
بسم الله الرحمن الرحیم

Title: Using the CRISPR/Cas9 technology to attenuate virulence factors and pathogenicity of bacteria

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Outline

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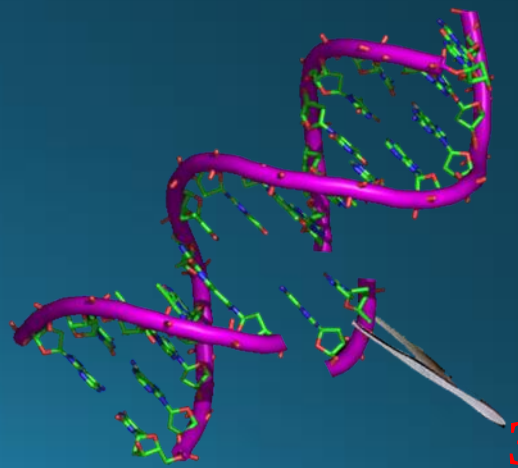
2. Mechanisms of Gene Editing

3. Platforms of genome-editing technology

4. CRISPR-Cas technology

5. Major steps in CRISPR genome editing

6. Conclusion and future perspectives



Introduction

Importance of genome editing

- Genome editing can be achieved in vitro or in vivo by delivering the editing machinery in situ.

- Gene editing is quite extraordinary technique because of its capability to alter DNA by utilizing engineered nucleases called as molecular scissors.

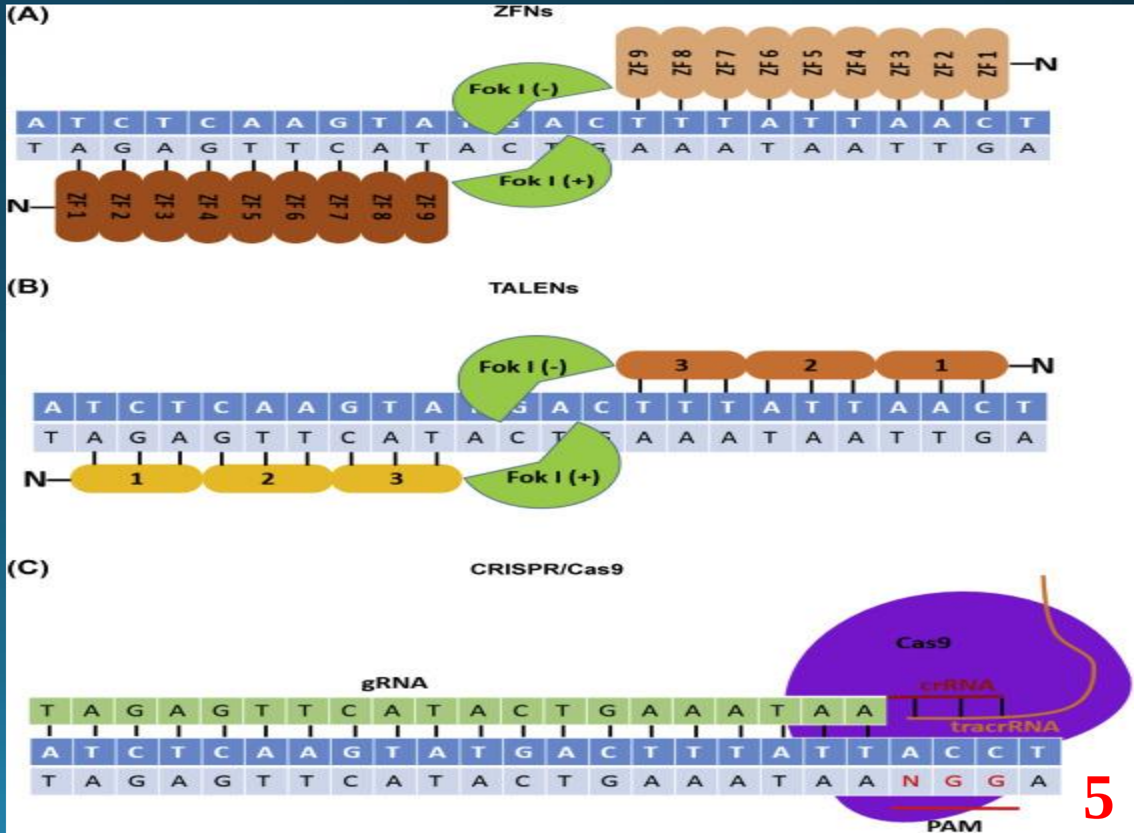
- Genome editing is the process of precisely modifying the nucleotide sequence of the genome.

Genome editing technology

Targeted DNA alterations begin from the generation of nuclease-induced double-stranded breaks (DSBs), which leads to the stimulation of highly efficient recombination mechanisms of cellular DNA.

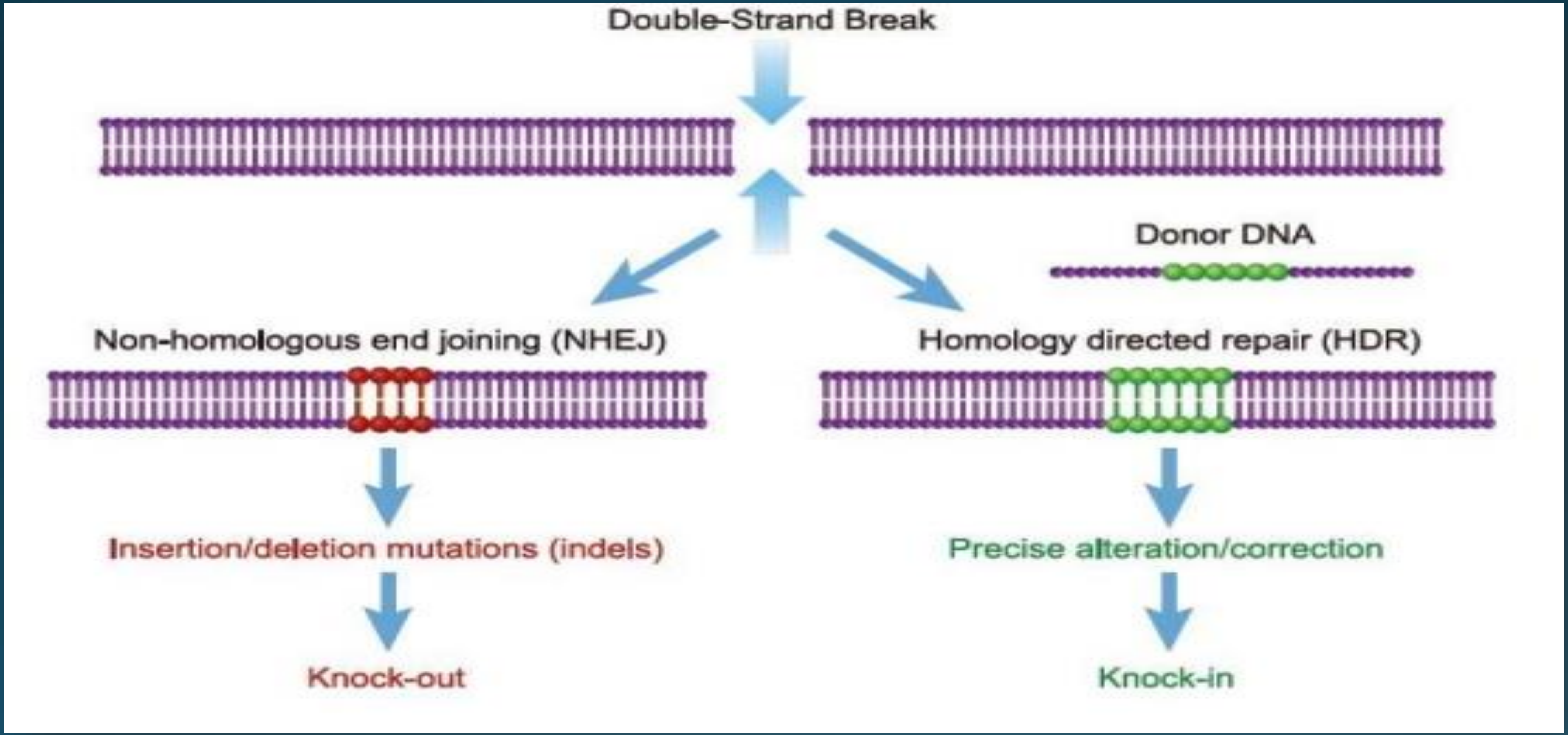
Nuclease-induced DNA DSBs:

1-Homology-directed repair (HDR)
2-Nonhomologous end-joining (NHEJ)



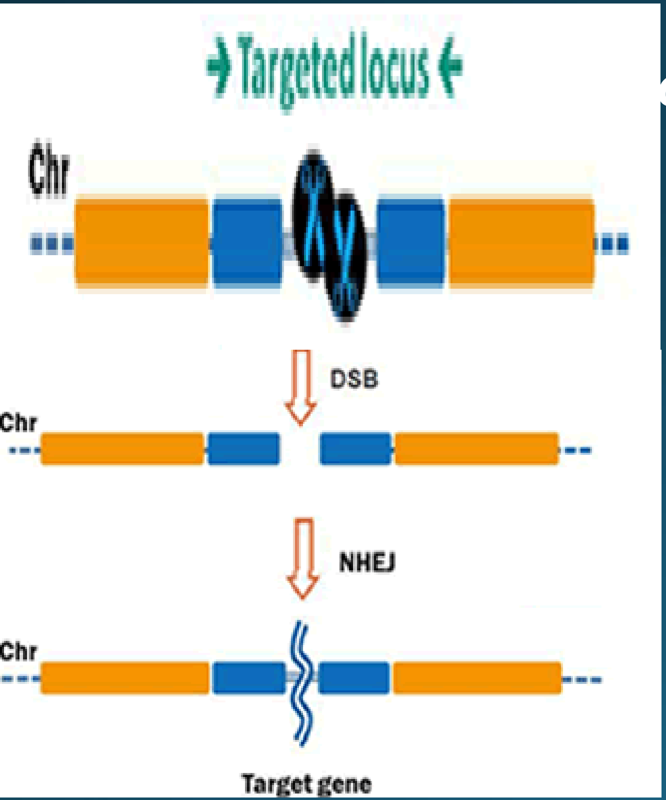
Mechanisms of Gene Editing

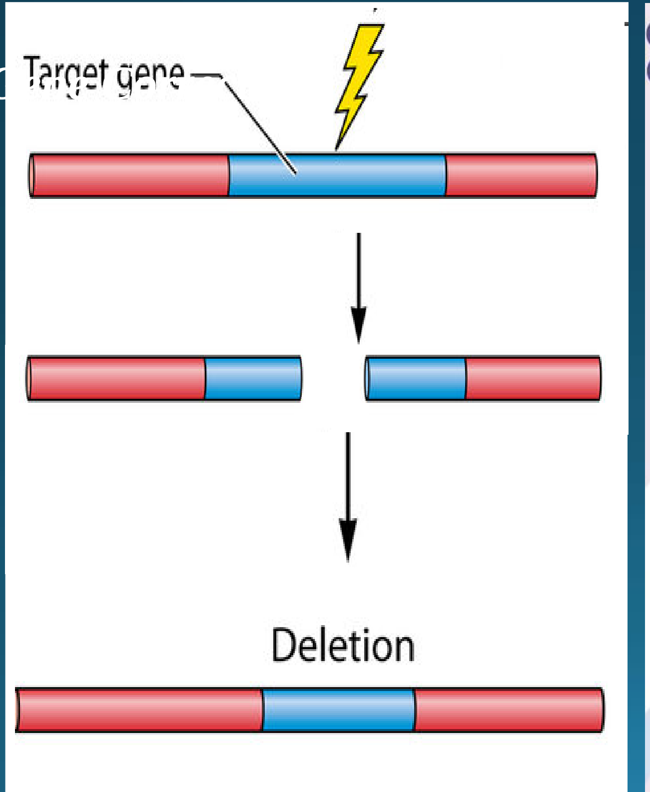
Foundational to the field of gene editing was the discovery that targeted DNA double strand breaks (DSBs) could be used to stimulate the endogenous cellular repair machinery.

