CRISPR/ CAS SYSTEM AND TARGETED GENOME EDITING

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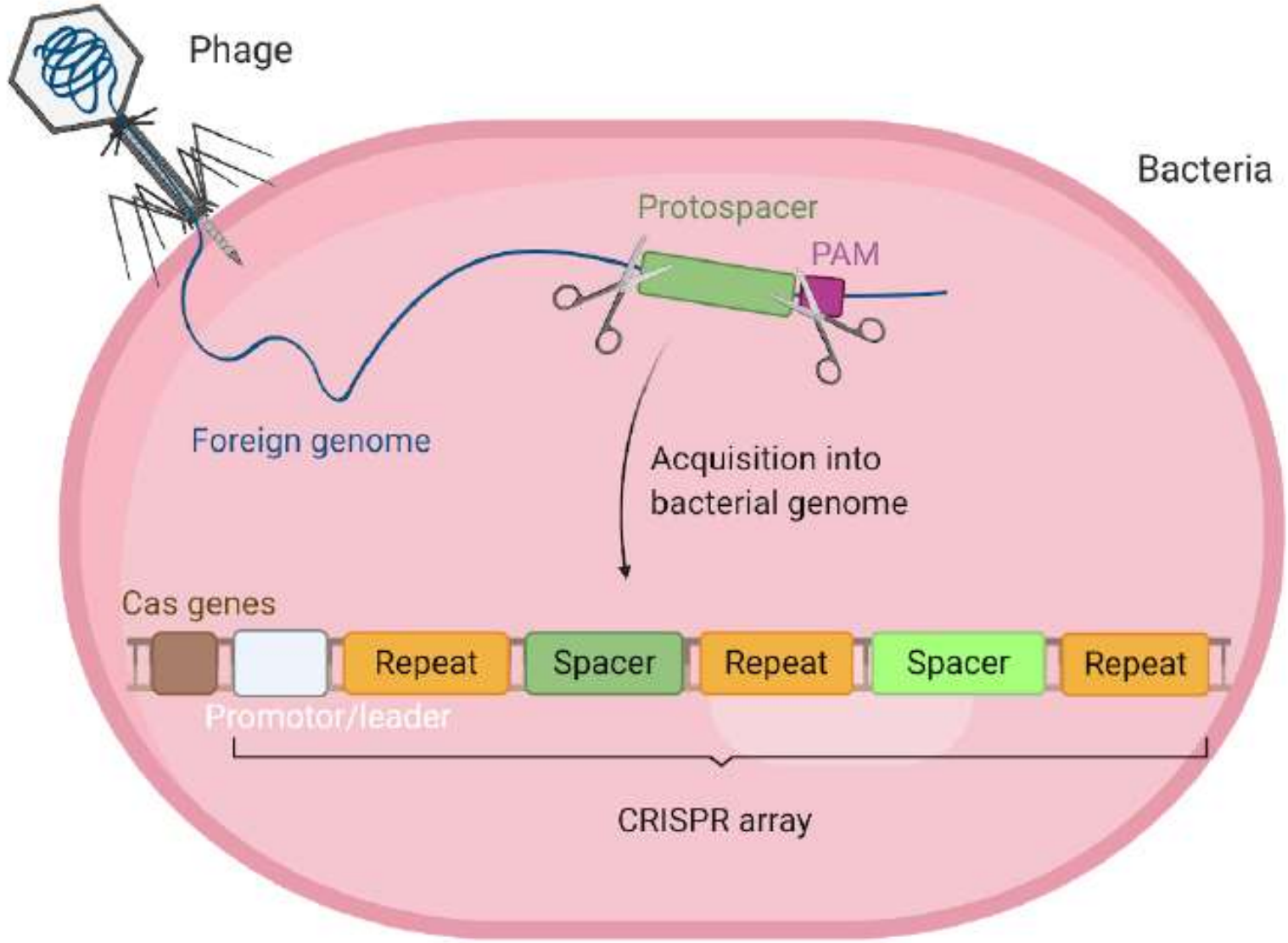
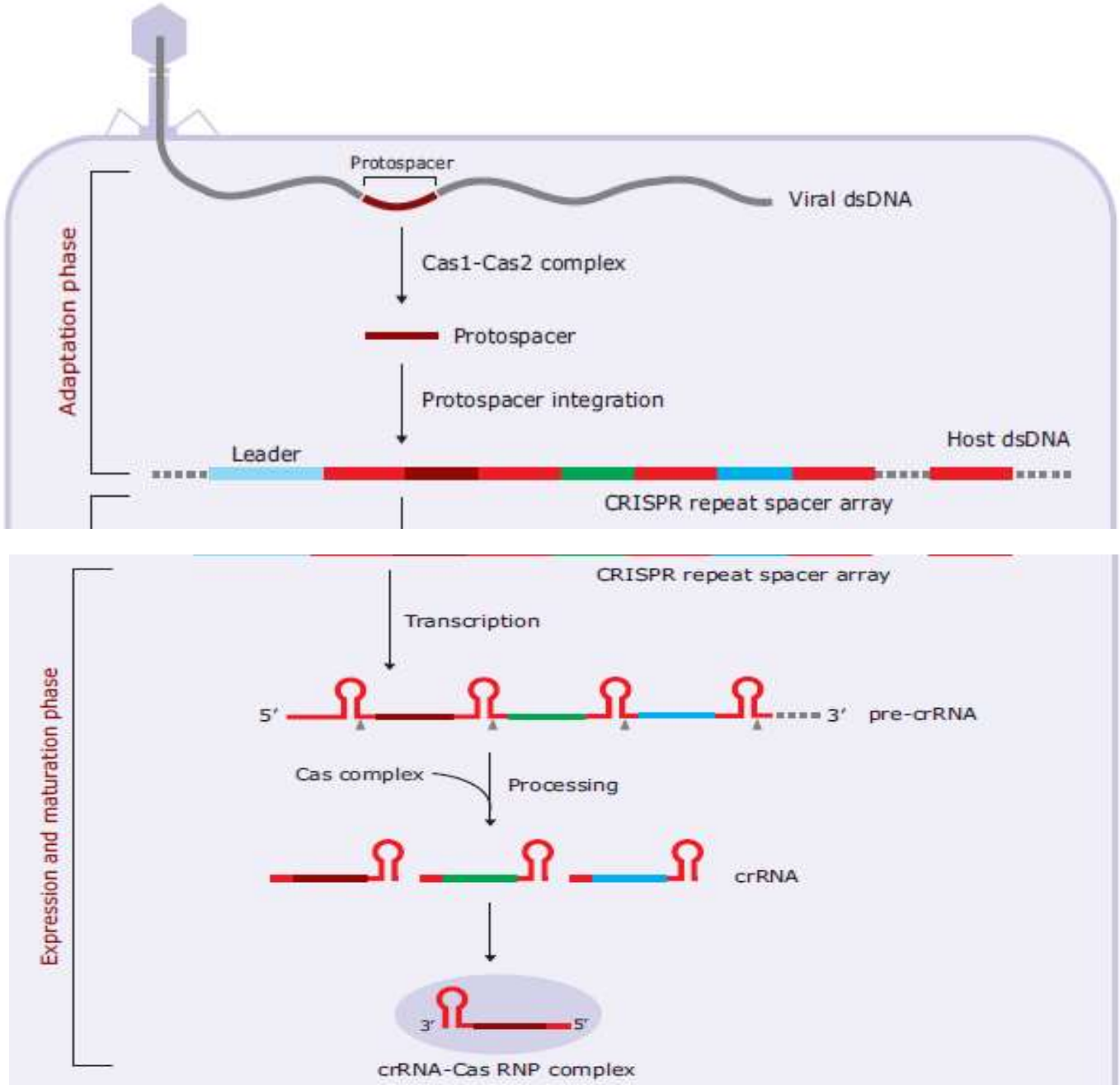
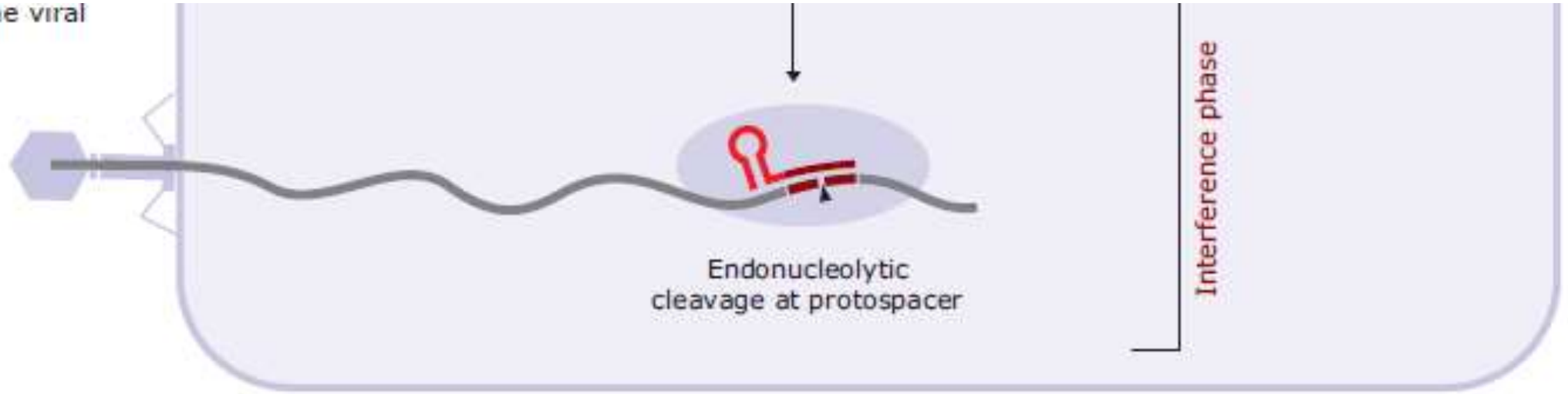
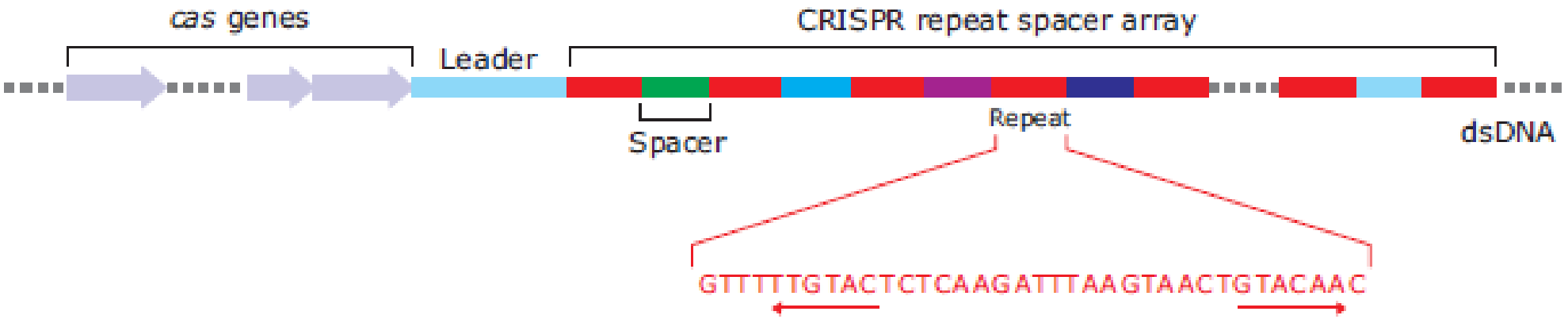


