Development of CRISPR-Cas13a-based antimicrobials capable of sequence-specific killing of target bacteria

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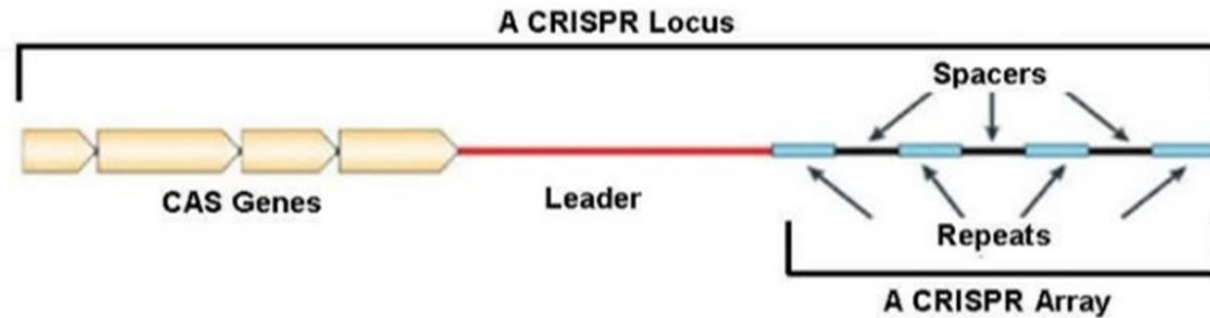
- Introduction
- HISTORY
- The mechanism of action
- Classification
- Review of tests and results
- Discussion

➢ CRISPR : سیستم تناوب کوتاه پالیندروم فاصله دار منظم خوشه ای



1) ژن رمز کننده آنزیم نوکلئازی Cas

2) توالی رهبر:(bp 500-200)

3) مکان ژنی آرایه های کریسپر

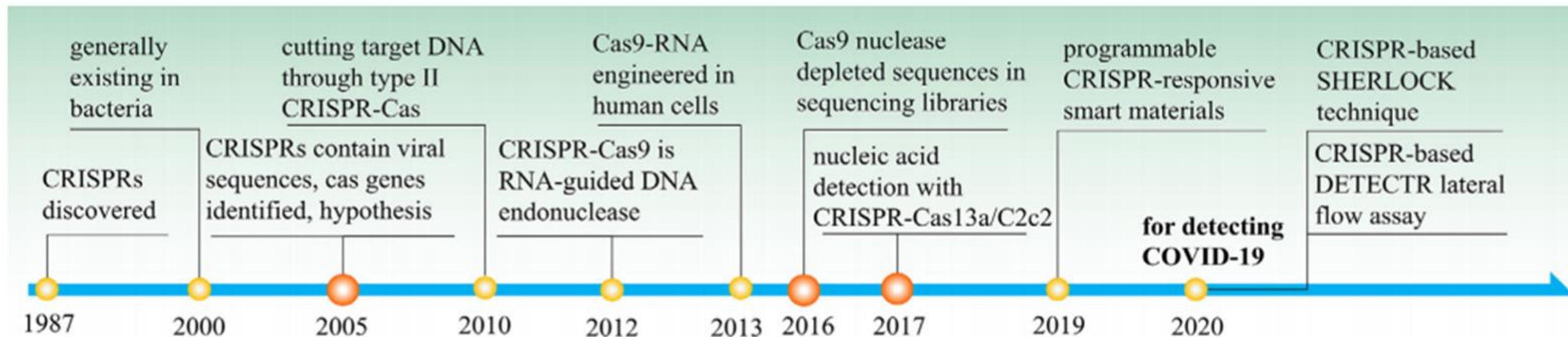
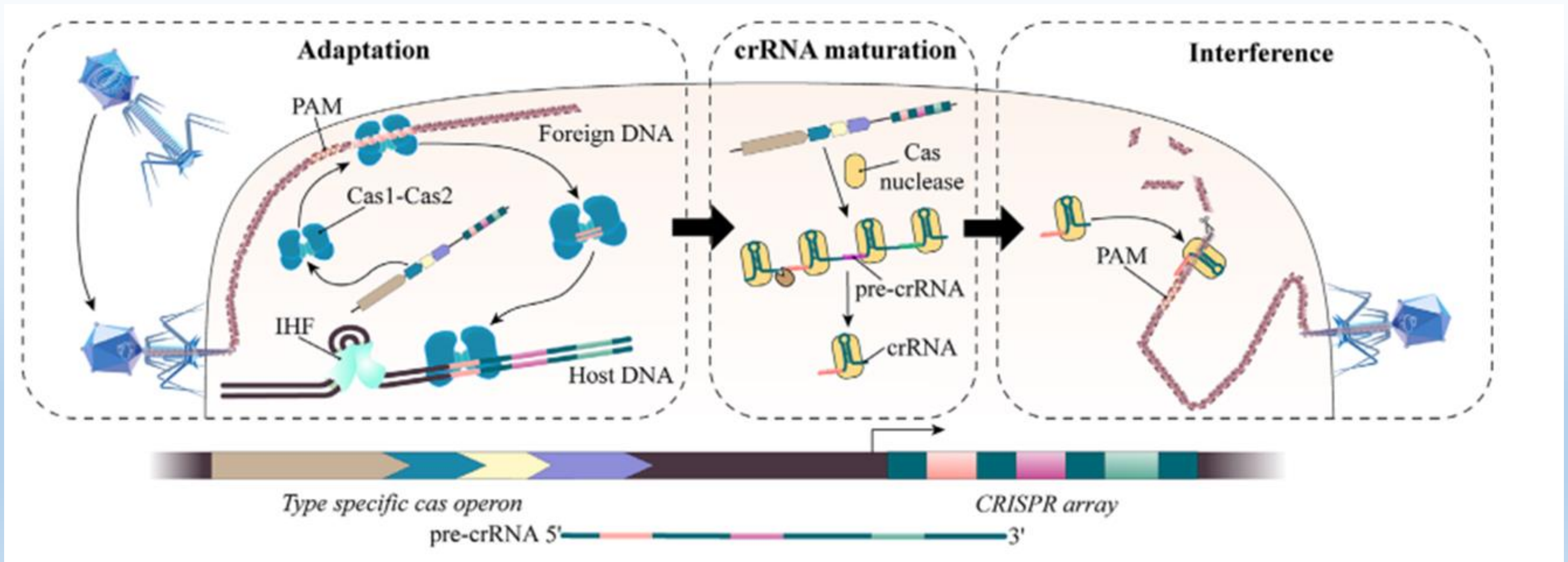


