Application of CRISPR/Cas System in Nucleic Acid Detection

1st Mphil student: Emily Lei

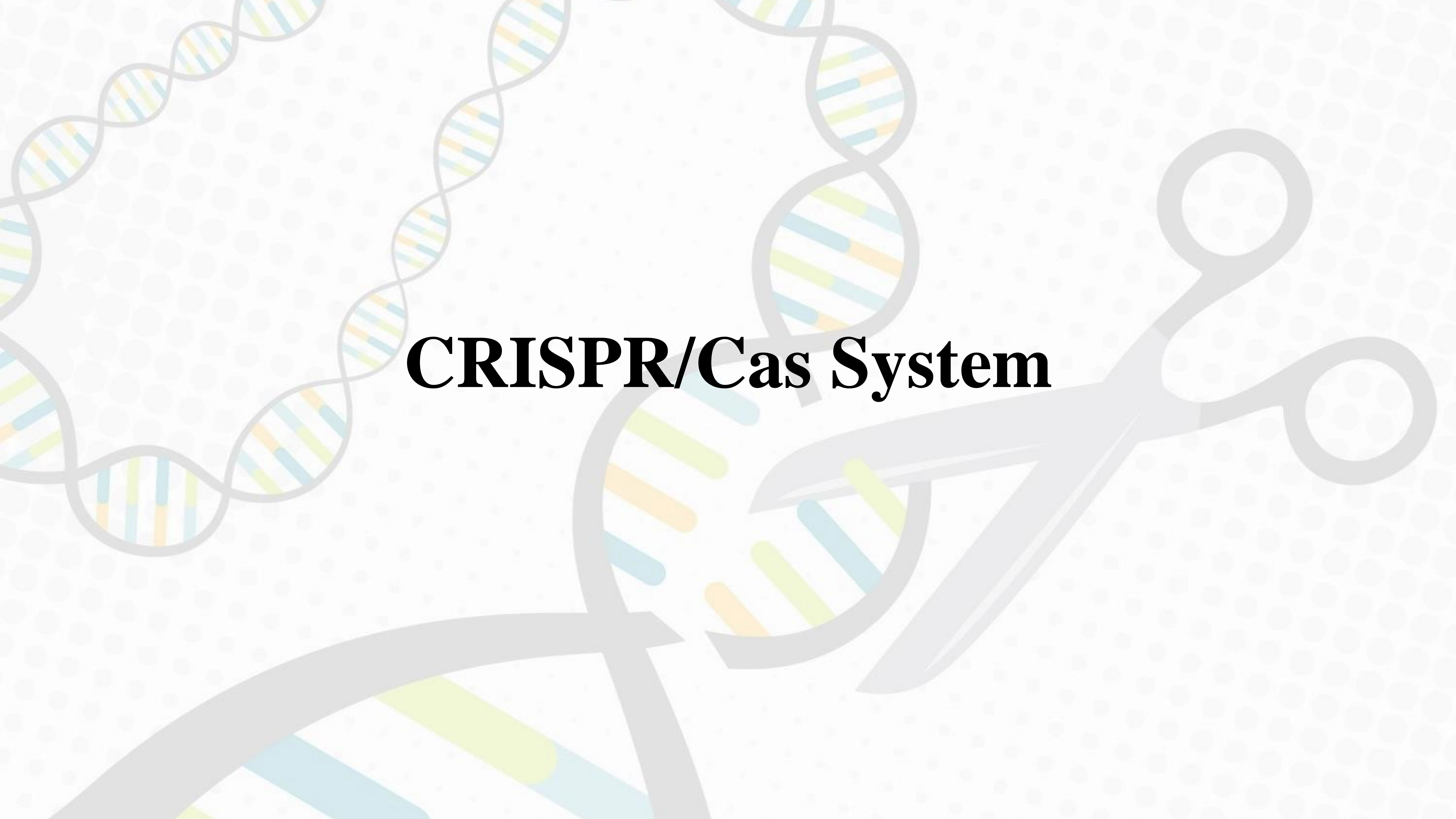
Supervisor: Professor Zigui Chen

Joint Graduate Seminar

14th Dec 2018

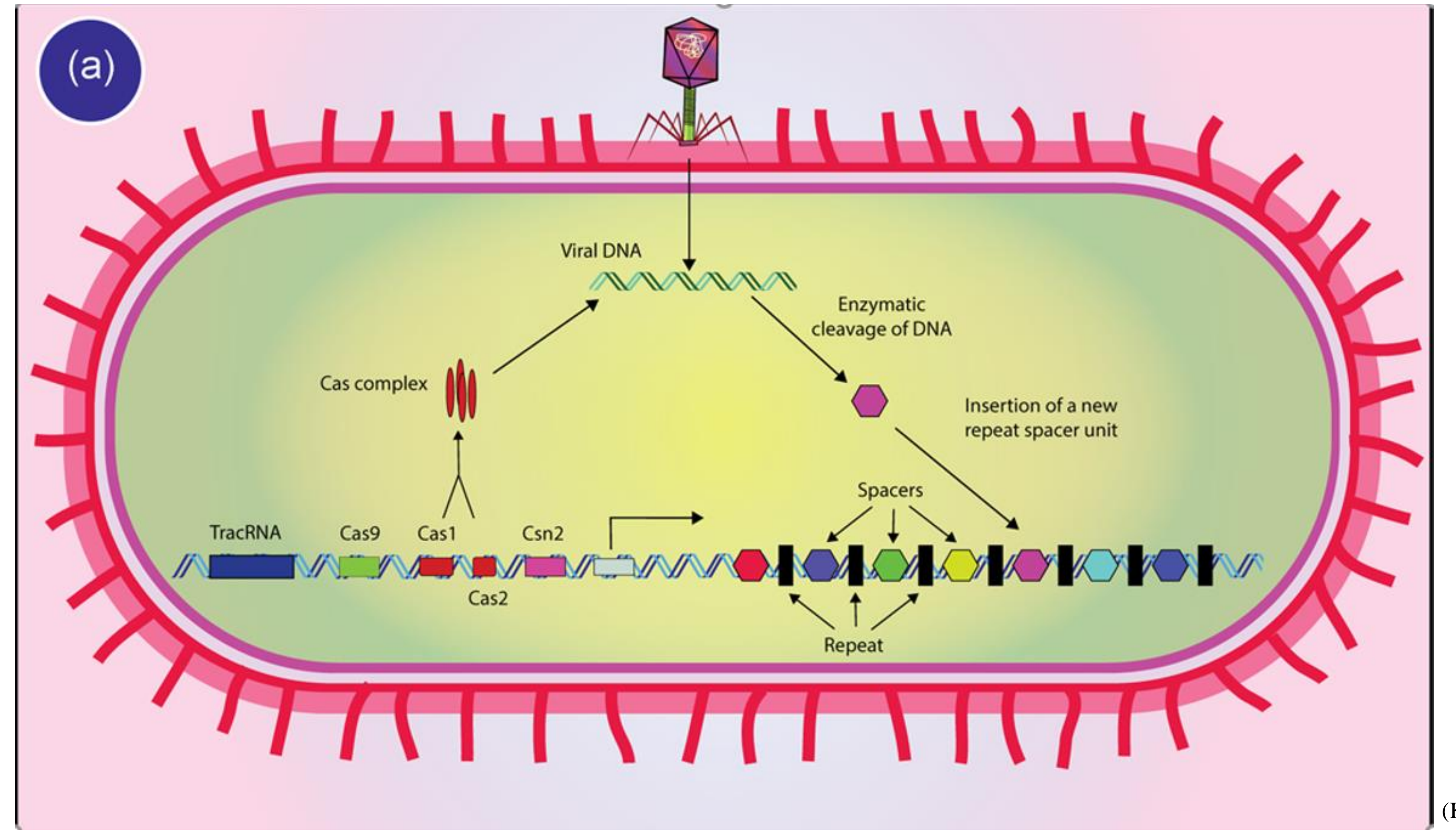
Contents

- 1.CRISPR/Cas System
- 2.CRISPR for Genome Editing
- 3.CRISPR for Detection



CRISPR/Cas System

CRISPR/Cas: An Adaptive Immune System

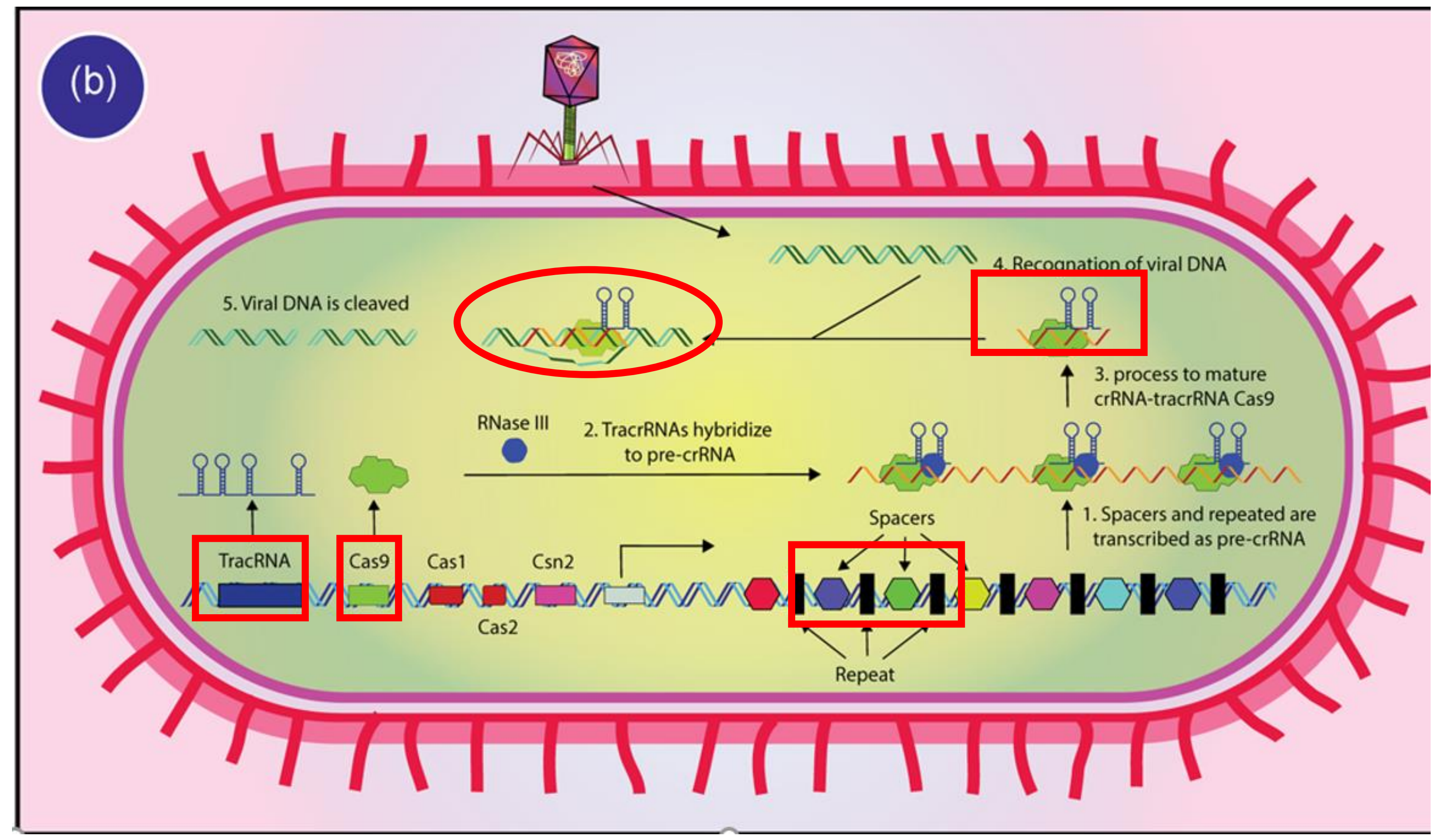


An overview of CRISPR/Cas as a bacterial adaptive immune system

