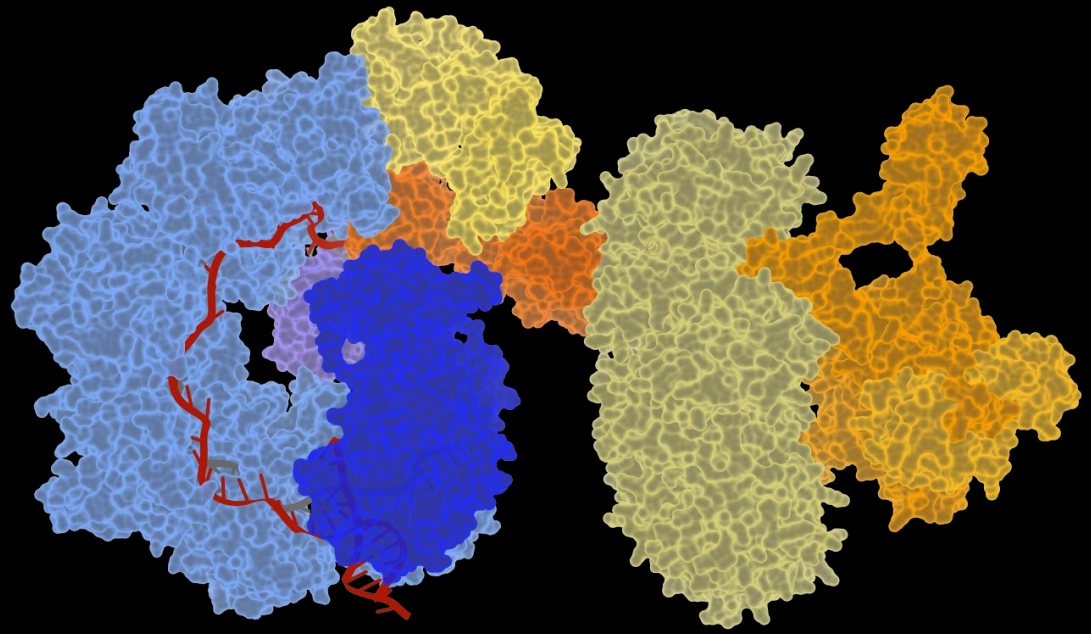
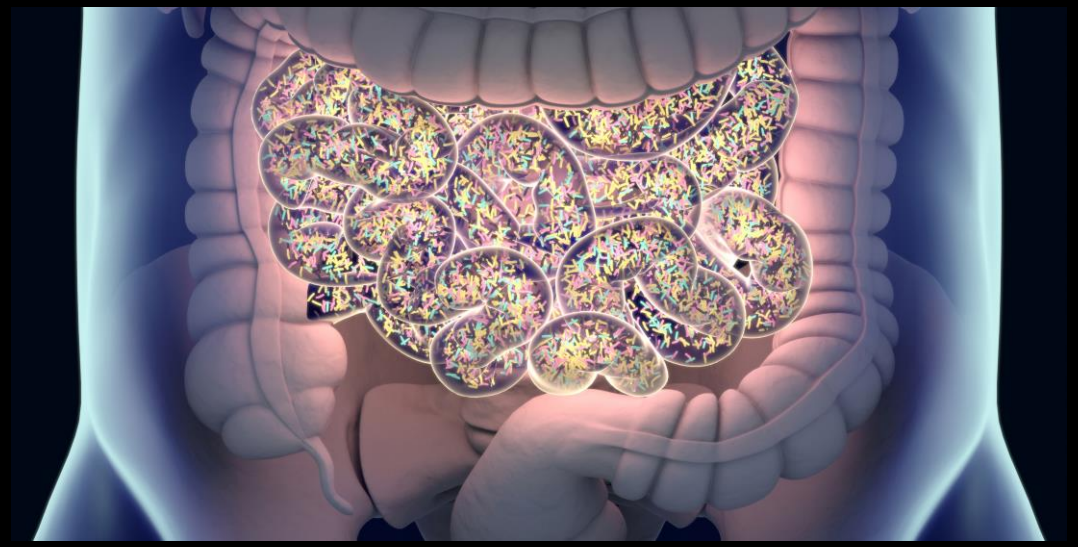
Precision Editing of Non-model Bacteria in Native Microbiomes using Mobile CRISPR-associated Transposases





Diego Rivera Gelsinger, Ph.D.

Harris Wang & Sam Sternberg labs

AIChE2023

December 10th, 2023

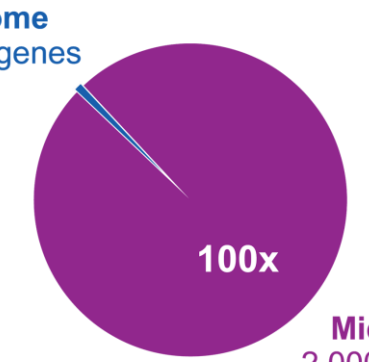
BURROUGHS WELLCOME FUND

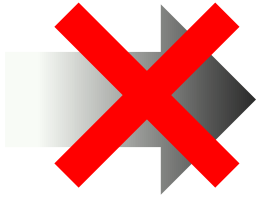
COLUMBIA UNIVERSITY IRVING MEDICAL CENTER

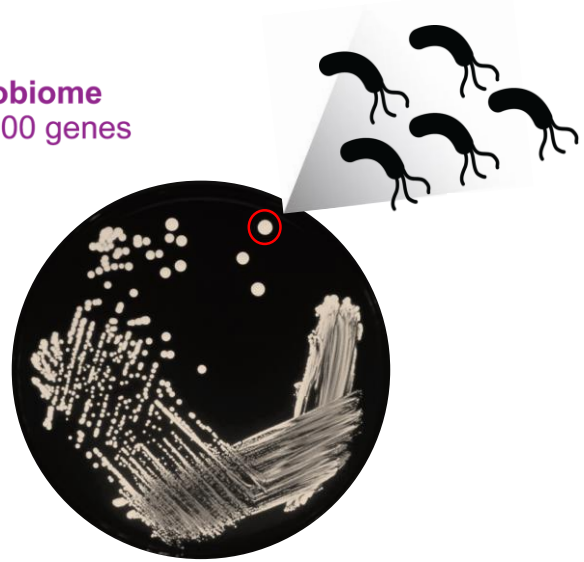
Deep Untapped Potential of Microbial Communities

In the wild

In our bodies

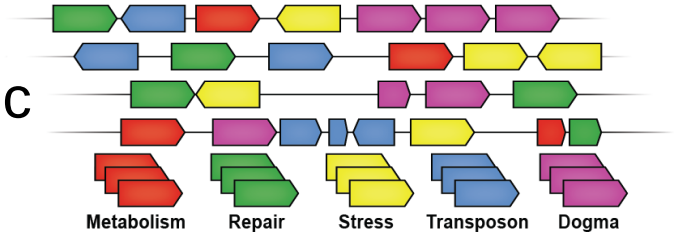






Limited species culturable

Metagenomic potential

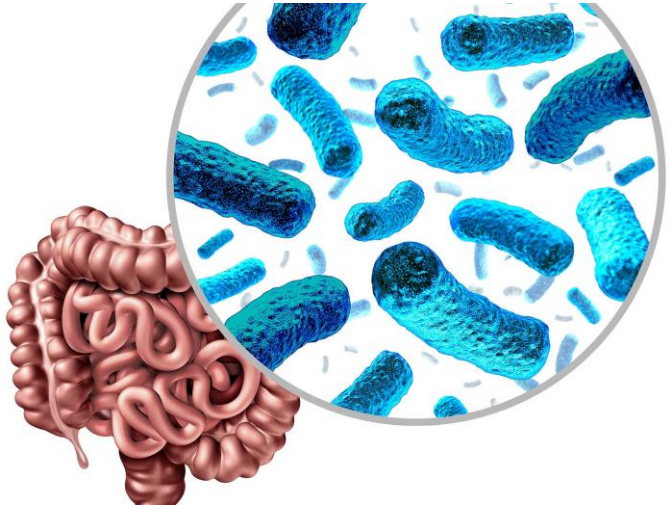


Goal: Harness microbes in their native communities

Approach: Culture-independent universal genetics

Motivation: Need a precise method to manipulate natural communities

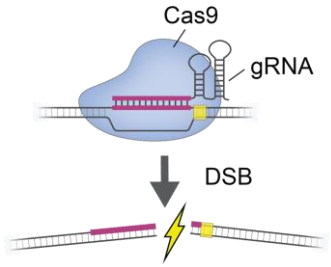
Fundamental biology:
the gut microbiome



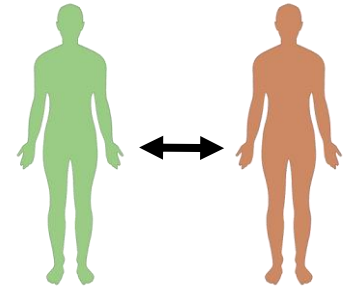
Broad changes in the ecosystem

Antibiotic	Probiotics
Nutrition	Prebiotics
FMT	

Current methods for manipulation too broad



Probiotic therapies



Precise manipulation
&
Predictable outcomes

How can we manipulate microbiomes with high precision?

Challenges and Unmet Needs in Microbiome Engineering

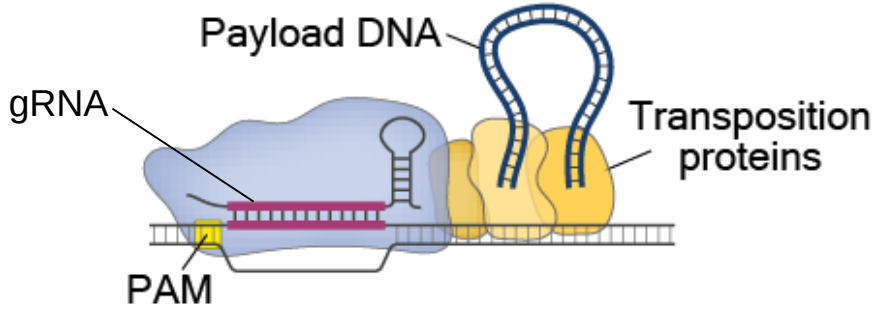
Objective: Edit the microbiome

What do we need?

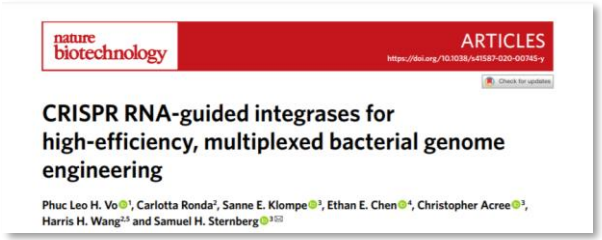
- Target any sequence
- High efficiency
- Non-deleterious
- Large edits (kilobases)

➢ Current methods lacking

CRISPR-associated transposon (CAST)



INTEGRATE


nature biotechnology ARTICLES

CRISPR RNA-guided integrases for high-efficiency, multiplexed bacterial genome engineering

Phuc Leo H. Vo, Carlotta Ronda, Sanne E. Klompe, Ethan E. Chen, Christopher Acree, Harris H. Wang and Samuel H. Sternberg

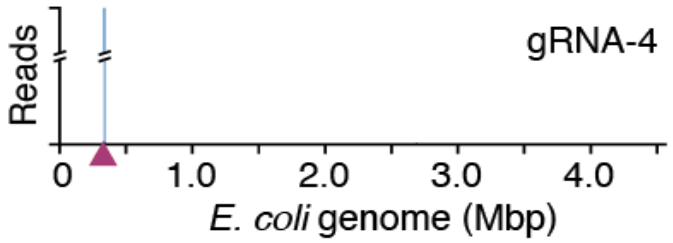
DART


nature microbiology ARTICLES

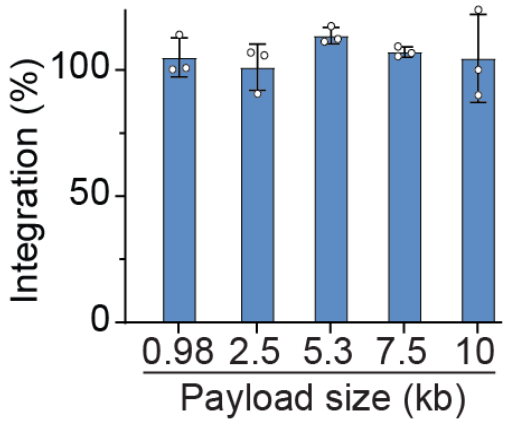
Species- and site-specific genome editing in complex bacterial communities

Benjamin E. Rubin, Spencer Diamond, Brady F. Cress, Alexander Crits-Christoph, Yue Clare Lou, Adair L. Borges, Haridha Shivram, Christine He, Michael Xu, Zeyi Zhou, Sara J. Smith, Rachel Rovinsky, Dylan C. J. Smock, Kimberly Tang, Trenton K. Owens, Netravathi Krishnappa, Rohan Sachdeva, Rodolphe Barrangou, Adam M. Deutschbauer, Jillian F. Banfield and Jennifer A. Doudna

Specific



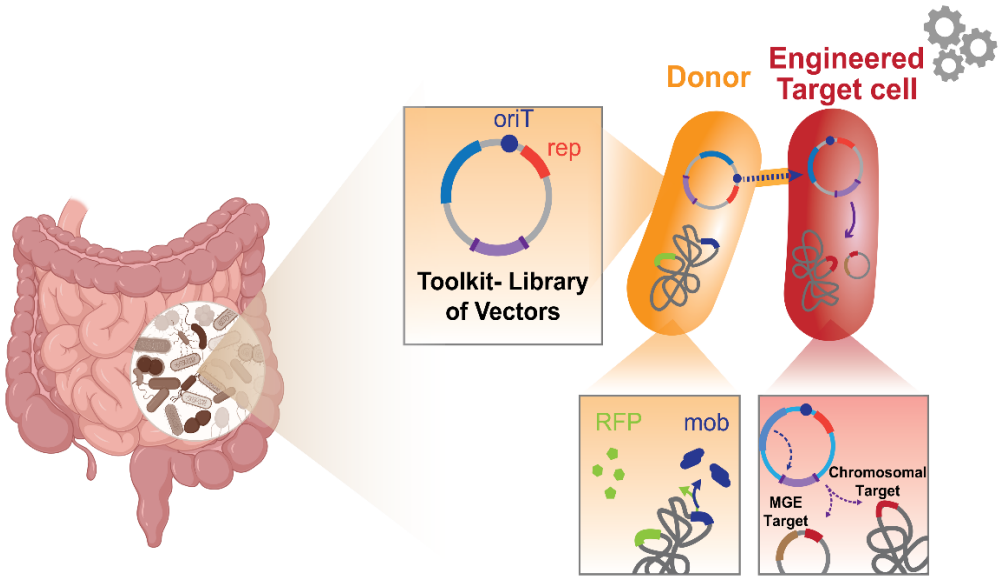
Efficient



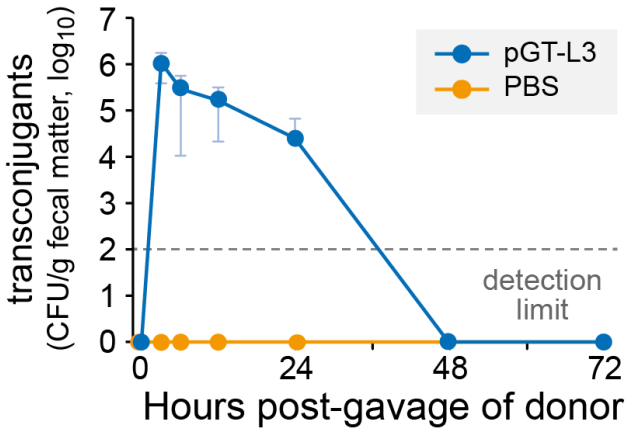
Klompe et al., *Nature* (2019)
Vo et al., *Nature Biotech* (2021)
Rubin, Diamond, Cress et al., *Nature Micro* (2021)

How can we deliver payloads to microbial communities?