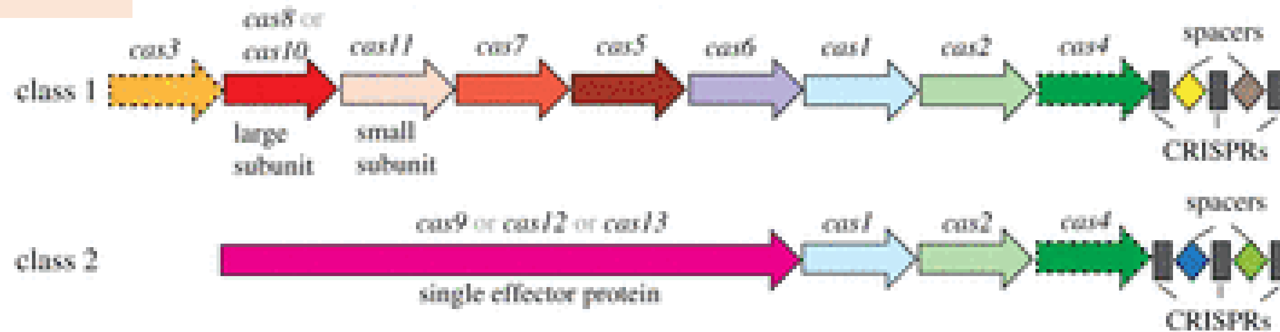
FIGURE 1 Brief summary of the development history of the CRISPR-Cas system

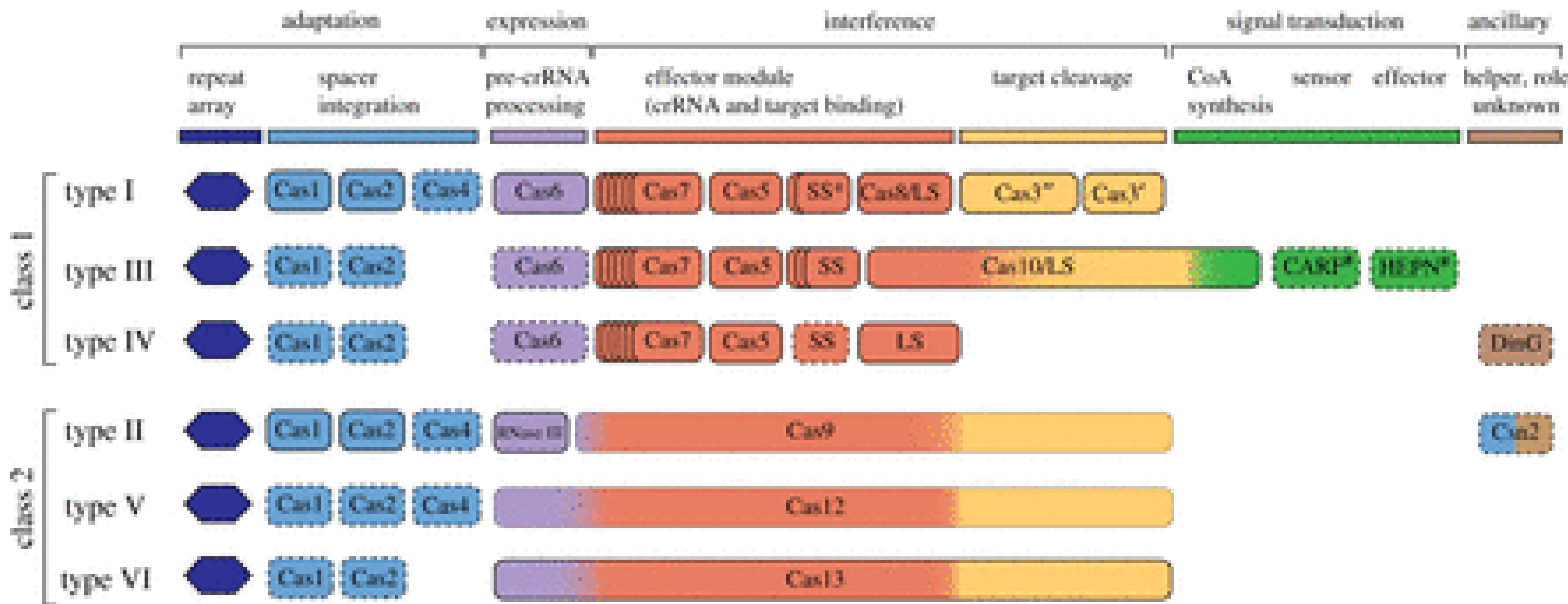
Mechanism

- Adaptation
- crRNA maturation
- Interference



Classification





Target	Requires tracrRNA?
DNA	no
DNA/RNA	no
--	--
DNA	yes
DNA	yes (1 subtype)
RNA	no

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ARTICLE

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Development of CRISPR-Cas13a-based antimicrobials capable of sequence-specific killing of target bacteria

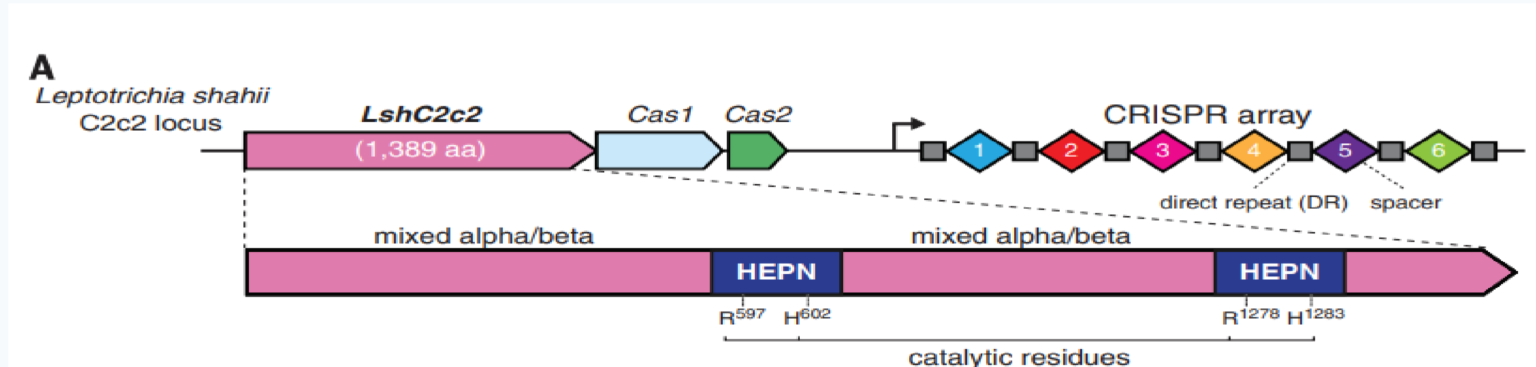
Kotaro Kiga[1], Xin-Ee Tan[1], Rodrigo Ibarra-Chávez[2], Shinya Watanabe[1], Yoshifumi Aiba[1], Yusuke Sato'o[1], Feng-Yu Li[1], Teppei Sasahara[1], Bintao Cui[1], Moriyuki Kawauchi[1], Tanit Boonsiri[1], Kanate Thitiananpakorn[1], Yusuke Taki[1], Aa Haeruman Azam[1], Masato Suzuki[3], José R. Penadés[2] & Longzhu Cui[1✉]

