Genome engineering methodologies



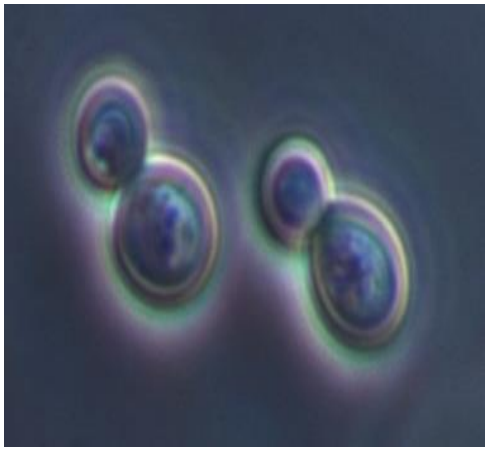




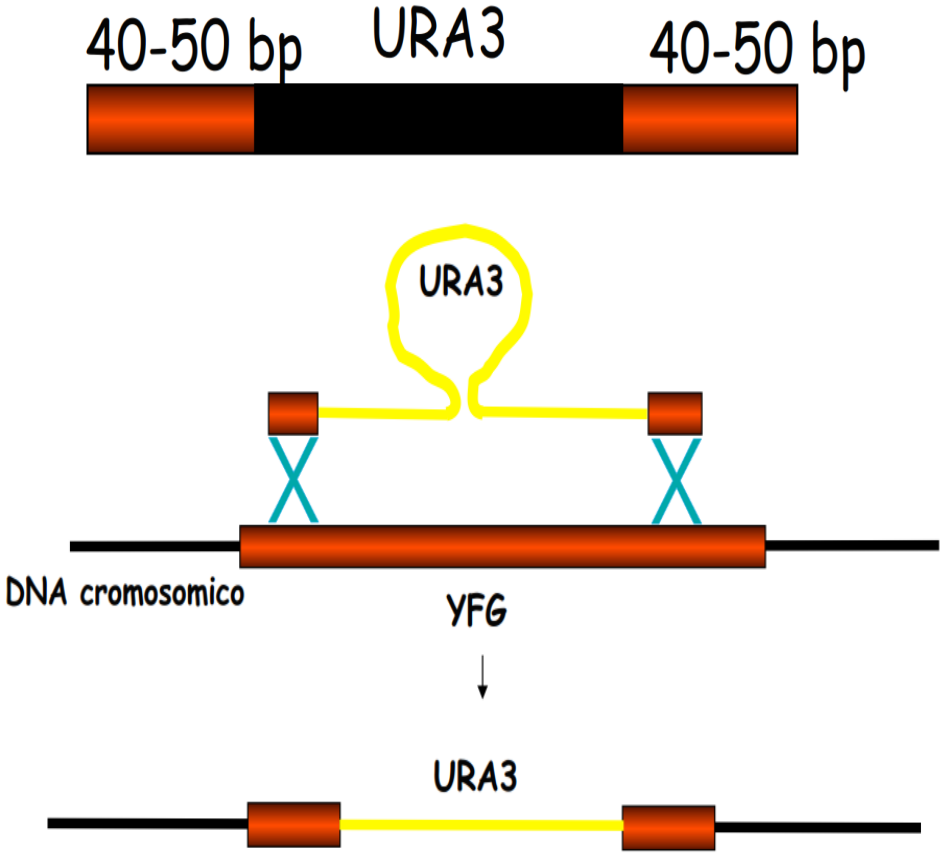
What does ''Genome engineering'' means?

''Genome engineering refers to all the m*ethodologies that allow to specifically change the genomic DNA sequence in a site-specific manner.''*

Homologous recombination in yeast

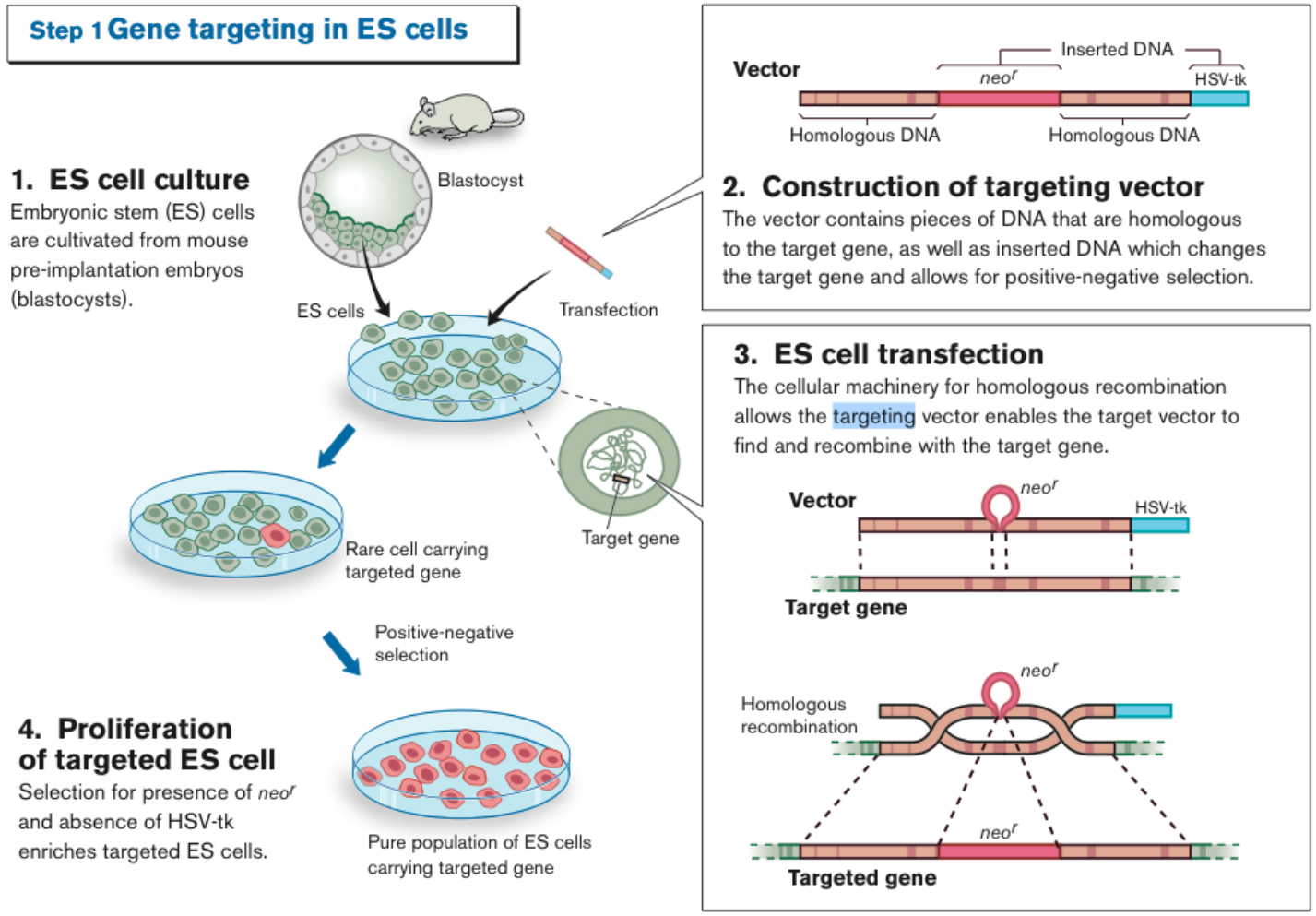


S.cerevisiae



Genome engineering

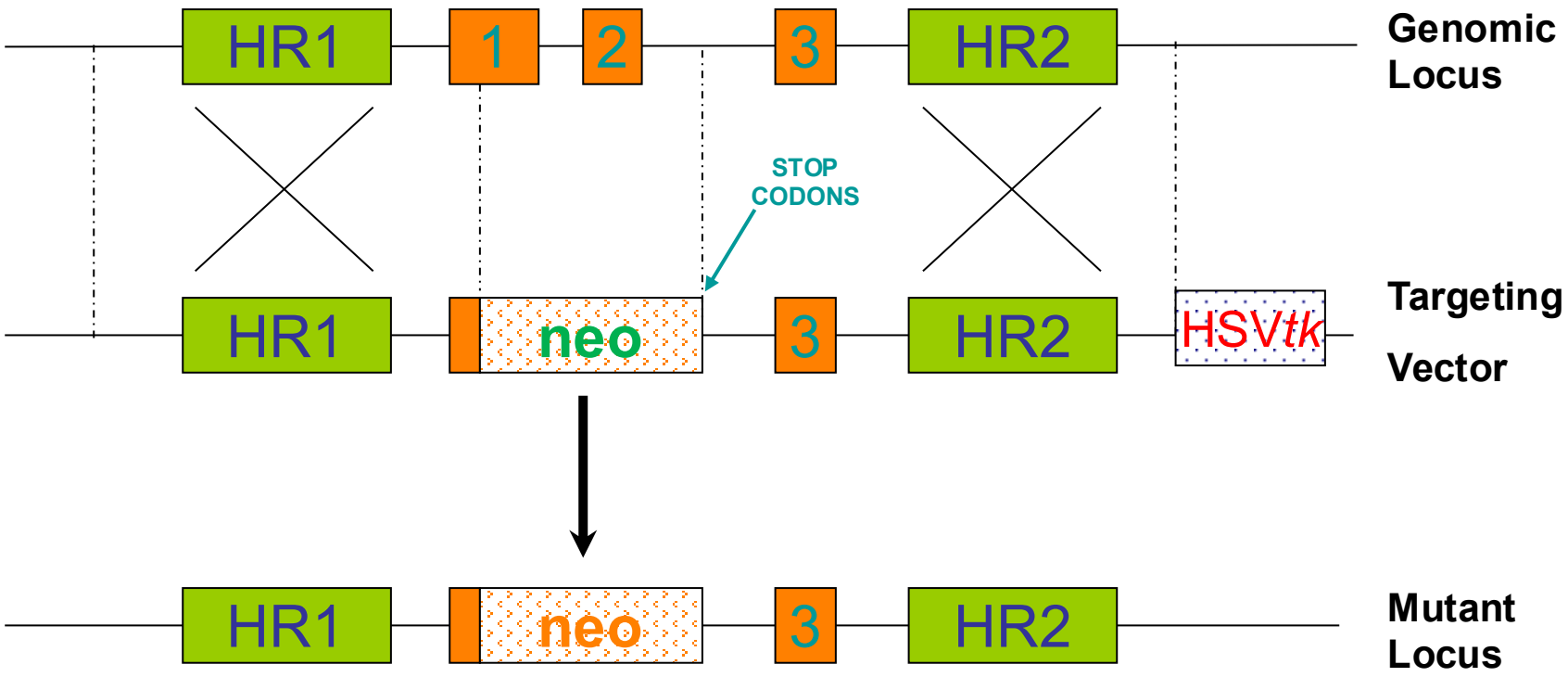




The efficiency of targeting is around 1%.

Positive-negative selection

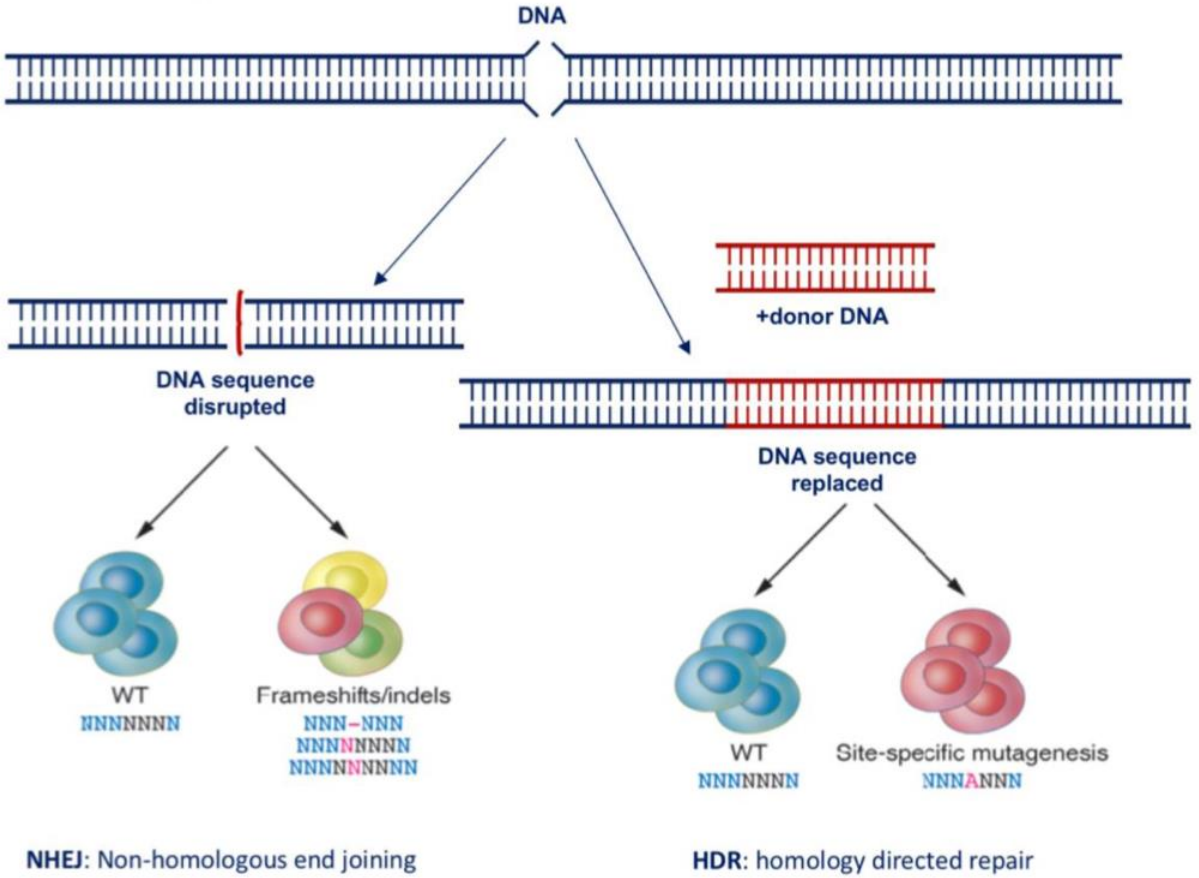
It allows to distinguish between homologous and heterologous recombination



Genome editing

Genome editing is a type of genetic engineering in which DNA is inserted, deleted or replaced in the genome of an organism using engineered nucleases.

These nucleases create site-specific double-strand breaks (DSBs) at desired locations in the genome. The induced double-strand breaks are repaired through non-homologous end-joining (NHEJ) or homologous recombination (HR), resulting in targeted mutations ('edits').



Repair of DNA double-strand breaks (DSBs)

Following DNA cleavage at a user-specified site, DSBs can be repaired by one of two highly conserved cellular processes to enable gene customization:

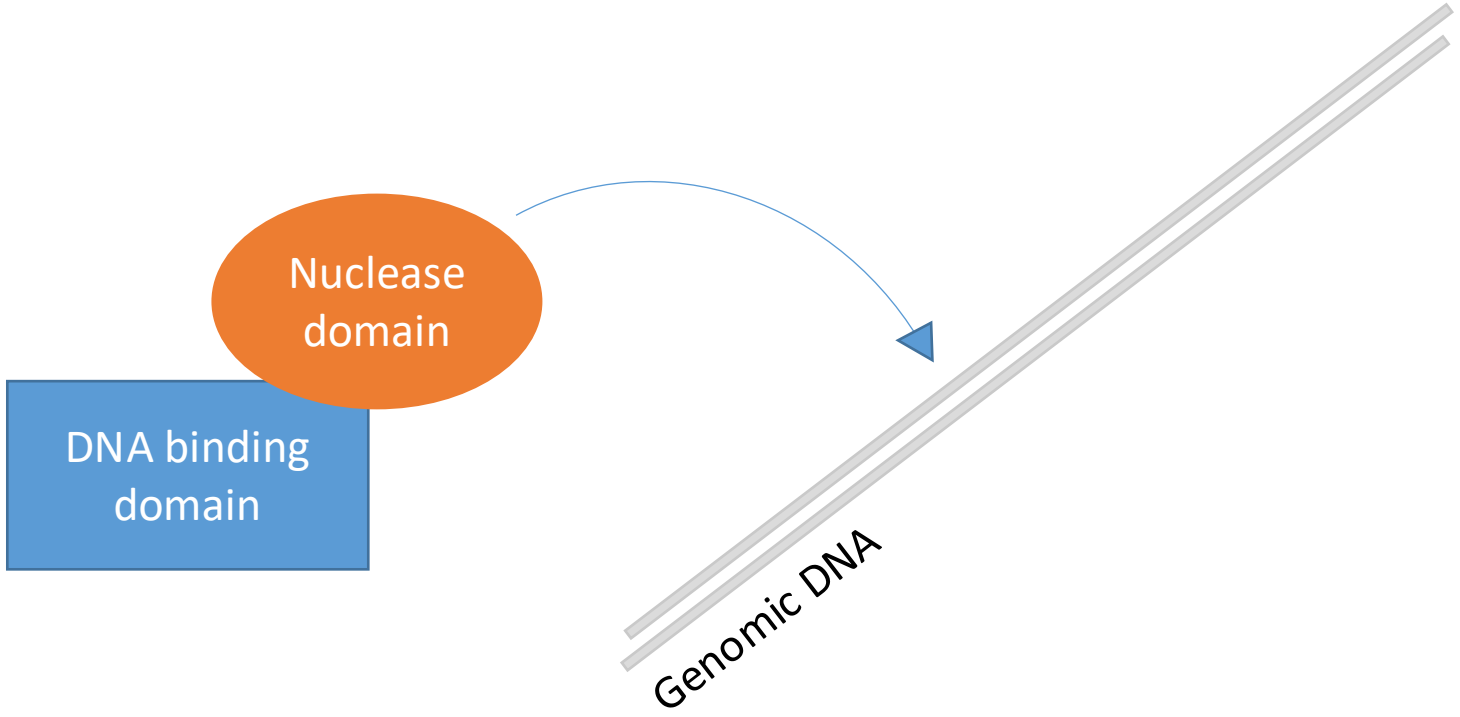
- **Homologous Recombination (HR),** in which nucleotide sequences are exchanged between two similar or identical molecules of DNA.

- **Non-homologous end-joining (NHEJ).** It is a cell natural process that repairs DSBs in DNA. NHEJ is referred to as "non-homologous" because the break ends are directly re-ligated without the need for a homologous template, in contrast to HR. Due to the **error-prone** nature of NHEJ, a proportion of DSBs will be misrepaired by the **addition and/or deletion of nucleotides**. These mutations within the genomic sequence occur at the site of the DSB, resulting in the loss of gene function and therefore achieving **gene knock-out.**

