Microbial allies in Disease Detection and Treatment

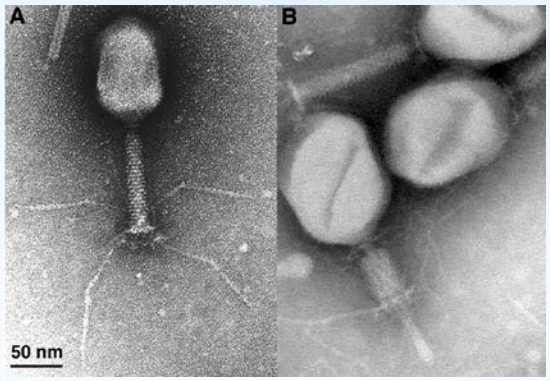
Journal club presentation
Vijay Chandrasekar
16.06.2015

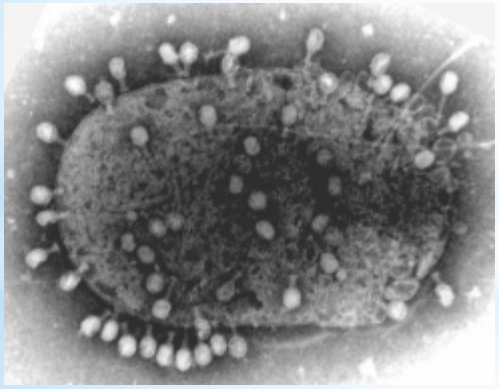
Our inner ecosystem

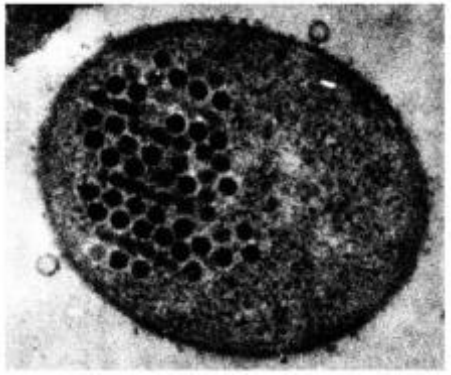
Over 100 TRILLION bacteria (including perhaps a billion or more E.Coli)

Individuals taking certain antibiotics may experience a nearly 3/4 reduction in Vitamin K production

Bacteriophages







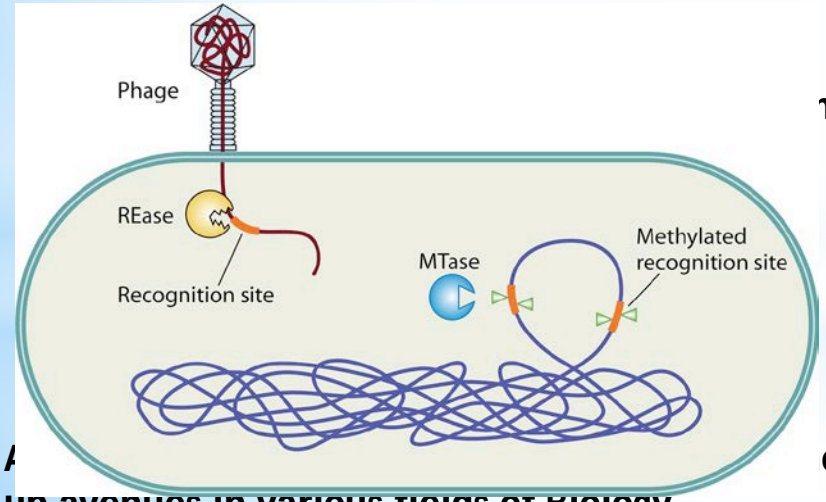
Bacterial Genetics:

Subfield of genetics devoted to the study of bacteria and bacteriophages

Bacterial defense system: Restriction modification systems:

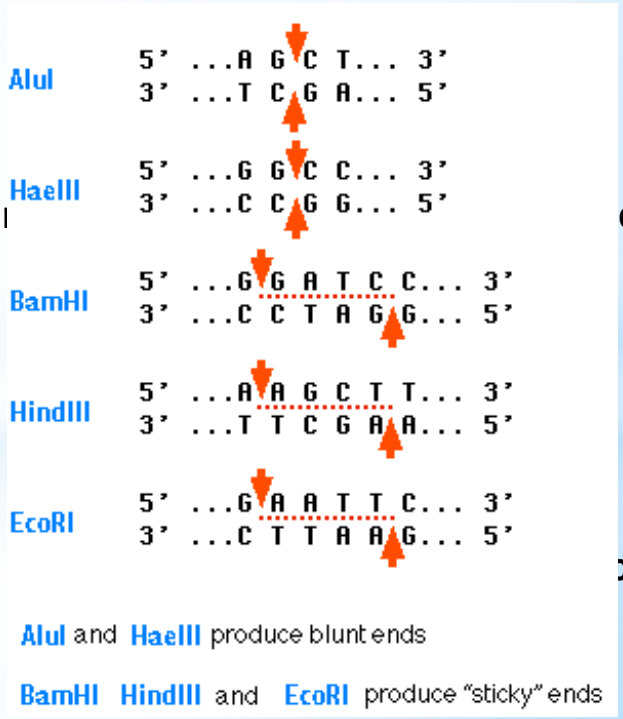
Type II : Over 3000 known and 600 commercially available Restriction enzymes

Type V: CRISPR-CAS9 prokaryotic immune system



nd the origi ce are

A e bacterial opening up avenues in various fields of Biology.



PNAS

Temperate and lytic bacteriophages programmed to sensitize and kill antibiotic-resistant bacteria

Ido Yosef[1], Miriam Manor[1], Ruth Kiro, and Udi Qimron[2]

Department of Clinical Microbiology and Immunology, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel

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LETTERS

nature biotechnology

Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials

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Science Translational Medicine AAAS

Programmable probiotics for detection of cancer in urine

Tal Danino *et al.*
Sci Transl Med **7**, 289ra84 (2015);
DOI: 10.1126/scitranslmed.aaa3519

Editor's Summary

Synthetic bacteria for tumor detection

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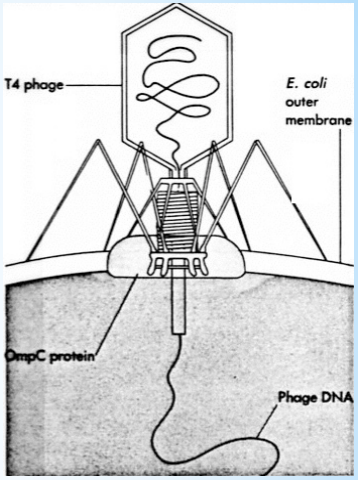
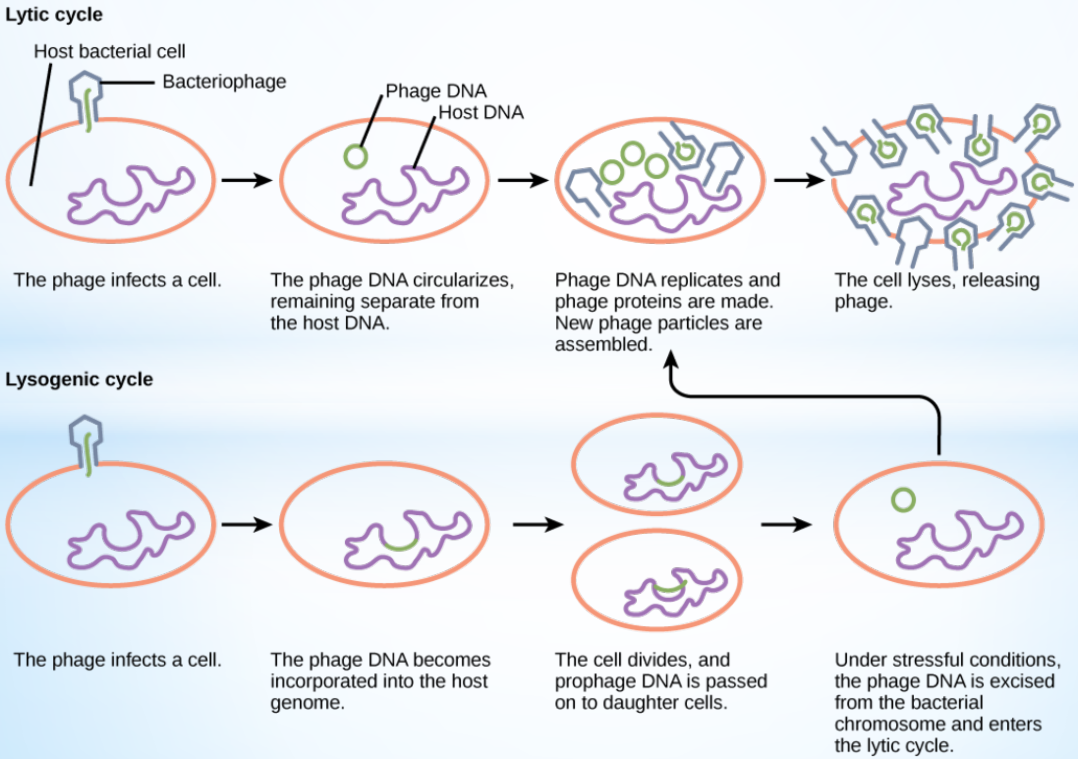
Bacteriophages

Bacteriophages were discovered independently by Twort (1915) in London & by d'Herelle (1917) at the Institut Pasteur in Paris.