MAGIC is a modular and expandable platform for highly efficient *in vivo* engineering

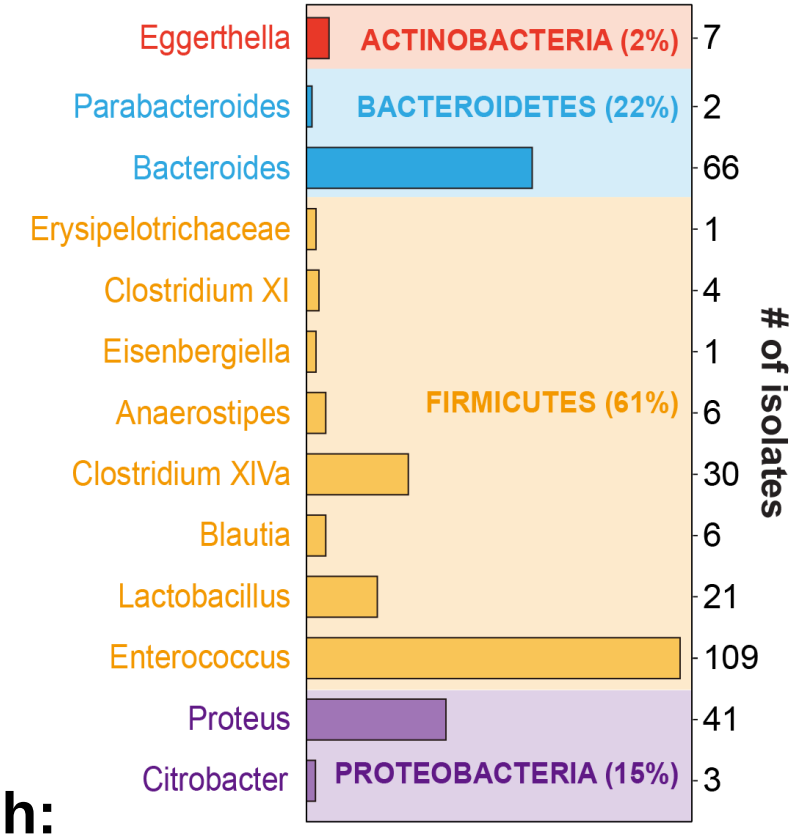


Ronda et al., *Nature Methods* (2019)



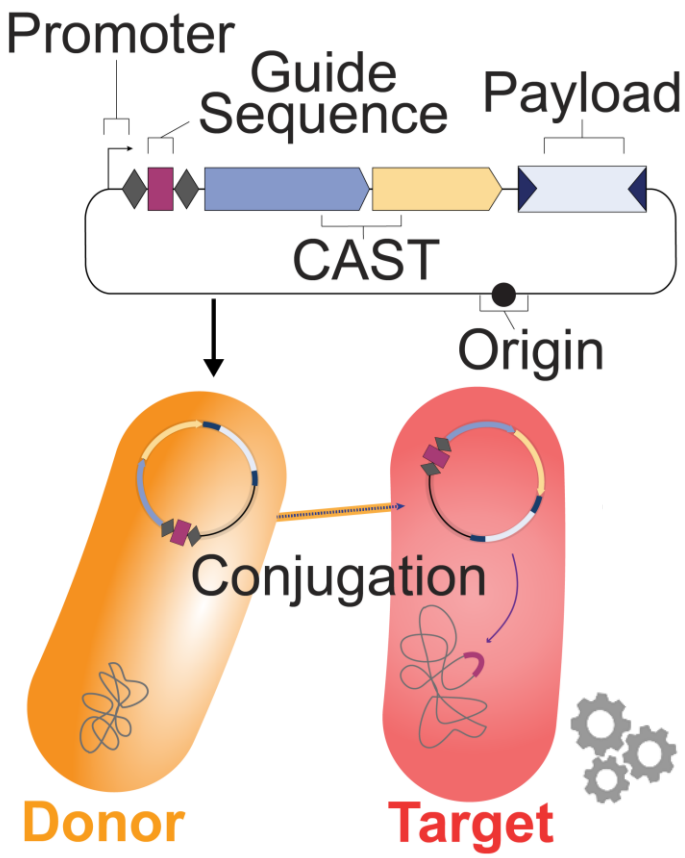
Limitations to approach: non-targeted, plasmid instability

Cultured and sequence verified isolates



MAGICAST: Versatile technology for precision microbiome engineering

Efficient *in vivo* delivery via conjugation (MAGIC)



Programmable CAST for payload integration

Phase 1

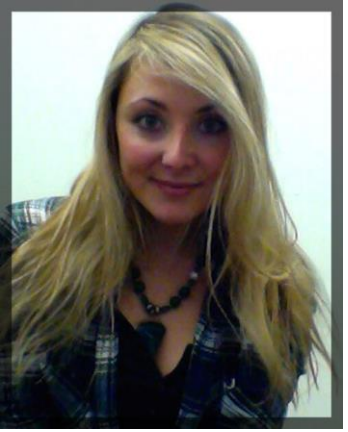
How can we deliver genetic payloads into wild bacteria?

Phase 2

How can we engineer microbial communities in vivo?

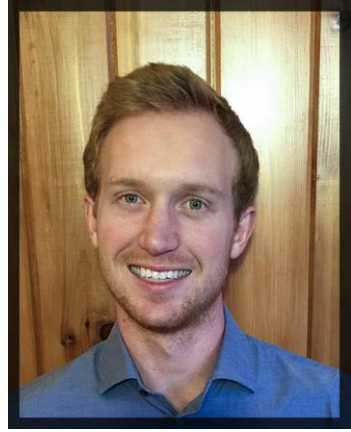
Phase 3

How can we ensure payloads retain and persist?



Carlotta Ronda

Poster



Tyler Perdue

Collaborative effort with the MAGICAST team

Logan Schwanz

Poster

COLUMBIA UNIVERSITY
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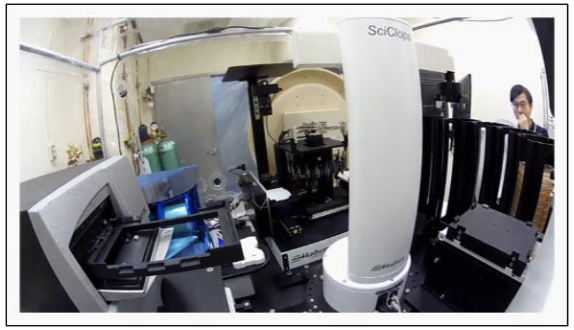
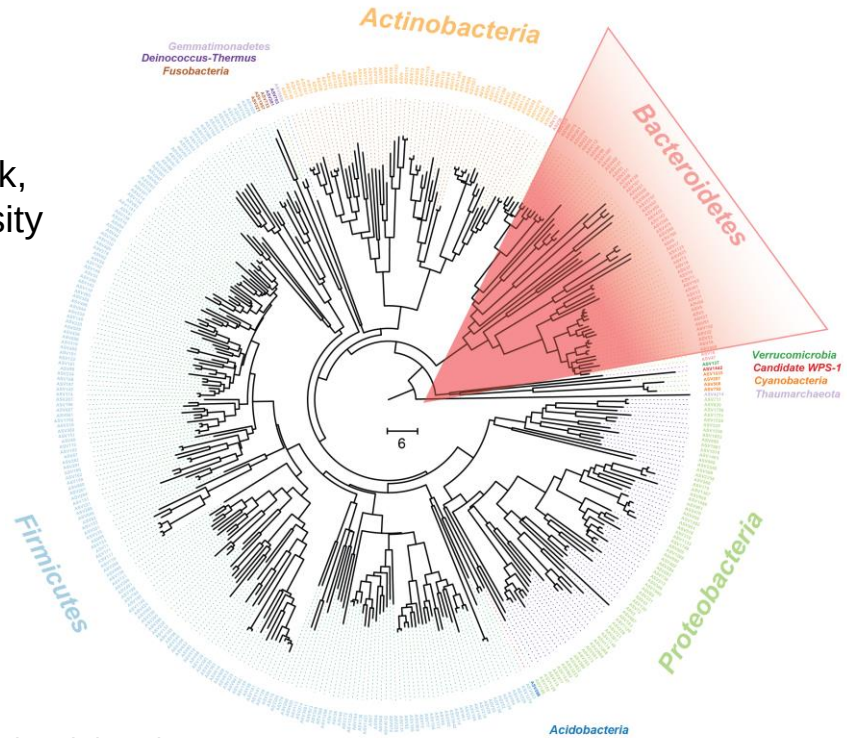
Phase I: Optimize delivery into diverse wild gut bacteria

Consortium of human isolates for editing

Culturomics by Automated Microbiome Imaging and Isolation (CAMII) System

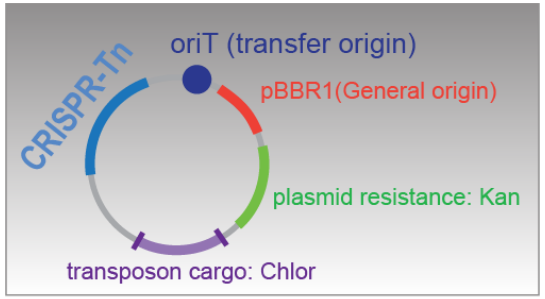


Gut biobank, ~80% diversity

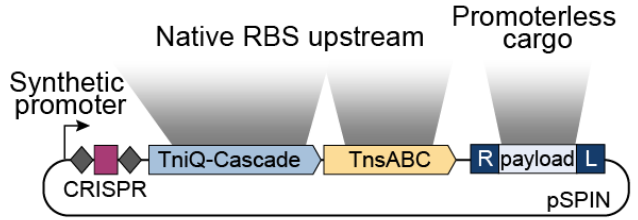


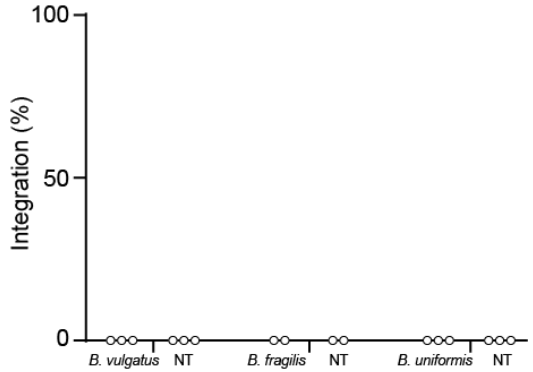
Huang, Sheth et al. *Nat Biotech* (2023)

General vectors do not edit wild *Bacteroides*

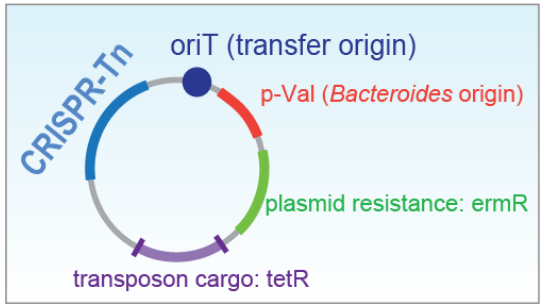


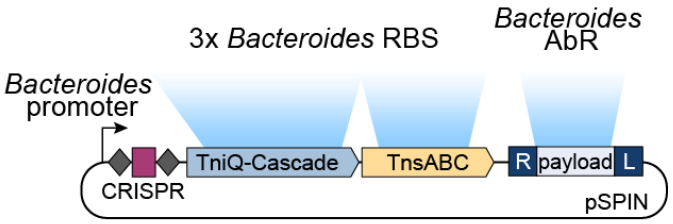
Vo et al., *Nature Biotech* (2021)





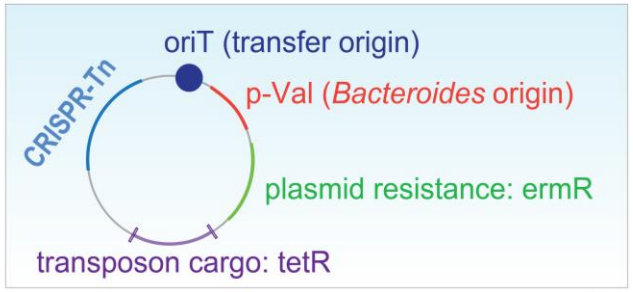
Bacteroides customized



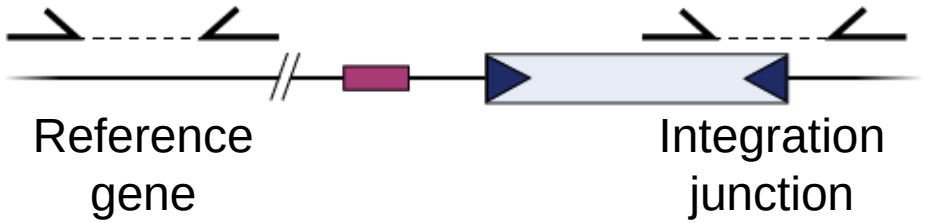


In submission

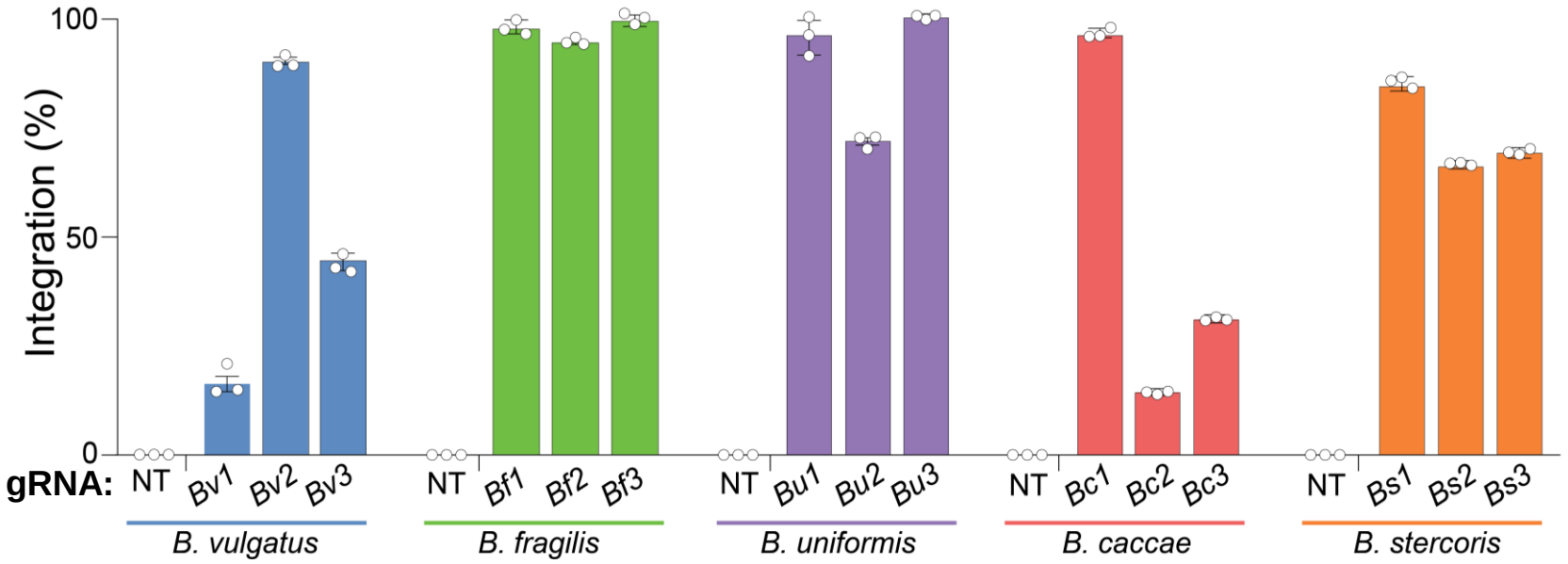
MAGICAST is customizable to engineer wild bacteria