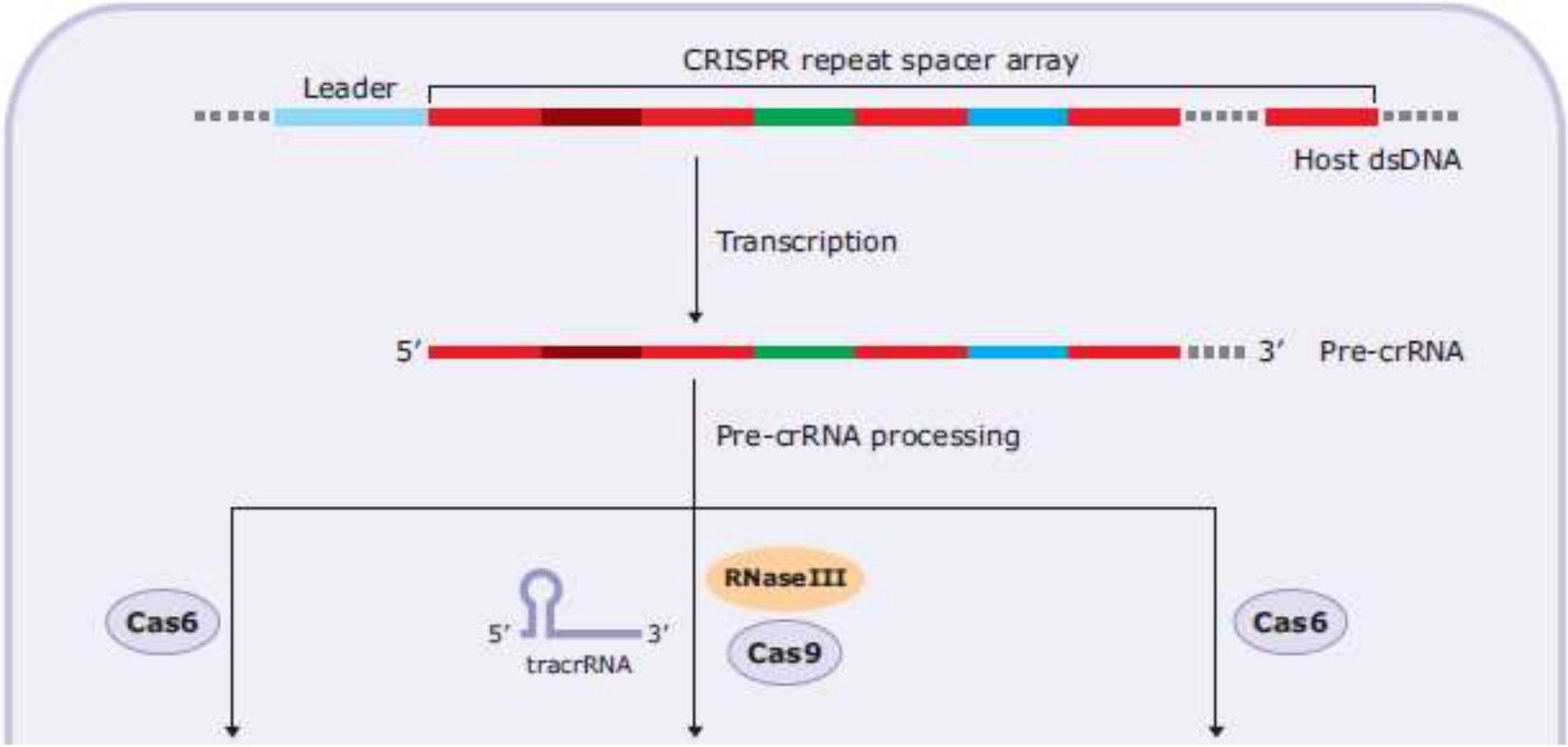
The bacterial adaptive immune system CRISPR. Here, a bacteriophage injects its DNA into the bacteria and the bacteria cuts out a piece of it, called a protospacer, and inserts it into the CRISPR array in a process called spacer acquisition. An inserted protospacer is called a spacer.