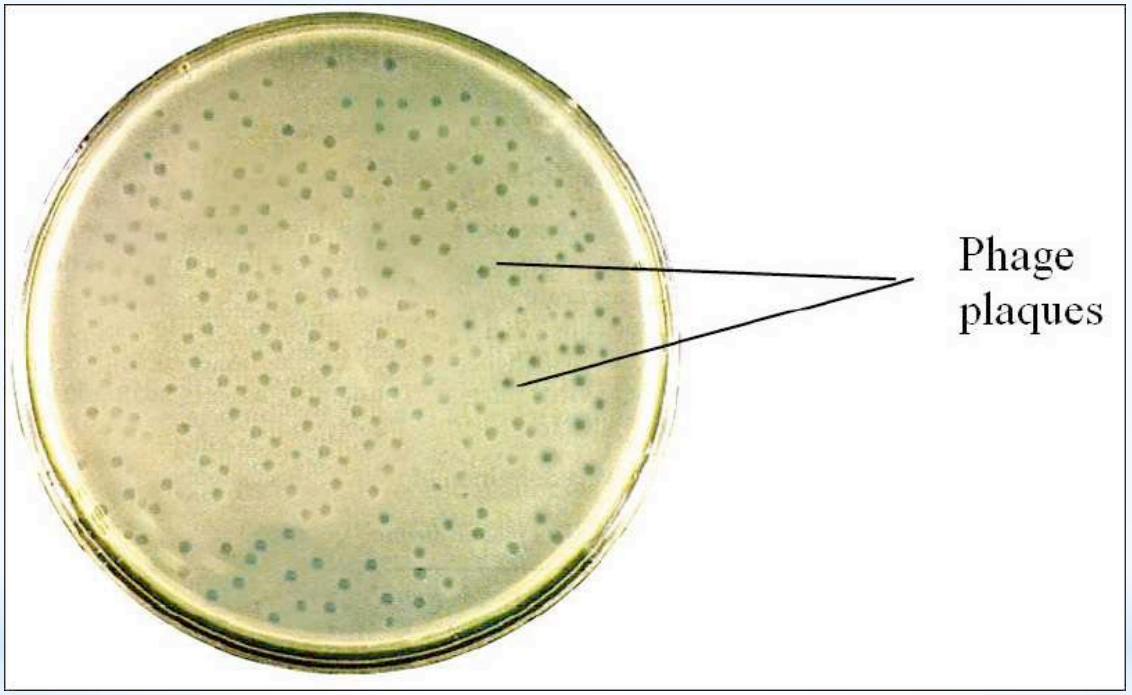
18 families infecting bacteria and Archea

Protein capsids that encapsulate a DNA or RNA genome

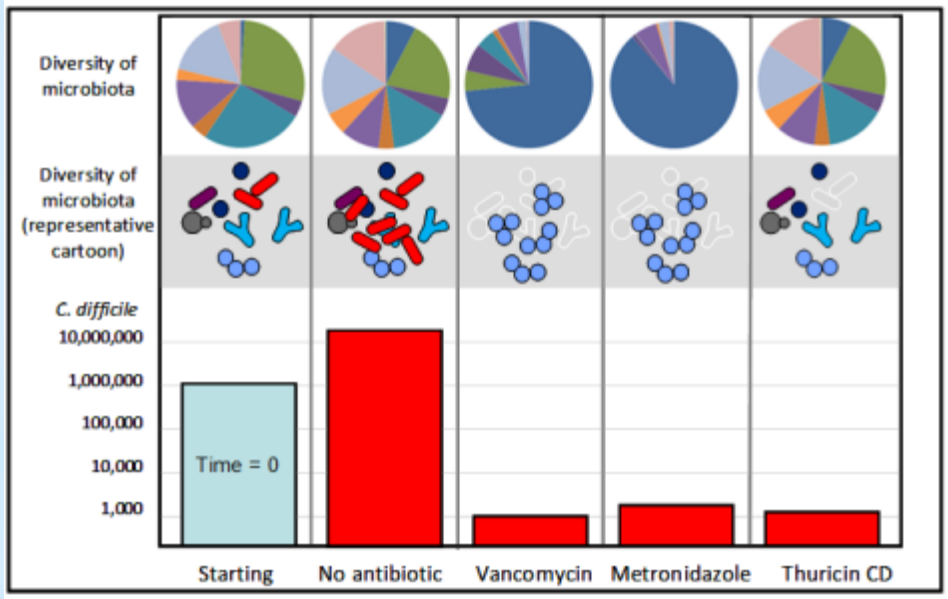




The life cycle of a T-phage takes about 25-35 minutes to complete.


Phage
plaques

Impact of antimicrobials



(all at 90 μM) after 24 hrs on the gut microbiota as revealed by high throughput sequencing-based analysis

ESKAPE

Enterococcus faecium,
Staphylococcus aureus,
Klebsiella pneumoniae,
Acinetobacter baumannii,
Pseudomonas aeruginosa
Enterobacter sp

Cotter, 2015

Phage therapy is widely used in Russia, Georgia and Poland

Phage therapy: mainly on treatment for wounds and intestinal infections (topically in ointments, sprays, and dressings or capsules)

Absence of “Herxheimer reaction”

FDA approved “Ecoshield”, Listshield”, “Salmofresh” – Phage mix against S.enterica, E.coli, L. monocytogenes. For targeted eradication of pathogens even in consumables.

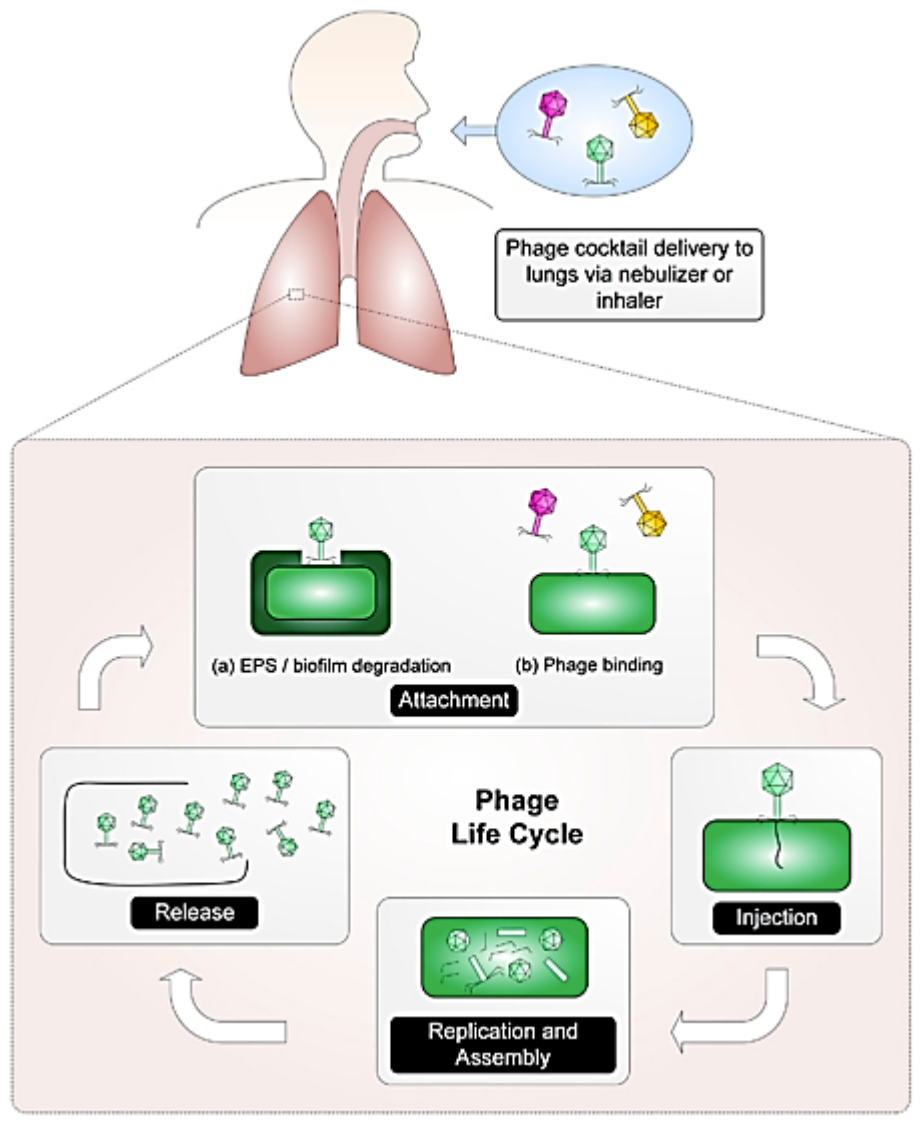
	Antibiotics	Phage therapy
Specificity	Broad spectrum, affecting more than the targeted organism	Generally species or strain specific
Side effects	Many, including allergies and intestinal disorders	No side effects (Bruttin and Brüssow, 2005; Merabishvili et al., 2009; Rhoads et al., 2009; Wright et al., 2009)
Resistance	Occurs and is not limited to targeted bacteria	Occurs, but can be linked to host virulence attenuation (Zahid et al., 2008). Also, phage can co-evolve with host
Development	Time-consuming and expensive	Rapid

A time period of only a few days or weeks is needed to acquire new phages for resistant strains of bacteria, whereas it can take years to obtain new antibiotics.

Phage activity was retained on average for 23 days after application

Semler et al., 2012

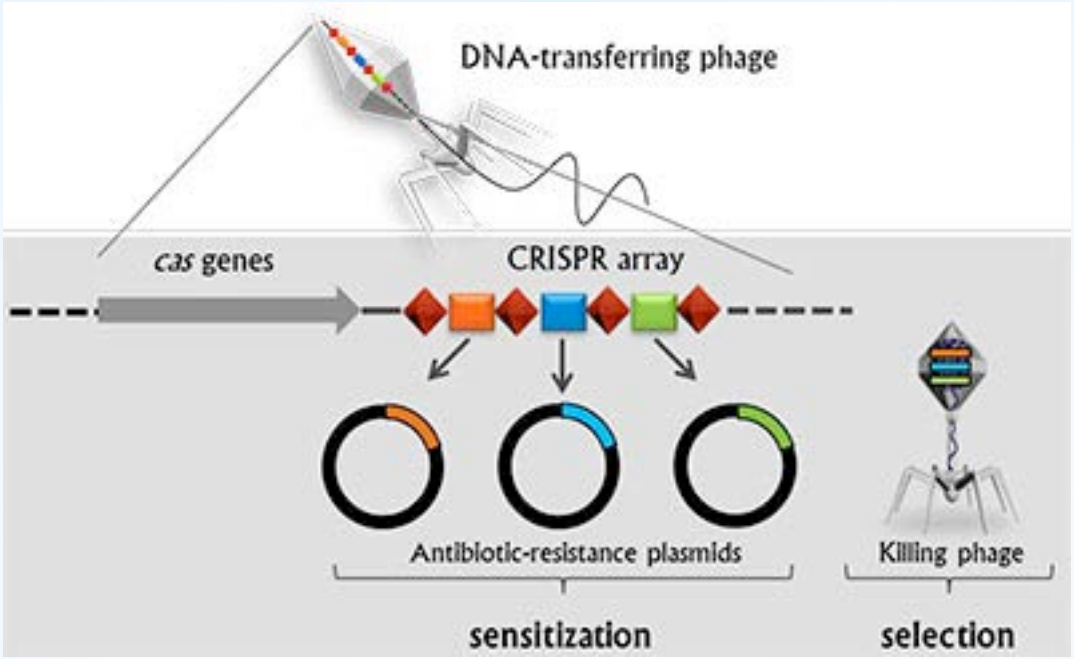
Bacteriophage therapy for Burkholderia cepacia complex respiratory infections



BCC (group of 17 genetically diverse, but phenotypically similar Gram-negative rod-shaped bacteria) are antibiotic pan-resistant