The emergence of antimicrobial-resistant bacteria is an increasingly serious threat to global health, necessitating the development of innovative antimicrobials. Here we report the development of a series of CRISPR-Cas13a-based antibacterial nucleocapsids, termed CapsidCas13a(s), capable of sequence-specific killing of carbapenem-resistant *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* by recognizing corresponding antimicrobial resistance genes. CapsidCas13a constructs are generated by packaging programmed CRISPR-Cas13a into a bacteriophage capsid to target antimicrobial resistance genes. Contrary to Cas9-based antimicrobials that lack bacterial killing capacity when the target genes are located on a plasmid, the CapsidCas13a(s) exhibit strong bacterial killing activities upon recognizing target genes regardless of their location. Moreover, we also demonstrate that the CapsidCas13a(s) can be applied to detect bacterial genes through gene-specific depletion of bacteria without employing nucleic acid manipulation and optical visualization devices. Our data underscore the potential of CapsidCas13a(s) as both therapeutic agents against antimicrobial-resistant bacteria and nonchemical agents for detection of bacterial genes.

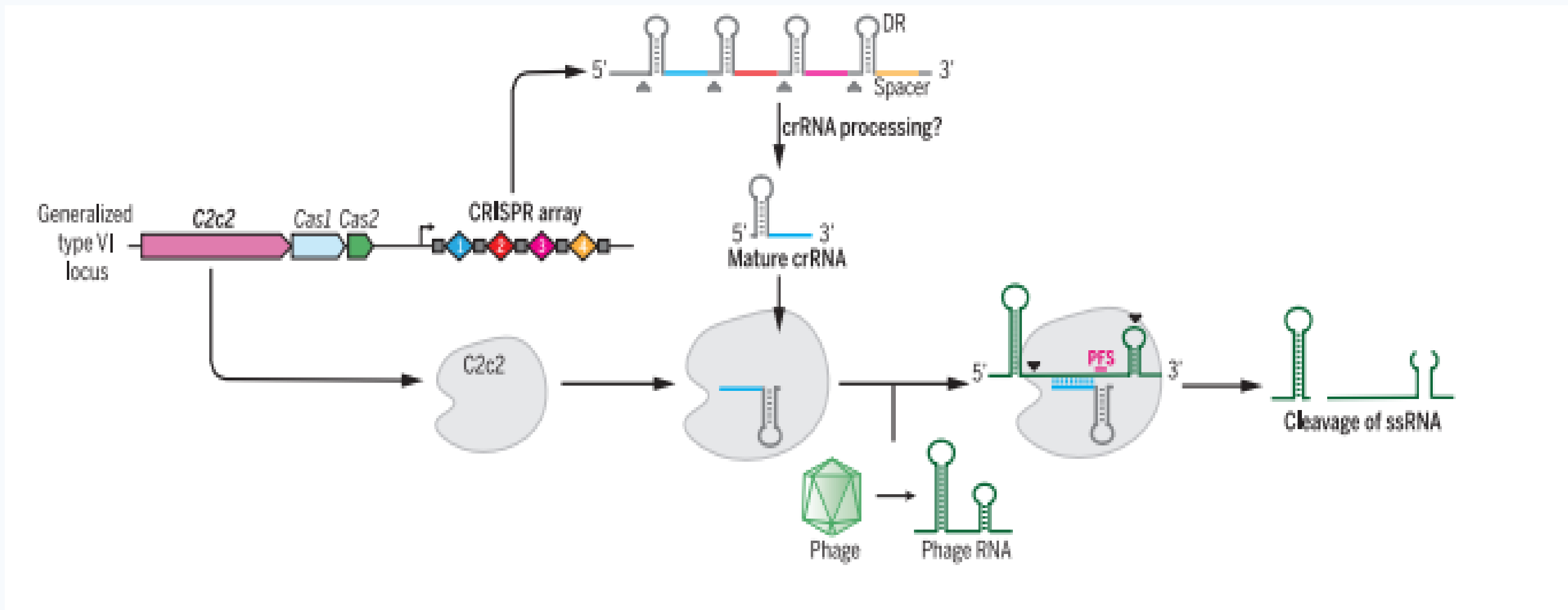
CRISPR-Cas13a (formerly C2c2), a Type VI Class 2 CRISPR-Cas effector,

- One RNase is responsible for pre-crRNA processing to help form mature Type VI interference complexes

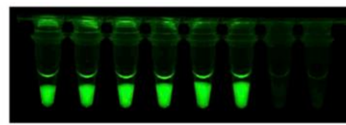
- the other RNase activity, provided by the two HEPN (Higher Eukaryotes and Prokaryotes Nucleotide binding) domains, is required for degradation of target RNA



anaerobic, Gram-negative



- Type VI CRISPR-Cas systems do not require tracrRNA
- type VI systems target ssRNA.
- The crRNA-Cas13 complex recognizes a protospacer flanking site (PFS) adjacent
- Binding of Cas13 to the PFS and the target induces cleavage of both target and nonspecific RNA within the protein's two HEPN-binding domains



