CRISPR/Cas-Based Biosensing Techniques

A nucleic acid-based **adaptive immune system protecting microorganisms** (Archaea and Bacteria) from viral infection and help their coexistence with phages.

CRISPR/Cas systems rely on the bacterial ability to **store a fragment of foreign phage genome in the CRISPR loci, as a memory** of past encounters.

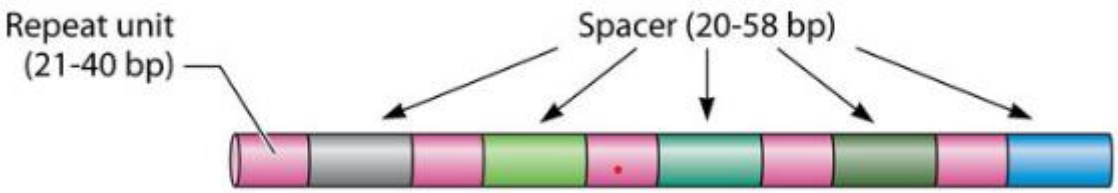
These loci, together with surrounding repeats, are then used by Cas endonuclease **as guides** to selectively recognize sequences in the foreign genomes and fight the invader.

CRISPR locus was discovered in *E. coli* in 1987 by Ishino et al. Osaka University
Present in 90% of Archaea and 50% of Bacteria

Some common terminology

CRISPR

:Clustered Regularly Interspaced Short Palindromic Repeats



crRNAs: CRISPR RNAs

Cas:

CRISPR associated proteins

tracer RNA: trans-activating CRISPR RNA

gRNA: Guide RNA

PAM: proto-spacer-adjacent motif sequence, NGG (N= A, T, C, or G)

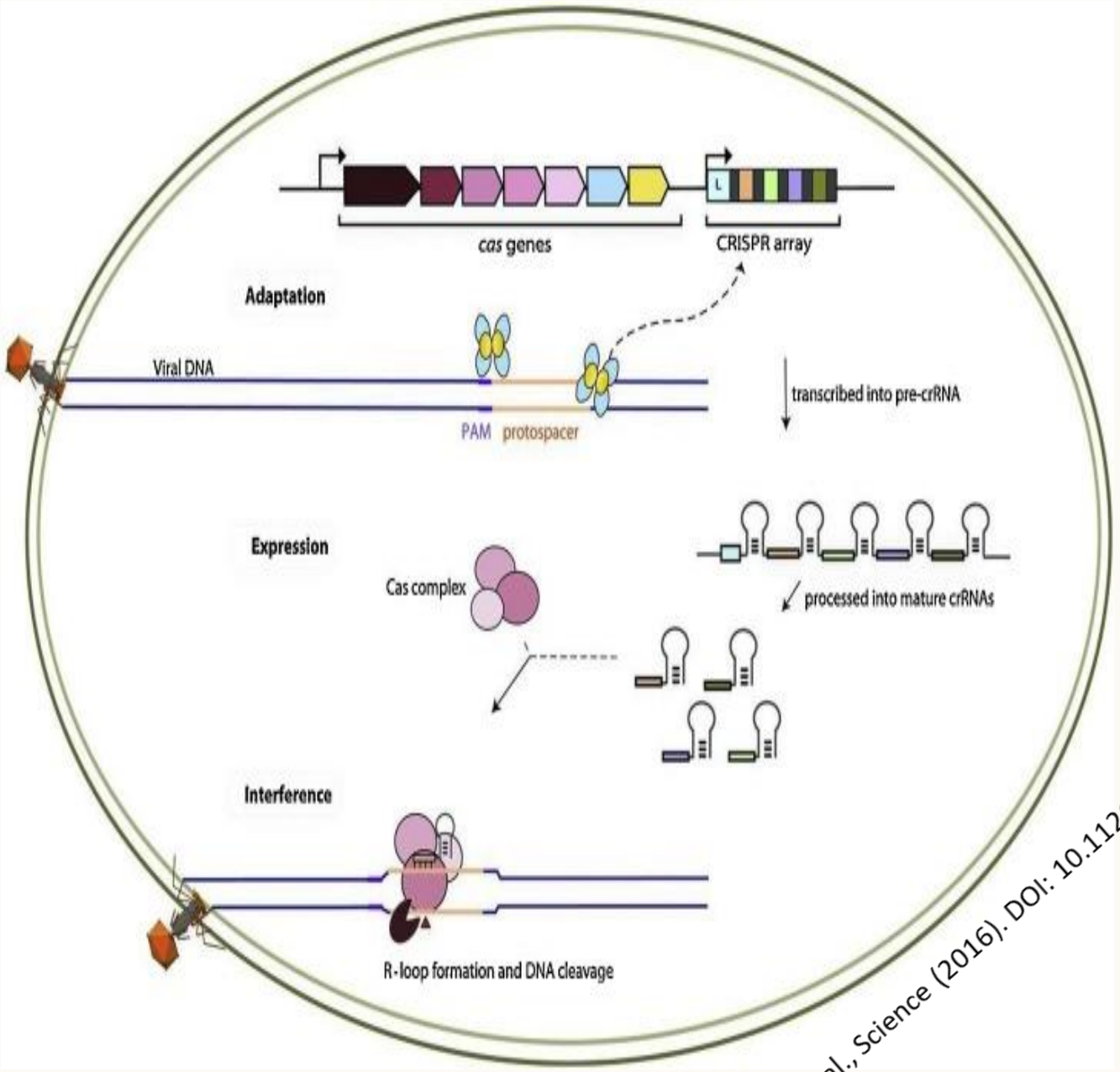
3 main stages in the CRISPR-Cas immune response:

