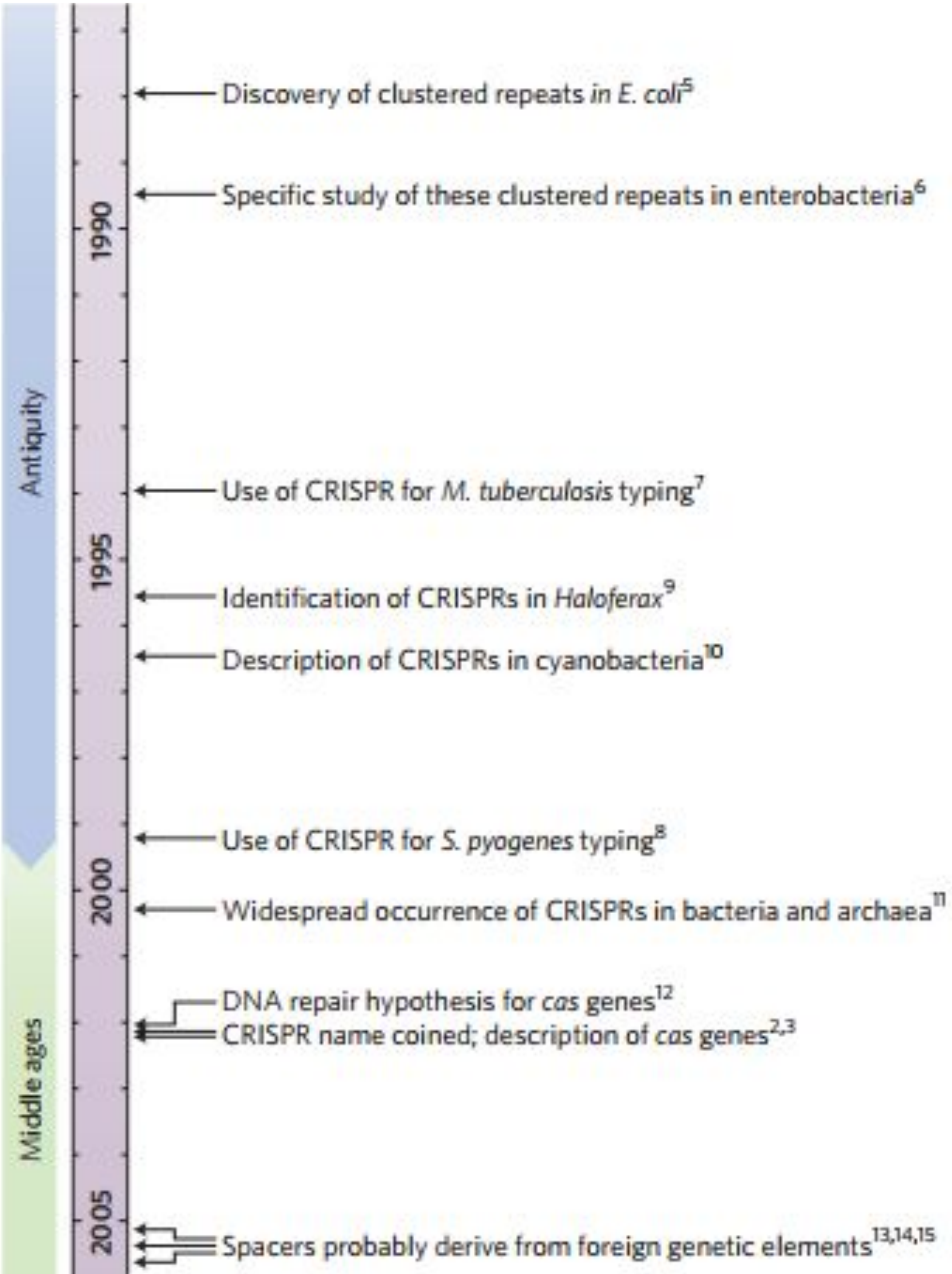
Genome Editing

CRISPR

Clustered Regularly Interspaced Short Palindromic Repeats

Короткие палиндромные кластерные повторы, или CRISPR



Discovery of clustered repeats *in E. coli*[5]

Specific study of these clustered repeats in enterobacteria[6]

Use of CRISPR for *M. tuberculosis* typing[7]

Identification of CRISPRs in *Haloferax*[9]

Description of CRISPRs in cyanobacteria[10]

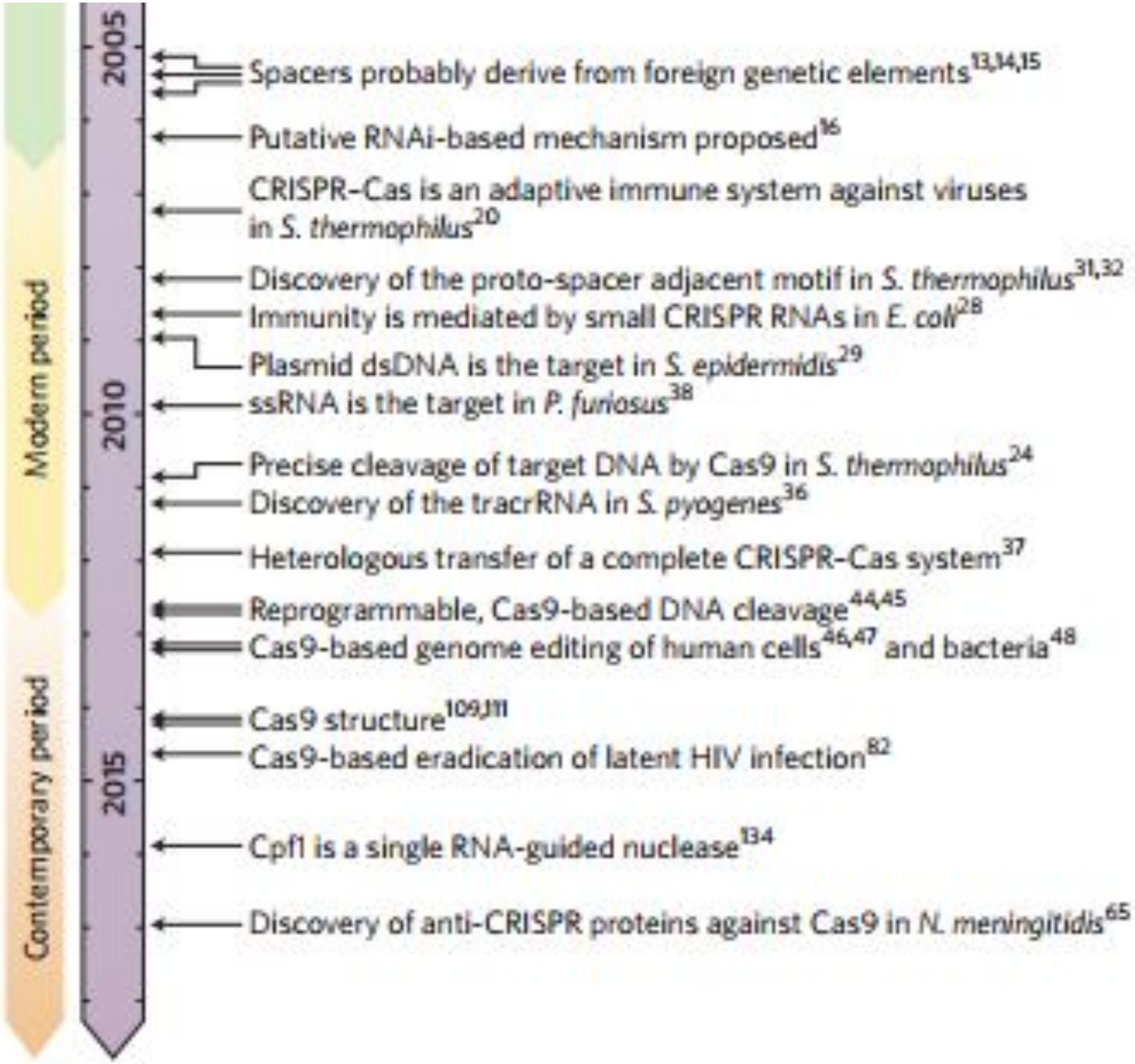
Use of CRISPR for *S. pyogenes* typing[8]

Widespread occurrence of CRISPRs in bacteria and archaea[11]

DNA repair hypothesis for *cas* genes[12]

CRISPR name coined; description of *cas* genes[2,3]

Spacers probably derive from foreign genetic elements[13,14,15]



Spacers probably derive from foreign genetic elements[13,14,15]

Putative RNAi-based mechanism proposed[16]

CRISPR–Cas is an adaptive immune system against viruses in *S. thermophilus*[20]

Discovery of the proto-spacer adjacent motif in *S. thermophilus*[31,32]

Immunity is mediated by small CRISPR RNAs in *E. coli*[28]

Plasmid dsDNA is the target in *S. epidermidis*[29]

ssRNA is the target in *P. furiosus*[38]

Precise cleavage of target DNA by Cas9 in *S. thermophilus*[24]

