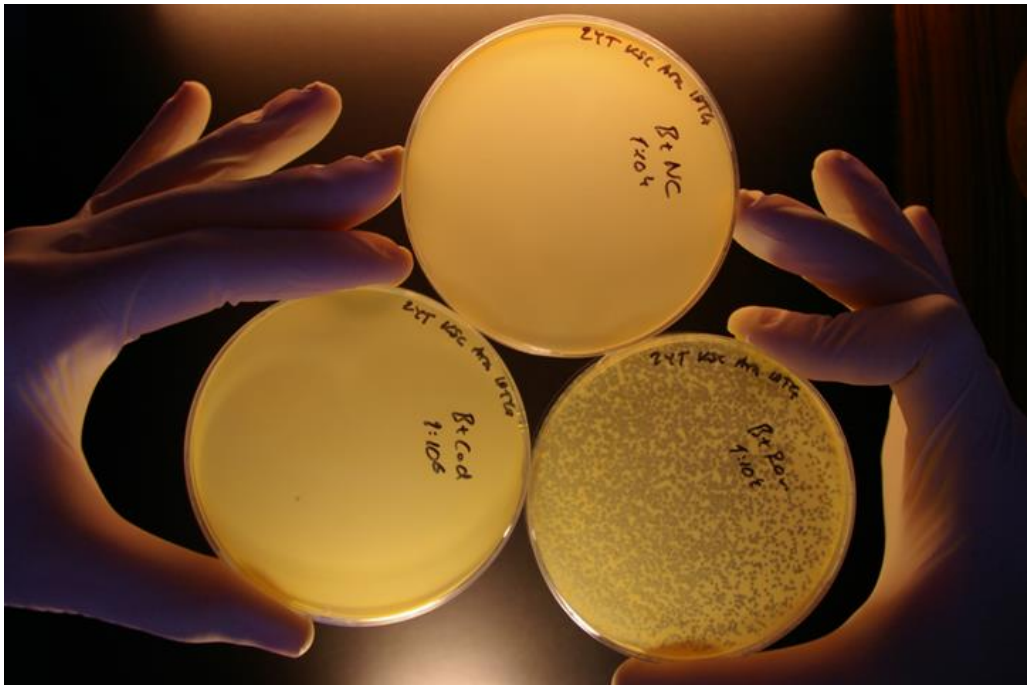
CRISPR-Cas Crash Course



John van der Oost

Laboratory of Microbiology
Wageningen University

CRISPR-Cas Crash Course

- Part 1 – CRISPR-Cas – from biology to application
- Part 2 – Crop engineering
- Part 3 – Panel Discussion

CRISPR-Cas - *discovery*

- CRISPR – clustered regularly interspaced palindromic repeats

Oshino (1987), She (2001), Jansen (2002)

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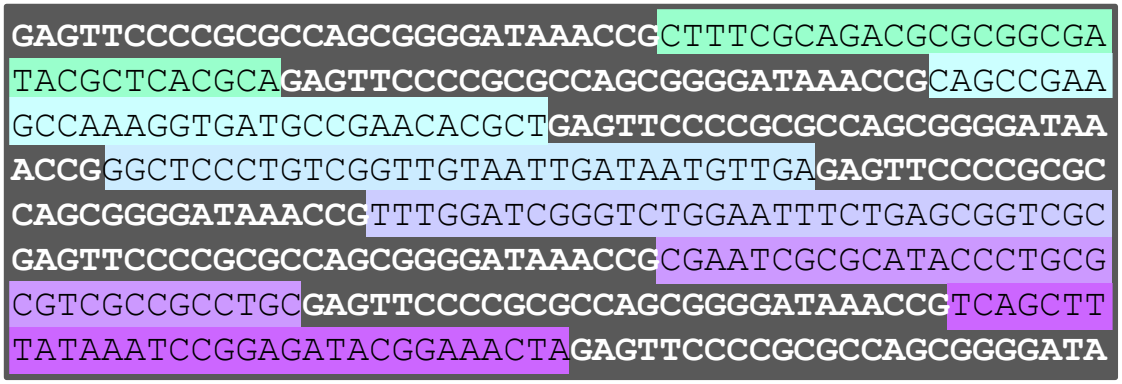

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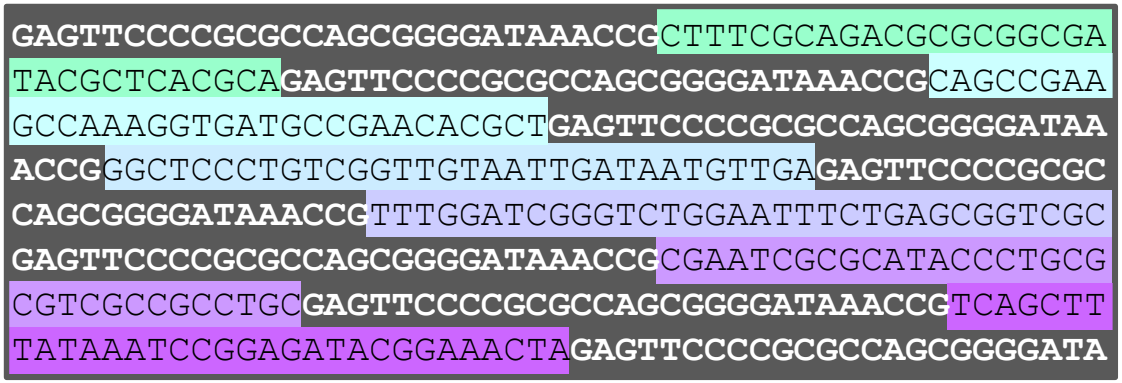

