CRISPR-Cas system

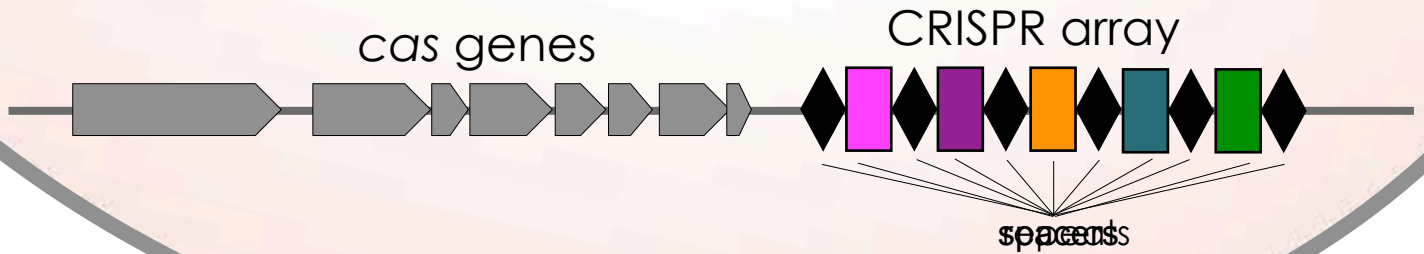
CRISPR (clustered regularly interspaced short palindromic repeats)

Cas (CRISPR-associated) proteins

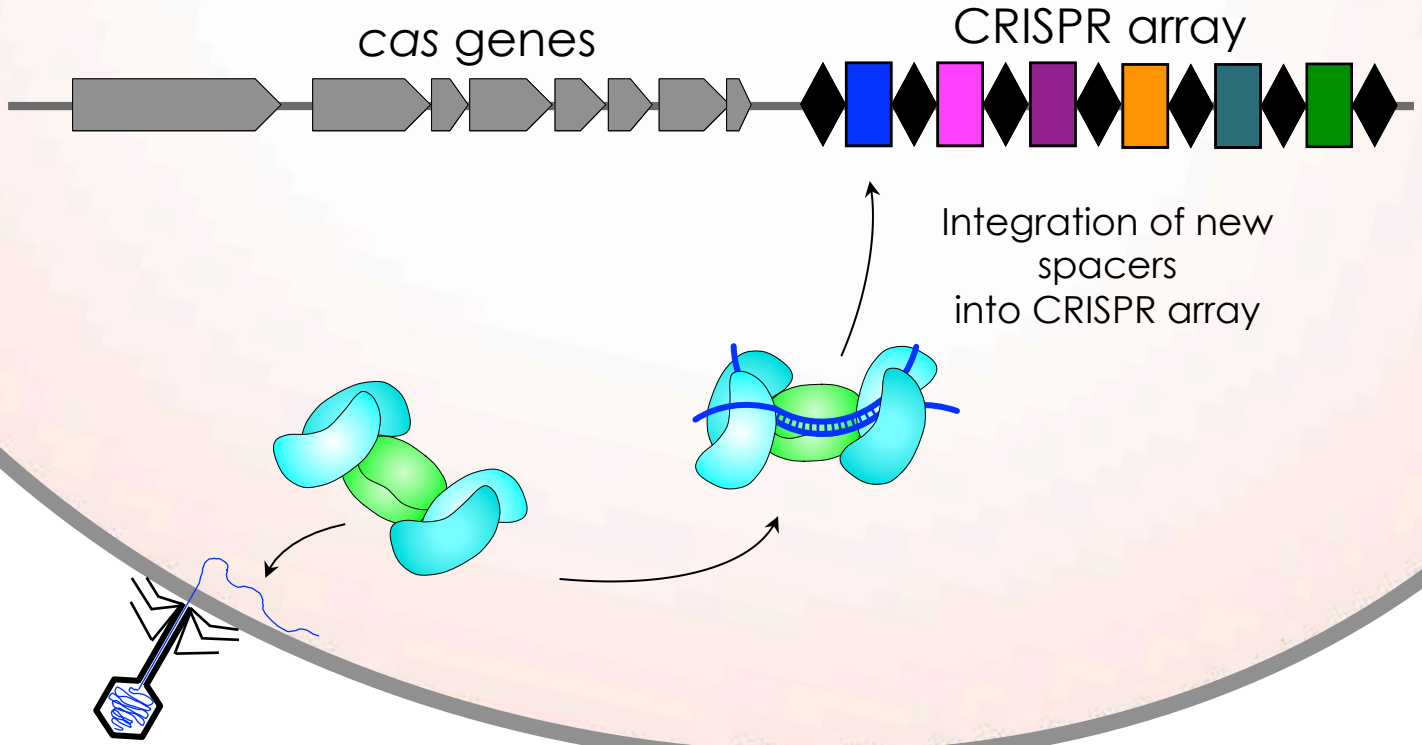
CRISPR-Cas system provide prokaryotes with adaptive immunity

- immunization – cells collect molecular records of encounters with genetic invaders;
- self versus non-self discrimination – cells must avoid autoimmunity but specifically target and destroy foreign genome

