CRISPR CAS9 and Gene Editing

for Ph.D students - Medical Students

(Foggia University)

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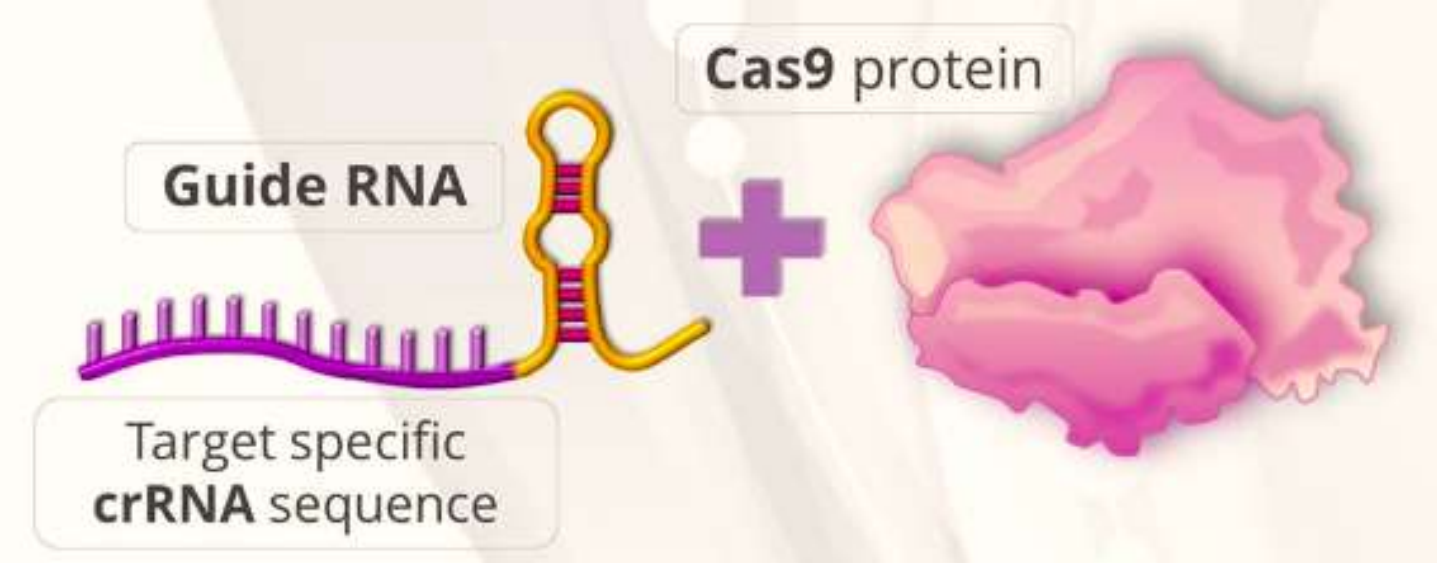
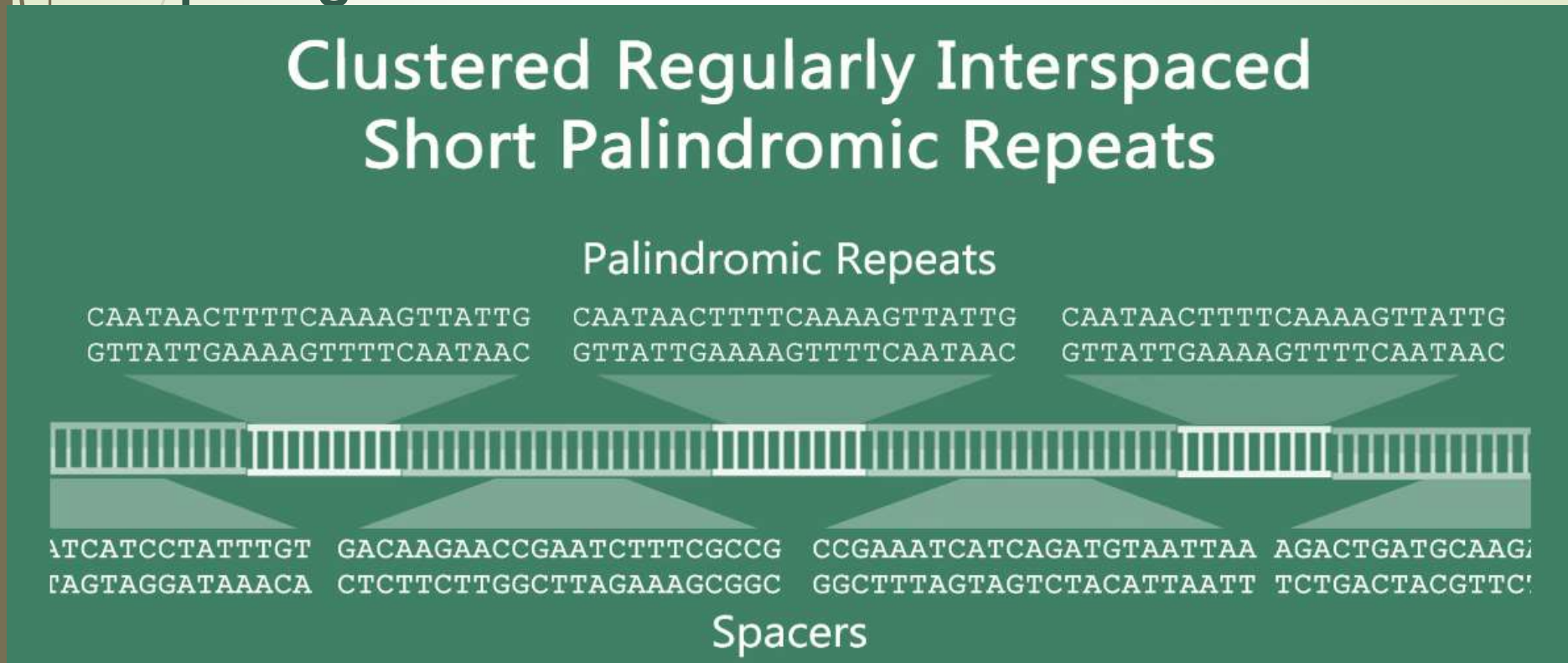


Objective of this Lecture:

1. Knowing the discovery of CRISPR-Cas9 and how it works, and the idea of applying it.
2. Importance of the use of CRISPR CAS9 in medical applications while in vitro and in vivo
3. To know some uses of CRISPR CAS9 in Gene Therapy.