(Khadempar, Familghadakchi et al. 2018)

The invasive foreign DNA is broken down by the **Cas nucleases**, and then part of it is placed in the CRISPR site between two repeated sequences, in which case it is referred to as a **spacer**.

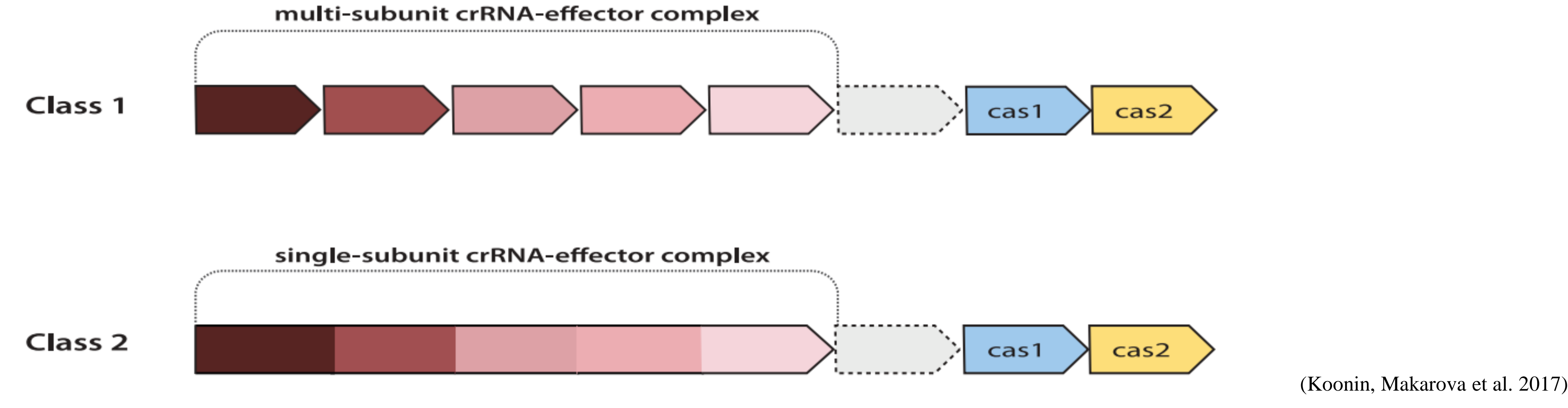
CRISPR/Cas: An Adaptive Immune System



(Khadempar, Familghadakchi et al. 2018)

An overview of CRISPR/Cas as a bacterial adaptive immune system

Classification of CRISPR/Cas system

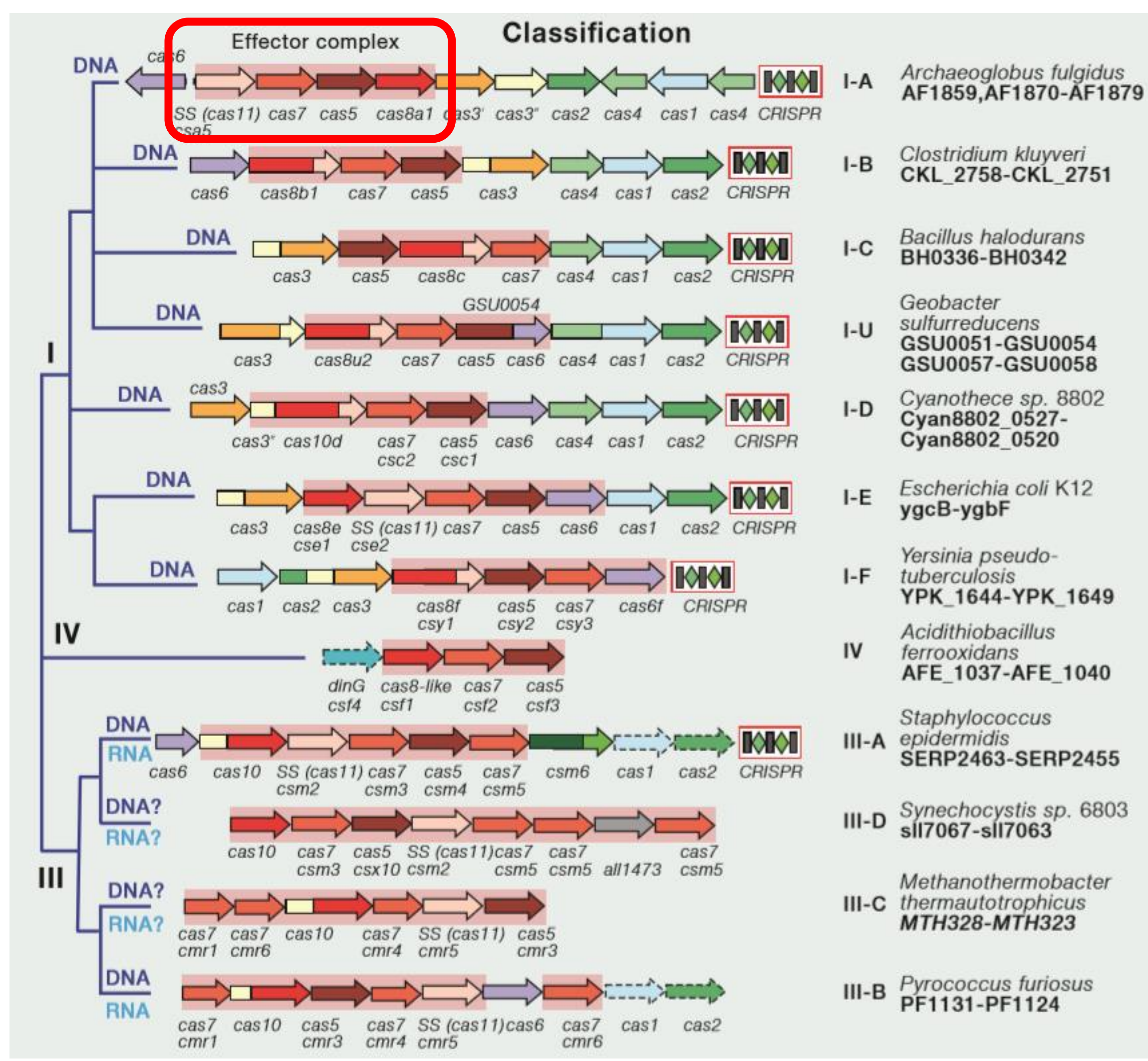


(Koonin, Makarova et al. 2017)

