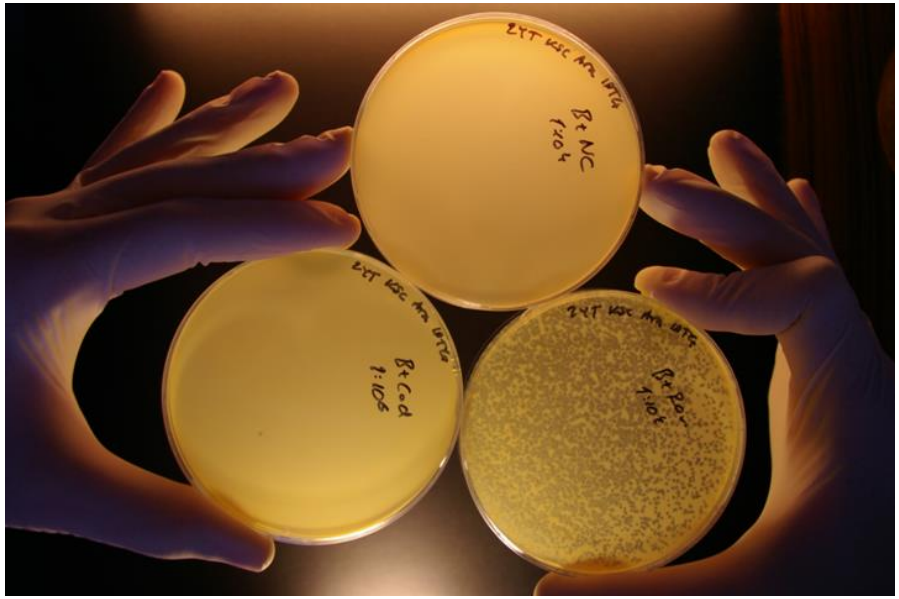
CRISPR-Cas systems

history, biology & applications



John van der Oost

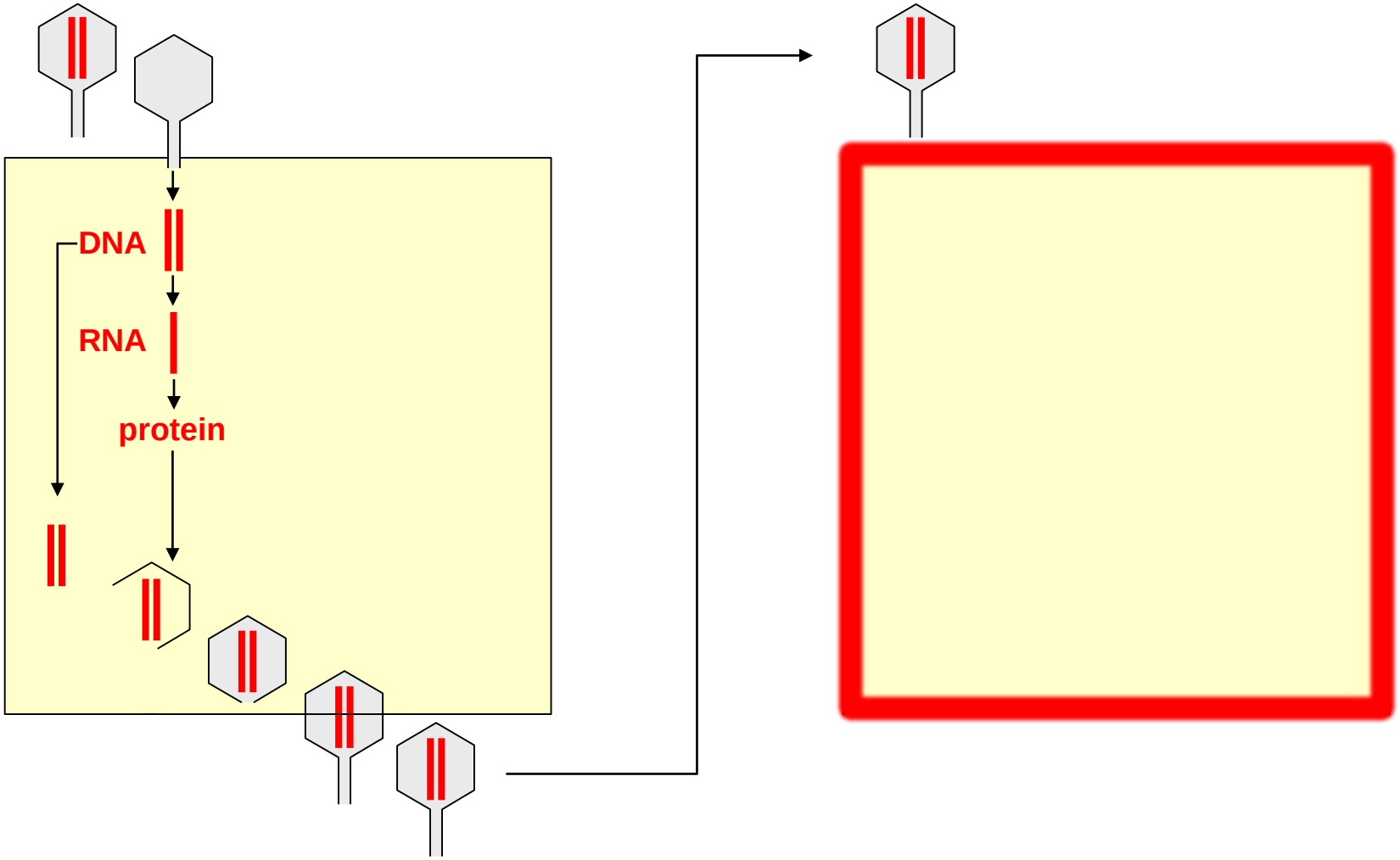
Laboratory of Microbiology
Wageningen University

outline

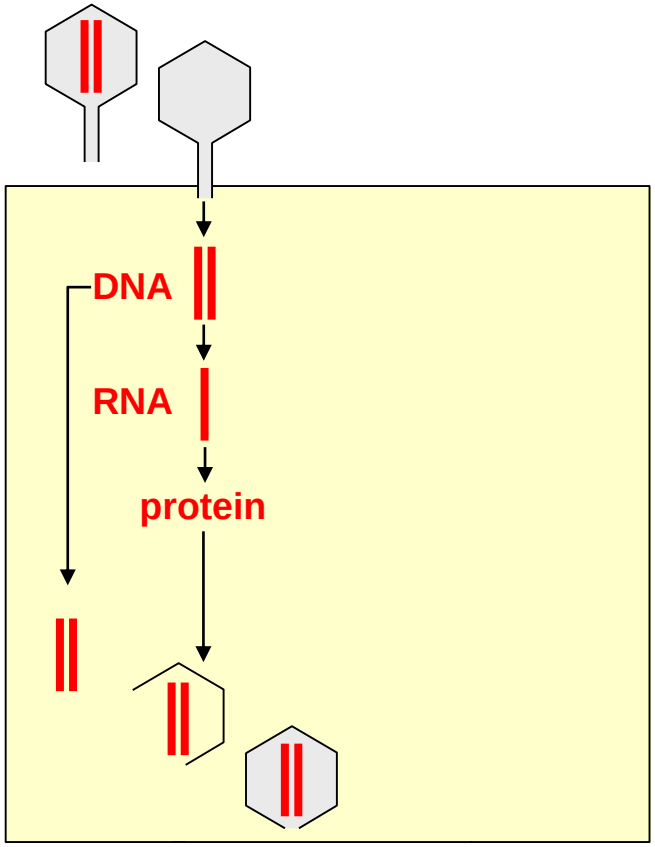
History - *discovery & diversity*

- Biology - *anti-virus defence mechanism*
- Applications - *genome editing & gene therapy*

