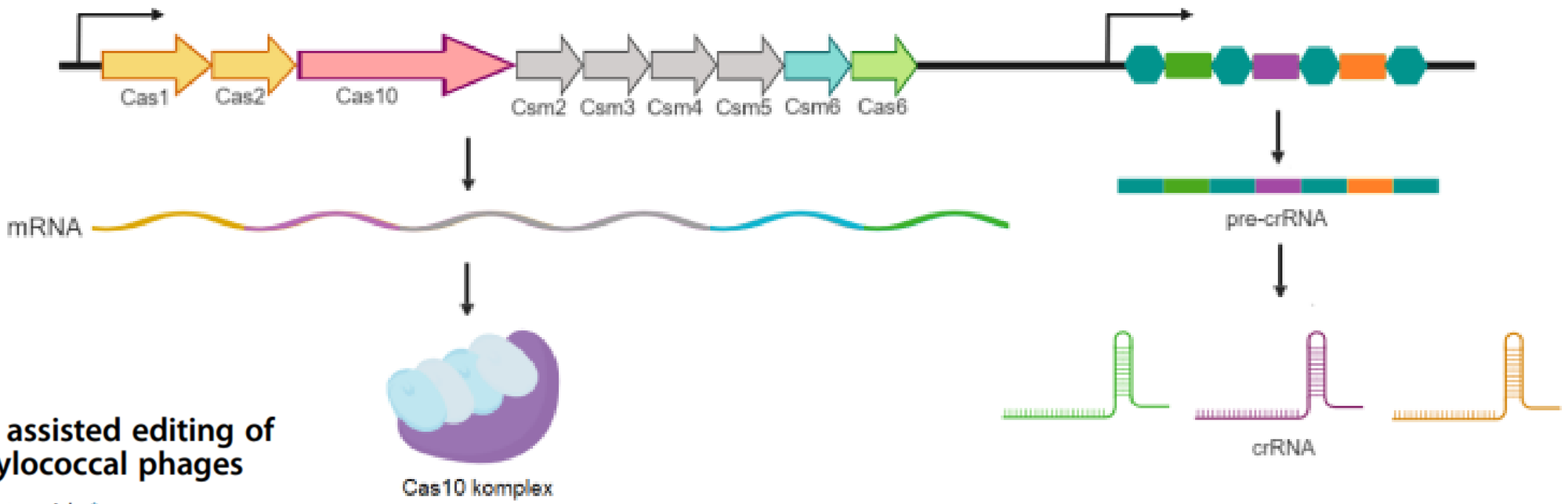
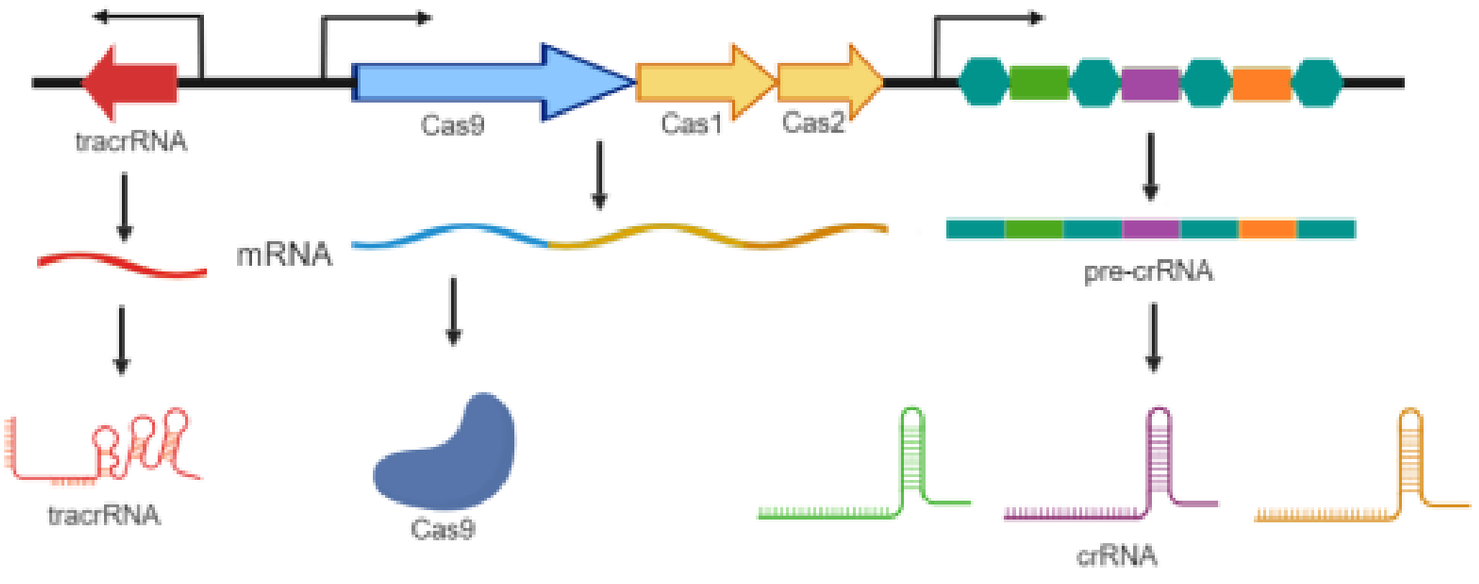
CRISPR/Cas10



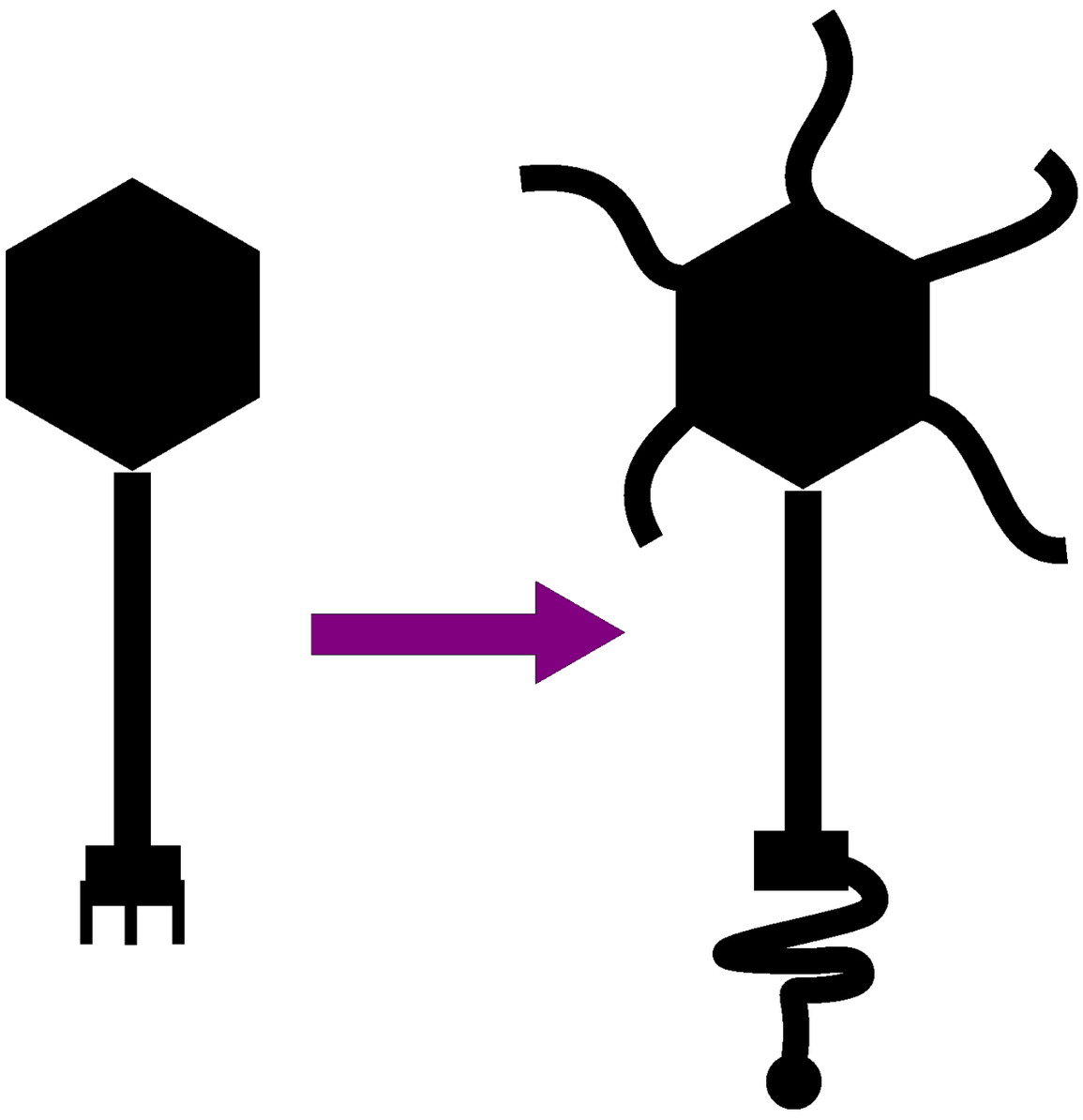
CRISPR–Cas10 assisted editing of virulent staphylococcal phages

S.M. Nayeemul Bari, Asma Hatoum-Aslan*
Department of Biological Sciences, University of Alabama, Tuscaloosa, AL, United States
*Correspondence author: e-mail address: ahatoum@ua.edu