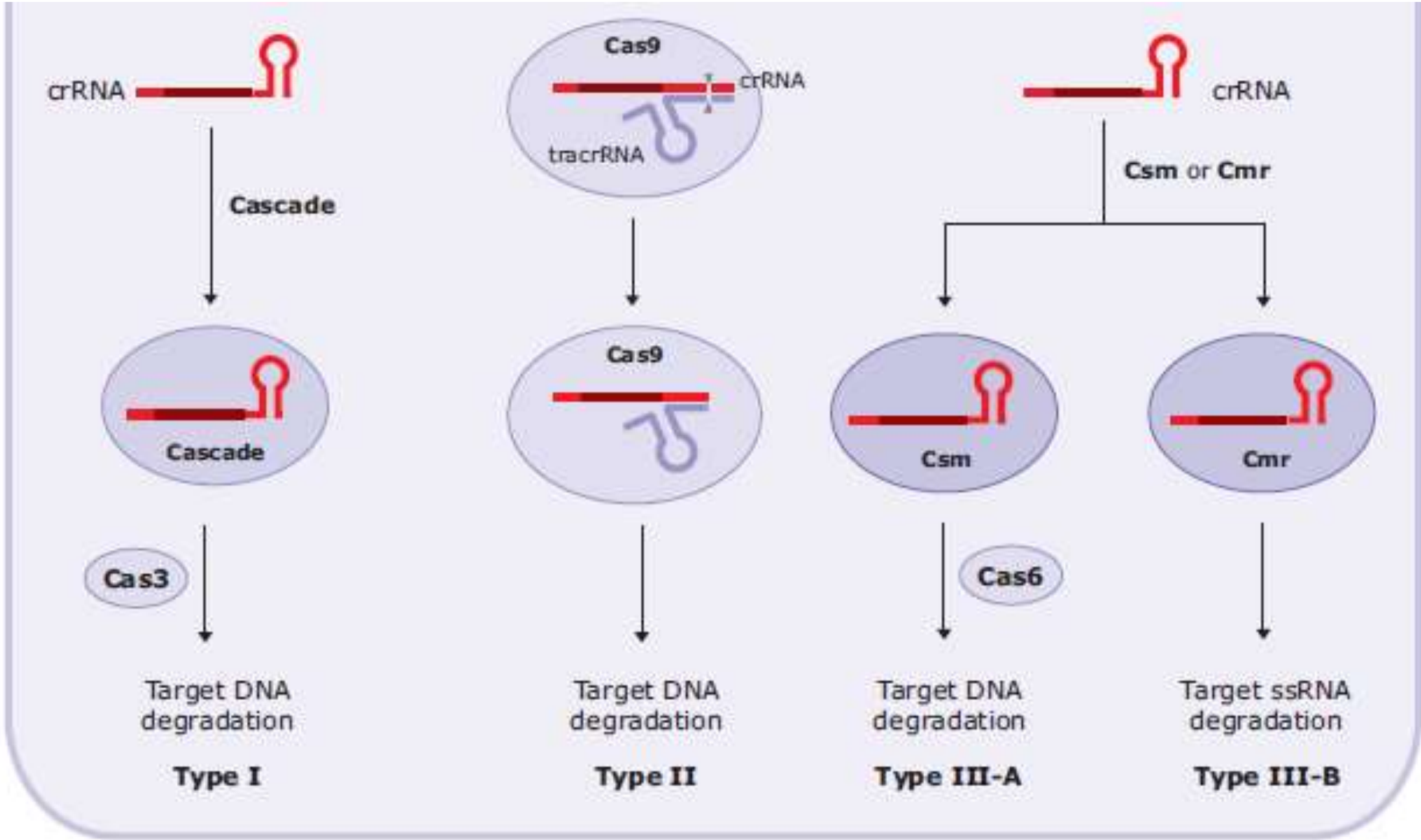

Protospacer
Viral dsDNA
Adaptation phase
Cas1-Cas2 complex
Protospacer
Protospacer integration
Host dsDNA
Leader
CRISPR repeat spacer array
CRISPR repeat spacer array
Transcription
5′
3′
pre-crRNA
Cas complex
Processing
crRNA
Expression and maturation phase
3′
5′
crRNA-Cas RNP complex

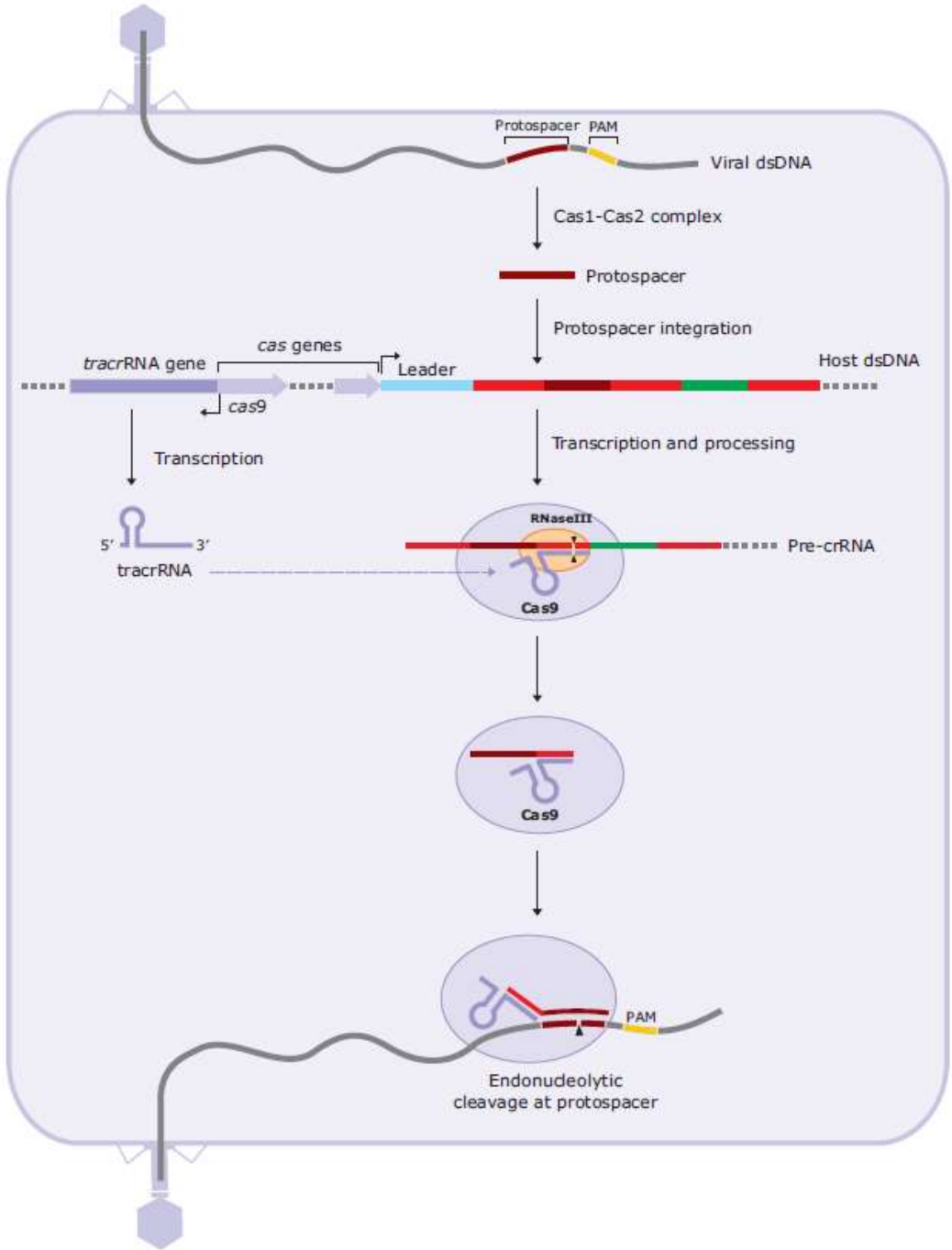
m the viral

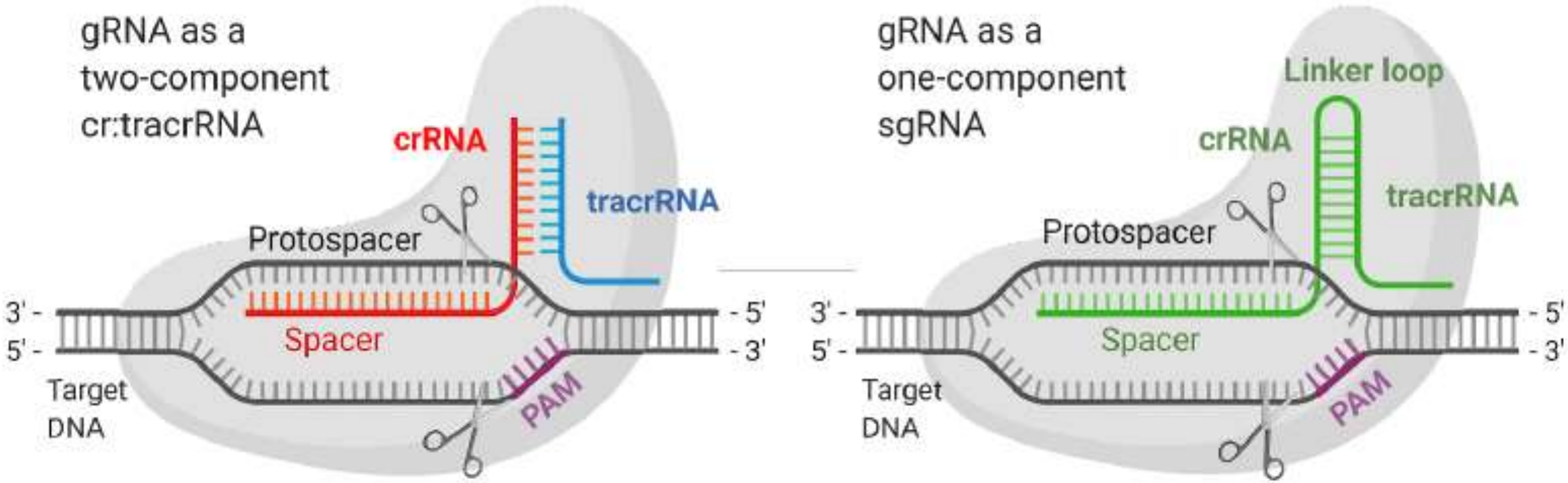

Endonucleolytic
cleavage at protospacer
Interference phase


cas genes
Leader
CRISPR repeat spacer array
Spacer
Repeat
dsDNA
GTTTTTGTACTCTCAAGATTTAAGTAACTGTACAAC


CRISPR repeat spacer array
Leader
Host dsDNA
Transcription
5′
3′
Pre-crRNA
Pre-crRNA processing
Cas6
5′
3′
tracrRNA
RNaseIII
Cas9
Cas6


crRNA
Cascade
Cascade
Cas3
Target DNA degradation
Type I
Cas9
crRNA
tracrRNA
Cas9
Target DNA degradation
Type II
crRNA
Csm or Cmr
Csm
Cas6
Target DNA degradation
Type III-A
Cmr
Target ssRNA degradation
Type III-B


Protospacer
PAM
Viral dsDNA
Cas1-Cas2 complex
Protospacer
Protospacer integration
cas genes
tracrRNA gene
Leader
Host dsDNA
cas9
Transcription
Transcription and processing
5′
3′
tracrRNA
RNaseIII
Pre-crRNA
Cas9
Cas9
PAM
Endonucleolytic
cleavage at protospacer



The CRISPR-Cas9 complex with two different gRNA configurations. The grey figure represents the Cas9 protein. To the left is a Cas9 protein attached to gRNA consisting of the two components crRNA in red and tracrRNA in blue, called a cr:tracrRNA complex. To the right is a Cas9 protein in complex with sgRNA in green.