(SHERLOCK): Specific High-sensitivity Enzymatic Reporter UnLOCKing

a

CRISPR Type VI-A Cas13

HEPN HEPN

Cas13a

ssRNA target

Spacer

DR sequence

b

RNA sensor

F

Q

Collateral RNase activity

Cleaved RNA sensor

c

dsDNA

RPA

T7 transcription

RT-RPA

RNA

Cas13a detection

Collateral cleavage of reporter release signal

Target sequence

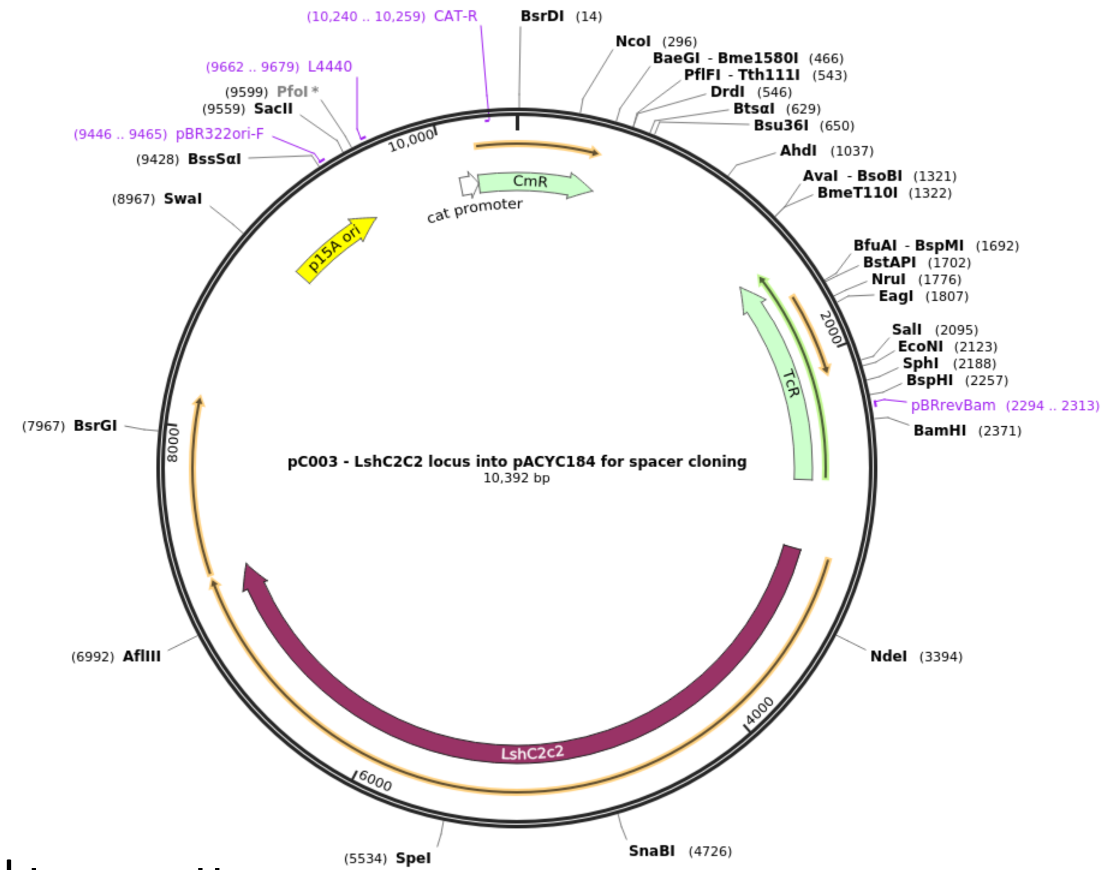
Cas13a–crRNA

Cleavage reporter

which combines isothermal recombinase polymerase amplification (RPA) or reverse transcription (RT)-RPA with Cas13a cleavage

In this article

The CRISPR-Cas13a expression vector (pC003), which carries LshC2c2 locus on pACYC184, was kindly provided by Dr. Feng Zhang



Bacterial strains were grown at 37 °C in LB medium ➡ added to growth

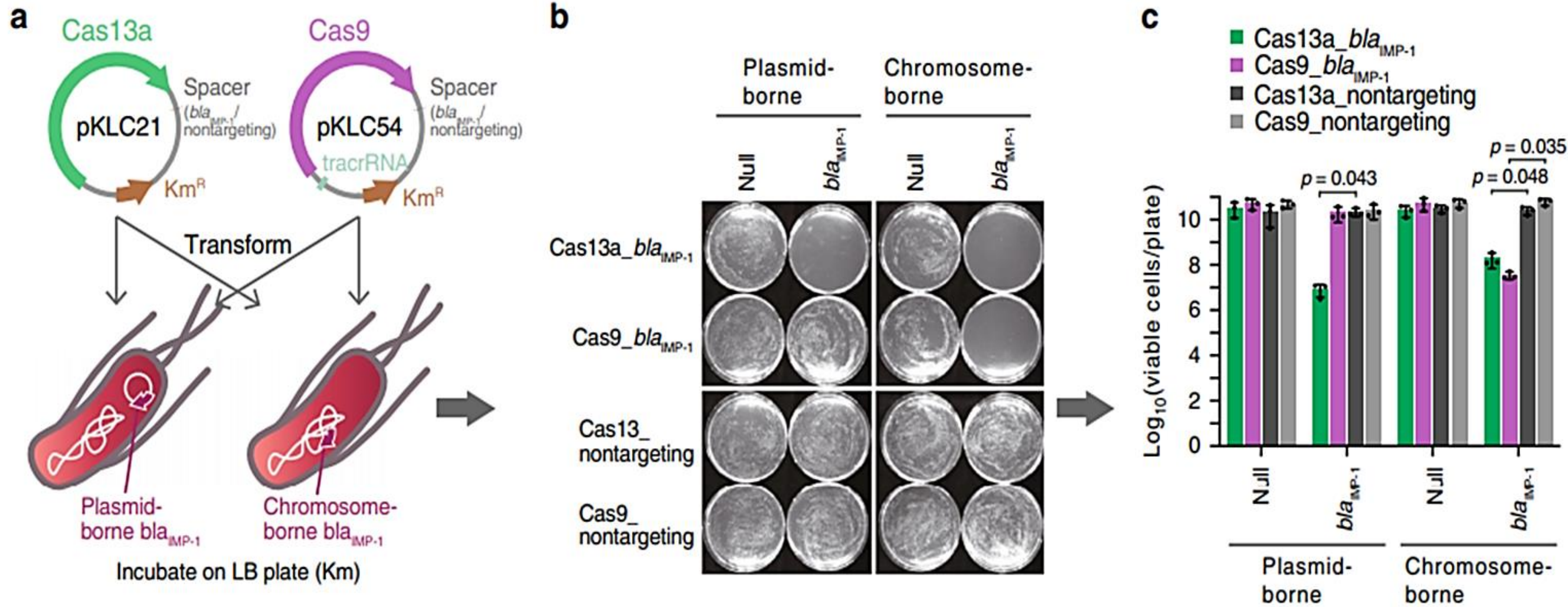
100 µg/mL for ampicillin (Amp)

30 µg/mL for Km

34 µg/mL for chloramphenicol (Cm)

