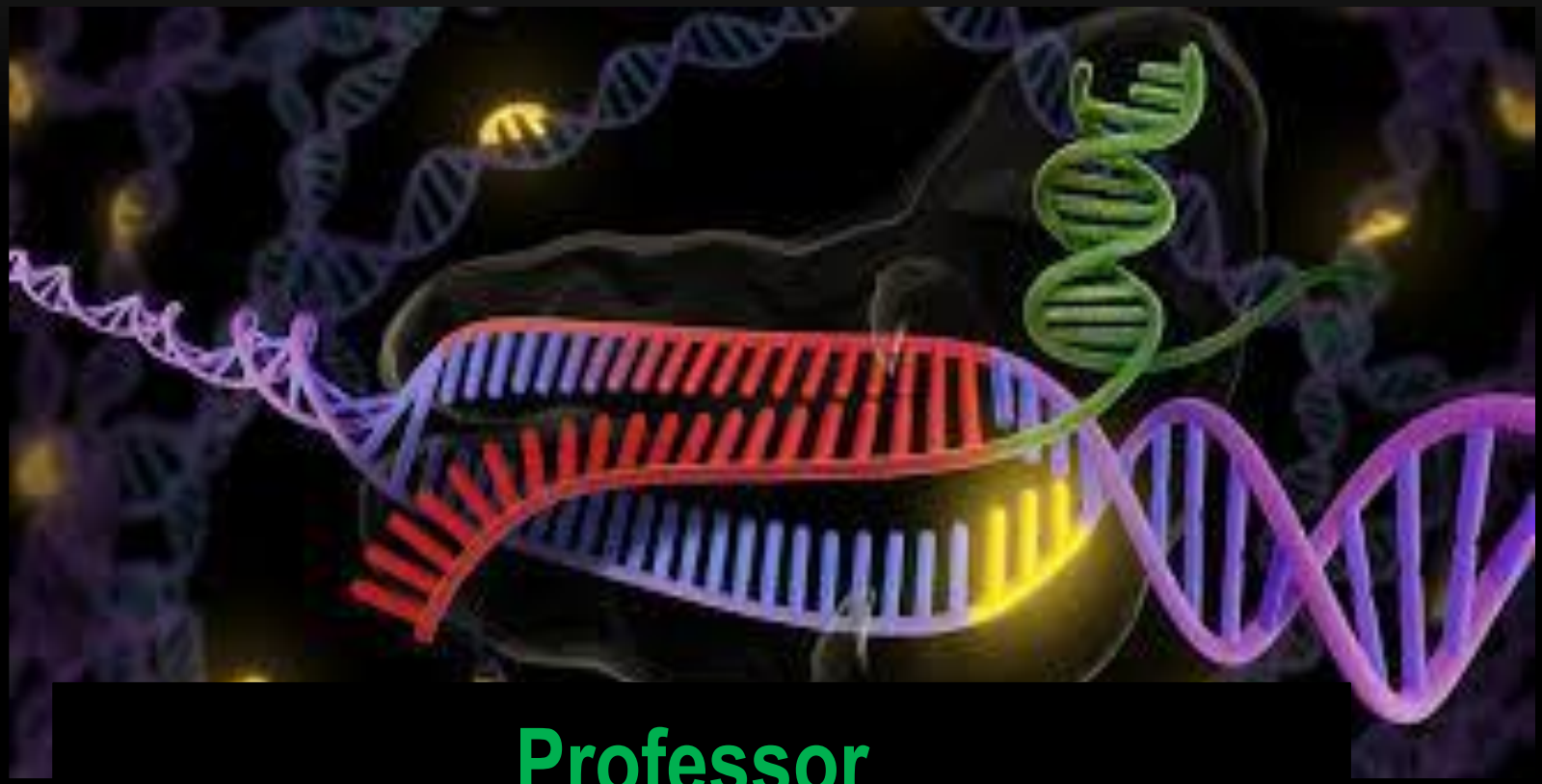
'CRISPR/CAS 9 TECHNOLOGY'