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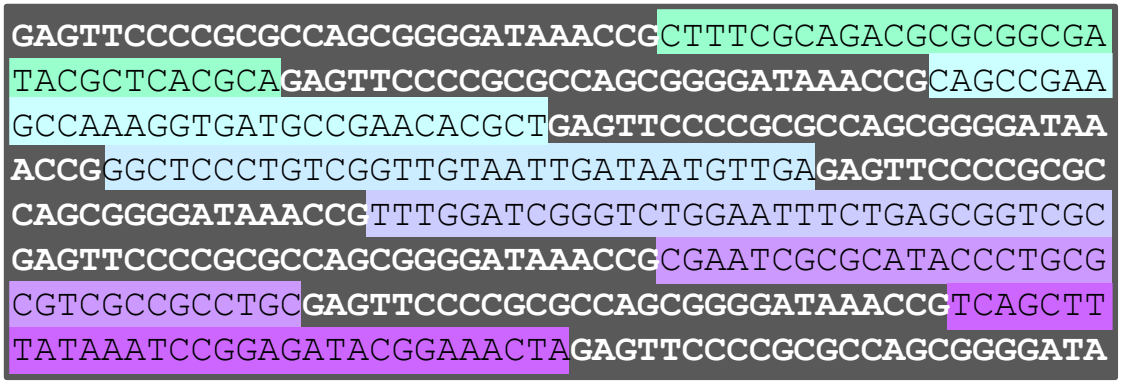
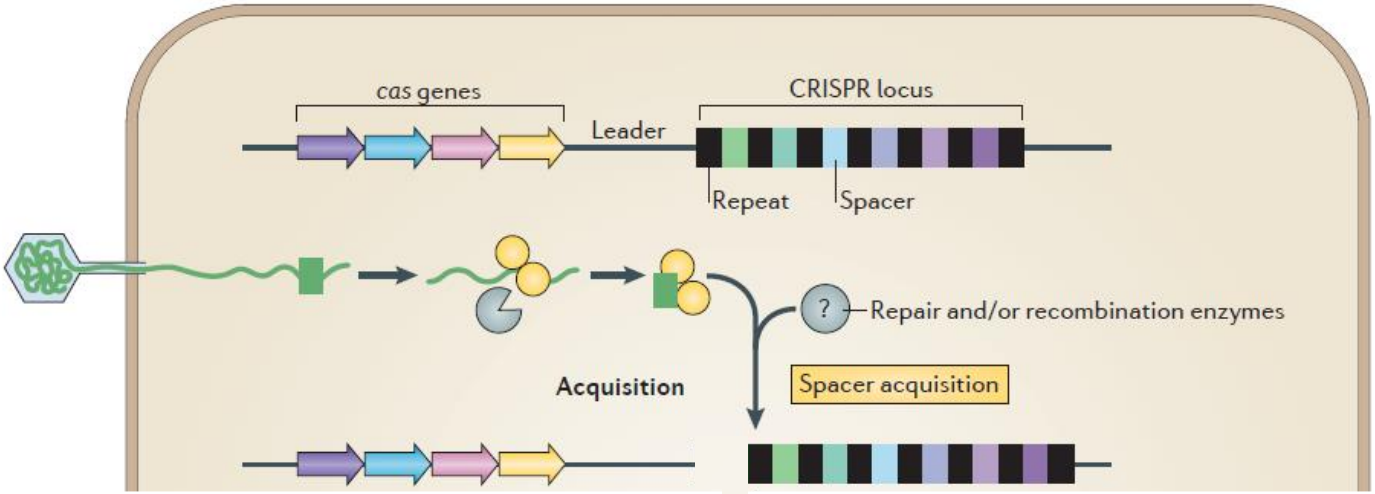


- CRISPR – clustered regularly interspaced palindromic repeats
- Cas – CRISPR-associated genes & proteins
- some CRISPR spacers resemble virus fragments

Mojica (2005), Pourcel (2005), Bolotin (2005)