Discovery of the tracrRNA in *S. pyogenes*[36]

Heterologous transfer of a complete CRISPR–Cas system[37]

Reprogrammable, Cas9-based DNA cleavage[44,45]

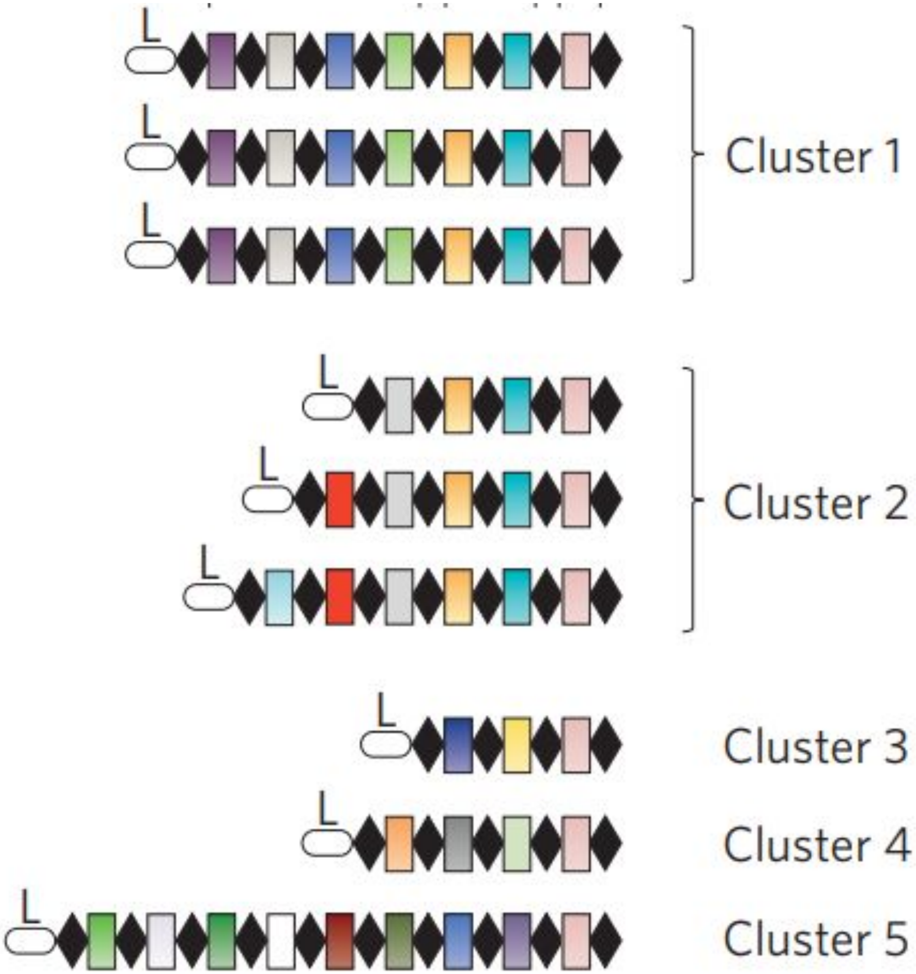
Cas9-based genome editing of human cells[46,47] and bacteria[48]

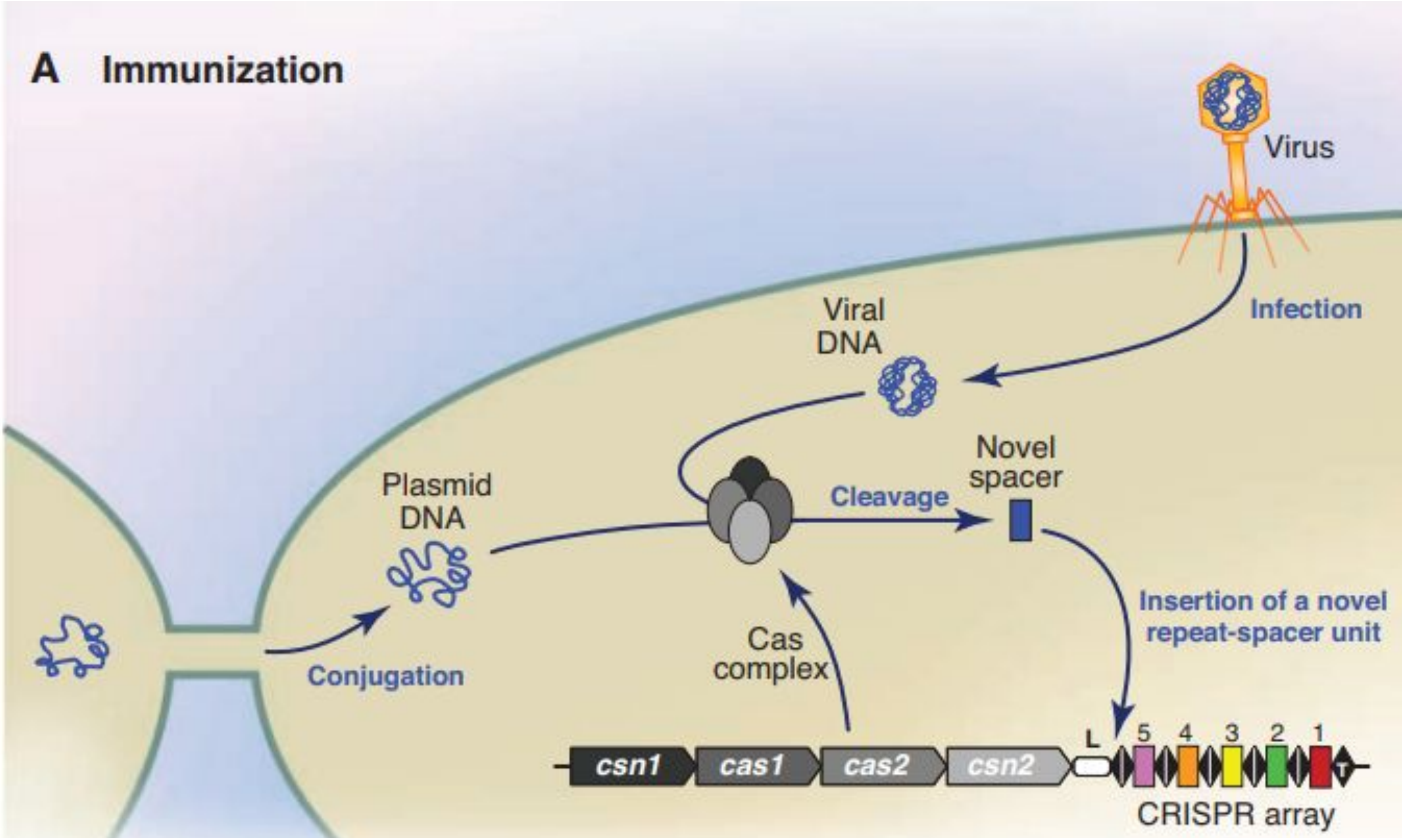
Cas9 structure[109,111]

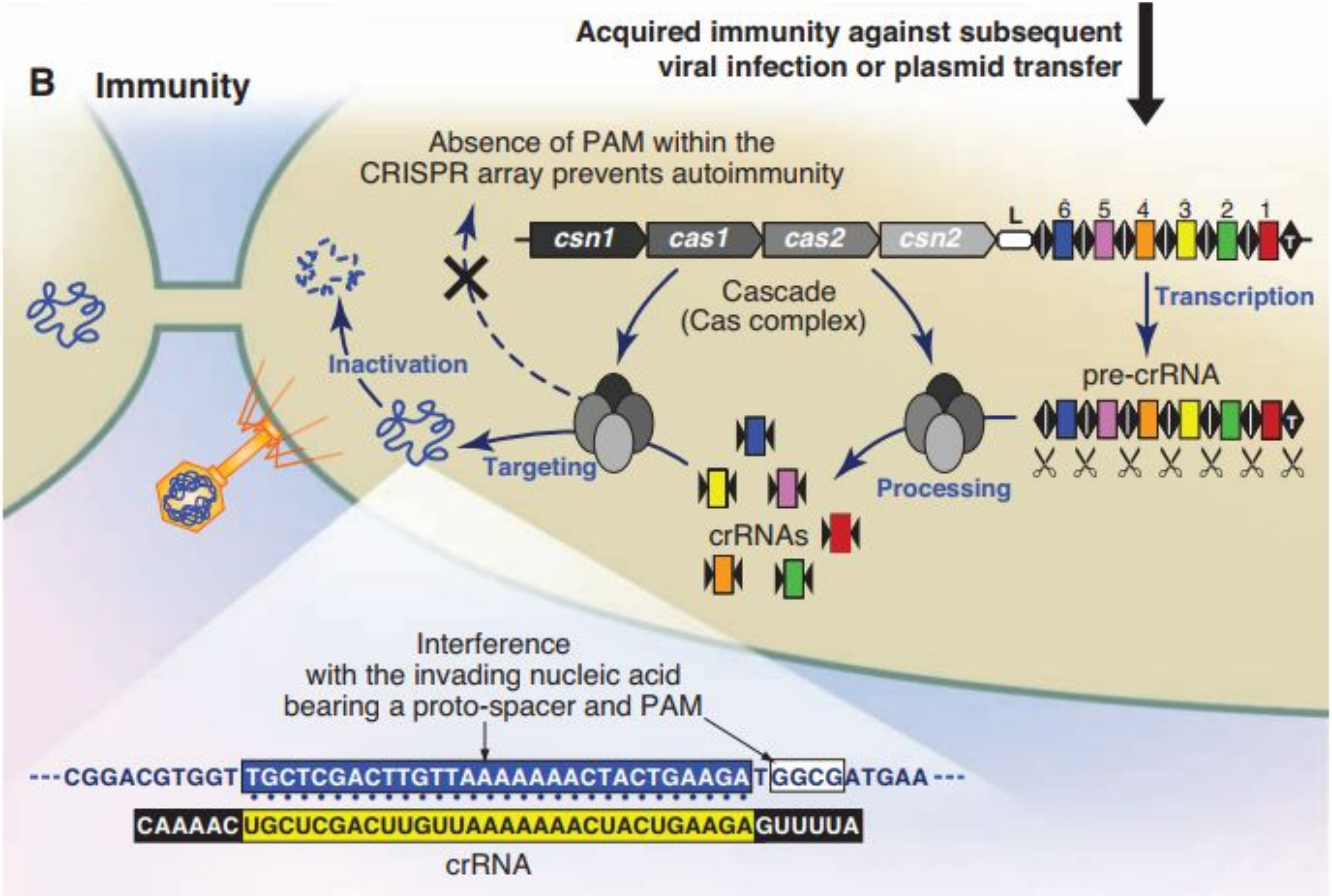
Cas9-based eradication of latent HIV infection[82]