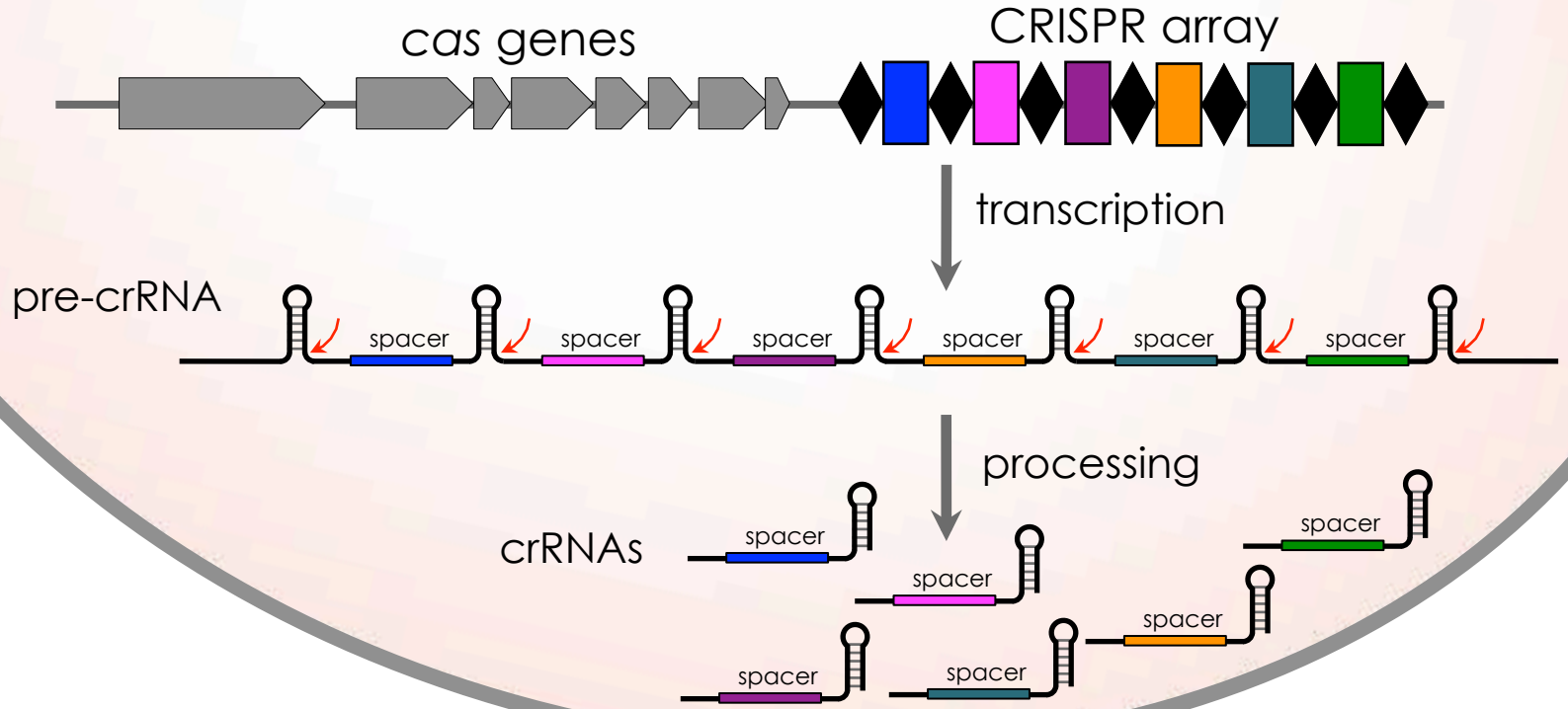
CRISPR-Cas locus structure



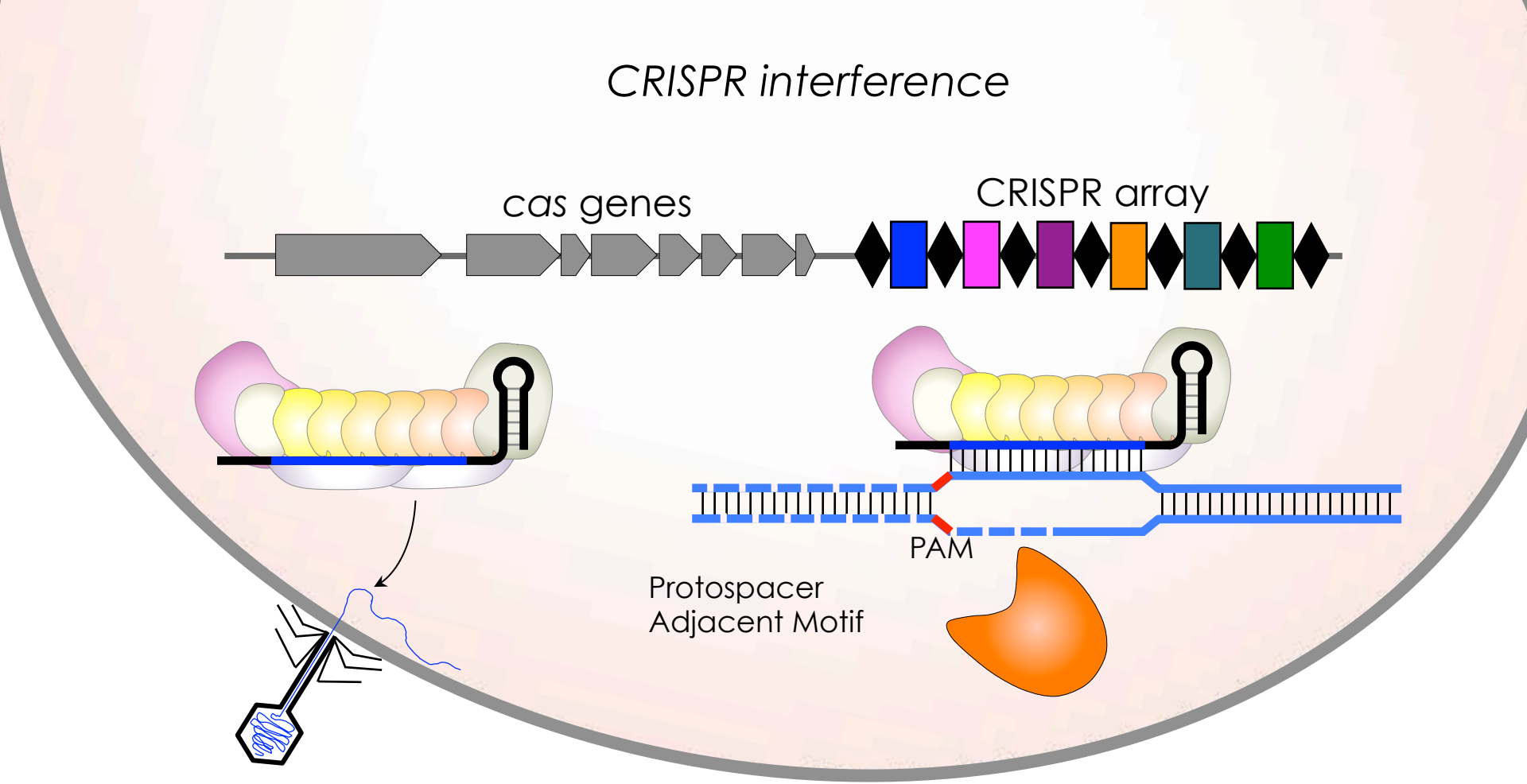
CRISPR adaptation



CRISPR RNA biogenesis



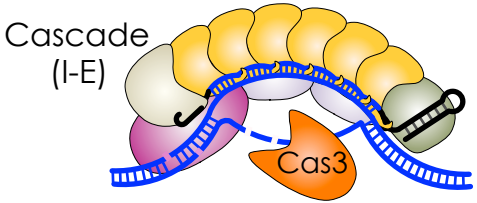
CRISPR interference



Classification of CRISPR-Cas systems

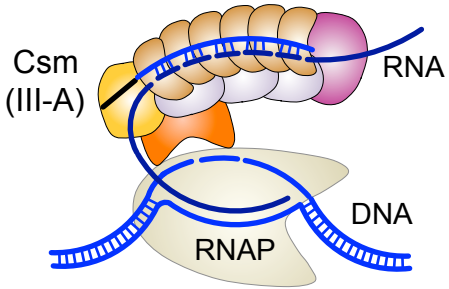
Class 1 (multi-subunit effector complex)