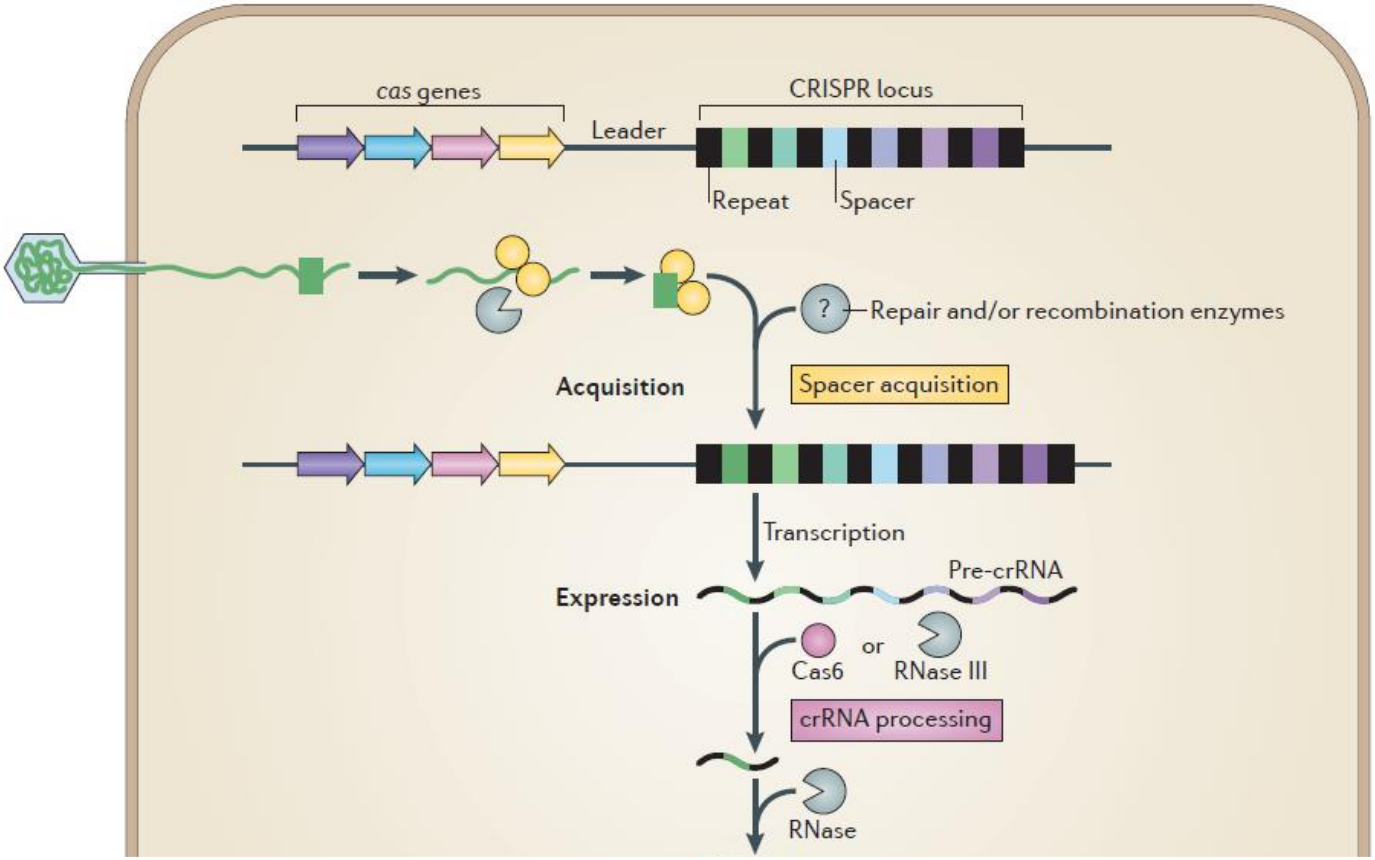
CRISPR-Cas mechanism – *3 steps*



1. Spacer acquisition

Van der Oost (2014) Nat Rev Microbiol

CRISPR-Cas mechanism – *3 steps*

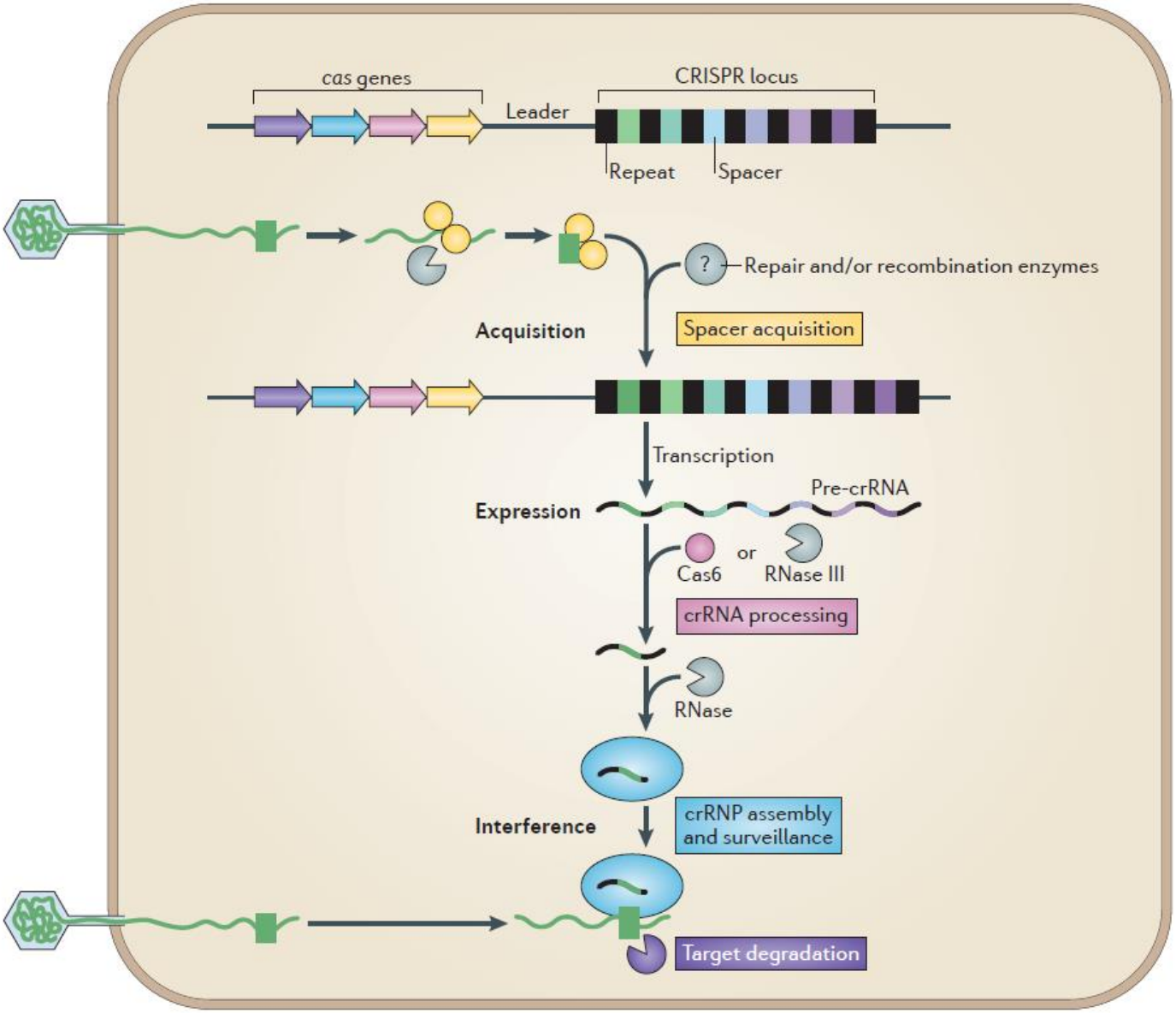


1. Spacer acquisition

2. Guide expression

Van der Oost (2014) Nat Rev Microbiol

CRISPR-Cas mechanism – *3 steps*



1. Spacer acquisition

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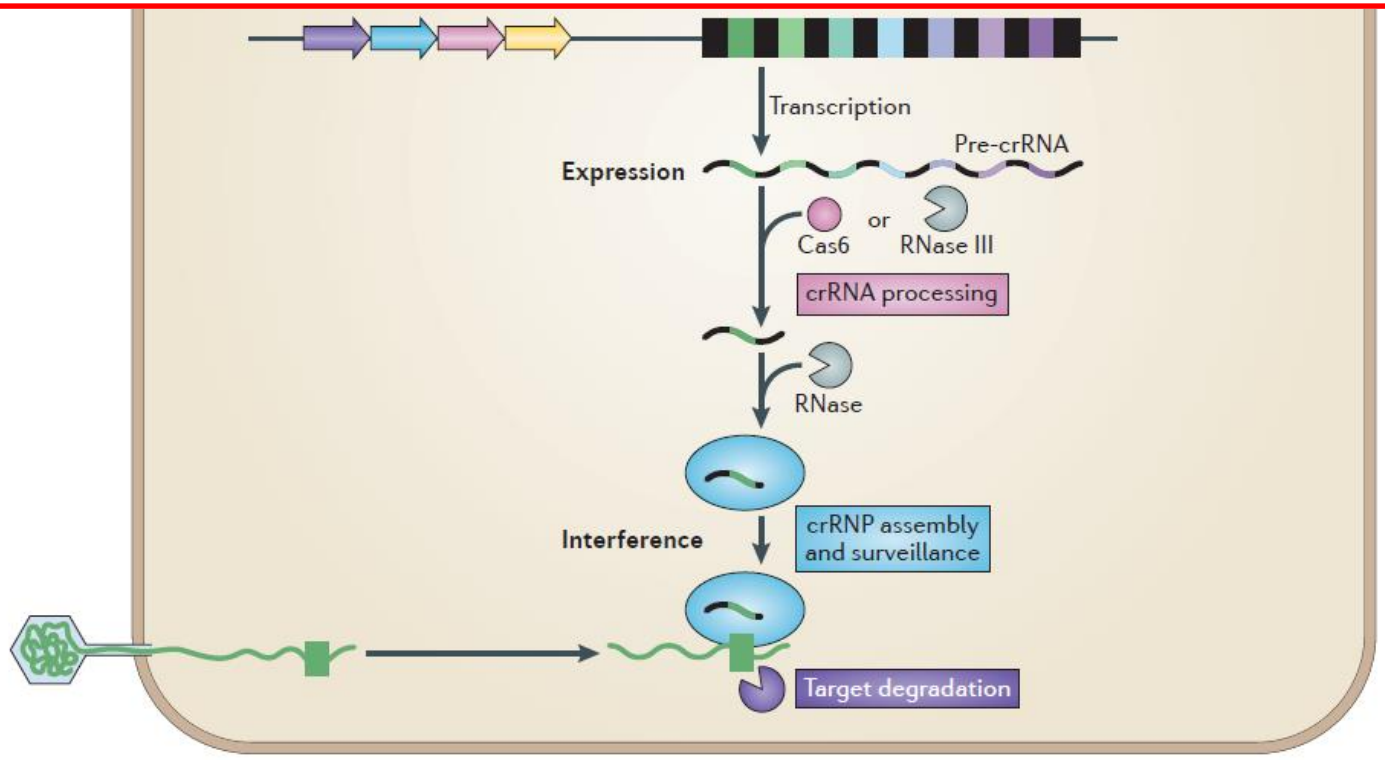
3. Target interference

