# Genome Editing Techniques in Plants 24.03.2020

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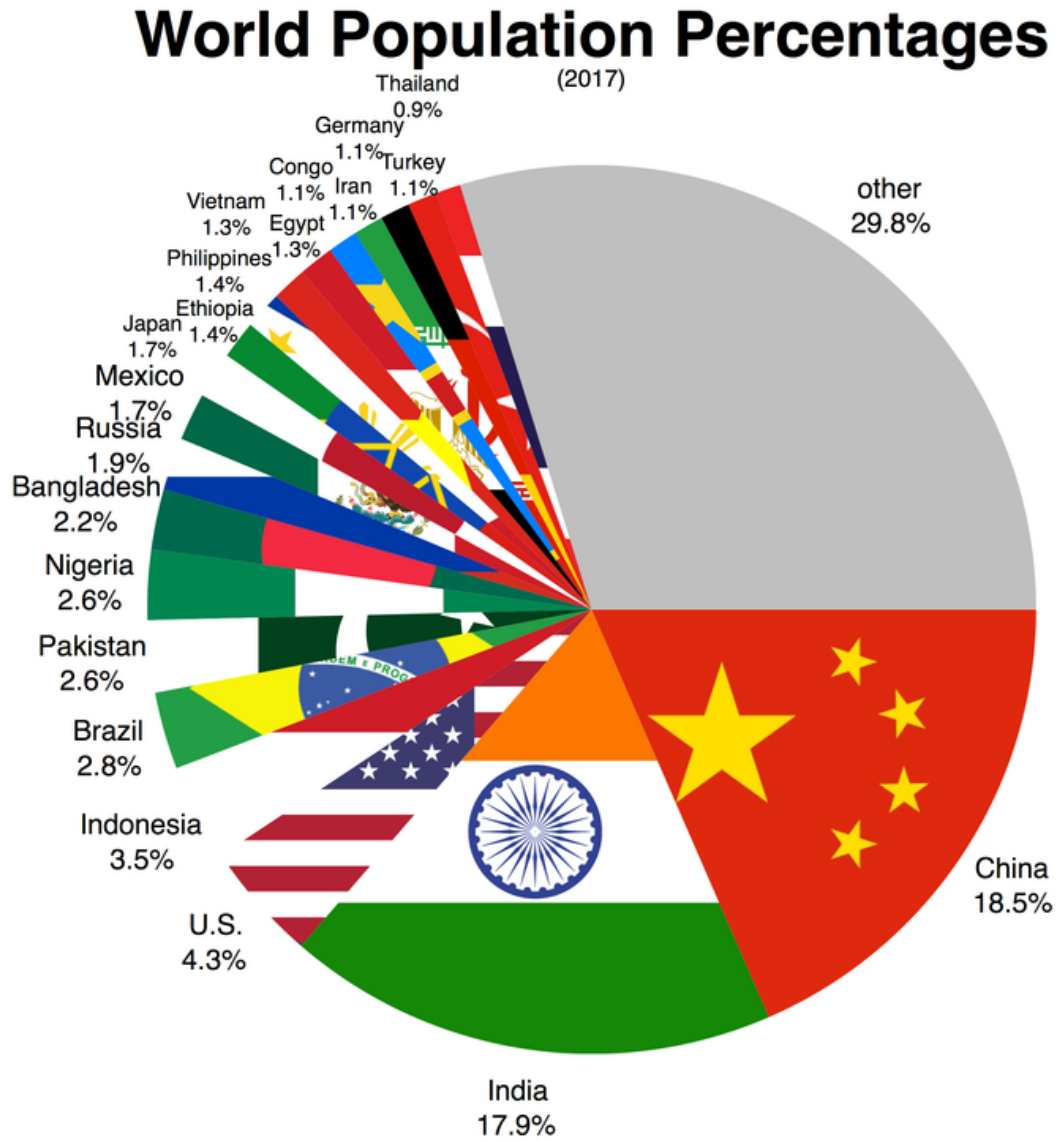
- The human population is growing rapidly and this figure is expected to reach 9.1 billion by 2050 [1].

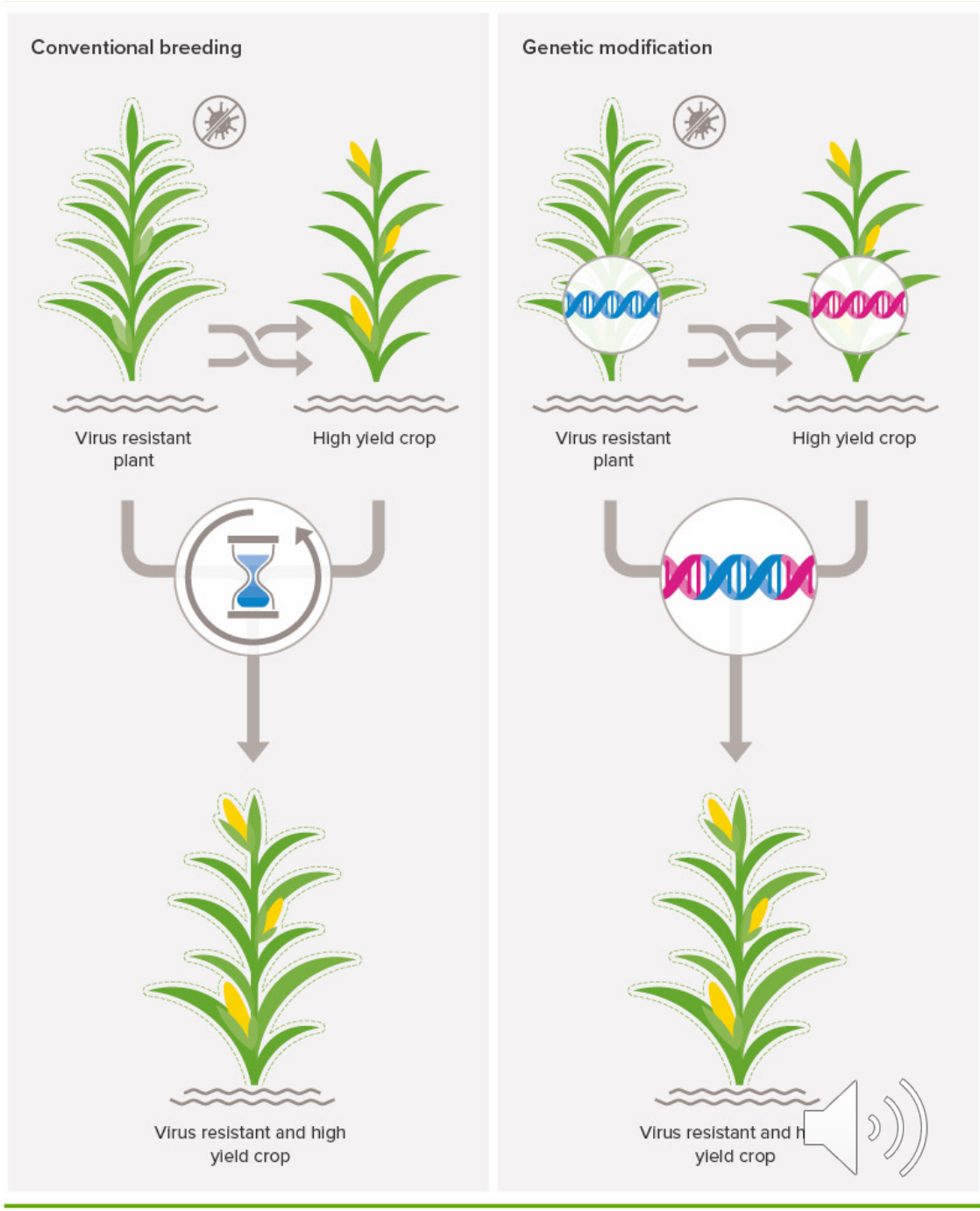
- Since agricultural productivity does not increase in line with population growth, food deficiencies are considered to be a major problem in the future [2].