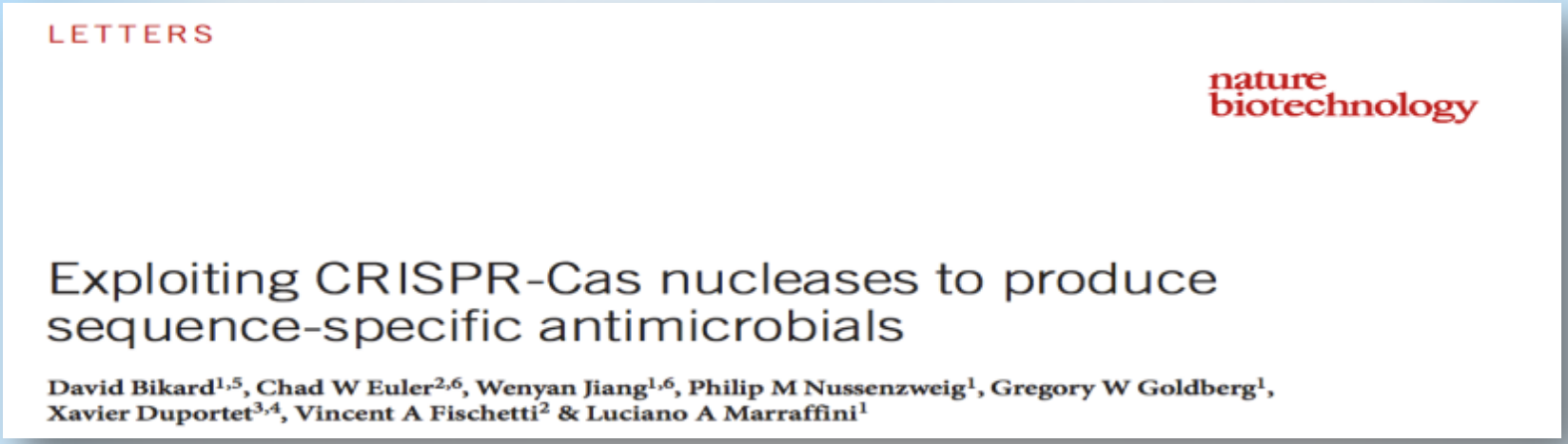
Cepacia syndrome in CF patients

BFC-1 had been tested on eight patients with no reported adverse effects (Merabishvili et al., 2009)



Characterized Phages targeting specifically Antibiotic resistant bacteria

Selective pressure (immunization) to retain loss of resistant to antibiotics



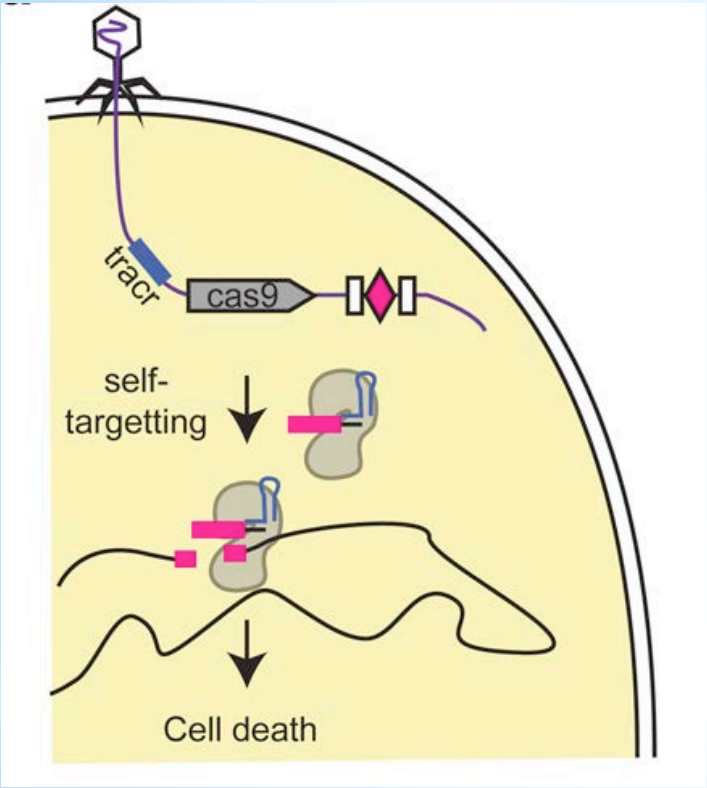
S. aureus

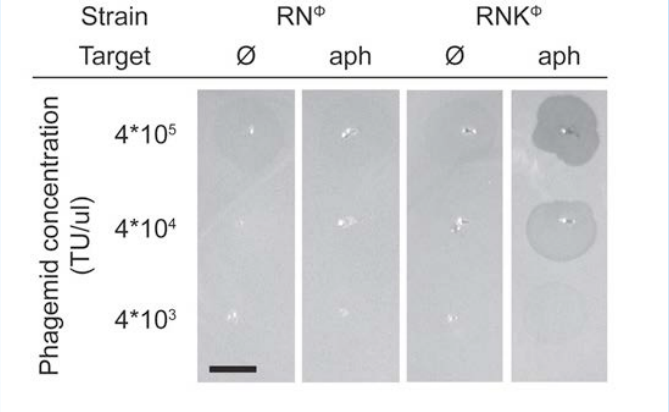
ΦNM1 phagemid

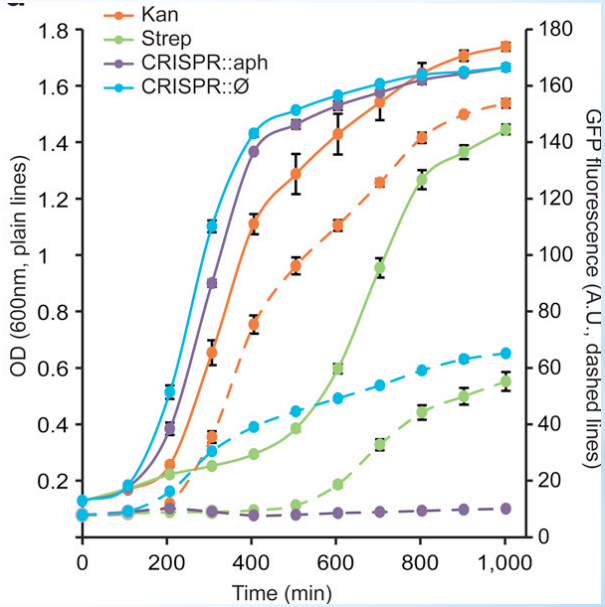
RNΦ-Lysogenic strain of S. aureus resistant to phage infection