4 µg/mL for colistin

Bactericidal activity of Cas13a



E. coli STBL3 expressing blaIMP-1 from a plasmid (plasmid-borne blaIMP-1) and chromosome (chromosome-borne blaIMP-1)

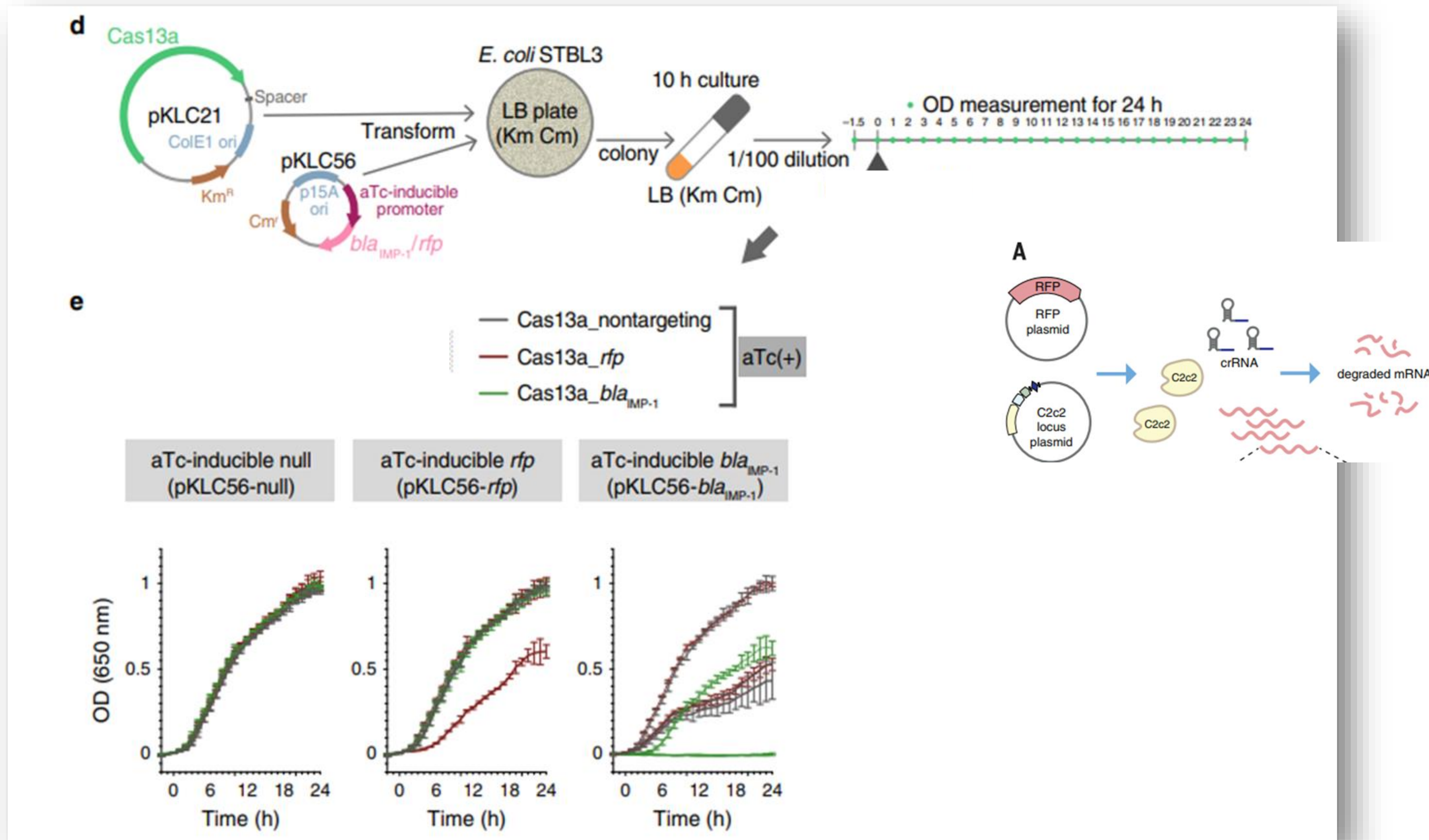
CRISPR-Cas13a

chromosome (from 2.6 × 1010 to 2.0 × 108 CFU/ ml)
plasmid (from 2.3 × 1010 to 8.7 × 106 CFU/ml)

CRISPR-Cas9

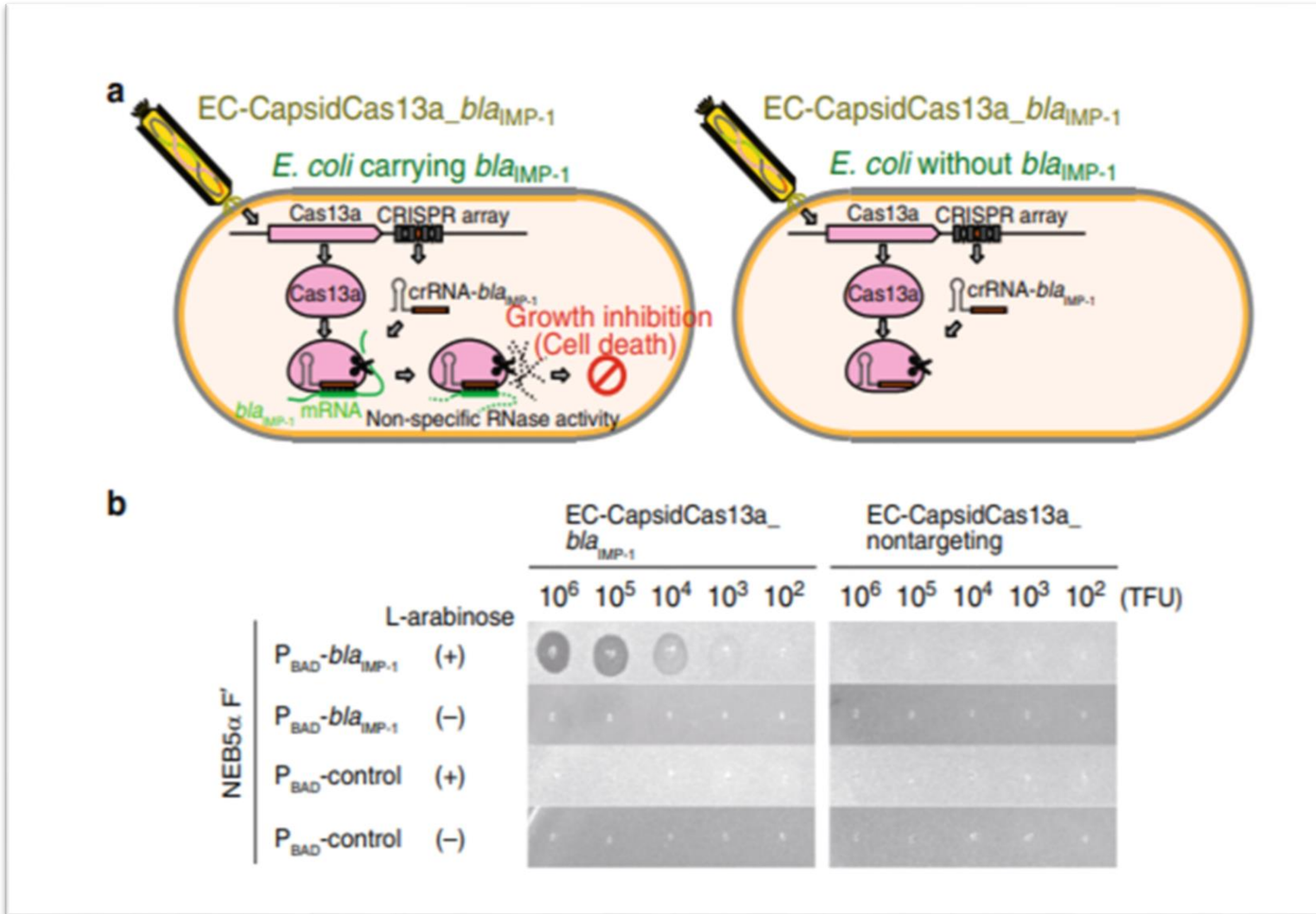
chromosome (from 6.3 × 1010 to 3.6 × 107 CFU/ml
plasmid (from 2.8 × 1010 to 2.2 × 1010 CFU/ml)