Adaptation: Cas protein recognizes and binds to the target DNA. Cas1 (blue) and Cas2 (yellow) proteins select and process the invading DNA, and thereafter, a protospacer (orange) is integrated as a new spacer at the leader end of the CRISPR array [repeat sequences (gray) that separate similar-sized, invader-derived spacers (multiple colors)].

Expression: the CRISPR array is transcribed first into pre-crRNA, then into mature crRNA guides by Cas (e.g., Cas6) or non-Cas proteins (e.g., RNase III).

Interference: crRNA guided Cas nuclease to cleave and inactivate the invading virus or plasmid genome. The Cas-crRNA **complex scans (search) invading DNA** for a complementary nucleic acid target, after which the target is degraded by a Cas nuclease.

When Cas9-gRNA complex recognizes PAM, the spacer of gRNA pairs with the target DNA strand to form an "R-loop" structure, after which the cleavage of DNA strands is accomplished with a blunt-end 3 bp upstream of the PAM into the protospacer.



Overview of the CRISPR/Cas immune system

Mohanraju et al., Science (2016). DOI: 10.1126/science.aad5147

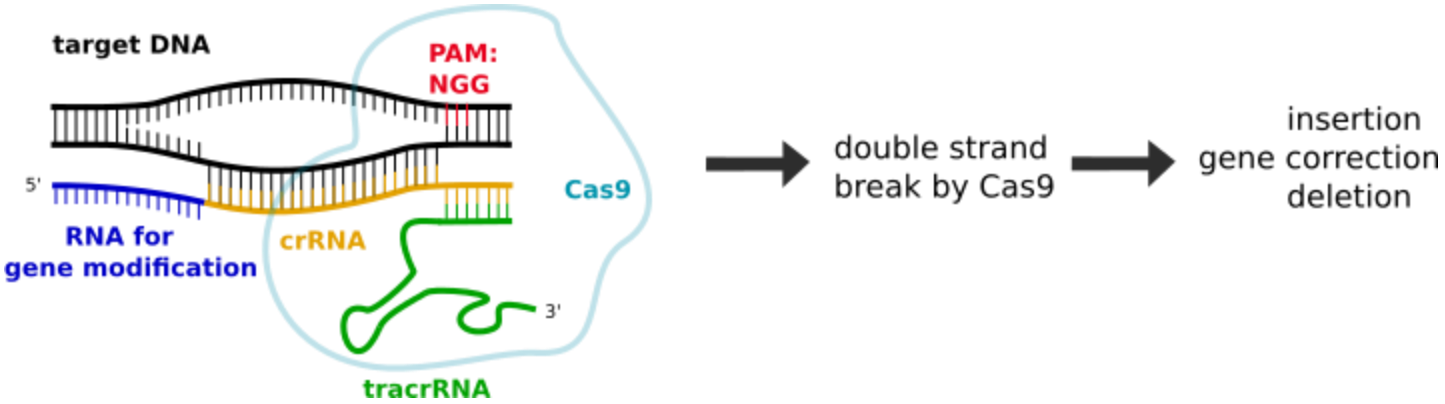
Some facts of CRISPR system:

Both the CRISPR array and the Cas enzymes differ from species to species, in length, composition and mode of action, respectively

CRISPR array is composed of a family of DNA repetitions (25–35 bp) interspaced by a spacer sequence (30–40 bp), which represents the recorded portion of DNA from the invader

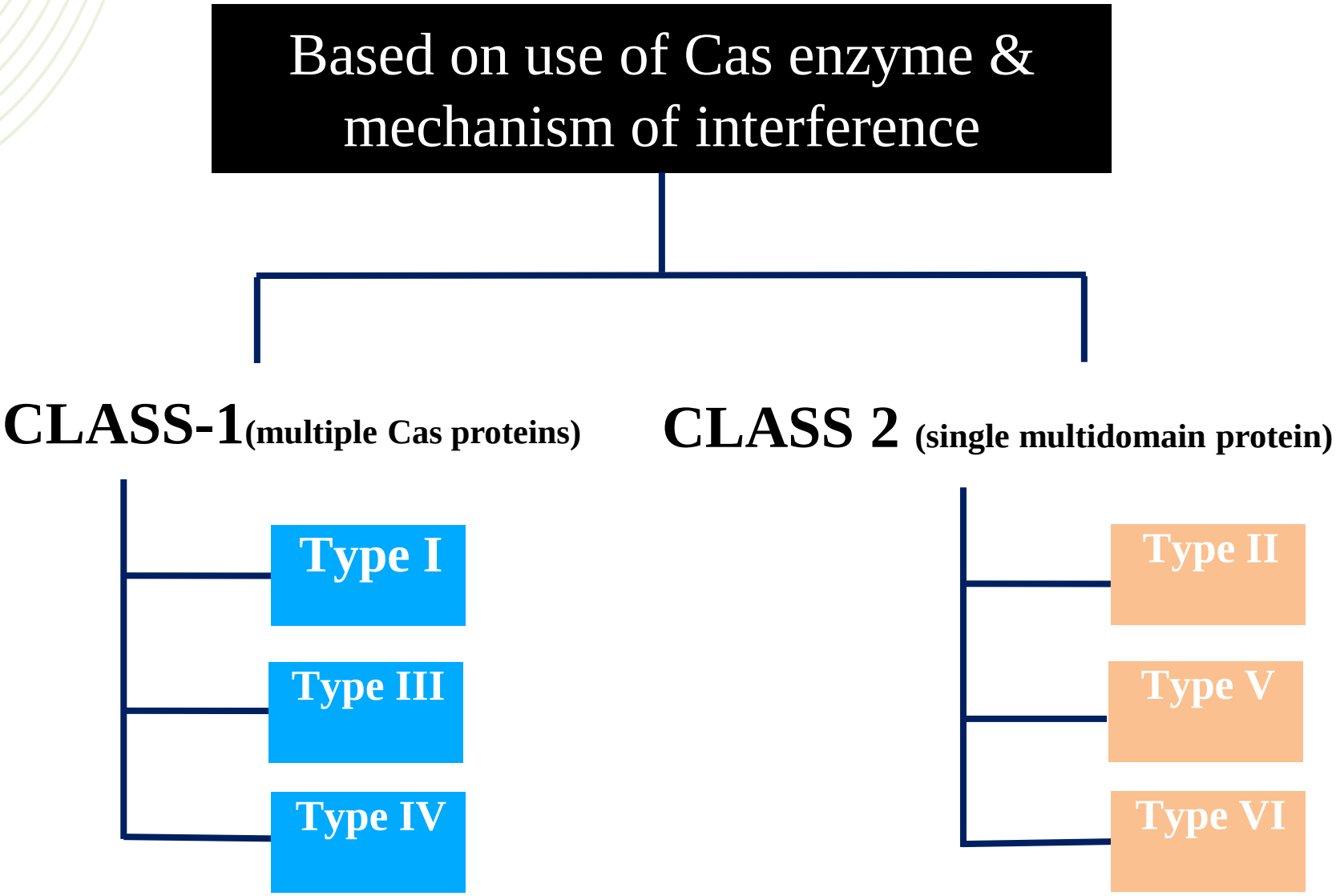
crRNAs and Cas proteins form multicomponent CRISPR ribonucleoprotein (crRNP) complexes.