Van der Oost (2014) Nat Rev Microbiol

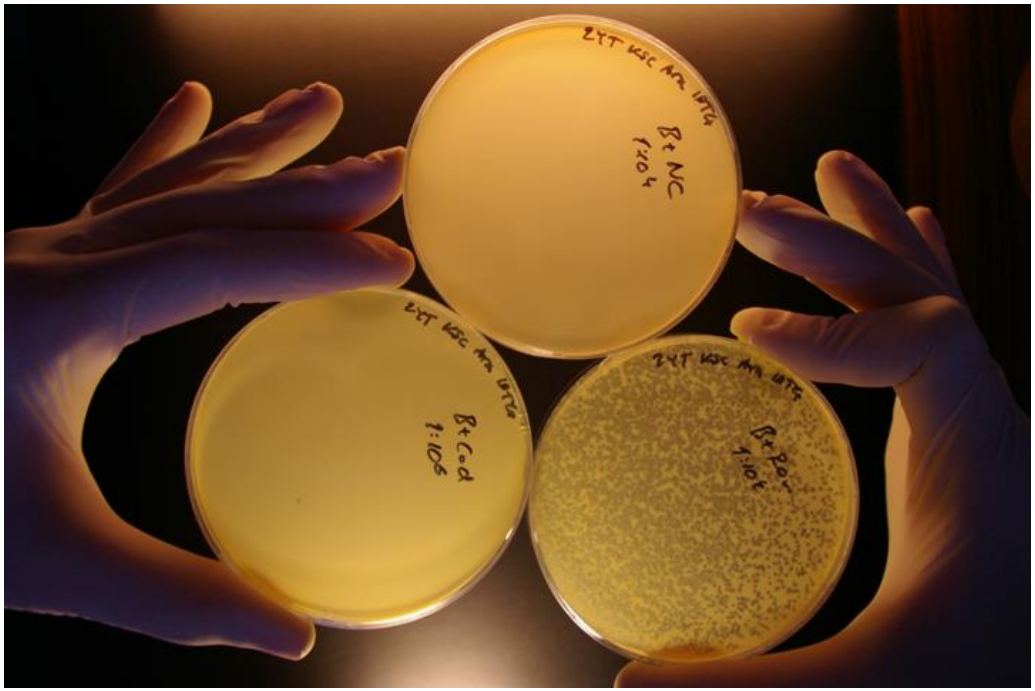
CRISPR-Cas mechanism – *3 steps*



2. Guide expression

3. Target interference

crRNA-guided DNA interference



Brouns et al. (2008) Science

crRNA-guided DNA interference

& genome editing

Molecular basis of CRISPR-Cas mechanism

Brouns et al. (2008) Science

Cascade & Cas3 – *target attack*

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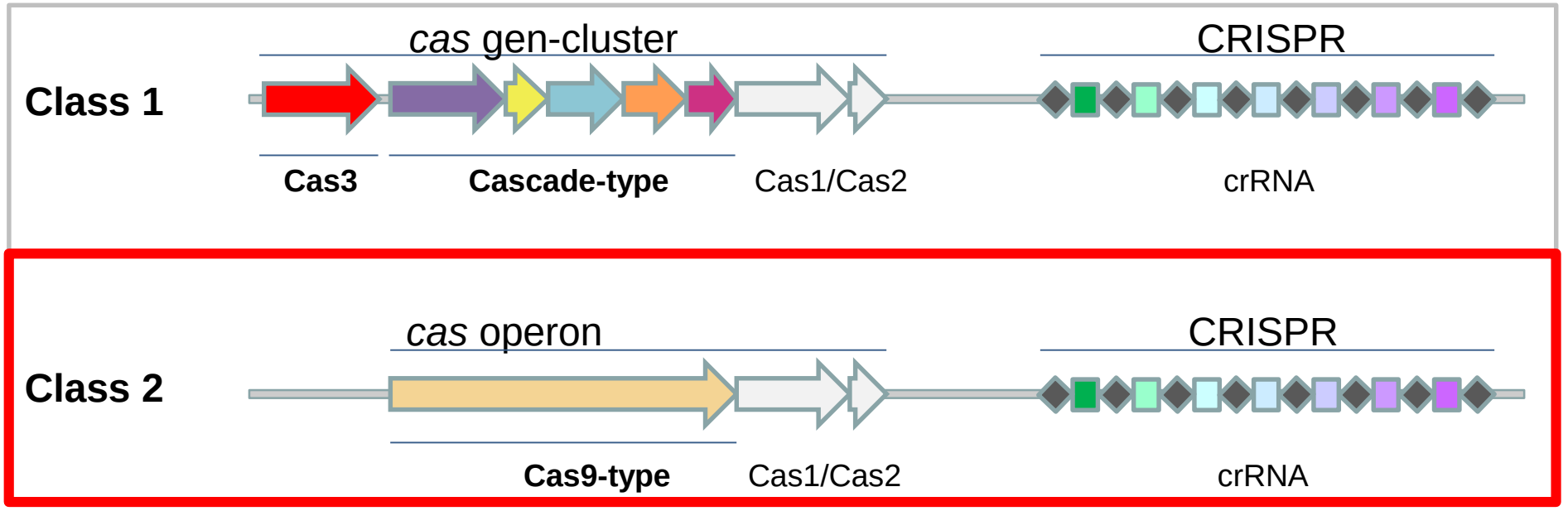
Jackson et al. (2014) Science, Gong et al. (2015) PNAS

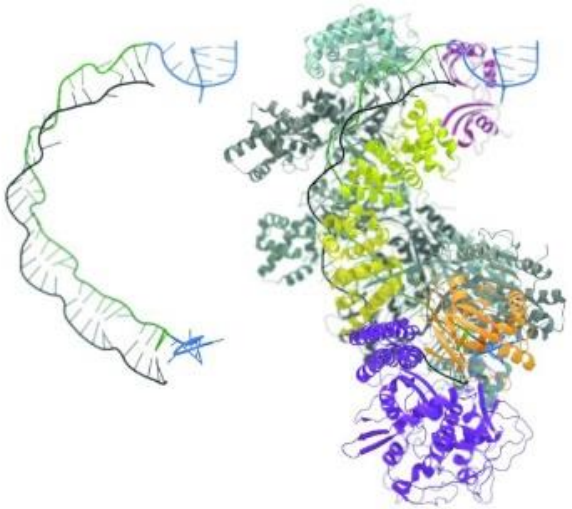
CRISPR-Cas – 2 classes



Makarova (2015) Nat Rev Microbiol

CRISPR-Cas – 2 classes

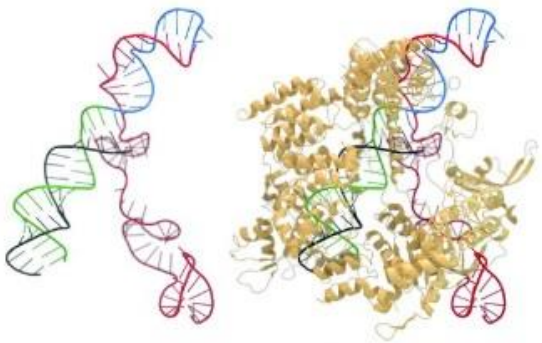
Class 1



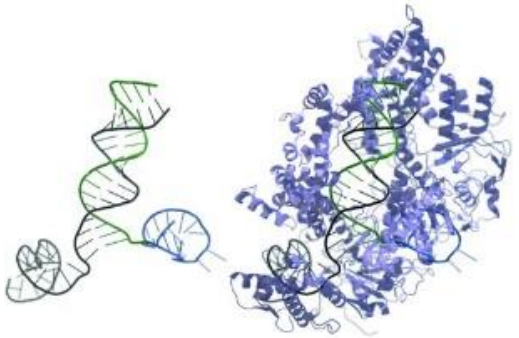
Cascade complex (Type I-E)

Cmr complex (Type III-B)

Class 2



Cas9 complex (Type II-A)