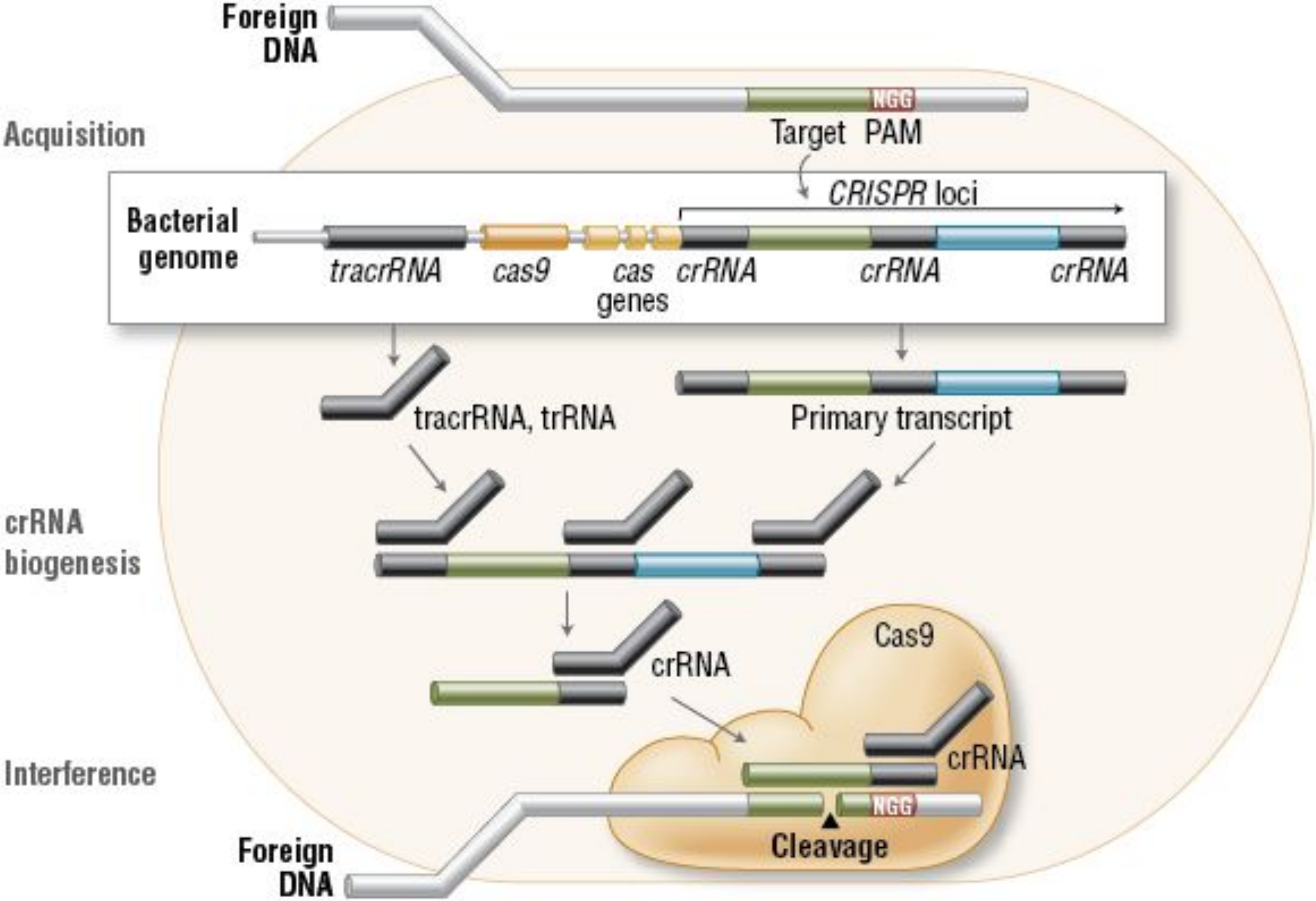
Cpf1 is a single RNA-guided nuclease[134]

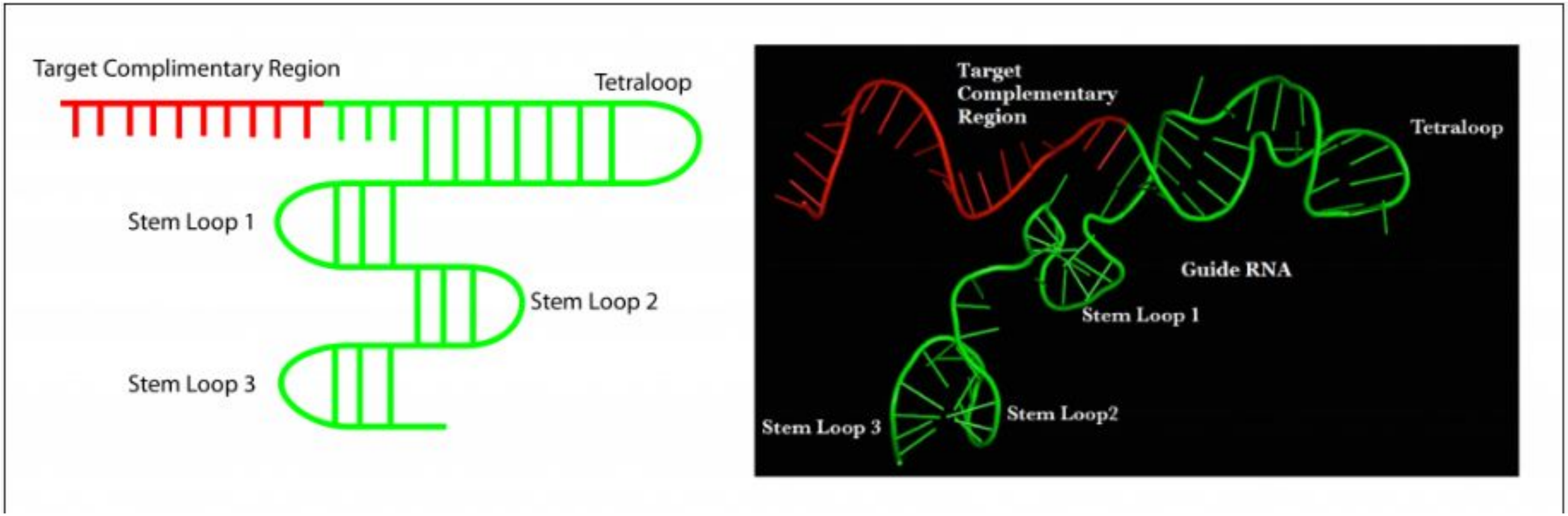
Discovery of anti-CRISPR proteins against Cas9 in *N. meningitidis*[65]

