Eligobiotiques : élimination « intelligente » des bactéries résistantes ?

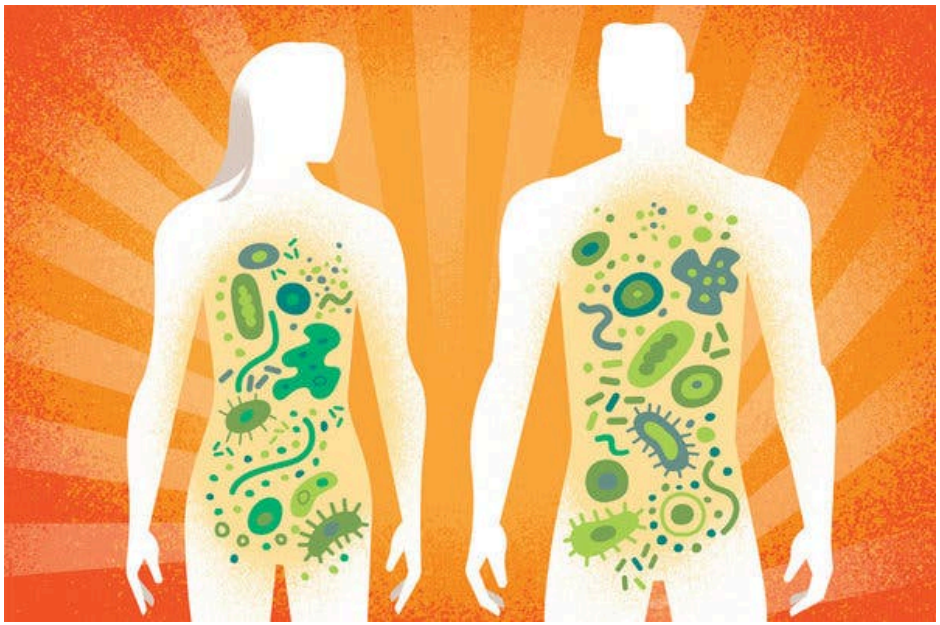
David Bikard

A la recherche d'antibiotiques spécifiques

Importance du microbiome:

- Nutrition
- Barrière contre les infections
- Interactions avec le système immunitaire

➔ Rôle dans de nombreuses pathologies: obésité, maladies auto-immunes, autisme …



➔ Les antibiotiques peuvent perturber le microbiome de manière durable

➔ Liens établis avec des maladies comme l'asthme, le diabète de type I, le psoriasis …

Cibler les gènes de résistance

- Séquençage massif des bactéries pathogènes

- ➢ >200 000 génomes bactériens sur NCBI
- ➢ Séquençage systématique des nouveaux isolats par les centres de référence

- Dans de nombreux cas, les gènes de résistance sont connus

Isolate fields																hvK2 multiplex PCR	MLST								Species			
id	isolate	aliases	taxonomic designation	phylogroup from ST	world region	host	source	infection	other source info	resistance info	virulence info	K typing method	clonal group	duplicate number	comments	accession number	Kpl50233a	gapA	infB	mdh	pgi	phoE	rpoB	tonB	ST	mtnC	Kpl50233a	KP1_2389 (KP888)	KP1 (yp
68	H117/5		K. pneumoniae	Kp1 (7 loci)	Europe	Human	Blood			CTX-M-15					clone II			3	4	6	1	7	4	38	147				
69	H149/5		K. pneumoniae	Kp1 (7 loci)	Europe	Human	Blood			CTX-M-15					HEC			1	1	1	1	1	1	1	15				
70	H442/5		K. pneumoniae	Kp1 (7 loci)	Europe	Human	Blood			CTX-M-15					clone III			3	3	1	1	1	1	4	11				
451	H219/6		K. pneumoniae	Kp1 (7 loci)	Europe	Human	Blood			CTX-M-15					clone IV			2	1	2	1	4	31	19	274				
1540	1191100241		K. pneumoniae	Kp1 (7 loci)	Europe	Human				CTX-M-15 & OXA-48			395			AFXH01	4	3	1	2	4	1	1	4	395		4		
1551	KpO3210		K. pneumoniae		Europe	Human	Blood			OXA-1, OXA-48, TEM-1, SHV-76, CTX-M-15					>6000 contigs; ST405	AMRH01			1	62				110					
1626	pKDO1		K. pneumoniae		Europe	Human				CTX-M-15					Plasmid	JX424423													
1871	PTE234		K. pneumoniae	Kp1 (7 loci)	Europe	Human	Urine	-		CTX-M ESBL								2	6	1	5	4	1	6	101				
1872	PTE738		K. pneumoniae	Kp1 (7 loci)	Europe	Human	Abdominal drainage	-		CTX-M ESBL, Epidemic Clone								1	1	1	1	1	1	1	15				
1873	PTE1173		K.	Kp1 (7 loci)	Europe	Human	Urine	-		CTX-M ESBL								3	4	6	1	7	4	38	147				

Est-il possible de combattre les gènes de résistance directement ?

➔ CRISPR-Cas: des ciseaux à ADN reprogrammables

History of CRISPR: first observation (1987)

JOURNAL OF BACTERIOLOGY, Dec. 1987, p. 5429–5433
0021-9193/87/125429-05$02.00/0

Vol. 169, No. 12

Nucleotide Sequence of the *iap* Gene, Responsible for Alkaline Phosphatase Isozyme Conversion in *Escherichia coli*, and Identification of the Gene Product

YOSHIZUMI ISHINO, HIDEO SHINAGAWA, KOZO MAKINO, MITSUKO AMEMURA, AND ATSUO NAKATA*

Department of Experimental Chemotherapy, The Research Institute for Microbial Diseases, Osaka University, 3-1 Yamadaoka, Suita, Osaka 565, Japan

(A)

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                                        -iap-TGAT GGGTTTGAAA ATGGGAGCTG    (1390)
GGAGTTCTAC CGCAGAGGCG GGGGAACTCC AAGTGATATC CATCATCGCA TCCAGTGCGC C  (1451)
CGGTTTATCC CCGCTGATGC GGGGAACACC AGCGTCAGGC GTGAAATCTC ACCGTCGTTG C  (1512)
CGGTTTATCC CTGCTGGCGC GGGGAACTCT CGGTTCAGGC GTTGCAAACC TGGCTACCGG G  (1573)
CGGTTTATCC CCGCTAACGC GGGGAACTCG TAGTCCATCA TTCCACCTAT GTCTGAACTC C  (1634)
CGGTTTATCC CCGCTGGCGC GGGGAACTCC CGGGGGATAA TGTTTACGGT CATGCGCCCC C  (1695)
CGGTTTATCC CCGCTGGCGC GGGGAACTCT GGGCGGCTTG CCTTGCAGCC AGCTCCAGCA G  (1756)
CGGTTTATCC CCGCTGGCGC GGGGAACTCA AGCTGGCTGG CAATCTCTTT CGGGGTGAGT C  (1817)
CGGTTTATCC CCGCTGGCGC GGGGAACTCT AGTTTCCGTA TCTCCGGATT TATAAAGCTG A  (1878)
CGGTTTATCC CCGCTGGCGC GGGGAACTCG CAGGCGGCGA CCGGCAGGGT ATGCGCGATT CG(1940)
CGGTTTATCC CCGCTGGCGC GGGGAACTCG CGACCGCTCA GAAATTCCAG ACCCGATCCA AA(2002)
CGGTTTATCC CCGCTGGCGC GGGGAACTCT CAACATTATC AATTACAACC GACAGGGAGC C  (2063)
CGGTTTATCC CCGCTGGCGC GGGGAACTCA GCGTGTTCGG CATCACCTTT GGCTTCGGCT G  (2124)
CGGTTTATCC CCGCTGGCGC GGGGAACTCT GCGTGAGCGT ATCGCCGCGC GTCTGCGAAA G  (2185)
CGGTTTATCC CCGCTGGCGC GGGGAACTCT CTAAAAGTAT ACATTTGTTC TTAAAGCATT    (2255)
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Direct Repeats (29 pb)

Variable region = spacers (32-33 pb)

History of CRISPR:
weird repeats in the genomes of bacterial and archaea
(1987-2002)

DVR (Direct Variable Repeats),
TREP (Tandem Repeats),
LTRR (Long Tandemly Repeated Repetitive Sequences),
SRSR (Short Regularly Spaced Repeats),
LCTR (Large Clusters of Tandem Repeats),
SPIDR (Spacer Interspersed Direct Repeats)

CRISPR
Clustered Regularly Interspaced Short Palindromic Repeats

Jansen et al., molecular microbiology 2002

History of CRISPR:
Spacers sequences match foreign DNA (2005)

Mojica, F. J., Diez-Villasenor, C., Garcia-Martinez, J. & Soria, E.
J. Mol. Evol. 2005

Pourcel, C., Salvignol, G. & Vergnaud, G.
Microbiology 2005

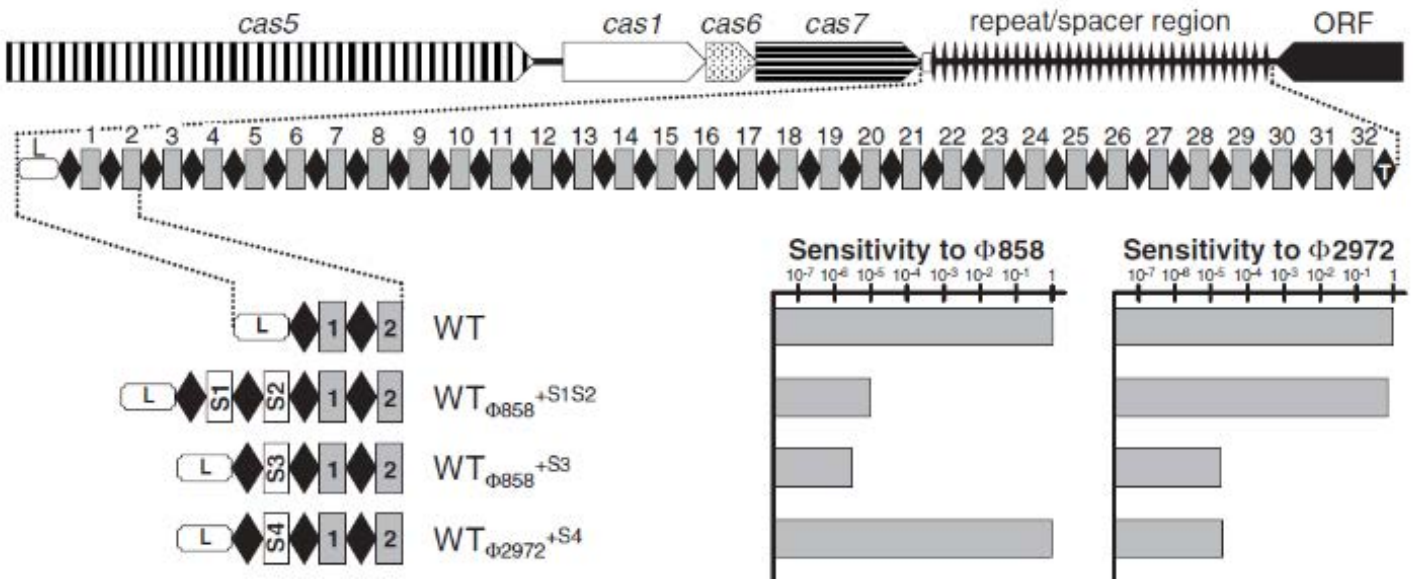
Bolotin, A., Quinquis, B., Sorokin, A. & Ehrlich, S. D.
Microbiology 2005

History of CRISPR: Experimental evidence (2007)

