



# **Application of CRISPR/Cas System in Nucleic Acid Detection**

**1<sup>st</sup> Mphil student: Emily Lei**

**Supervisor: Professor Zigui Chen**

**Joint Graduate Seminar**

**14<sup>th</sup> Dec 2018**

# **Contents**

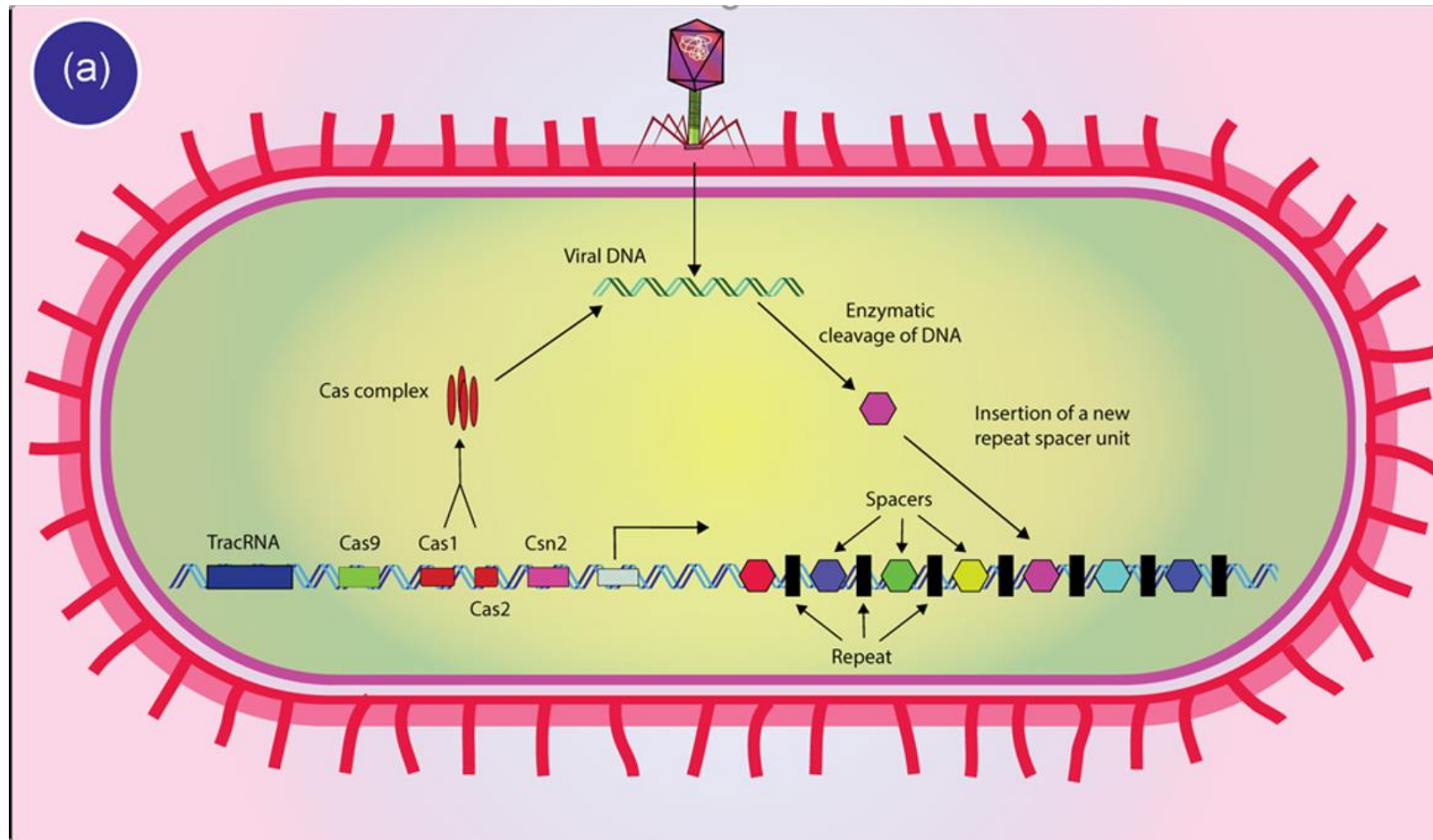
The background features several stylized DNA double helix structures in shades of blue, green, and orange. A large, light gray pair of scissors is positioned on the right side, symbolizing genome editing. The background is filled with a pattern of small, light gray dots.

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

The background features a repeating pattern of DNA double helix structures. Each helix is rendered in a light gray color, with the base pairs represented by colored bars in shades of blue, green, and orange. A large, semi-transparent gray pair of scissors is positioned on the right side of the image, with its blades pointing towards the center, symbolizing the gene-editing process. The entire scene is set against a light gray background with a subtle pattern of small white dots.