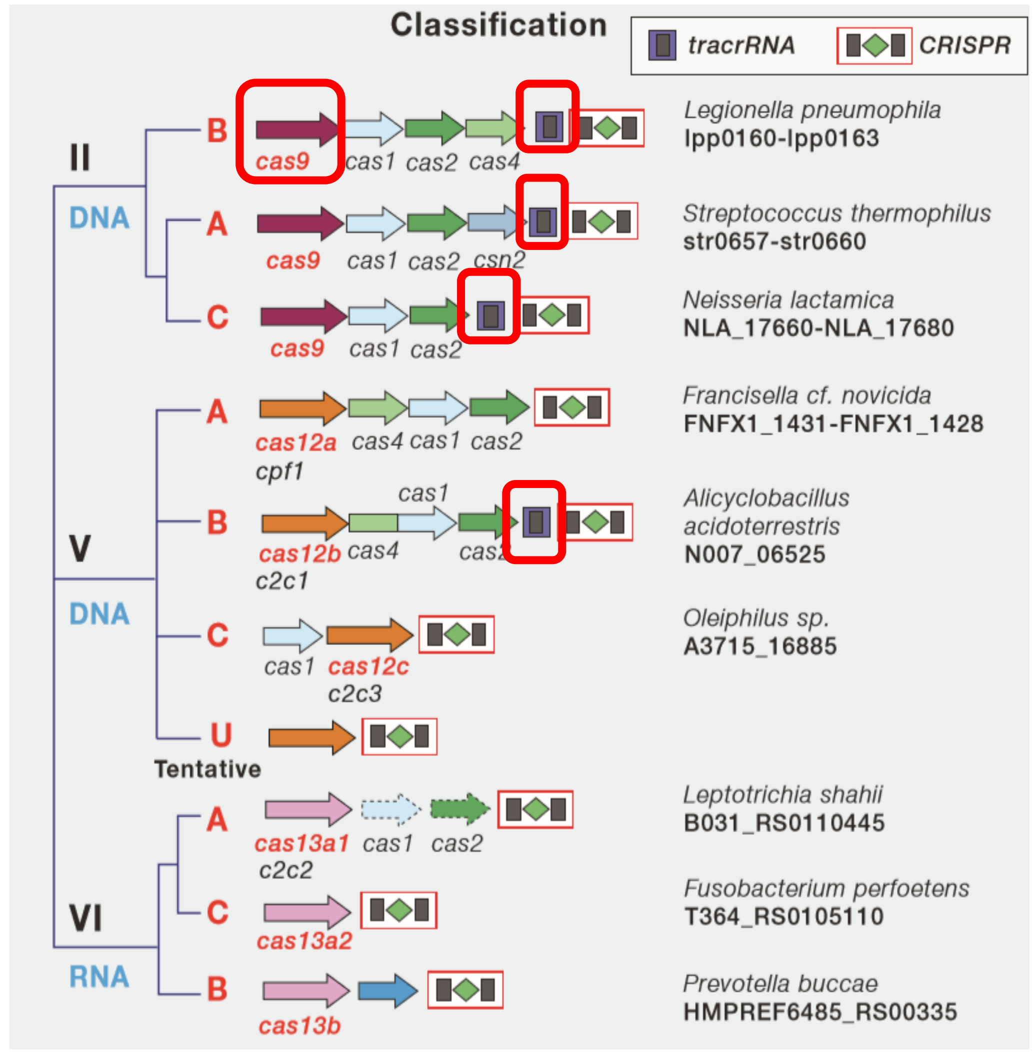
CRISPR-Cas system split into two distinct classes based on effector module organization.

Class 1 CRISPR-Cas systems utilize **multi-protein effector** complexes, whereas **class 2** utilize **single-protein effectors**.