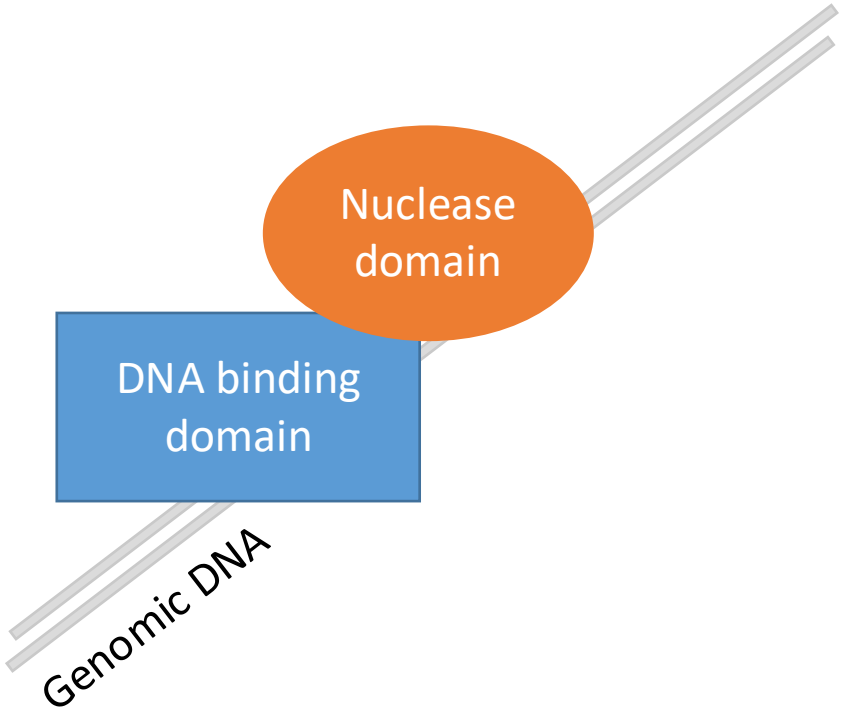
Genome editing

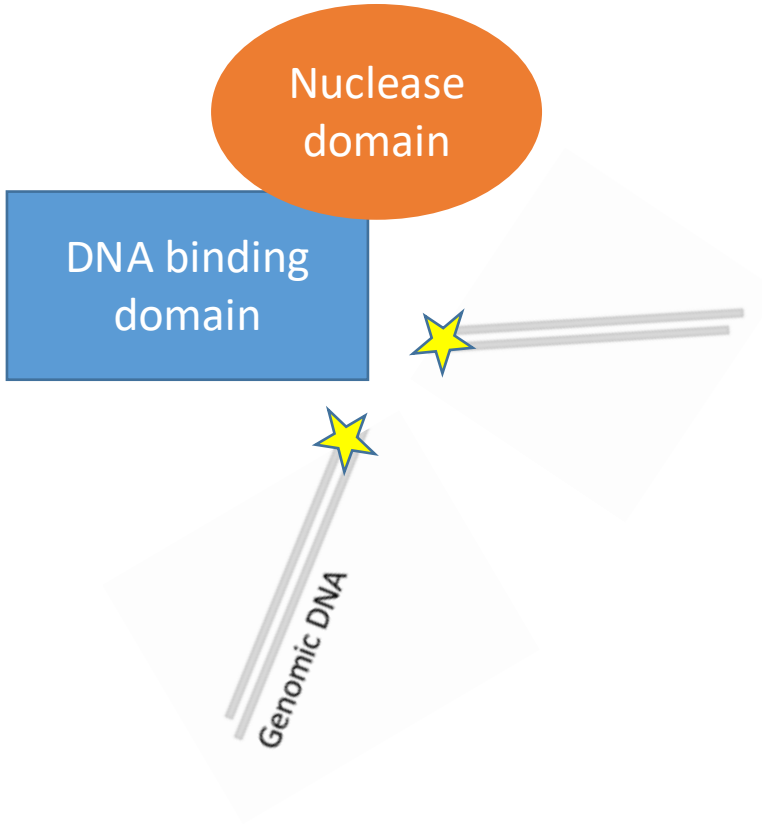


Genome editing

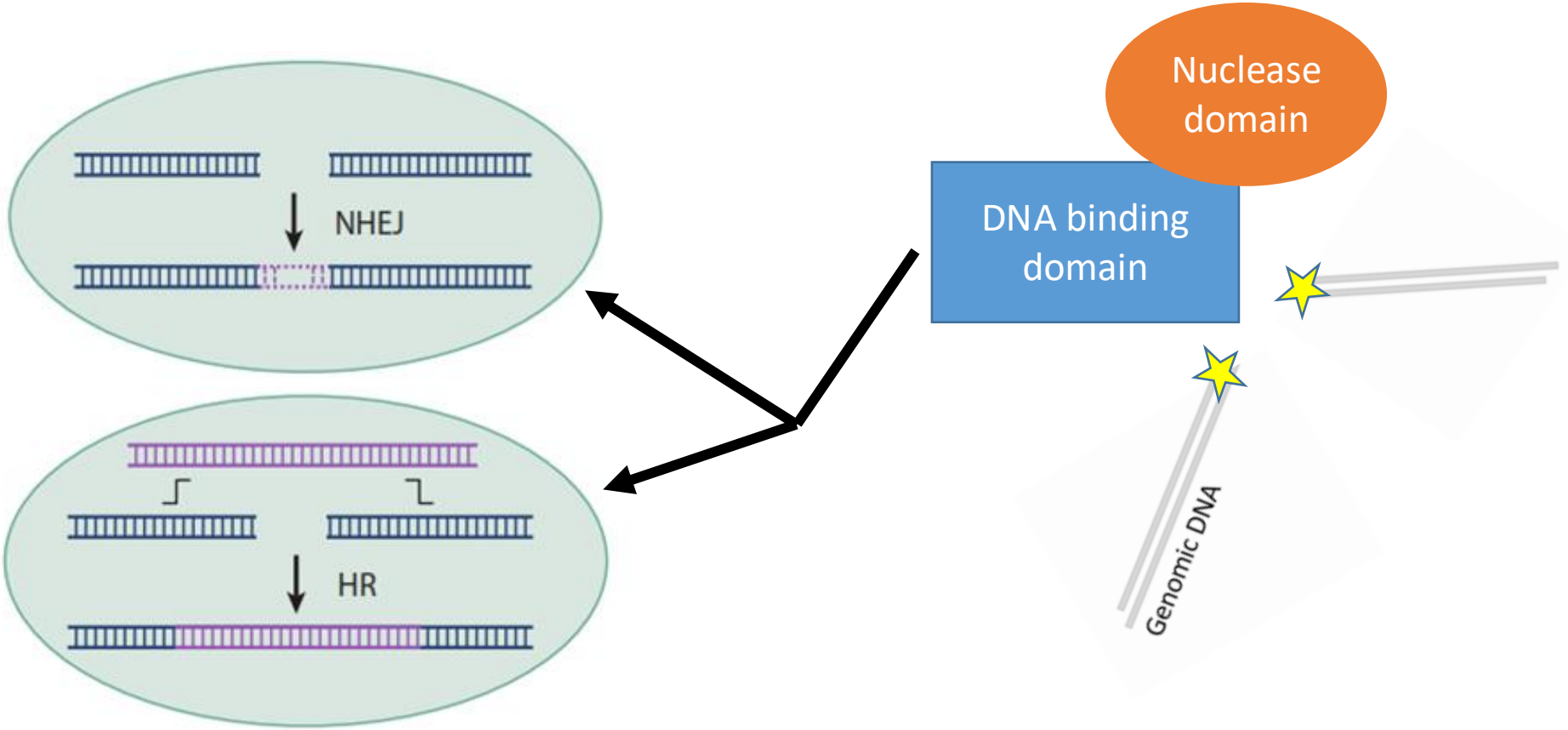


Genome editing

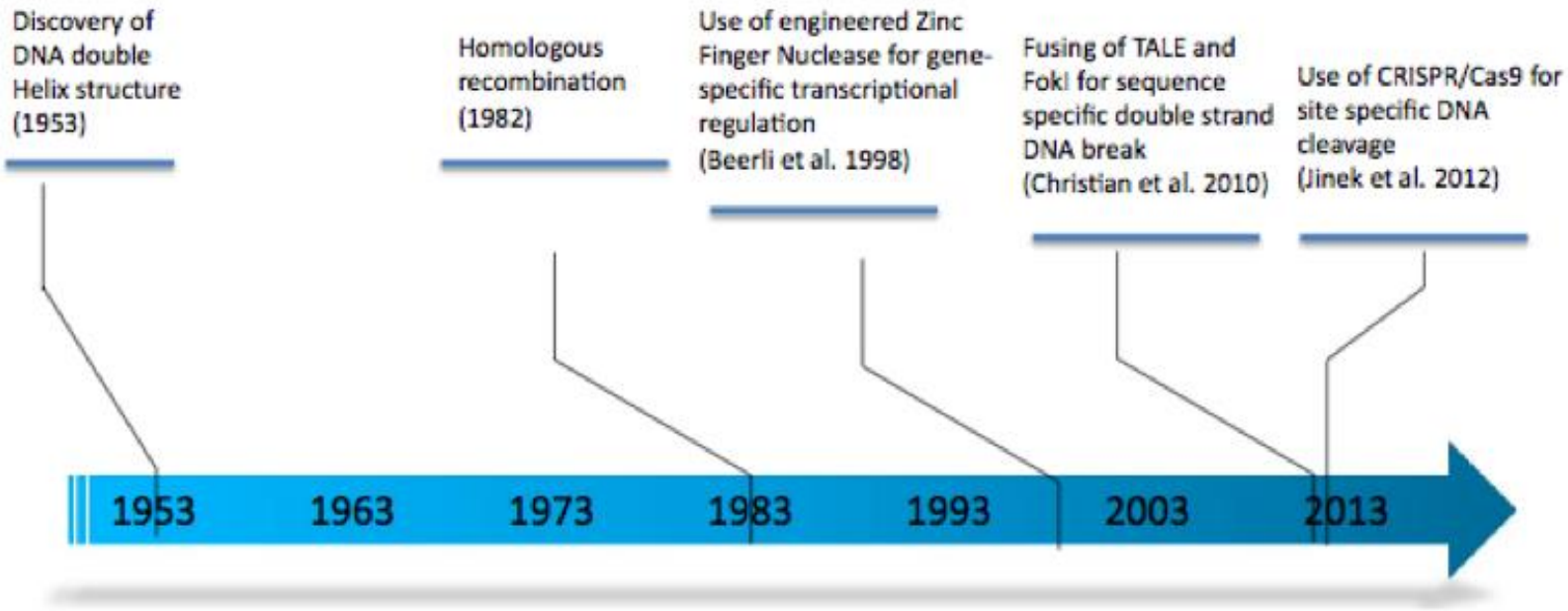



Nuclease domain
DNA binding domain
Genomic DNA

Genome editing



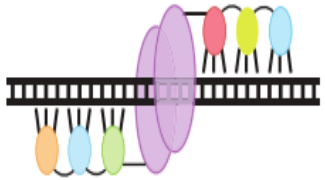
Timeline of genome engineering research



These technology can be used to investigate the function of a gene of interest or to correct gene mutations in cells via genome editing, paving the way for future therapy approaches.

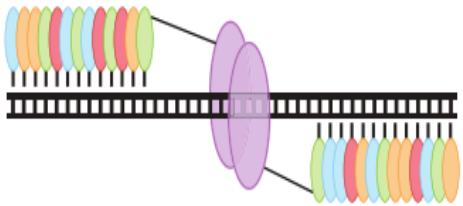
Genome editing

1°Generation:



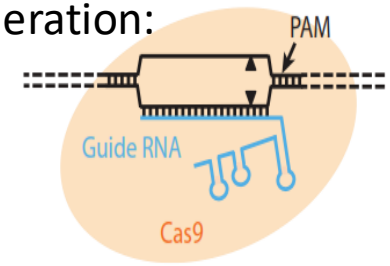
ZFNs

2° Generation:

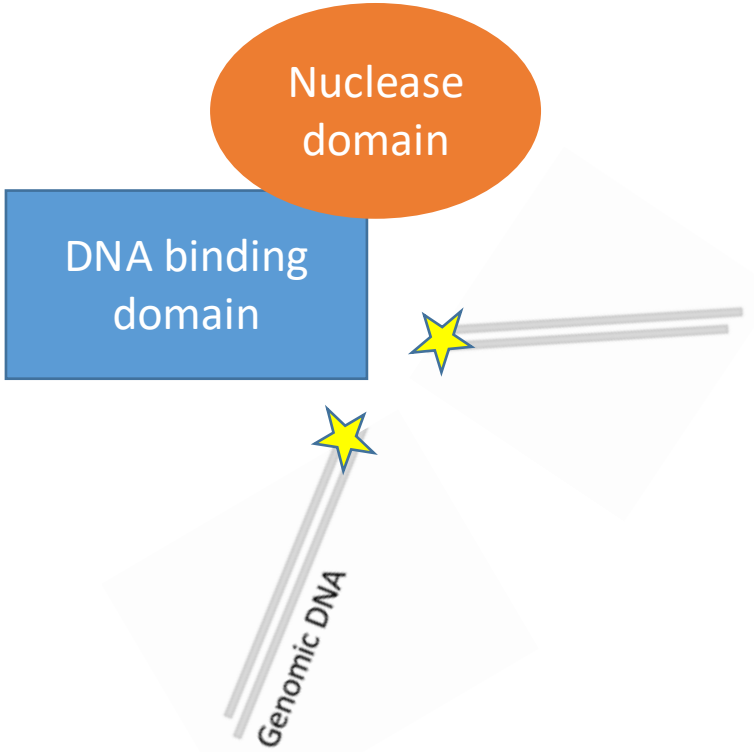


TALENs

3° Generation:

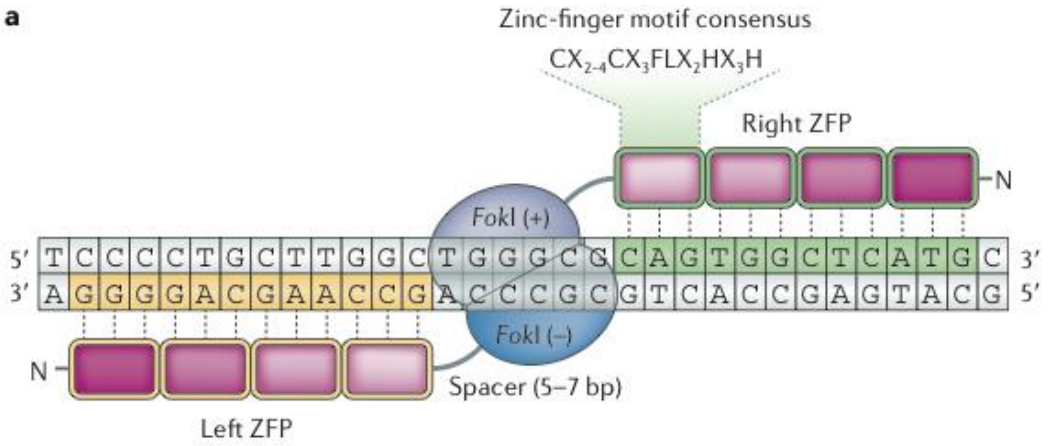


CRISPR/Cas



Zinc-finger nucleases (ZFNs)

A ZFN has a modular structure that is composed of two domains: a **DNA-binding zinc-finger protein (ZFP) domain** and the **nuclease domain** derived from the FokI restriction enzyme. Two ZFN monomers are required to form an active nuclease.

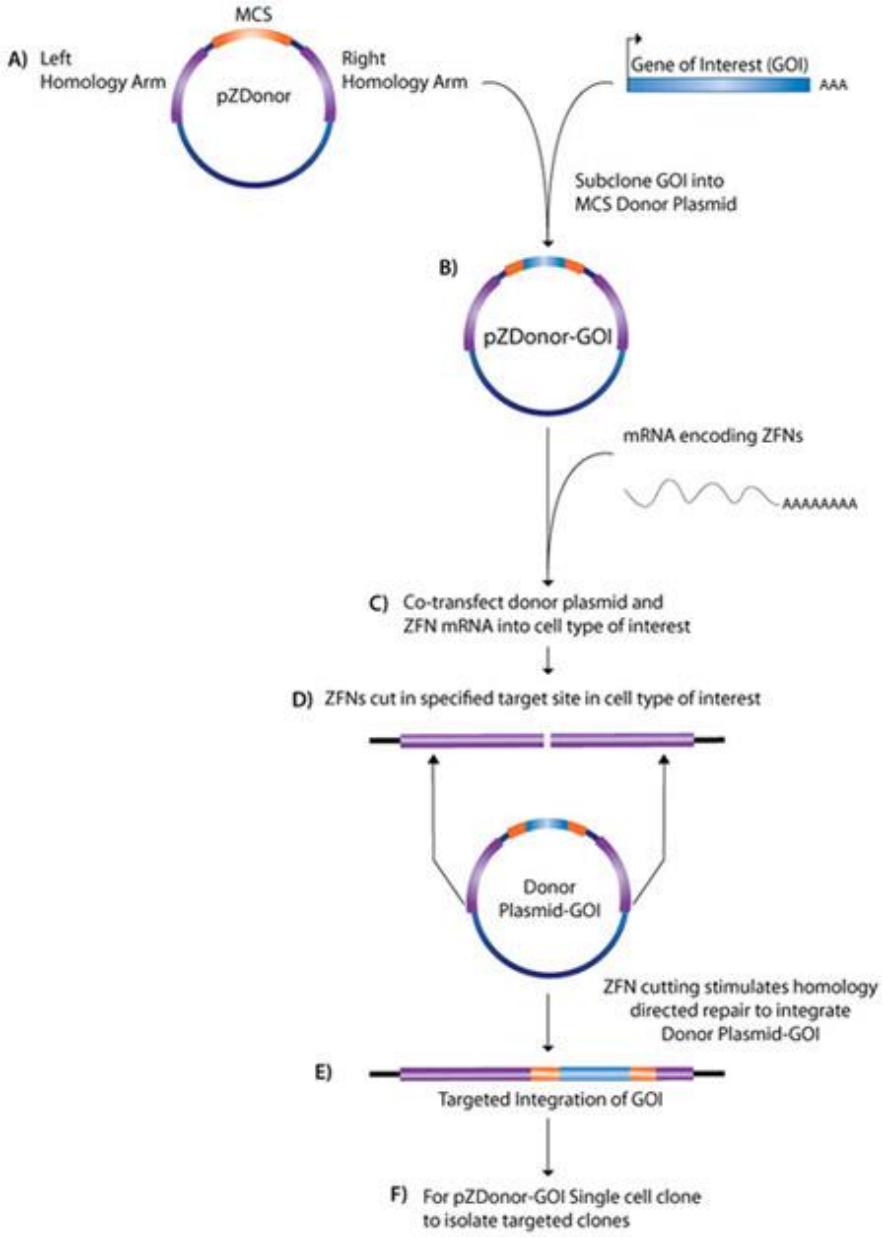


The sequence specificity of ZFNs is determined by ZFPs, which consist of tandem arrays of C2H2 zinc-fingers, the most common DNA-binding motif in higher eukaryotes. Each zinc-finger recognizes a 3-bp DNA sequence, and 3–6 zinc-fingers are used to generate a single ZFN subunit that **binds to DNA sequences of 9–18 bp**. Various computer programs are available that search for possible ZFN target sites in a given DNA sequence.

Zinc-finger nucleases (ZFNs)

Targeted Integration

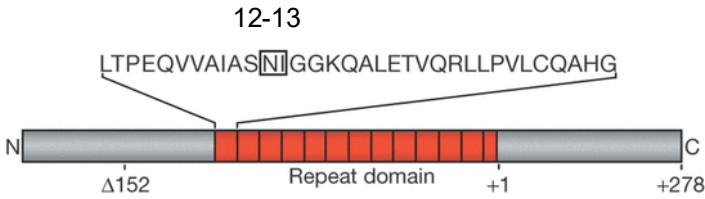
ZFNs are used to rapidly integrate a user-specified gene of interest (GOI) into preferred sites of integration in either the human, mouse or rat genome. In the human genome **the adeno-associated virus (AAVS1) locus** on human chromosome 19 is the preferred site, while the **Rosa26 locus** is located on chromosome 6 in the mouse and chromosome 4 in the rat. The ZFNs target each site with high specificity, creating a double strand break. ZFN treated cells then use a Donor plasmid with homology arms to either the AAVS1 or Rosa26 genomic regions to replace the natural locus by the repair process of homology directed repair. The Donor plasmid can be engineered to contain your GOI and will direct targeted integration of your GOI into the AAVS1 or Rosa26 loci.



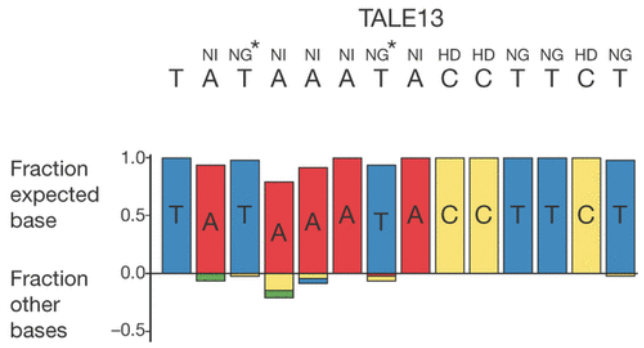
Transcription Activator-Like Effector Nucleases (TALEN)

The first component is the **TALE** domain. TALEs are naturally-occurring transcription factors first discovered in the plant pathogen *Xanthomonas*. In the nucleus they bind specific sequences within gene promoters to modulate host resistance mechanisms.

Each TALE contains a highly conserved central region consisting of varying numbers of repeat units of typically 33 to 35 amino acids that confer specific DNA sequence recognition to the TALE protein. Remarkably, these repeat units contain high levels of identity with the exception of two variable amino acids at positions 12 and 13. These residues, termed the **Repeat-Variable Di-residues (RVDs)**, confer DNA-binding specificity to the repeat region.



RVD:	NI	HD	NN	NG
Base:	A	C	G/A	T



N= Apsparagine
I=Isoleucine
H= Histidine
D=Aspartic acid
G=Glycine

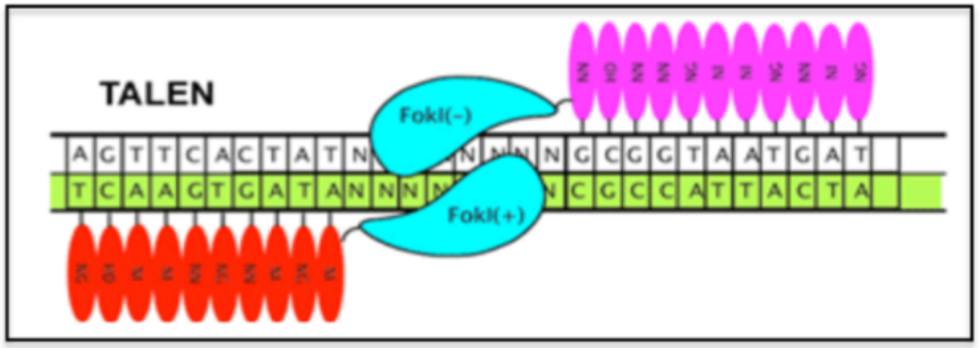
Transcription Activator-Like Effector Nucleases (TALEN)

- **TALENs** are similar to ZNFs in that they use **DNA binding motifs** to direct the same non-specific nuclease to cleave the genome at a specific site, but instead of recognizing DNA triplets, each domain recognizes a **single nucleotide**.

- Functional endonuclease **FokI** is artificially fused to DNA-binding domains to cut the targeting sequences and generate a DSB

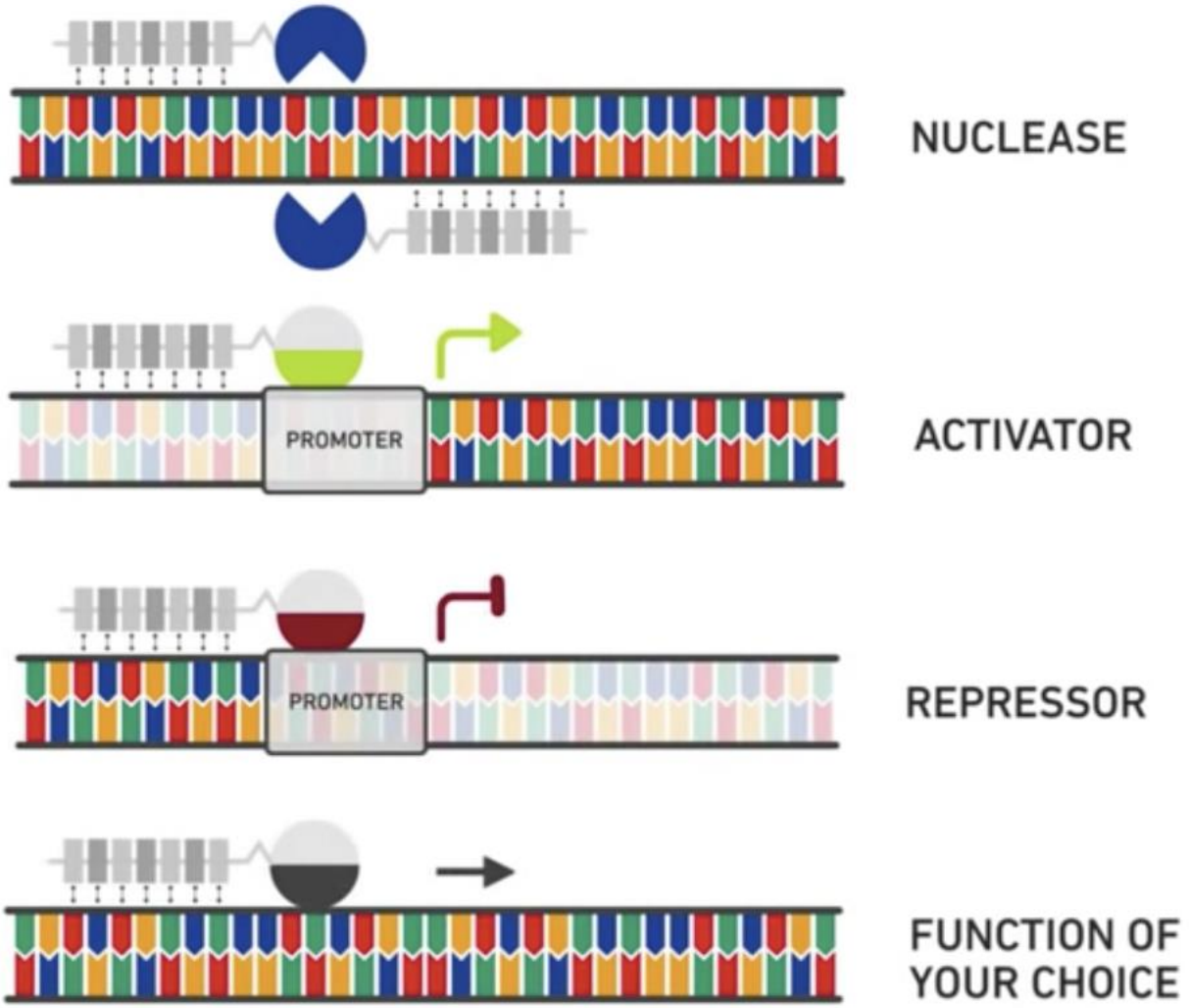
- Since FokI works as a **dimer**, two TALE binding sites which separated by a spacer sequence are needed to locate opposing DNA target sites.