Science

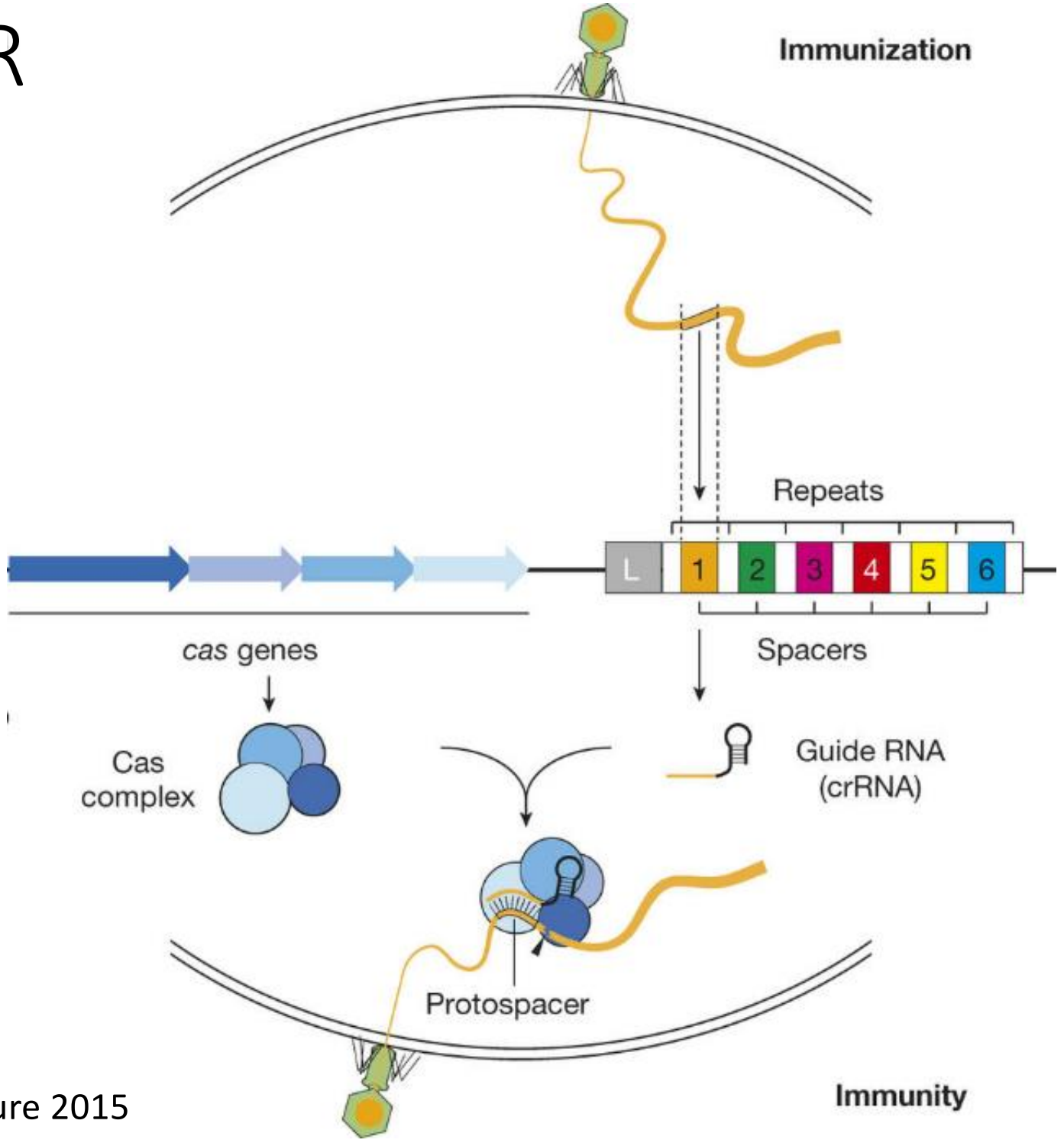
CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes

Rodolphe Barrangou,[1] Christophe Fremaux,[2] Hélène Deveau,[3] Melissa Richards,[1] Patrick Boyaval,[2] Sylvain Moineau,[3] Dennis A. Romero,[1] Philippe Horvath[2]*



SCIENCE VOL 315 23 MARCH 2007

CRISPR

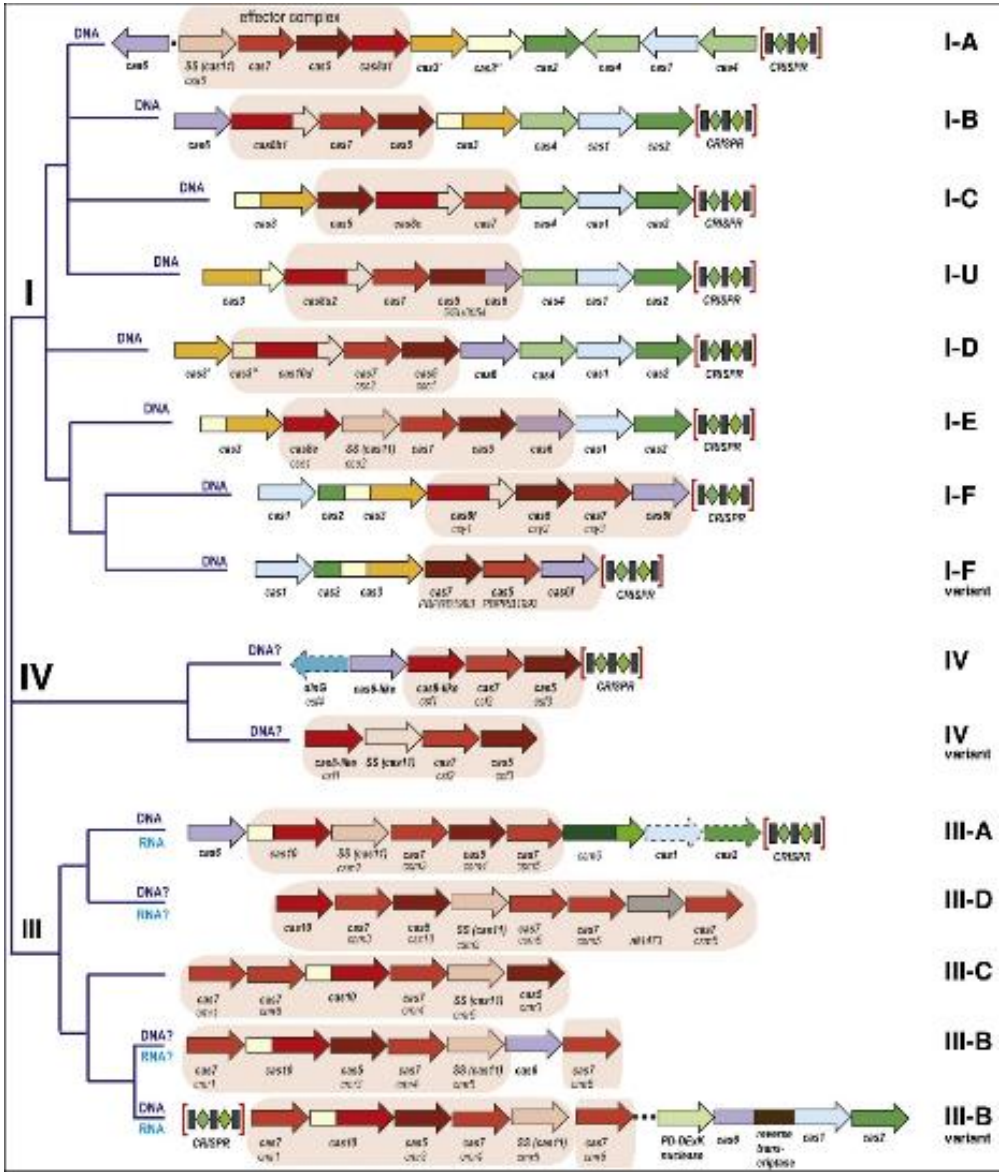


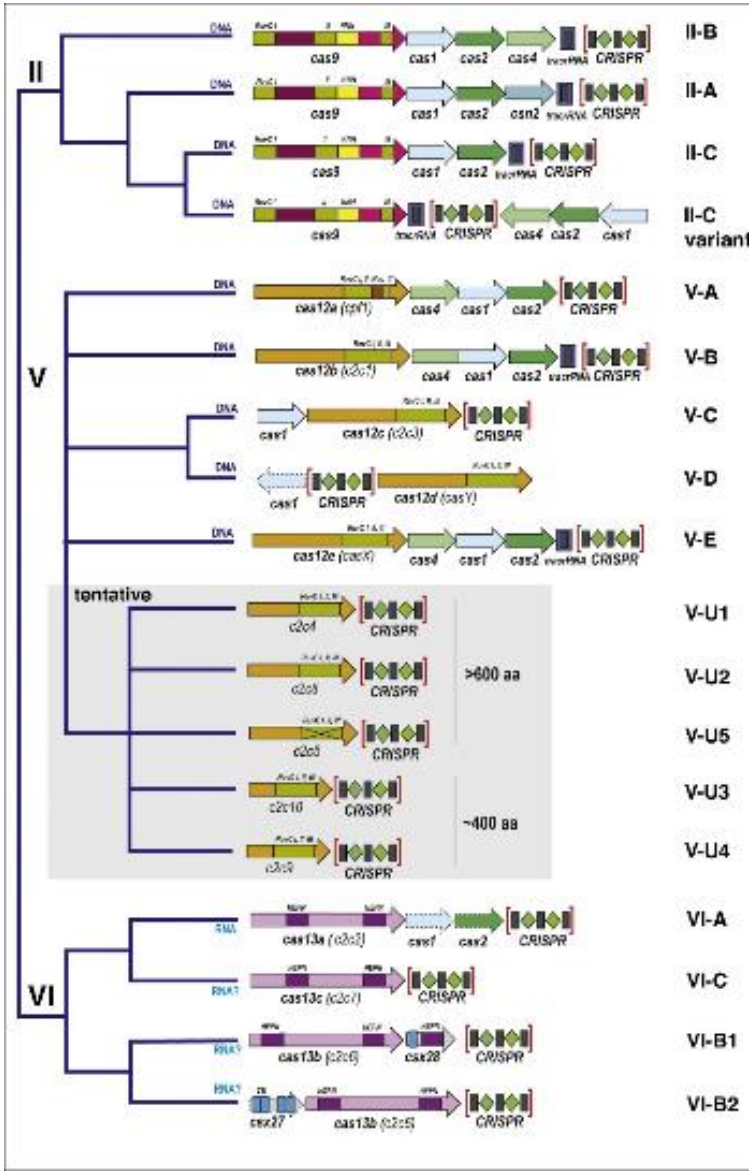
Marraffini, Nature 2015

The diversity of CRISPR-Cas system

Class 1

Class 2





Koonin & al., Current Opinion in Microbiology 2017

S. pyogenes CRISPR02

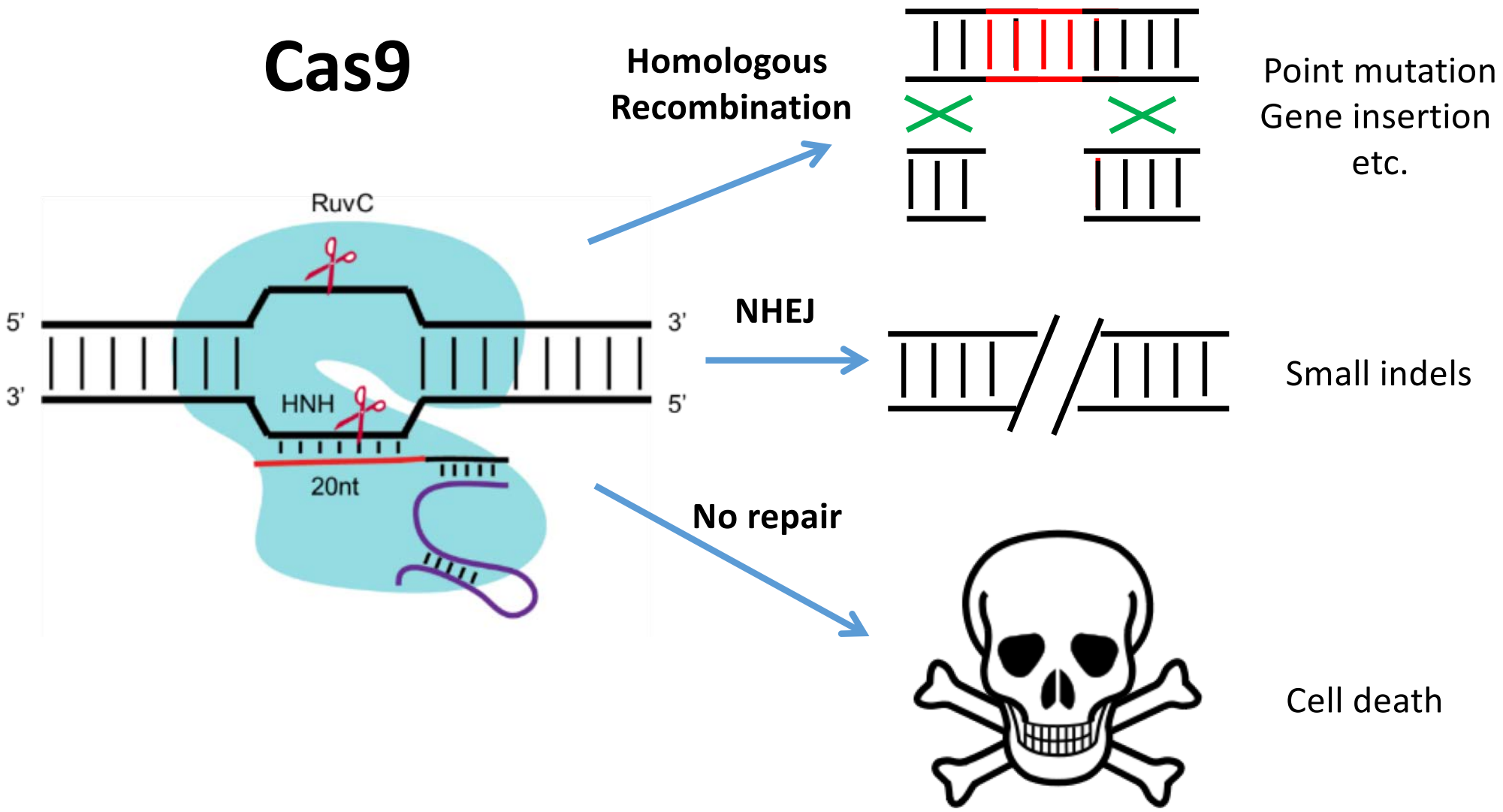
tracrRNA

NA
cursor
NA

Garneau & al., Nature 2010
Sapranauskas & al., PNAS 2011
Deltcheva & al., Nature 2011

Jinek & al., Science 2012
Gasiunas & al., PNAS 2012
Sternberg & al., Nature 2014

CRISPR as a biotechnological tool



Genome Editing

- *S. pneumoniae* (Jiang, Bikard *et al*, **2013**, *Nat. Biotechnol.*)
- *E. coli* (Jiang, Bikard *et al*, **2013**, *Nature Biotechnol.*)