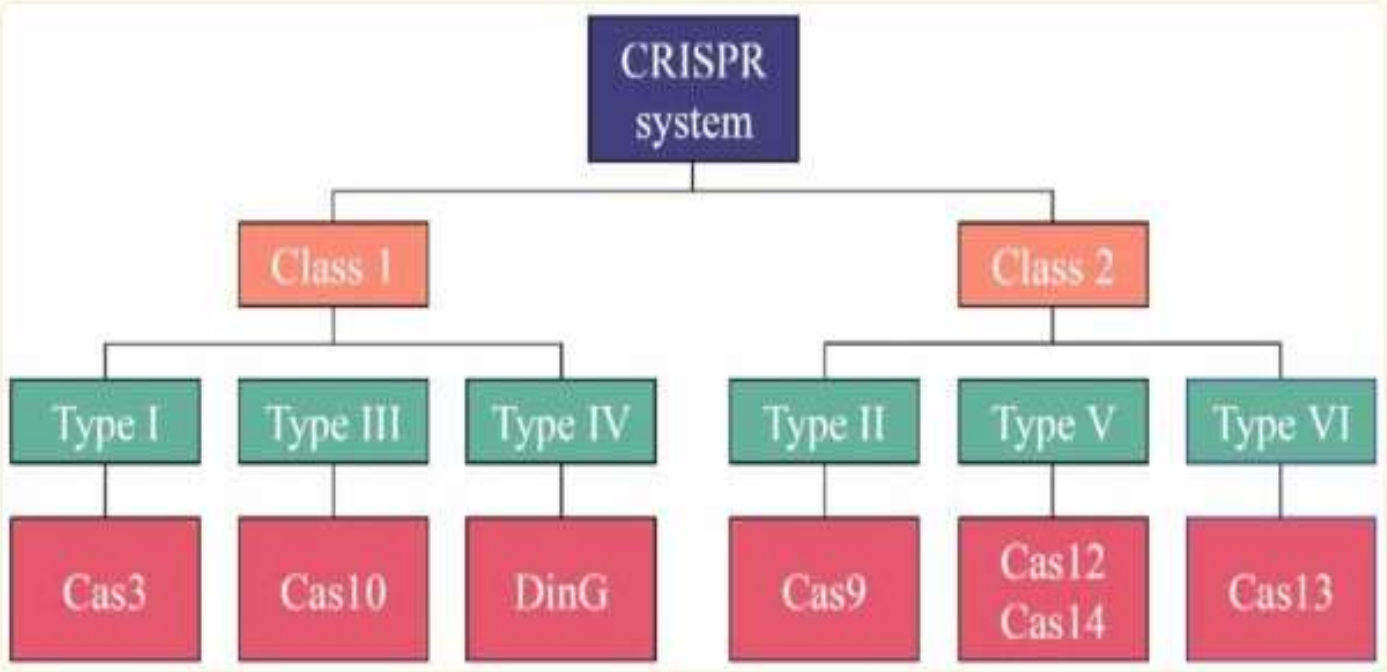
Understanding the CRISPR CAS9

CRISPR-Cas9 have been found primitively in *E. coli* and these sequences involved in archaea and bacterial immune system toward terminating invader DNA (Bacterial Viruses) by stimulating RNA-guided DNA splitting.



CRISPR Classes

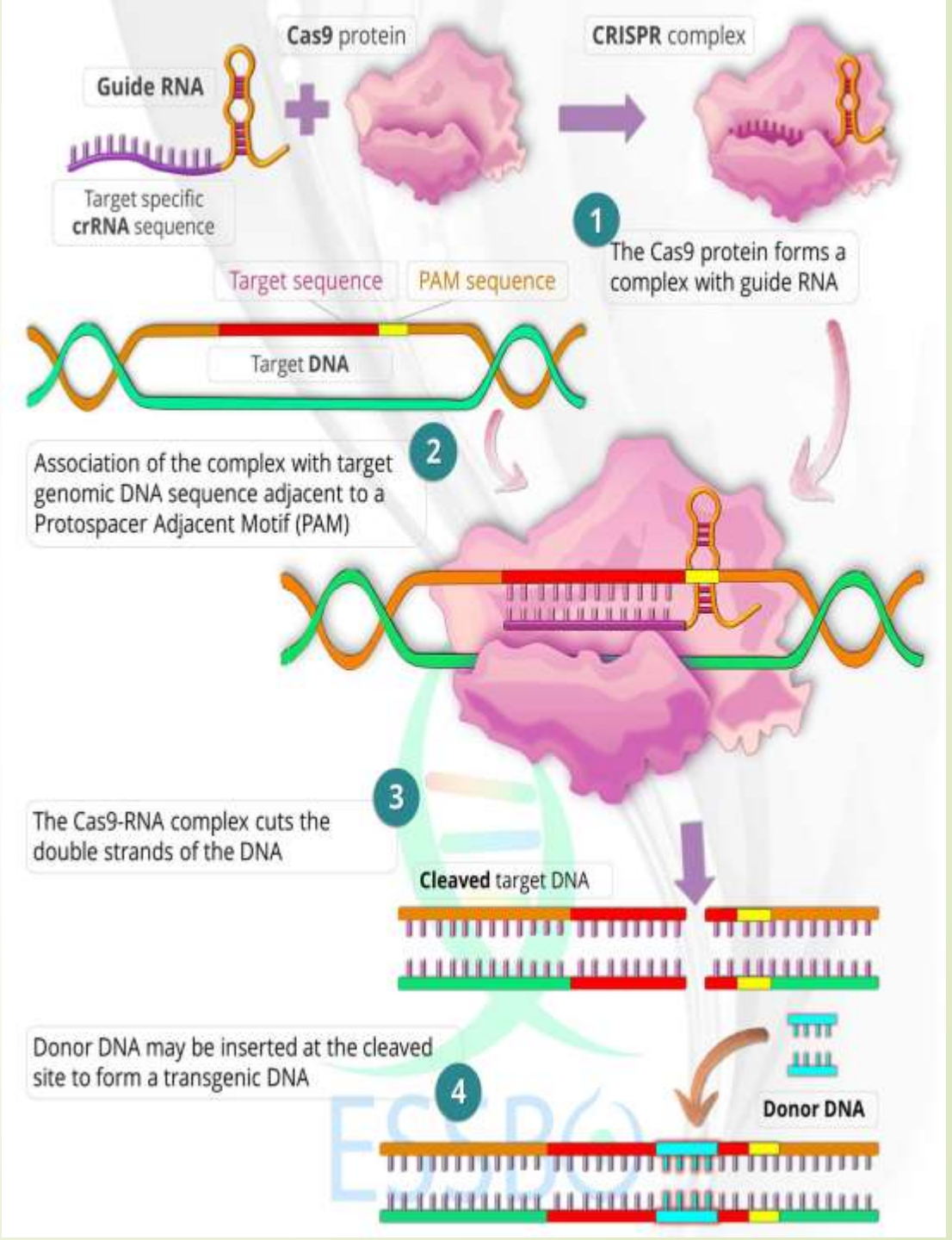
Currently two class of CRISPR system are present, Class 2 comprises of a great type 2 CRISPR method, and frequently use gene editor endonuclease Cas9 protein.