- The interactions between the TALEN-derived DNA binding domains and their target nucleotides are **less complex** than those between ZNFs and their target trinucleotides, and designing TALENs is generally **more straightforward than ZNFs.**

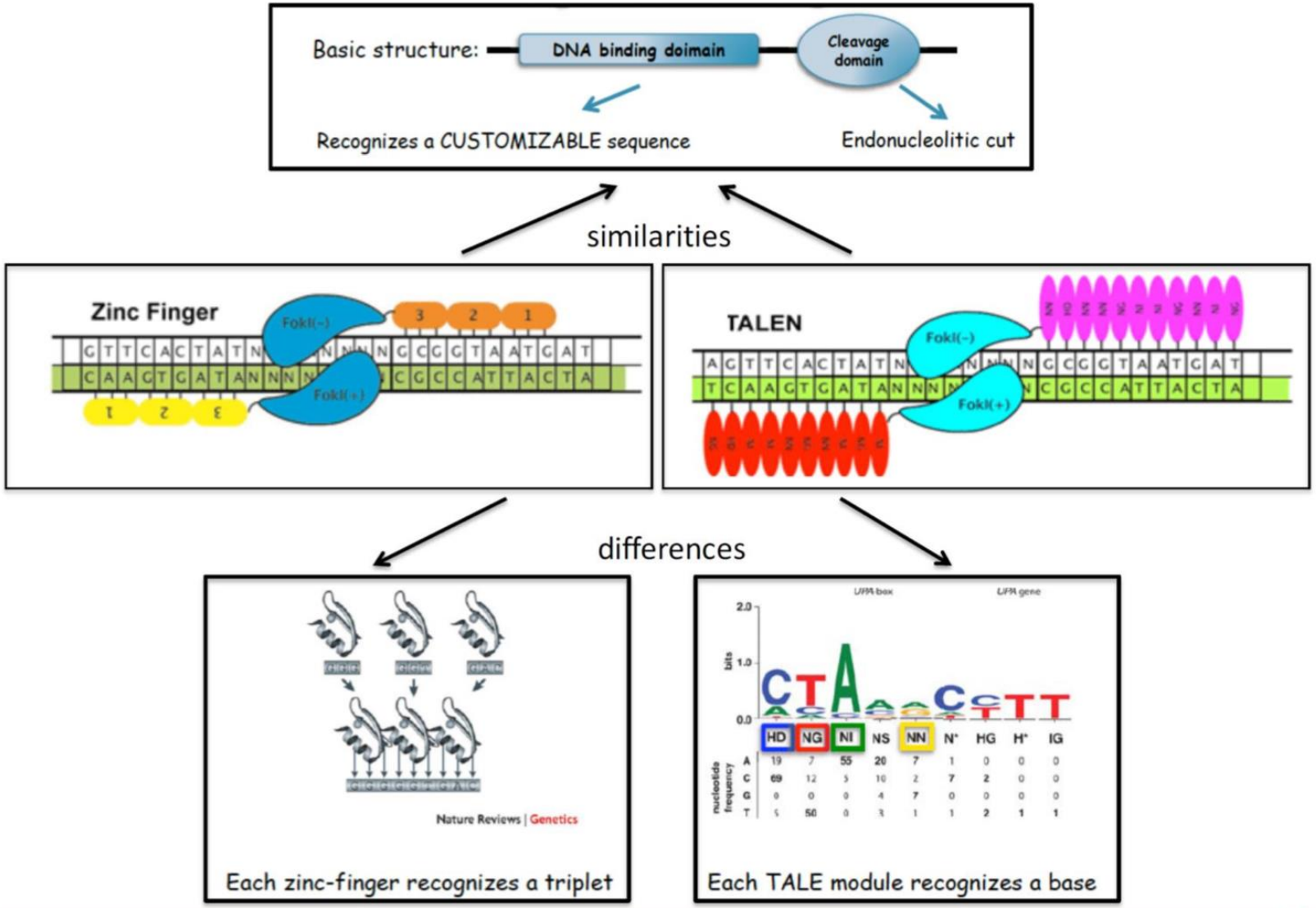


Mussolino et al., 2013

Transcription Activator-Like Effector Nucleases (TALEN)



TALEN vs ZNFs



Clustered Regurarly interspaced Short Palyndronic Repeats (CRISPR) and CRISPR-assoctaed (Cas) genes

The CRISPR/Cas system is a prokaryotic immune system that confer resistance to phages, and provide a form of **acquired immunity**.

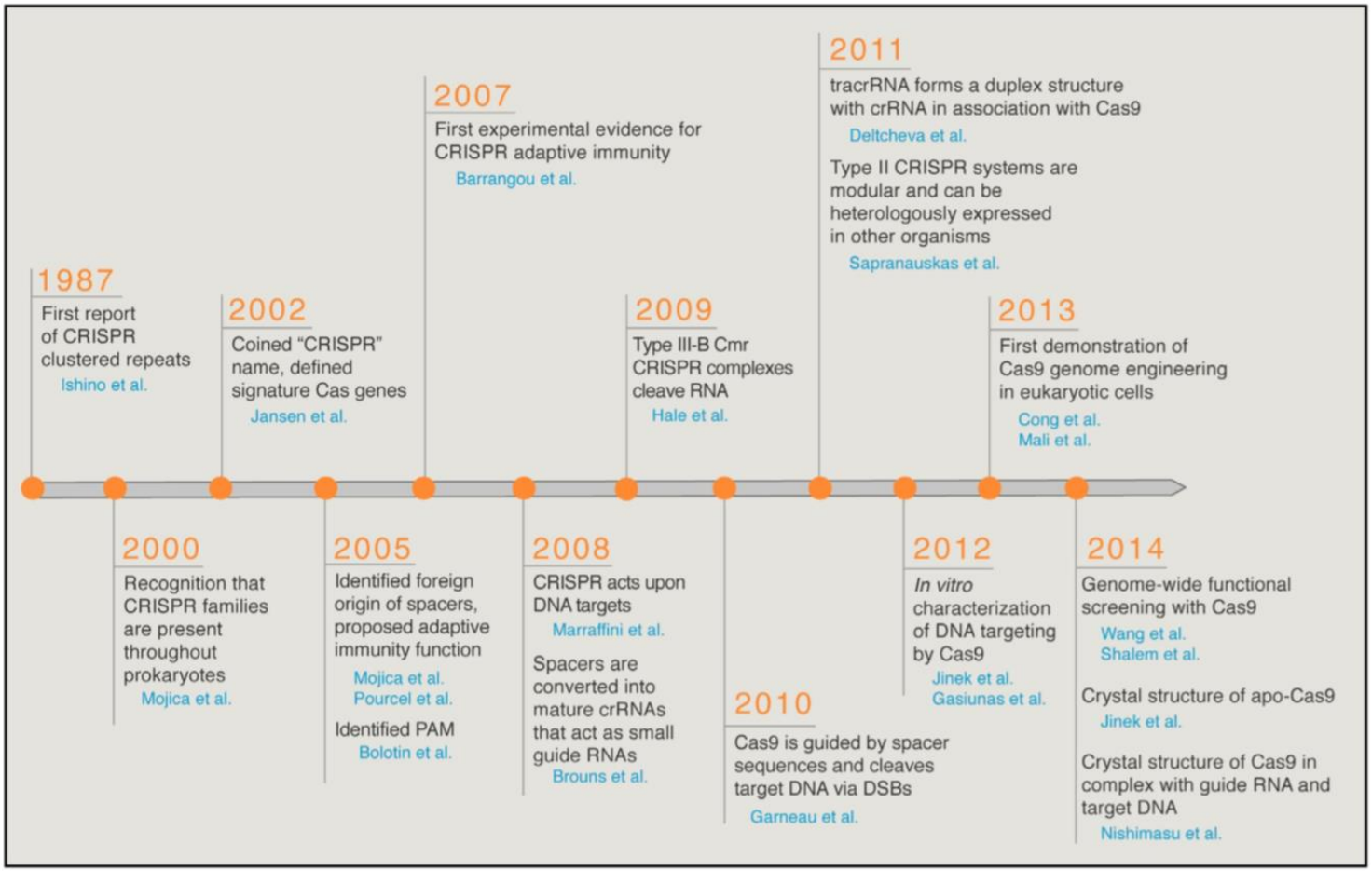


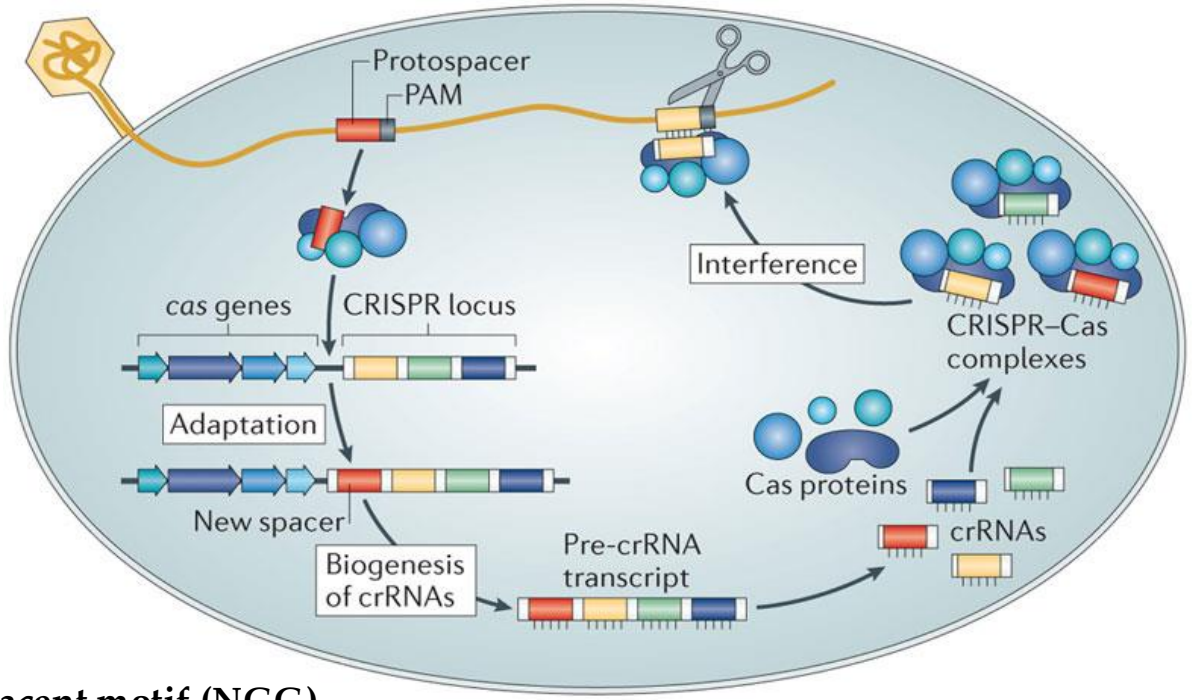
Figure: Hsu, Lander, Zhang: Development and Applications of CRISPR-Cas9 for Genome Engineering; Cell 157, June 5, 2014

Emmanuelle Charpentier and **Jennifer Doudna** have been awarded the 2020 Nobel Prize in Chemistry for their development of CRISPR/Cas9 genetic editing.



RNA-guided engineered nucleases (RGENs)

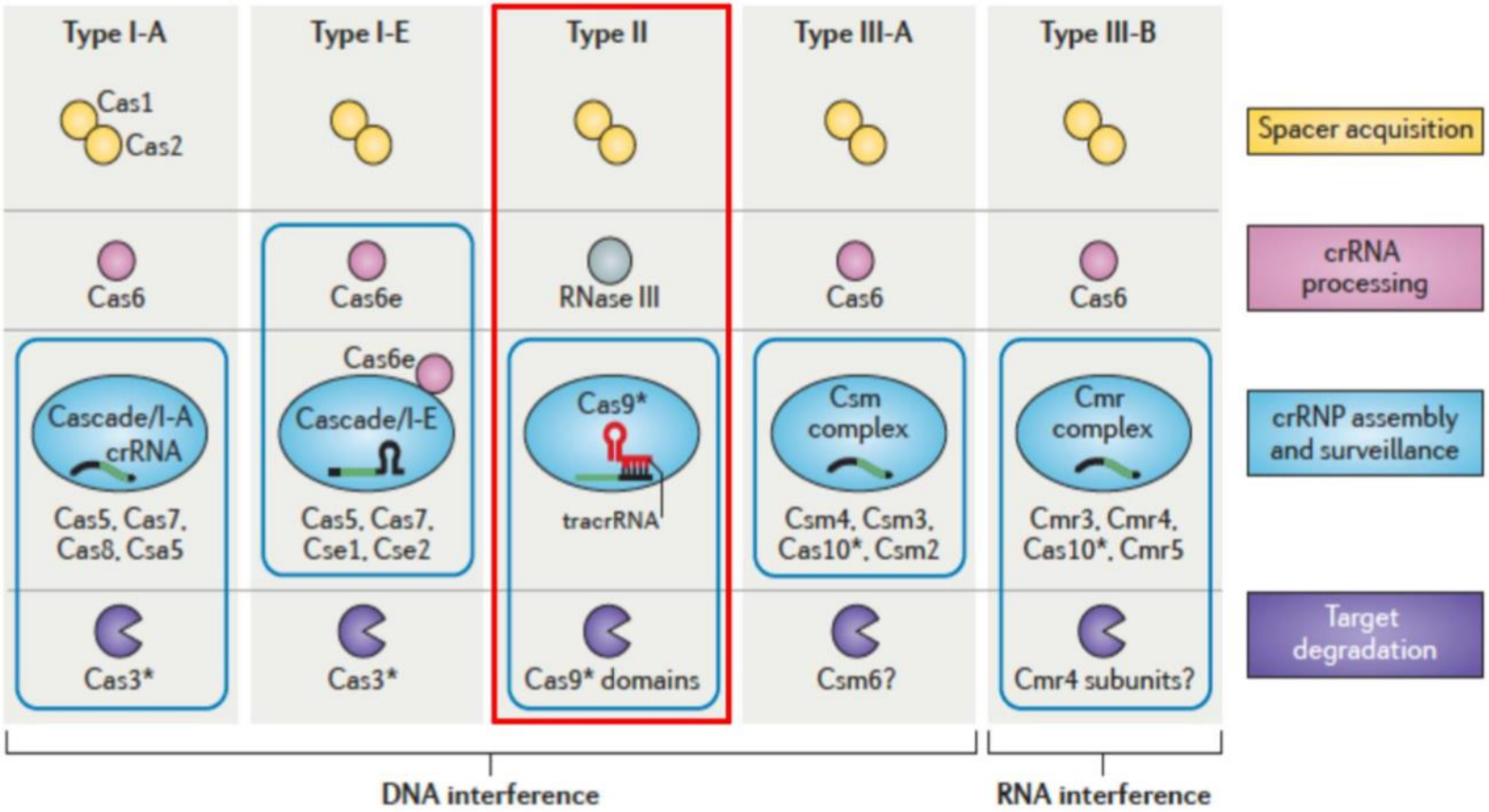
In bacteria, the **CRISPR system** provides acquired immunity against invading foreign DNA via RNA-guided DNA cleavage. In **the type II CRISPR/Cas system**, short segments of foreign DNA, termed **'spacers'** are integrated within the CRISPR genomic loci and transcribed and processed into short **CRISPR RNA (crRNA)**. These crRNAs direct sequence-specific cleavage and silencing of pathogenic DNA by Cas proteins.



PAM: Protospacer adiacent motif (NGG)

CRISPR-Cas systems

There are at least 11 different CRISPR-Cas systems, which have been grouped into three major types (I-III)

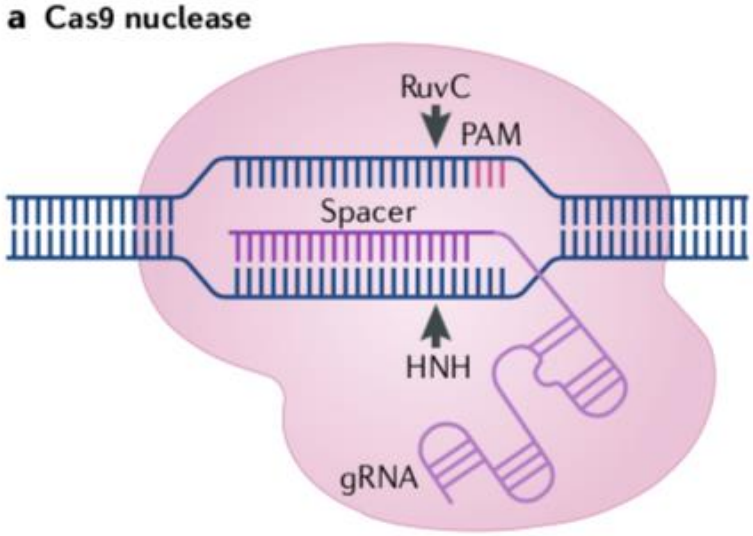


The type II CRISPR system from *Streptococcus pyogenes* has been adapted for inducing sequence-specific double stranded breaks and targeted genome editing.

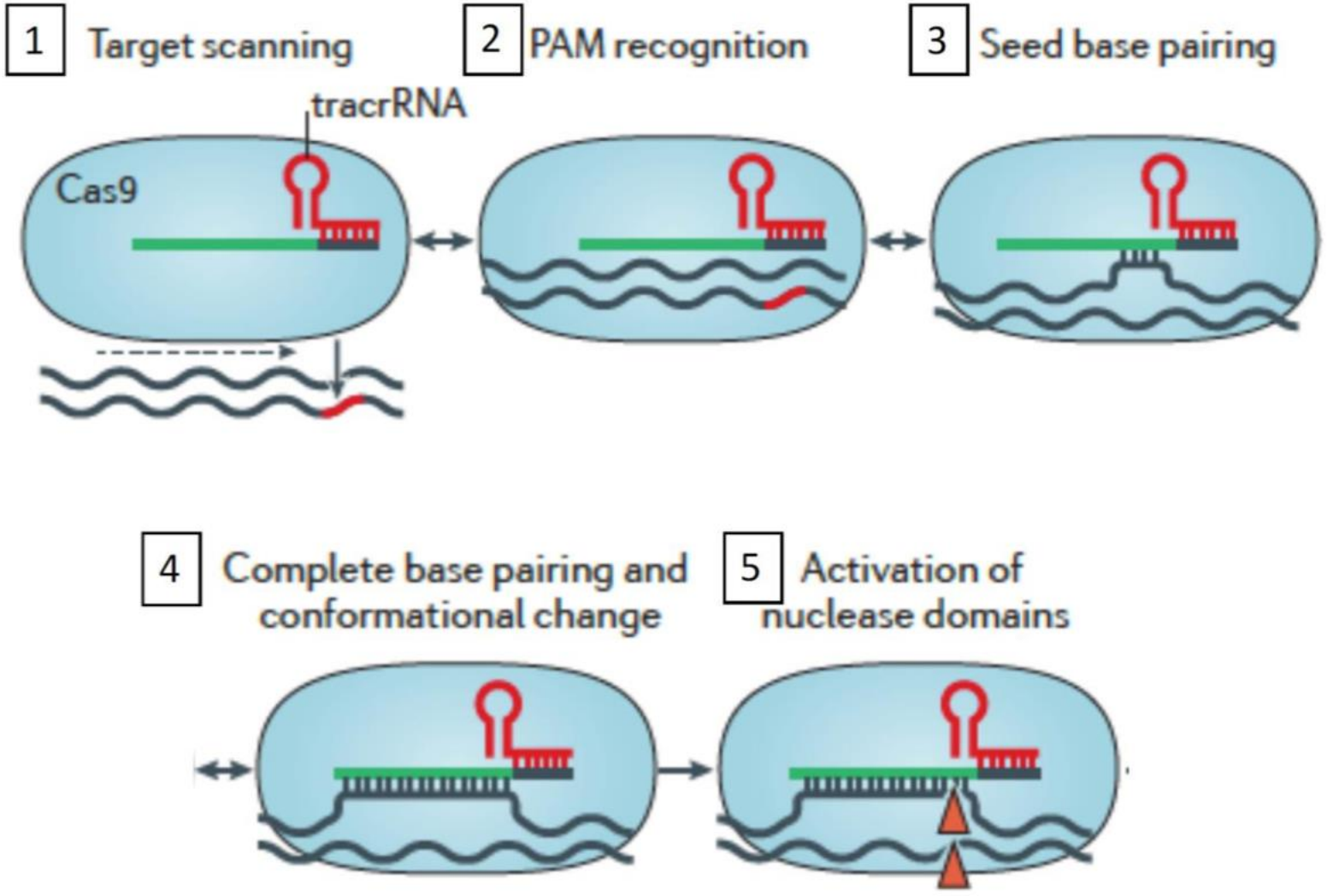
John van der Oost et al. ,Nature Reviewers 2014

CRISPR-Cas gene editing tools

Cas9 proteins rely on RNA guidance for targeting specificity. In engineered CRISPR–Cas9 systems, Cas9 interacts with the backbone of the guide RNA (gRNA). Complementary pairing of the spacer portion of the gRNA to a DNA target sequence positioned next to a PAM results in generation of a blunt DNA double-strand break by the two Cas9 nuclease domains, RuvC and HNH.

