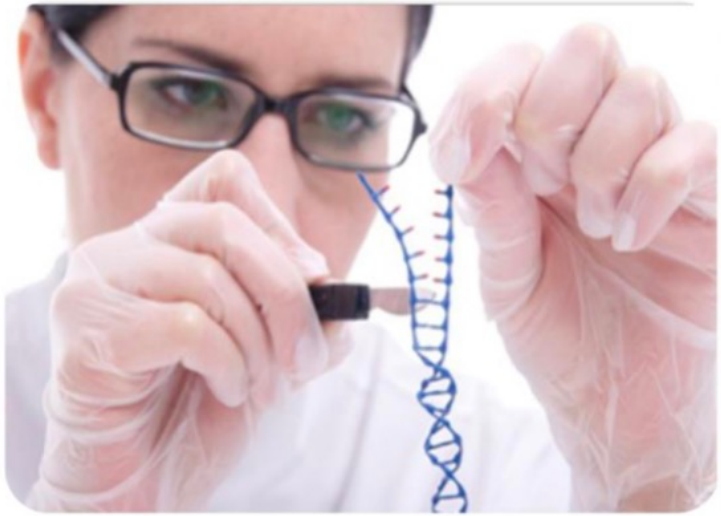
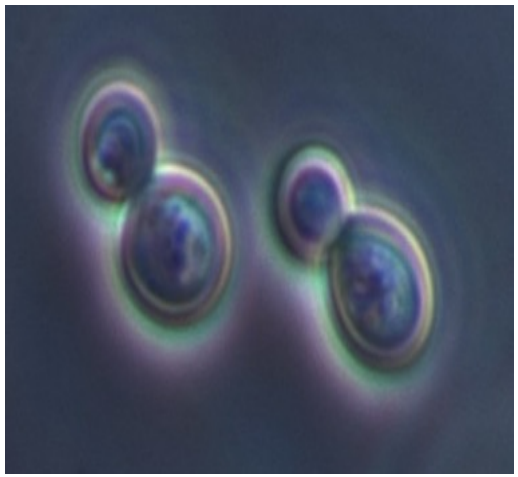
# Genome engineering methodologies



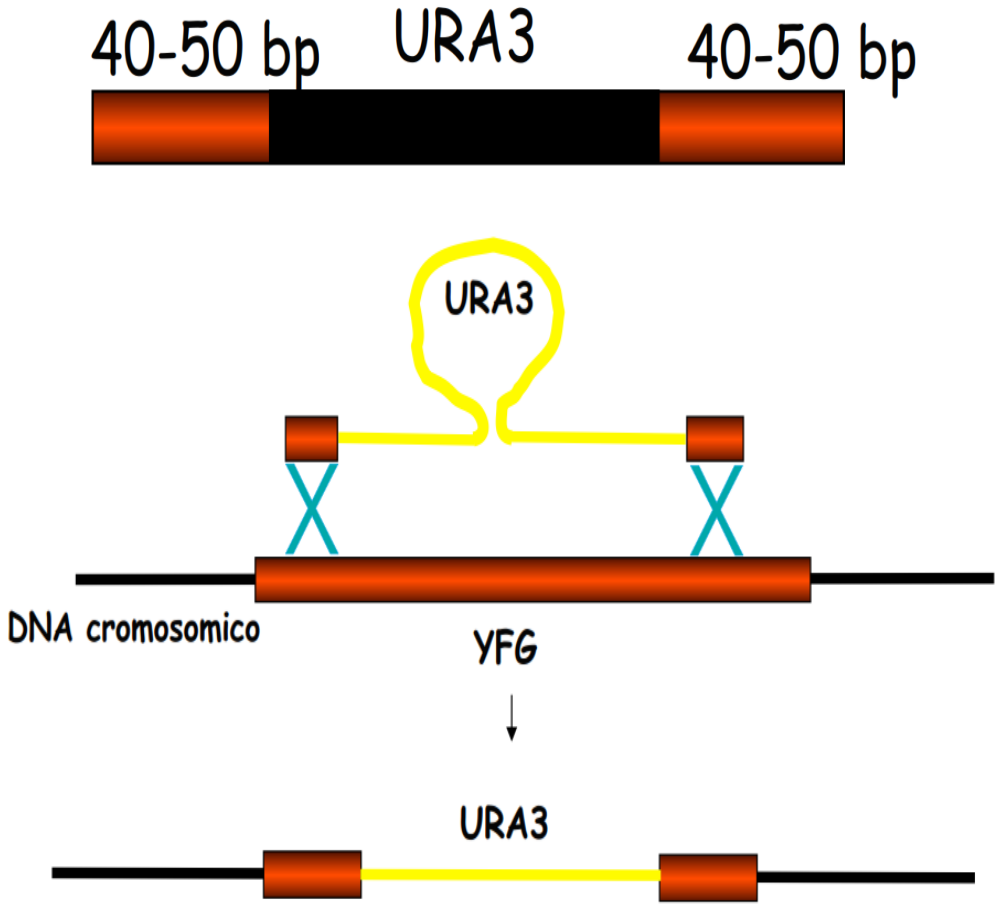
# What does ''Genome engineering'' means?

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

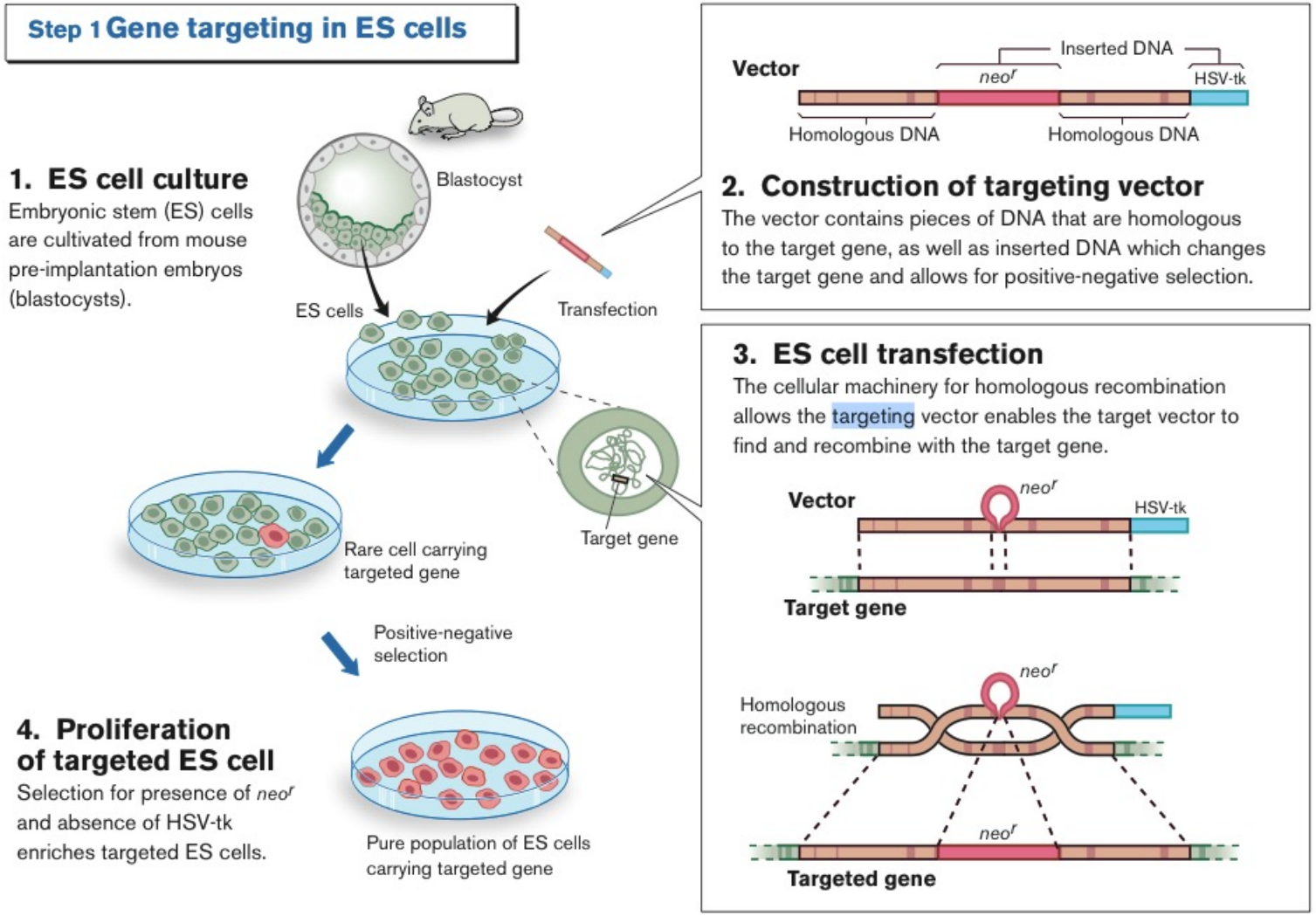
# Homologous recombination in yeast



*S.cerevisiae*



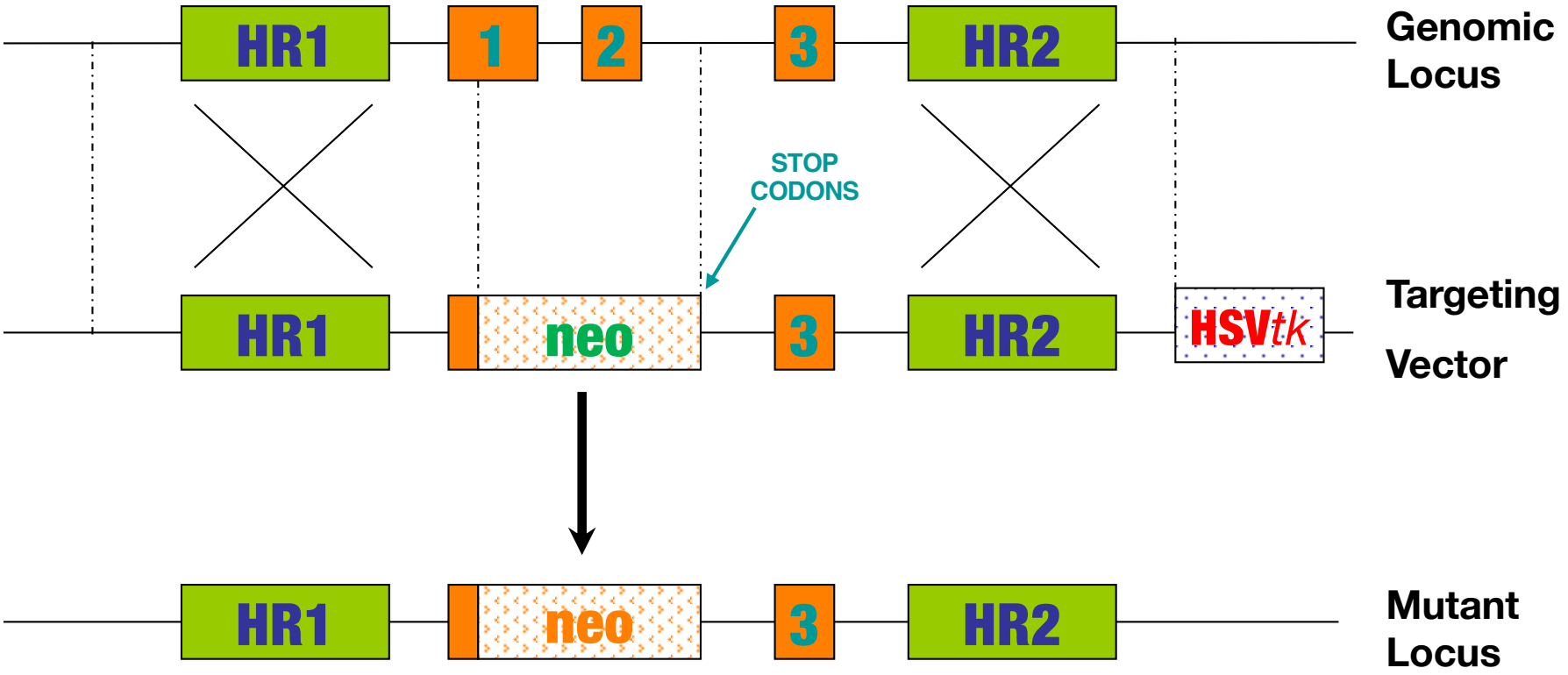
# Genome engineering




The efficiency of targeting is around 1%.

# Positive-negative selection

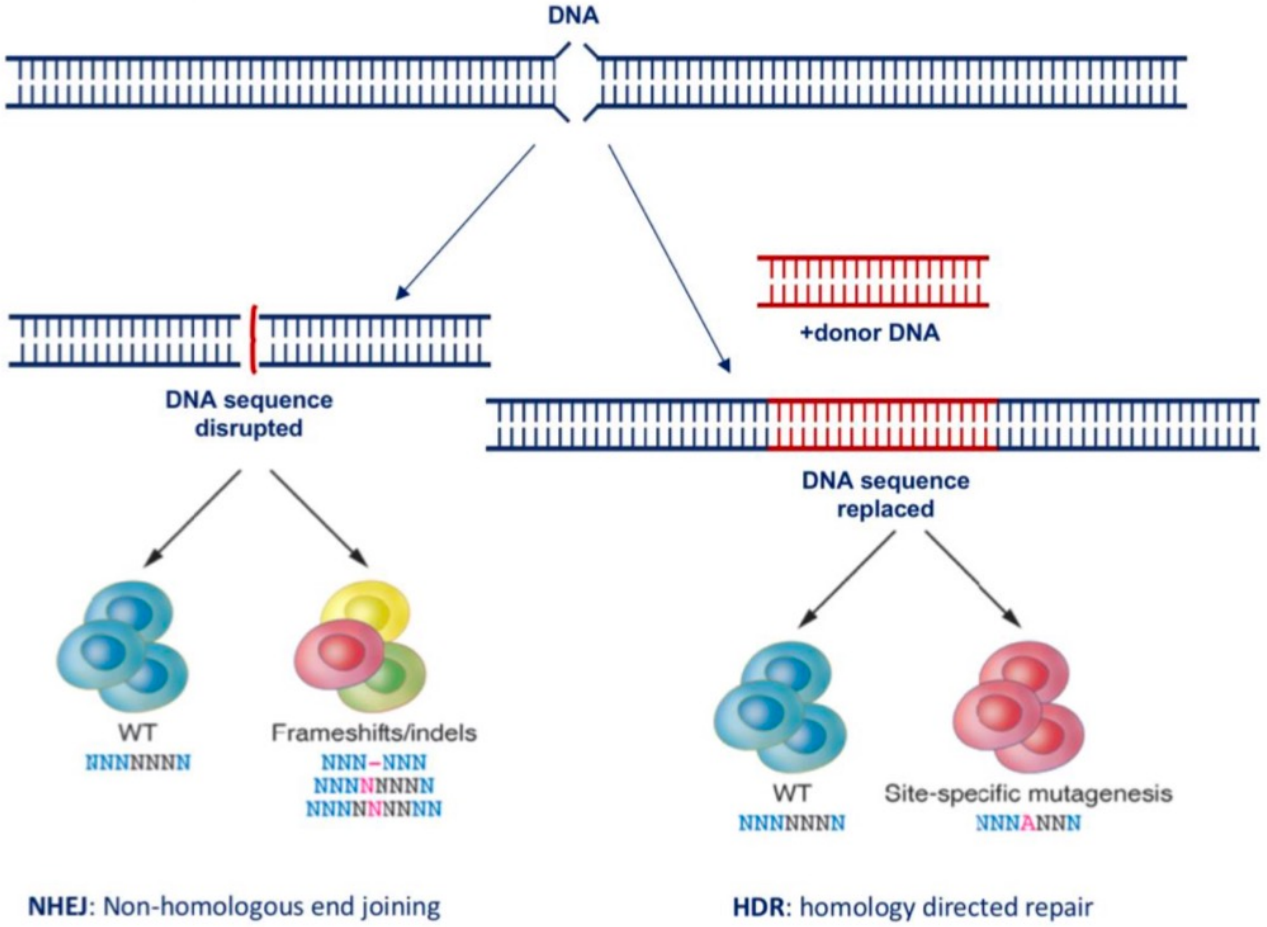
It allows to distinguish between homologous and heterologous recombination



# 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').




**NHEJ**: Non-homologous end joining

**HDR**: homology directed repair

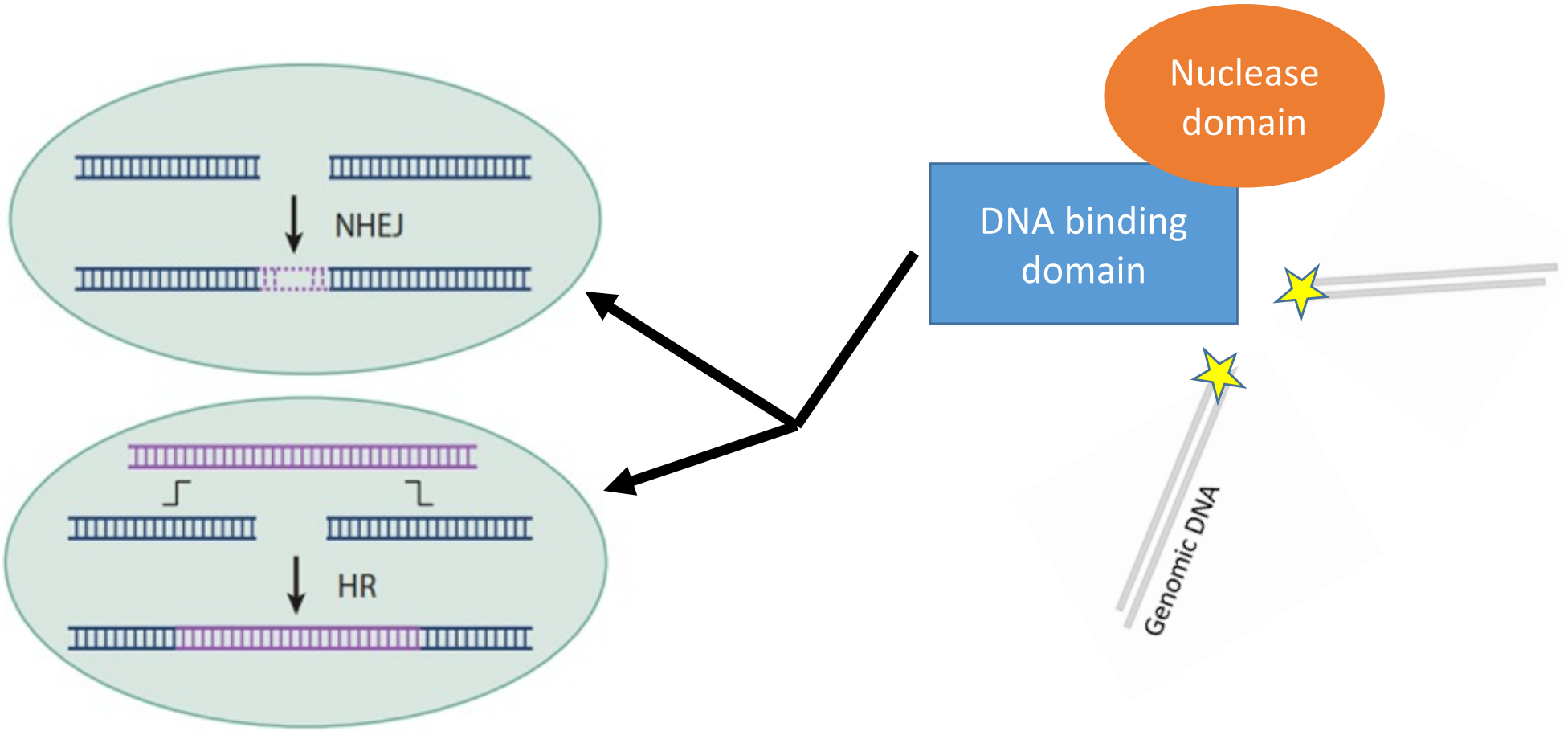
# Repair of DNA double-strand breaks (DSBs)

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

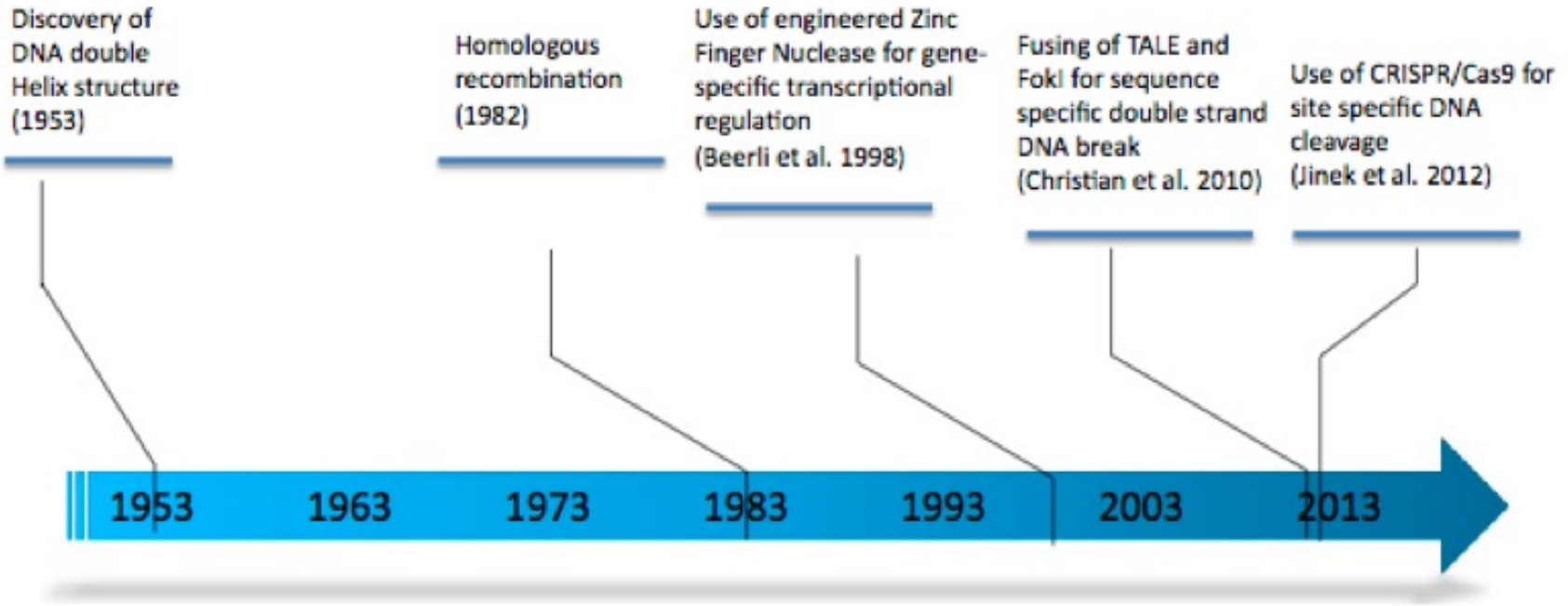
- **Homologous Recombination (HR),** in which nucleotide sequences are exchanged between two similar or identical molecules of DNA.

- **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



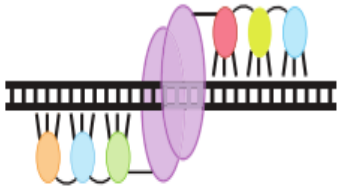
# Timeline of genome engineering research




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.

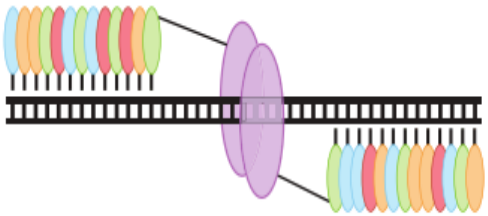
# Genome editing

1°Generation:



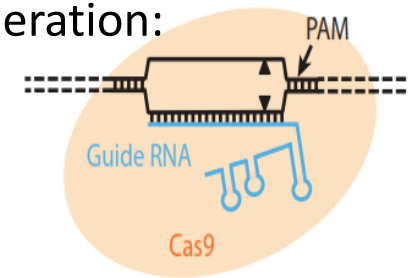
ZFNs

2° Generation:

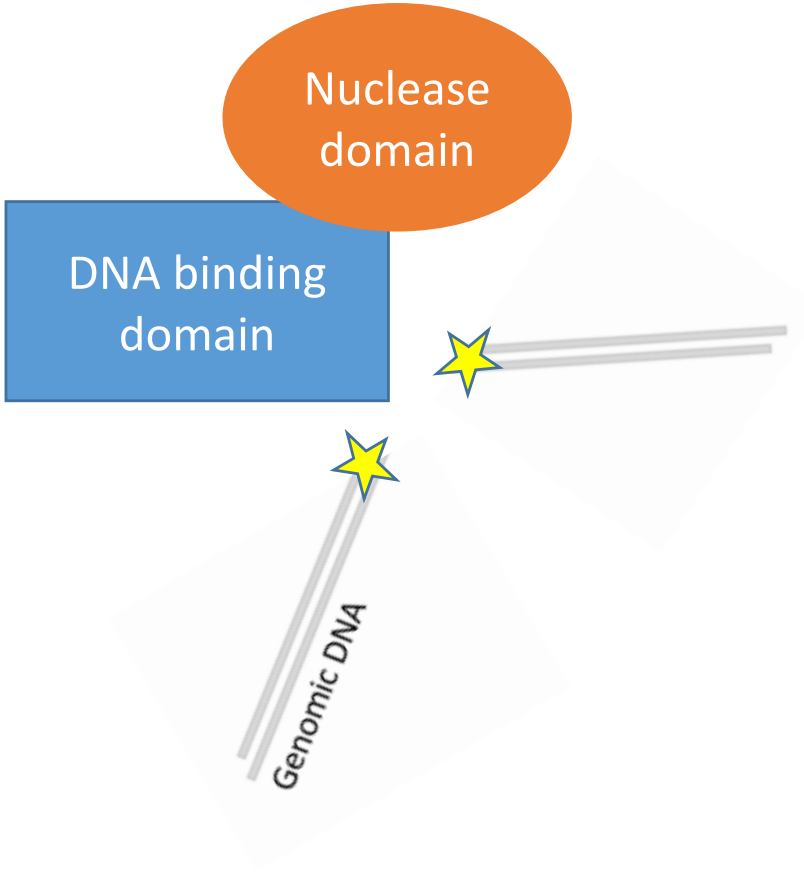


TALENs

3° Generation:

