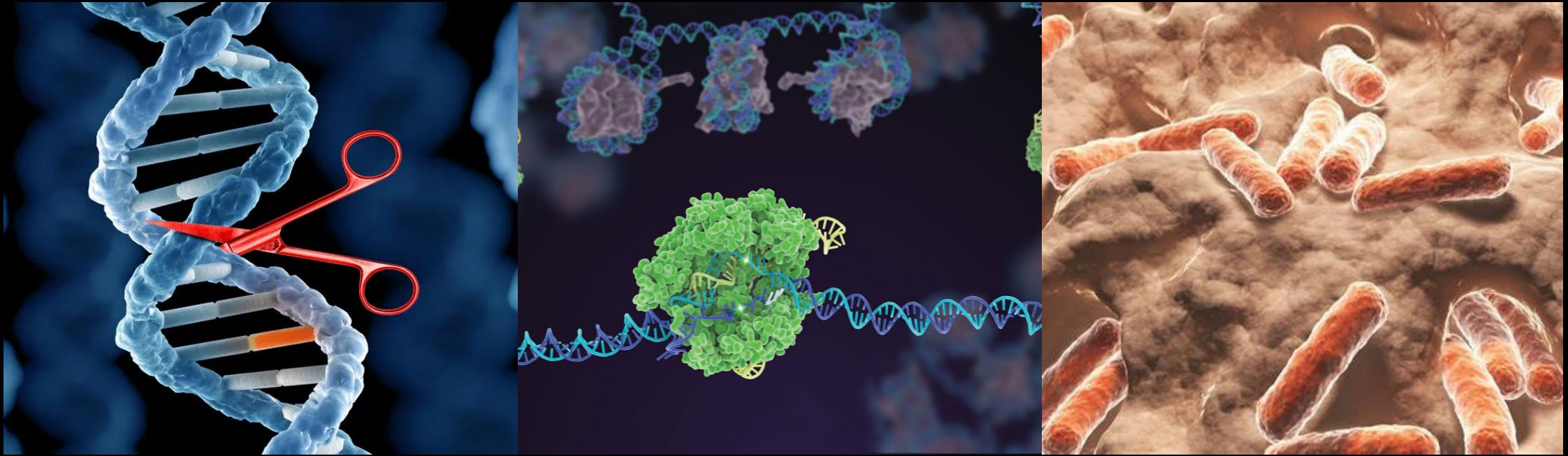




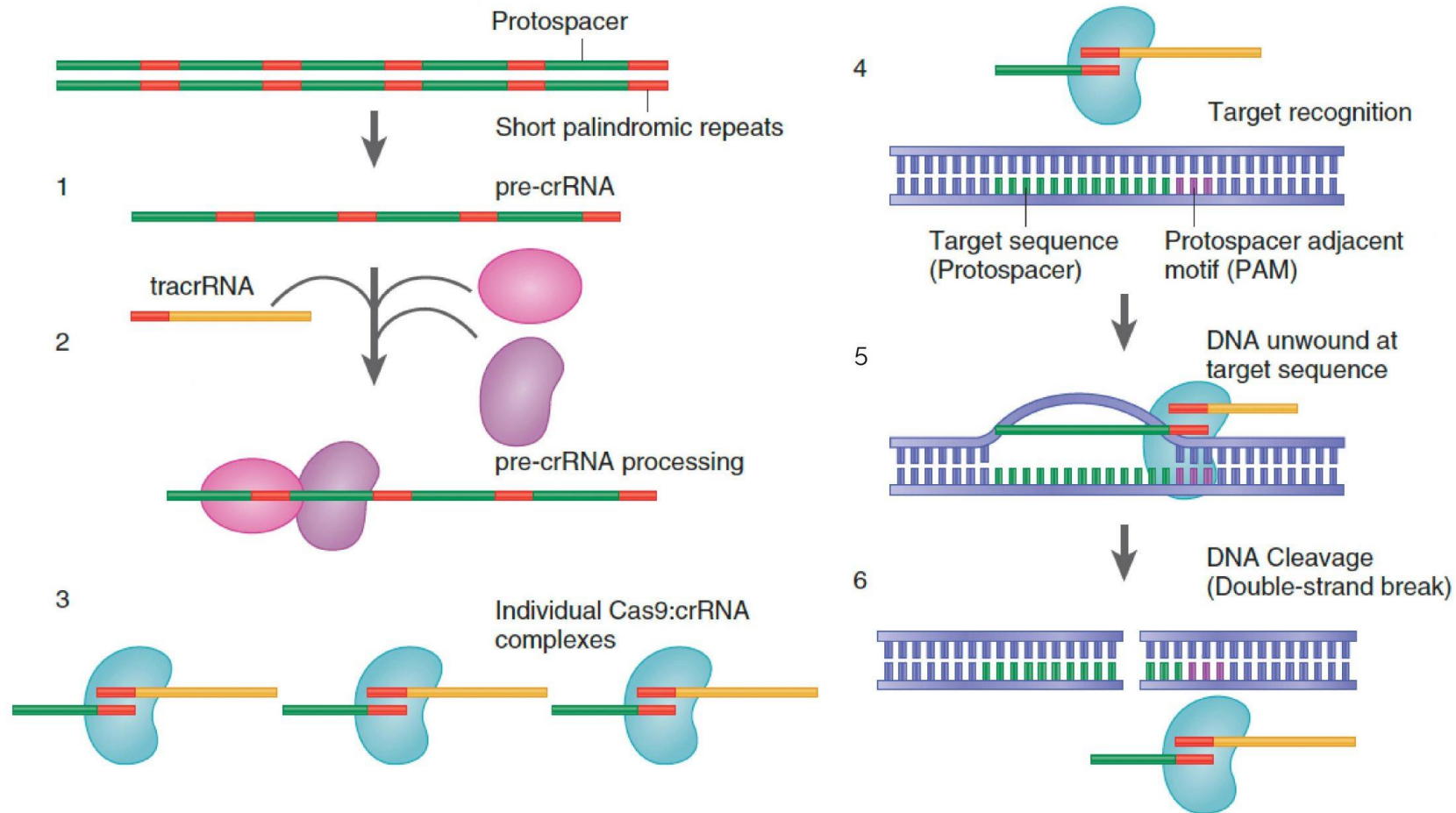
Presenting a Method in Environmental Microbiology and Microbial Ecology