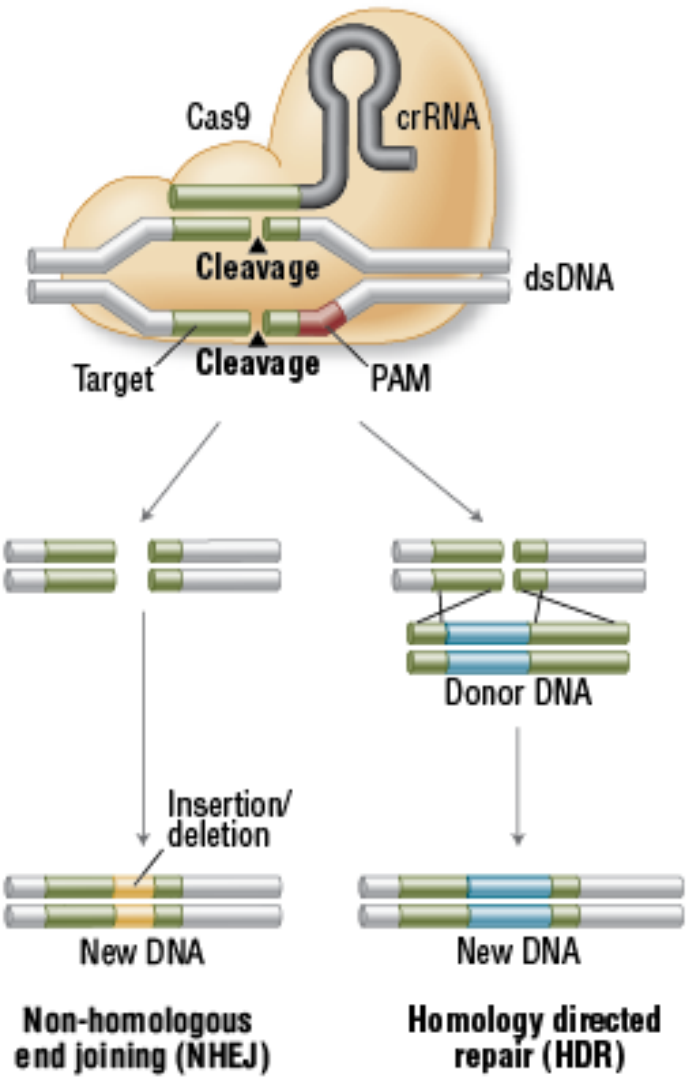


The process

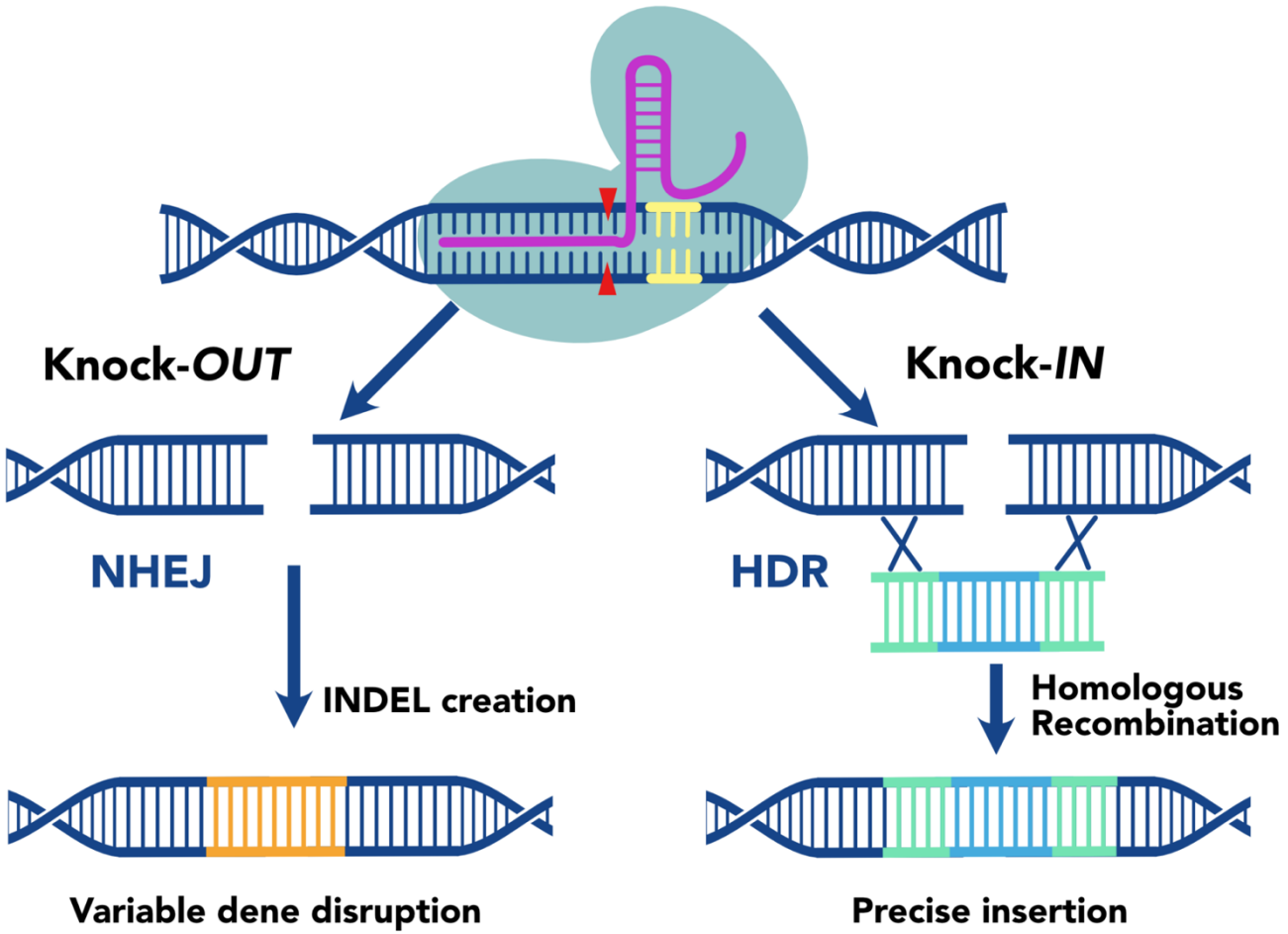

1
Target scanning
2
PAM recognition
3
Seed base pairing
tracrRNA
Cas9
4
Complete base pairing and conformational change
5
Activation of nuclease domains

The CRISPR/Cas9 system

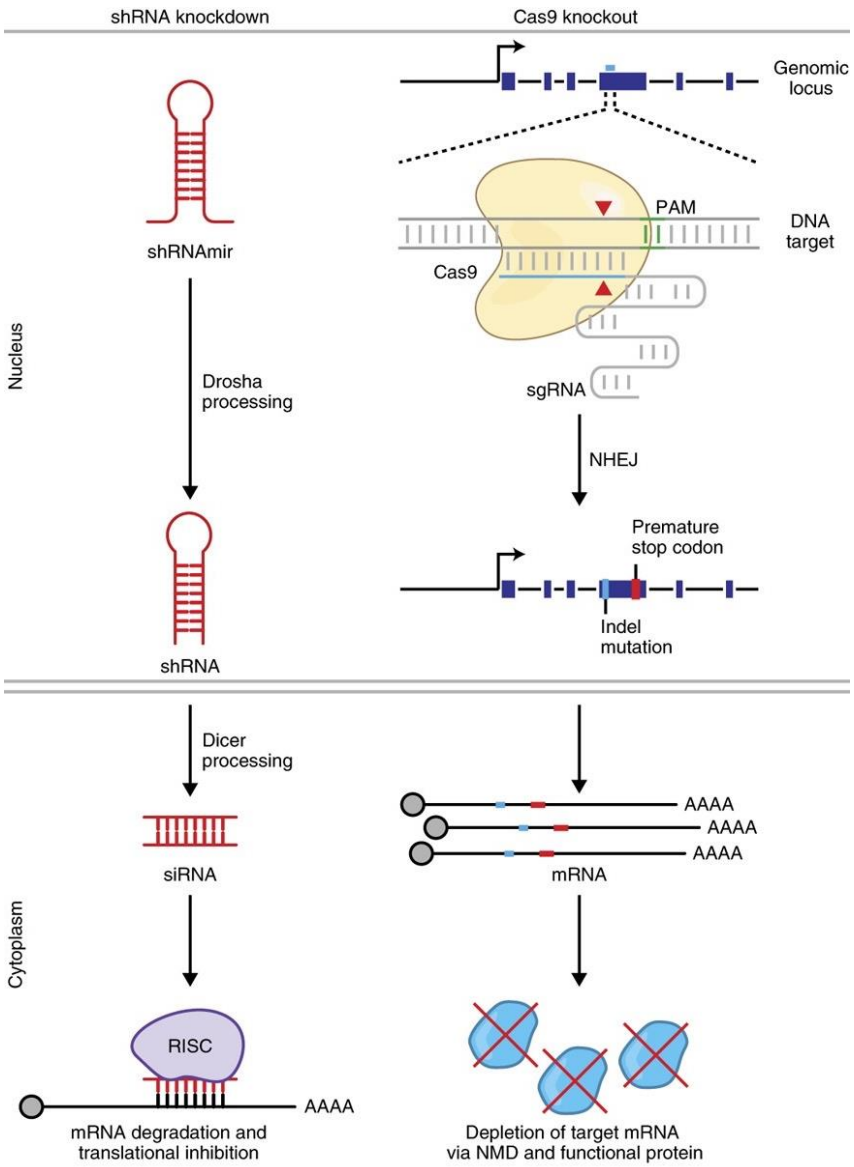
The **CRISPR/Cas system** can thereby be retargeted to cleave virtually any DNA sequence by redesigning the crRNA. Significantly, the CRISPR/Cas system has been shown to be directly portable to human cells by co-delivery of plasmids expressing the Cas9 endonuclease and the necessary crRNA components. New plasmids for genome editing are easily prepared by cloning 20-bp guide DNA sequences in a vector that encodes crRNA. Complicated protein engineering is not necessary for making new RGENs because Cas9 remains the same.



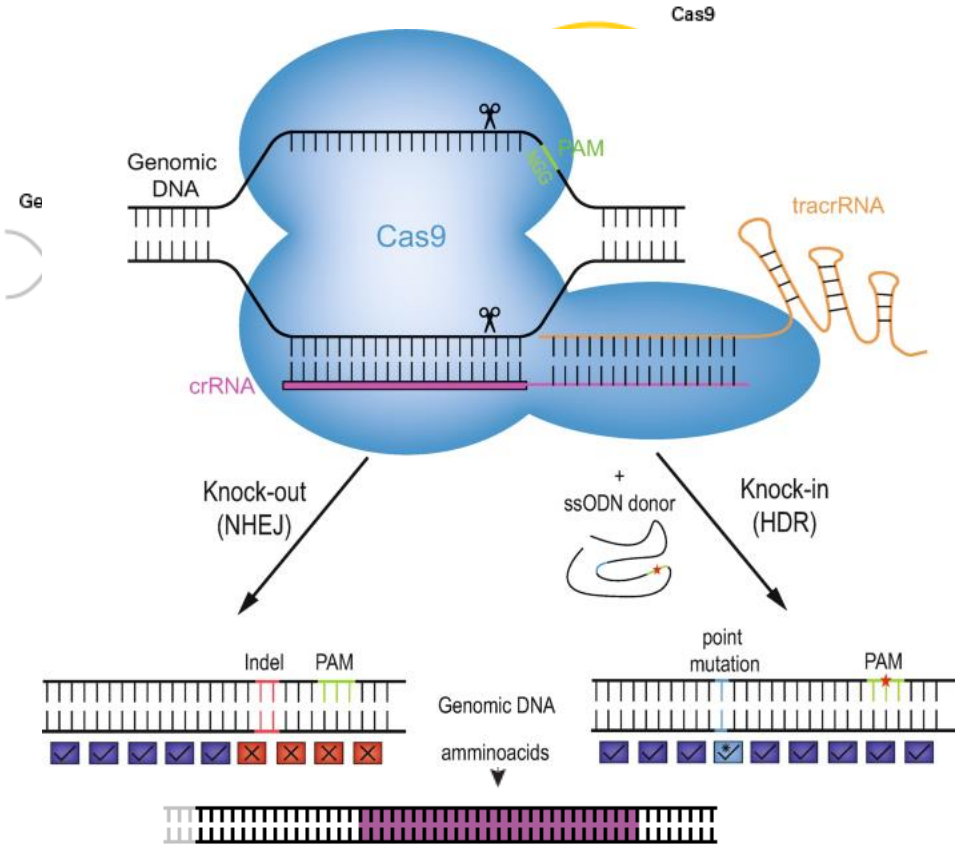
The CRISPR/Cas9 system



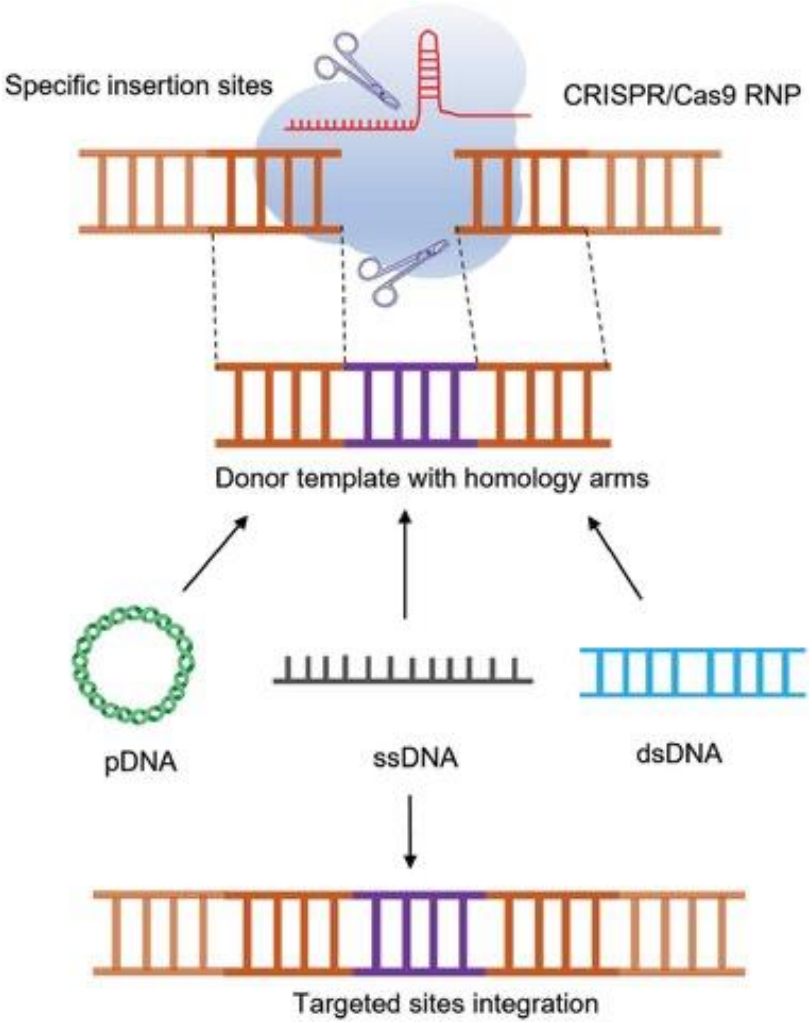
RNAi *vs* Cas9 mediated KO



CRISPR/Cas9-mediated knock-in

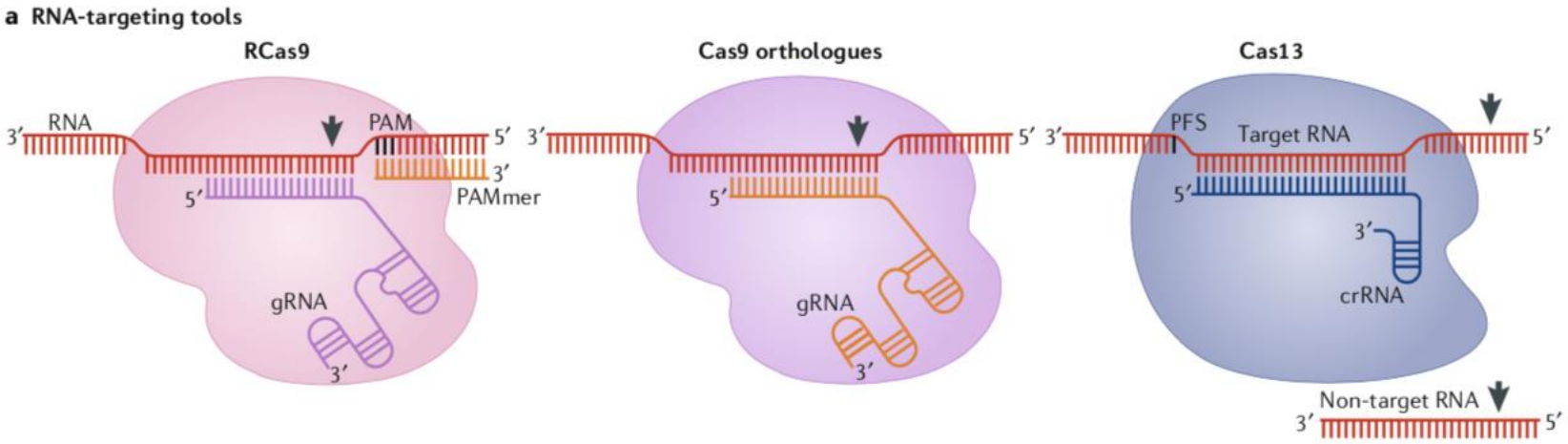


CRISPR/Cas9-mediated knock-in



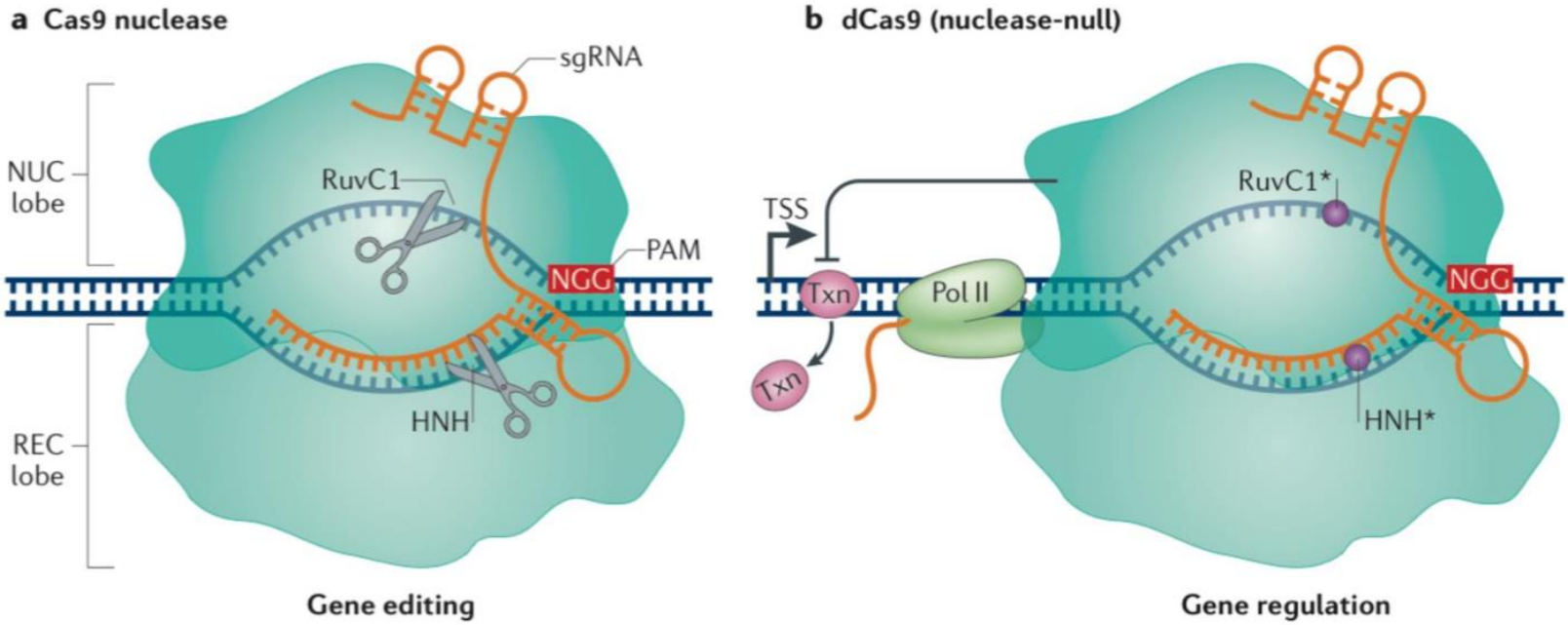
RNA-targeting tools

Streptococcus pyogenes Cas9 was repurposed to target RNA (**RCas9**) by providing it with a matching guide RNA (gRNA) and a complementary protospacer adjacent motif (PAM)-presenting oligonucleotide (PAMmer). Cas9 orthologues such as *Staphylococcus aureus* Cas9 and *Campylobacter jejuni* Cas9 can target RNA in the absence of a PAMmer, thereby demonstrating PAM-independent RNA cleavage. **Cas13** proteins are RNA-guided RNA-targeting nucleases, some requiring recognition of a protospacer flanking sequence (PFS). Cas13 generates cuts along target and non-target RNA molecules using two HEPN domains, which are nucleotide-binding domains with RNA-cutting activity.



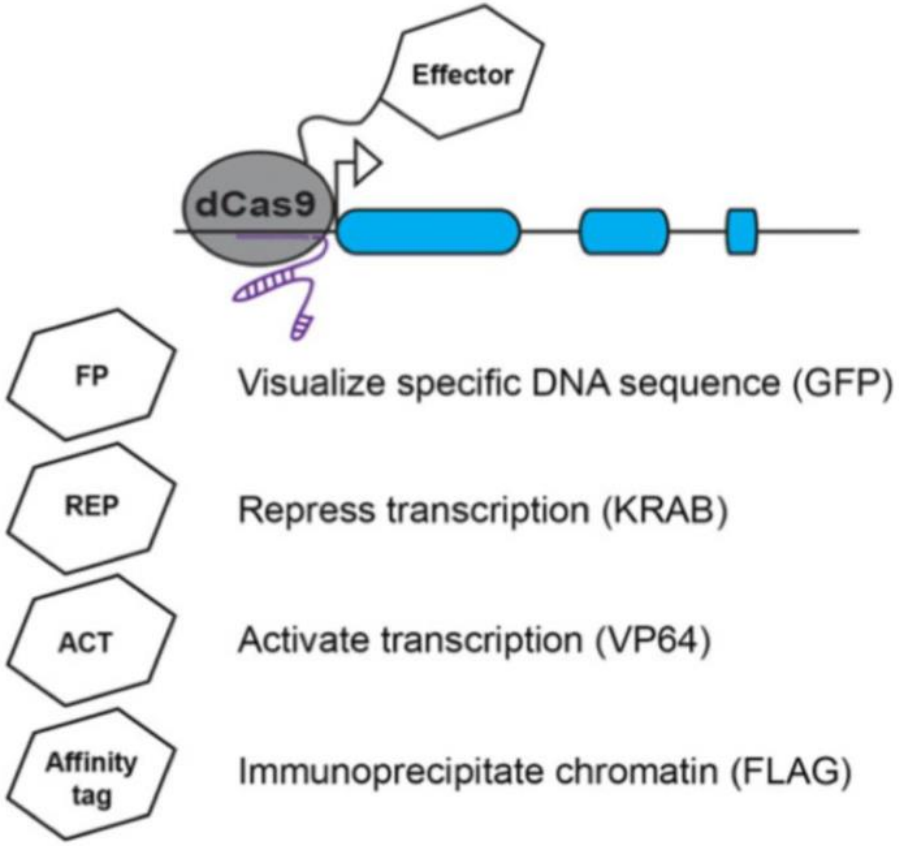
Nuclease-deficient Cas9

dCas9 protein contains mutation in its RuvC1 and HNH domanis, which inactive its nuclease function. dCas9 retains the ability to target specific sequnces through the sgRNA and Pam.



