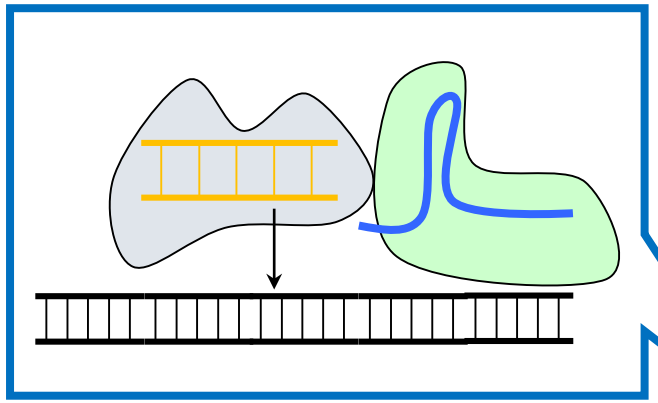
Leveraging Type I-F CRISPR-Associated Transposase Regulators to Improve Editing Efficiency

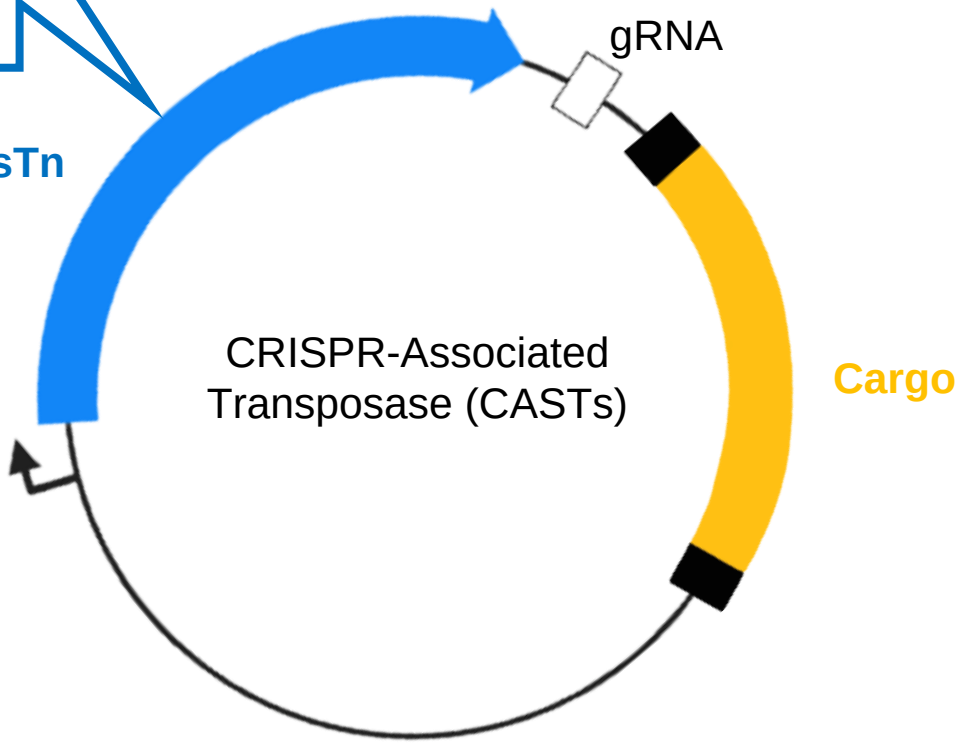
Leo Song, Amanda Alker, **Sophia E. Swartz**, Jigyasa Arora, Sara Smith, Rachel Rovinsky, Abby Wang, Agnès Oromí-Bosch, Robin Herbert, Brady F. Cress, Jennifer A. Doudna, Benjamin E. Rubin

CRISPR-associated transposases (CASTs)