# **CRISPR/Cas System**

# CRISPR/Cas: An Adaptive Immune System

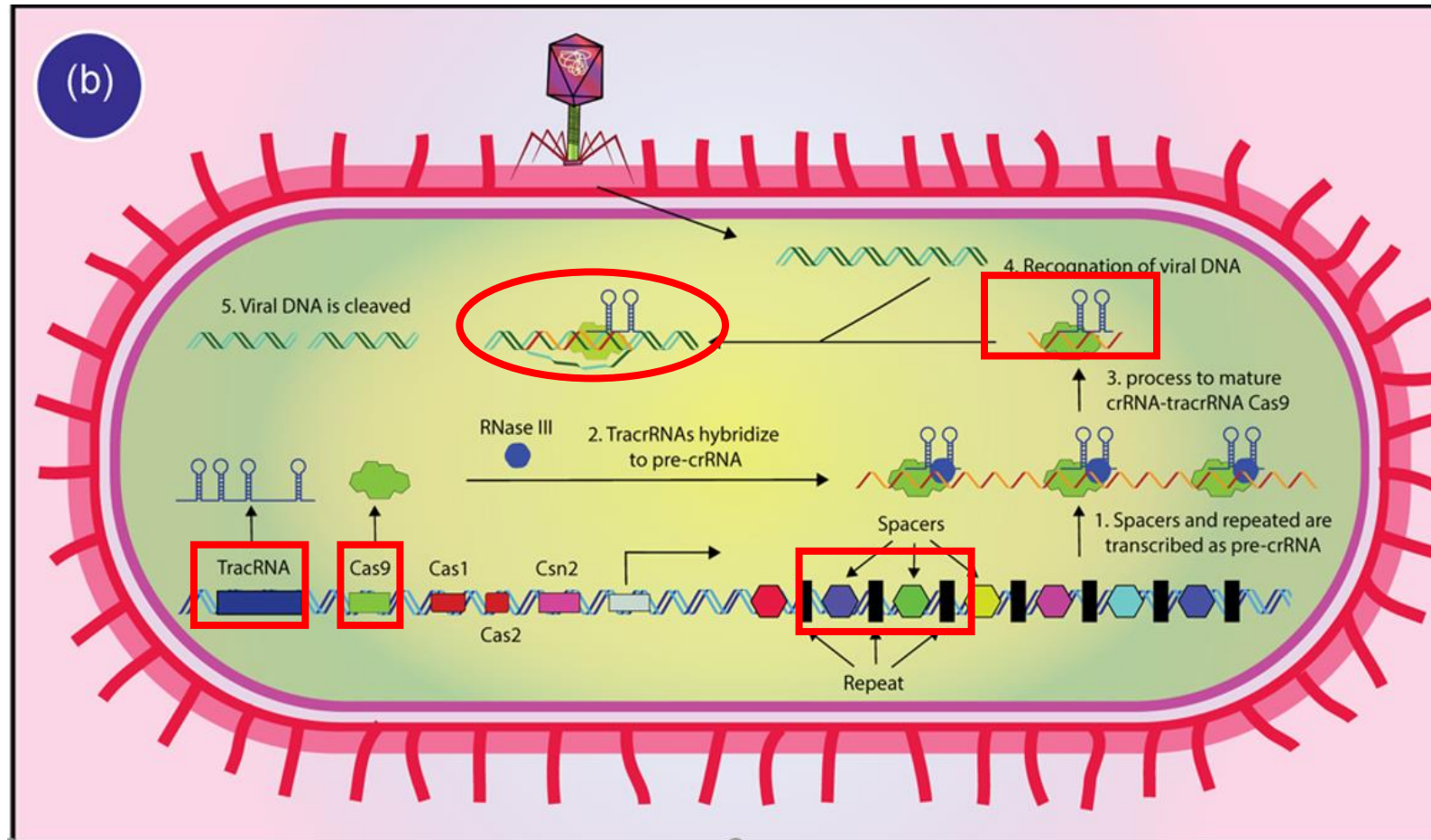


An overview of CRISPR/Cas as a bacterial adaptive immune system

(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**.

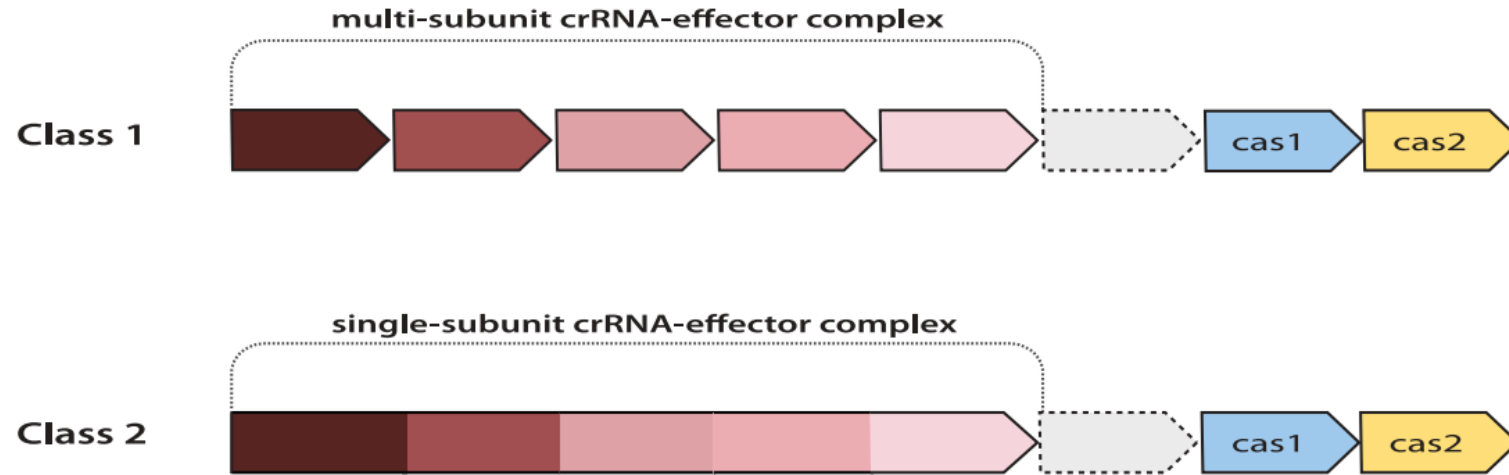
# CRISPR/Cas: An Adaptive Immune System



(Khadempar, Familghadakchi et al. 2018)

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

# Classification of CRISPR/Cas system

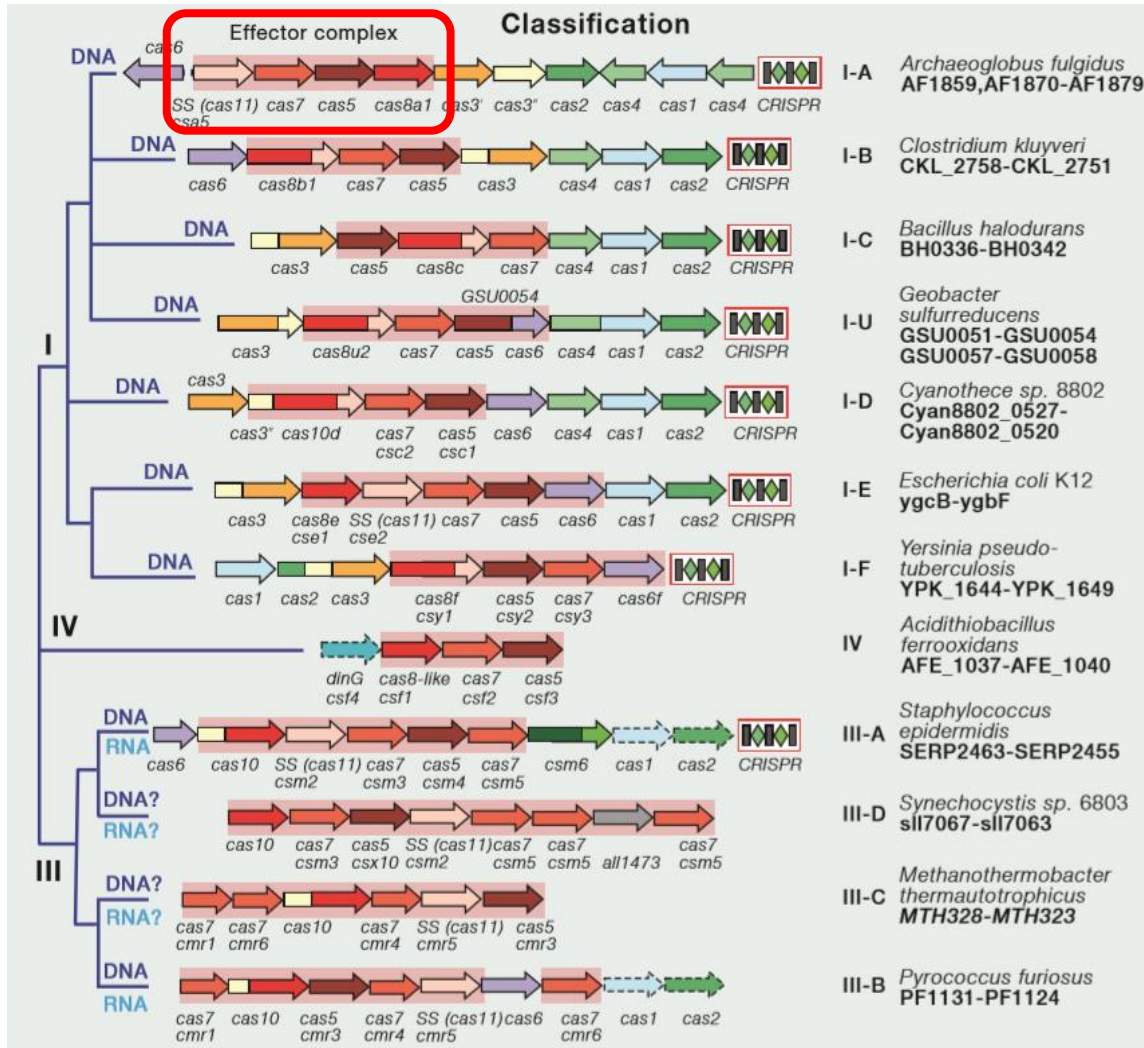


(Koonin, Makarova et al. 2017)