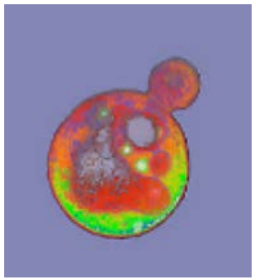
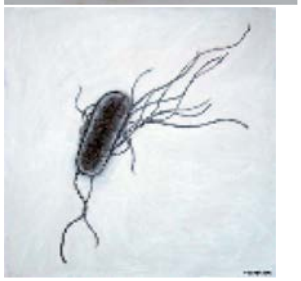
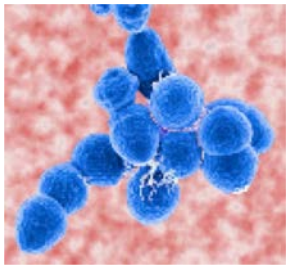
- Human cell
- Monkeys (N
- Livestocks (
- Mice (Wang
- *Frogs* (Blitz , *Genetics*)
- Zebrafish (H chnol.)
- Insects (Wa
- Plants (Li *et* iotechnol.):
- Flies (Gratz
- Nematodes (Lo *et al.*, **2013**, *Genetics*)
- Yeast (DiCarlo *et al.*, **2013**, *NAR)*

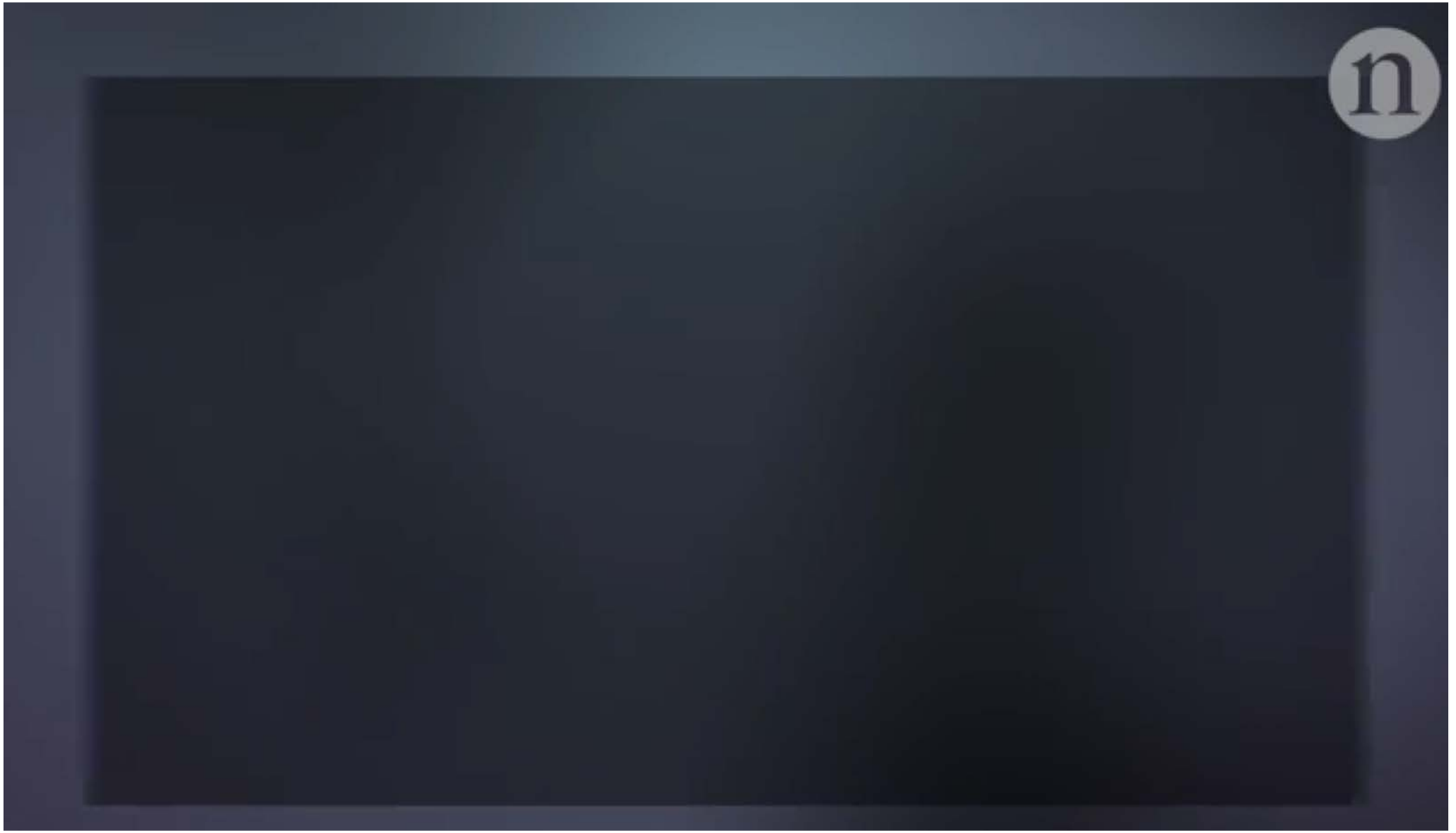






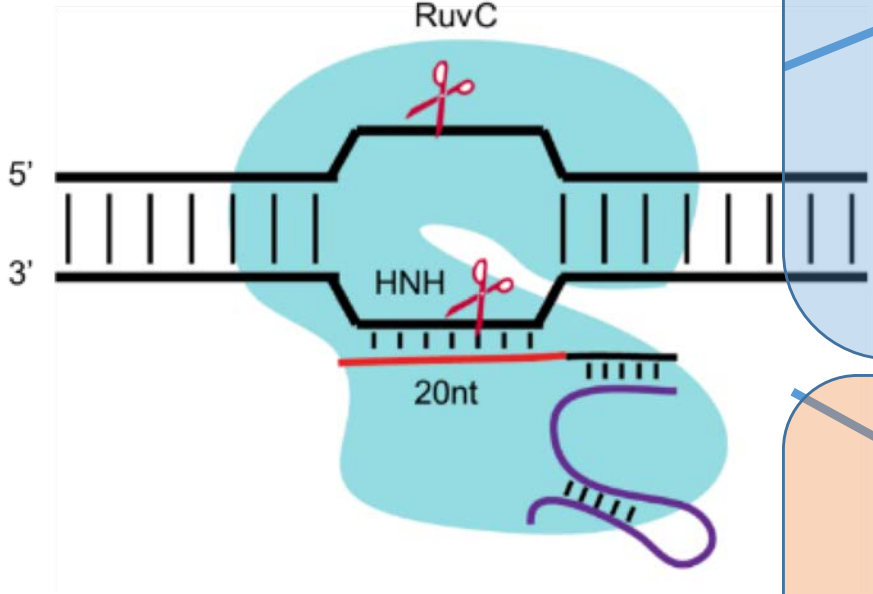


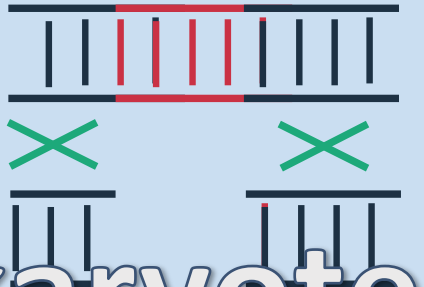


CRISPR as a biotechnological tool

Cas9



Homologous Recombination



Point mutation
Gene insertion
etc.

Eukaryotes ?

NHEJ

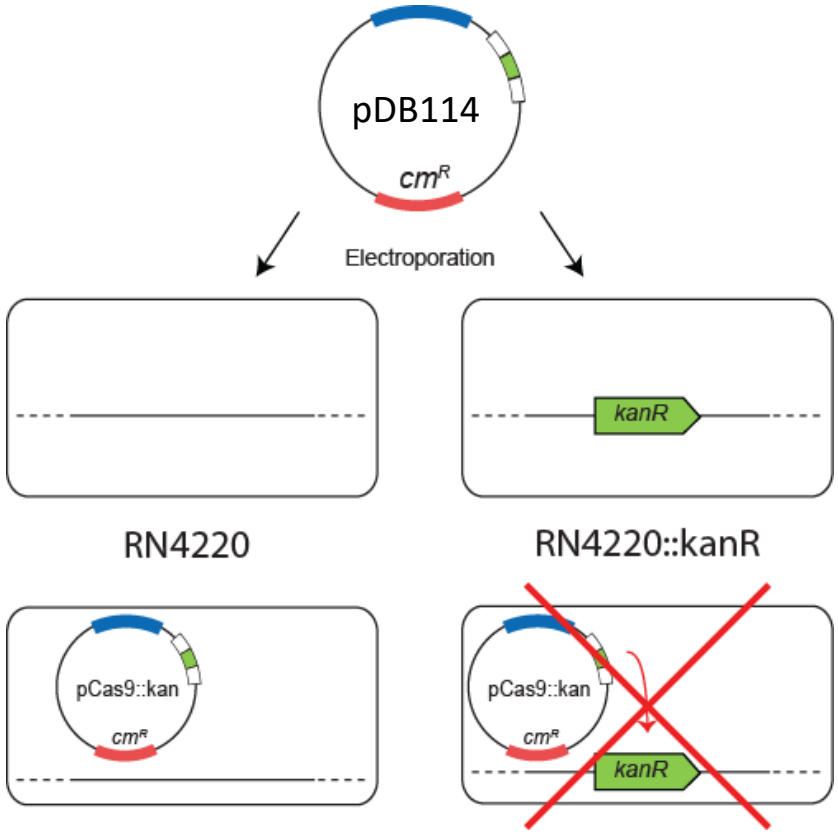
Small indels

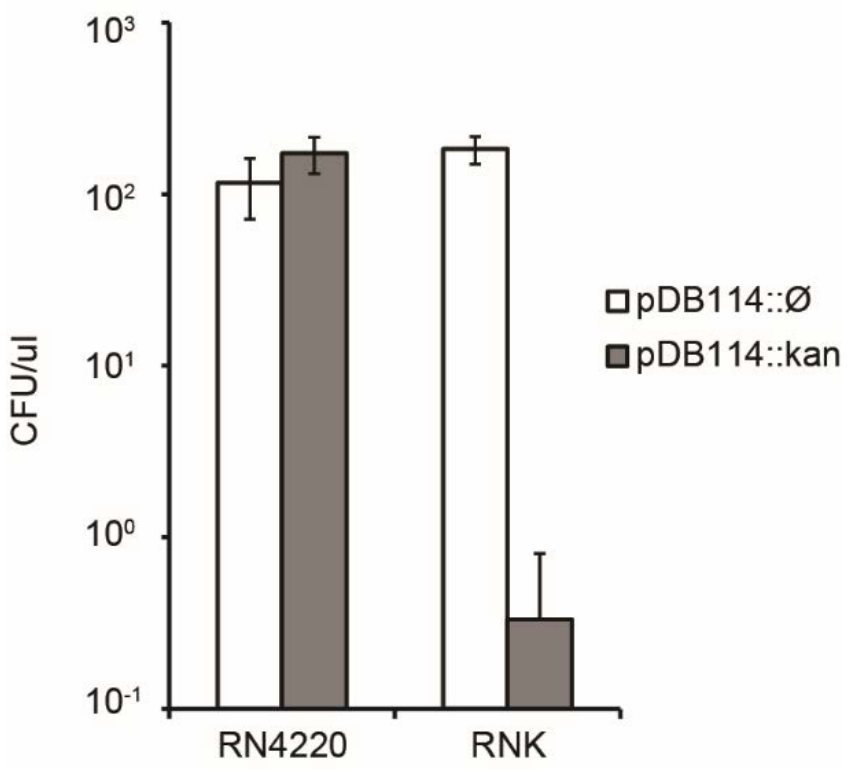
No repair

Bacteria ?