virus infection of bacteria



anti-virus systems of bacteria



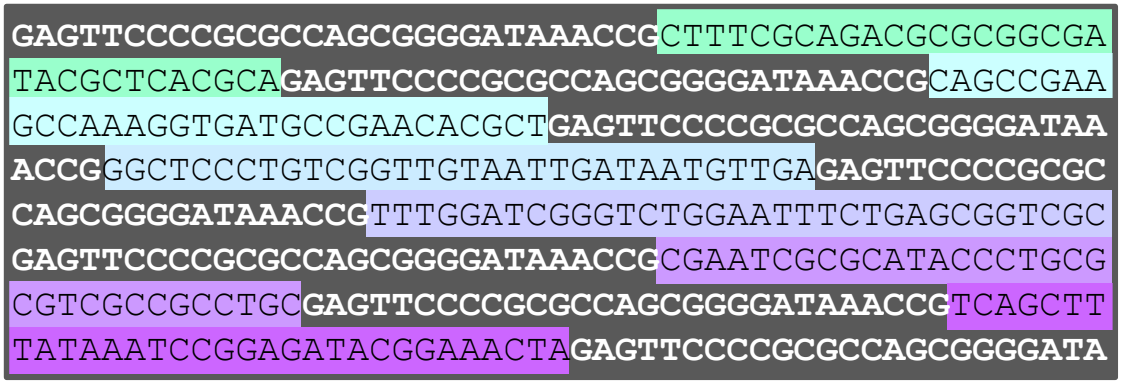
known anti-virus systems

- attachment
- inhibit DNA injection
- degradation of alien DNA
- suicide system

new anti-virus system

- CRISPR-Cas

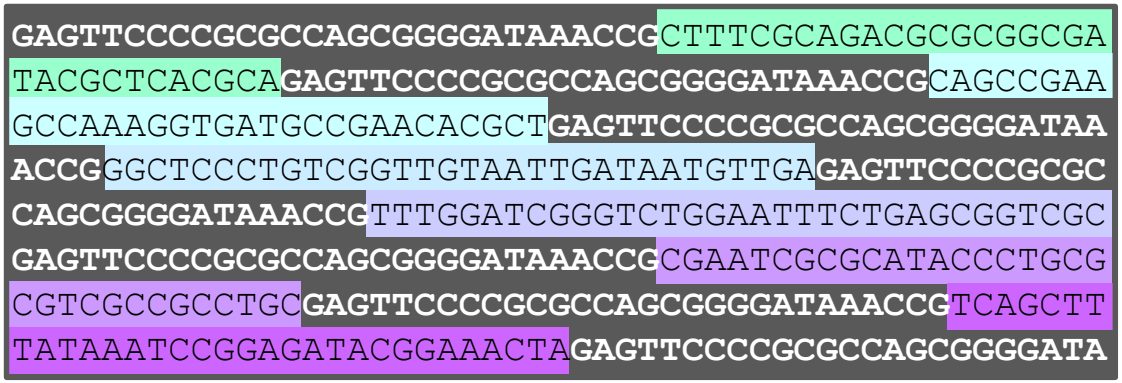
CRISPR-Cas - *discovery*



- CRISPR – clustered regularly interspaced palindromic repeats
- Cas – CRISPR-associated genes & proteins

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

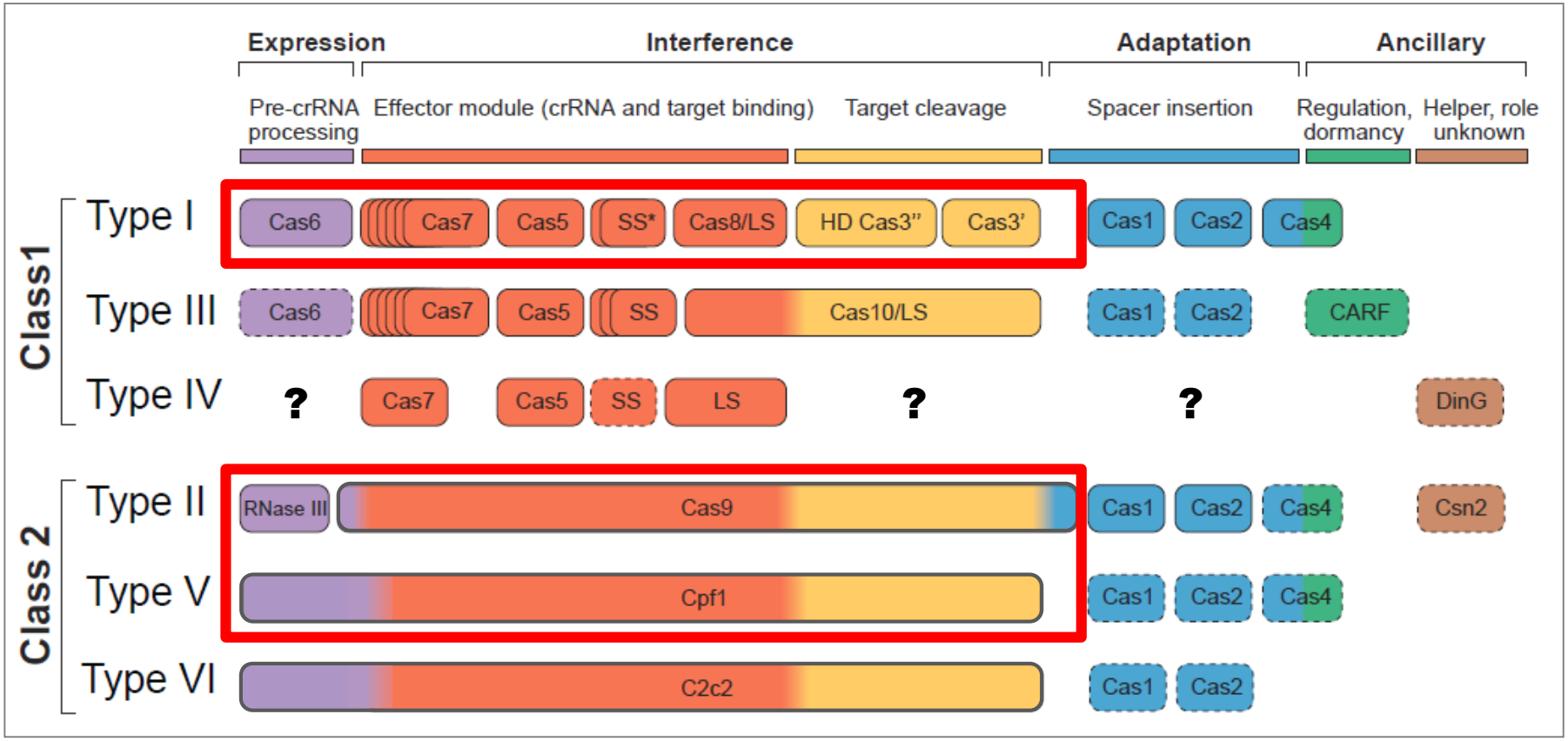
CRISPR-Cas system – *adaptive immunity*



- many CRISPR spacers are homologous to viruses or plasmids
- hypothesis: novel prokaryotic defence system – RNA interference ?
- present in genomes of 40% of bacteria and 85% of archaea

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

CRISPR-Cas diversity – *2 classes / 6 types*



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

outline

- History - *discovery & diversity*
- Biology - *anti-virus defence mechanism*
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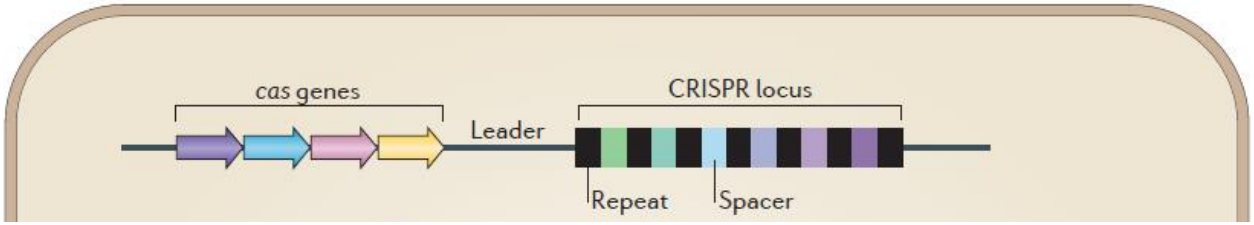
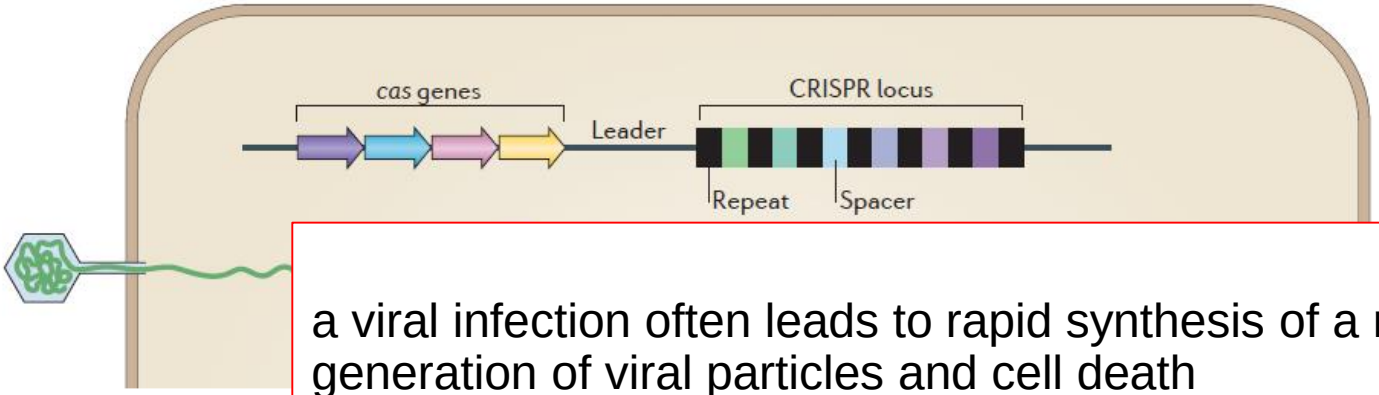
CRISPR-Cas mechanism