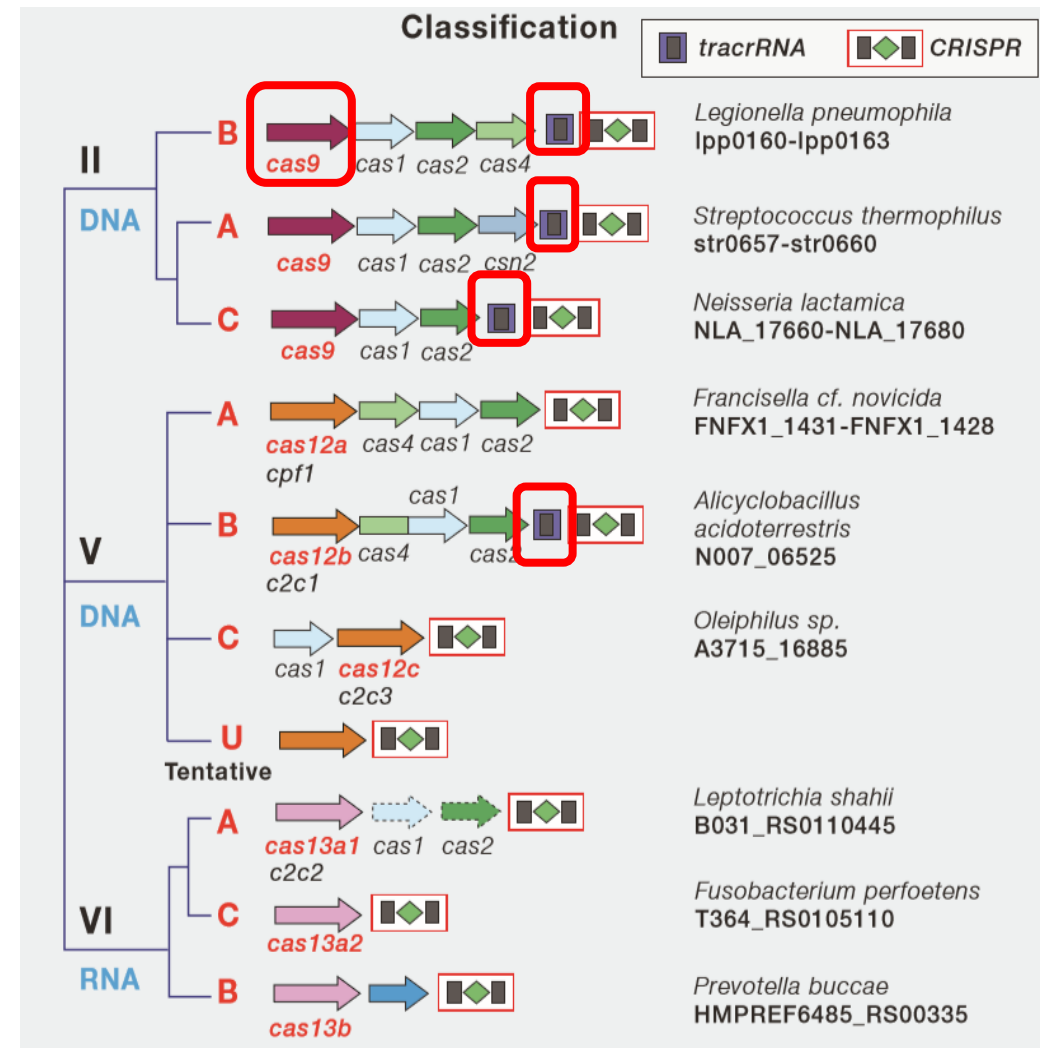
CRISPR-Cas system split into two distinct classes based on effector module organization.

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

# Classification of CRISPR/Cas system

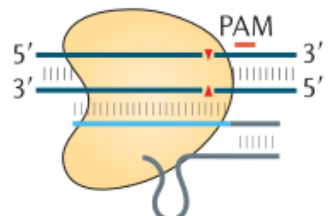
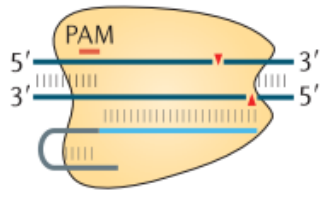
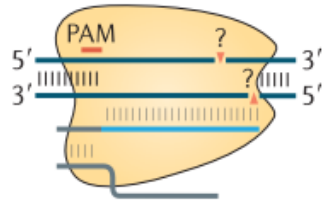
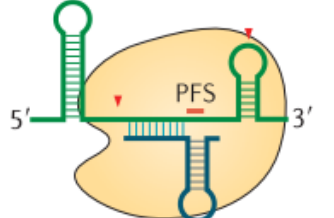