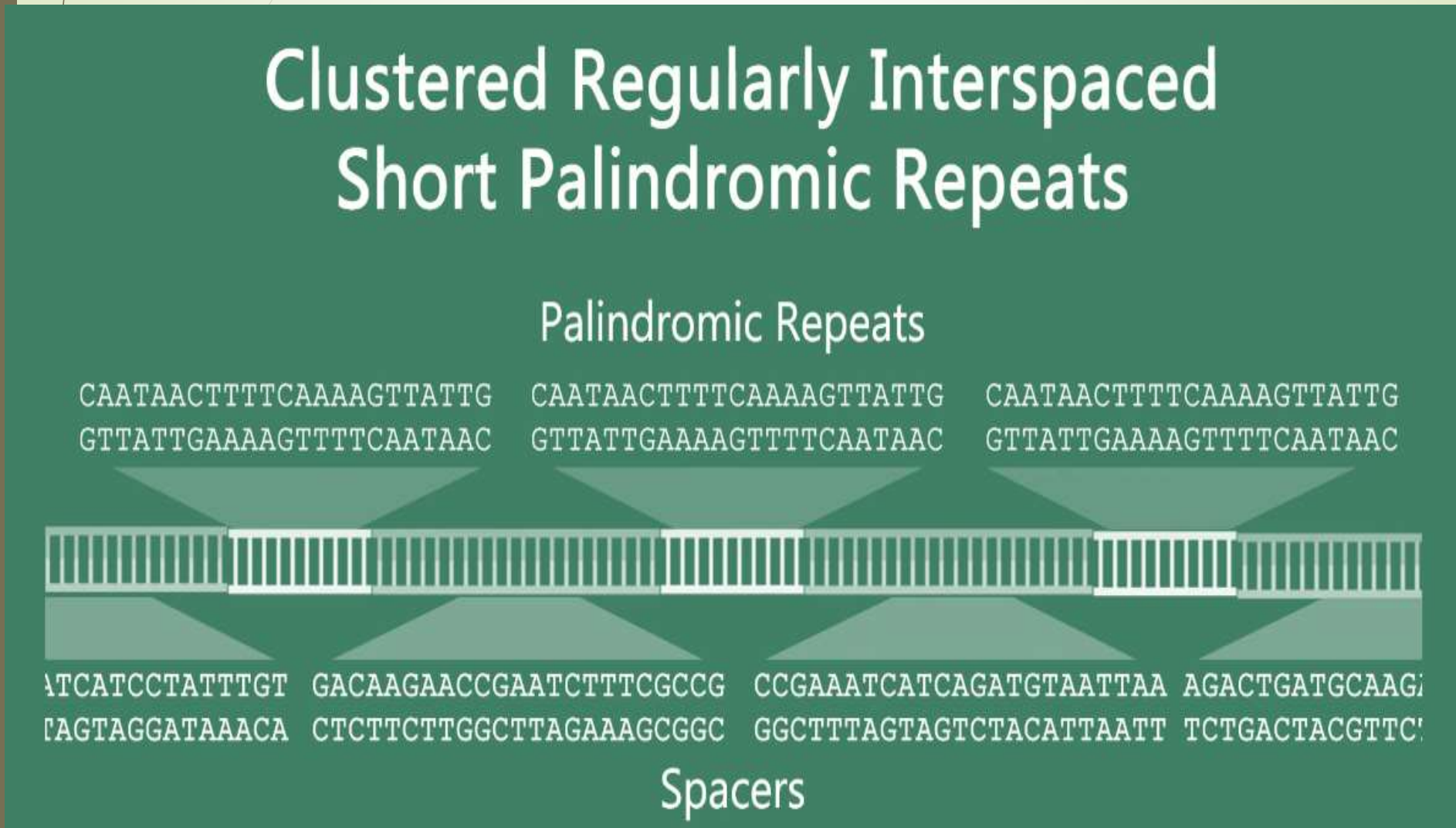
(Liu W, Li L, Jiang J, Wu M, Lin P. Applications and challenges of CRISPR-Cas gene-editing to disease treatment in clinics. Precis Clin Med. 2021)

How it works?