Type I



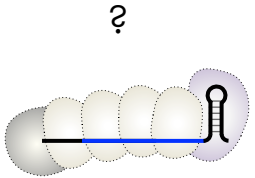
Target: dsDNA

Type III



Target: ssRNA/ssDNA

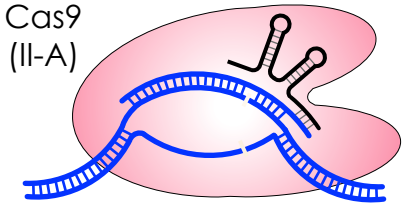
Type IV



Target unknown

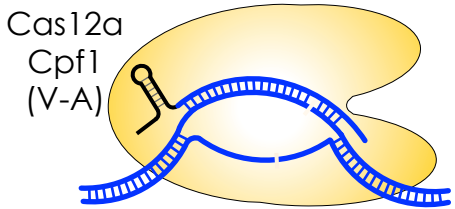
Class 2 (single-subunit effector complex)

Type II



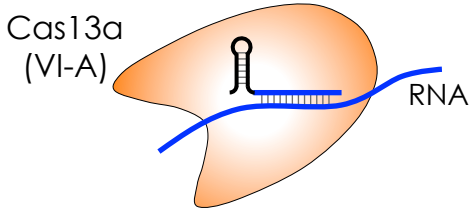
Target: dsDNA

Type V



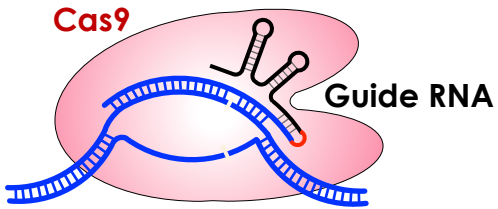
Target: dsDNA

Type VI

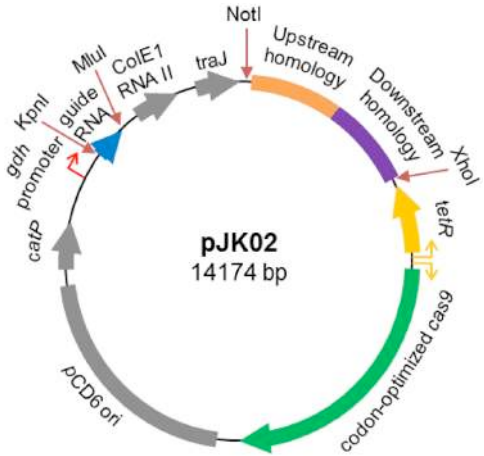


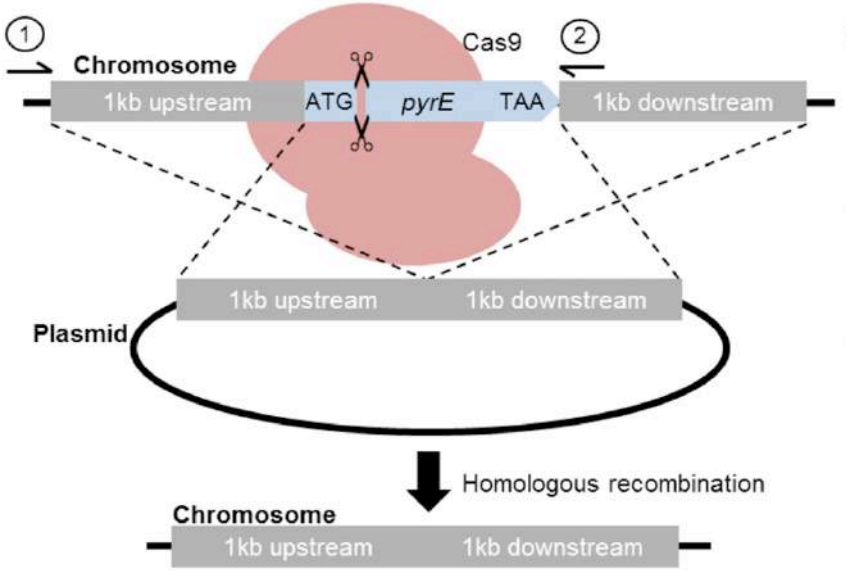
Target: ssRNA

CRISPR-Cas9-based tool

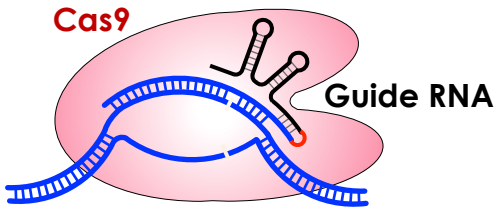


McAllister et al. Sci Reports 2017





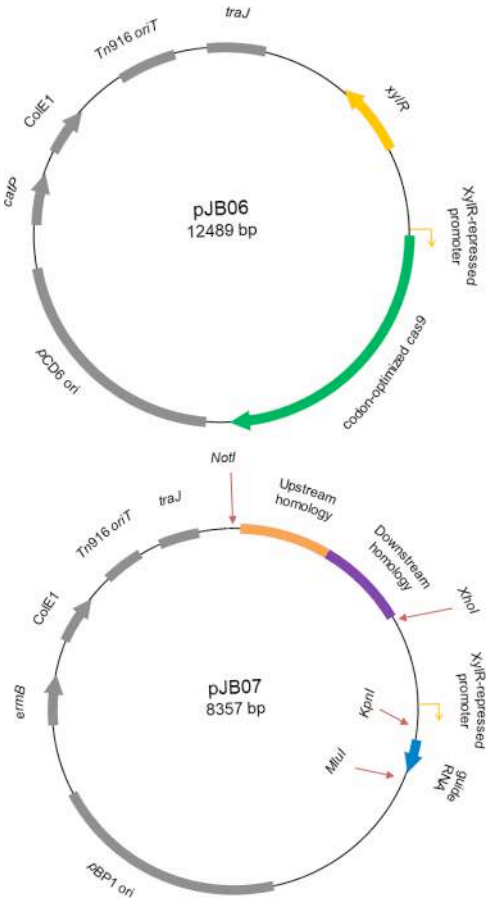
Optimized CRISPR-Cas9-based tool



A two-plasmid mutagenesis system to avoid toxicity of CRISPR-Cas9 :