**Class 1**



**Class 2**

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

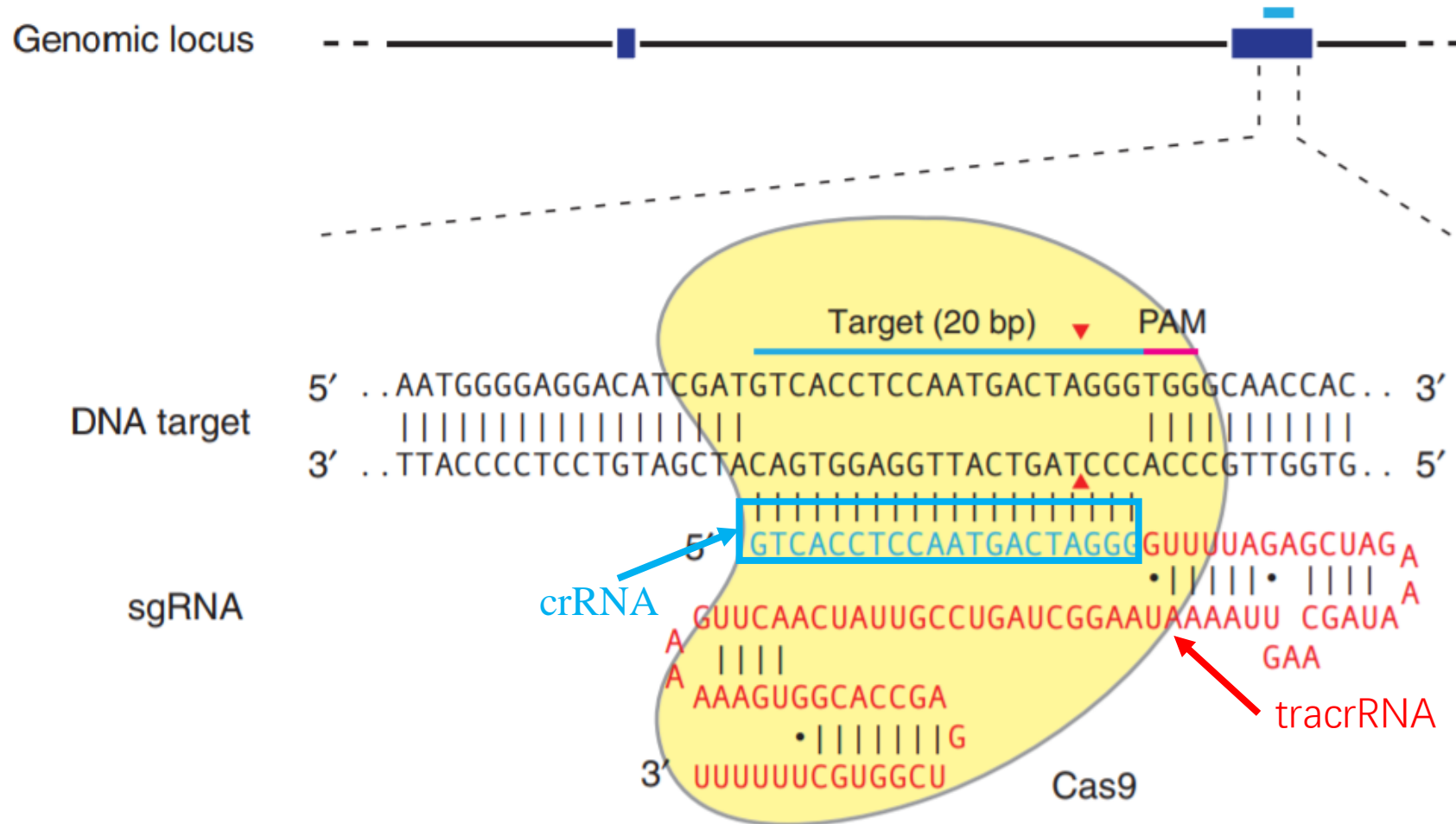
		Nuclease domains	tracrRNA	PAM	Substrate	Cleavage pattern
Genome editing	<b>Type II</b> Cas9 	RuvC and HNH	Yes	3', GC-rich	dsDNA	Blunt ends
	<b>Type V-A</b> Cas12a (Cpf1) 	RuvC and Nuc	No	5', AT-rich	dsDNA	Staggered ends, 5' overhangs
	<b>Type V-B</b> Cas12b (C2c1) 	RuvC	Yes	5', AT-rich	dsDNA	Staggered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



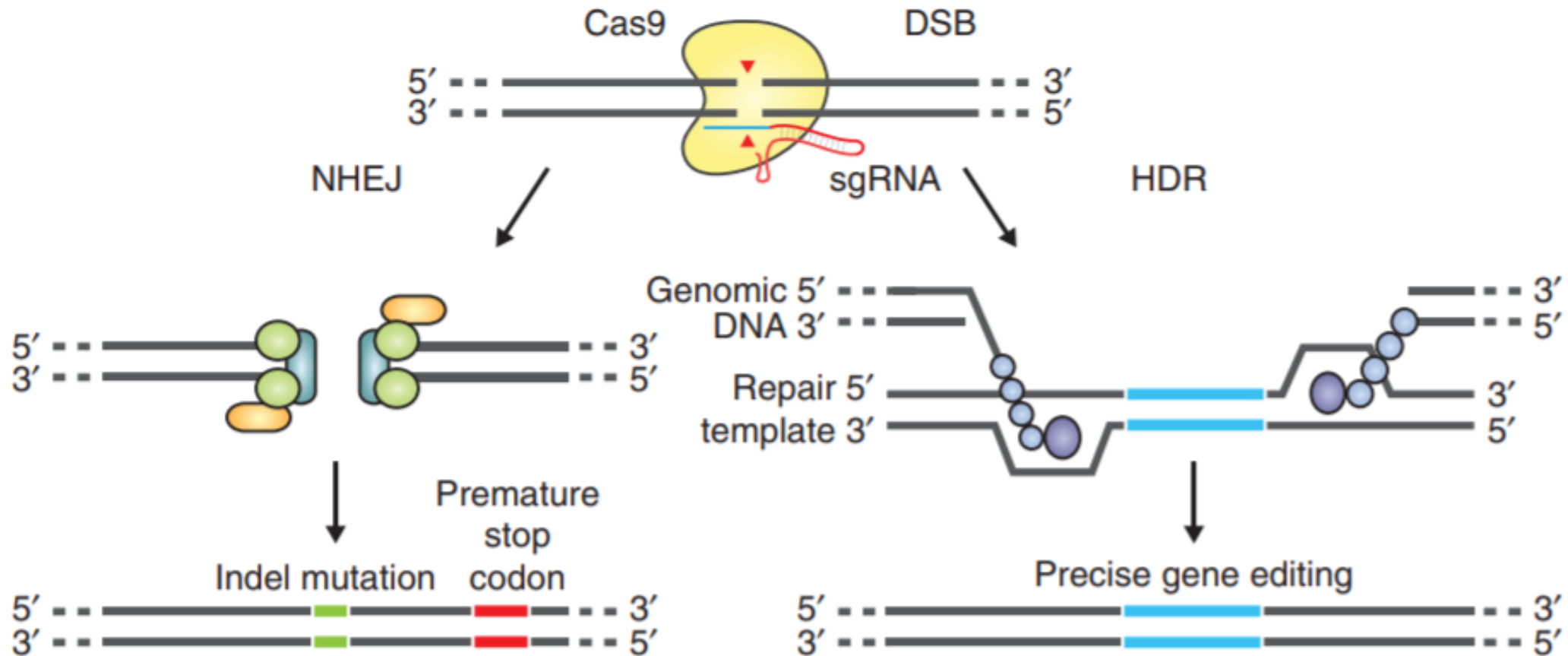
The background features a light gray pattern of small dots. Overlaid on this are several stylized DNA double helix structures. Some are smaller and positioned in the upper left, while a larger one is in the lower right. A large, light gray pair of scissors is also depicted, with its blades positioned as if cutting through the DNA helix. The text 'CRISPR for Genome Editing' is centered in a bold, black, serif font.