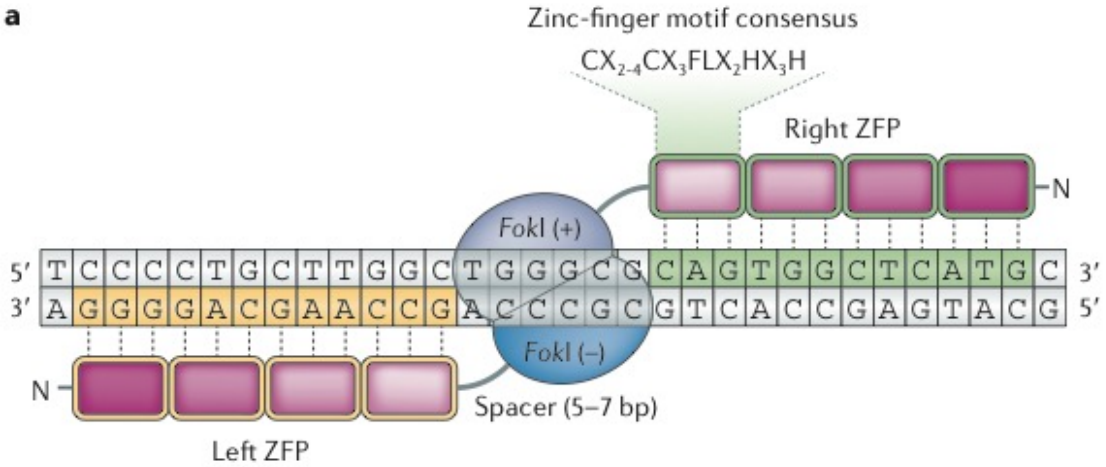



CRISPR/Cas



# **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.

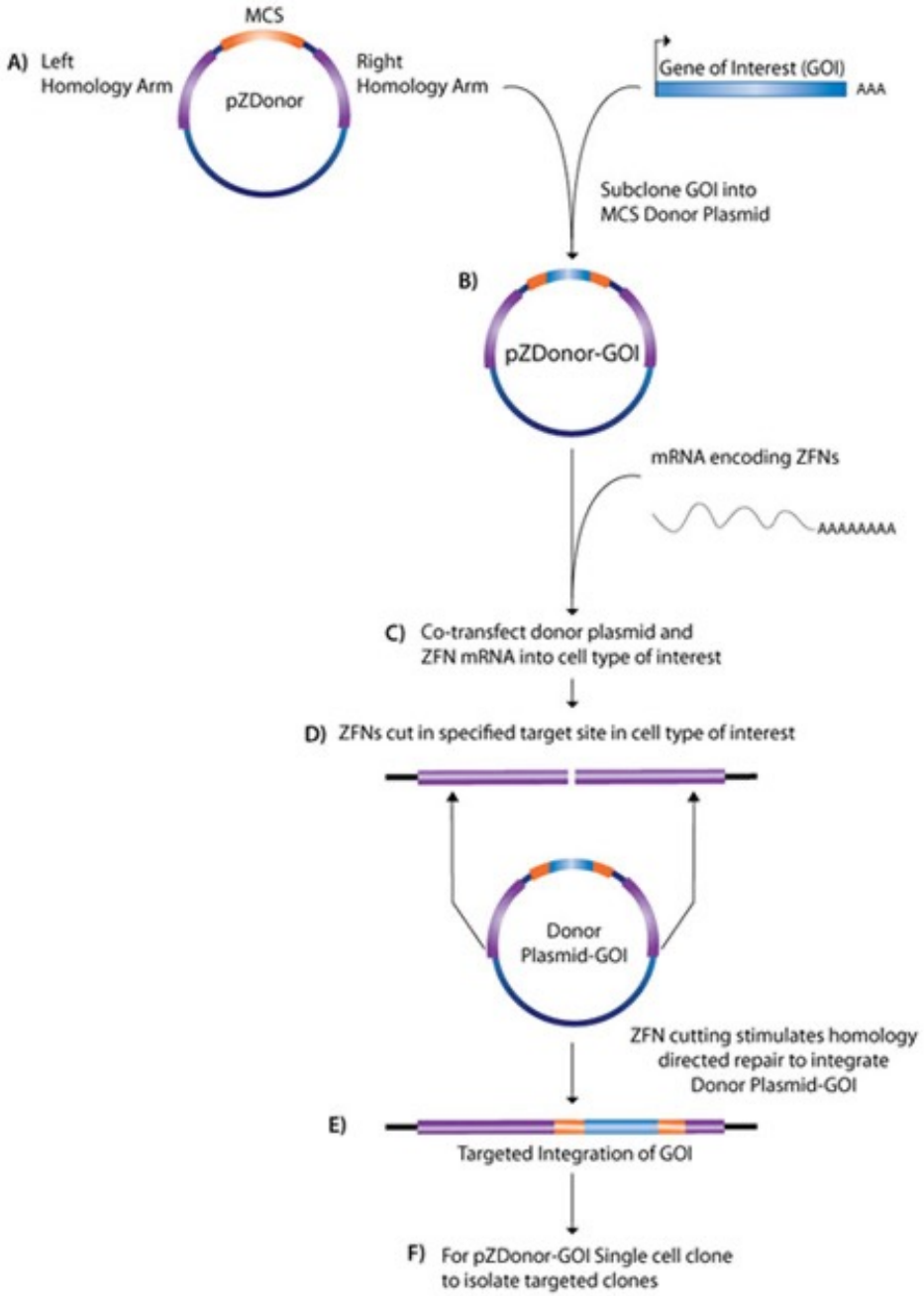



The sequence specificity of ZFNs is determined by ZFPs, which consist of tandem arrays of C2H2 zinc-fingers, the most common DNA-binding motif in higher eukaryotes. Each zinc-finger recognizes a 3-bp DNA sequence, and 3–6 zinc-fingers are used to generate a single ZFN subunit that **binds to DNA sequences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

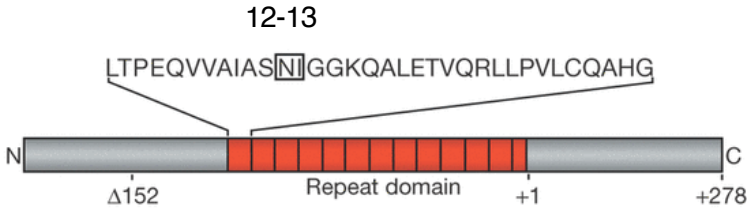
ZFNs are used to rapidly integrate a user-specified gene of interest (GOI) into preferred sites of integration in either the human, mouse or rat genome. In the human genome **the adeno-associated virus (AAVS1) locus** on human chromosome 19 is the preferred site, while the **Rosa26 locus** is located 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.



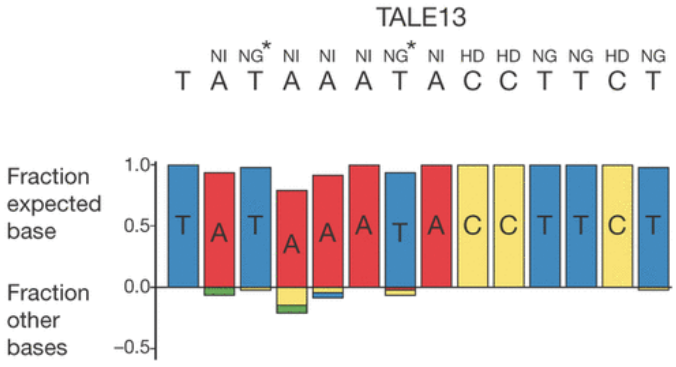
# Transcription Activator-Like Effector Nucleases (TALEN)

**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

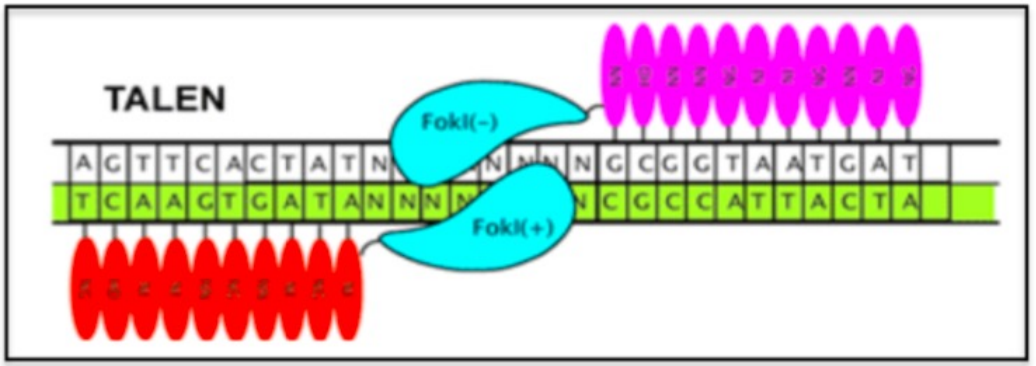
# Transcription Activator-Like Effector Nucleases (TALEN)

- **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

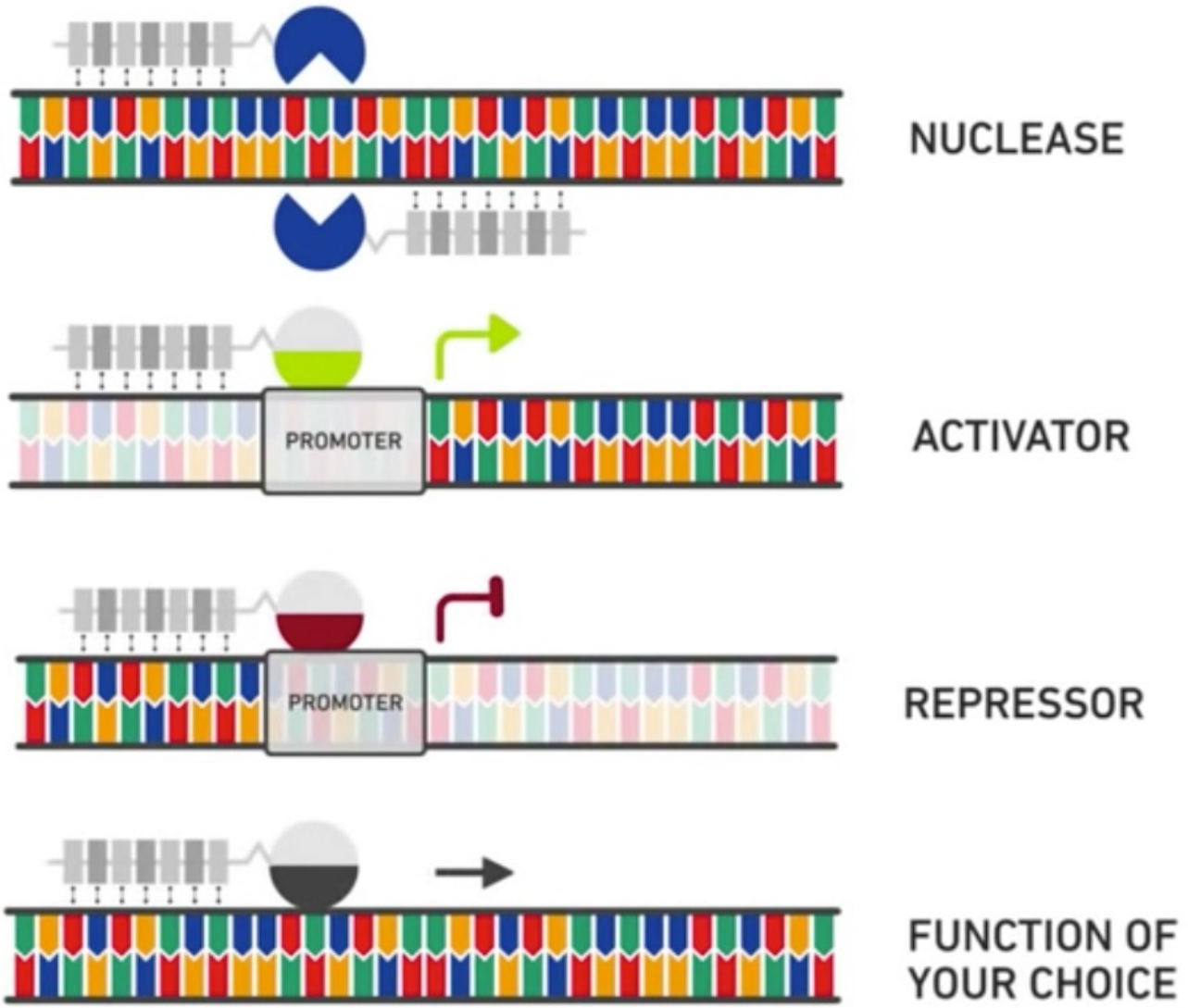
- Since FokI works as a **dimer**, two TALE binding sites which separated by a spacer sequence are needed to locate opposing DNA target sites.

- 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.**

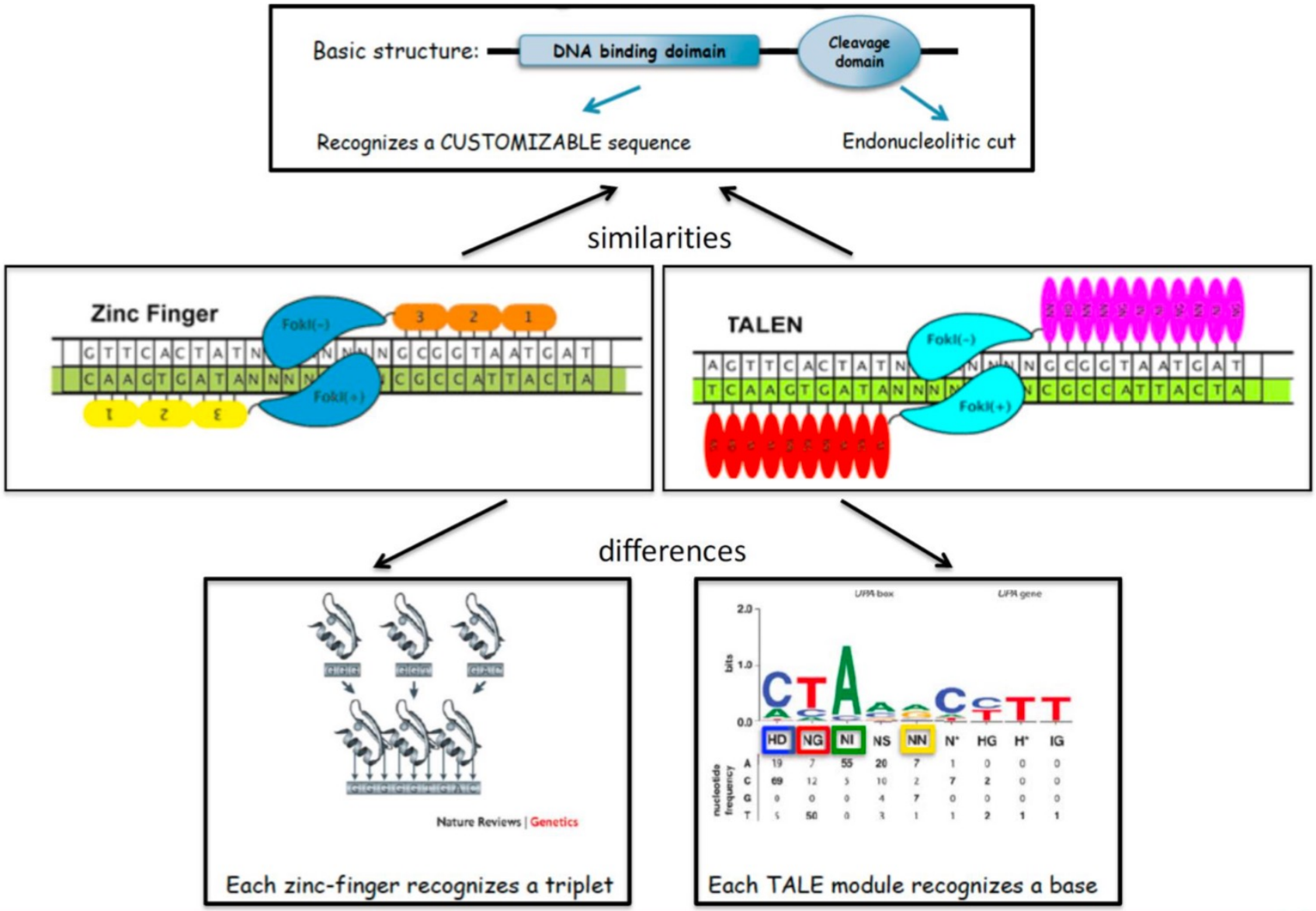



Mussolino et al., 2013

# 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**.

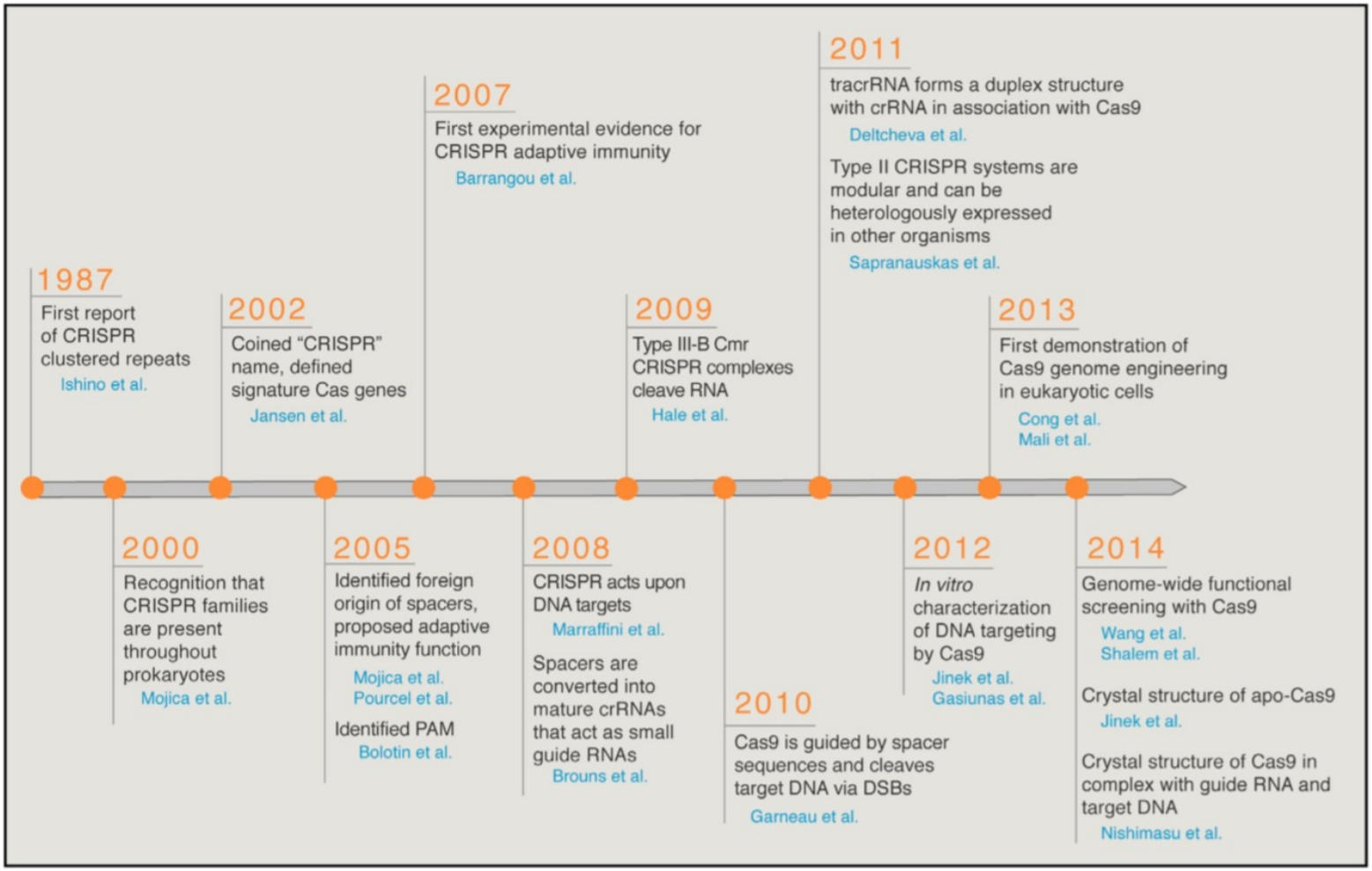
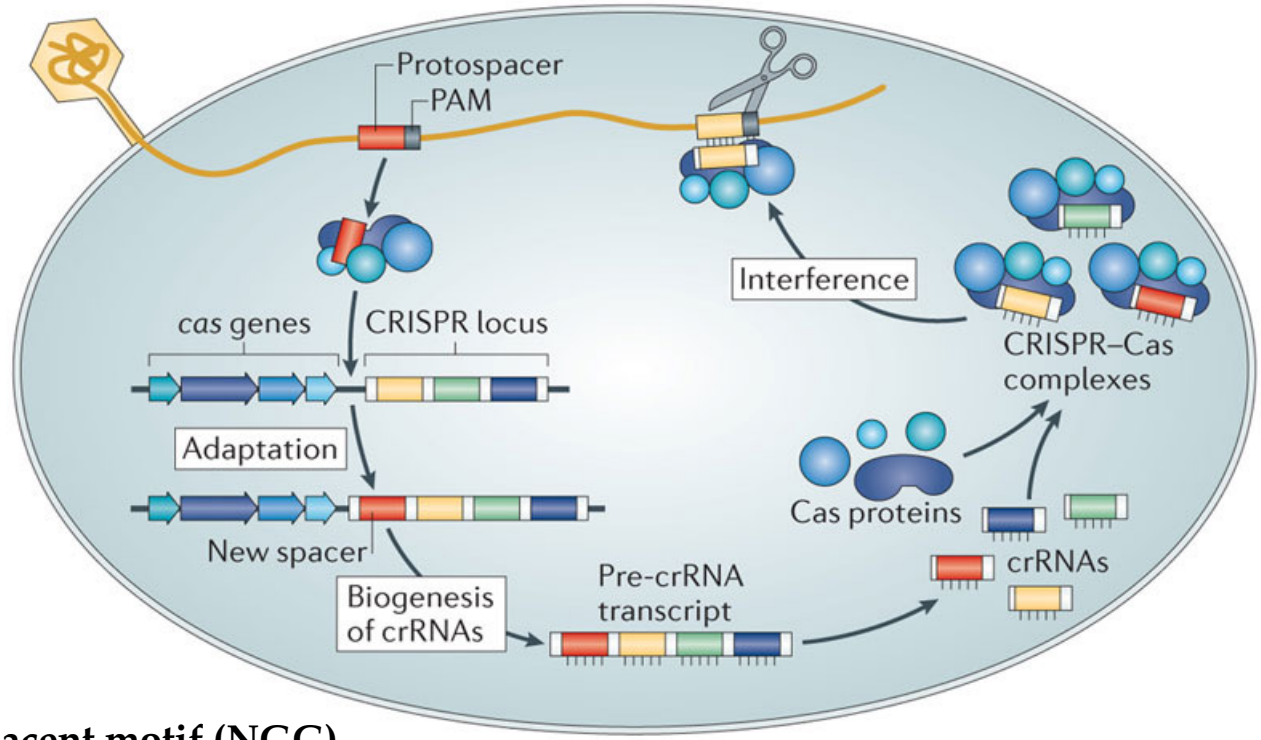



**Figure:** Hsu, Lander, Zhang: Development and Applications of CRISPR-Cas9 for Genome Engineering; Cell 157, June 5, 2014

**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)

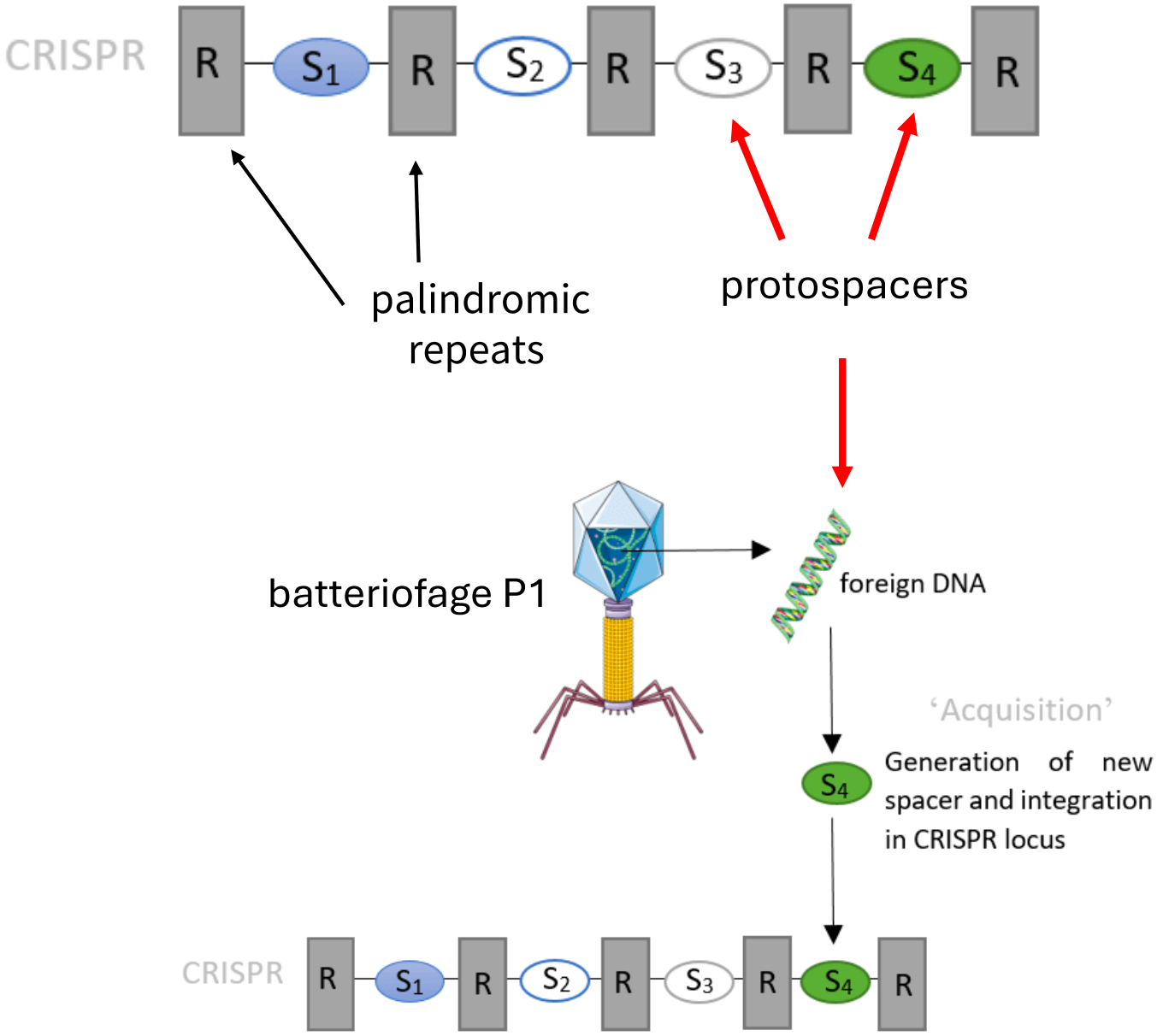
In bacteria, the **CRISPR system** provides acquired immunity against invading foreign DNA via RNA-guided DNA cleavage. In **the type II CRISPR/Cas system**, short segments of foreign DNA, termed **'spacers'** are integrated within the CRISPR genomic loci and transcribed and processed into short **CRISPR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