METHOD

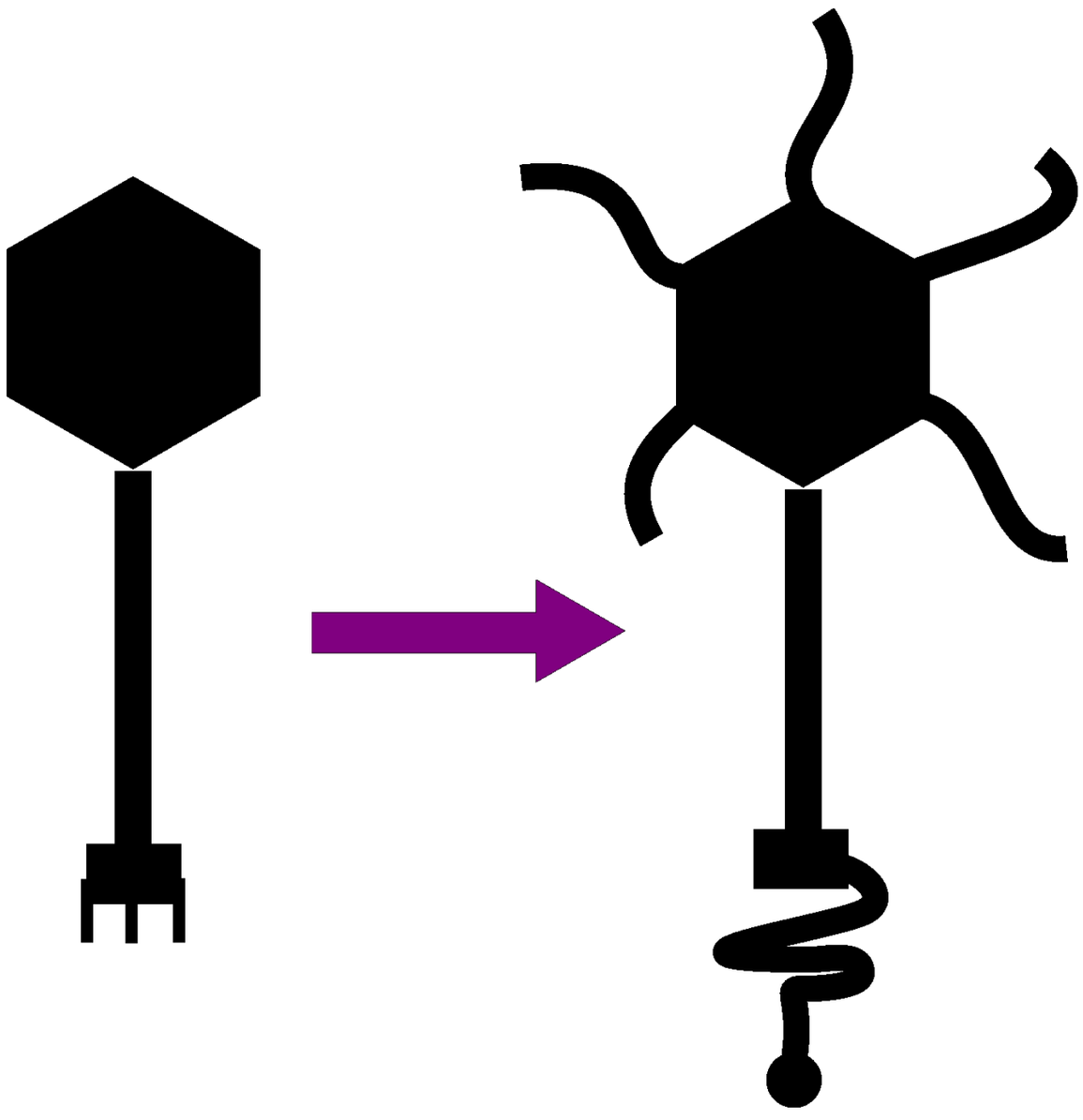
Virus editing by Cas10 protein



1. Identify gene(s) of interest
2. Create spacers targeting these genes
3. Prove that spacer abolish phage infection

METHOD

Virus editing by Cas10 protein

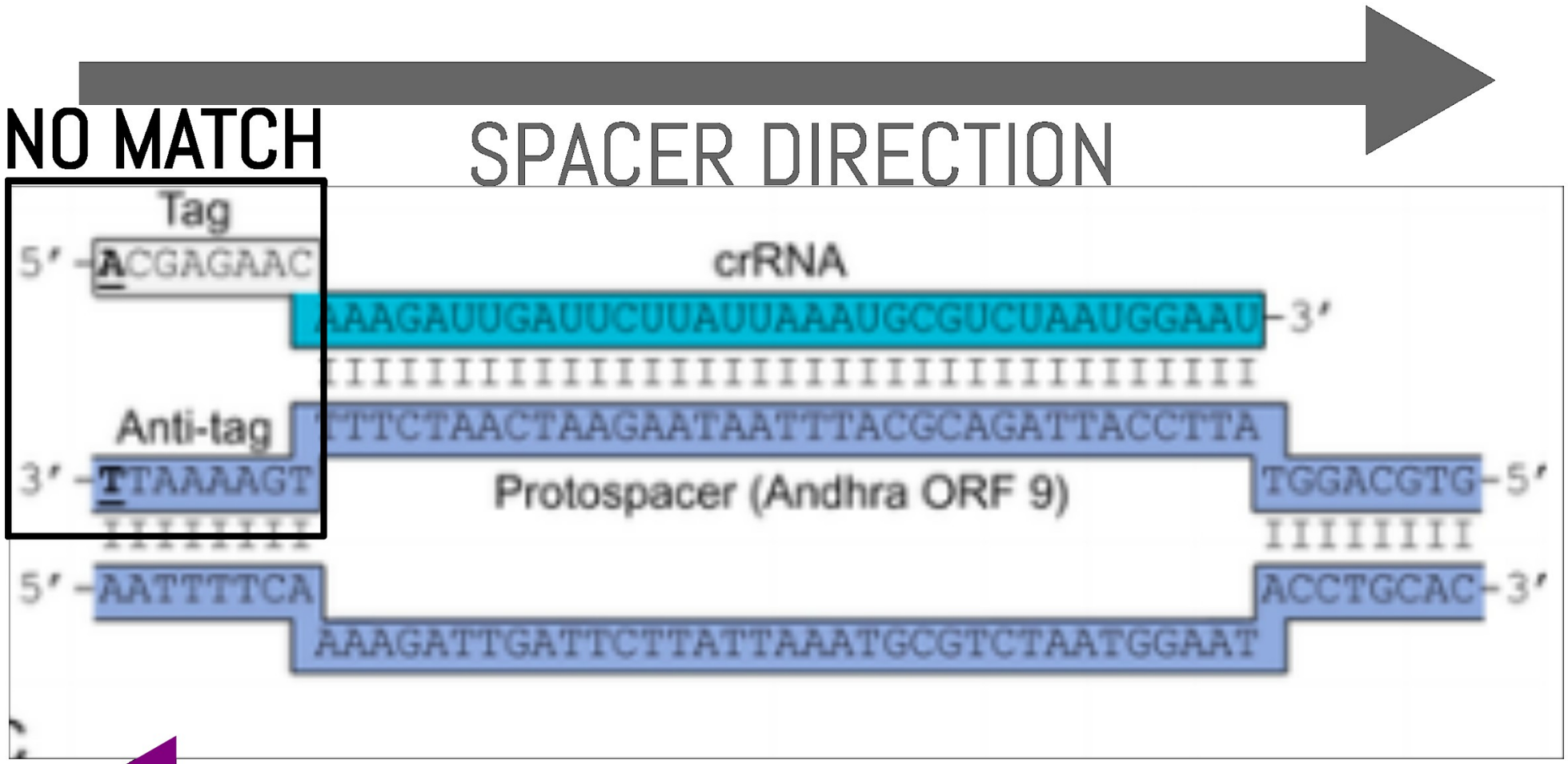


4. Design repairing region with mutation of interest

5. Create repairing-targeting system