RNKΦ-Lysogenic strain of S. aureus containing Kan gene in Chrosmosome

Sequence-specific killing of *S. aureus* by a phagemid-delivered CRISPR system

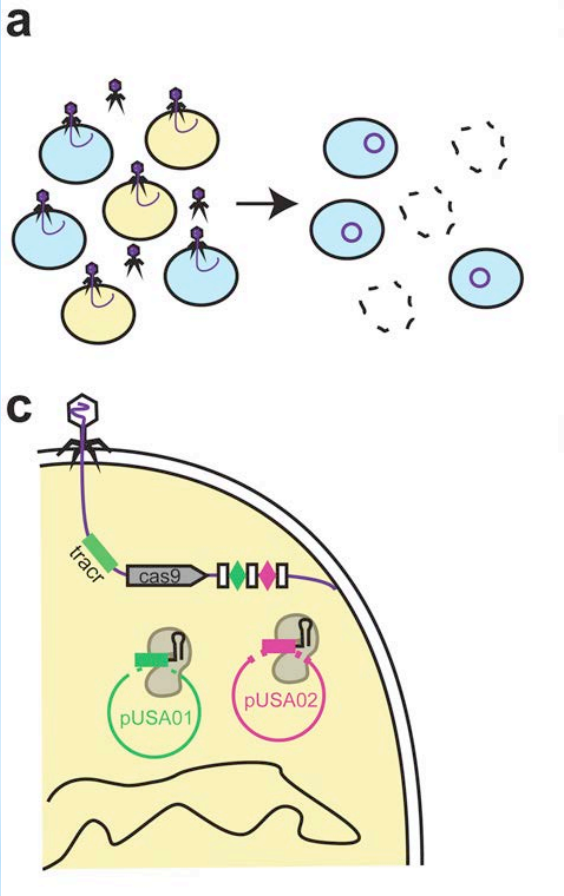


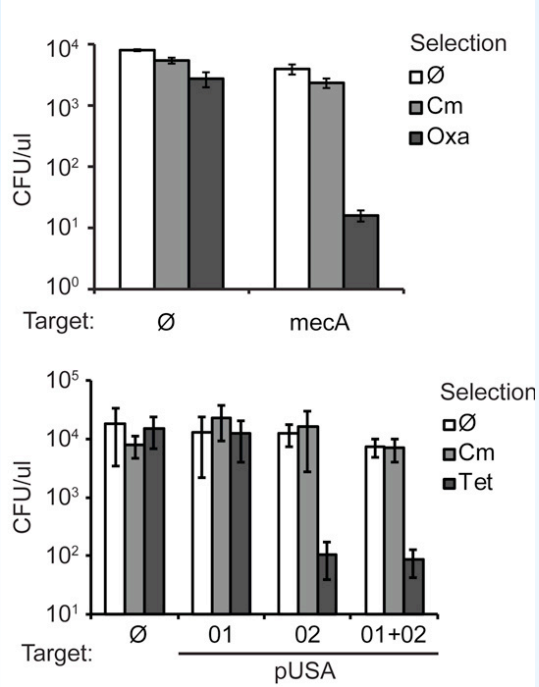


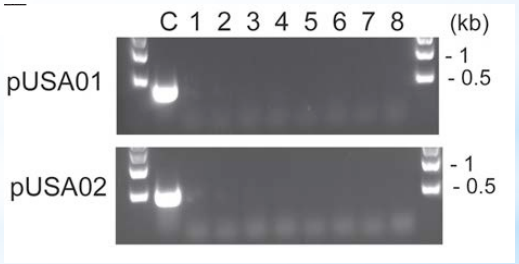


phagemid carries the tracrRNA, *cas9* and a programmable CRISPR array sequence

Targeting antibiotic resistance genes and plasmids in an MRSA strain





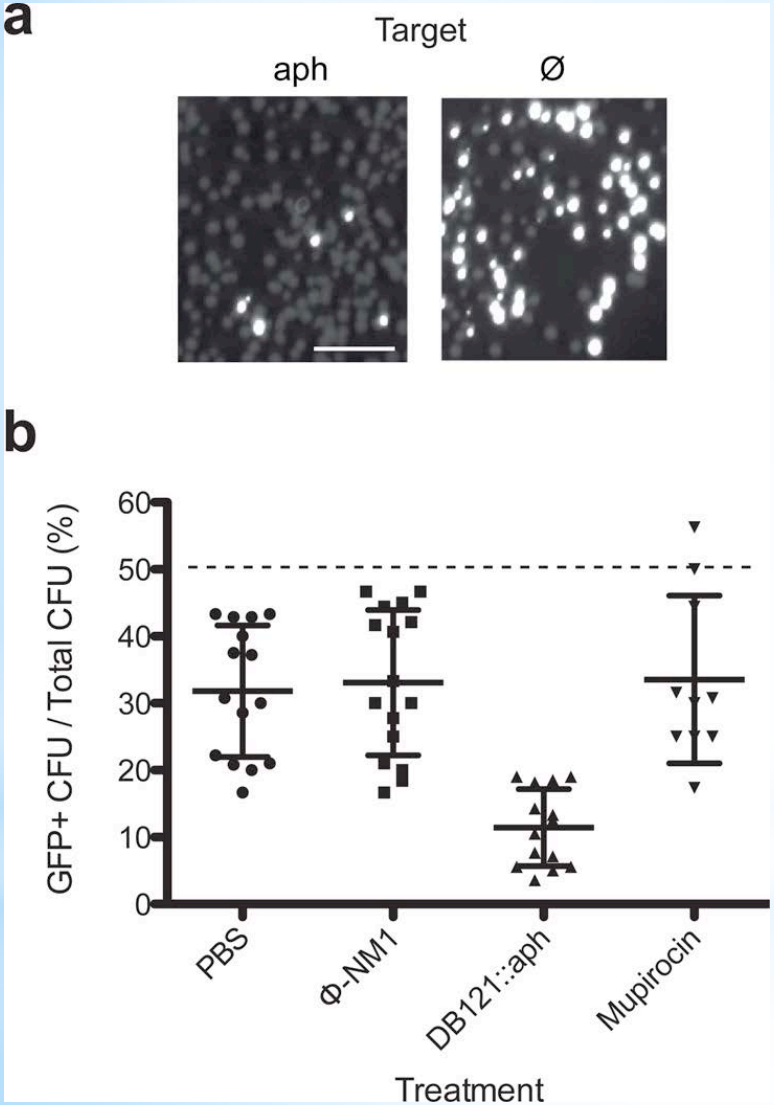


Exponentially growing USA300Φ and RNΦ cells were mixed 1:1 and treated with pDB121mecA at an MOI of ~5.

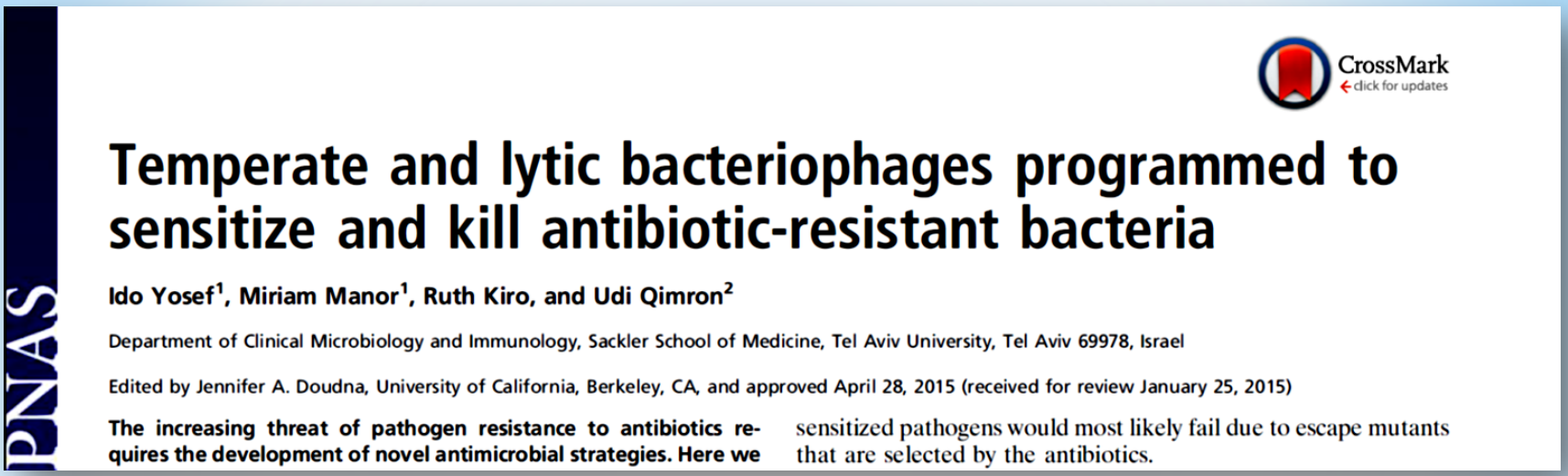
Plasmid curing was confirmed by the lack of PCR amplification with plasmid specific oligonucleotides

Immunization of naive staphylococci against pUSA02 transfer

Sequence-specific killing of kanamycin resistant S. aureus in a mouse skin colonization model.



Mice skin was colonized with a 1:1 mixture of 10^5 RNΦ and RNKΦ cells carrying the pCN57 GFP reporter plasmid, followed by treatment for 1 hour



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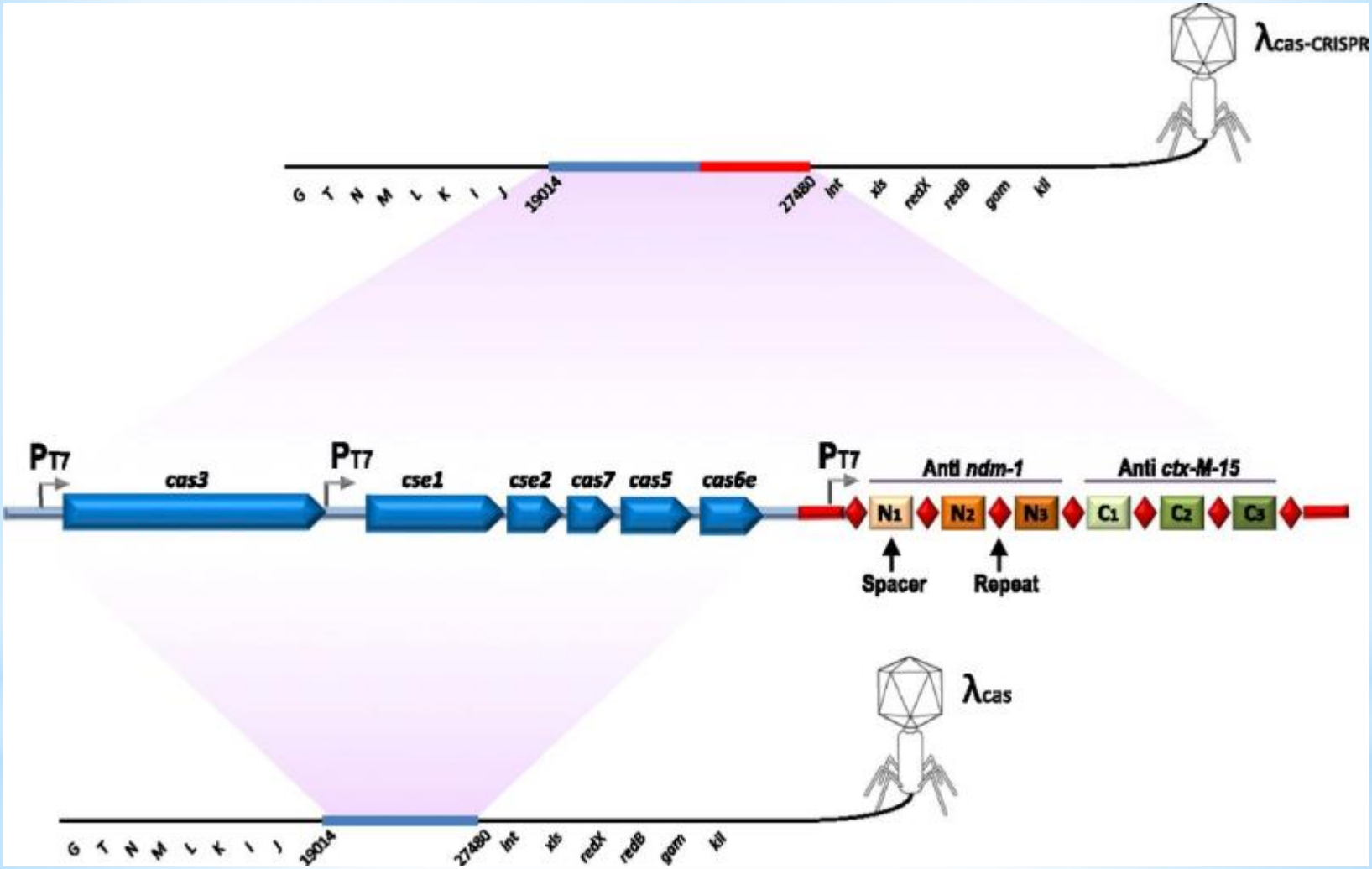
The increasing threat of pathogen resistance to antibiotics requires the development of novel antimicrobial strategies. Here we sensitized pathogens would most likely fail due to escape mutants that are selected by the antibiotics.

E. coli K12

λ and T7 bacteriophage (WT and engineered)

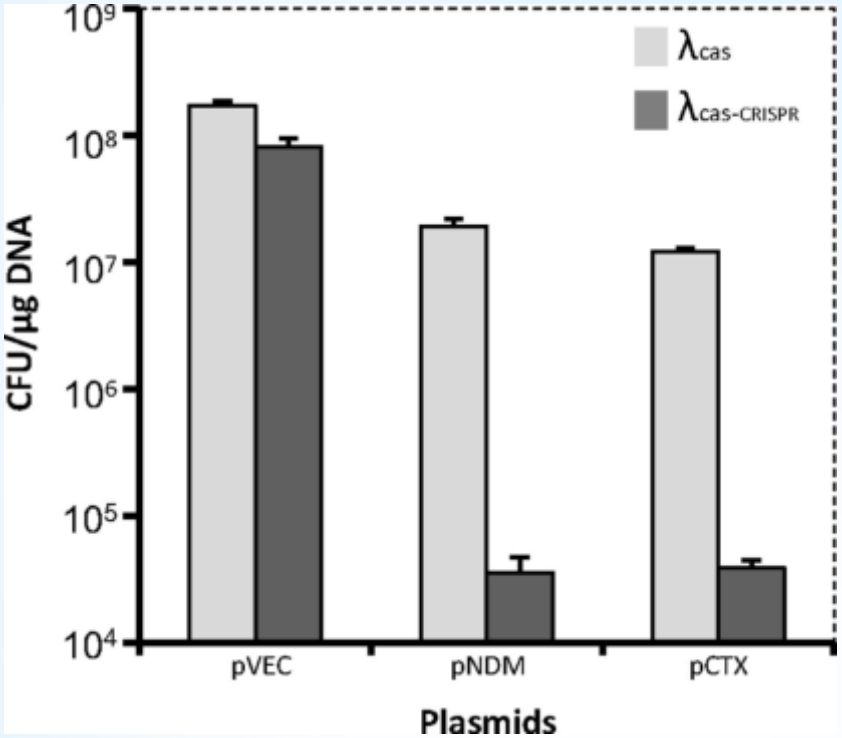
Schematics of the lysogenizing phages.