In the CRISPR-Cas system (in type II and subtype V-B CRISPR-Cas systems) the tracrRNA (42 nucleotides) base pairs with the crRNA to form gRNA. **Cas9 uses the tracrRNA portion of the guide as a handle, while the crRNA spacer sequence directs the complex to a matching viral sequence**.



CRISPR-Cas9 Genome Editing Technology - YouTube

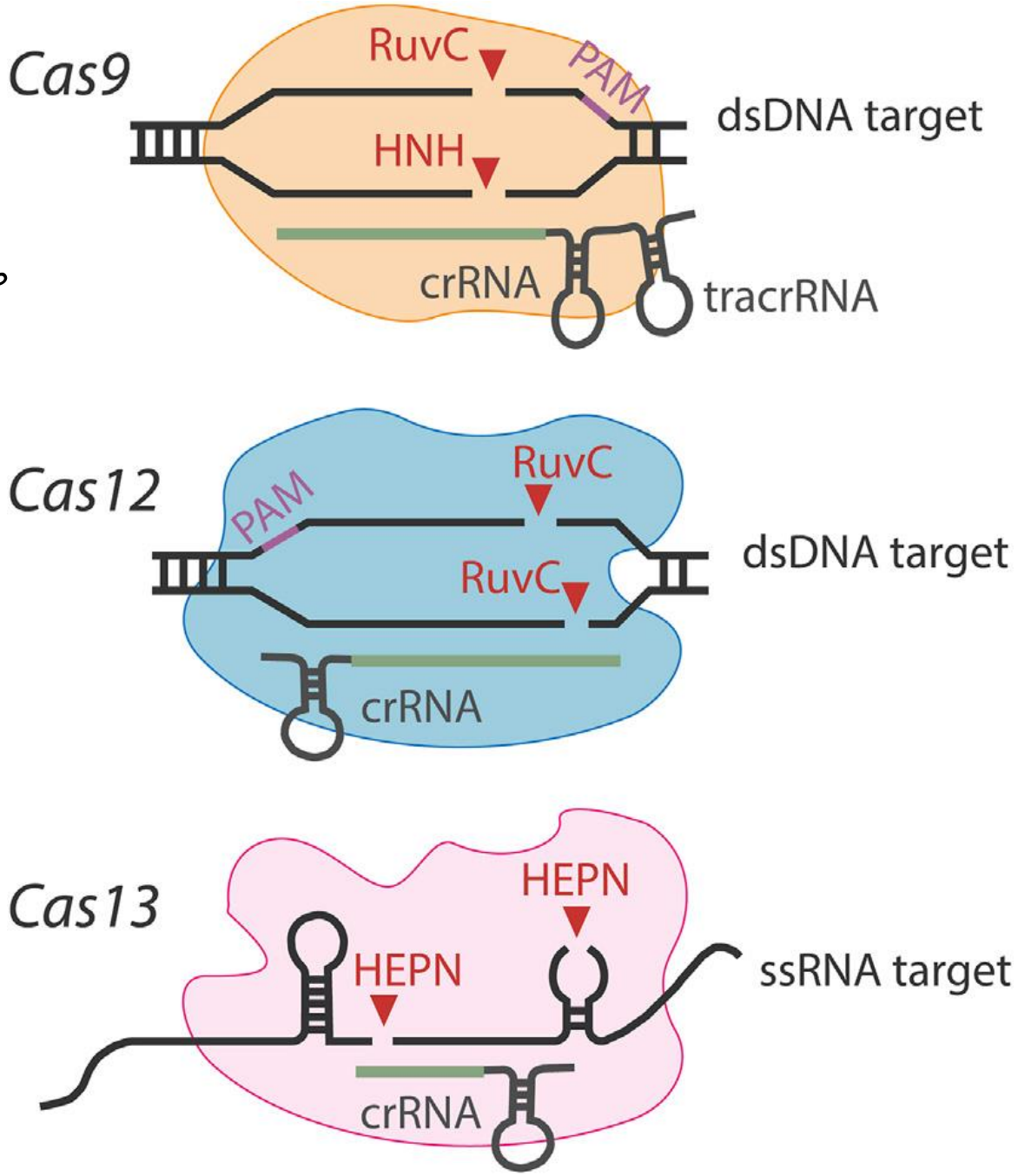
Classification of Crispr/cas system



Main characteristics of Cas endonucleases **(Class 2)** used in developing biosensor

