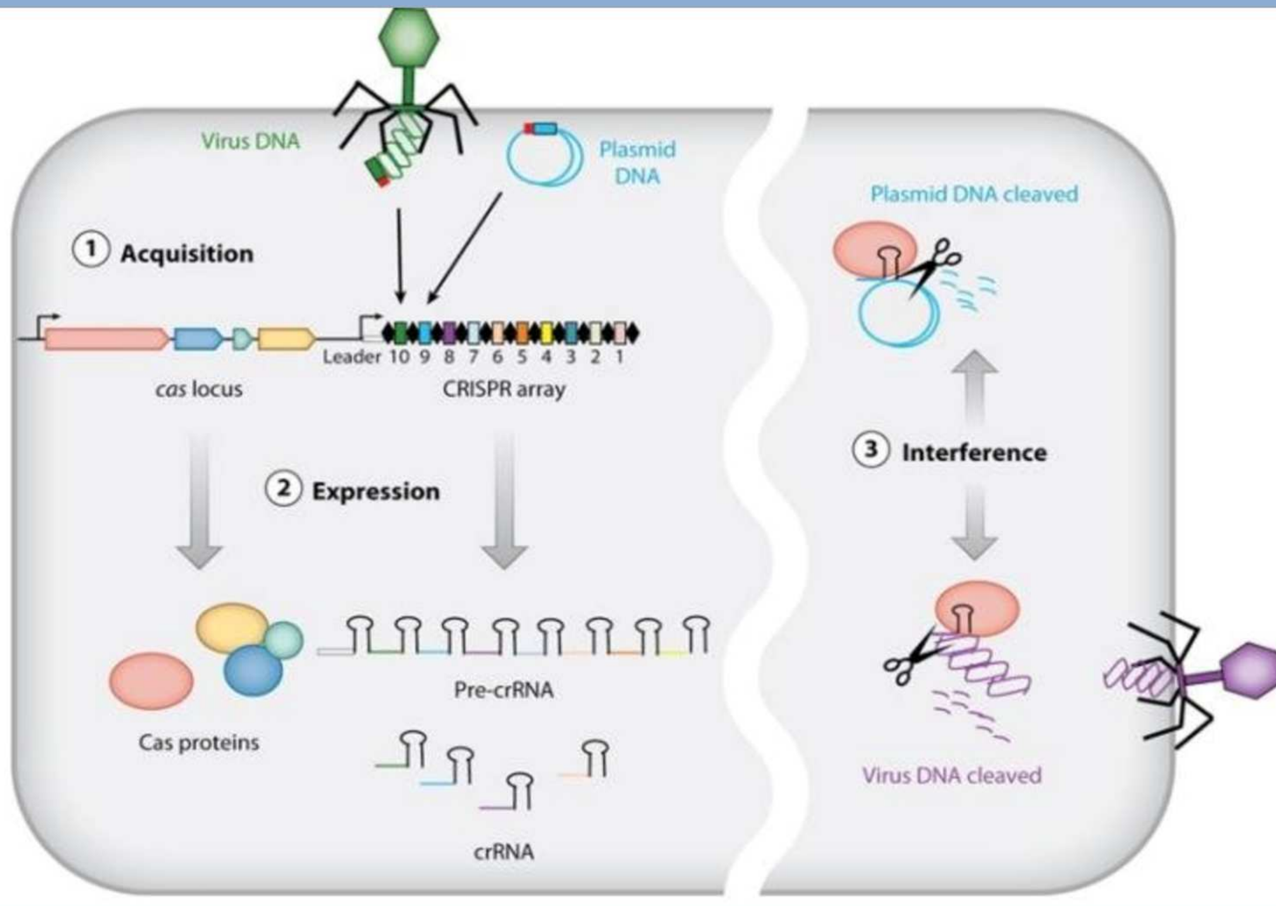
Clustered **R**egularly **I**nterspaced **S**hort **P**alindrome **R**epeats (**CRISPR**)
Archaea and Bacteria Immune system



(1) acquisition of foreign DNA

(2) synthesis and maturation of CRISPR RNA (crRNA) followed by formation of RNA-Cas nuclease protein complexes

(3) target recognition by crRNA and destruction of foreign DNA by Cas nuclease cleavage

CRISPR-Cas9 Applications

Genome editing
- indels (NHEJ)
- precise changes (HDR)
- chromosomal rearrangements