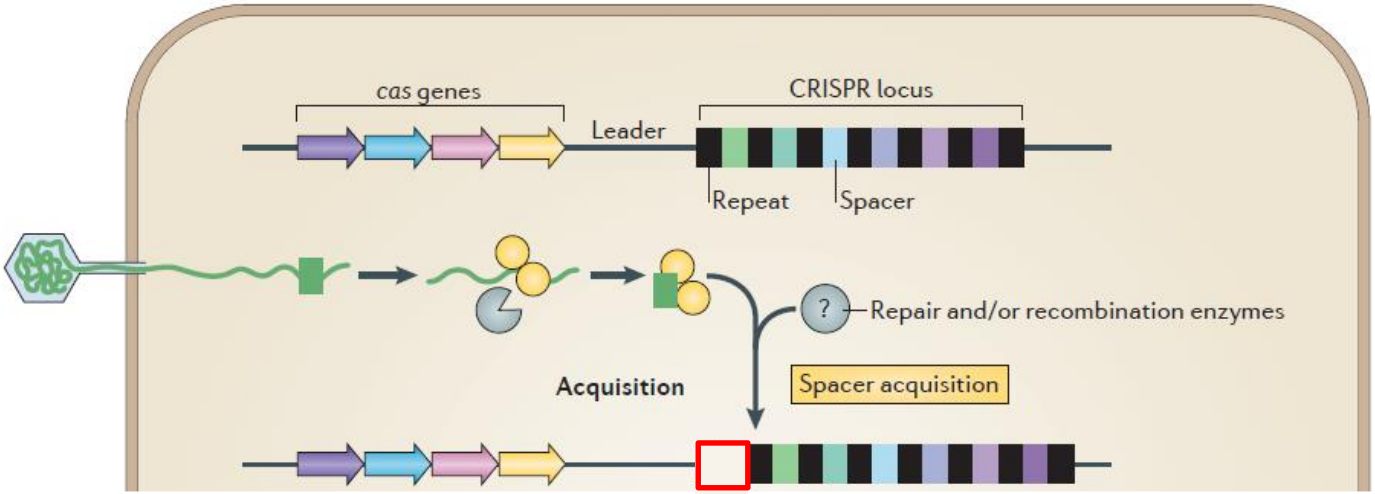
CRISPR-Cas mechanism



a viral infection often leads to rapid synthesis of a new generation of viral particles and cell death

in a sub-fraction of a bacterial population, for instance due to infection by an inactive virus mutant, immunity can be gained through CRISPR-Cas