Cell death

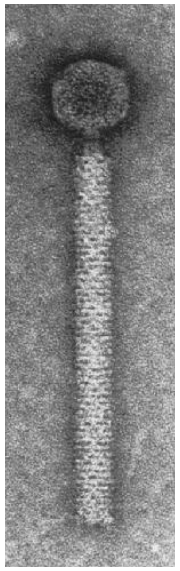
Sequence-specific killing of S. aureus

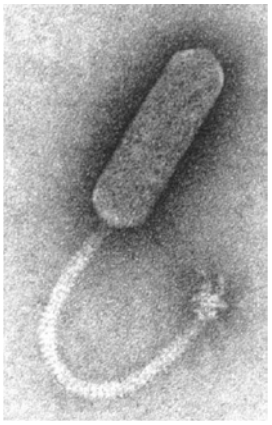


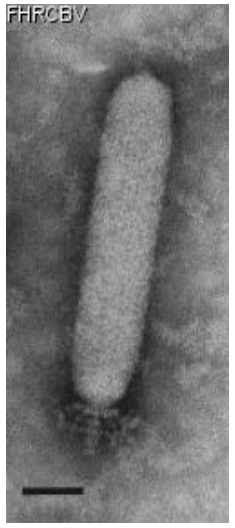


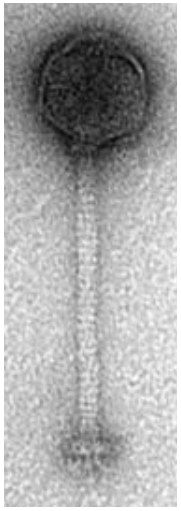
Bikard & al., Nat Biotech, 2014

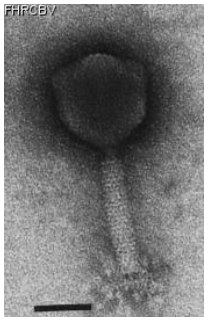
Bacteriophages



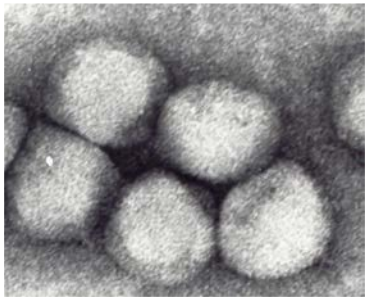


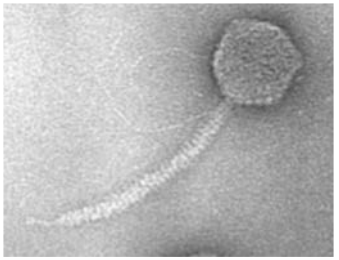






- Number: >10^{30}: A trillion phages for every grain of sand in the world (*Keen E., Bioessays 2015)*
- Size: from 3.4 kb to almost 500 kb
- Phages kill and lyse between 15% and 40% of the ocean's bacteria every day (*Danovaro R et al., 2011)*





(www.phage.ulaval.ca)

Figure 4 T4 phages adsorbed to an E. coli bacteria. The phages' sheaths are contracted, and their baseplates are 300-400 angstroms from the cell wall revealing in several individuals the tail cores

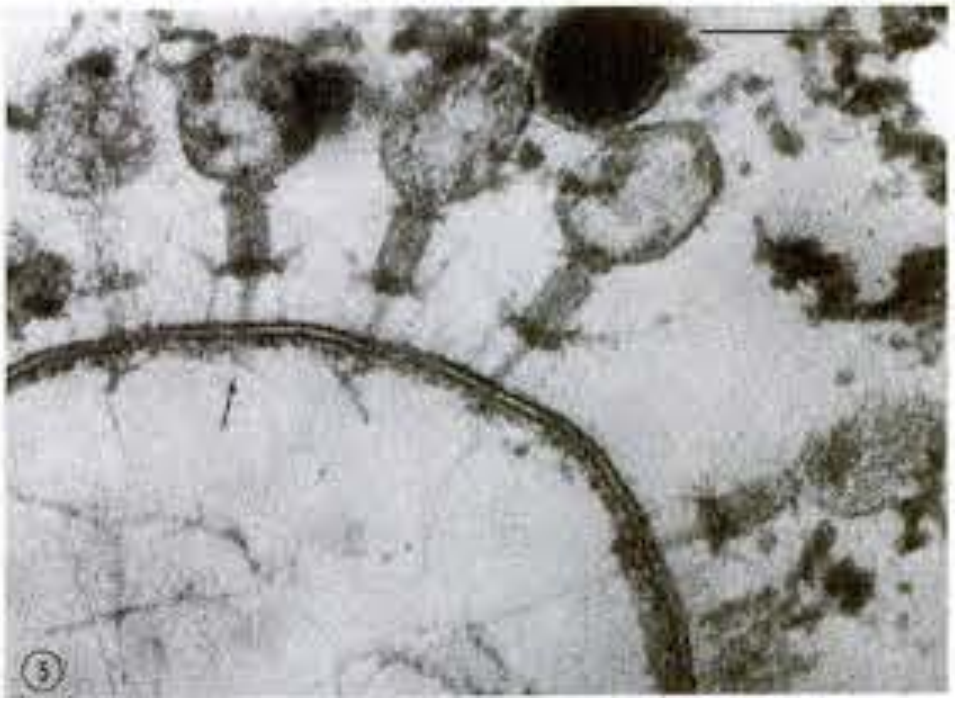
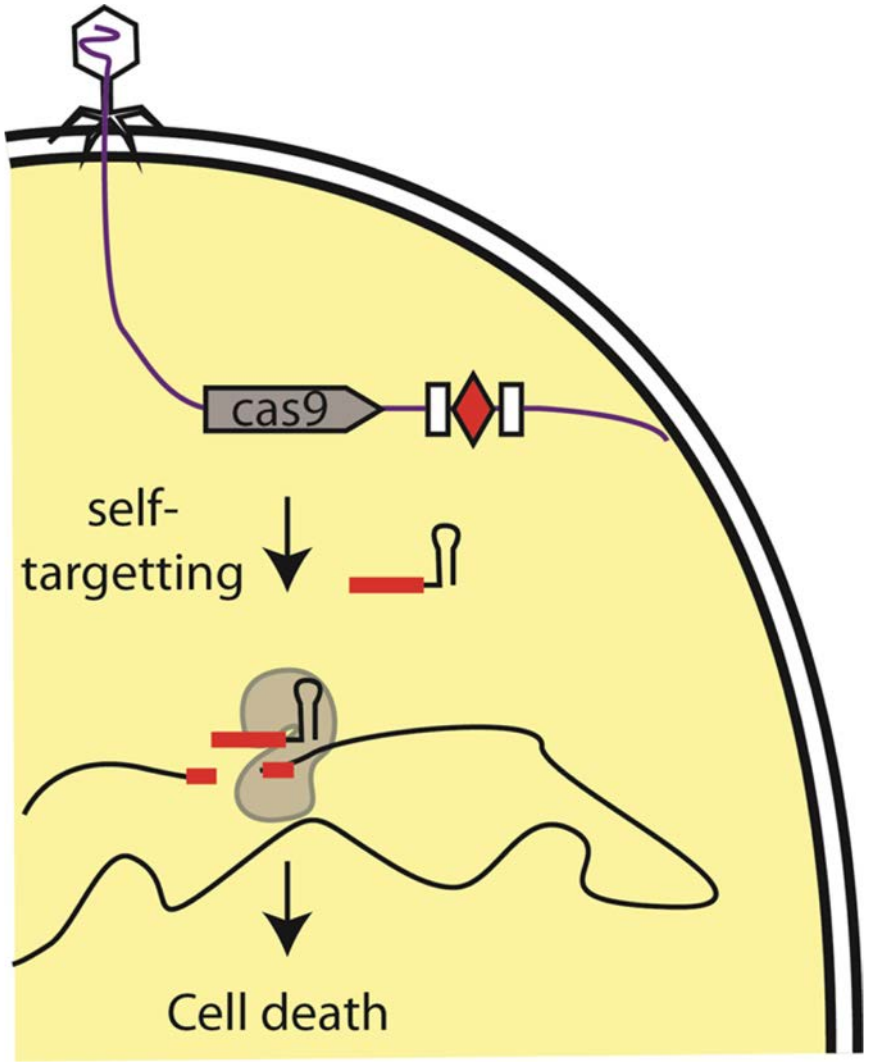
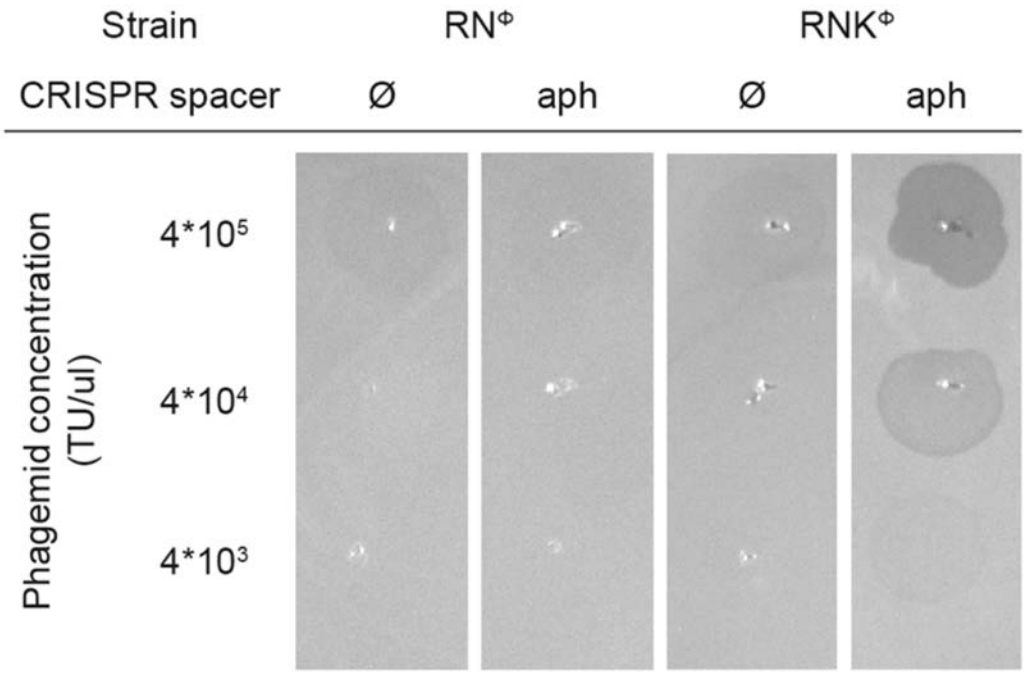


Figure 5 In this thin section of T4 phages each can be seen to be bound directly to the bacterial wall by short tail fibres extending from their baseplates. The tail cores of the phages have just penetrated through the wall (arrow), and dark fibres of DNA extend from the tail tips within the cell

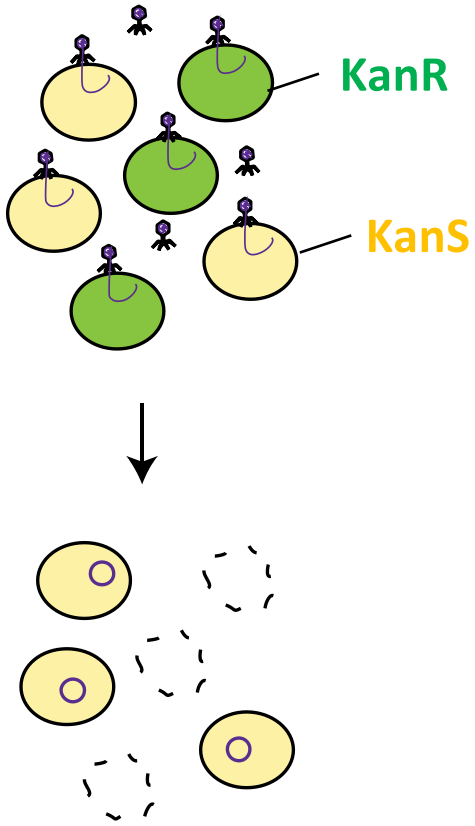
Sequence specific killing with Cas9

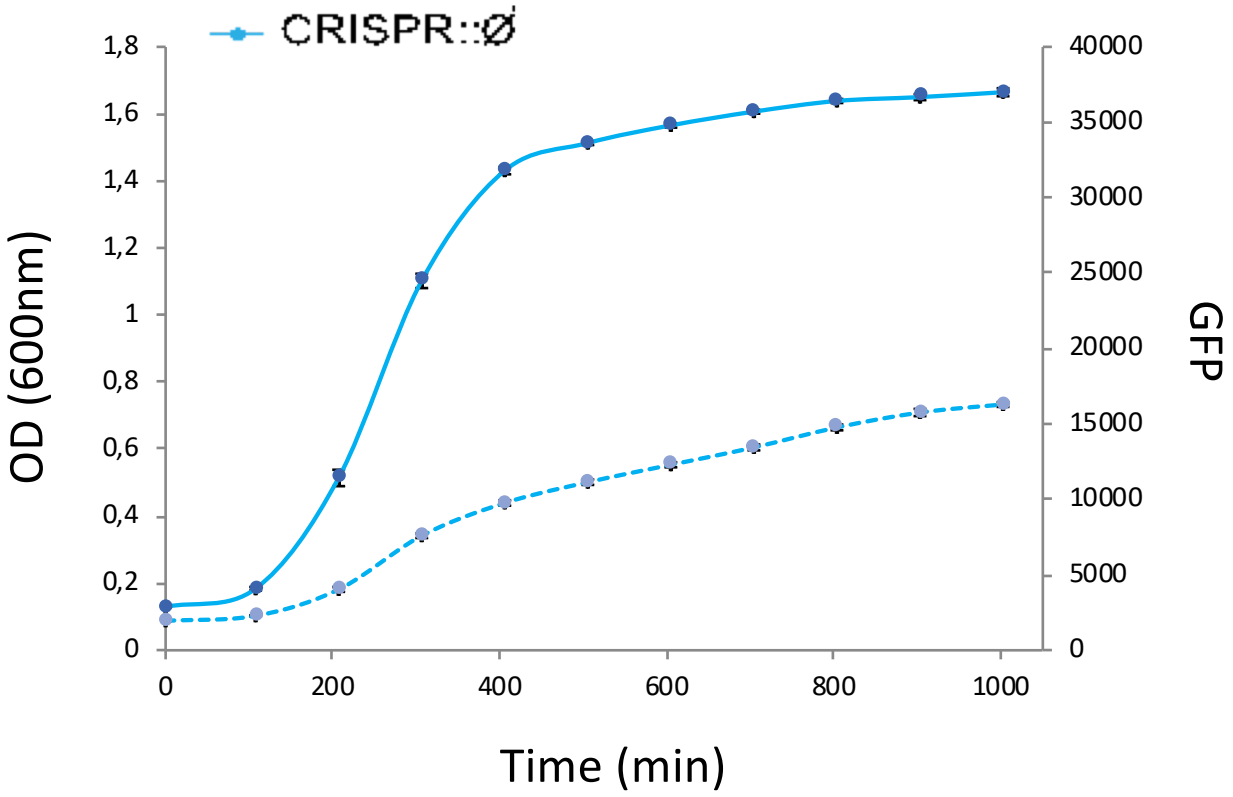




Bikard & al., Nat Biotech, 2014

Competition with non-targeted strain





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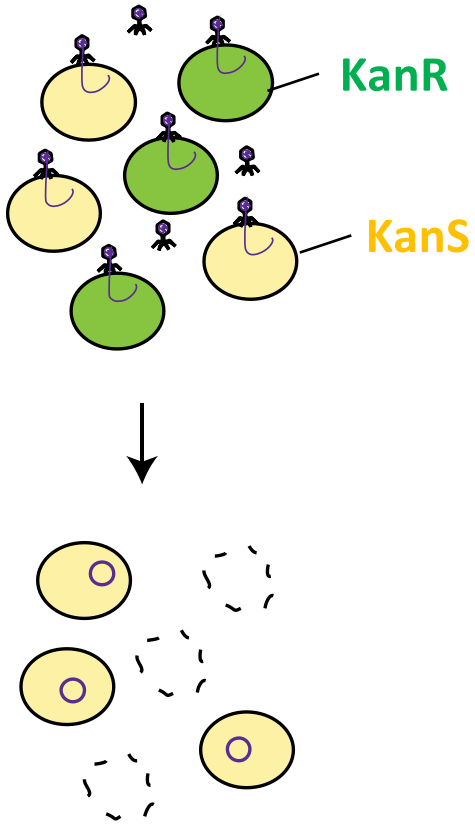