Improving specificity
- paired nickases
- FokI fusions
- truncated sgRNAs

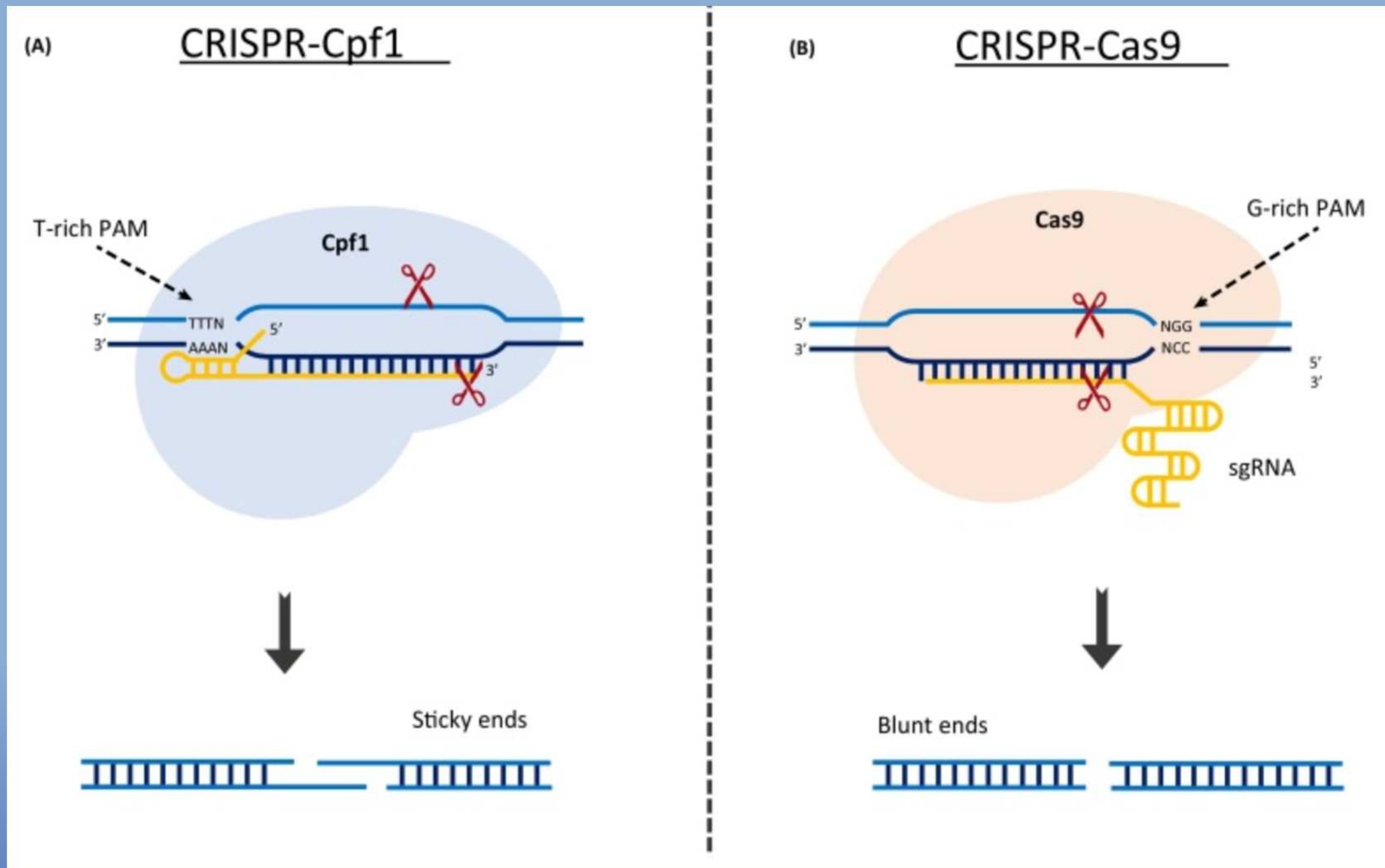
Genome-wide screening
- knockout libraries
- loss-of-function screens
- gain-of-function screens

Other uses of dCas9
- genomic locus imaging
- synthetic genetic circuits
- RNA manipulation