Nuclease-deficient Cas9

Catalytically inactive dCas9 provides a platform for probing genomic function. **dCas9 can be fused to any number of different effectors** to allow for the visualization of where specific DNA sequences localize, the repression or activation of transcription, or the immunoprecipitation of the bound chromatin.

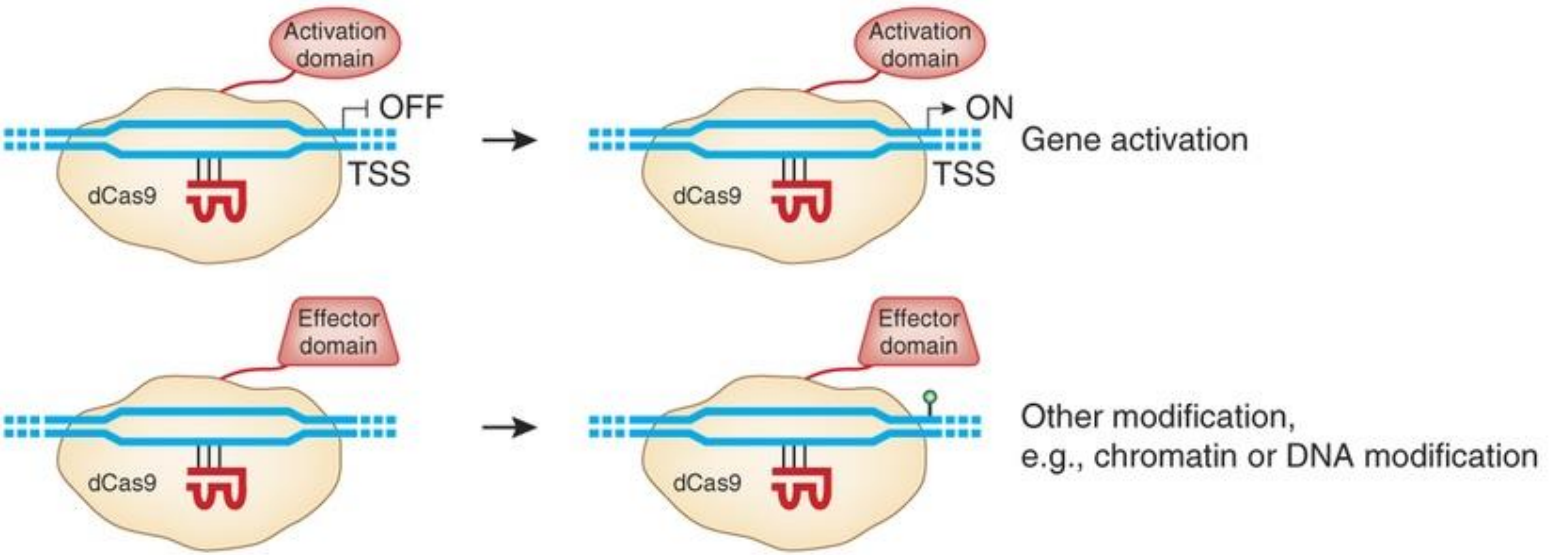


Melissa M. et al., GENES & DEVELOPMENT 2014

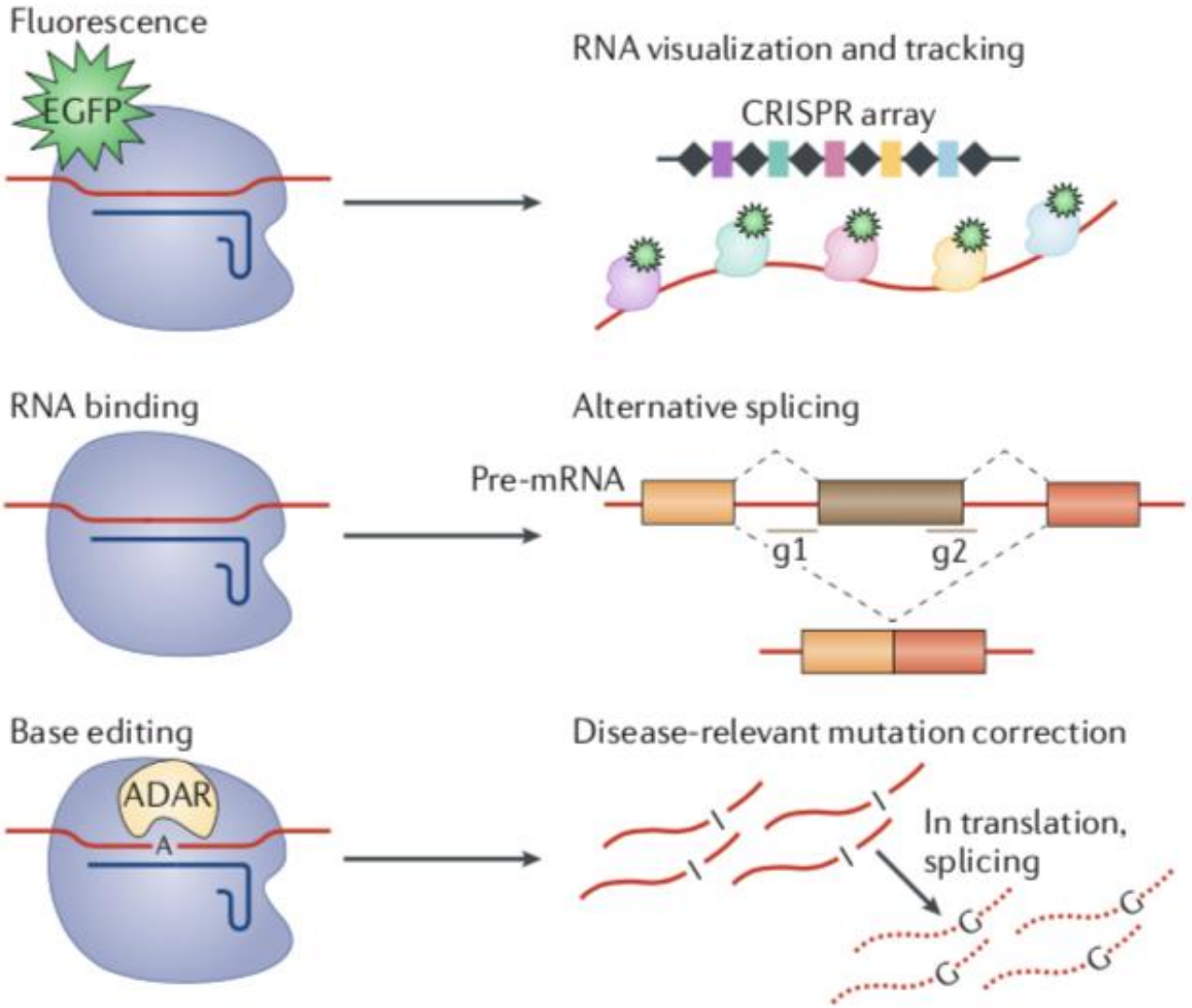
Catalytically inactive applications

A programmable DNA-binding protein that can recruit an effector domain to turn transcription on and off in a dynamic and quantitative manner offers, in principle, a more flexible tool for interrogating the many transcripts in complex genomes.

dCas9 fusion proteins guided by gene-specific sgRNAs con be use to localize effector domains to specific DNA sequences to either repress or activate transcription of target genes.



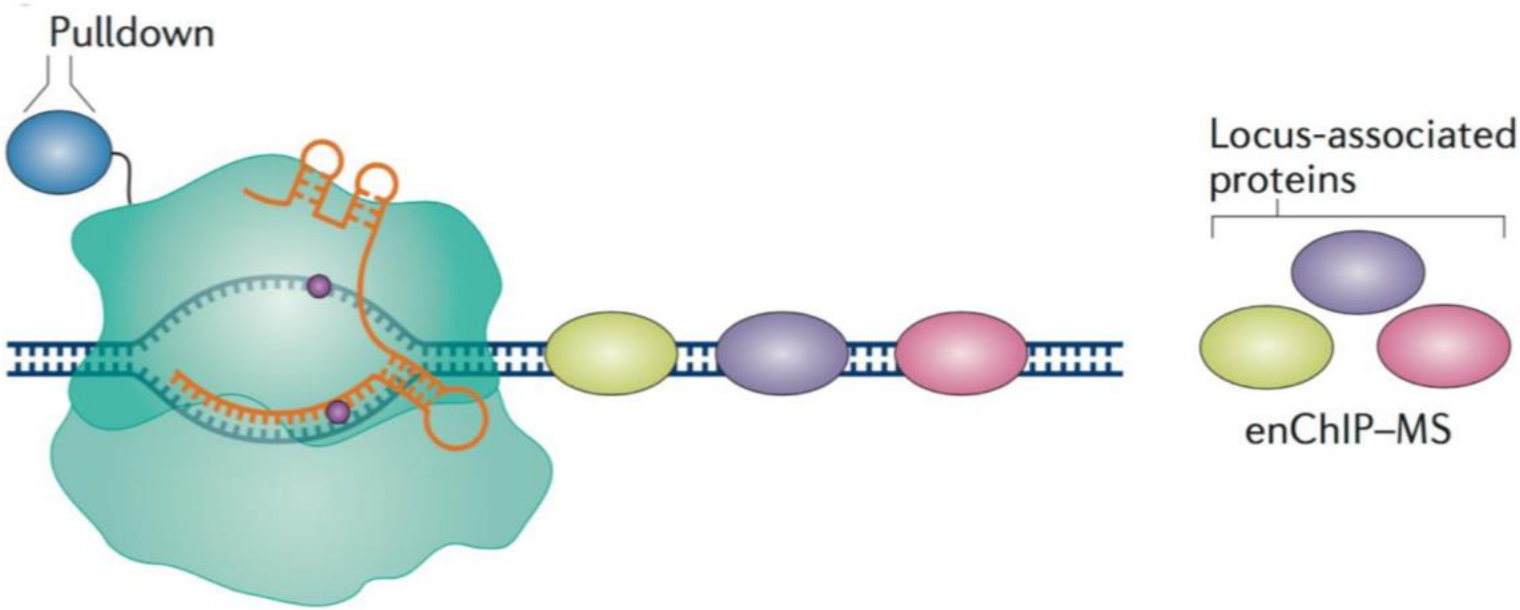
Catalytically inactive applications



Catalytically inactive applications

Identification of target specific interacting proteins

Immunoprecipitation with an antibody against **tagged dCas9** targeted to a specific genomic locus by a sgRNA (known as engineered DNA-binding molecule-mediated chromatin immunoprecipitation) followed by mass spectrometry (enChIP–MS), allowed the identification of target-specific interacting proteins



Cas9 proteins

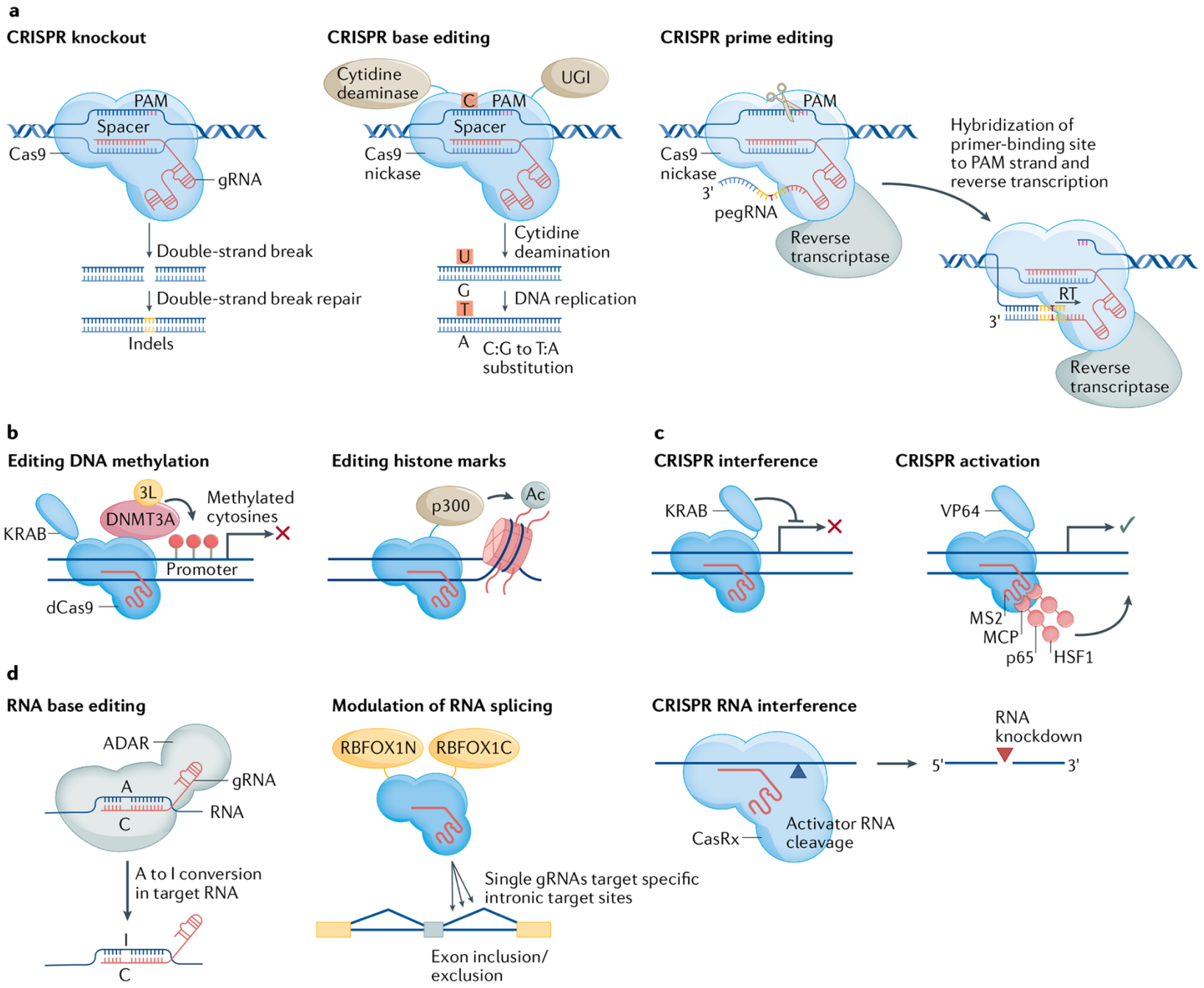
	Cut	Wild type Cas9 efficiently generates double strand breaks (DSBs) at sequences homologous to co-expressed gRNA.
	Nick	A mutated "nickase" version of the Cas9 enzyme generates a single-strand DNA break (Nick), instead of a double-strand DNA break (Cut).
	Interfere	A catalytically inactive Cas9 (dCas9) can knockdown gene expression by interfering with transcription. The dCas9 can sometimes be fused to an additional repressor peptide.
	Activate	A catalytically inactive Cas9 (dCas9) fused to an activator peptide can activate or increase gene expression.
	dCas9-FokI	A catalytically inactive Cas9 (dCas9) fused to FokI nuclease to generate double strand breaks (DSBs, Cut) at sequences homologous to two co-expressed gRNA.
	Purify	Isolate specific genomic regions of interest using a catalytically inactive Cas9 (dCas9) fused with an epitope tag(s).
	Visualize	Visualize specific genomic regions of interest using a catalytically inactive Cas9 (dCas9) fused to a fluorescent protein.
	Tag	Find the tools for tagging your endogenous protein of interest.
	Screen	Use pooled CRISPR libraries to screen for genes involved in specific biological processes.

Cas9

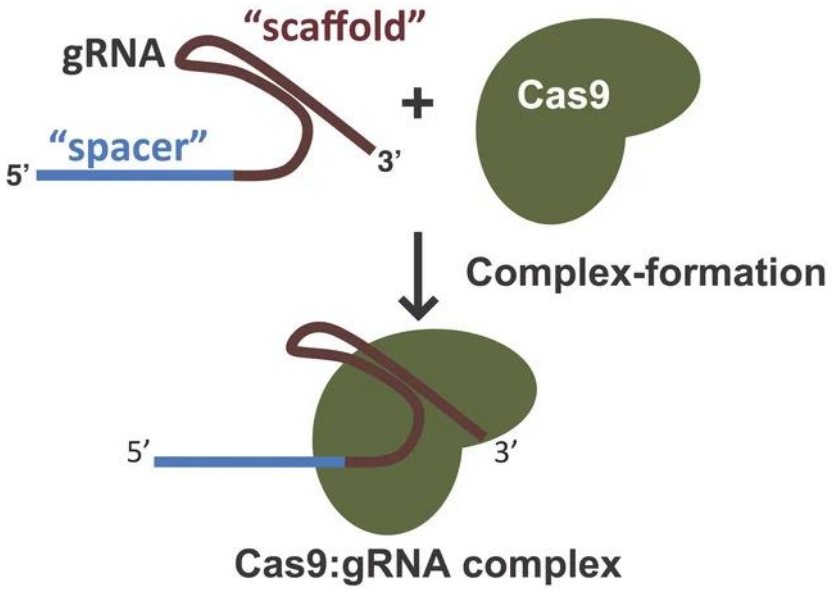
nCas9

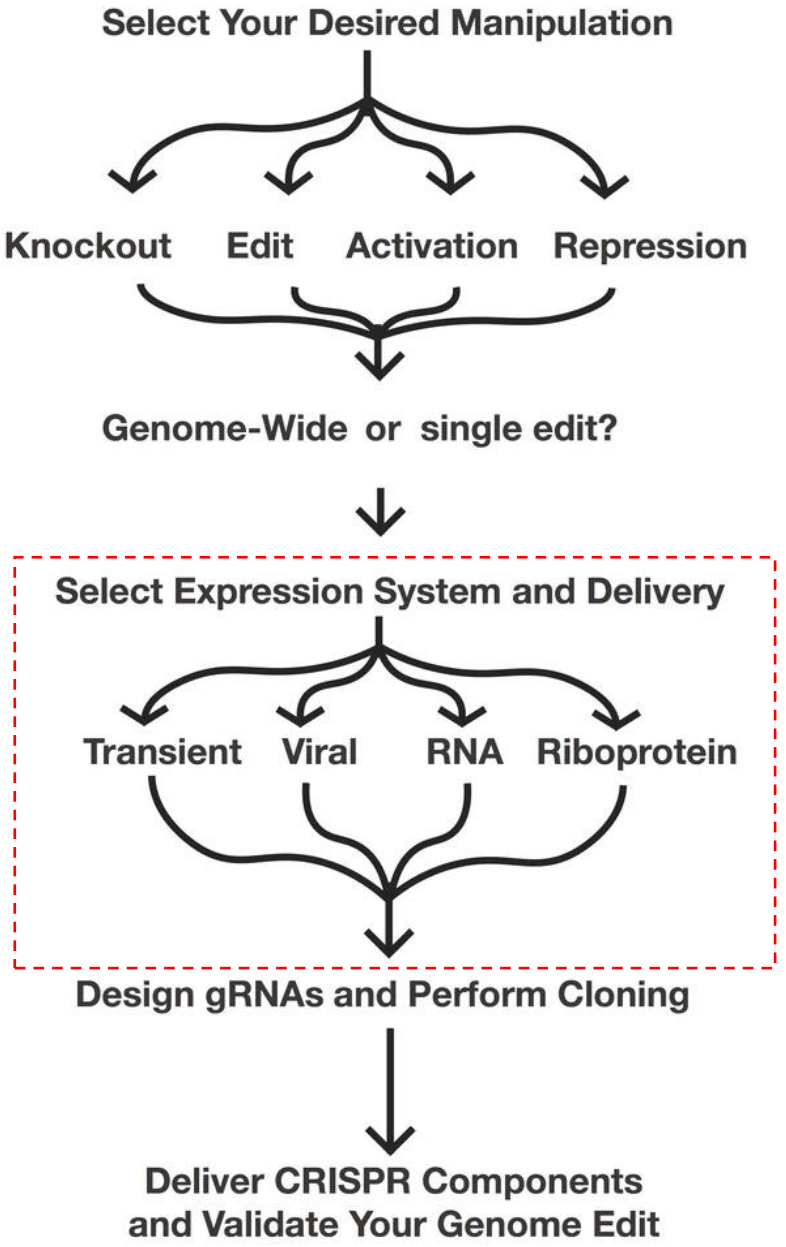
dCas9

Modified by Addgene

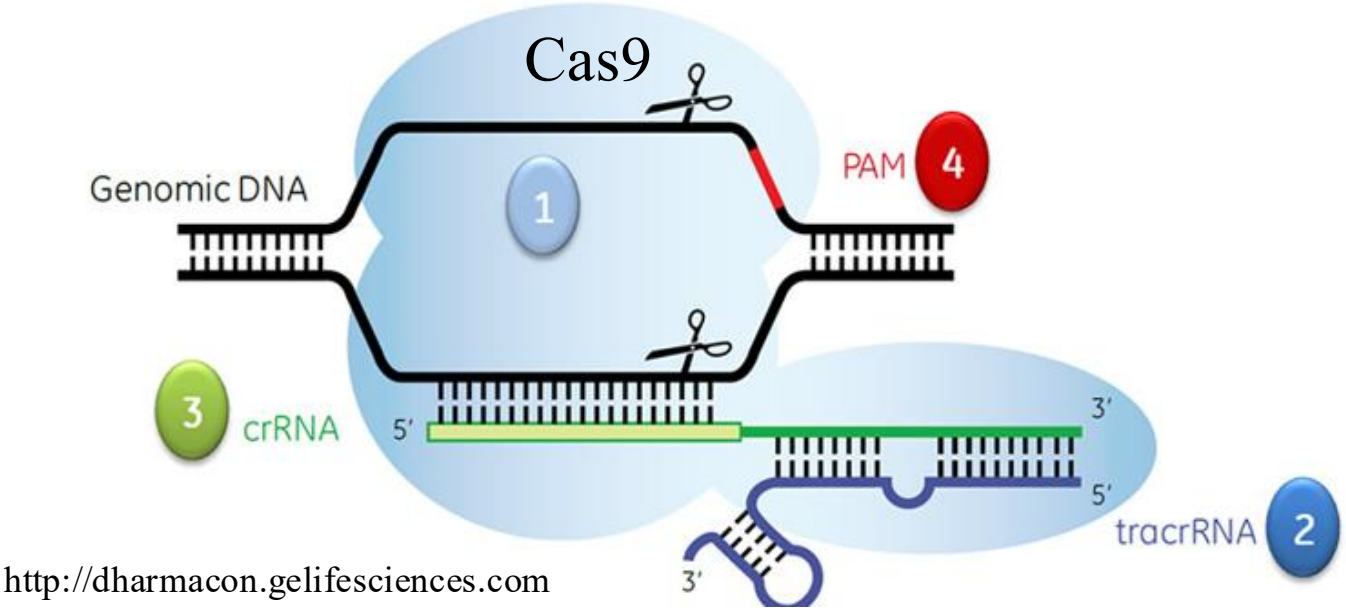

a
CRISPR knockout
PAM
Spacer
Cas9
gRNA
Double-strand break
Double-strand break repair
Indels
CRISPR base editing
Cytidine deaminase
UGI
C
PAM
Spacer
Cas9 nickase
Cytidine deamination
U
G
T
DNA replication
A
C:G to T:A substitution
CRISPR prime editing
PAM
Cas9 nickase
3'
pegRNA
Reverse transcriptase
Hybridization of primer-binding site to PAM strand and reverse transcription
RT
3'
Reverse transcriptase
b
Editing DNA methylation
3L
DNMT3A
Methylated cytosines
KRAB
Promoter
dCas9
Editing histone marks
p300
Ac
c
CRISPR interference
KRAB
CRISPR activation
VP64
MS2
MCP
p65
HSF1
d
RNA base editing
ADAR
A
gRNA
RNA
C
A to I conversion in target RNA
I
C
Modulation of RNA splicing
RBFOX1N
RBFOX1C
Single gRNAs target specific intronic target sites
Exon inclusion/ exclusion
CRISPR RNA interference
CasRx
Activator RNA cleavage
RNA knockdown
5'
3'

What do you need?