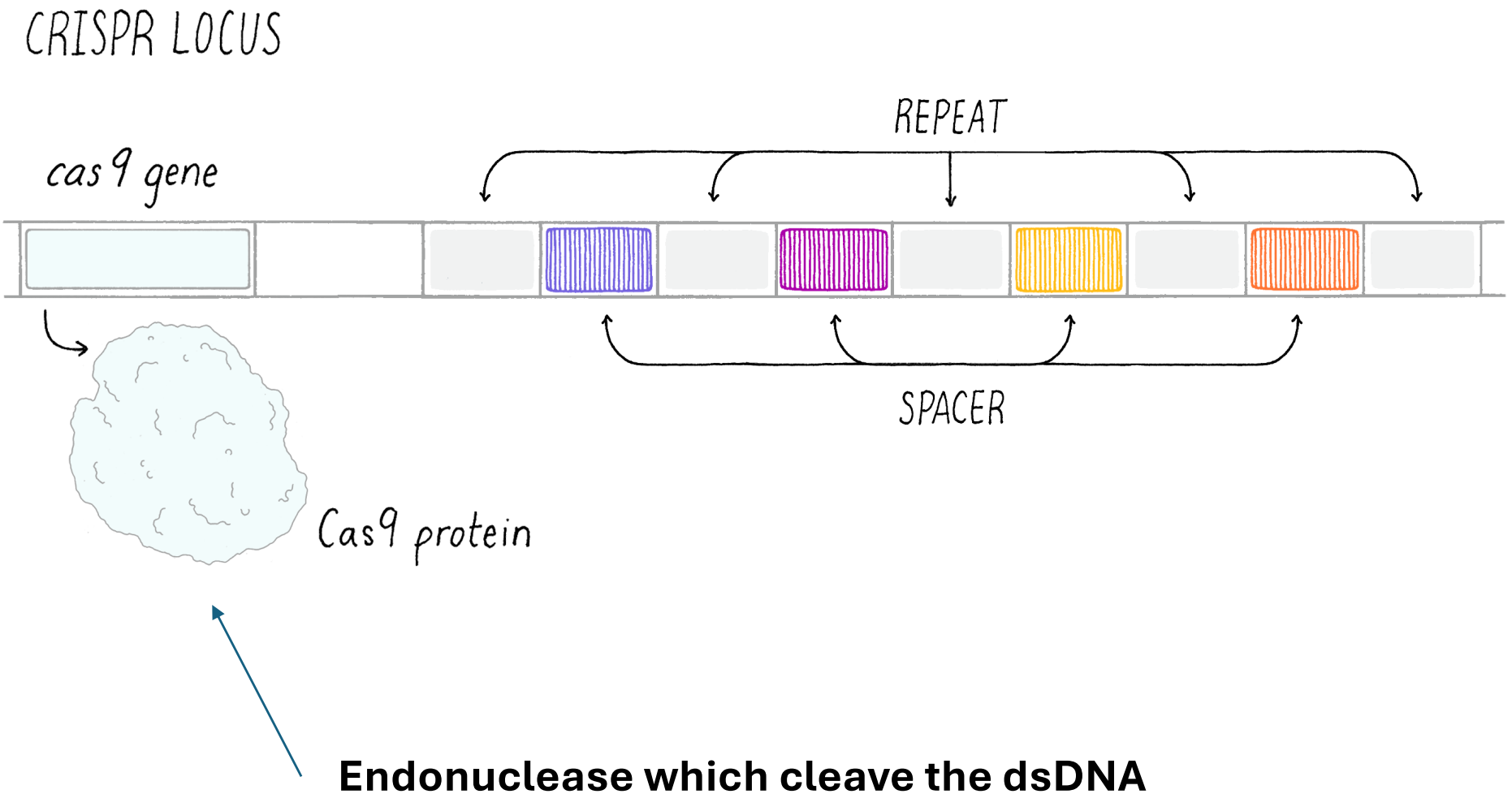



**PAM: Protospacer adiacent motif (NGG)**

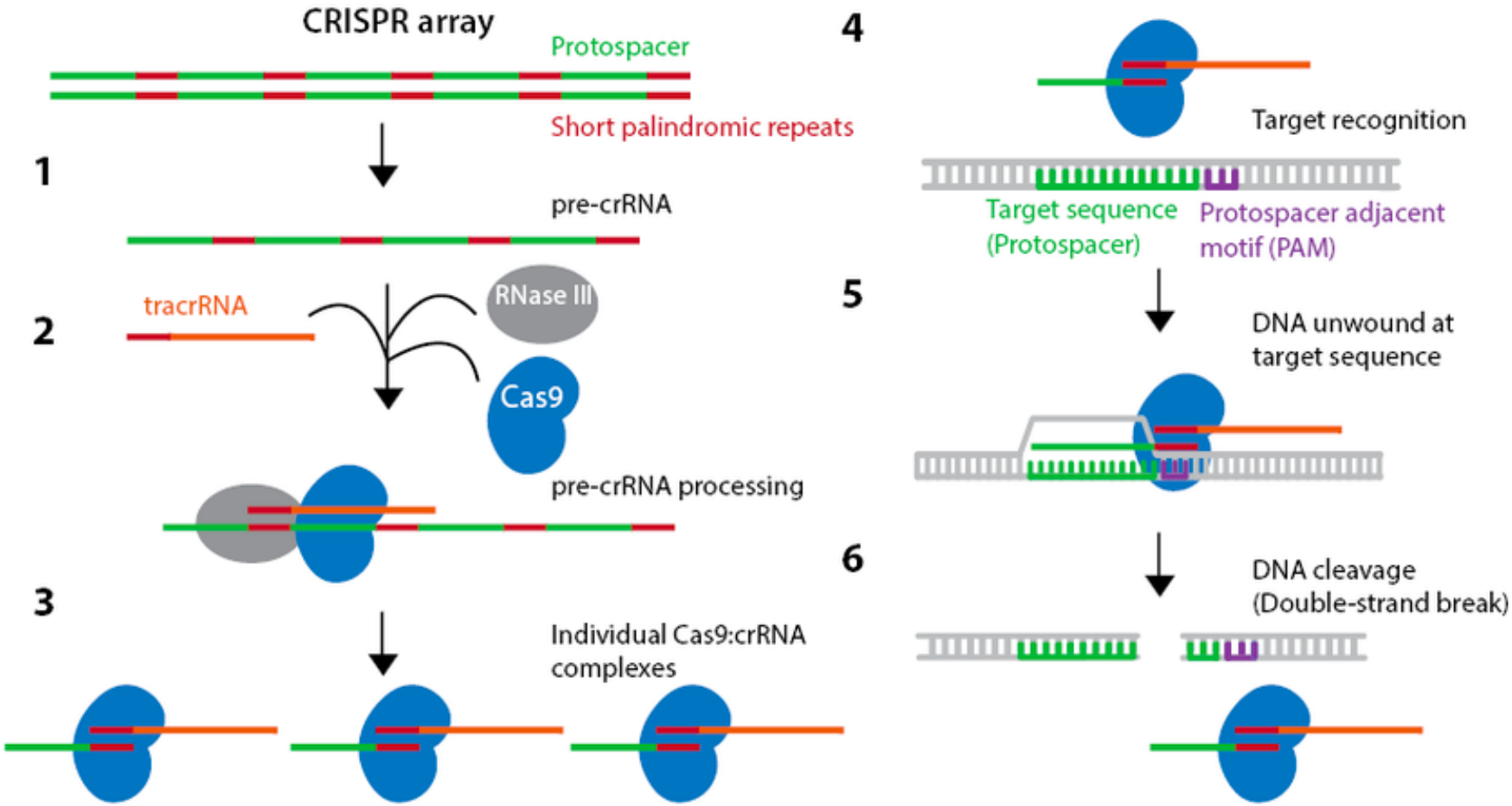
# How this system works

*CRISPR locus di Escherichia Coli*



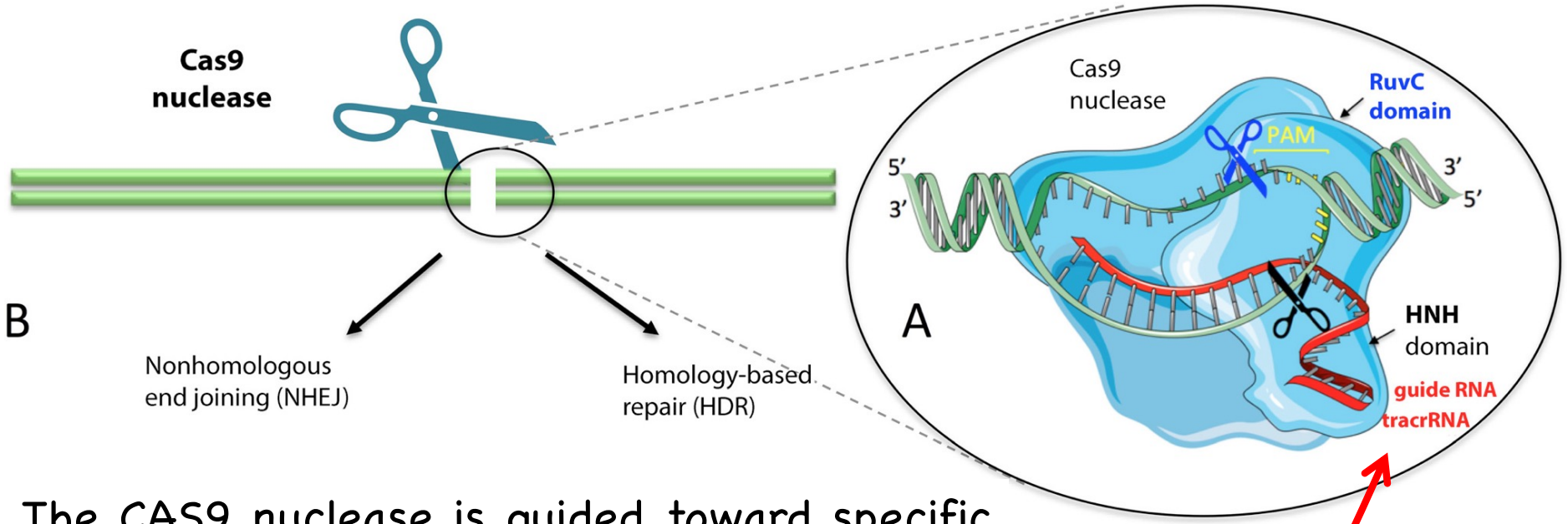

CRISPR LOCUS
REPEAT
cas 9 gene
SPACER
Cas9 protein
Endonuclease which cleave the dsDNA

# CRISPR/CAS9




DNA is recognised through sequence complementarity with the crRNA and the **PAM sequence** (2-6 nt downstream) that is recognised by CAS9. In particolar Cas9 cleave the DNA 3-4 nt downstream the **PAM.**

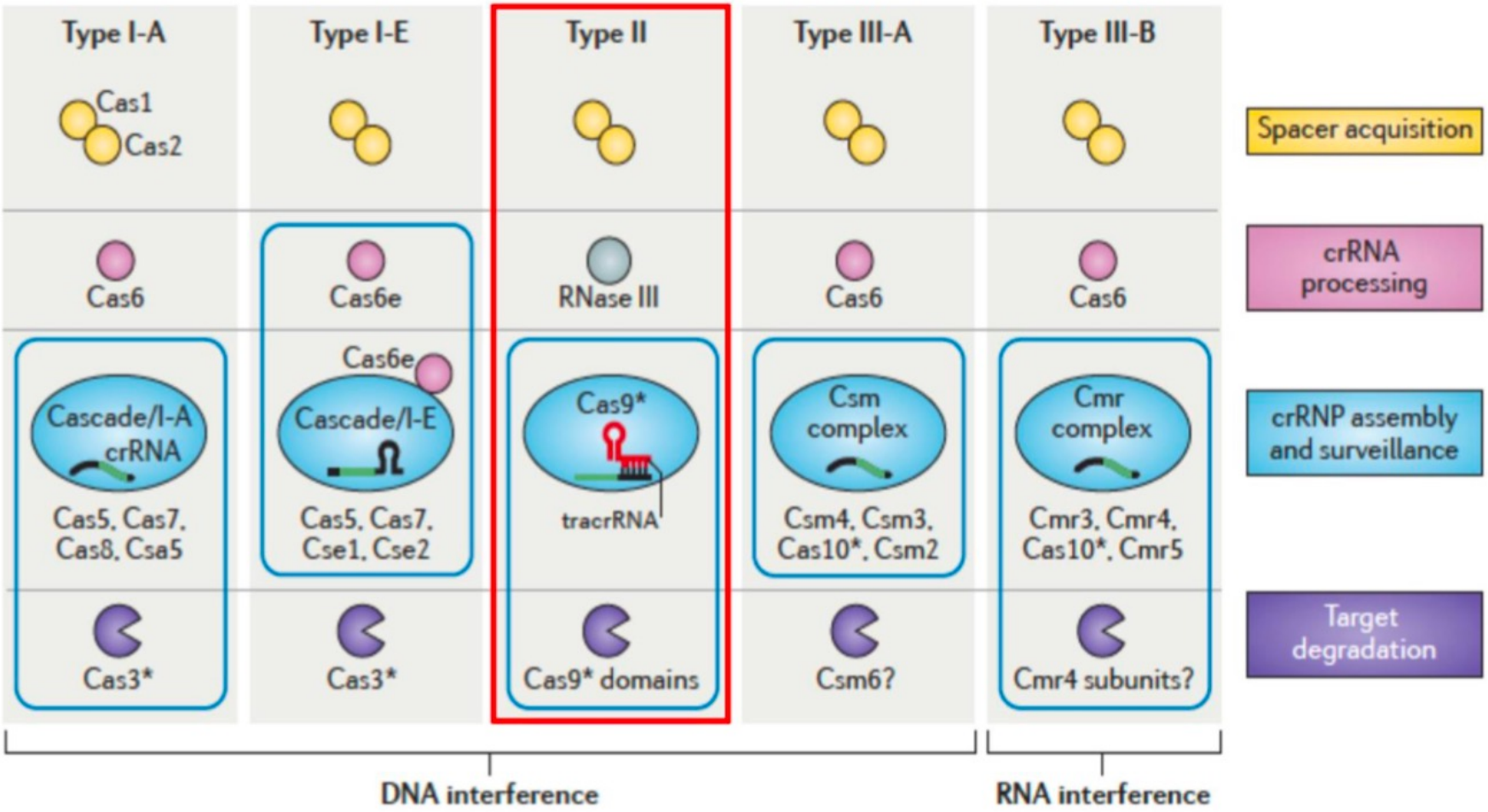
# CRISPR-Cas9 tool




The CAS9 nuclease is guided toward specific sites of the genoma by a guide RNA complementary to the site to be edited

# CRISPR-Cas systems

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

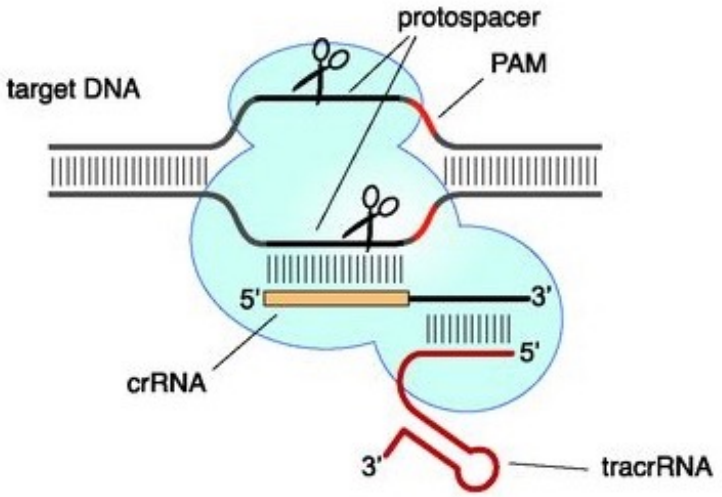



**The type II CRISPR system from *Streptococcus pyogenes* has been adapted for inducing sequence-specific double stranded breaks and targeted genome editing.**

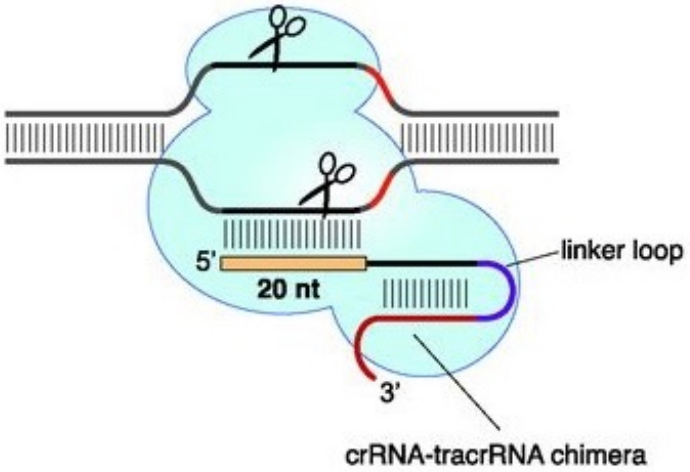
John van der Oost et al. ,Nature Reviewers 2014

# From Bacterial CRISPR-cas to a genome editing technique

Cas9 programmed by crRNA:tracrRNA duplex




Cas9 programmed by single chimeric RNA




## A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

Martin Jinek,[1,2]* Krzysztof Chylinski,[3,4]* Ines Fonfara,[4] Michael Hauer,[2]† Jennifer A. Doudna,[1,2,5,6]‡ Emmanuelle Charpentier[4]‡

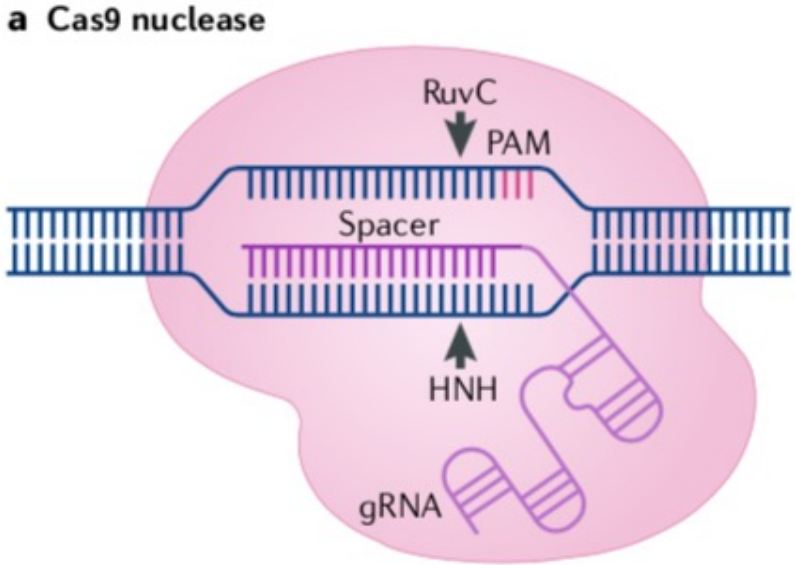
In bacterial type 2 CRISPR cas system, the site specificity is defined by complementery base pairing of a small CRISPR RNA(crRNA

➢After annealing to a transactiviting CRISPR RNA(tracrRNA) the crRNA directly guides the cas9 endonuclease to cleave the targeted DNA sequence.

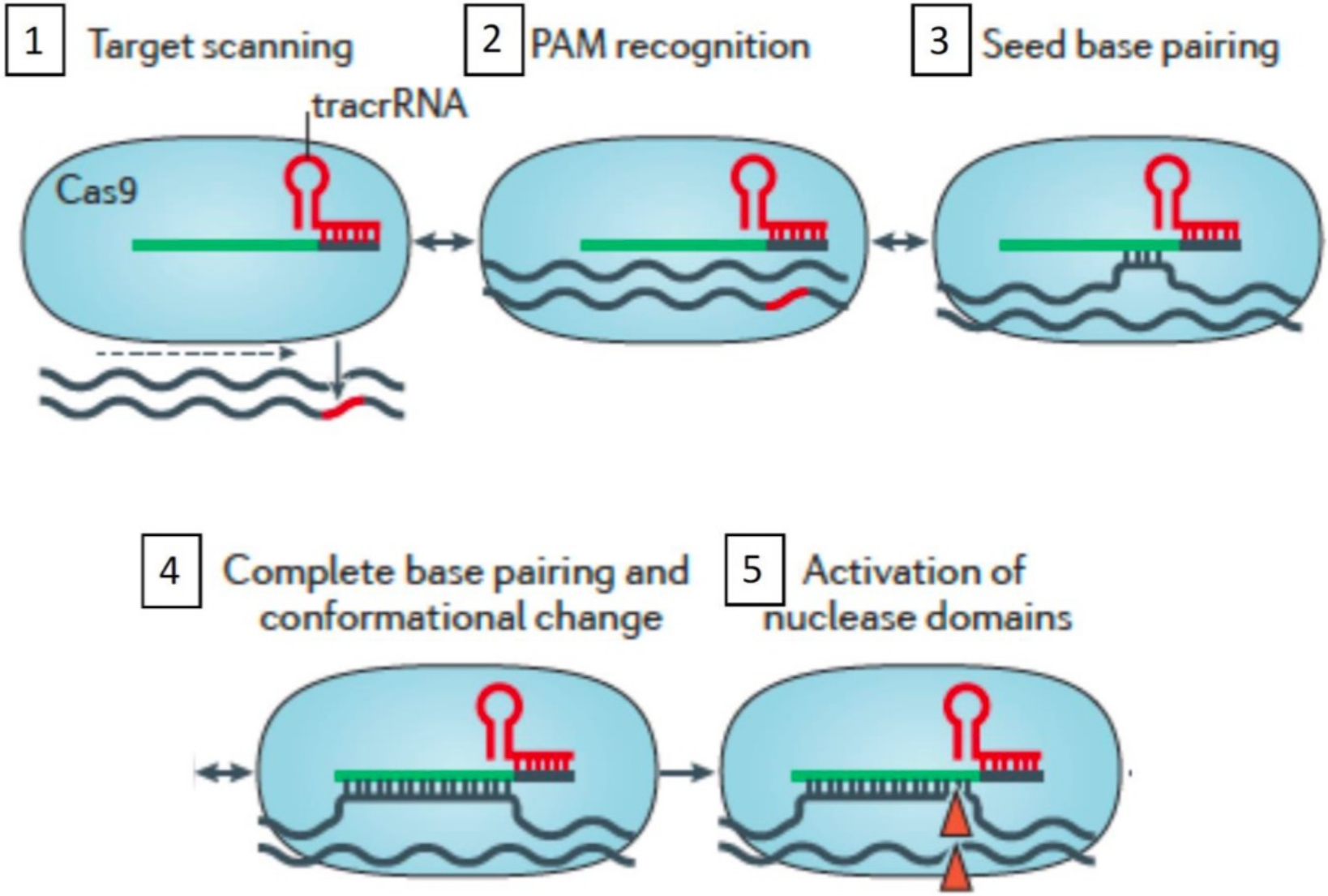
➢The crRNA–transcr- RNA heteroduplex could be replaced by one chimeric RNA (so-called guide RNA (gRNA)) and the gRNA could be programmed to target specific sites.

# CRISPR-Cas gene editing tools

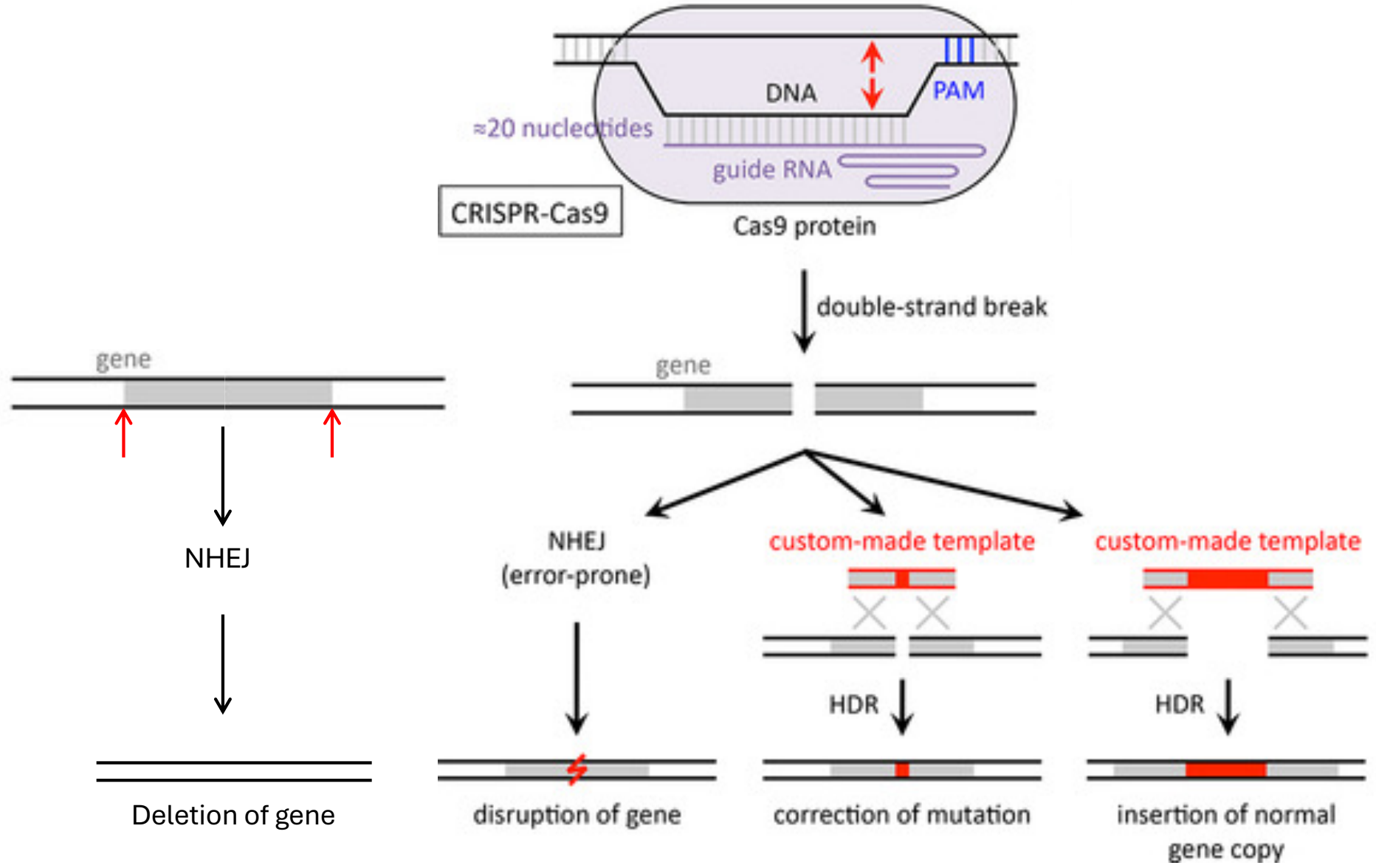
**Cas9** proteins rely on RNA guidance for targeting specificity. In engineered CRISPR–Cas9 systems, Cas9 interacts with the backbone of the guide RNA (gRNA). Complementary pairing of the spacer portion of the gRNA to a DNA target sequence positioned next to a PAM results in generation of a blunt DNA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



# The process

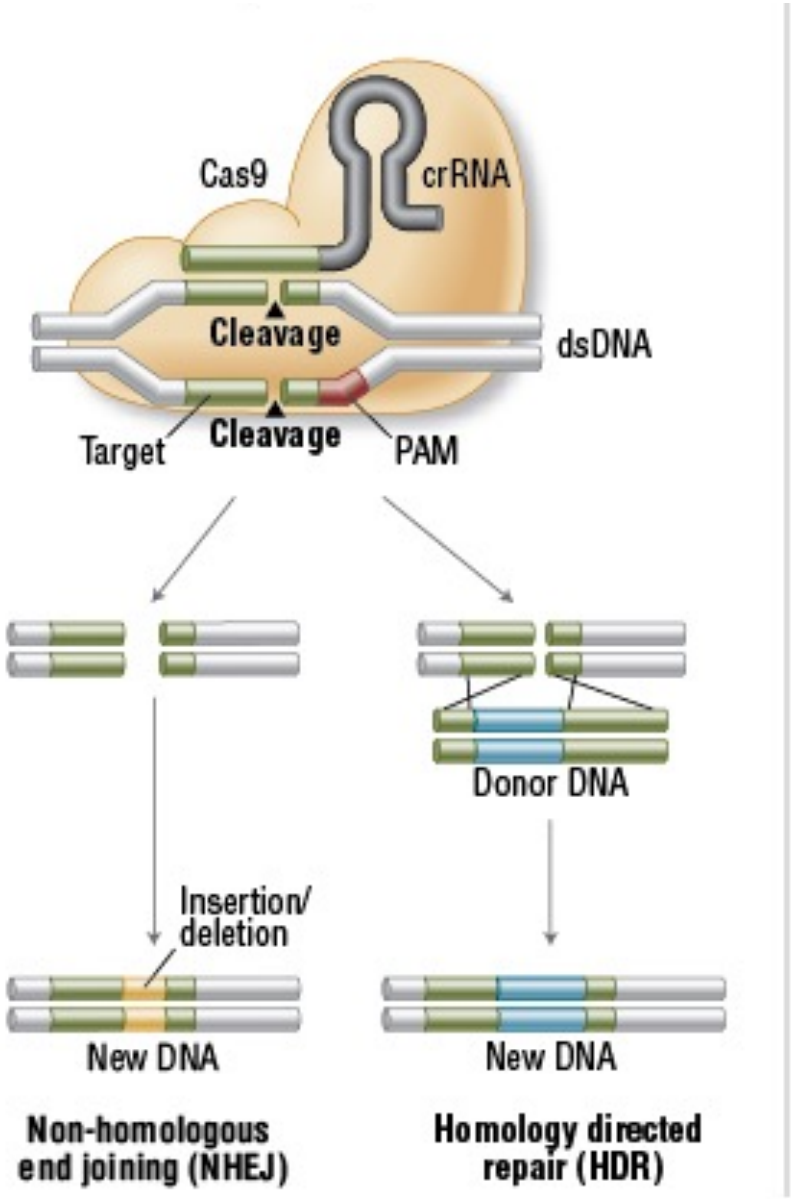

1 Target scanning
2 PAM recognition
3 Seed base pairing
tracrRNA
Cas9
4 Complete base pairing and conformational change
5 Activation of nuclease domains