Classification of CRISPR/Cas system



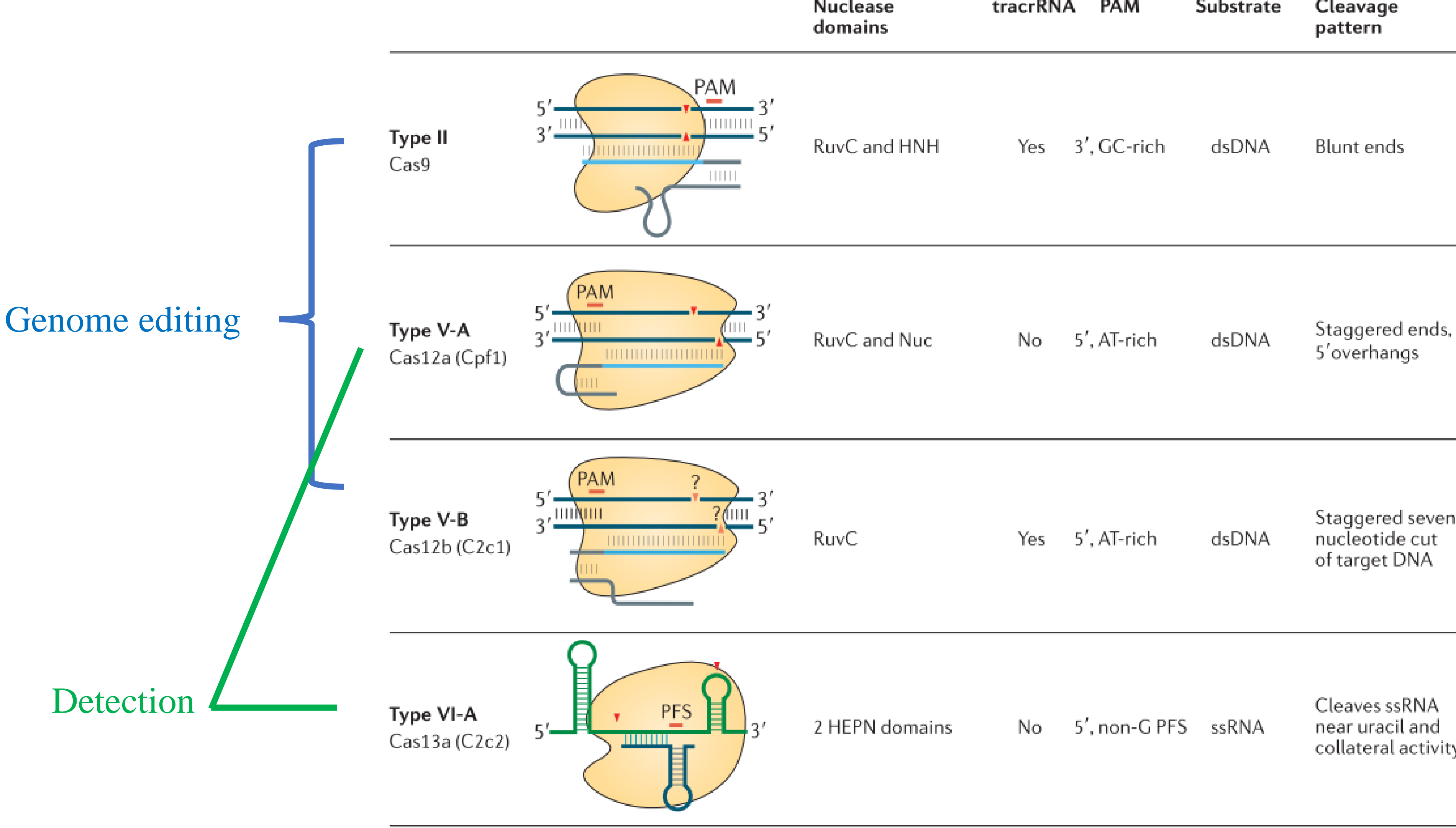
Class 1



Class 2

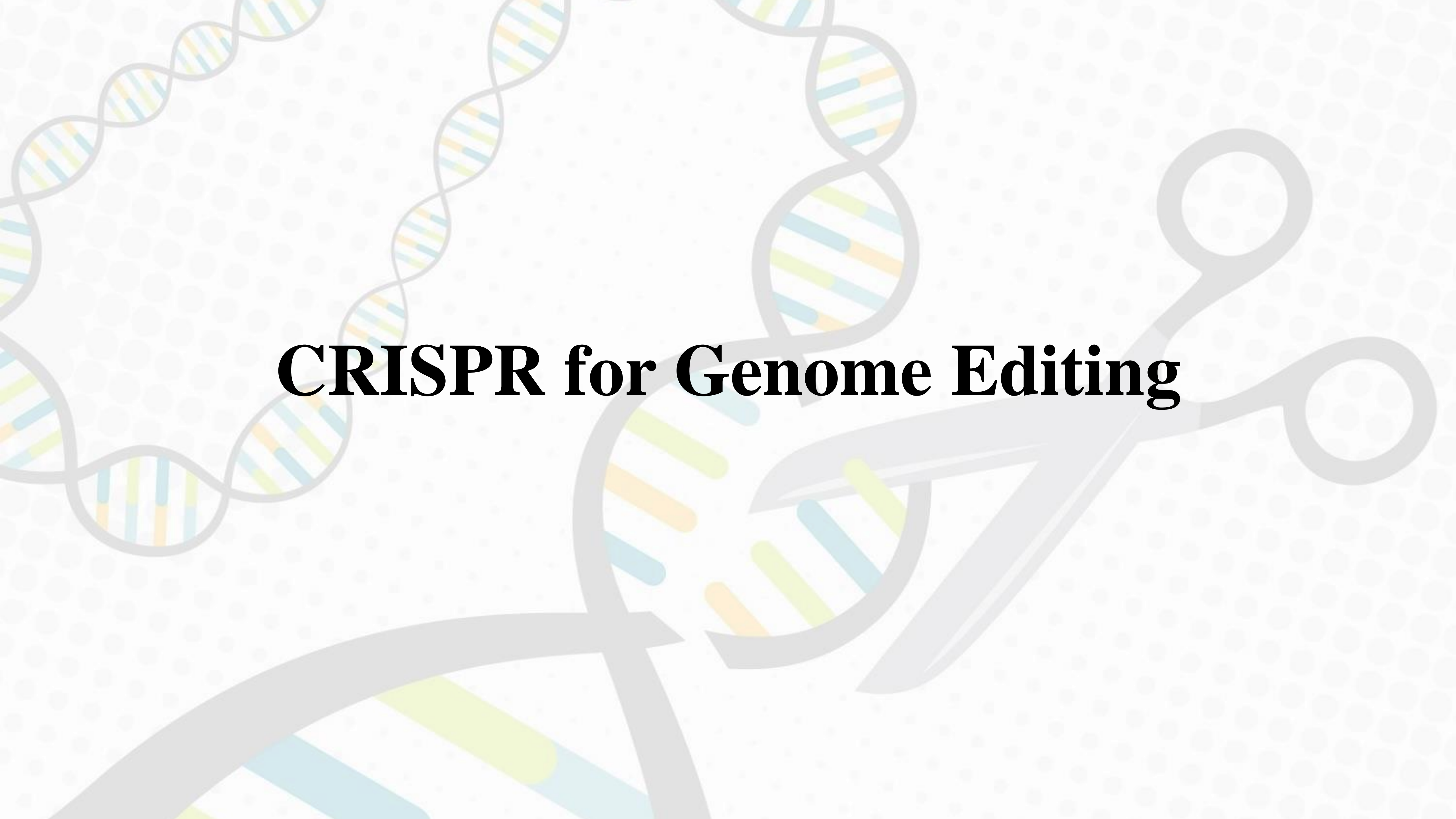
(Koonin, Makarova et al. 2017)

Functional diversity of the experimentally characterized class 2 CRISPR/Cas systems

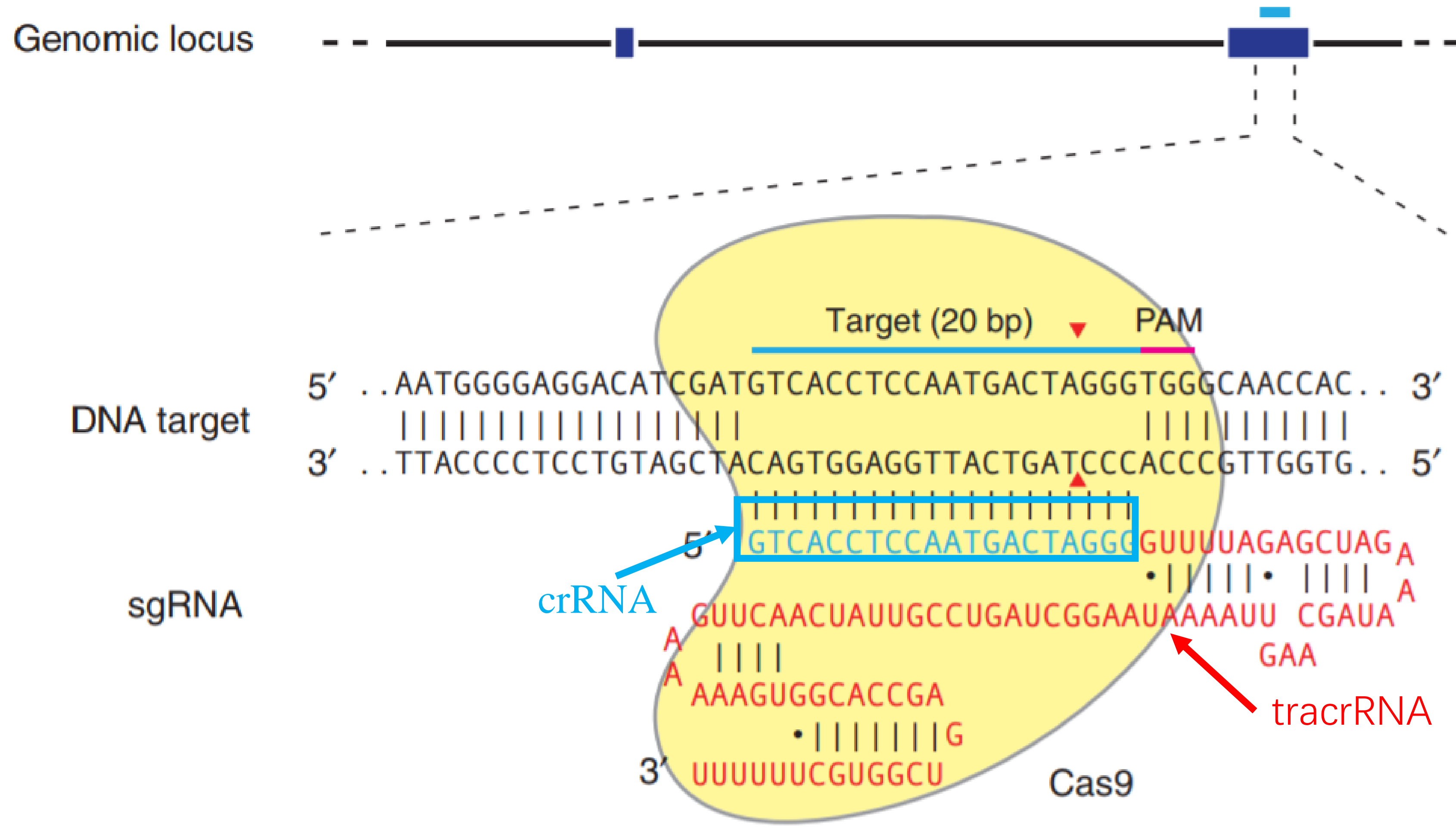


	Nuclease domains	tracrRNA	PAM	Substrate	Cleavage pattern
Type II Cas9	RuvC and HNH	Yes	3′, GC-rich	dsDNA	Blunt ends
Type V-A Cas12a (Cpf1)	RuvC and Nuc	No	5′, AT-rich	dsDNA	Staggered ends, 5′overhangs
Type V-B Cas12b (C2c1)	RuvC	Yes	5′, AT-rich	dsDNA	Staggered seven-nucleotide cut of target DNA
Type VI-A Cas13a (C2c2)	2 HEPN domains	No	5′, non-G PFS	ssRNA	Cleaves ssRNA near uracil and collateral activity

(Shmakov, Smargon et al. 2017)