CRISPR ASSOCIATED PROTEIN 9

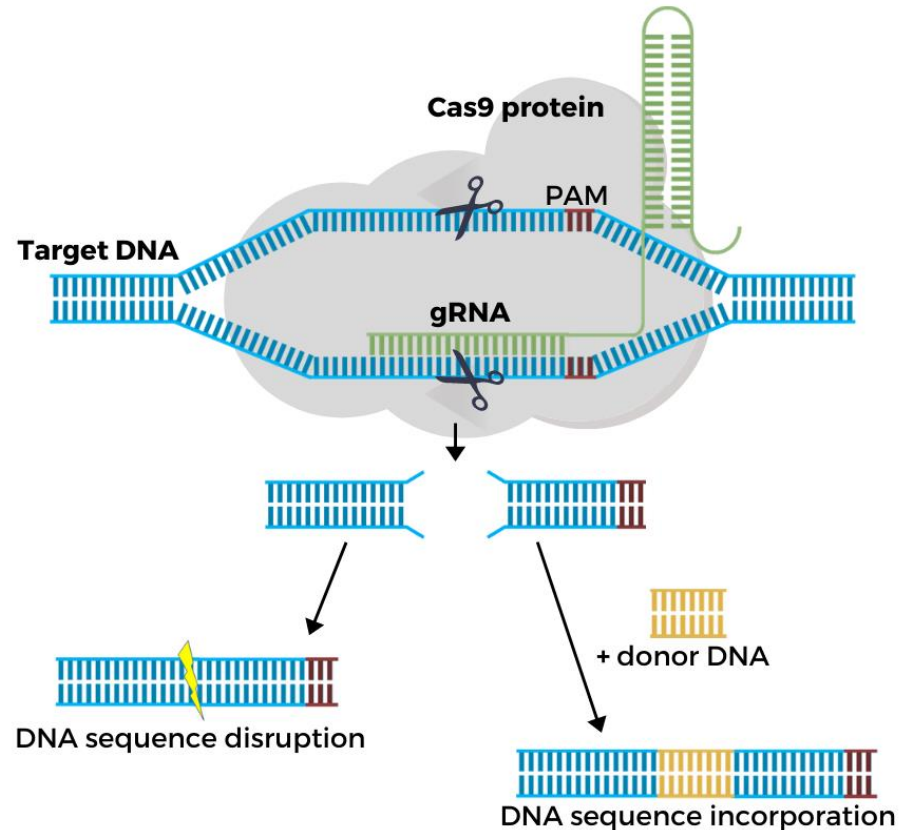
CAPUCINE MONDHARD, ADÈLE SOULIER

Microbial Ecology, Master SIE

CRISPR-Cas9 as a natural bacterial defense mechanism

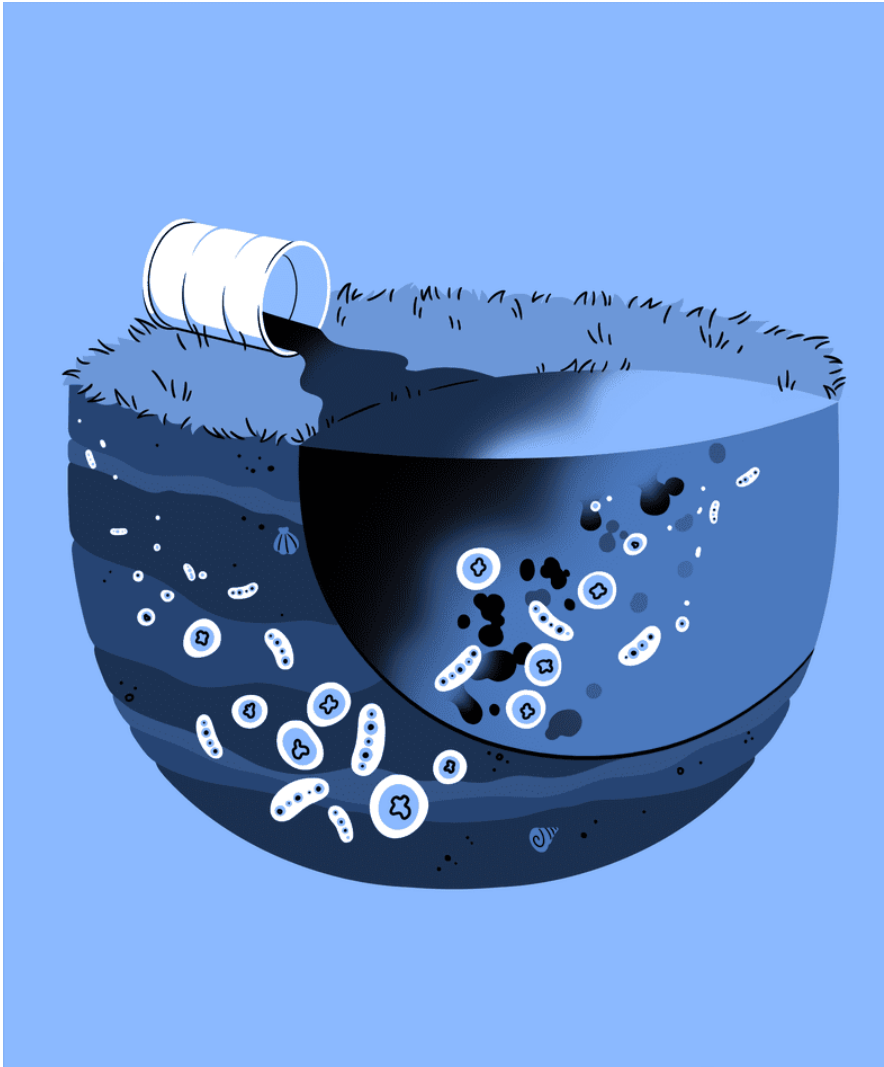


How do scientists use CRISPR-Cas9?



Clustered
Regularly
Interspaced
Short
Palindromic
Repeats





CRISPR-cas9 in applied environmental microbiology : BIOREMEDIATION

Enhancing the performance of *Methyloburum extorquens* AAZ-1 by using CRISPR/Cas9 for the degradation of hydrocarbon compounds

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Abstract. An efficient expression method was used to create a genetic modification strategy for methylotrophic bacteria to improve the capacity of bioremediating bacteria to absorb oil in contaminated environments. Using the plasmid-treated CRISPR-Cas9 system, *Methyloburum extorquens* AAZ-1(OR226417.1) was treated, and qPCR analysis revealed that the expression of the *MxaF* gene (encoding methanol dehydrogenase enzyme) rose six-fold. To increase the biodegradation effectiveness of hydrocarbons (n-alkane and PAH), CRISPR-Cas9 technology was selected for the investigation and use in the laboratory. The biodegradation rate of n-alkane in the modified *M. extorquens* AAZ-1 rose from 61.14% to 74.35% during a seven-day incubation period when compared to the control. The proportion of polycyclic aromatic hydrocarbons in the altered *M. extorquens* AAZ-1 rose from 65.69% to 78.23%. To the best of the authors' knowledge, this work is the first time that a CRISPR-Cas9 system has been employed to improve the efficiency of these bacteria in the biodegradation of hydrocarbon molecules.

Keywords: biodegradation, CRISPR-Cas9, methanol dehydrogenase, *Methyloburum extorquens* AAZ-1

INTRODUCTION