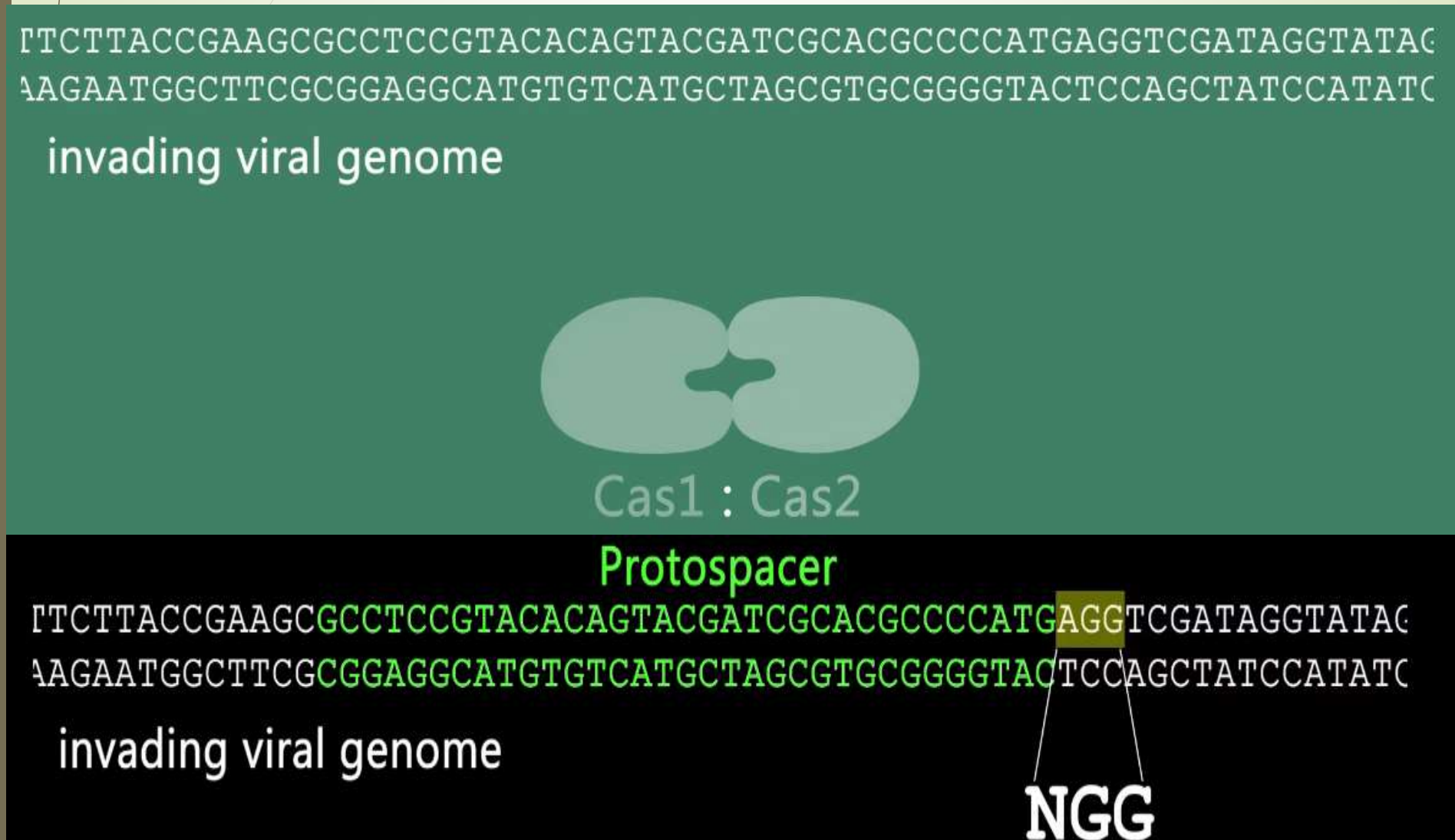
The CRISPR-Cas9 is created by the interaction of two critical elements, the Cas9 nuclease and the appropriate gRNA. With base-pair mediated binding to complementary DNA sequences crRNA which determines the specificity of a target DNA sequence. The binding of the gRNA then co-localizes Cas9 at the same specific-site, which leads to cuts in the DNA sequence and the creation of a DSB at the site (Hsu et al., 2014). Cas9 behaves as a pair of scissors to split the DNA sequence. Cas9 protein split three to four nucleotides ahead of the PAM region.



