

**CRISPR**

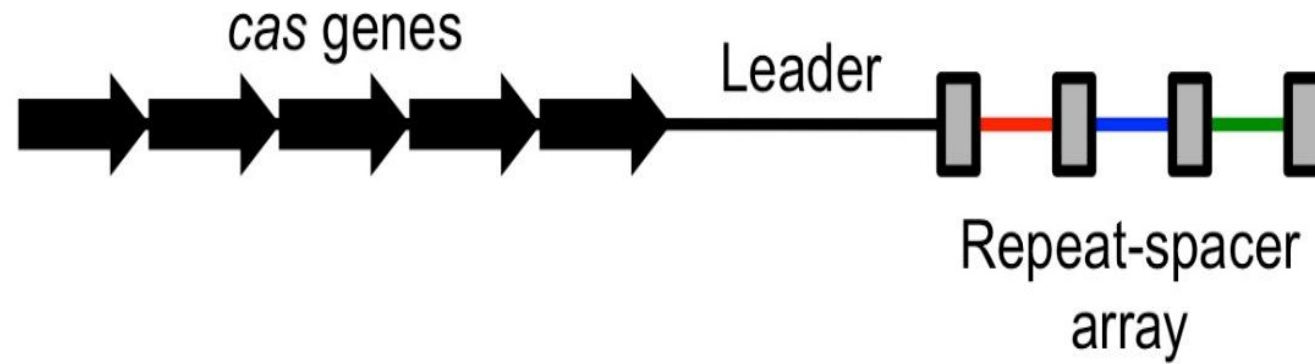
CRISPR      **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats

Familie de secvențe ADN prezente în cromozomul organismelor procariote (Eubacteria și Archaea)

Provin din fragmente ADN de origine virală sau plasmidială

Sunt folosite pentru detectarea și distrugerea unor alte genomuri virale / ADN plasmidial / ADN străin  
Intrate în celula bacteriană în infecții ulterioare

Reprezintă un sistem imunitar al celulelor procariote, protejându-le împotriva “invaziei” unor molecule ADN străine (infecție virală, transformare bacteriană cu ADN plasmidial sau cromozomal, conjugare bacteriană)



### Un element CRISPR conține

- un set **gene *cas*** (crispr-associated)
- o secvență ***leader*** – bogat în AT
- un set de segmente în **repetiții palindromice** – de câte 20-40 bp
- un set de secvențe "**spacer**" cu secvență ADN unică – de câte 30-50 bp

Elemente de tip CRISPR au fost identificate la

- > 60% din genomuri Eubacteria
- > 90% din genomuri Archaea

# Mecanismul procesului de imunitate

Se desfășoară în mai multe etape

## 1. Achiziția de segmente ADN

- a. ADN străin (viral/plasmidial/cromozomal) intrat în celula bacteriană este recunoscut de proteinele Cas1 și Cas2 și este clivat de către acestea → *protospacer*
- b. *Protospacer*-ul este introdus în genomul gazdă, la nivelul unui element CRISPR, înaintea secvenței *Leader* → o nouă secvență *spacer* și are loc și duplicarea unei secvențe repetate

Cas1 = nucleaze/integraze

Cas2 = endonucleaze situs-specifice

Procesul de integrare depinde și de proteinele IHF, ce se atașează specific la secvența Leader

## 2. Producerea moleculelor ARNcr (crRNA = crisprRNA)

- a. Transcrierea regiunii formate din repetiții și din spacere → transcript ARN primar, lung
- b. Procesarea transcriptului primar → generarea moleculelor ARNcr

## 3. Producerea complexelor Cas9-ARNcr

## 4. Recunoașterea ADN străin nou intrat prin complementaritate cu ARNcr

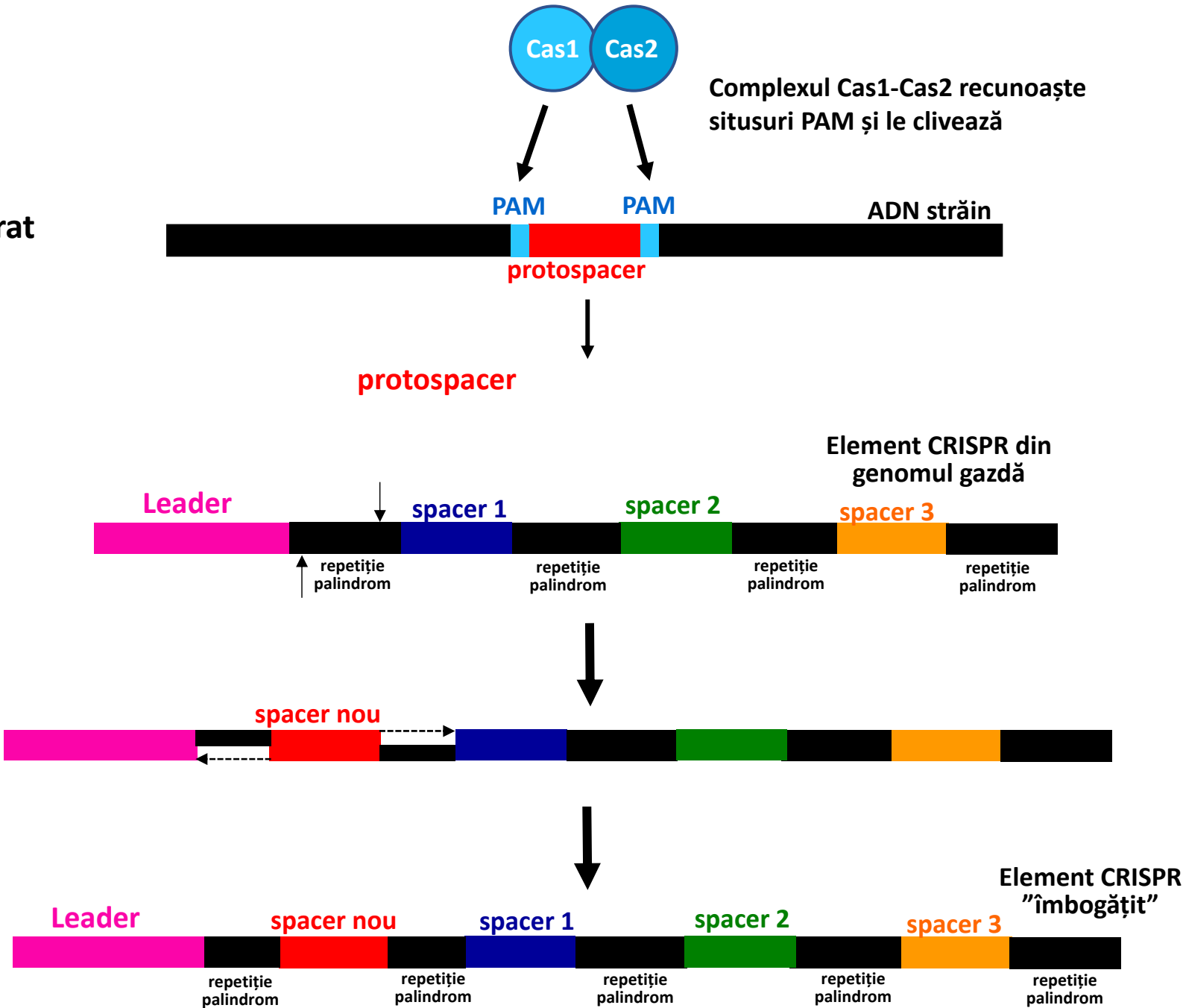
## 5. Digestia endonucleazică a ADN străin de către Cas9

1. Achiziția de segmente ADN

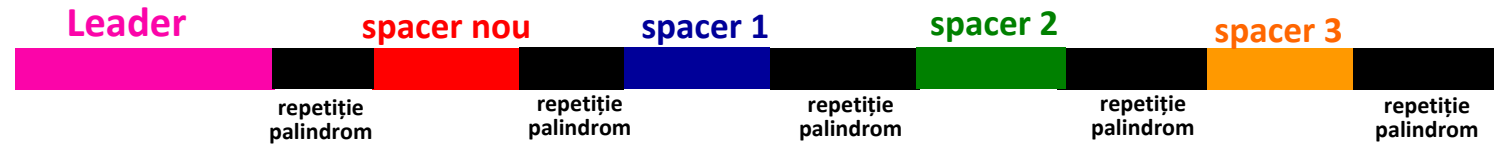
ADN străin (viral/plasmidial/cromozomal) intrat în celula bacteriană este recunoscut de proteinele Cas1 și Cas2 și este clivat de către acestea → *protospacer*