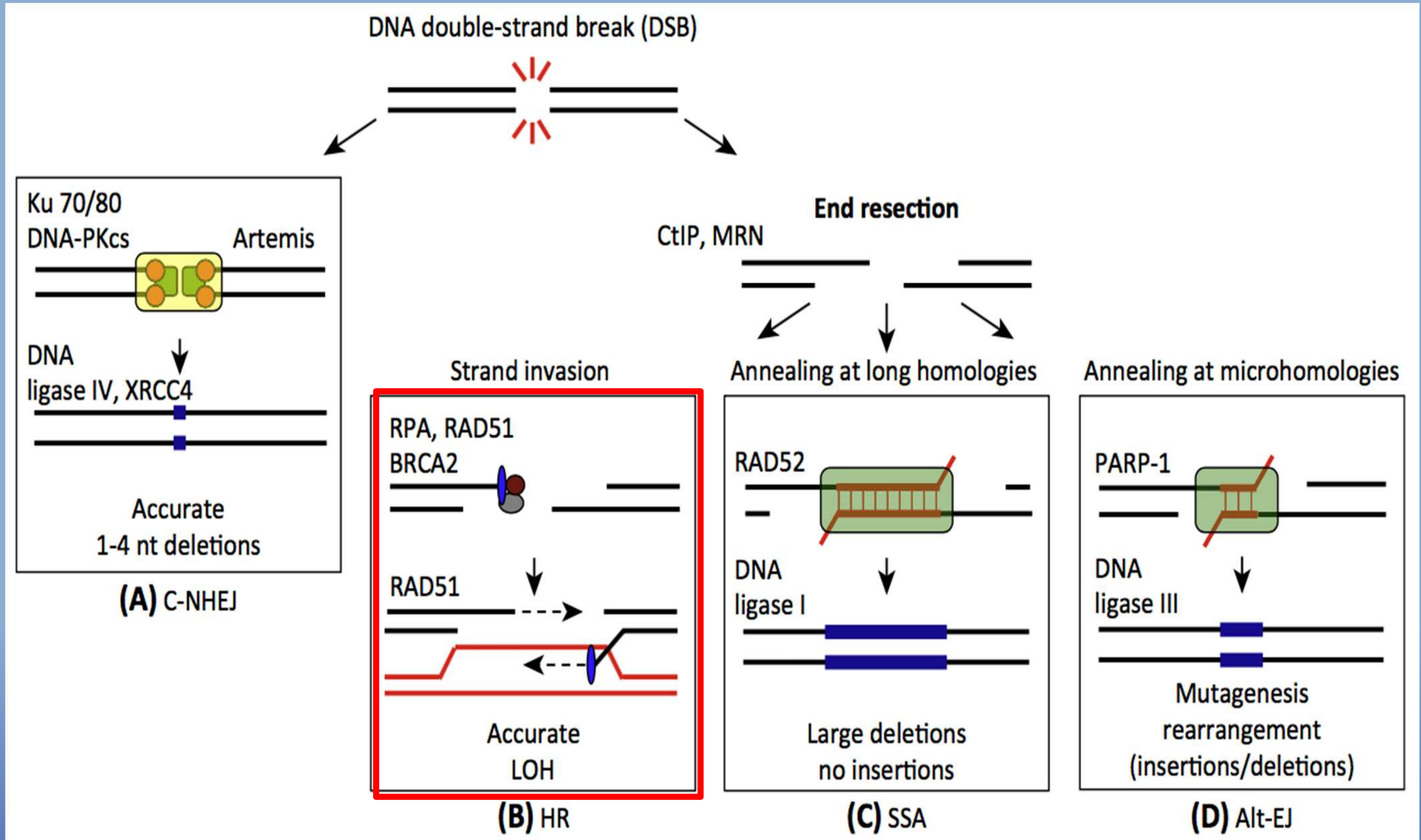
Future directions
- human therapeutics
- ecological engineering
- tool development

Gene regulation (dCas9)
- transcriptional repression
- transcriptional activation
- epigenetic modification

Two of the CRISPR effectors



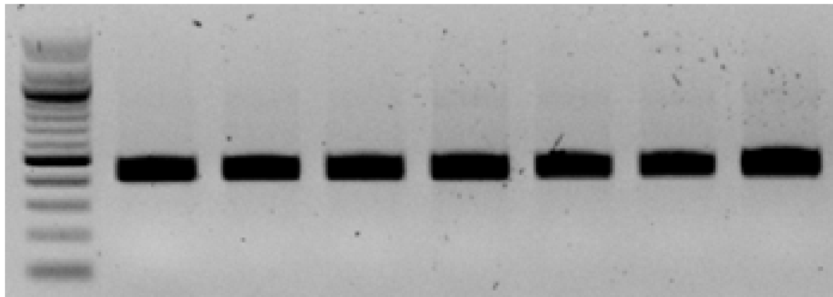
What happens after inducing a Double Strand Break?