CRISPR-Cas mechanism – *step 1*

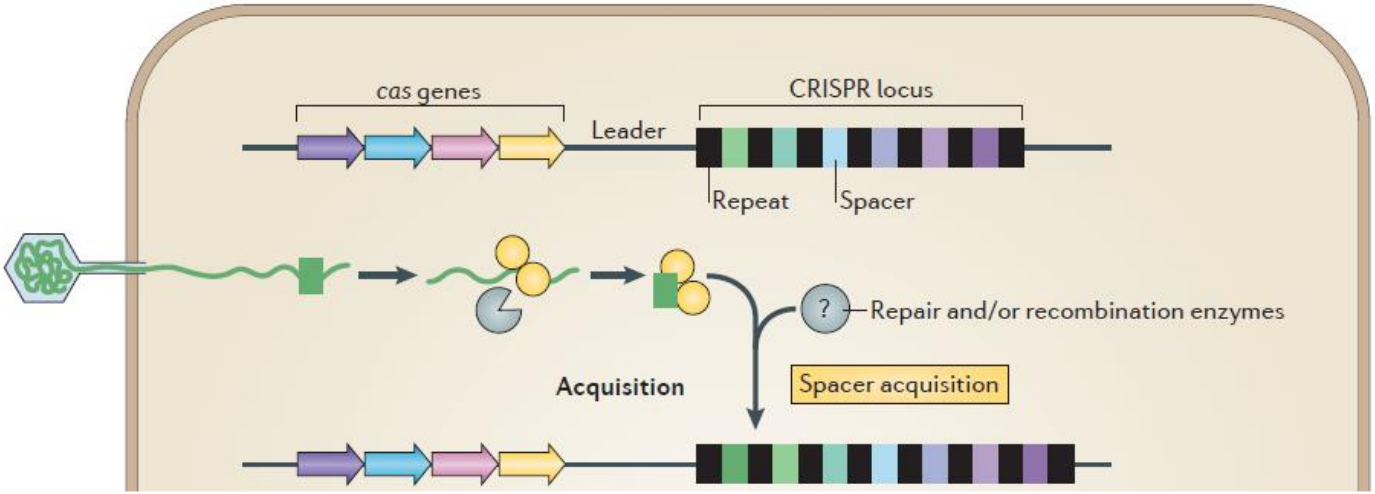


Spacer acquisition

CRISPR array = memory of adaptive immune system

Barrangou (2007) Science

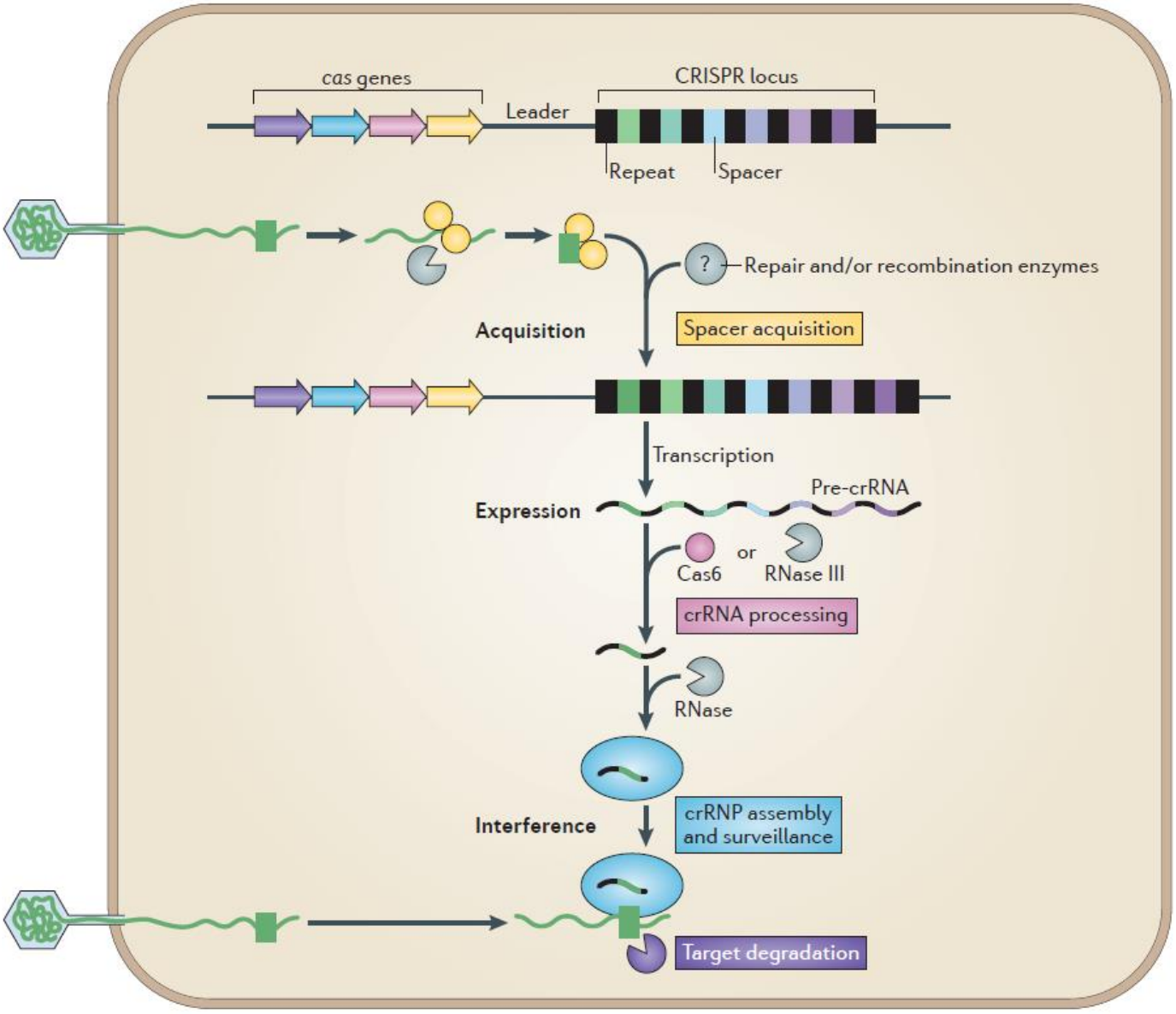
CRISPR-Cas mechanism – *steps 2 & 3*



Spacer acquisition

Van der Oost (2014) Nat Rev Microbiol

CRISPR-Cas mechanism – *steps 2 & 3*



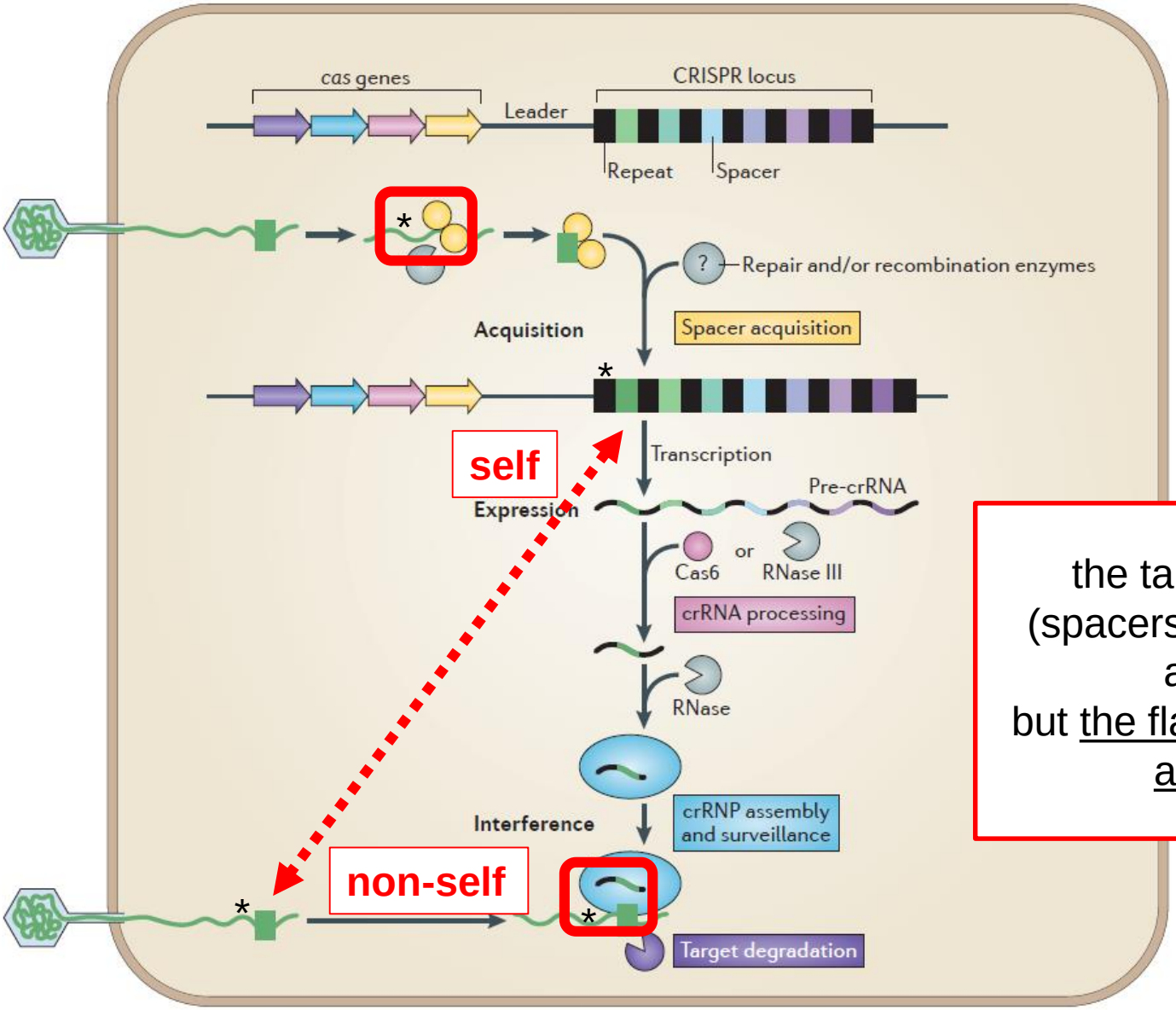
Spacer acquisition

Guide expression

Target interference

Van der Oost (2014) Nat Rev Microbiol

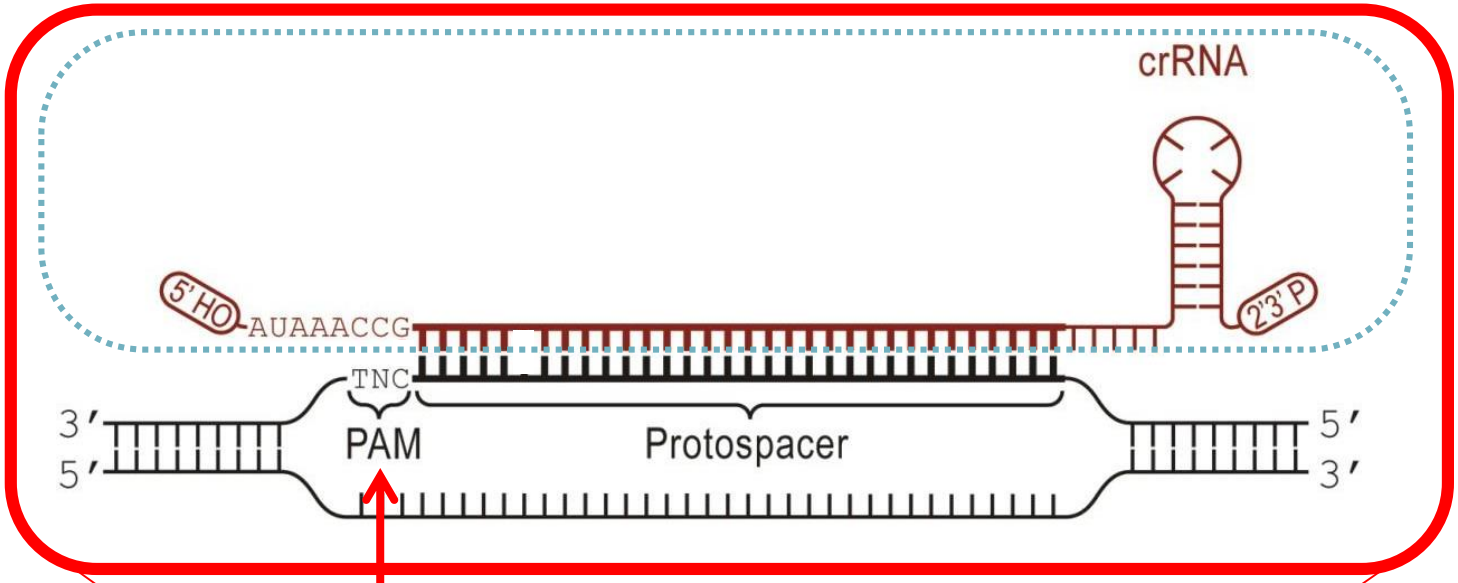
CRISPR-Cas – *self / non-self discrimination*



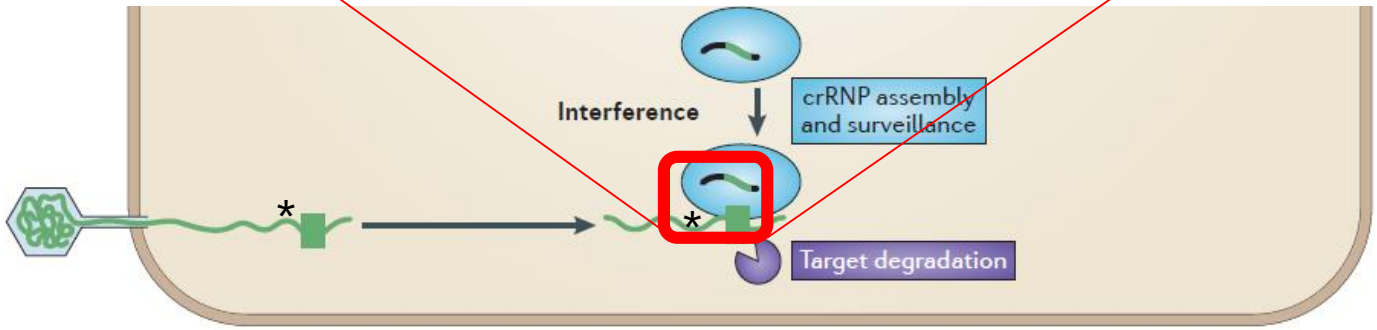
the target sequences
(spacers & proto-spacers)
are similar,
but the flanking sequences*
are different