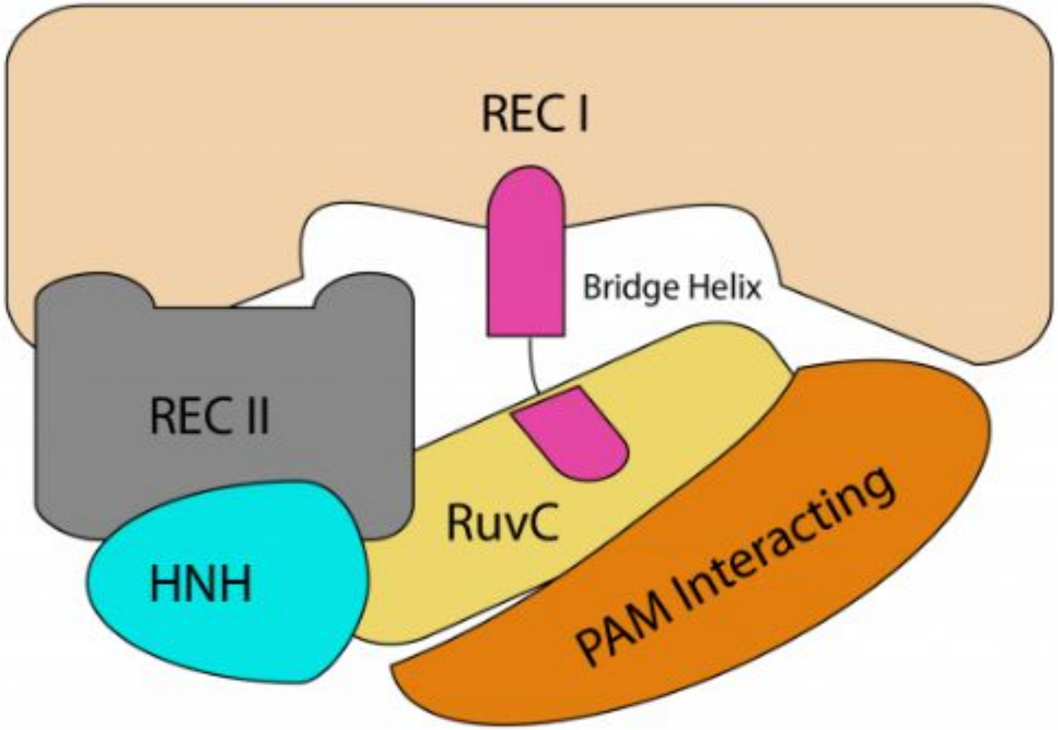

L
L
L
Cluster 1
L
L
L
Cluster 2
L
Cluster 3
L
Cluster 4
L
Cluster 5

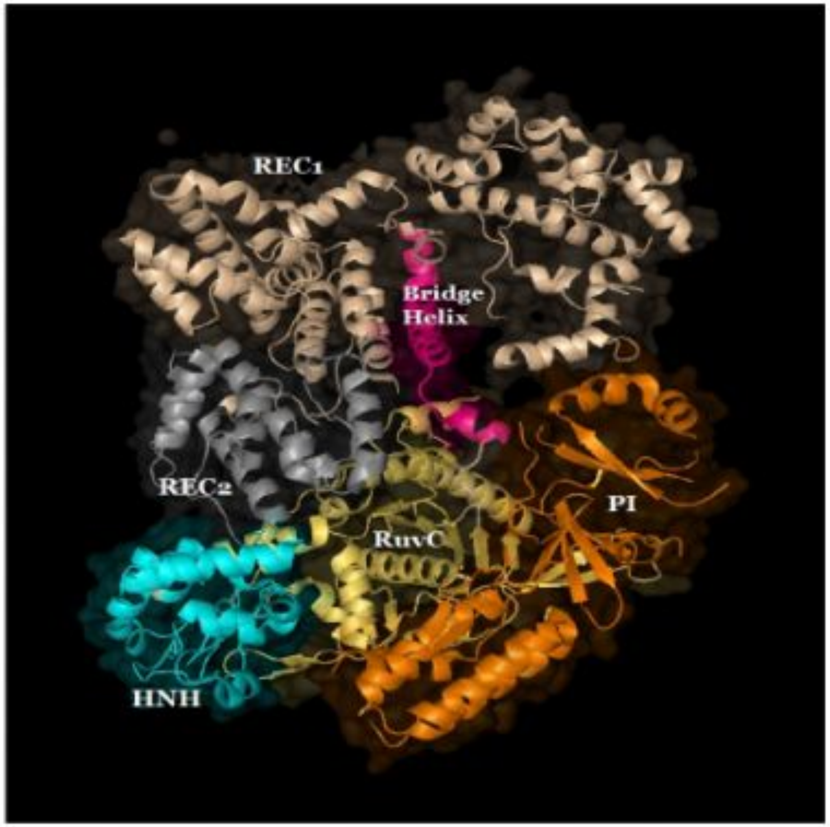

A
Immunization
Virus
Infection
Viral DNA
Plasmid DNA
Cleavage
Novel spacer
Insertion of a novel repeat-spacer unit
Conjugation
Cas complex
csn1
cas1
cas2
csn2
L
5
4
3
2
1
T
CRISPR array

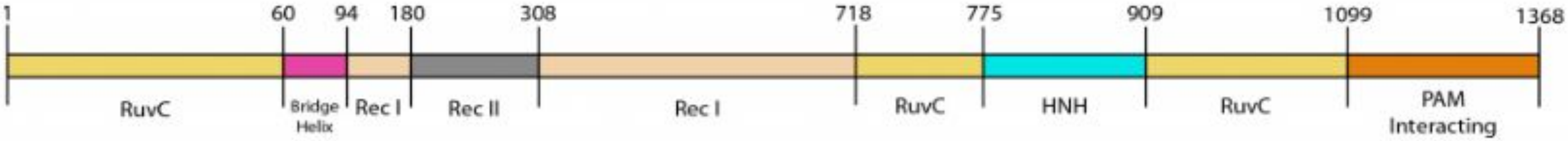

B
Immunity
Acquired immunity against subsequent viral infection or plasmid transfer
Absence of PAM within the CRISPR array prevents autoimmunity
csn1
cas1
cas2
csn2
L
6
5
4
3
2
1
T
Transcription
Cascade (Cas complex)
pre-crRNA
Inactivation
Targeting
Processing
crRNAs
Interference with the invading nucleic acid bearing a proto-spacer and PAM
---CGGACGTGGT TGCTCGACTTGTTAAAAAACTACTGAAGA T GGCG ATGAA---
CAAAAC UGCUCGACUUGUUAAAAAACUACUGAAGA GUUUUA
crRNA

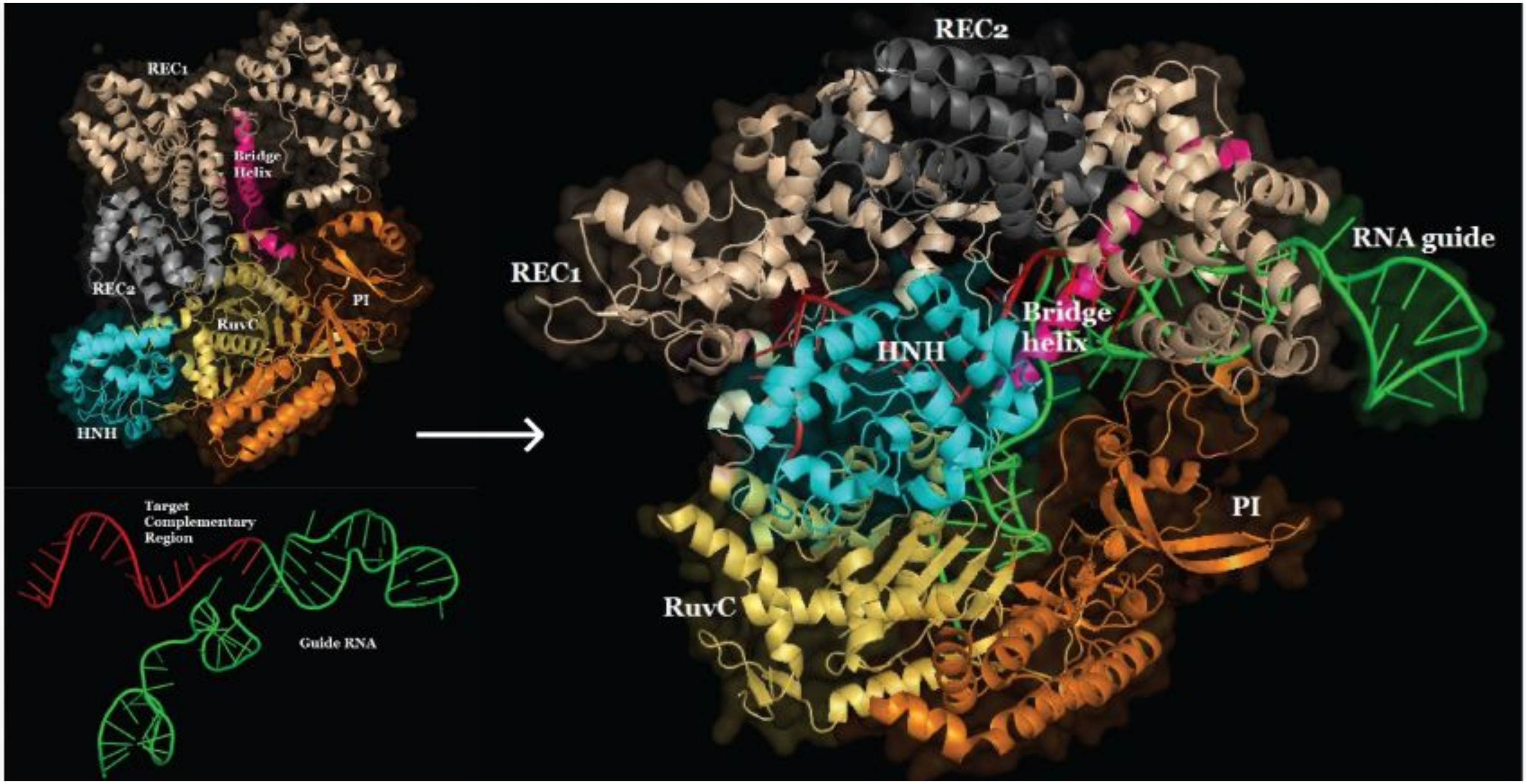

Foreign DNA
NGG
Target
PAM
Acquisition
CRISPR loci
Bacterial genome
tracrRNA
cas9
cas genes
crRNA
crRNA
crRNA
tracrRNA, trRNA
Primary transcript
crRNA biogenesis
Cas9
crRNA
crRNA
Interference
NGG
Cleavage
Foreign DNA