Professor
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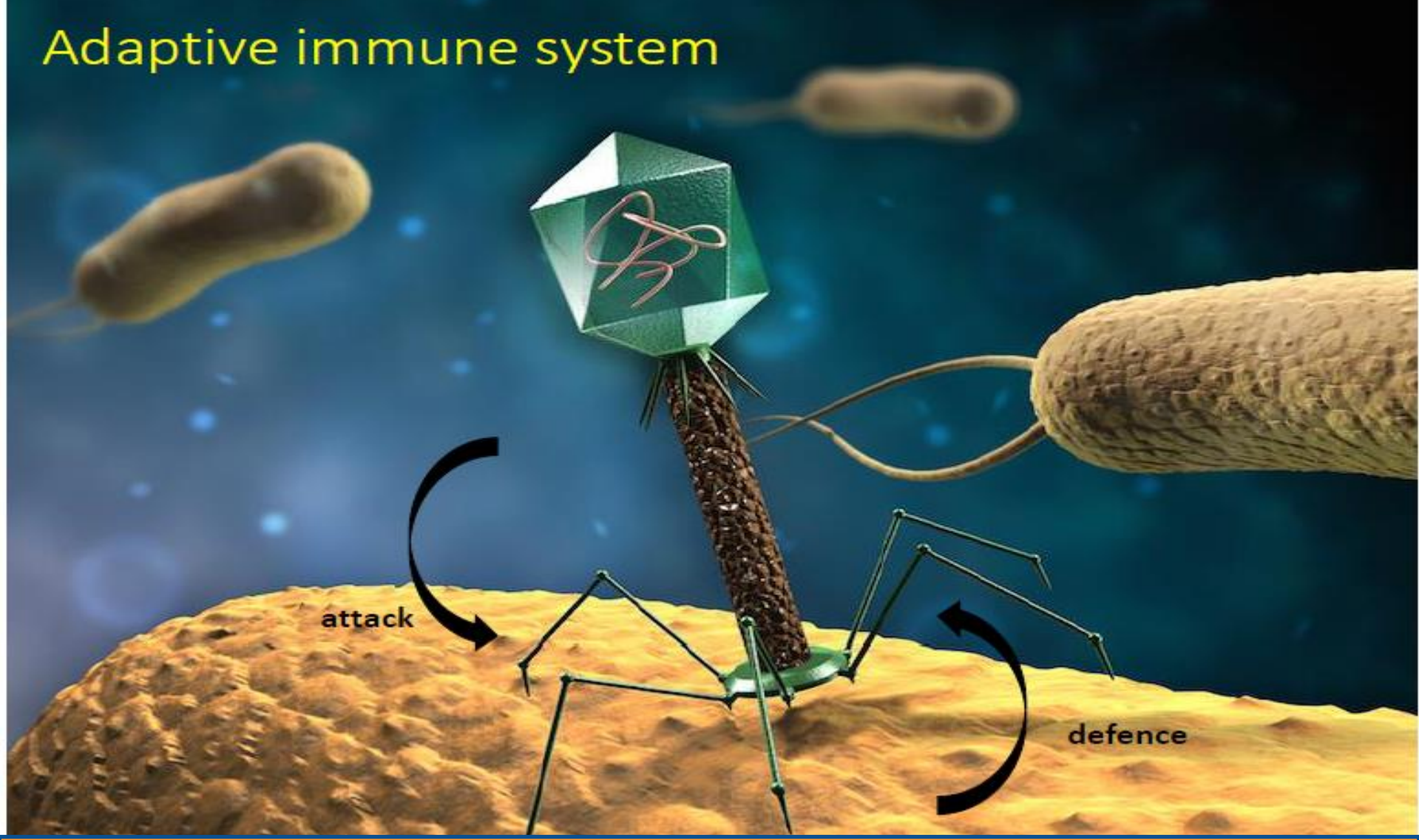
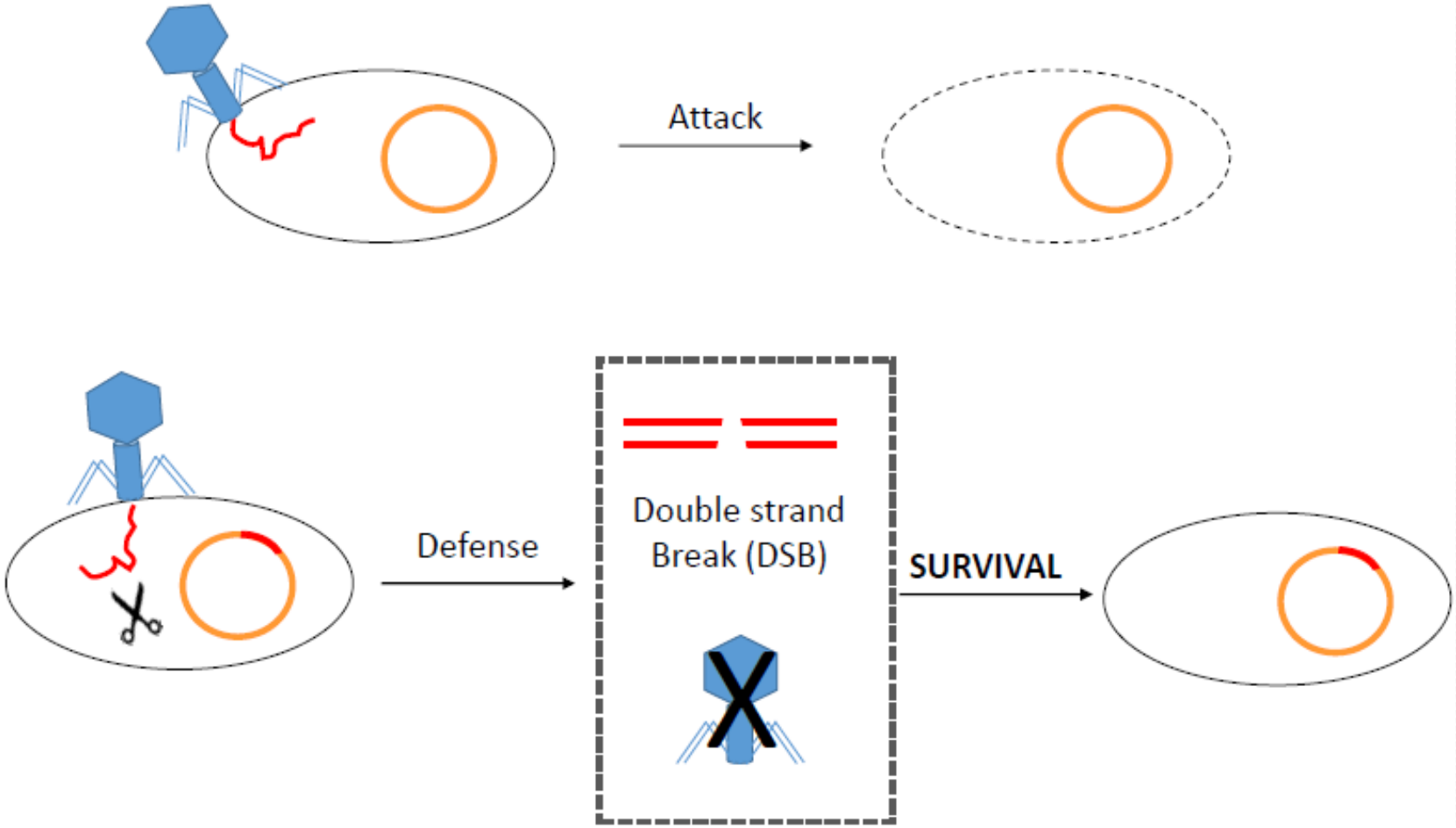
INTRODUCTION