Van der Oost (2014) Nat Rev Microbiol

CRISPR-Cas – *self / non-self discrimination*

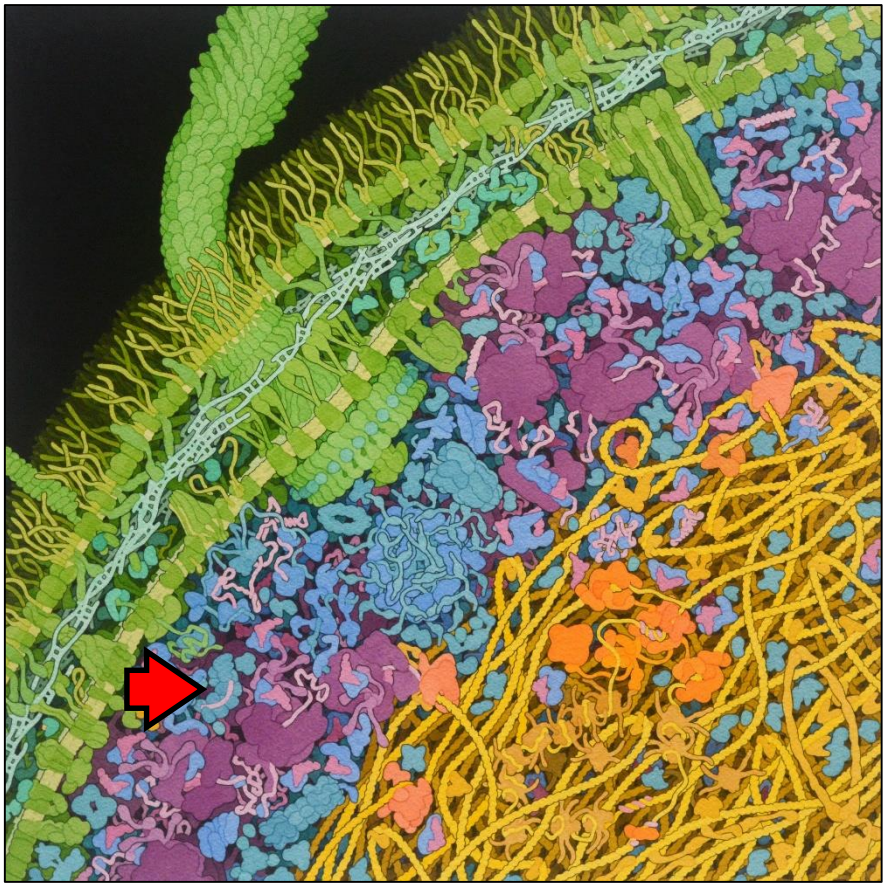


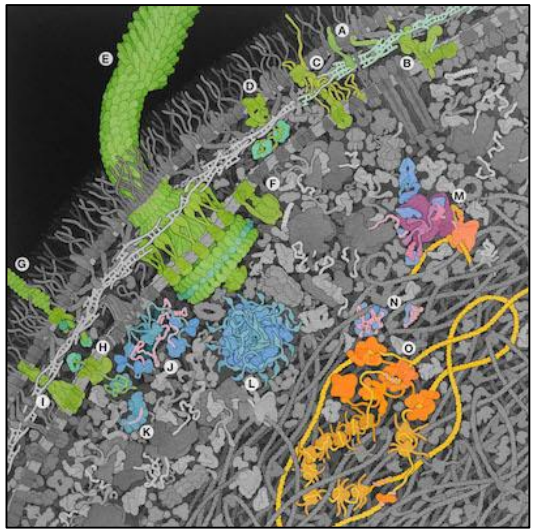
Protospacer Adjacent Motif (PAM)



Jore et al. (2011) NSMB, Semanova et al. (2011) PNAS, Westra et al. (2012) PLoS Gen.

Cascade in *E. coli* cell

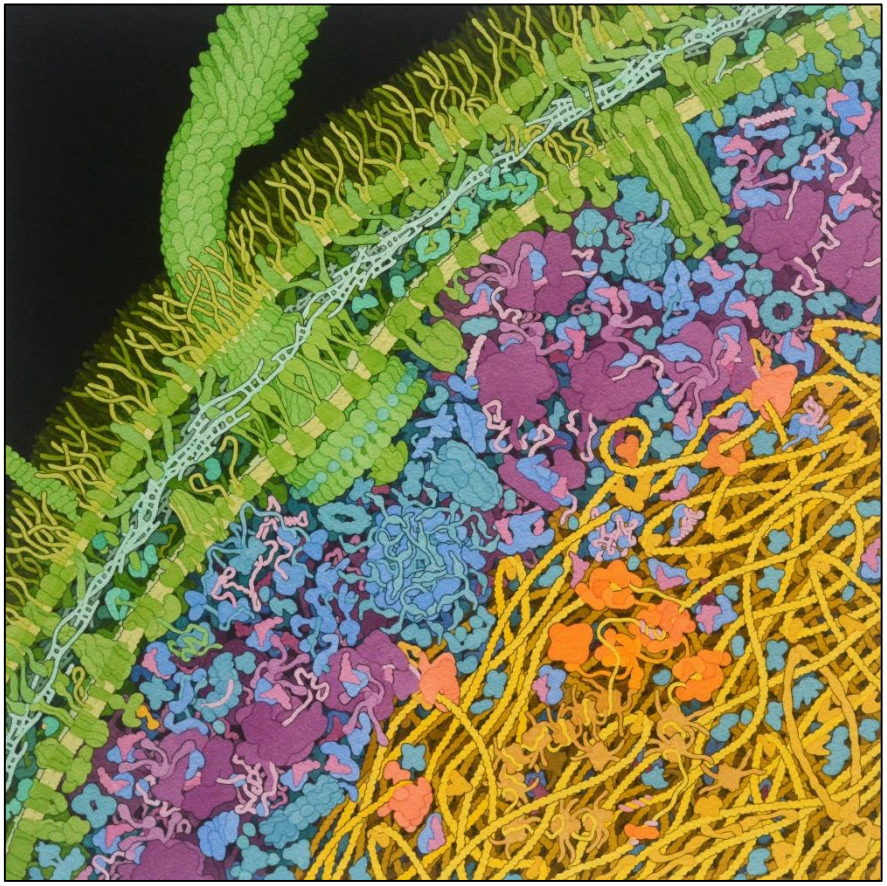


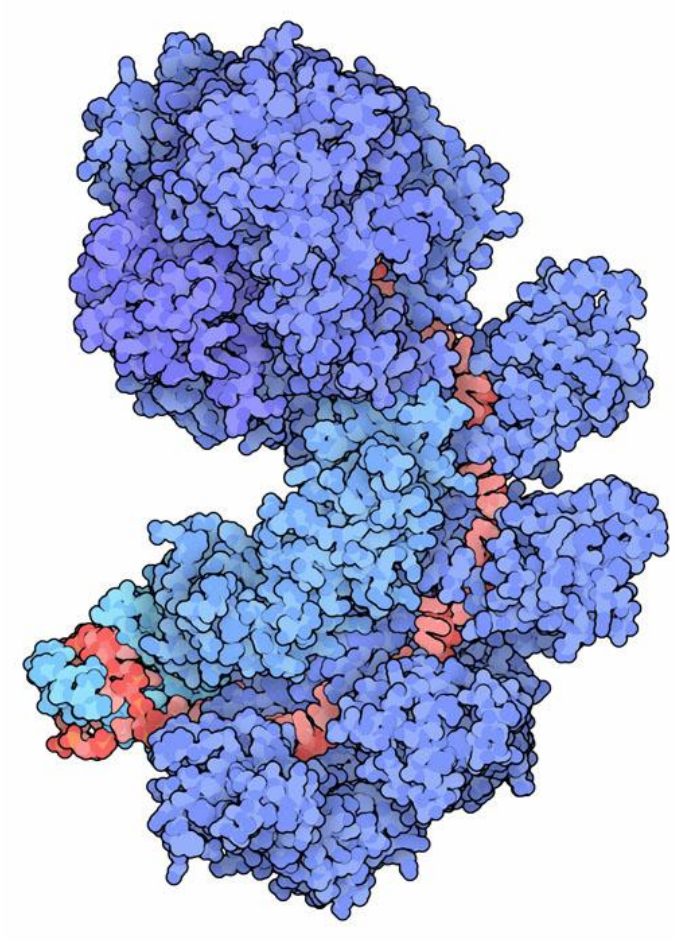


A. Lipoprotein, the most abundant protein in the cell, and OmpA. Two structural proteins that connect the outer membrane and the peptidoglycan network.
B. A complex of proteins that synthesize the peptidoglycan network, including penicillin-binding proteins that are the target of penicillin.
C. The Lpt system that transports lipopolysaccharides from the inner membrane (where they are synthesized) to the outer membrane.
D. The Bam system that chaperones the folding of outer membrane proteins.
E. Flagellum and flagellar motor.
F. ATP synthase.
G. Fimbria and fimbrial usher. One subunit is being transported to the usher by a chaperone.
H. Secretory translocon, being assisted by chaperones.
I. AcrAB/TolC multi-drug efflux pump.
J. Degradosome, a complex of proteins for degrading obsolete RNA.
K. Cascade and CRISPR.
L. Pyruvate dehydrogenase complex.
M. Expressome. The flexible arms on the ribosome are L12 subunits, which help coordinate the binding of tRNA and elongation factors.
N. Two sRNA with their associated proteins.
O. Replisome including several DNA polymerases and single-strand DNA-binding proteins protecting the single-stranded intermediates formed during the process of DNA replication.