Gene editing with the CRISPR/Cas9 system

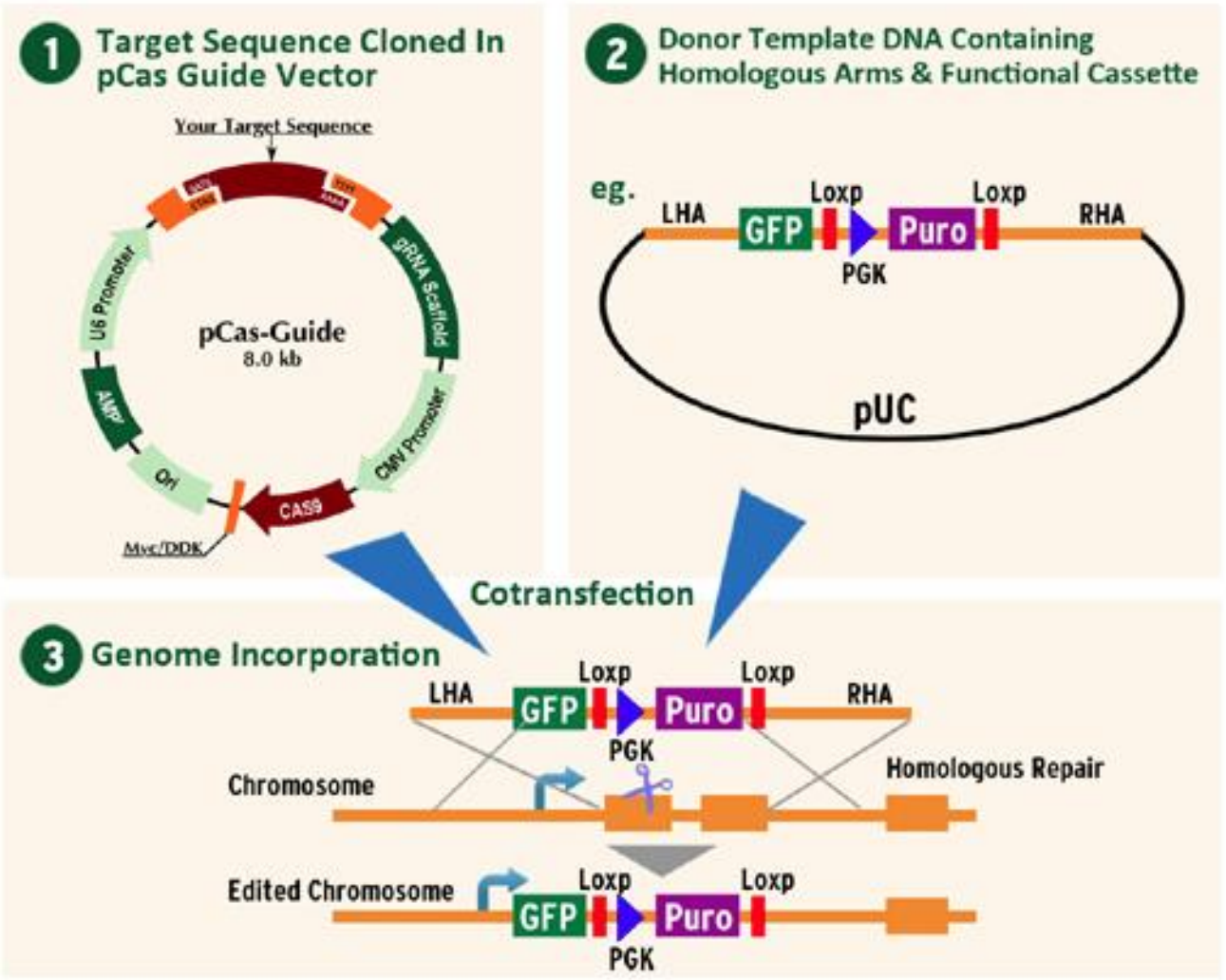


http://dharmacon.gelifesciences.com

1. A plasmid expressing a mammalian codon-optimized gene sequence encoding Cas9 nuclease or Cas9 protein
2. A chemically synthesized trans-activating CRISPR RNA (tracrRNA)
3. A chemically synthesized CRISPR RNA (crRNA) designed to cleave the gene target site of interest (the chosen 20-base target sequence in the gene must be immediately upstream of a PAM in the genomic DNA, the predominant PAM nucleotide sequence is NGG)

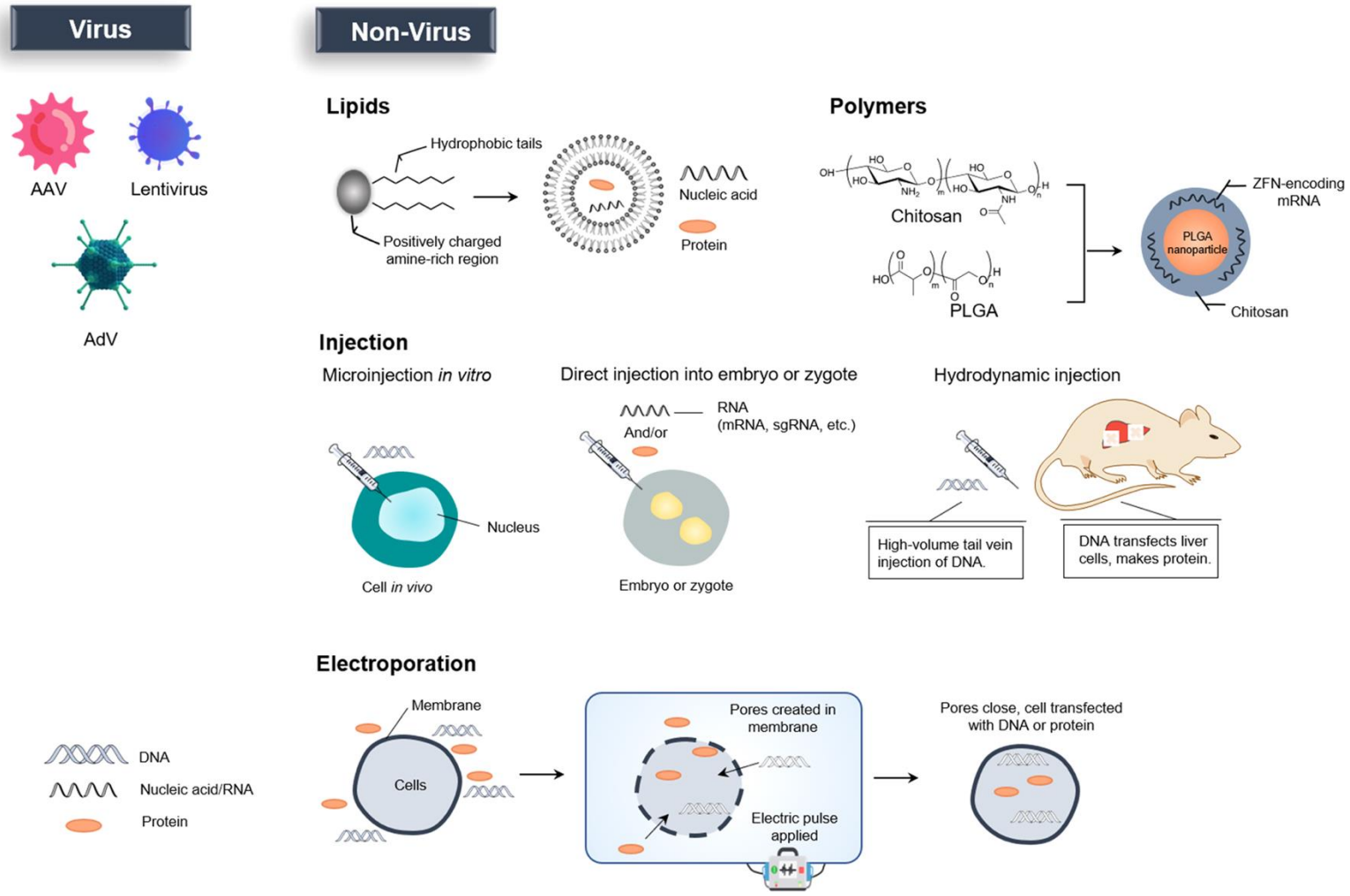
Gene editing with the CRISPR/Cas9 system

The Cas9, tracrRNA and crRNA can be combined in a single plasmid (1)

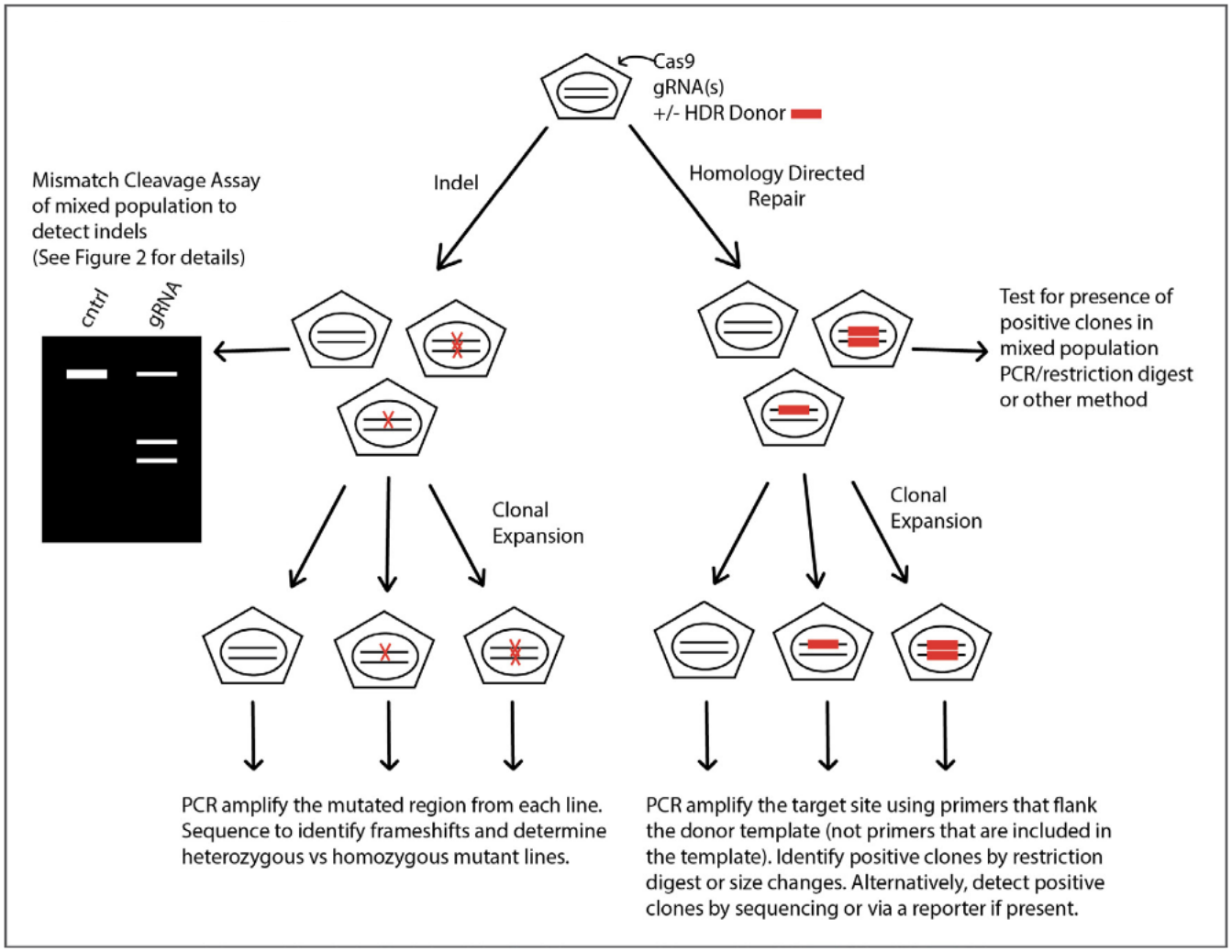


www.origene.com

Viral and nonviral delivery systems for genome editing technology



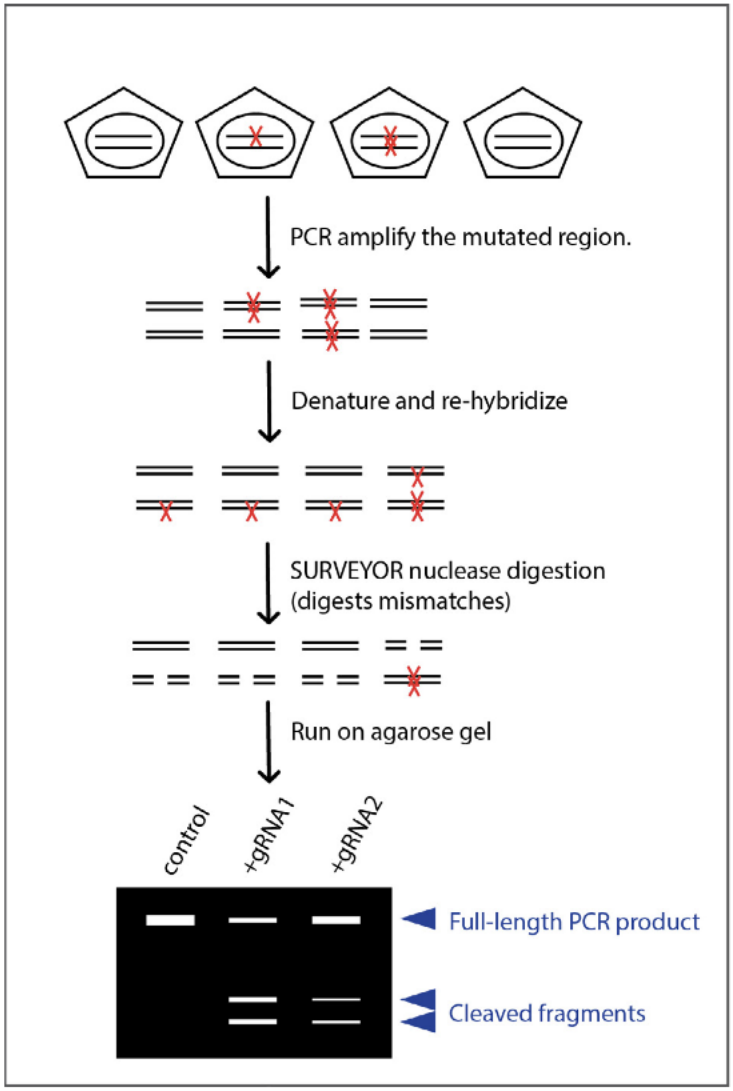
Validating your genome editing



Validating your genome editing

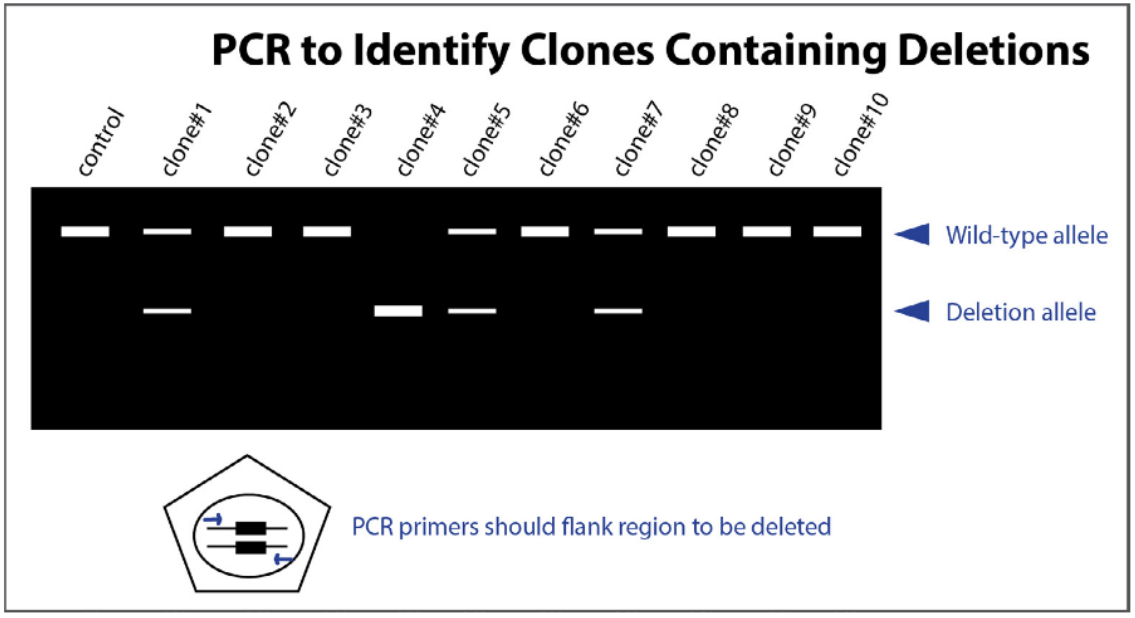
Mismatch cleavage assay:

Surveyor nuclease is commonly used for this purpose, as it cleaves both DNA strands 3' to any mismatches.
Mismatch cleavage assays typically consist of four steps: 1) PCR amplify the region of interest, 2) denature the strands and rehybridize to allow for the mutant and wild-type strands to anneal, 3) treat annealed DNA with Surveyor nuclease to cleave heteroduplexes, and 4) analyze DNA on an agarose gel or other instrument that separates DNA based on size.
This assay is often used semi-quantitatively, and in this case, gRNA1 appears to be more efficient at producing indels than gRNA2.

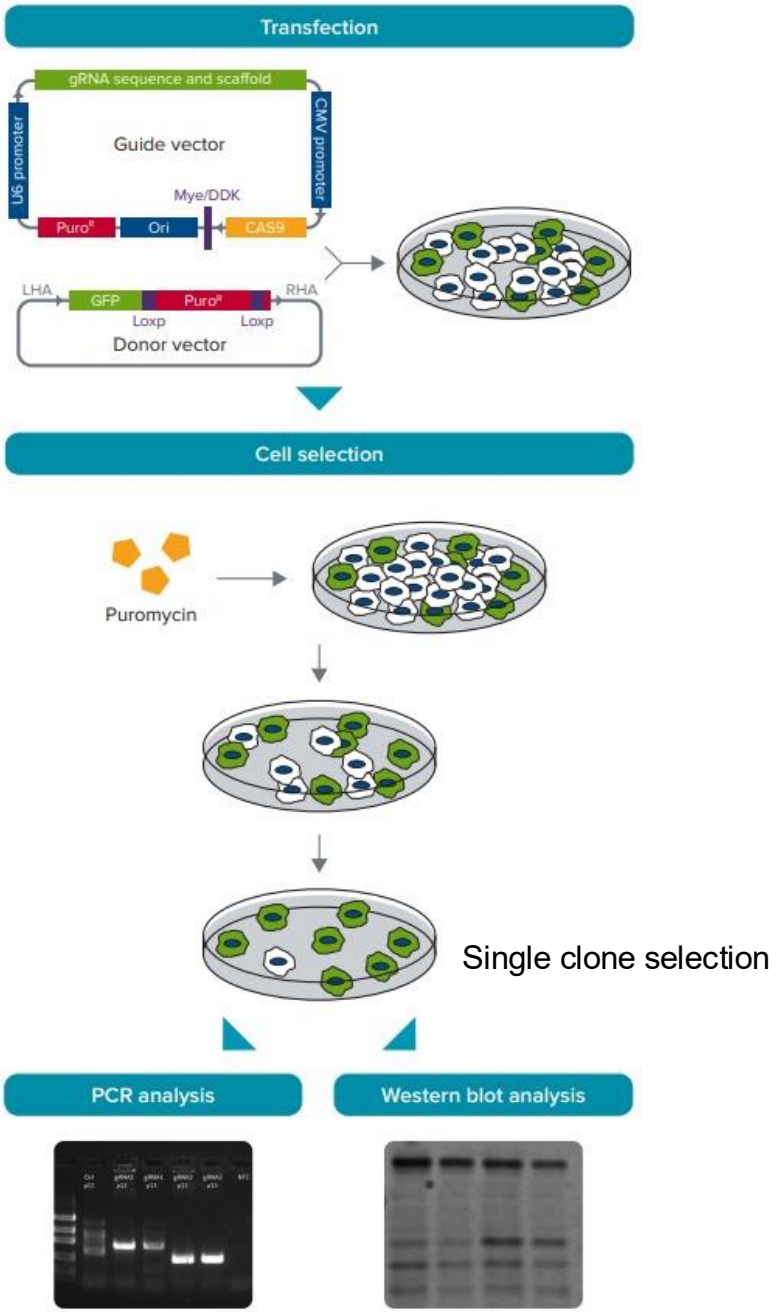


Validating your genome editing

HR events are generally less frequent than indels, so you will likely need to screen a larger number of colonies to create a clonal line. The number of clones that need to be screened will depend on both your transfection/transduction efficiency and HR frequency. For example, if you have 40% transfection efficiency and 5% HR efficiency, approximately 0.4 x 0.05= 0.02 or 2% of your cells will have the recombined region. Thus, you should plan to screen at least 50 colonies. If you are able to select cells that have been successfully transfected/transduced using a marker, then you may be able to test fewer colonies.