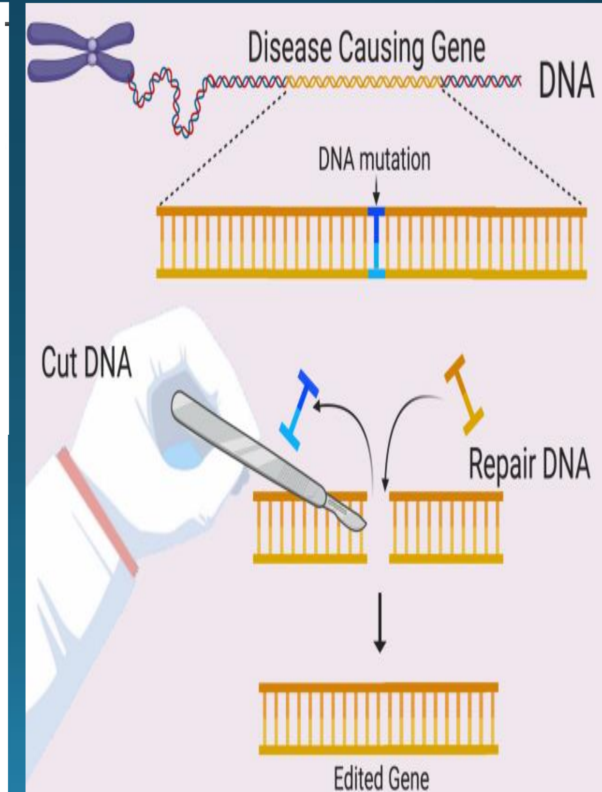


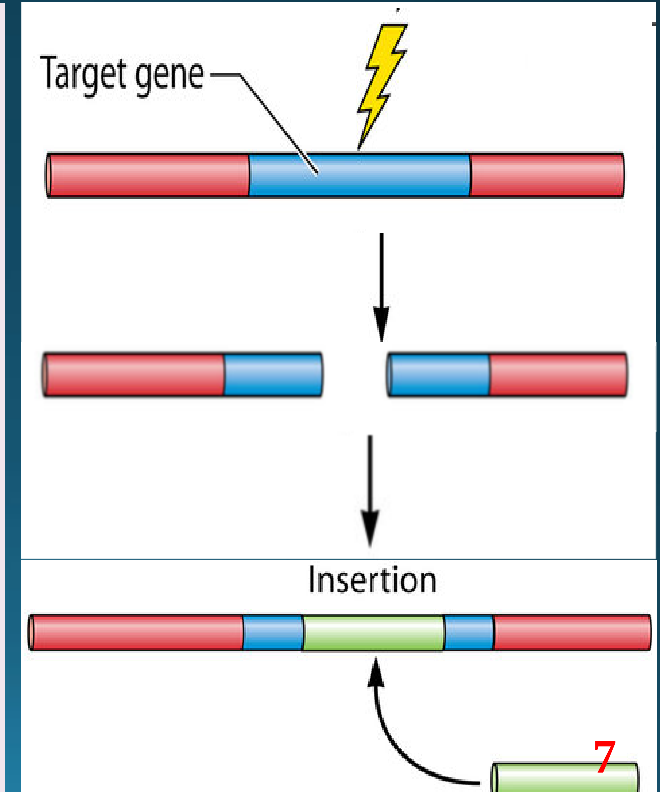
Types of genetic changes

Gene Knockout | **Gene Deletion** | **Gene Correction** | **Gene Insertion**

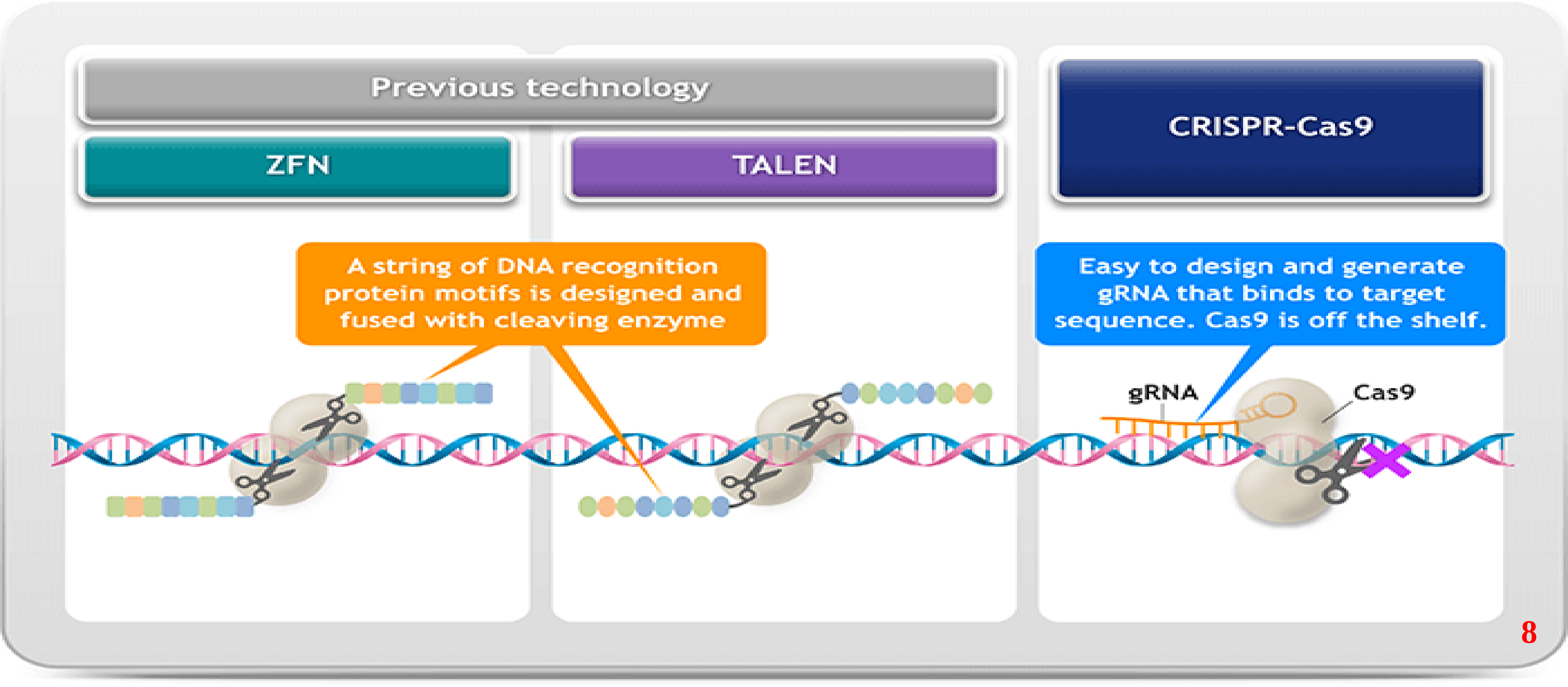






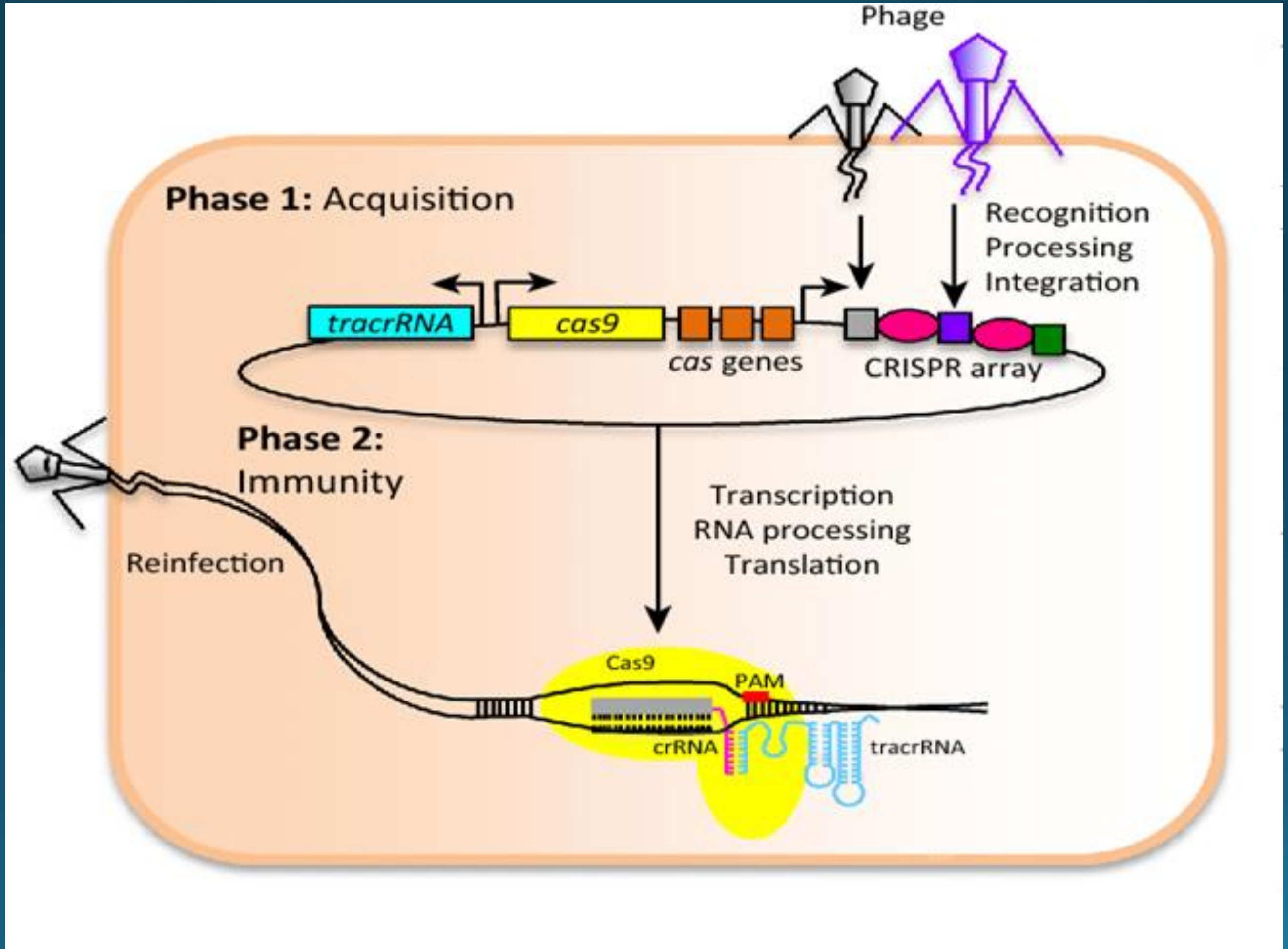


Platforms of genome-editing technology

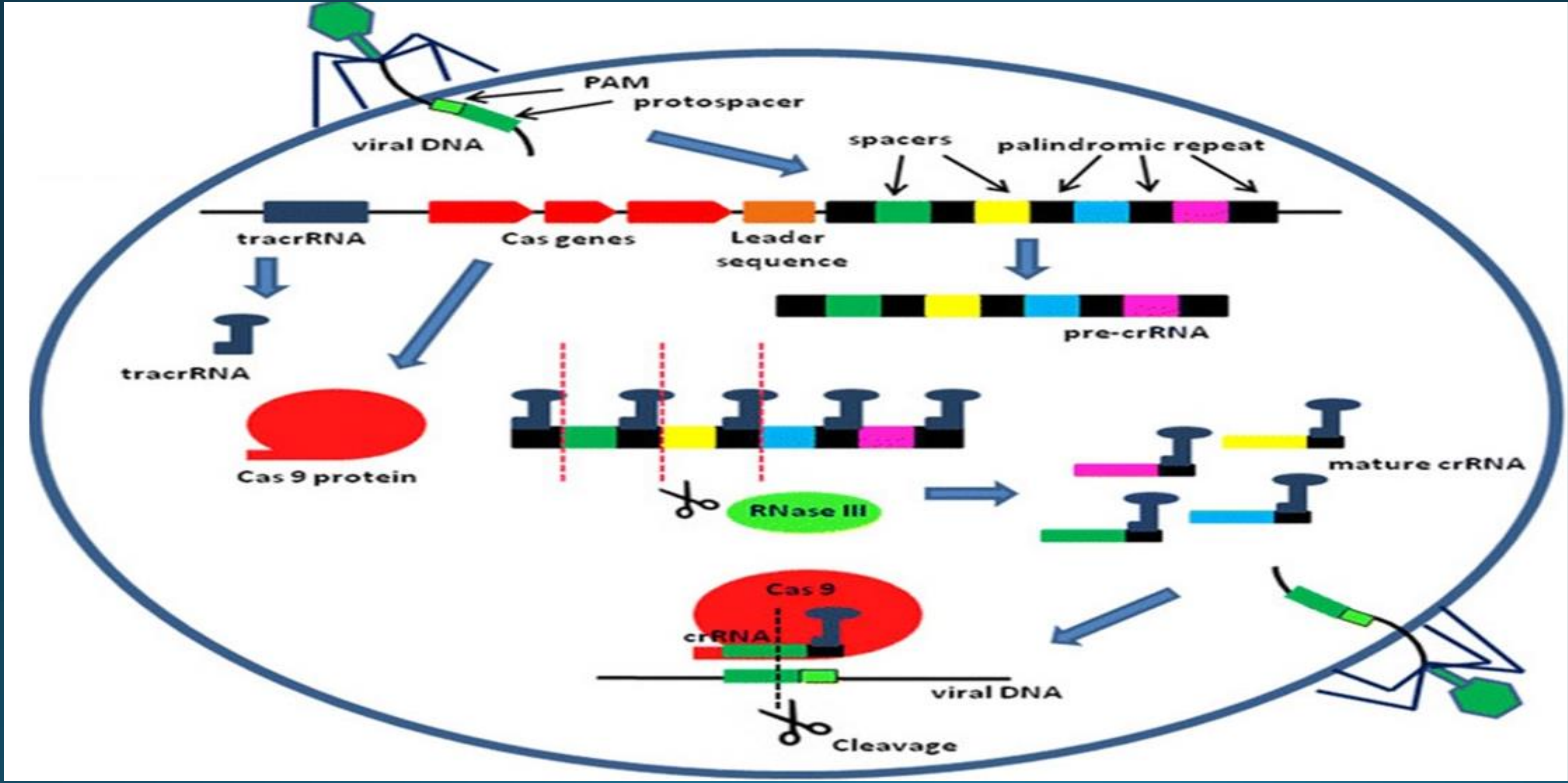


The CRISPR-Cas immune system

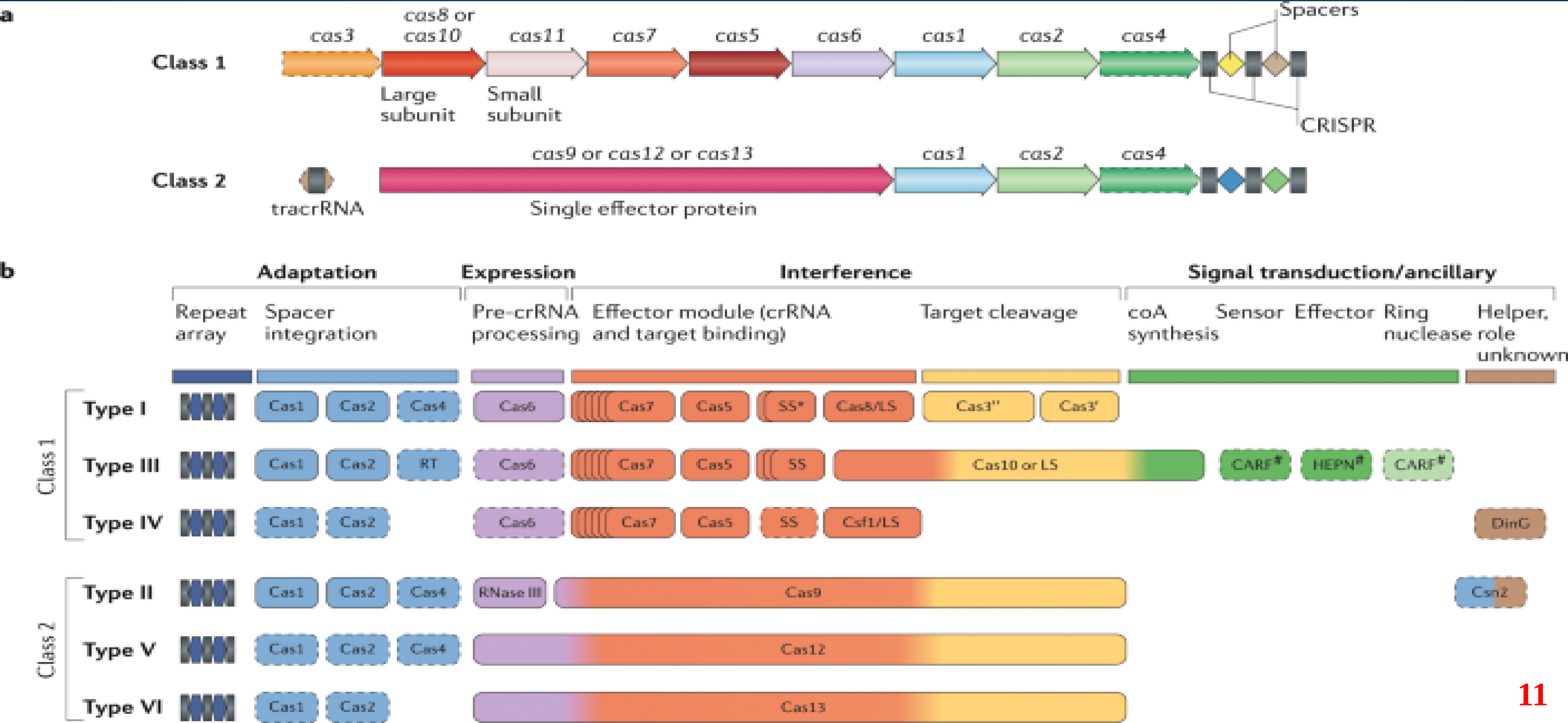
CRISPR (clustered regularly interspaced short palindromic repeats) were firstly identified in the *Escherichia coli* genome in 1987 as an unusual sequence element consisting of a series of 29-nucleotide repeats separated by unique 32-nucleotide “spacer” sequences.