http://pdb101.rcsb.org/sci-art/goodsell-gallery/escherichia-coli-bacterium

Cascade in *E. coli* cell

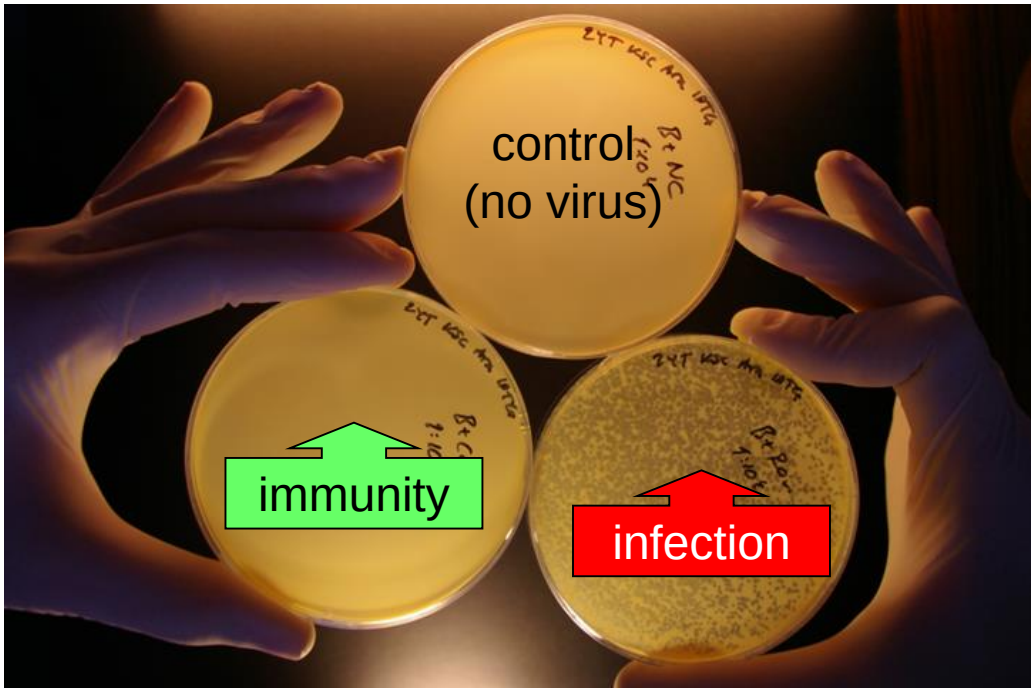




http://pdb101.rcsb.org/sci-art/goodsell-gallery/escherichia-coli-bacterium

crRNA-guided DNA interference

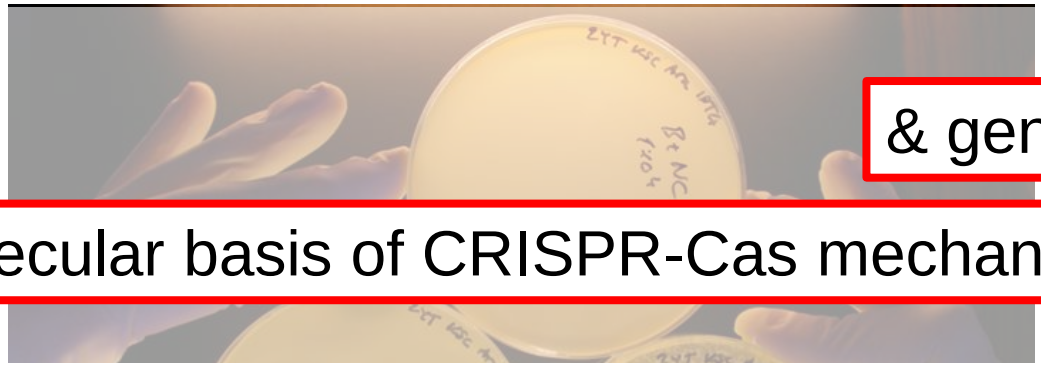
***E.coli* BL21**
<u>design</u>
CRISPR-Cas



Brouns et al. (2008) Science

crRNA-guided DNA interference

***E.coli* BL21**
<u>design</u>
CRISPR-Cas



& genome editing

Molecular basis of CRISPR-Cas mechanism

- Specific maturation of CRISPR-derived RNA guides
- Binding of crRNA guides by Cas effector complex
- Anti-viral defense by crRNA-guided DNA interference
- Transplantation of CRISPR-Cas system to another host
- CRISPR design allows for specific (multiplex) DNA targeting

Brouns et al. (2008) Science

outline

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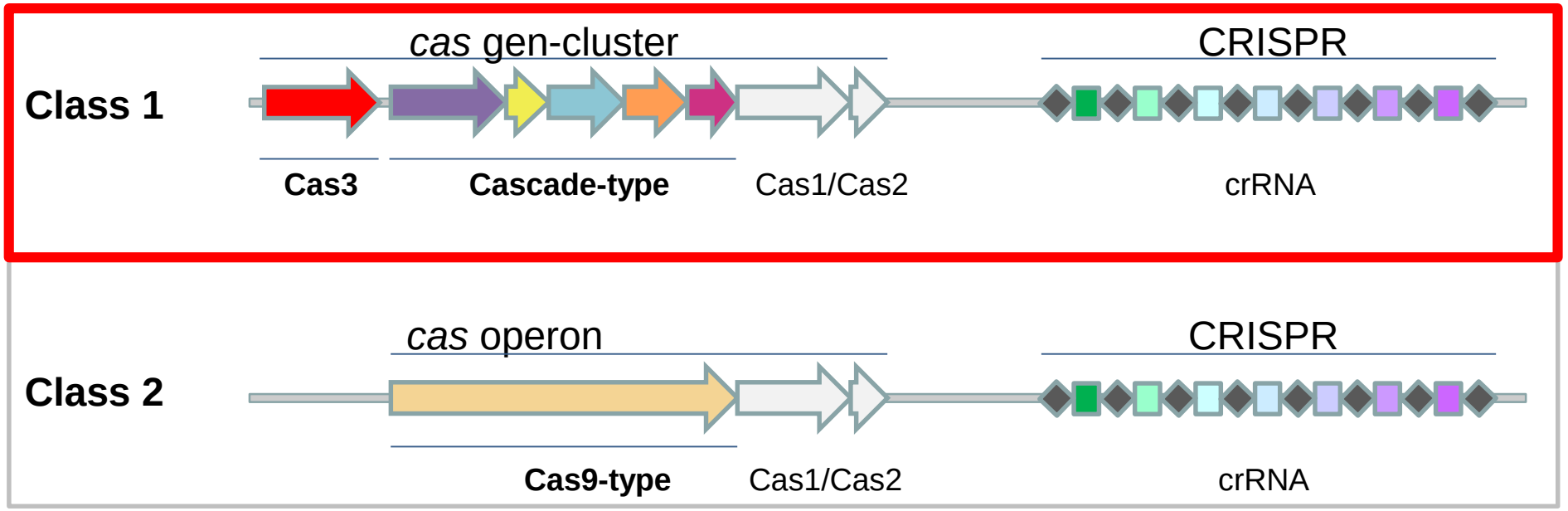
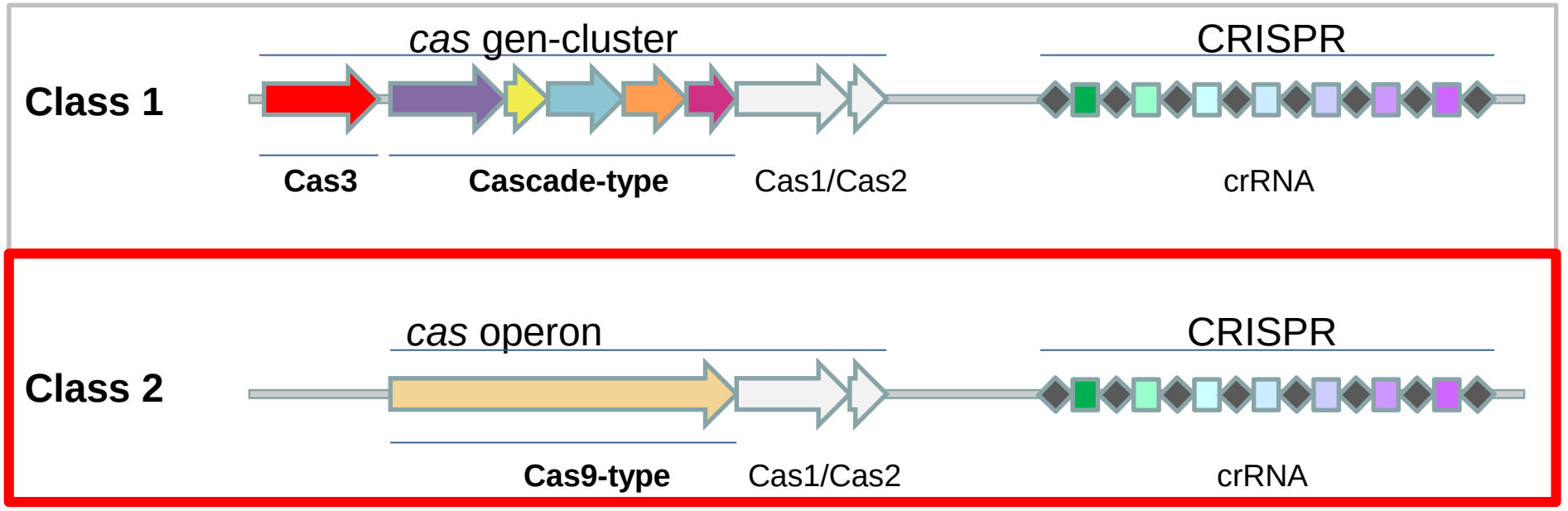
CRISPR-Cas – 2 classes