- (clustered regularly interspaced short palindromic repeats) is a family of DNA sequences found within the genomes of prokaryotic organisms such as bacteria and archaea.
- These sequences are derived from DNA fragments from viruses that have previously infected the prokaryote and are used to detect and destroy DNA from similar viruses during subsequent infections. Hence these sequences play a key role in the antiviral defense system of prokaryotes.
- termed by Francis Mojica in 1990



The CRISPR-Cas system is a prokaryotic immune system that confers resistance to foreign genetic elements such as those present within plasmids and phages that provides a form of acquired immunity

Adaptive immune system



Cas9 (CRISPR associated protein 9)

is an **RNA-guided DNA endonuclease enzyme** associated with the CRISPR adaptive immunity system in Streptococcus pyogenes, among other bacteria. S. pyogenes utilizes Cas9 to memorize and later interrogate and cleave foreign DNA, such as invading bacteriophage DNA or plasmid DNA.

Cas9 performs this interrogation by unwinding foreign DNA and checking for sites complementary to the 20 base pair spacer region of the guide RNA.

- Cas9 enzymes together with CRISPR sequences form the basis of a technology known as CRISPR-Cas9 that can be used to edit genes within organisms.
- This editing process has a wide variety of applications including **basic biological research**, **development of biotechnology products**, and **treatment of diseases**

CRISPR COMPONENTS

- **crRNA/ CRISPR RNA :** Guide RNA present in Host DNA that bind with tracrRNA and form hairpin

complex

.

- **tracrRNA :** Trans active RNA that

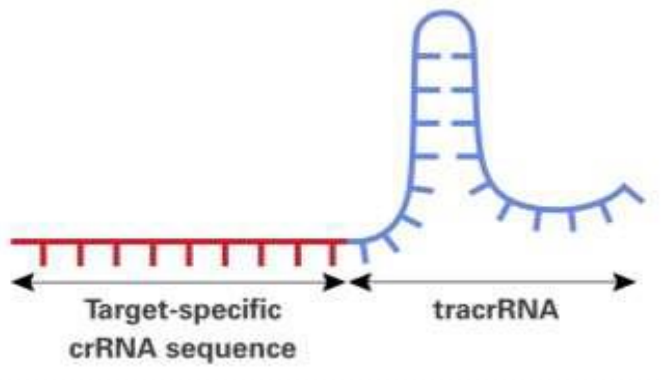
bind with crRNA form active complex

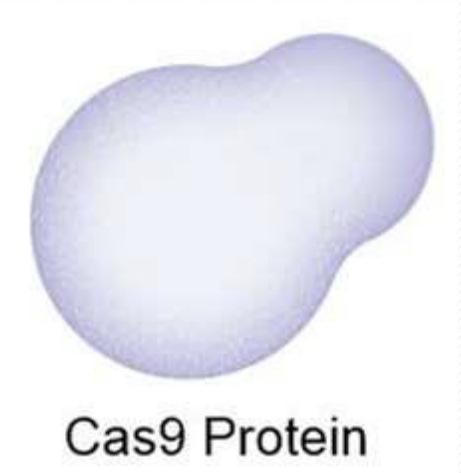
.