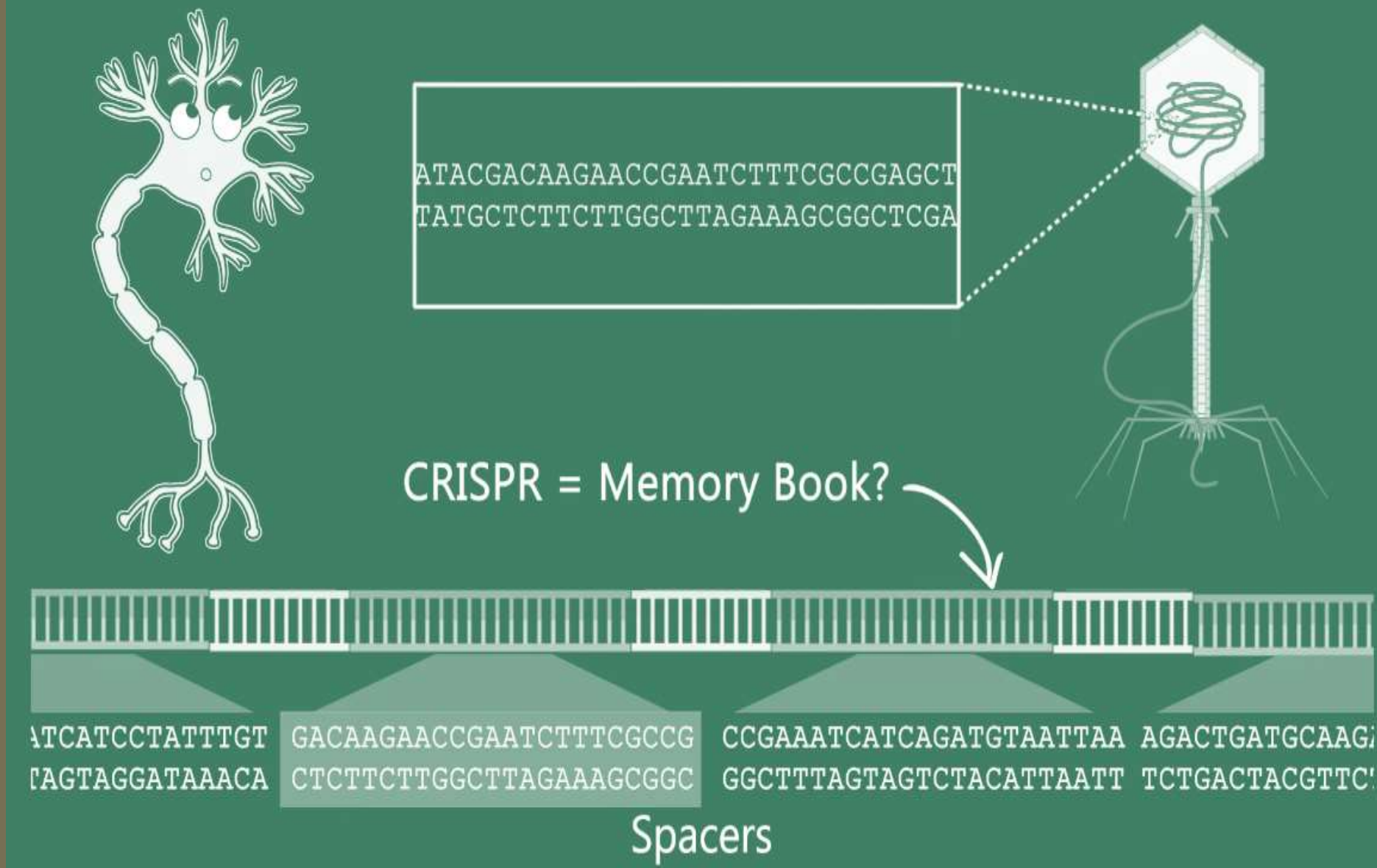
Understanding the CRISPR CAS9



Reading the invador Viral DNA, then inserting it to the CRISPR sites on Bacterial DNA (Protospacer)

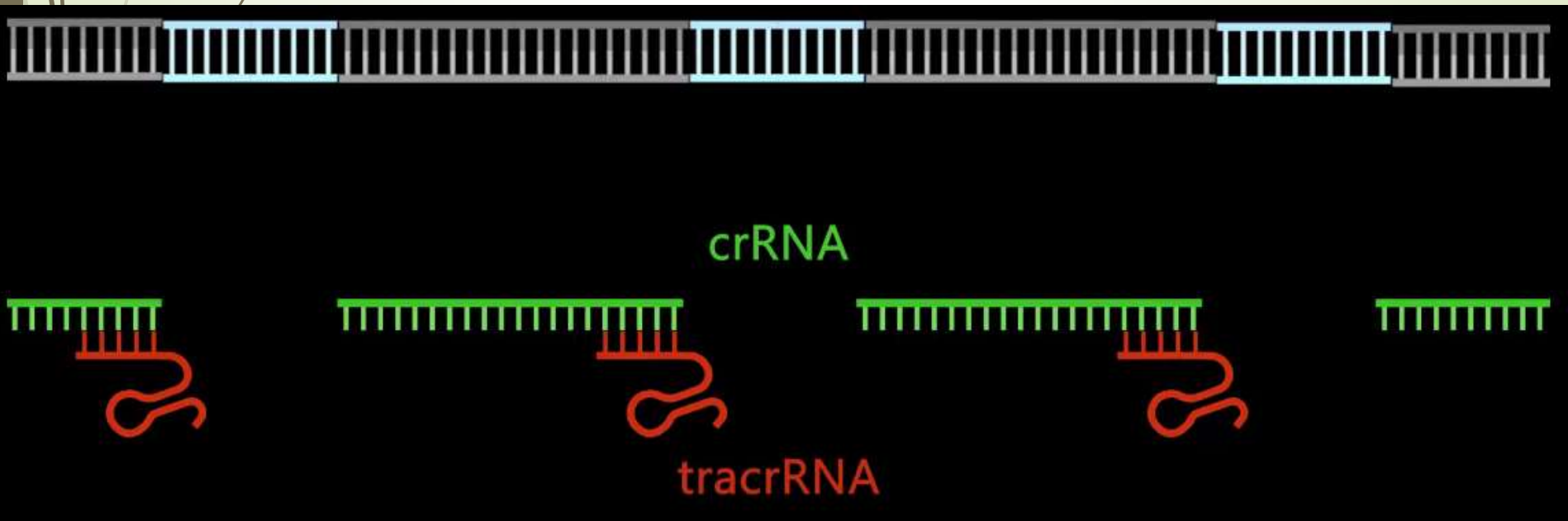


Inserted invador Viral DNA into the CRISPR sites (Protospacer) as an immune memory