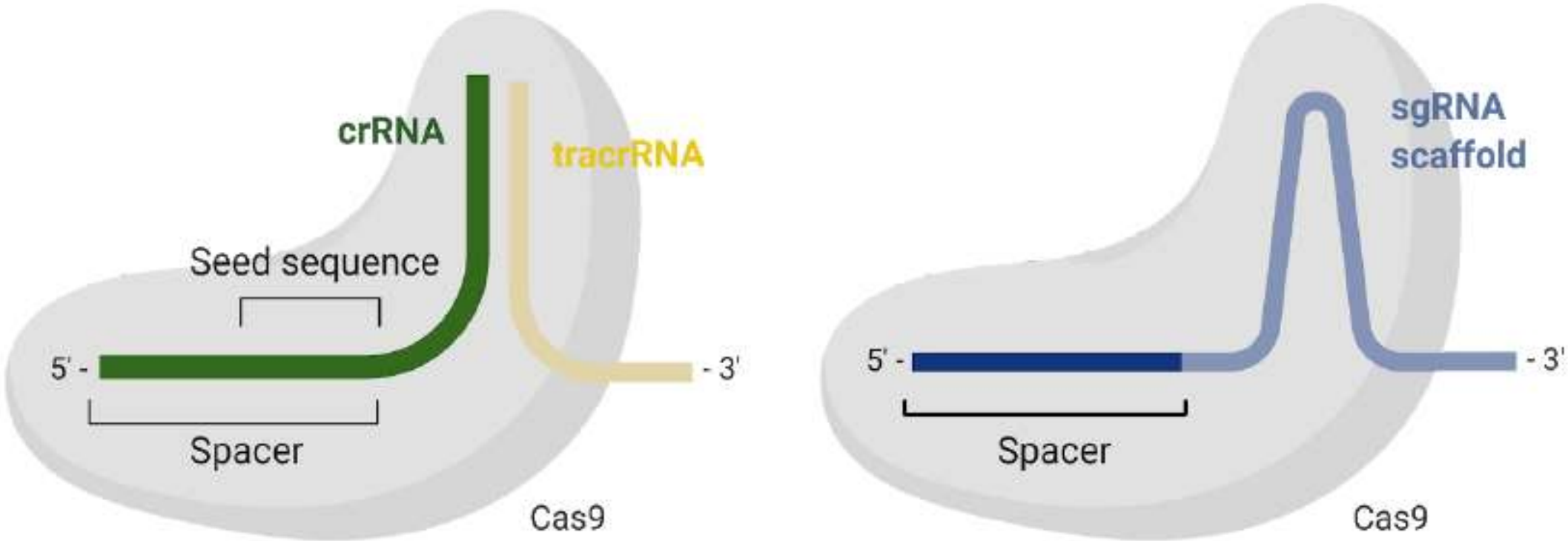
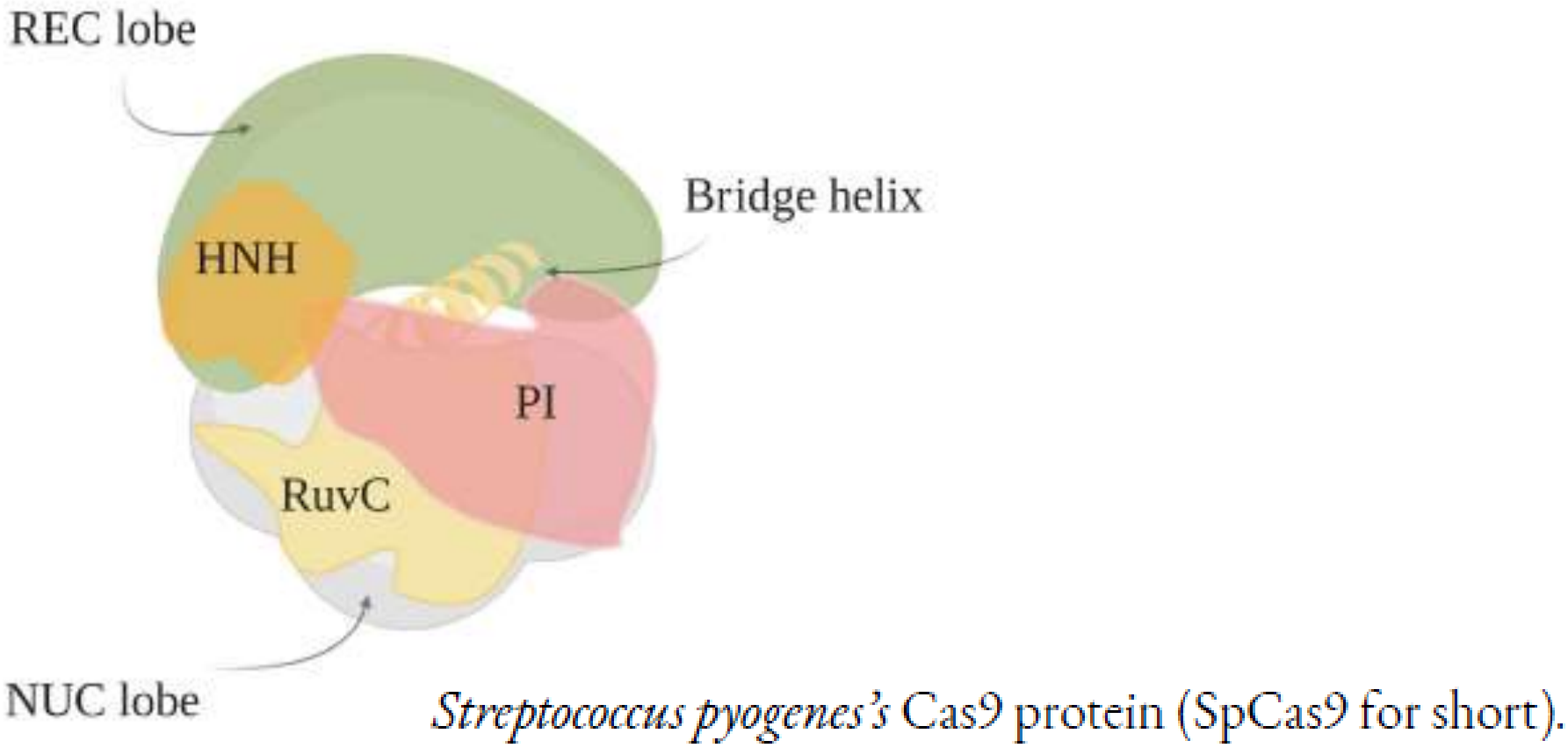
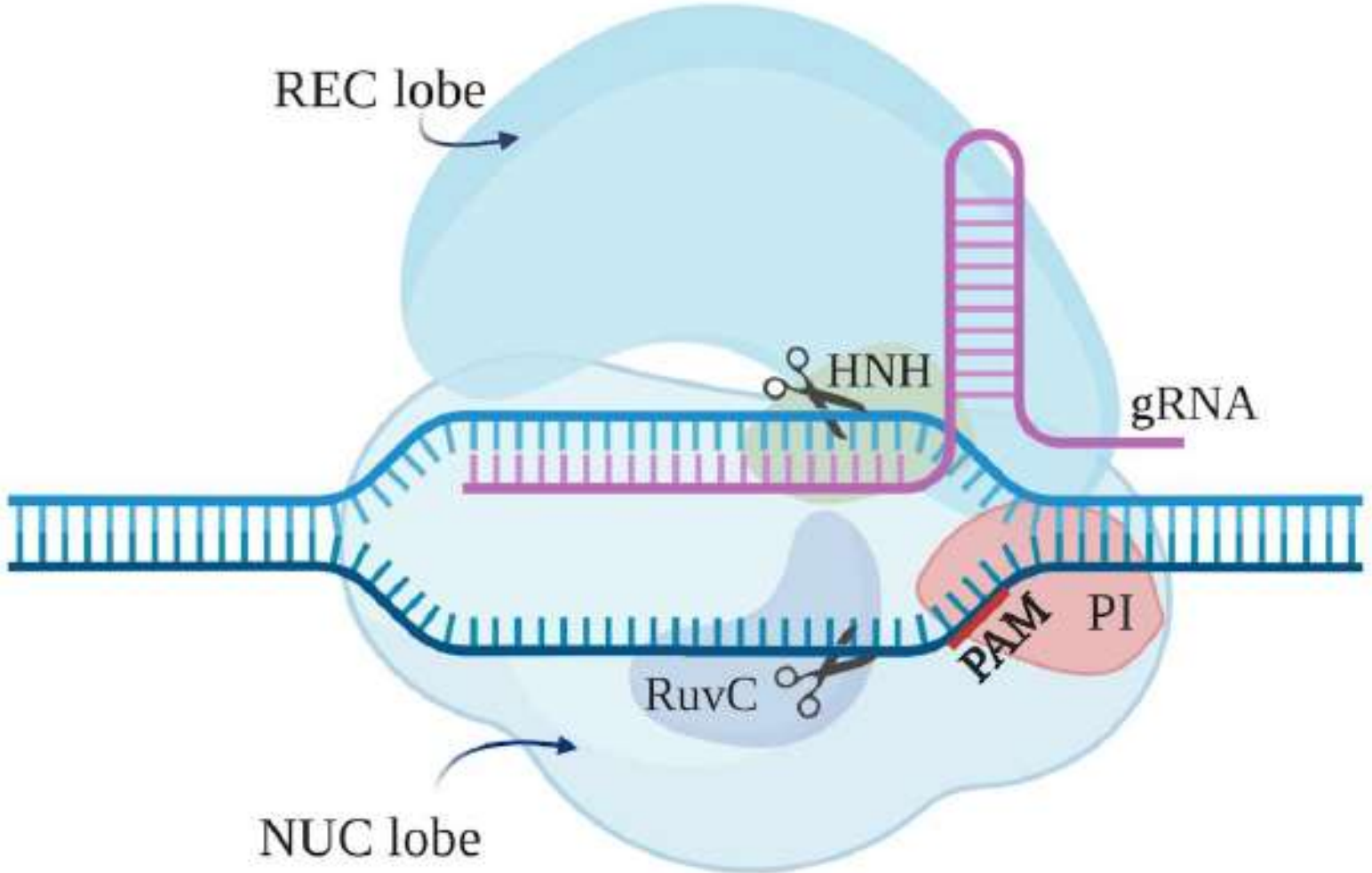