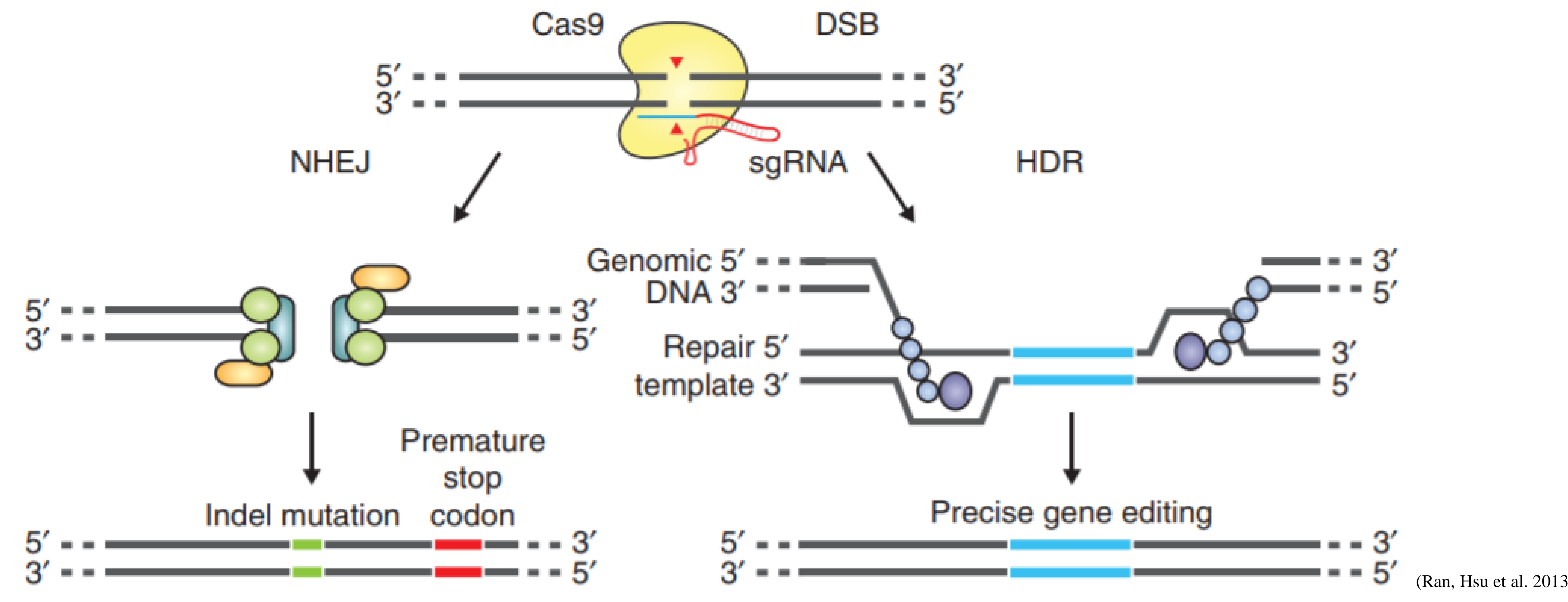

CRISPR for Genome Editing

Schematic of Cas9-sgRNA complex



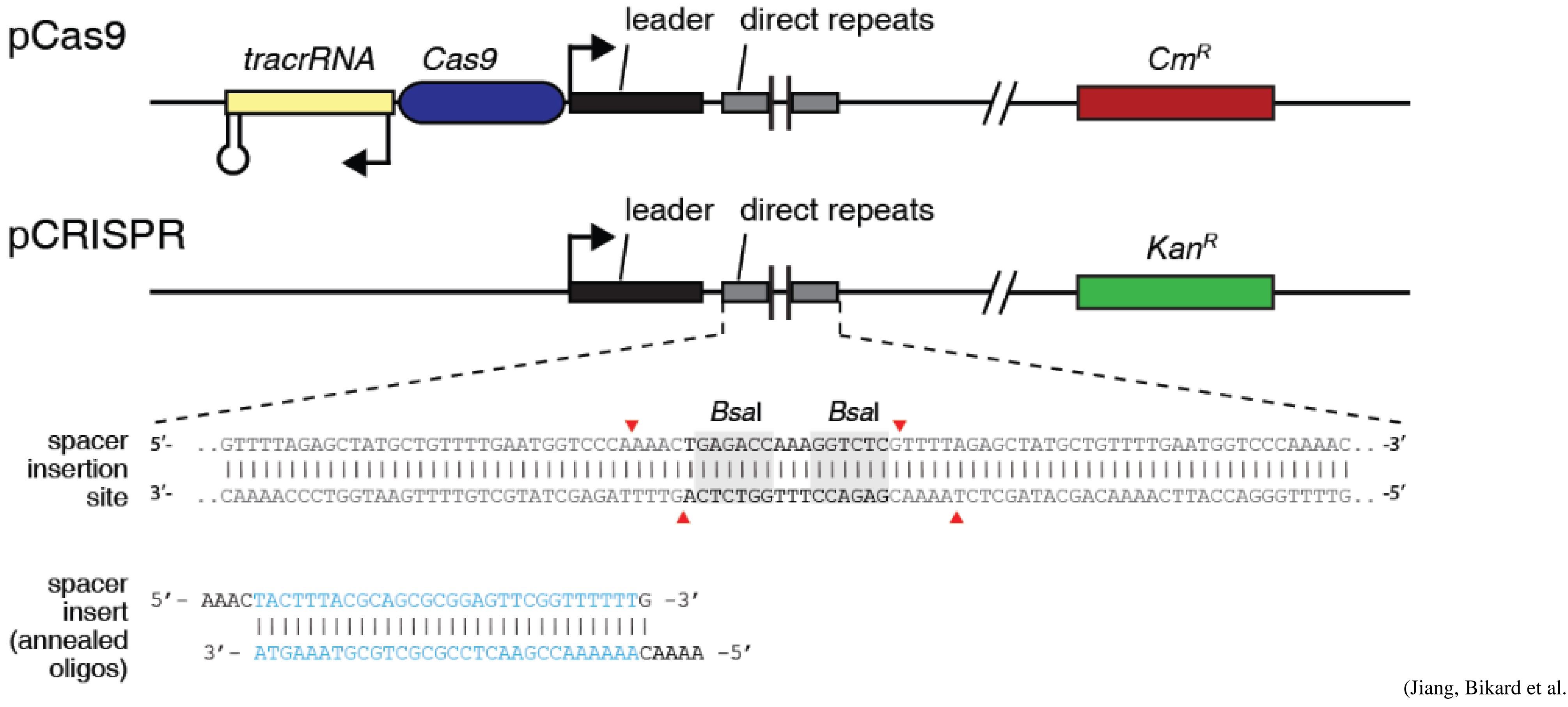
(Ran, Hsu et al. 2013)

Two general repair pathways



Non-Homologous End Joining (**NHEJ**) pathway : **eficient** but **error-prone**;
Homology Directed Repair (**HDR**) pathway: **less efficient** but **high-fidelity**

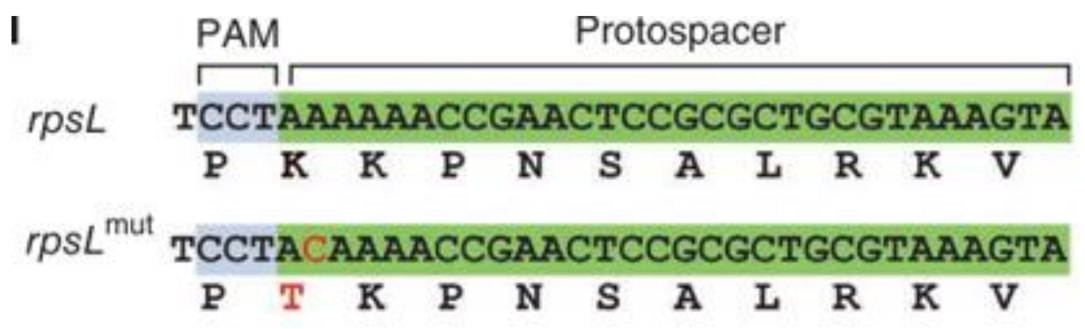
Example: Genome editing with dual-RNA:Cas9 in E. coli



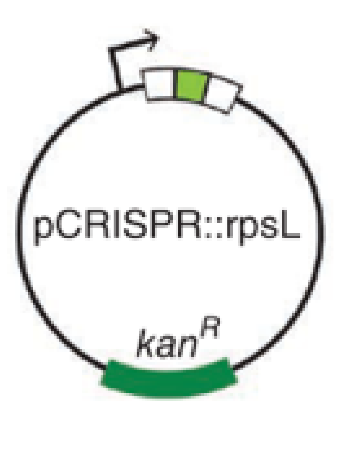
(Jiang, Bikard et al. 2013)

Spacers can be inserted into the crRNA array between **BsaI sites** using annealed **oligonucleotides.** Oligonucleotide design is shown at bottom.

Example: Genome editing with dual-RNA:Cas9 in E. coli



First, the scientists introduced an **A to C** transversion in **rpsL** that confers **streptomycin** resistance.

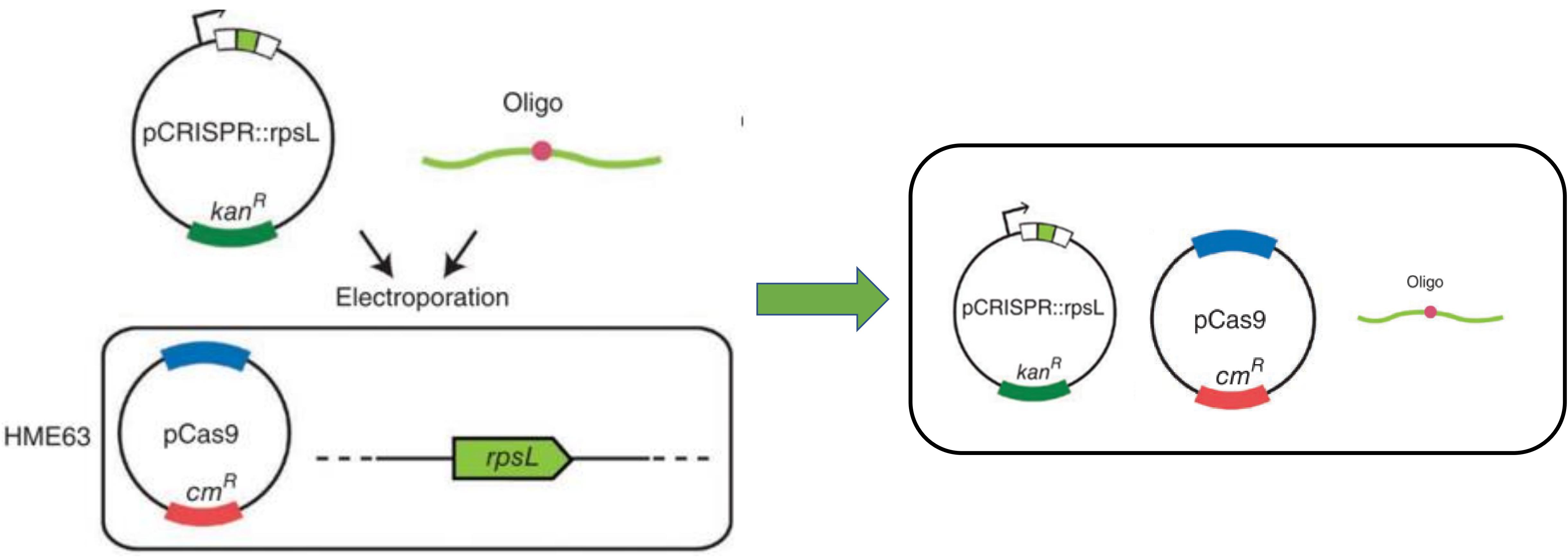


Then they constructed a **pCRISPRørpsL** plasmid harboring a spacer that would guide dual-RNA:Cas9 cleavage of the **wild-type**, but not the mutant rpsL allele.