A serious global environmental problem is the pollution of soil by crude oil and its compounds. Many hydrocarbon compounds have the potential to change the natural soil or water conditions. They can permeate ecosystems and build up in both plant and animal tissues, resulting in a variety of harmful effects, including cancer induction, mutations, as well as respiratory and brain system problems (Asemoloye *et al.*, 2020; Naveed *et al.*, 2020). Microorganisms may use hydrocarbons as an energy source by decomposing them, so their use in bioremediation procedures is a logical outcome (Ławniczak *et al.*, 2020). Methylotrophs are microorganisms that can produce biomass from single-carbon substances like methane, methanol, or formate, as well as multicarbon substances that lack carbon-carbon bonds. Methylotrophic bacteria have enzymes that seem suitable to employ in the processes of bioremediation and biodegradation due to their

ability to oxidize CH₃OH to HCHO and HCOO⁻, as well as their non-formation of unwanted products (Klein *et al.*, 2022; Qiu *et al.*, 2014).

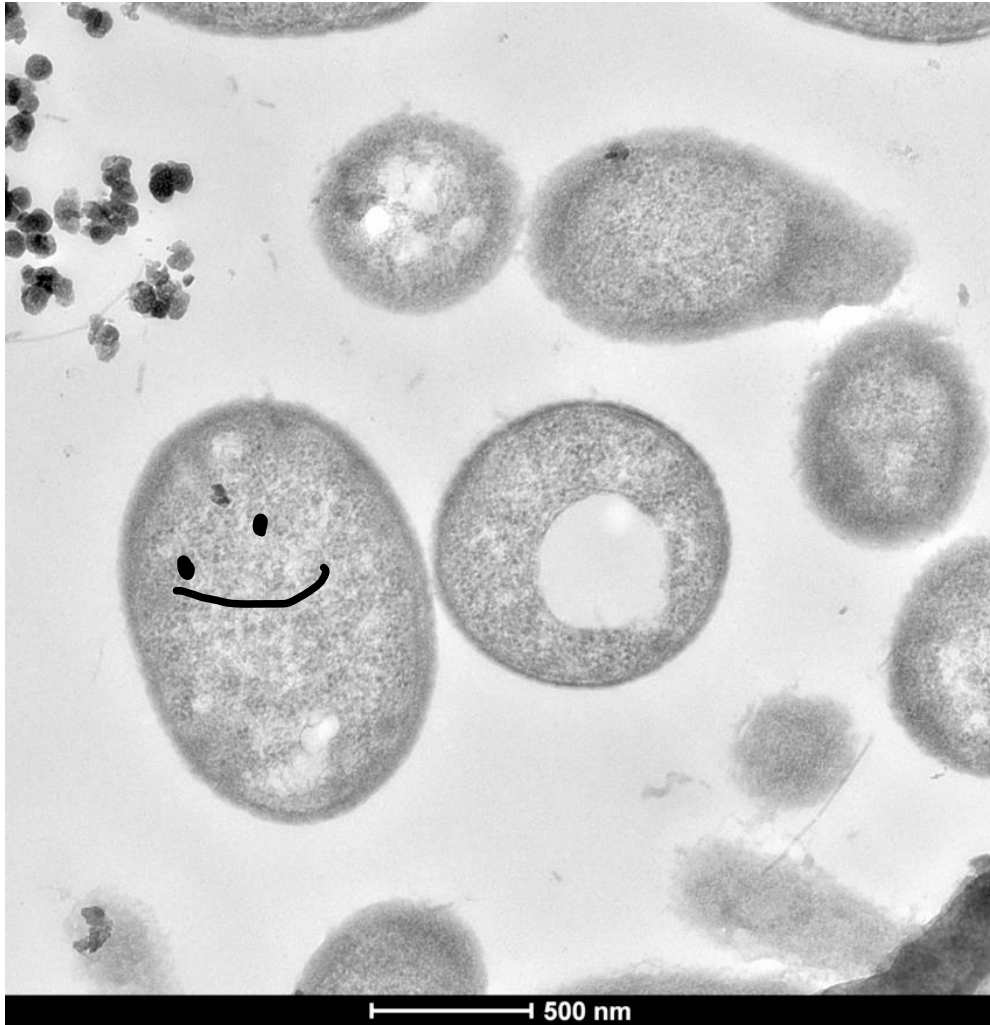
Bioremediation, also known as self-cleaning, relies on bacterial populations already present in contaminated soil to remove oil without the use of any specific technology. Oil bioremediation is acknowledged to be a practical and eco-friendly method on a global scale (Ejaz *et al.*, 2021). The gold standard for researchers studying the genetic underpinnings of physiological and metabolic processes of any organism, particularly the important bacteria used in science and industry, is genome editing (Arroyo-Olarte *et al.*, 2021). The gene-editing approach supplies the best microbe with complex genes, resulting in a more distinct microorganism (Dangi *et al.*, 2019; Basu *et al.*, 2018). As a result, it is possible to obtain changed genetic sequences of distinct wild types (Dai *et al.*, 2018; Stein *et al.*, 2018).