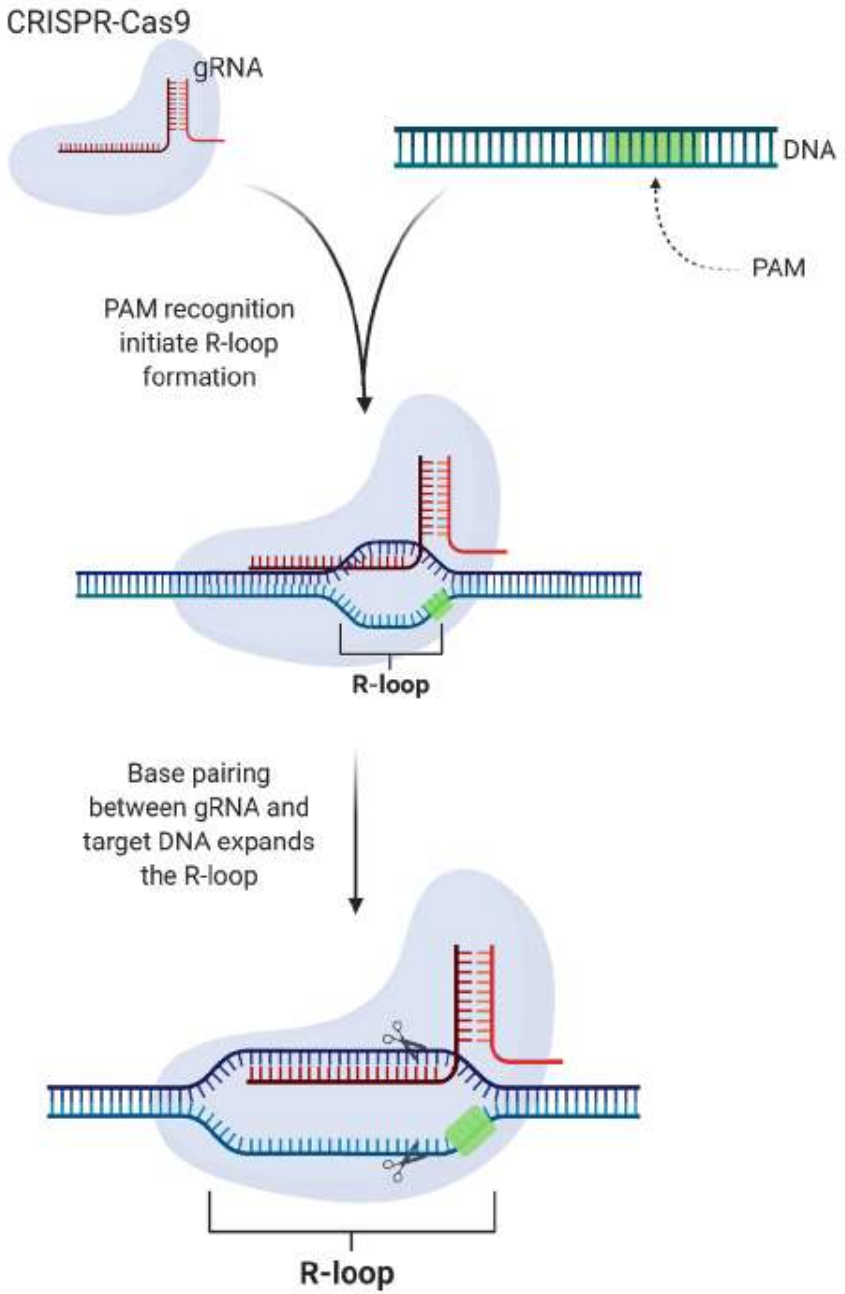
Figure 3: The left picture emphasizes where the spacer and seed sequence are located on the crRNA strand in a Cas9 complex. Right picture illustrates a Cas9 complex in a simplified manner as an sgRNA scaffold and a spacer.



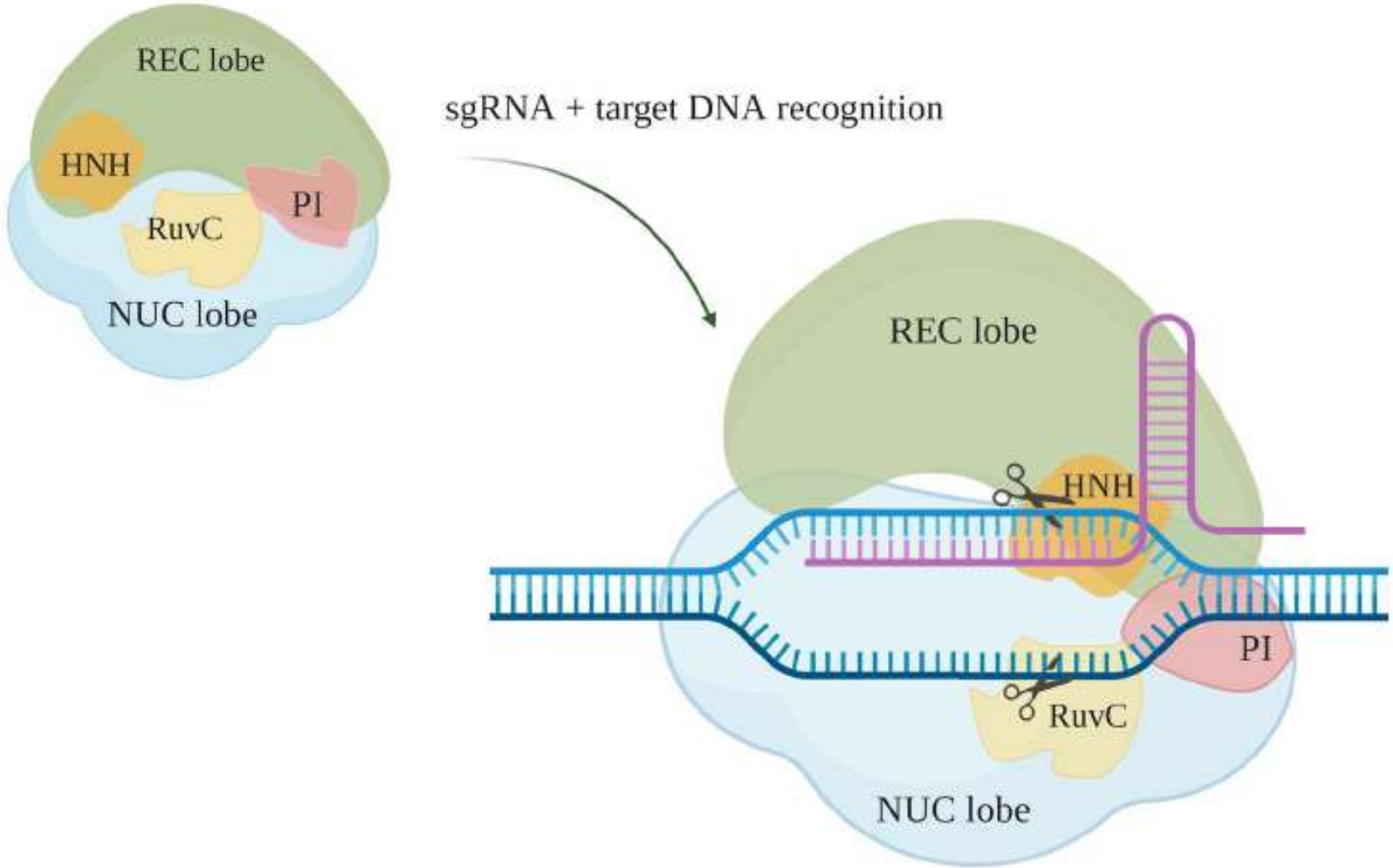
The bilobed Cas9 structure consisting of the NUC and REC lobe is connected by a bridge helix. The NUC lobe (grey) has the nuclease activity of the Cas9 protein, and contains two endonuclease domains called HNH (orange) and RuvC (yellow), and a PI domain (pink) responsible for PAM recognition. The REC lobe (green) recognizes the gRNA.