# Il principio della tecnologia CRISPR-Cas9

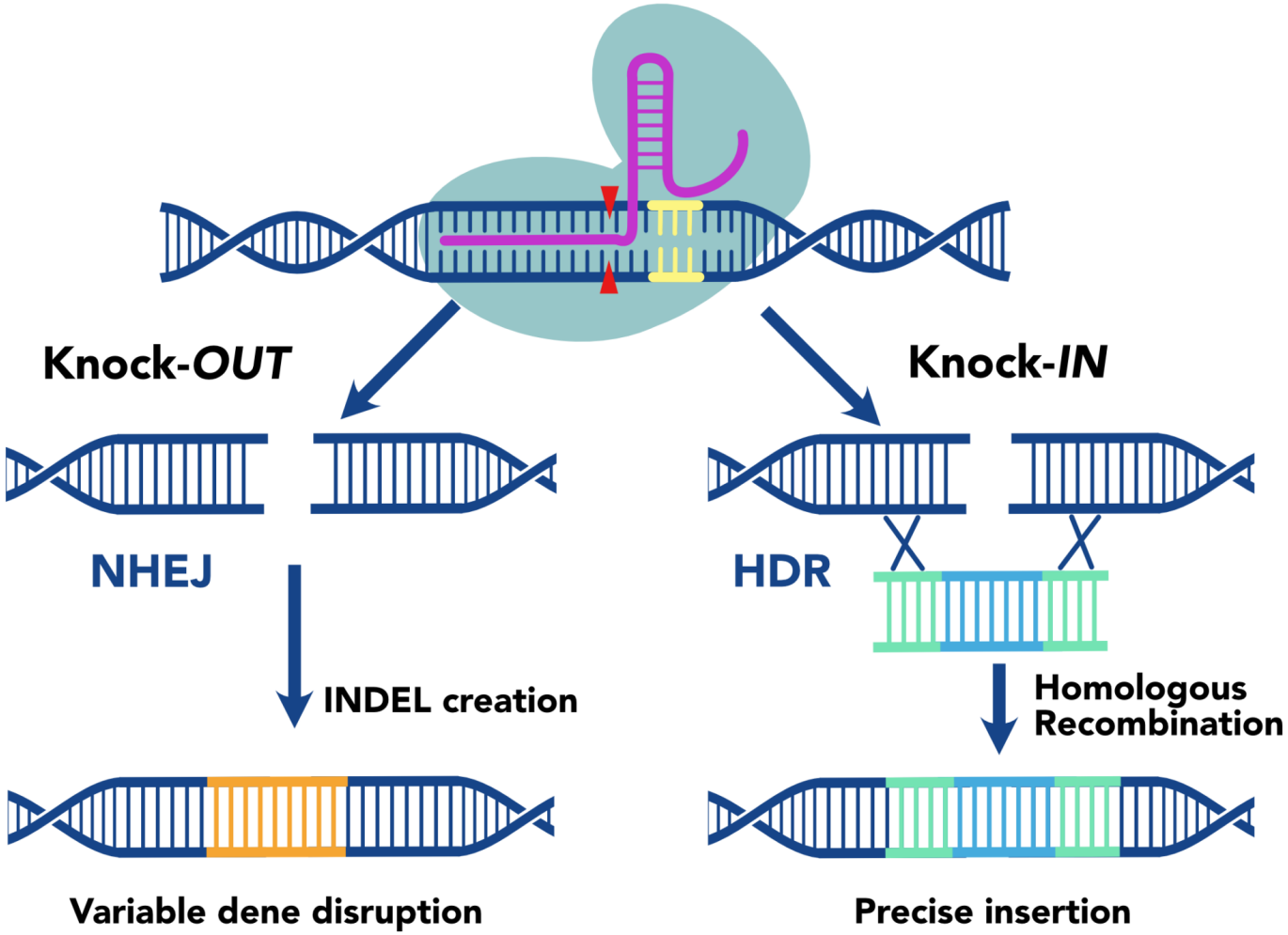


# The CRISPR/Cas9 system

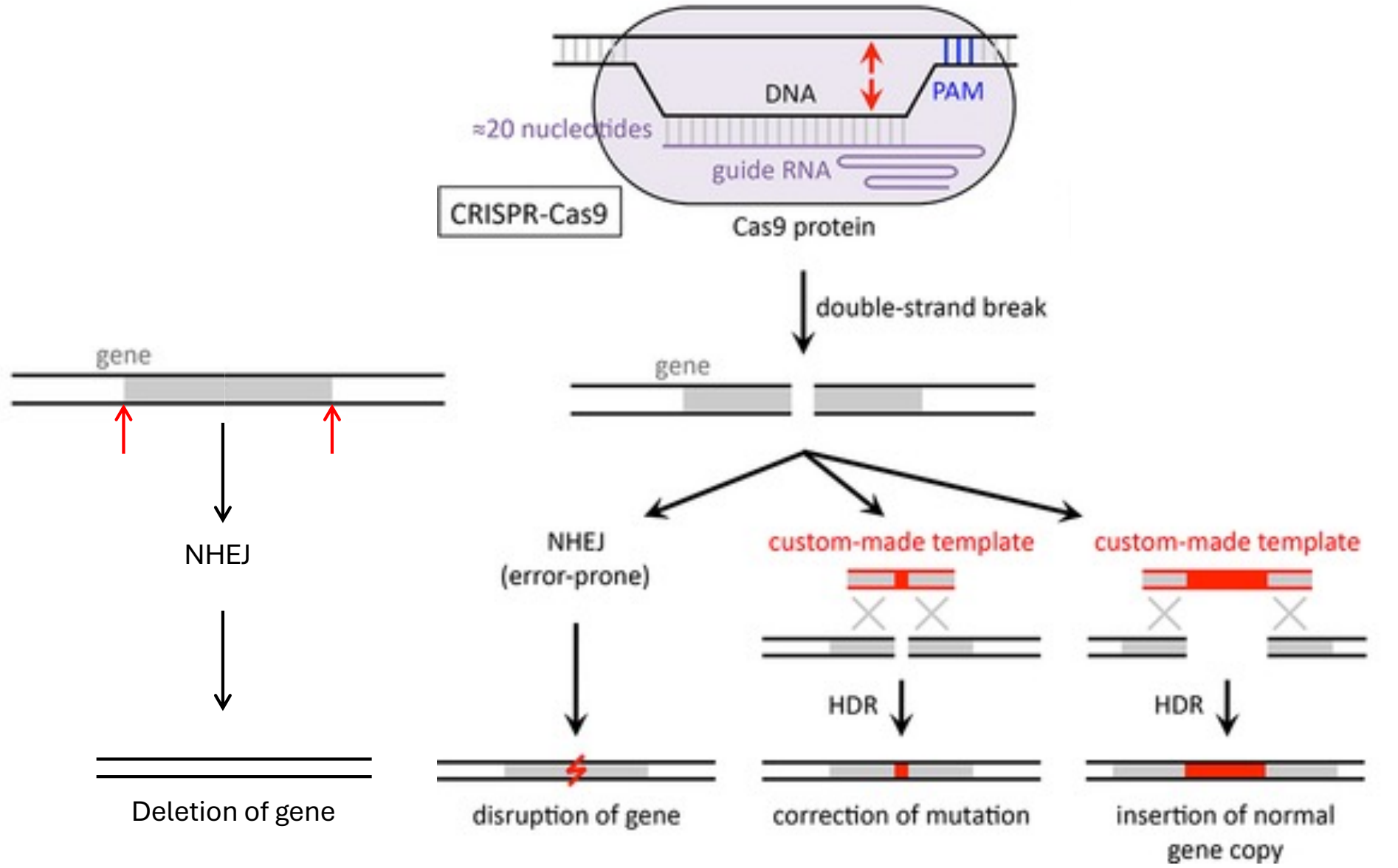
The **CRISPR/Cas system** can thereby be retargeted to cleave virtually any DNA sequence by redesigning the crRNA. Significantly, the CRISPR/Cas system has been shown to be directly portable to human cells by co-delivery of plasmids expressing the Cas9 endonuclease and the necessary crRNA components. 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.



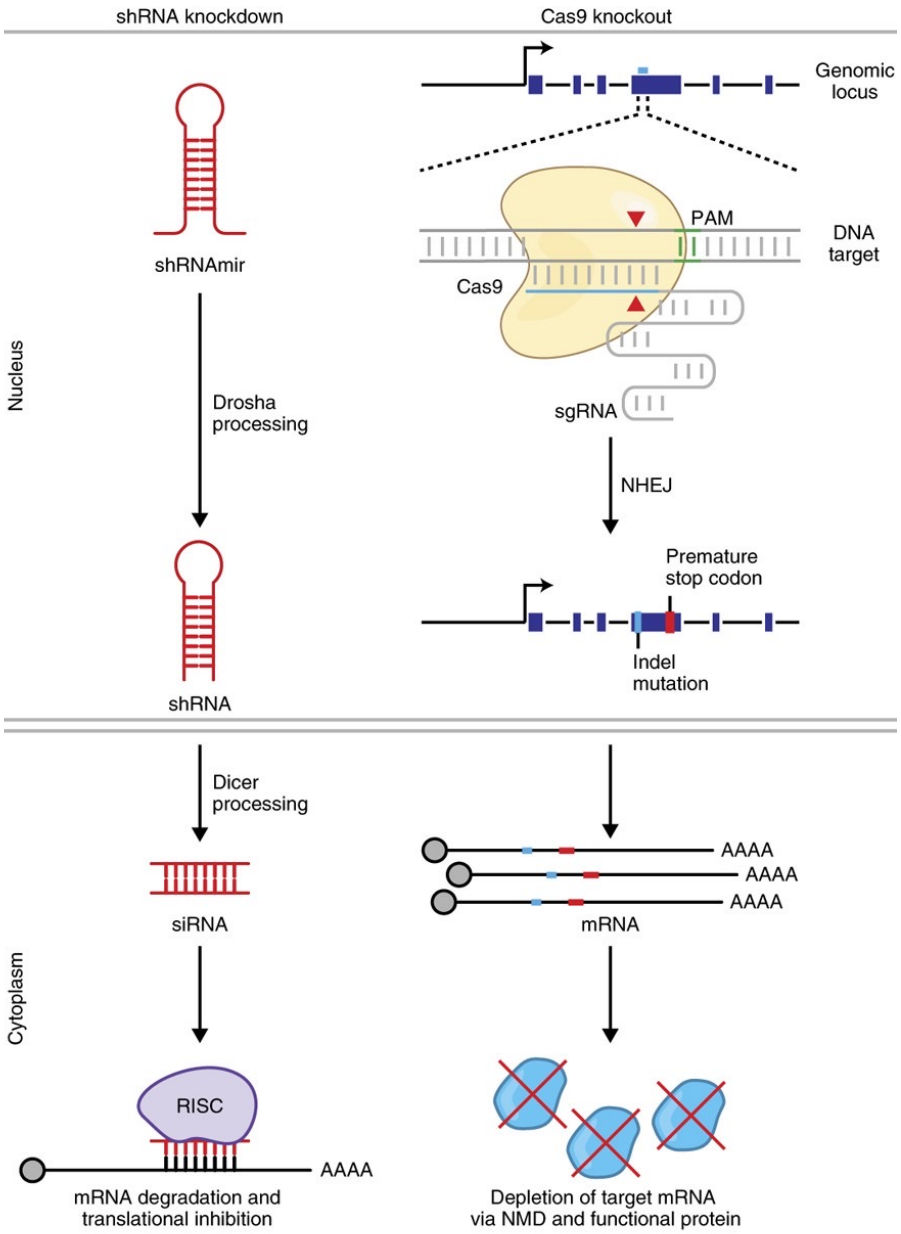
# The CRISPR/Cas9 system



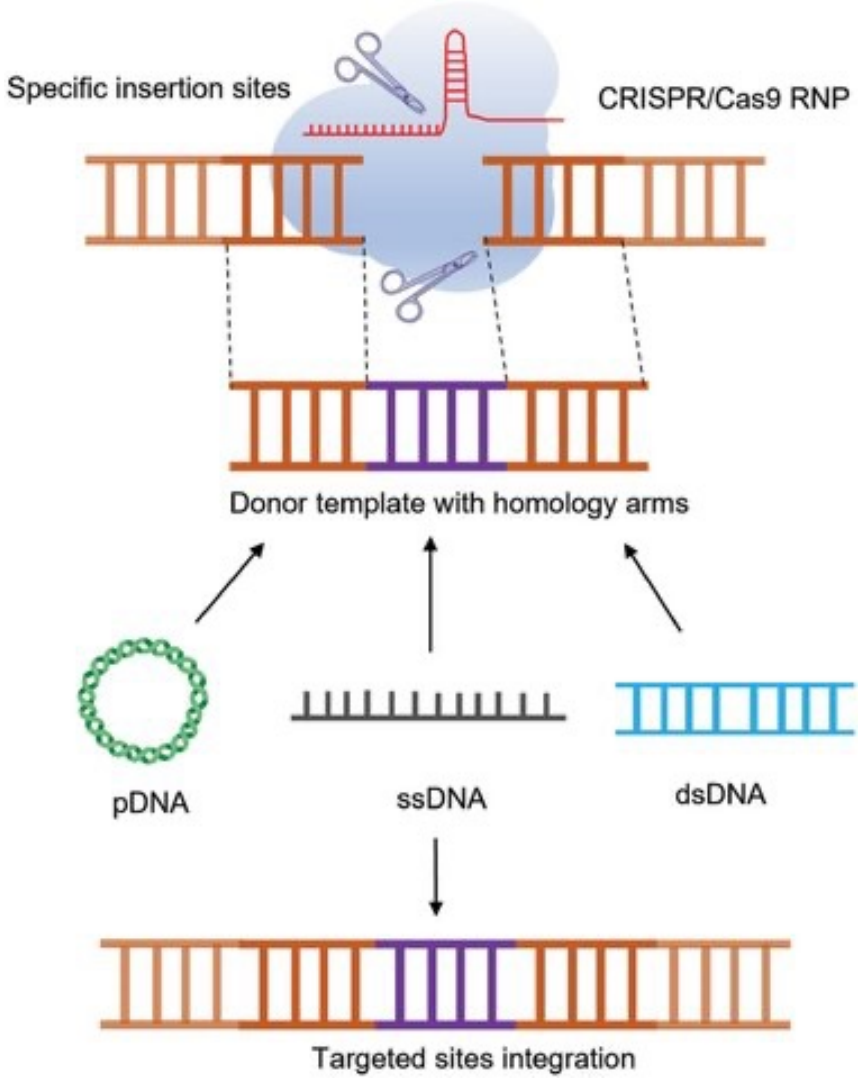
# The CRISPR-Cas9 system



# RNAi *vs* Cas9 mediated KO

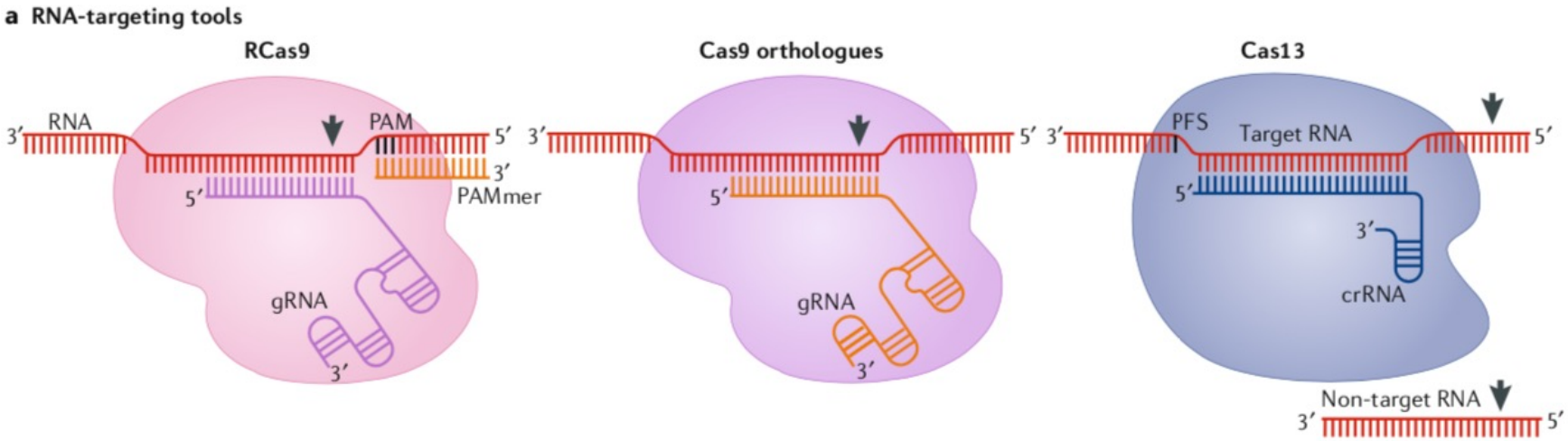


# CRISPR/Cas9-mediated knock-in

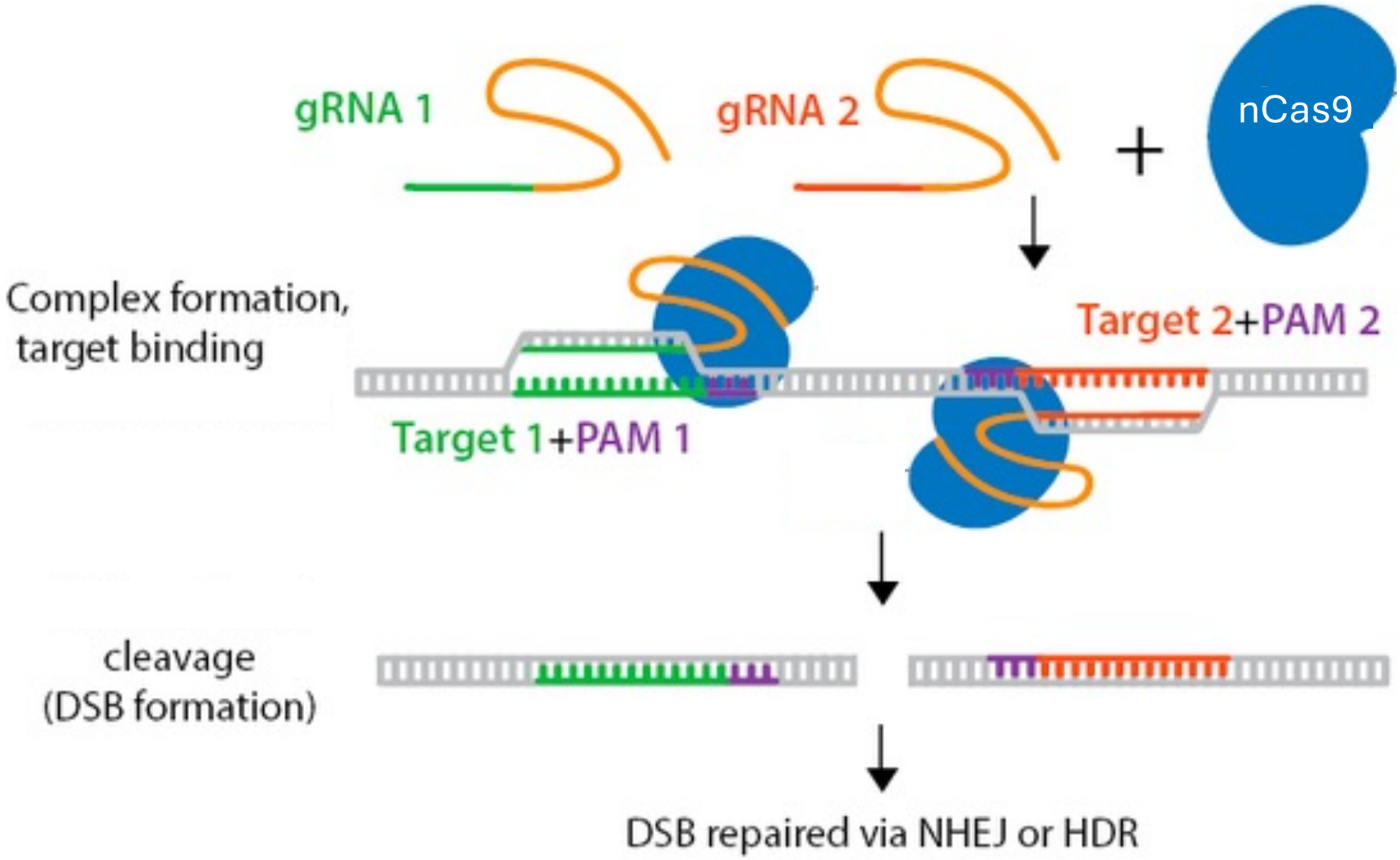


# RNA-targeting tools

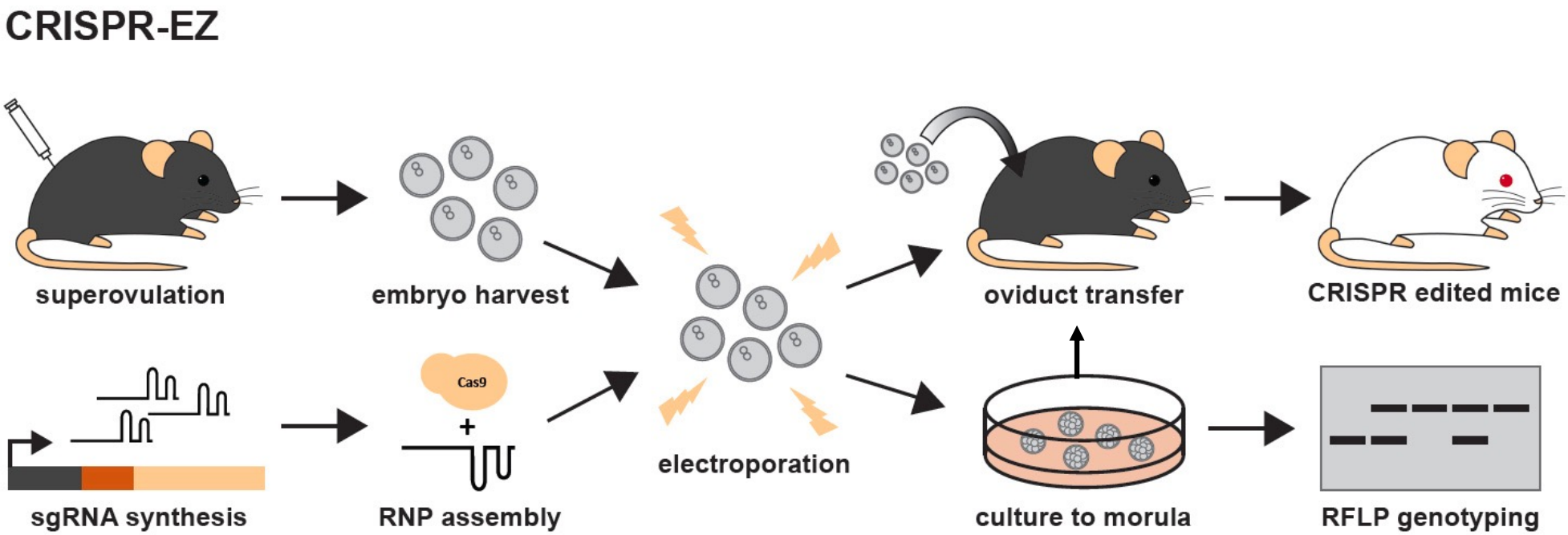
*Streptococcus pyogenes* Cas9 was repurposed to target RNA (**RCas9**) by providing it with a matching guide RNA (gRNA) and a complementary protospacer adjacent motif (PAM)-presenting oligonucleotide (PAMmer). Cas9 orthologues such as *Staphylococcus aureus* Cas9 and *Campylobacter jejuni* Cas9 can target 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.



# Modifications of CRISPR-Cas9 tool to improve specificity



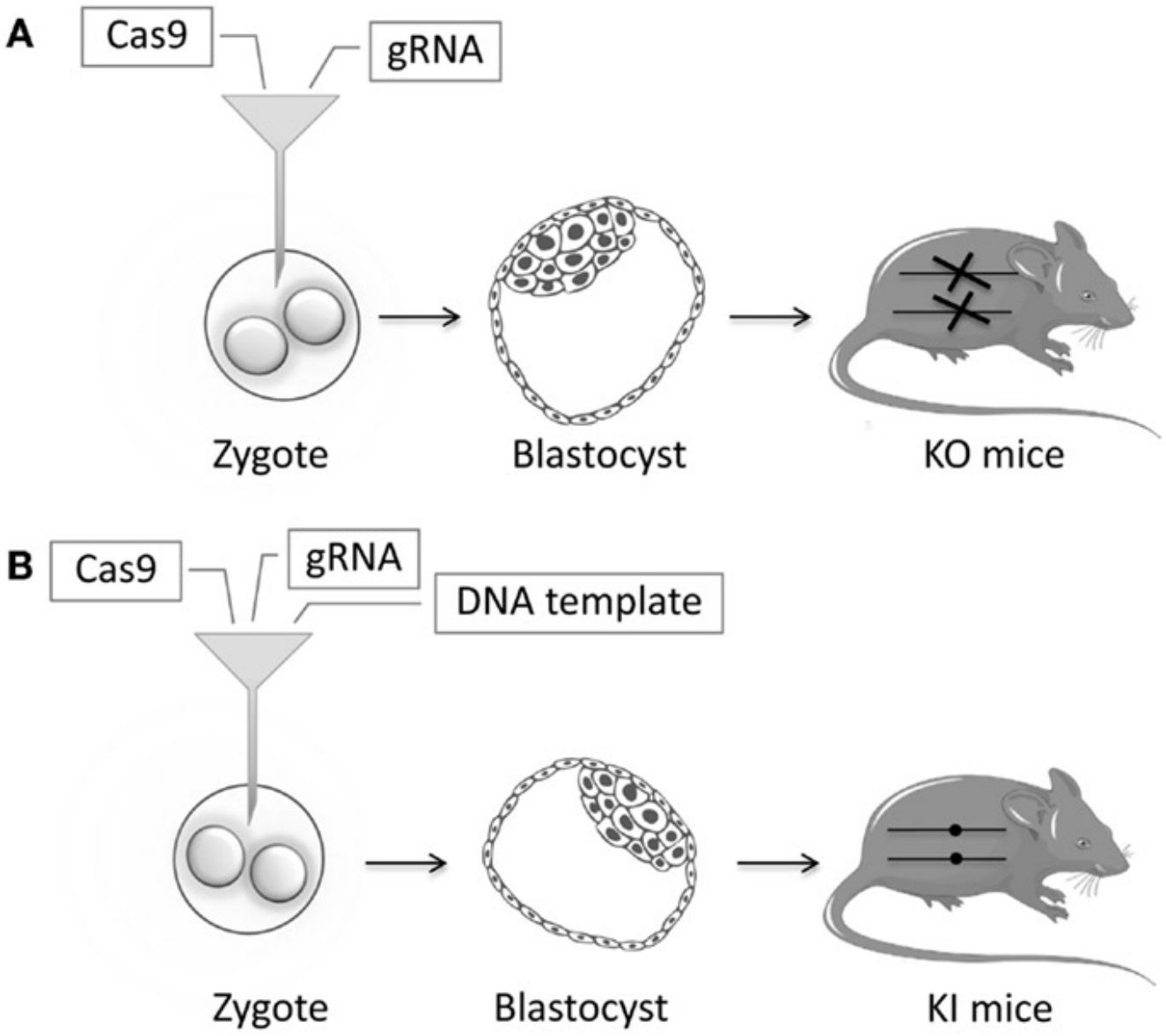
# An efficient tool for the generation of animal model




Procedure for the generation of gene-modified mice by CRISPR-Cas9

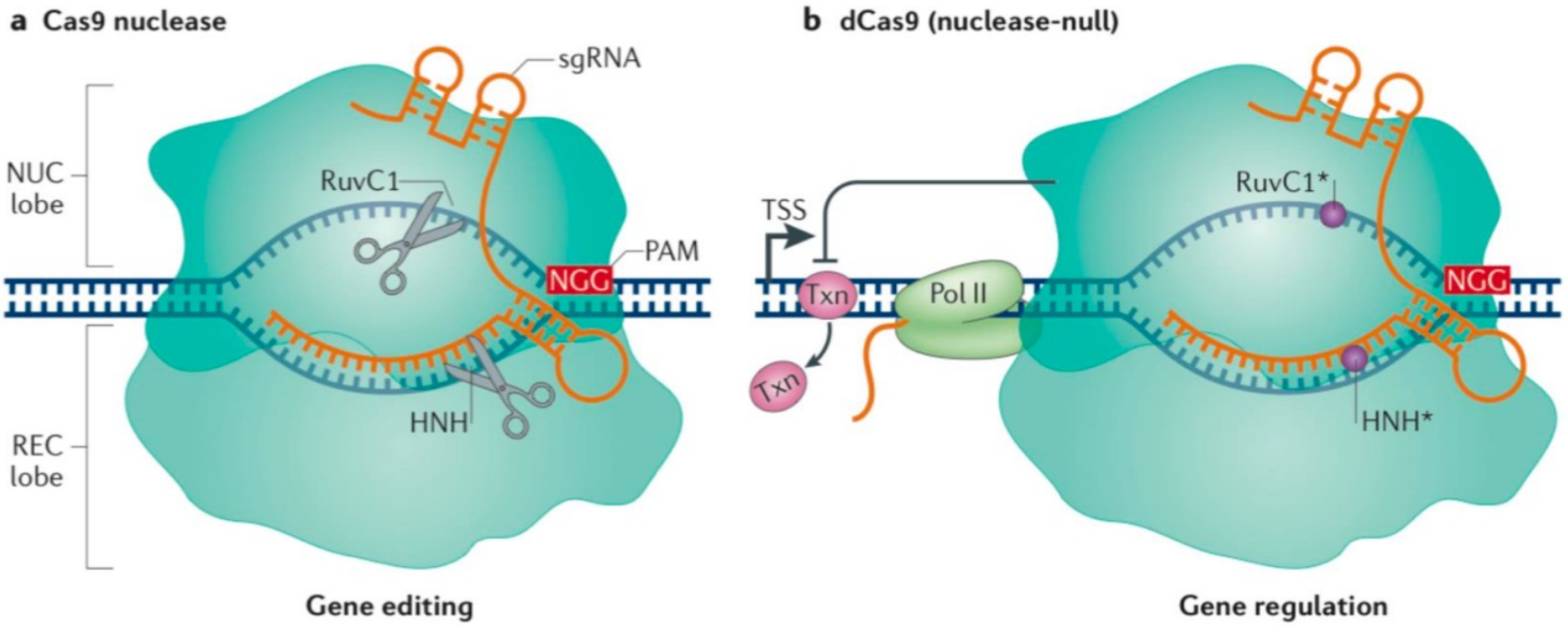
Yang et al., Nature protocol 2014

# An efficient tool for the generation of animal model



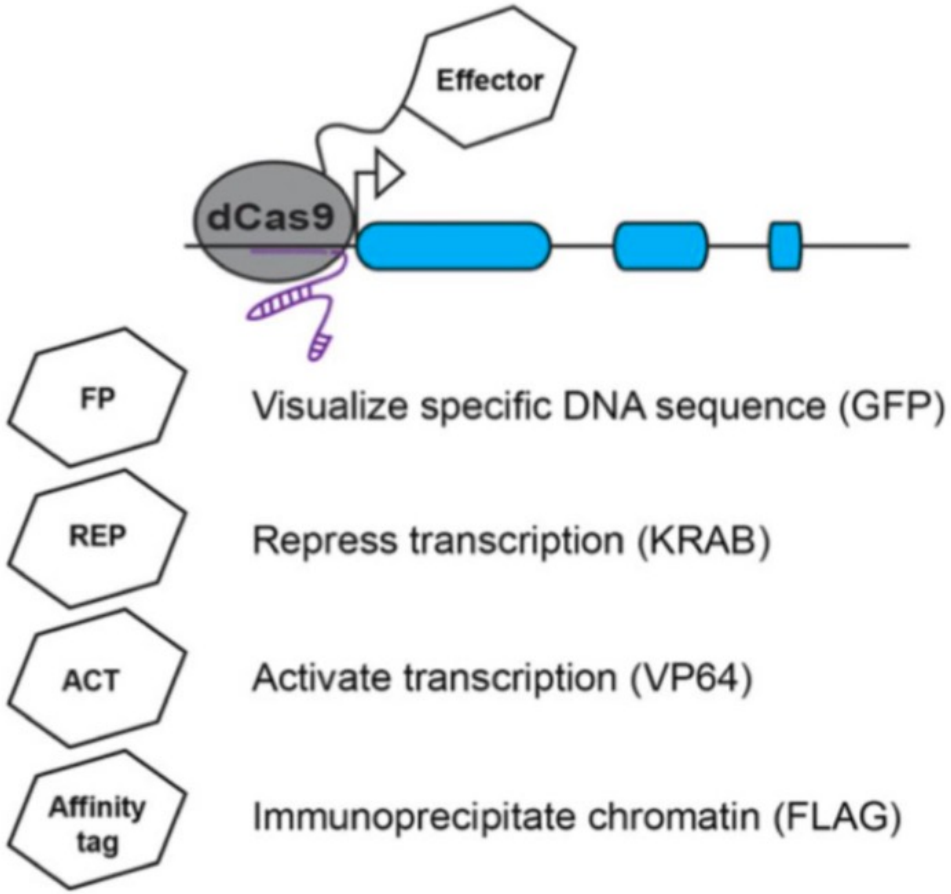
# Nuclease-deficient Cas9

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.