- Xylose-inducible expression of *cas9*

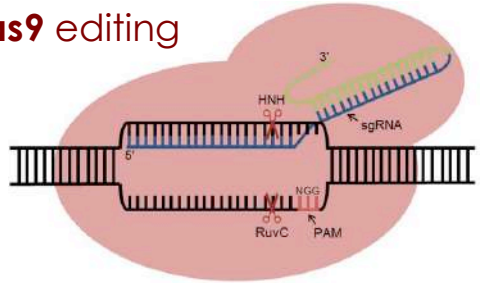
- Xylose-inducible expression of guide RNA



Brehn & Sorg. Microbiology Resources announcement 2022

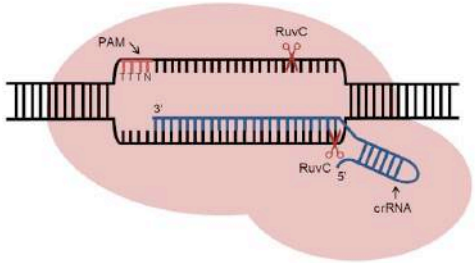
Variety of CRISPR-Cas-based tools

Cas9 editing

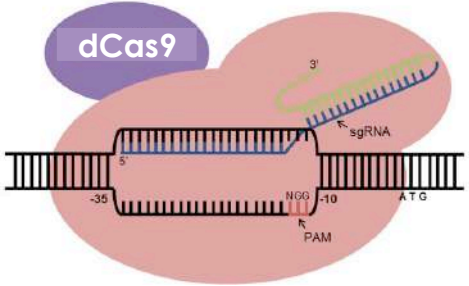


Mutant Alleles

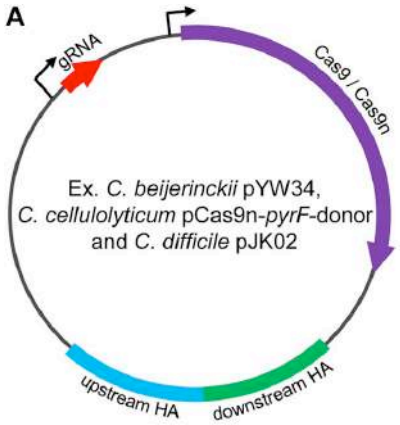
Mutation	Catalytic activity	Name
D10A	ΔRuvC	Cas9n, "nickase"
D10A / H840A	Dead	dCas9 / CRISPRi

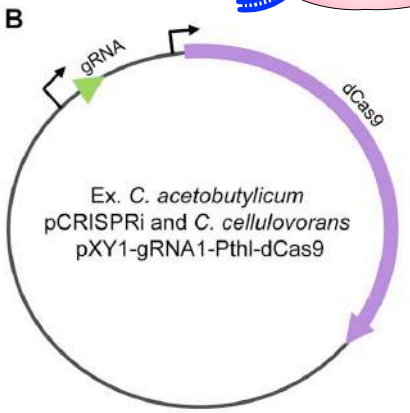


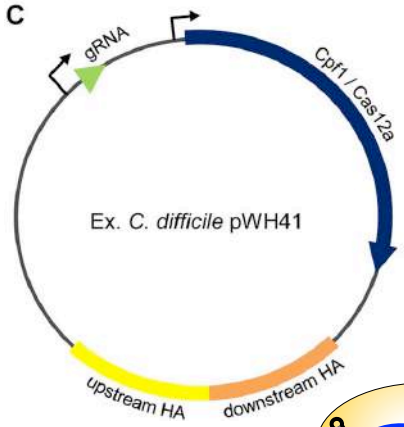
Cpf1/Cas12a editing

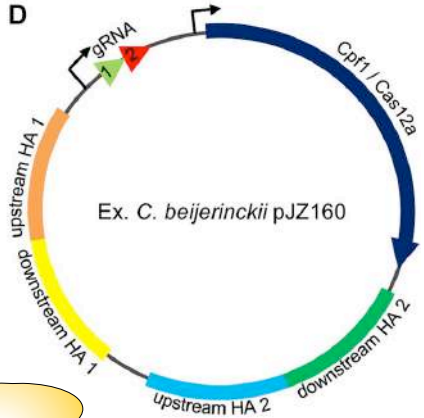


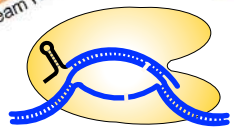
dCas9 CRISPRi gene knock-down











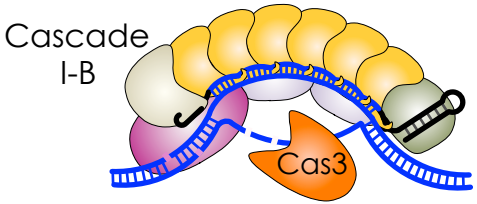
McAllister & Sorg. J. Bacteriology 2019

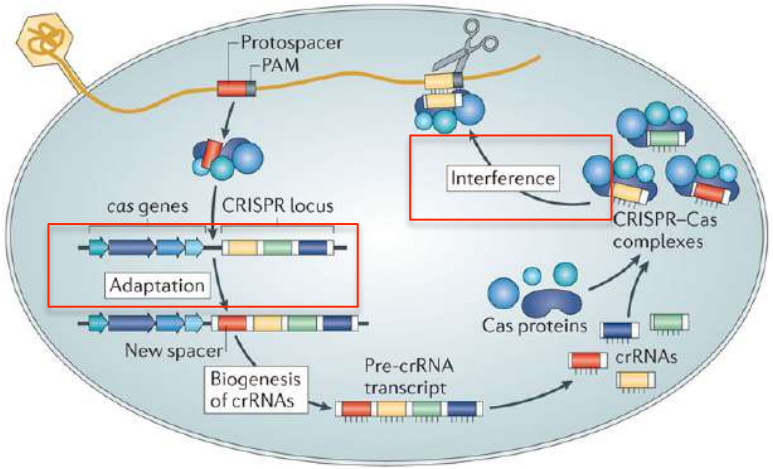
Endogenous *C. difficile* CRISPR-Cas system

UNIVERSITÉ DE SHERBROOKE

Hôpitaux Universitaires Est Parisien
SAINT-ANTOINE

Boudry et al. mBio. 2015
Maikova et al. NAR. 2018
Maikova et al. Front Microbiol. 2018
Maikova. PhD thesis
Maikova et al. mBio. 2021
Muzyukina et al. mSphere. 2023