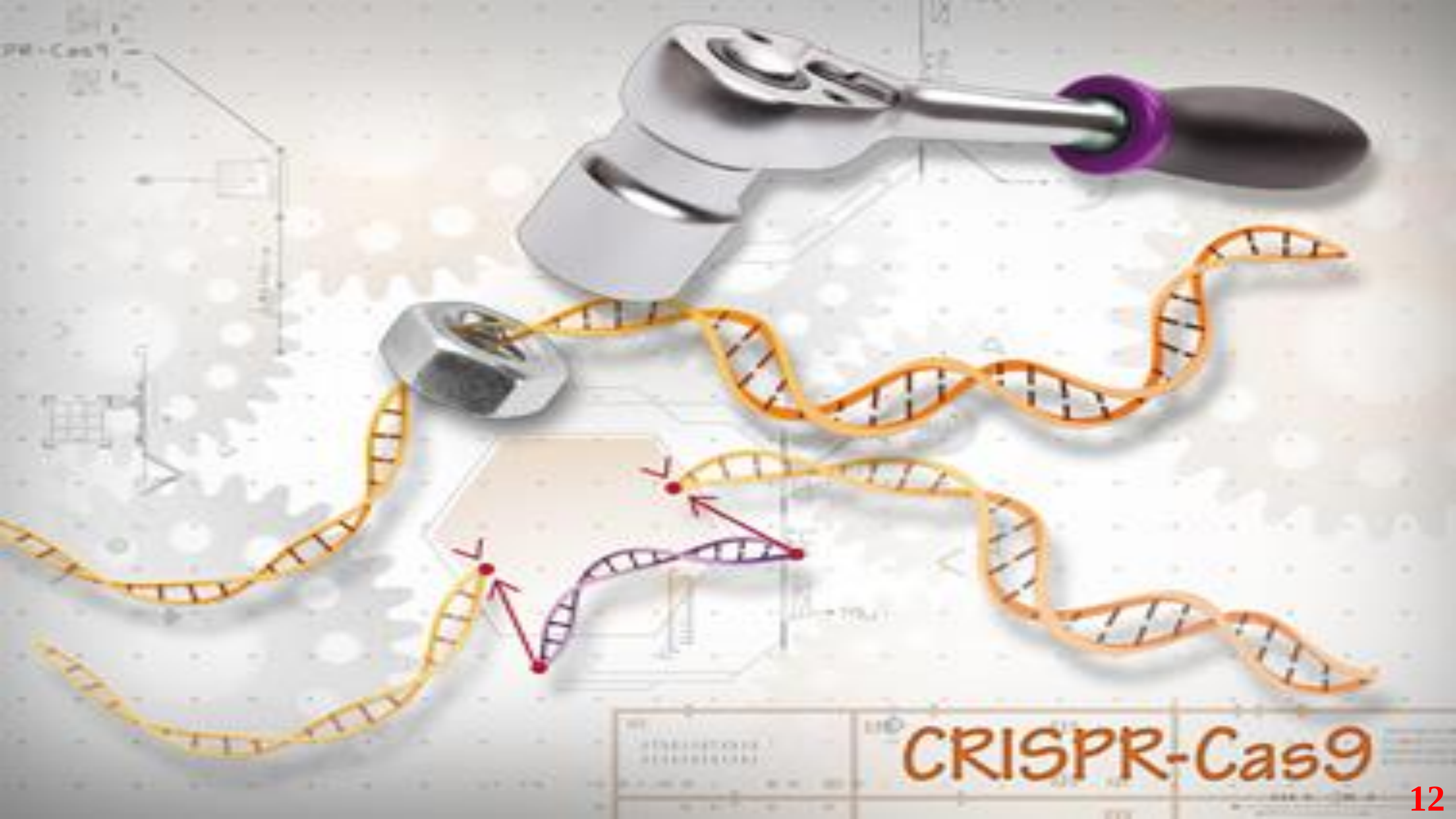


Mechanisms of the acquired immune system mediated by the CRISPR-Cas system



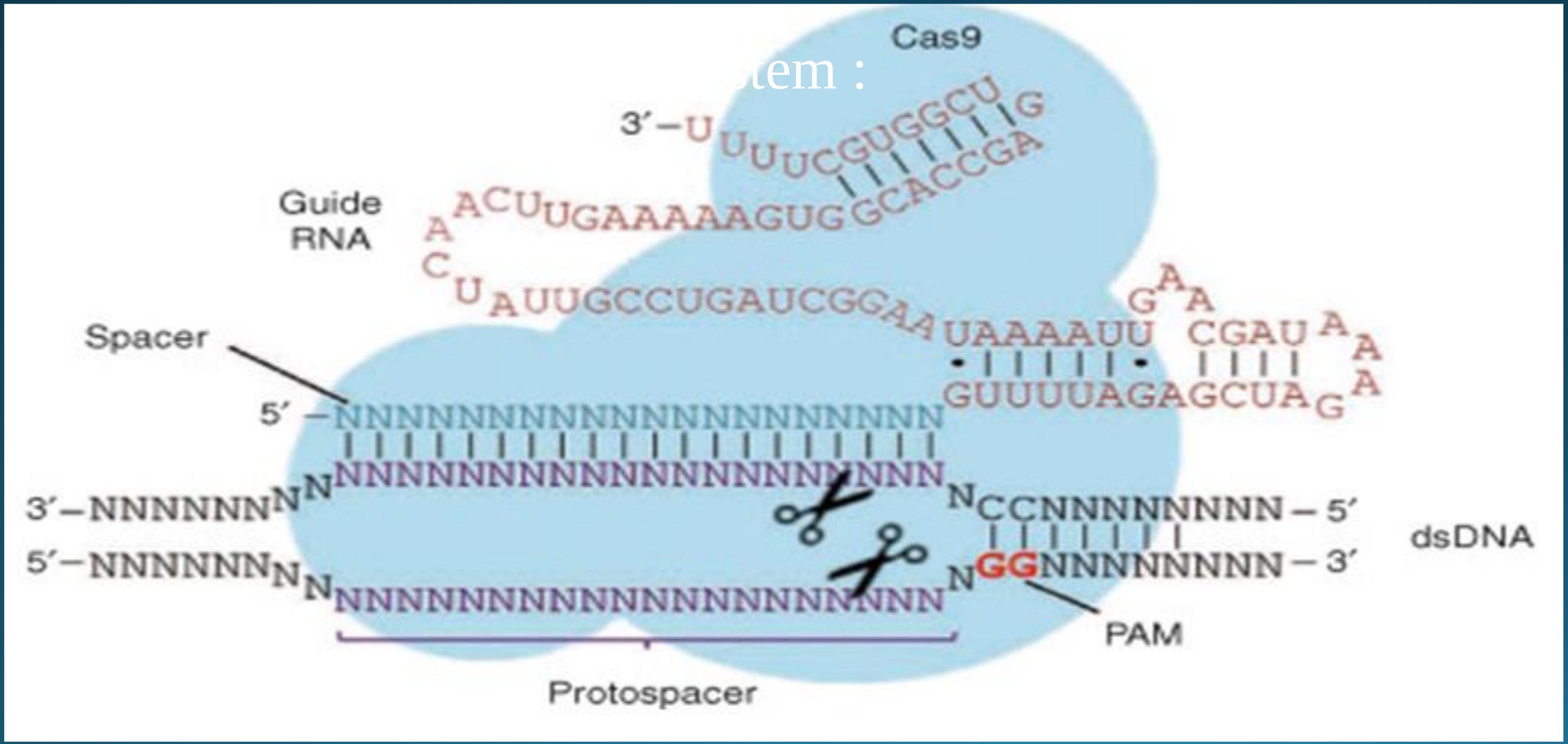
Evolutionary classification of CRISPR–Cas systems




CRISPR-Cas9

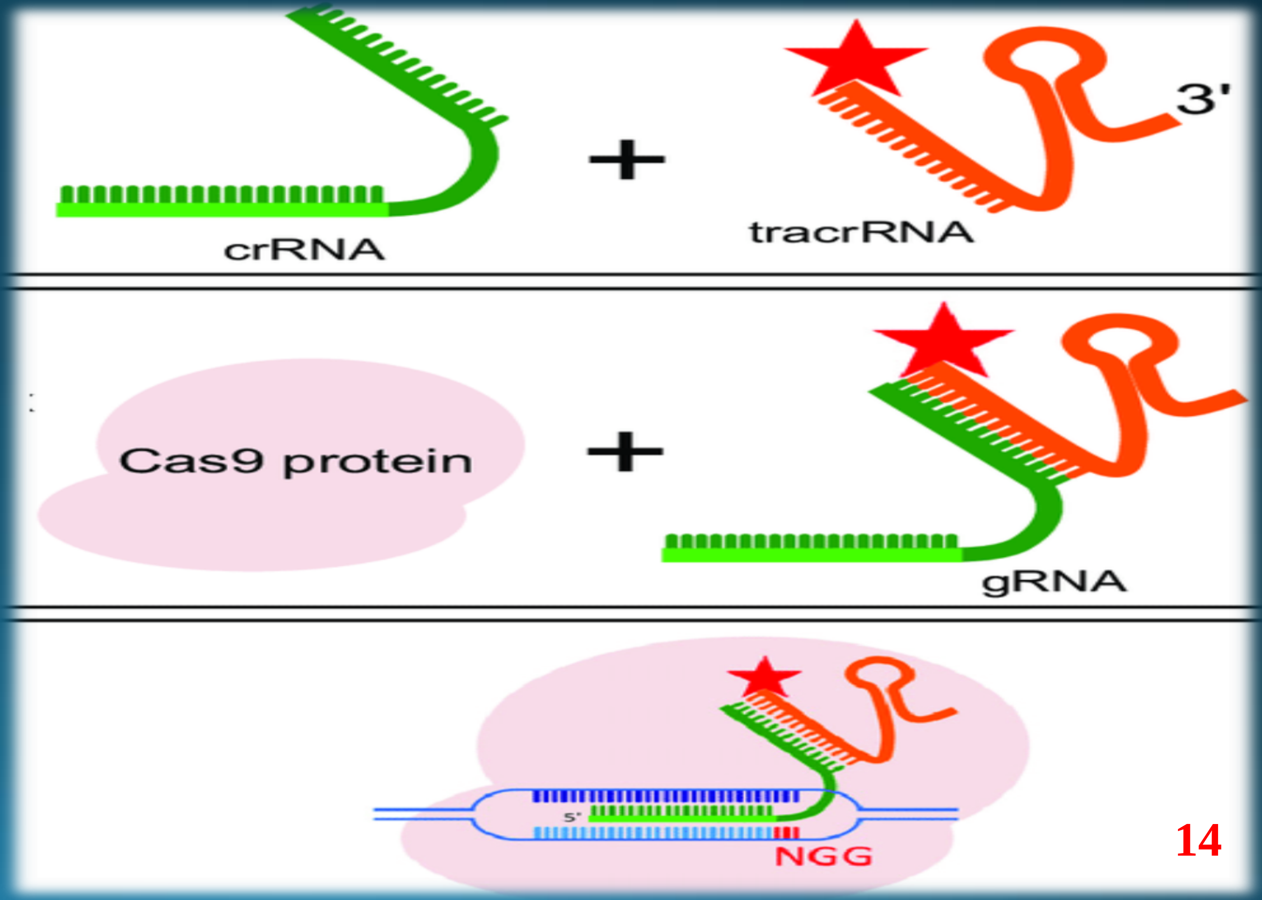
CRISPR-Cas technology

❖ CRISPR-Cas9 system :
1) Cas9 protein (RuvC and HNH)
2) CRISPR RNA (crRNA)
3) Trans-activating crRNA (tracrRNA)



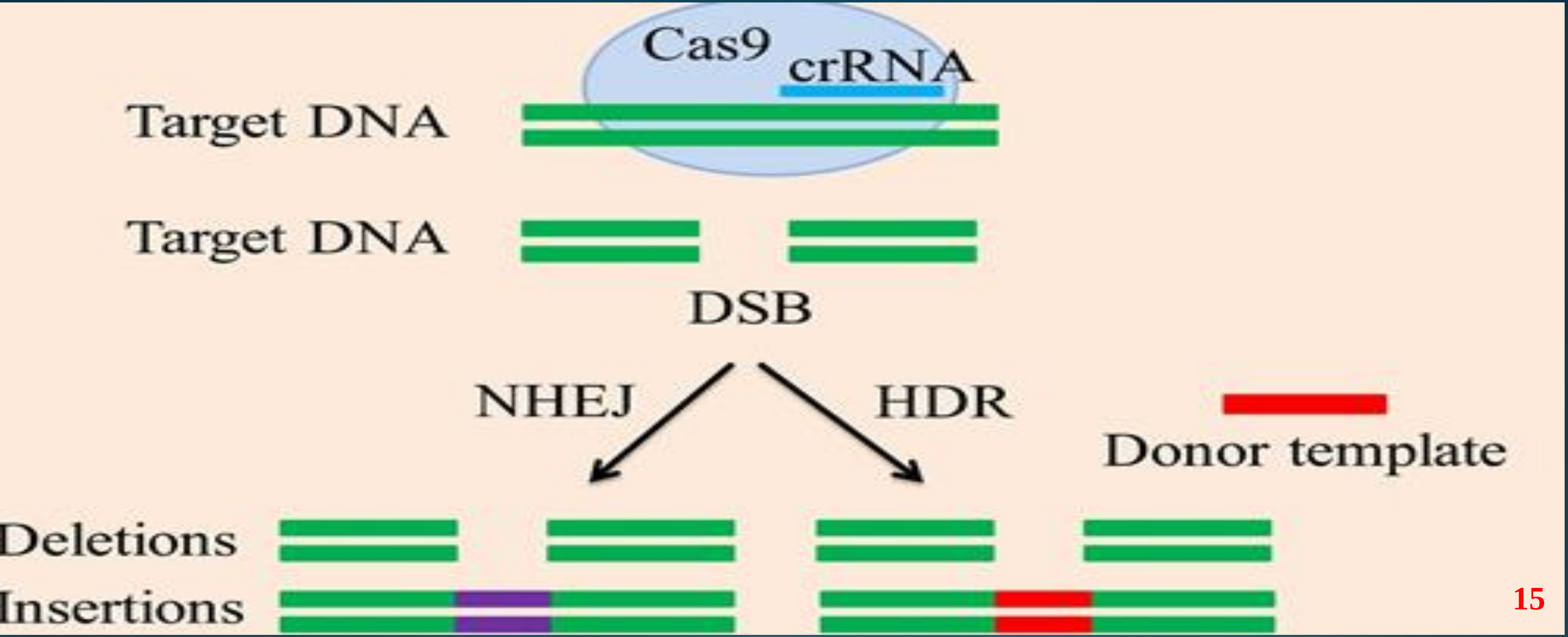
CRISPR-Cas technology

Doudna, Charpentier and colaleagues showed through in vitro DNA cleavage experiments that this system could be reduced to two components by fusion of the crRNA and tracrRNA into a single guide RNA (gRNA).