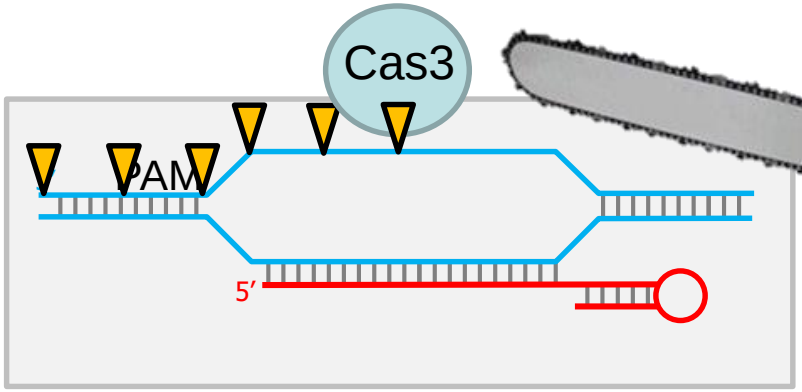


Cpf1 complex (Type V-A)

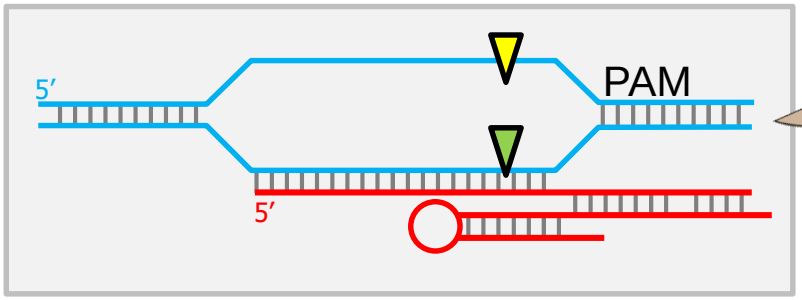
Mohanraju, Zhang, Koonin, Van der Oost (2016) Science

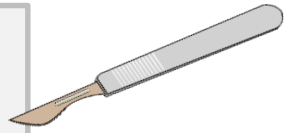
Cascade - Cas9 - Cas12

Type I
Cascade

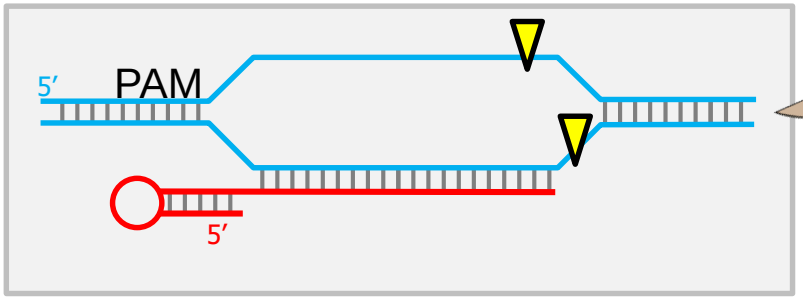


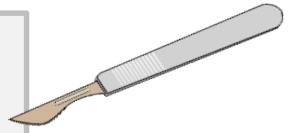
Type II
Cas9





Type V
Cas12

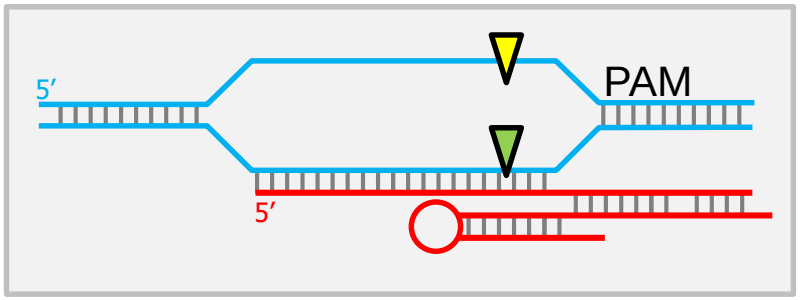




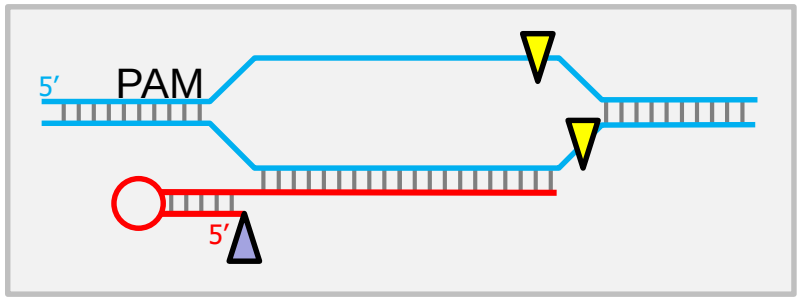
Sinkunas & Siksnys (2011), Garneau & Moineau (2010), Zetsche & Zhang (2015)

CRISPR application – genome editing

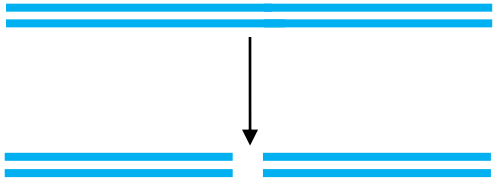
CRISPR-Cas9



CRISPR-Cpf1



specific cleavage by Cas9 / Cpf1



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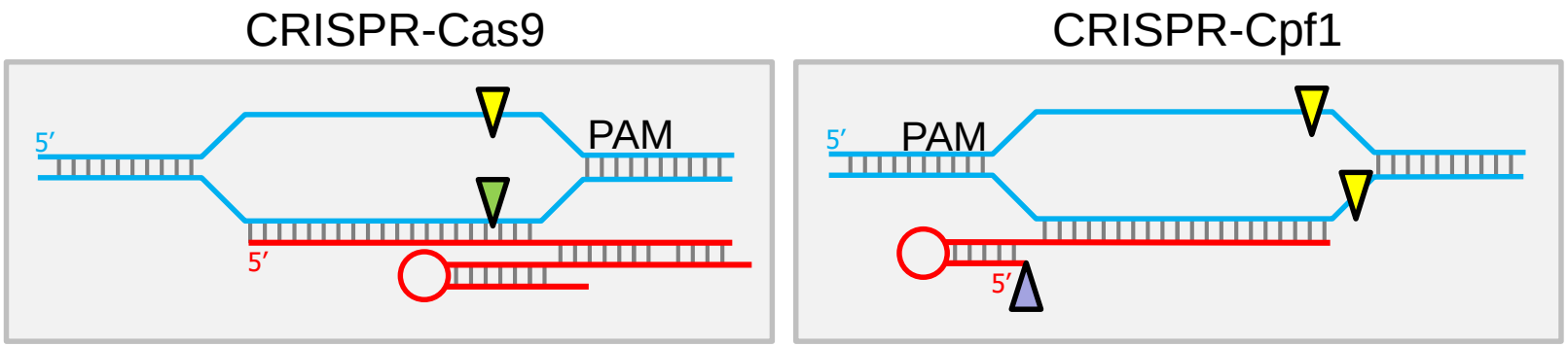