In order to determine whether Cas13a causes cell death or not

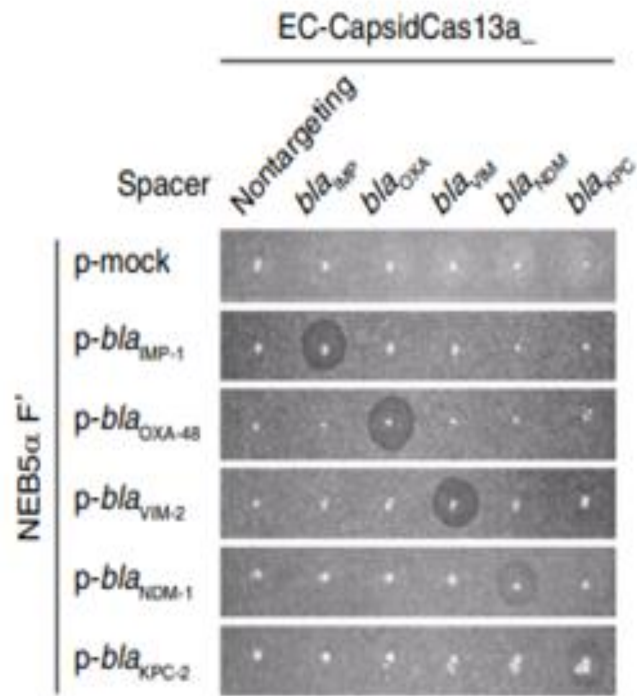


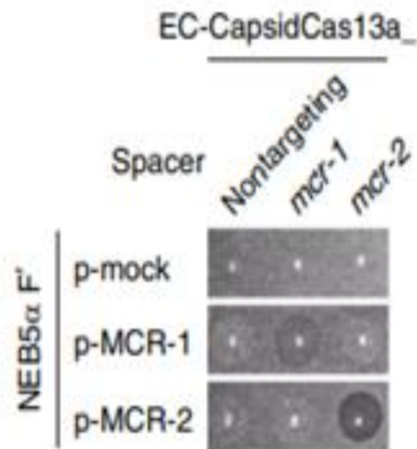
Anhydrotetracycline (aTc)-inducible blaIMP-1- or rfp-expression plasmid (pKLC56) was co-transformed with pKLC21 plasmid

Packaging of CRISPR-Cas13a into bacteriophage capsid



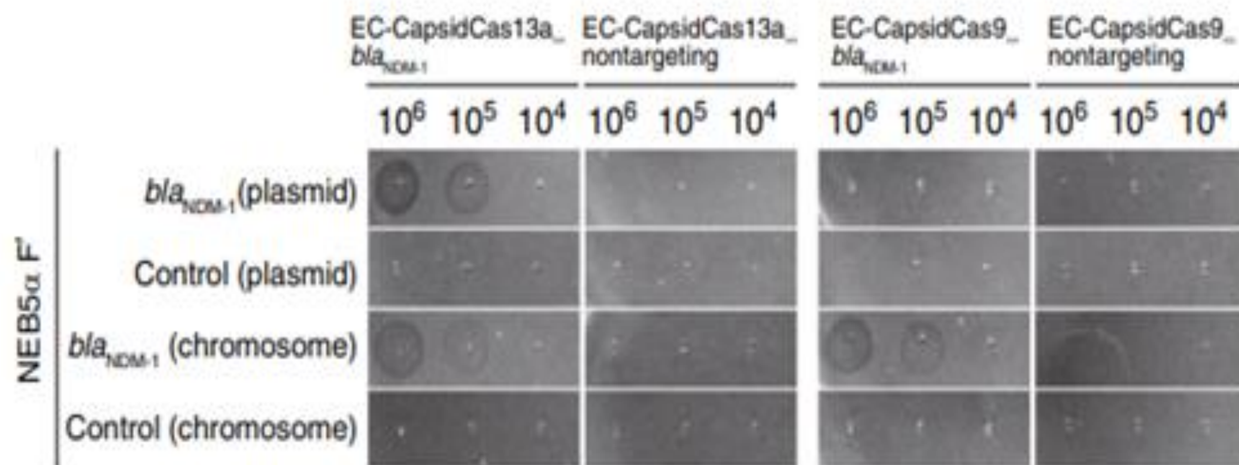
CapsidCas13a, with blaIMP-1-targeting CRISPR-Cas13a packaged into E. coli M13 phage capsid (EC-CapsidCas13a-blaIMP-1)





different carbapenem resistance genes

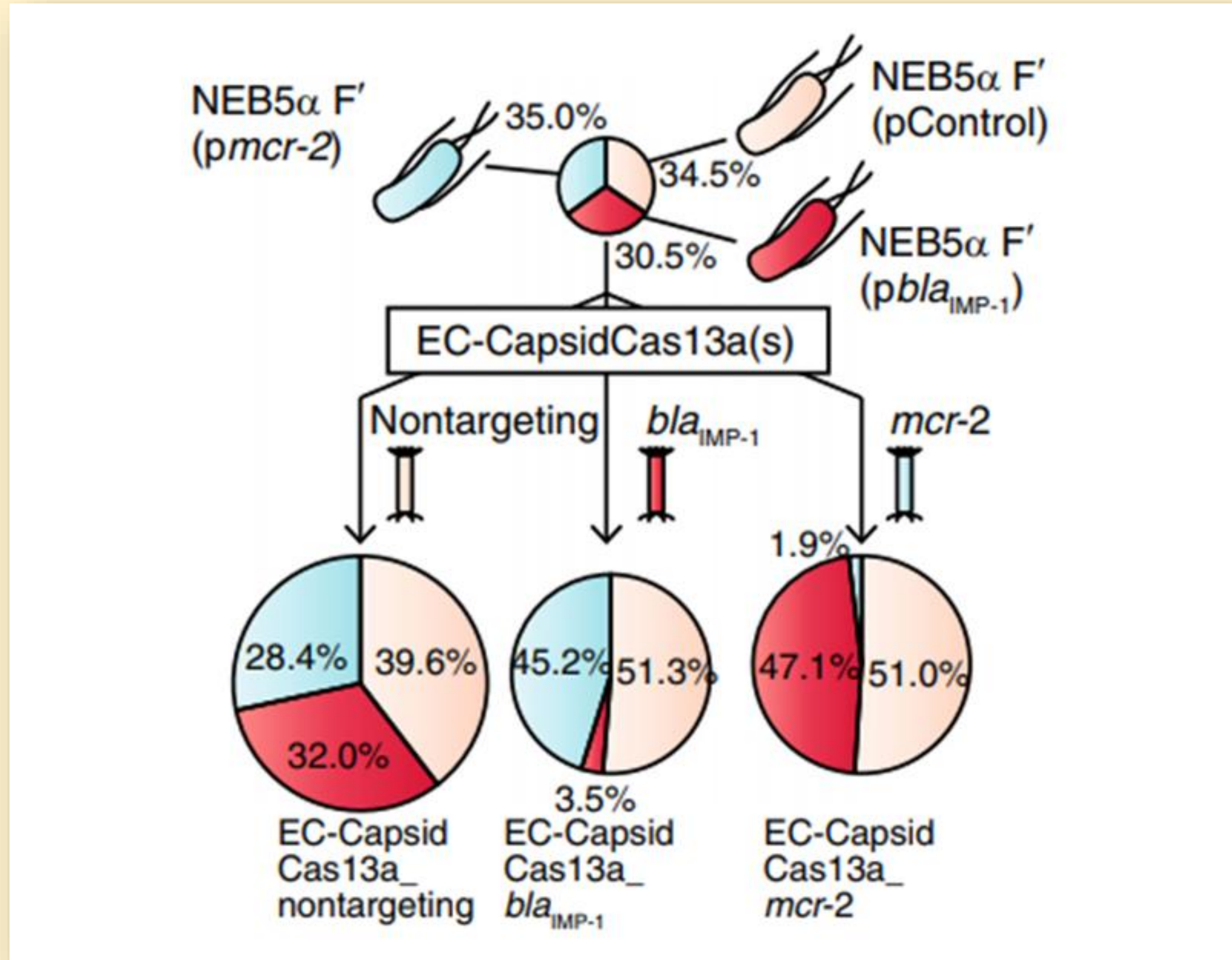
(blaIMP-1, blaOXA-48, blaVIM-2, blaNDM-1, and blaKPC-2)