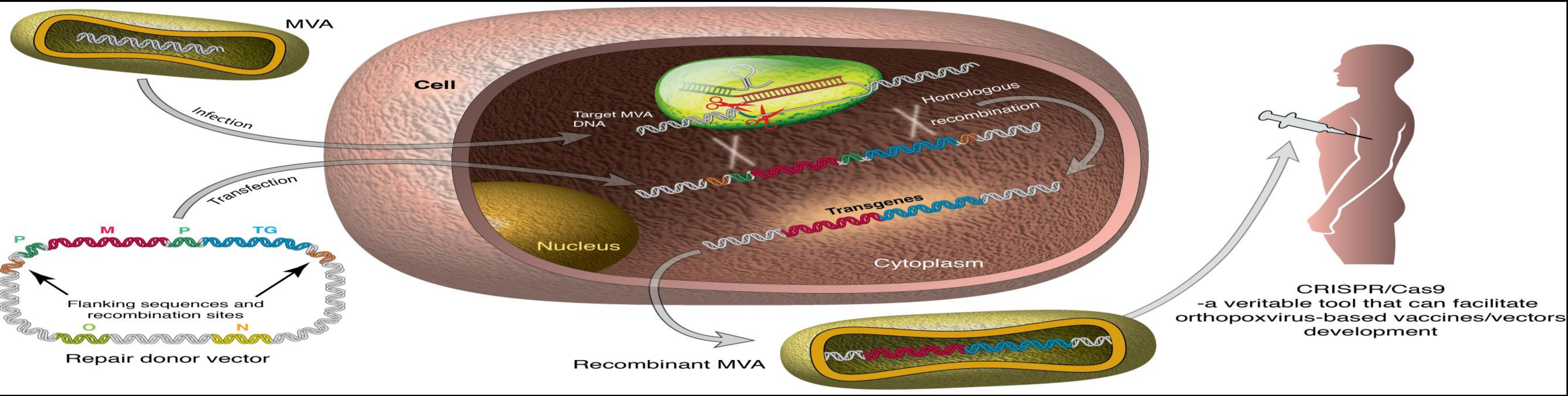


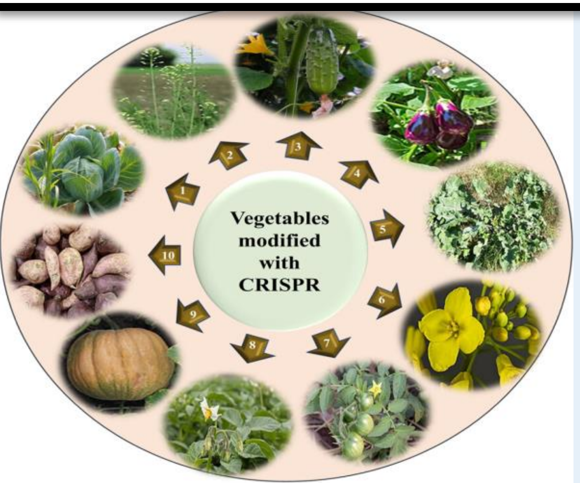
CRISPR-Cas technology

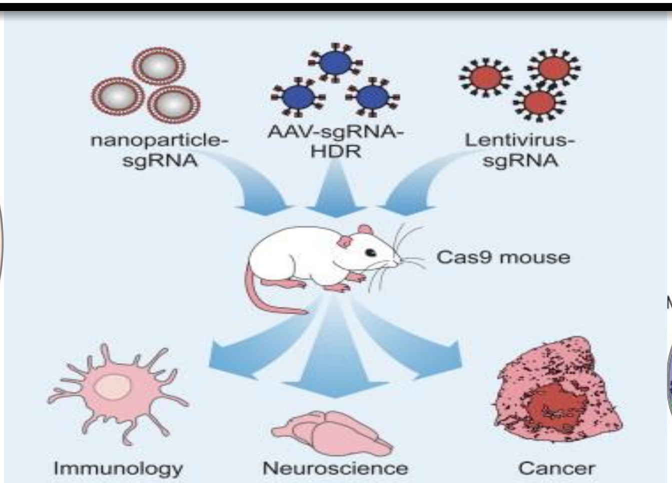
- CRISPR-Cas9 system bases on simple base pairing rules between a specific guide RNA (gRNA) and the target genome site, offers simple yet effective methods of genome editing.

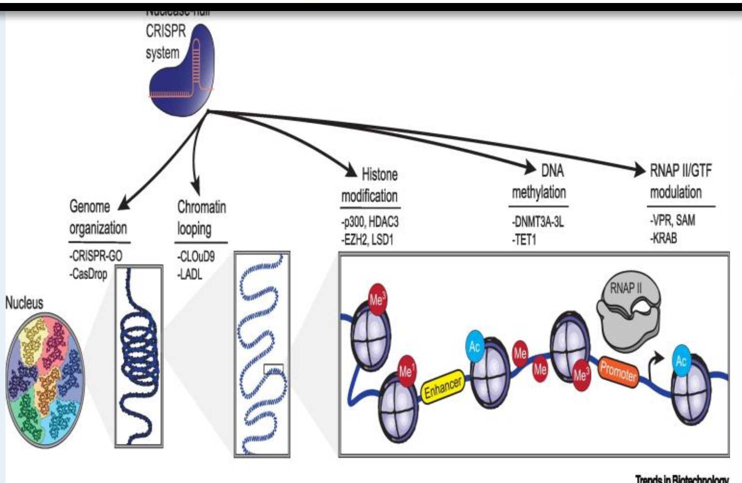


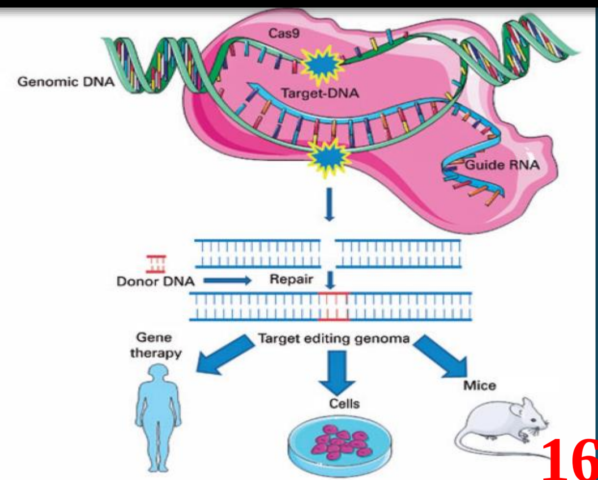
Applications of crispr-cas9 technology

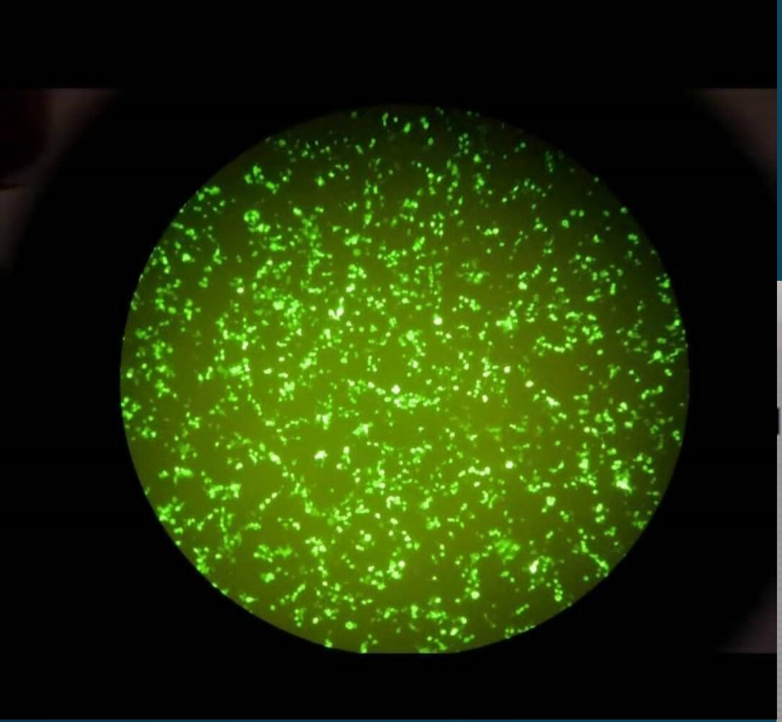


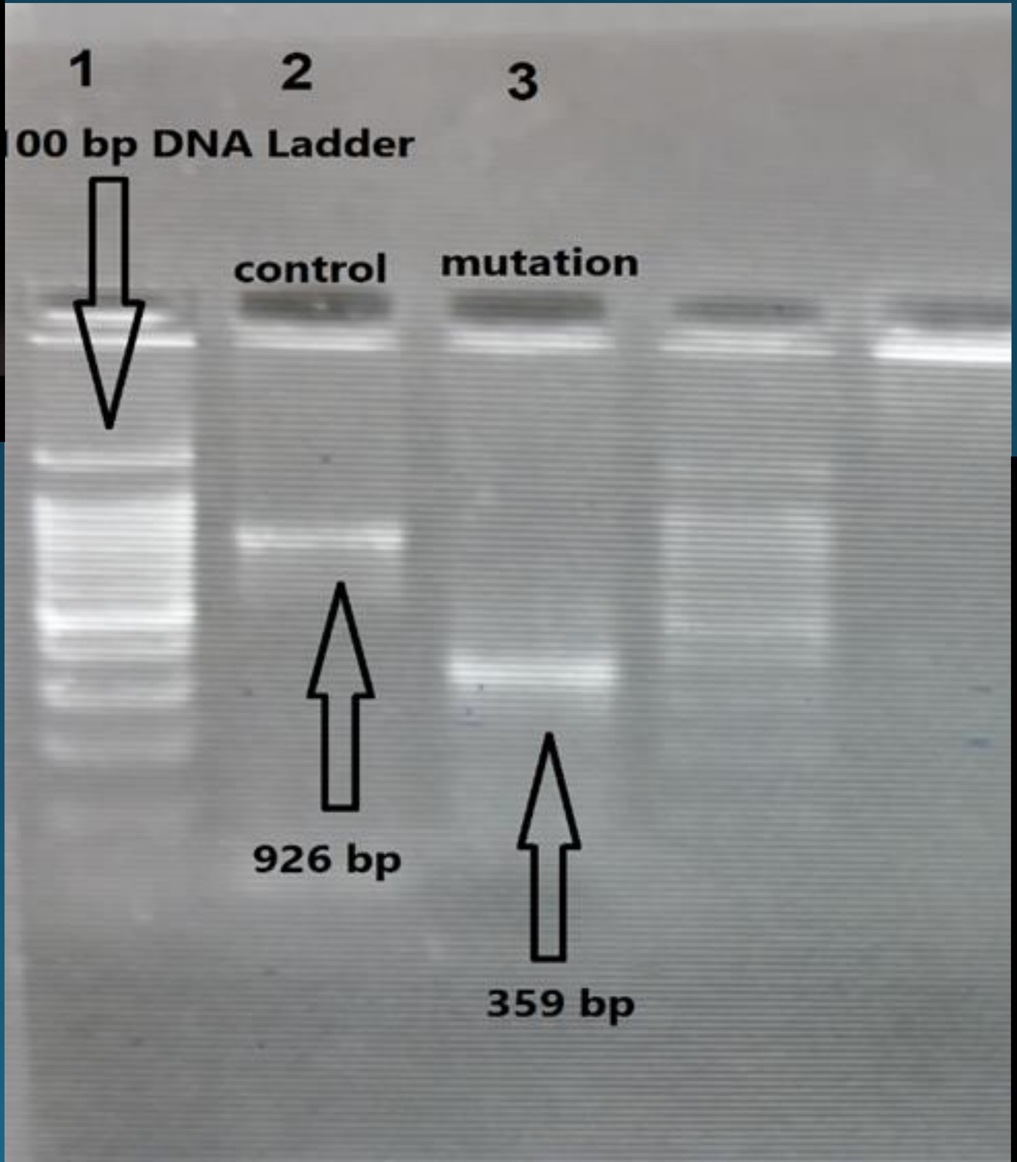


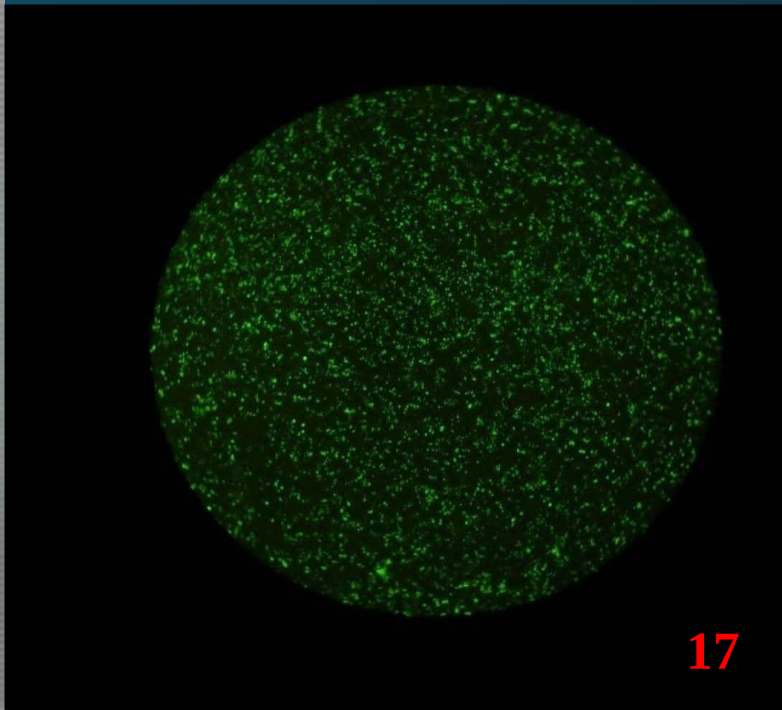









1
2
3
100 bp DNA Ladder
control
mutation
926 bp
359 bp



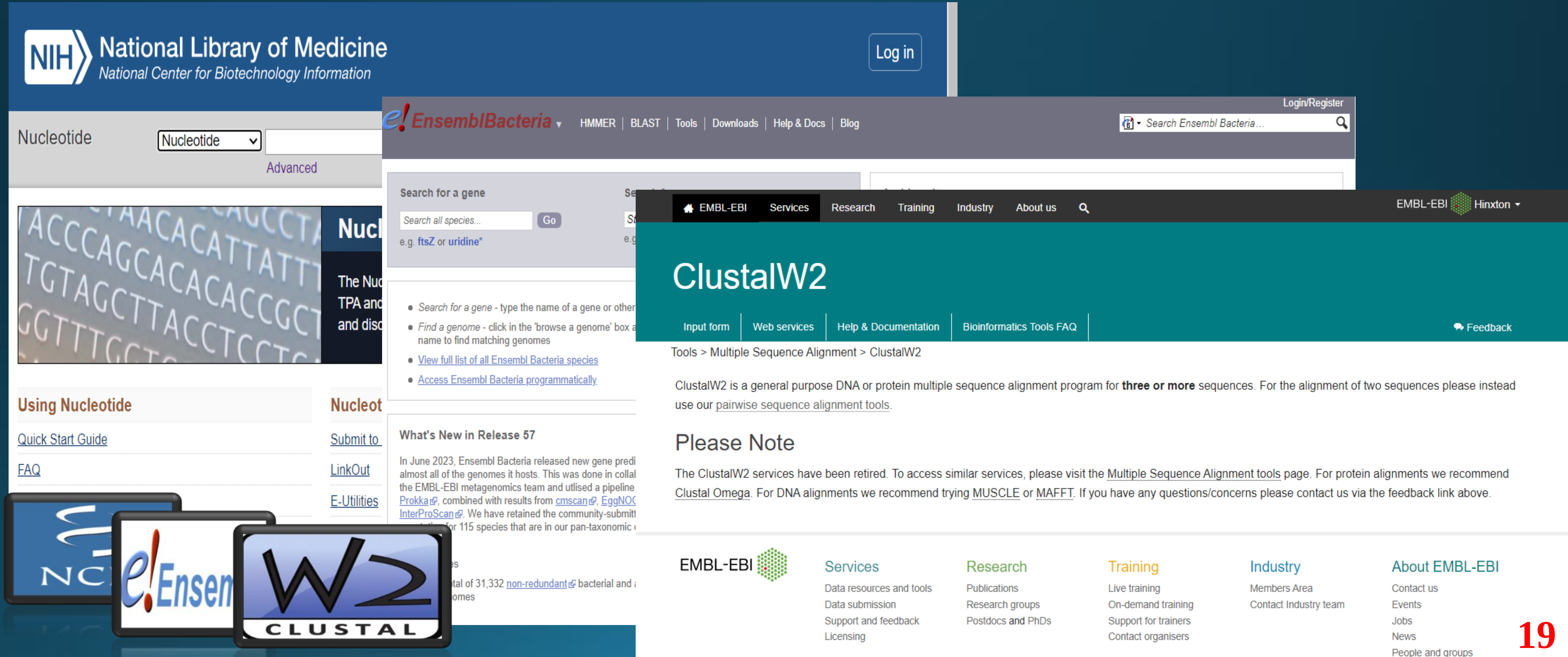
Using the CRISPR/Cas9 technology to attenuate virulence factors and pathogenicity of bacteria