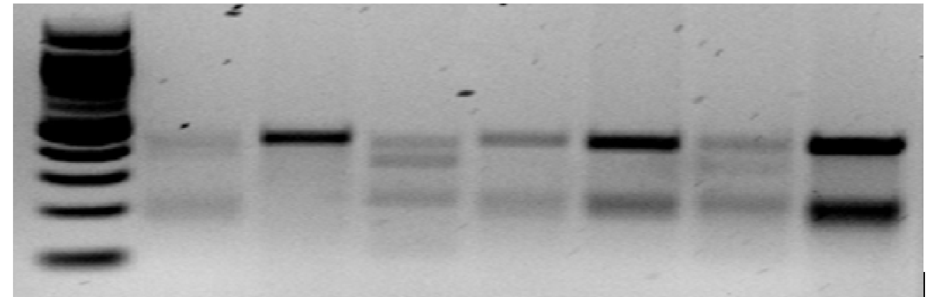
Premise:

- knock-in mutated donor DNA template → create cellular model
- The mutation in the donor introduced a KpnI cut site

KpnI digestion



T7E1 assay



Conclusions:

No incorporation of the donor DNA

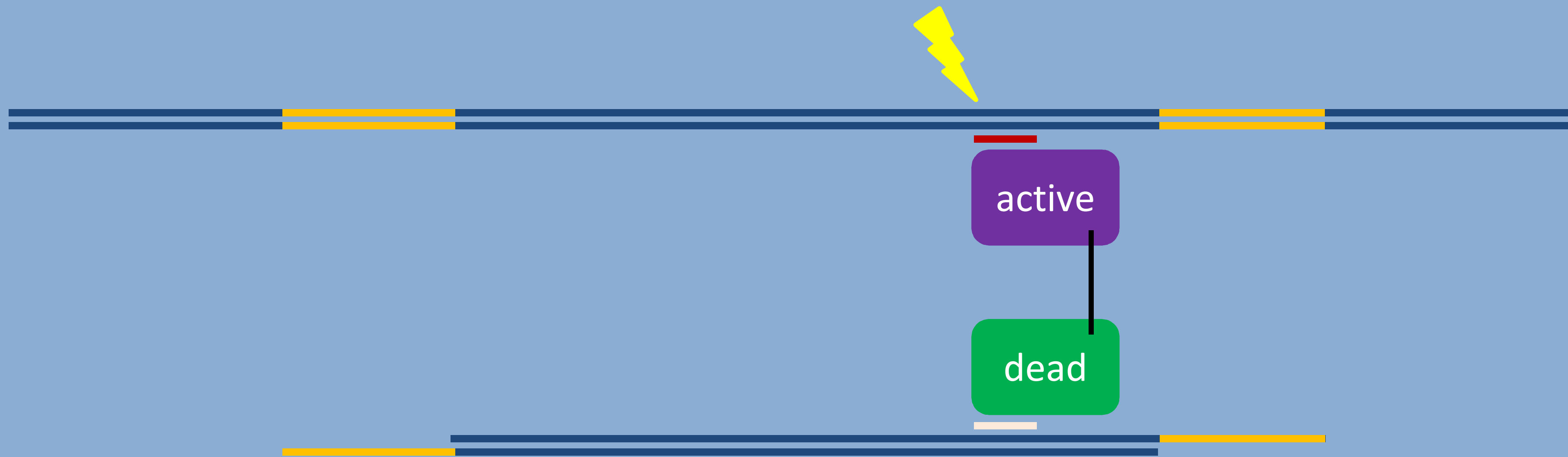
The targeting was efficient though

- **Incorporation of donor DNA is the limiting step !!!**

One big question

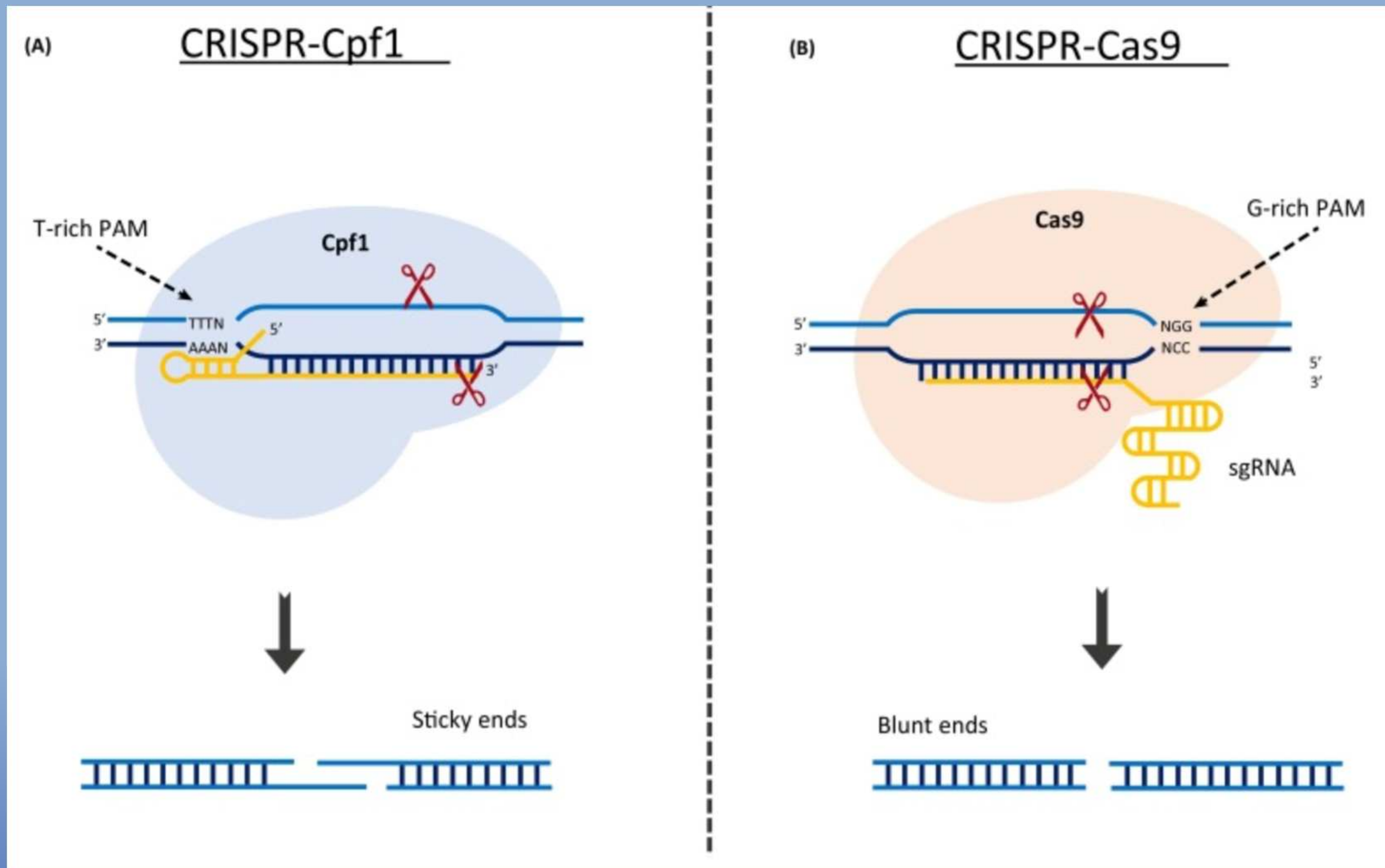
How to make the donor available?

Concept



- Dimerisation/fusion domain
- CRISPR effectors ! HETERODIMER not HOMODIMER !
- gRNA's
- Homology arms

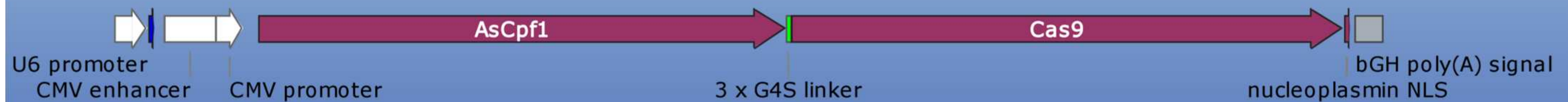
Two of the CRISPR effectors



Strategy

1. Post – translational dimerization: AsCpf1 – Nintein + Cintein – Cas9

2. Express fusion dimer: AsCpf1 – linker – Cas9



Come legare due proteine covalemtemente: Le inteine

Inteins are auto-processing domains found in housekeeping genes of unicellular organisms
Mostly prokaryotes and completely absent in multicellular organisms.