Target Complimentary Region
Tetraloop
Stem Loop 1
Stem Loop 2
Stem Loop 3
Target Complementary Region
Tetraloop
Guide RNA
Stem Loop 1
Stem Loop2
Stem Loop 3

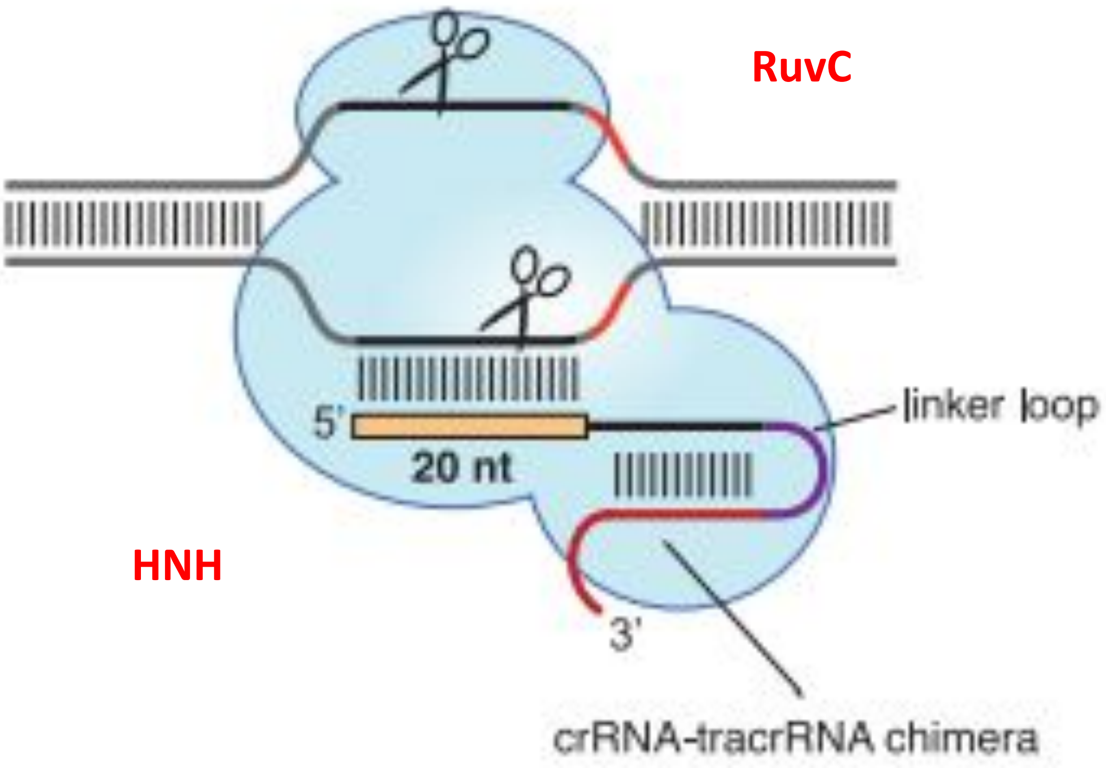

REC I
Bridge Helix
REC II
RuvC
HNH
PAM Interacting


REC1
Bridge
Helix
REC2
PI
RuvC
HNH


1
60
94
180
308
718
775
909
1099
1368
RuvC
Bridge
Helix
Rec I
Rec II
Rec I
RuvC
HNH
RuvC
PAM
Interacting


REC1
Bridge Helix
REC2
RuvC
PI
HNH
Target Complementary Region
Guide RNA
REC2
REC1
RNA guide
Bridge helix
HNH
PI
RuvC