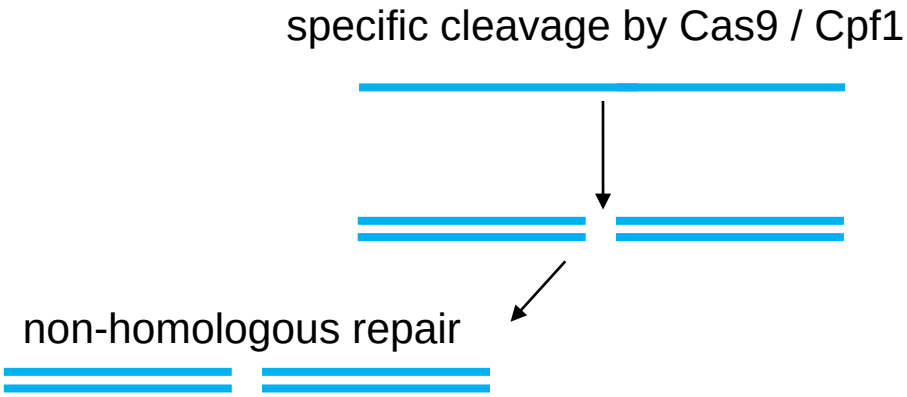

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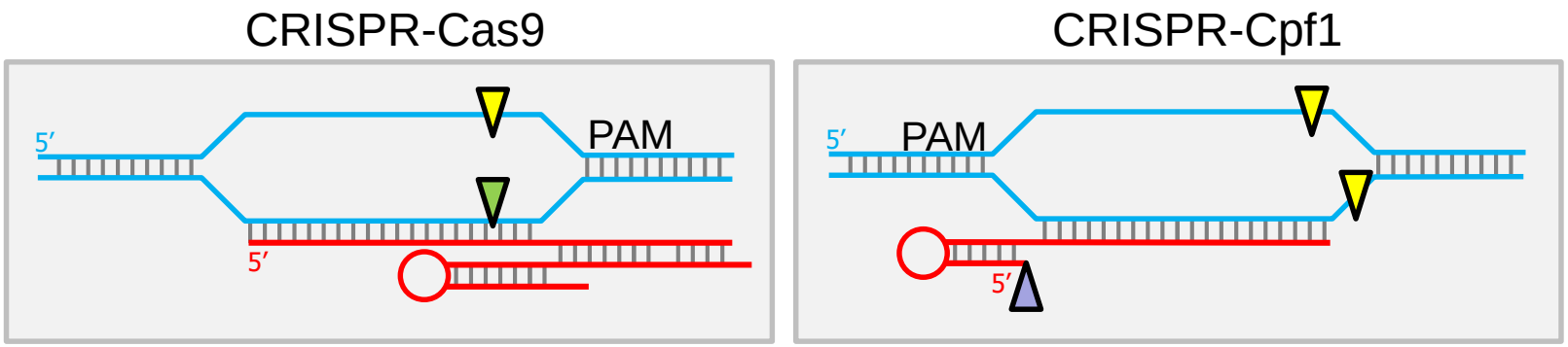
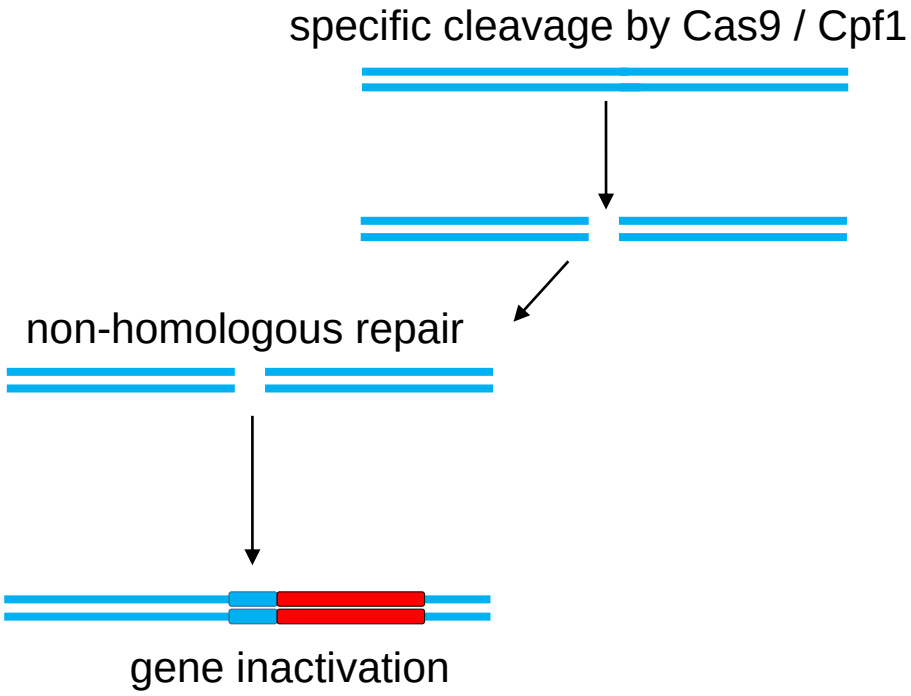
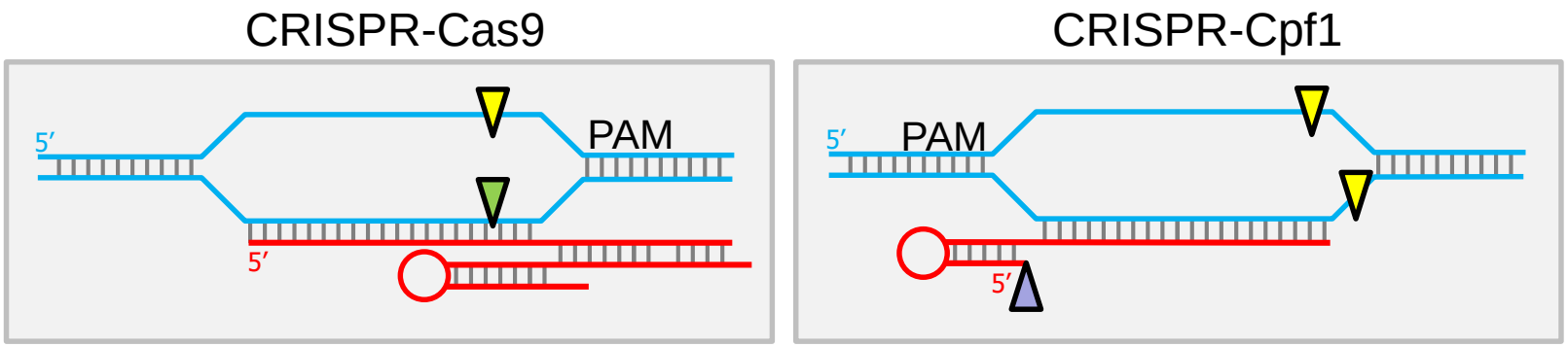
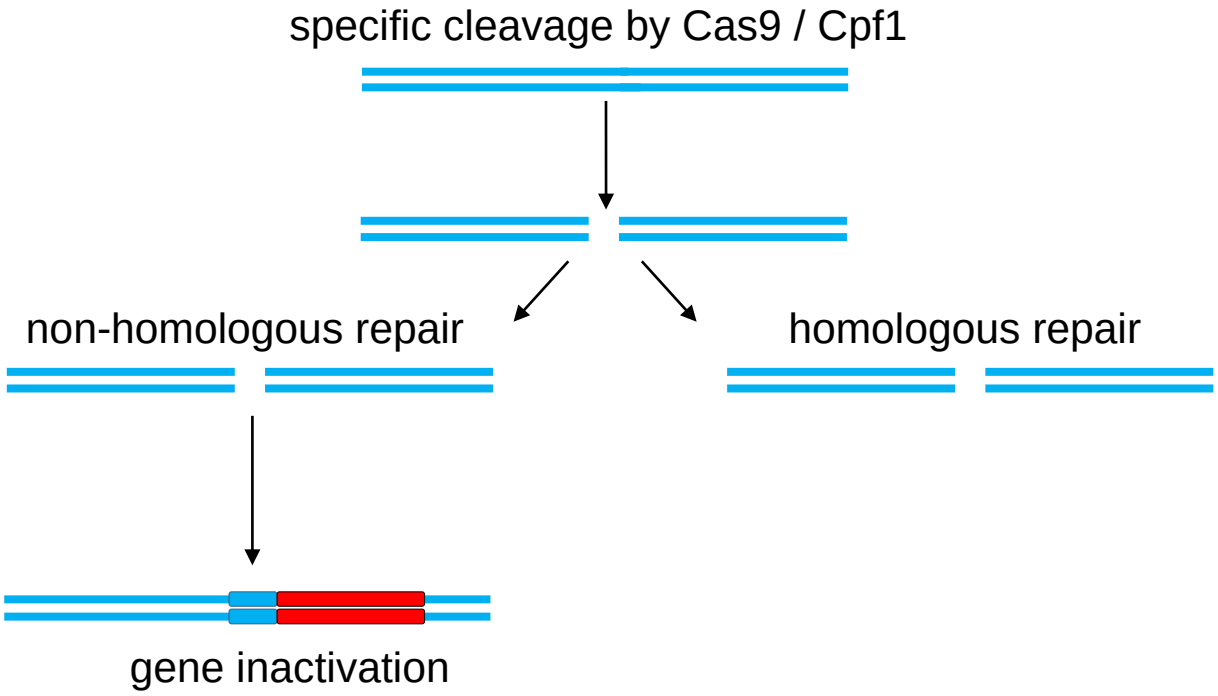