- **sgRNA :** Single guide RNA

(crRNA + tracrRNA)

- **Cas9 :** Protein / nuclease that can modify DNA



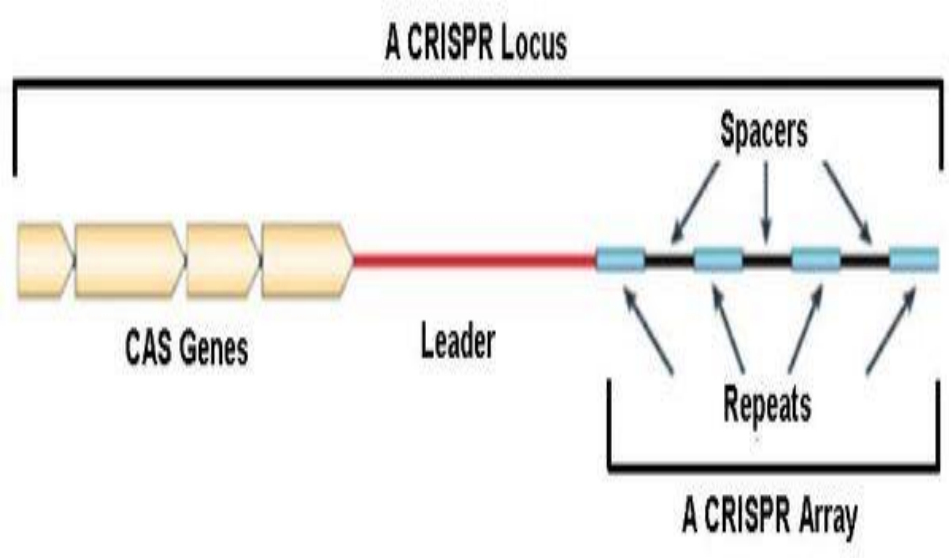


- Cas9 participates in the processing of crRNAs , and is responsible for the destruction of the target DNA . Cas9's function in both of these steps relies on the presence of two nuclease domains
- To achieve site-specific DNA recognition and cleavage, Cas9 must be **complexed** with both a crRNA and a separate trans-activating crRNA (tracrRNA or trRNA), that is partially complementary to the crRNA .
- The tracrRNA is required for crRNA maturation from a primary transcript encoding multiple pre-crRNAs. This occurs in the presence of RNase III and Cas9

CRISPR locus

- It occur in a bacterial chromosome

- ❖ array of identical repeats
- ❖ Invader target DNA spacer
- ❖ Operon of CAS genes that encoding Cas protein components
- ❖ Leader



- The locus includes an array of alternating spacers and palindromic direct repeats. The identical repeats range between 21 and 47 bp in different loci; the spacers are of constant length but are hypervariable in sequence, their sequences having been derived from previously encountered DNA phages or plasmids. The entire array is transcribed as a single mRNA under the direction of a promoter located in the leader sequence.
- Other CRISPR-associated genes (CAS genes) encode the CAS proteins that add new spacer-repeat pairs, process the CRISPR transcript, and cleave the recognized foreign DNA.
- In a single array, repeats are almost always identical with respect to size and sequence

Leader

- . A sequence of up to 550 bp is located 5′ to most CRISPR loci, directly adjoining the first repeat. This common sequence has been denoted the ‘leader’ and is usually AT-rich.

The leader has also been suggested to act as the promoter of the transcribed CRISPR array, as it is found directly upstream of the first repeat

- Spacers. In any CRISPR system, spacers are generally unique, with a few exceptions that are thought to have resulted from segmental duplications.

Mechanism

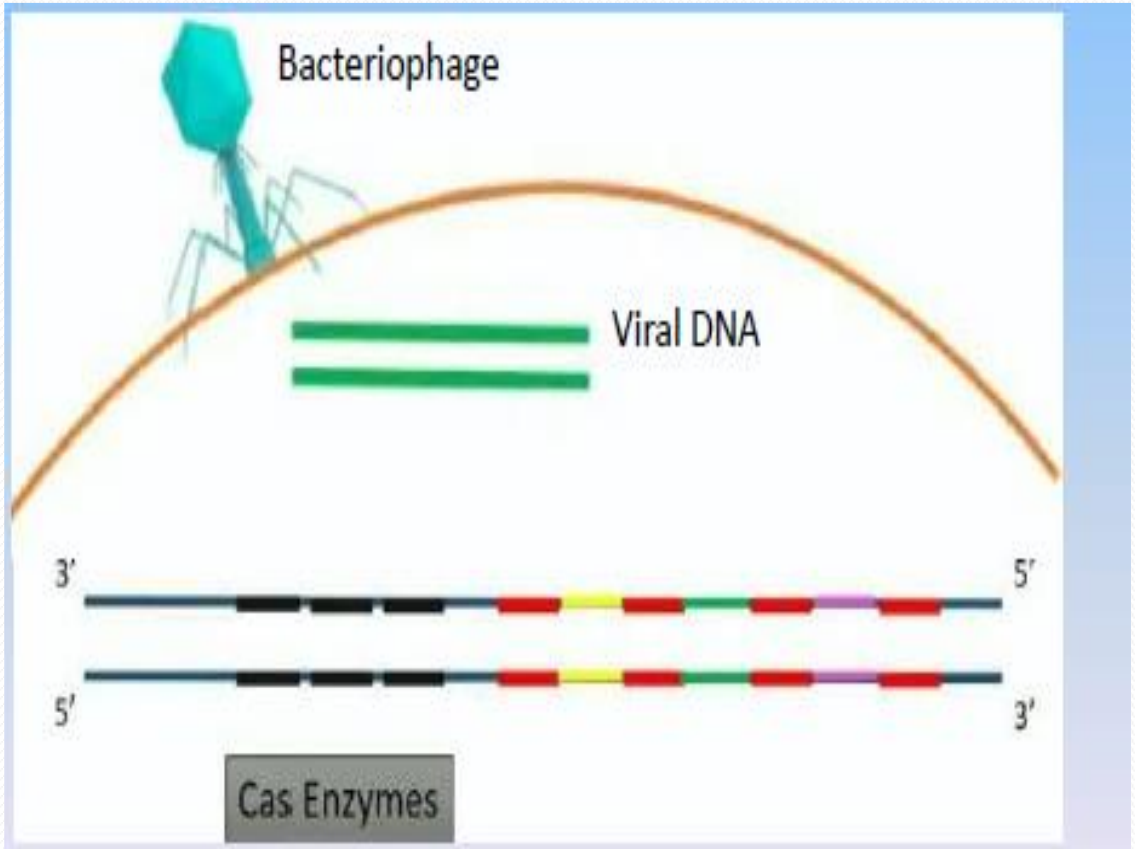
- The CRISPR-Cas mediated defense process can be divided into three stages .