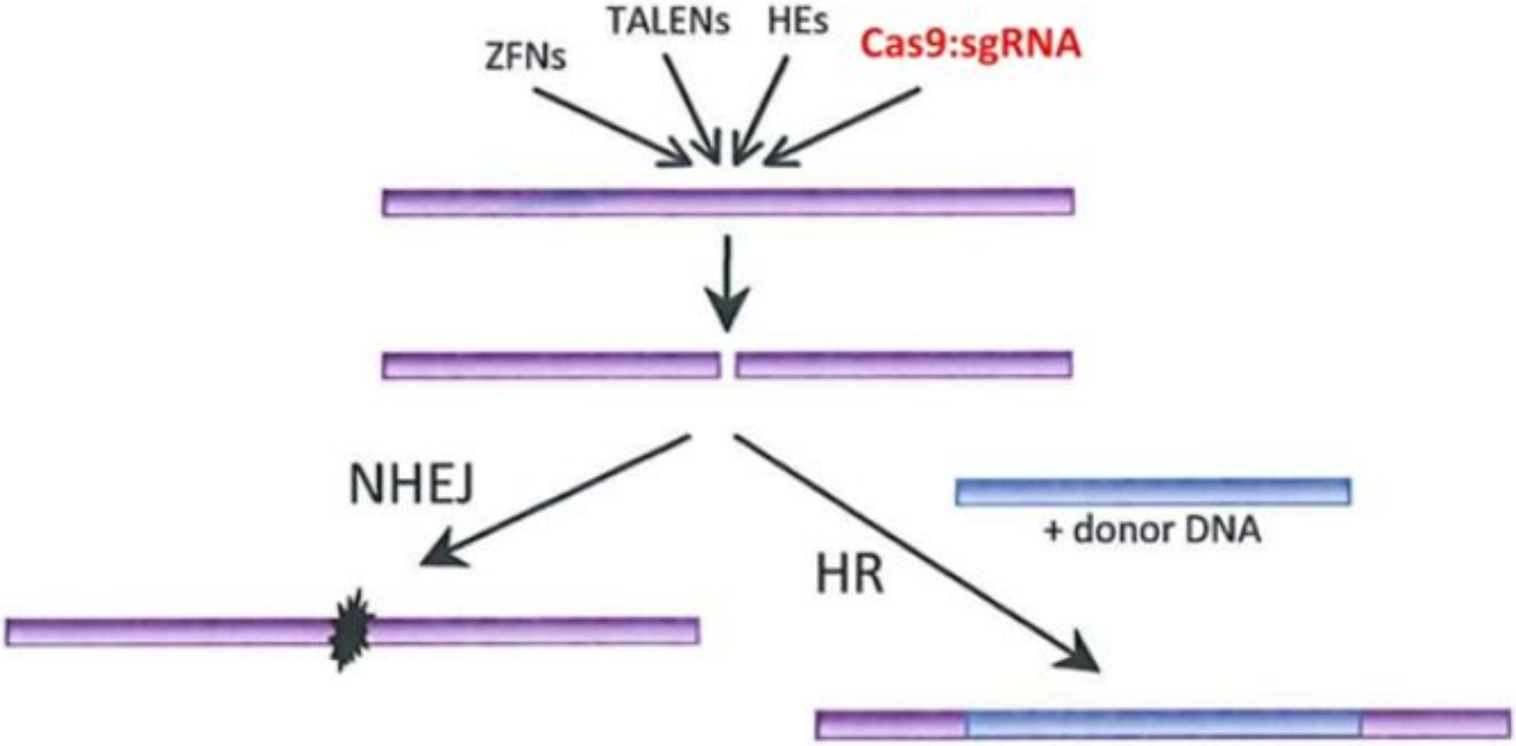
Two major domains

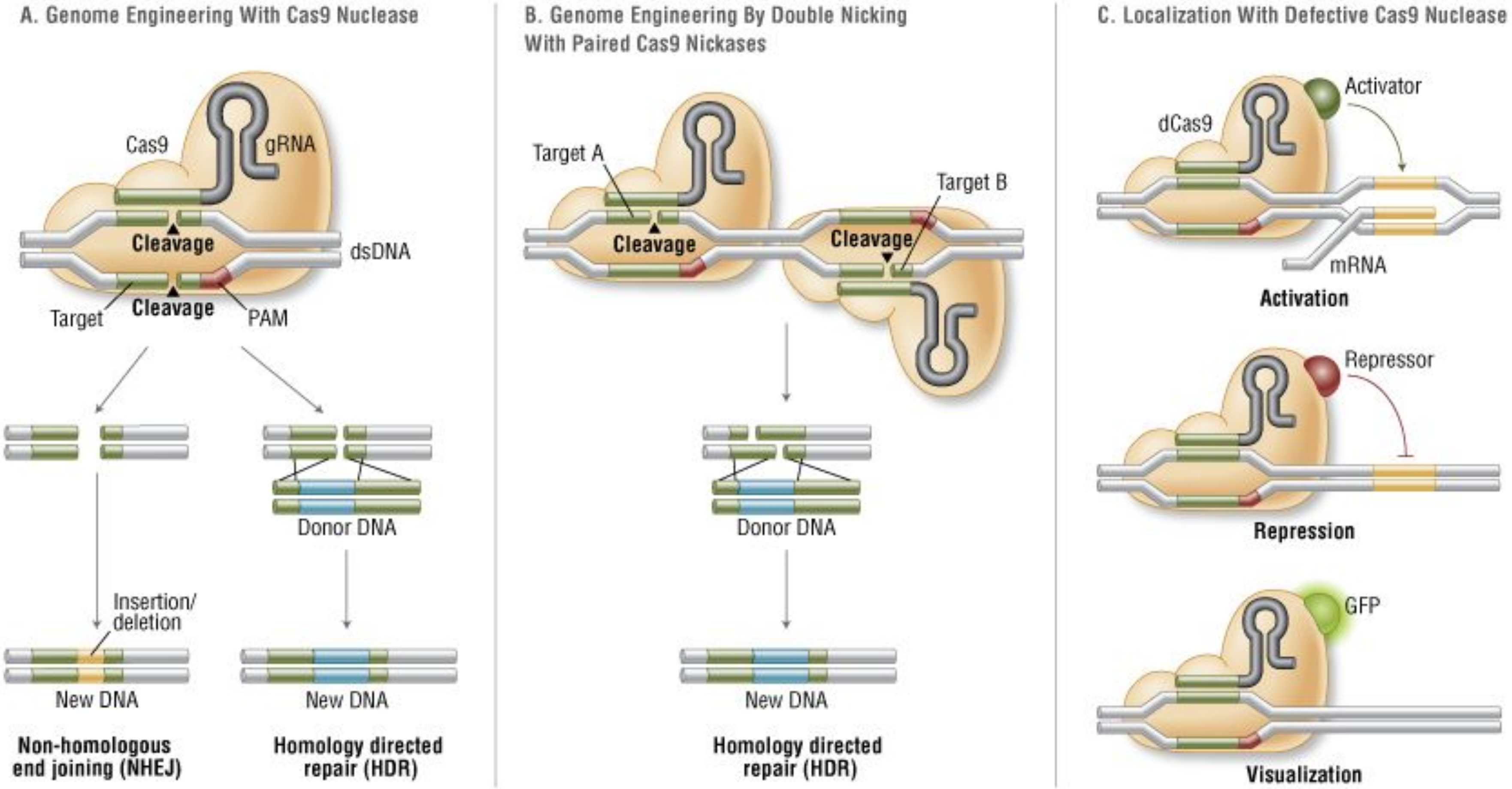


RuvC

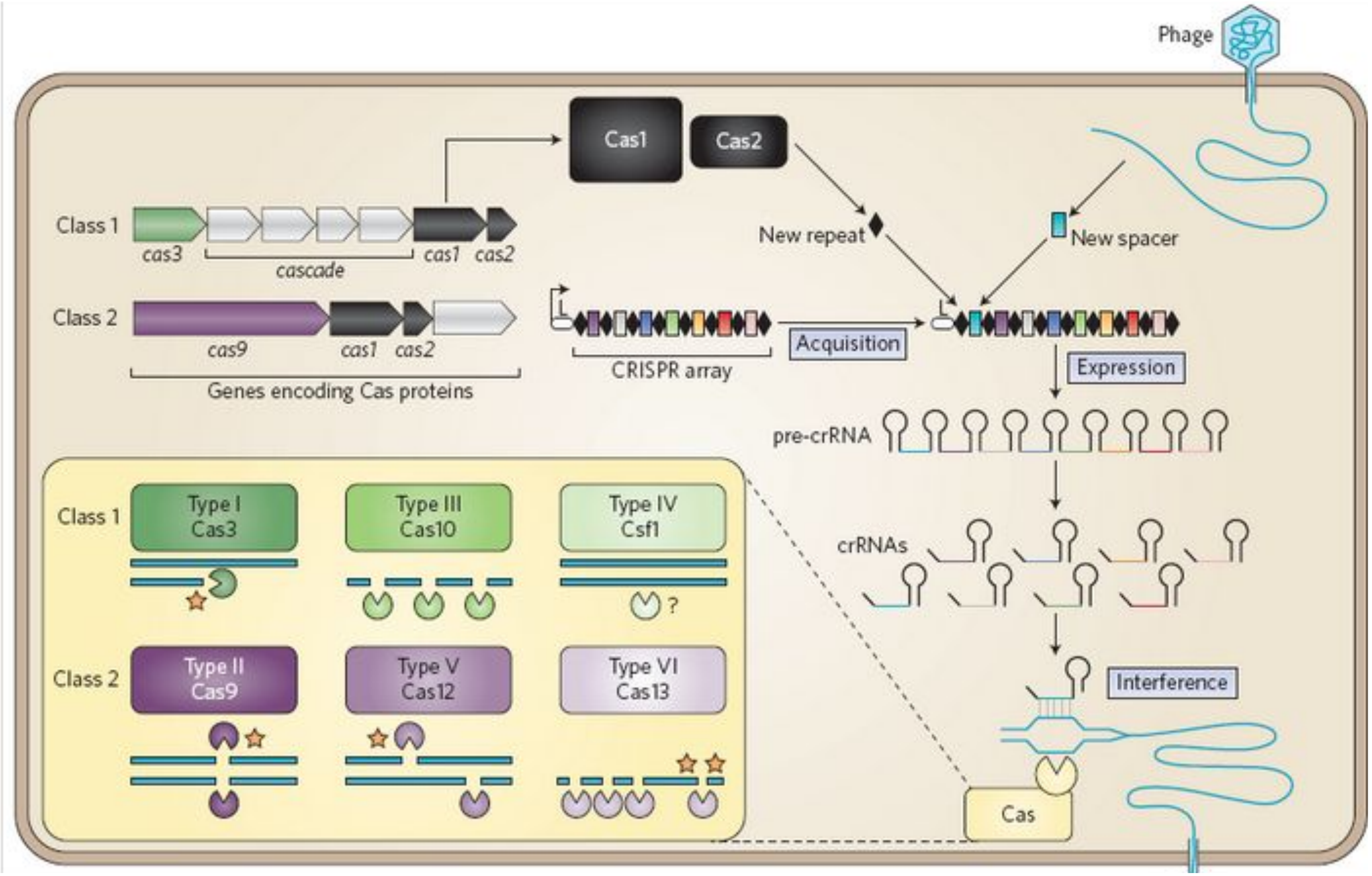
HNH

Genome editing begins with dsDNA cleavage

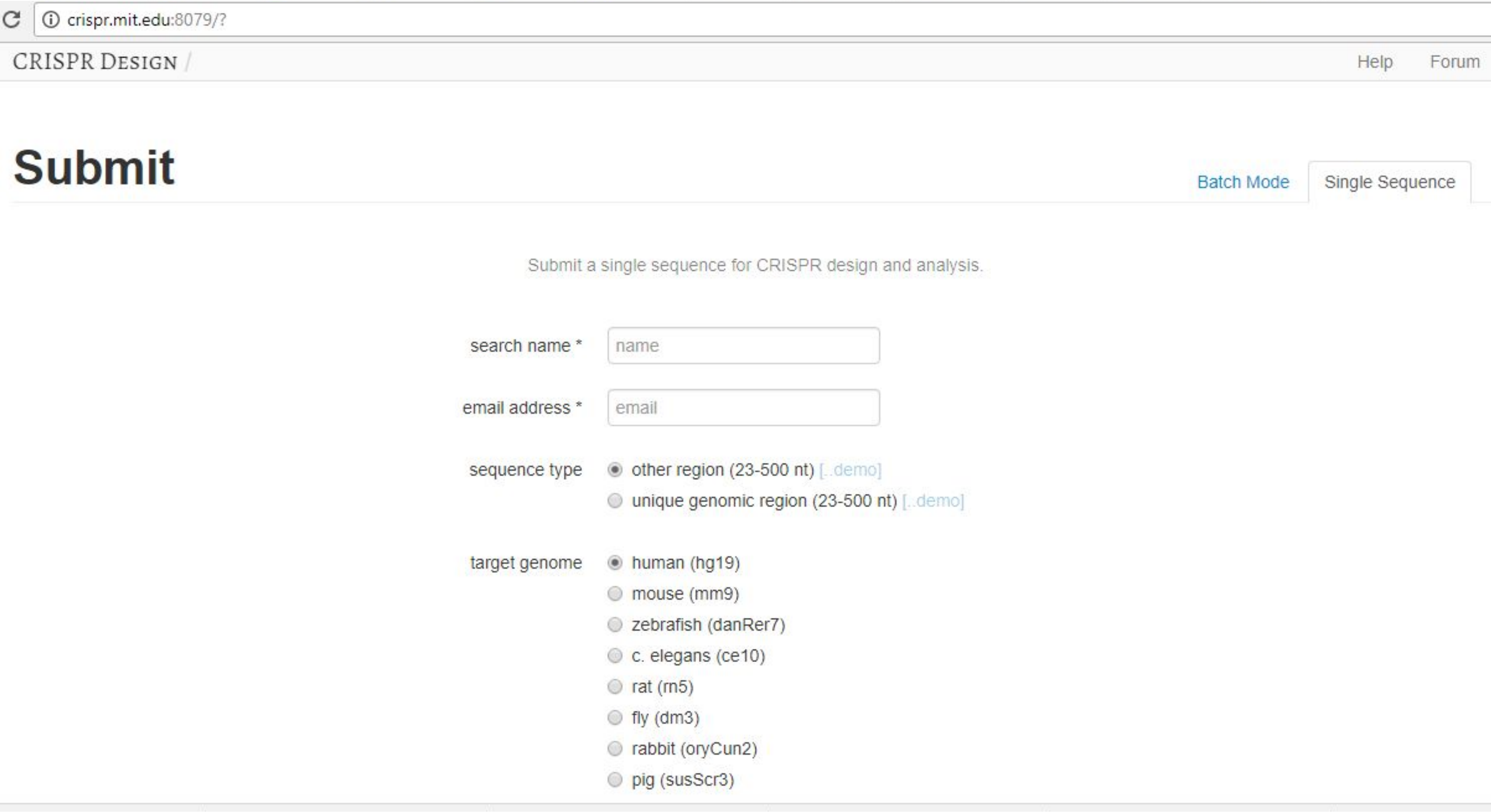



A. Genome Engineering With Cas9 Nuclease
Cas9
gRNA
Cleavage
dsDNA
Target
Cleavage
PAM
Insertion/ deletion
New DNA
Non-homologous end joining (NHEJ)
Donor DNA
New DNA
Homology directed repair (HDR)
B. Genome Engineering By Double Nicking With Paired Cas9 Nickases
Target A
Cleavage
Target B
Cleavage
Donor DNA
New DNA
Homology directed repair (HDR)
C. Localization With Defective Cas9 Nuclease