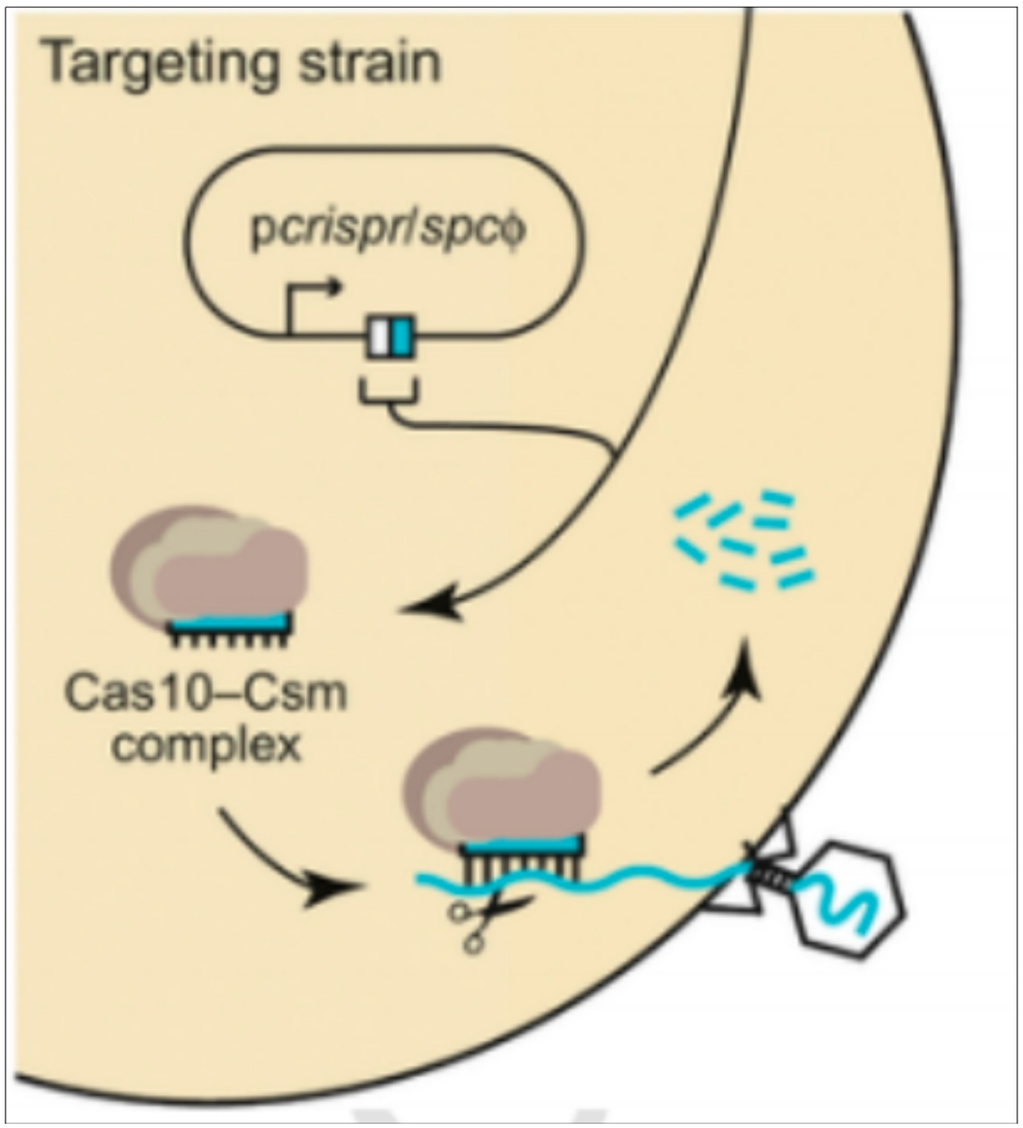
6. Isolate and sequence recombinants

2. Create spacer targeting the gene



METHOD

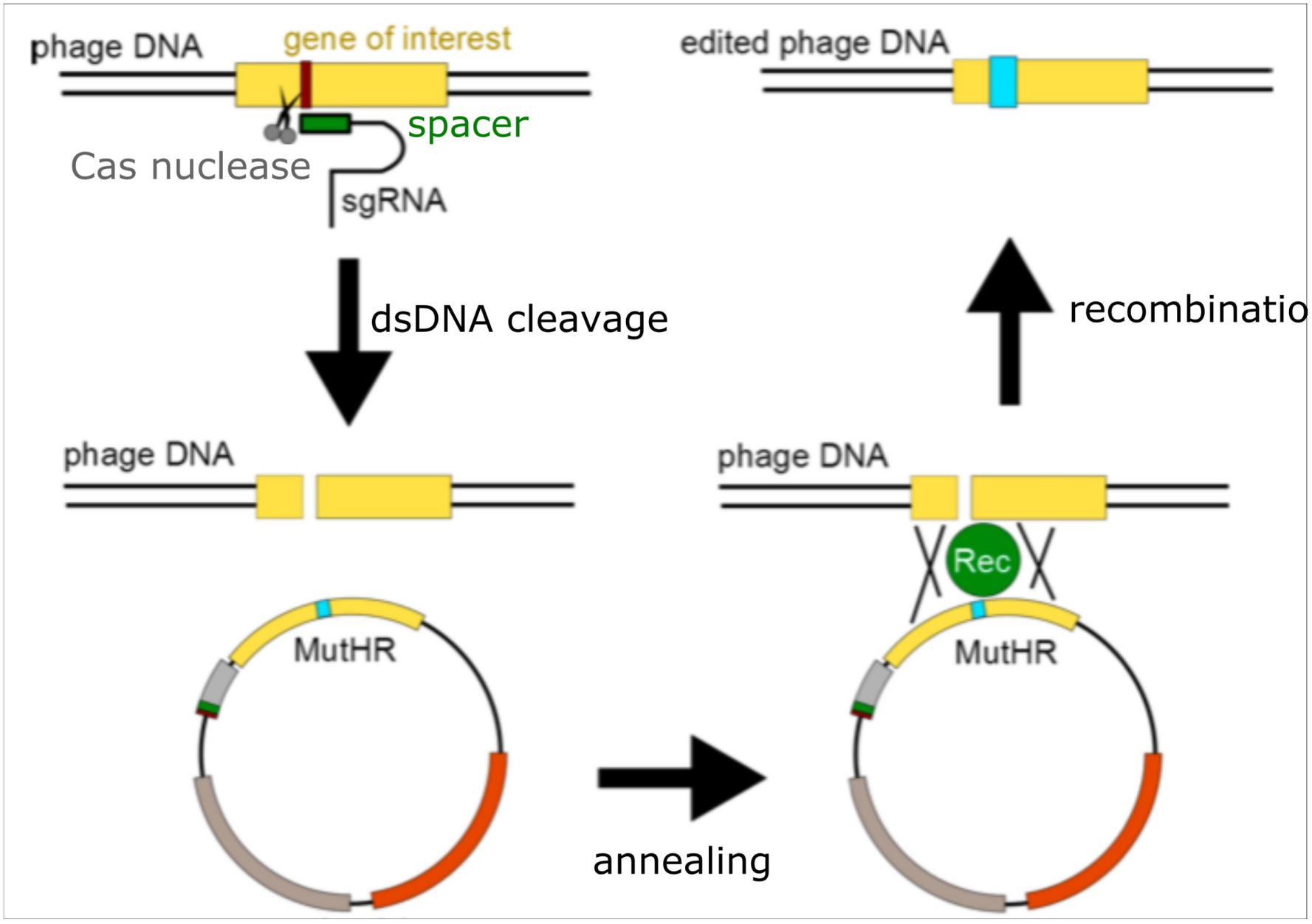
3. Prove spacer killing ability



Strain with empty Cas10 vector
= phage grows

Strain with Cas10 plus spacer vector
= no plaques

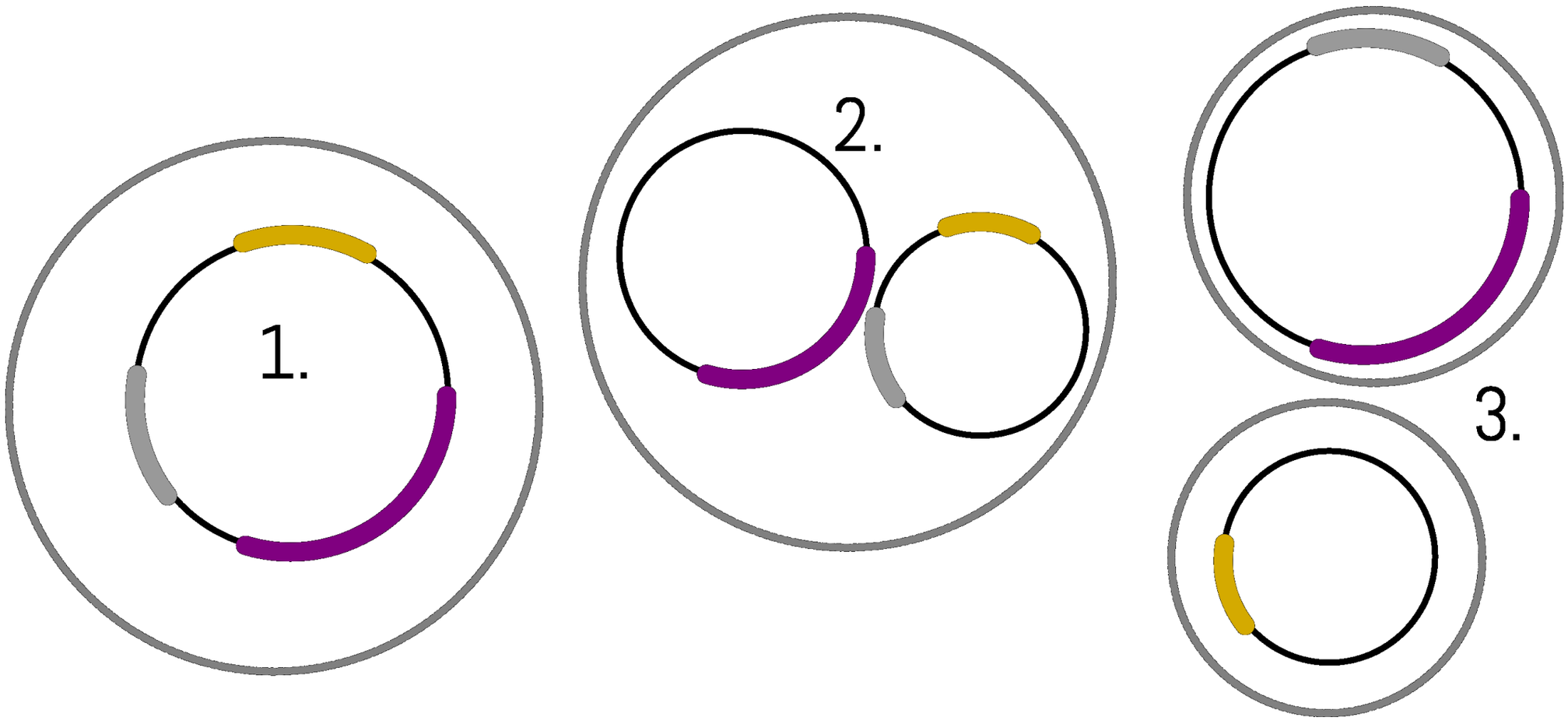
4. Design repairing region with mutation of interest