PAM = Protospacer Adjacent Motif

*Protospacer*-ul este introdus în genomul gazdă, la nivelul unui element CRISPR, înaintea secvenței *Leader* → o nouă secvență *spacer* și are loc și duplicarea unei secvențe repetate



## 2. Producerea moleculelor ARNcr (crRNA = crisprRNA)



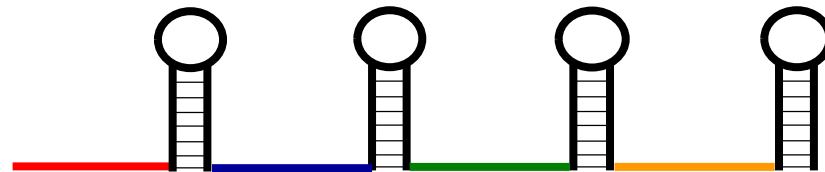
a. Transcrierea regiunii formate din repetiții și din *spacere* → transcript ARN primar, lung

Transcriere

ARN transcript primar, lung

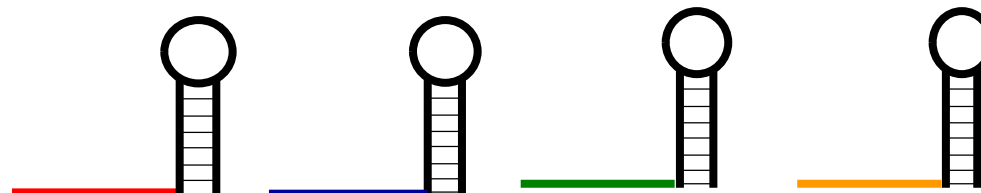


Datorită repetițiilor palindromice, transcriptul formează structuri hairpin și chiar și bucle



b. Procesarea transcriptului primar prin clivare de către Cas6 → generarea moleculelor ARNcr

Cas6 – excizia transcriptului



Molecule de ARNcr

### 3. Producerea complexelor Cas9-ARNcr

Fiecare moleculă ARNcr se atașează la o moleculă de proteină Cas9 → complex Cas9-ARNcr

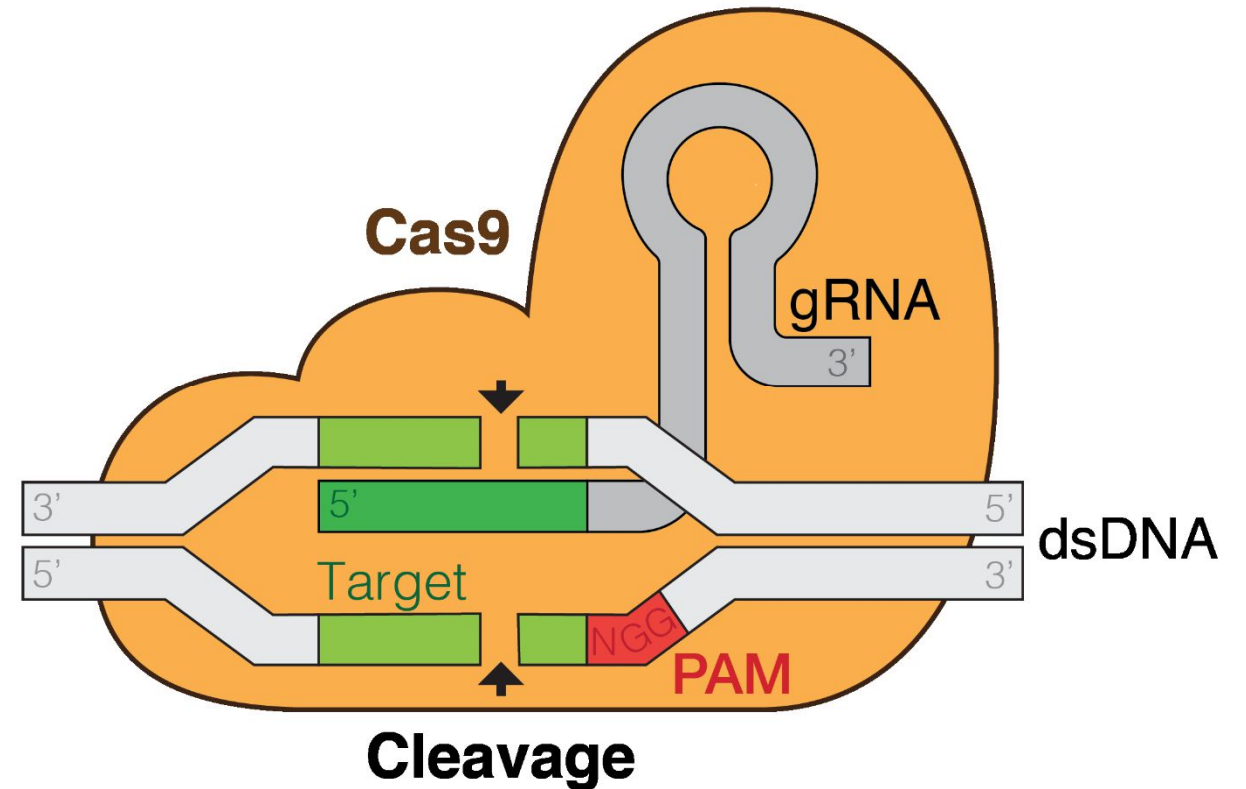
### 4. Recunoașterea ADN străin nou intrat prin complementaritate cu ARNcr

Complexul Cas9-ARNcr astfel format recunoaște secvențe ADN străin intrat în celulă, prin complementaritate cu secvența *spacer* din ARNcr

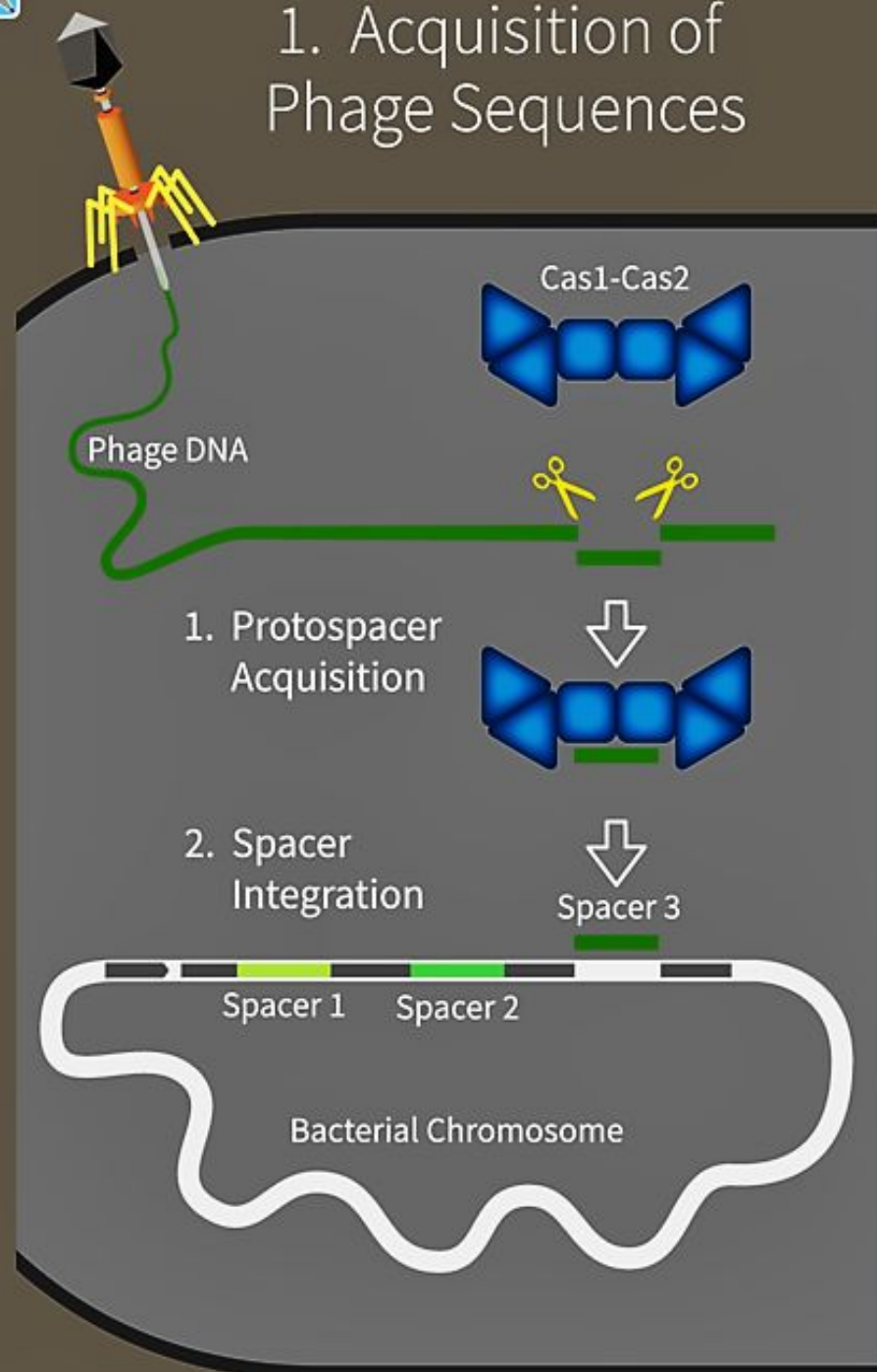
### 5. Digestia endonucleazică a ADN străin de către Cas9