dCas9
Activator
mRNA
Activation
Repressor
Repression
GFP
Visualization

Variety of CRISPR system



CRISPR design tool



Submit

Submit a single sequence for CRISPR design and analysis.

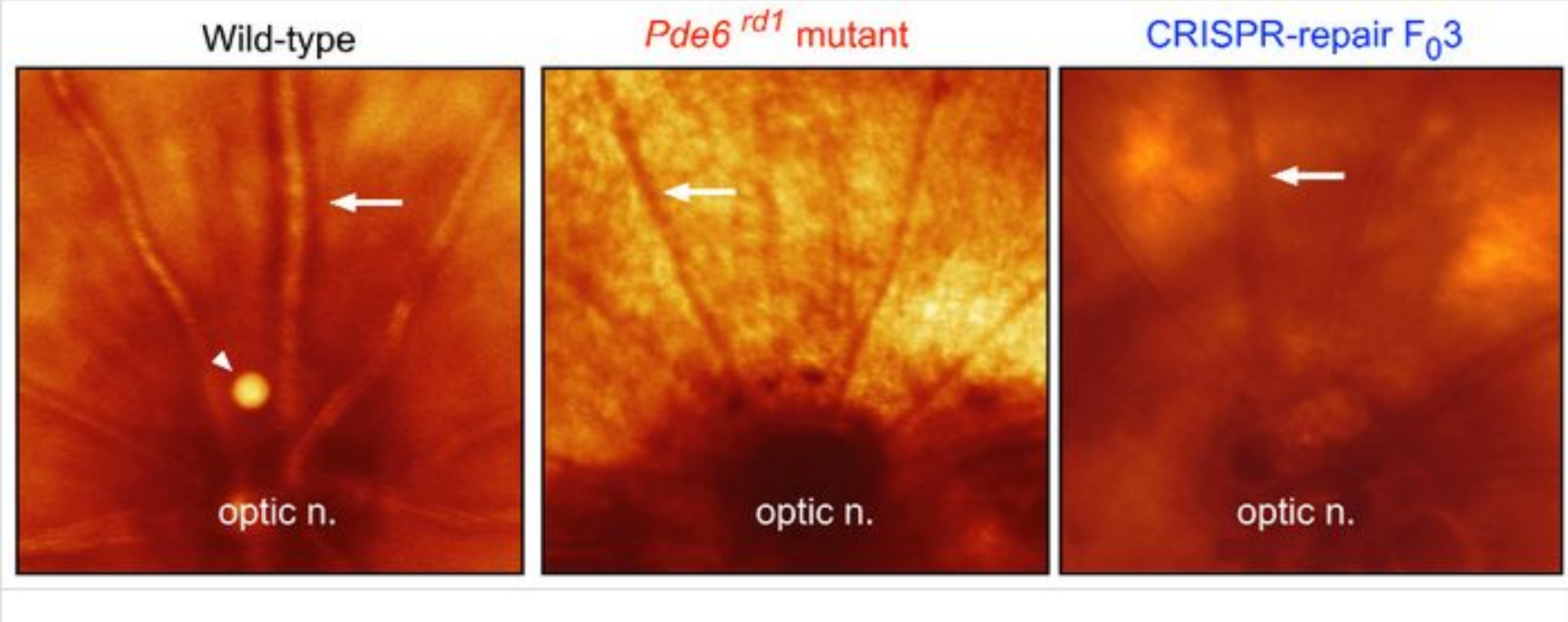
search name * name

email address * email

sequence type
● other region (23-500 nt) [..demo]
○ unique genomic region (23-500 nt) [..demo]

target genome
● human (hg19)
○ mouse (mm9)
○ zebrafish (danRer7)
○ c. elegans (ce10)
○ rat (rn5)
○ fly (dm3)
○ rabbit (oryCun2)
○ pig (susScr3)

W.-H. Wu et al., 2016. CRISPR repair reveals causative mutation in a preclinical model of retinitis pigmentosa.