Makarova (2015) Nat Rev Microbiol

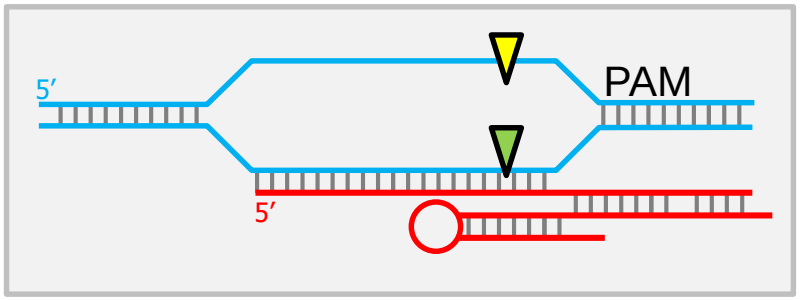
CRISPR-Cas – 2 classes



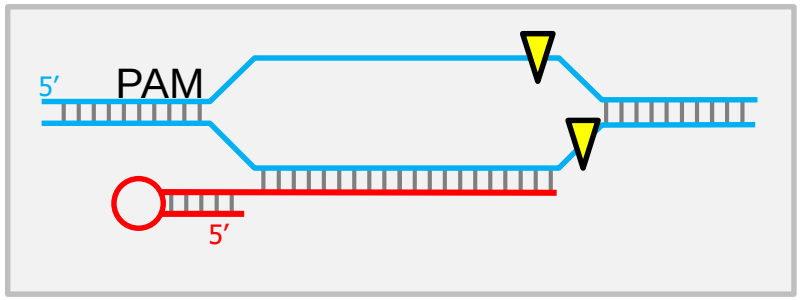
Makarova (2015) Nat Rev Microbiol

Class 2 – Cas9

CRISPR-Cas9



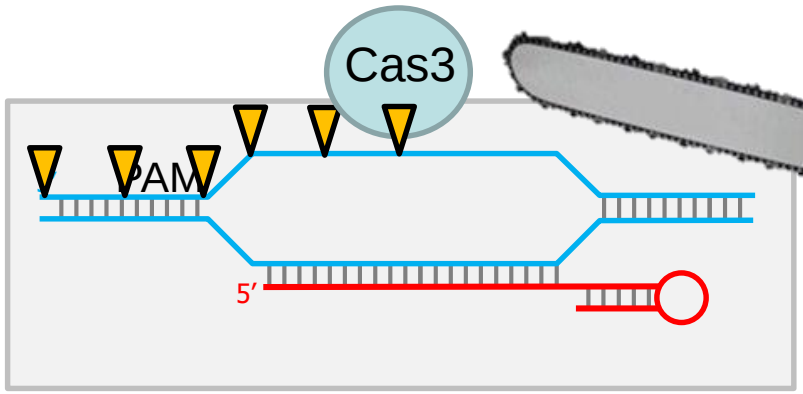
CRISPR-Cas12



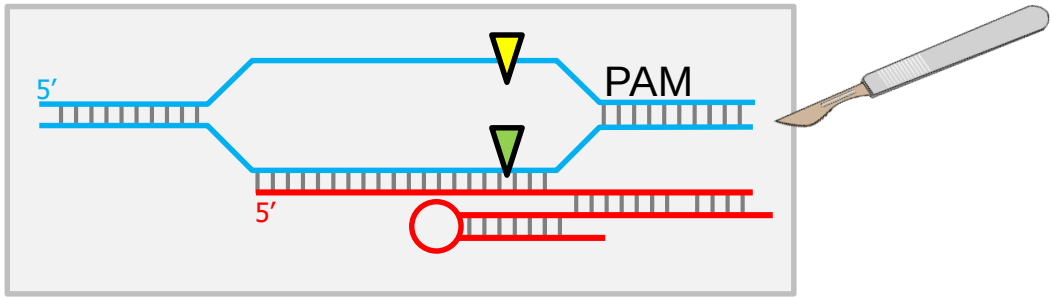
class 2 Cas nucleases are multi-functional