They also constructed **W542**, an editing oligonucleotide containing the **A to C** mutation.

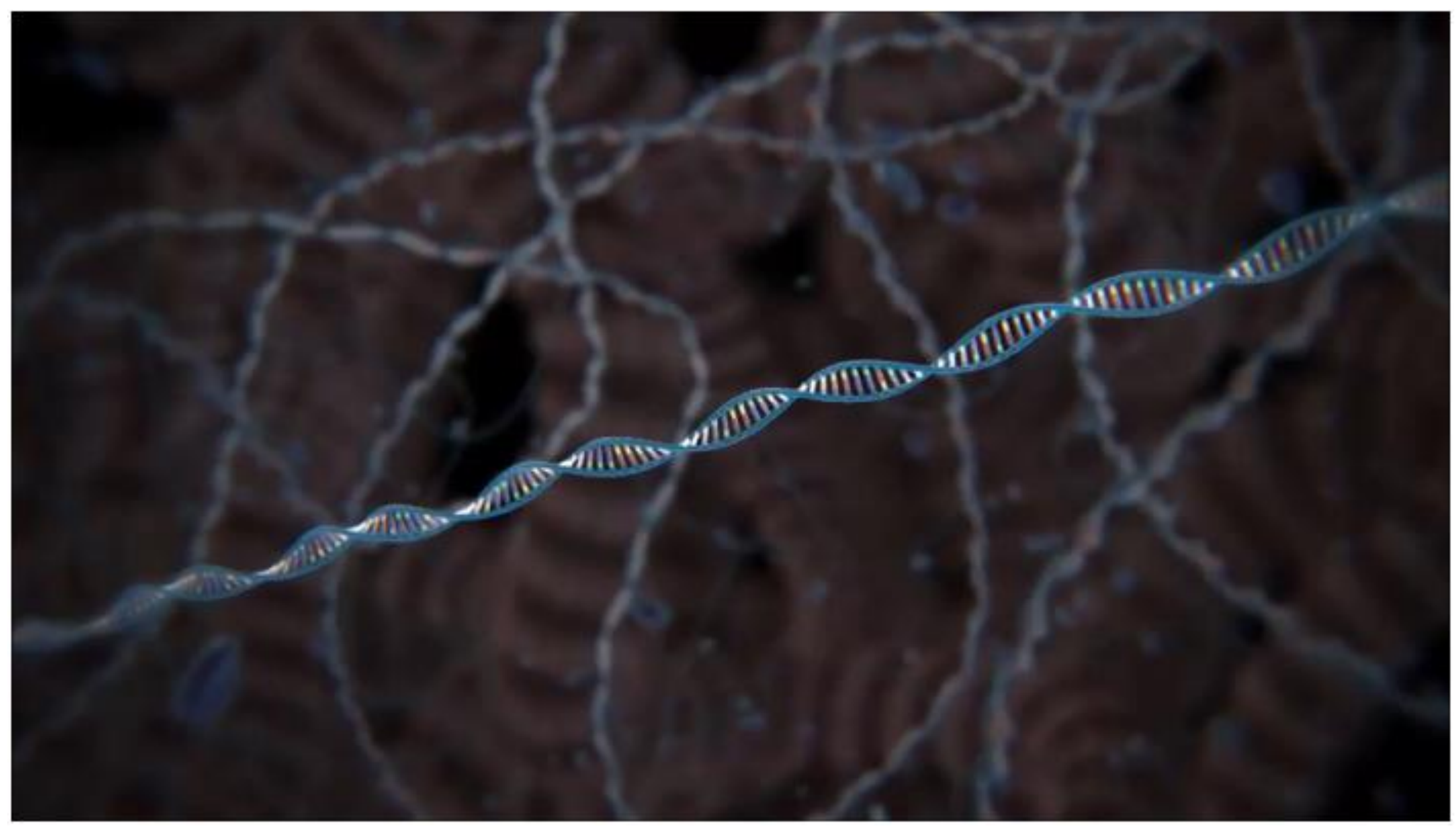
(Jiang, Bikard et al. 2013)

Example: Genome editing with dual-RNA:Cas9 in E. coli

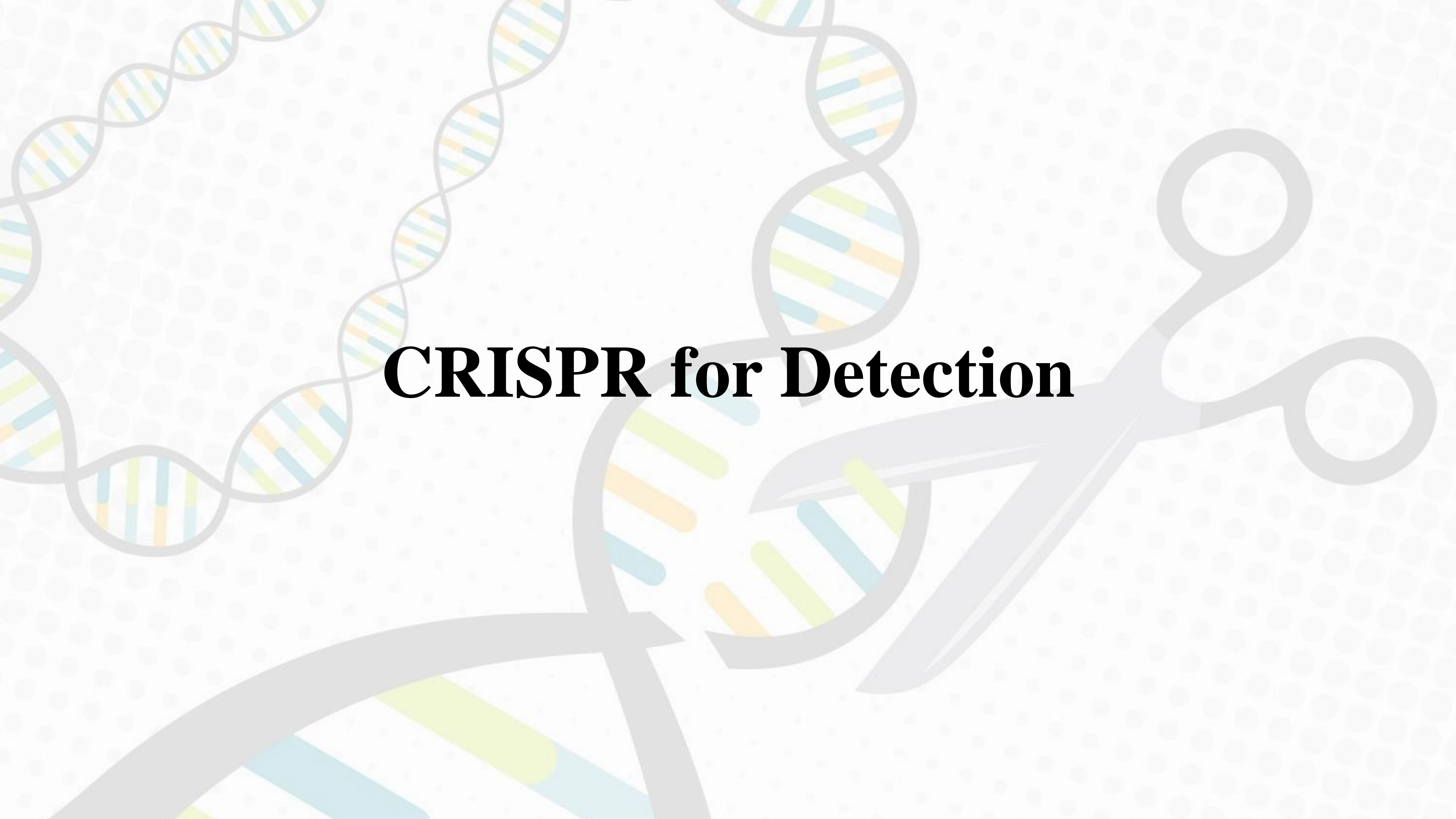


(Jiang, Bikard et al. 2013)

Genome editing with CRISPR-Cas9 system



https://www.youtube.com/watch?v=2pp17E4E-O8



CRISPR for Detection

Background of detection using CRISPR/Cas system

◆CRISPR-Cas systems contain programmable **endonucleases** that can be leveraged for **CRISPR-based diagnostics**.