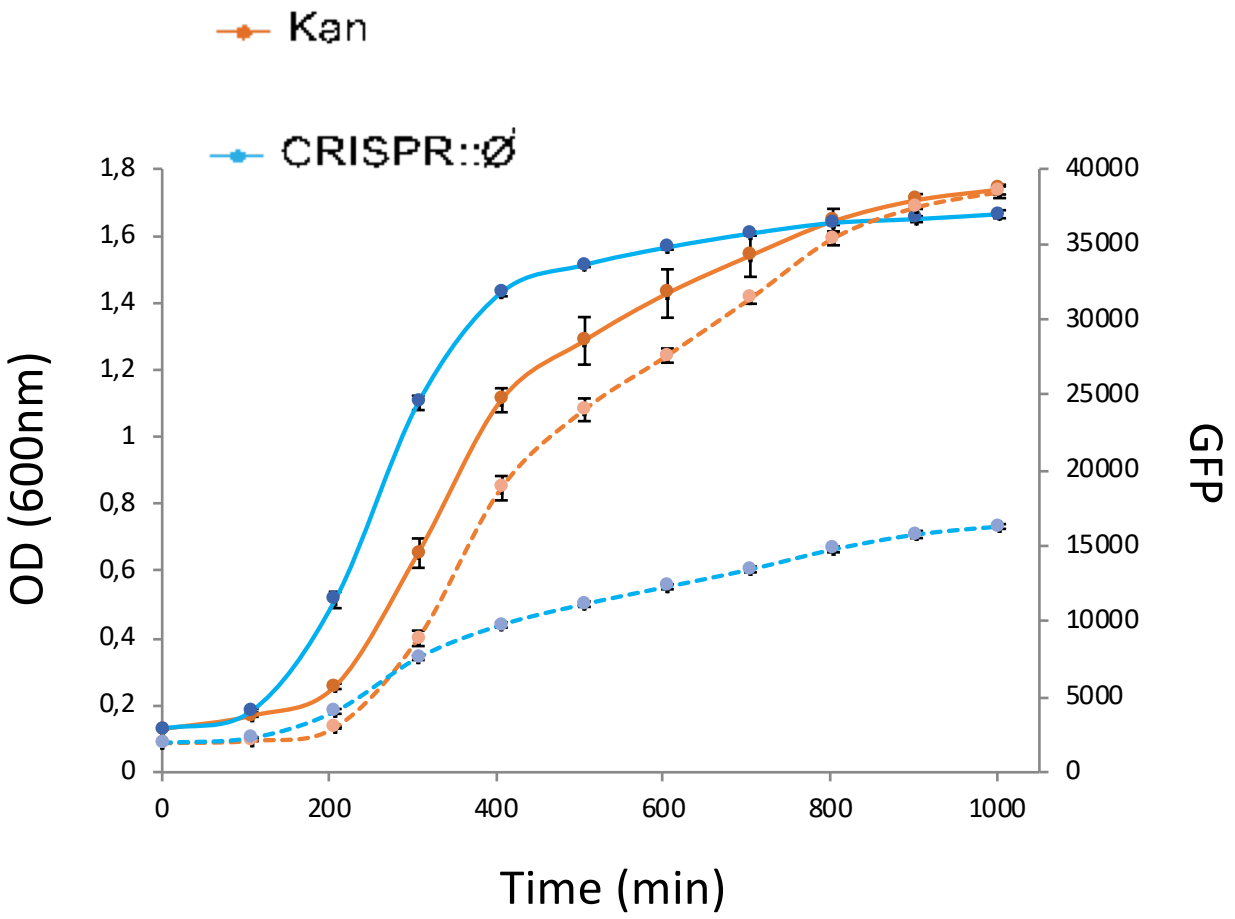

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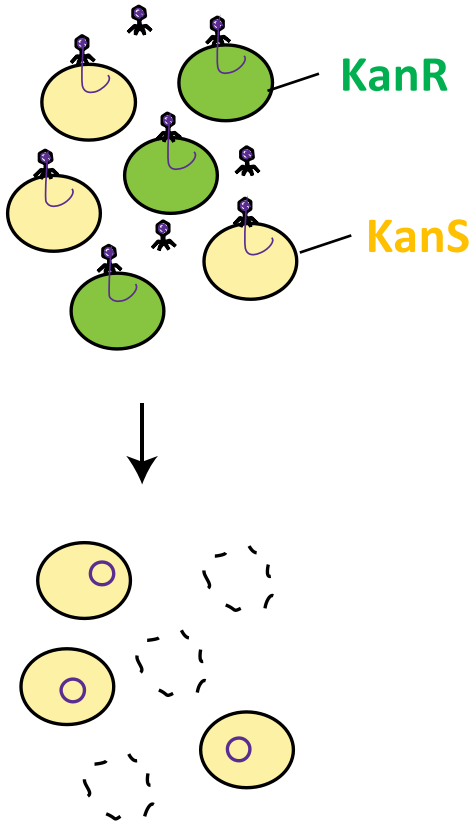
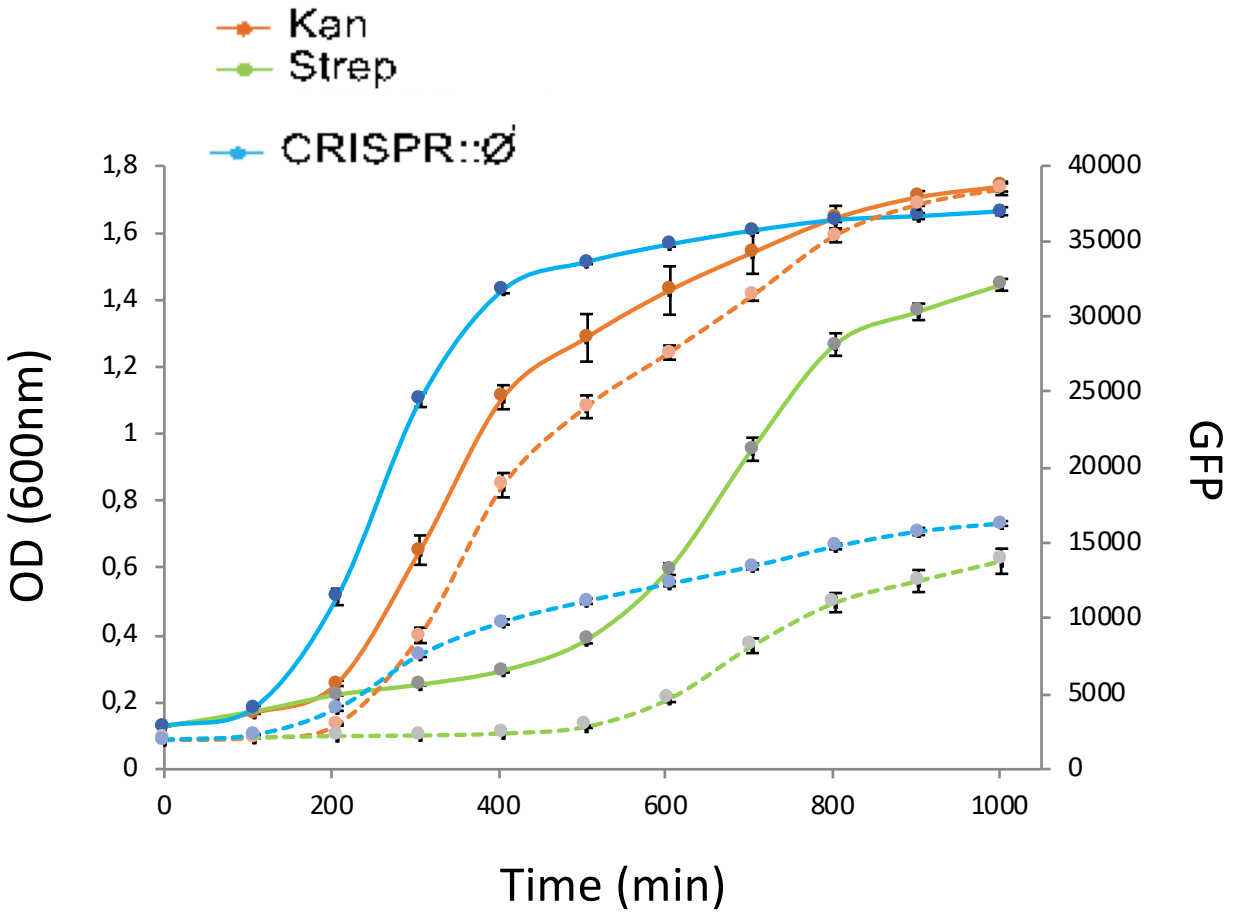
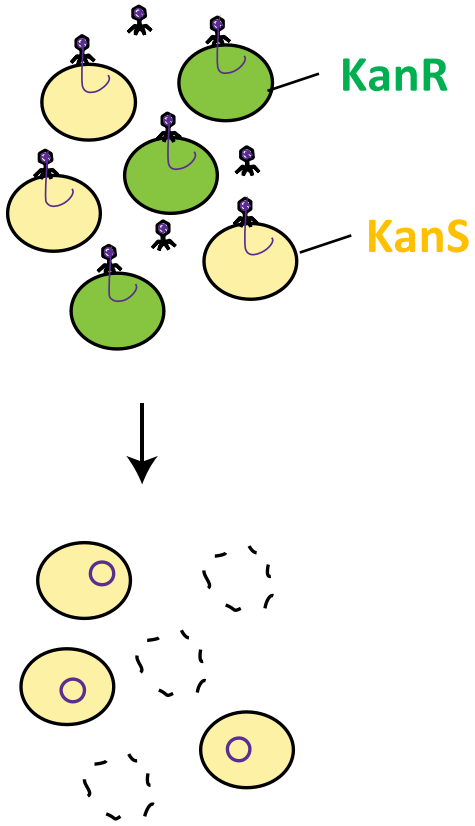
Competition with non-targeted strain