1- CRISPR-Cas Bioinformatics

2- CRISPR-Cas Experiments

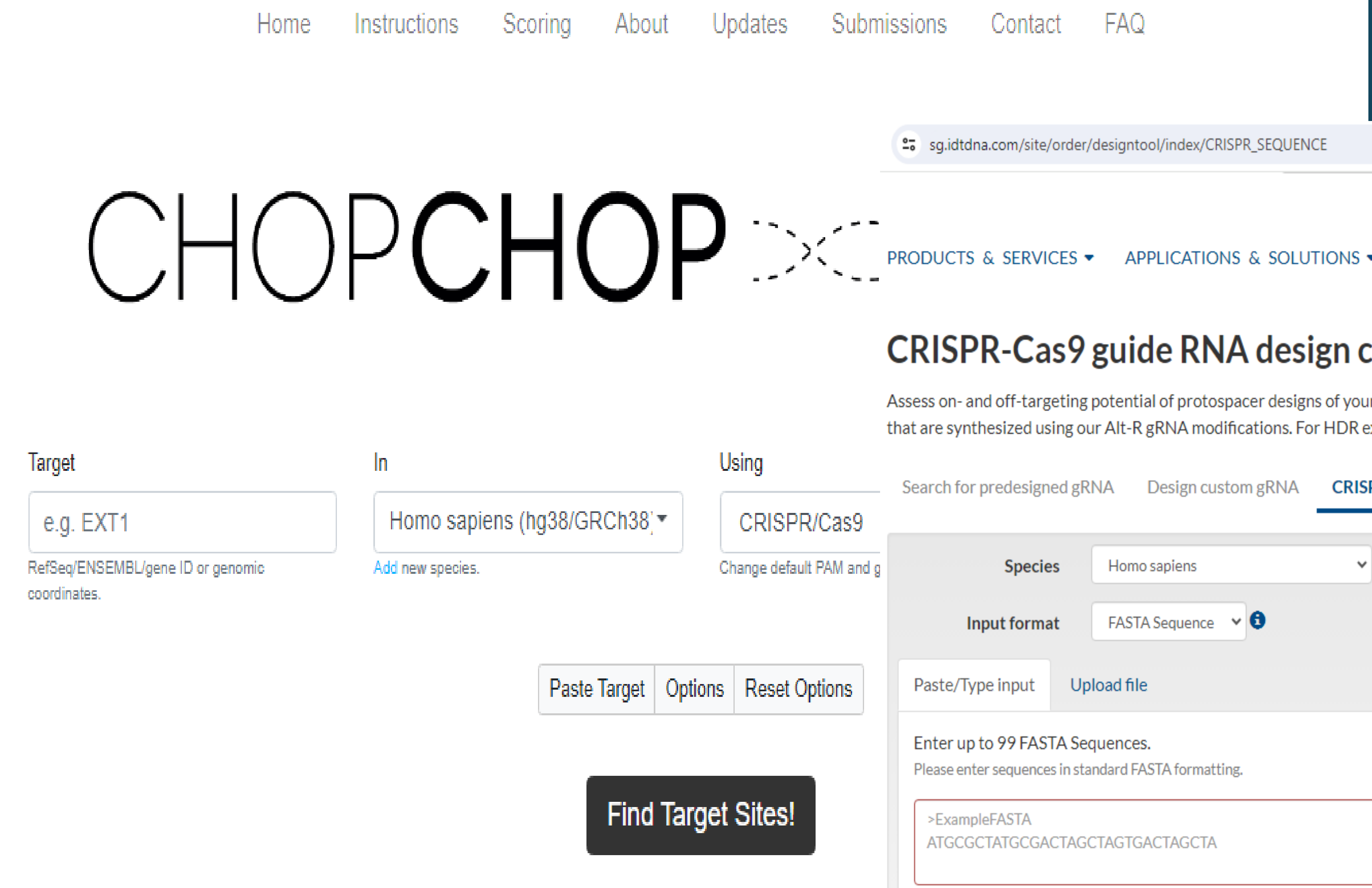
CRISPR-Cas bioinformatics

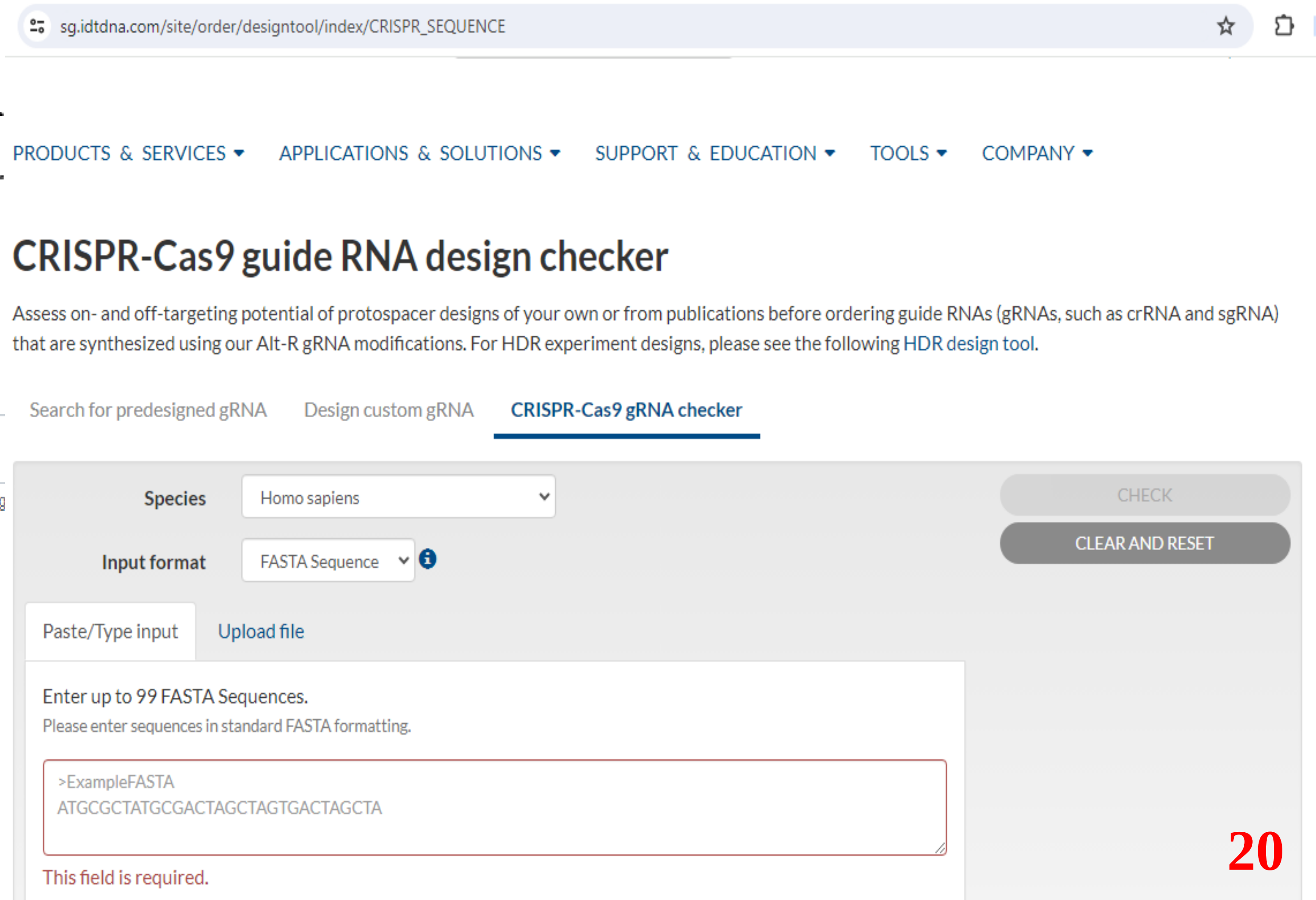
Step 1: Obtaining the required gene sequence



CRISPR-Cas bioinformatics

Step 2: gRNA Oligonucleotide Design





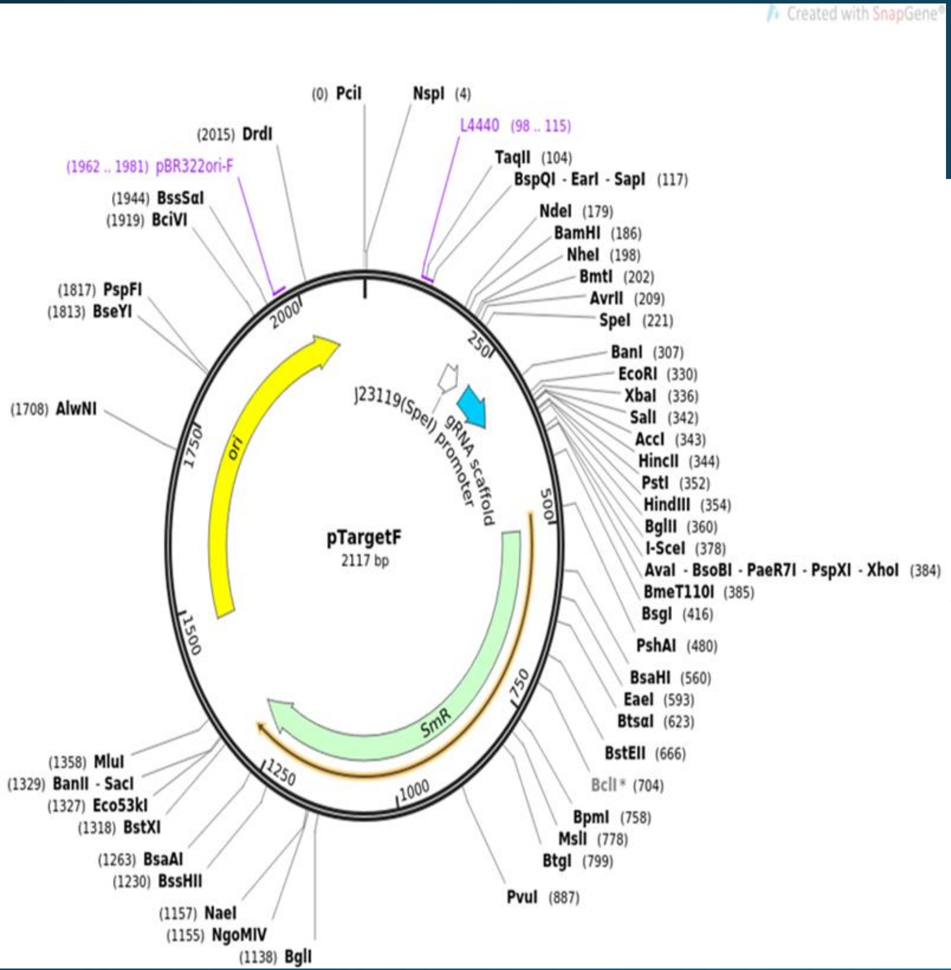
CRISPR-Cas bioinformatics

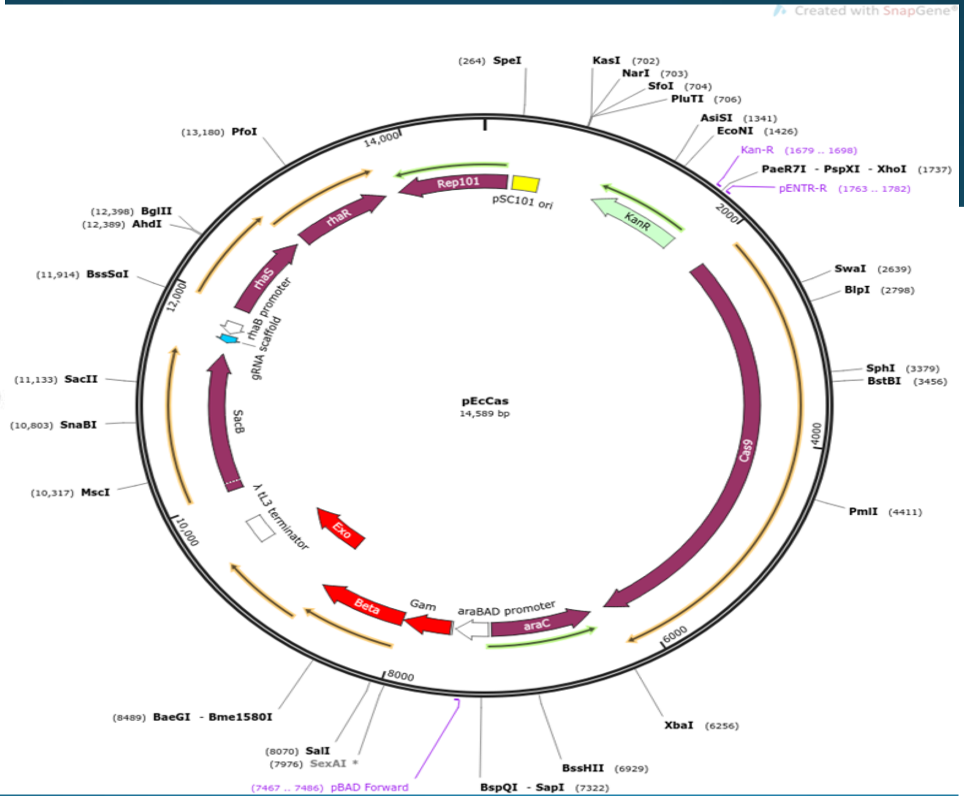
Step 3: Primer Design

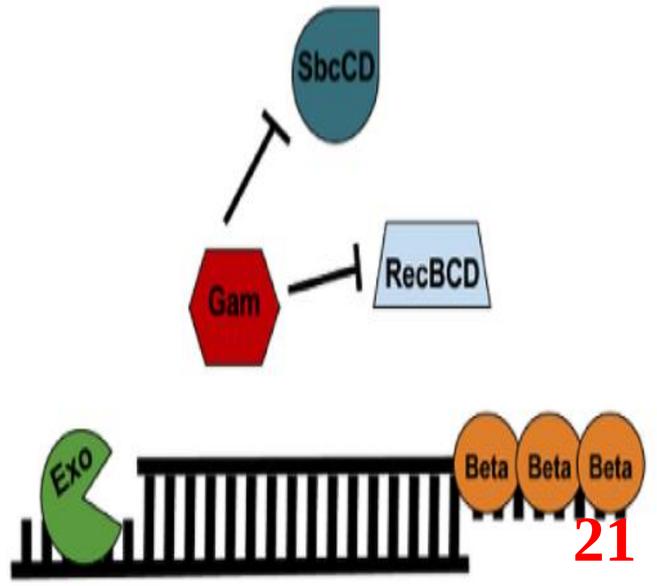
1-gRNA expressing plasmid

1- Confirmation of Positive Clones by colony PCR (Plasmid backbone)