The success of traditional plant breeding techniques remains limited in the development of sustainable agricultural products due to the complexity of plant stress resistance mechanisms [3].

Therefore, new and strong approaches should be developed to meet the increasing food demand in the world.

GENOM EDITING TECHNOLOGY, which has recently become a popular research area; has many functions such as mutation in specific genes, reprogramming epigenetic markers and making sequence-specific Changes [4].



Genome editing begins with the breaking the double strand DNA using sequence-specific endonucleases [5].

Among these nucleases;

- Zinc finger nucleases (**ZFN**),
- Transcription activator like nucleases (**TALEN**),
- Clustered regularly spaced short palindromic repeats (**CRISPR**) are found.

Recently discovered, **CRISPR** has become widely used in the field of plant genome editing thanks to its easy design, high efficiency and flexibility.


In addition, plants produced with this approach cannot be distinguished from plants produced by classical breeding and mutagenesis techniques.

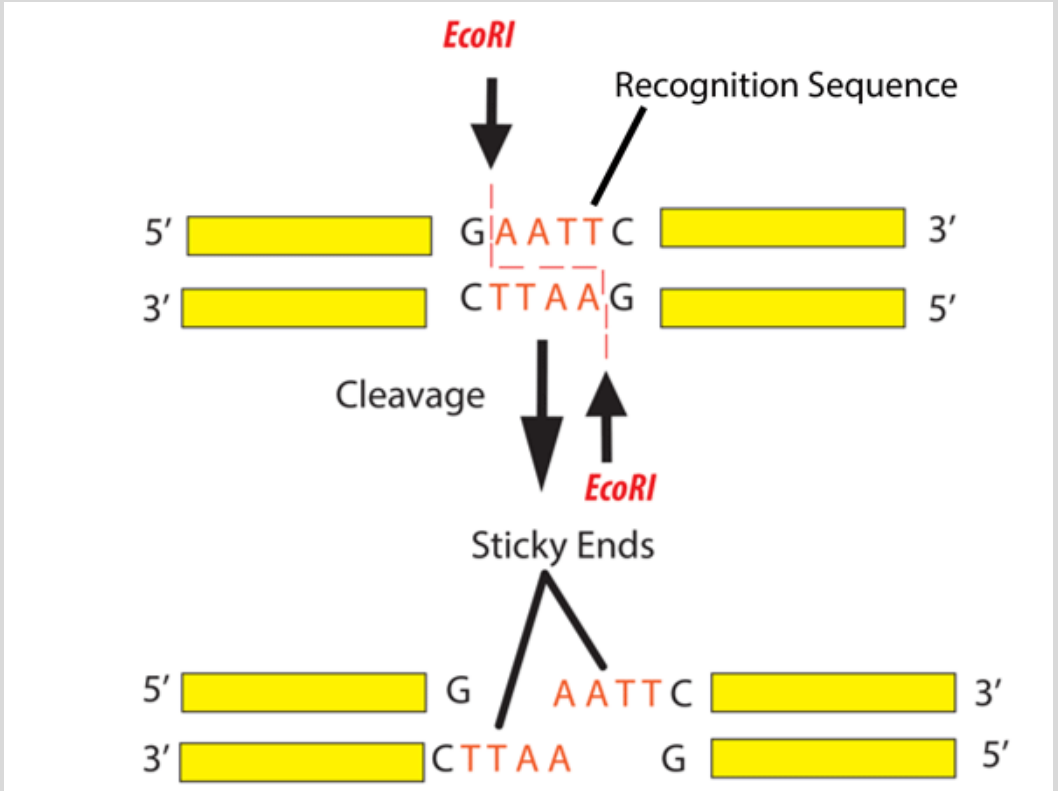
In 2018, the Court of Justice of the European Union ruled that the genome correction should also be classified as GMO and subject to the same strict regulations [6].

However, in August 2018, the Government of Japan decided otherwise, declaring that genome editing techniques cannot be counted as GMOs, and therefore stated that genetically modified organism producers do not need approval from the government [7].

# Genome Editing Tools

- Sequence-specific nuclease is at the heart of ZFNs, TALENs and CRISPR technologies used as genome correction tools.
- These nucleases have found wide use in medicine, molecular biology and plant breeding for the past 10 years.
- These nucleases cut DNA by creating double chain fractures in the region where the genome will be corrected, similar to restriction enzymes [8].

Restriksiyon Enzim Kesimi



DNA breaks as a result of cutting the nuclease are repaired by two DNA repair mechanisms, called **Homologous Recombination (HR)** and **Non-homologous end joining (NHEJ)** [9].

Depending on the repair mechanism used by the cell, different modifications occur in the genome.