The gene editing procedure begins with the creation of a complementary guide sequence to

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CRISPR-cas9 in applied environmental microbiology : BIOREMEDIATION

"Enhancing the performance of *Methyloburum extorquens* AAZ-1 by using CRISPR/Cas9 for the degradation of hydrocarbon compounds" 2024, A. Maki et al.



DOI:[10.1002/bit.27595](https://doi.org/10.1002/bit.27595)

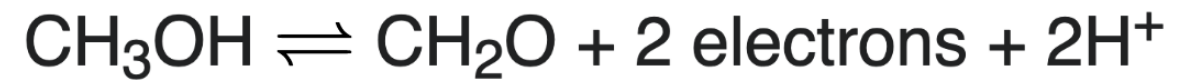
Methylobacterium extorquens

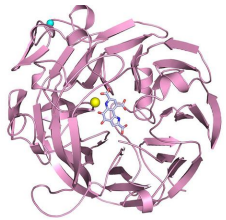


MxaF gene

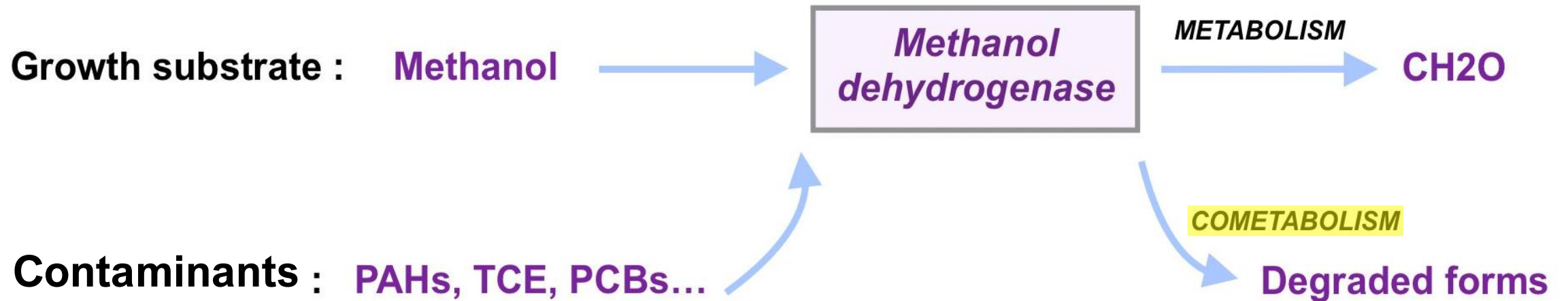


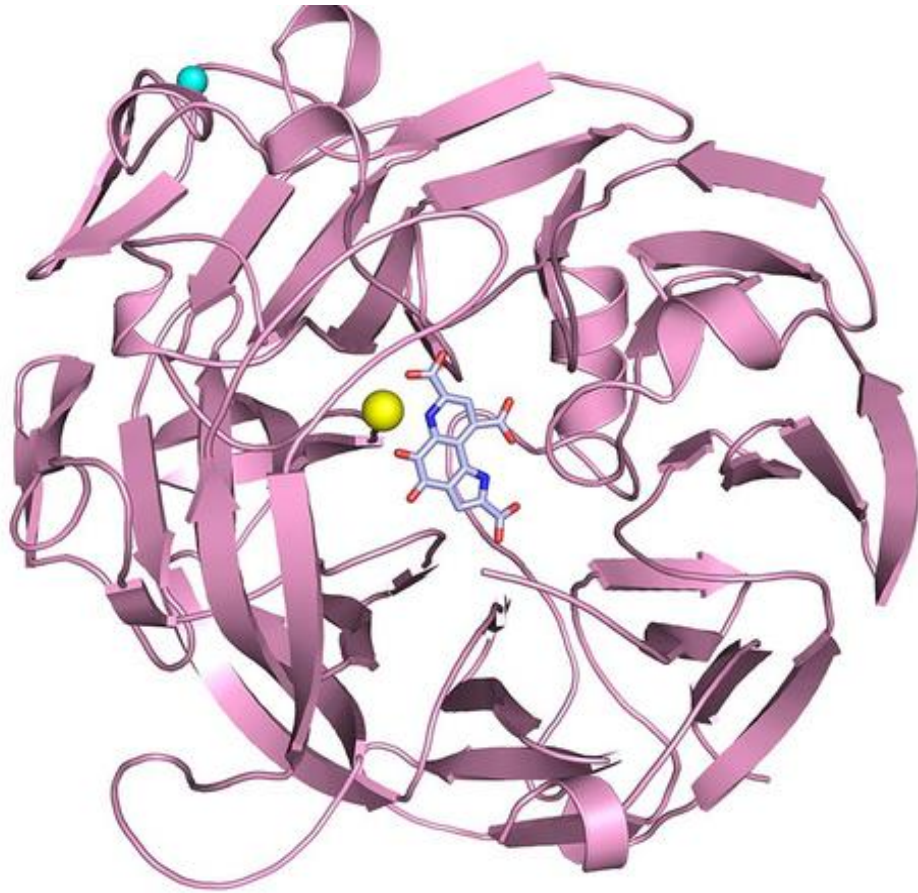
Methanol dehydrogenase enzyme





Methanol dehydrogenase enzyme :





With CRISPR cas9 :



+ expression of *MxaF gene*



Methanol dehydrogenase enzyme



+ degradation of contaminants

The MxaF gene :

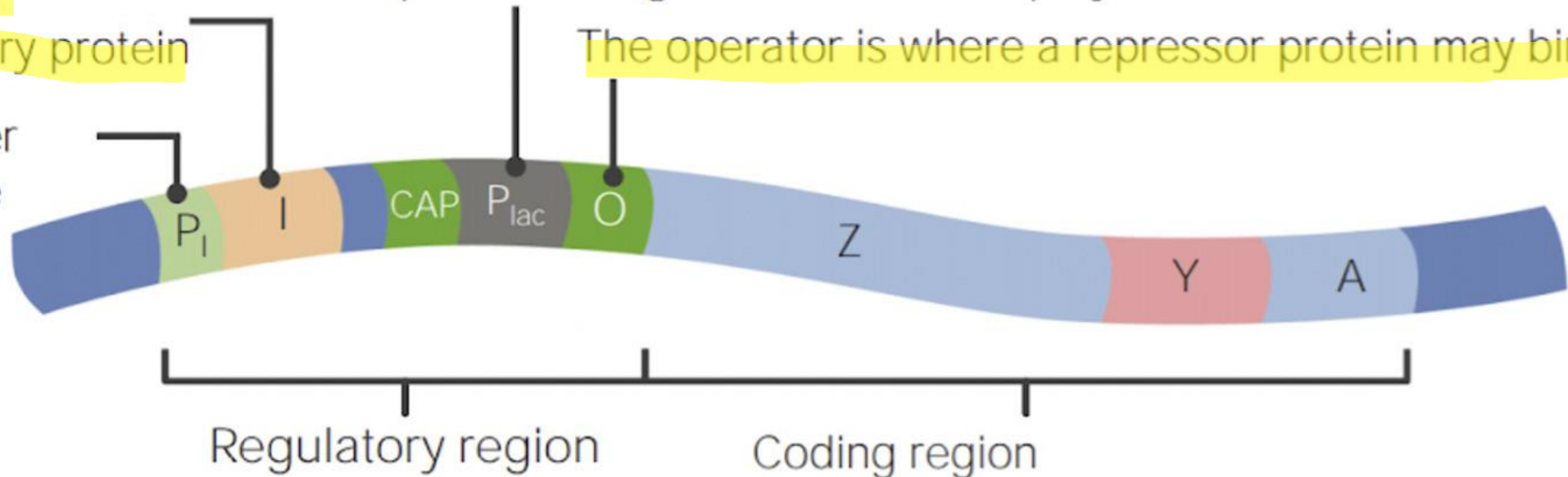
Prokaryotic gene expression is primarily regulated at the level of transcription.

The promoter region is where RNA polymerase binds.