NDM-1 (New Delhi Metallo Beta lactamase
CTX-M15 (Ceftazidime-hydrolysing Beta lactamase)

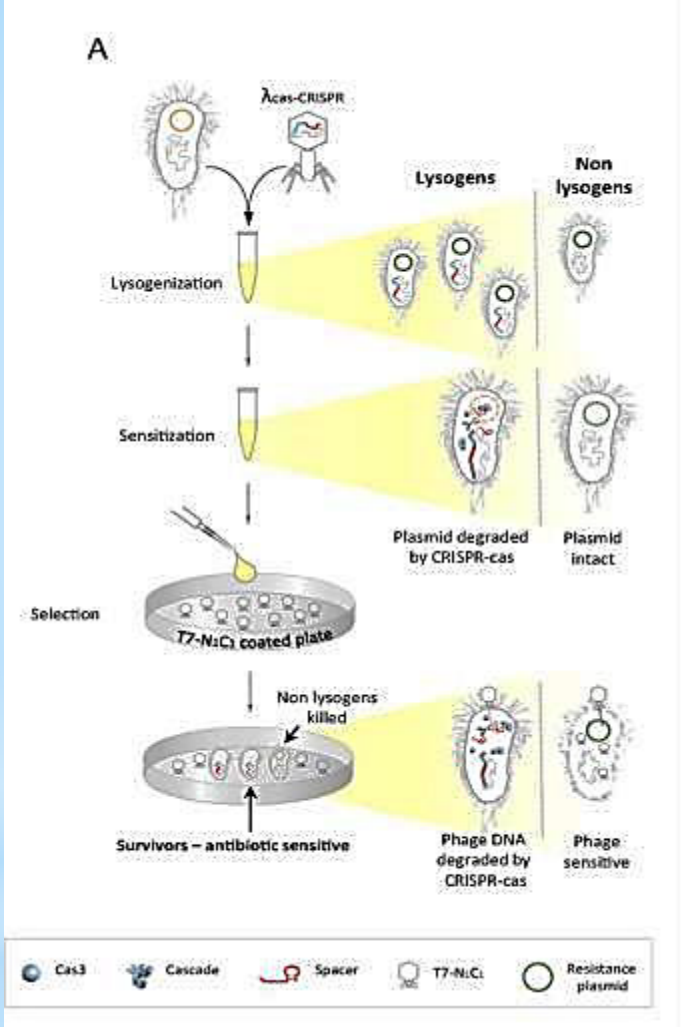
Ido Yosef et al. PNAS 2015

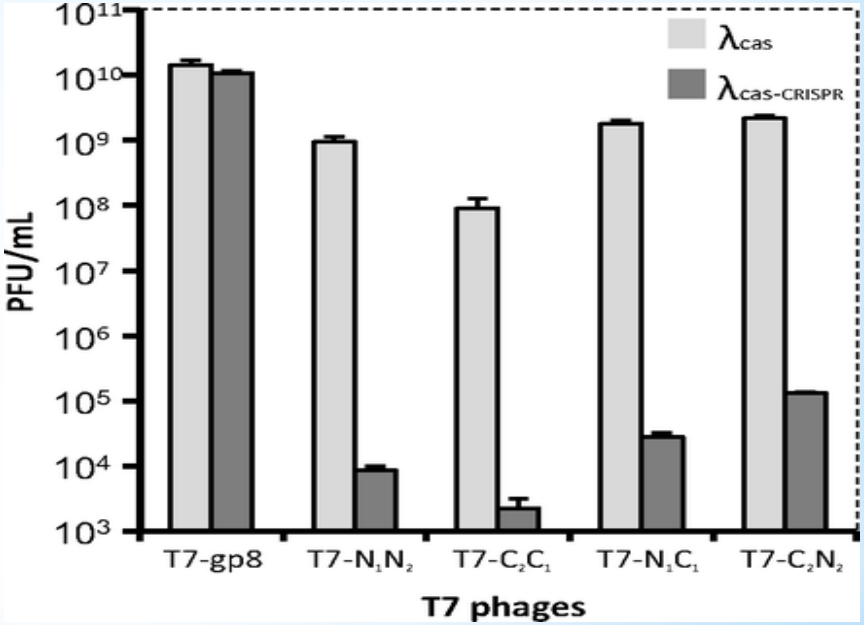
Lysogenization effect on transformation of antibiotic resistance plasmids



E. coli **K-12 lysogens were transformed with a control (pVEC),** ***ndm-1*** **(pNDM), or** ***ctx-M-15*** **(pCTX) encoding**

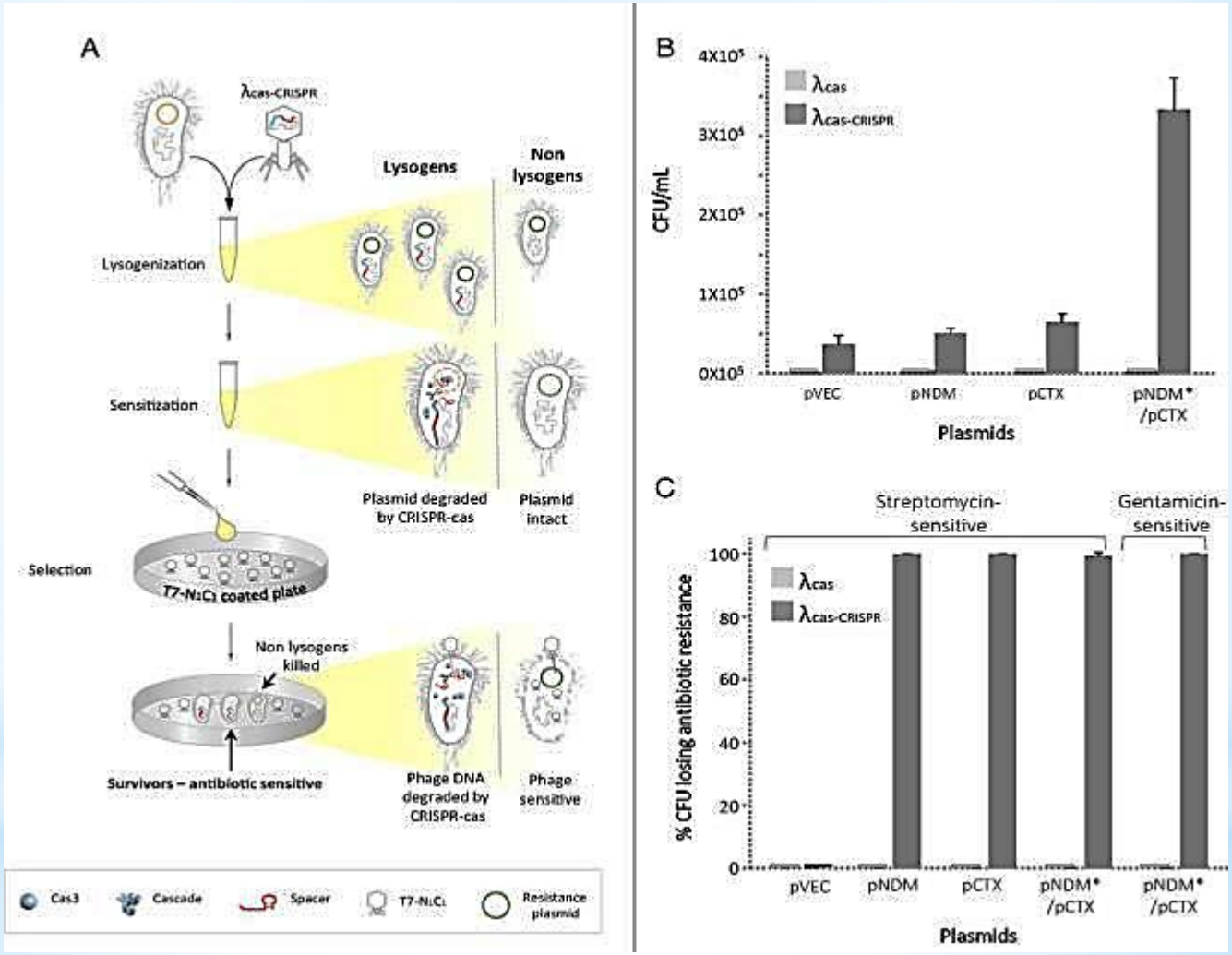
Lysogenization effect on protection against lytic phages.





E. coli K-12 were lysogenized with λcas (light gray bars) or λcas-CRISPR (dark gray bars). These lysogens were infected with a control T7-gp8 lacking targeted protospacers, or with T7 phages encoding two protospacers from ndm-1 (T7-N1N2) or two protospacers from ctx-M-15 (T7-C2C1) or one spacer from each gene (T7-N1C1 and T7-C2N2).

Enrichment of antibiotic-sensitized bacteria by lytic phages.



Ido Yosef et al. PNAS 2015

Limitations

Both carbohydrates and bile salts can interfere with bacteriophages ability to replicate in the stomach.

Therapeutic phages must be entirely lytic and cannot carry toxic or housekeeping genes associated with lysogeny, to avoid transfer of bacterial virulence genes into other bacteria.

Outlook

Combinatorial application with antibiotics

Grégory Resch of the University of Lausanne- “Phagoburn”- The first large, multi-centre clinical trial of phage therapy for human infections, funded by the European Commission.

Burn victims whose wounds have become infected with the common bacteria E. coli or P. aeruginosa will be given phage preparations from a company called Pherecydes Pharma (France)

Application of phages is using them on surgical equipment and clinical surfaces

Science Translational Medicine
AAAS

Programmable probiotics for detection of cancer in urine

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Editor's Summary

Synthetic bacteria for tumor detection

Organ- Liver

Probiotic strain E. coli Nissle 1917

Engineered to express bacterial luciferin (IVIS-luminescence detection)

Stabilized plasmid expressing lacZ (for calorimetric (CPRG) or luminescence (LuGal)

Organ of choice - Liver

Many types of cancer eg., colon and pancreatic tend to metastasize to the liver. Early detection critical

Liver is hard to image with conventional imaging techniques like CT scanning or magnetic resonance imaging (MRI), making it difficult to diagnose metastatic tumors

Bacteria thrive in tumors, especially necrotic core in cancerous tumors

Tumors are filled with nutrients released from dying cells and relatively free of immune cells

Orally delivered bacteria zero in on liver tumors because the hepatic portal vein carries them from the digestive tract to the liver (reticuloendothelial filtration of gut derived venous outflow to liver)

Tests in mice with colon cancer that has spread to the liver, the probiotic bacteria colonized nearly 90 percent of the metastatic tumors.