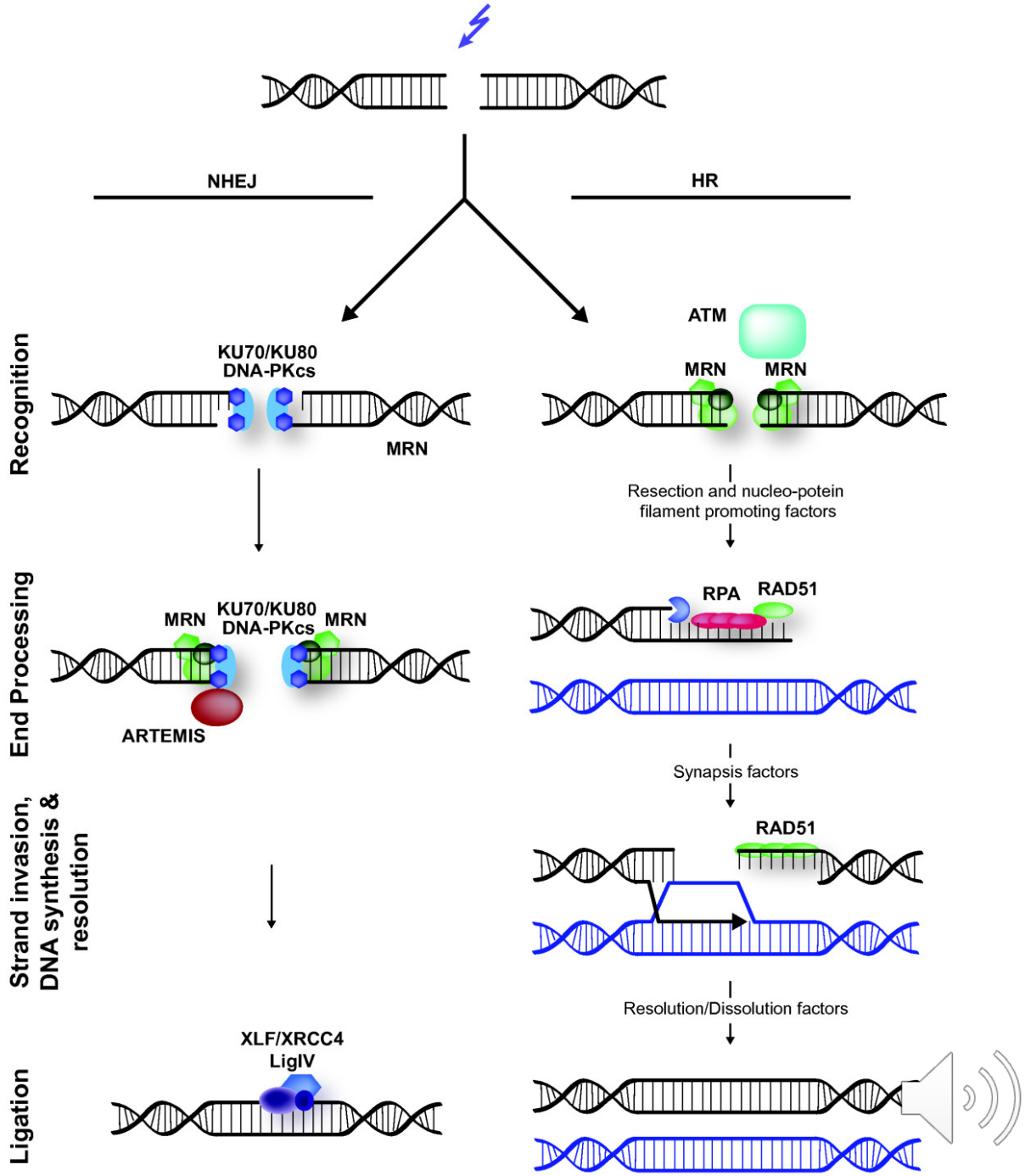
**For example**;

In the HR mechanism, knock out silencing of the gene can be achieved as a result of inducing insertion or deletion events.

In the case of repair templates showing homology to double chain fractures, **homology directed repair (HDR)**, including HR repair mechanisms such as gene replacement or gene insertion, is used to create error-free and gene-specific modifications.

**NHEJ**, on the other hand, is a repair mechanism frequently seen in advanced eukaryotes, especially humans and plants. It is often used to correct damage caused by environmental mutations to the genome. In this way, nucleotides can be added to and removed from a genomic region and as a result, frame shift mutations can be observed in the genome [9].

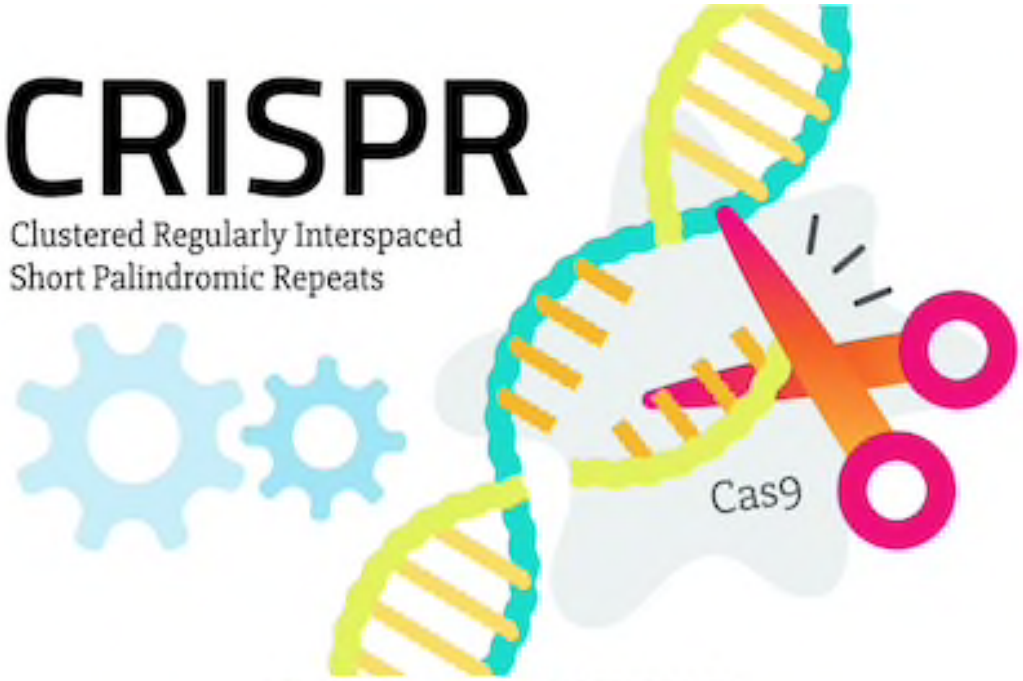
# Homologous Recombination (HR) and Non-homologous end joining (NHEJ)