Hargreaves et al. 2014
Andersen et al. 2016

In *CD* strain 630 : 12 CRISPR regions exist and are expressed

In epidemic *CD* strain 027: 9 active CRISPR arrays

↓

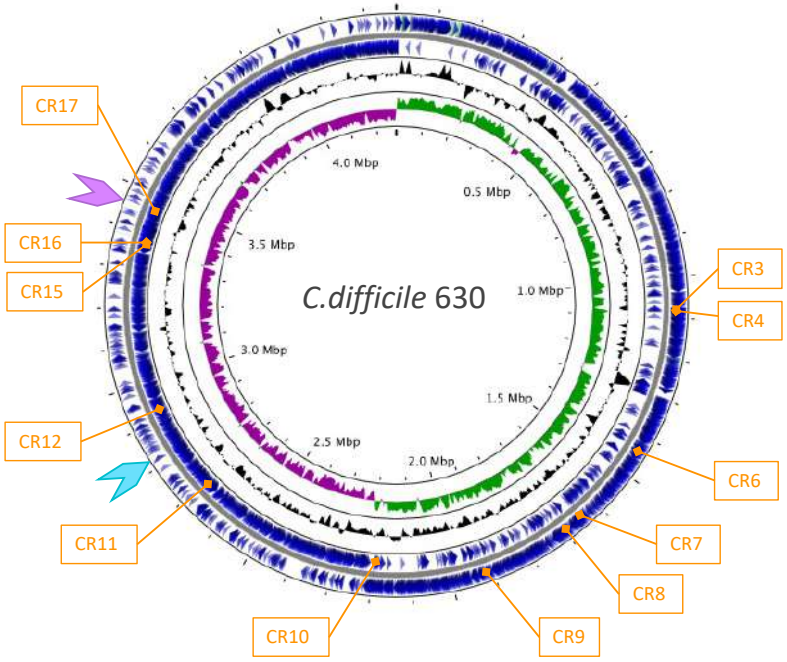
…. A total of 819 spacers from nine *CD* strains

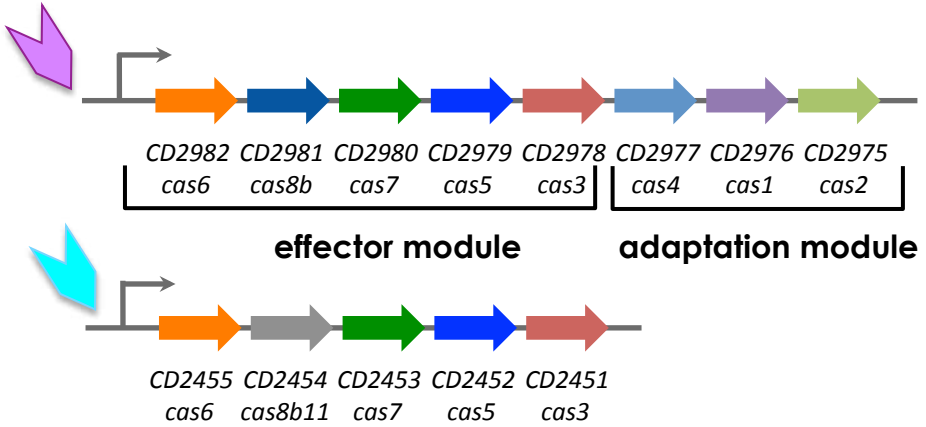
Large defence capacity within phage-rich gut communities

CRISPR analysis in *C. difficile*

- **Type I-B CRISPR-Cas system** (active for interference and adaptation)
- **Large number of arrays** (the average of 8 array/genome, **12** in 630 strain)
- **2-3 *cas* gene sets (type I-B)** present in the majority of strains (about 2000)
- Location of arrays in **phage** regions **(5** in 630 strain, 3 highly expressed**)**

C.difficile chromosome 630
4,290,252 bp

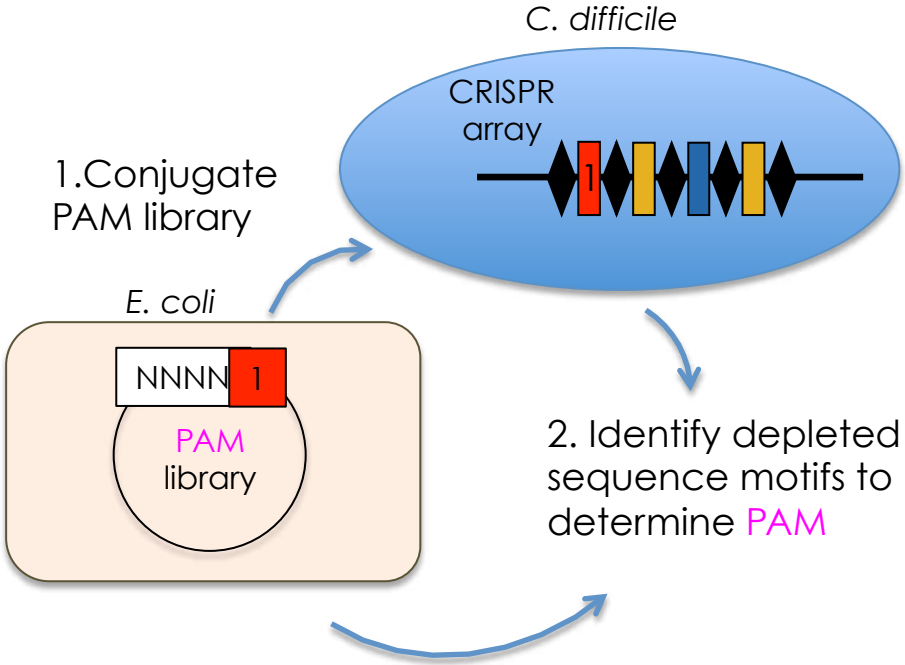




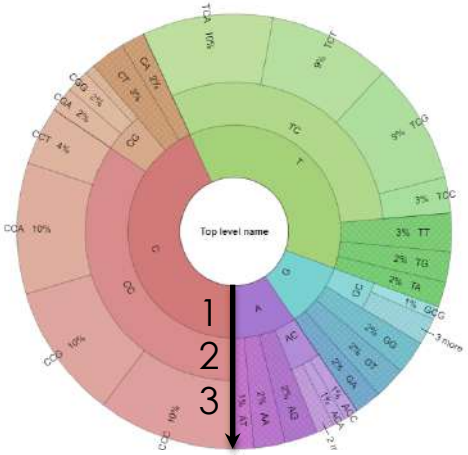
Boudry et al. mBio. 2015
Maikova et al. NAR. 2018
Maikova et al. Front Microbiol. 2018
Maikova et al. AEM 2019
Maikova. PhD thesis
Maikova et al. mBio. 2021
Muzyukina et al. mSphere. 2023