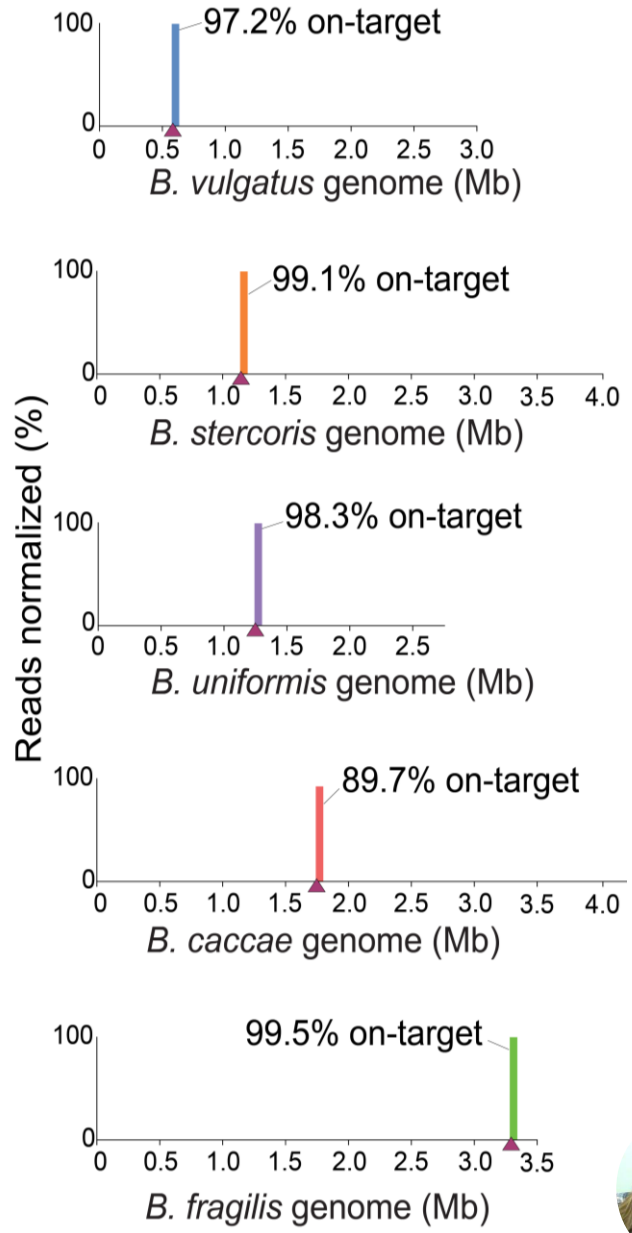


Measuring efficiency of integration by qPCR



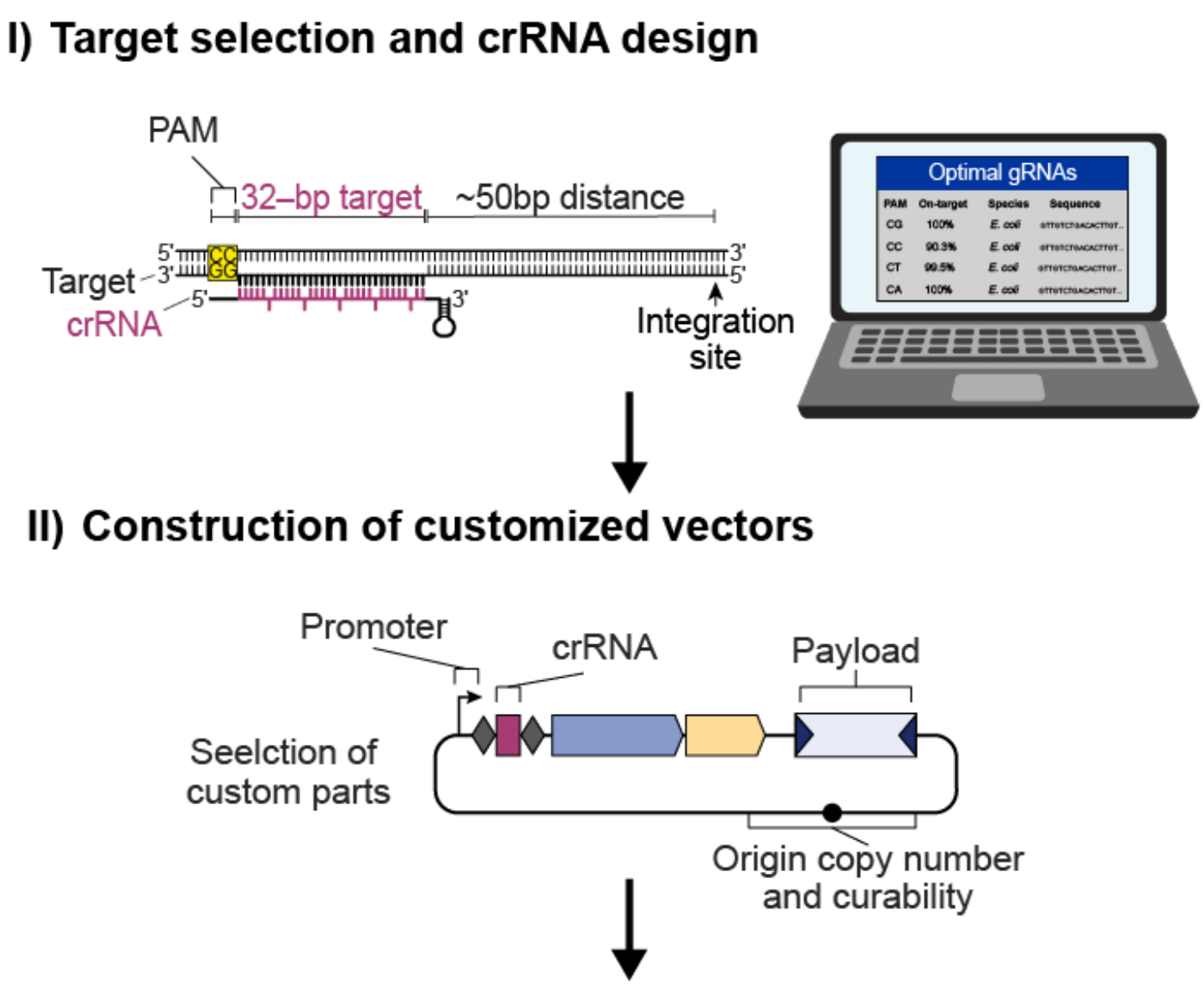
Successful editing of wild *Bacteroides* isolates from humans

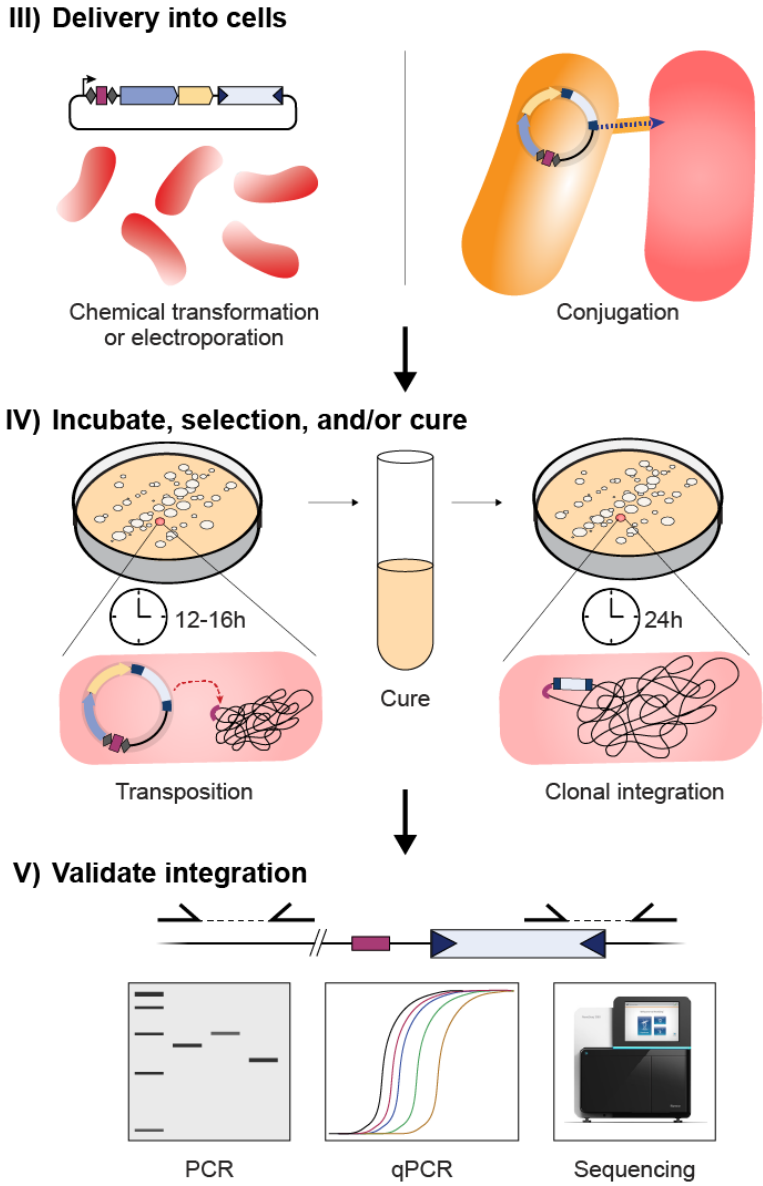




In submission

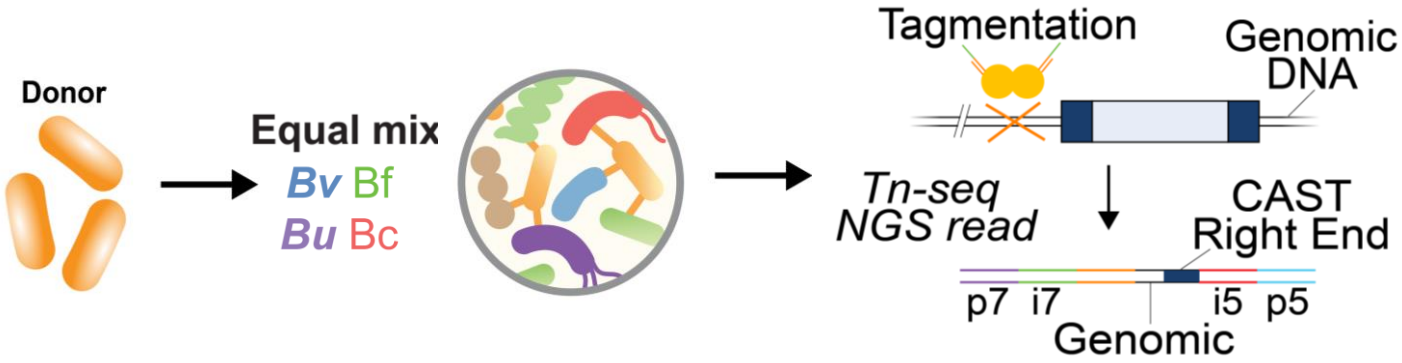
Nature Protocols: streamlined protocol for strain engineering



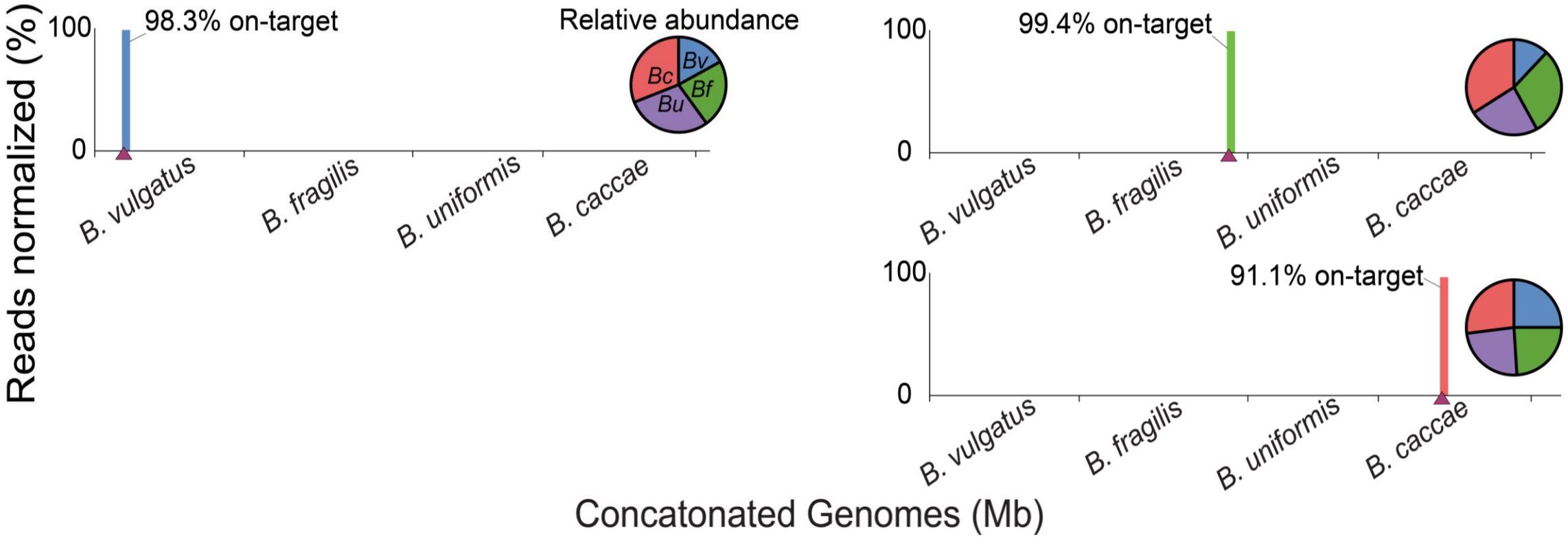


Gelsinger et al. *Nature Protocols* (2023)

MAGICAST is highly specific in communities of bacteria



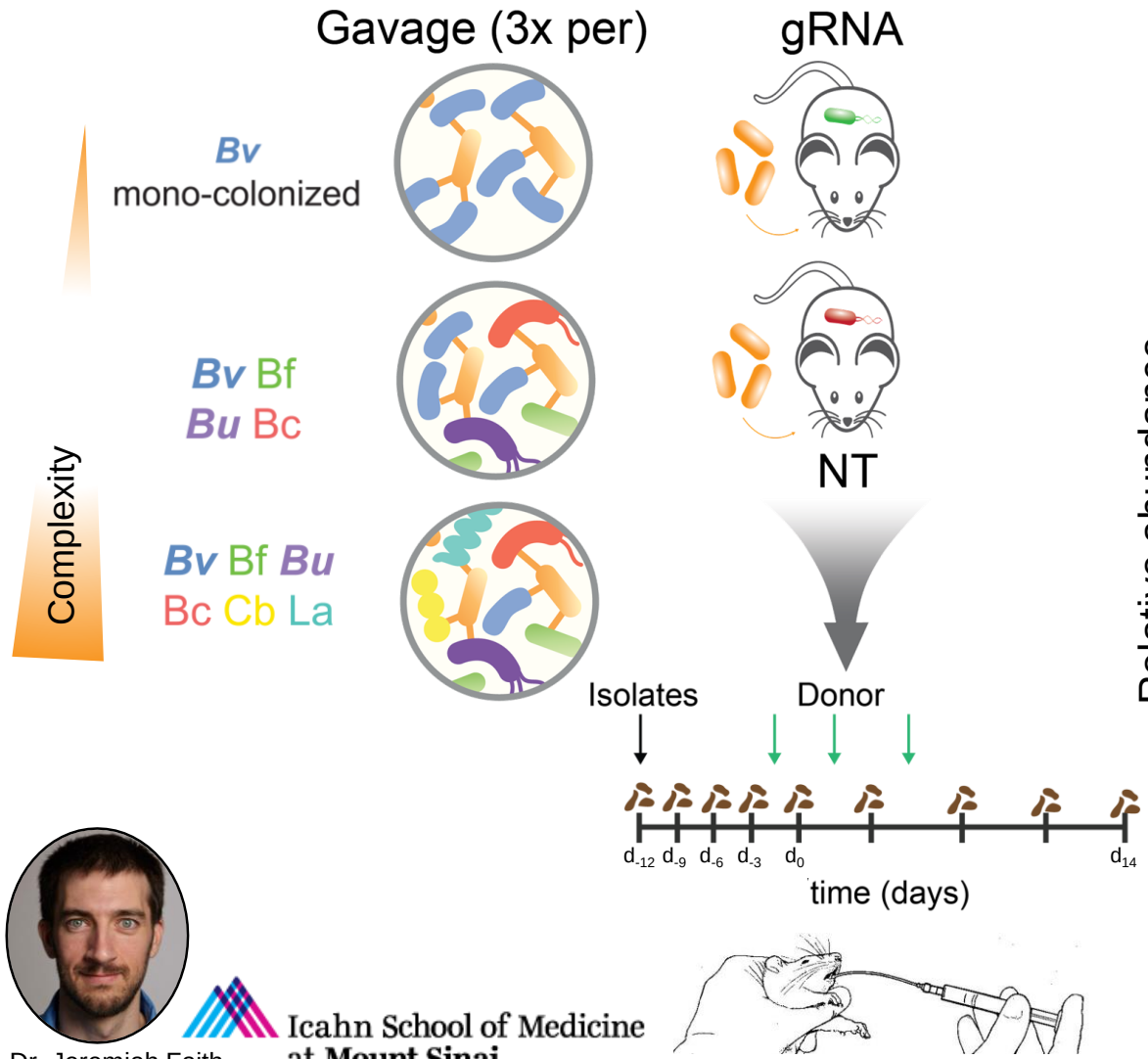
High specificity of insertions in community of wild *Bacteroides*