# **CRISPR for Genome Editing**

# Schematic of Cas9-sgRNA complex



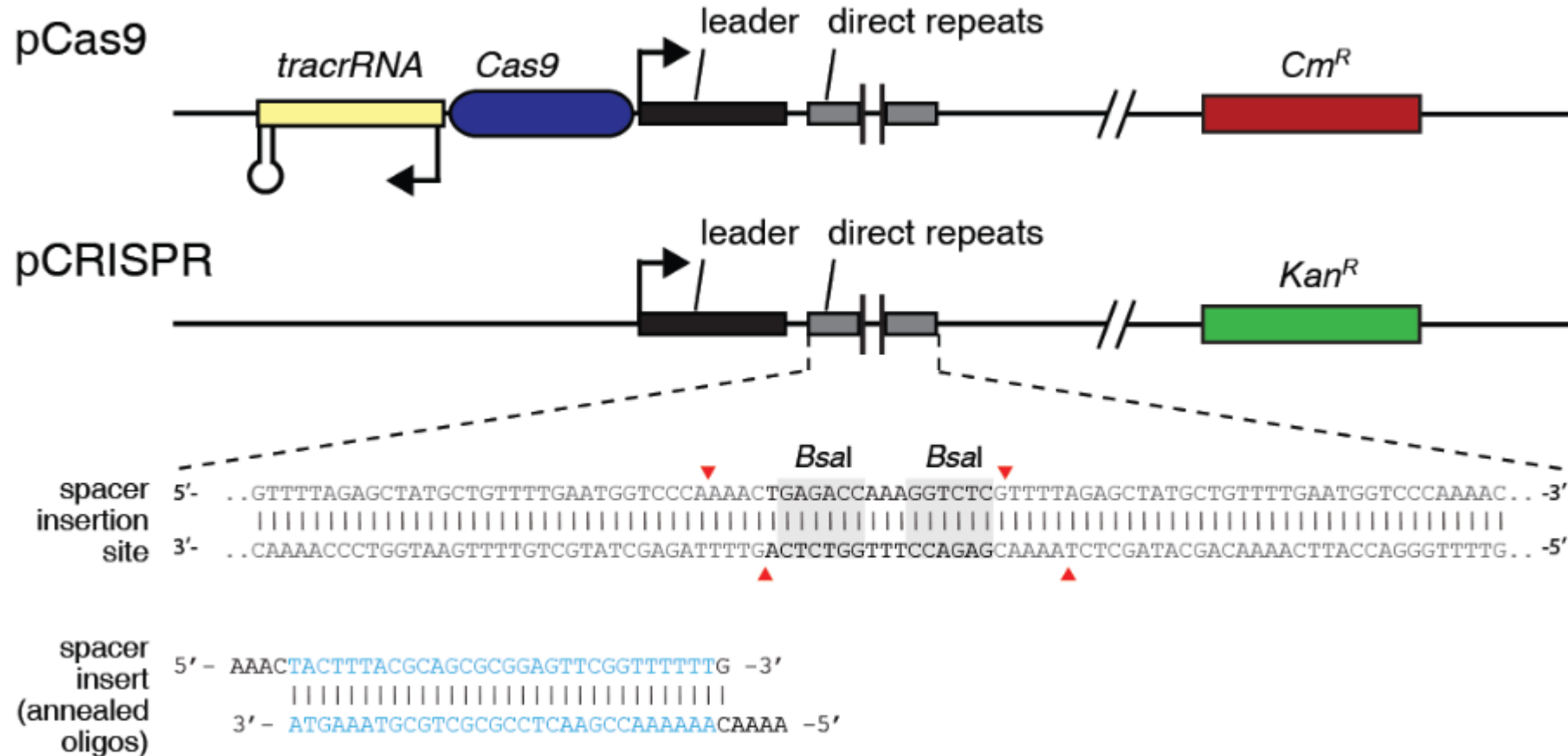
# Two general repair pathways



(Ran, Hsu et al. 2013)

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

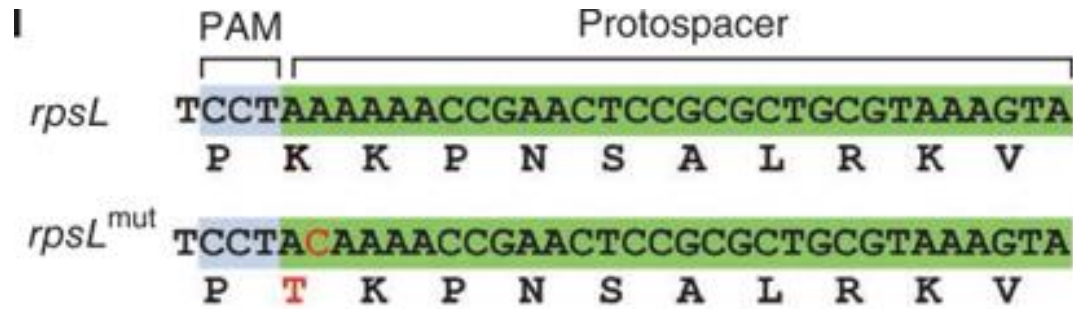
# Example: Genome editing with dual-RNA:Cas9 in E. coli



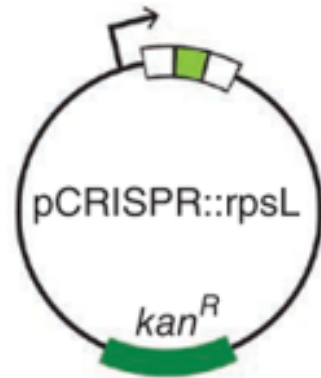
(Jiang, Bikard et al. 2013)

**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.

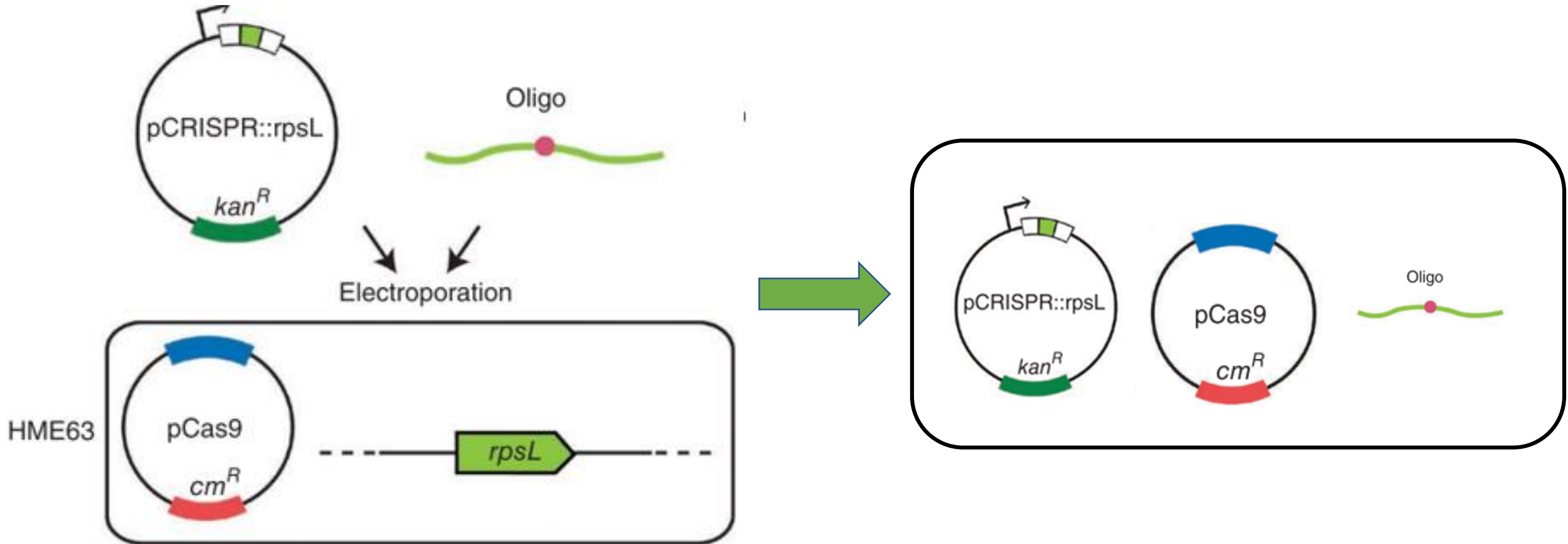


Then they constructed a **pCRISPR $\phi$ rpsL** plasmid harboring a spacer that would guide dual-RNA:Cas9 cleavage of the **wild-type**, but not the mutant *rpsL* allele.