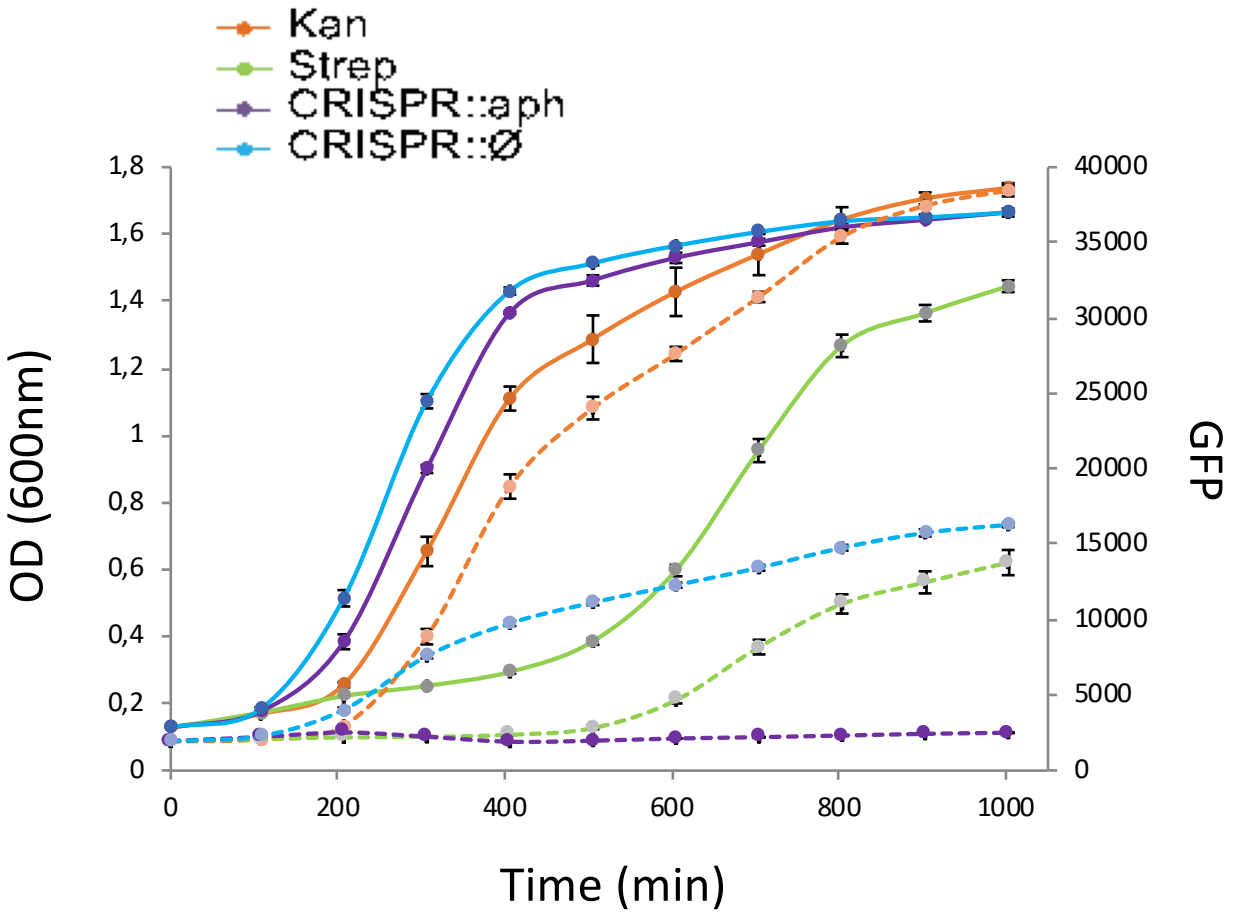




Bikard & al., Nat Biotech, 2014

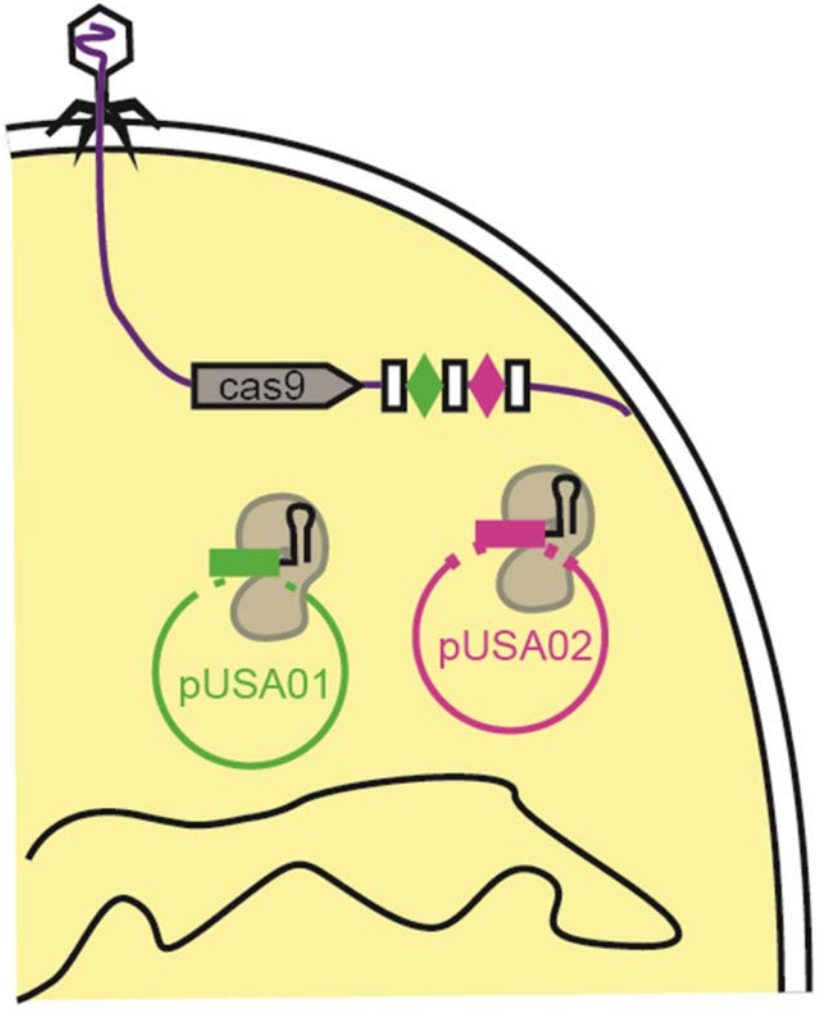
Competition with non-targeted strain

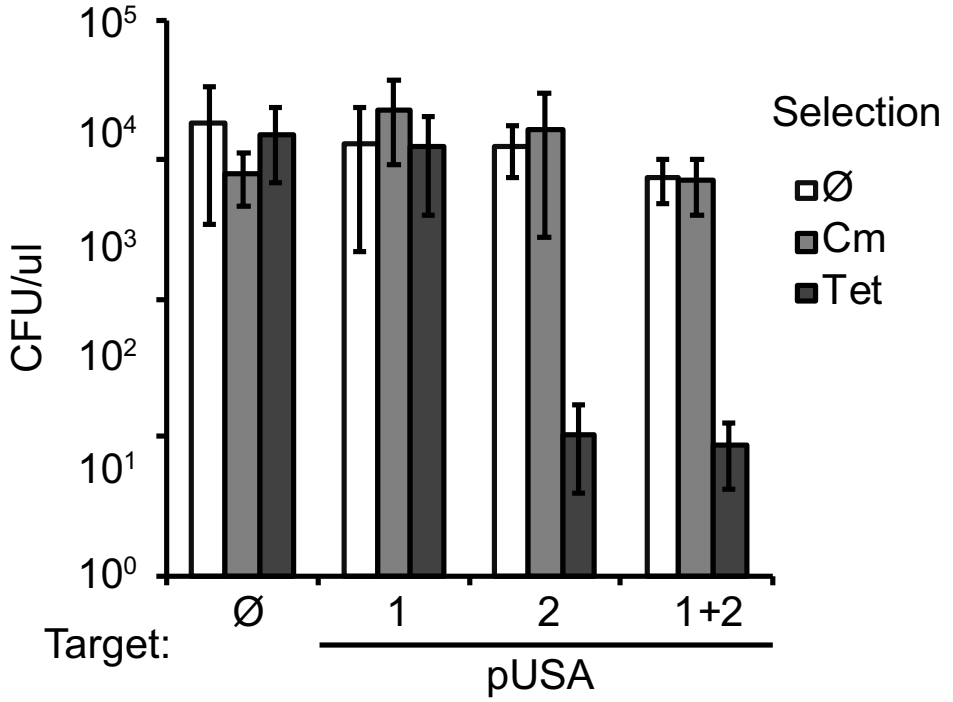


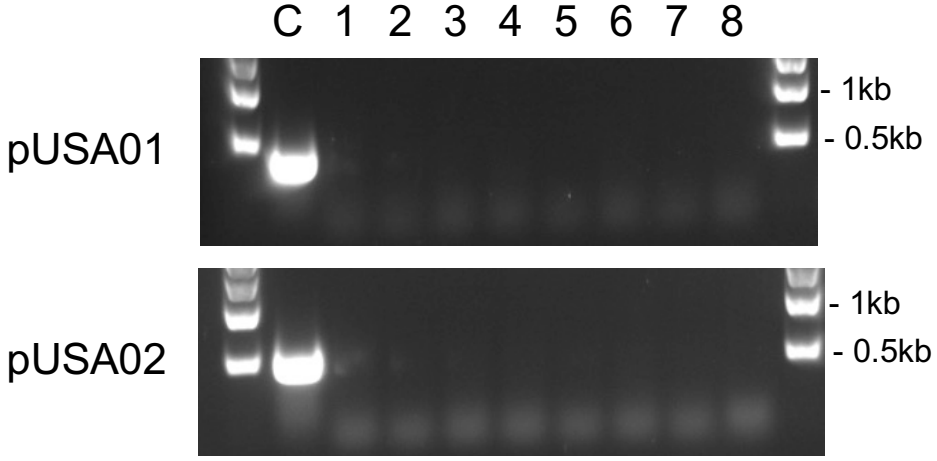


Bikard & al., Nat Biotech, 2014

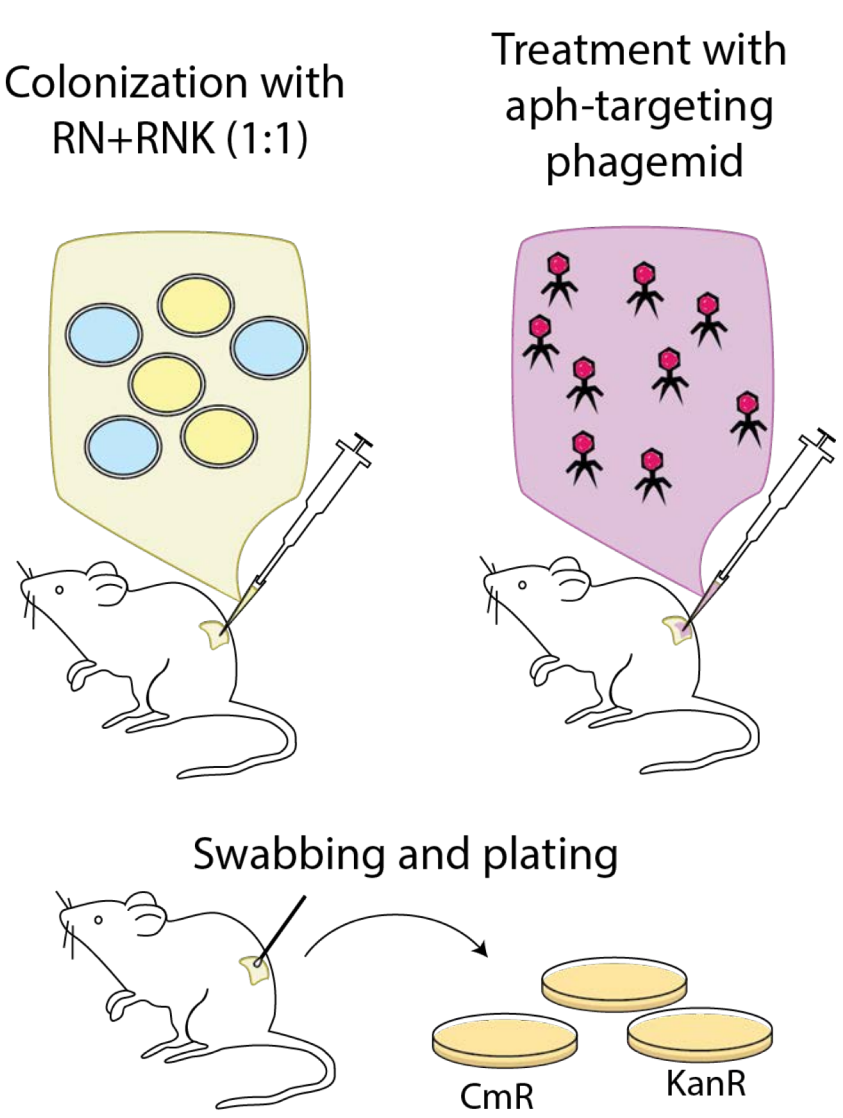
Plasmid curing:
re-sensitizing bacteria to antibiotics