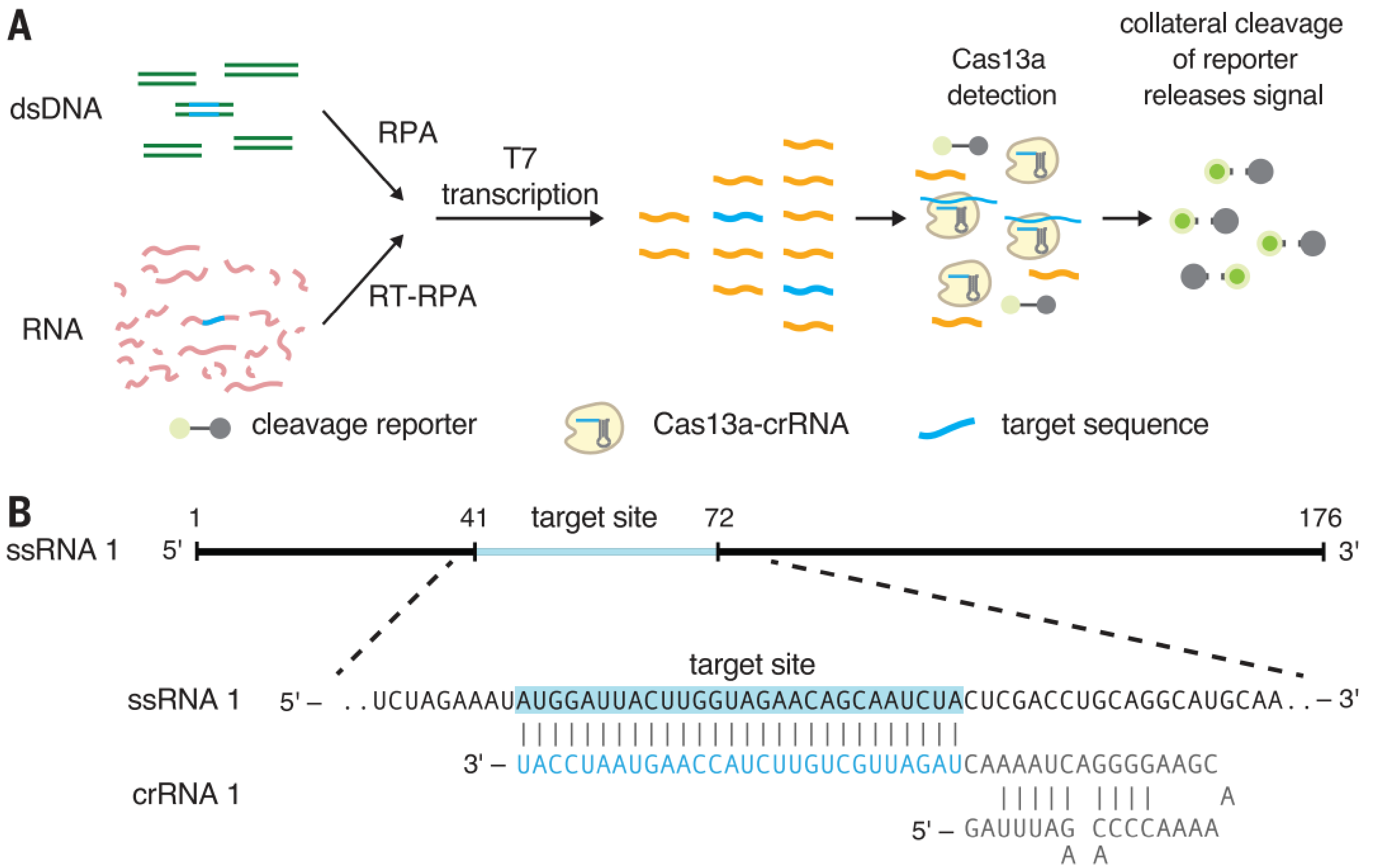
◆**Cas13a**,RNA-guided ribonucleases (**RNases**), can be reprogrammed with crRNAs to provide a platform for specific RNA detection.

◆On recognition of its RNA target, activated Cas13a engages in **“collateral” cleavage** of nearby non-targeted **reporter RNA.**

Schematic of SHERLOCK

Specific High-Sensitivity Enzymatic Reporter UnLOCKing

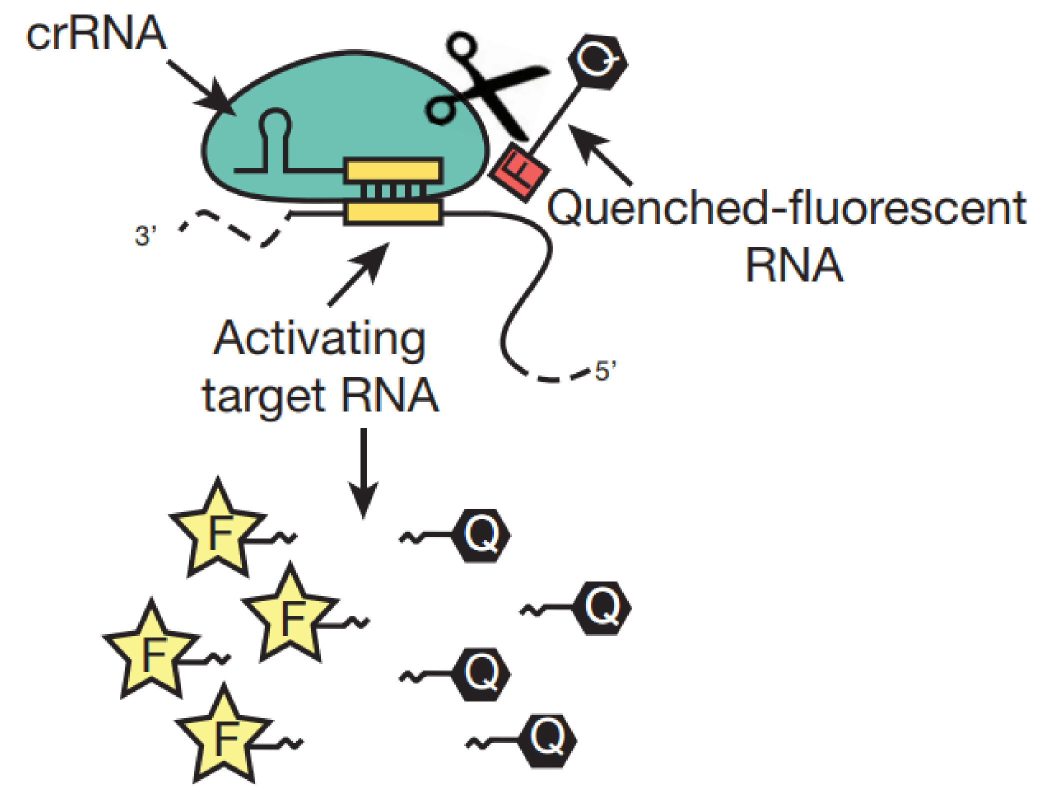
RPA: recombinase polymerase amplification



(Gootenberg, Abudayyeh et al. 2017)

Collateral Cleavage of Reporter

(crRNA-directed, nonspecific RNA degradation)



(East-Seletsky, O'Connell et al. 2016)

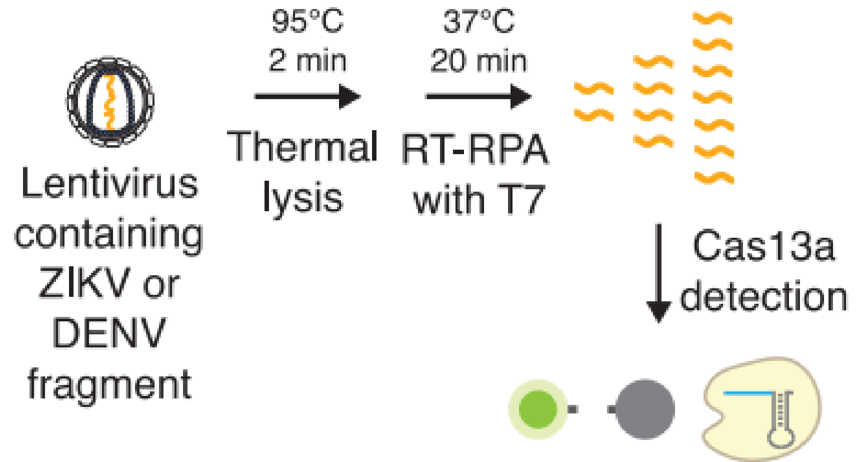


Figure 1.Schematic of ZIKV RNA detection by SHERLOCK

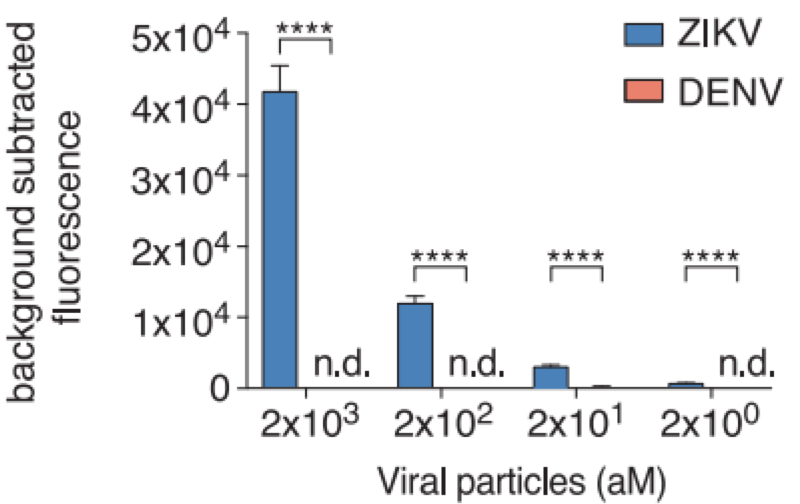


Figure 2. Highly sensitive detection of the ZIKV lentiviral particles.