CRISPR application – genome editing





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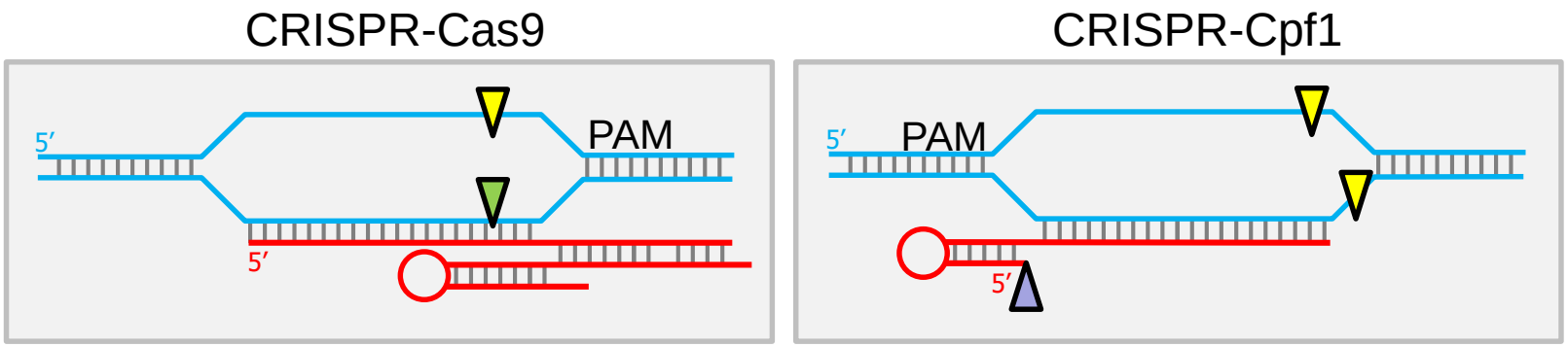