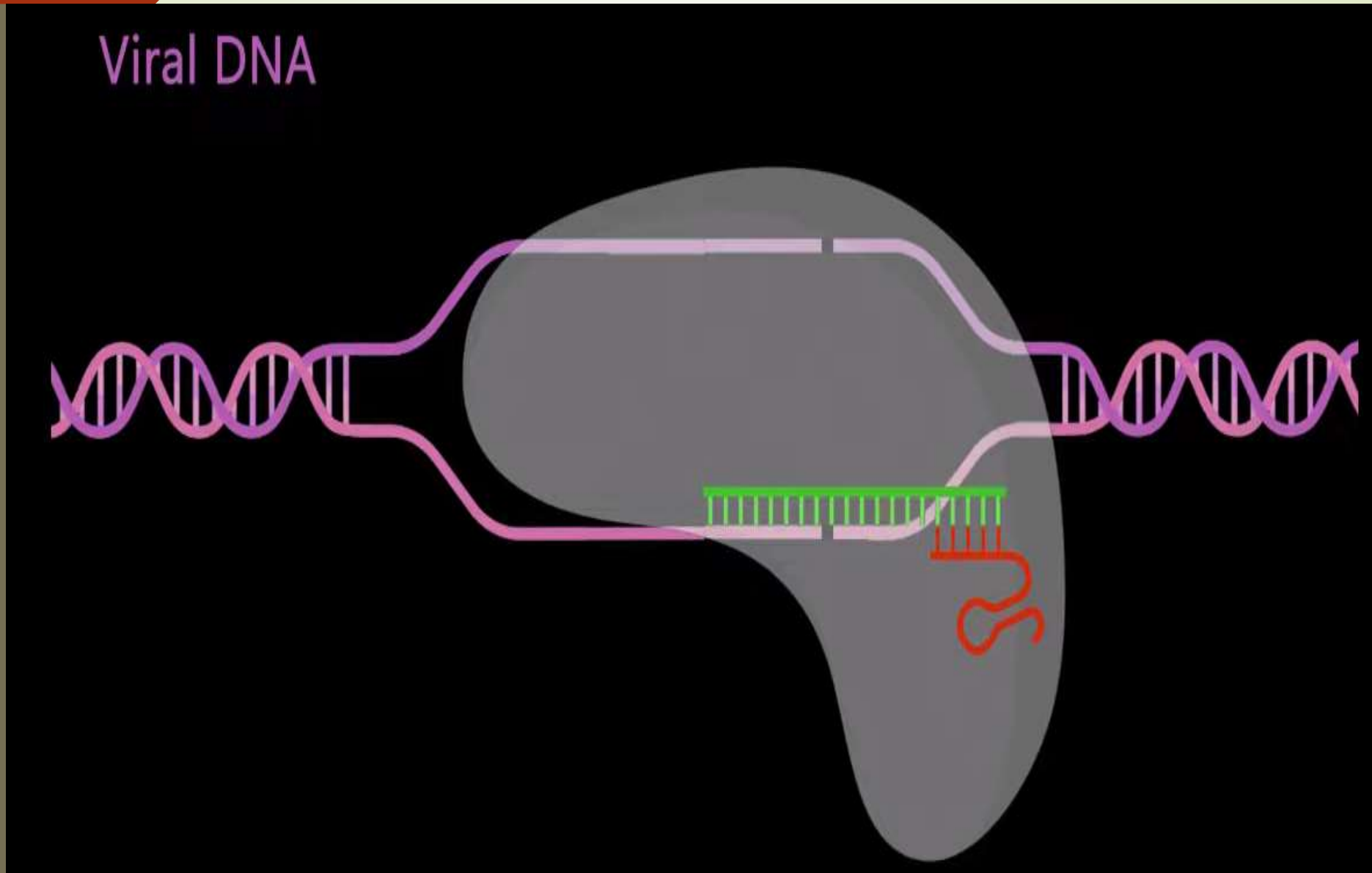


Inactivating the Invador Viral DNA

During next Viral invason, the inserted Viral DNAs within the CRISPR sites (Protospacer) will be copied to a crRNA and then bind with tracrRNA forming the guide sgRNA, then bind to the CAS9 nuclease and the specific site of the the invading viral DNA to distroy it.



Inactivating the Invador Viral DNA

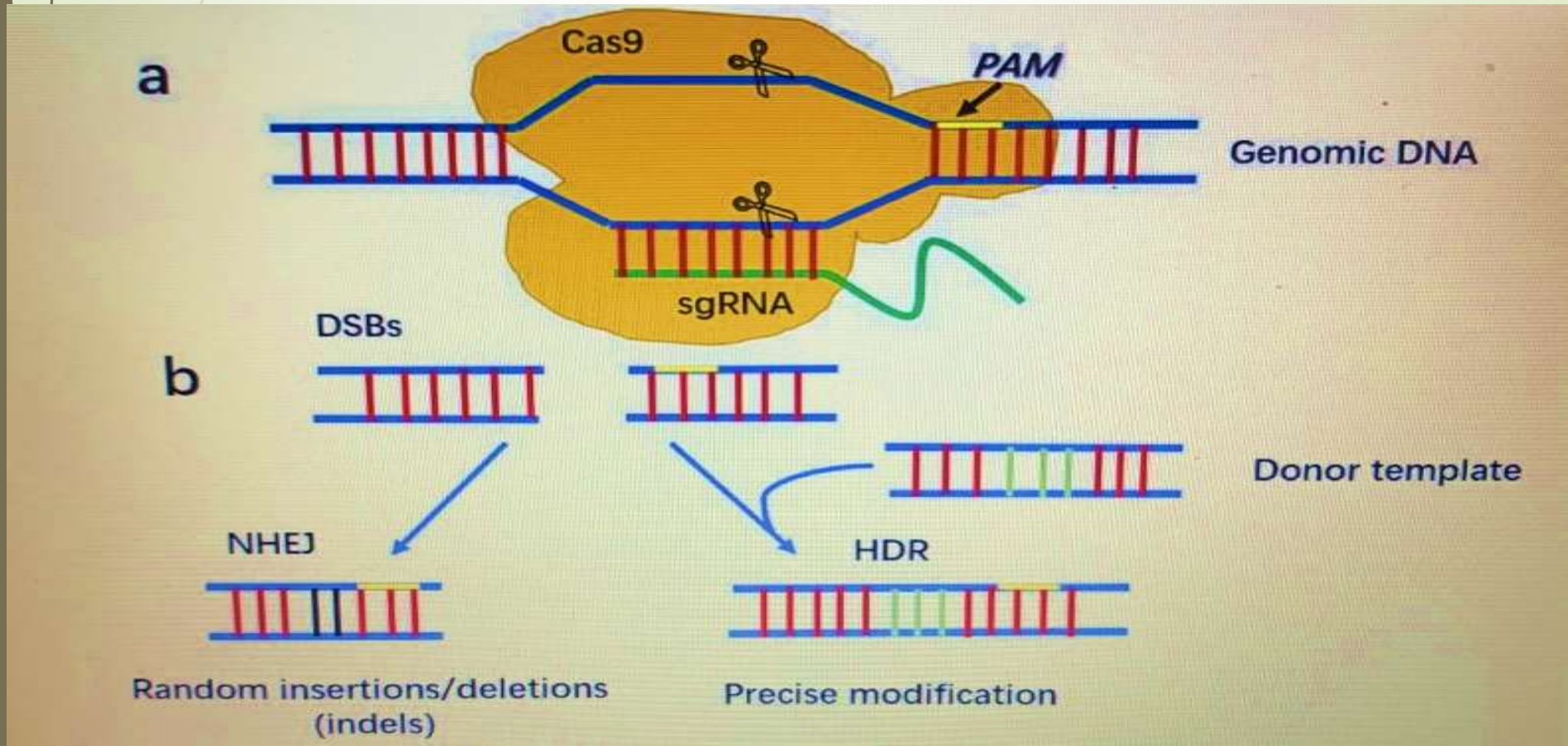


CRISPR CAS9 Animation

Genome Editing with CRISPR-Cas9.mkv

The genome editing processes of CRISPR-Cas9.

a. The single guide RNA (sgRNA) guides the Cas9 nuclease to specific genomic sequences.
b. b. Double strand breaks (DSBs) repaired by non-homologous end joining (NHEJ) or by homology-directed repair (HDR) (Chen et al., 2019).