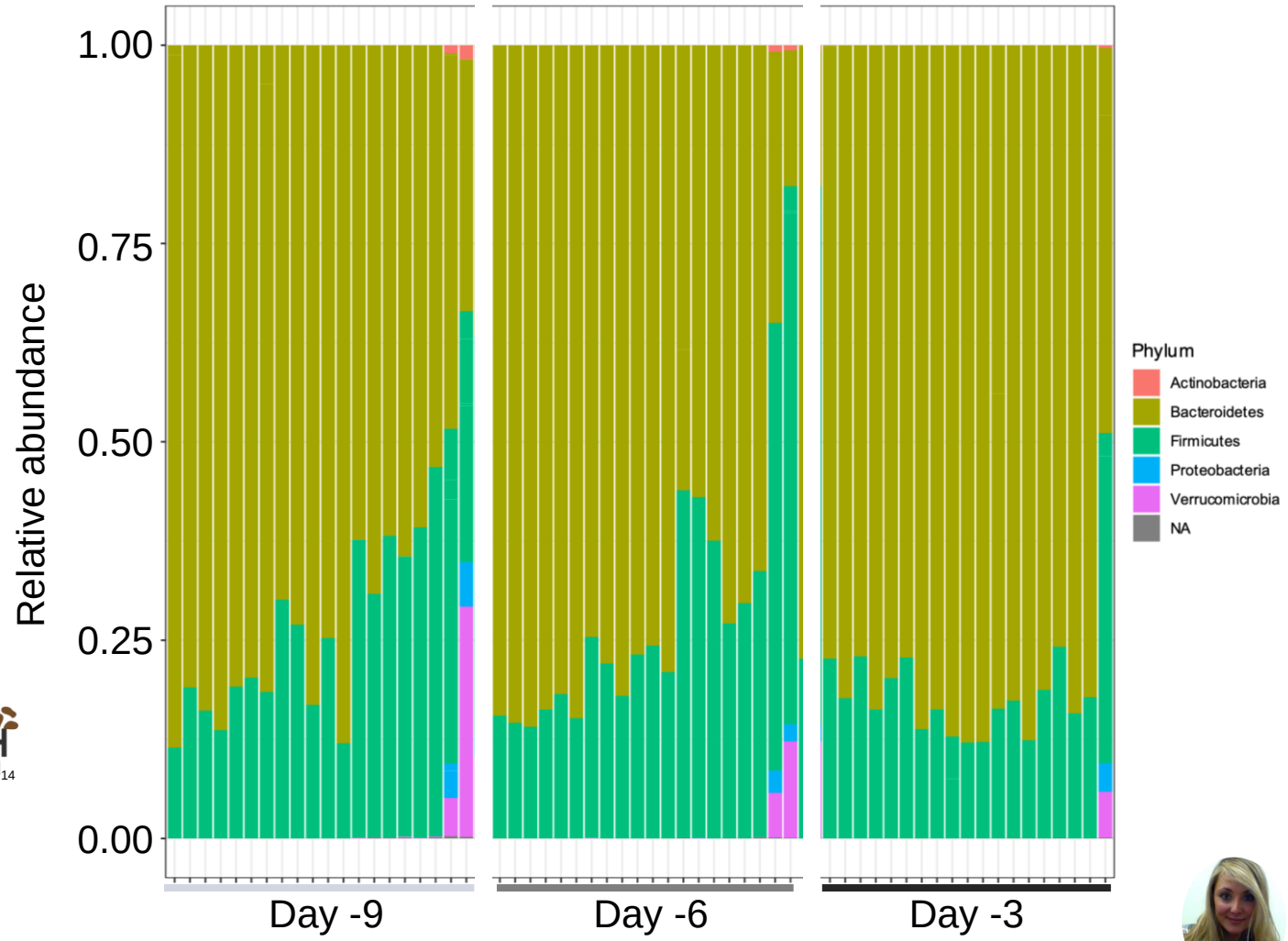
In submission

Phase II: Gnotobiotic mice to study human commensal bacteria

Gnotobiotic experimental pipeline



Colonization with human isolates is stable after several days

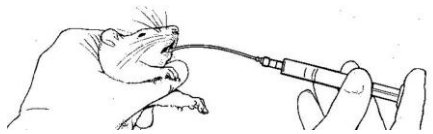


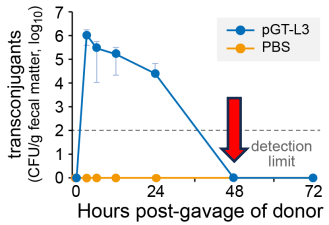
Dr. Jeremiah Faith

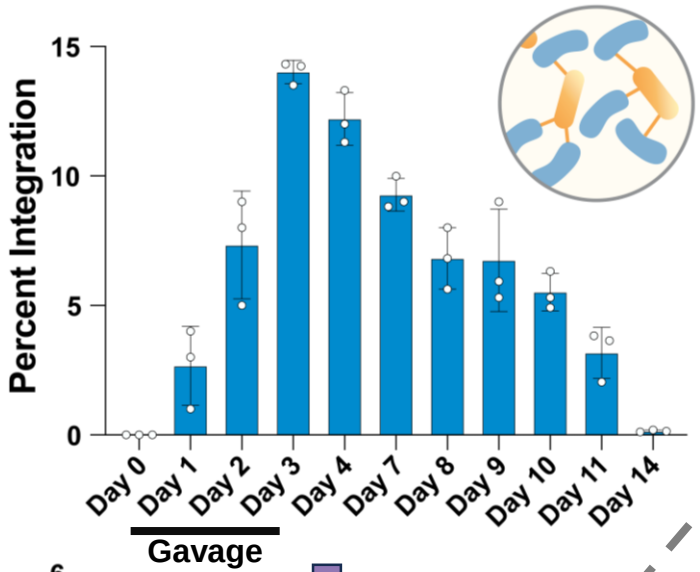
Icahn School of Medicine at Mount Sinai

In submission

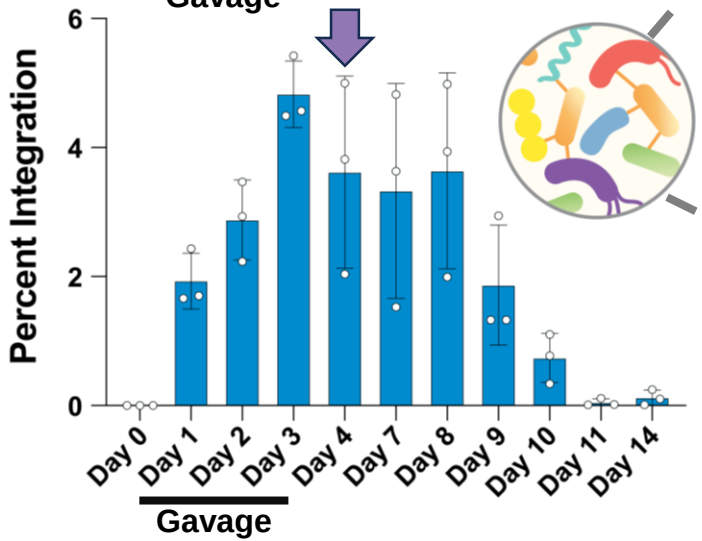
Detectable *in vivo* on-target integration over several days





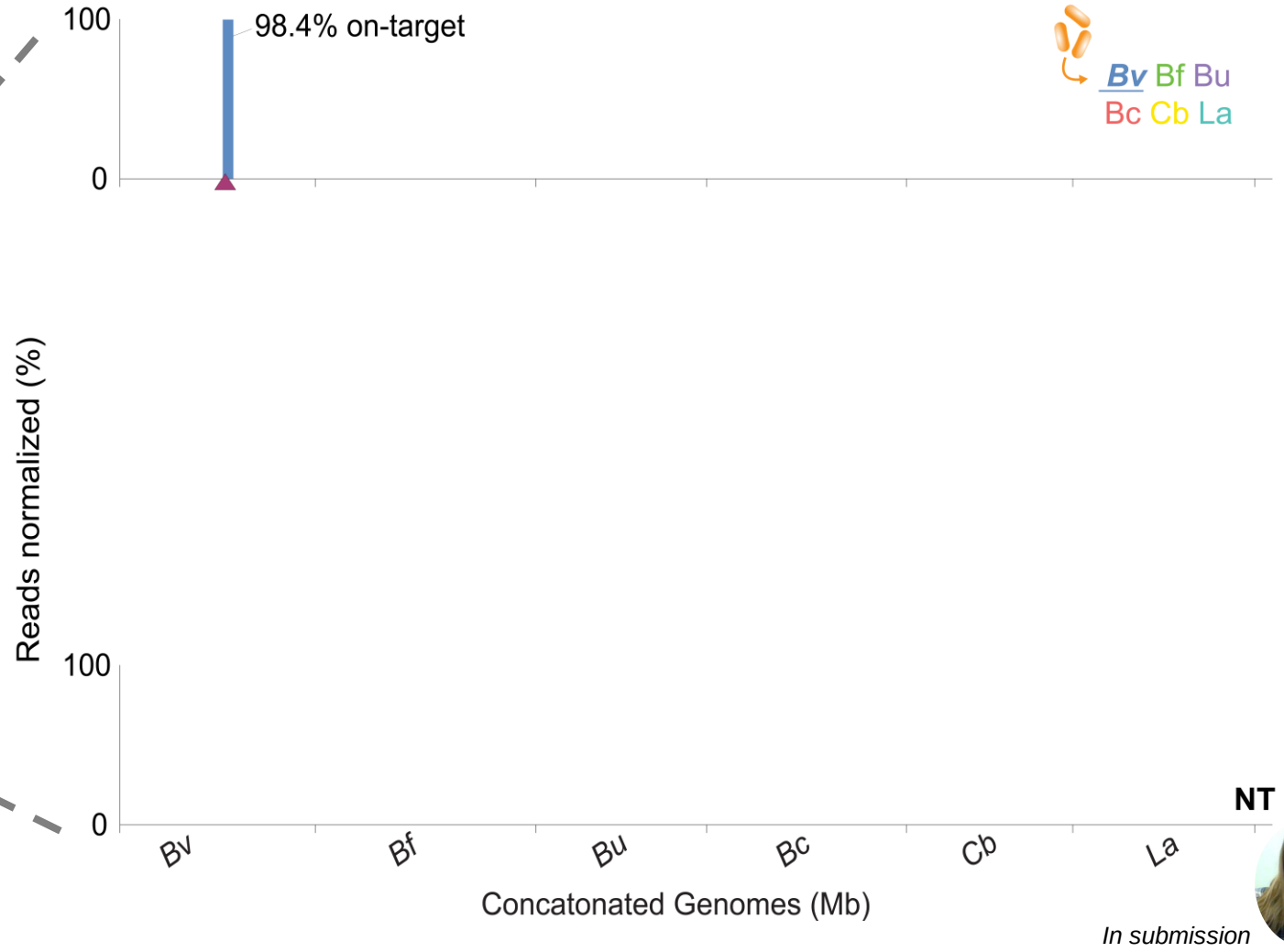


Bv
mono-colonized



Bv Bf *Bu*
Bc Cb La

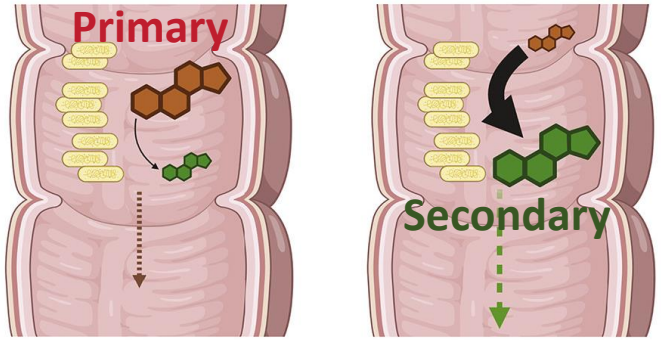
In vivo targeting of bacteria in community with high specificity



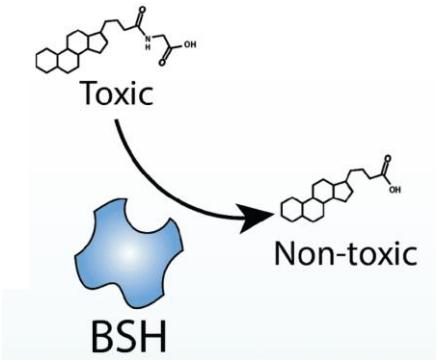
In submission

Can we modulate gut metabolites with MAGICAST?

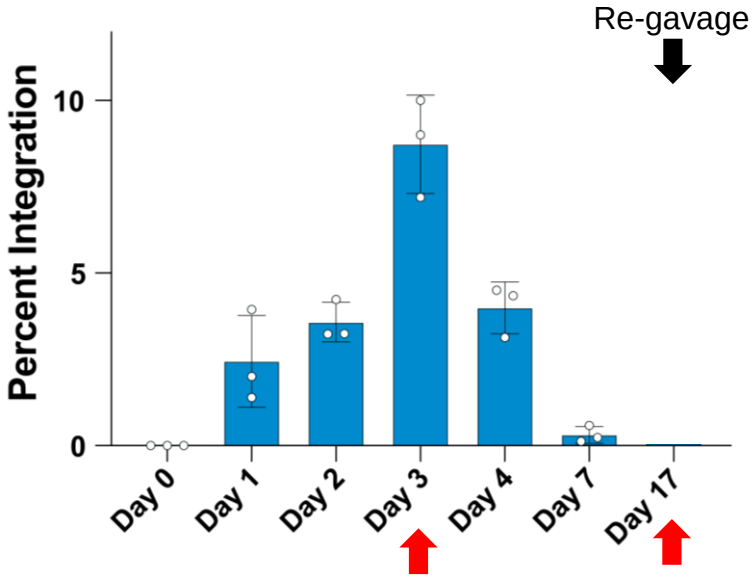
Bile acids: “digestive surfactant” metabolites



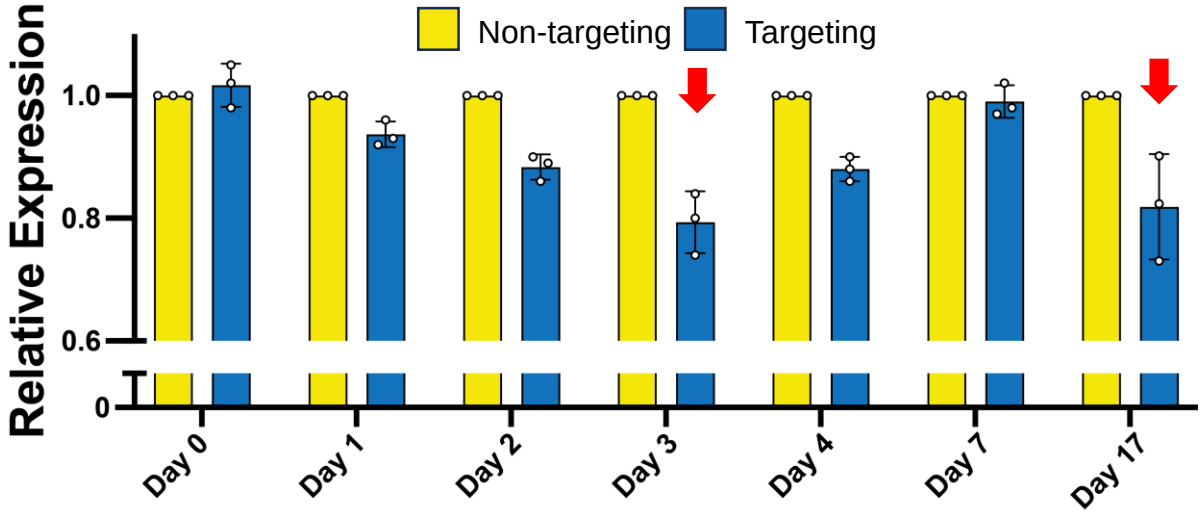
Come in 2 flavors



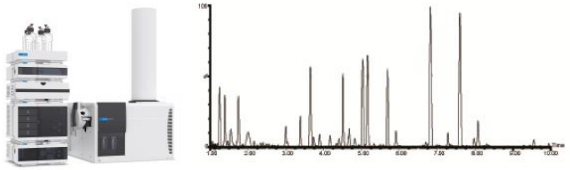
Integration in Bile Salt Hydrolase (BSH)