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

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



# Genome editing with CRISPR-Cas9 system



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

The background features a light gray pattern of small dots. Overlaid on this are several stylized DNA double helix structures. One large helix is positioned in the lower right, with a pair of gray scissors cutting through it. Other smaller helices are scattered in the upper left and center. The DNA strands are represented by gray lines, and the base pairs are shown as colored bars in shades of blue, green, and orange.

# **CRISPR for Detection**



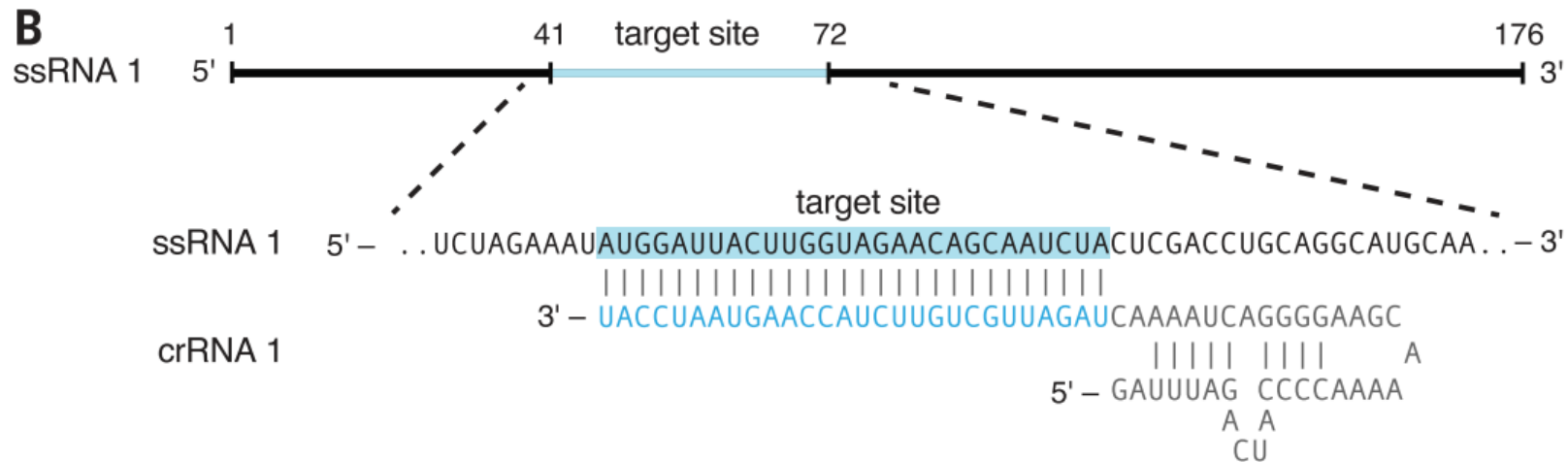
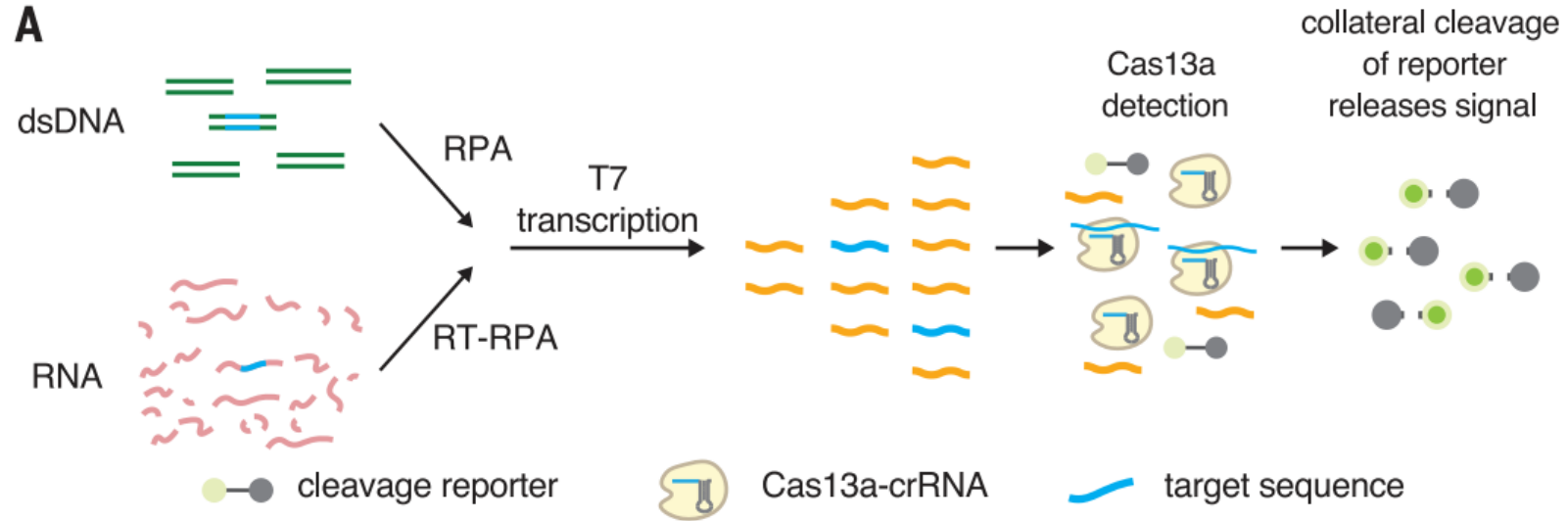
# Background of detection using CRISPR/Cas system