1. adaptation, leads to insertion of new spacers in the CRISPR locus
2. expression, the system gets ready for action by expressing the cas genes and transcribing the CRISPR into a long precursor CRISPR RNA (pre-crRNA). The pre-crRNA is subsequently processed into mature crRNA by Cas proteins and accessory factors
3. interference, target nucleic acid is recognized and destroyed by the combined action of crRNA and Cas proteins

adaptation

Acquisition of spacer DNA with the insertion of pieces of viral genome in between CRISPR repeats.

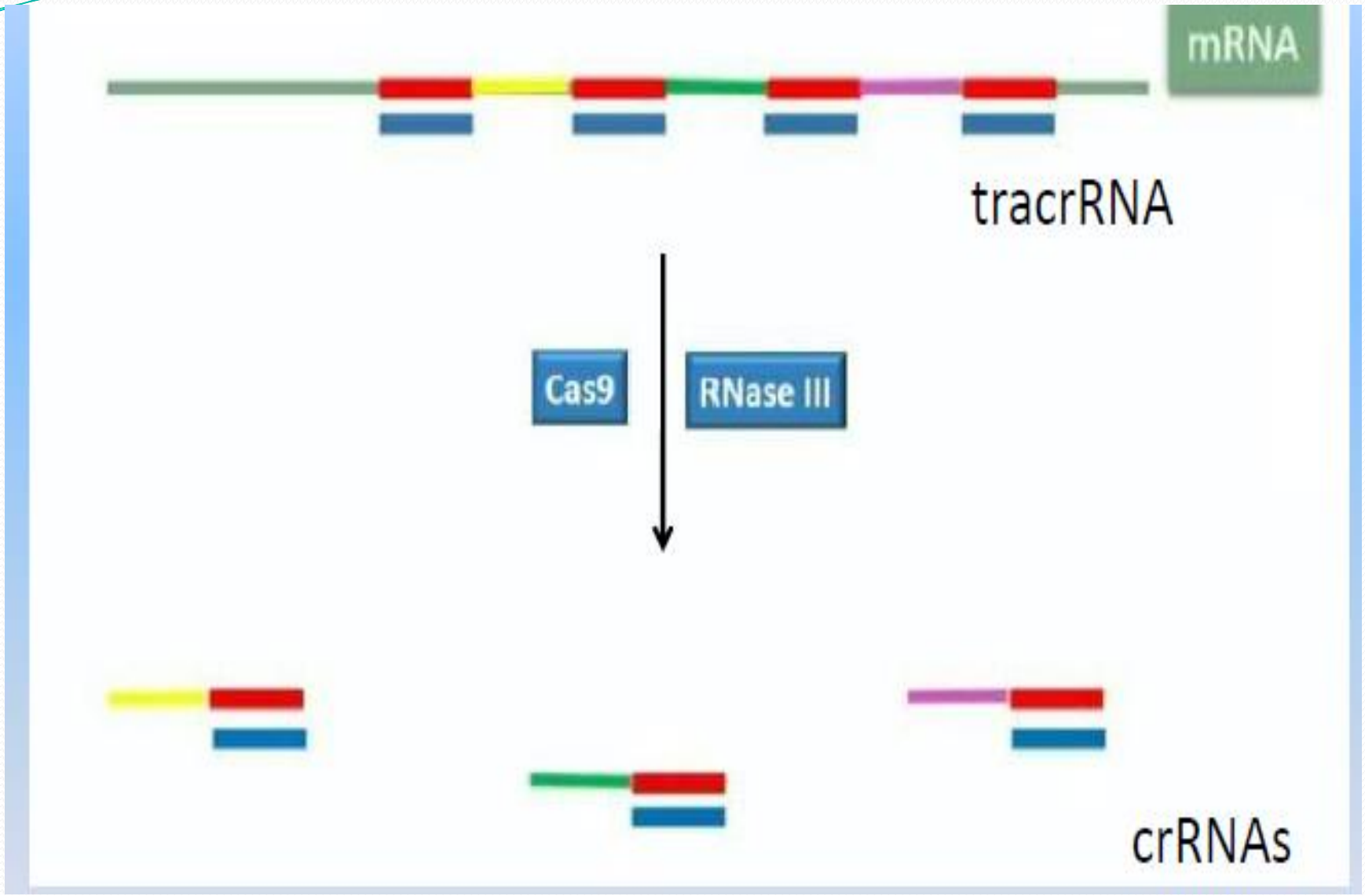
Repeats = 28-37 bp
Spacer DNA = 32-38 bp