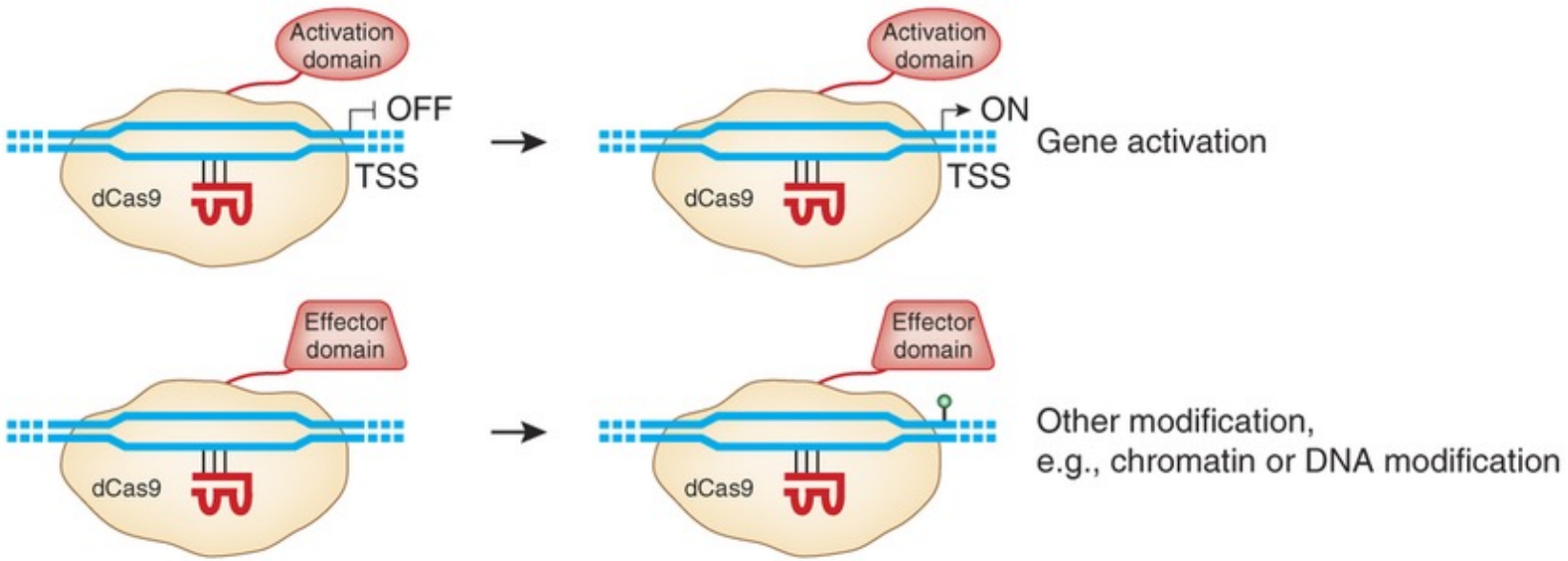



Melissa M. et al., GENES & DEVELOPMENT 2014

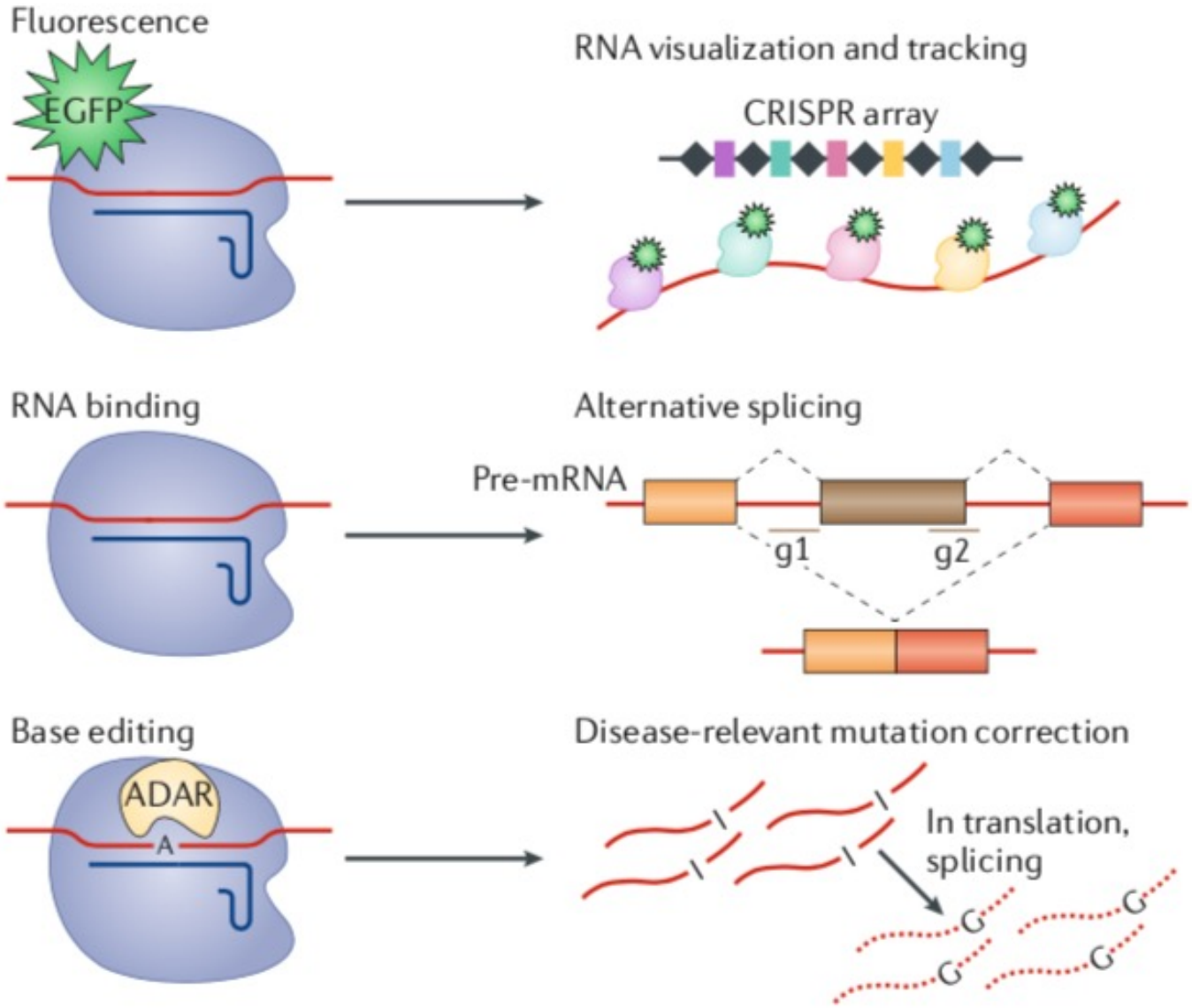
# Catalytically inactive applications

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.



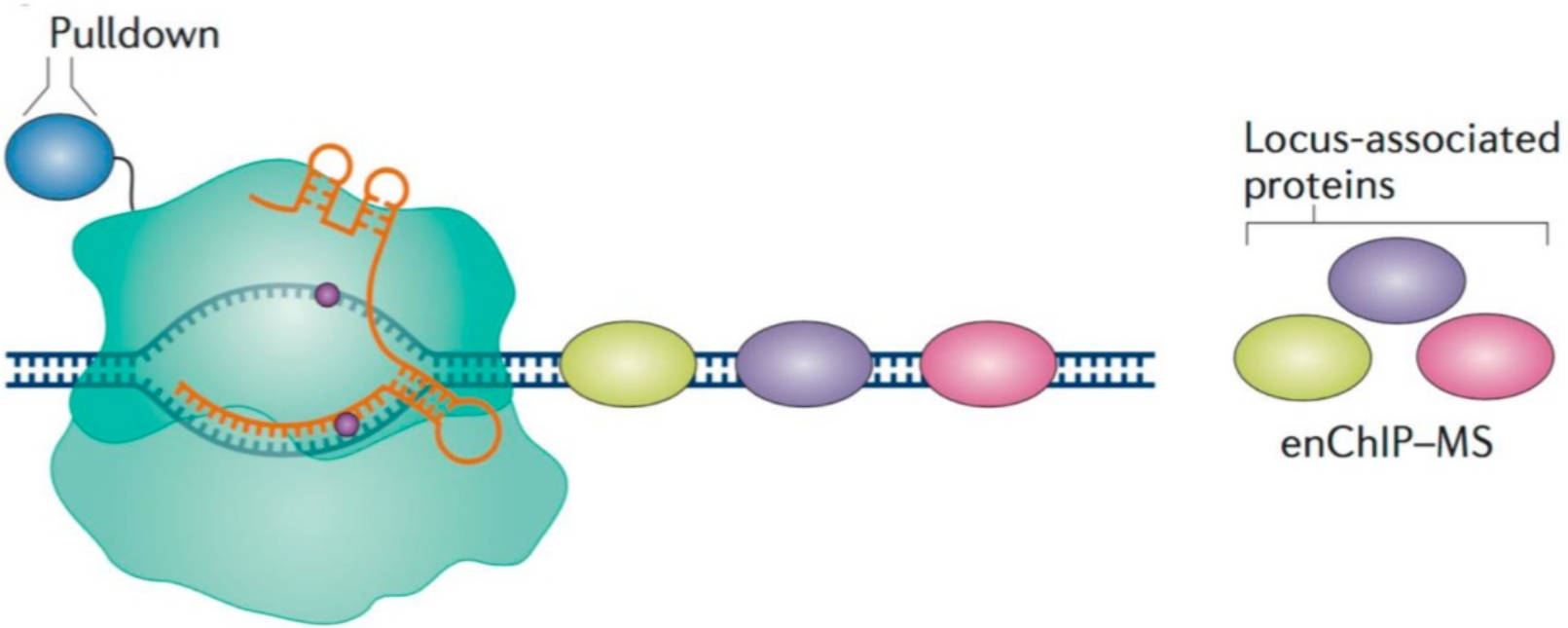
# Catalytically inactive applications



# 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

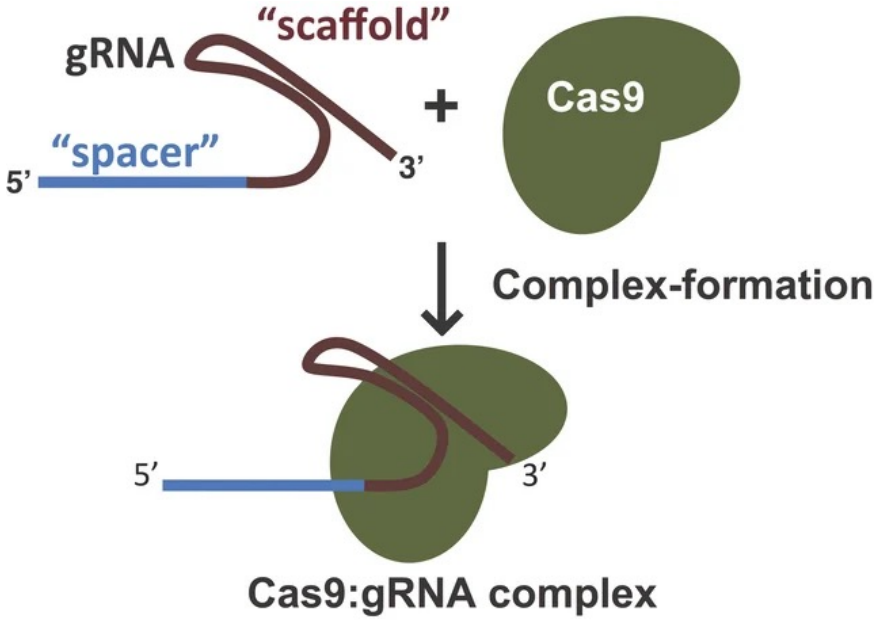


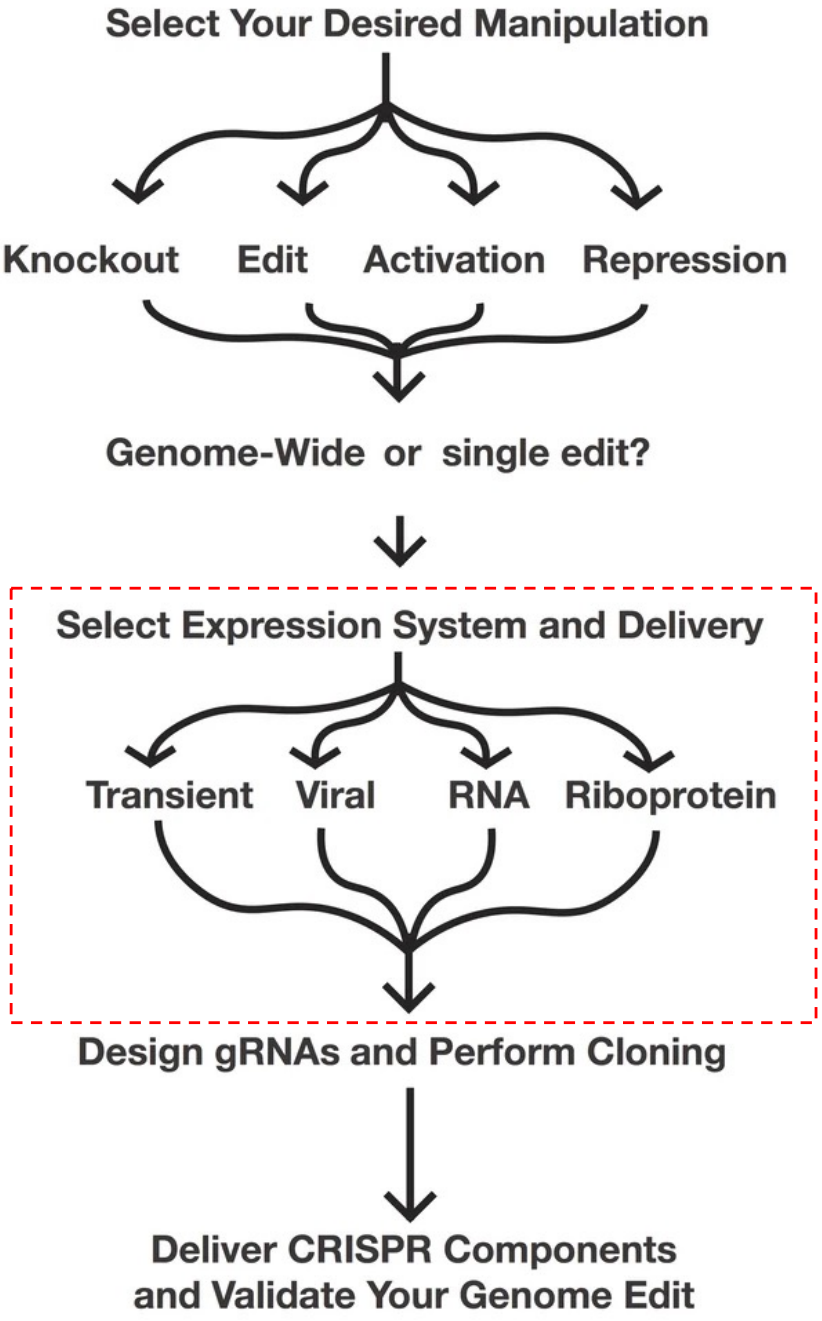
# Cas9 proteins

| | | | |
|---|---|---|---|
|  | Cut | Wild type Cas9 efficiently generates double strand breaks (DSBs) at sequences homologous to co-expressed gRNA. | Cas9 |
|  | Nick | A mutated "nickase" version of the Cas9 enzyme generates a single-strand DNA break (Nick), instead of a double-strand DNA break (Cut). | nCas9 |
|  | 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. | dCas9 |
|  | 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. | |

Modified by Addgene

# What do you need?



# Introducing the CRISPR/Cas9 system in the cell

Transfection is the introduction of DNA, RNA, or proteins into eukaryotic cells and is used in research to study and modulate gene expression. Transient transfection techniques involve the introduction of DNA into cells, but in this method, the DNA does not integrate with the cellular chromosomes. Stable transfection techniques involve the integration of the transfected DNA into cellular chromosomes or the formation of episomes. The stably transfected cell can be subsequently identified using selectable markers

**Transient**

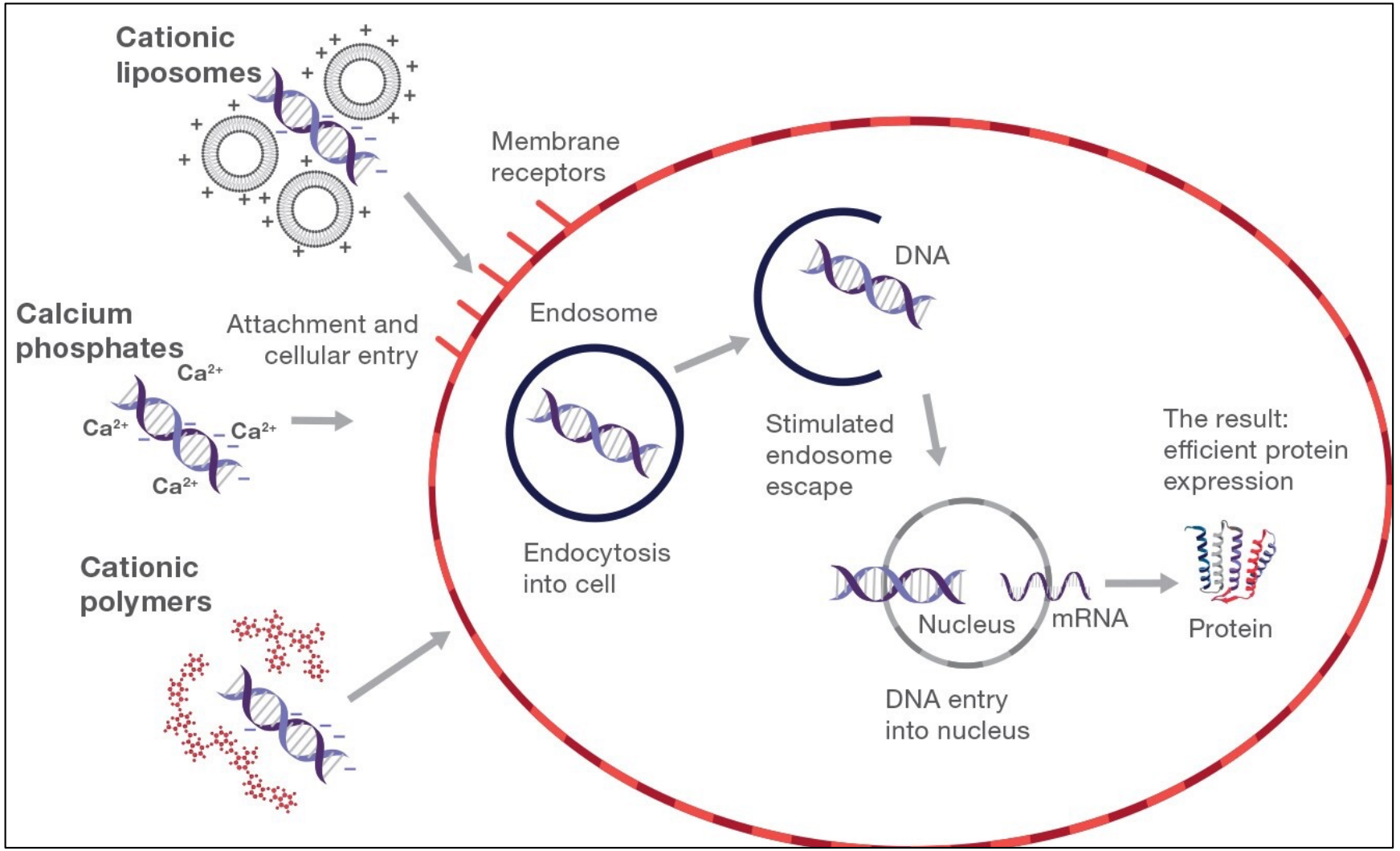
- **Calcium phospate method**
- **Catyonic polymers**
- **Electroporation**
- **Lipofection**

**Stable**

- **Viral trasduction**
- **Trasposons**

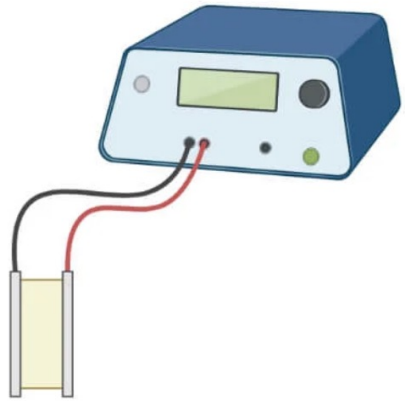
# Transient transfection techniques

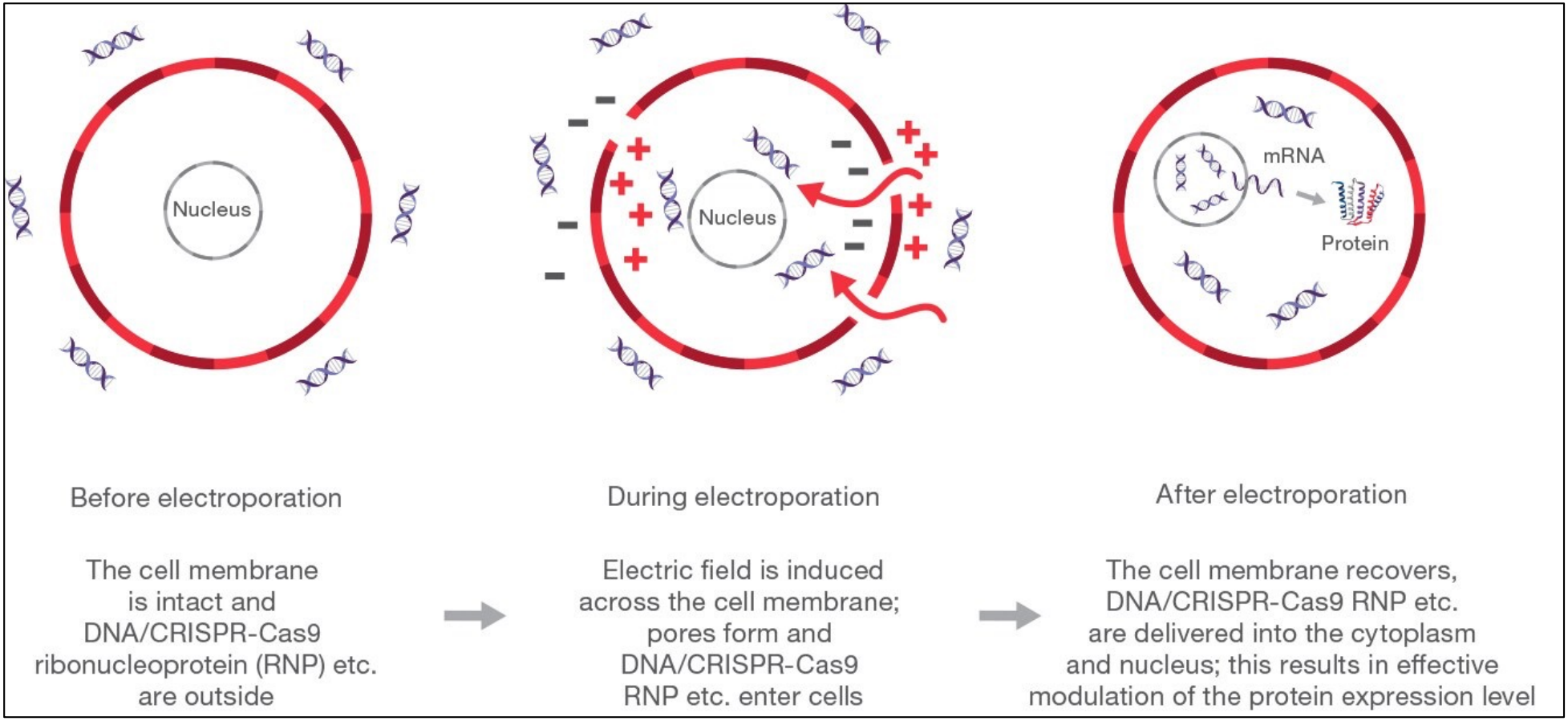
Chemical transfection methods



# Transient transfection techniques

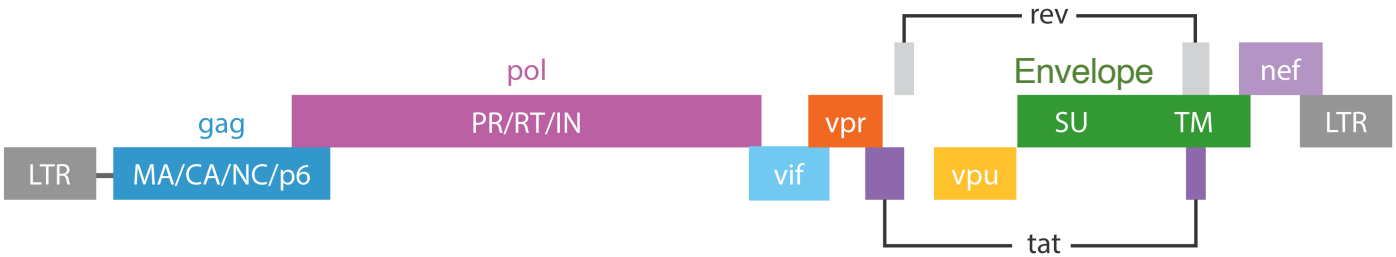
Physical transfection methods: electroporation