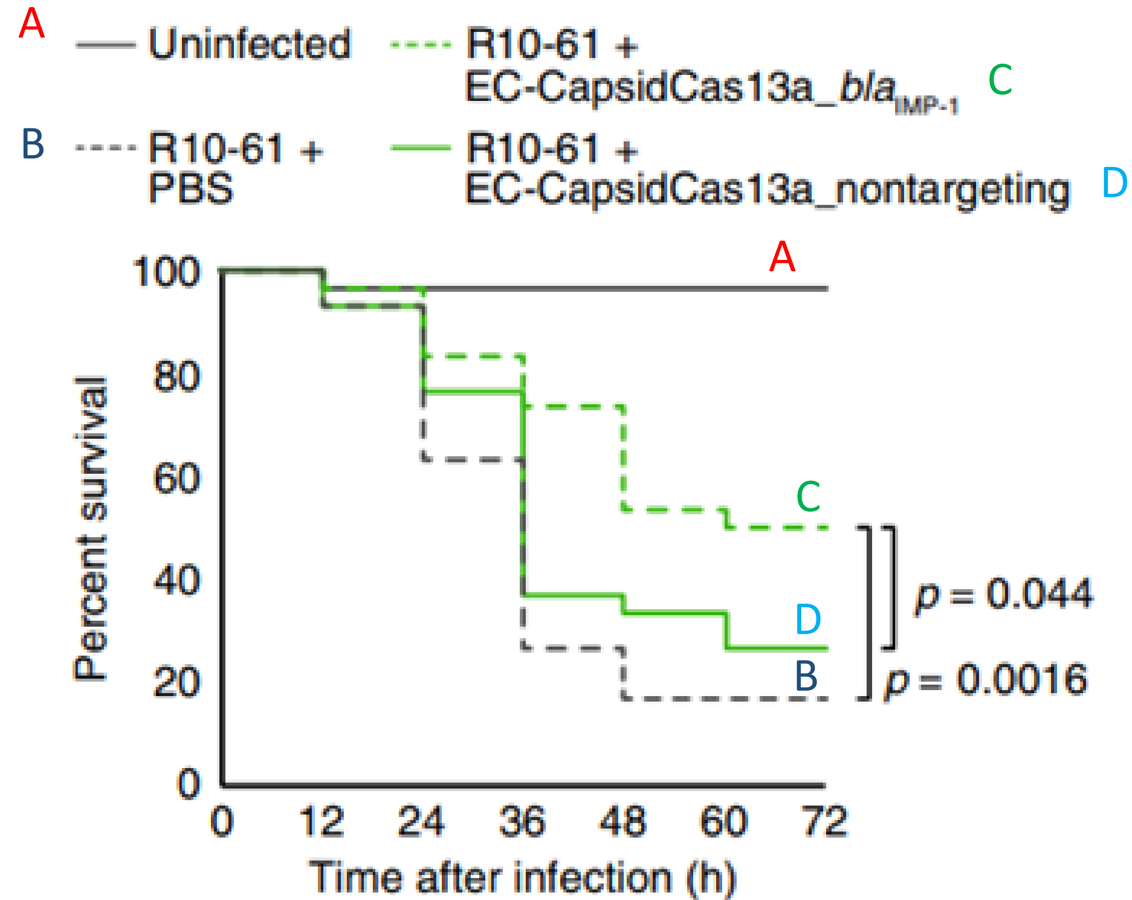
colistin resistance genes

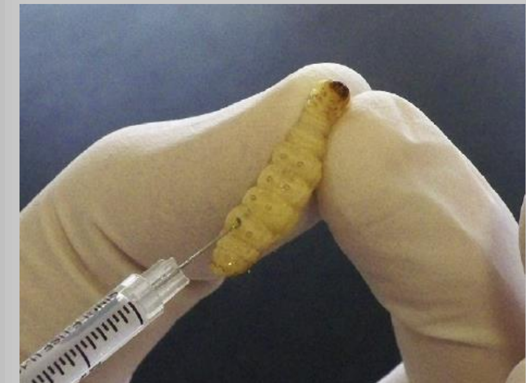
(mcr-1 and mcr-2

In order to determine whether the CapsidCas13a can selectively kill target bacteria among a mixed population of AMR bacteria



Potential of CapsidCas13a as a therapeutic against AMR bacteria infections





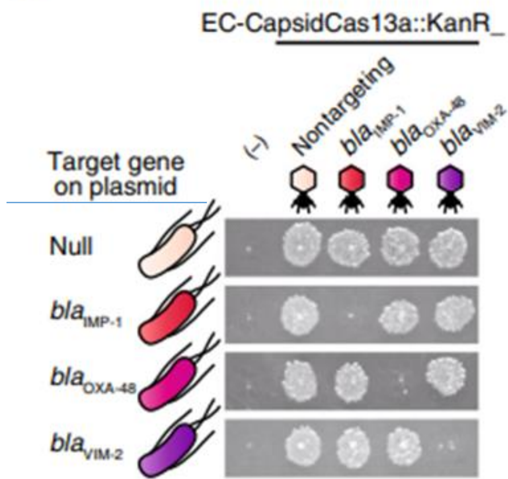
Galleria mellonella larvae (Model)