Complexul Cas9-ARNcr se atașează la secvențele străine complementare, desfăcând astfel cele 2 catene ale ADN.

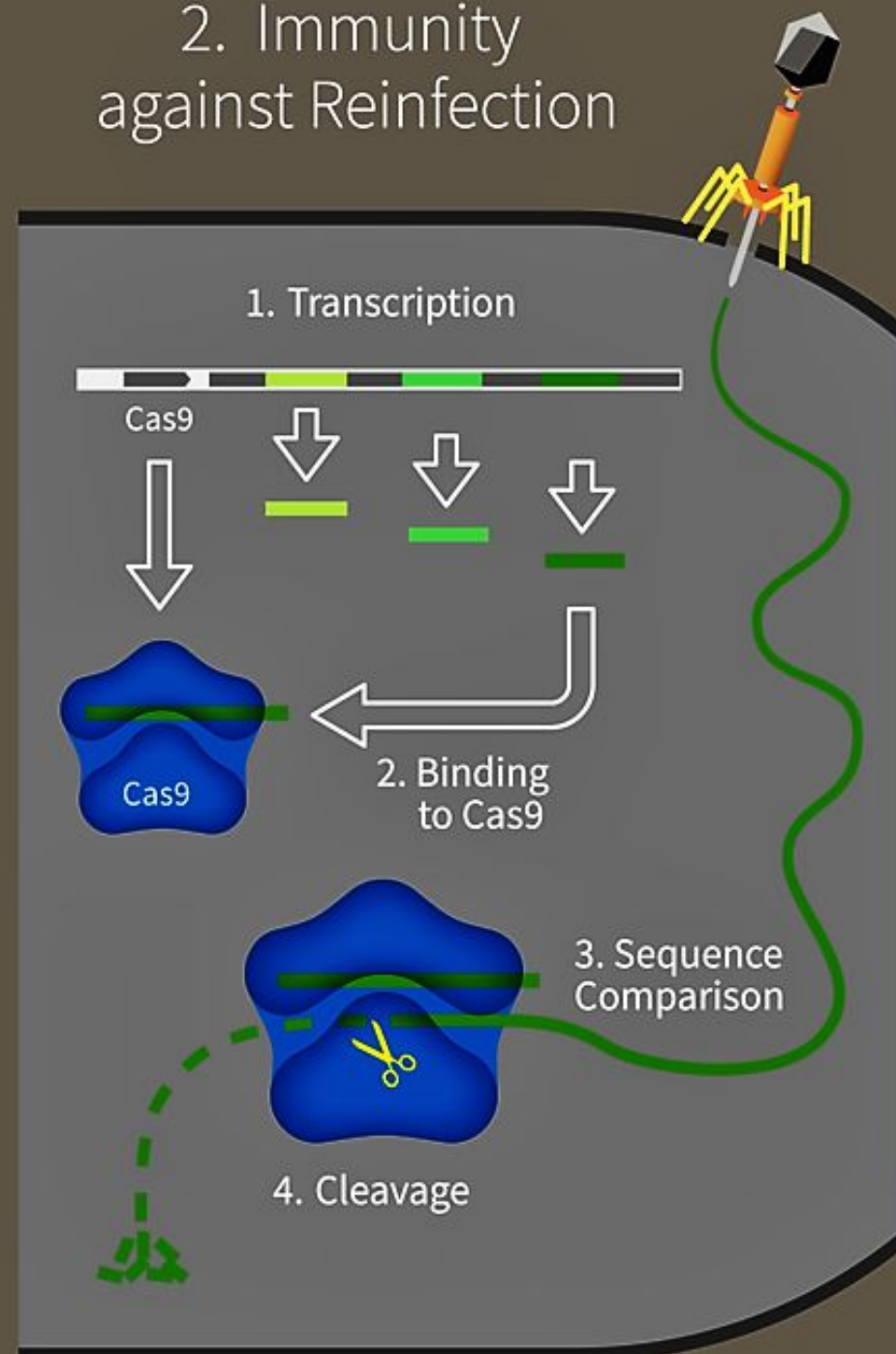
Cas9 taie cele 2 catene ADN → ADN străin este distrus