2-Cas9-expressing plasmid

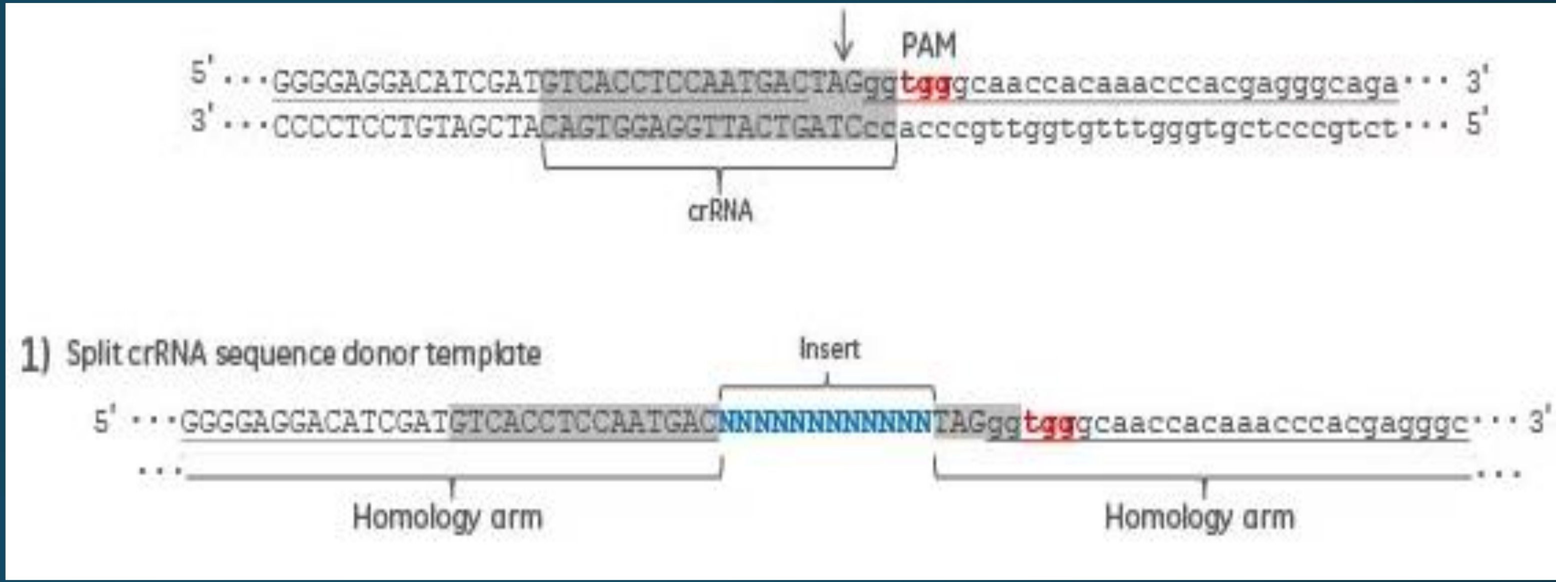






Step 3: *Primer Design*

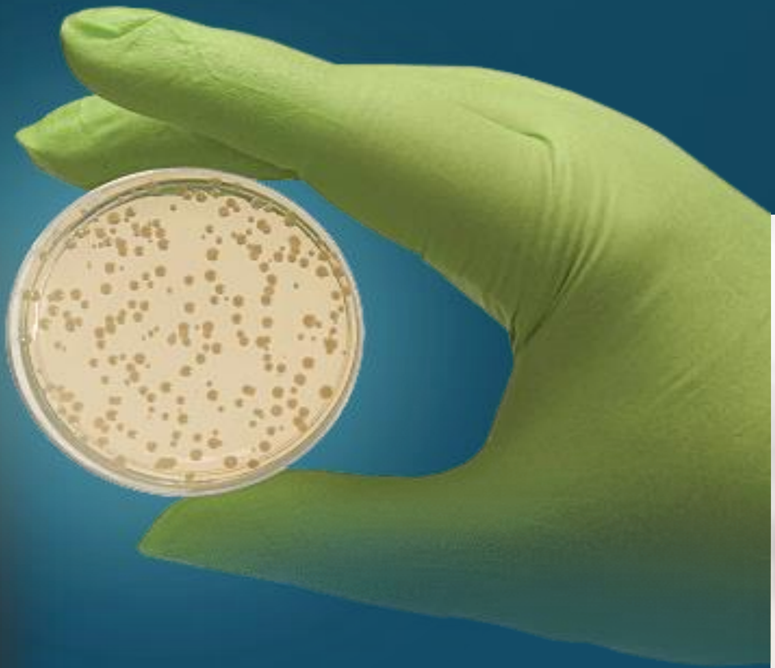
2- Construction of HDR oligo fragment



3- Confirmation of the recombination

4- Confirmation of the Sequencing

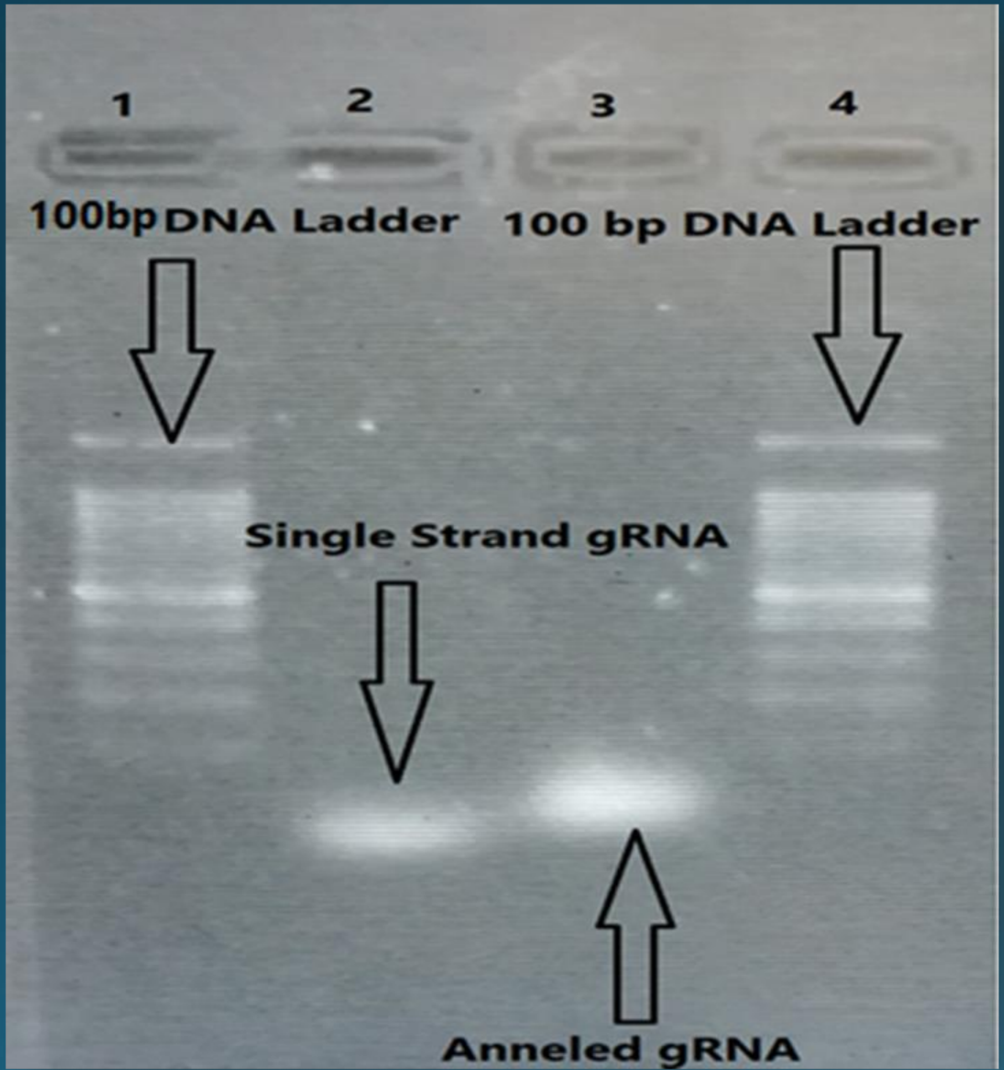
CRISPR-Cas Experiments



CRISPR-Cas Experiments

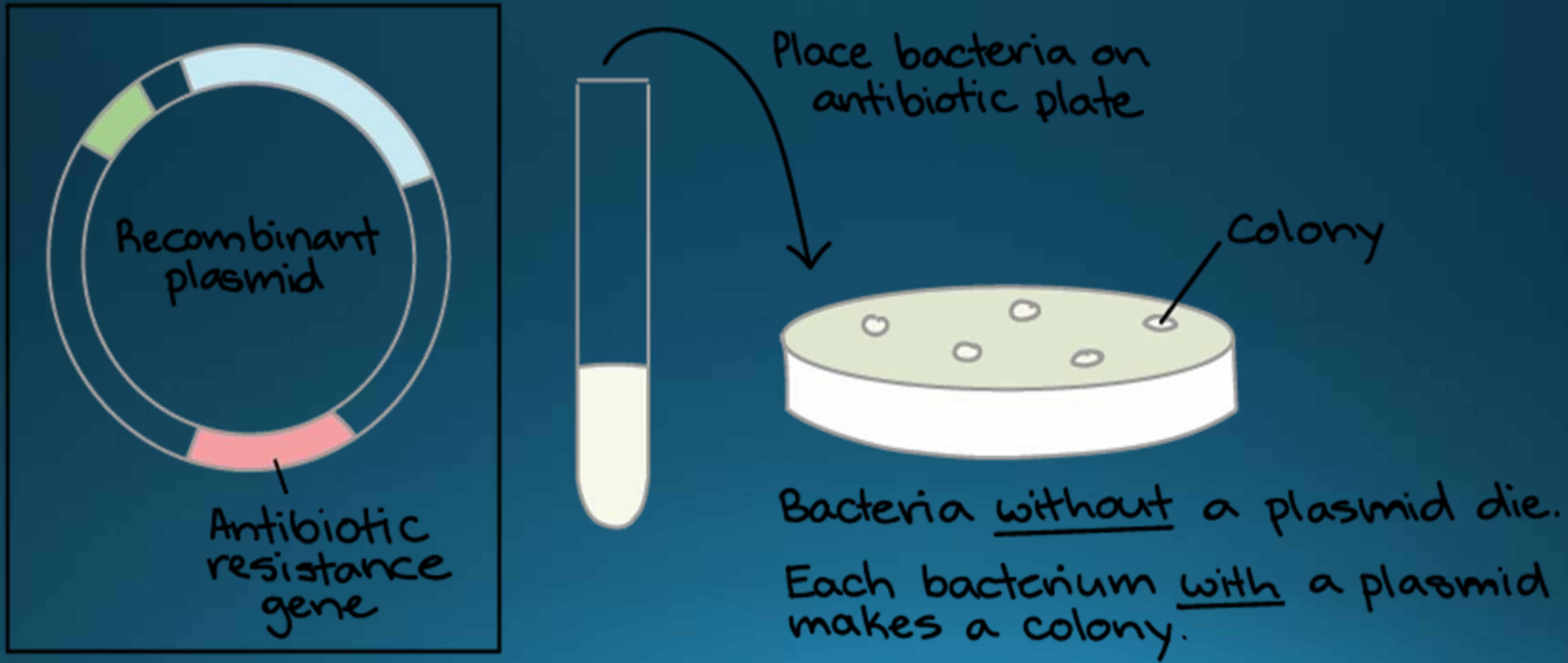
Step 1: gRNA oligo annealing

- Anneal two single-stranded DNA oligonucleotides to produce double-stranded DNA fragments.