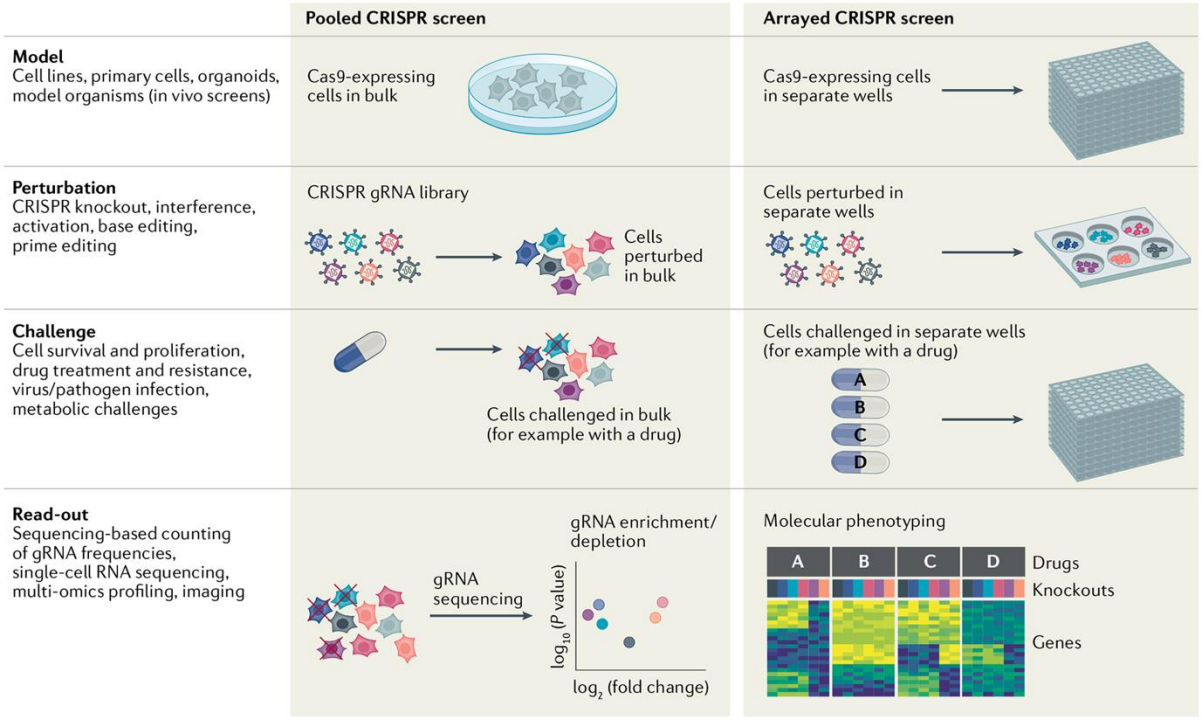


CRISPR/Cas9 knock-in experimental workflow



CRISPR screens

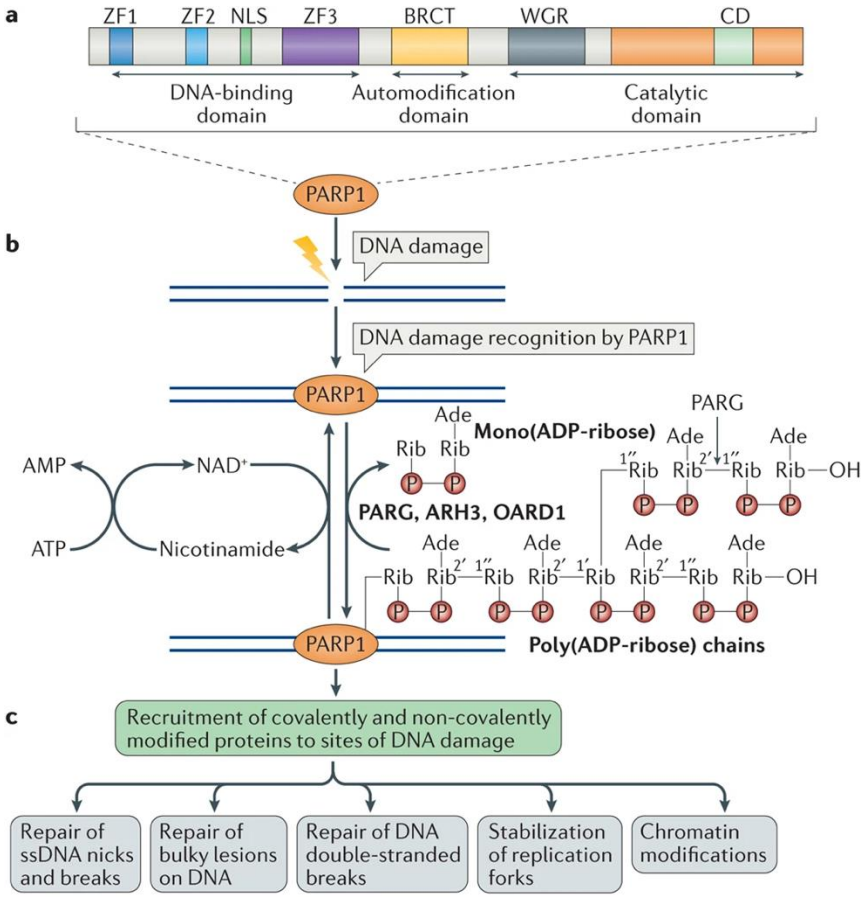
Pooled CRISPR screening is a high-throughput functional genomics strategy to systematically interrogate gene function across a population of cells. A gRNA library is introduced at a low multiplicity of infection via lentiviral integration, pairing specific genetic perturbations with stable nucleic acid barcodes. Following Cas9-mediated gene disruption, cells undergo phenotypic or selective pressure (e.g., drug exposure, pathogen challenge, or long-term proliferation). Next-generation sequencing (NGS) quantifies gRNA abundance post-selection: depleted gRNAs reveal essential genes or drug sensitizers (negative selection), while enriched gRNAs highlight tumor suppressors or resistance factors (positive selection).



CRISPR screens

Experimental Scenario

Identify genes modulating sensitivity to the PARP1 inhibitor Olaparib in BRCA1-mutant cells stably expressing SpCas9.



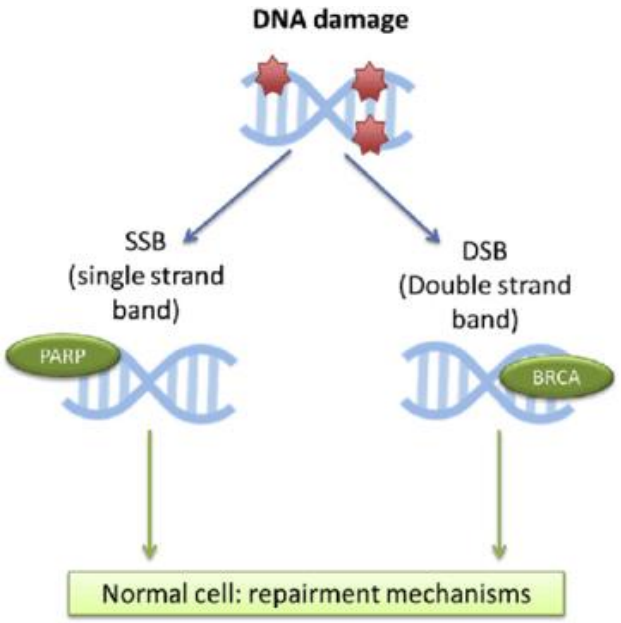
Nature Reviews | Molecular Cell Biology

CRISPR screens

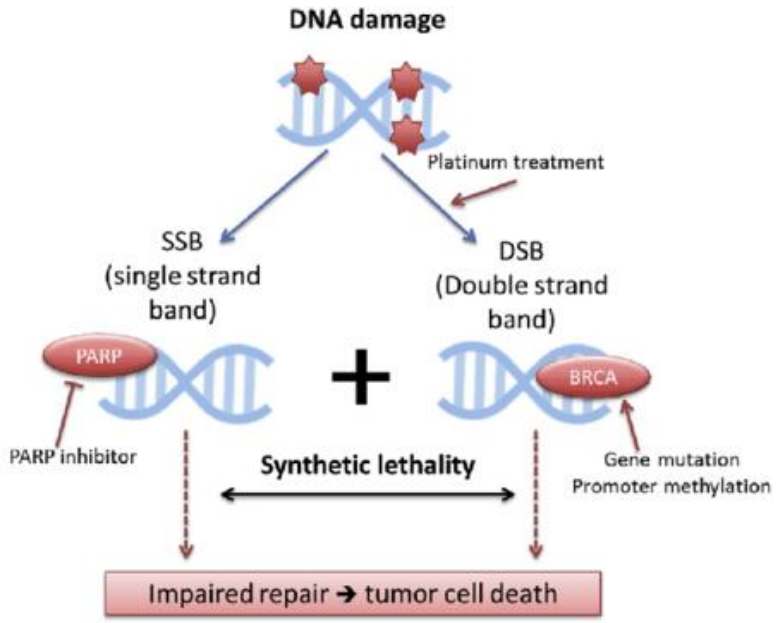
Experimental Scenario

Identify genes modulating sensitivity to the PARP1 inhibitor Olaparib in BRCA1-mutant cells stably expressing SpCas9.

Homologous recombination proficiency (HRP) status



Homologous recombination deficiency (HRD) status



CRISPR screens

Experimental Scenario

Identify genes modulating sensitivity to the PARP1 inhibitor Olaparib in BRCA1-mutant cells stably expressing SpCas9.

Genome-wide lentiviral library with 6 gRNAs per gene plus 500 non-targeting controls.

Unique gRNAs 10,000 genes + 500 NTCs	**60,000**

Screening Workflow

01

DAY 0

Transduction

Bulk lentiviral delivery at MOI 0.3.

02

48 HOURS

Selection

Puromycin removes uninfected cells.

03

14 DAYS

Drug Challenge

T0 baseline vs. DMSO or Olaparib IC30.

04

ENDPOINT

NGS Readout

gDNA isolation and gRNA sequencing.

CRISPR screens

NGS Differential Abundance

Gene Target	Hit gRNAs	log2FC (DMSO vs. T0)	log2FC (Olaparib vs. DMSO)	FDR
NTC (Controls)	0 / 6	-0.05	+0.02	0.94
RPL11	6 / 6 (vs. T0)	**-4.60**	-0.15	0.78
RNASEH2A	5 / 6	-0.25	**-3.85**	**< 0.0001**
TP53BP1	6 / 6	+0.10	**+3.70**	**< 0.0001**
OR2A1	0 / 6	+0.04	-0.08	0.91
GENE_X	1 / 6	-0.10	-4.90 (1 gRNA)	0.34

NTC & OR2A1: Neutral controls confirming absence of Cas9 cutting toxicity.

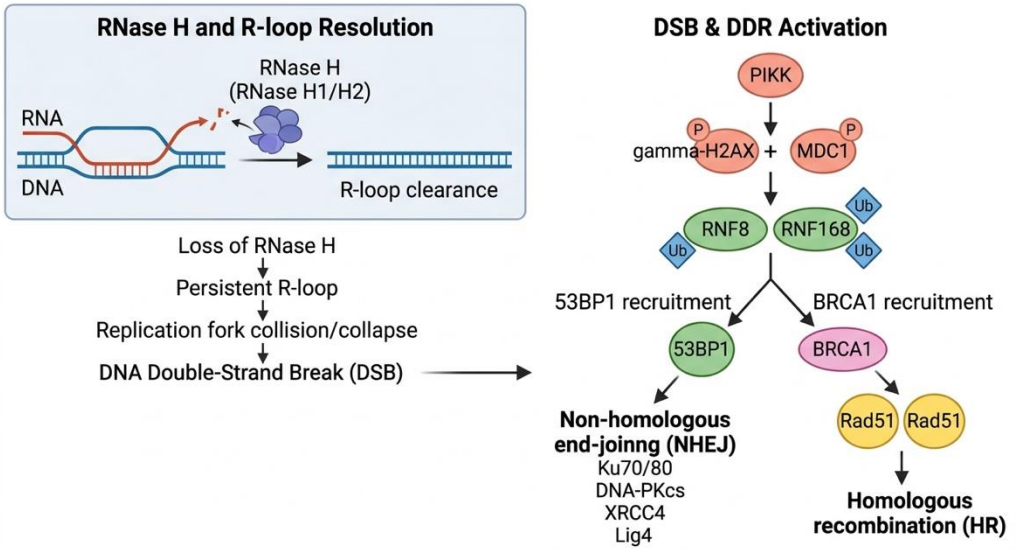
RPL11: Core essential ribosomal gene depleted independently of Olaparib.

RNASEH2A: Synthetic lethal sensitizer depleted selectively under Olaparib.

TP53BP1: Resistance factor enriched under Olaparib selective pressure.

GENE_X: Off-target false positive (single gRNA hit, FDR 0.34).

CRISPR screens



TP53BP1 LOSS → RESISTANCE

Restoration of End-Resection

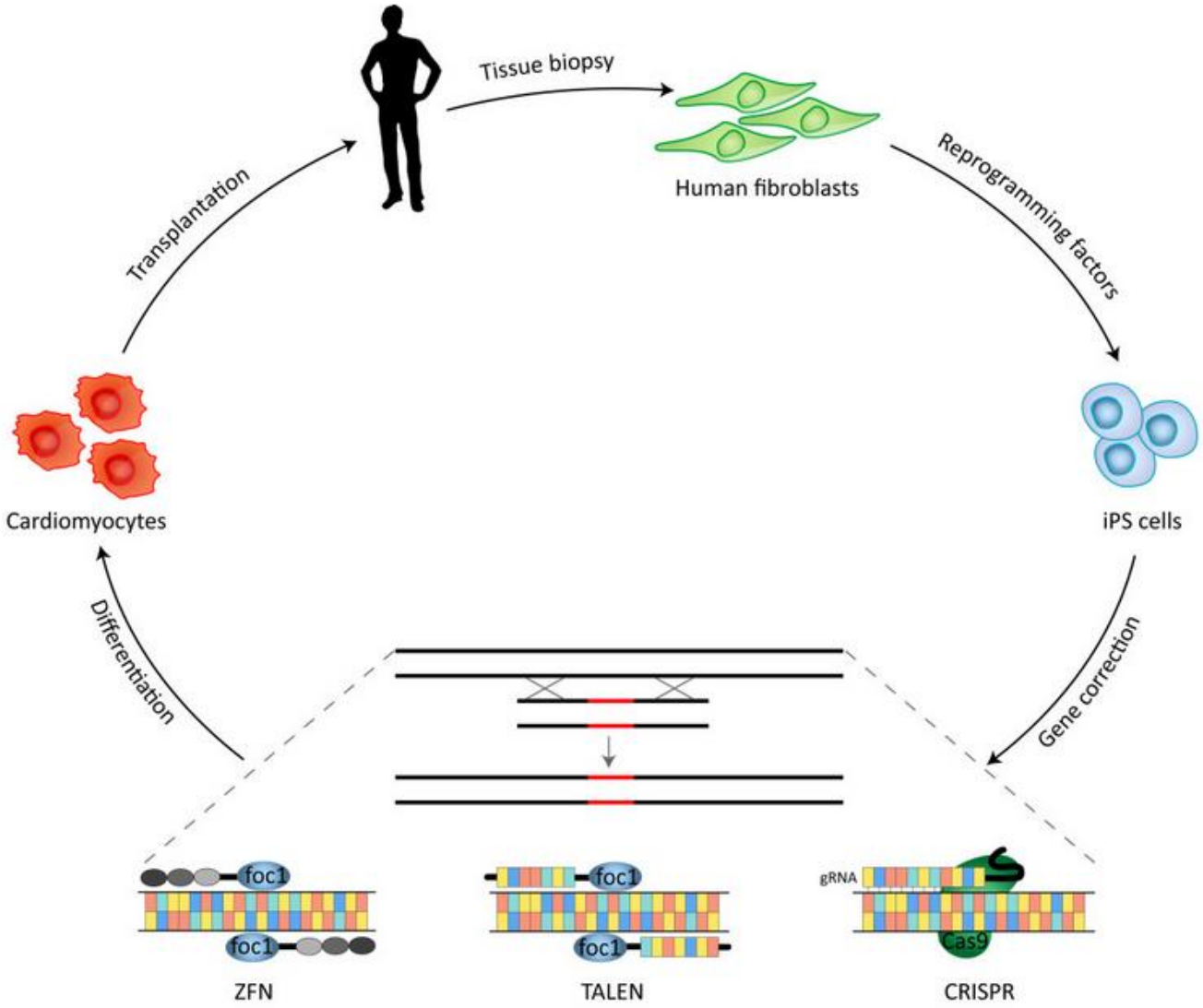
- **Resection Block Removed:** Loss of 53BP1 permits DNA end-resection.
- **HR Reactivation:** Homologous recombination resumes without BRCA1.
- **Drug Survival:** Cells repair DSBs and resist Olaparib.

RNASEH2A LOSS → SENSITIVITY

Defective Ribonucleotide Excision

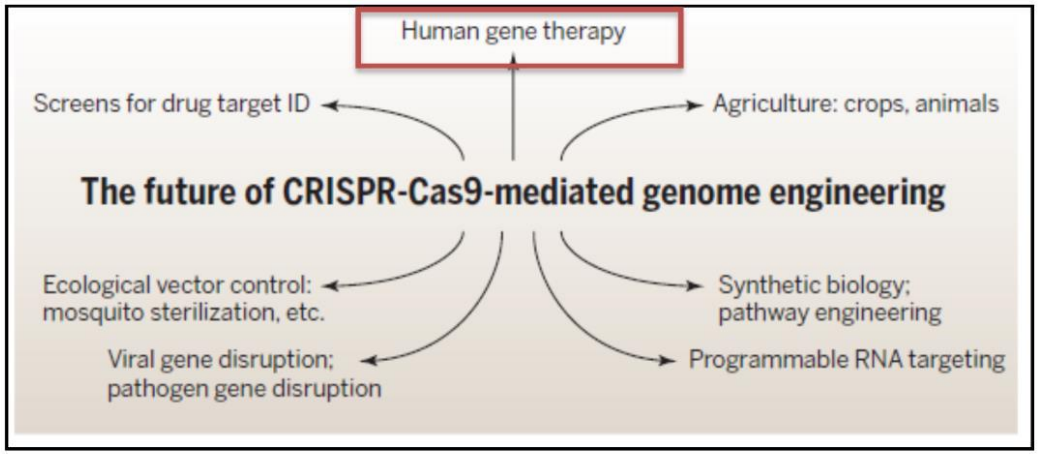
- **RNA:DNA hybrid resolution:** Embedded RNA persist in genomic DNA.
- **Top1 Cleavage:** Generates nicks requiring PARP1 for resolution.
- **PARP Trapping:** Olaparib stalls forks and triggers synthetic lethality.

Clinical applications?



Ben-David, 2013, ‘’Molecular and Cellular Therapies’’





Doudna and Charpentier, Science 2014

Application of CRISPR/Cas9 genome editing to the study and treatment of disease.

Application of genome editing technologies to the study and treatment of hematological disease

[Application progress of CRISPR/Cas9 genome editing technology in the treatment of HIV-1 infection].

[Article in Chinese]

Application Progress of CRISPR/Cas9 System for Gene Editing in Tumor Research

Chao LIU, Zhiwei LI, Yanqiao ZHANG

In vivo gene therapy potentials of CRISPR-Cas9

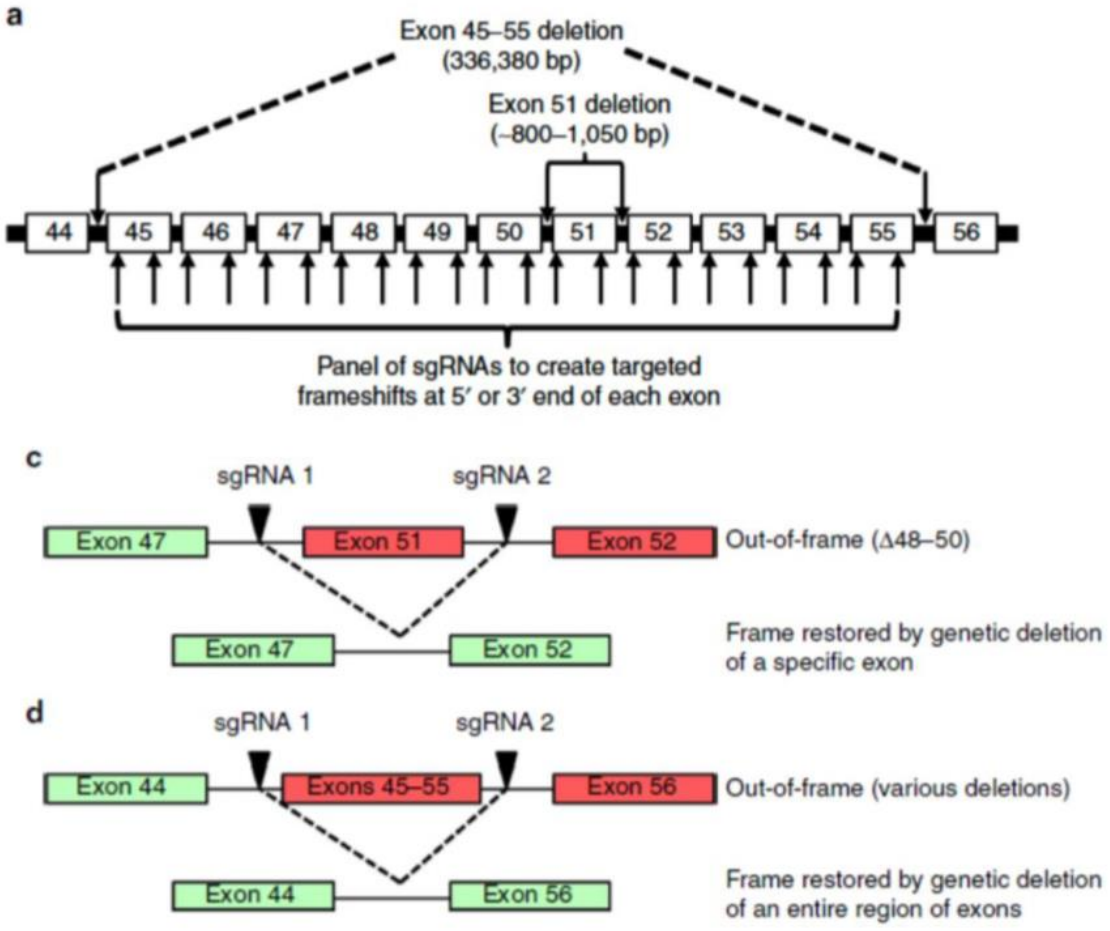
H-Y Xue[1,6], X Zhang[2,6], Y Wang[3,6], L Xiaojie[3], W-J Dai[4] and Y Xu[5]

nature COMMUNICATIONS

Multiplex CRISPR/Cas9-based genome editing for correction of dystrophin mutations that cause Duchenne muscular dystrophy

David G. Ousterout, Ami M. Kabadi, Pratiksha I. Thakore, William H. Majoros, Timothy E. Reddy & Charles A. Gersbach

Received 16 November 2014 | Accepted 08 January 2015 | Published 18 February 2015



Cell-based studies

Following gene editing in DMD Patient myoblast, dystrophin expression is restored *in vitro*. Human dystrophin is also detected *in vivo* after transplatation of genetically corrected patient cells into immunodeficient mice.