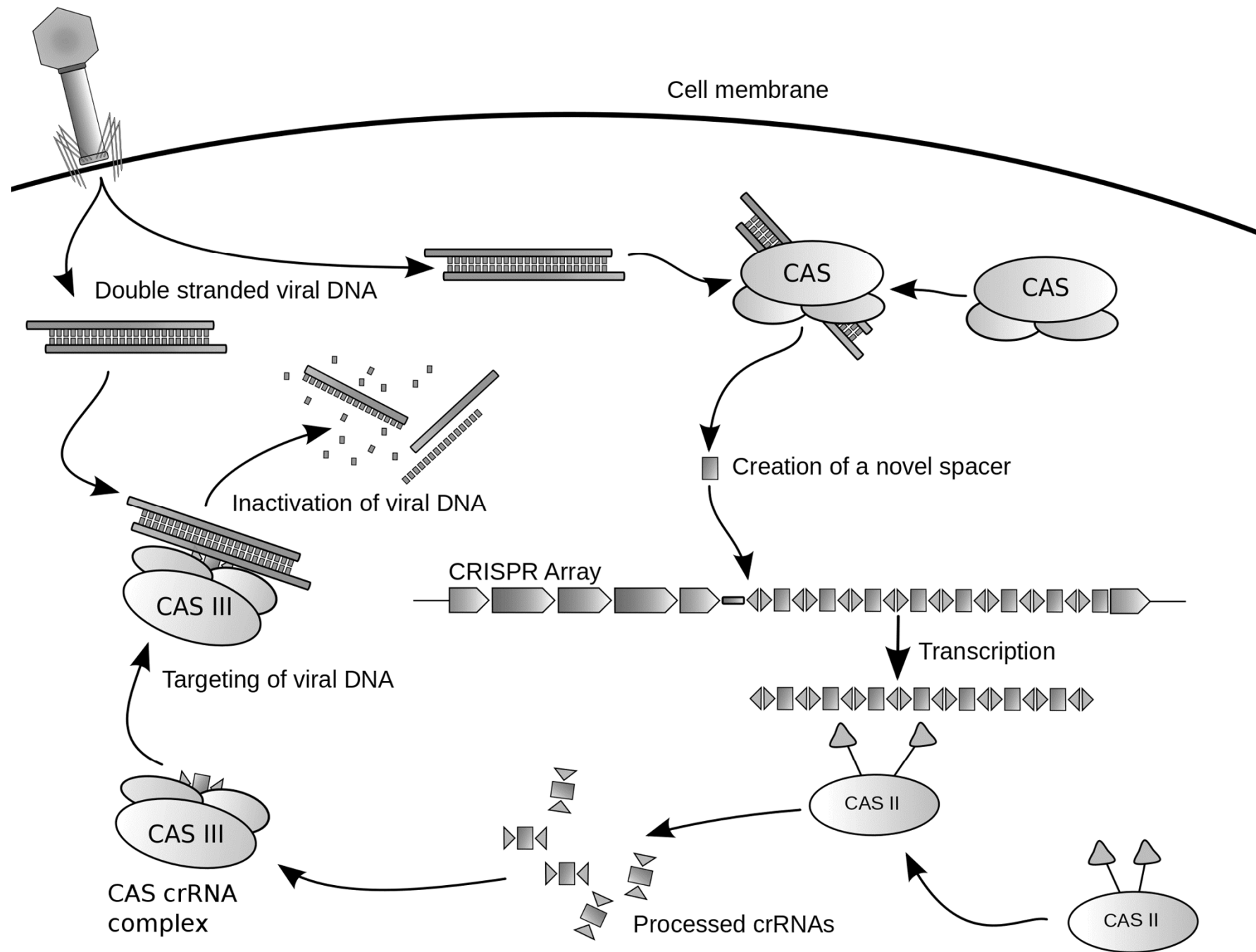
## 1. Acquisition of Phage Sequences



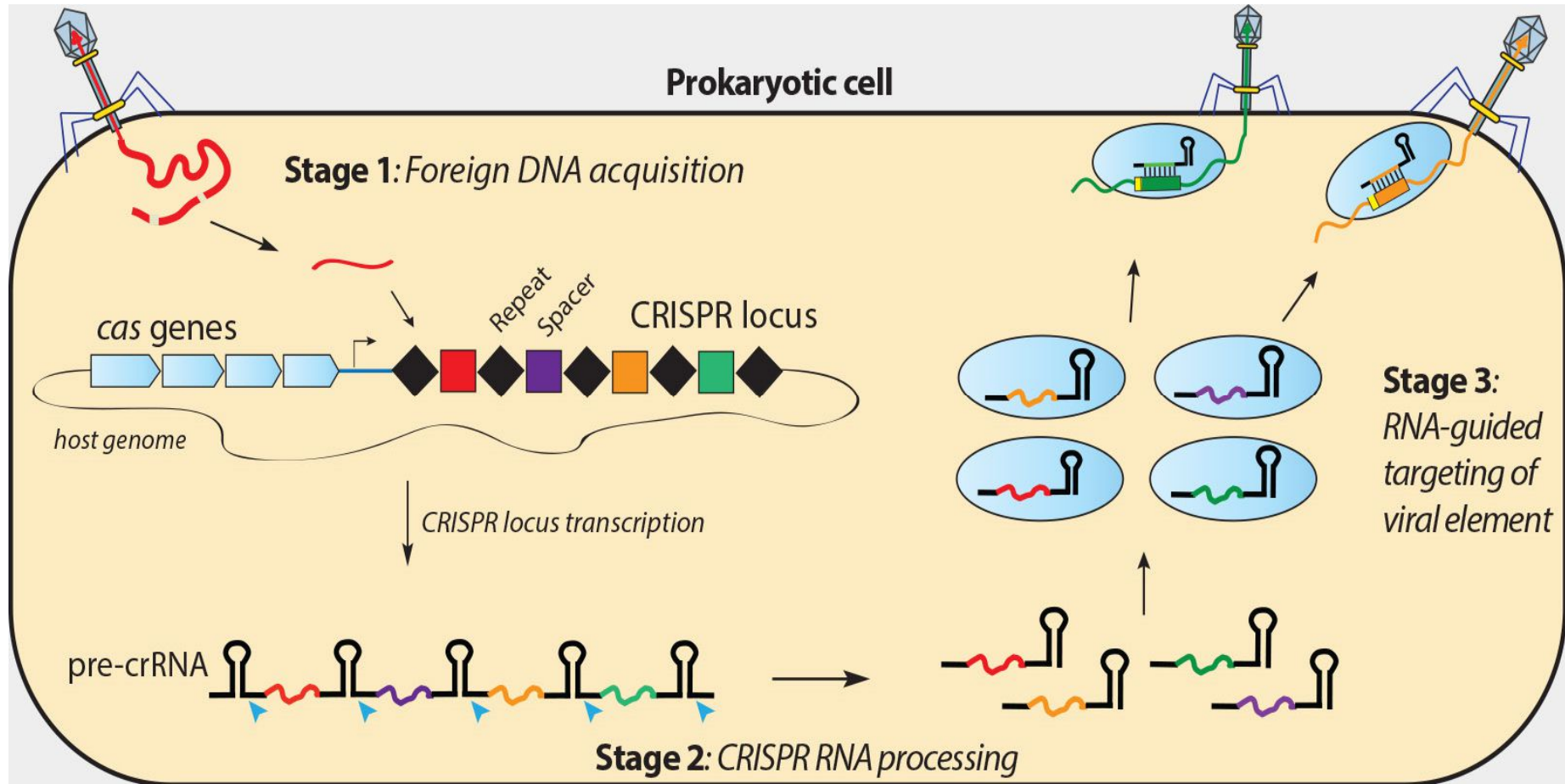
## 2. Immunity against Reinfection





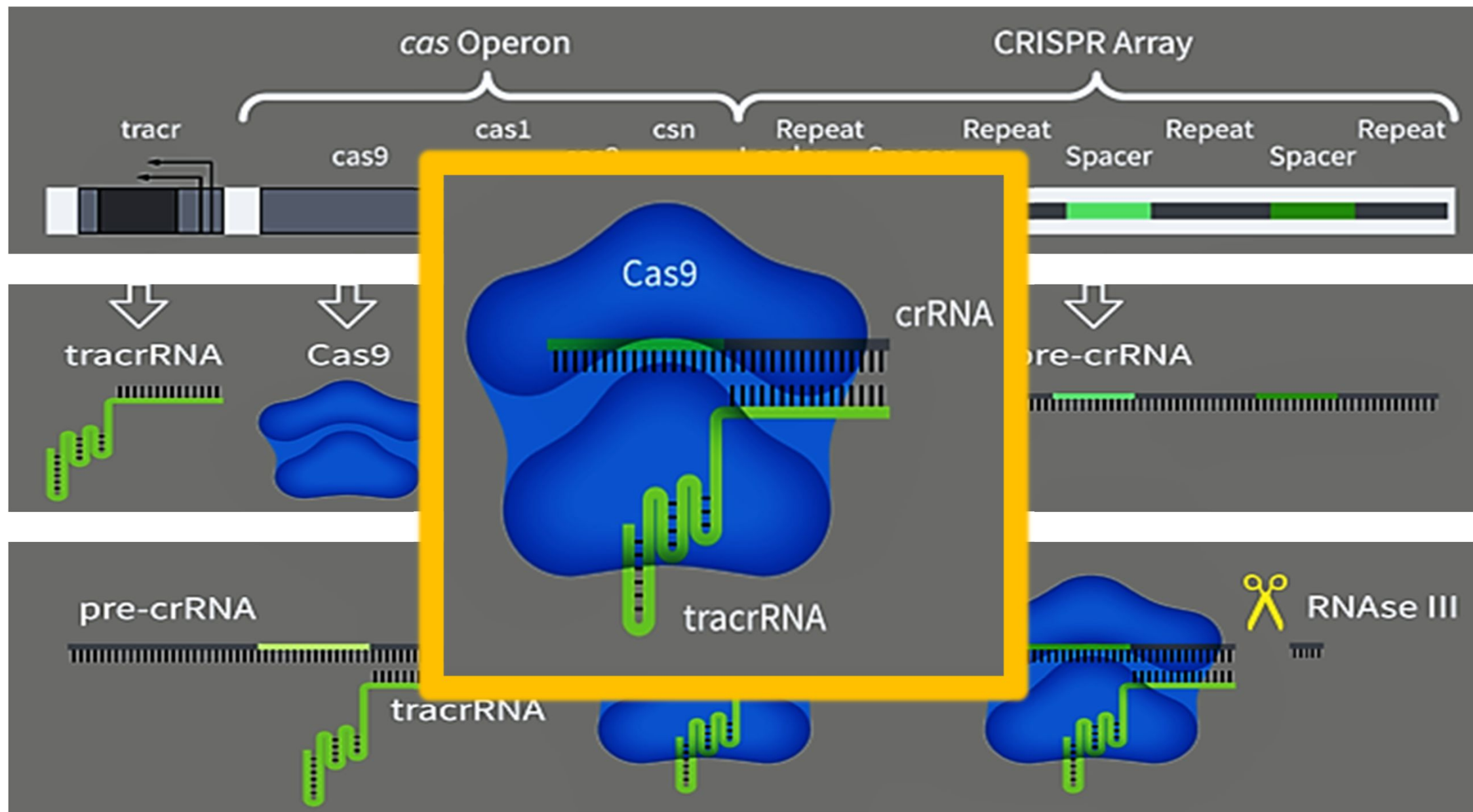


# CRISPR/Cas9 bacterial immune system



## CRISPR tip II

În afară de ARNcr (ce conține 1 secv. repetată+ 1 secv. spacer) are și o moleculă ARN tracr (trans-activating) ce este complementară parțial cu o repetiție și absolut necesar pentru stabilizarea complexului Cas9-ARNcr



The mechanism for distinguishing self from foreign DNA during interference is built into the crRNAs and is therefore likely common to all three systems. Throughout the distinctive maturation process of each major type, all crRNAs contain a spacer sequence and some portion of the repeat at one or both ends. It is the partial repeat sequence that prevents the CRISPR-Cas system from targeting the chromosome as base pairing beyond the spacer sequence signals self and prevents DNA cleavage.<sup>[128]</sup> RNA-guided CRISPR enzymes are classified as [type V restriction enzymes](#).