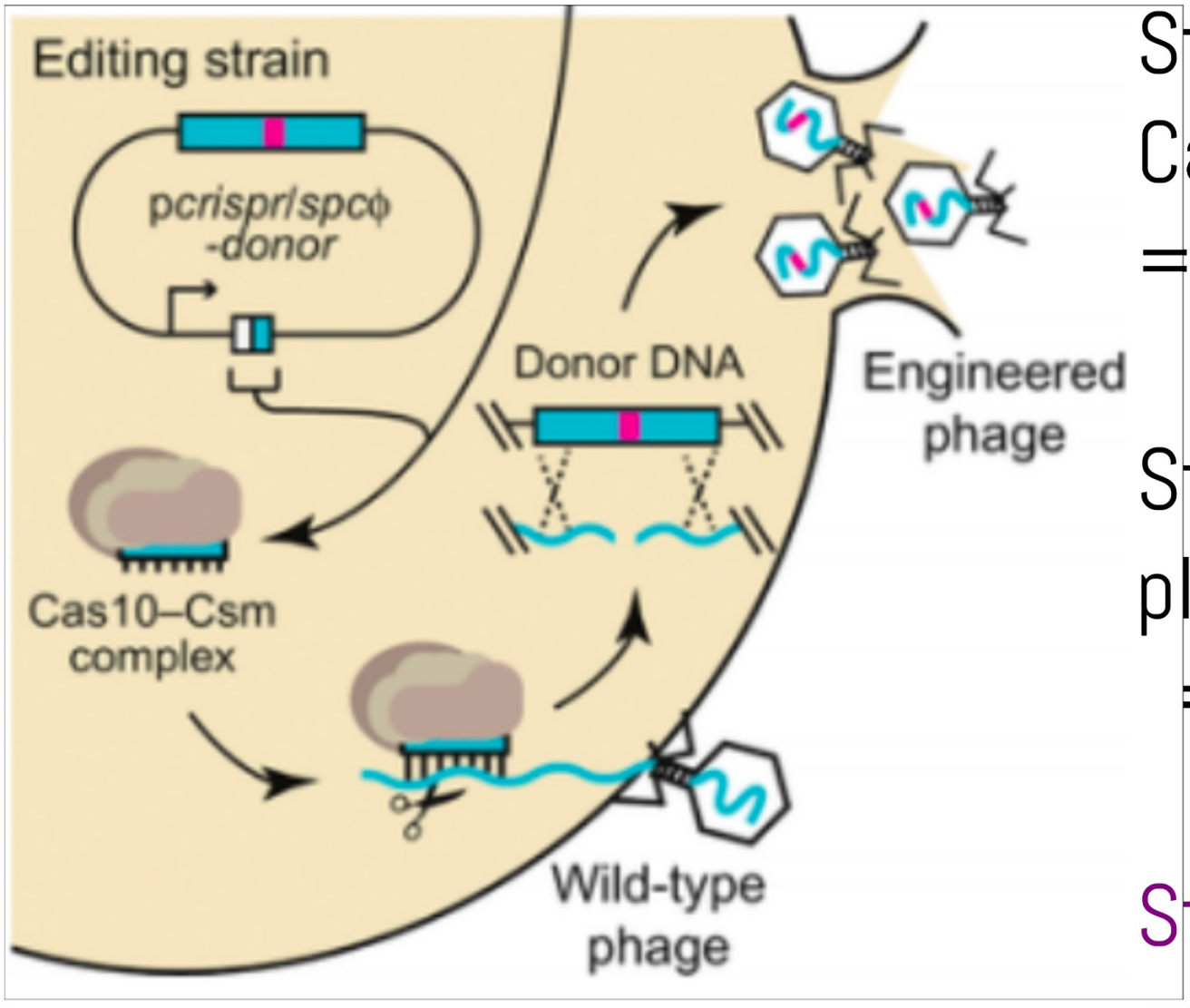
5. Create repairing-targeting system

1. One plasmid system
2. Two plasmid system
3. Two strain system

Cas10
spacer cassette
repairing region



5. Create repairing-targeting system



Strain with empty Cas10 vector
= phage grows

Strain with Cas10 plus spacer vector
= no plaques

Strain with Cas10-spacer plus RR
= a few plaques

METHOD

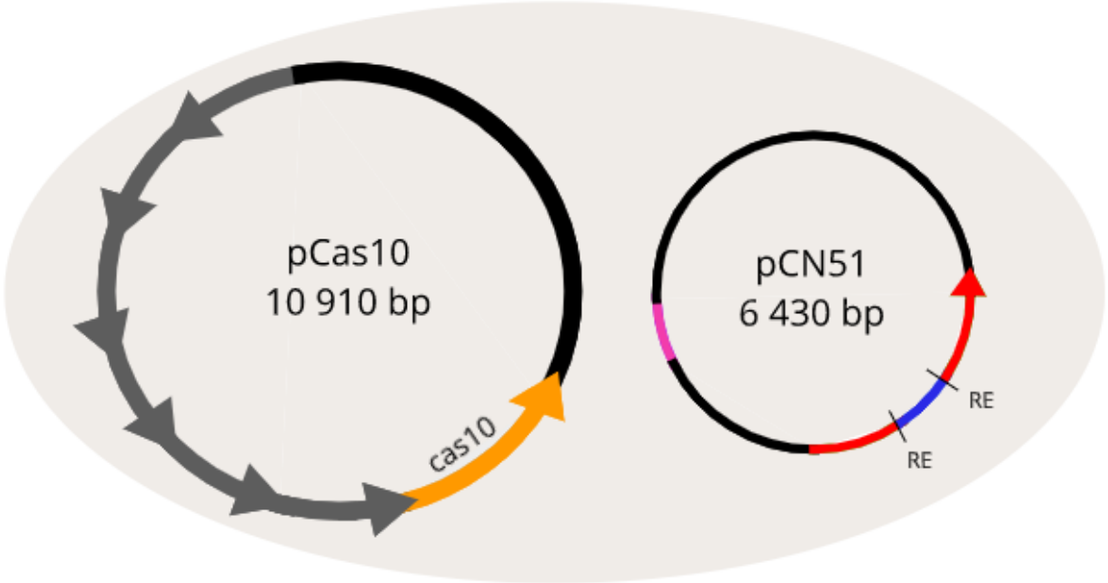
6. Isolate and sequence recombinants

1. Identify mutants
2. Multiply each to hight titre
3. Isolate genome and do PCR of the gene
4. Do restriction degistion/ sequencing to confirm your mutation

More information:

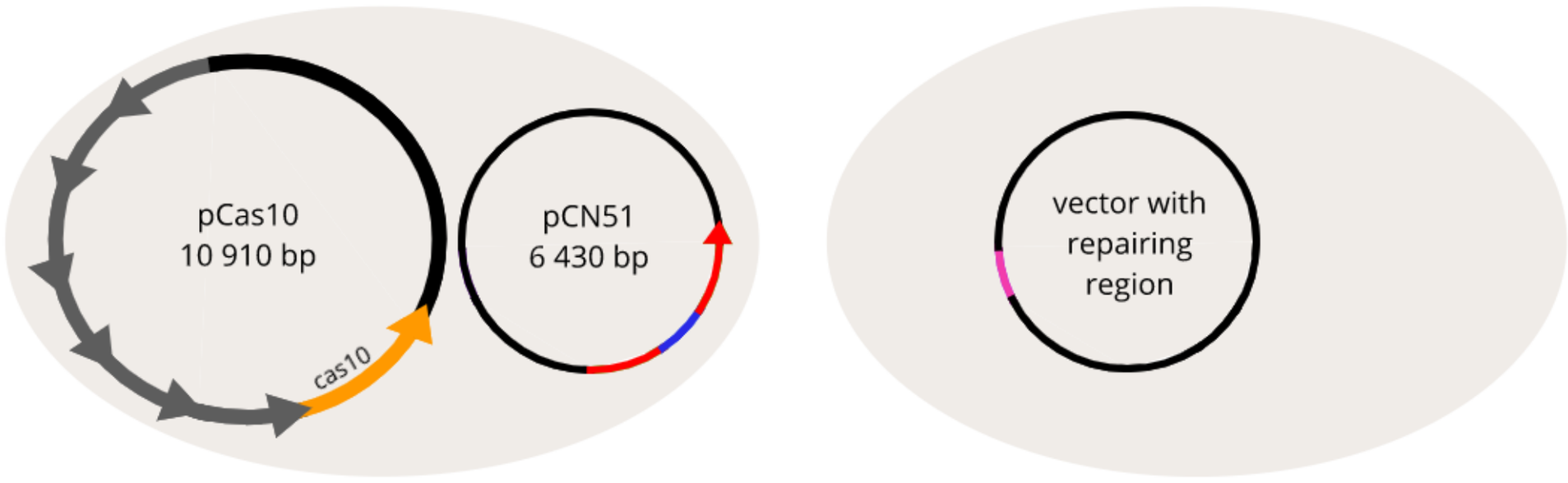
https://www.ncbi.nlm.nih.gov/pubmed/28885820
https://doi.org/10.1016/bs.mie.2018.10.023