- ◆ 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

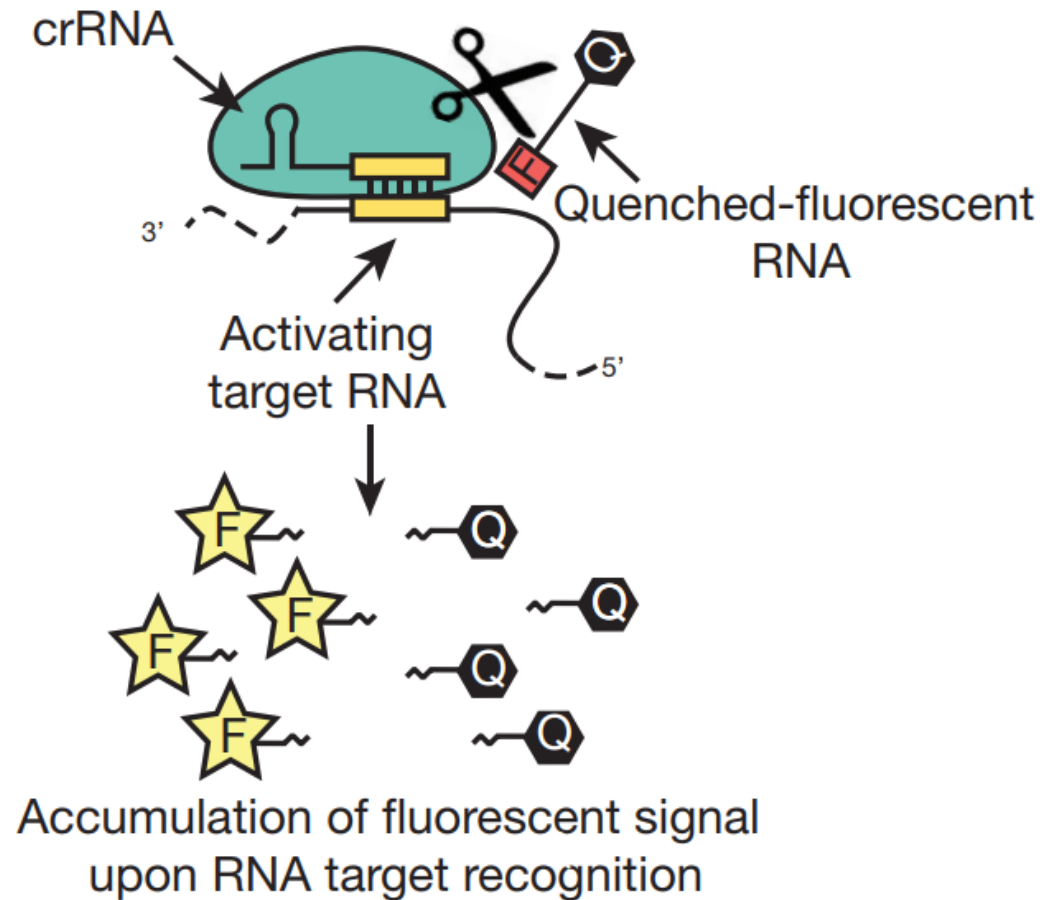
RPA: recombinase polymerase amplification



# Collateral Cleavage of Reporter

(crRNA-directed, nonspecific RNA degradation)

*trans* cleavage of fluorescent RNA oligo



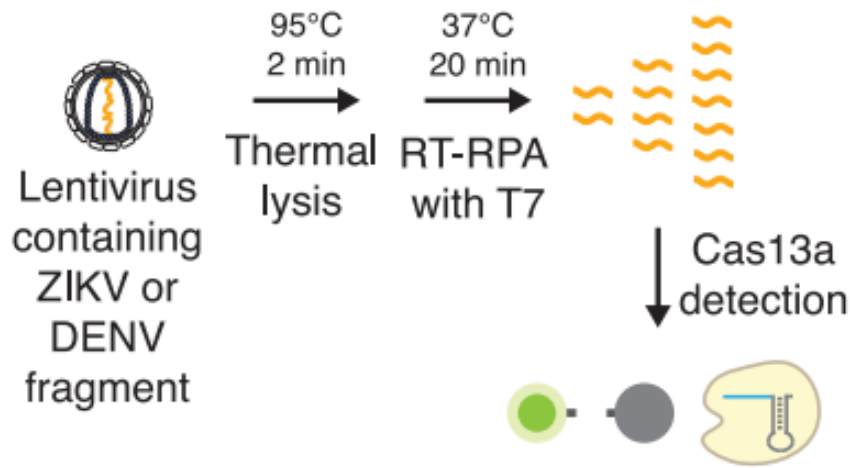


Figure 1. Schematic of ZIKV RNA detection by SHERLOCK

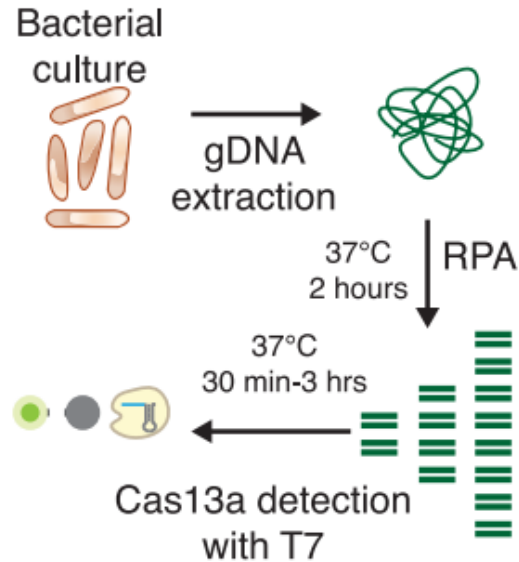


Figure 3. SHERLOCK used to distinguish bacterial strains

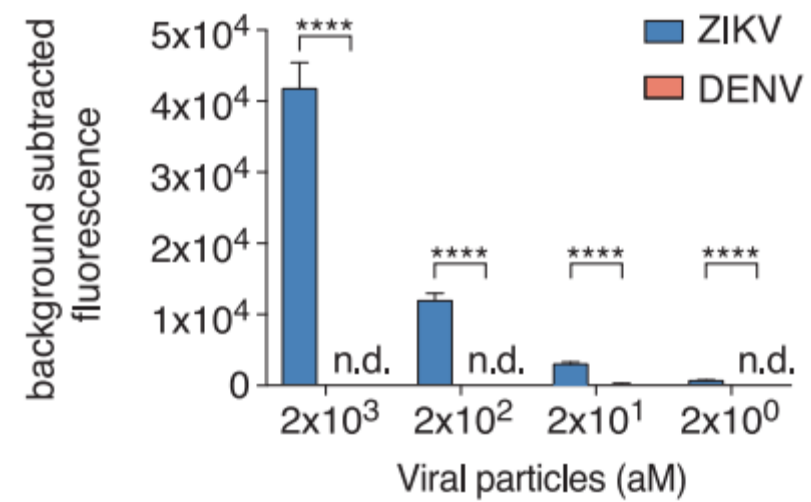


Figure 2. Highly sensitive detection of the ZIKV lentiviral particles.