## Evolution

The cas genes in the adaptor and effector modules of the CRISPR-Cas system are believed to have evolved from two different ancestral modules. A transposon-like element called casposon encoding the Cas1-like integrase and potentially other components of the adaptation module was inserted next to the ancestral effector module, which likely functioned as an independent innate immune system.<sup>[129]</sup> The highly conserved cas1 and cas2 genes of the adaptor module evolved from the ancestral module while a variety of class 1 effector module genes evolved from the ancestral effector module.<sup>[130]</sup> The evolution of these various class 1 effector module cas genes was guided by various mechanisms, such as duplication events.<sup>[131]</sup> On the other hand, each type of class 2 effector module arose from subsequent independent insertions of mobile genetic elements.<sup>[132]</sup> These mobile genetic elements took the place of the multiple gene effector modules to create single gene effector modules that produce large proteins which perform all the necessary tasks of the effector module.<sup>[132]</sup> The spacer regions of CRISPR-Cas systems are taken directly from foreign mobile genetic elements and thus their long term evolution is hard to trace.<sup>[133]</sup> The non-random evolution of these spacer regions has been found to be highly dependent on the environment and the particular foreign mobile genetic elements it contains.<sup>[134]</sup>

## CRISPR gene / genome editing

is a method by which the [genomes](#) of living organisms may be edited. It is based on a simplified version of the bacterial [CRISPR](#)/Cas (CRISPR-Cas9) antiviral defense system. By delivering the [Cas9](#) nuclease complexed with a synthetic [guide RNA](#) (gRNA) into a cell, the cell's [genome](#) can be cut at a desired location, allowing existing genes to be removed and/or new ones added.

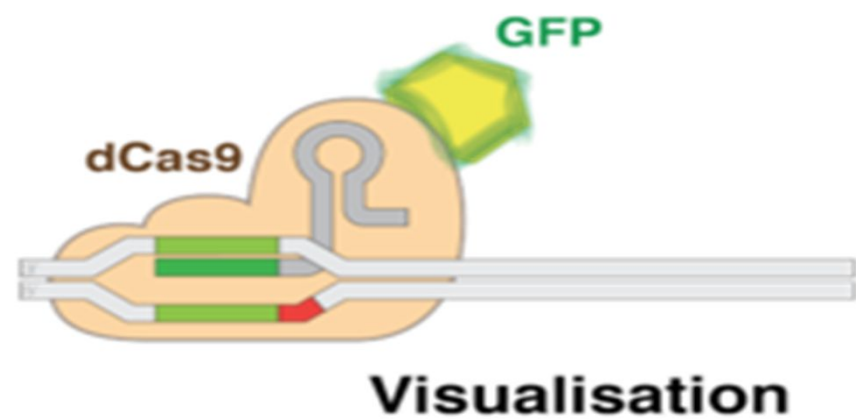
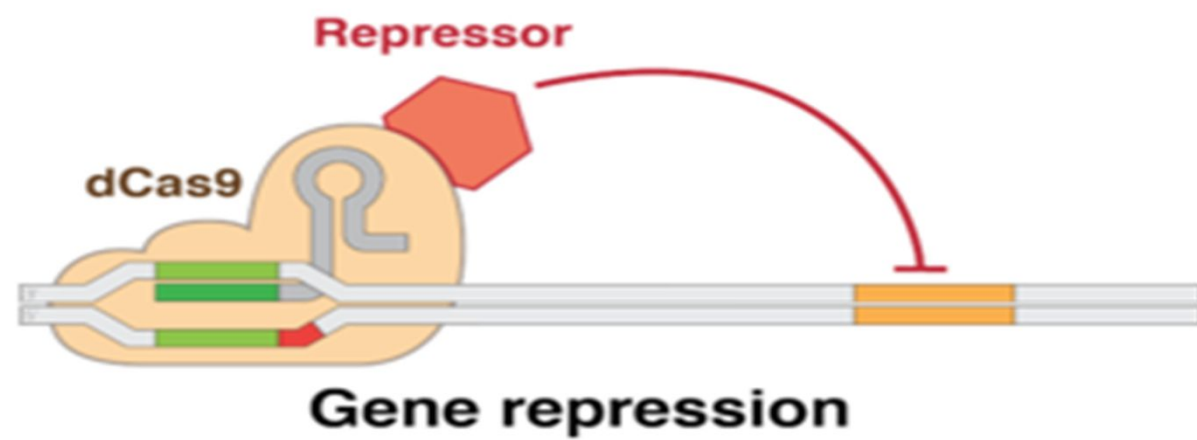
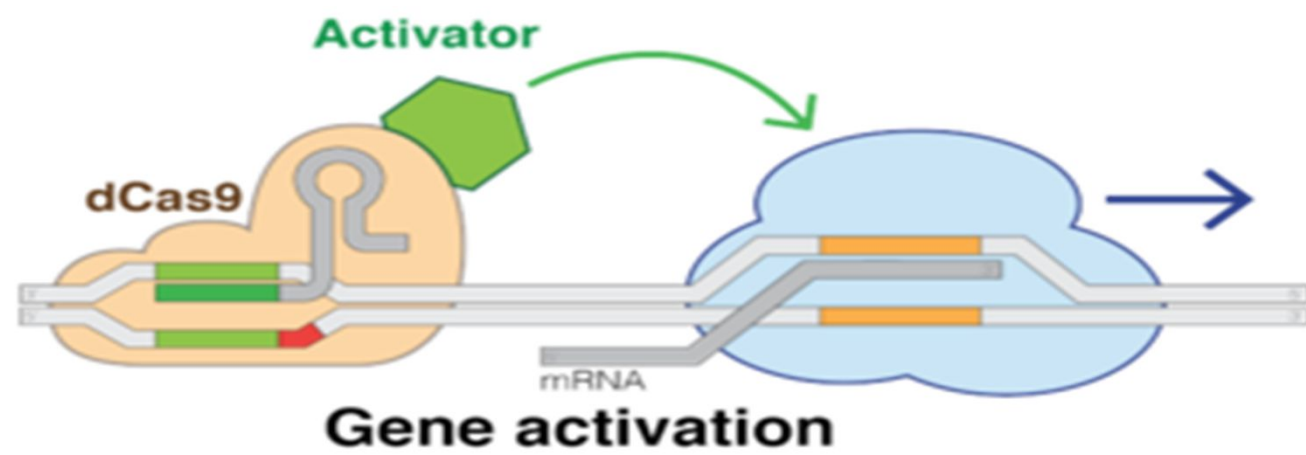
### 2. Blood disorders

The [first CRISPR trial in Europe and the US](#), which enrolled its first patient in February this year, aims to treat beta-thalassemia and sickle cell disease, two blood disorders that affect oxygen transport in the blood. The therapy, developed by CRISPR Therapeutics and Vertex Pharmaceuticals, consists in harvesting bone marrow stem cells from the patient and using CRISPR technology to make them produce fetal hemoglobin, a natural form of the oxygen-carrying protein that binds oxygen much better than the adult form.