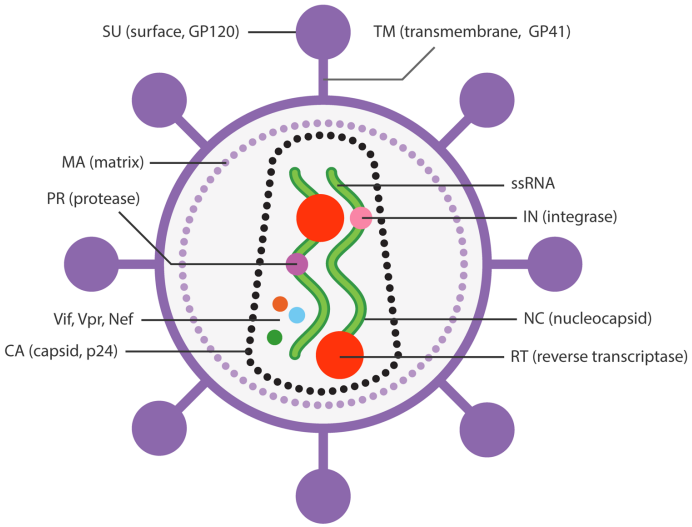


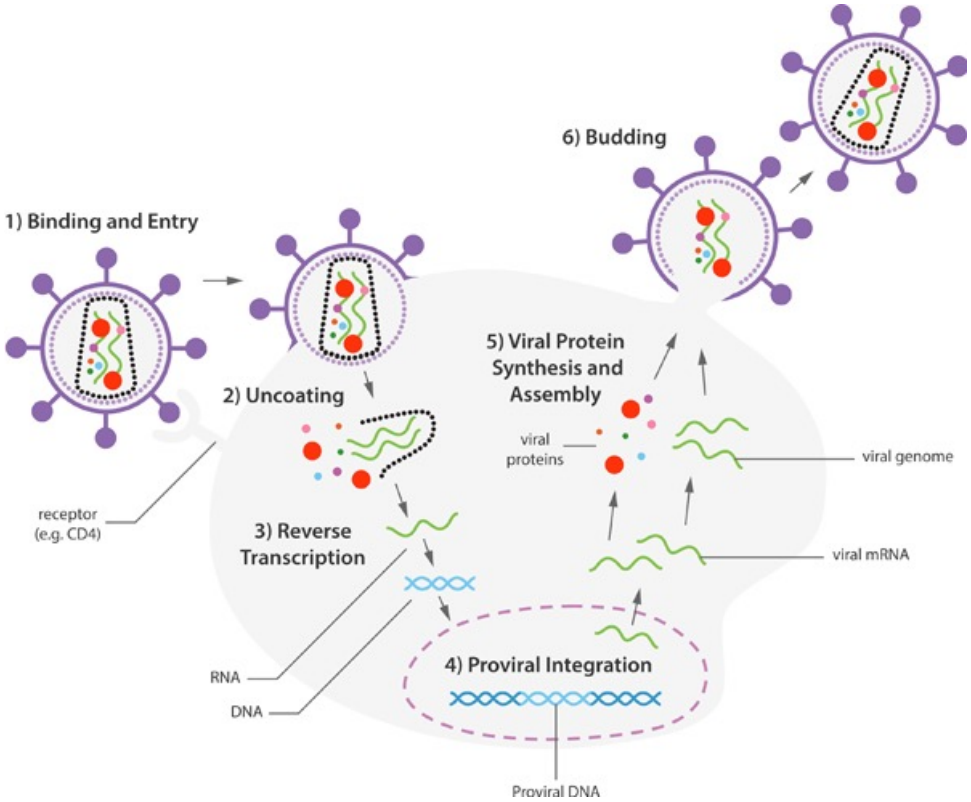


# Stable transfection techniques

## Lentivirus

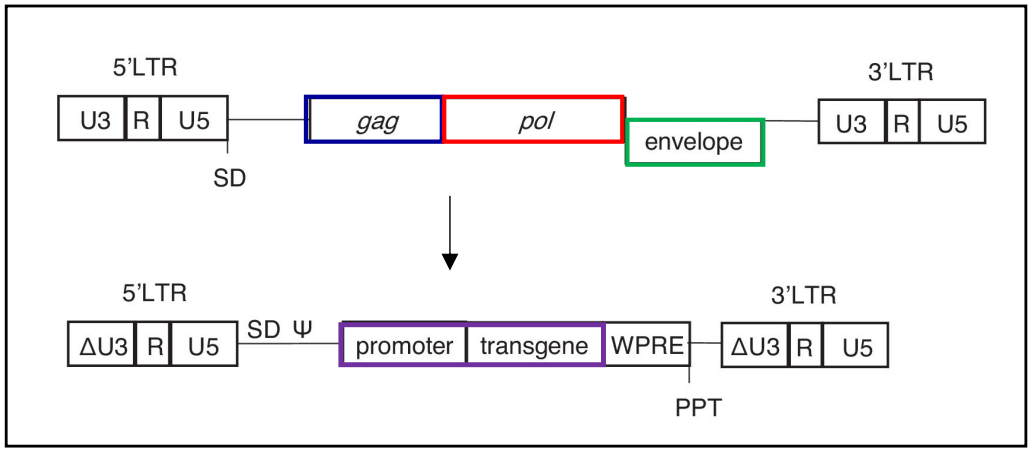


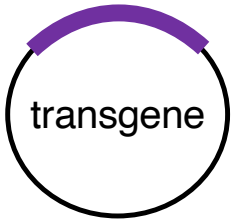



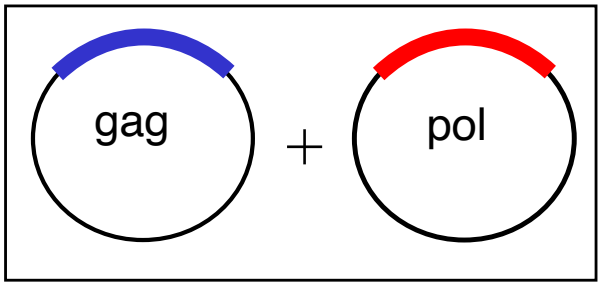
# Stable transfection techniques

Lentiral expressing vector

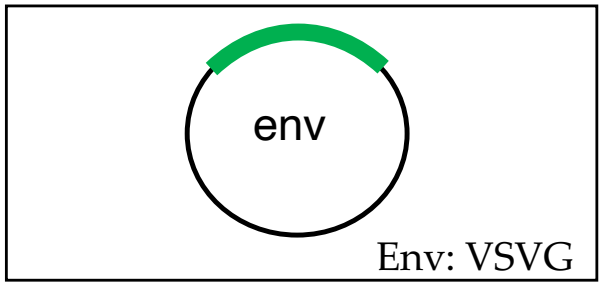



Packaging plasmids

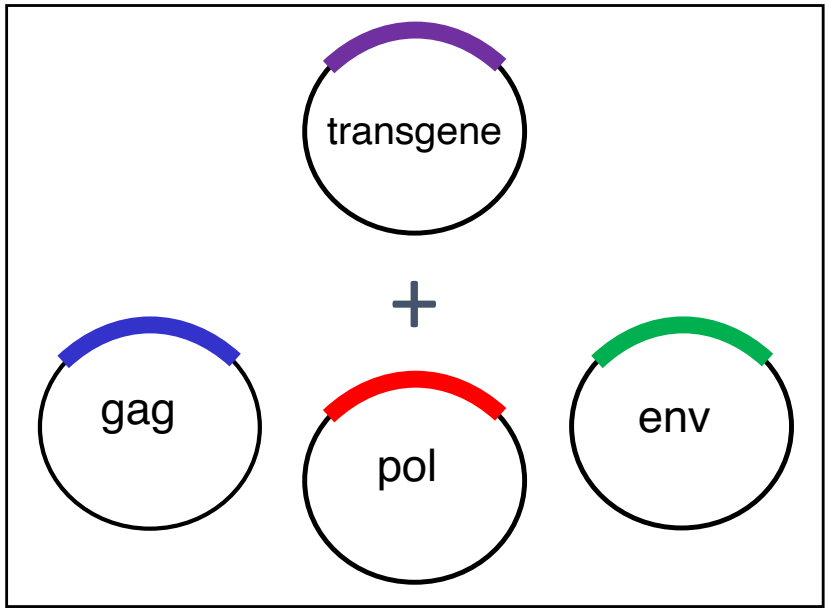



Envelope plasmid

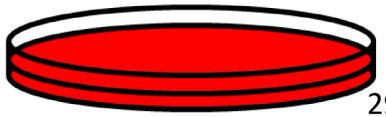


# Stable transfection techniques

Packaging

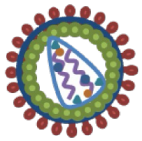



1) Transfect packaging cells

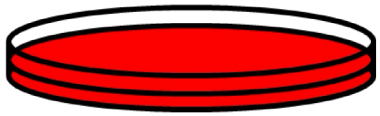


293T/293FT

2) Collect virus particles

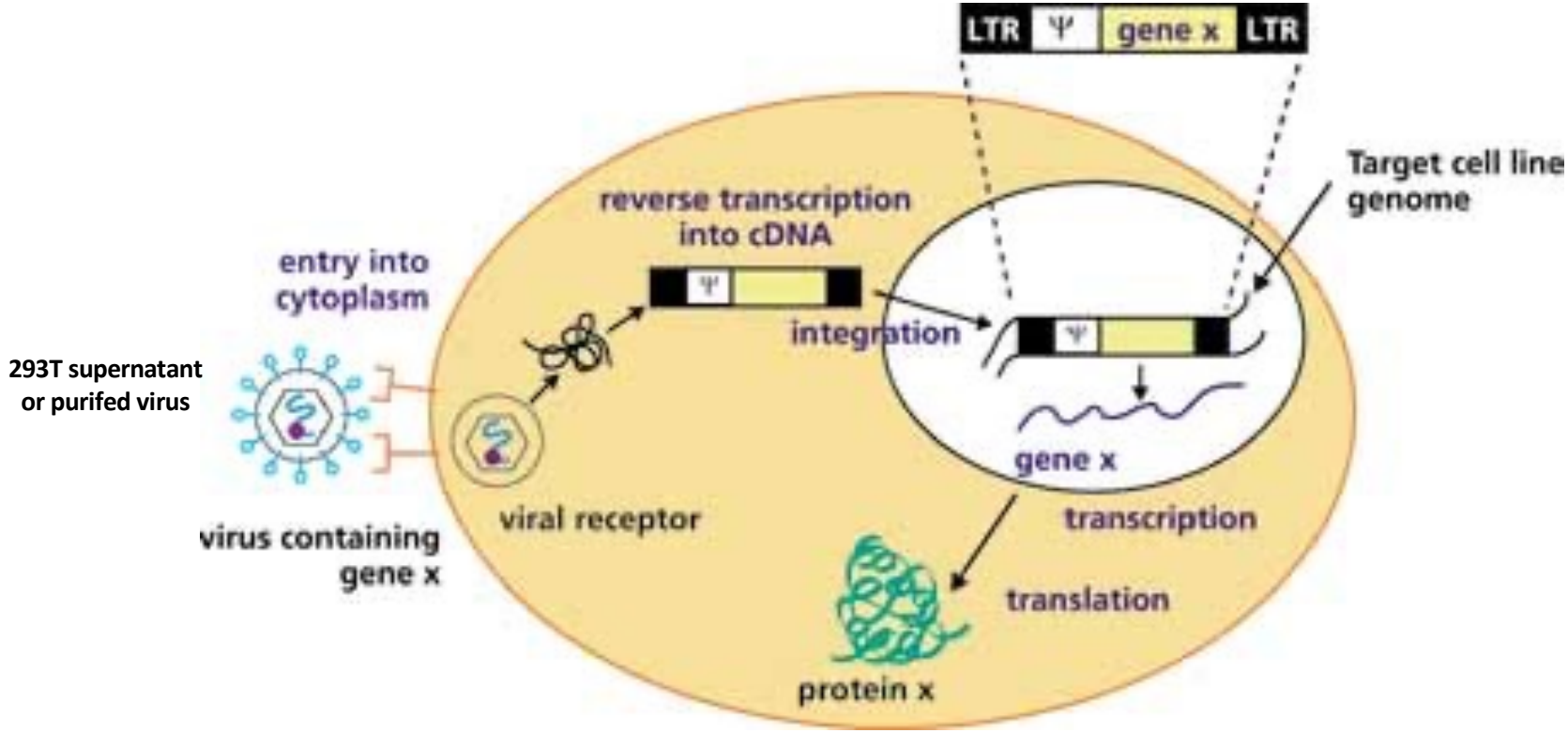


3) Transduce target cells



# Stable transfection techniques

## Transduction

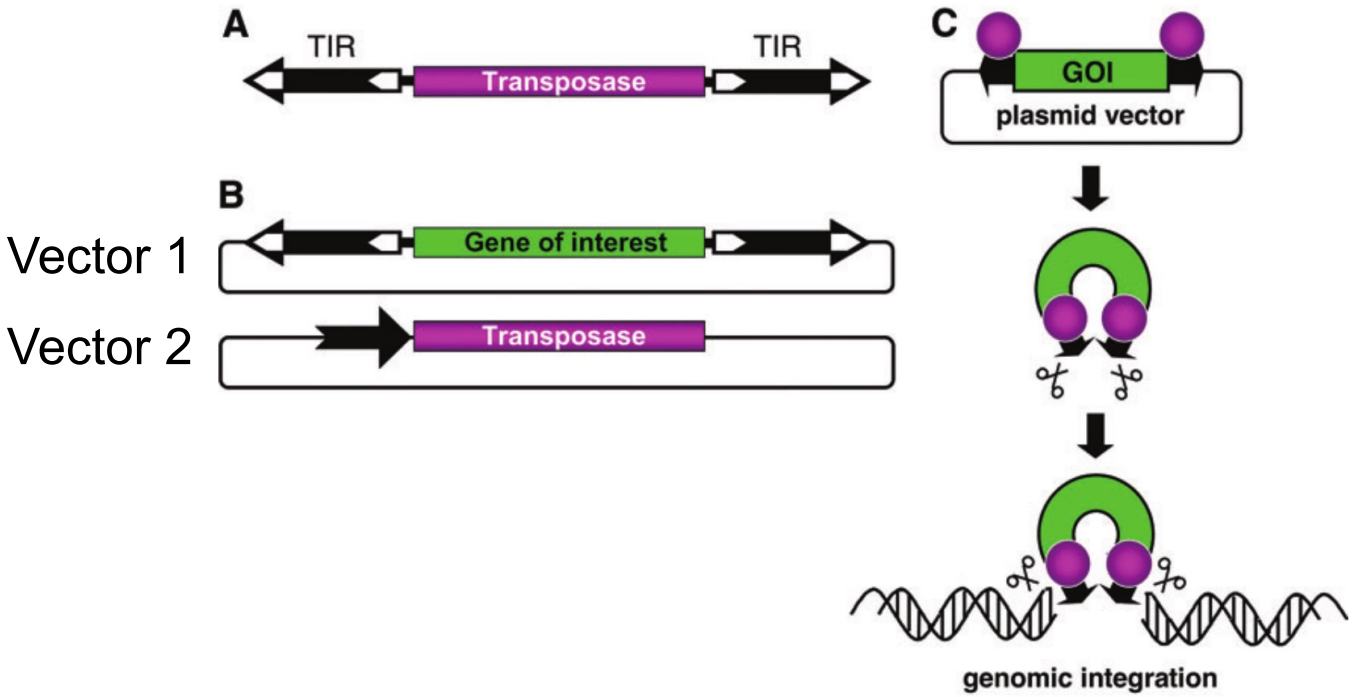


# Trasposable vectors

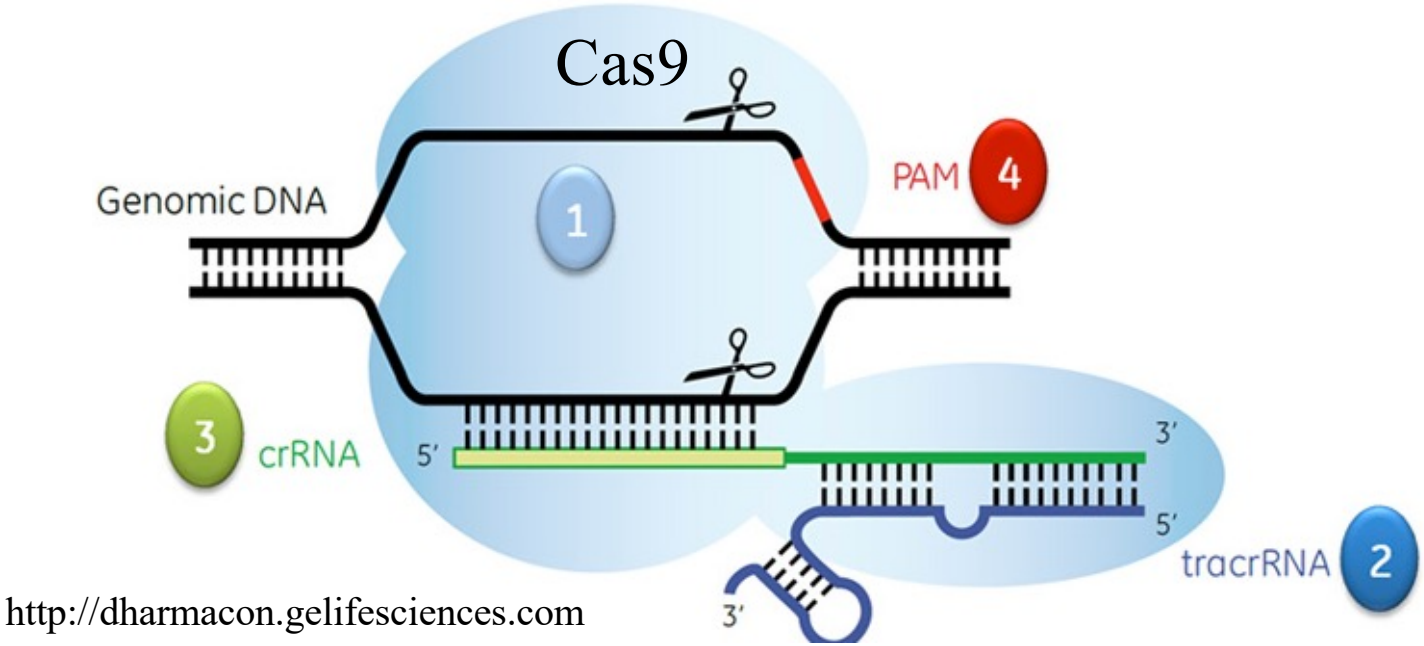
(A) Autonomous transposable elements consist of Terminal Inverted Repeats (TIRs; black arrows) flanking the transposase gene.

(B) Vector system for transposition. One component contains the DNA of interest flanked by the TIRs in the plasmid vector, while the other component is a transposase expression plasmid, where the black arrow represents the promoter driving transposase expression.

C) The transposon carrying the DNA of interest is excised from the donor plasmid and integrated into a chromosomal site by the transposase.



# Gene editing with the CRISPR/Cas9 system

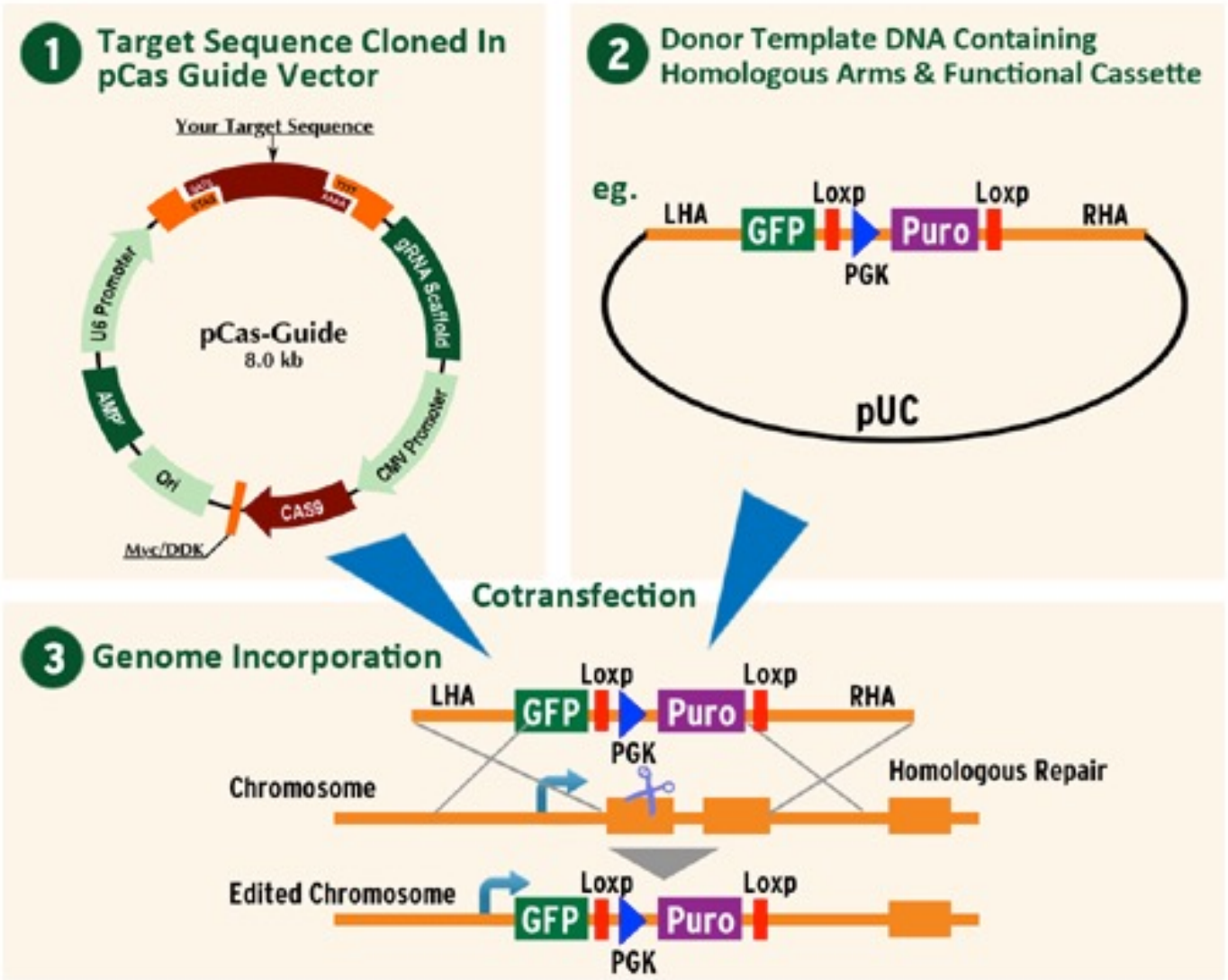



http://dharmacon.gelifesciences.com

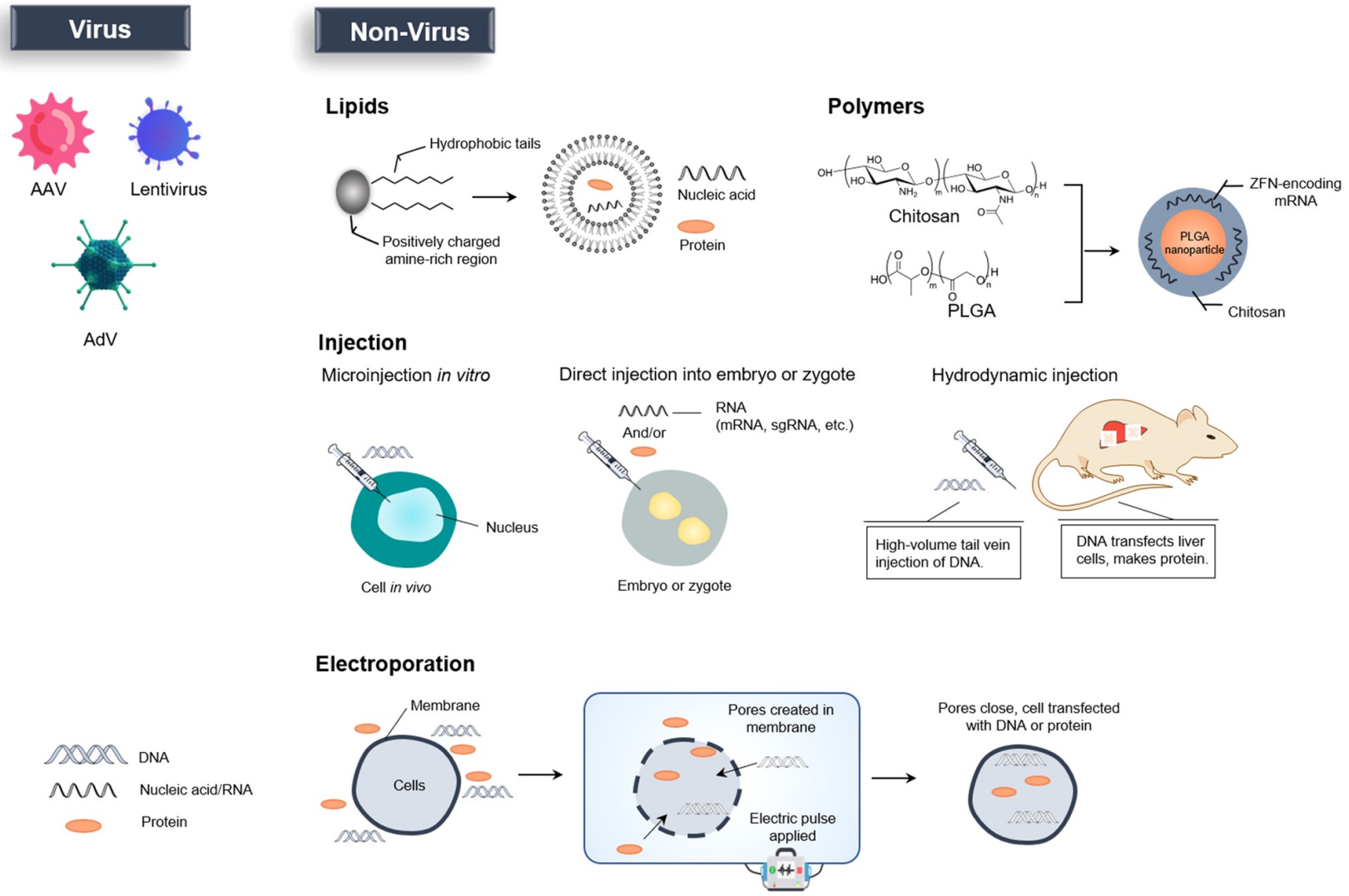
1. A plasmid expressing a mammalian codon-optimized gene sequence encoding Cas9 nuclease or Cas9 protein
2. A chemically synthesized trans-activating CRISPR RNA (tracrRNA)
3. A chemically synthesized CRISPR RNA (crRNA) designed to cleave the gene target site of interest (the chosen 20-base target sequence 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)



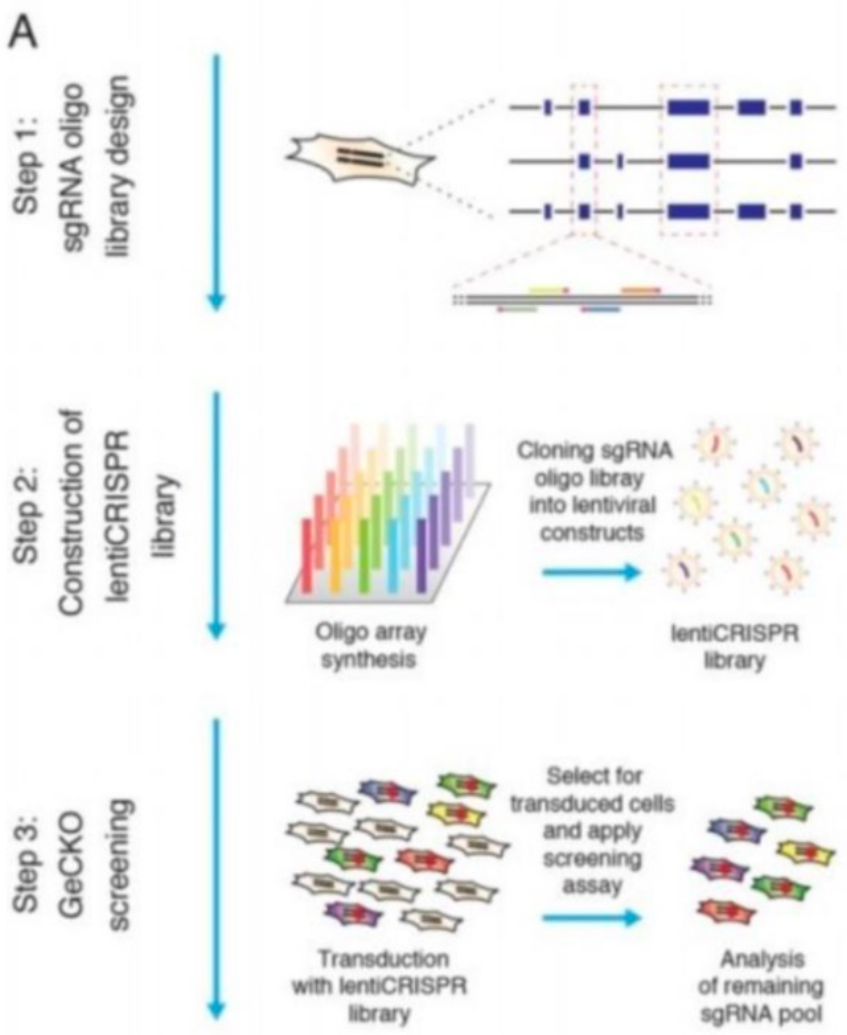
# Viral and nonviral delivery systems for genome editing technology