➡ Two-vector editing system

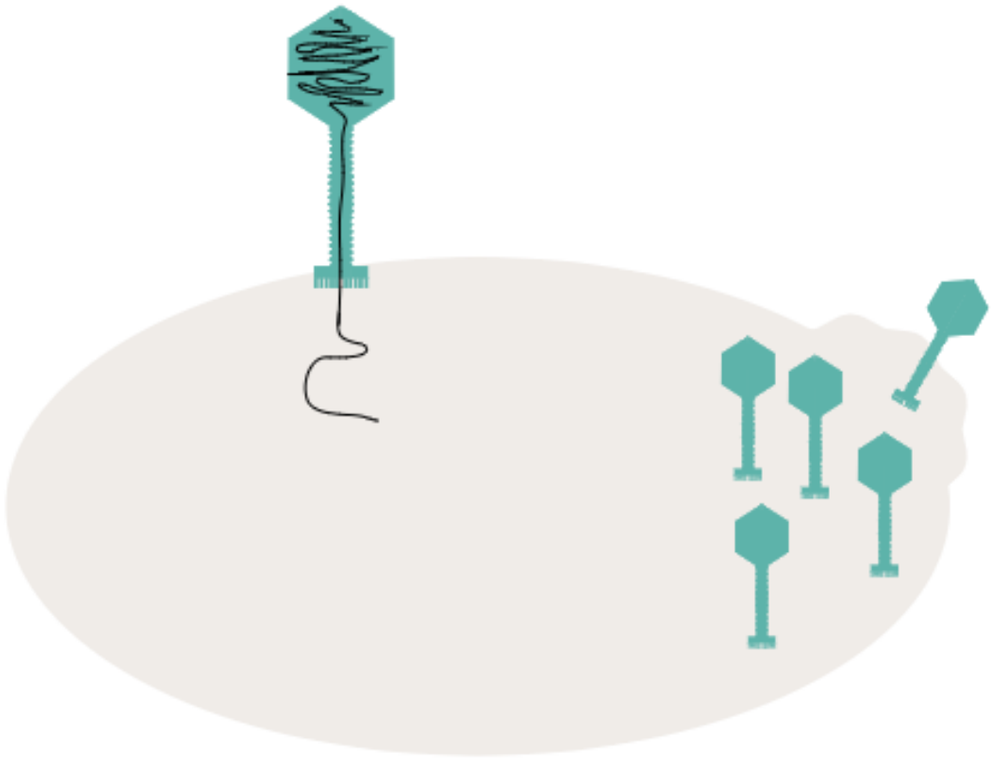


gRNA
programmable spacer sequence
template DNA

➡ Two-strain editing system

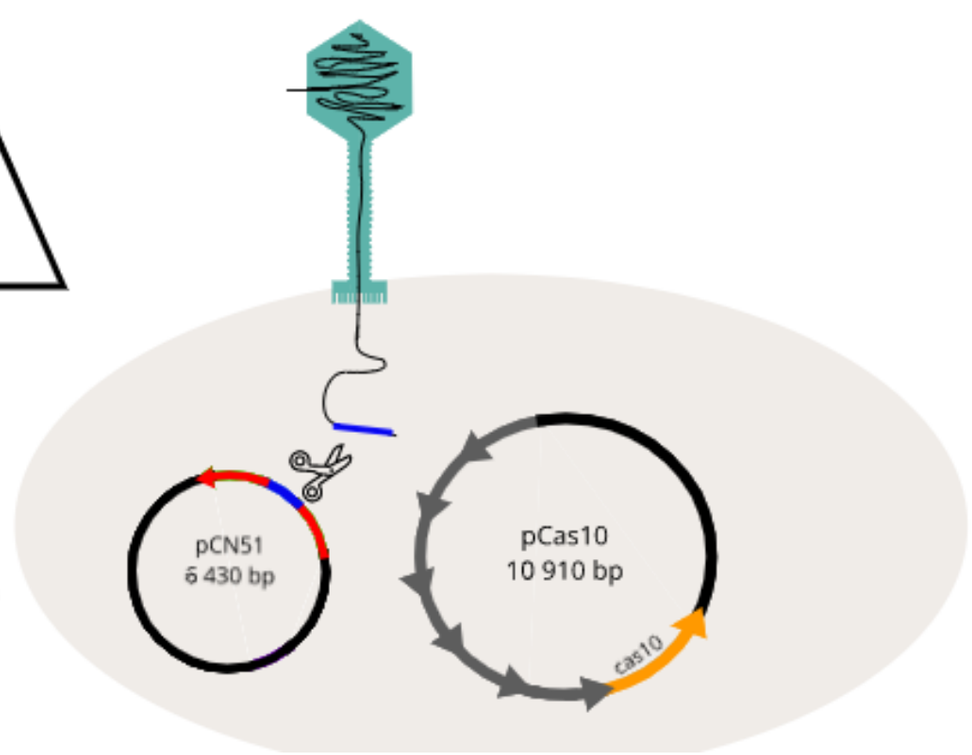


Two-strain editing system in detail

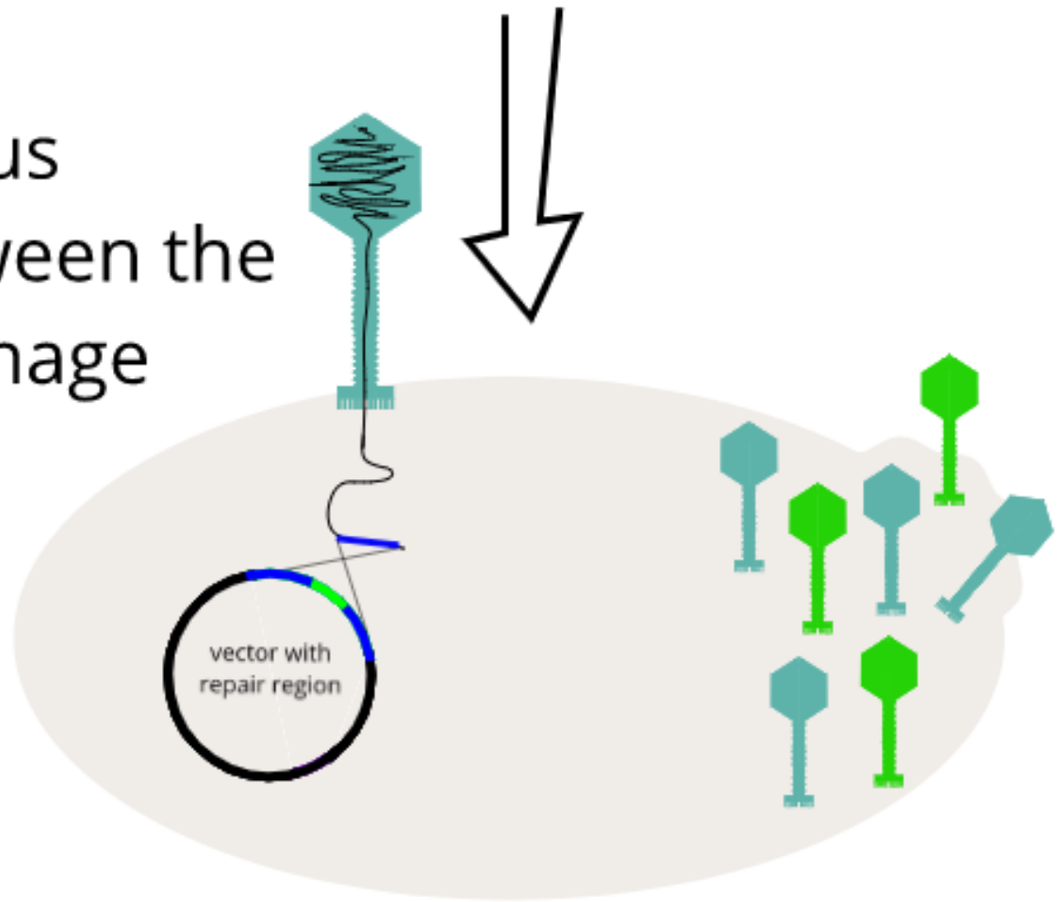


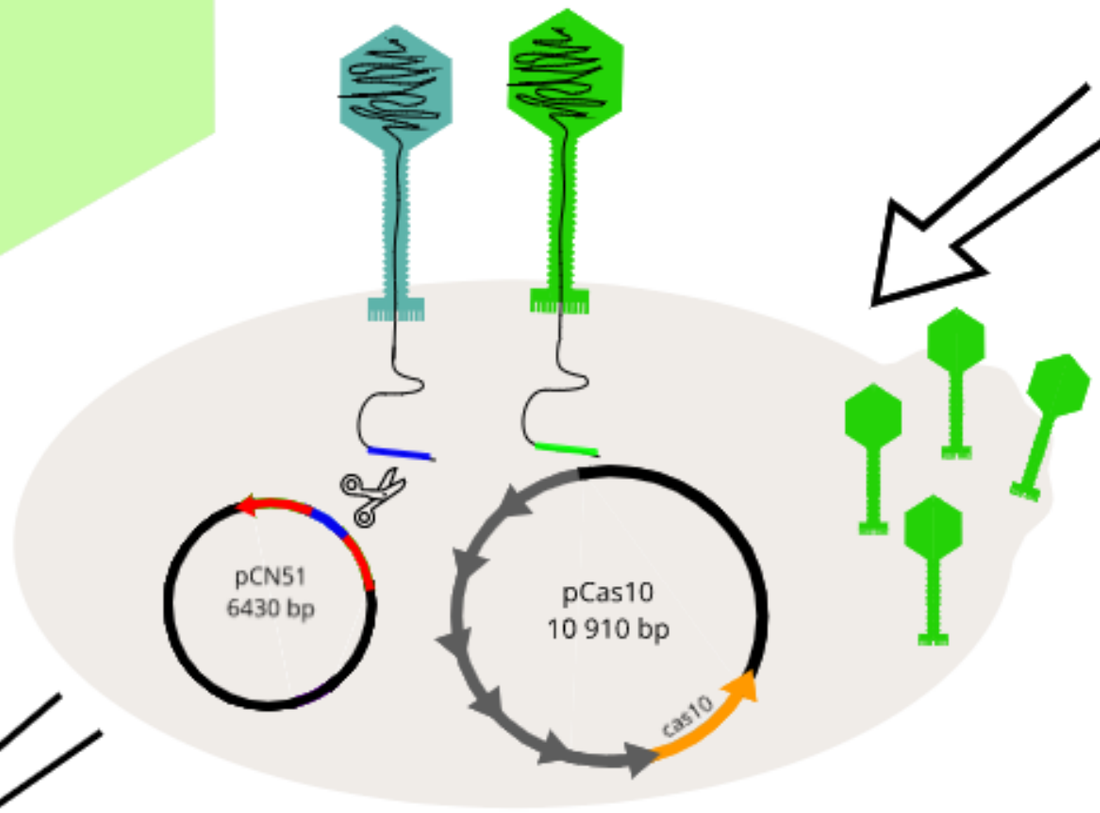
S. aureus infected with wild type bacteriophage 812.

S. aureus with CRISPR/Cas10. Prevention of phage infection.



Random homologous recombination between the repair region and phage DNA.