## Knockdown/activation

Using "dead" versions of Cas9 ([dCas9](#)) eliminates CRISPR's DNA-cutting ability, while preserving its ability to target desirable sequences. Multiple groups added various regulatory factors to dCas9s, enabling them to turn almost any gene on or off or adjust its level of activity.<sup>[81]</sup> Like RNAi, CRISPR interference (CRISPRi) turns off genes in a reversible fashion by targeting, but not cutting a site. The targeted site is methylated, [epigenetically](#) modifying the gene. This modification inhibits transcription. These precisely placed modifications may then be used to regulate the effects on gene expressions and DNA dynamics after the inhibition of certain genome sequences within DNA. Within the past few years, epigenetic marks in different human cells have been closely researched and certain patterns within the marks have been found to correlate with everything ranging from tumor growth to brain activity.<sup>[5]</sup> Conversely, CRISPR-mediated activation (CRISPRa) promotes gene transcription.<sup>[85]</sup> Cas9 is an effective way of targeting and silencing specific genes at the DNA level.<sup>[86]</sup> In bacteria, the presence of Cas9 alone is enough to block transcription. For mammalian applications, a section of protein is added. Its guide RNA targets regulatory DNA sequences called [promoters](#) that immediately precede the target gene.<sup>[87]</sup>

Cas9 was used to carry synthetic [transcription factors](#) that activated specific human genes. The technique achieved a strong effect by targeting multiple CRISPR constructs to slightly different locations on the gene's promoter.<sup>[87]</sup>



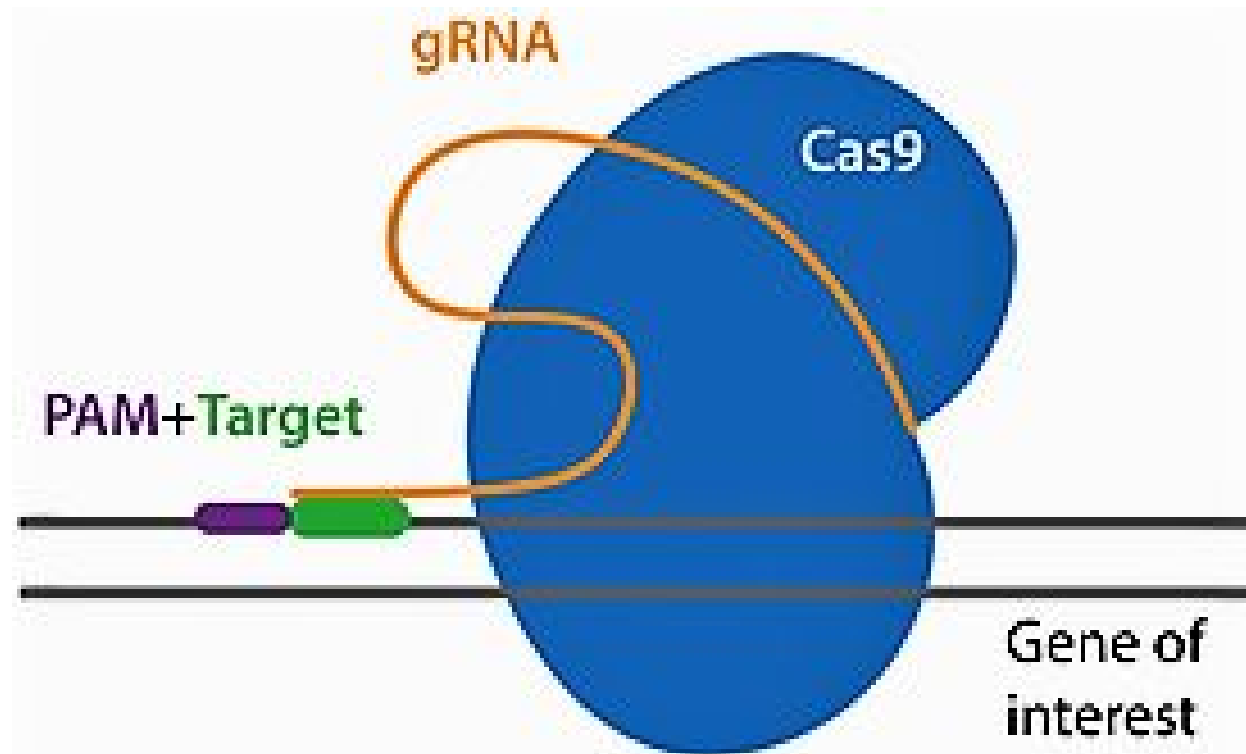


# CRISPR pentru *genome editing*

În general, se folosesc sisteme derivate din CRISPR tip II

Este sintetizat artificial o moleculă ARN, denumită **ARNg** (guide) ce cuprinde

— o zonă complementară cu secvența de interes  
(ARNcr)  
— o zonă necesară stabilizării sistemului  
(ARNtracr)



Genome Engineering  
Transcriptional Regulation  
Other Applications

Complexul Cas9-ARNg este introdus în celule prin transfecție / microinjecție etc



# Inactivarea unei gene cu ajutorul CRISPR

## Introducerea unei mutații

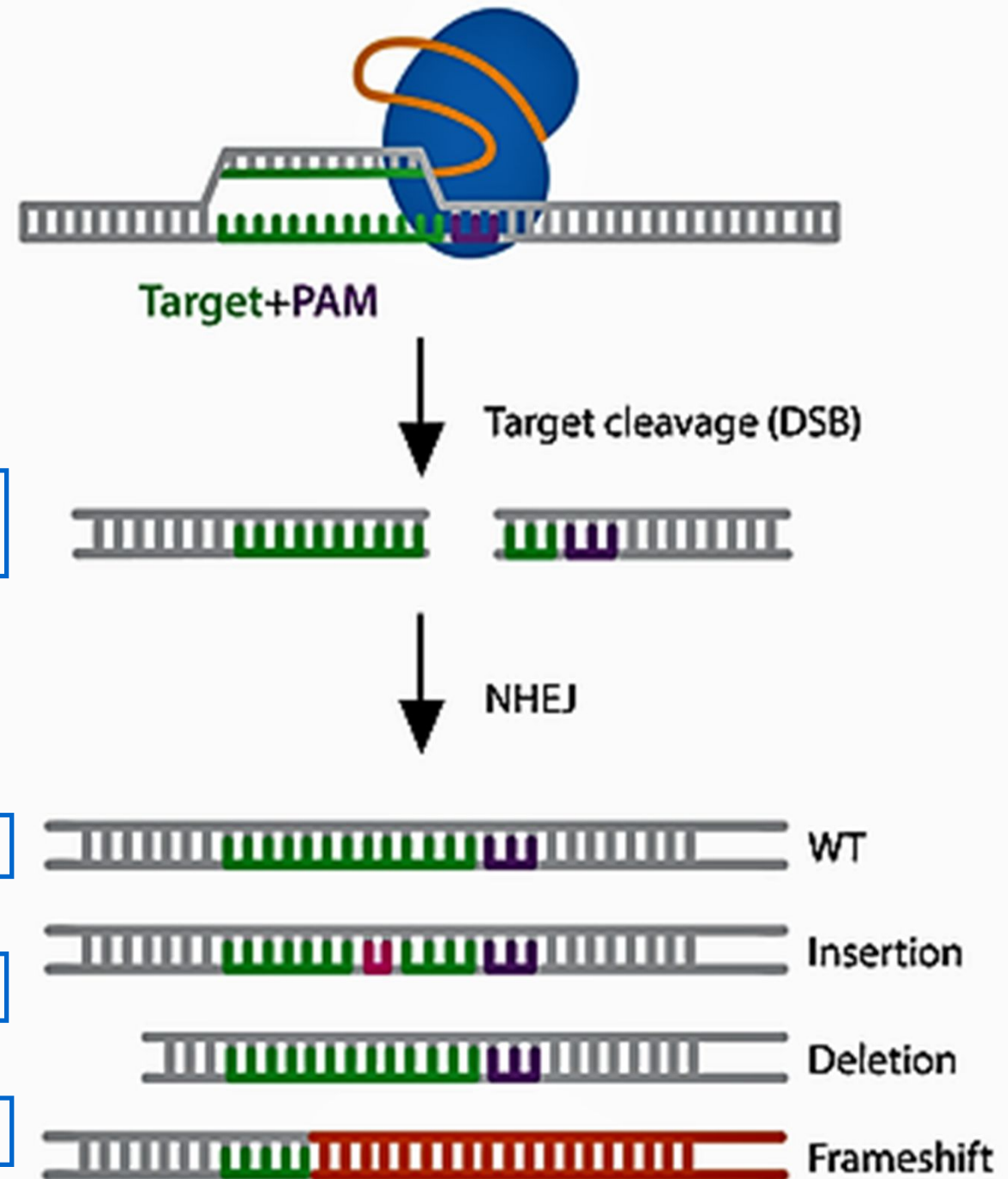
secvența *spacer* din ARNg este complementară cu secvența de interes  
ARNg se atașează la țintă