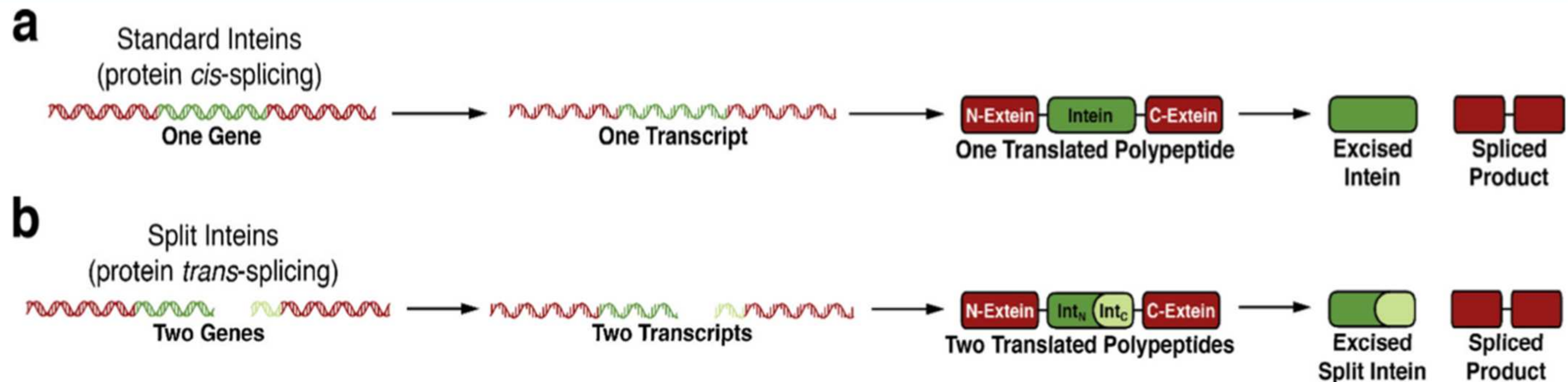
Only one copy in host organisms

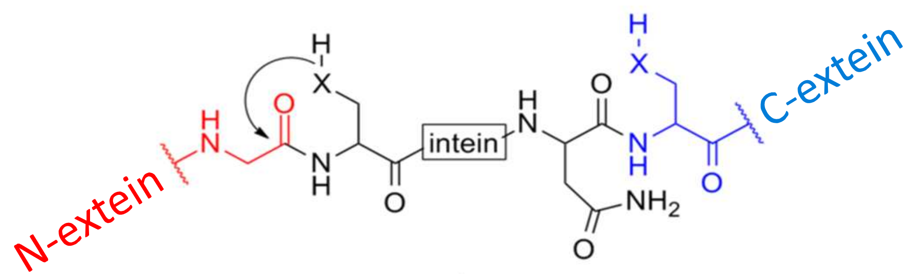
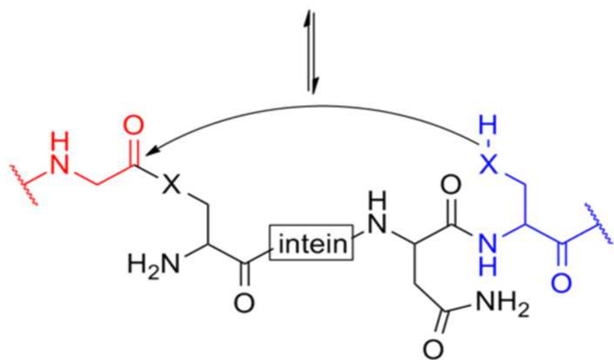
Inteins – nature's gift to protein engineers

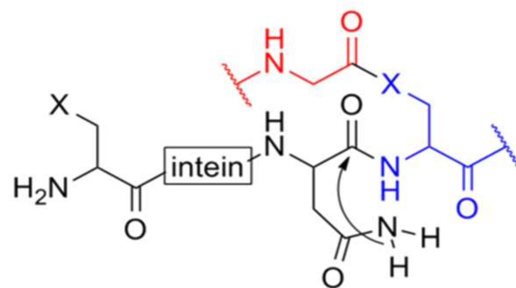
Upon interaction the split inteins assume the exact structure observed for contiguous ones.