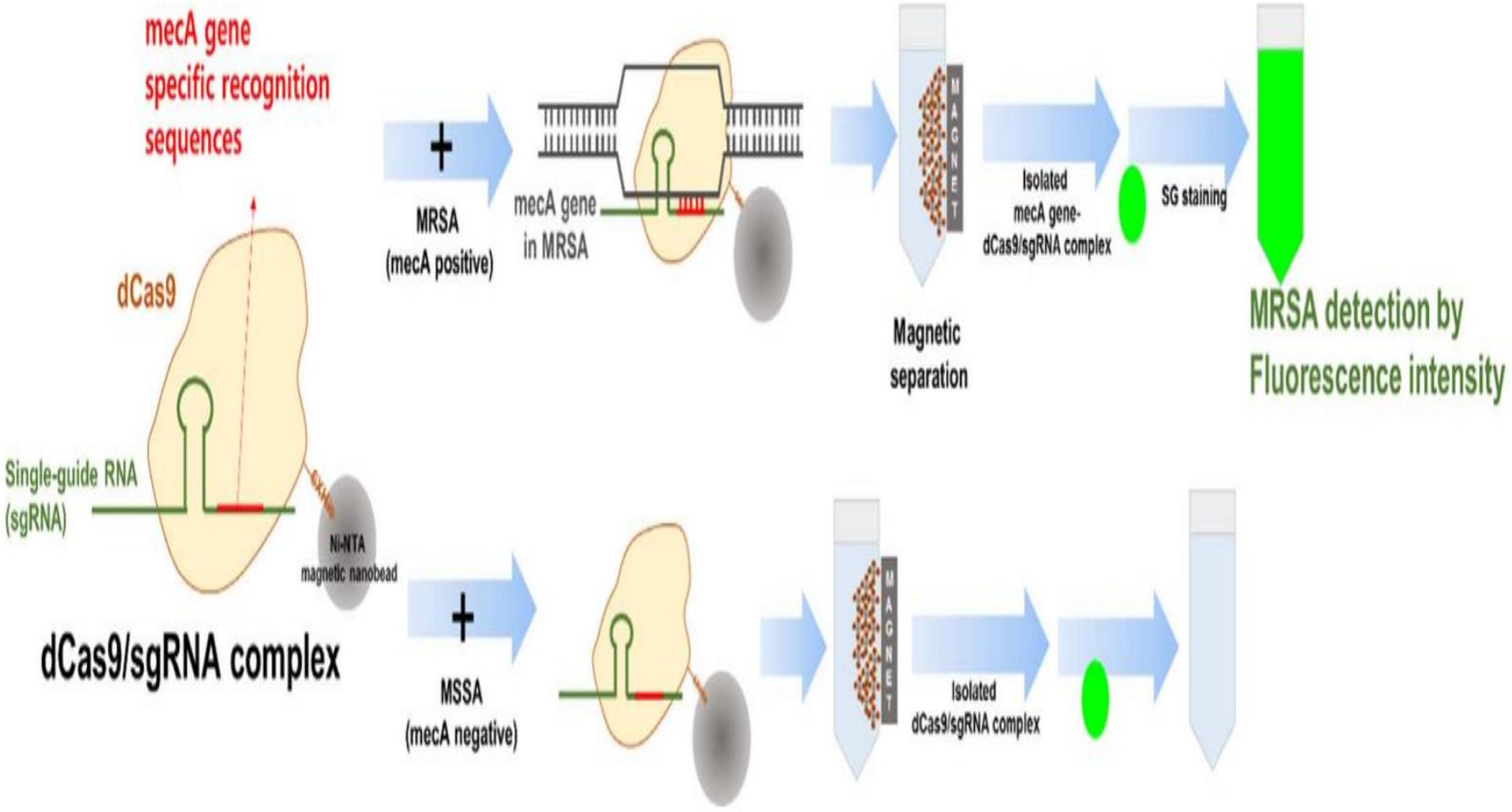
NAME	Cas9	Cas12a	Cas13a
Type	II	V	VI
tracrRNA	Required	Not required	Not required
PAM	5′ – NGG – 3′	5′-TTTV-3′	Not required
Target	dsDNA	dsDNA/ssDNA	ssRNA
Collateral activity	Not present	Present	Present
Pre-crRNA processing	Requires host RNaseIII	Self processing	Self processing

RuvC , HNH, HEPN are nuclease domains



Chen et al. (2022)
Front. Bioeng. Biotechnol. doi:
10.3389/fbioe.2022.851712

Example 1: MRSA(methicillin-resistant *Staphylococcus aureus*) detection using the dCas9/sgRNA-SYBR Green I based system



Note: SYBR Green I is a dsDNA binding dye, used to quantify amplicon amount during the course of the PCR by tracking overall fluorescence emission. The dye binds into the minor groove of dsDNA, and does not bind to ssDNA. When bound, it increases its fluorescence by up to 100 fold. NTA: Nitriloacetic acid

Due to their selectivity and programmability, CRISPR/Cas systems have been rapidly adapted as a recognition element in the development of biosensors for the detection of nucleic acids, which are important targets in diagnosis such as: pathogenic microorganisms(e.g. Sepsis, Ebola, Zika, Dengue, Tuberculosis, COVIT19), , antimicrobial resistance genes, gene mutations or as biomarkers (e.g. microRNA (miRNA)) for specific pathologies such as cancer and Duchenne muscular dystrophy.

Class 2 Cas endonucleases are used for developing biosensing system‘Collateral activity’ of Cas enzyme is employed for nucleic acid detection Crispr/Cas systems are amenable to multiplexing

CRISPR/Cas era has just paved the road for a significant leap to be made in BIOSENSING APPLICATIONS.

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