PAM sequences in *C. difficile* 630E and R20291 strains

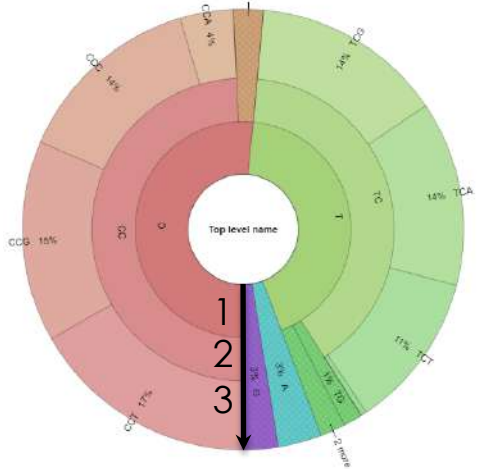
Maikova. PhD thesis
Maikova et al. mBio 2021



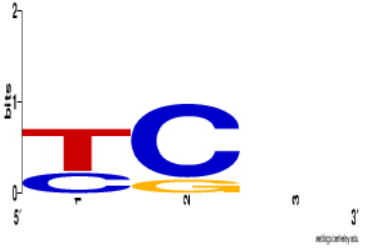
Functional PAMs **CCN/TCN** in accordance with interference efficiency experiments



630 strain



R20291 strain

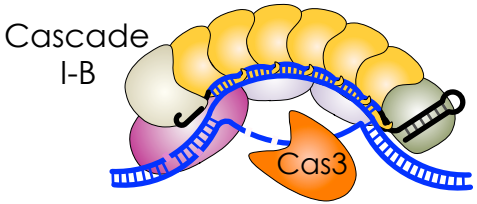


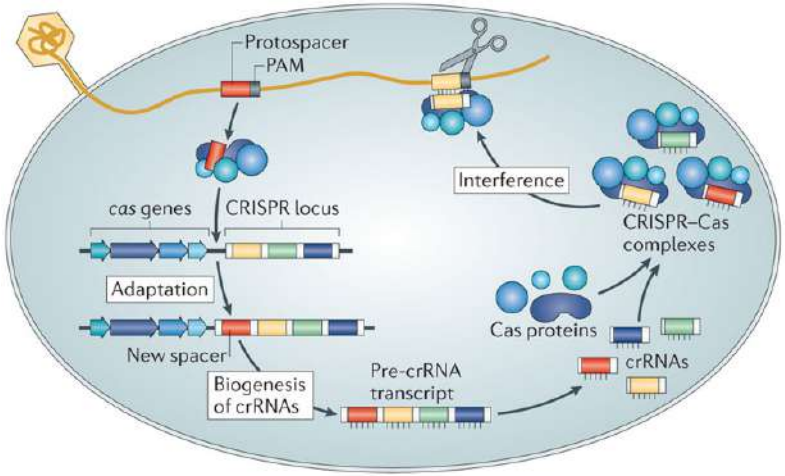
C. difficile CRISPR-Cas system applications

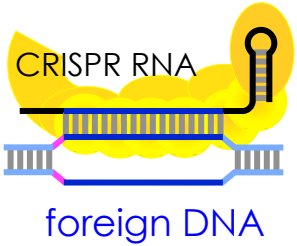
UNIVERSITÉ DE SHERBROOKE

Hôpitaux Universitaires Est Parisien
SAINT-ANTOINE

Boudry et al. mBio. 2015
Maikova et al. NAR. 2018
Maikova et al. Front Microbiol. 2018
Maikova. PhD thesis
Maikova et al. AEM. 2019
Muzyukina et al. In preparation
Andersen et al. 2016







Sensitive high-resolution CRISPR-based typing for epidemiology & *CD* microevolution survey

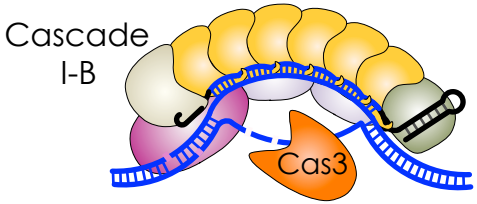
Endogenous system for *CD* genome editing

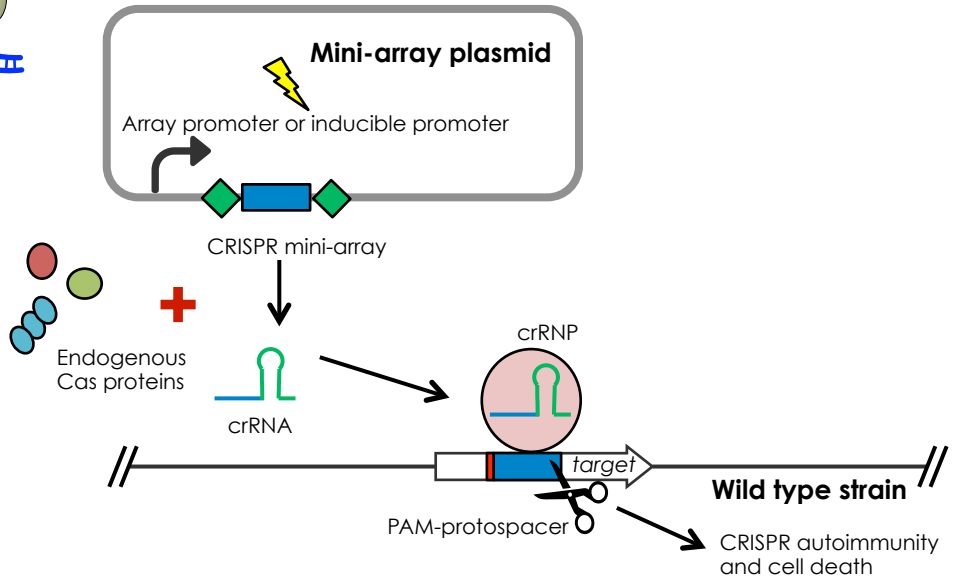
Self-targeting, autoimmunity

Great potential for genome editing and therapeutic applications

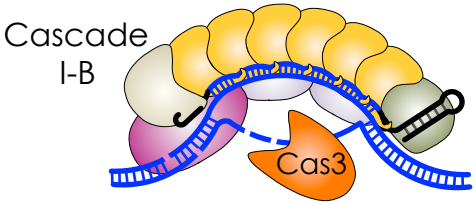
Genome editing in *C. difficile* using the endogenous CRISPR-Cas system