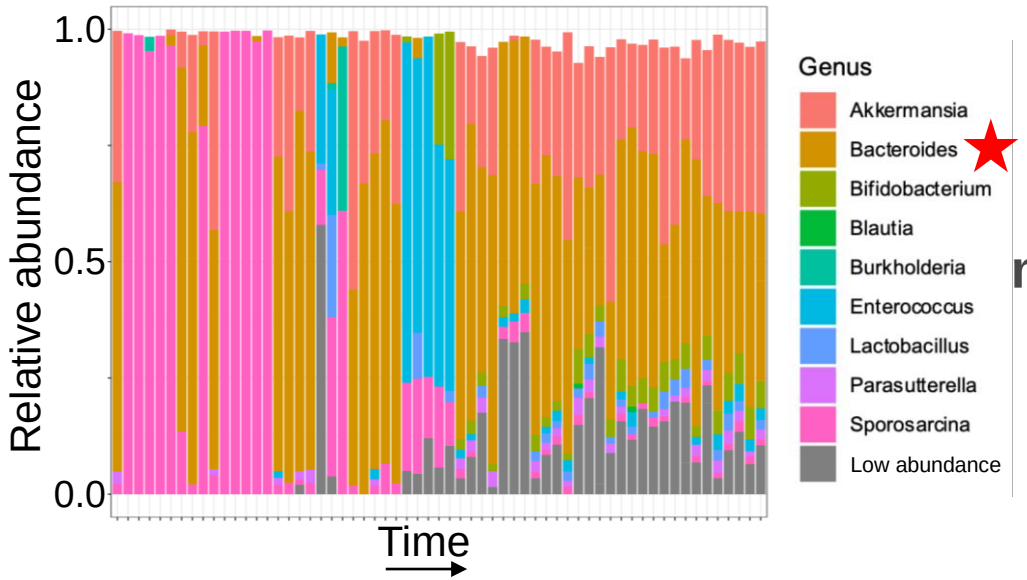
RT-qPCR reveals decreased expression of BSH *in vivo*



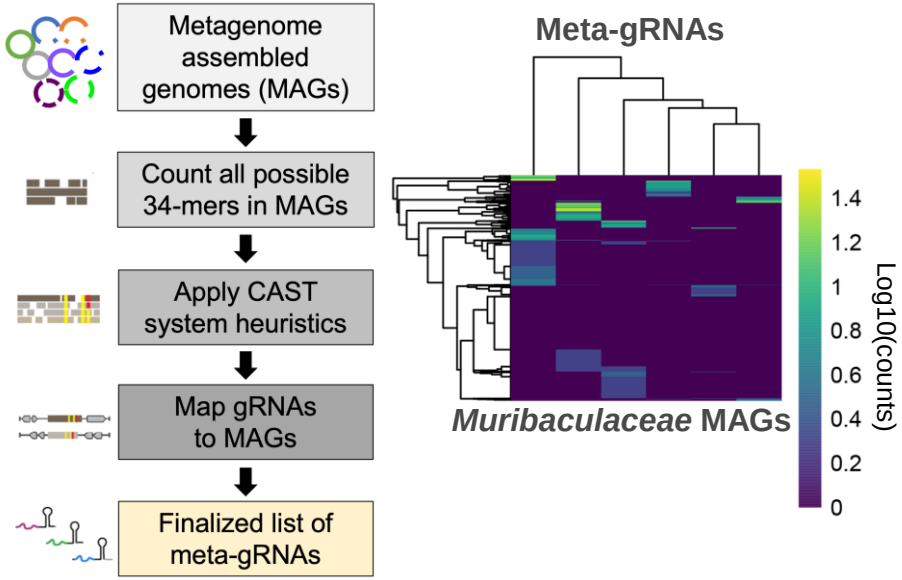
Next step: quantify host bile acid pool



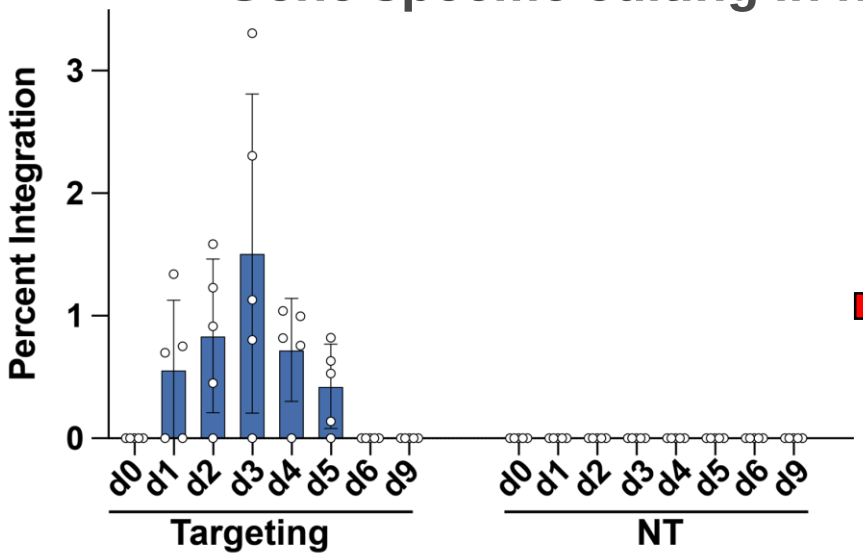
MAGICAST precisely edits native gut bacteria *in vivo*

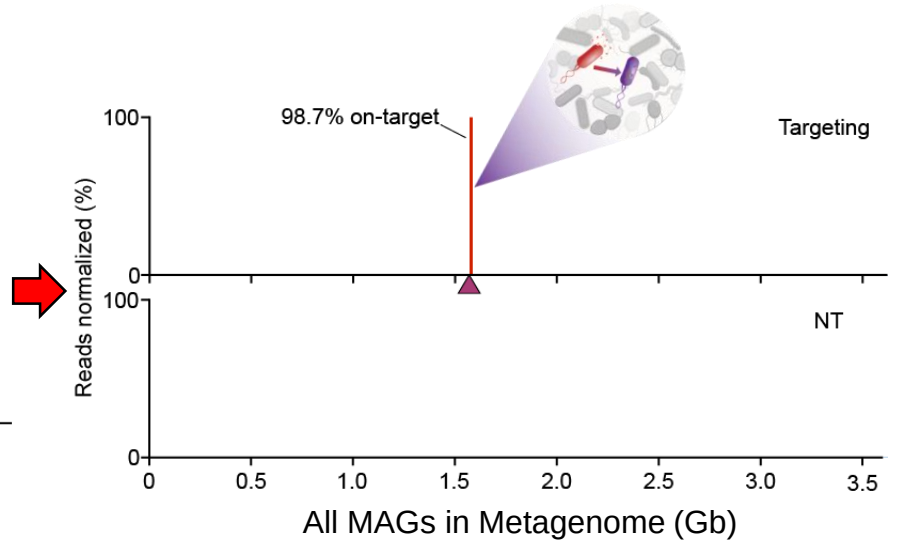


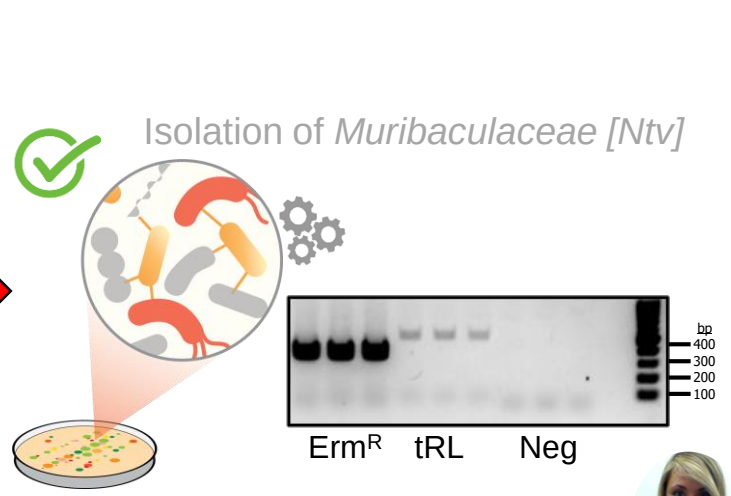
Native murine microbiomes are highly diverse



Gene specific editing in native murine *Muribaculaceae (*aka ”mouse *Bacteroides”)*



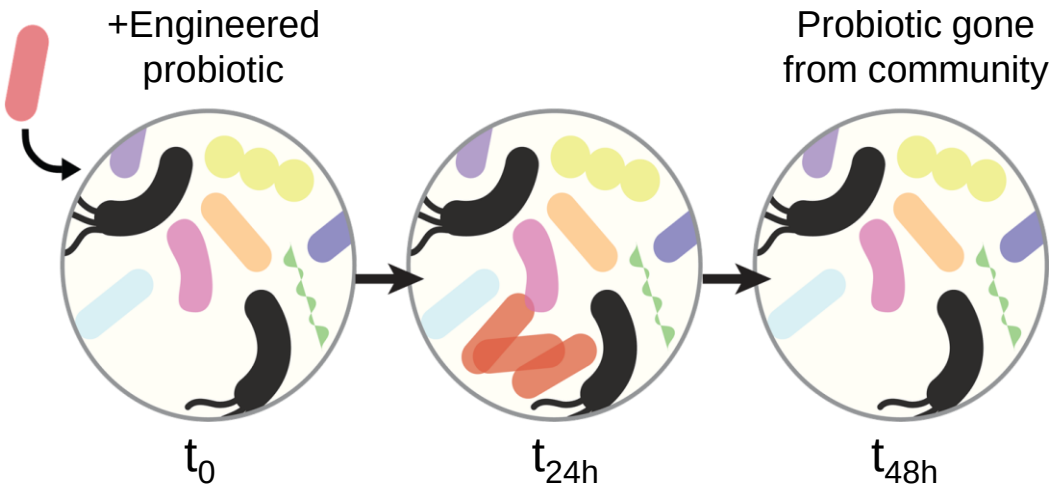




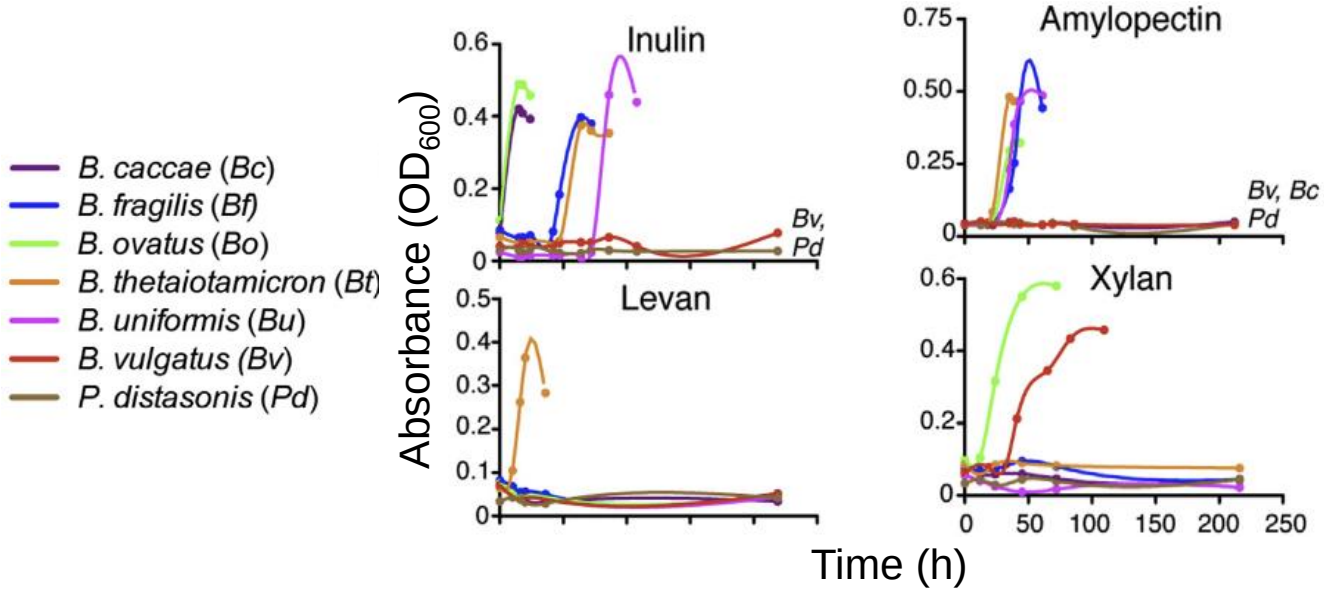
In submission

Phase III: Microbiomes use colonization factors to engraft in the gut

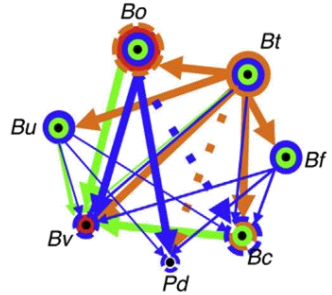
Non-grafting *in vitro* engineered probiotics



Bacteroides are polysaccharide utilization locus (PULs) specialists



Can we increase engraftment with metabolic payloads?

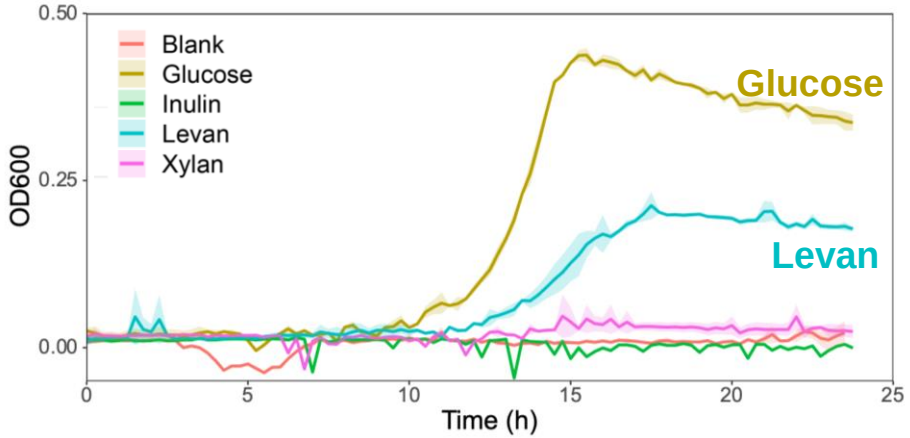


Comstock, et al. (2014)

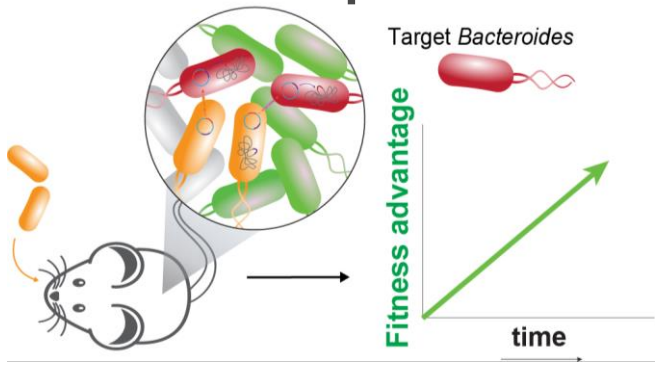
PULs as a functional payload for metabolic engineering