Integrated their diagnostic circuits into a harmless Probiotic strain of E. coli called Nissle 1917

Bacteria can grow in tumors as small as 1 millimeter, so the urine test has the potential to detect liver tumors — which tend to be small and dispersed — very early on, which would improve survival rates for patients.

Current imaging techniques, such as CT scans flag tumors at 1 centimeter.

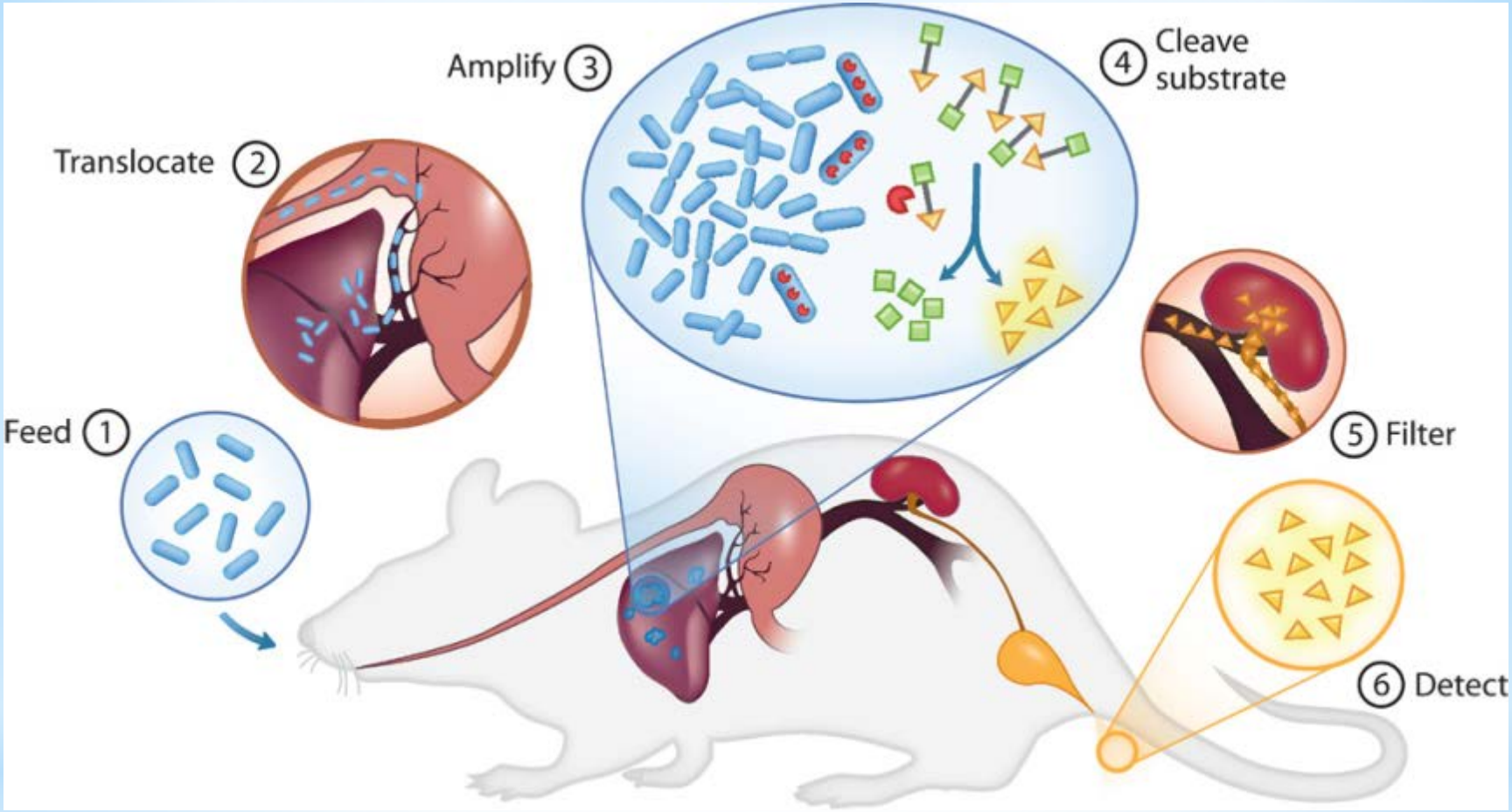
Oral administration is safer and more practical, particularly for a diagnostic that will likely be used more than once.

Table 1. Bacterial strains and mammalian cell types used in this study.

AHL, N-(3-oxohexanoyl)-L-homoserine lactone; GEMM, genetically engineered mouse model

Label	Bacterial strain or mammalian cell	Genomic/plasmids	Use(s)
PROP-Z	EcN	luxCDABE (genomic), IPTG-inducible lacZ (stabilized plasmid)	Bioluminescent imaging of bacteria, urine diagnostic assay
PROP-Luc	EcN	luxCDABE (genomic), luxCDABE (plasmid)	Bioluminescent imaging of bacteria
PROPi-Luc	EcN	AHL-inducible luxCDABE (plasmid)	Bioluminescent imaging of bacteria
Non-lacZ	Mach One	lacZΔM15 (genomic mutant)	Non-lacZ control for urine diagnostic assay
MC26-LucF	Metastatic colorectal mouse cell line	Firefly luciferase (genomic)	Subcutaneous xenografts and liver metastasis models
LS174T-LucF	Human colorectal adenocarcinoma cell line	Firefly luciferase (genomic)	Subcutaneous xenograft models
393M1-LucF	GEMM lung mouse cell line	Firefly luciferase (genomic)	Subcutaneous xenografts and liver metastasis models

PROP-Z probiotics for noninvasive cancer detection

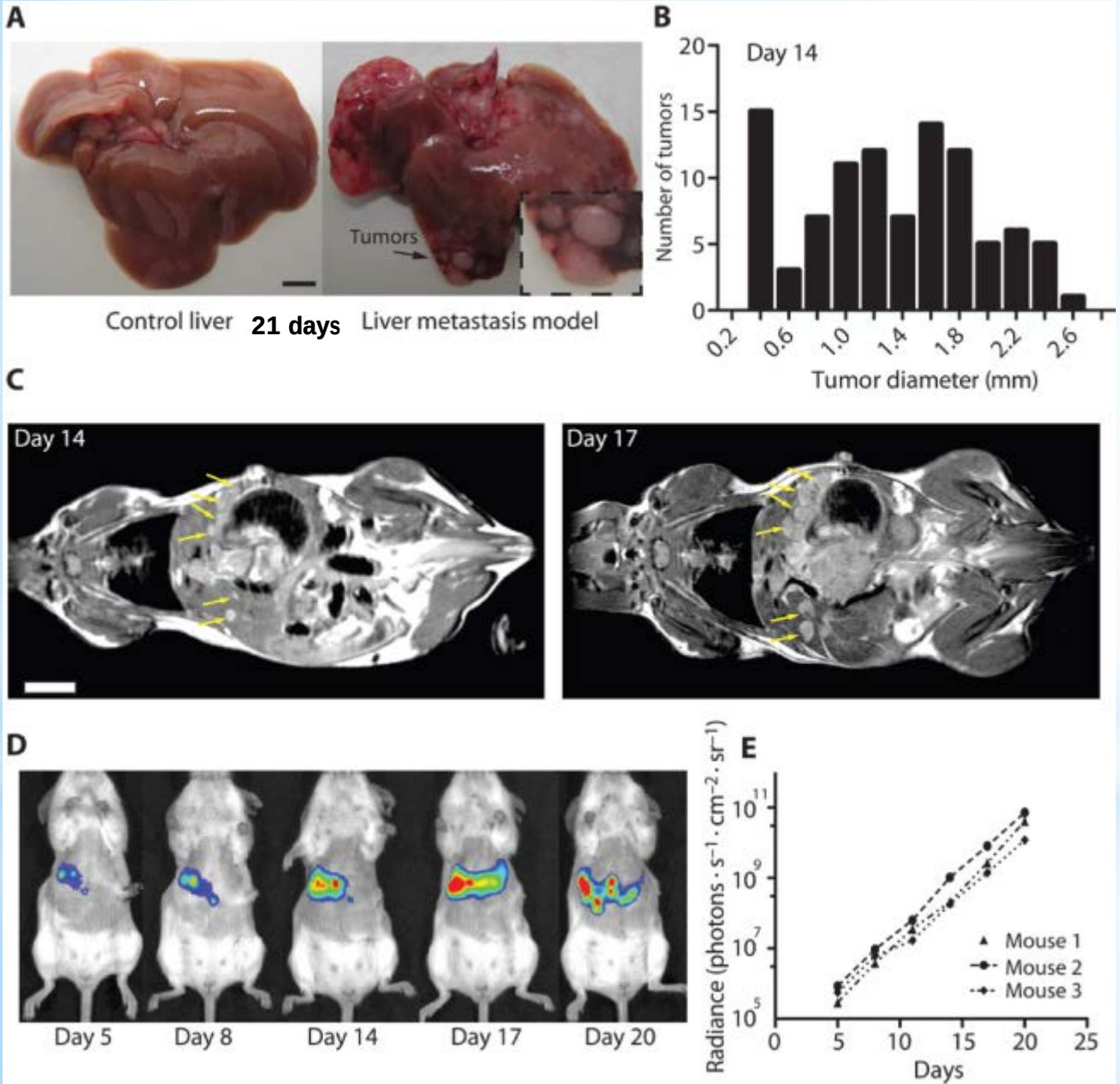


PROP-Z (**E. coli Nissle 1917)** dual-stabilized, high-expression lacZ vector as well as a genomically integrated luxCDABE cassette that allows for luminescent visualization without providing exogenous luciferin (blue).