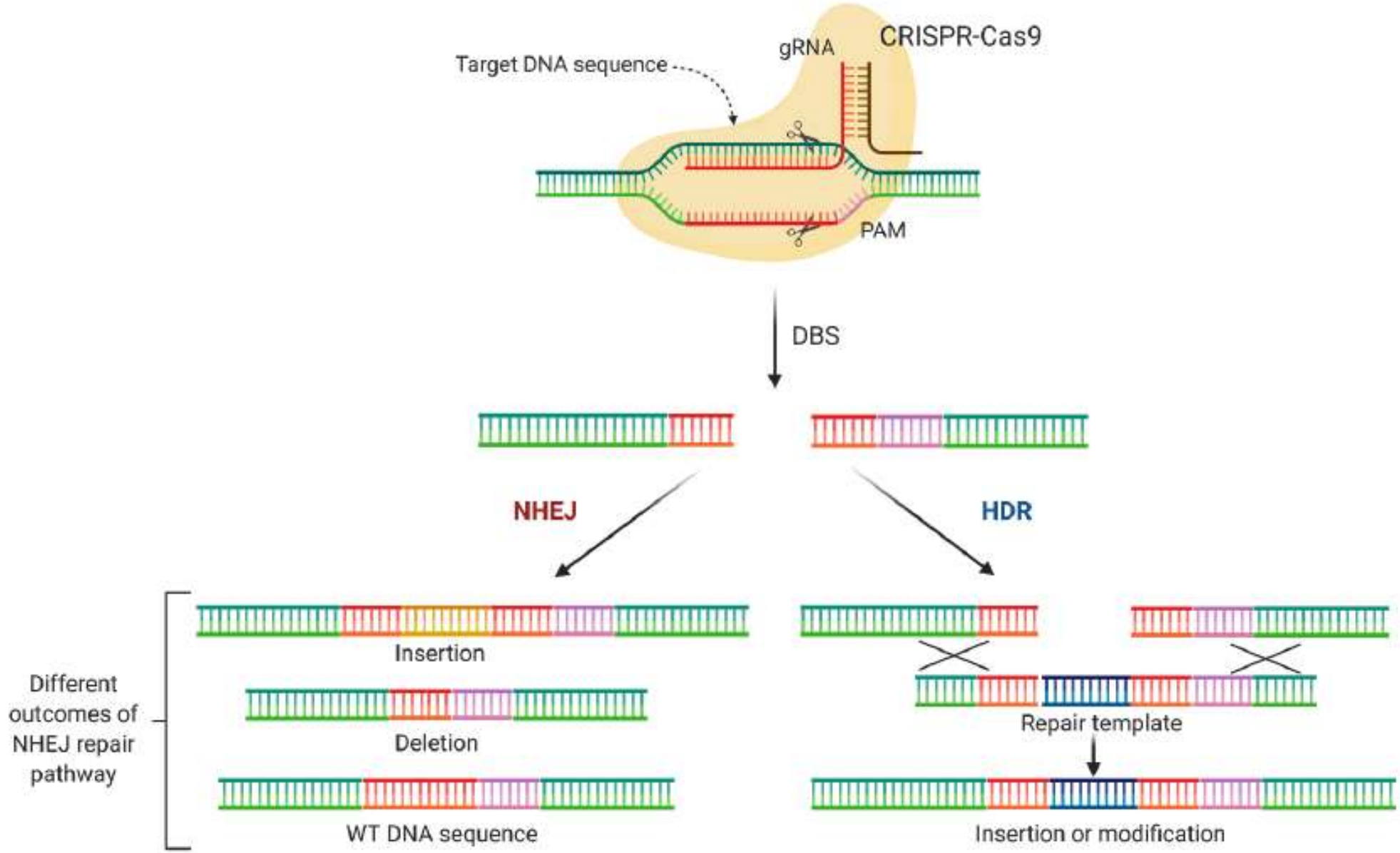
Binding of gRNA to the REC lobe initiates a series of conformational changes, resulting in a fully activated protein. The target DNA can be attached when the complex of Cas9 and gRNA has connected. The PI domain recognizes the PAM region and is followed by pairing of the gRNA seed sequence. The NUC-lobe's HNH and RuvC domains are responsible for the cleavage of target dsDNA.



When the Cas9 recognizes the PAM sequence, the target DNA starts to unwind, forming an R-loop structure. The R-loop expands when bases between gRNA and target DNA match. When the entire spacer region of the gRNA is bound to the target DNA, Cas9 makes a double stranded cut.



Illustrates the critical, dynamical changes of the Cas9 protein structure upon target binding and R-loop formation. HNH (orange) changes position into the spacious room created during the R-loop expansion, fully activating the nuclease activity of Cas9.


Target DNA sequence
gRNA
CRISPR-Cas9
PAM
DBS
NHEJ
HDR
Different outcomes of NHEJ repair pathway
Insertion
Deletion
WT DNA sequence
Repair template
Insertion or modification

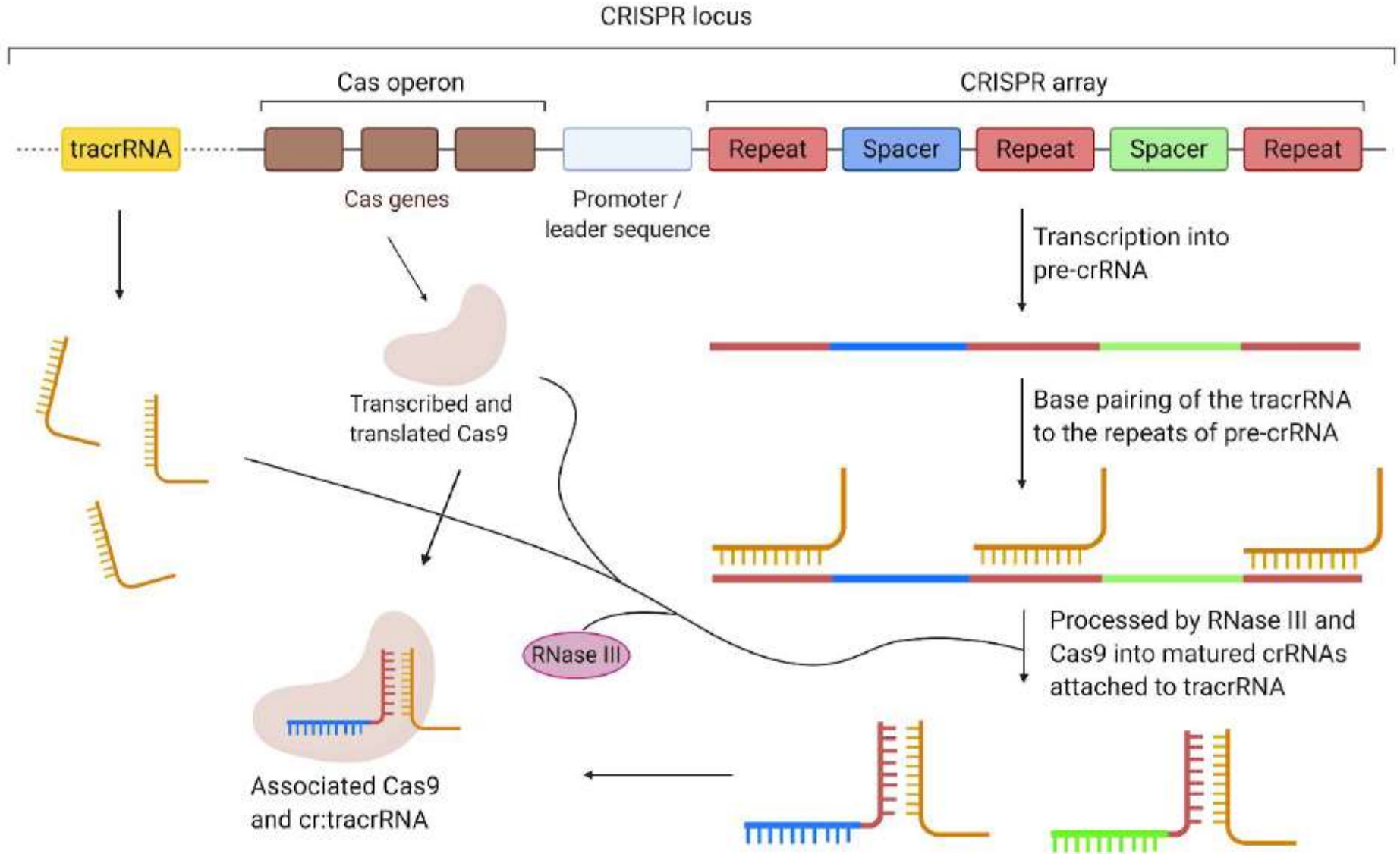


Figure 11: An overall description of the CRISPR-Cas9 locus, its components, its products after transcription/translation and the final product of a Cas9 complex in the left corner. The tracrRNA attaches to pre-crRNA by its anti-repeat sequence. In order to achieve mature cr:tracrRNA, RNase III and Cas9 are required.