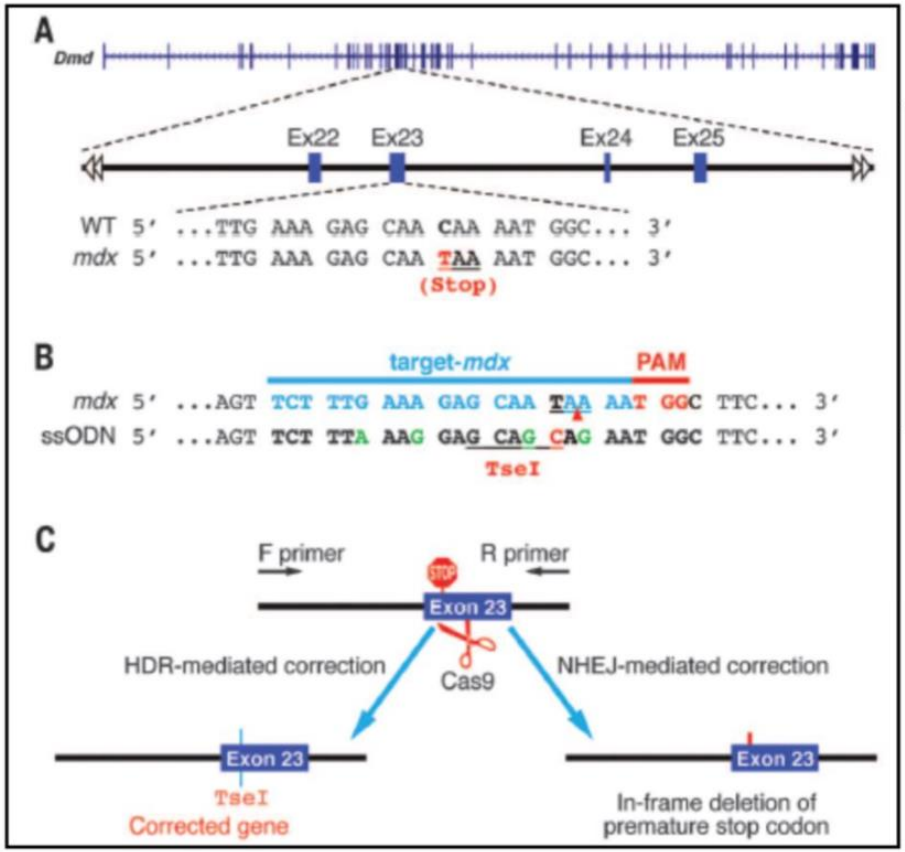
Importantly, the unique multiplex gene-editing capabilities of CRISPR-Cas9 system facilitate the generation of a single large deletion that can correct up to 62% of DMD mutations.

Science
AAAS

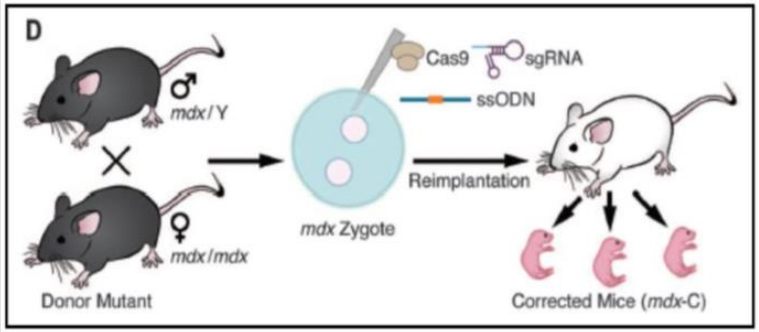
Prevention of muscular dystrophy in mice by CRISPR/Cas9–mediated editing of germline DNA

Chengzu Long *et al.*

Science **345**, 1184 (2014);
DOI: 10.1126/science.1254445



In vivo animal studies



Our results show that CRISPR/Cas9-mediated genomic editing is capable of correcting the primary genetic lesion responsible for muscular dystrophy and preventing development of characteristic features of this disease in *mdx* mice.

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]*

SCIENCE 22 JANUARY 2016 • VOL 351 ISSUE 6271

In Vivo CRISPR/Cas9 Gene Editing Corrects Retinal Dystrophy in the S334ter-3 Rat Model of Autosomal Dominant Retinitis Pigmentosa

Benjamin Bakondi[1], Wenjian Lv[1,2], Bin Lu[1], Melissa K Jones[1], Yuchun Tsai[1], Kevin J Kim[1], Rachelle Levy[1], Aslam Abbasi Akhtar[1], Joshua J Breunig[1], Clive N Svendsen[1] and Shaomei Wang[1]

www.moleculartherapy.org vol. 24 no. 3, 556–563 mar. 2016

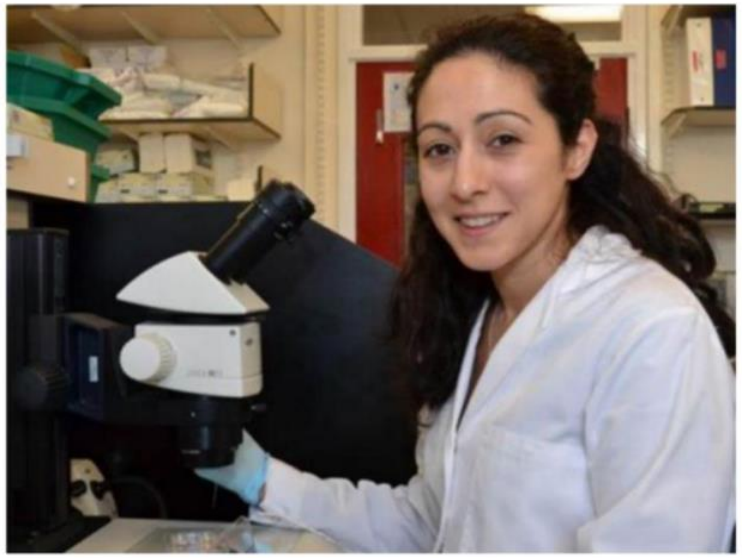
Genome editing in human embryos

UK approves CRISPR for editing human embryonic genome

Francis Crick Institute, London, 01/02/2016

Kathy Niakan, a biologist at the Francis Crick Institute, will use the Crispr/Cas9 gene-editing system to study donated embryos in the first seven days after fertilization, then discard them. Tweaking the genes in those cells will help her team understand how an embryo develops into a healthy baby, research that could lead to improved success rates for in vitro fertilization.

The licence was granted by the UK's independent Human Fertilisation and Embryology Authority (HFEA).



Dr Kathy Niakan says her research will help explain the genes needed for a human embryo to develop successfully into a healthy baby

It remains illegal for the scientists to implant the altered embryos into women, but the decision represents a huge landmark in the use of the revolutionary gene-editing technology

Genome-edited baby claim provokes international outcry

In November 2018, **He Jiankui**, a Chinese biology professor at Southern University of Science and Technology (SUST) in Shenzhen (Guangdong Province) announced that he and his team had created **the World's first "genetically edited babies"** to make them resistant to HIV: twin babies Lula and Nana.



The Infamous 'CRISPR Babies' Experiment

Jiankui and his colleagues were targeting a gene called CCR5, which is necessary for the HIV virus to enter into lymphocytes and infect our body.

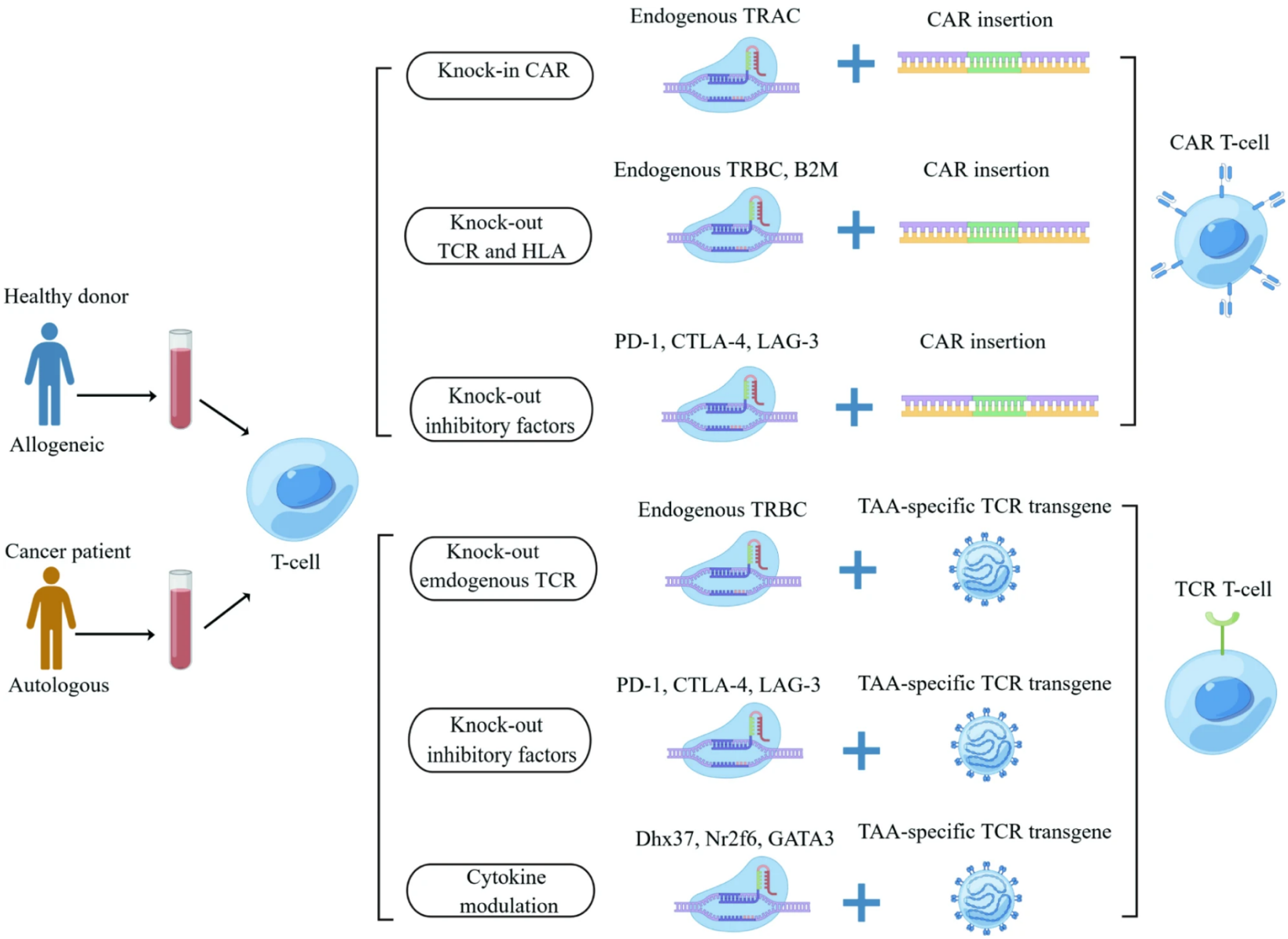
One variant of CCR5, called CCR5 Δ32, results in a high level of resistance to the most common type of HIV virus.

despite claiming in the abstract of their **unpublished article** that they reproduced the human CCR5 mutation, in reality the team tried to modify CCR5 *close* to the Δ32 mutation.

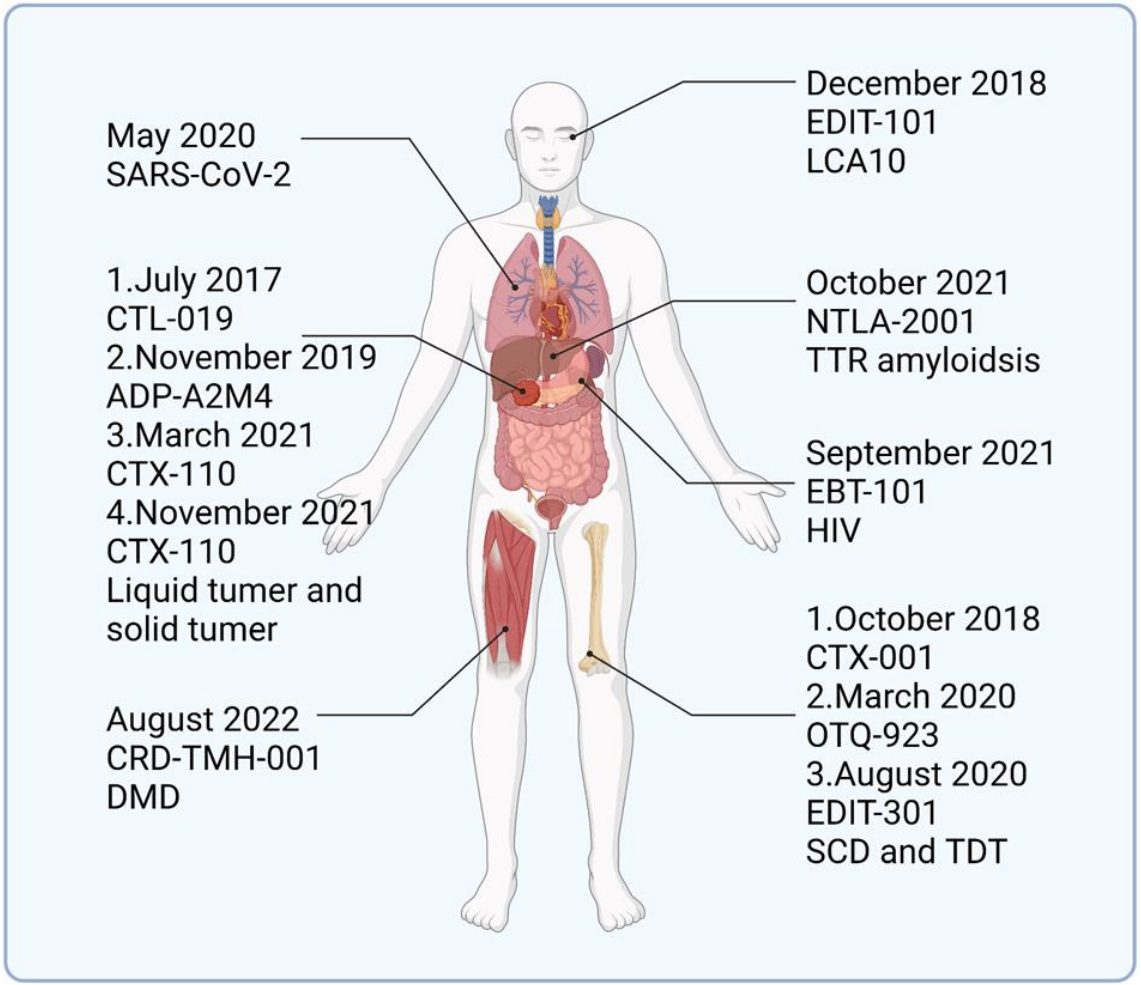
As a result, they generated different mutations, of which the effects are unknown. It may or may not confer HIV resistance, and may or may not have other consequences.

A second source of errors could have been that the editing was not perfectly efficient. This means that not all cells in the embryos were necessarily edited.
When an organism has a mixture of edited and unedited cells, it is called a "mosaic". While the available data are still limited, it seems that both Lulu and Nana are mosaic.

Application of CRISPR in immuno-oncology



FDA-approved CRISPR therapies that can be used in clinical treatments



DMD Duchenne muscular dystrophy, SCD sickle cell disease, TDT transfusion-dependent β-thalassemia, LCA10 Leber congenital amaurosis type 10, TTR transthyretin.

Article

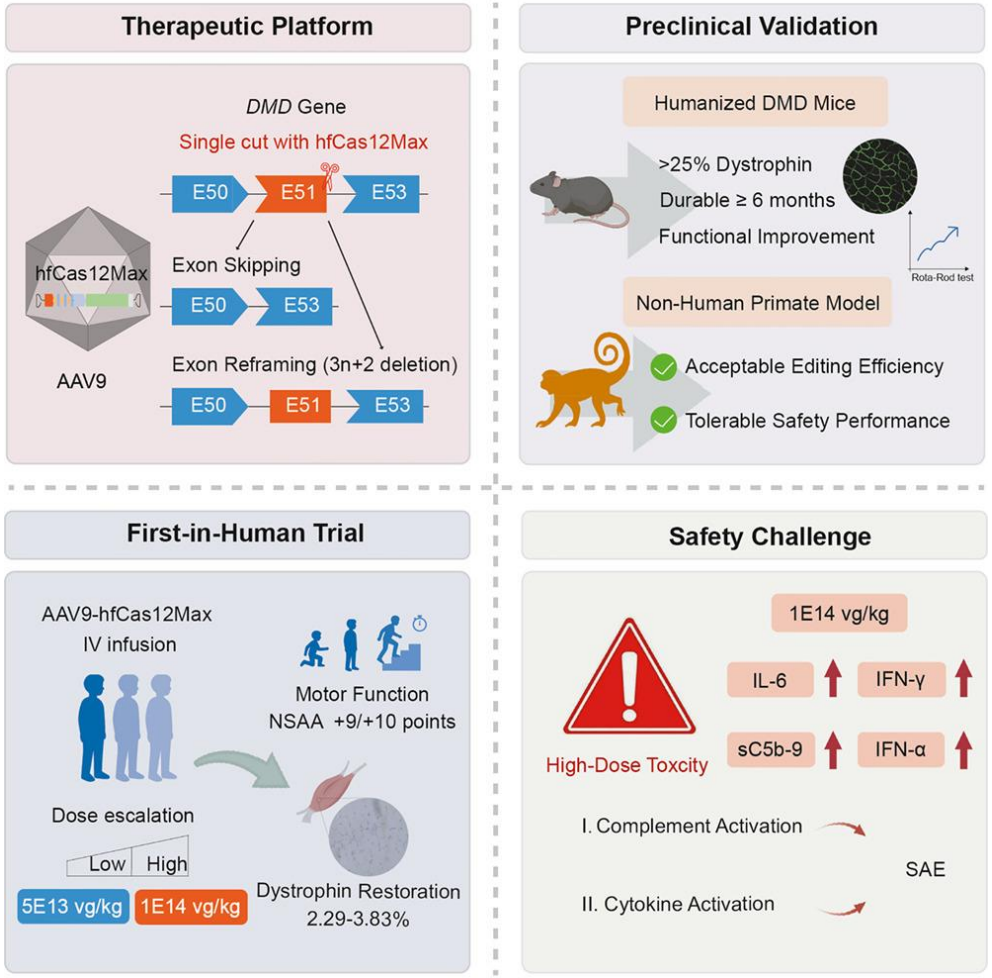
In vivo genome-editing therapy for Duchenne muscular dystrophy: Preclinical studies and first-in-human trial

Cell
2026

Guoling Li,[2,3,4,8] Feng Han,[1,8] Cuijin Wang,[1,8] Ming Jin,[5,8] Jiajia Lin,[5,8] Hainan Zhang,[2] Xiudong Yan,[2] Xing Wang,[2] Xinyan Sun,[6] Yiqun Yuan,[2] Siyuan Zhang,[2] Shuaishuai Zhang,[2] Jingyan Zhu,[2] Yichao Xu,[7] Yingyan Wang,[1] Yujiao Deng,[1] Fangxin Li,[2] Linyu Shi,[2] Alvin Luk,[2,*] Ning Wang,[5,*] Hui Yang,[2,3,4,9,*] and Jiwen Wang[1,*]

Summary

We report the preclinical and early clinical evaluation of in vivo genome editing for Duchenne muscular dystrophy (DMD), delivered via a single AAV9 vector. In humanized DMD mice, systemic administration achieved targeted genome editing, partial dystrophin restoration, and improved muscle function. In non-human primates, efficient on-target editing was achieved with no dose-limiting toxicity identified under the conditions tested. In an open-label, dose-escalation, first-in-human clinical trial (NCT06594094), post-treatment muscle biopsies provided early molecular evidence of hfCas12Max-mediated genome editing and detectable dystrophin expression in participants with DMD. However, at the higher dose level, one participant experienced a treatment-related fatal serious adverse event, characterized by marked complement activation and systemic cytokine response shortly after dosing. This work provides initial human proof-of-concept for muscle genome editing while crucially highlighting the critical role of immunologic risks and the narrow therapeutic window of systemic adeno-associated virus (AAV)-CRISPR therapies, underscoring the need to develop safer dosing and immunomodulation strategies.



Statement from the Organising Committee of the Third International Summit on Human Genome Editing (2023)

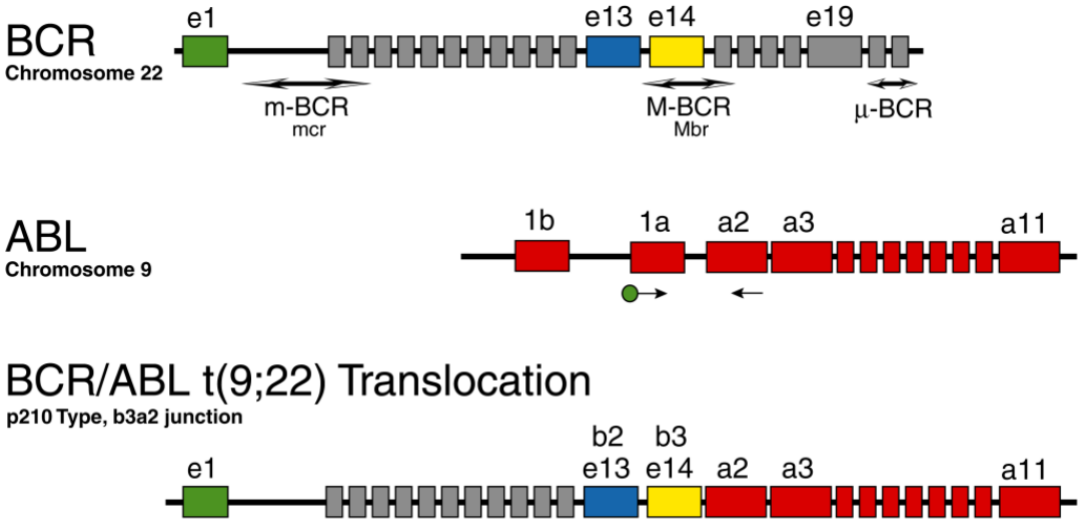
….."Remarkable progress has been made in somatic human genome editing, demonstrating it can cure once incurable diseases. To realise its full therapeutic potential, research is needed to expand the range of diseases it can treat, and to better understand risks and unintended effects. The extremely high costs of current somatic gene therapies are unsustainable. A global commitment to affordable, equitable access to these treatments is urgently needed.

Heritable human genome editing remains unacceptable at this time. Public discussions and policy debates continue and are important for resolving whether this technology should be used. Governance frameworks and ethical principles for the responsible use of heritable human genome editing are not in place. Necessary safety and efficacy standards have not been met."…….

Genome Editing Exercises

1.

Chronic myeloid leukemia (CML) is characterized by the t(9;22) translocation, which produces the BCR-ABL1 fusion protein. We aim to express BCR-ABL1 in human hematopoietic stem cells (HSCs) to demonstrate whether the presence of the fusion protein is sufficient to transform HSCs.



Genome Editing Exercises

2.

The YTHDF2 protein interacts with proteins involved in mRNA degradation. We aim to demonstrate that its deletion in 293T cells increases global mRNA levels. The gene is present in two copies in 293T cells and is non-essential.