Extract bacteria from microbiome

Native mouse microbiome unable to metabolize inulin & xylan

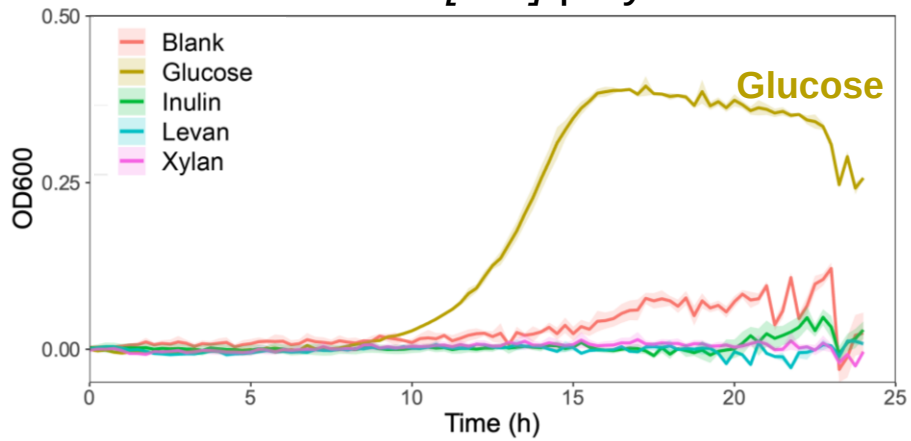


Next step: *in vivo*

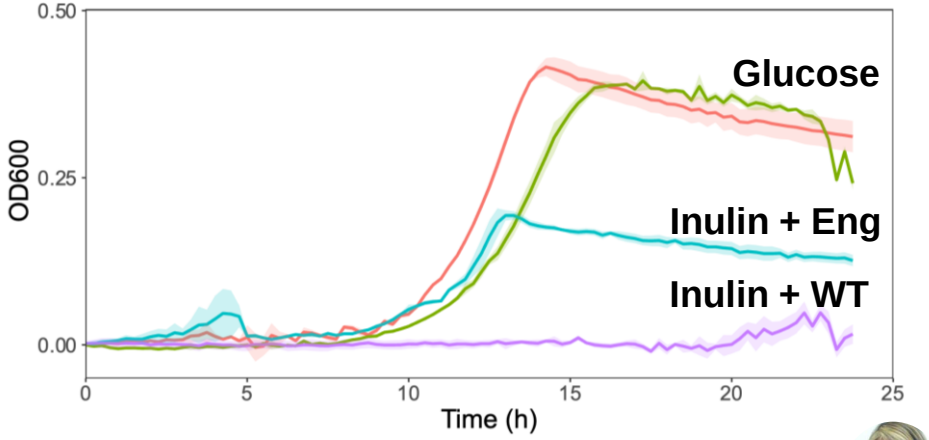


Genetic engineering rewires colonization

Muribaculaceae [Ntv] polysac screen



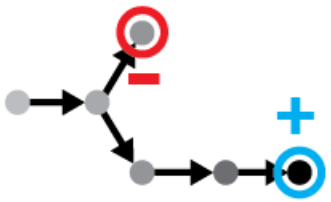
MAGICAST inulin PUL editing



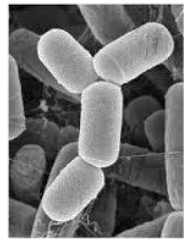
In submission

MAGICAST: Novel platform for targeted microbiome engineering

Interactions & Structure
- Colonization
- Microbe-Microbe
- Microbe-Host



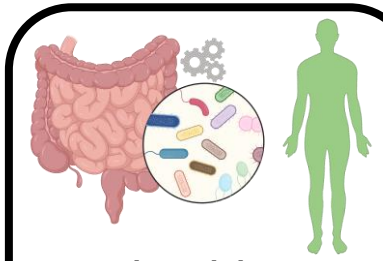
Targeted knockouts



Access rare species

Methanogenic archaea
- Low abundance
- CH_4 metabolic niche

MAGICAST Platform



Microbiome gene therapy

Amplify functions
- Anti-Inflammation: SCFA
- Detoxification: BSH

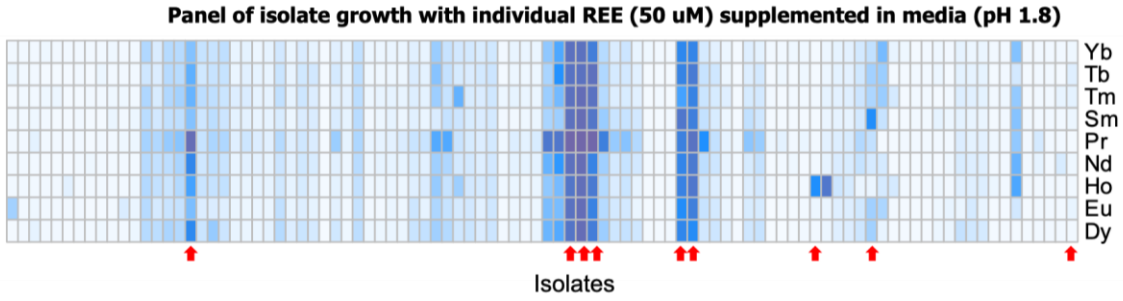
Infiltrate new payloads
- Phenylketonuria (PKU)
- *Self-destruction genes*

Expanding MAGICAST Engineering Into Extreme Environments

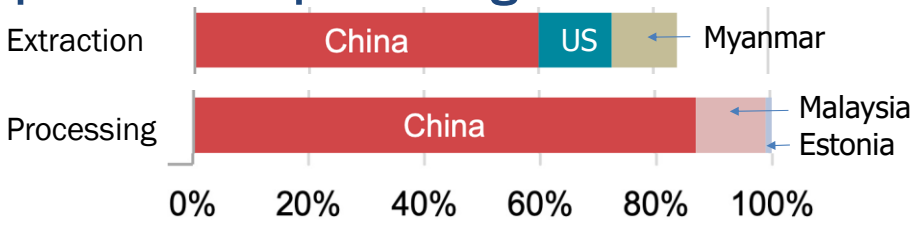
Bioprospecting and Bioengineering Extremophiles for Rare Earth Elements (REE)

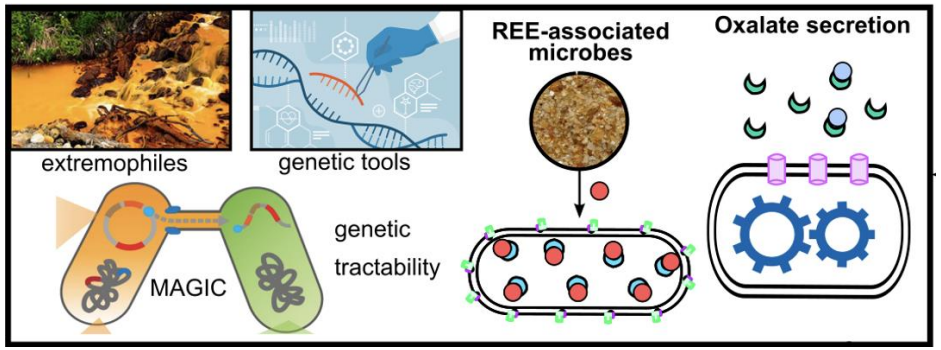
Acid mine drainage pH < 2

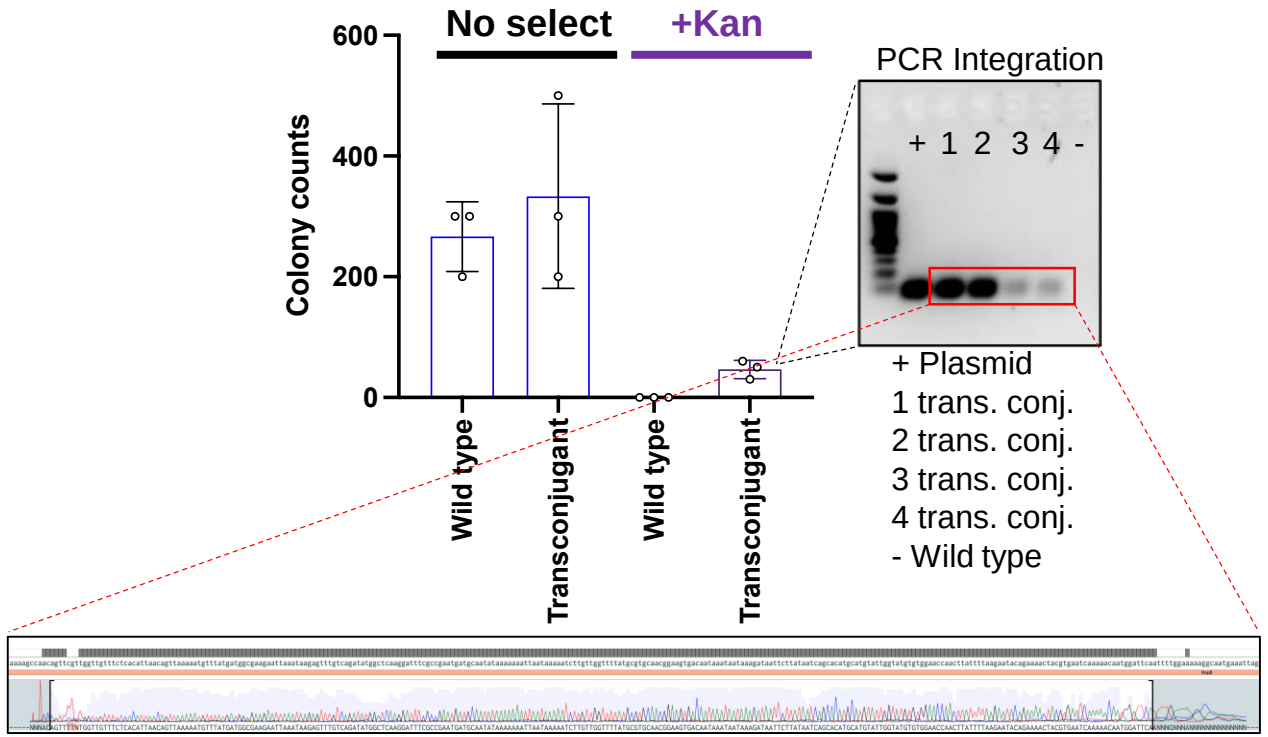
Economical & sustainable REE biomining process



Top three REE-producing countries in 2019







Acknowledgements & Questions

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Sanne
George
Rebeca

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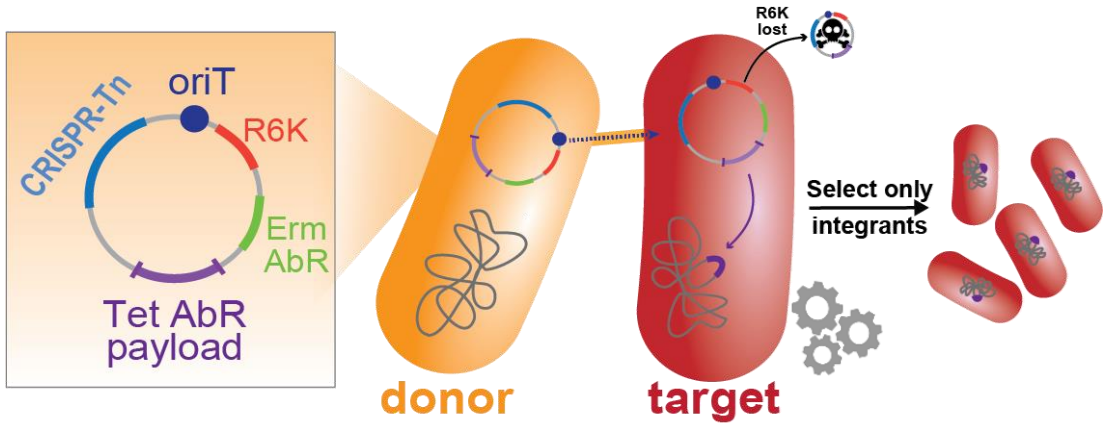
COLUMBIA UNIVERSITY IRVING MEDICAL CENTER

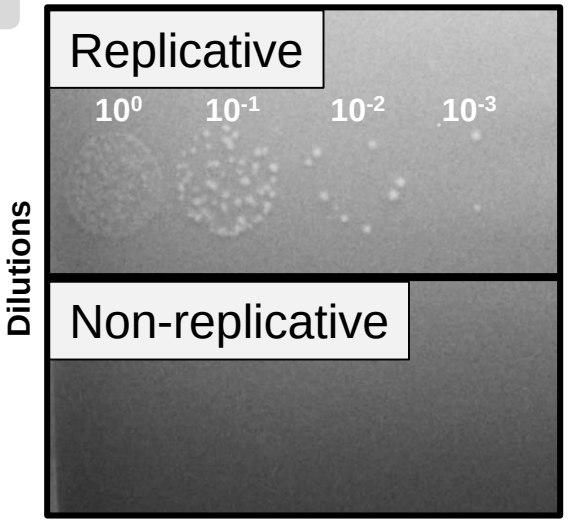
Questions?

Phase I: Expanding the engineering toolkit in *Bacteroides*

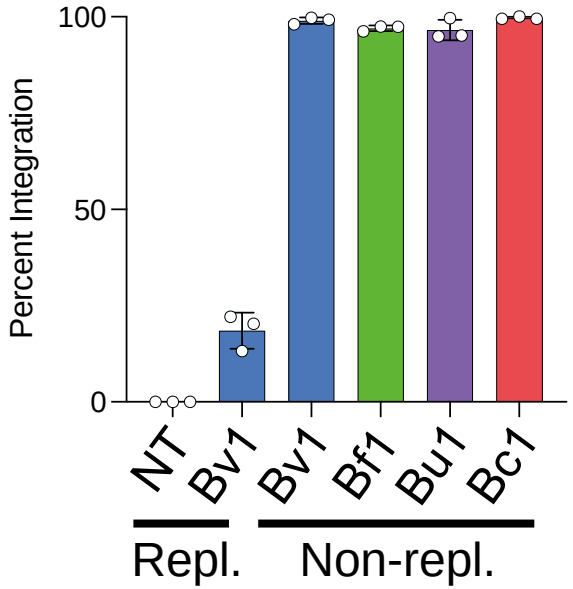
Bypass origins with non-replicative CAST vectors