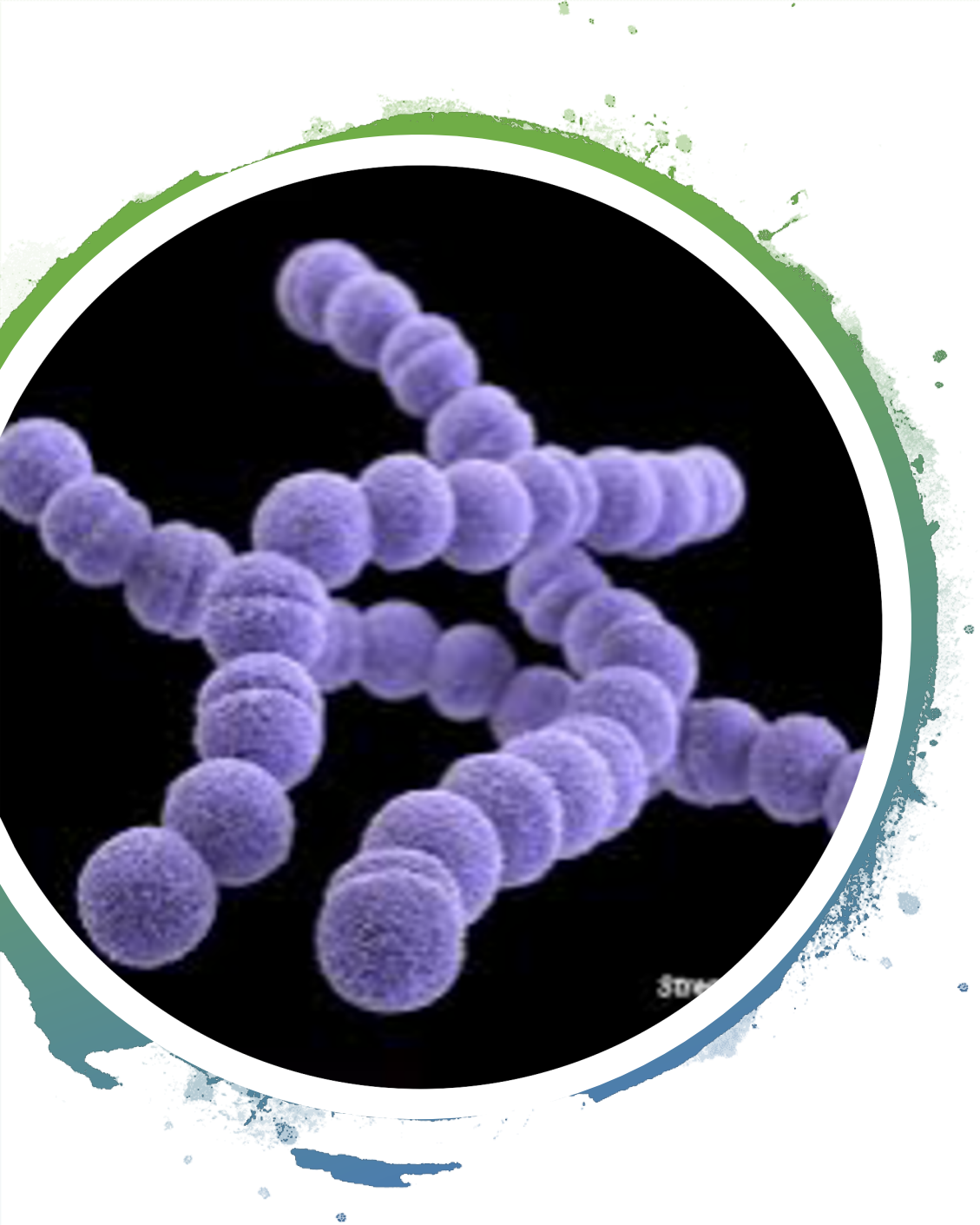
| FEATURE | ZFN | TALEN | CRISPR |
|---|---|---|---|
| **The principle of attachment** | RNA-DNA | Protein-DNA | Protein-DNA |
| **Methylation sensitivity** | Not Responsive | Responsive | Responsive |
| **Cost** | High | Medium | **Low** |
| **Targeted effect** | Limited | Average | **High** |
| **Basic components** | Zinc finger protein<br>FOK I Fusion Protein | TALE ve FOK I Fusion Protein | Guide RNA<br>Cas protein |
| **Design time** | Long (7-15 days) | Long (5-7 days) | **Short (1-3 days)** |
| **Multiple gene mutations** | Limited | Limited | **Unlimited** |
| **Non-target effect** | High | Low | **Low** |
| **The length of the target sequence** | 18-24 base pair<br>4-7 spacers | 30-60 base pairs<br>13-33 spacer | 20 base pairs |
| **Origin** | Zinc finger proteins commonly found in nature | TAL effector proteins in plant pathogens | S. pyogenes from bacterial immune system |
| **Cytotoxicity** | Variable / High | Low | **Low** |
| **Designed state** | Very complex | Complex | **Simple** |
| **The nuclease used** | Fok I | Fok I | Cas |
| **Success rate** | Low (%24) | Low (>%99) | **High (>%90)** |
| **Mutation rate** | Low / Variable (%10) | High (>%20) | **Low (<%10)** |

[10]

# CRISPR / Cas Technology







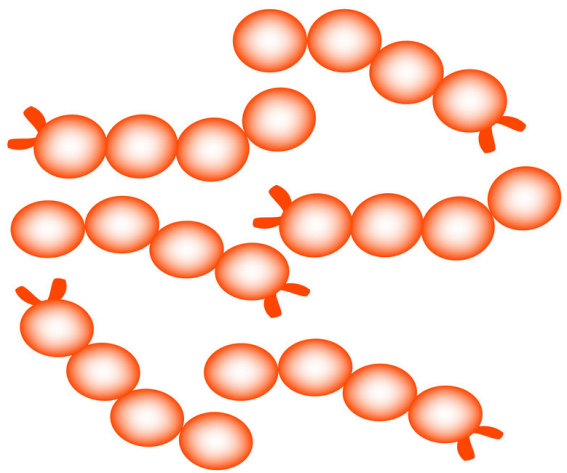
**Discovery of the CRISPR / Cas System**

- First, in 1987, Ishino et al. (1987), while examining the Apoptosis Inhibitor Protein (IAP) gene sequence in the E. coli genome, 29 nt repeat sets and 32 nt spacer DNA regions were discovered between these repeats. However, these regions were not named and their functions were not defined.

- Studies in the following years have shown that these sequences are found in many organisms other than viruses and eukaryotes.