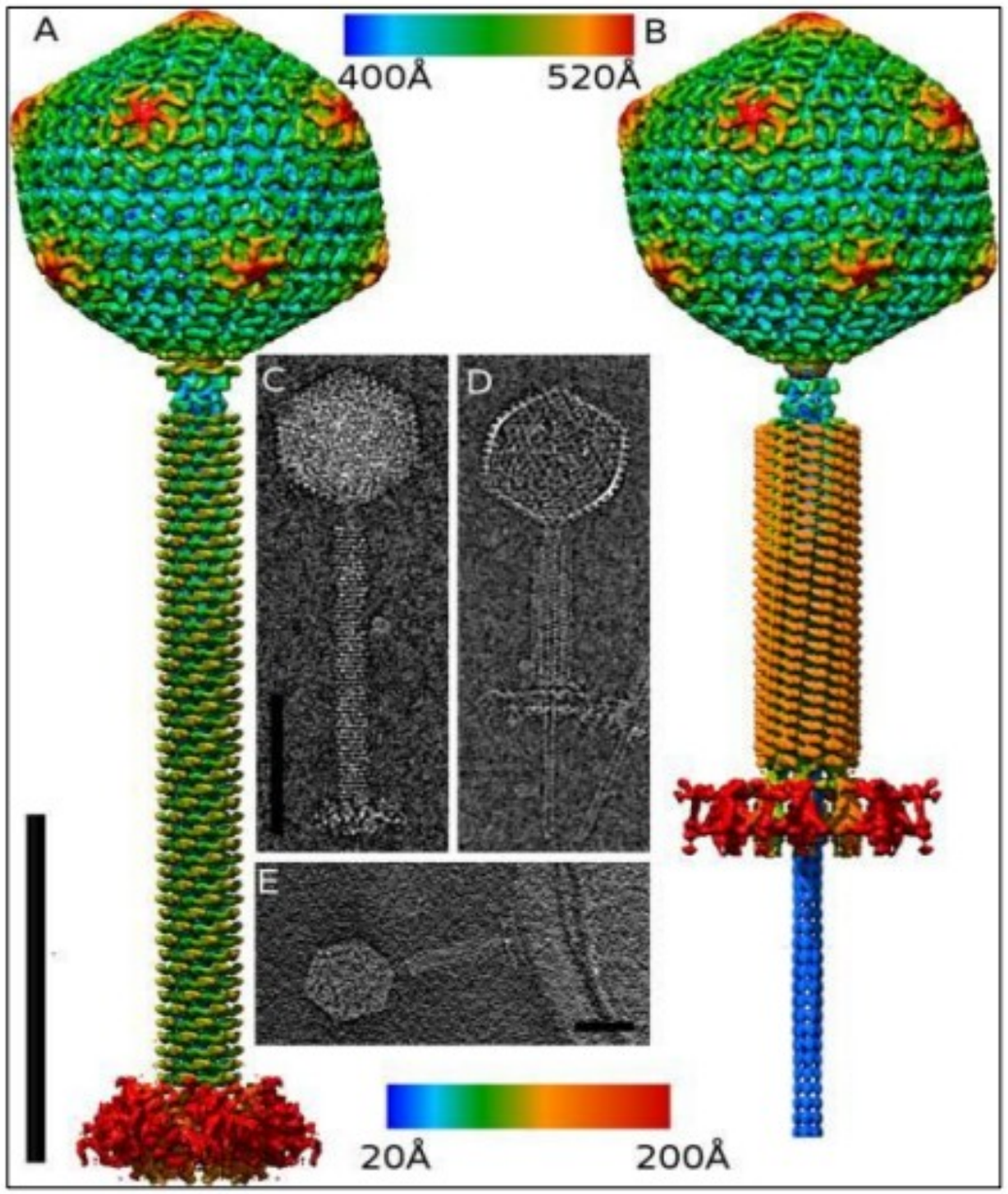
Counter-selection of modified non-GMO phages on editing strain.

Cíle praktické úlohy

- Upravit vektor pCN51
    - RE štěpení, ligace inzertu = mezerník cílící na fága 812
- Transformovat připravený vektor do buněk *E. coli* a *S. aureus*
- Provést infekci fágem 812 a 812a – pozorovat rozdíly

Bakteriofág 812

- Polyvalentní fág s širokým rozmezím hostitelů
- Lytický fág



Bakteriofág 812

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ORF 812_192

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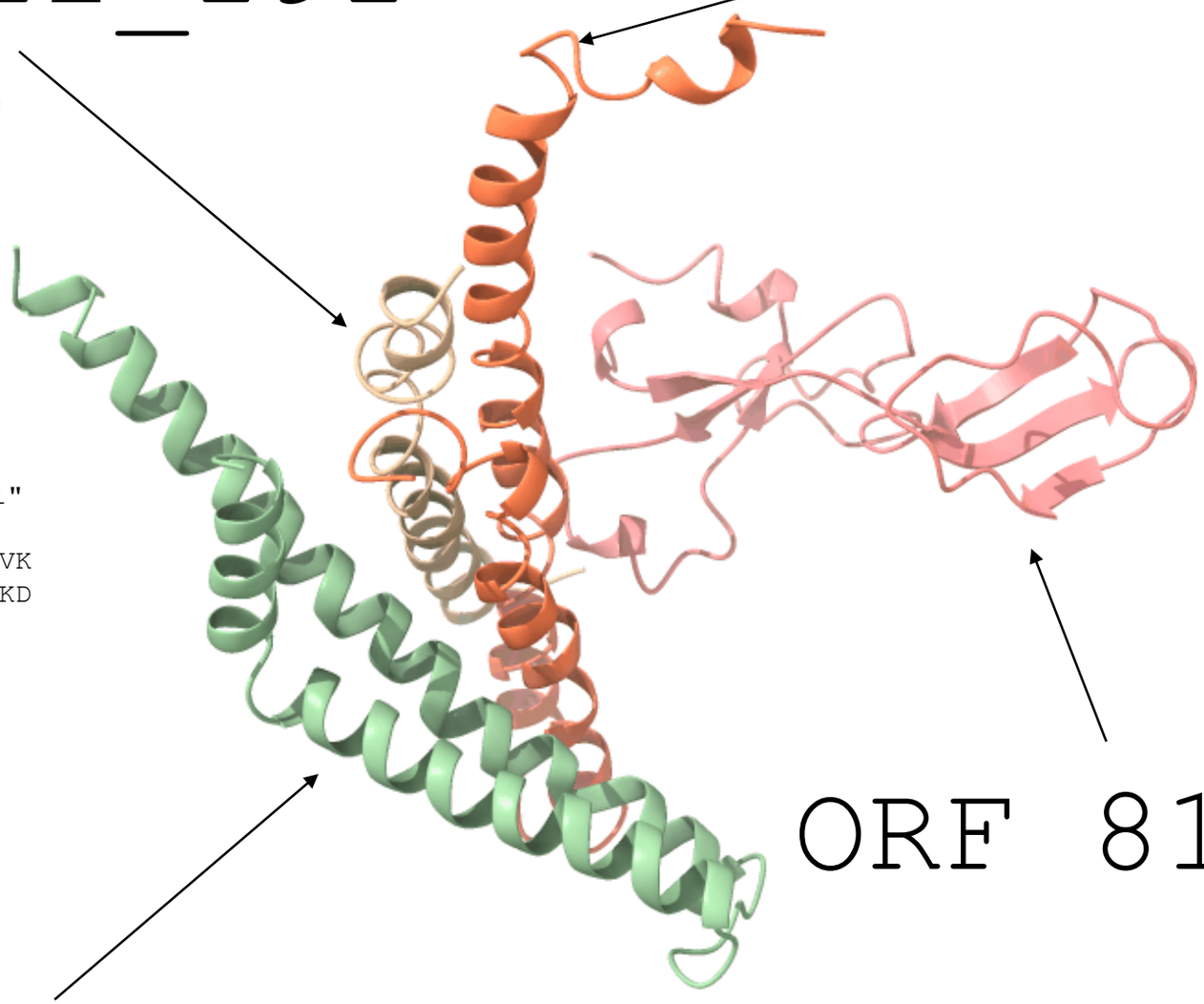