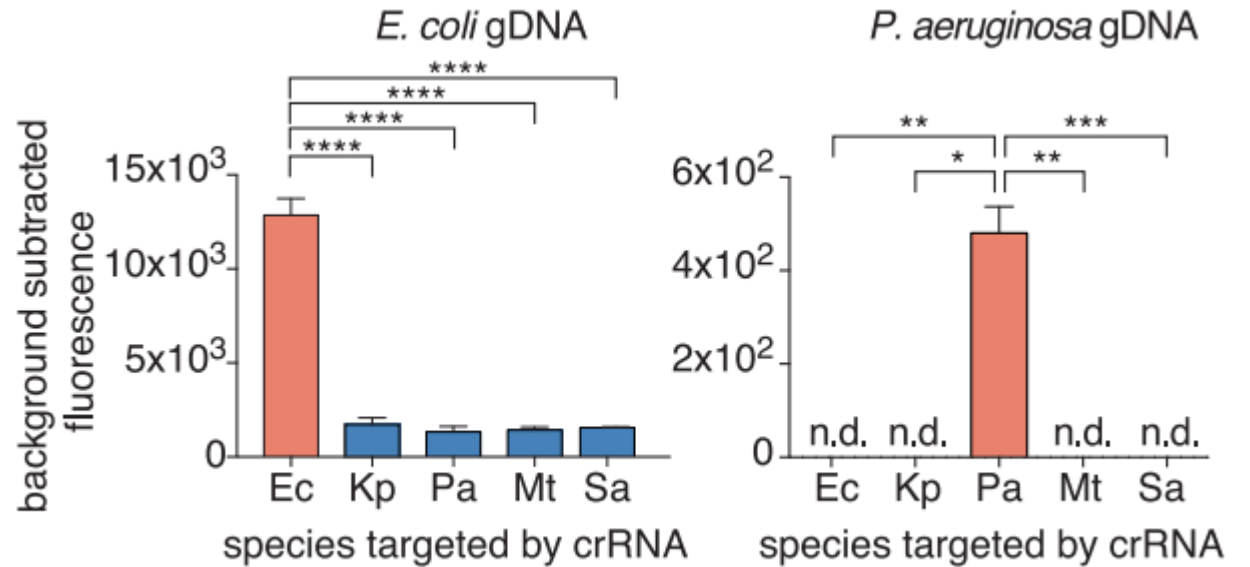
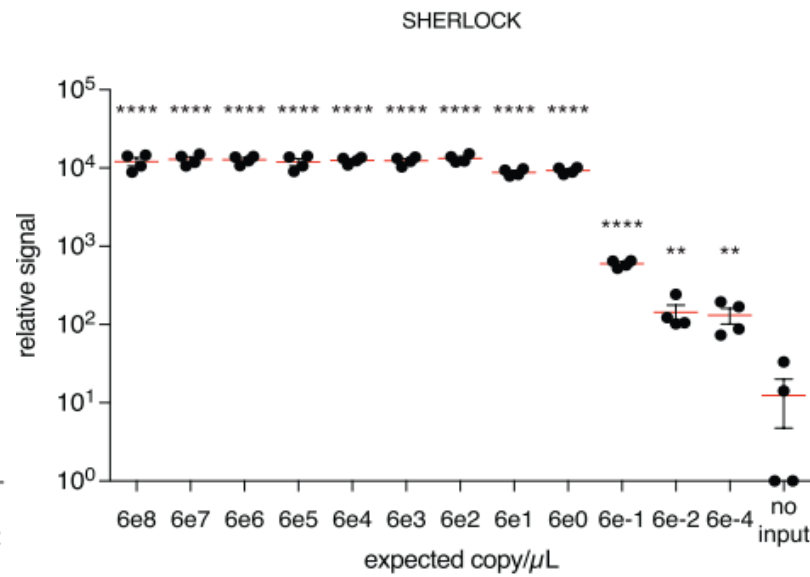
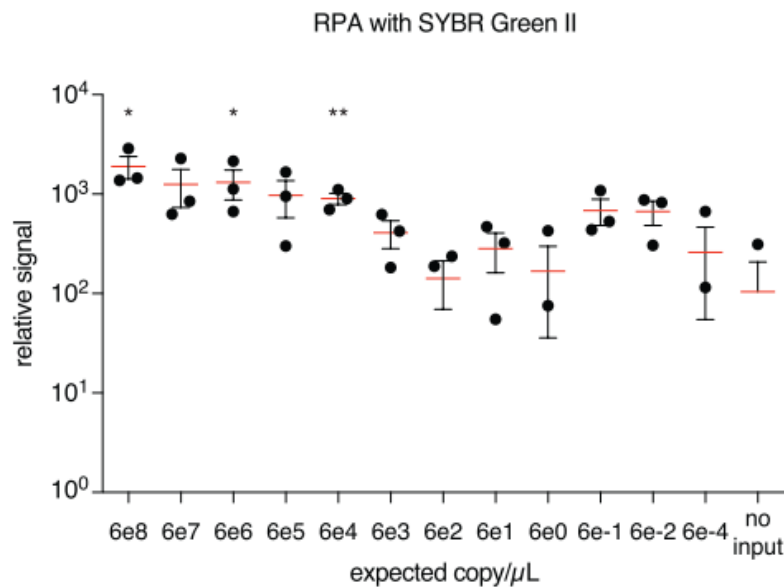
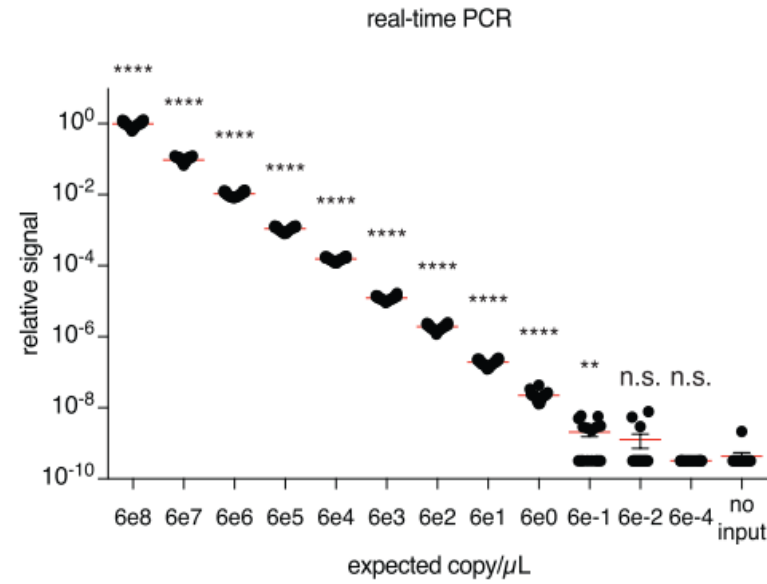
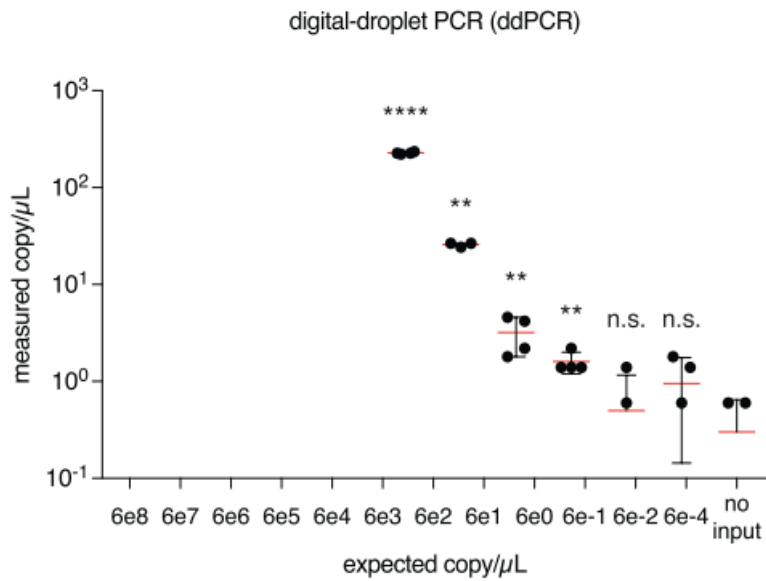


Figure 4. Sensitive and specific detection of *E. coli* or *P. aeruginosa* DNA

## Cas13a detection can be used to sense viral and bacterial pathogens

# 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.

# 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

- Khadempour, 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.

The background features a light gray pattern of small dots. Overlaid on this are several stylized DNA double helix structures. One large helix is positioned on the right side, with a pair of gray scissors cutting through it. Other smaller helices are scattered on the left and top. The DNA strands are represented by gray lines, and the base pairs are shown as colored bars in shades of blue, green, and orange.

**Thank you!**