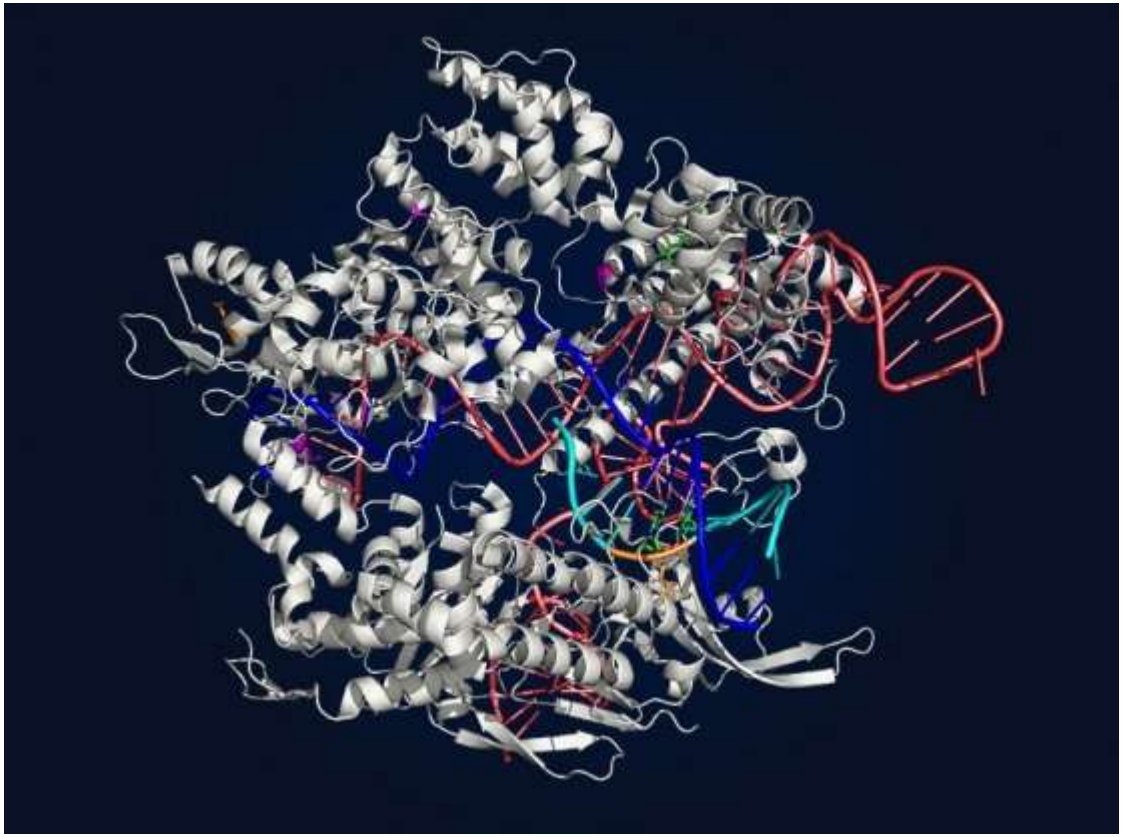
CRISPR-Cas rescues Fragile X syndrome



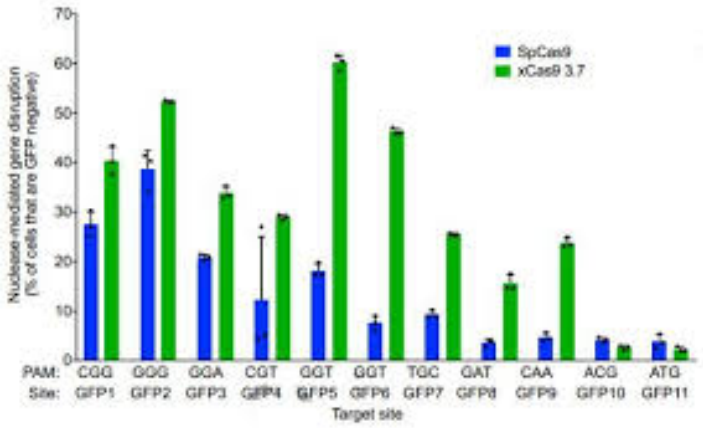
Liu et al. Cell 2018

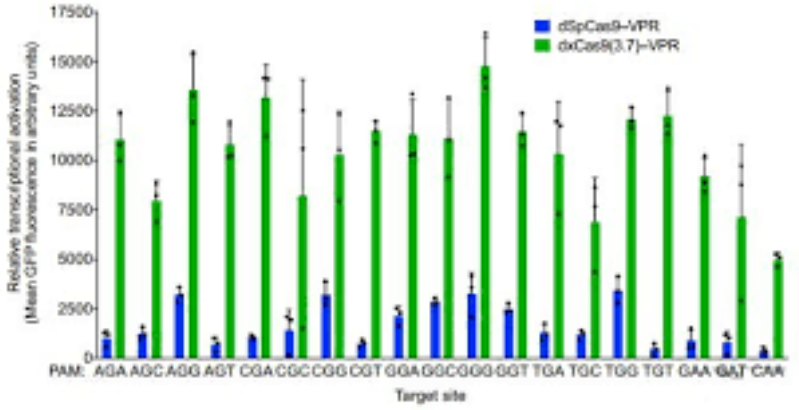


Liao et al. Cell 2017



Evolved Cas9 variants with broad PAM compatibility and high DNA specificity



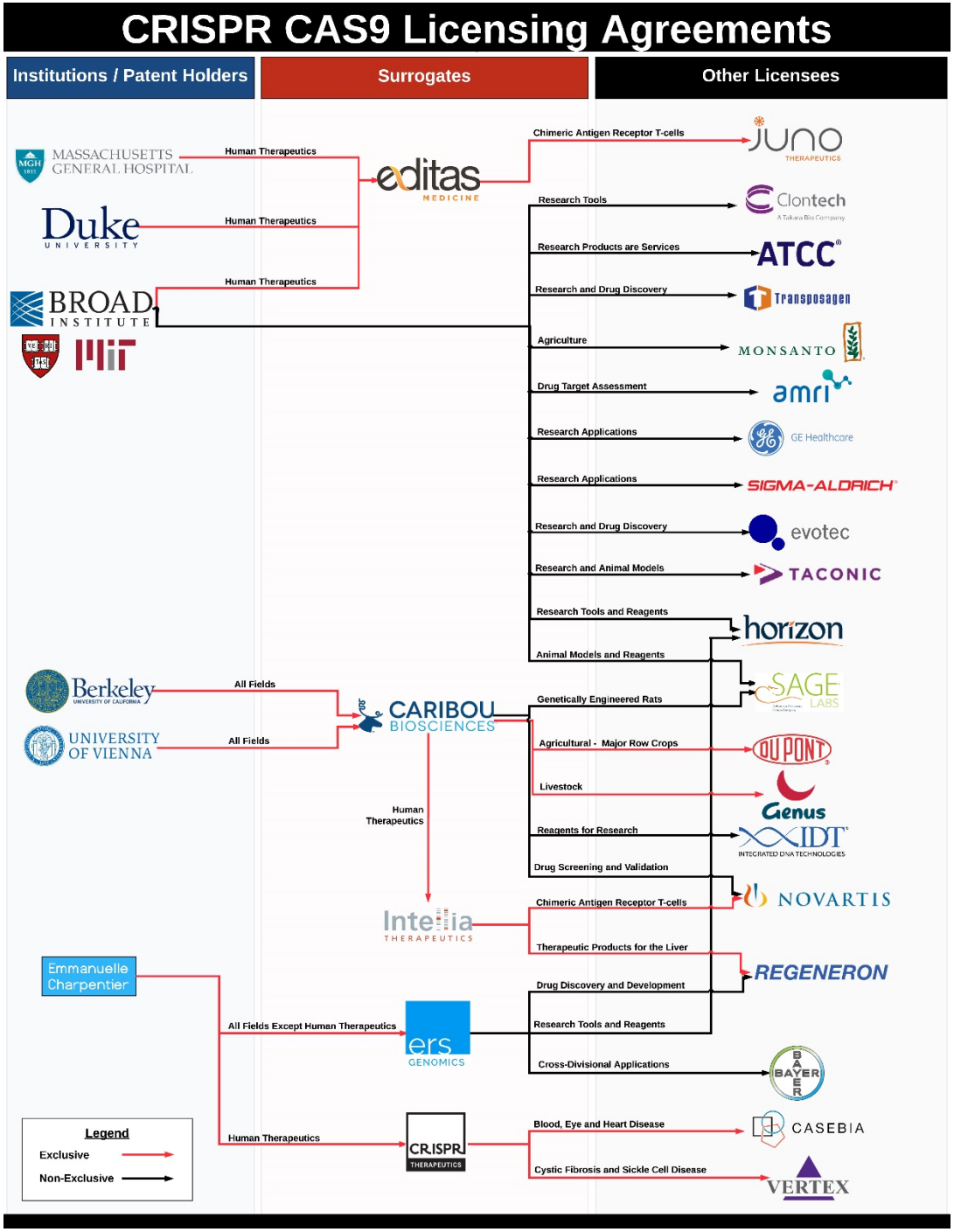


Hu et al. Nature 28 Feb 2018


CRISPR
EVERYWHERE
Illustration by Chris Labrooy ©nature

BROAD / MIT
Patent filed in December 2012
Paper published January 2013
Patent granted in April 2014
"CRISPR applications in all eukaryotic cells"

UC Berkeley
Patent filed in May 2012
Paper published in August 2012
Interference filed in January 2016
Interference denied in February 2017
Patent still under evaluation
"CRISPR applications in all cells"



http://www.cnb.csic.es/~montoliu/

Fotos: Ana Ytyrralde

Almudena Fernández
Marcos Rubio
Diego Muñoz
Santiago Josa
Andrea Montero
Julia Fernández
Marta Cantero
Marta Sánchez

Cryopreservation and Histology teams: Julia Fernández, María Jesús del Hierro, Marta Castrillo, Soledad Montalbán, Óscar Sánchez

Former members: Davide Seruggia, Mónica Martínez, Irene Robles, Irene Sánchez, Esther Zurita, Magdalena Hryhorowicz, Barbara Franck, Rafael Jiménez, Cesar Echeverria, Celia de Lara, Iván Caballero, Yaiza López …

Pawel Pelczar (Univ. Zürich / Univ. Basel) Transgenic Unit
Belén Pintado (CNB-CSIC, Madrid) Transgenic Unit
Juan Carlos Oliveros (CNB-CSIC, Madrid) Bioinformatics

The CRISPR web page at CNB

www.cnb.csic.es/~montoliu/CRISPR/

Google for CNB + CRISPR