Cas9 clivează ambele catene ADN  
(DSB = ruperi dublu-catenare)

Celula activează sistemele reparatorii → Reparare neomoloagă, error-prone

este introdusă o mutație (inserție/deleție)

La următoarea rundă de transcriere + traducere → o altă proteină



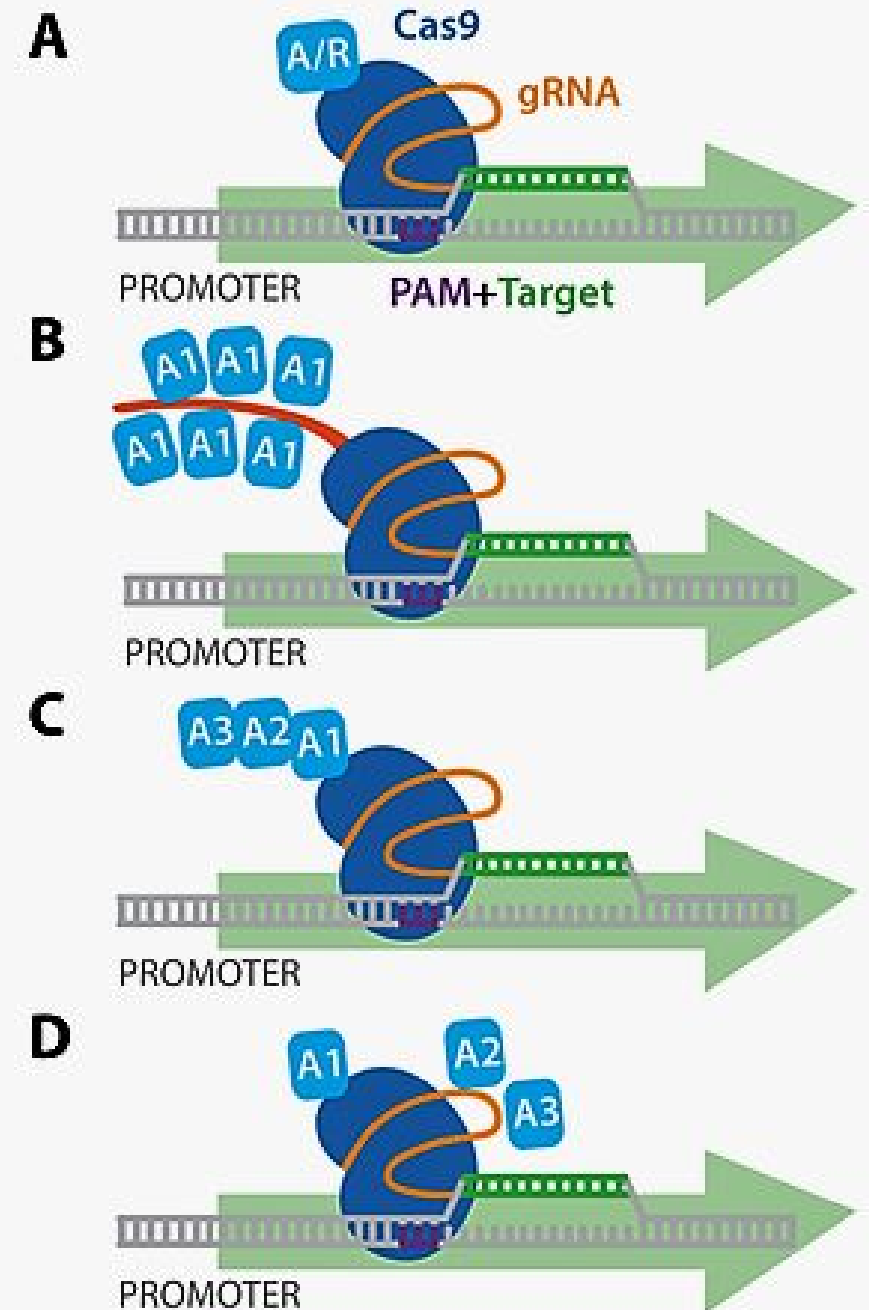
# Activarea și represia unor gene folosind CRISPR

Cas9 a fost modificată = **dCas9** ("dead") → nu mai are funcție endonucleazică

Au fost create **complexe proteice**

- **dCas9**
- proteine **activatori** / **represori** de transcriere

ARNg are secvența *spacer* complementară cu un anumit promotor al genei de activat / represat

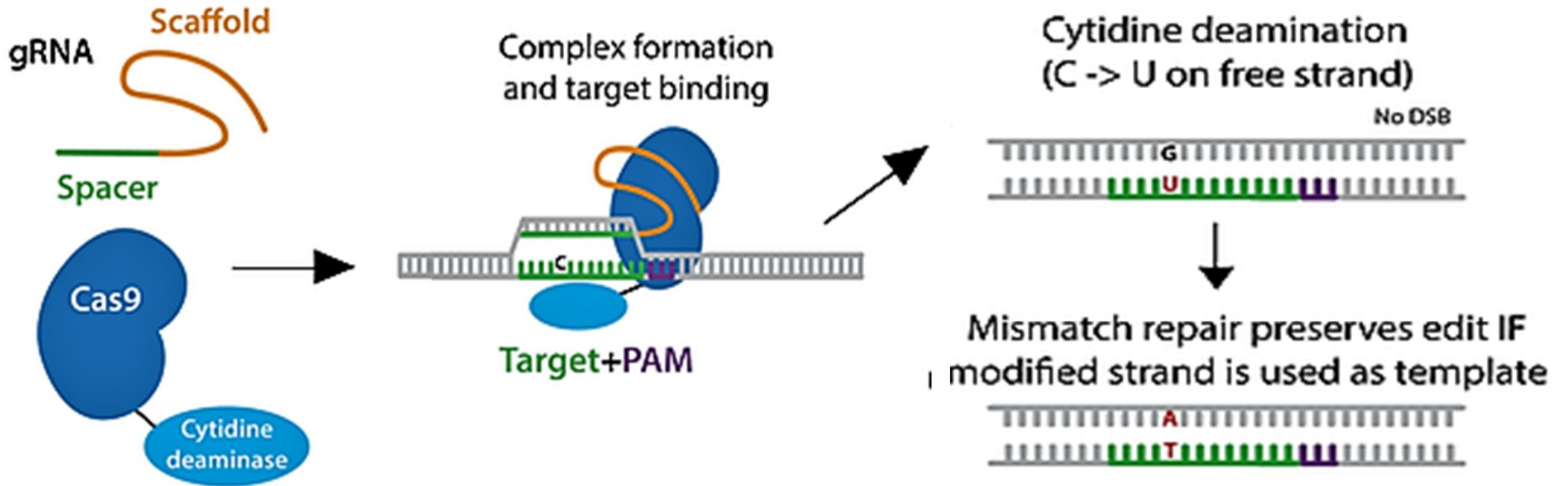


## CRISPR Base Editors

Eficiența inducerii de mutații punctiforme este mai mare folosind sistemul CRISPR Base Editors  
Specificitatea de secvență țintă este dată de ARNg ; dCas9 este fuzionată cu o citidin-dezaminază.