# Lentiviral CRISPR libraries

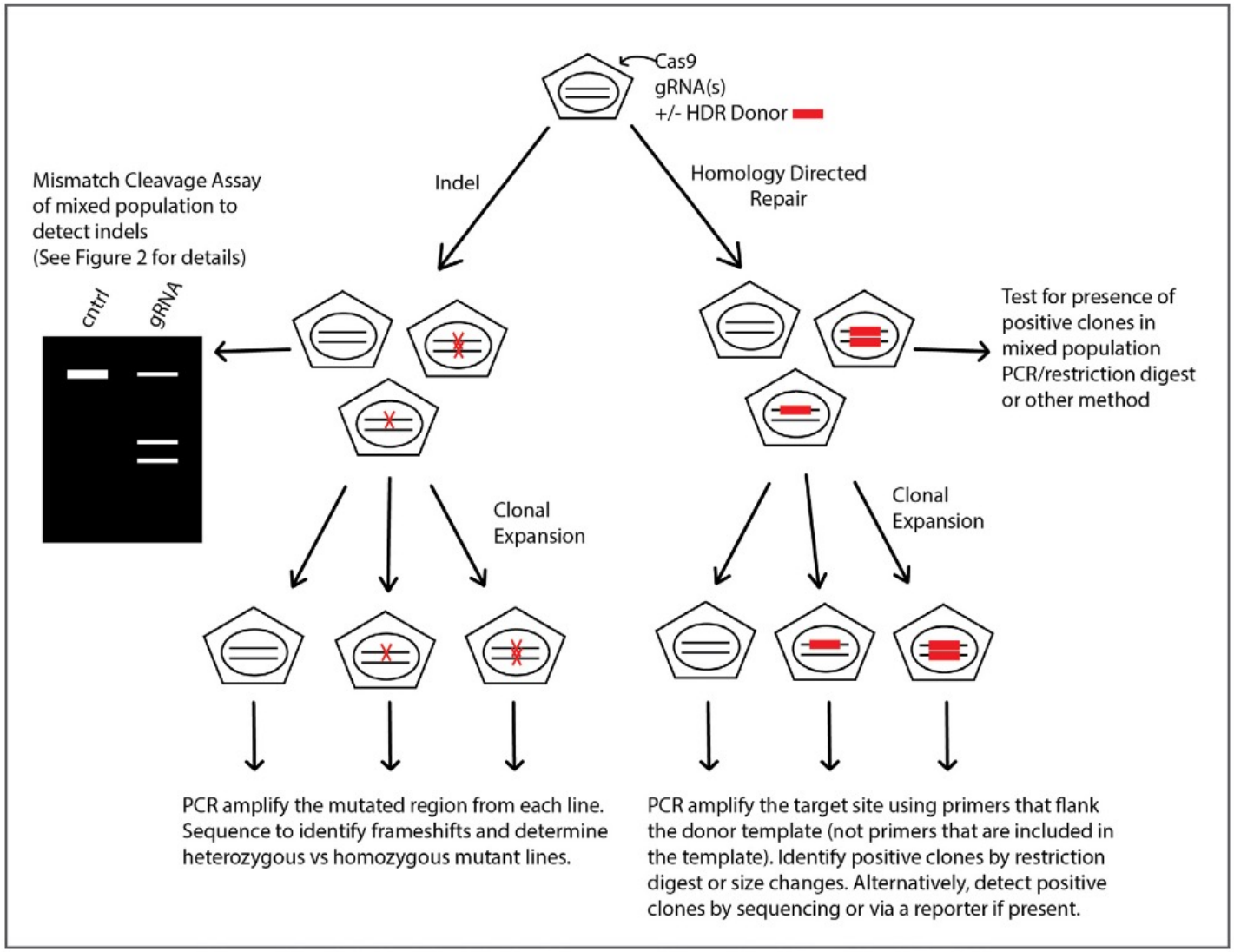
Pooled lentiviral CRISPR libraries (heretofore referred to as CRISPR libraries) are a heterogenous population of lentiviral transfer vectors, each containing an individual gRNA targeting a single gene in a given genome.

In CRISPR-KO screening, **a pool of synthetic single guide RNAs (sgRNAs, or simply gRNAs) that targets every gene in the genome under interrogation is transduced into the cells under study**, usually at the stoichiometry of a single gRNA per cell. **The final cell population that exhibits the phenotype of interest are then recovered and the integrated gRNAs, which reveal the identity of the knocked-out genes, are sequenced in parallel**. Analysis of the differences in the abundance of gRNAs between the controls and the treated cells would unveil the relationship between genes and the phenotype of interest[22]




Modified by Addgene

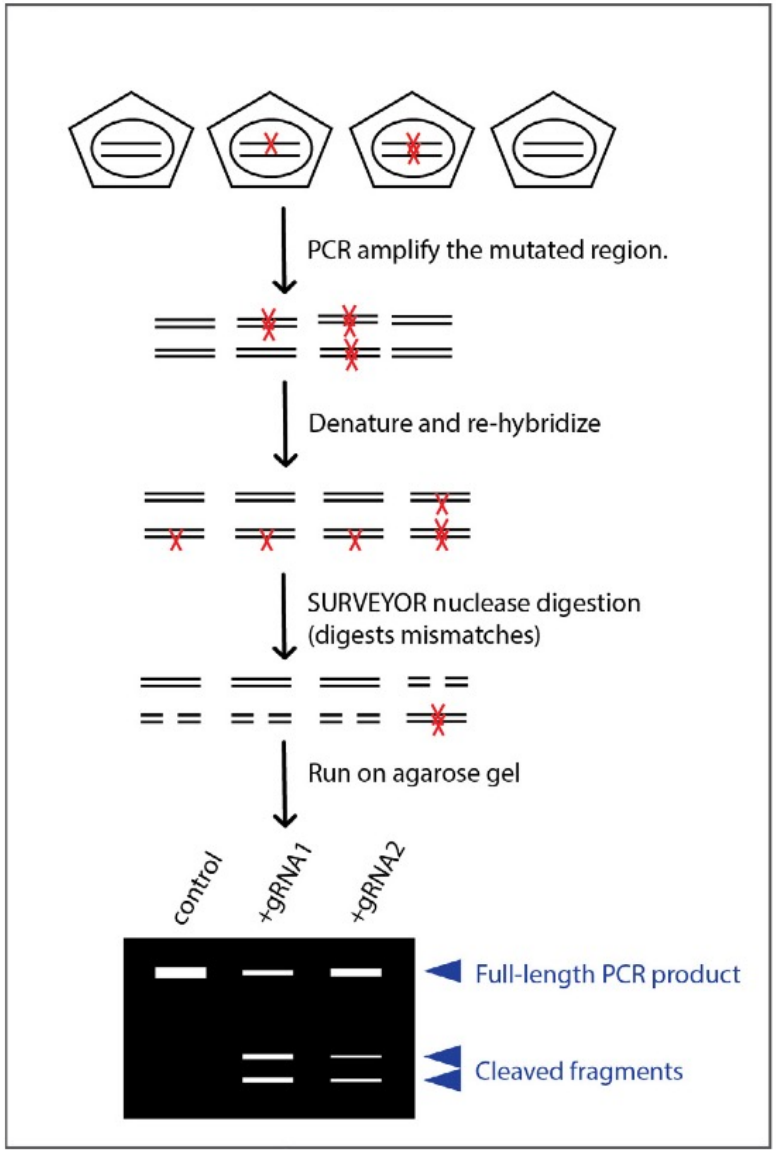
# Validating your genome editing



# Validating your genome editing

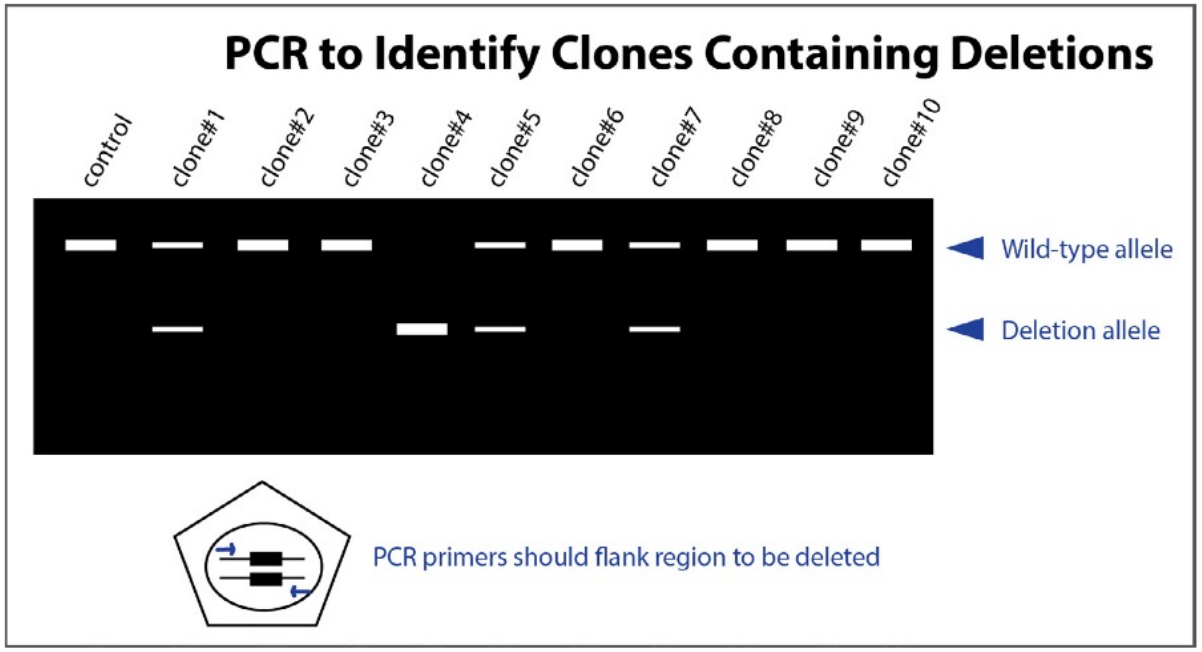
**Mismatch cleavage assay:**

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.

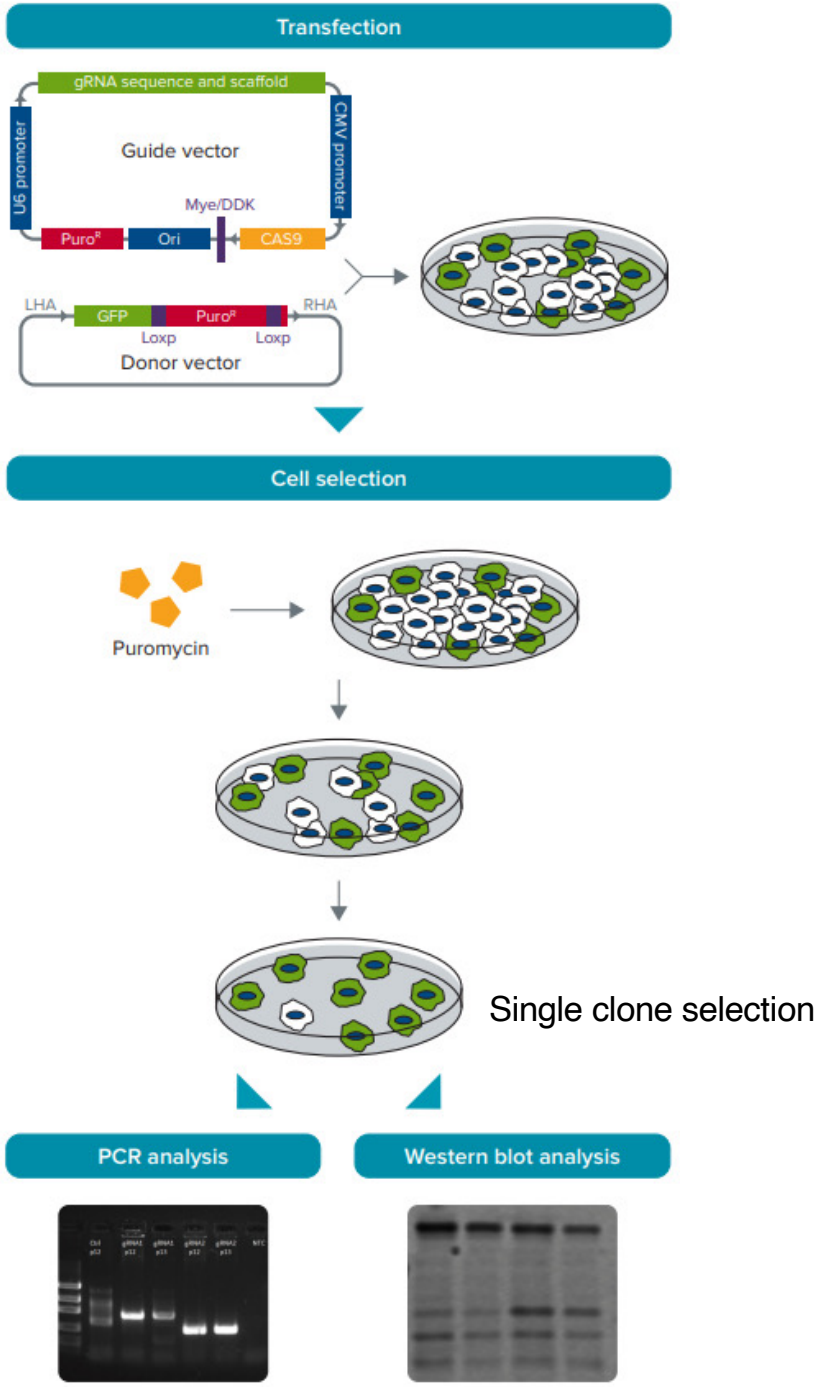


# Validating your genome editing

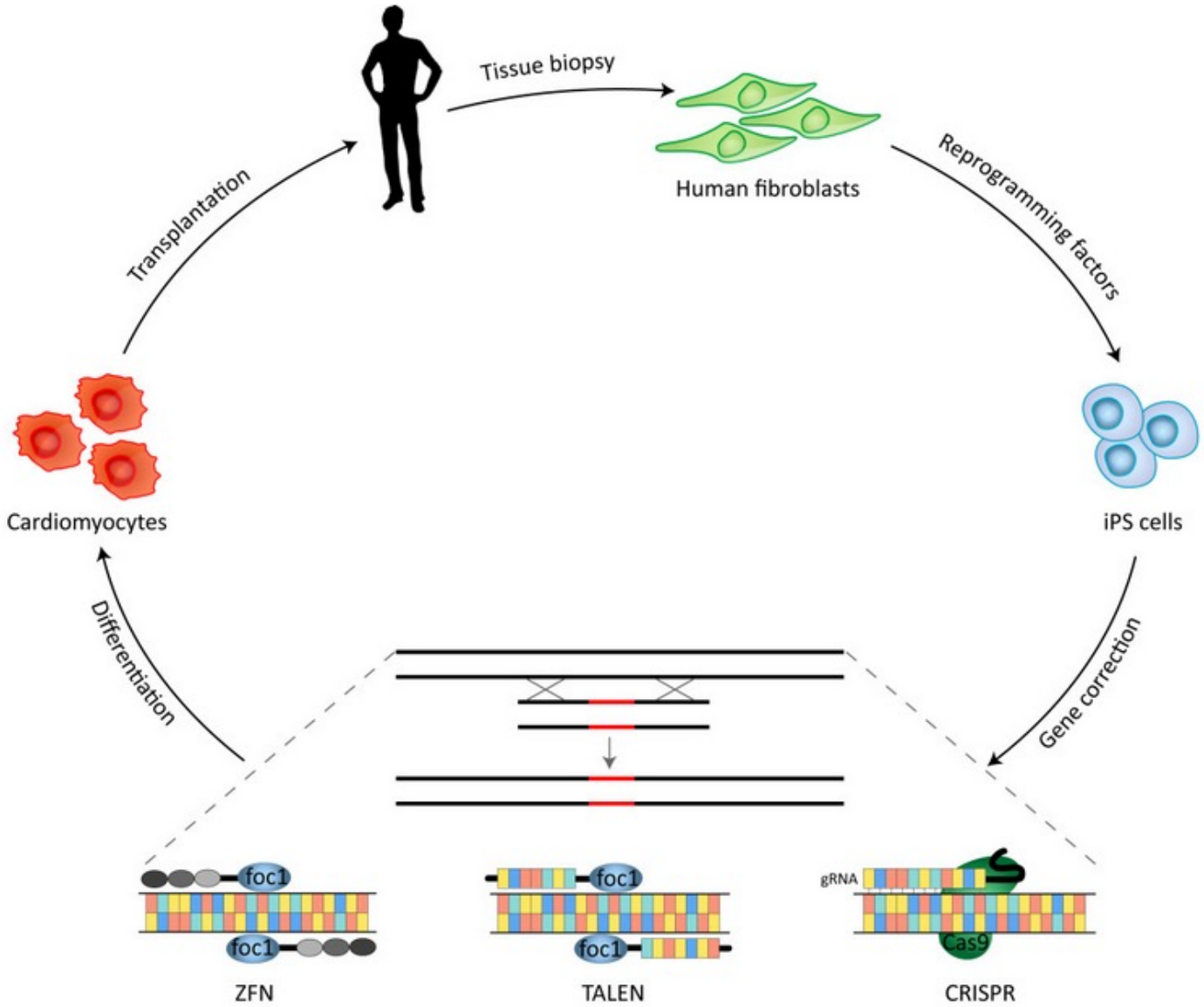
HR events are generally less frequent than indels, so you will likely need to screen a larger number of colonies to create a clonal line. The number of clones that need to be screened will depend on both your transfection/transduction efficiency and HR frequency. For example, if you have 40% transfection 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.



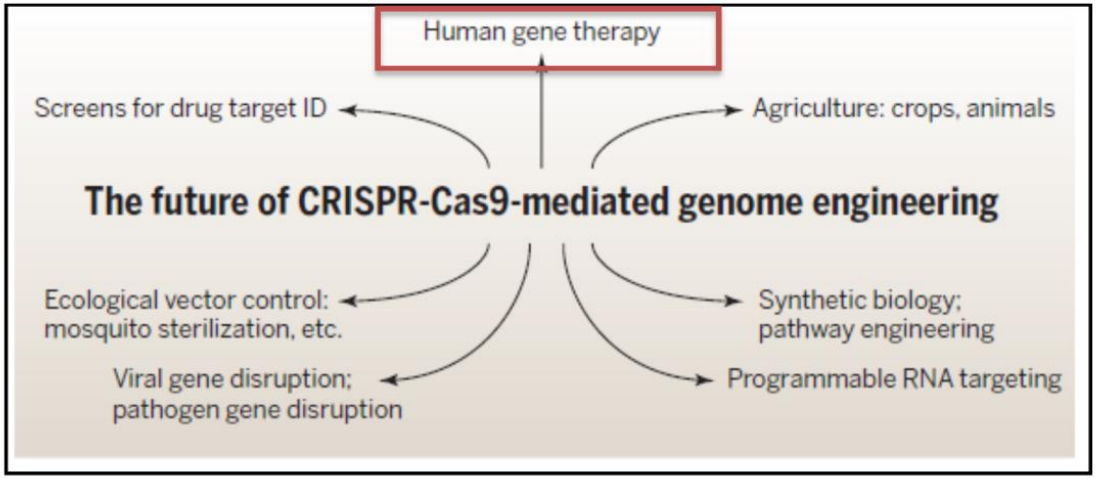
# CRISPR/Cas9 knock-in experimental workflow



# 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, Yangiao ZHANG

*In vivo* gene therapy potentials of CRISPR-Cas9

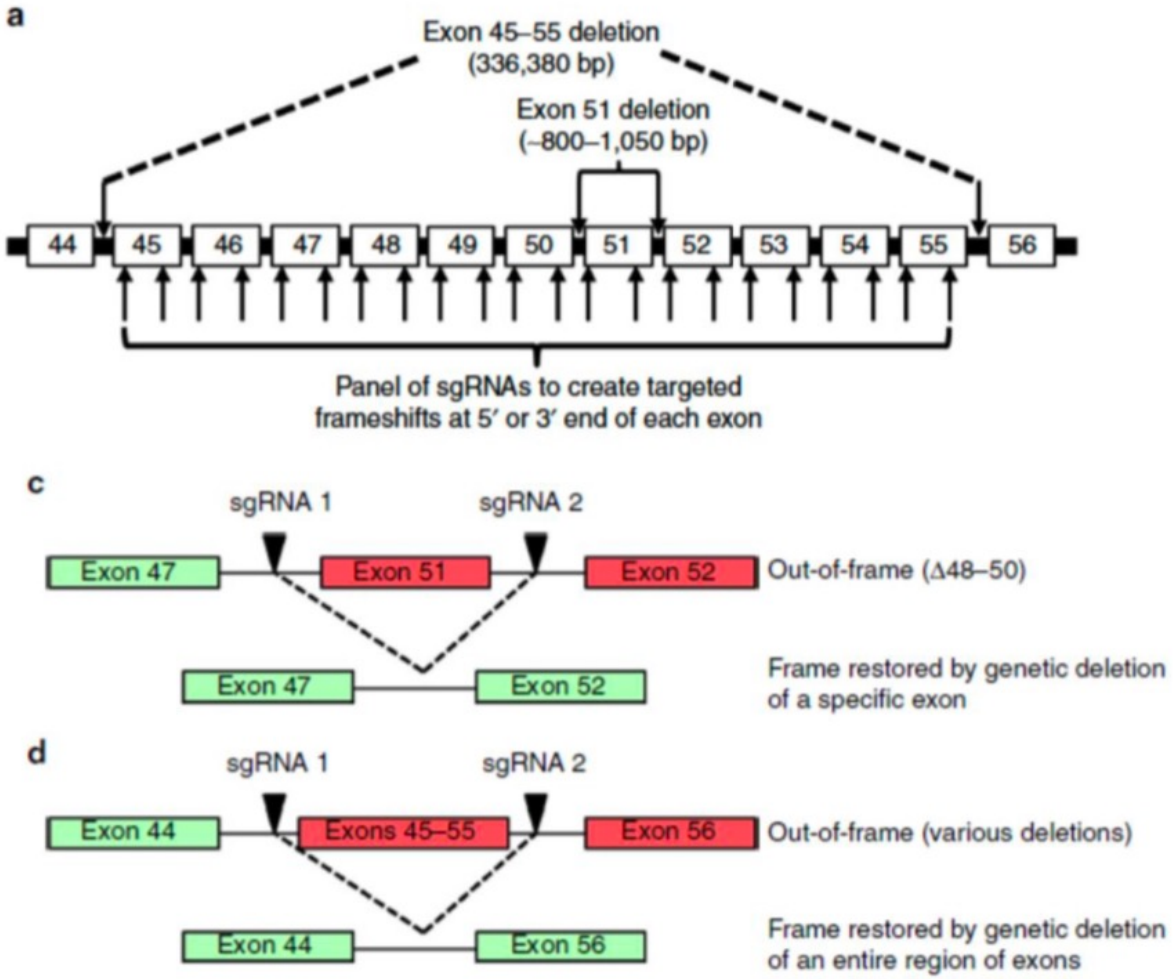
H-Y Xue[1,6], X Zhang[2,6], Y Wang[3,6], L Xiaojie[3], W-J Dai[4] and Y Xu[5]

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.

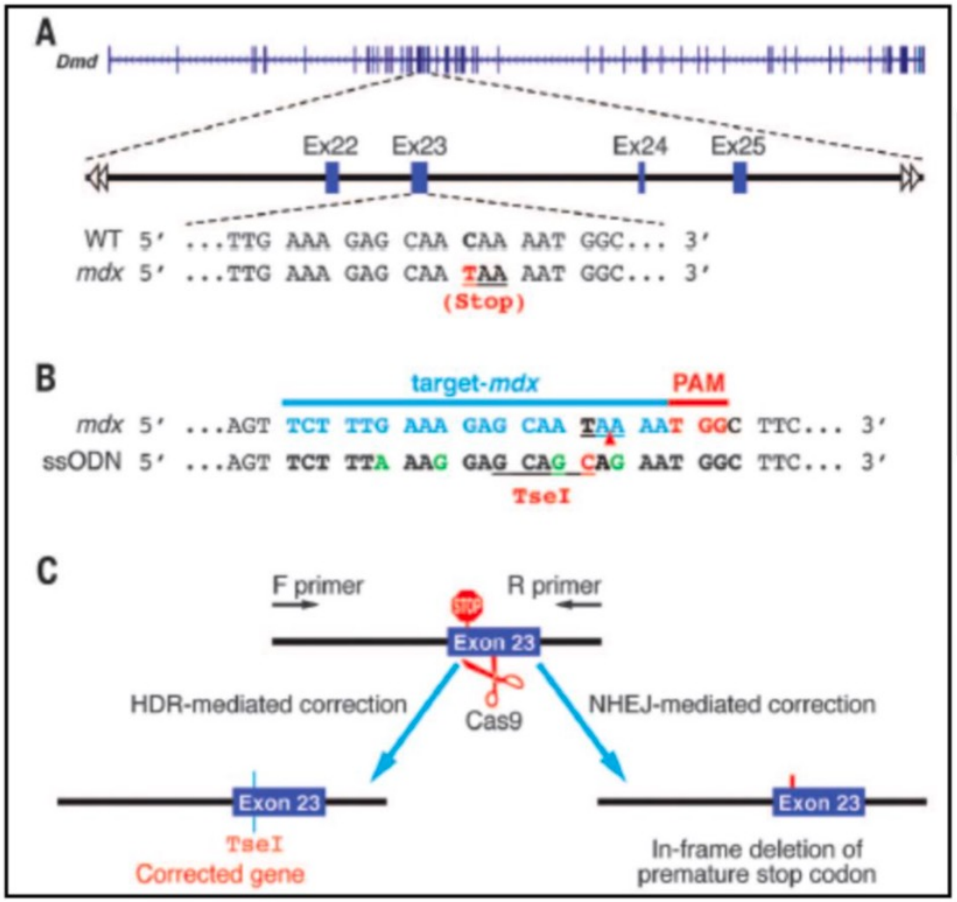
**Importantly, the unique multiplex gene-editing capabilities of CRISPR-Cas9 system facilitate the generation of a single large deletion that can correct up to 62% of DMD mutations.**

Science
AAAS

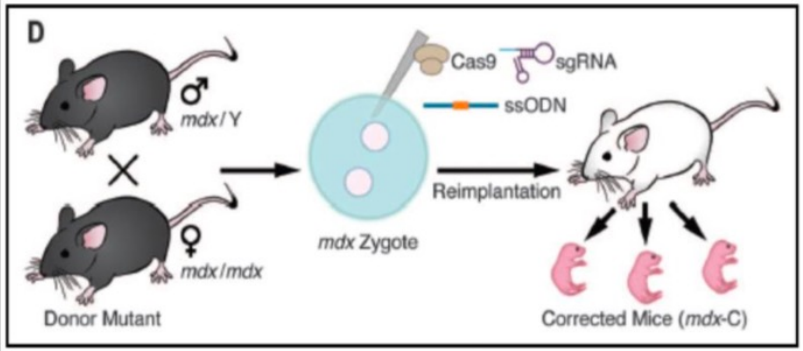
**Prevention of muscular dystrophy in mice by CRISPR/Cas9–mediated editing of germline DNA**

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

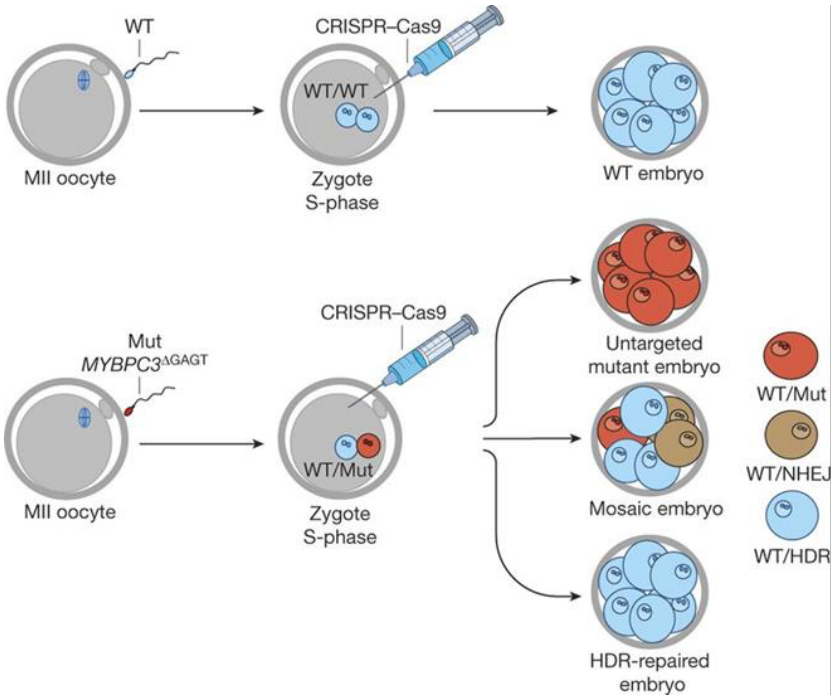
MENU nature
International journal of science

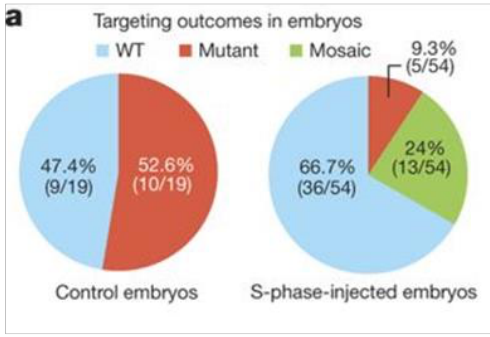
Article | Published: 02 August 2017

# Correction of a pathogenic gene mutation in human embryos

Hong Ma, Nuria Marti-Gutierrez […] Shoukhrat Mitalipov

*Nature* **548**, 413–419 (24 August 2017) | Download Citation




MII (hypertrophic cardiomyopaty) oocytes were fertilized by sperm from a heterozygous patient with equal numbers of mutant and wild-type (WT) spermatozoa. CRISPR–Cas9 was then injected into **one-cell zygotes (**97.1%) survival rate after injection and development rates comparable to controls). Embryos at the 4–8-cell stage were collected for genetic analysis. Injection during S-phase resulted in mosaic embryos consisting of non-targeted mutant, targeted NHEJ-repaired and targeted HDR-repaired blastomeres.