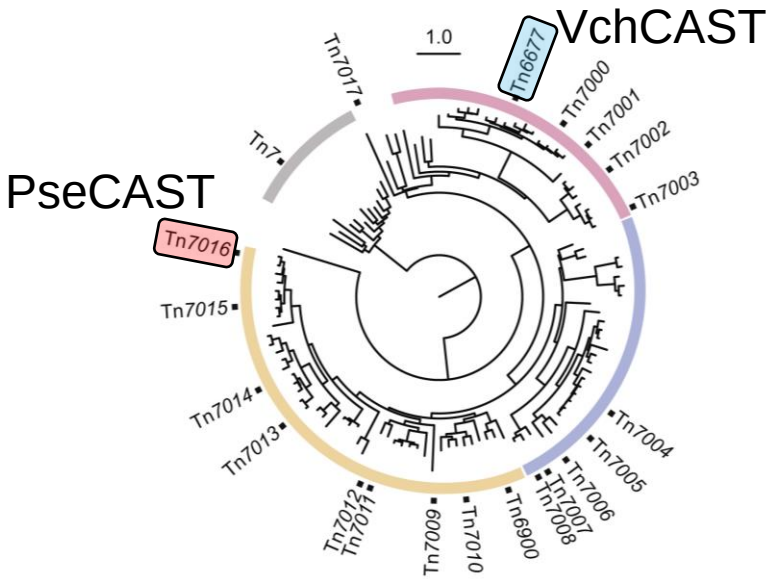
Select backbone AbR

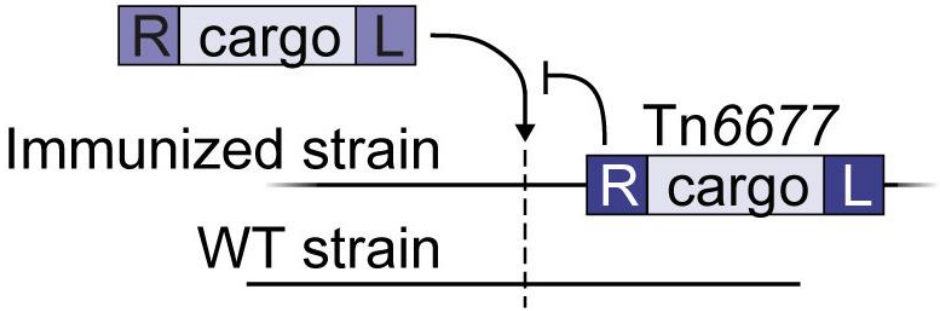


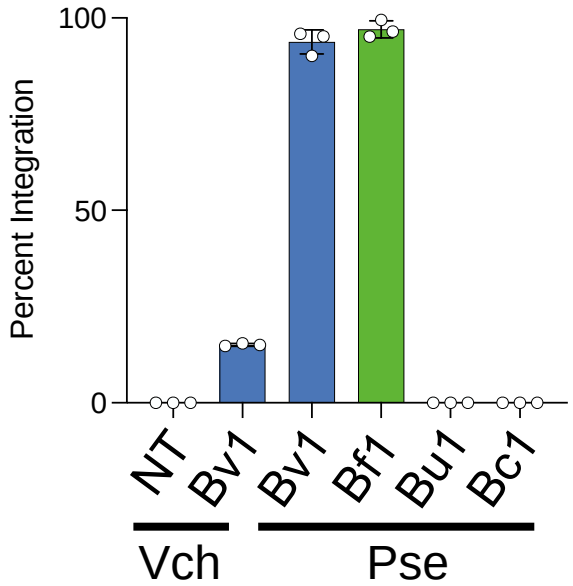
Select payload AbR



Orthogonal CAST homologs in wild *Bacteroides*

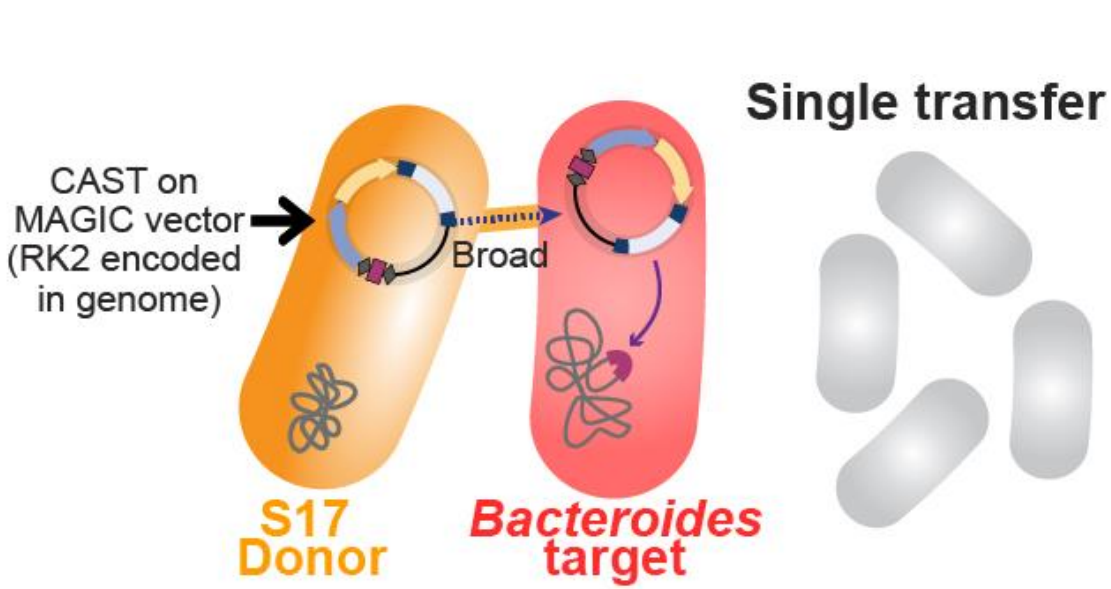


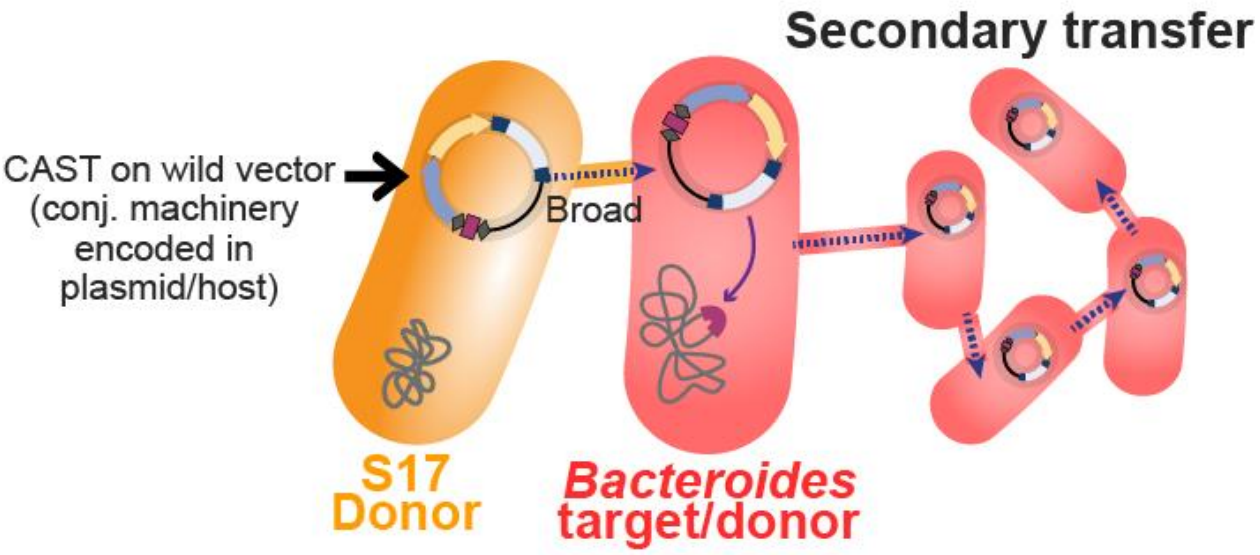


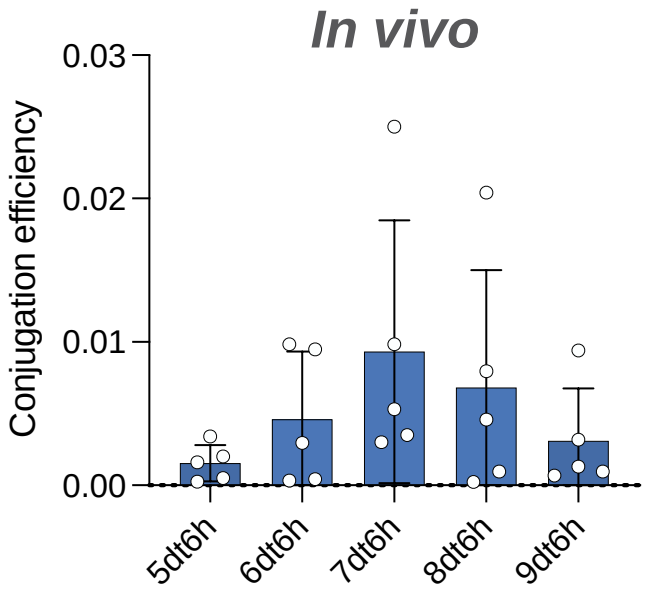


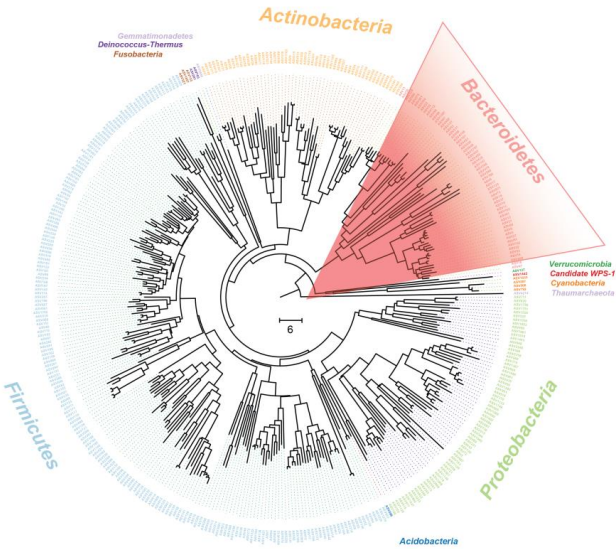
Klompe et al. *Molecular Cell* (2022)

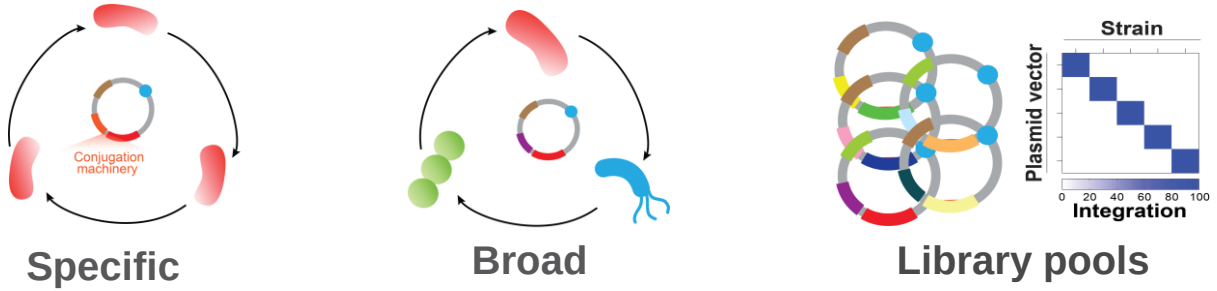
Future Directions: Improved delivery with native conjugative vectors







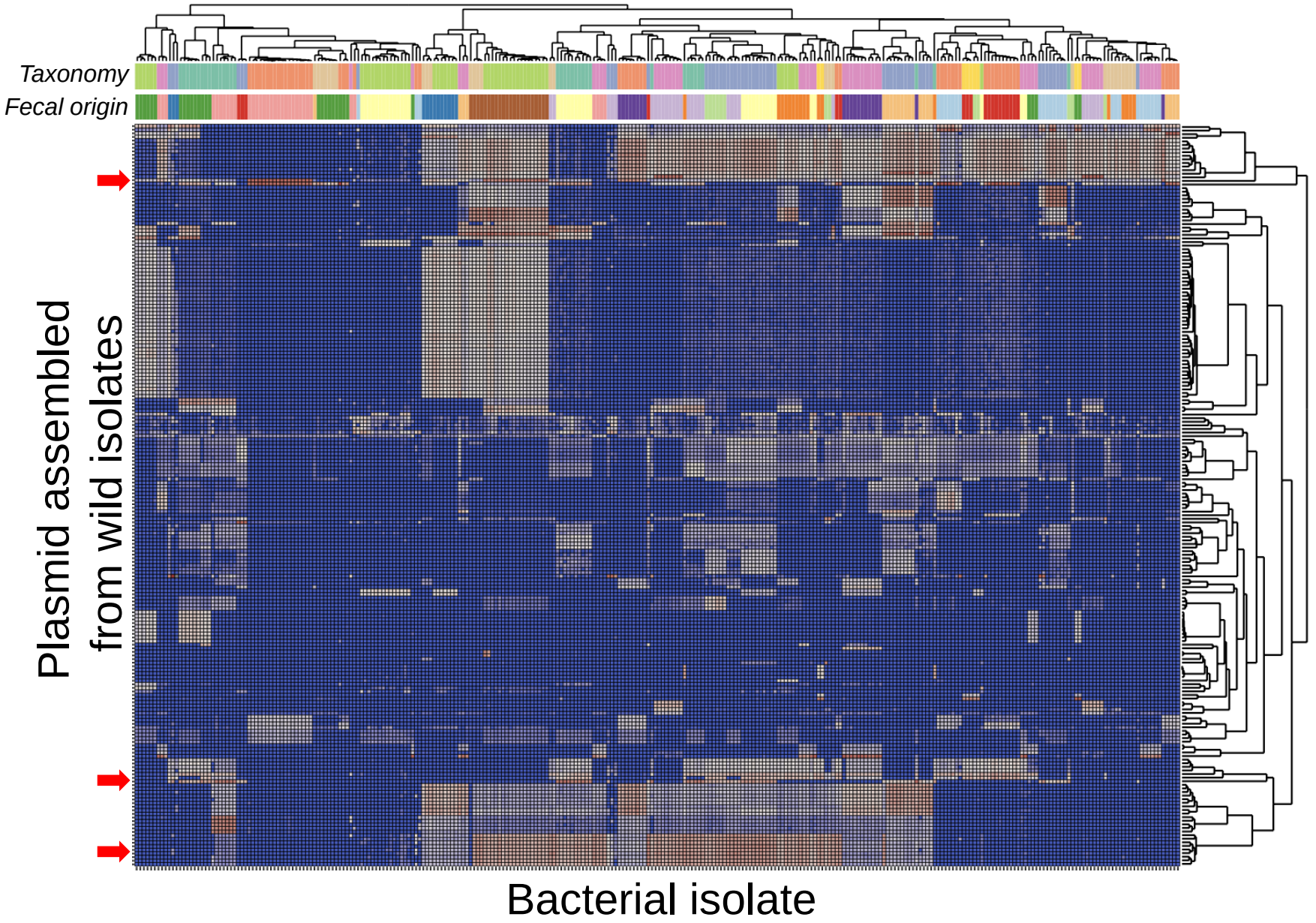




Universal toolkit for wild bacteria

Huang, Sheth et al. *Nat Biotech*

Future Directions: Identification of pervasive plasmids in wild isolates



Log_{10}(relative abundance) of plasmid

5
4
3
2
1
0

Fecal origin

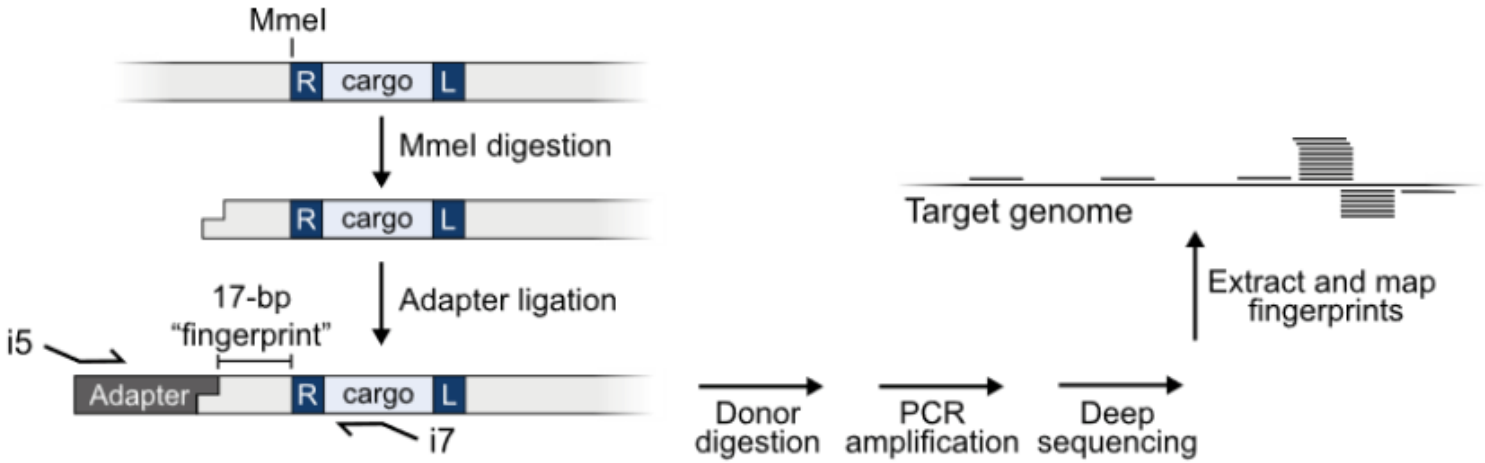
H1T4
H2
H3
H4
H5
H6
H9
H10
H11
H12
H13
H14