ORF 812_190

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ORF 812_189

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/translation="MKQRDFEFEEDFVLTYECEDCKHFEDWGHDEEPEECSECGSS
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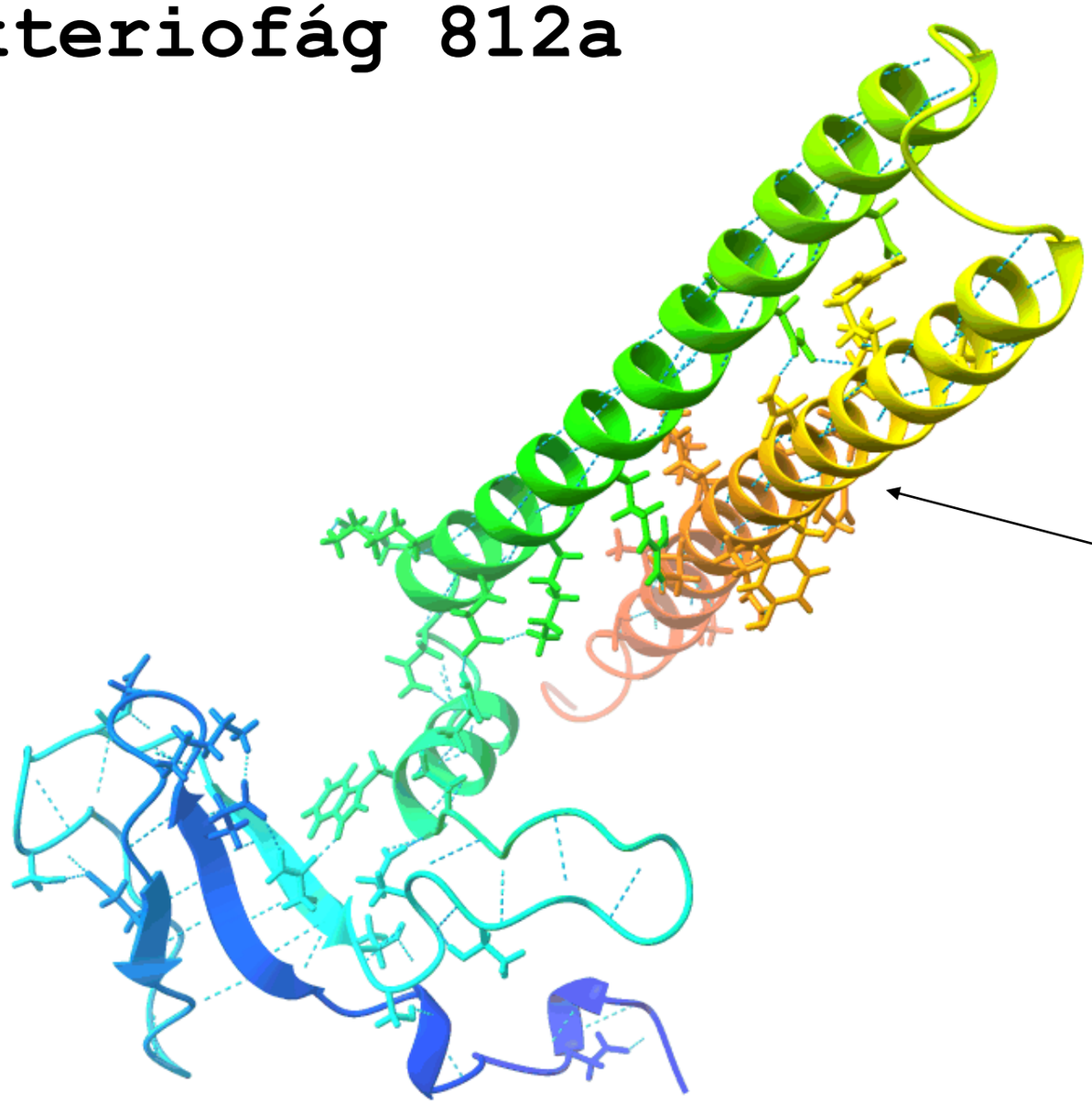
Bakteriofág 812a



ORF 812a_191

Phage 812a (KJ206560.1)
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EEKQKMNSREKKILTLTVNNF
LMLALDIVALVRYKKGKIKQENYNTGQISRTIVTTAN
SLGILYLEEQERKEKKSVKIGTLESGTLRGFKNK"

Bakteriofág 812

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Bakteriofág 812a

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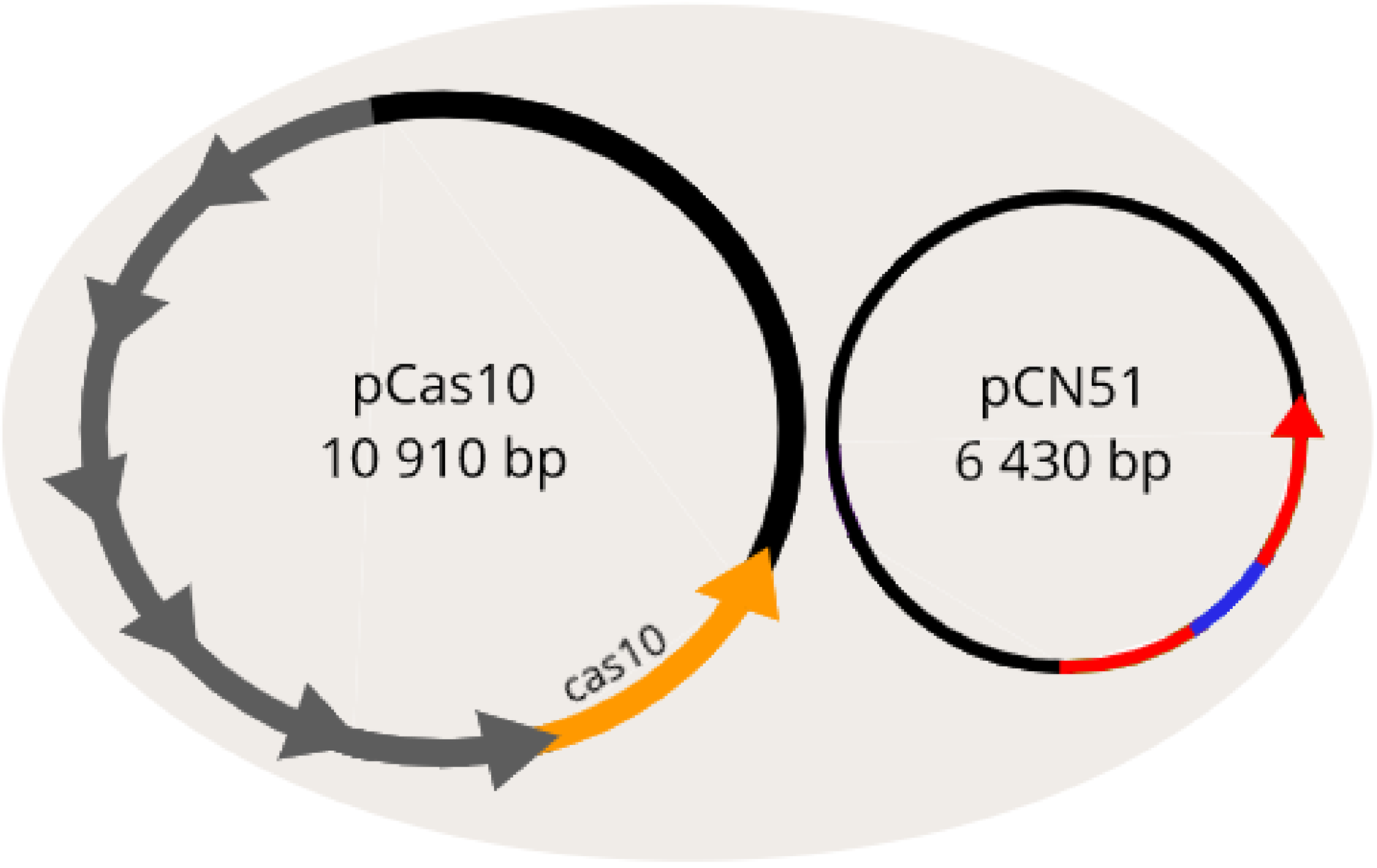
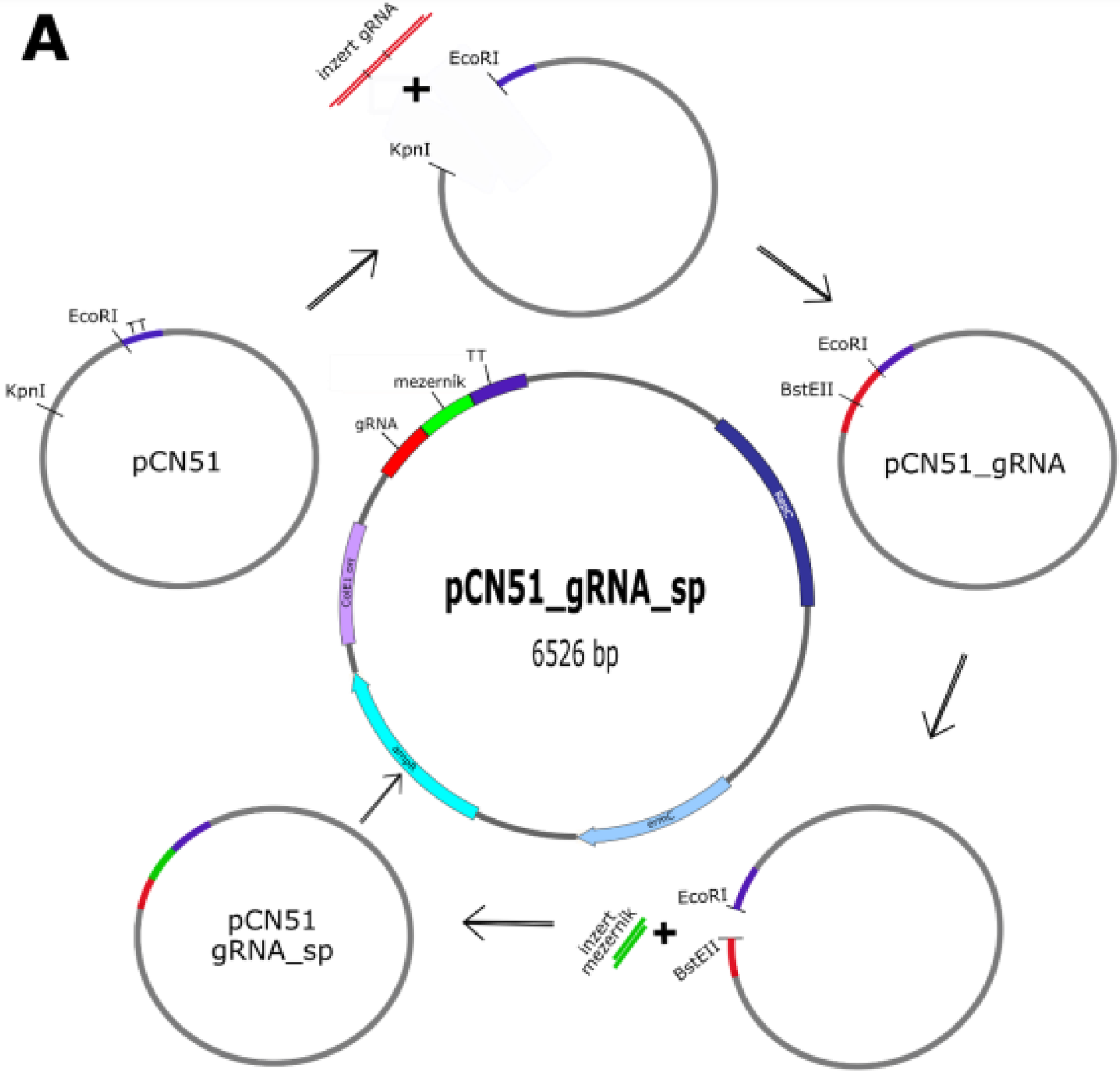