Probiotics rapidly (within 24 hours) translocate across the gastrointestinal tract and

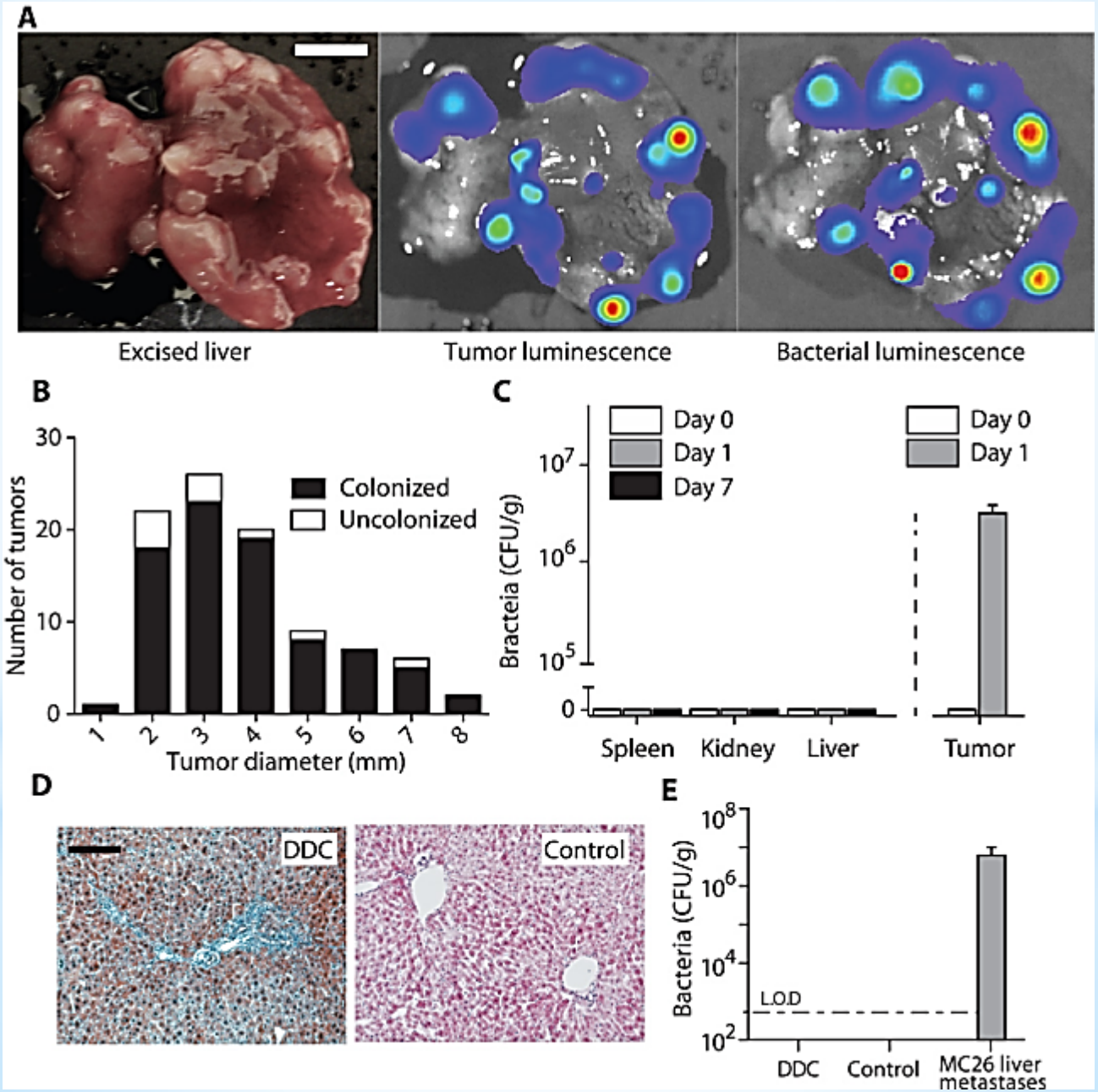
Specifically amplify within metastatic tumors present in the liver.
The enzyme lacZ (red), which can cleave systemically injected, cleavable substrates (green and yellow).
Urine for detection

Tumor-bearing livers from Balb/c mice, excised after intrasplenic injection



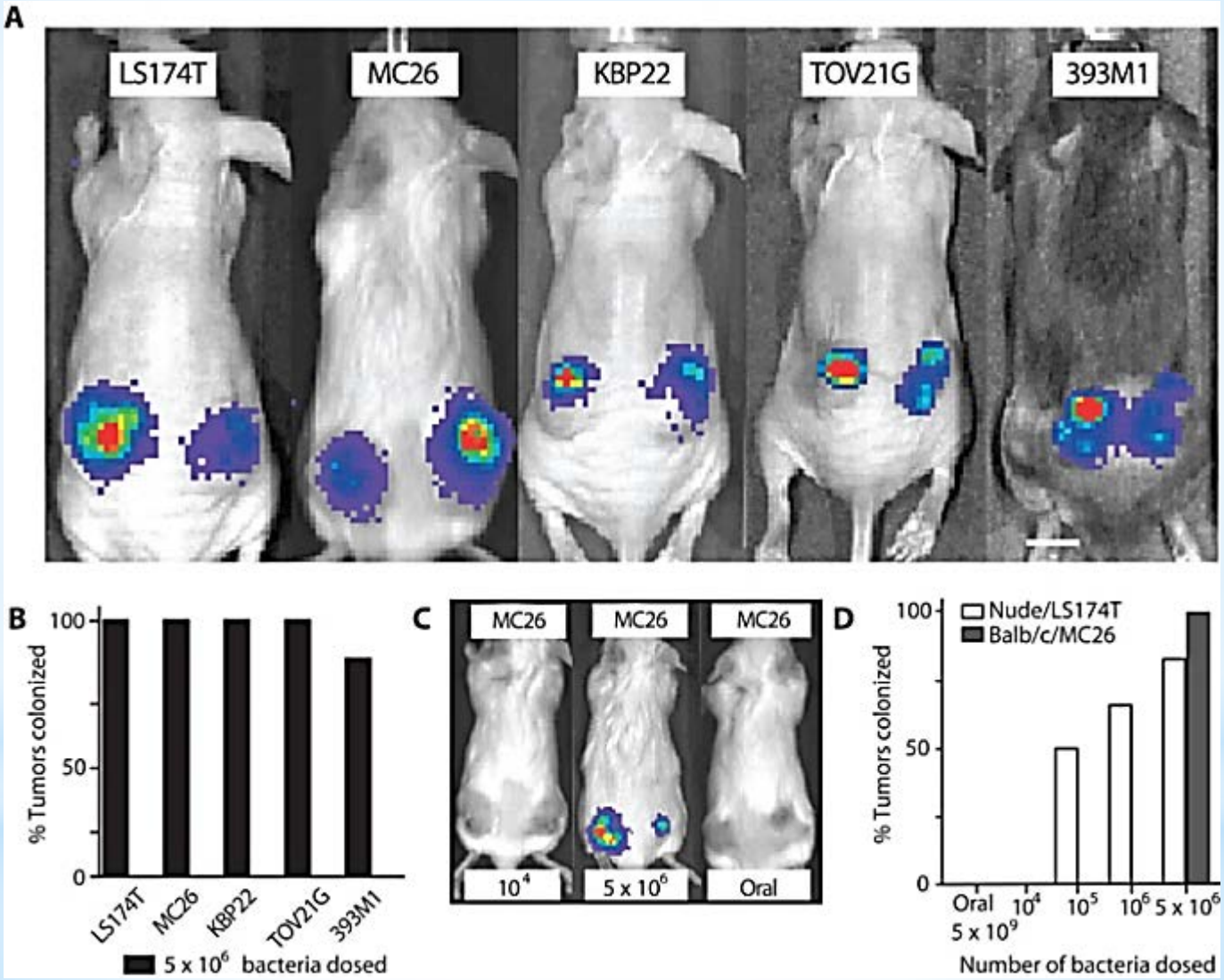
MC26-LucF cells bearing a firefly luciferase transgene

Colonization of liver metastases by orally delivered PROP: Quantitation and specificity



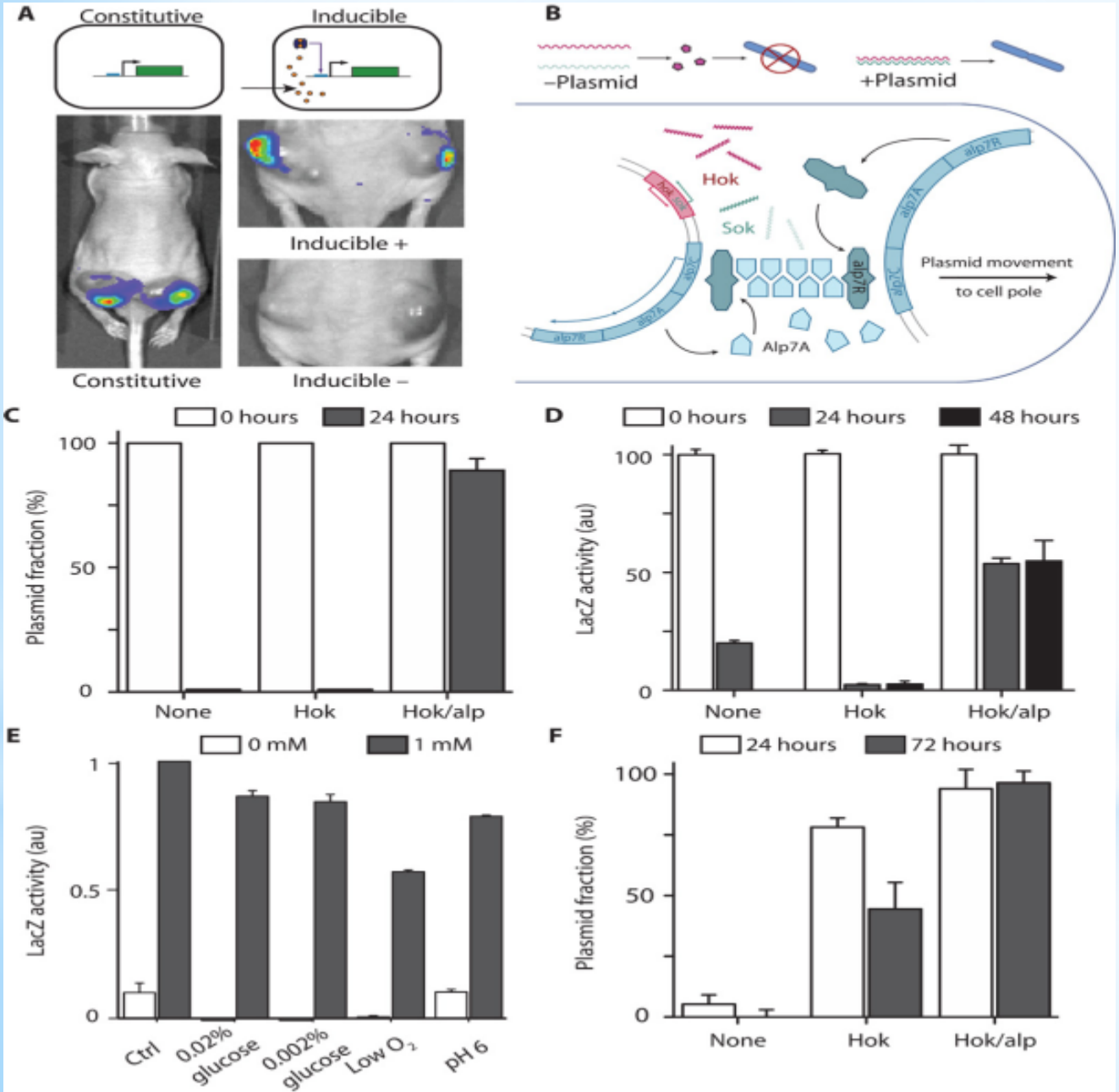
5-diethoxycarbonyl-1,4-dihydrocollidine

Colonization in tumor models and different modes of administration



IVIS images showing colonization of subcutaneous human (LS174T-LucF, TOV21G) or mouse (MC26, KBP22, 393M1-LucF) tumors with 5 × 106 CFU PROP-Z via i.v