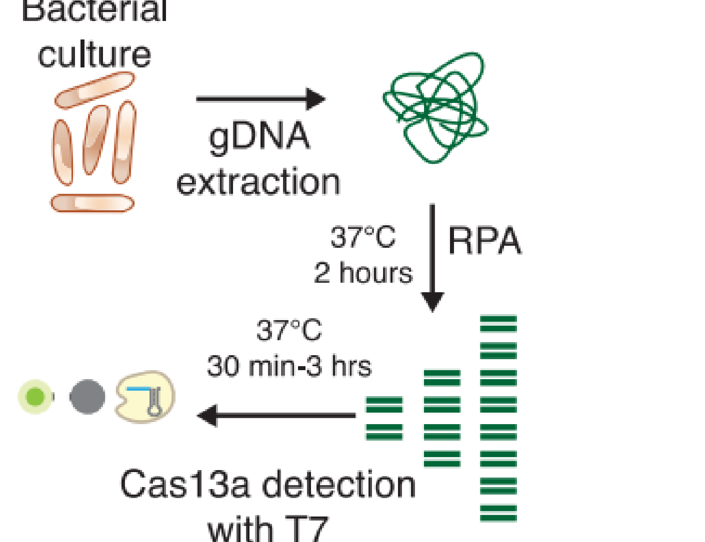


Figure 3. SHERLOCK used to distinguish bacterial strains

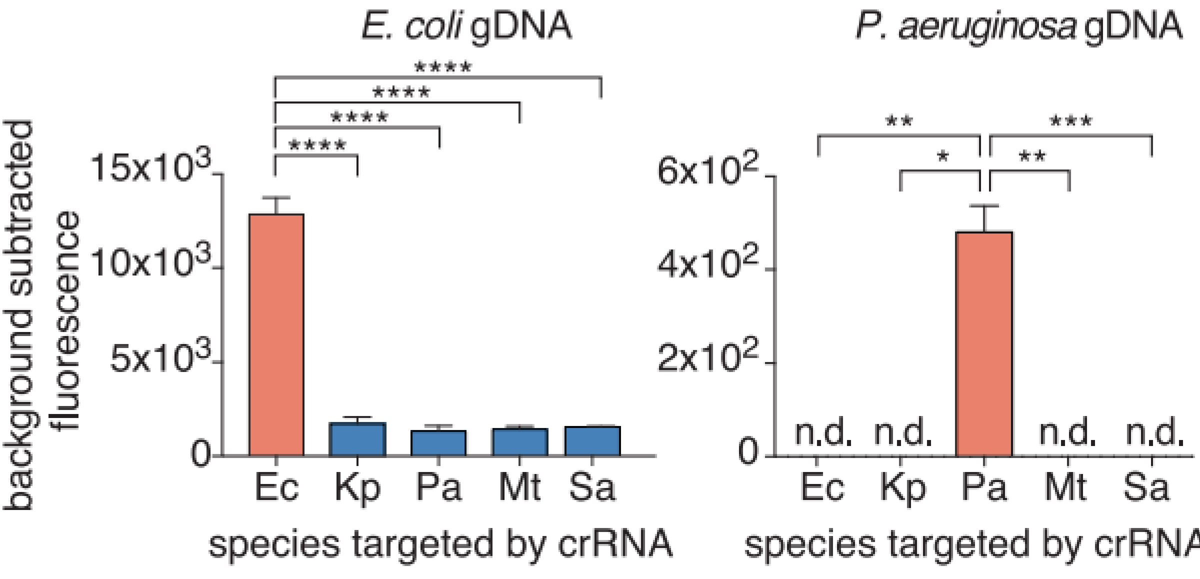
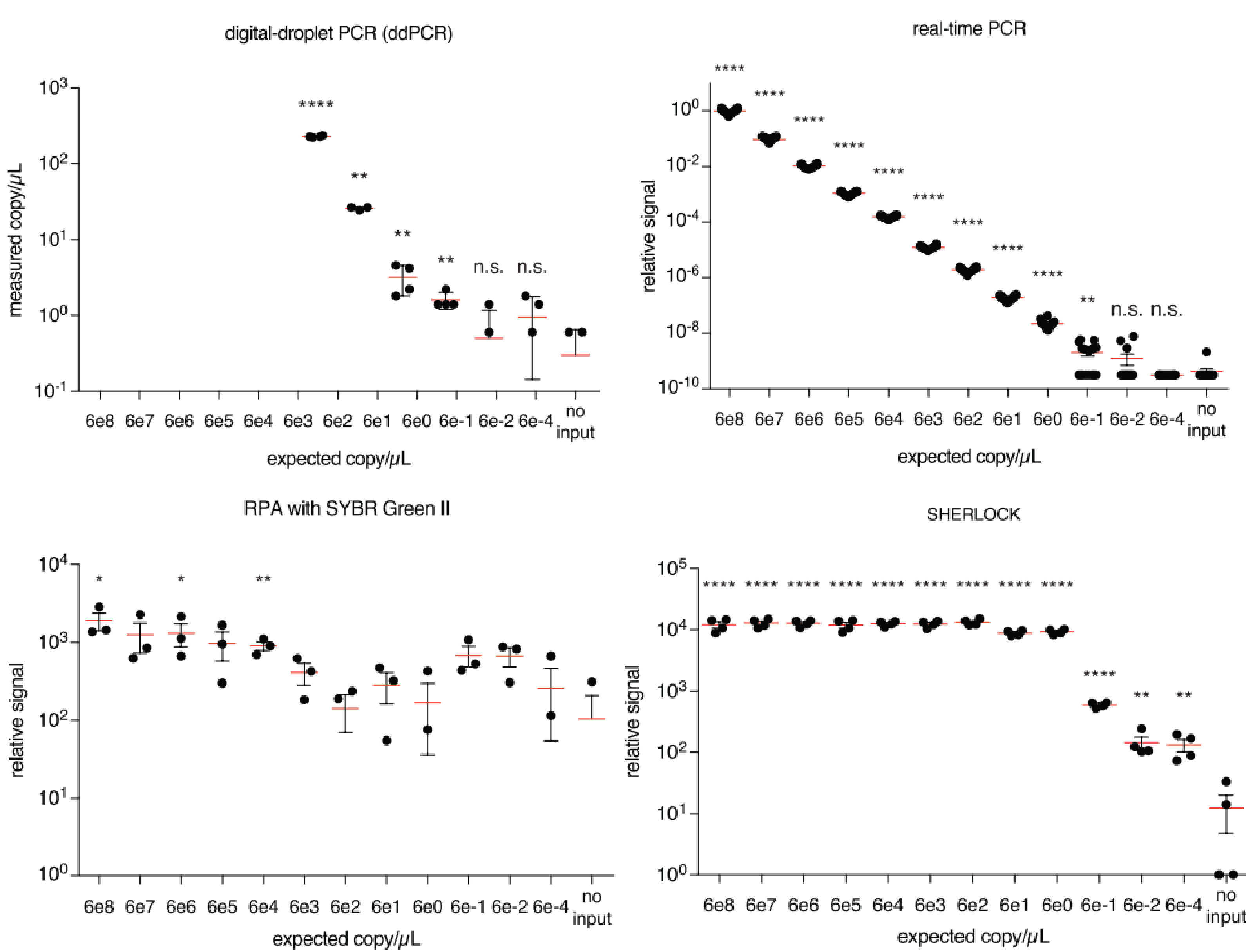


Figure 4. Sensitive and specific detection of E. coli or P. aeruginosa DNA

Cas13a detection can be used to sense viral and bacterial pathogens

(Gootenberg, Abudayyeh et al. 2017)

Comparison of SHERLOCK to other sensitive nucleic acid detection tools



SHERLOCK has similar levels of sensitivity to those of **ddPCR** and quantitative PCR(**qPCR**),two established sensitive nucleic acid detection approaches, whereas **RPA** alone was not sensitive enough to detect low levels of target.

(Gootenberg, Abudayyeh et al. 2017)

Conclusion

◆CRISPR/Cas9 system has brought forth revolutionary changes in genomic research, including genome editing, regulation, and imaging.

◆SHERLOCK can rapidly detect single molecules of DNA or RNA.

◆But CRISPR/Cas system also has some well-known problems, such as off-target effects.

References

- Khadempar, S., et al., *CRISPR–Cas9 in genome editing: Its function and medical applications*. Journal of cellular physiology, 2018.
- Koonin, E.V., K.S. Makarova, and F. Zhang, *Diversity, classification and evolution of CRISPR-Cas systems*. Current opinion in microbiology, 2017. **37**: p. 67-78.
- Shmakov, S., et al., *Diversity and evolution of class 2 CRISPR–Cas systems*. Nature Reviews Microbiology, 2017. **15**(3): p. 169.
- Ran, F.A., et al.,*Genome engineering using the CRISPR-Cas9 system*. Nature protocols,2013. **8**(11): p. 2281.
- Jiang, W., et al., *RNA-guided editing of bacterial genomes using CRISPR-Cas systems*. Nature biotechnology, 2013. **31**(3): p. 233.
- Gootenberg, J.S., et al., *Nucleic acid detection with CRISPR-Cas13a/C2c2*. Science, 2017: p. eaam9321.
- East-Seletsky, A., et al., *Two distinct RNase activities of CRISPR-C2c2 enable guide-RNA processing and RNA detection*. Nature, 2016. **538**(7624): p. 270.

Thank you!