- In order to eliminate the complexity of names, it was decided to call these series CRISPR in 2016.

- Today, it is reported that this system is present in 90% of the arches and approximately 45% of the bacteria [11].

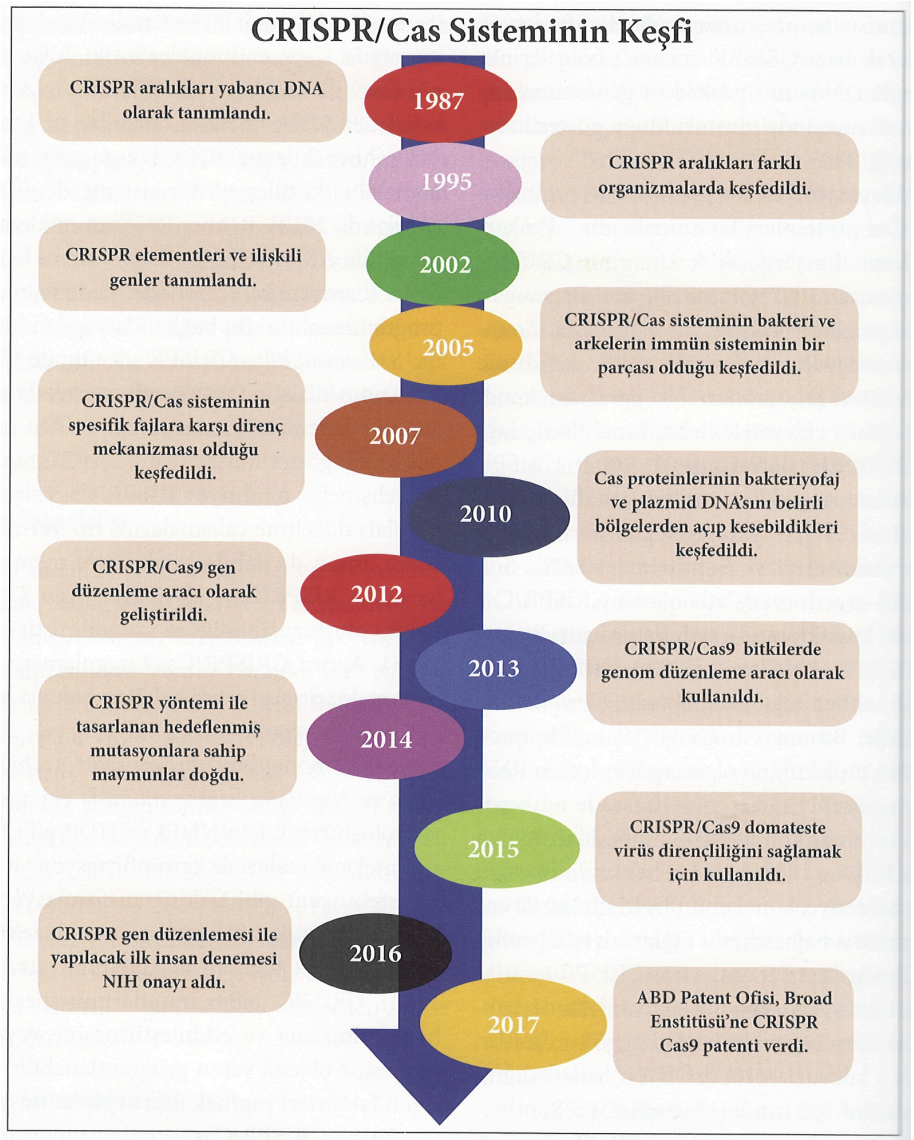


*Streptococcus thermophilus*

After examining the DNA sequences surrounding CRISPR regions in different organisms, it was determined that the genes encoding Cas proteins before or after CRISPR regions and CRISPR regions were associated with 4 Cas genes..

After the discovery that most Cas genes show sequence similarity to nuclease and helicase gene families, the CRISPR / Cas system is thought to be an RNA-mediated defense system developed by prokaryotes against viruses.

The CRISPR / Cas mechanism of the immune system acquired in prokaryotes was first shown by Barrangou et al. in 2007. In this study, it has been discovered that bacteria named Streptococcus thermophilus provide resistance to the virus by adding a part of the genome of the virus attacking it to its CRISPR locus.

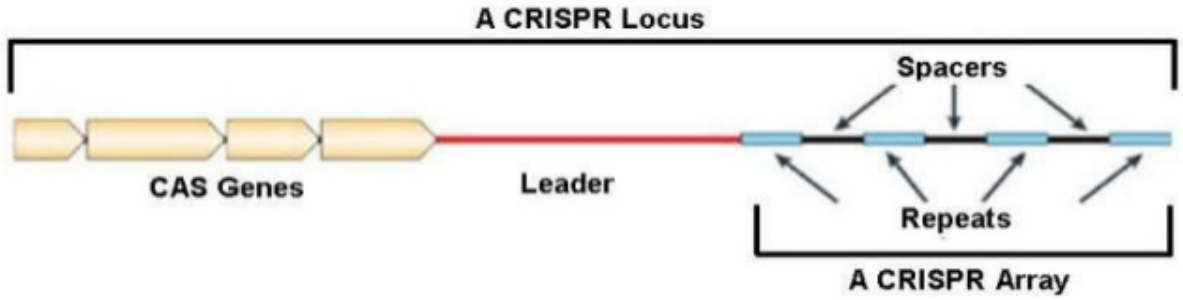

CRISPR/Cas Sisteminin Keşfi
CRISPR aralıkları yabancı DNA olarak tanımlandı.
1987
1995
CRISPR aralıkları farklı organizmalarda keşfedildi.
CRISPR elementleri ve ilişkili genler tanımlandı.
2002
2005
CRISPR/Cas sisteminin bakteri ve arkelerin immün sisteminin bir parçası olduğu keşfedildi.
CRISPR/Cas sisteminin spesifik fajlara karşı direnç mekanizması olduğu keşfedildi.
2007
2010
Cas proteinlerinin bakteriyofaj ve plazmid DNA'sını belirli bölgelerden açıp kesebildikleri keşfedildi.
CRISPR/Cas9 gen düzenleme aracı olarak geliştirildi.
2012
2013
CRISPR/Cas9 bitkilerde genom düzenleme aracı olarak kullanıldı.
CRISPR yöntemi ile tasarlanan hedeflenmiş mutasyonlara sahip maymunlar doğdu.
2014
2015
CRISPR/Cas9 domateste virüs dirençliliğini sağlamak için kullanıldı.
CRISPR gen düzenlemesi ile yapılacak ilk insan denemesi NIH onayı aldı.
2016
2017
ABD Patent Ofisi, Broad Enstitüsü'ne CRISPR Cas9 patenti verdi.