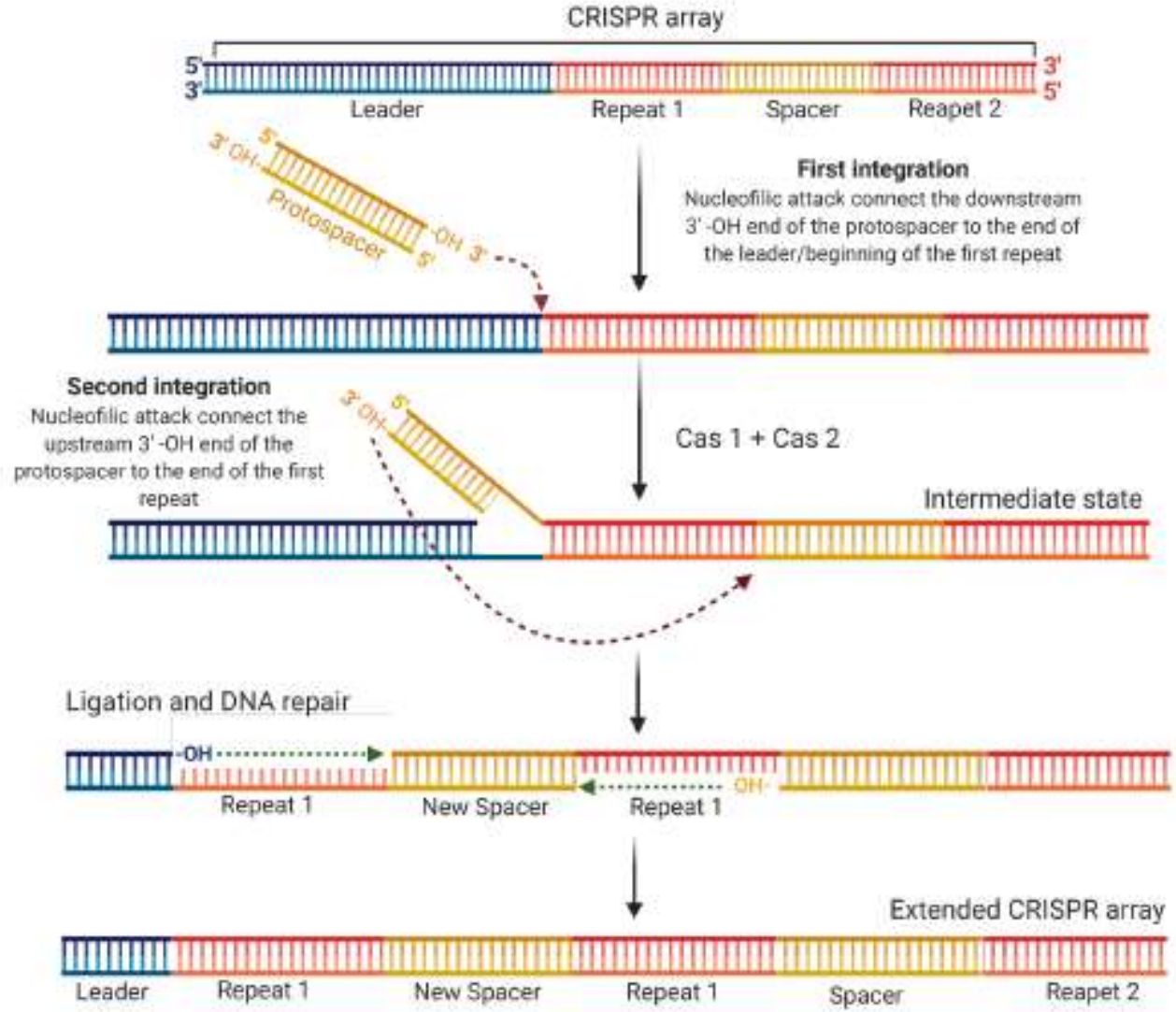


Figure 12: The spacer acquisition starts at the leader sequence which is followed by repeats and spacers. The Cas1 and Cas2 proteins find, cut and incorporate a new piece of viral DNA (protospacer) into the CRISPR array. First, the downstream 3'-end of the protospacer connects to the 5'-end of the first repeat by a nucleophilic attack. In the second integration, the upstream 3'-end of the protospacer attaches to the end of the first repeat. Repeat 1 is hereby separated into ssDNA at each side of the newly incorporated protospacer (now termed a spacer). Ligation and DNA repair mechanisms fill in the remaining gaps and the final extended CRISPR array is acquired.