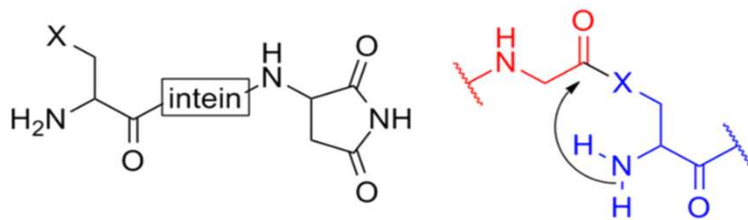
The **N-intein** has a **well organized region** of secondary structure **and a disorganized domain**
The **C-intein is completely disorganized.**

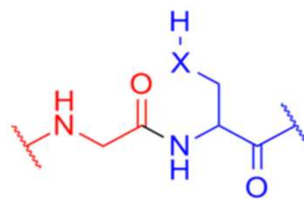
There's a marked charge separation between the two split inteins that it is not found in contiguous inteins.




N-extein
C-extein
intein

intein

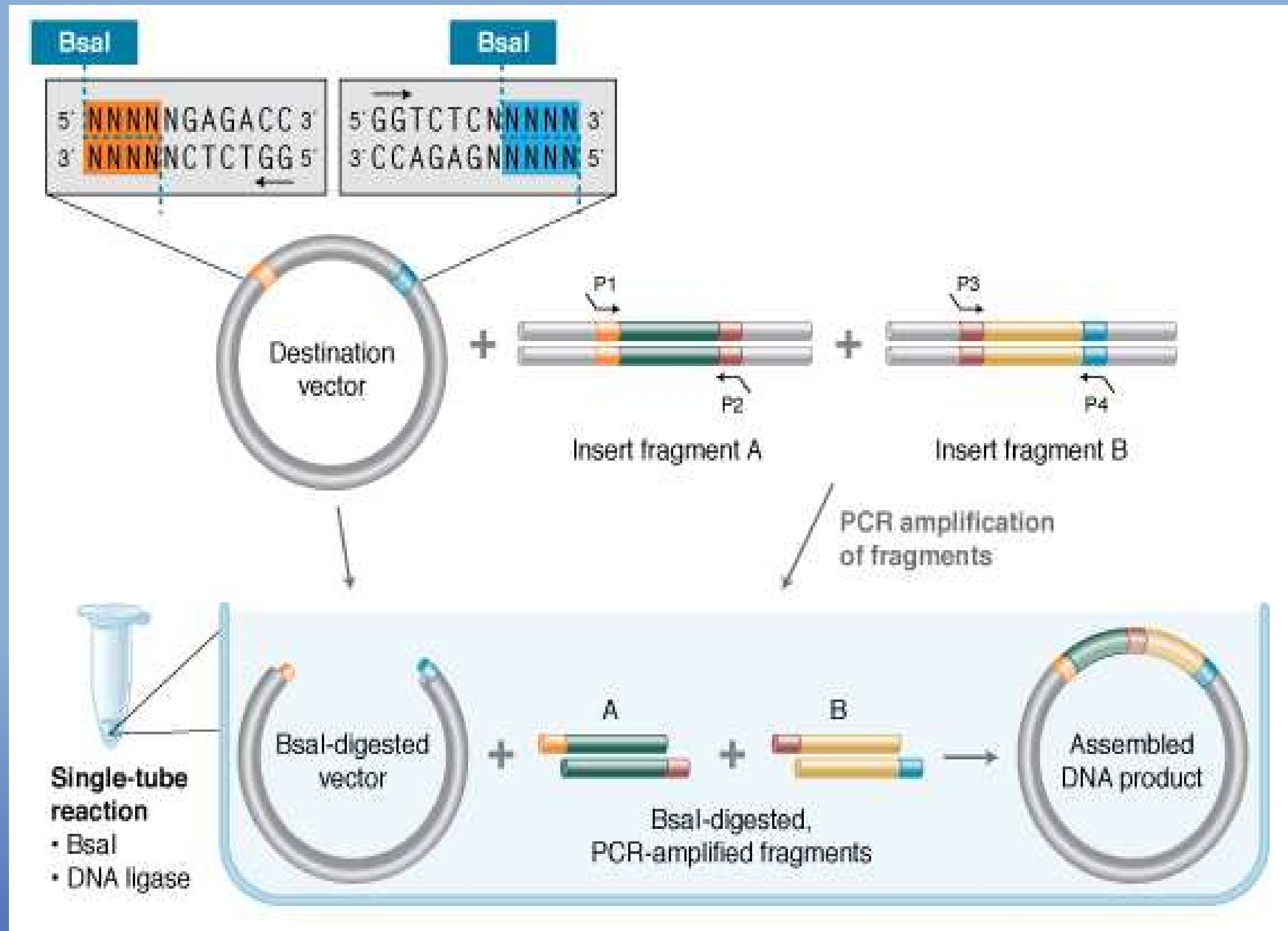

intein


intein



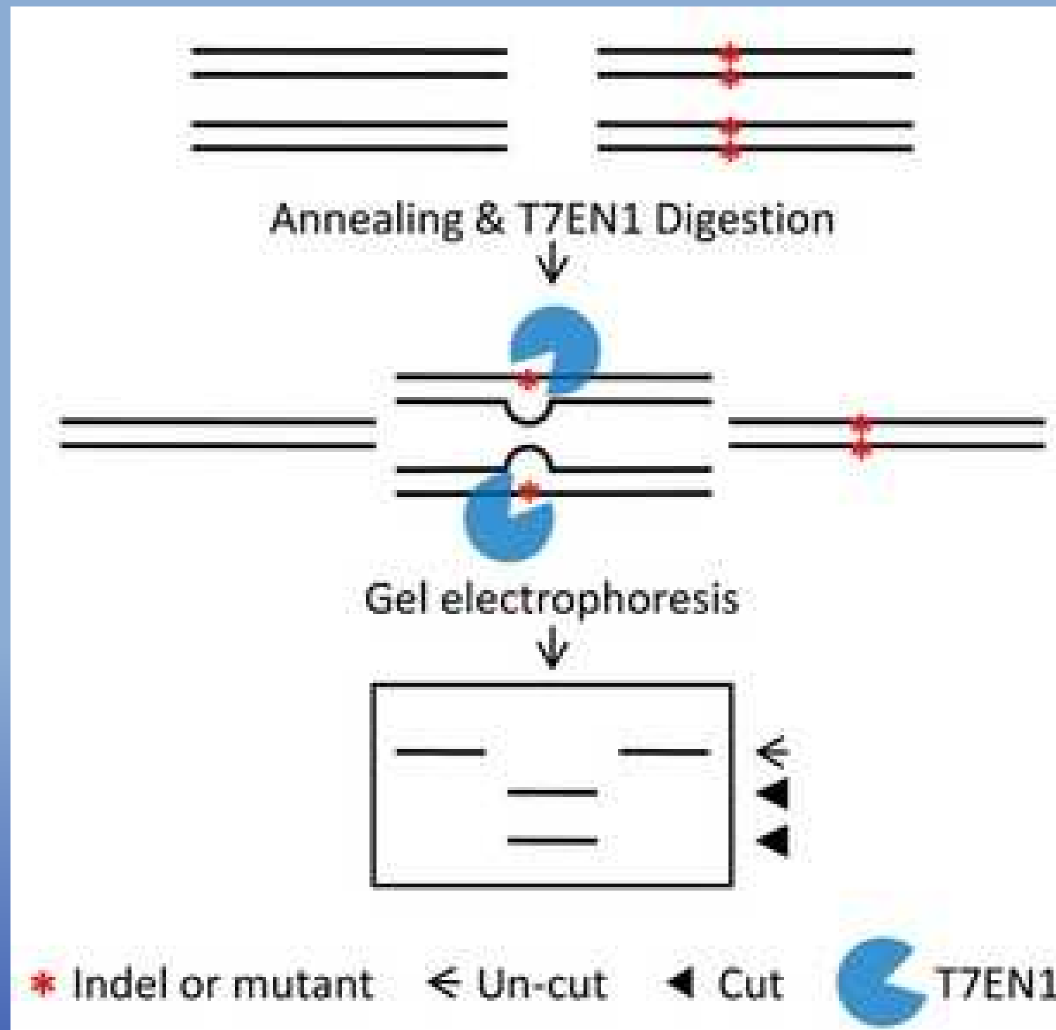
Come clonare efficientemente in modo direzionale

Golden Gate Assembly



Come testare l’efficienza di taglio

T7 Endonuclease I Assay



CONCLUSIONS:

- We have managed to build a functional dimer
- Both designs were successful
- In our intein based strategy the inactive monomer should be Cpf1-Nint

CONCLUSIONS:

- High impact applications can now be tested with the dimer:
  - ➢ Increment the efficiency of Homologous Directed Repair
  - ➢ Large chromosomal rearrangements (discern between deletions and inversions, translocations, create TADs, transactivations)