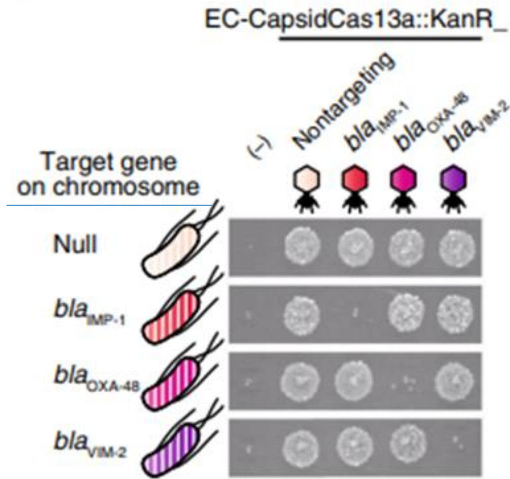
Optimize conditions to achieve better results

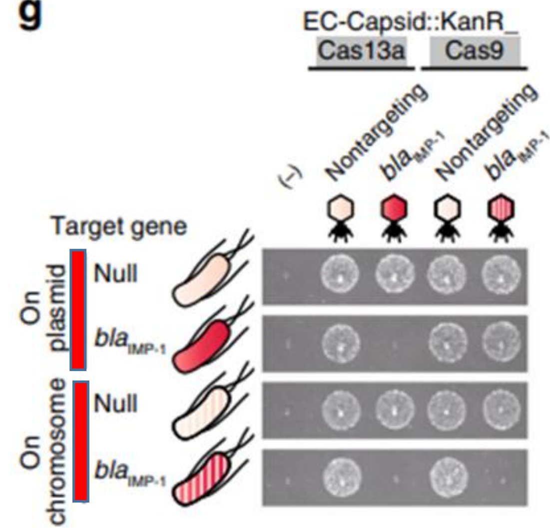
- ✓ the spacer sequence of EC-CapsidCas13a_blaIMP-1 targeting the blaIMP-1 gene was optimized in order to improve the killing efficiency → blaIMP-1_563 spacer sequence GACTTTGGCCAAGCTTC TATATTTGCGT

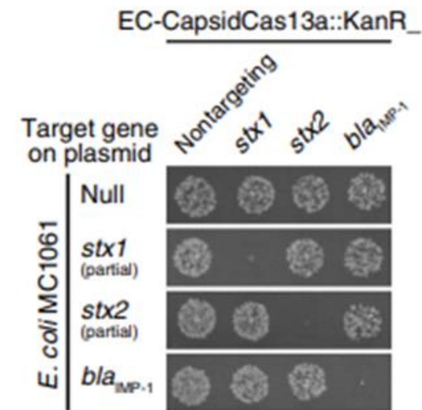
- ✓ the carrier M13 phage was replaced with the lysogenic phage Φ80

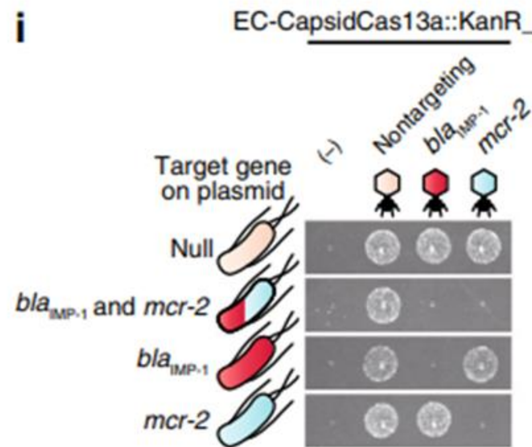
- ✓ the kanamycin resistance gene (KanR) was inserted as a selection marker to generate the constructs EC-CapsidCas13a:: KanR_blaIMP-1 and EC-CapsidCas13a::KanR_nontargeting (control)

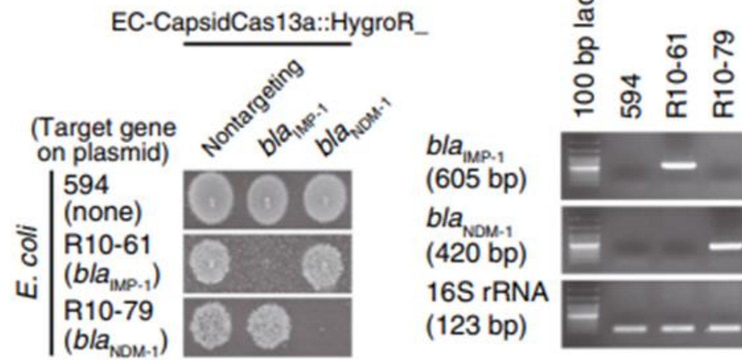

e
EC-CapsidCas13a::KanR_
(–)
Nontargeting
bla IMP-1
bla OXA-48
bla VIM-2
Target gene on plasmid
Null
bla IMP-1
bla OXA-48
bla VIM-2


f
EC-CapsidCas13a::KanR_
(–)
Nontargeting
bla IMP-1
bla OXA-48
bla VIM-2
Target gene on chromosome
Null
bla IMP-1
bla OXA-48
bla VIM-2


g
EC-Capsid::KanR_
Cas13a
Cas9
(–)
Nontargeting
bla IMP-1
Nontargeting
bla IMP-1
Target gene
On plasmid
Null
bla IMP-1
On chromosome
Null
bla IMP-1


h
EC-CapsidCas13a::KanR_
Target gene on plasmid
Nontargeting
stx1
stx2
bla IMP-1
E. coli MC1061
Null
stx1 (partial)
stx2 (partial)
bla IMP-1


i
EC-CapsidCas13a::KanR_
(–)
Nontargeting
bla IMP-1
mcr-2
Target gene on plasmid
Null
bla IMP-1 and mcr-2
bla IMP-1
mcr-2


j
EC-CapsidCas13a::HygroR_
Nontargeting
bla IMP-1
bla NDM-1
(Target gene on plasmid)
E. coli
594 (none)
R10-61 (bla IMP-1)
R10-79 (bla NDM-1)
100 bp ladder
594
R10-61
R10-79
bla IMP-1 (605 bp)
bla NDM-1 (420 bp)
16S rRNA (123 bp)

Discussion

- Although there are still many questions to be answered concerning practical application

❖ phage capsids
❖ catalytic mode of Cas13a
❖ The efficiency of phage capsid packaging
❖ ethical issues regarding(genetic recombinants)

- our strategy demonstrated that the CapsidCas13a antimicrobials are promising to be developed for at least three application categories:

Advantages

- as promising antibacterial therapeutic agents targeting any bacterial gene, including AMR genes, or selectively killing targeted toxin-producing bacteria

- as a simple and inexpensive bacterial gene detection system for bacterial identification and efficient molecular epidemiological investigations without the need for the amplification of nucleic acids or optic devices

- as tools to manipulate the bacterial flora by targeting and eliminating a specific bacterial population without disrupting other irrelevant bacterial populations

Limitations

- it is necessary to construct corresponding CapsidCas13a for each bacterial species and gene
- turnaround time for test results can be long since interpretation of the results is dependent on bacterial growth
- it cannot be used when the bacteria cannot be cultured or the target gene is not transcribed.

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Thank You
For Your Attention

Questions & Comments are Welcome!

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