Cascade - Cas9 - Cas12

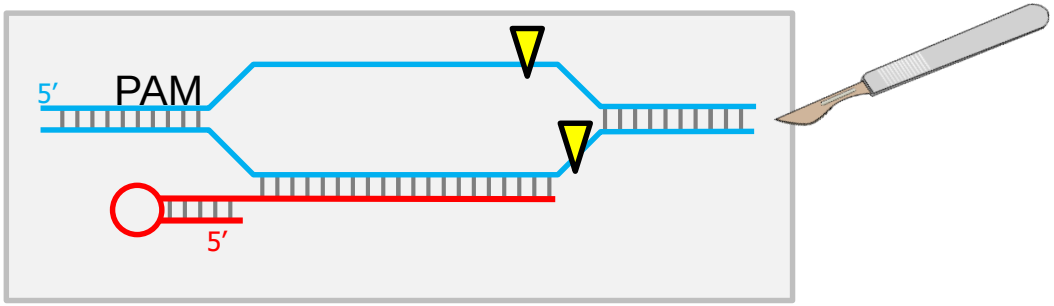
Type I
Cascade



Type II
Cas9

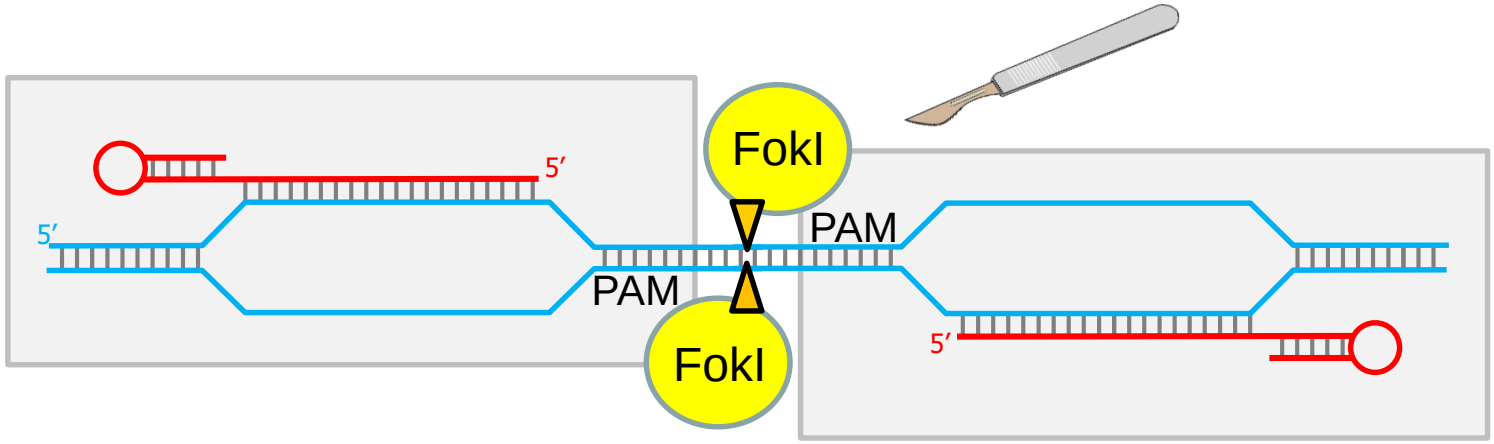


Type V
Cas12

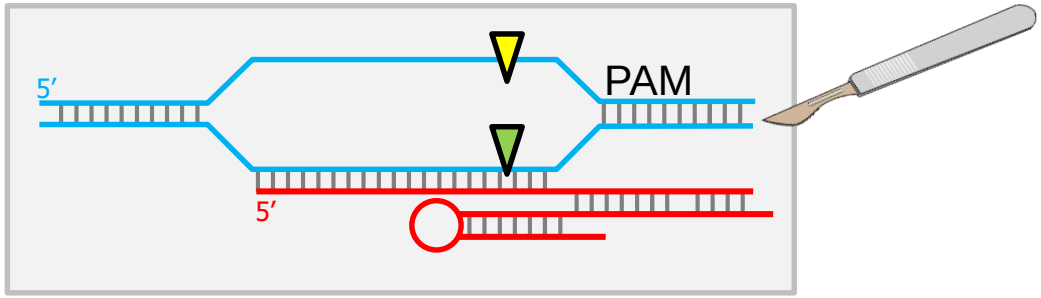


Cascade - Cas9 - Cas12

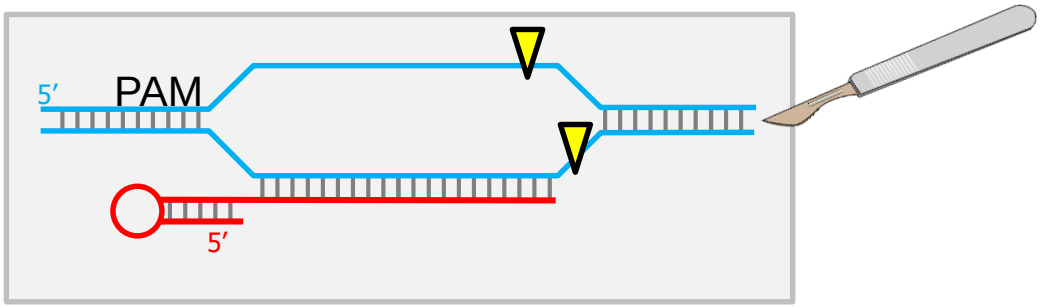
Type I
Cascade



Type II
Cas9

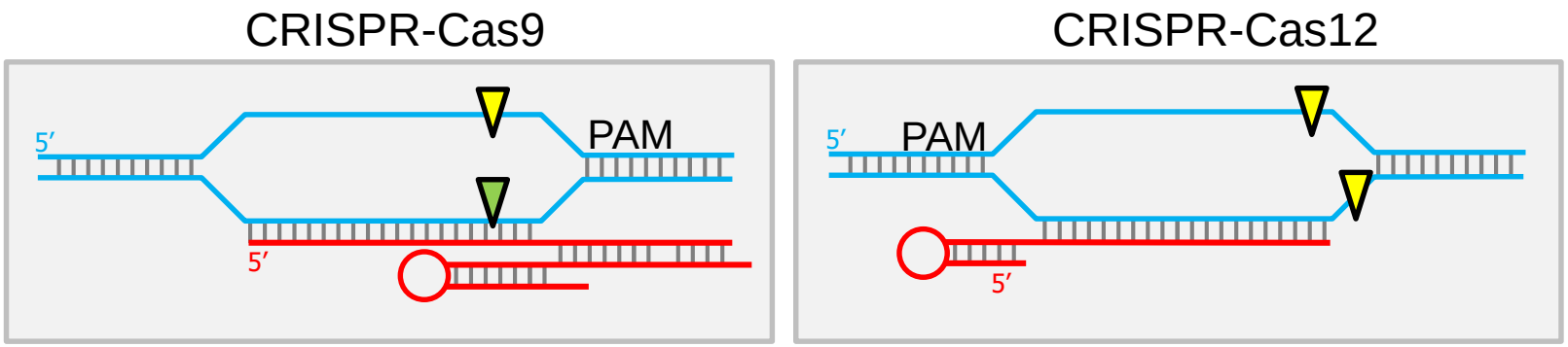


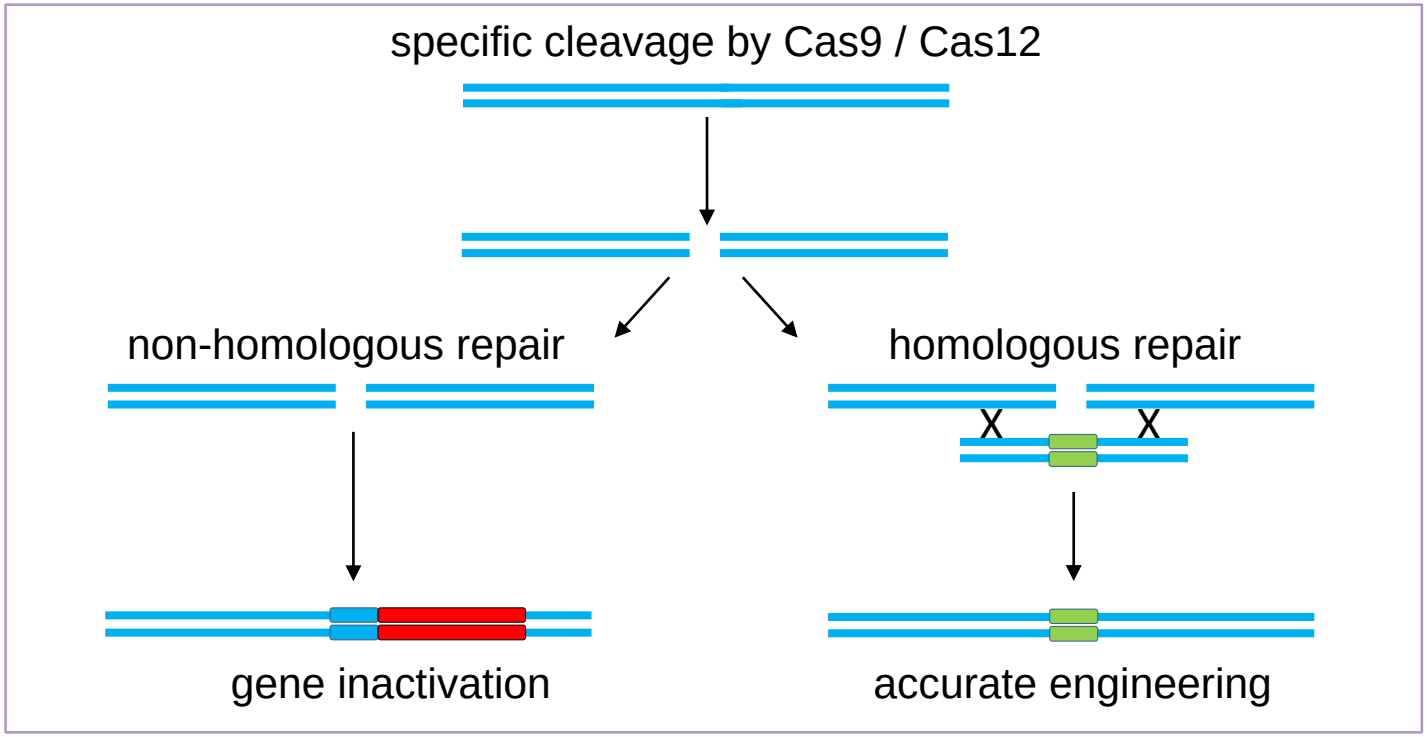
Type V
Cas12



Cameron (2019) Nat Biotech

CRISPR-Cas – DNA editing



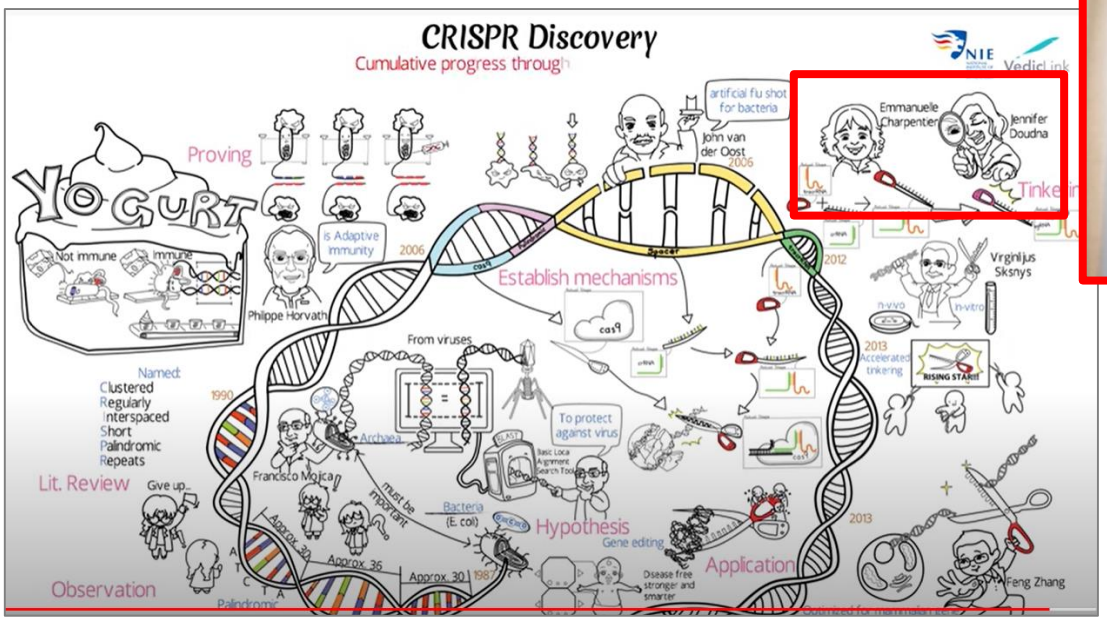


Conclusions

- CRISPR-Cas is an adaptive defense system in bacteria and archaea
- CRISPR RNAs guide nucleases to complementary **DNA*** (or RNA) target sequences
- CRISPR-Cas systems are highly diverse (2 classes, 6 types, >20 subtypes)
- Cas nucleases (**Cas9, Cas12a, Cascade-FokI**)* led to genome editing revolution
- Genome engineering in **Biotechnology**: bacteria, yeast, algae & plants
- **Clinical trials** of natural & synthetic Cas nucleases to cure human genetic diseases

Conclusions

- CRISPR discoveries – youtube : https://www.youtube.com/watch?v=RKh2mi3tsmc



Collaborations

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Yifan Zhu*
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NWO
Netherlands Organisation for Scientific Research

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