Assisting enzymes – Cas1 (endonuclease), and Cas2 (endoribonuclease)

Expression

- Transcription into mRNA that is complementary to Template.
- tracrRNA bind to CRISPR repeats.
- mRNA gets chopped off by Cas9 and RNAse III into individual crRNAs.


mRNA
tracrRNA
Cas9
RNase III
crRNAs

Interference

- crRNAs get integrated with a Cas9 protein to form effector

complexes.

CRISPR repeats take hairpin like looped form.

[Fact: The bacterial genome preferably takes in a part of the viral

genome only from the portion adjacent to PAM

sequence(Protospacer Adjacent Motif) which is targeted by the

Cas9 nuclease]

On finding a viral genome containing a strand complementary to

the crRNA itself, it binds to viral DNA.

- Cas9 induces a double strand cut in the viral DNA

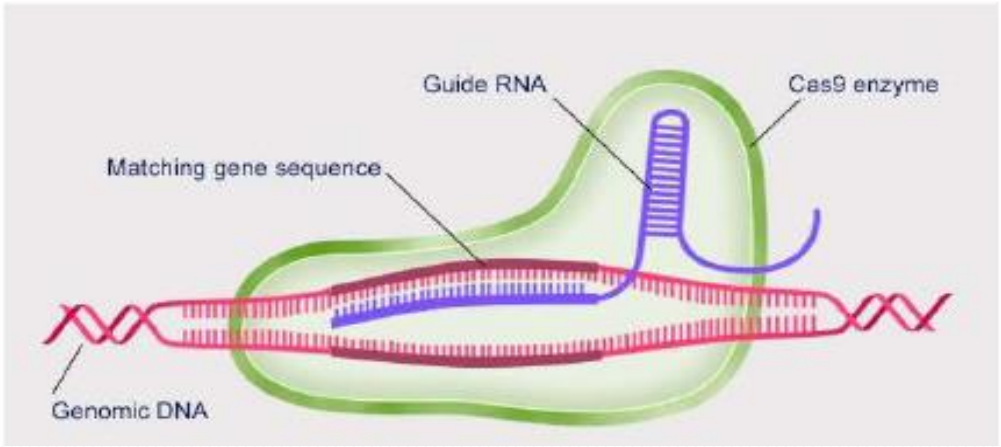
crRNAs

Cas Protein

Effector Complex

PAM

Genomic DNA
Break apart



Adaption

Expression

Interference

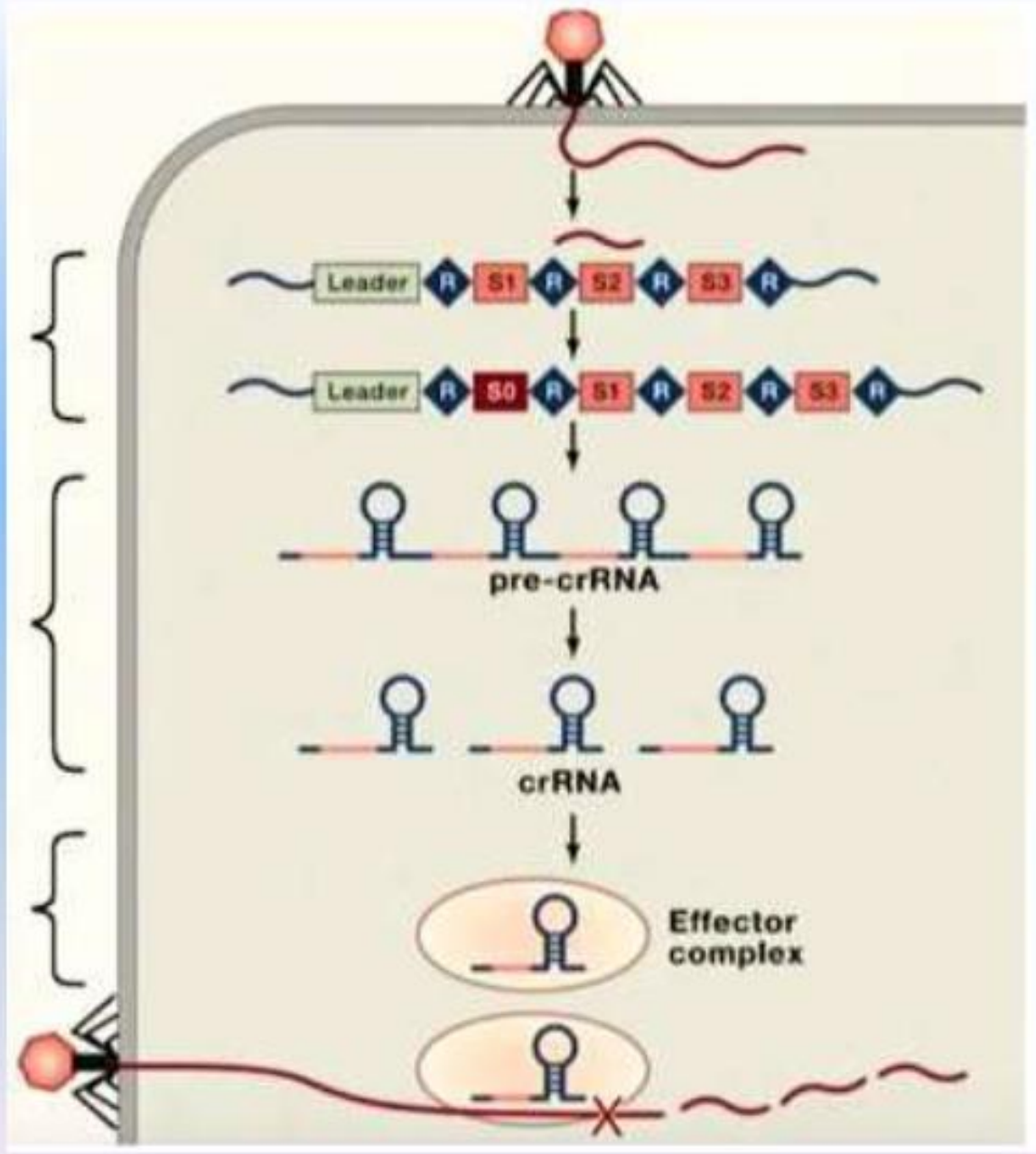


Fig: Mechanism Flowchart

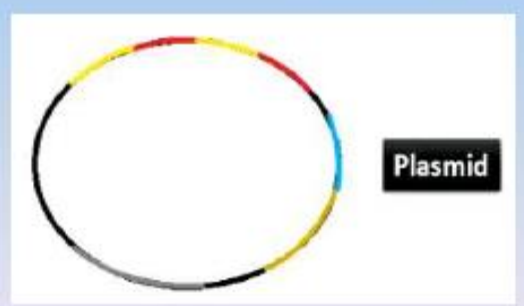
CRISPR – CAS9 TECHNIQUE

- Technique that can be used to make targeted cuts in DNA, applicable in genetic engineering.

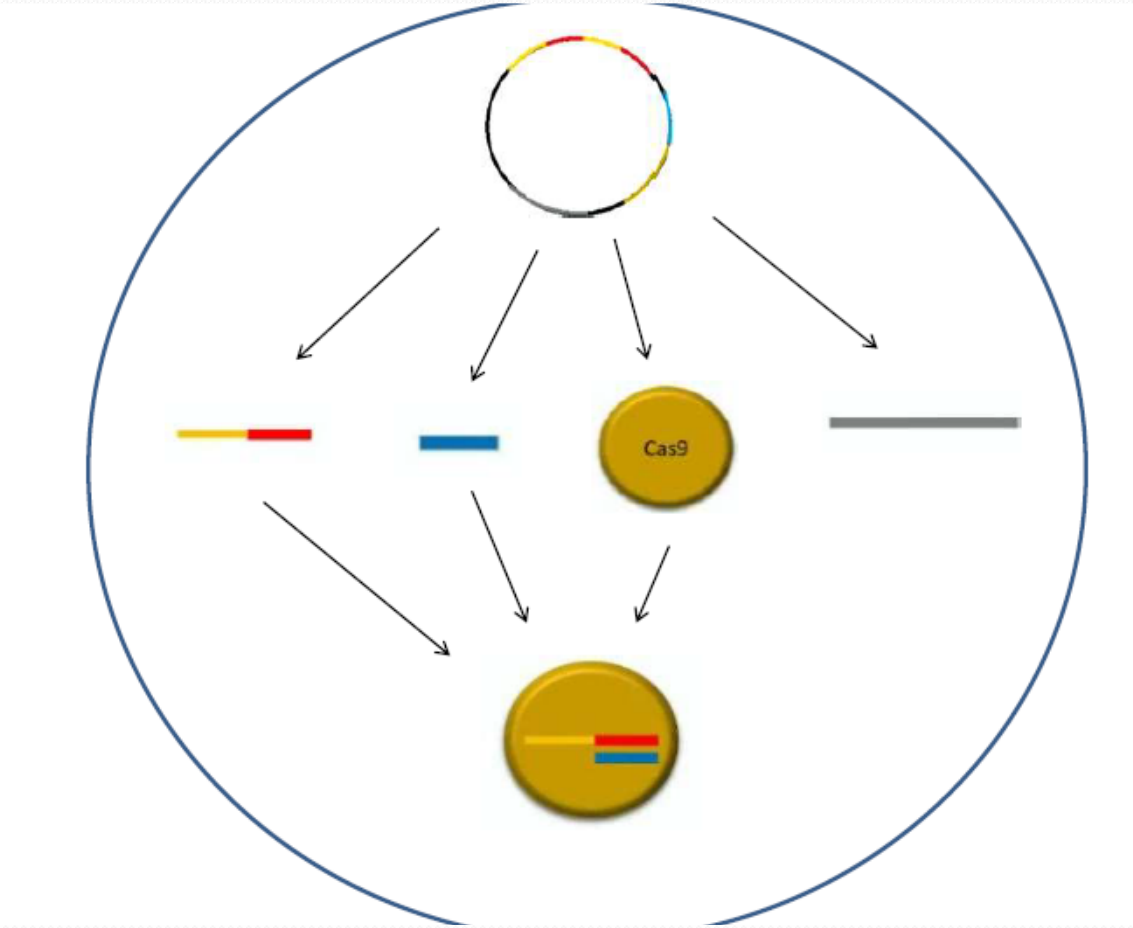
- Can be delivered in embryonic stem cells of animals.