C, prin dezaminare → U

U, prin reparare prin excizie → T

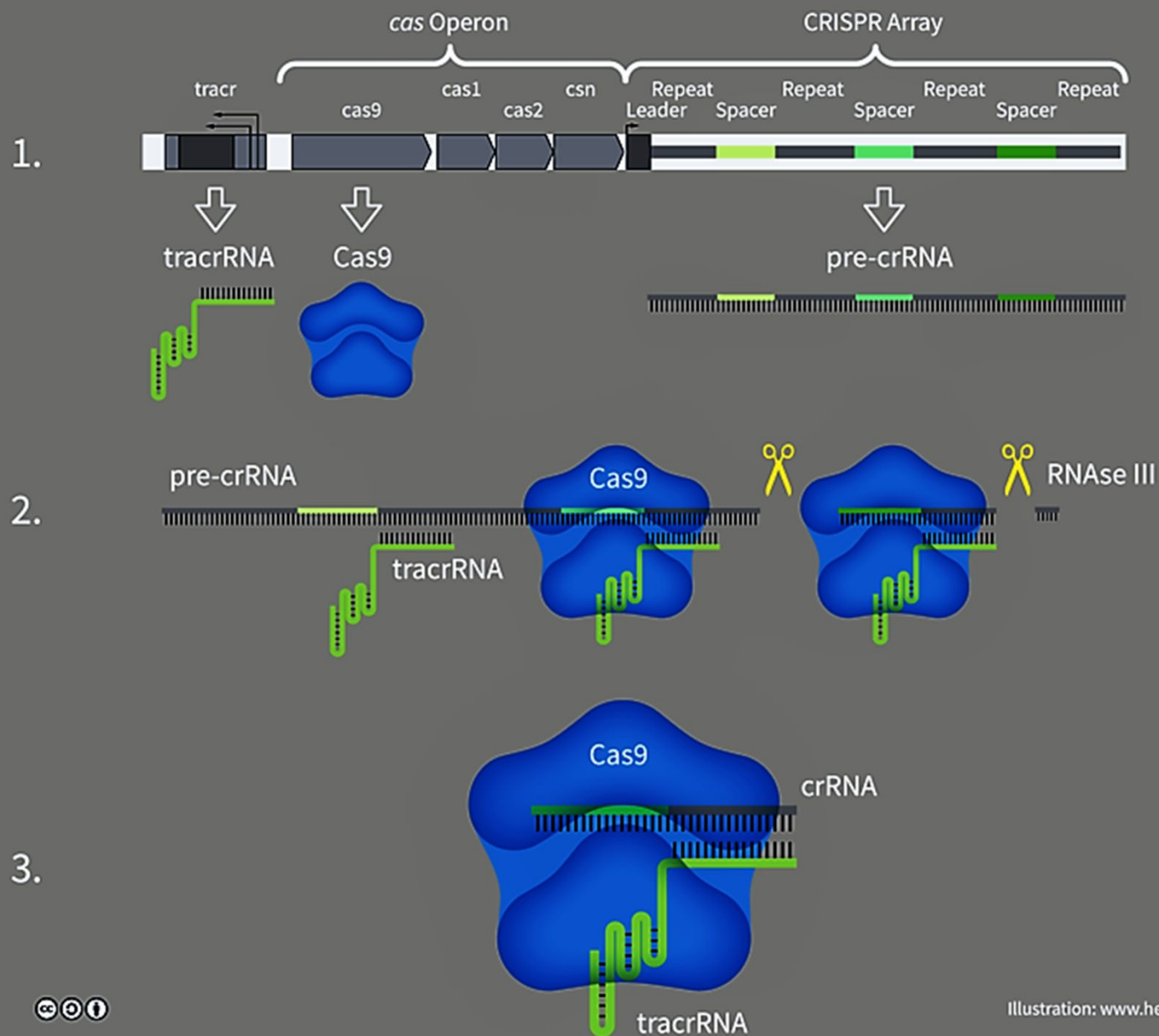


dCas9 or Cas9 nickase  
fused to a cytidine deaminase

*Base excision repair inhibitor is  
also present in fusion protein*



# CRISPR Gene Locus and pre-crRNA Maturation



In November 2018, [Jiankui He](#) announced that he had edited two human embryos, to attempt to disable the gene for [CCR5](#), which codes for a receptor that [HIV](#) uses to enter cells. He said that twin girls, Lulu and Nana, had been born a few weeks earlier. He said that the girls still carried functional copies of CCR5 along with disabled CCR5 ([mosaicism](#)) and were still vulnerable to HIV. The work was widely condemned as unethical, dangerous, and premature.<sup>[120]</sup> An international group of scientists called for a global moratorium on genetically editing human embryos.<sup>[121]</sup>

**C-C chemokine receptor type 5**, also known as **CCR5** or **CD195**, is a [protein](#) on the surface of [white blood cells](#) that is involved in the [immune system](#) as it acts as a [receptor](#) for [chemokines](#).<sup>[5]</sup> In humans, the *CCR5* gene that encodes the CCR5 protein is [located](#) on the short (p) arm at position 21 on [chromosome 3](#). Certain populations have inherited the *Delta 32* mutation, resulting in the [genetic deletion](#) of a portion of the CCR5 gene. [Homozygous](#) carriers of this mutation are resistant to [M-tropic strains](#) of [HIV-1 infection](#).

## In A 1st, Doctors In U.S. Use CRISPR Tool To Treat Patient With Genetic Disorder

• July 29, 2019 5:18 AM ET

For the first time, doctors in the U.S. have used the powerful gene-editing technique CRISPR to try to treat a patient with a genetic disorder.

"It is just amazing how far things have come," says Victoria Gray, 34, of Forest, Miss. "It is wonderful," she told NPR in an exclusive interview after undergoing the landmark treatment for [sickle cell disease](#).

Gray is the first patient ever to be publicly identified as being involved in a study testing the use of CRISPR for a genetic disease. "I always had hoped that something will come along," she says from a hospital bed at the Sarah Cannon Research Institute in Nashville, Tenn., where she received an infusion of billions of genetically modified cells. "It's a good time to get healed."



Victoria Gray, 34, of Forest, Miss., volunteered for one of the most anticipated medical experiments in decades: the first attempt to use the gene-editing technique CRISPR to treat a genetic disorder in the U.S.



## What is Sickle Cell Disease?

SCD is a group of inherited red blood cell disorders. Healthy red blood cells are round, and they move through small blood vessels to carry oxygen to all parts of the body. In someone who has SCD, the red blood cells become hard and sticky and look like a C-shaped farm tool called a “sickle”. The sickle cells die early, which causes a constant shortage of red blood cells. Also, when they travel through small blood vessels, they get stuck and clog the blood flow. This can cause pain and other serious problems such as infection, acute chest syndrome and stroke.

### Types of SCD

Following are the most common types of SCD:

#### HbSS

People who have this form of SCD inherit two sickle cell genes (“S”), one from each parent. This is commonly called *sickle cell anemia* and is usually the most severe form of the disease.

#### HbSC

People who have this form of SCD inherit a sickle cell gene (“S”) from one parent and from the other parent a gene for an abnormal hemoglobin called “C”. Hemoglobin is a protein that allows red blood cells to carry oxygen to all parts of the body. This is usually a milder form of SCD.

#### HbS beta thalassemia

People who have this form of SCD inherit one sickle cell gene (“S”) from one parent and one gene for beta thalassemia, another type of anemia, from the other parent. There are two types of beta thalassemia: “0” and “+”. Those with HbS beta 0-thalassemia usually have a severe form of SCD. People with HbS beta +-thalassemia tend to have a milder form of SCD.

There also are a few rare types of SCD:

#### HbSD, HbSE, and HbSO

People who have these forms of SCD inherit one sickle cell gene (“S”) and one gene from an abnormal type of hemoglobin (“D”, “E”, or “O”). Hemoglobin is a protein that allows red blood cells to carry oxygen to all parts of the body. The severity of these rarer types of SCD varies.

## Cure

The only cure for SCD is bone marrow or stem cell transplant.

For the study, doctors are using cells taken from patients' own bone marrow that have been genetically modified with CRISPR to make them produce a protein that is usually only made by fetuses and by babies for a short time following birth.

The hope is this protein will compensate for the defective protein that causes sickle cell disease and will enable patients to live normally for the rest of their lives.

This CRISPR treatment starts with doctors extracting bone marrow cells from patients' blood. Company scientists then use CRISPR to edit a gene in the cells to make the cells produce fetal hemoglobin.

"That will help the cells make more fetal hemoglobin and make them happier and healthier,"

The patients then undergo the same kind of grueling chemotherapy administered as part of a standard bone marrow transplant. That wipes out the existing cells that are carrying the genetic defect. But instead of receiving new cells from a donor, the patients get billions of their own cells that have been edited with CRISPR.

The hope is that it will provide a treatment option for all patients, including those who can't find a matched donor. The approach hopefully will be safer because the cells come from patients' own bone marrow. So they shouldn't attack patients' bodies



G-What is CRISPR? – 7 min

<https://www.youtube.com/watch?v=MnYppmstxls>

CRISPR Gene editing and beyond – 4:35

<https://www.youtube.com/watch?v=4YKFw2KZA5o>

Genetic Engineering Will Change Everything Forever – CRISPR – 17 min

<https://www.youtube.com/watch?v=jAhjPd4uNFY>