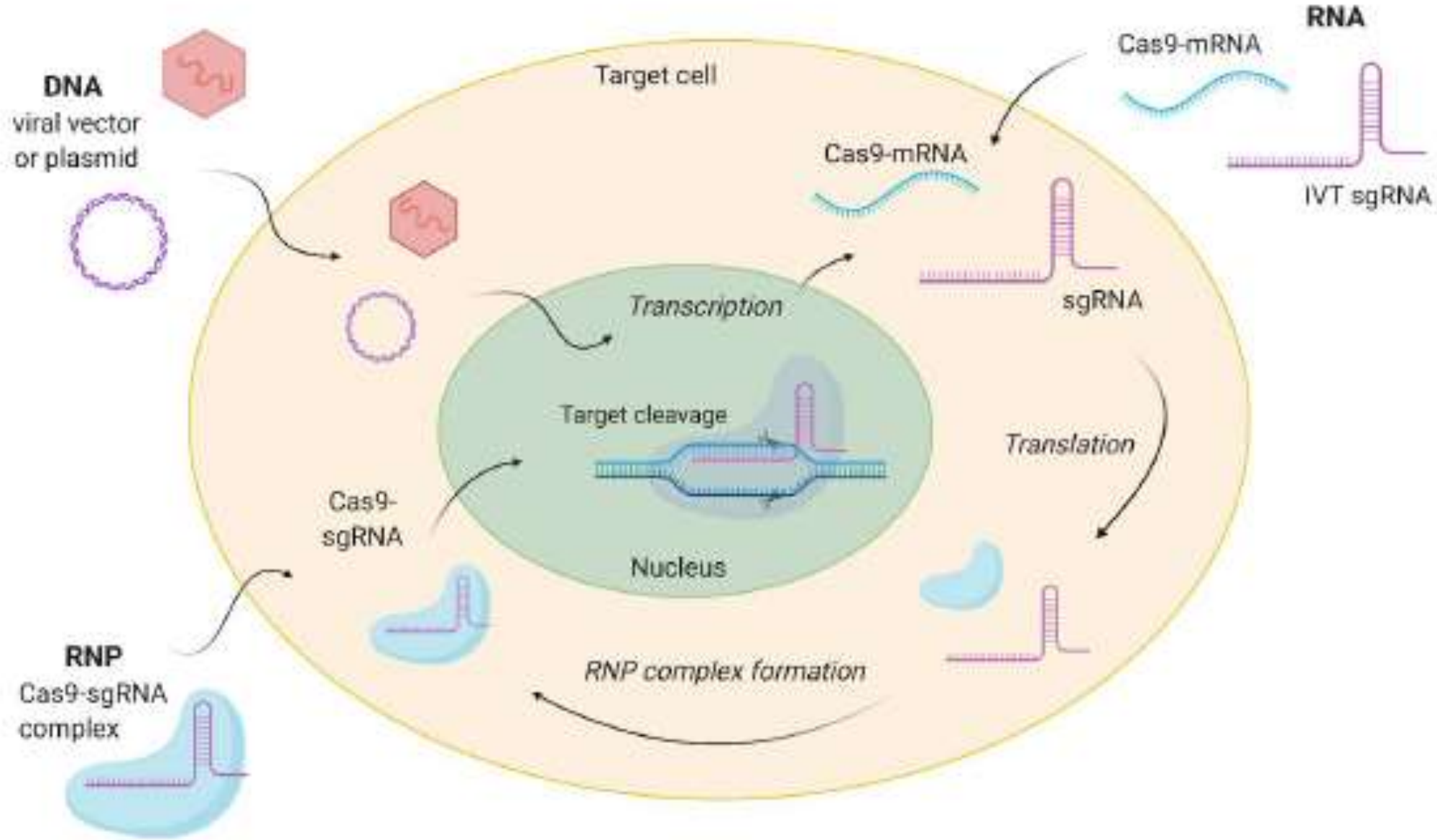
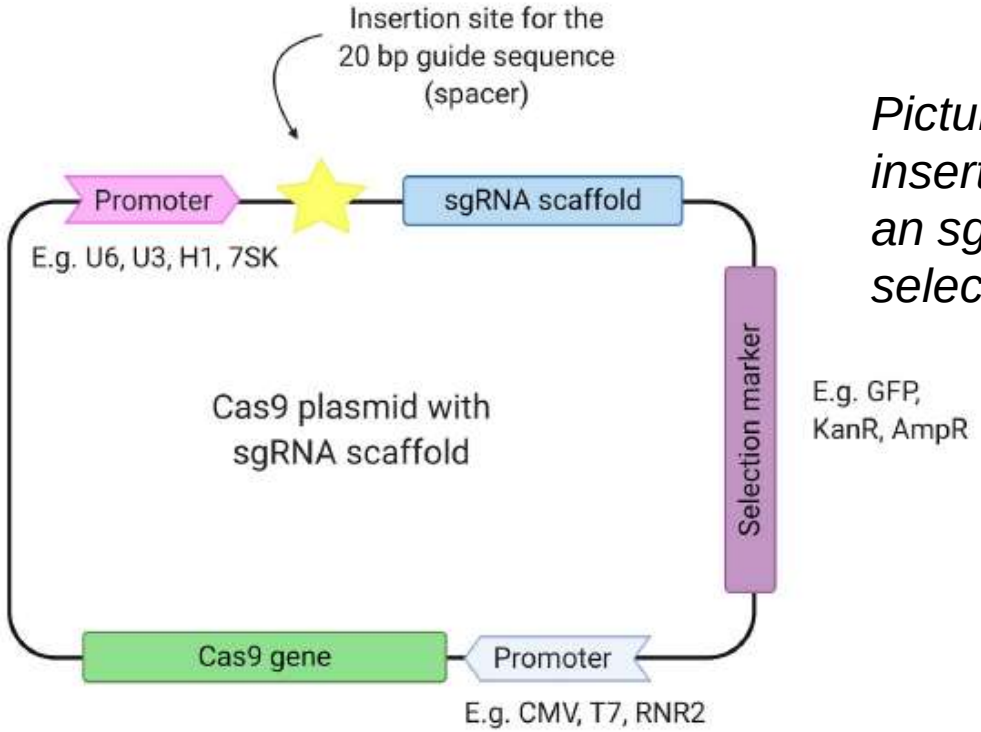
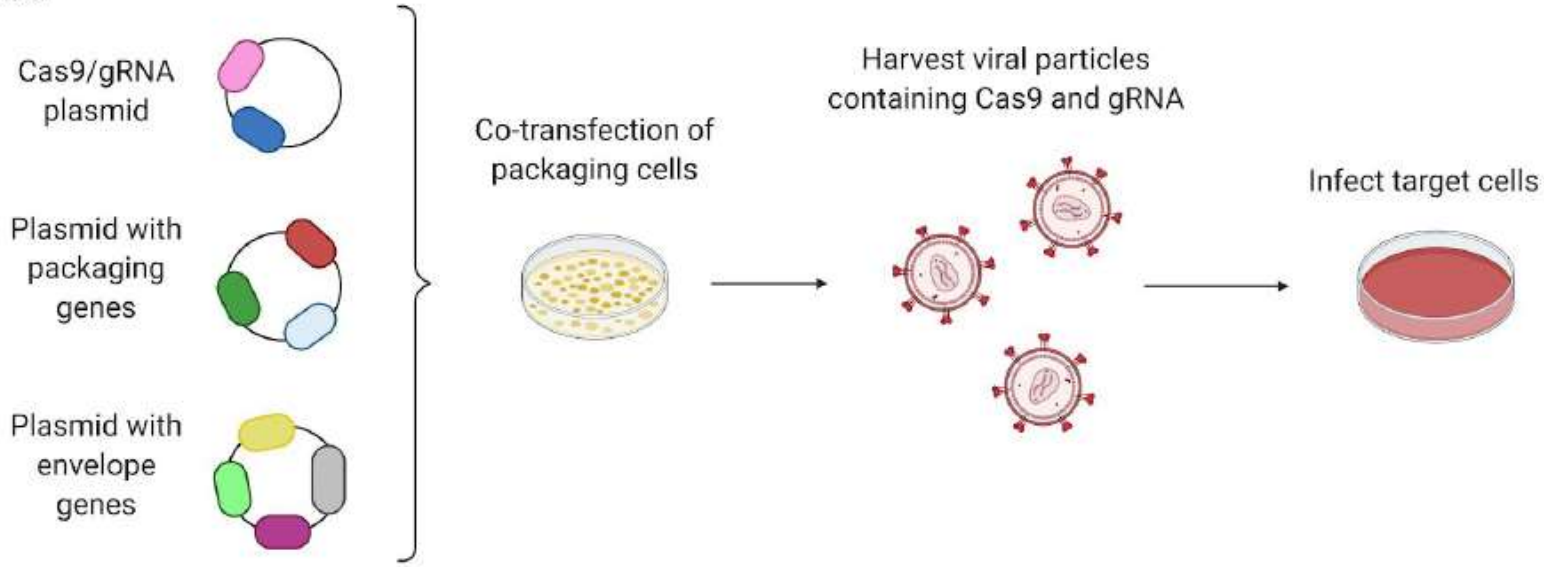


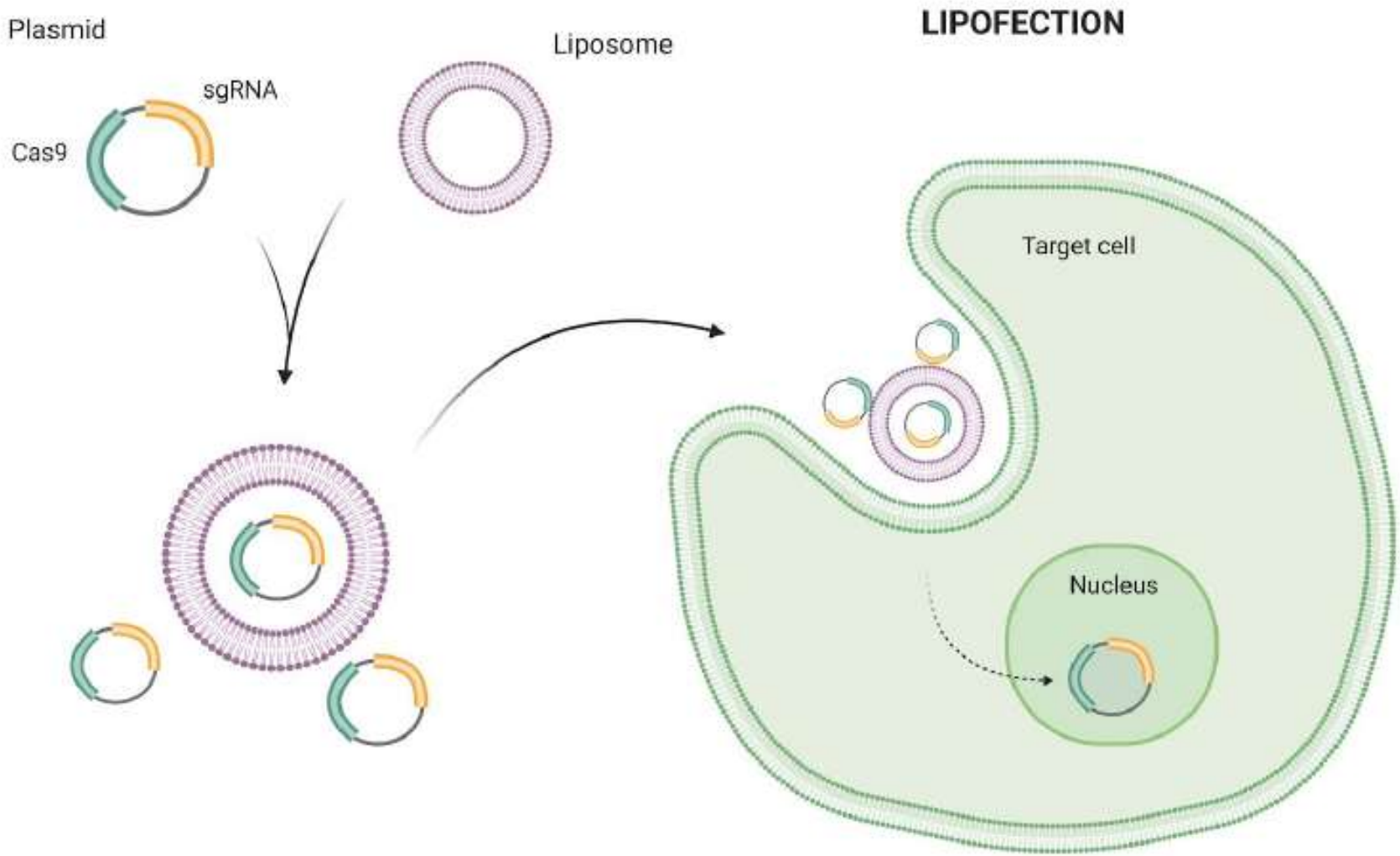
Figure 13 : Transcriptional and translational pathways of the CRISPR components (Cas9 and sgRNA) inserted as DNA, RNAs or RNP complexes into cells.



Pictures a simplified Cas9 plasmid containing promoters, insertion site for 20 bp guide RNA (spacer), an sgRNA backbone, the Cas9 sequence and a potential selection marker gene.

Production of lentiviral vectors containing Cas9 and gRNA genes. Several plasmids containing Cas9, gRNA, packaging genes and envelope genes are necessary for the production of viral particles





By mixing a Cas9:sgRNA plasmid with liposomes, CRISPR components can cross the lipid bilayer and be introduced into the cell. The lipid coat acts as a vehicle and protector of the components across the cell membrane