Maikova et al. Frontiers Microbiol. 2018
Maikova. PhD thesis
Maikova et al. AEM. 2019

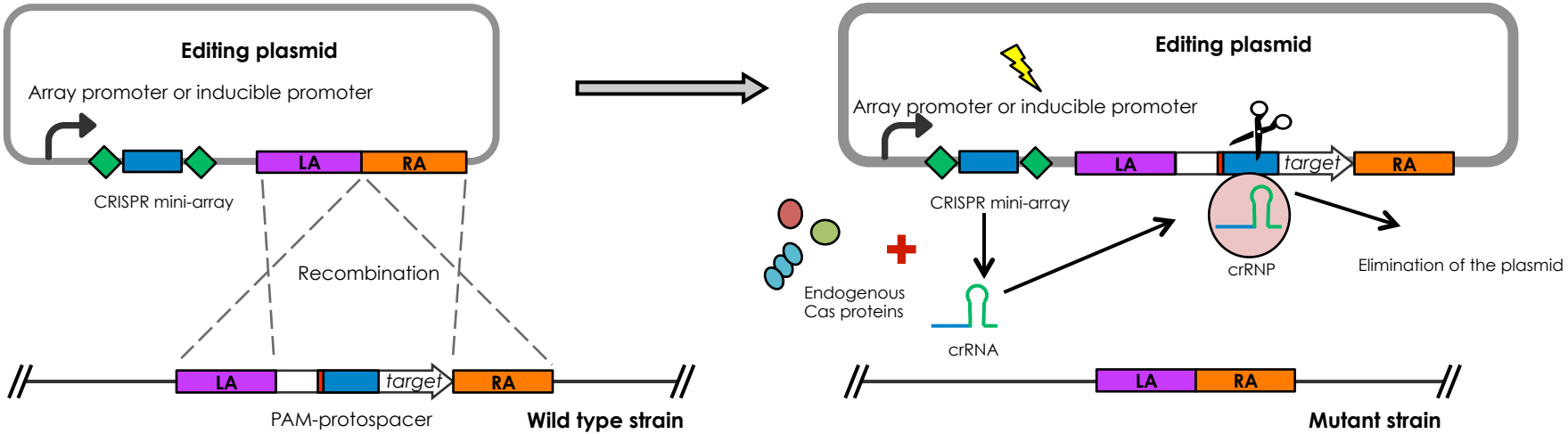




Genome editing in *C. difficile* using the endogenous CRISPR-Cas system



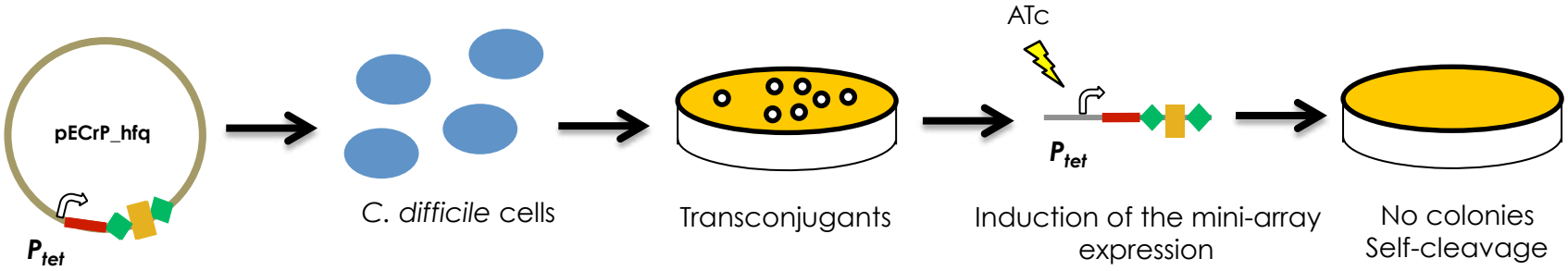
Maikova et al. Frontiers Microbiol. 2018
Maikova. PhD thesis
Maikova et al. AEM. 2019



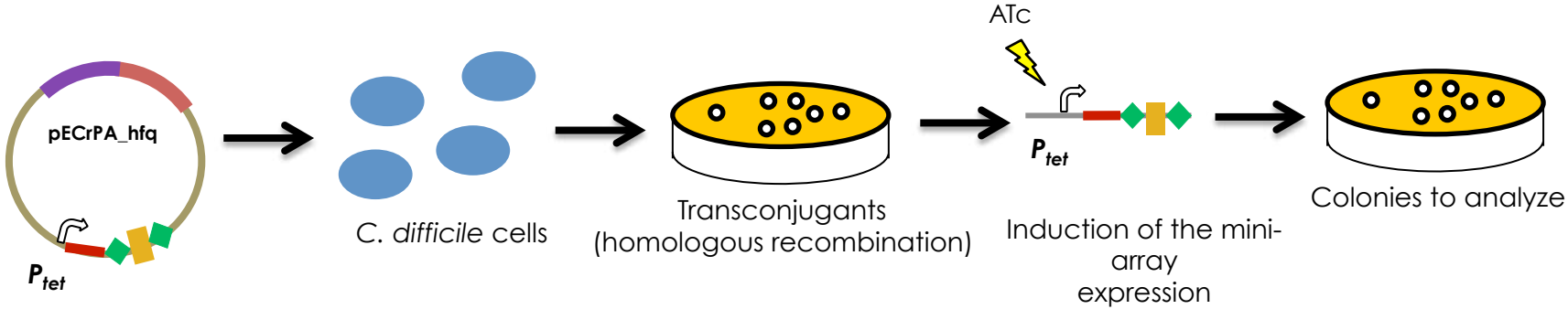
No need of unstable plasmid:
CRISPR-Cas cleavage

Genome editing in *C. difficile* using the endogenous CRISPR-Cas system

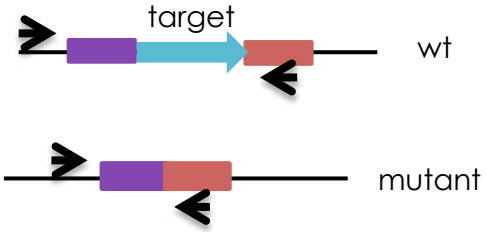
Induced endogenous **CRISPR-Cas autoimmunity** **to check CRISPR spacer efficiency**