# General Structural Features of CRISPR / Cas System

In the original CRISPR locus system in microorganisms;

There are two RNAs: non-coding RNA elements called CRISPR RNA (crRNA) that guide the Cas9 protein, Small trans-encoded trans-activating crRNA (tracrRNA) sequences.

After transcription of the CRISPR sequence, enzymatic processing of precursor-CRISPR transcripts by endonucleolytic cleavage yields crRNAs [14].

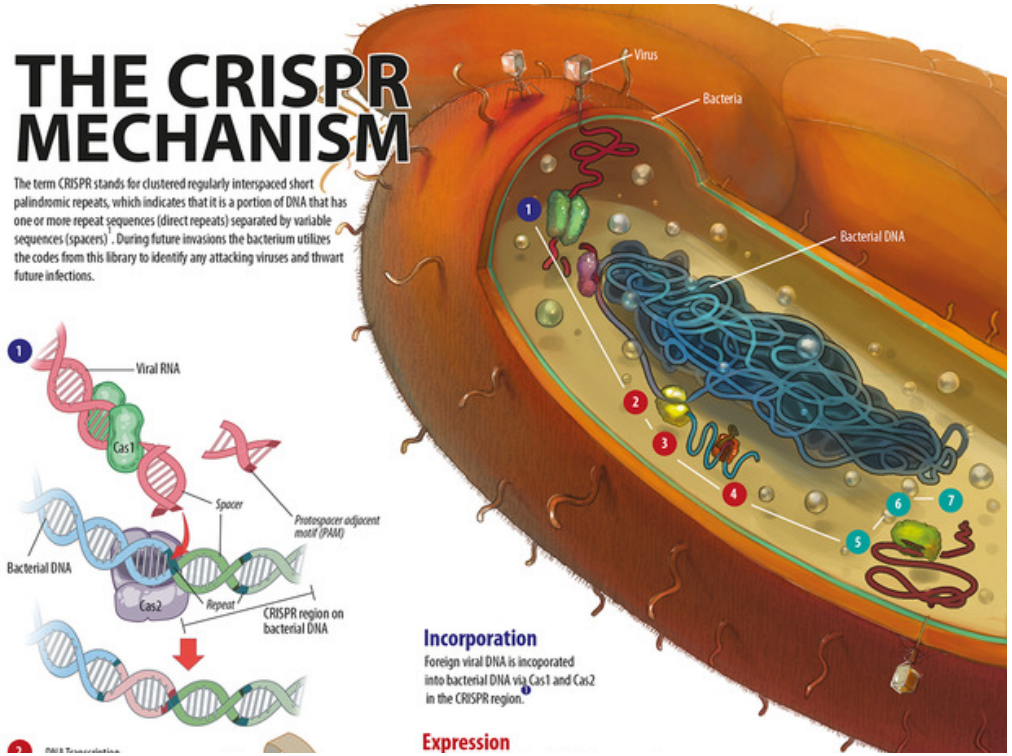


# General Structural Features of CRISPR / Cas System

At the 5 'end of the crRNA, there is a short RNA fragment that completes a sequence from foreign genetic material, and at the 3' end, there are regions called spacers that contain a part of the CRISPR repeat sequence (palindromic sequence).

After the organism is exposed to phage or plasmid infestation, the spacer regions trigger the creation of a genetic record of infection by integrating short pieces of foreign DNA. Thus, spacers provide sequence-specific destruction of invader DNA or RNA with Cas nucleases to ensure that the next attack of the same invader is prevented.

In addition, conserved sequences of the invasive virus or phage, called the motif adjacent to the protocol (PAM), located in the initial region of the CRISPR locus, allow the direction of transcription to be determined [15].



# Natural CRISPR Pathway

**1. DNA Invasion**
Foreign DNA from a virus or plasmid invades the cell.

**2. Invading DNA is Incorporated Into CRISPR Array**
DNA fragments from the invading DNA are incorporated into the CRISPR locus as spacers. The exact mechanism of incorporation remains unkonwn.

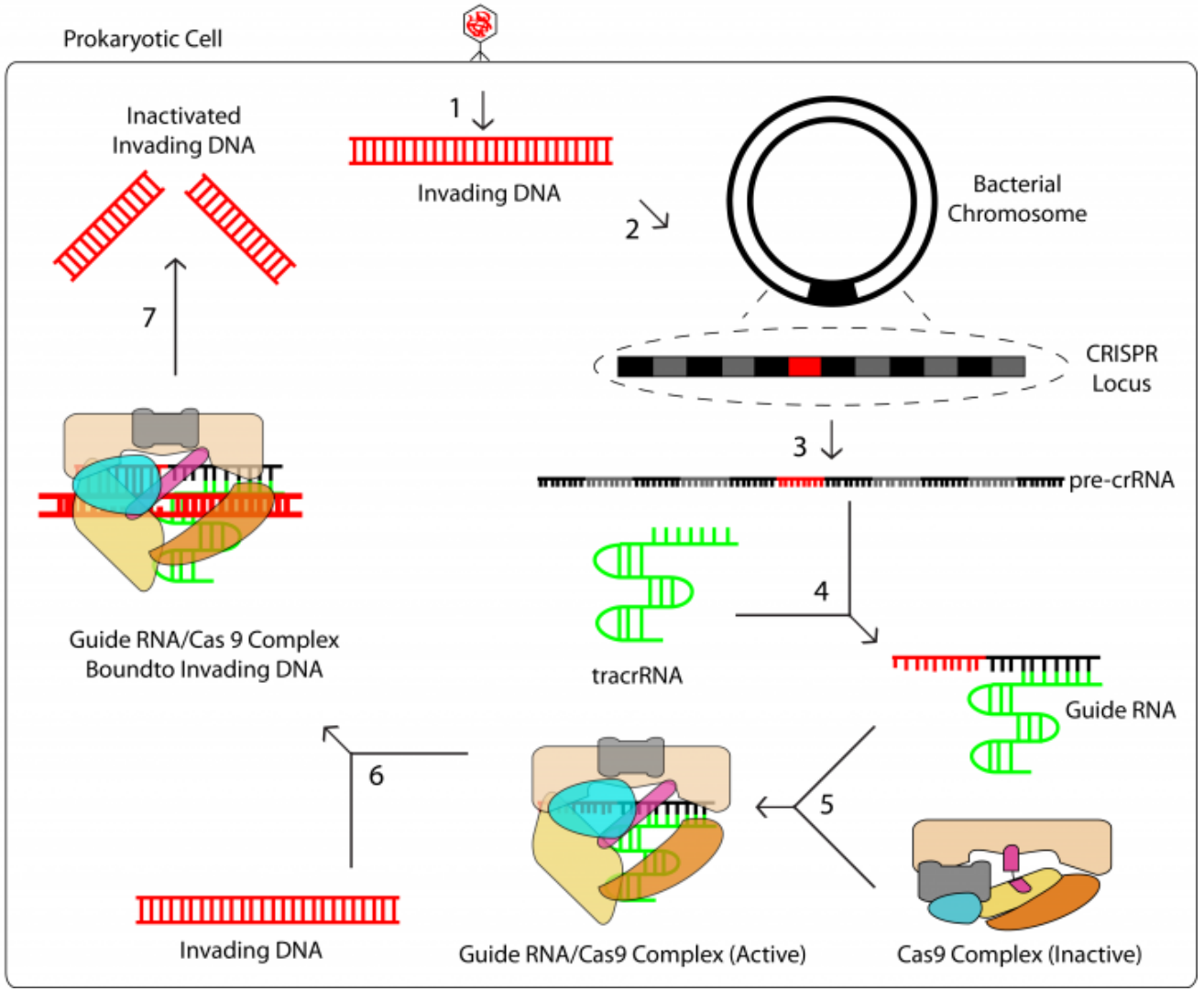
**3. Pre-crRNA Transcription**
The cell constitutively transcribes a repeat/spacer group into pre-crRNA. Black boxes represent repeats. Grey boxes represent spacers. The red box represents the spacer corresponding to the invading DNA.