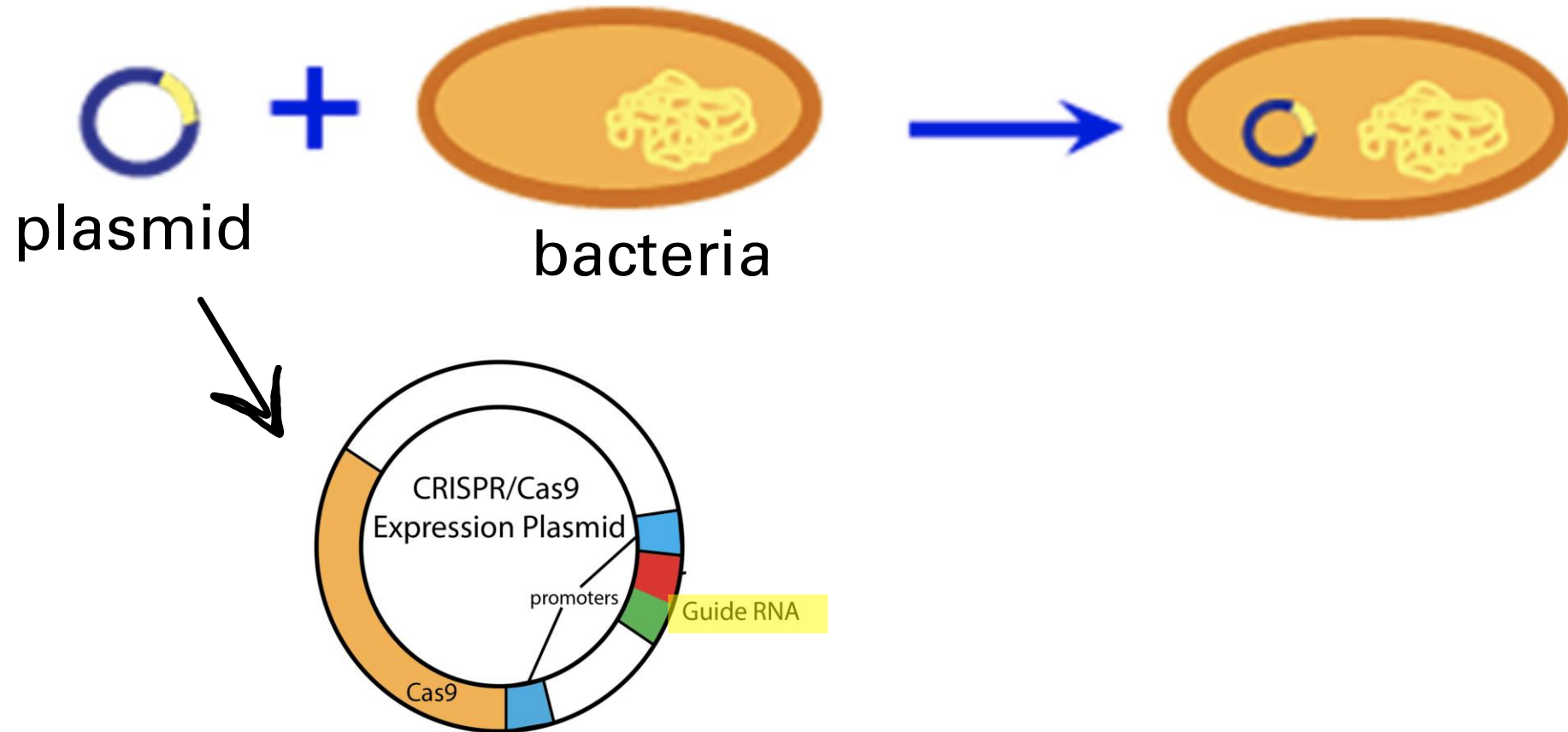
The operator is where a repressor protein may bind.