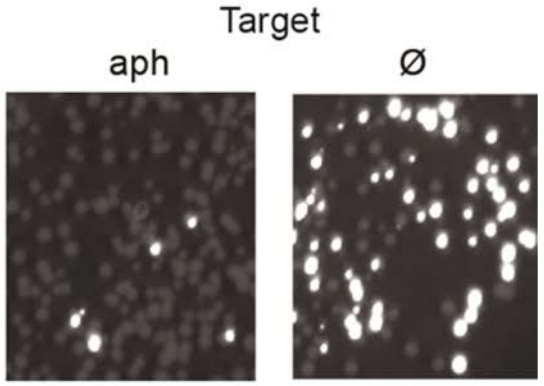


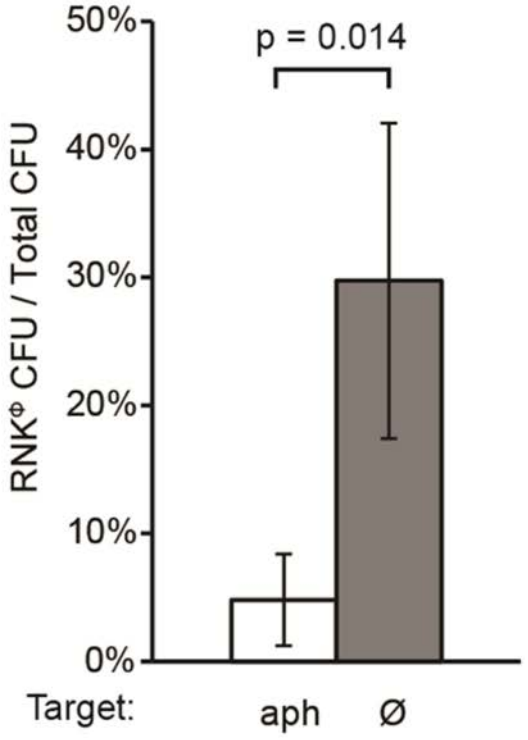




Specific decolonization of antibiotic resistant *S. aureus* on the mouse skin







Chad Euler, Rockefeller
Vince Fischetti, Rockefeller

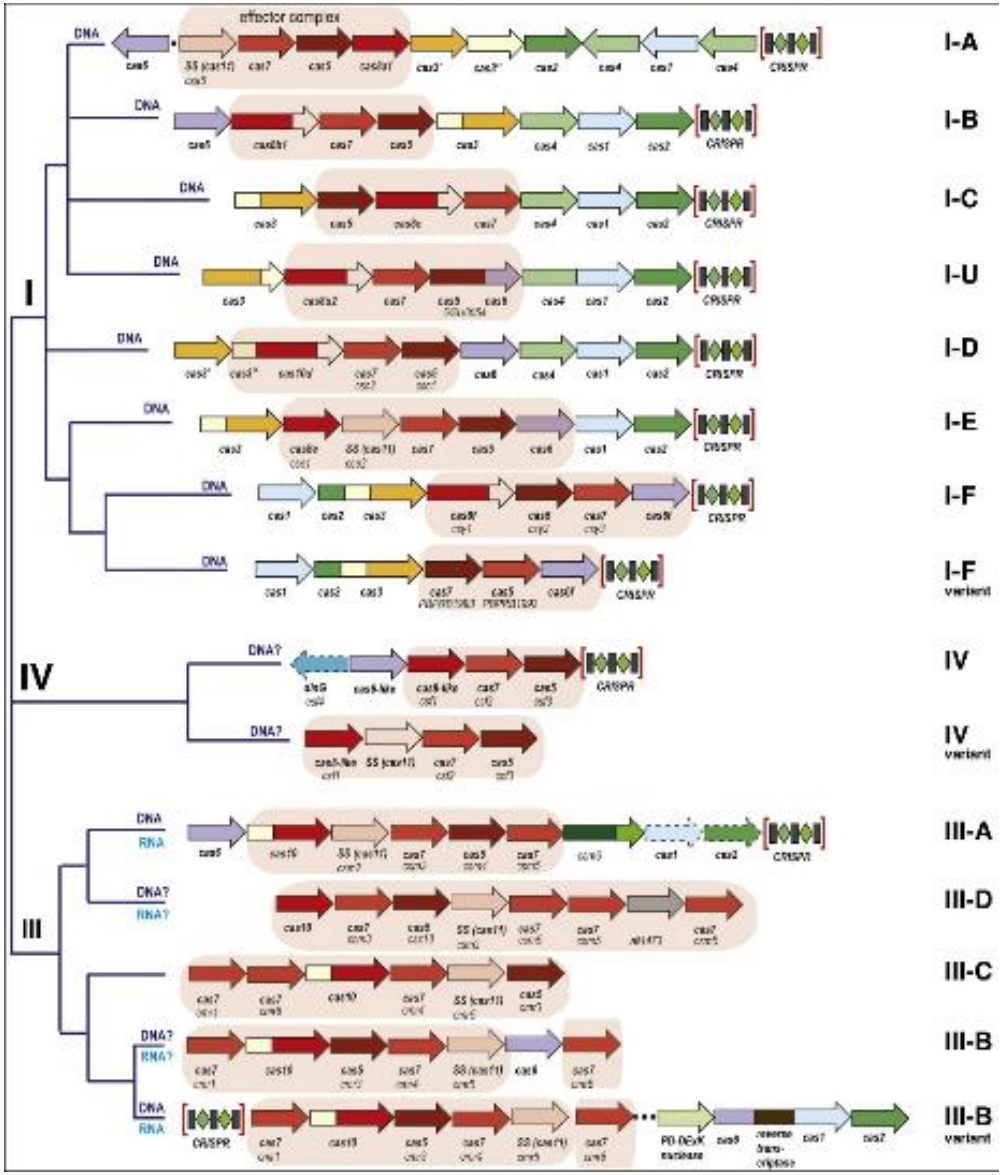

eligo
bioscience

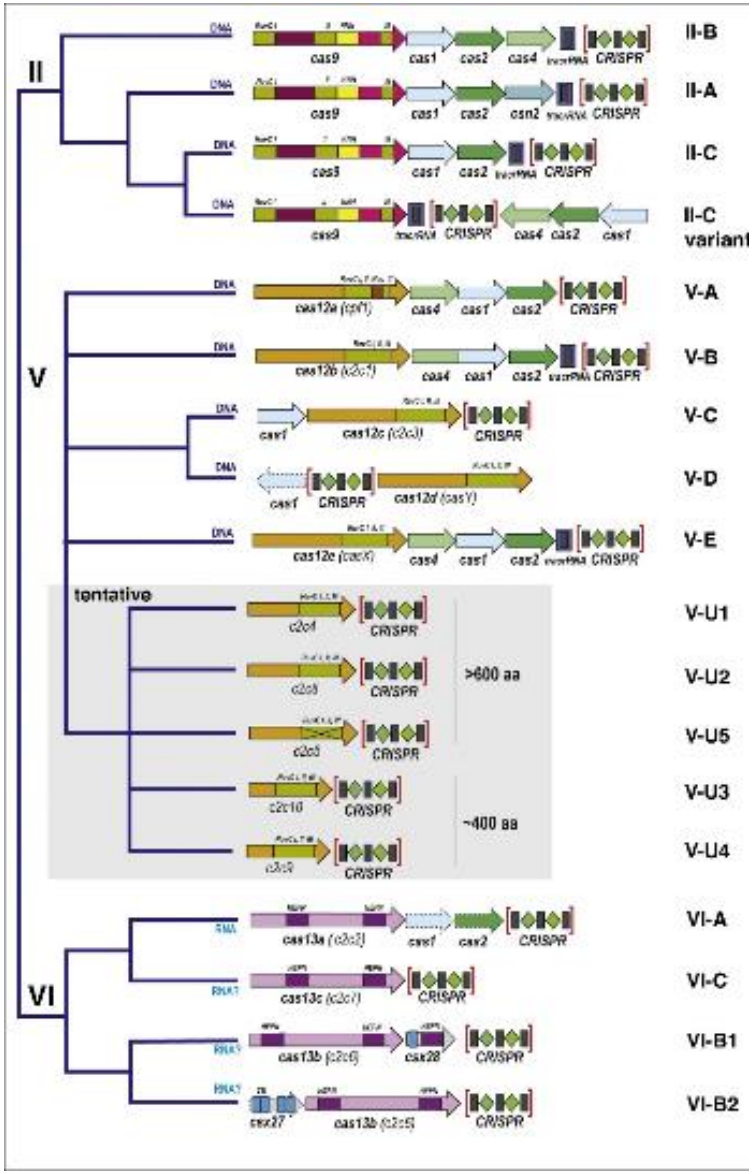
CRISPR diagnostics

The diversity of CRISPR-Cas system

Class 1

Class 2



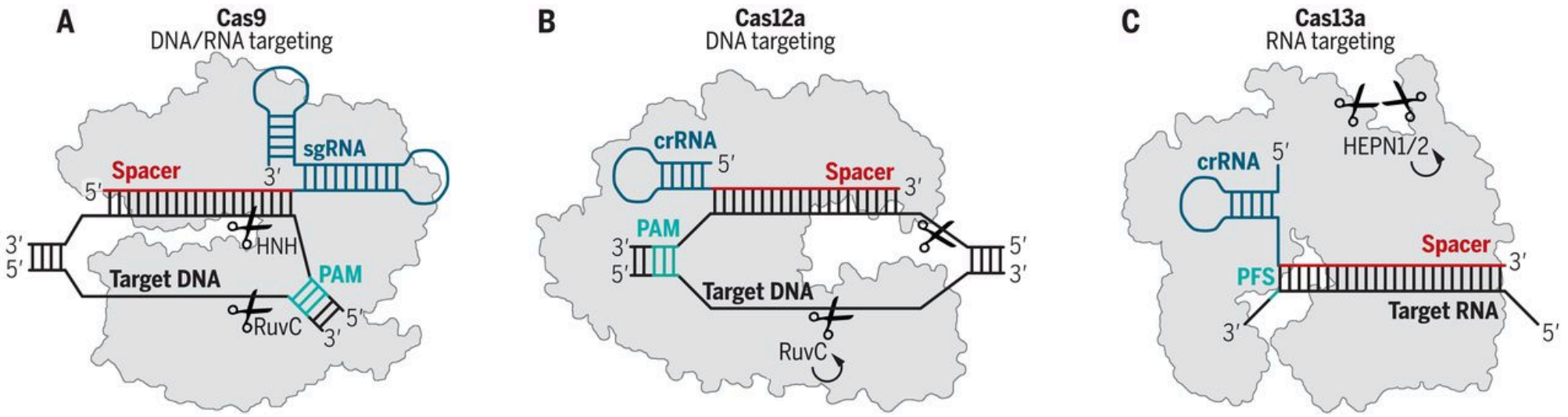


Koonin & al., Current Opinion in Microbiology 2017

Cas9

Cas12

Cas13



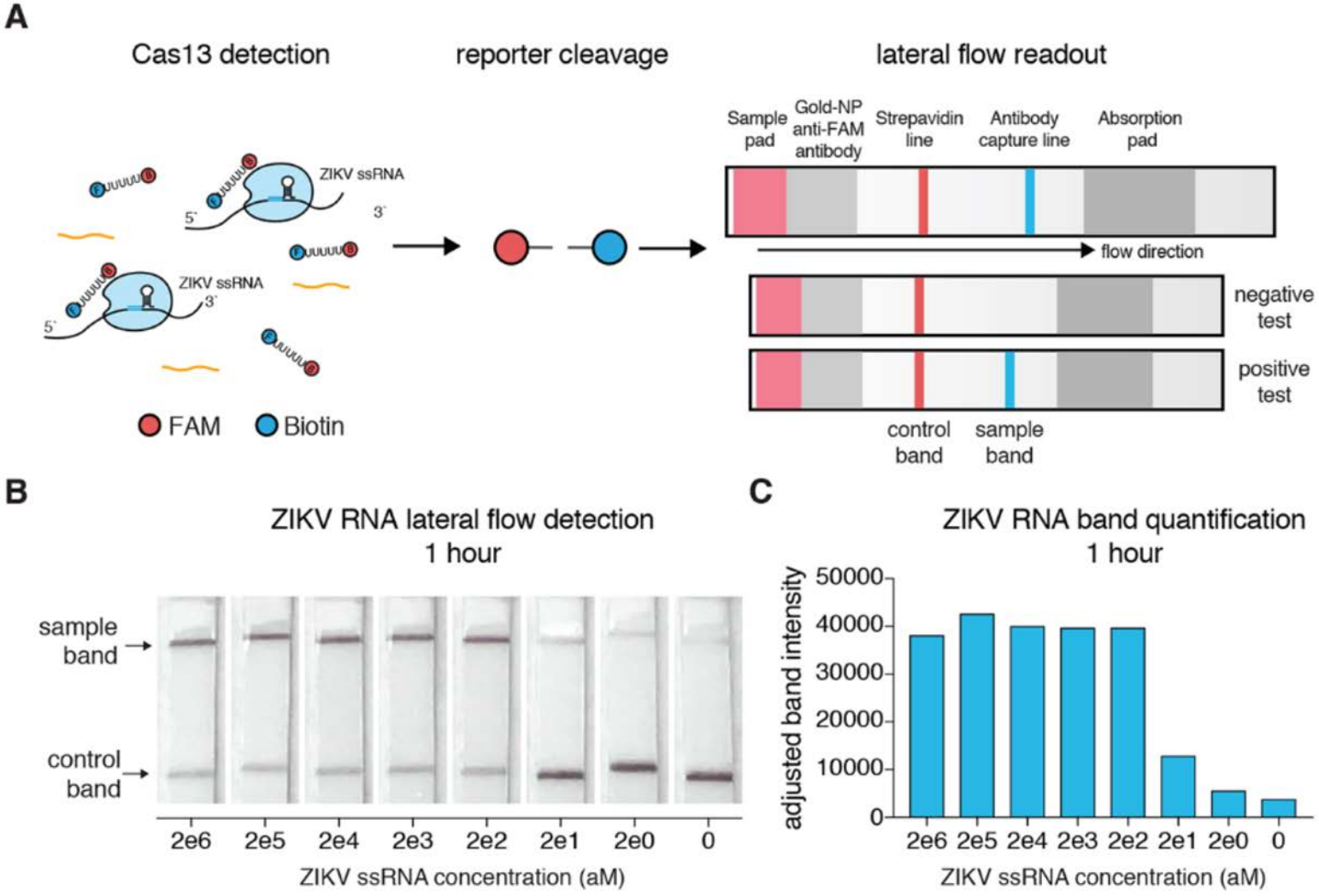
Science

REPORTS

Cite as: J. S. Gootenberg *et al.*, *Science* 10.1126/science.aaq0179 (2018).

Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6

Jonathan S. Gootenberg,[1,2,3,4,7*] Omar O. Abudayyeh,[1,2,3,4,5*] Max J. Kellner,[1] Julia Joung,[1,2,3,4] James J. Collins,[1,4,5,6,8] Feng Zhang[1,2,3,4†]



RESEARCH ARTICLE

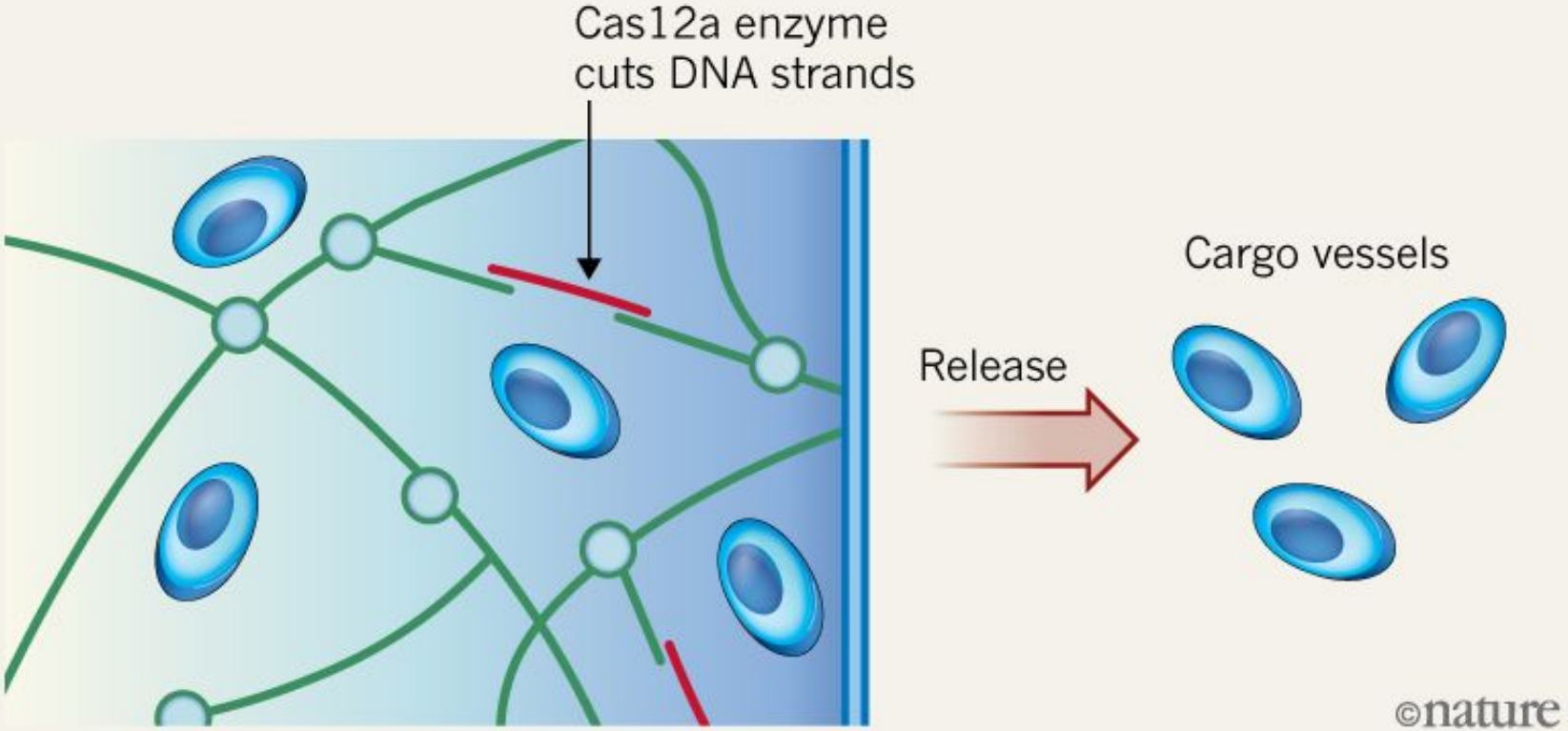
Programmable CRISPR-responsive smart materials

Max A. English[1,2,*], Luis R. Soenksen[2,3,4,*], Raphael V. Gayet[1,2,5,*], Helena de Puig[2,4,*], Nicolaas M. Angenent-Mari[1,2,4,†], An...
+ See all authors and affiliations

Science 23 Aug 2019:
Vol. 365, Issue 6455, pp. 780-785
DOI: 10.1126/science.aaw5122

CRISPR-CONTROLLED GEL

Researchers have created a smart hydrogel material that is held together by DNA. The CRISPR–Cas12a protein cuts the DNA strands, changing the gel's shape, which can be controlled to release drugs, particles or even switch an electronic circuit.

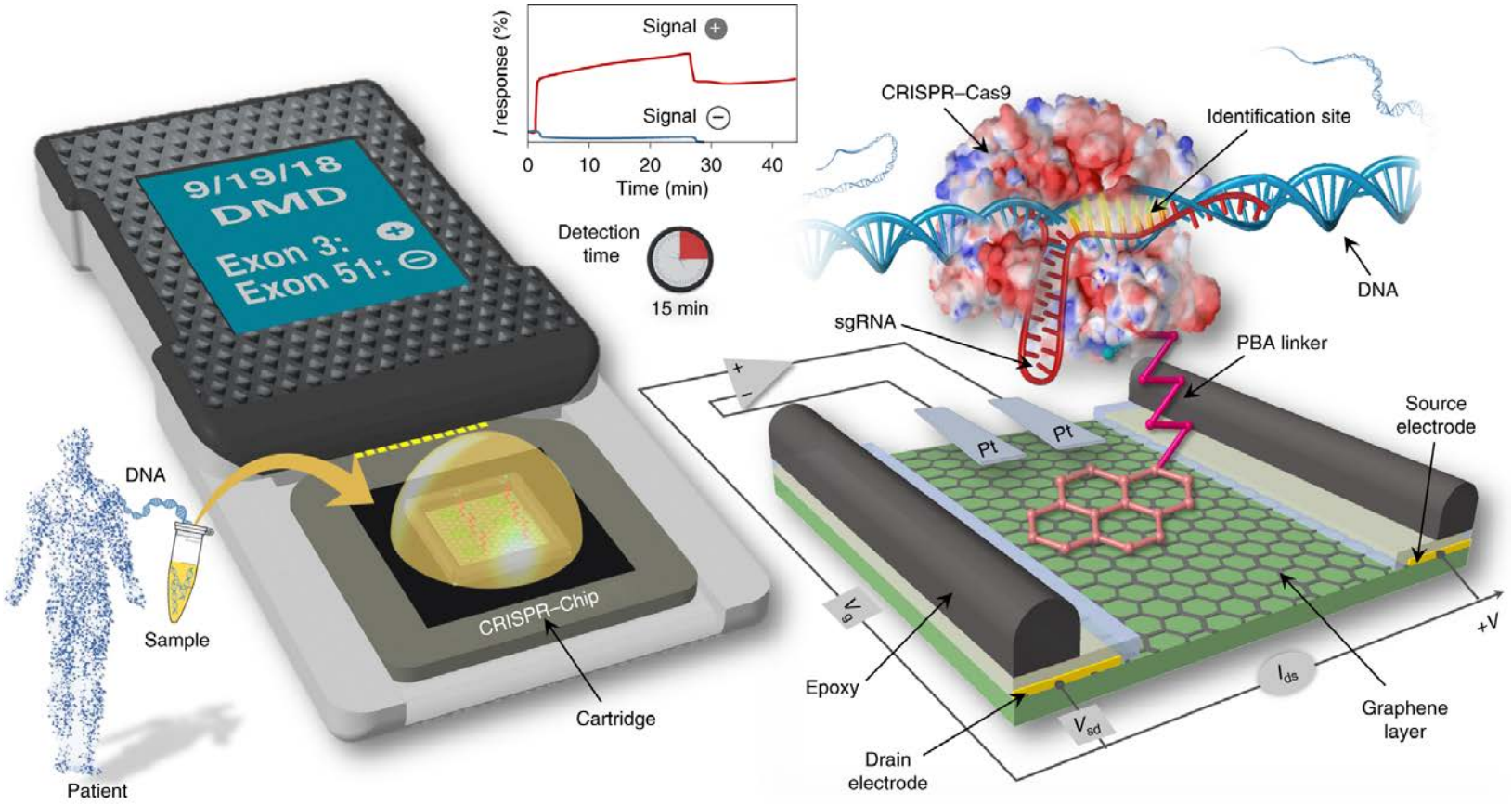


Article | Published: 25 March 2019

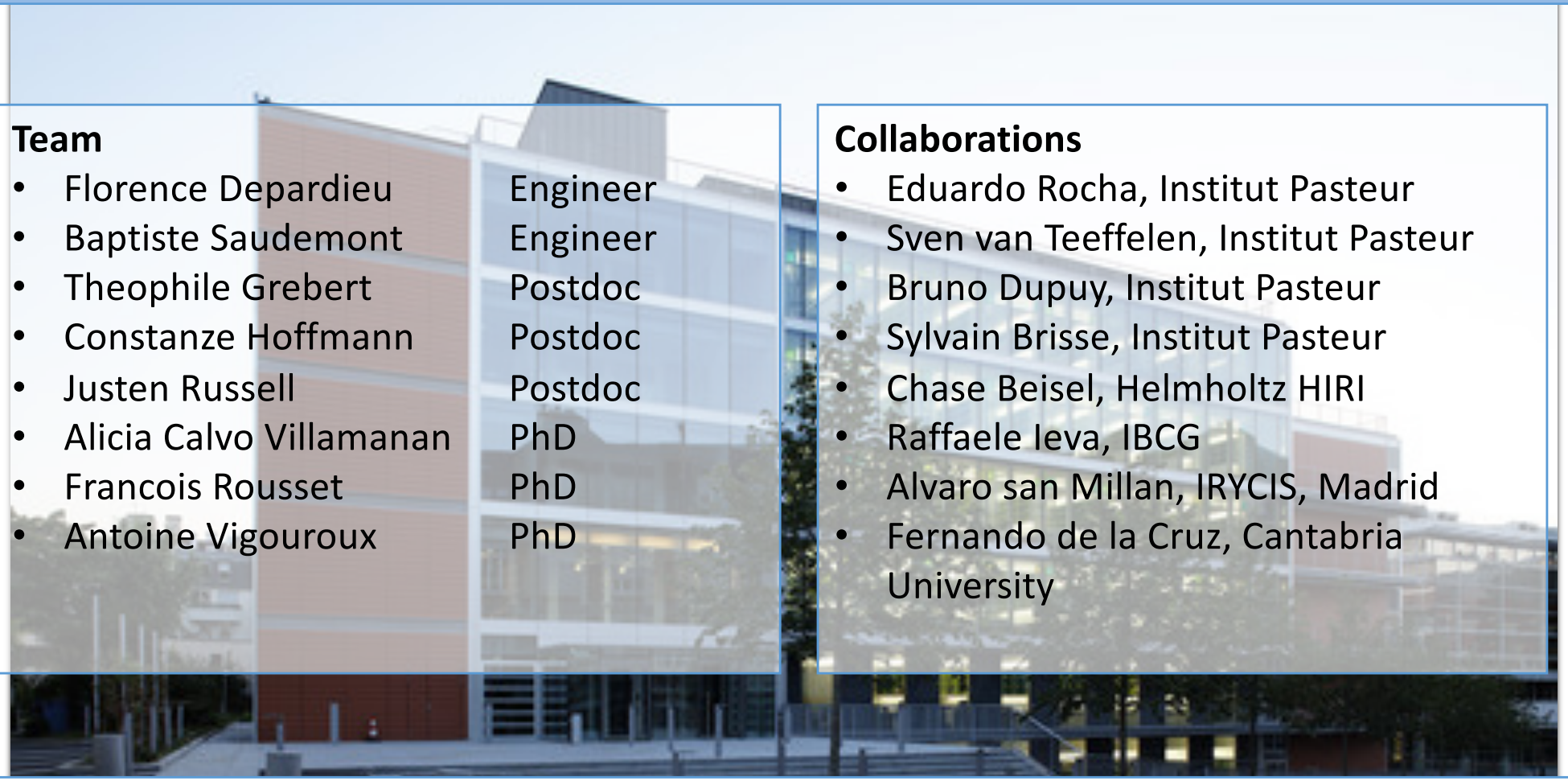
Detection of unamplified target genes via CRISPR–Cas9 immobilized on a graphene field-effect transistor

Reza Hajian, Sarah Balderston, Thanhtra Tran, Tara deBoer, Jessy Etienne, Mandeep Sandhu, Noreen A. Wauford, Jing-Yi Chung, Jolie Nokes, Mitre Athaiya, Jacobo Paredes, Regis Peytavi, Brett Goldsmith, Niren Murthy, Irina M. Conboy & Kiana Aran

Nature Biomedical Engineering **3**, 427–437 (2019) | Download Citation



The Synthetic Biology Group



Team

• Florence Depardieu	Engineer
• Baptiste Saudemont	Engineer
• Theophile Grebert	Postdoc
• Constanze Hoffmann	Postdoc
• Justen Russell	Postdoc
• Alicia Calvo Villamanan	PhD
• Francois Rousset	PhD
• Antoine Vigouroux	PhD

Collaborations

- Eduardo Rocha, Institut Pasteur
- Sven van Teeffelen, Institut Pasteur
- Bruno Dupuy, Institut Pasteur
- Sylvain Brisse, Institut Pasteur
- Chase Beisel, Helmholtz HIRI
- Raffaele Ieva, IBCG
- Alvaro san Millan, IRYCIS, Madrid
- Fernando de la Cruz, Cantabria University

Funding

LabEx IBEID

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European Research Council
Established by the European Commission