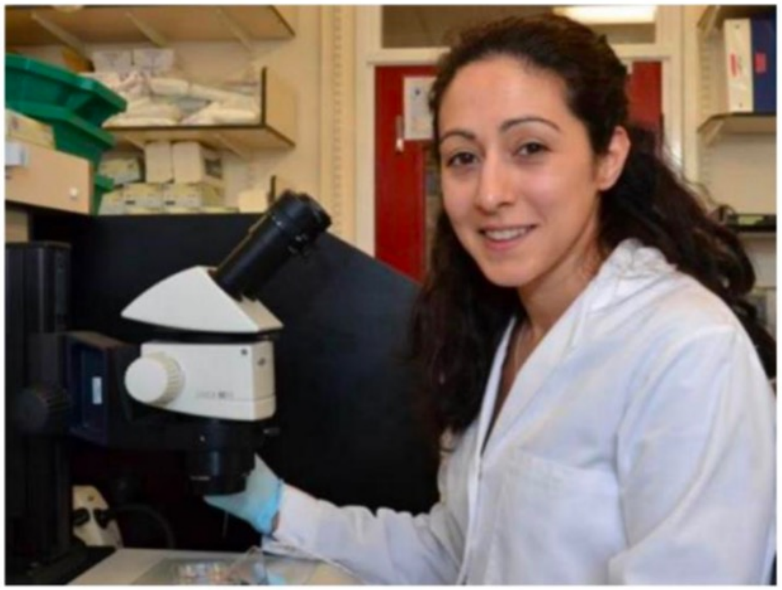
# Genome editing in human embryos

## 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.

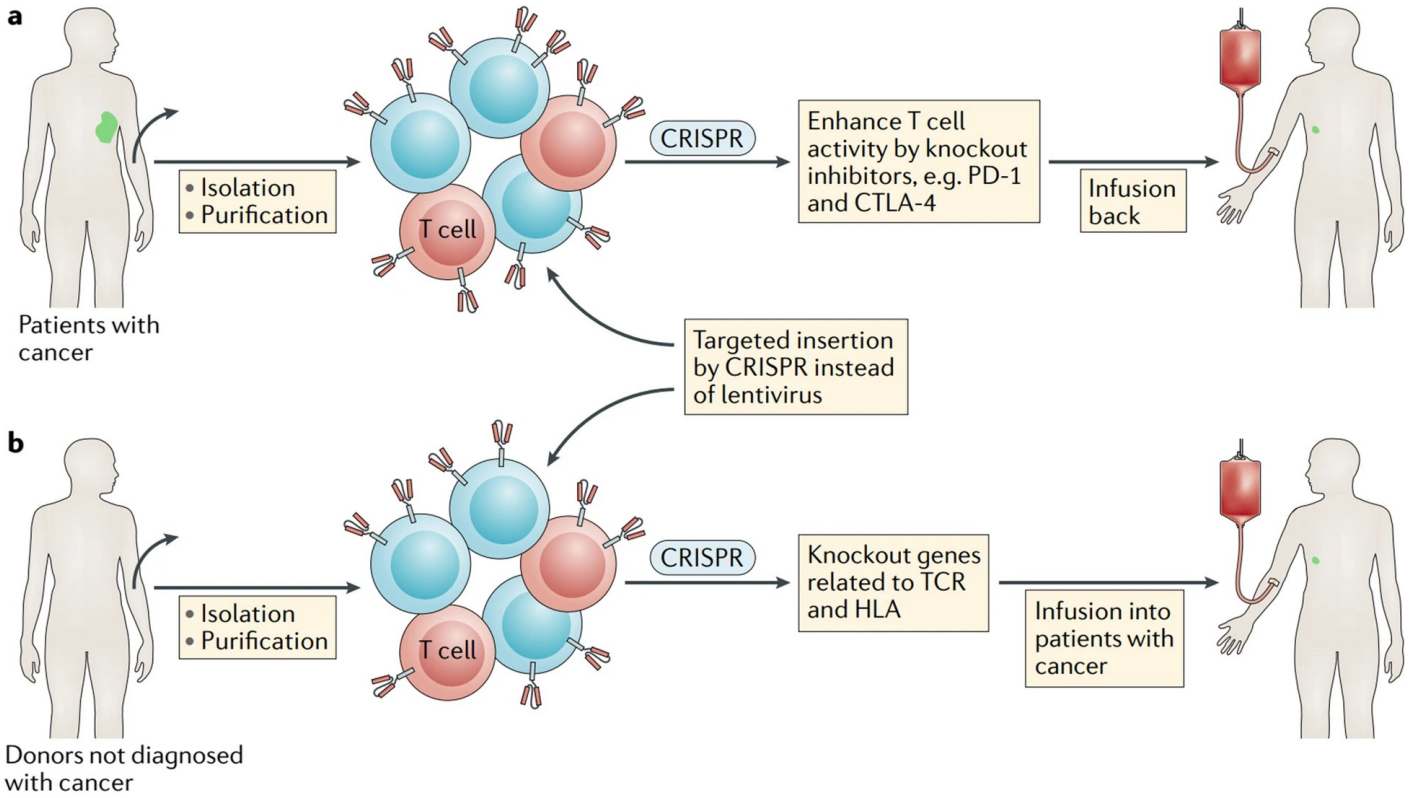
One variant of CCR5, called CCR5 Δ32, results in a high level of resistance to the most common type of HIV virus.

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.

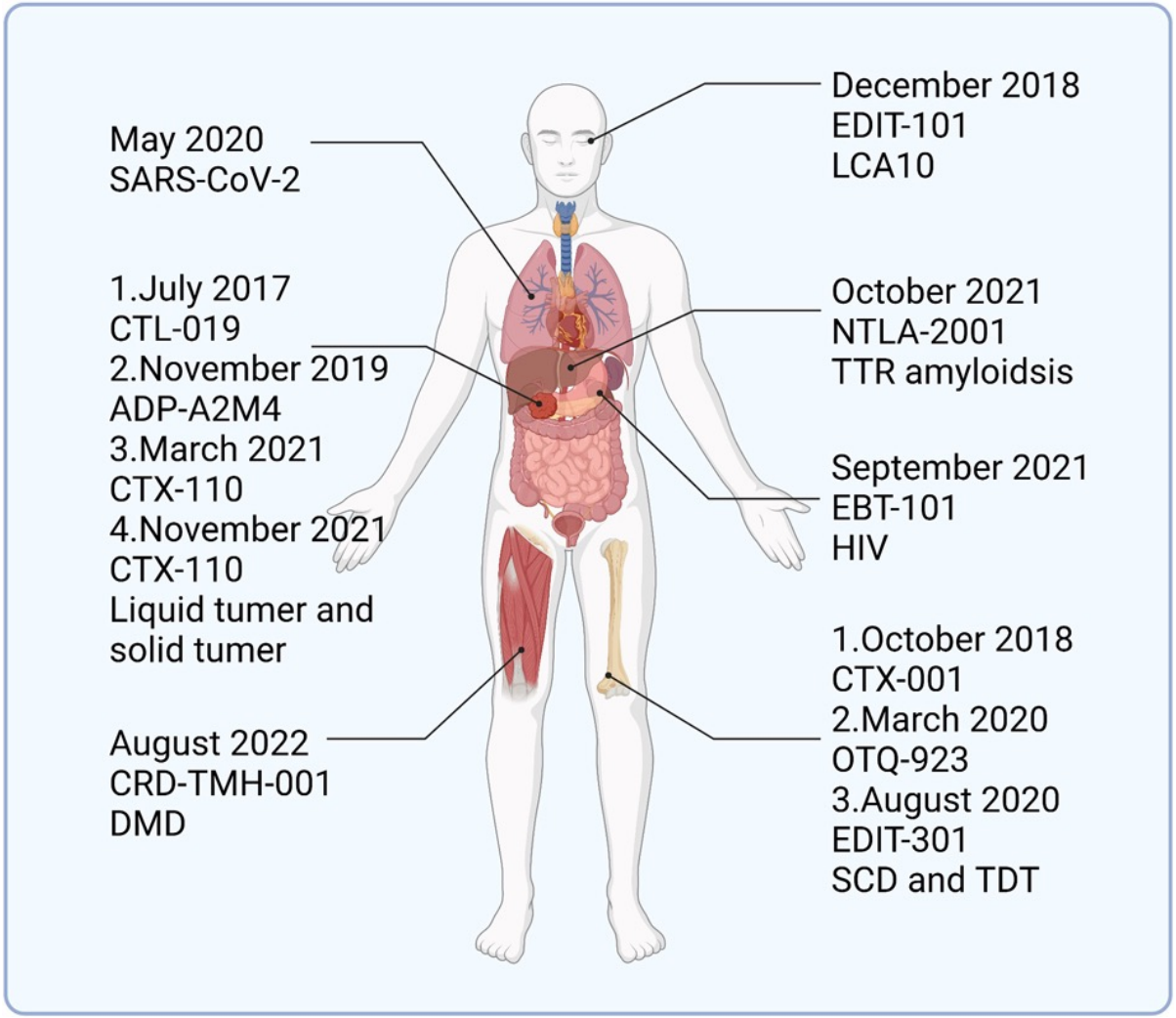
As a result, they generated different mutations, of which the effects are unknown. It may or may not confer HIV resistance, and may or may not have other consequences.

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.

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Dipartimento di Oncoematologia e Terapia Cellulare e Genica

Bambino Gesù
OSPEDALE PEDIATRICO

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

# CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia

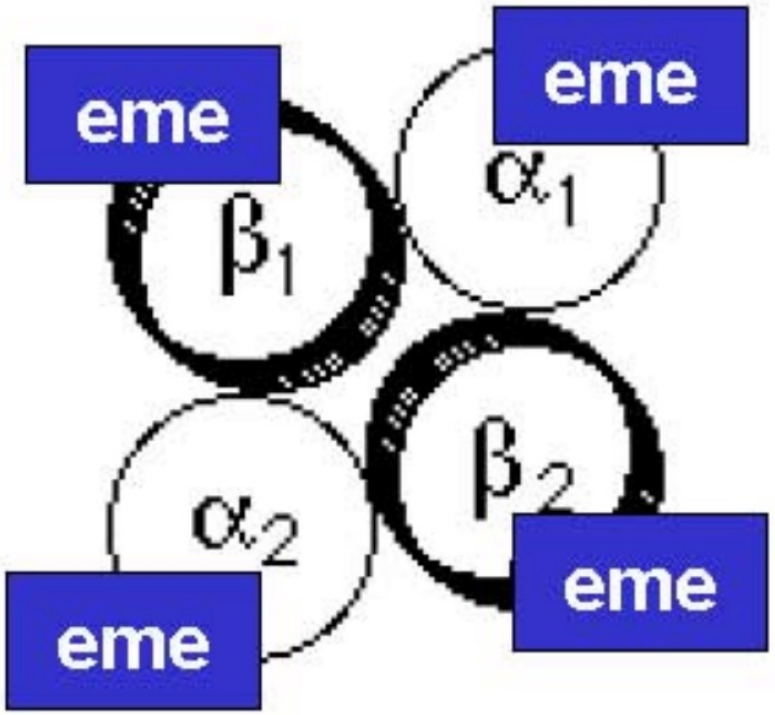
H. Frangoul, D. Altshuler, M.D. Cappellini, Y.-S. Chen, J. Domm, B.K. Eustace, J. Foell, J. de la Fuente, S. Grupp, R. Handgretinger, T.W. Ho, A. Kattamis, A. Kernytsky, J. Lekstrom-Himes, A.M. Li, F. Locatelli, M.Y. Mapara, M. de Montalembert, D. Rondelli, A. Sharma, S. Sheth, S. Soni, M.H. Steinberg, D. Wall, A. Yen, and S. Corbacioglu

SUMMARY

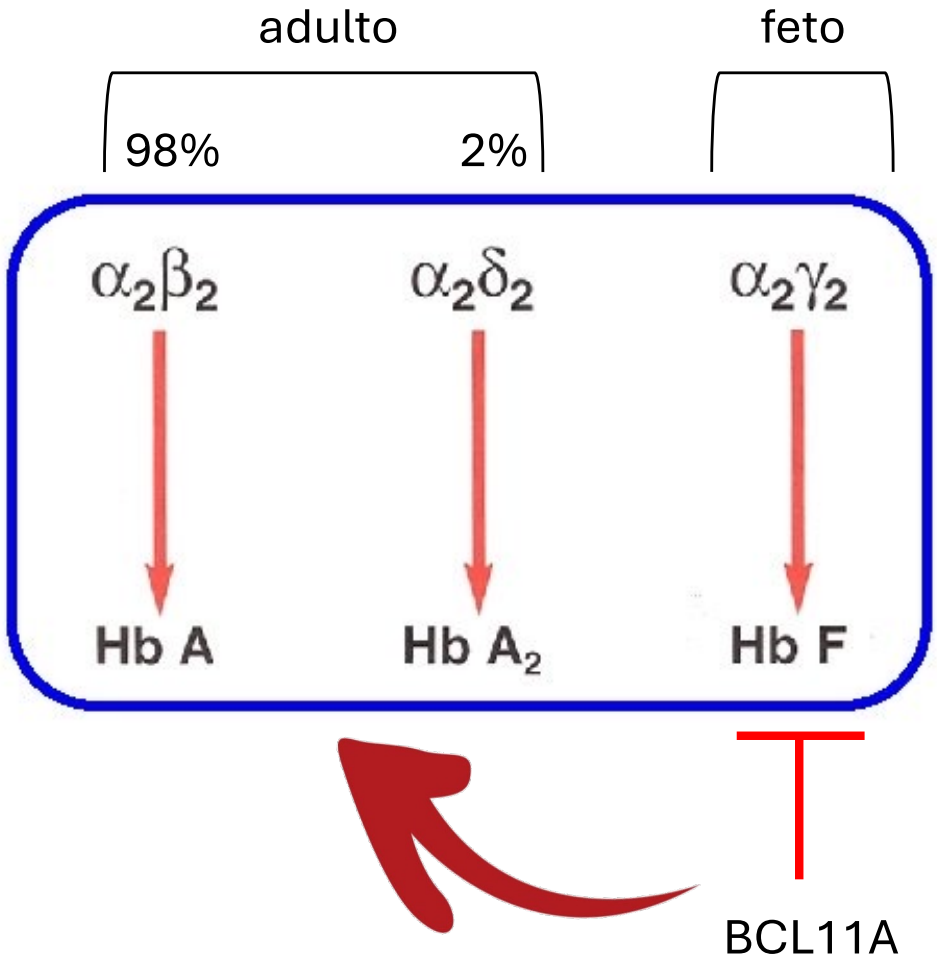
Transfusion-dependent β-thalassemia (TDT) and sickle cell disease (SCD) are severe monogenic diseases with severe and potentially life-threatening manifestations. BCL11A is a transcription factor that represses γ-globin expression and fetal hemoglobin in erythroid cells. We performed electroporation of CD34+ hematopoietic stem and progenitor cells obtained from healthy donors, with CRISPR-Cas9 targeting the *BCL11A* erythroid-specific enhancer. Approximately 80% of the alleles at this locus were modified, with no evidence of off-target editing. After undergoing myeloablation, two patients — one with TDT and the other with SCD — received autologous CD34+ cells edited with CRISPR-Cas9 targeting the same *BCL11A* enhancer. More than a year later, both patients had high levels of allelic editing in bone marrow and blood, increases in fetal hemoglobin that were distributed pancellularly, transfusion independence, and (in the patient with SCD) elimination of vaso-occlusive episodes. (Funded by CRISPR Therapeutics and Vertex Pharmaceuticals; ClinicalTrials.gov numbers, NCT03655678 for CLIMB THAL-111 and NCT03745287 for CLIMB SCD-121.)

La beta talassemia (BT) è caratterizzata dal deficit (B+) o dall'assenza (B0) della sintesi delle catene della beta-globina che codificano per la proteina dell'emoglobina (Hb).

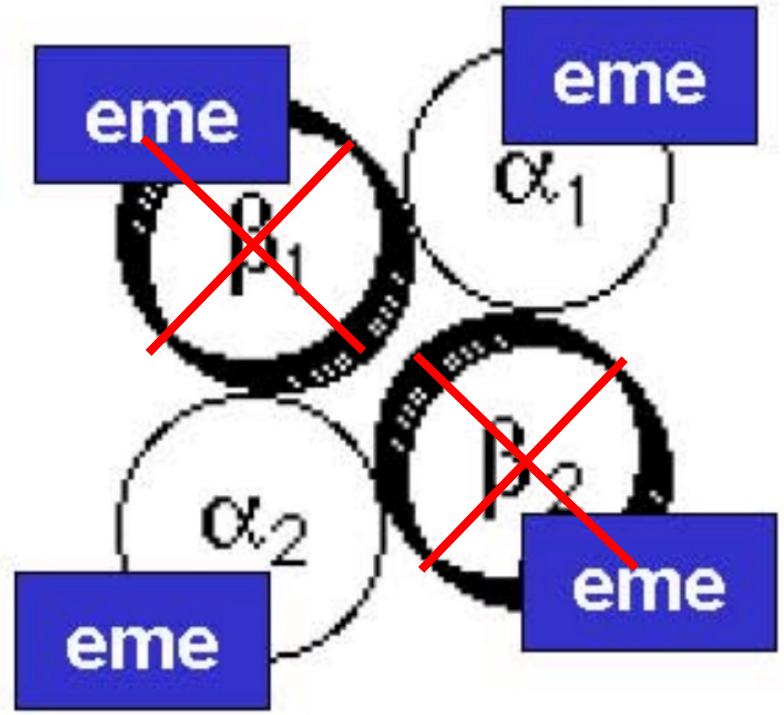
# Le globine umane normali




emoglobina



# Le globine mutate

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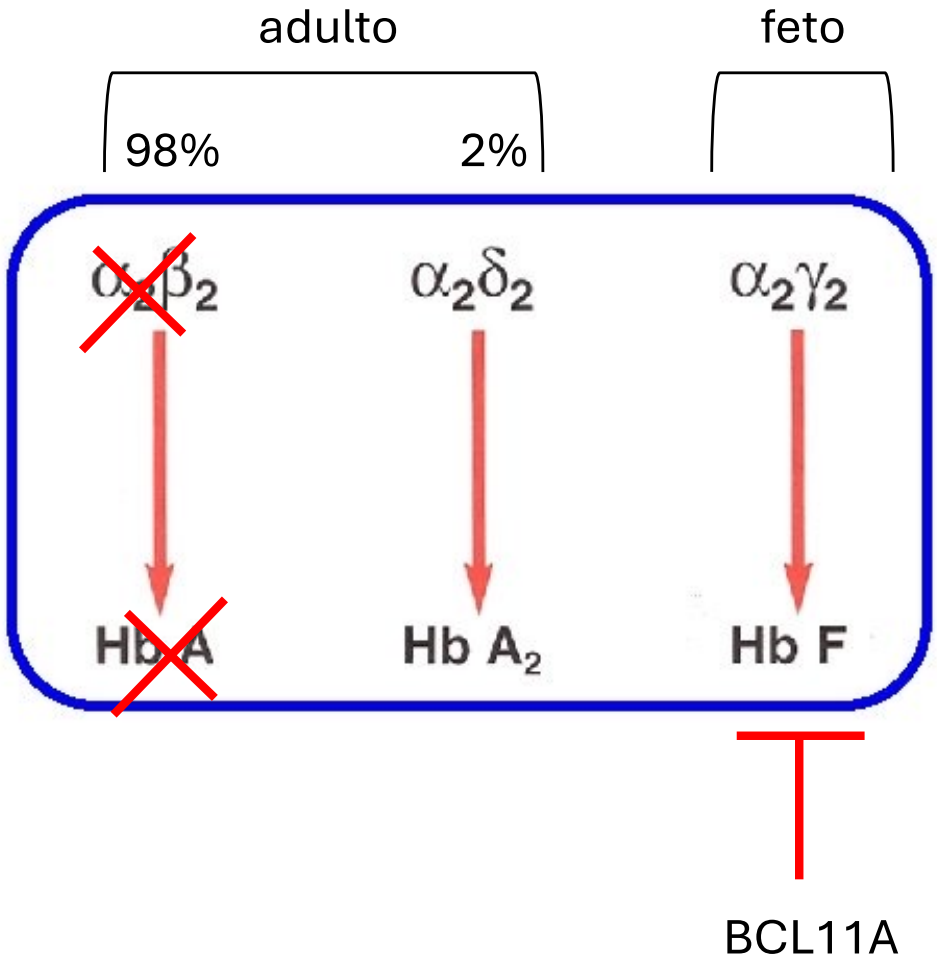
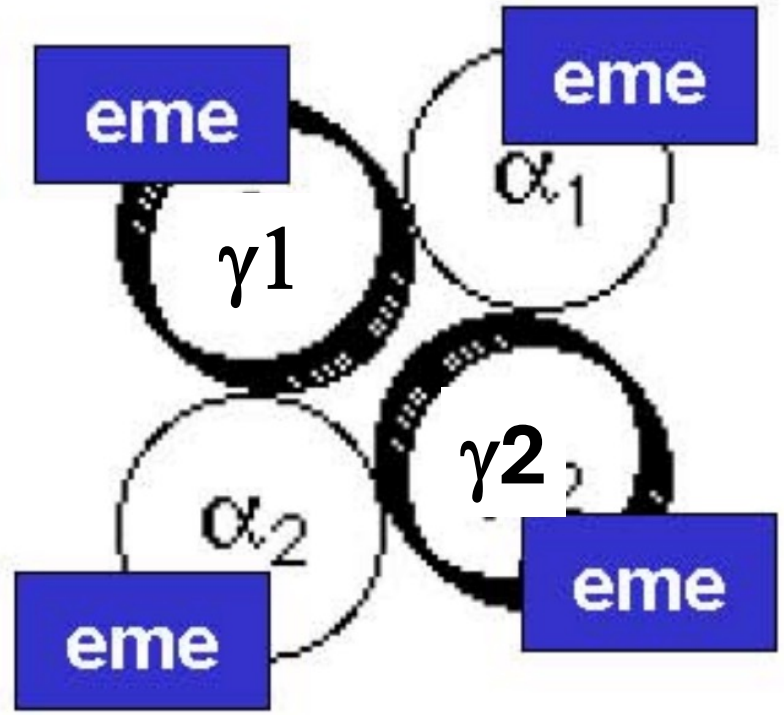
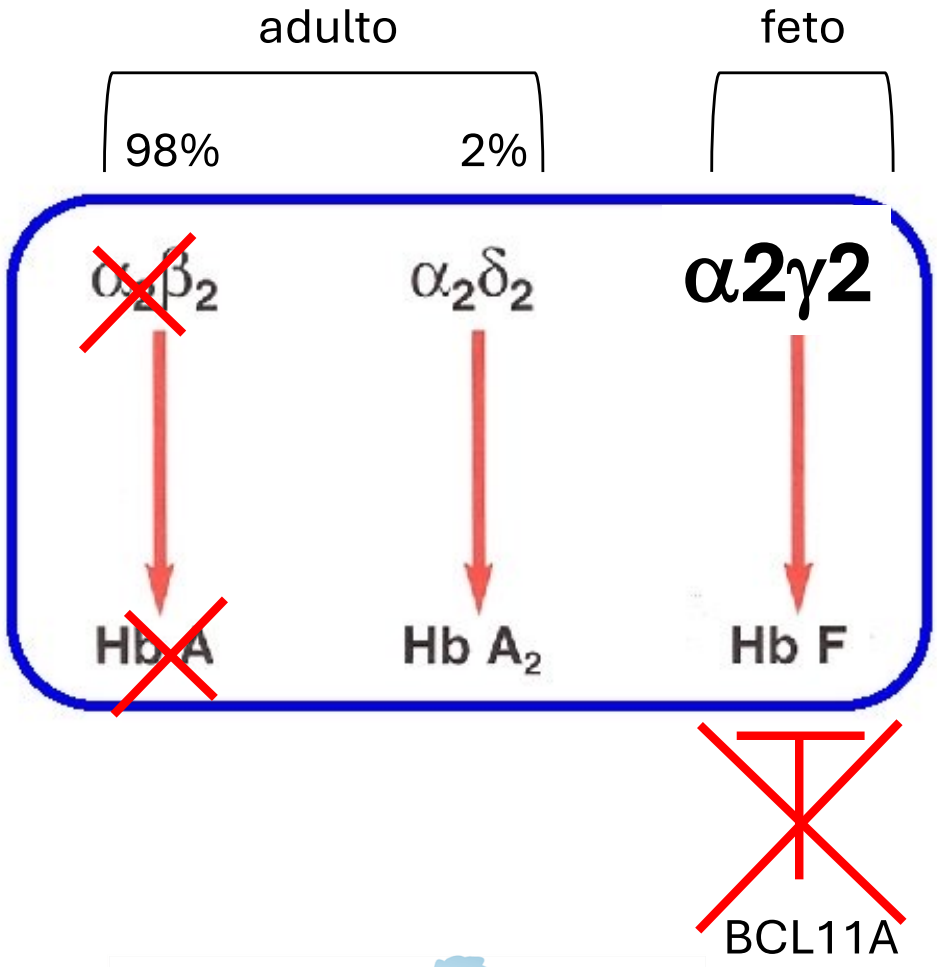
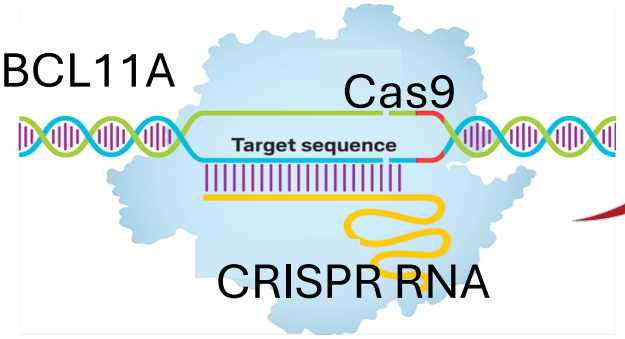


# Le CISPR-Cas9 per rispristinare la sintesi di emoglobina




emoglobina



# 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."…….*