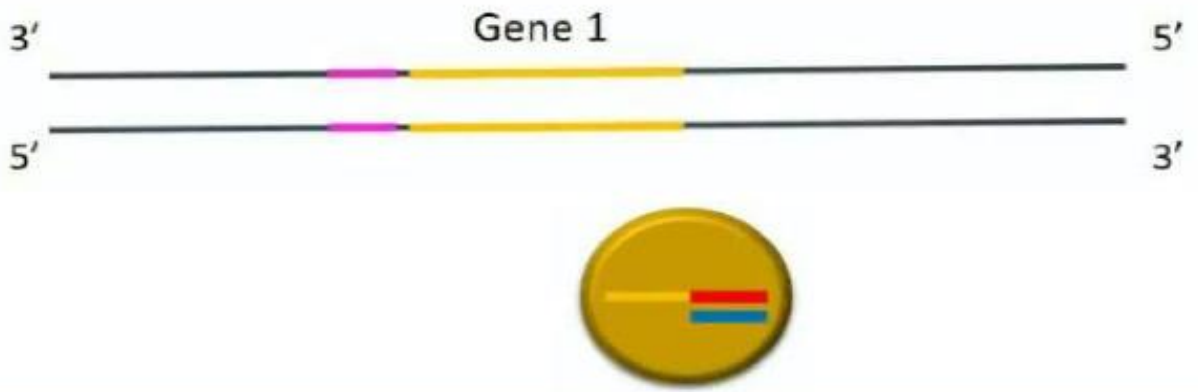
Requirements:

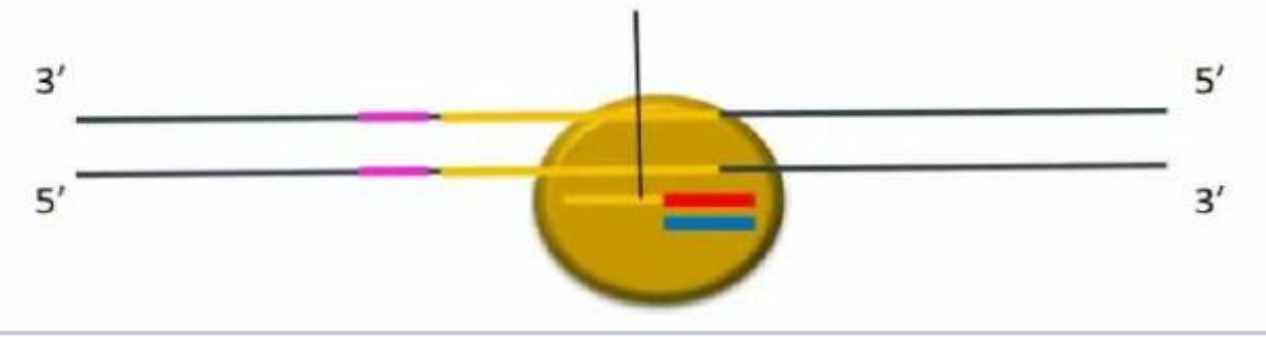
- Design a plasmid that can transcribe every single piece of the required components



- Can be induced through – (a) Electroporation
  (b) Viruses (lentivirus, adenovirus)


Cas9


Gene 1
3′
5′
5′
3′


3′
5′
5′
3′


3′
5′
5′
3′


3′
5′
5′
3′


3′
5′
5′
3′
Ligase


3′
5′
5′
3′

Applications of CRISPR

- **1. Fighting cancer**
- **2. Extracting HIV**
- **3. Making diseases self destruct**
- **4. Improving IVF(Humane Genome Editing)**
- **5. Eliminating malaria**
- **elimination of malaria in mosquitos, in the hope of stopping human infection. Through CRISPR Cas 9, scientists can snip out genes that are vital to the spread of malaria within the mosquito population**
- **6. Protecting plants**
- **7-Producing food**
- **8. Creating biofuel**
- **9. Reviving extinct mammals**
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