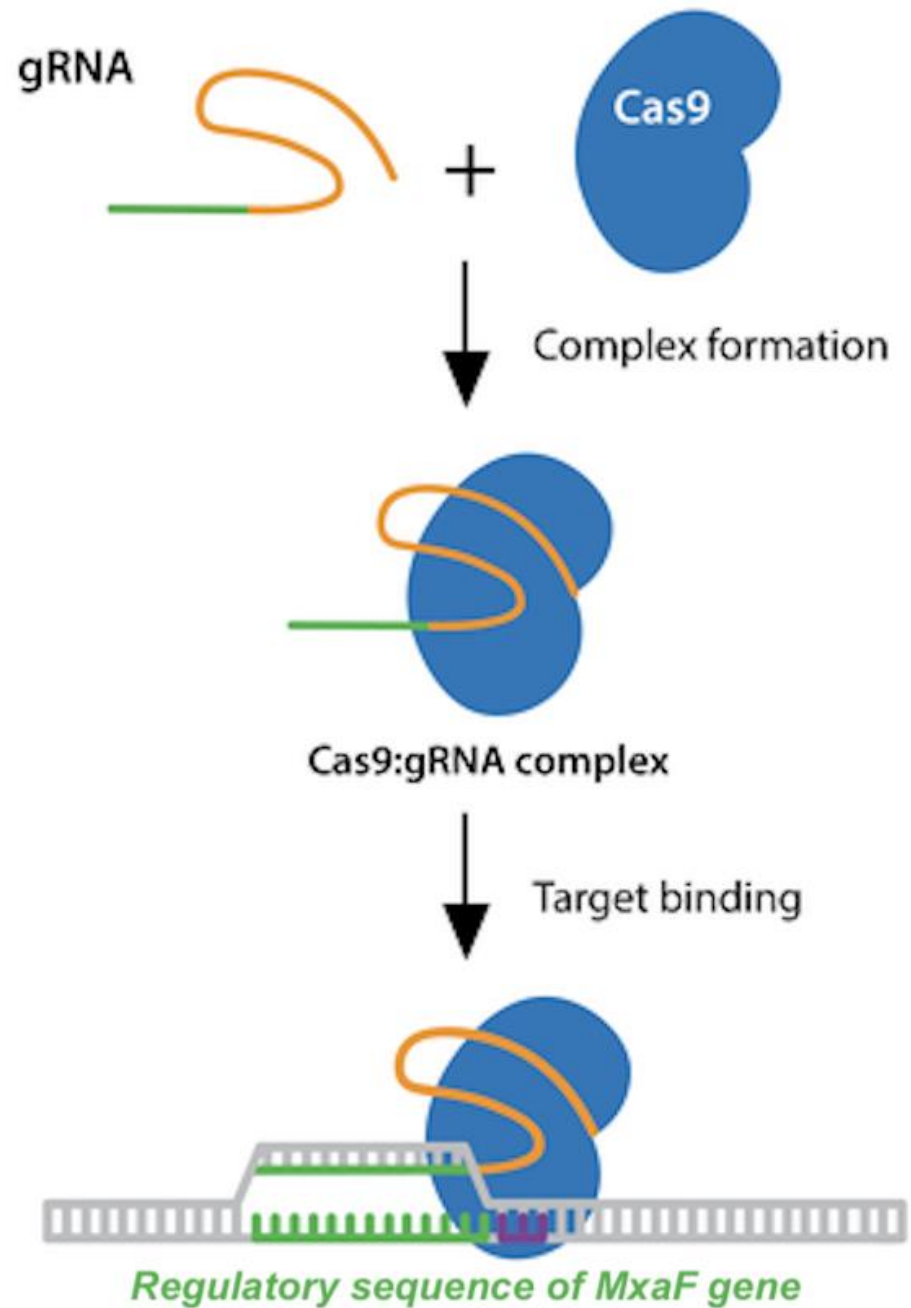
Gene for
regulatory protein

Promoter
for gene

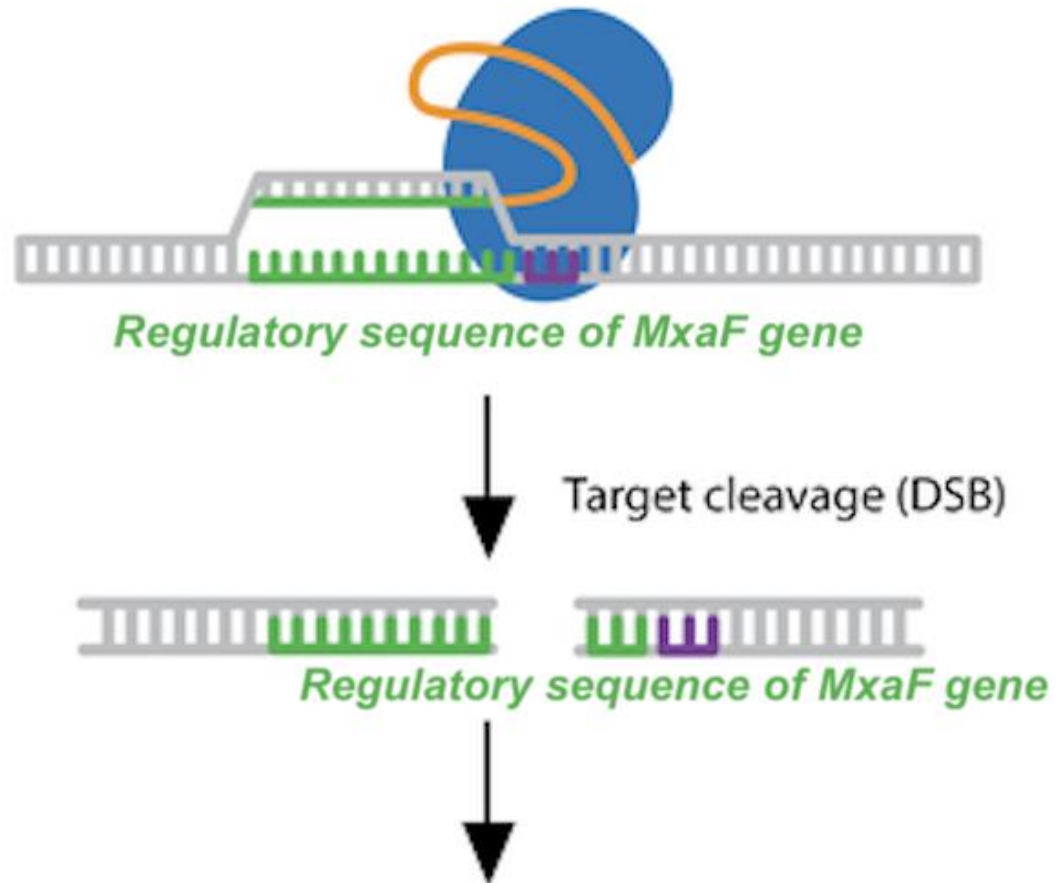


Method : CRISPR-cas9 plasmid insertion





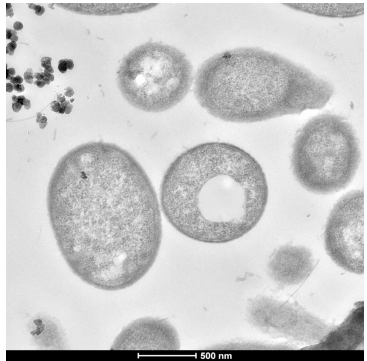
**Method : how does
CRISPR-cas9 act ?**



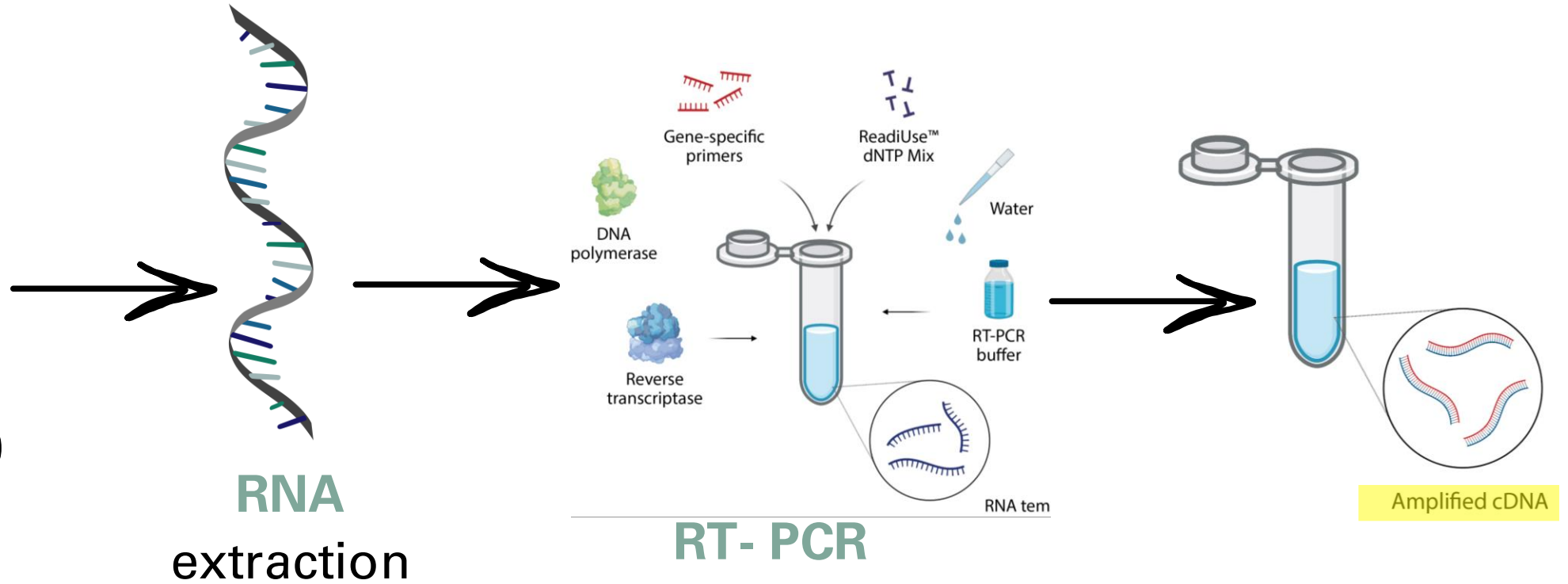
- Removal or editing of repressors that limit the gene expression