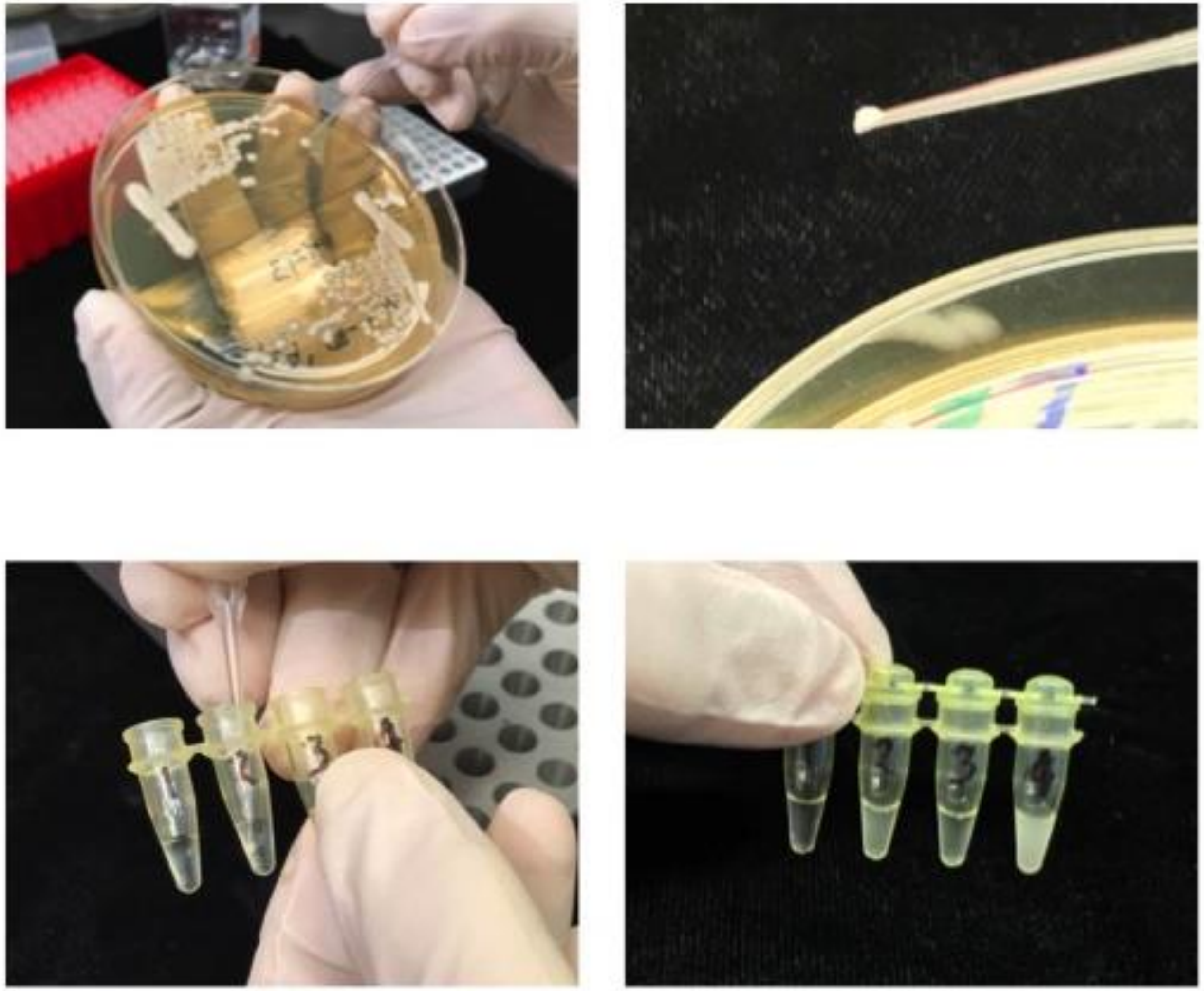


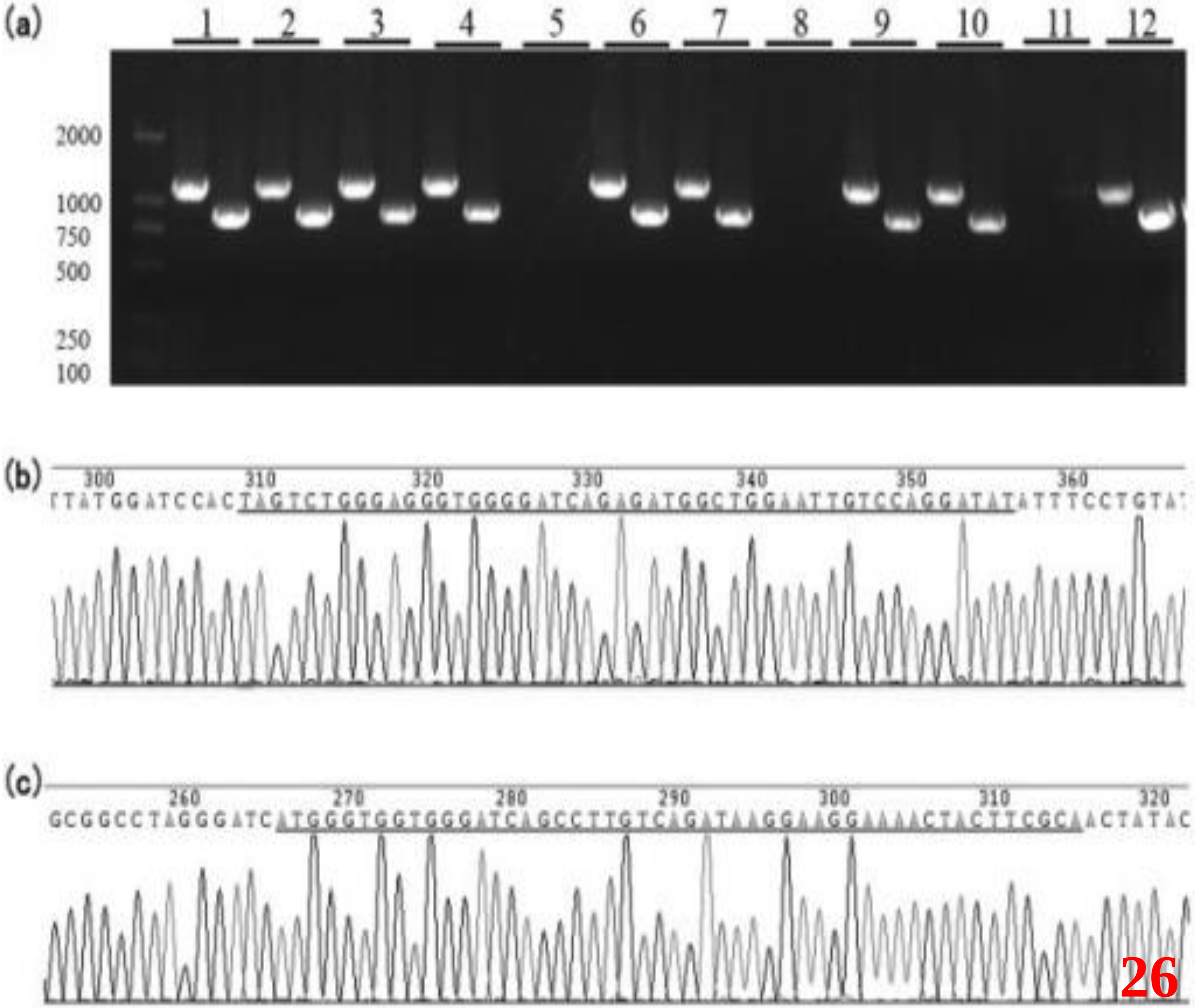
CRISPR-Cas Experiments

Step 2: Transformation



Step 3: Confirmation of Positive Clones by colony PCR and Sequencing

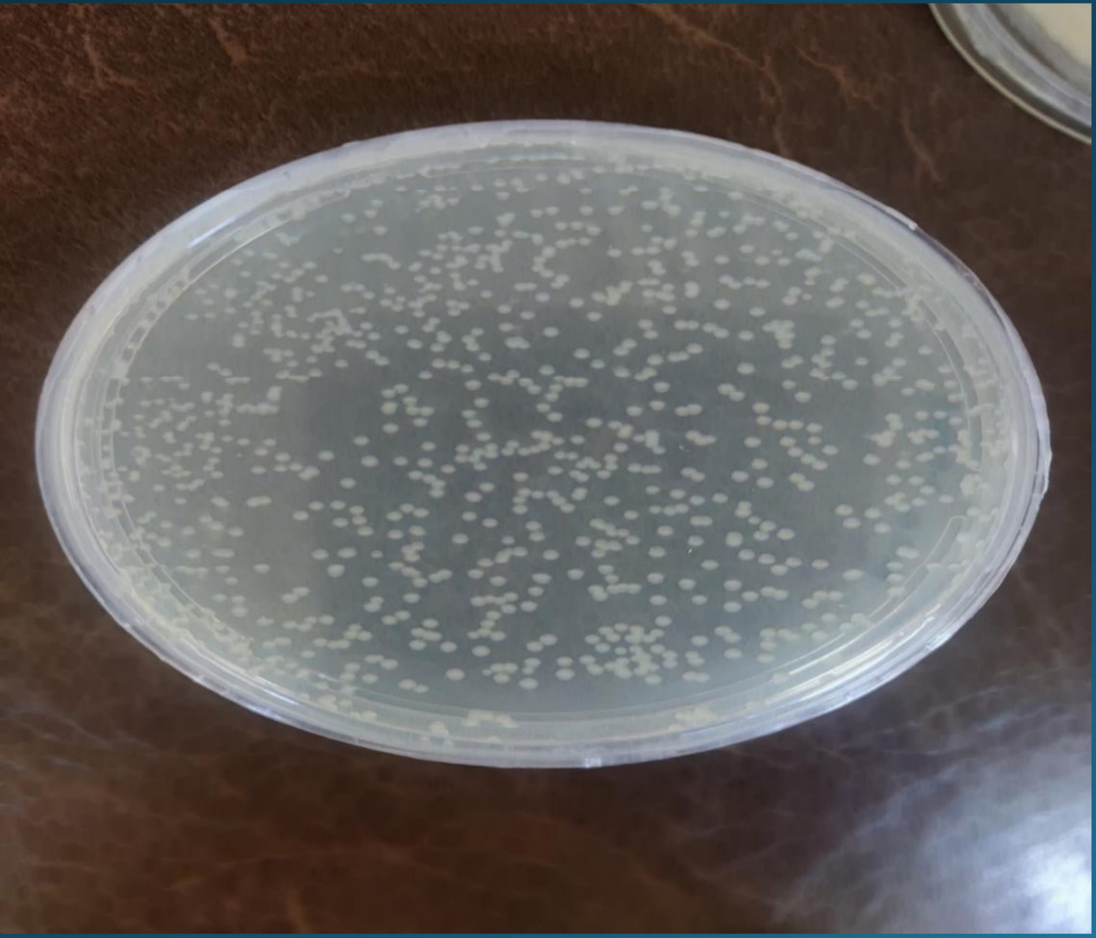


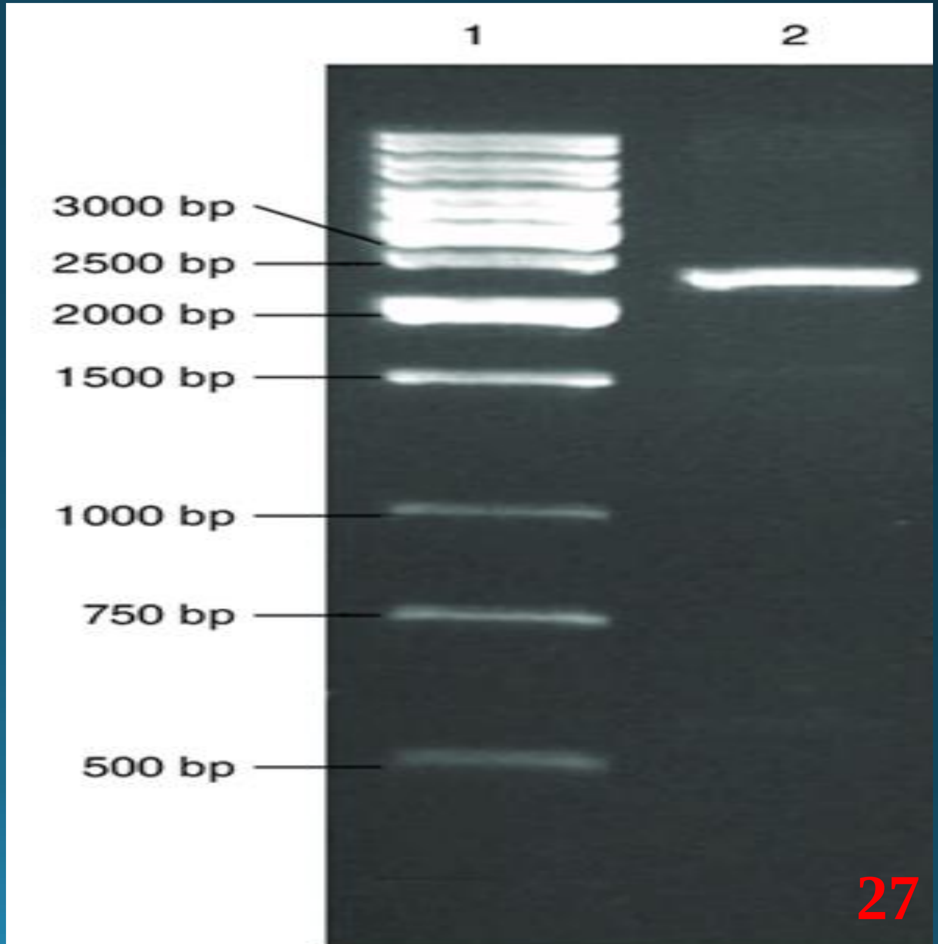


CRISPR-Cas Experiments

Step 4: Introducing cas9 plasmid into bacterial cells through electroporation
Confirmation of Positive Clones by colony PCR

Generation of bacteria carrying pCas9





CRISPR-Cas Experiments

Step 5: induction with arabinose and Preparation of competent cell

CRISPR-Cas Experiments

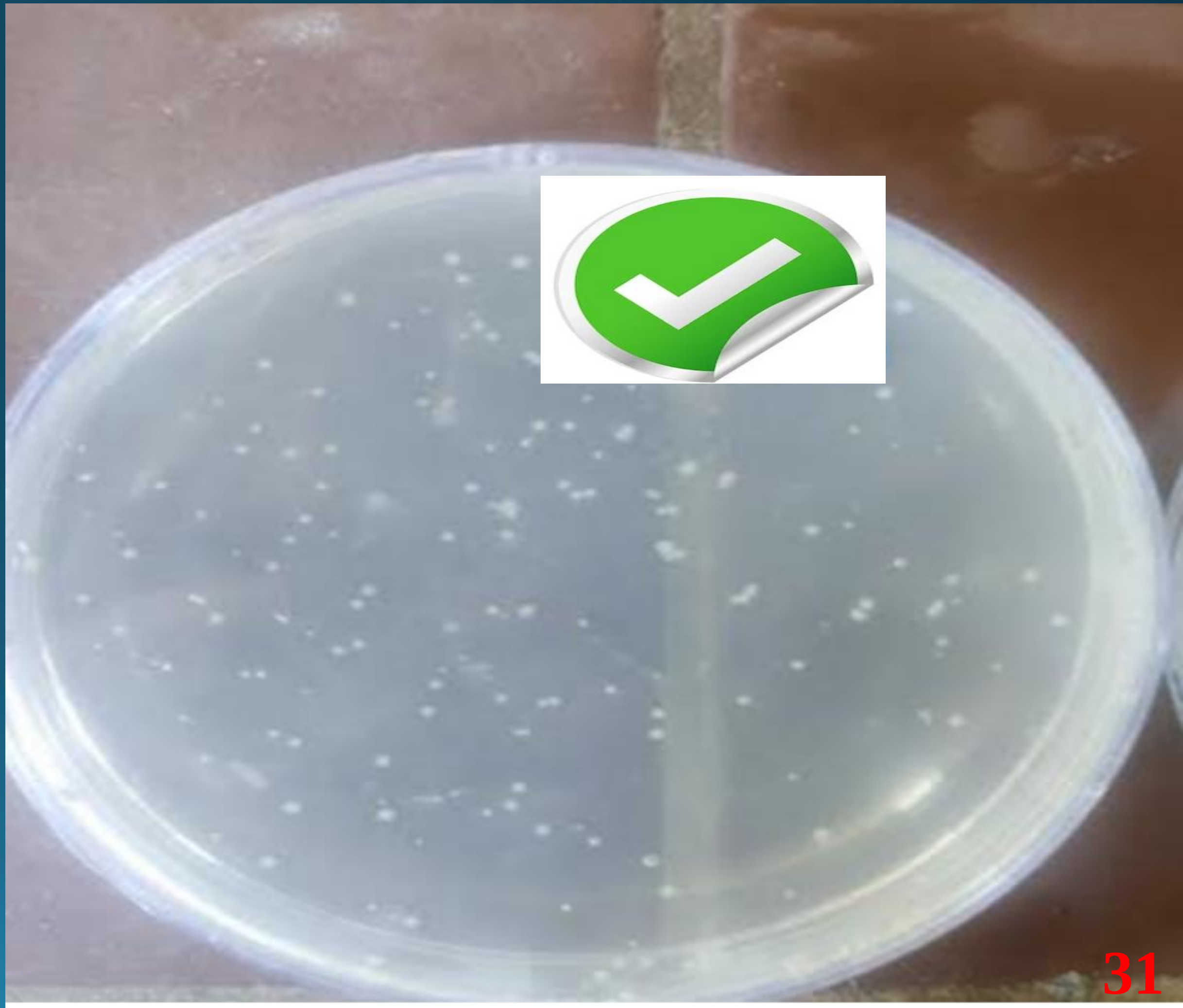
Step 6: Construction of HDR oligo fragment

overlap extension pcr

CRISPR-Cas Experiments

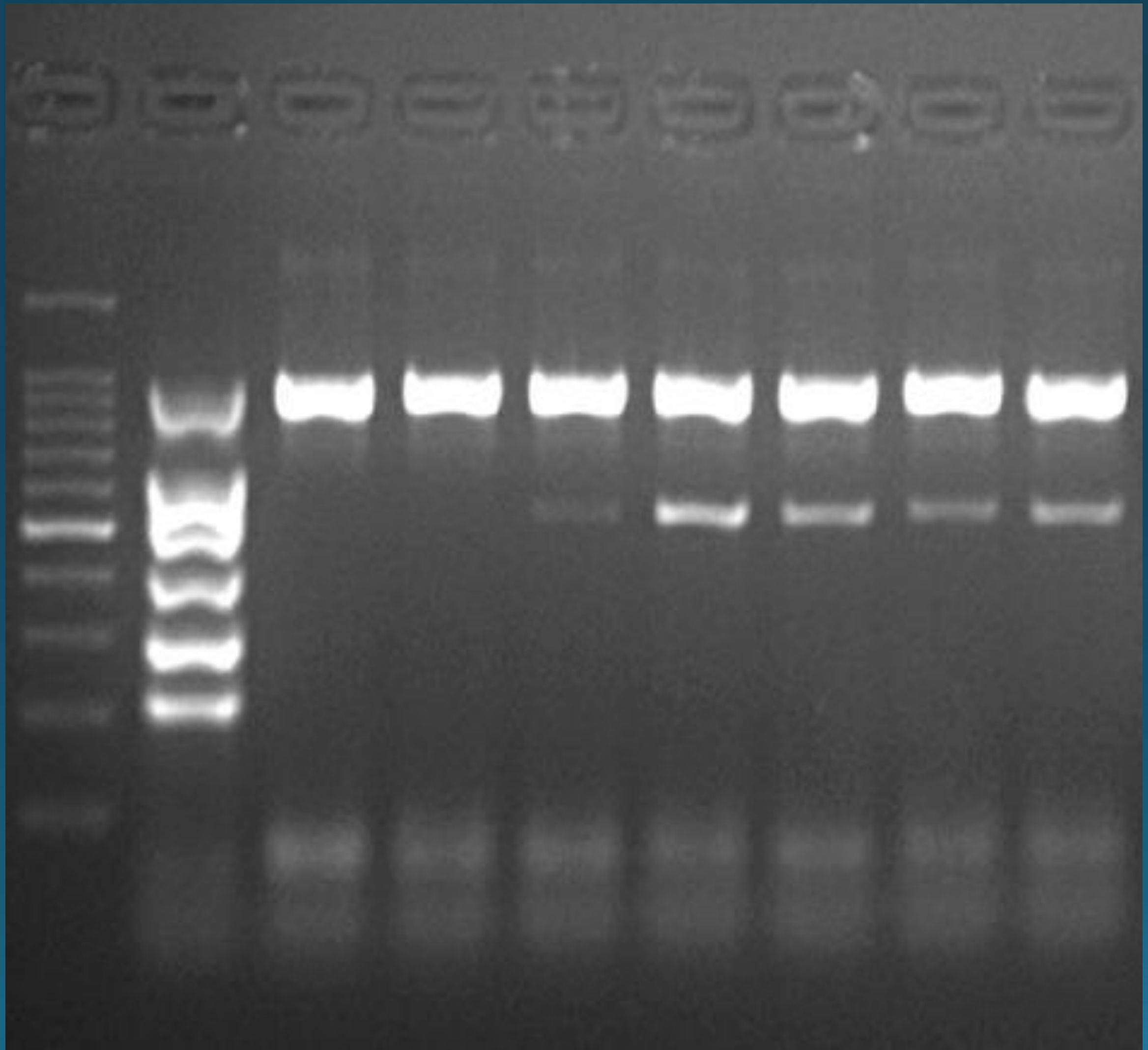
Step7: Transfer of sgRNA plasmid and HDR fragment to Cas9 Competent Cells