pCas10
10 910 bp
cas10
pCN51
6 430 bp

A

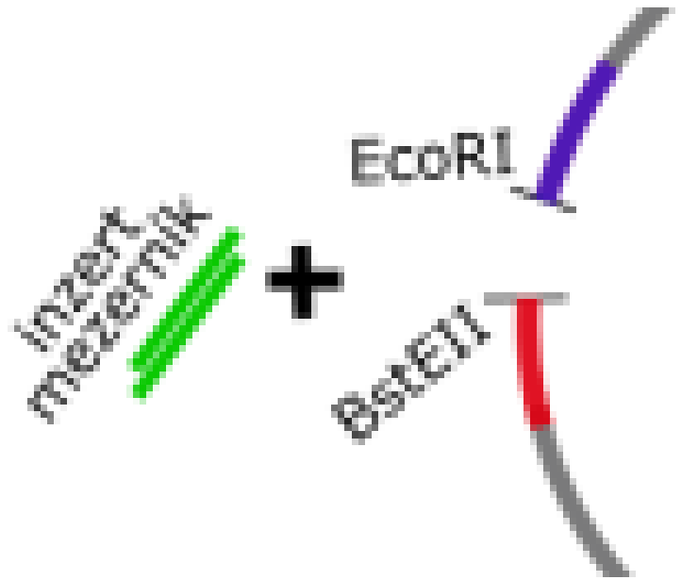

insert gRNA
EcoRI
KpnI
EcoRI
TT
KpnI
pCN51
TT
mezernik
gRNA
pCN51_gRNA_sp
6526 bp
EcoRI
BstEII
pCN51_gRNA
pCN51
gRNA_sp
insert
mezernik
EcoRI
BstEII

Spacer cílící na seq ORF_190

seq ORF 190 (protein není přítomen v 812a)
=> zdroj spaceru pro editaci Cas10
>MH844528.1:130545-130832 Staphylococcus phage 812, complete genome
ATGAATAAAGCAGTAGAACAAGCAAGTAATGCATTAGGTCAAGGATTTTCAGCTATGGTATGGCATCAAGTATTAGTAGGGTTAGGGTTTATTTTATTAGGATTGGTATTATCTTTACTGGTT
TGGGTATTAGTAAAAAAATTCCATGTACCTTTTAATCACCCGACAGCTTTTGTAGTGTACTCAATTATGTTAGTGAGTATTGTTGCTAGTTTTATTTGGGGCGGTTTACATGTAATTAACCCT
GAGTATTATGCTATTTTAGAACTTAAAGGTTTTATAAAGTAG

Sekvence Spaceru:

CTAATACTTGATGCCATACCATAGCTGAAAATCCT



Oligonukleotidy pro vložení kazety
CR_812_Cas10_F:
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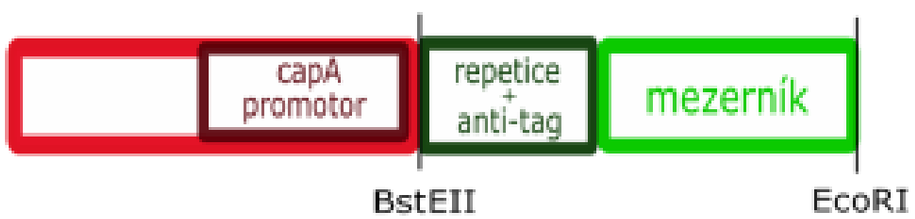
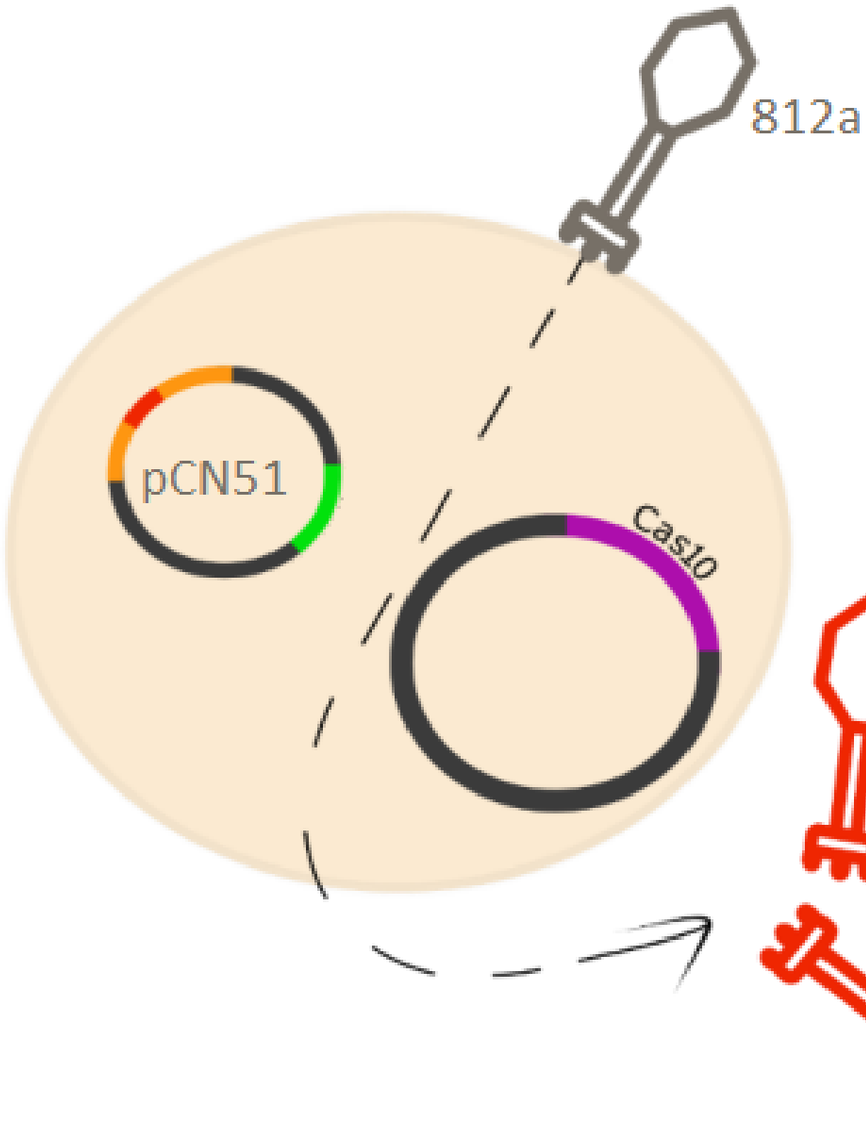
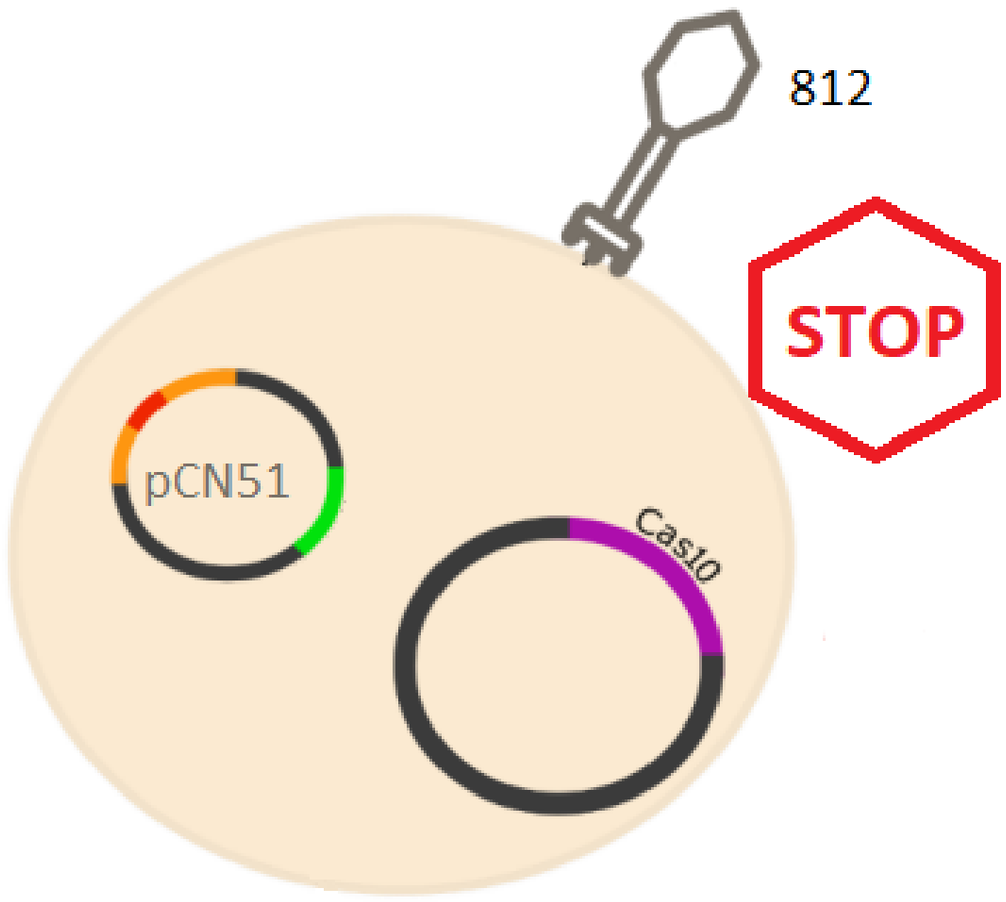



812a
pCN51
Cas10
812a


812
STOP
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