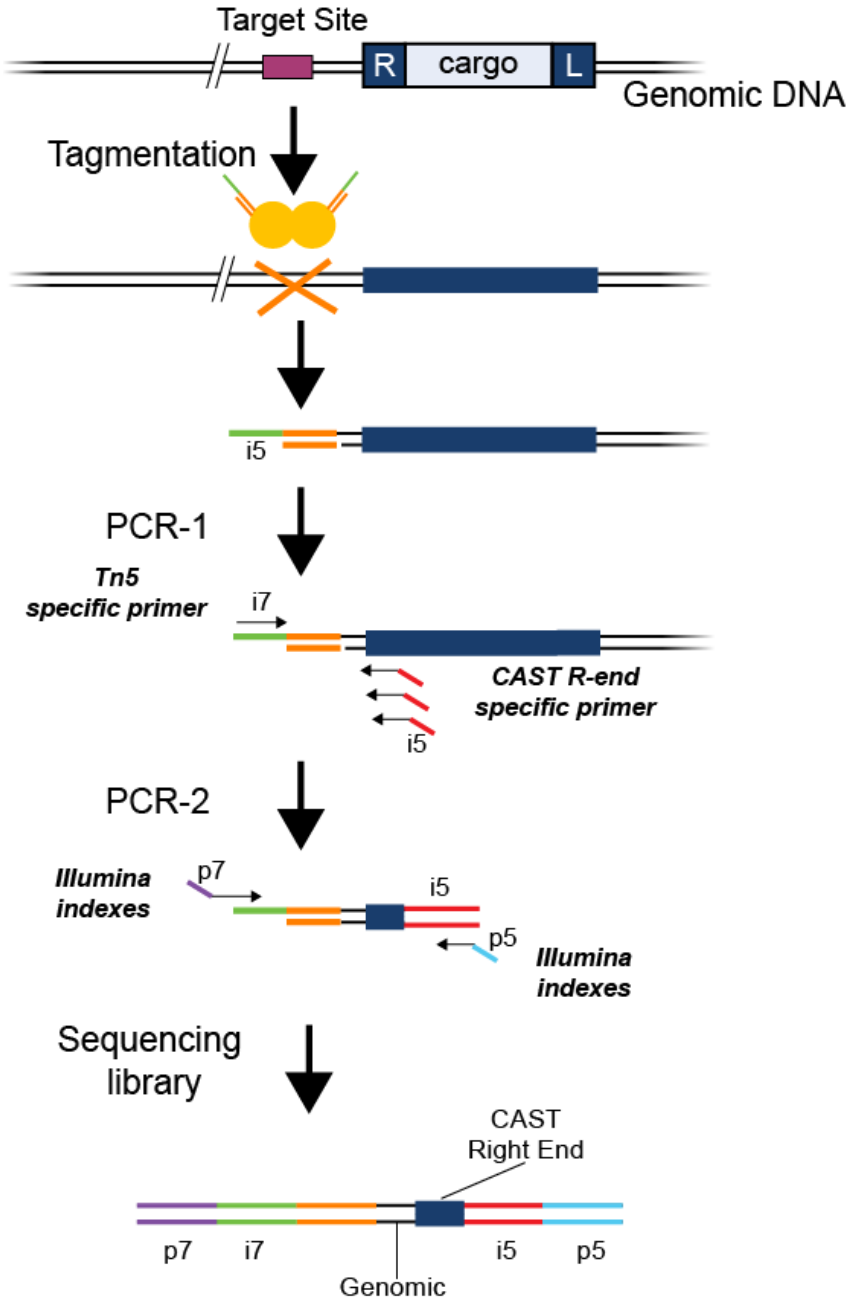
Taxonomy of isolate

Otu4, Bacteroides vulgatus
Otu6, Bacteroides stercoris
Otu9, Uncultured Otu9
Otu10, Bacteroides thetaiotaomicron
Otu11, Uncultured Otu11
Otu23, Bacteroides ovatus
Otu26, Bacteroides caccae

Phase II: Tagmentation Transposon sequencing (Tag Tn-seq)

- How do we map the location of transposon insertions in the metagenome with high resolution and fidelity?
- Sequencing without the need of restriction digest and ligation of genomic DNA

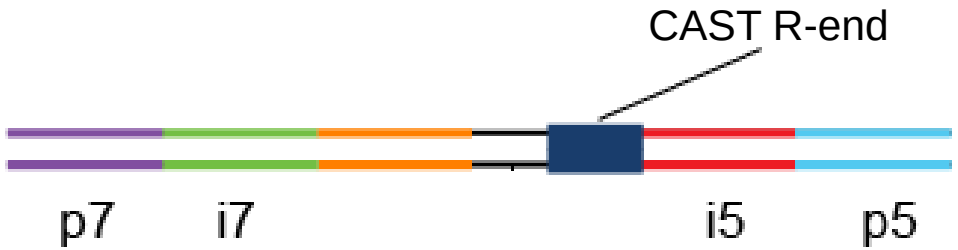




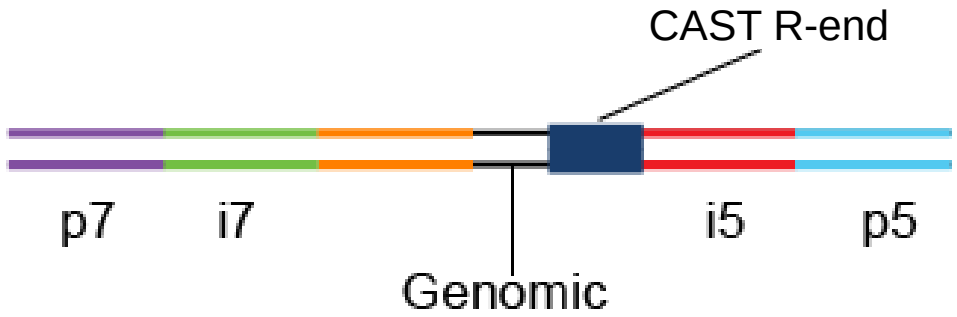
Klompe et al. *Nature* (2019)
Vo et al. *Nature Biotech* (2021)

Phase II: Computational pipeline for mapping integration events

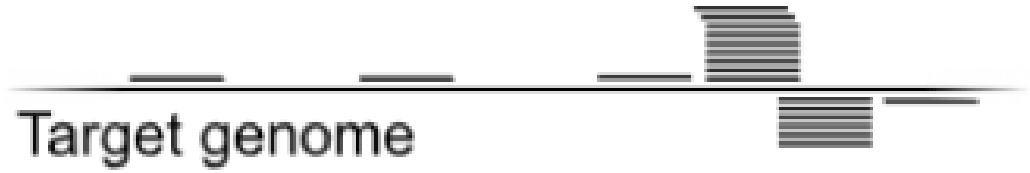
Step 1: Identify number of reads with transposon ends



Step 2: Extract 17bp flanks next transposon end

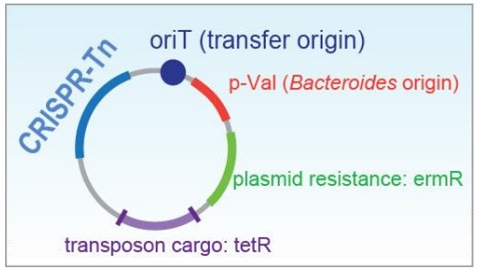


Step 3: Map flanks to target genomes/metagenome



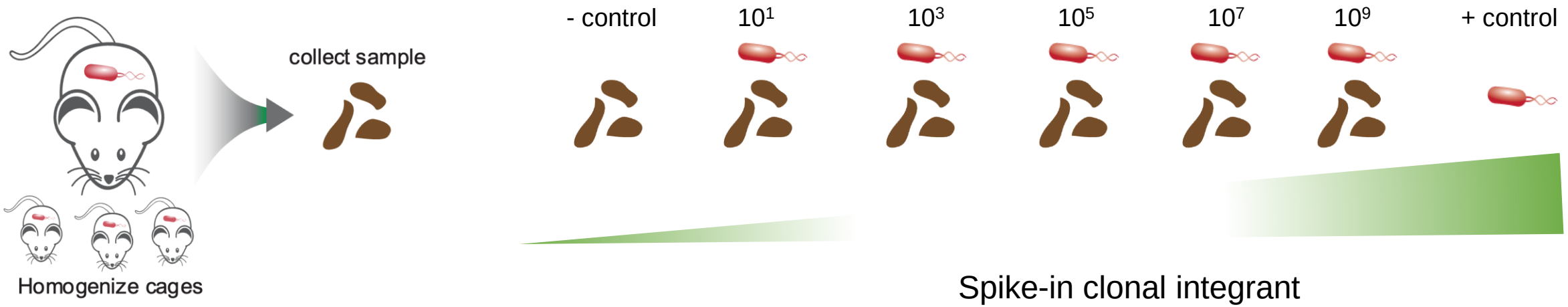
Sources of flanks:

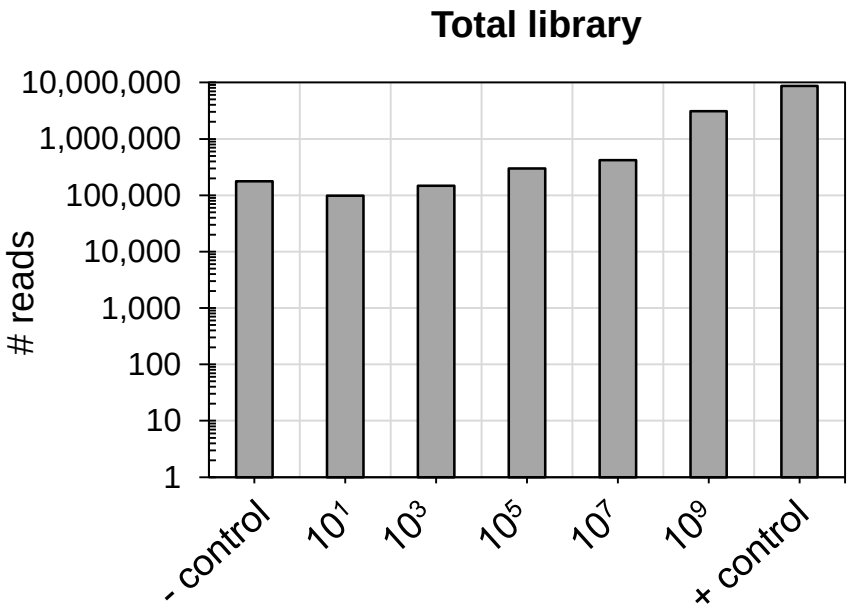
1. Genomic insertions
2. Plasmid donor
3. Other (mispriming, etc)

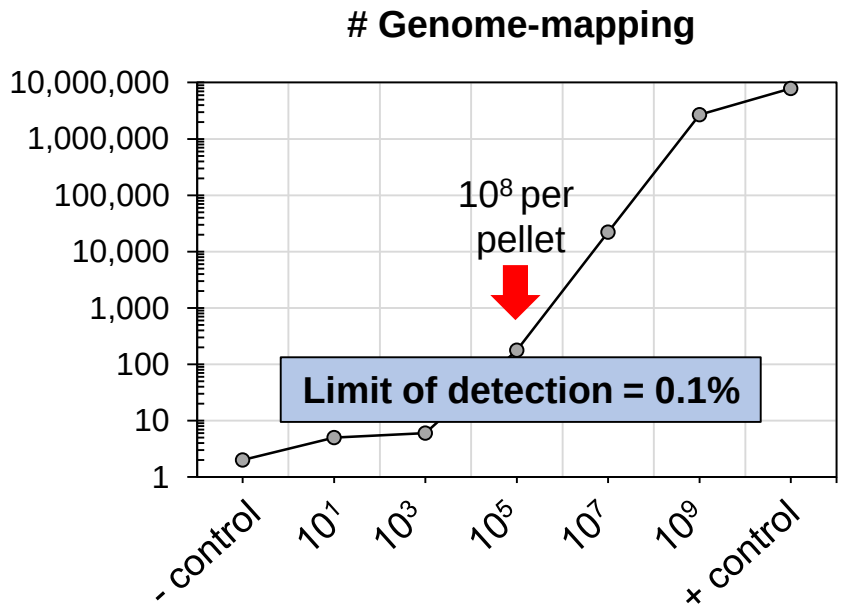


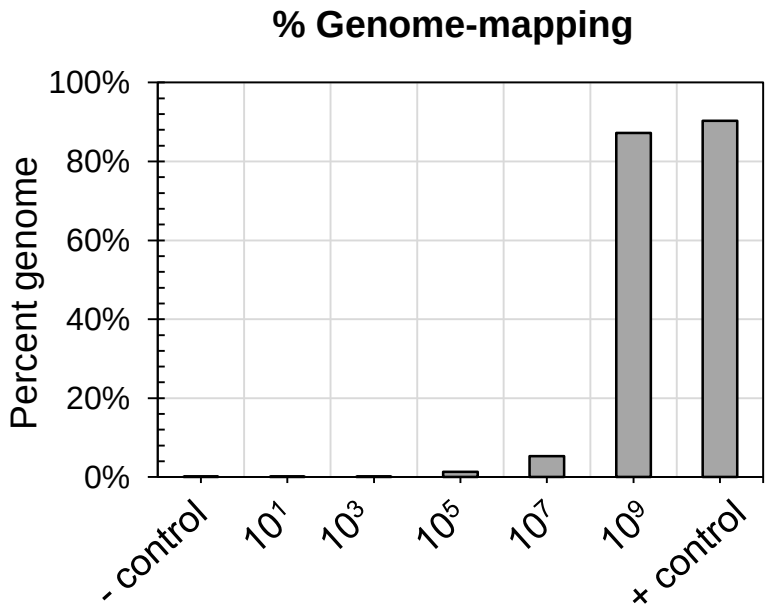
Gelsinger, Ronda et al., *In preparation*

Phase II: Tag Tn-seq limit of detection for metagenomic integration





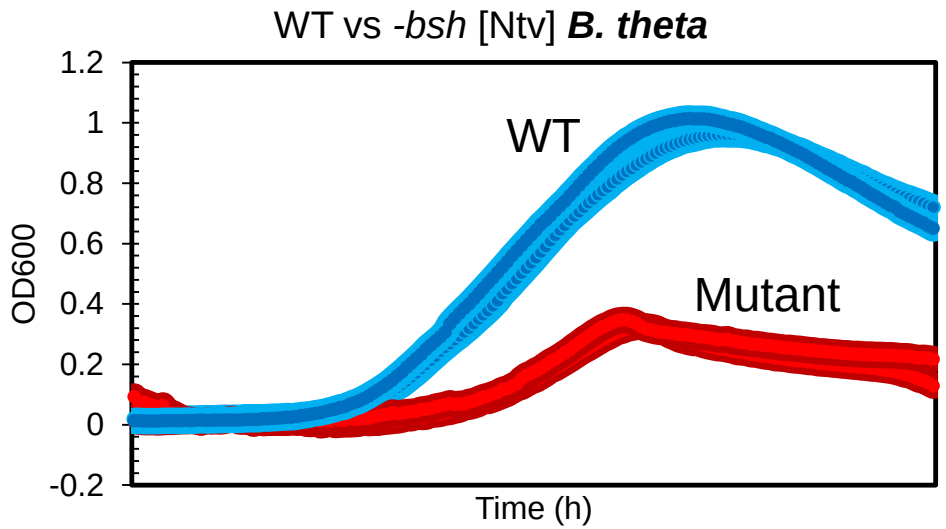


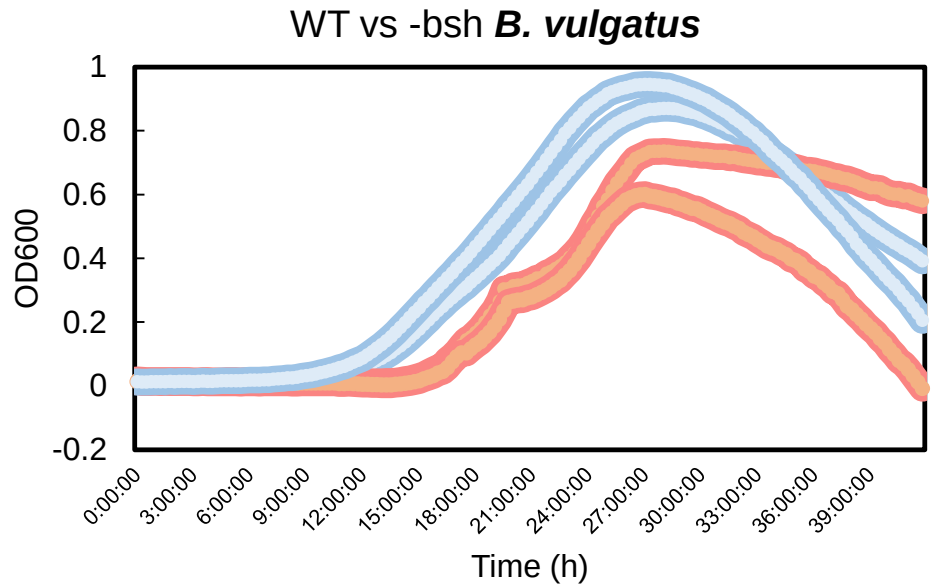


Gelsinger, Ronda, et al. *In preparation*

Phase III: PUL payloads can enable the study of BSH mutants

Comparison of WT vs *bsh*- mutant





Comparison of *bsh*- vs *bsh*- + PUL mutant