CRISP CAS9 Gene knockout

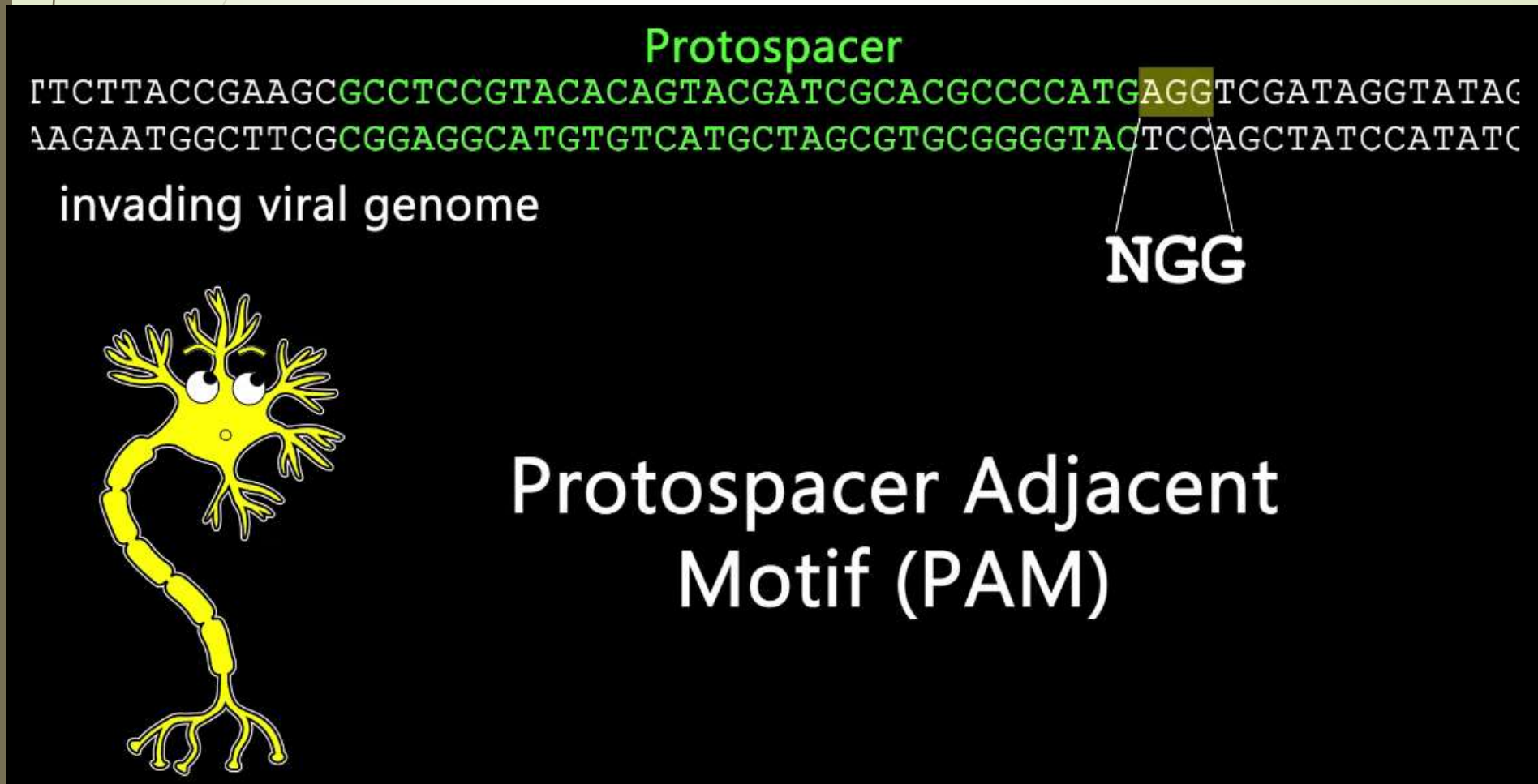
After double strand breaks are formed by CAS9; either one repairing mechanisms will be carried out:

1. Non-Homologous End Joining (NHEJ) repair mechanism or
2. Homology Directed Repair (HDR) mechanism

-The NHEJ repair mechanism contributing to frameshifts in the open reading frames of certain genes by the production of insertion or deletion and ultimately, gene knockout.

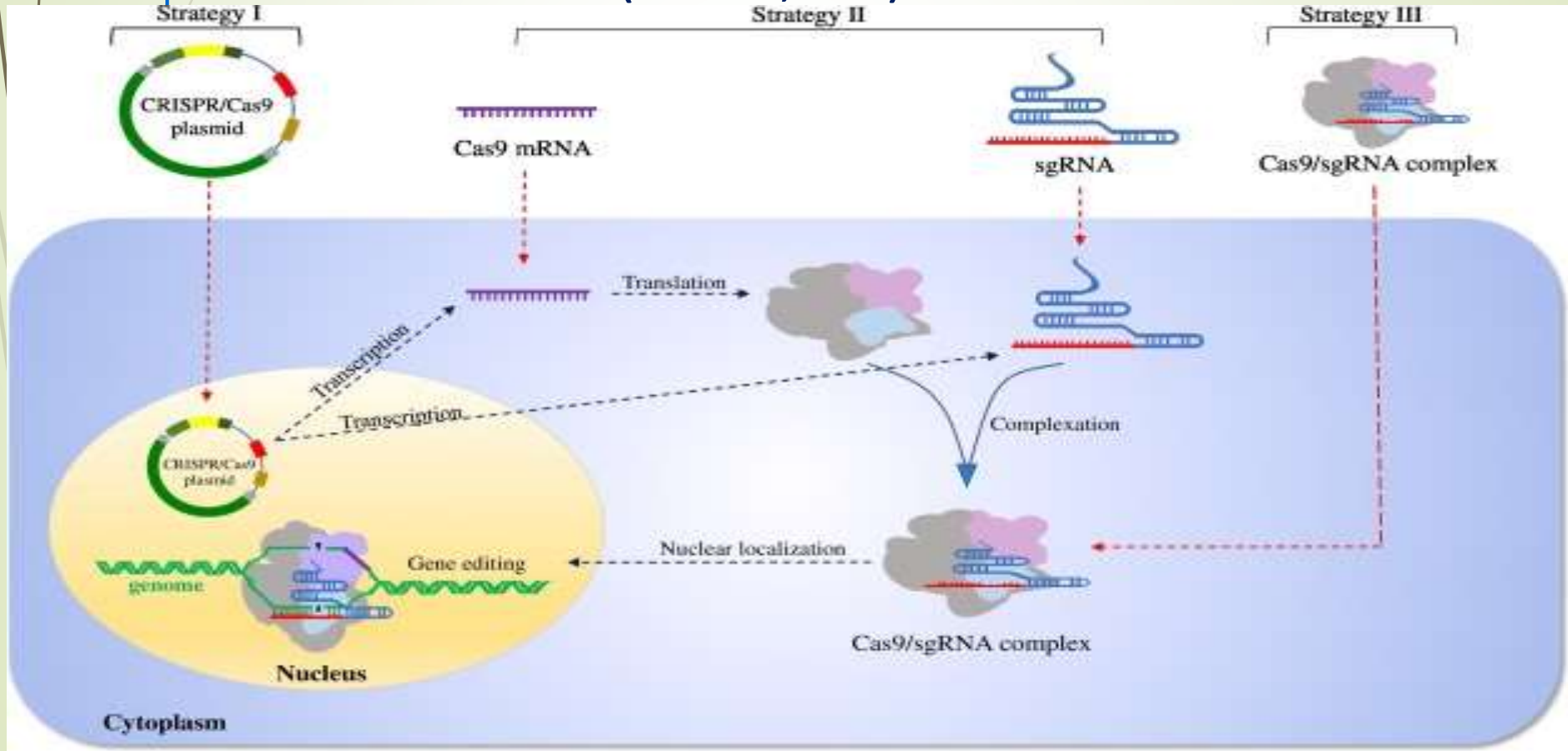
-Conversely to NHEJ, to correct the double strand breaks in the HDR mechanism, a donor DNA template is required. Correct DNA sequences are accurately inserted into the target site using a donor DNA template.