**4. Guide RNA Formation**
Constitutively expressed transactivating RNA (tracrRNA) base pairs with the CRISPR repeat sequences on the pre-crRNA. RNase III, Csn 1, and other unidentified CRISPR-associated pro-tiens modify the pre-crRNA/tracrRNA duplex to form a guide RNA. (Deltcheva et al. 2011)

**5. Cas9 Activation**
Inactive Cas9 protien binds to the guide RNA and becomes activated.

**6. Target Binding**
The activated guide RNA/Cas9 complex binds with the target DNA. The localization occurs stochastically (Sternberg et al. 2014).

**7. Target Cleavage**
The Cas9 protein cleaves the invading DNA and inactivates it.



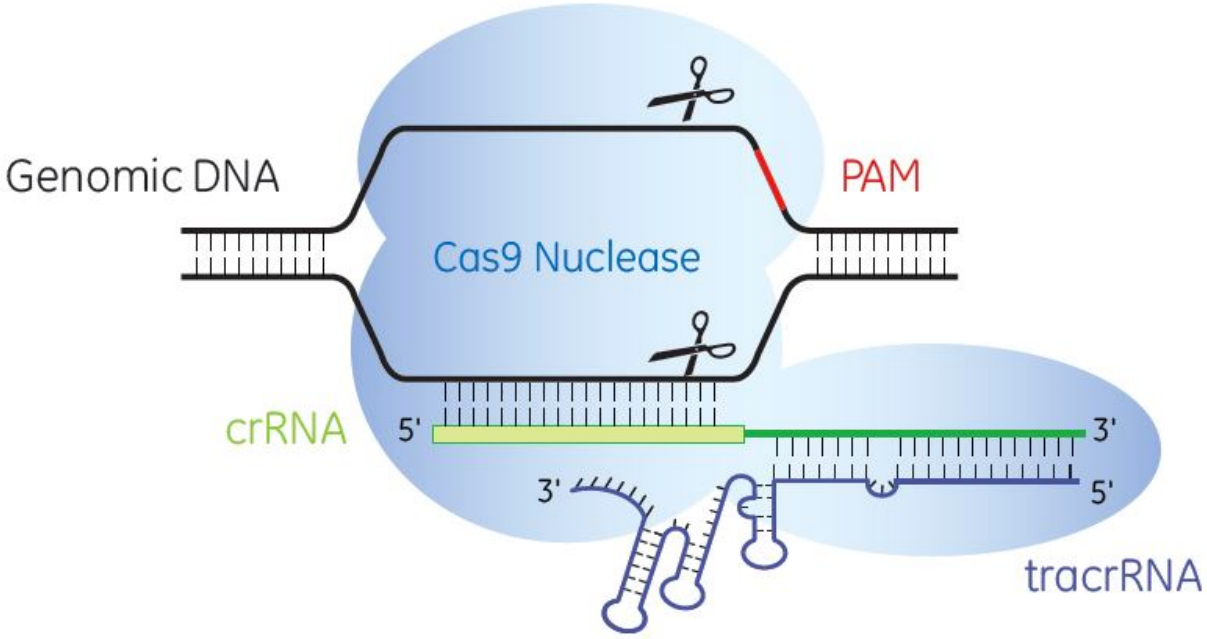
# **sgRNA**: single guide RNA

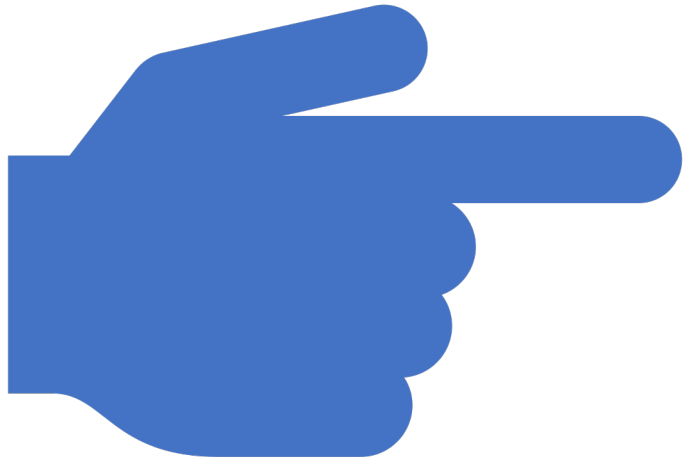
In the reprogrammed CRISPR system for genomic editing, there is a single RNA, called the single guide RNA, created by combining the 5 ‘end of the tracrRNA with the 3' region of the crRNA.

sgRNA can mimic the original crRNA-tracrRNA pair. In this way, the CRISPR / Cas9 system undergoes considerable change.

The Cas nuclease complex consists of the combination of Cas9 protein and sgRNA.