- Insertion or modification of sequences to strengthen the binding of RNA polymerase [...]

Method : how does
CRISPR-cas9 act ?

Results : Enhanced expression of MxaF Gene



CRISPR cas9
modified
bacteria



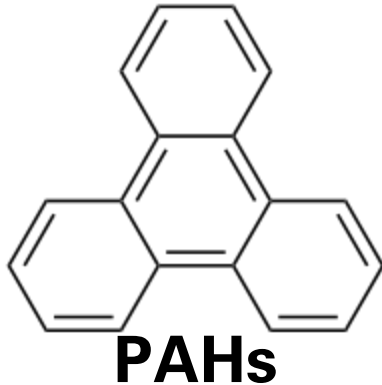
Results : Enhanced expression of MxaF Gene

MDH Relative Expression before and after CRISPR modification
in *M.extorquens*



Results : Enhanced degradation of PAHs

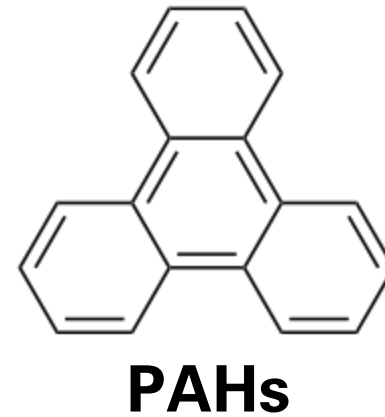
CRISPR cas9 **modified** bacteria



74.35% degradation

VS

unmodified bacteria



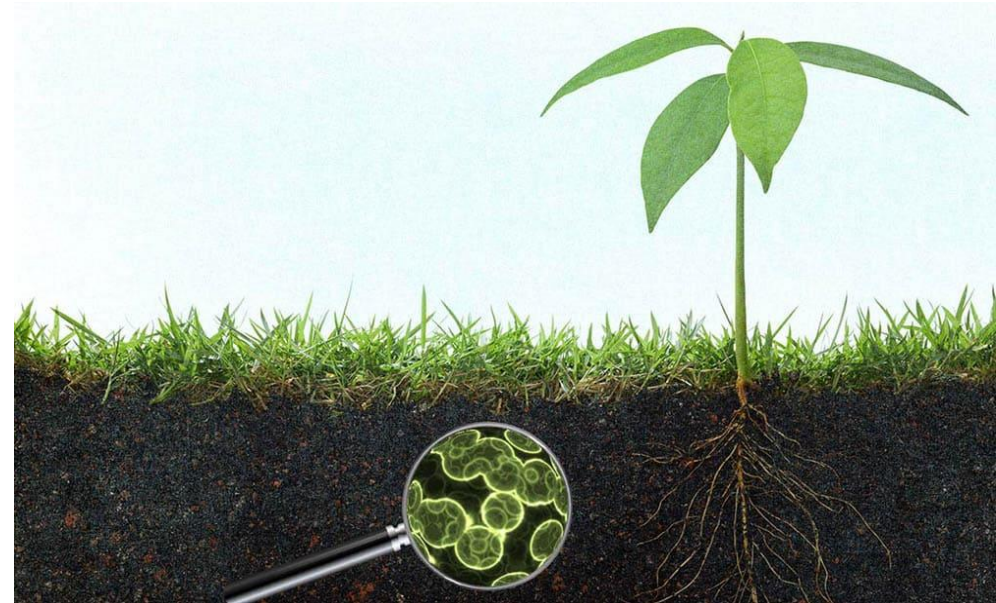
61.14% degradation

Discussion:

Are GMO microbes acceptable ?



What are the ecological risks of their use ?



**Thank you for
your attention !**
We accept questions.