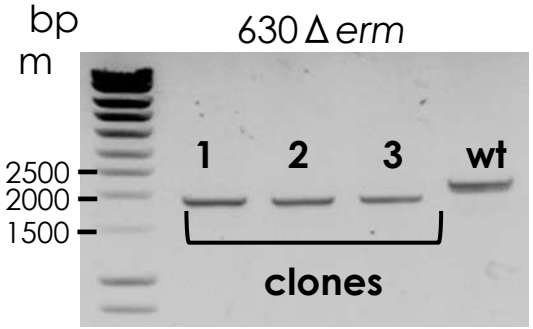


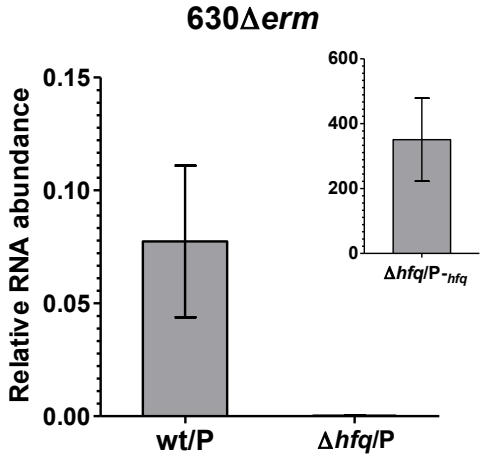
Mutant construction using plasmid with homologous arms

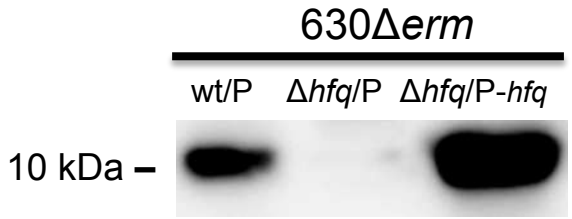


Genome editing in *C. difficile* using the endogenous CRISPR-Cas system







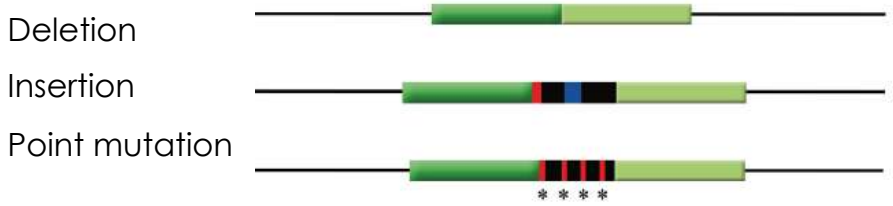


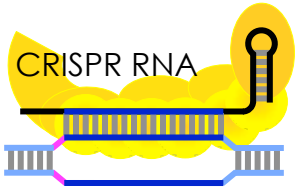
- **Δ*hfq* mutants** in 630 and R20291 strains
- **Could not be obtained** using **other** genome editing methods (*codA* allele exchange and ClosTron)
- **Efficient plasmid** loss

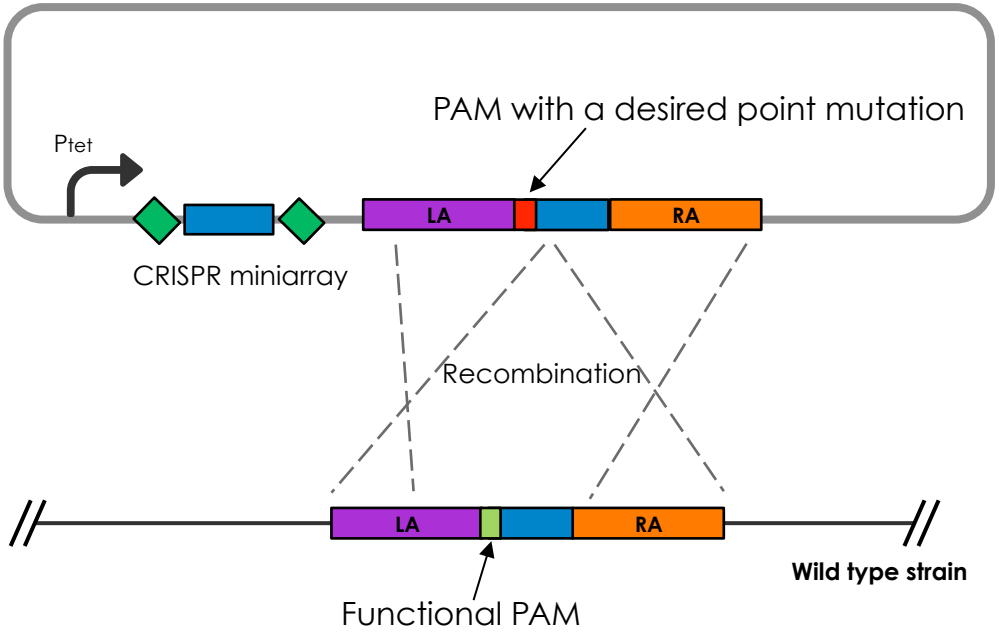
Maikova. PhD thesis
Maikova et al. AEM. 2019

Various mutagenesis types using CRISPR-Cas

Arms variants







C. difficile functional PAMs:
CCN and TCN

Mutations in PAM for example:
C**T**N, **A**CN

Mutations in first protospacer position
(seed region from +1 to +6)

Combination of mutations

CRISPR-Cas-based tools

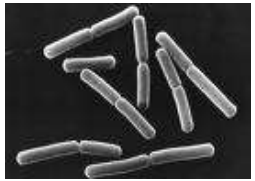
Types of mutations:

- Deletions (marker-less gene inactivation)
- Substitution (promoter replacement …)
- Insertion (tag for chromosomal gene copy, complementation …)
- Point mutations
- Gene knockdown by CRISPRi
- Multiple targeting (several crRNAs or gRNAs)

Remaining challenges:

- Conjugation efficiency in epidemic strains
- Homologous recombination efficiency

Clostridioides difficile



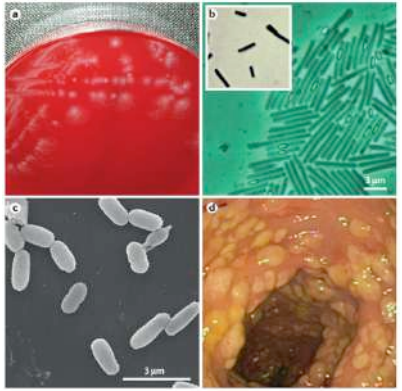
Challenges

✓Difficult to isolate

✓Difficult to culture requiring strictly anaerobic conditions

✓Difficult to manipulate genetically

Lack of efficient genetic tools



Smits et al. Nat Rev Dis Primers 2016