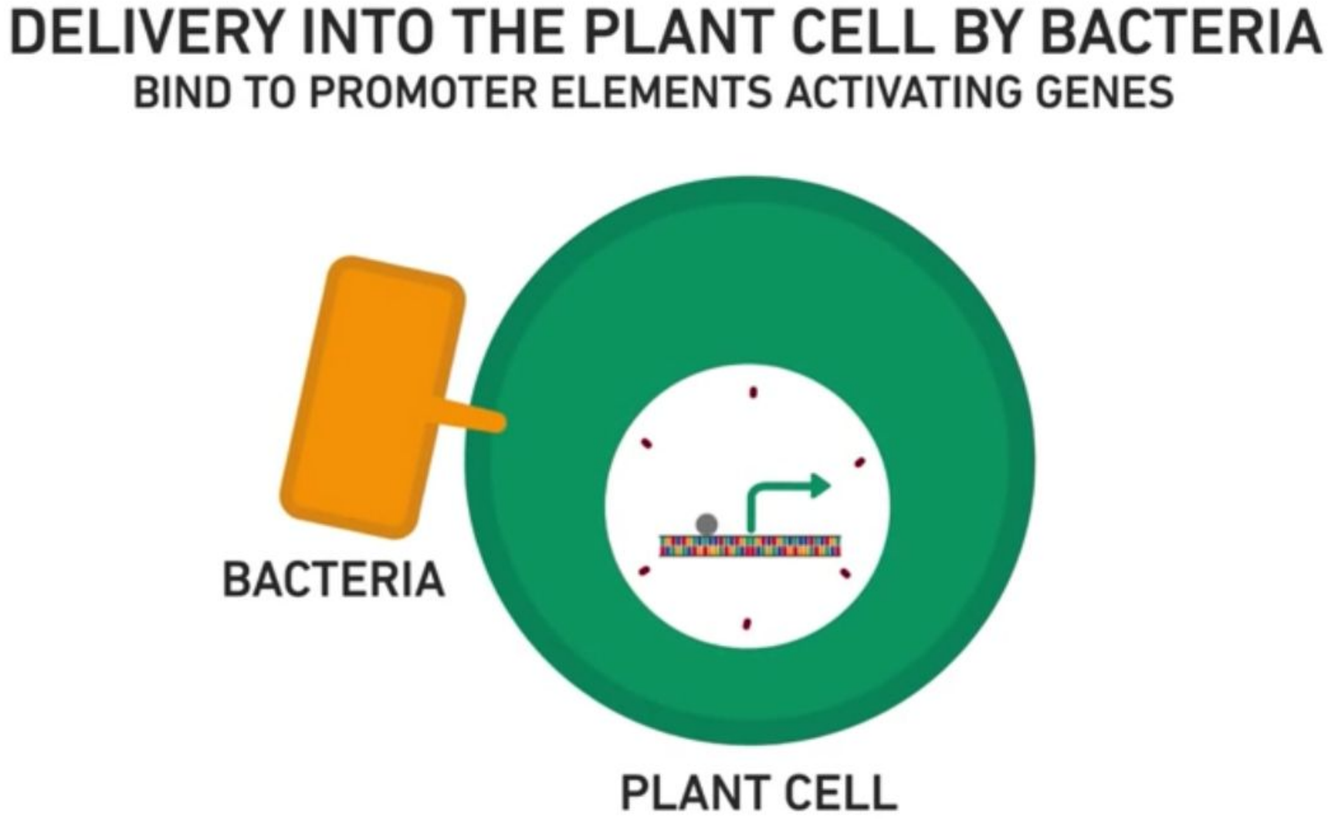


K. A. Schaefer et al., 2017. Unexpected mutations after CRISPR-Cas9 editing in vivo

117 insertions/delitions

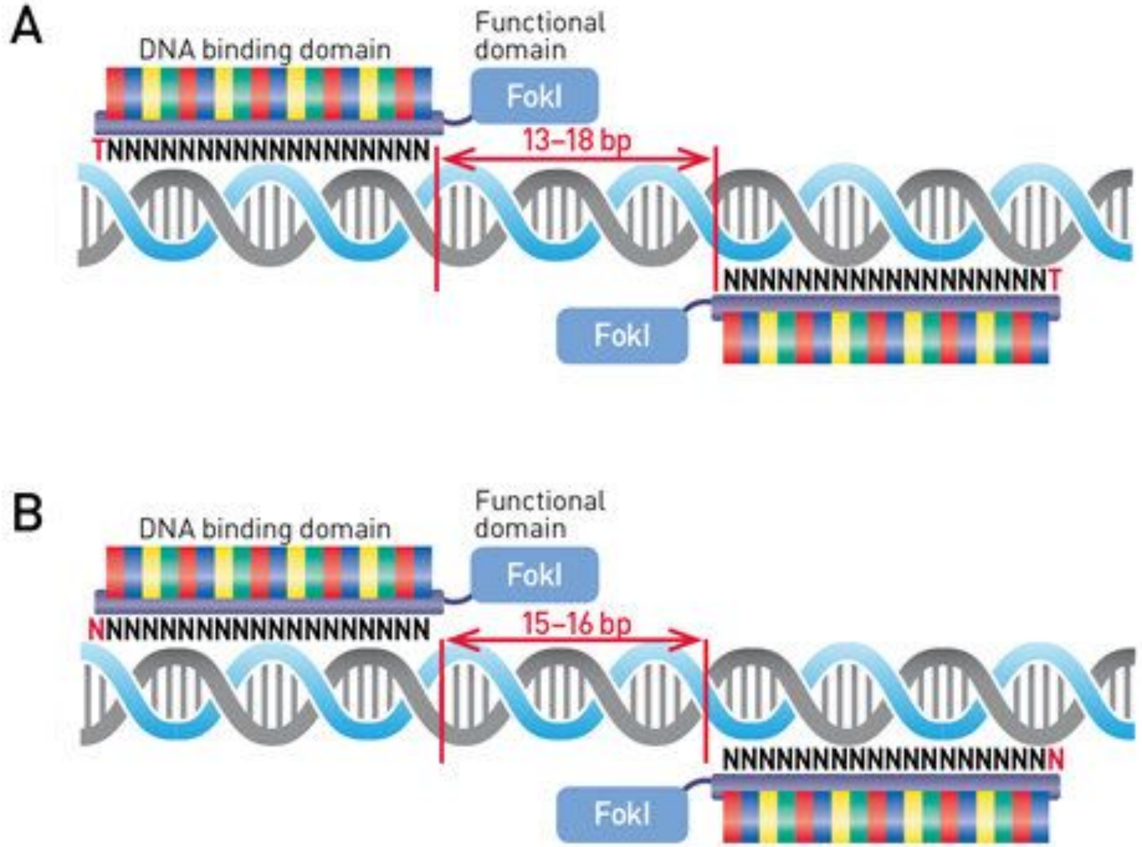
1397 SNP

TALEN
transcription activator-like nuclease

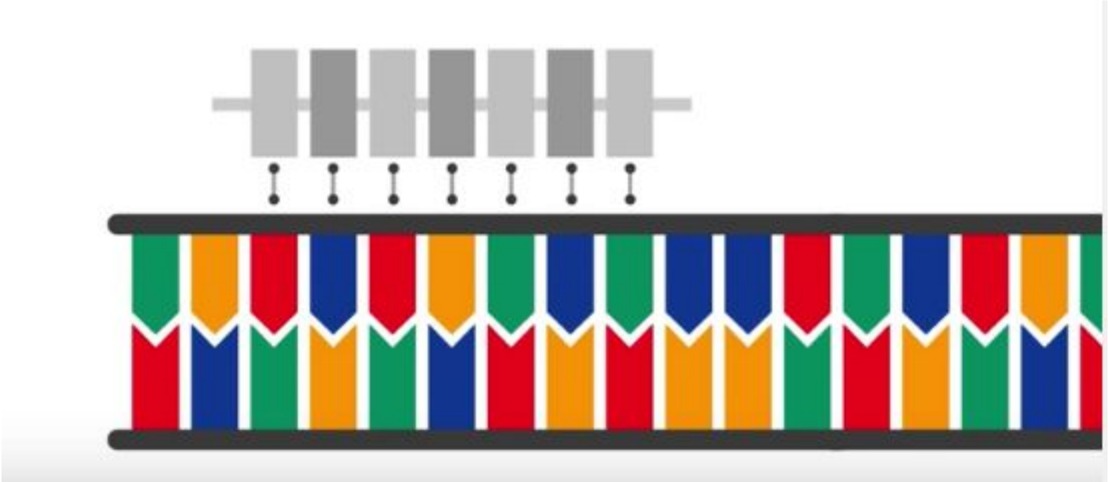


From *Xanthomonas* genus – crops pathogen

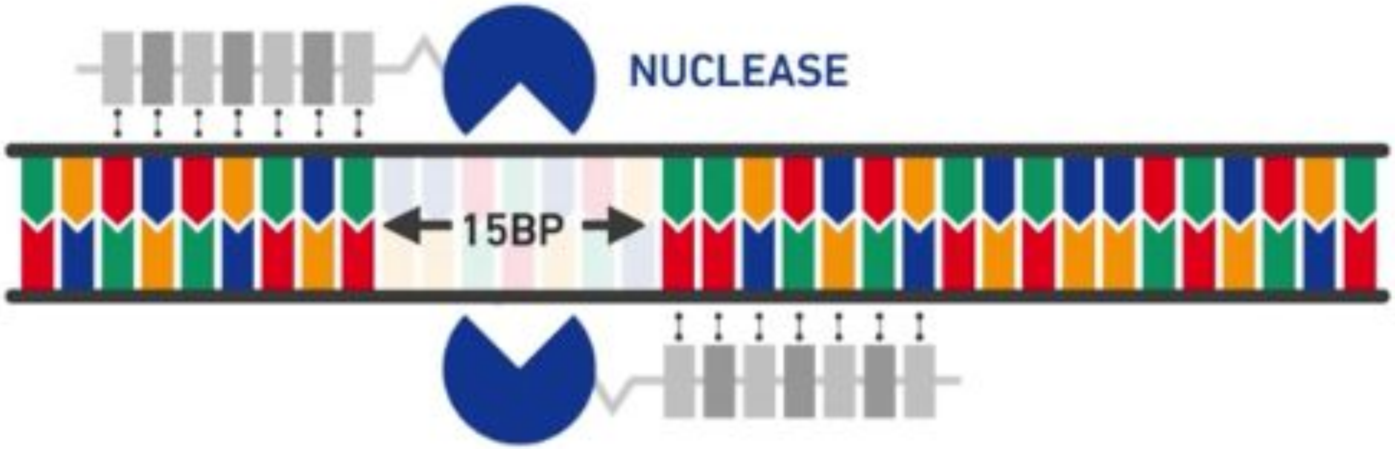
TALE proteins are composed of a central domain responsible for DNA binding, a nuclear localization signal, and a domain that activates the target gene transcription



DNA-binding domain was demonstrated to consist of monomers, each of them binds one nucleotide in the target nucleotide sequence



Monomers are tandem repeats of 34 amino acid residues, two of which are located at positions 12 and 13 and are highly variable (repeat variable diresidue, RVD), and it is they that are responsible for the recognition of a specific nucleotide


NUCLEASE
15BP

Property	TALEN	CRISPR-Cas9
Type of recognition	Protein-DNA	RNA-DNA
Methylation sensitive?	Sensitive	Not sensitive
Off-target effects	Fewer observed off-target effects	More potential for off-target effects than TALENs & ZFNs