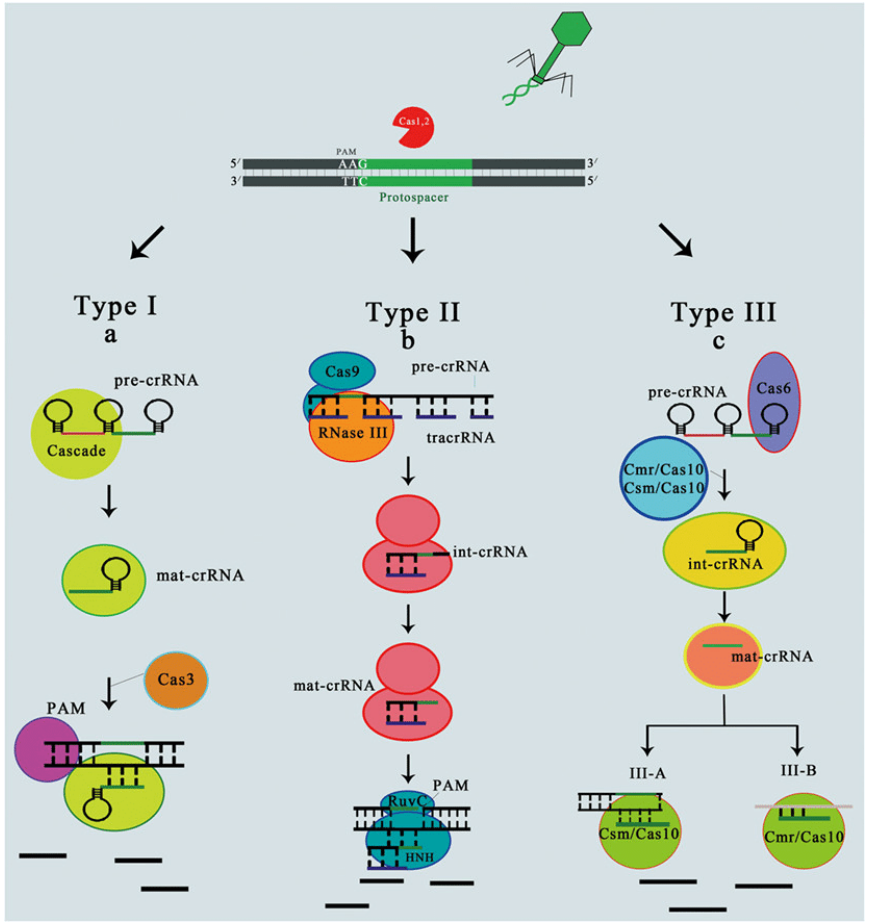
The PAM region at the 3 'end of the target region of an NGG sequence is the only requirement for the Cas9 / sgRNA complex to identify the target region and cut DNA [17].



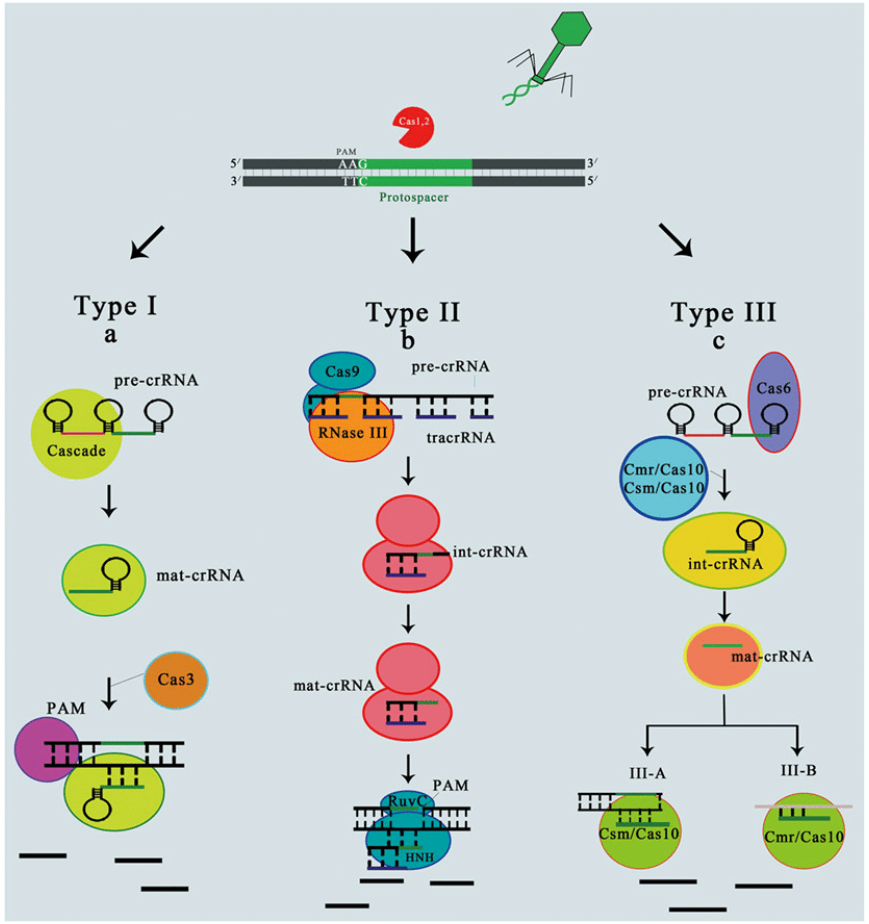


# The types of CRISPR/Cas system

- Type 1 system
- Type 2 system
- Type 3 system




- **Cas3** is especially important in the **Type 1 system**, which was first discovered in ***E. coli***. The complex contains Cse1, Cse2, Cas7, Cas5 and Cas6e proteins that are effective in anti-viral immunity.
- On the other hand, **Type2** systems use a single large Cas protein for the same purpose. Cas1, Cas2, Cas9 and, in some cases, Csn2 or Cas4 proteins work in the Type2 system, which was first discovered in ***Streptococcus thermophilus***. The non-coding trans-activating crRNA (tracrRNA) contained in this system hybridizes with crRNA to form a scaffold and thus facilitates Cas9's guidance.
- Cas9 assists in the adaptation phase, participates in the processing of crRNA, and allows the target DNA to be cut with the help of crRNA and tracrRNA.




In addition to the Type1 and Type2 systems, a new CRISPR / Cas system, consisting of a complex in which **Cas10** protein functions and containing other Cas proteins, has been identified and defined as **Type3**.

While Type1 and Type2 systems target DNA, Type3 CRISPR / Cas system can target **RNA** along with DNA.

thank you