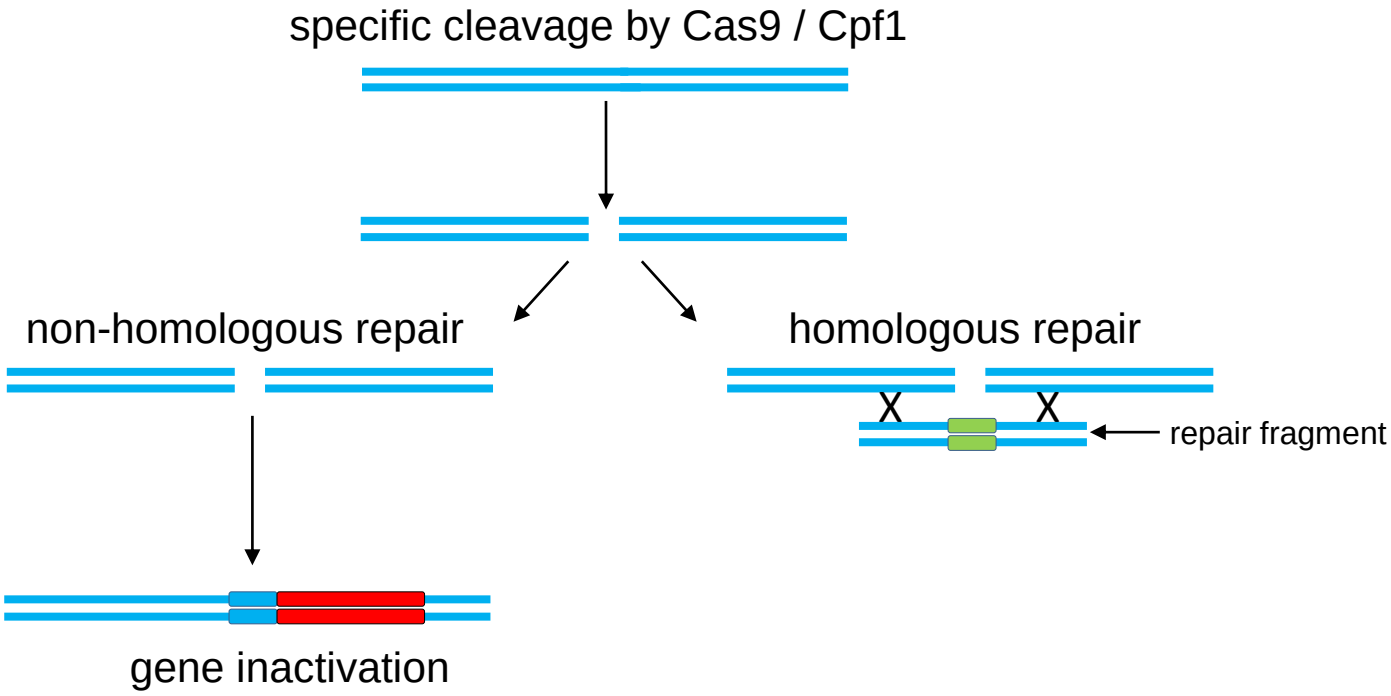

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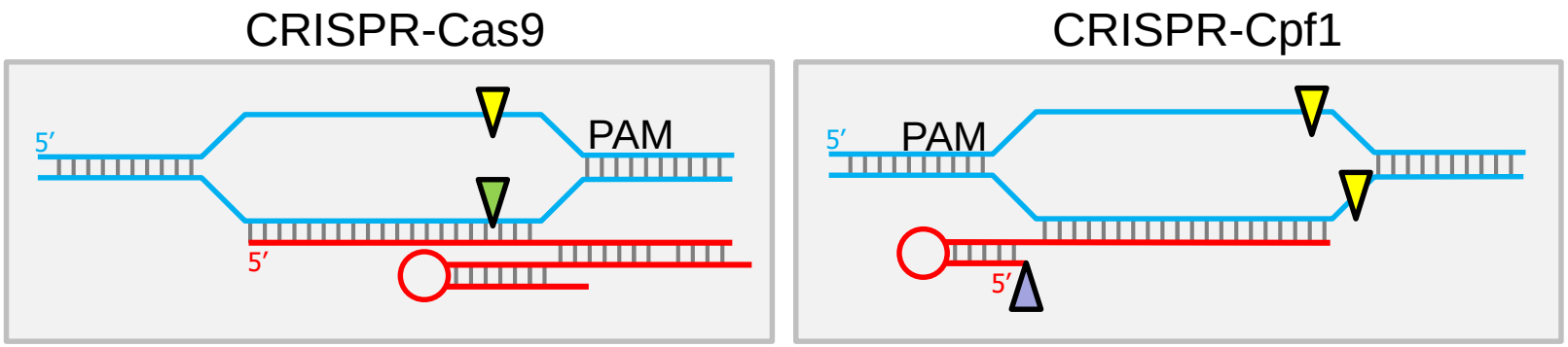

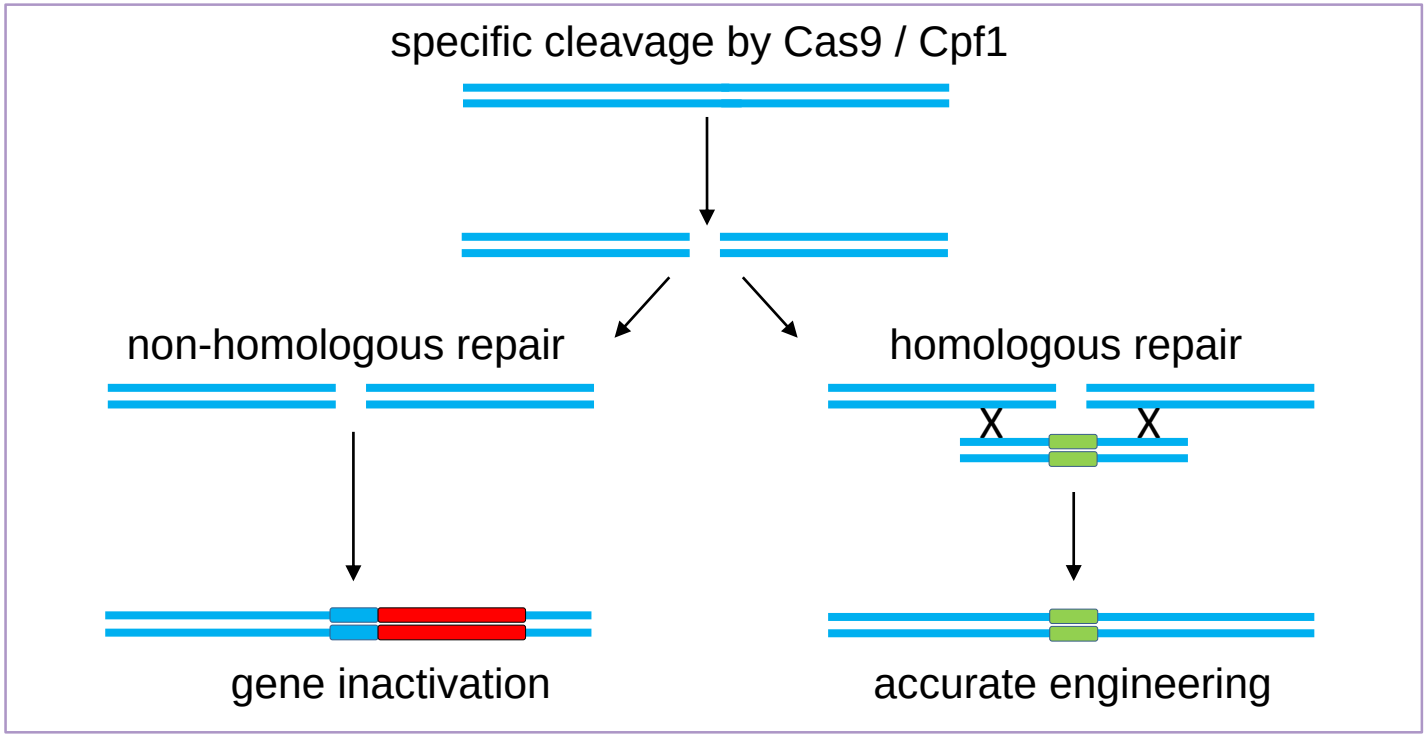

Conclusions - 1

- CRISPR-Cas is an adaptive defense system in bacteria and archaea
- Cas nucleases use crRNA guides to find complementary DNA (or RNA) targets
- CRISPR-Cas systems are highly diverse (2 classes, 6 types, >20 subtypes)
- Class-2 nucleases (Cas9, Cpf1) are ‘repurposed’ for gene editing applications
- Cas9 and Cpf1 are successful in biotechnology (bacteria, fungi, algae & plants)
- Clinical trials using CRISPR-Cas are on-going to cure genetic diseases

CRISPR-Cas Crash Course

- Part 1 – CRISPR-Cas – from biology to application
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Crop engineering

- Cas9 and Cpf1 (Cas12a) have been used successfully for editing of a range of plants, introducing dedicated changes exactly at desired genomic locations

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- in countries like the US and Japan, introduction of minor changes in plant genomes with CRISPR (point-mutations that also occur in Nature) are considered non-GMO

CRISPR engineering – arguments against

- Ethics - unnatural
- Safety – no long history of safe use
- Power – Biotech companies are in charge

CRISPR engineering – arguments for

- Ethics – genetic change is a **natural process** that occurs spontaneously during cell division (point mutations, insertions, deletions, rearrangements), through acquisition of exogenous genetic material across species or even kingdoms, or after induction by external factors (e.g. UV light, viral infection) – changes can be neutral, harmful or beneficial

- Safety – precision engineering is as safe as can be, **at least as safe as the accepted classical mutagenesis approach** (the random approach generally leads to numerous point mutations, deletions and major rearrangements; after lengthy screening for desirable features, selected variants carry many unintended mutations) – as always, genetic changes can be neutral, harmful or beneficial; however, the end product is much better characterized (hence safer) in case of directed engineering – for safety, **the product matters, not the production process**

- Power – **complex rules** take a lot of time, labor and money for pre-market evaluation and approval; hence, this is a **major hurdle for SMEs**, and can only be afforded by the bigger Biotech companies

Conclusions – 2

CRISPR-Cas has major potential for safe & useful Crop improvement

- Less – water, fertilizers, herbicides/pesticides & energy
- More – enhanced production of safe, healthy & tasty food

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