CRISPR-Cas Experiments



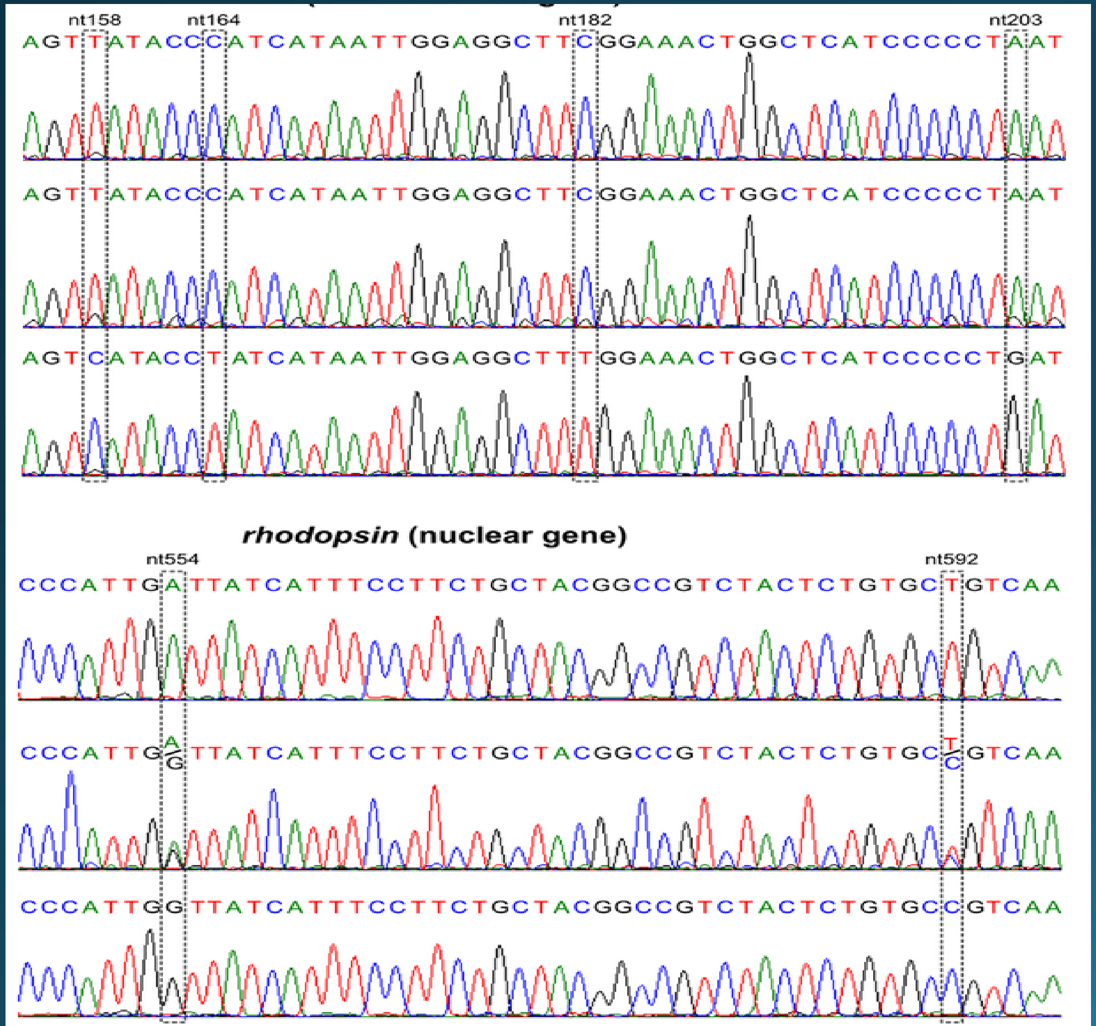
CRISPR-Cas Experiments

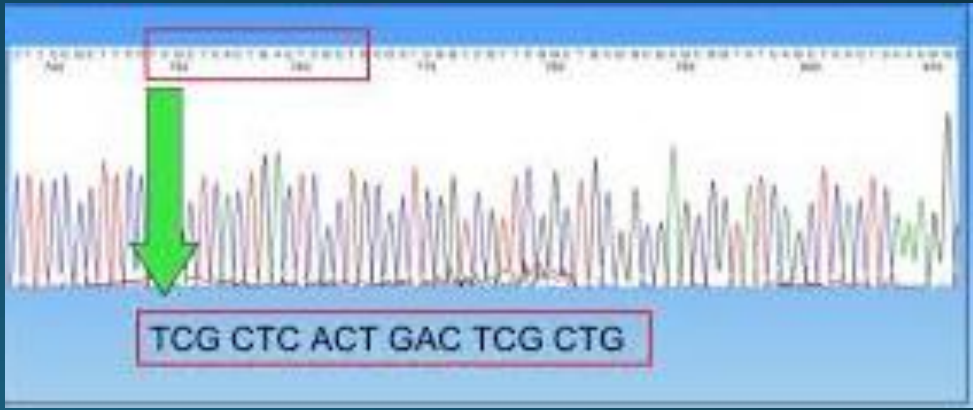
Step8: Screening of mutant strains by colony PCR



CRISPR-Cas Experiments

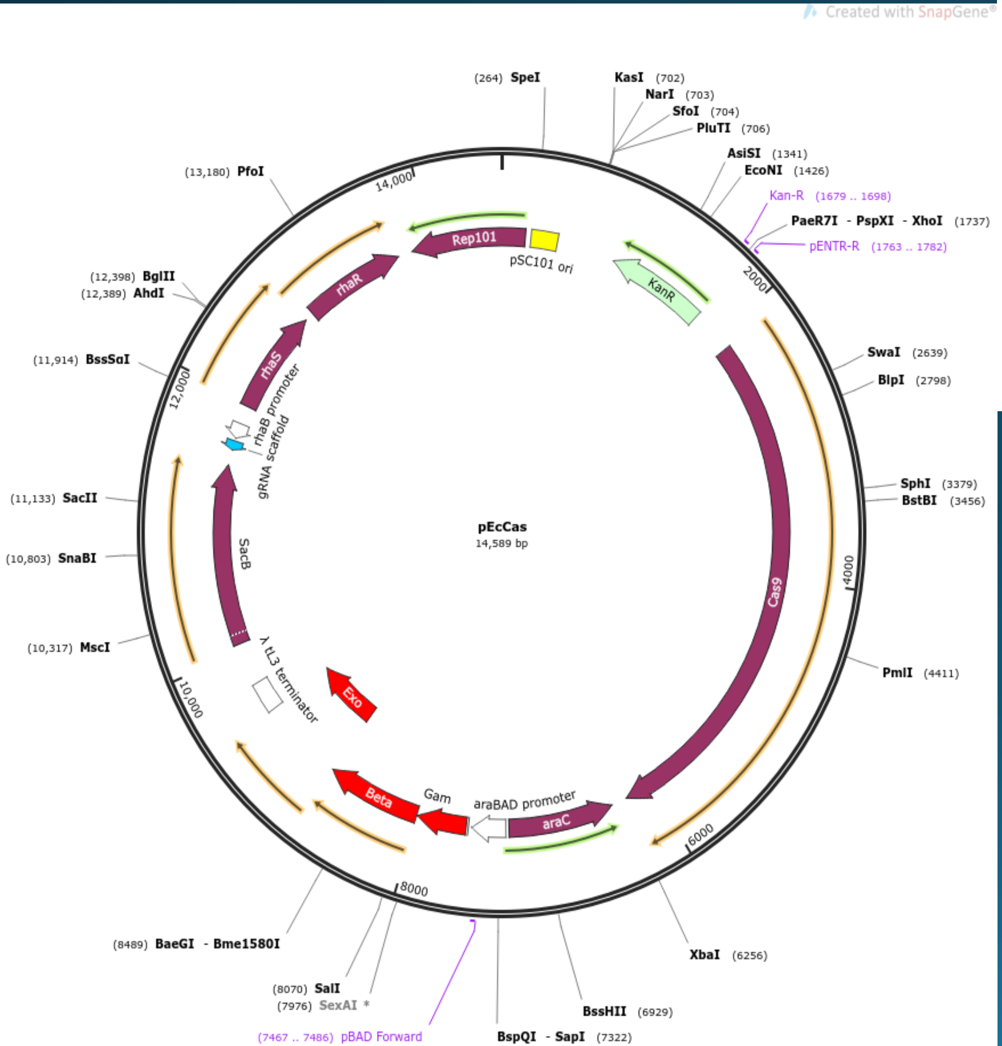
Step9: Analysis of modified bacterial genetic structures

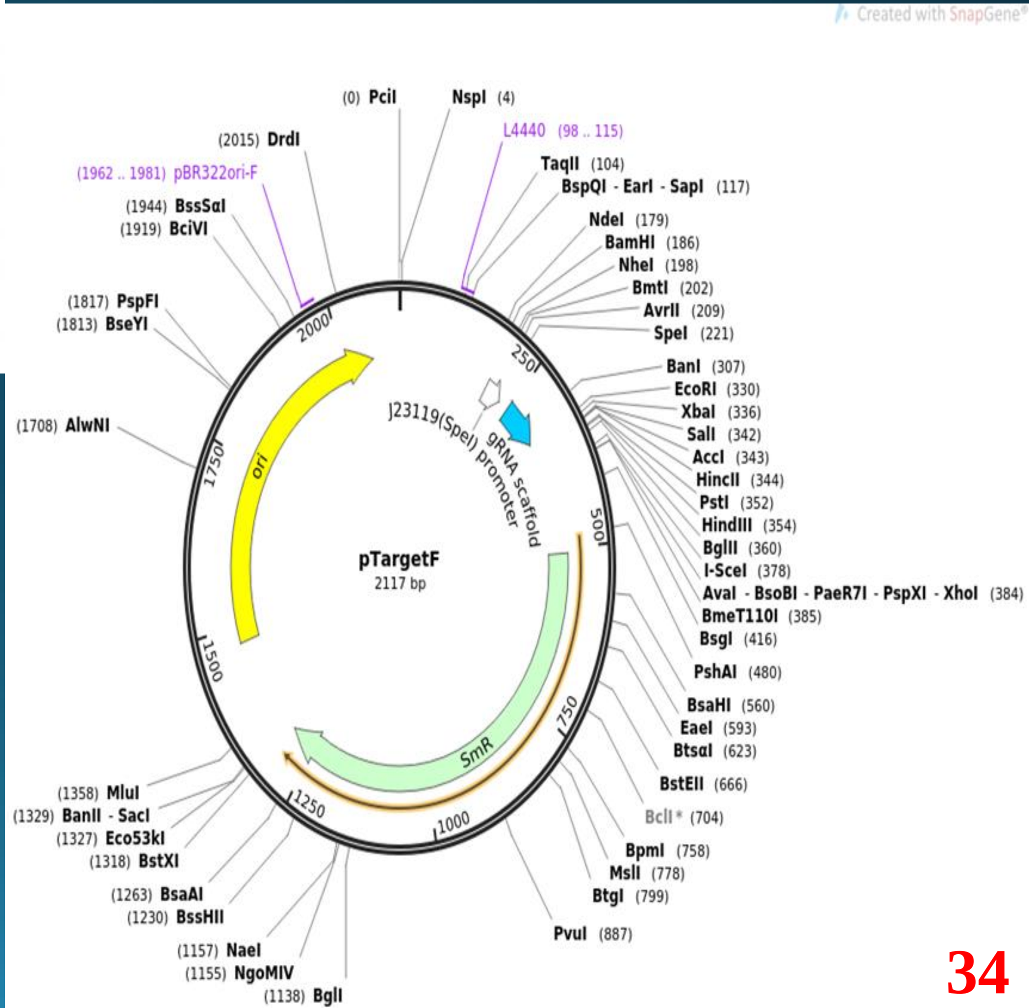




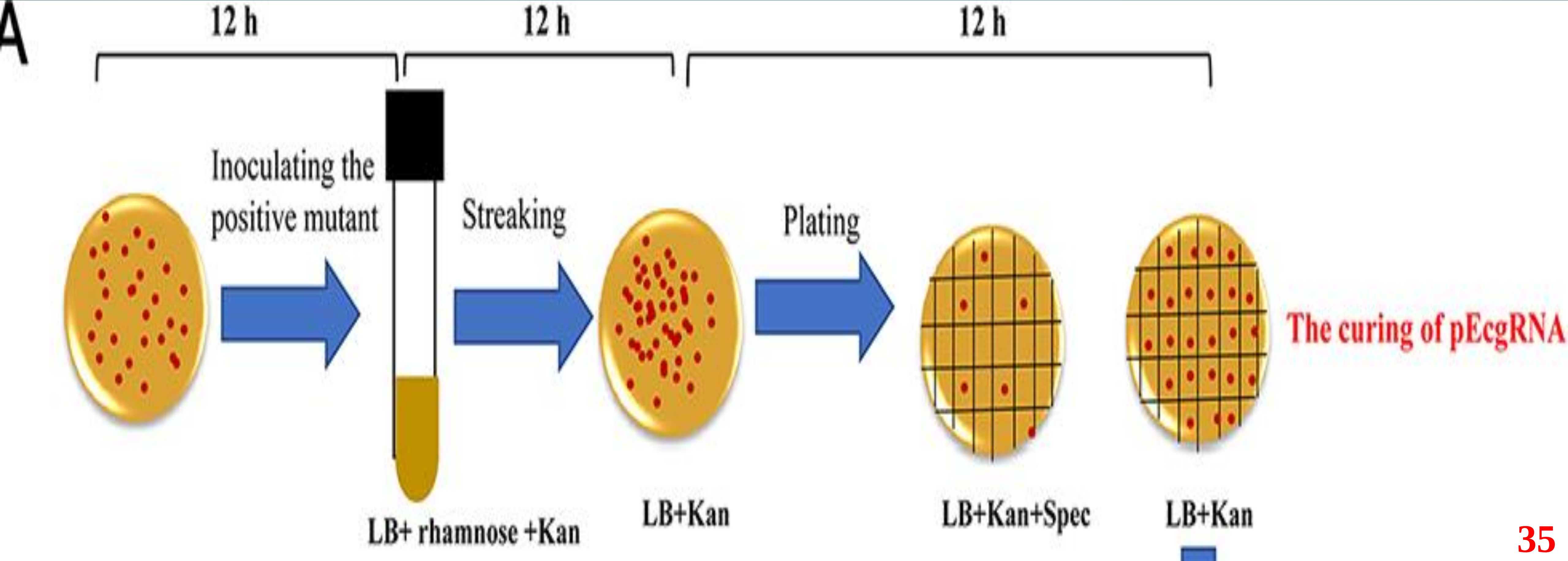
CRISPR-Cas Experiments

Step10: Plasmid curing





Step11: Replica plating



CRISPR-Cas Experiments

***Step 12**: induction with arabinose and Preparation of competent cell*

***Step13**: Screening of mutant strains by colony PCR*

***Step14**: Analysis of modified bacterial genetic structures*

Additional Tests

Western blot

Cellular assays

in vivo test

Conclusion and future perspectives

Through the implementation of the CRISPR Cas9 strategy, the modified bacteria can find applications in various research contexts. This includes potential use as a live-attenuated vaccine candidate, exploration of the impact of the target gene on bacterial pathogenicity, assessment of the gene's influence on bacterial morphology, and more. This strategy opens avenues for studying the functionality of the bacterium genes and even holds the potential for the creation of novel industrial strains.

Thanks for your attention