CRISP CAS9 how not affecting its own CRISP sites?

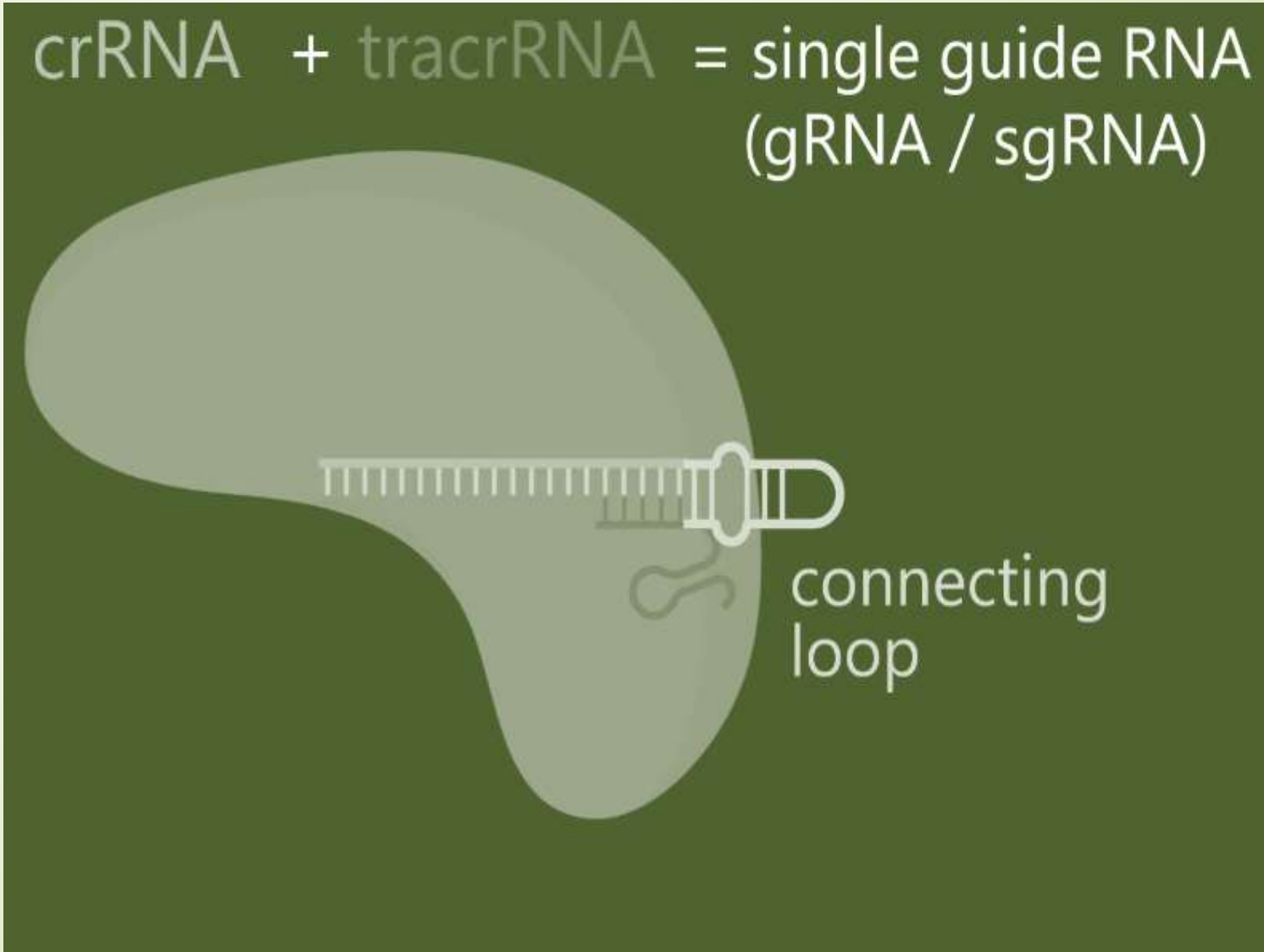


Various strategies for gene editing using the CRISPR-Cas9 method.

1. plasmid-based CRISPR-Cas9 system, the vector will transmit the Cas9 protein and sgRNA.
2. insert the combination of the Cas9 messenger RNA and the single-stranded guide RNA. Cas9 messengerRNA will be translated to Cas9 enzyme inside of the cell to produce the Cas-sgRNA combination.
3. to diametrically deliver the Cas9/single-stranded guide RNA combination into the inside of cell (Liu et al., 2017).



Simplified Gene editing using the CRISPR-Cas9 method



Application of CRISPR CAS9 in Medicine:

1. Cancer therapy and cancer immunotherapy,
2. Treatment of diseases i.e. gene therapy (treatment of genetic, viral, and hematological diseases)
3. Anticancer drug development.
4. Fertilized Egg Gene Therapy.

Any Questions?