Dually stabilized vector efficiently maintains PROP-Z activity in vivo



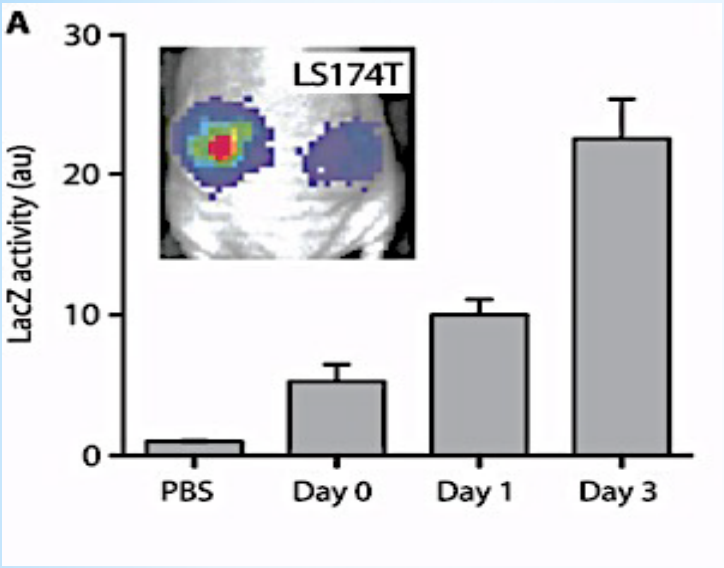
Plasmid stabilization systems used:

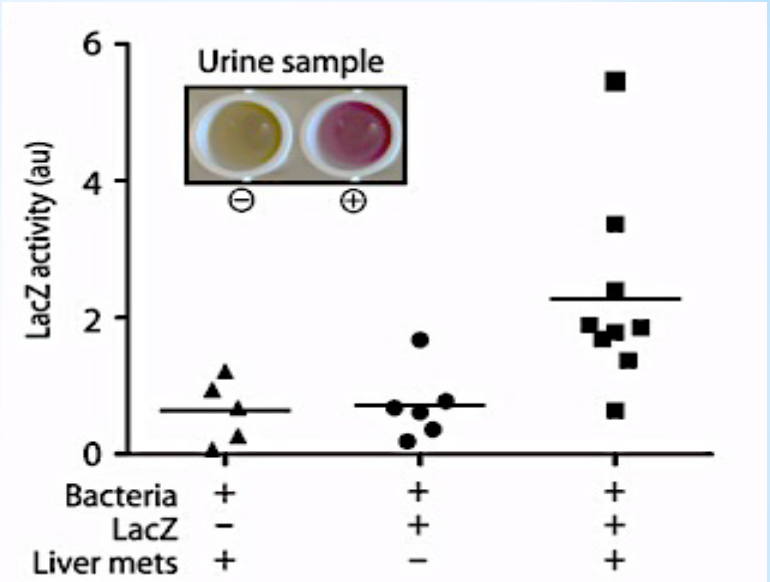
Hok-
host killing (a long lived toxin)

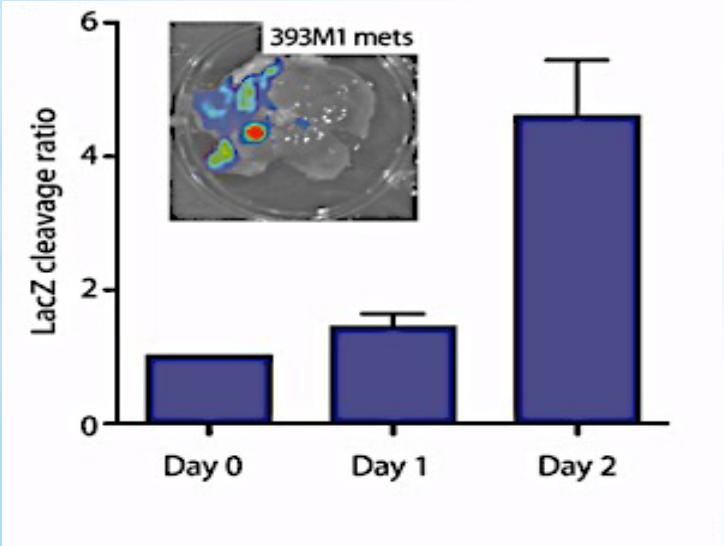
Sok-
suppression of killing

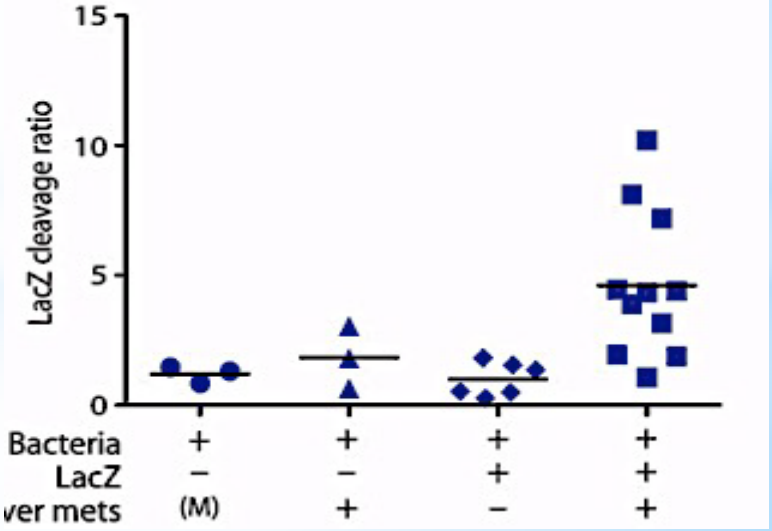
Alp7A-
Altered Polarity microtubule protein

Detection of metastatic tumors by PROP-Z urine diagnostic









Chlorophenol red ß-D-galactopyranoside

Limitations:

Needs improved sensitivity

Potential interference of PROP on PET imaging

Gut microbiome and tumor specific microbiome in humans might differ and affect PROP

Specificity of the test and further safety data before moving it into clinical trials

Outlook

Engineered bacteria to express receptors that would target them to tissues, cause genetic disruption of cancer cell function, deliver drugs, or reactivate the immune system

Far earlier than current techniques and for a fraction of the price.

Thank you for your attention

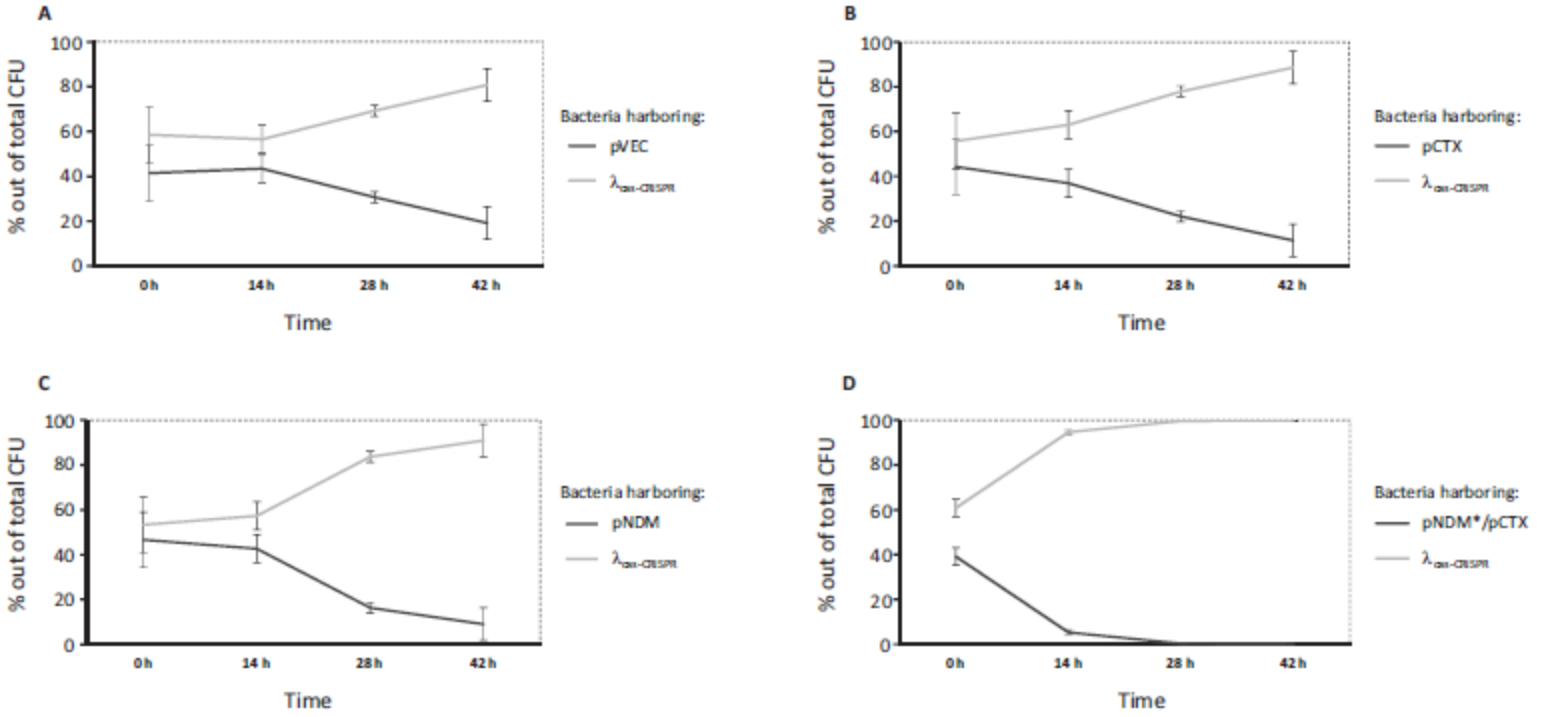


Fig. S1. Competitive fitness of a lysogen compared with bacteria harboring resistance plasmids. Bacteria encoding the $\lambda_{cas\text{-}CRISPR}$ prophage and (*A*) pVec, (*B*) pCTX, (*C*) pNDM, and (*D*) pNDM*+pCTX plasmids were mixed at a 1:1 ratio. They were then cultured together in LB at 32 °C for 14 h. The cells were then diluted 1/800 in LB and grown for an additional 14 h at 32 °C; this procedure was repeated once more. Samples from the mixed cultures were taken at the indicated time points and plated on either kanamycin or streptomycin or streptomycin+gentamicin agar plates to differentiate between lysogens (kanamycin^r) and plasmid-harboring bacteria (streptomycin^r for *A*–*C* or streptomycin^r+gentamicin^r for *D*). The CFU ratio of each strain was then determined by calculating the number of each type of resistant CFU out of the total resistant CFU.