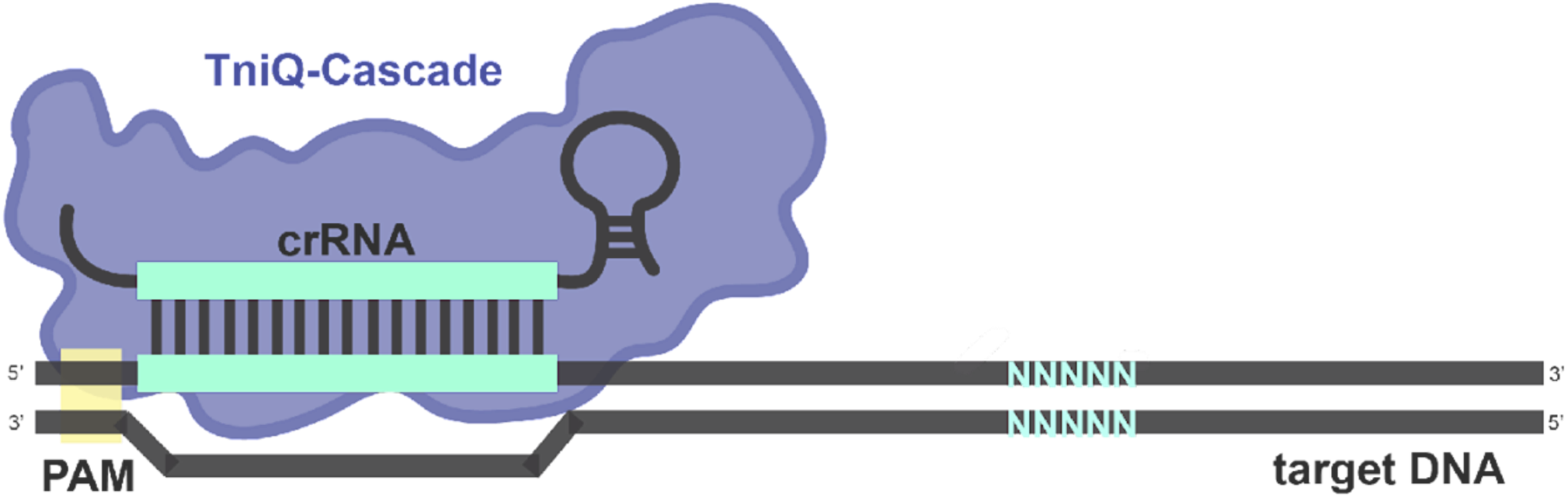


VcDART:

Vibrio **c**holerae (derived) **D**NA-editing-**a**ll-in-one **R**NA-guided CRISPR-Cas **T**ransposase



CASTs permit programmable insertion of large cargos



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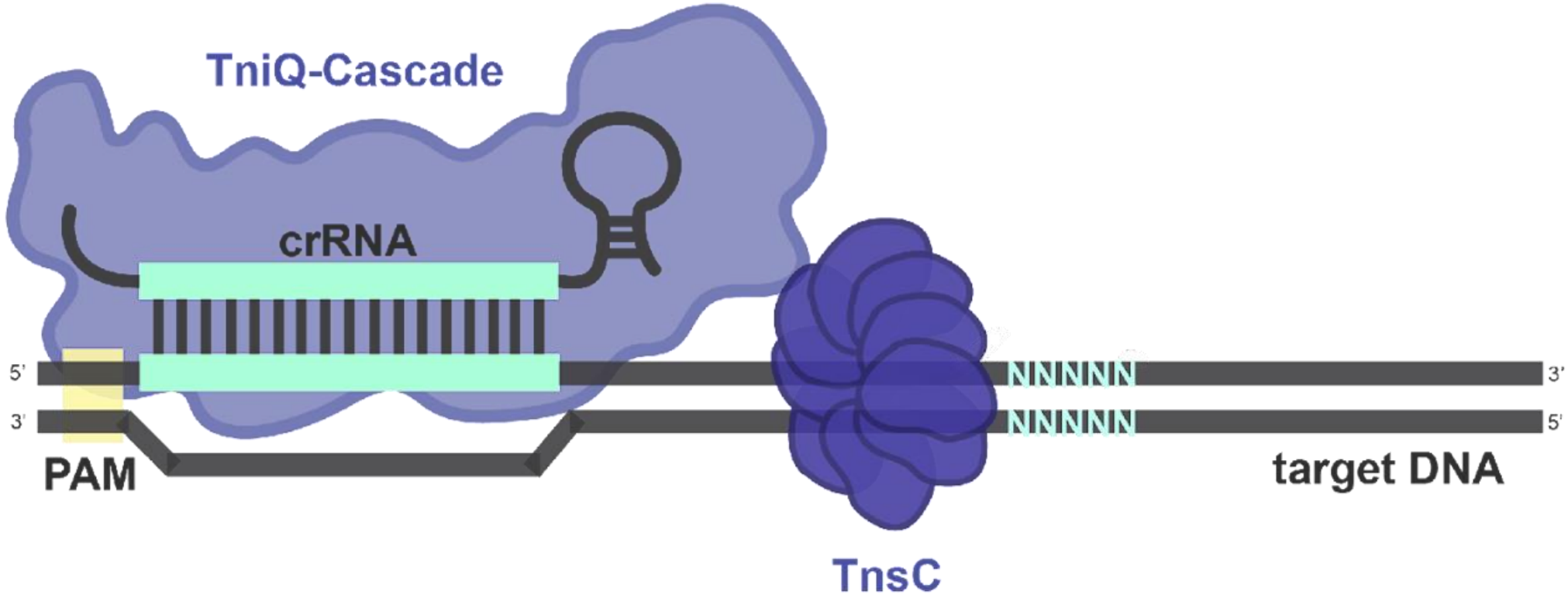

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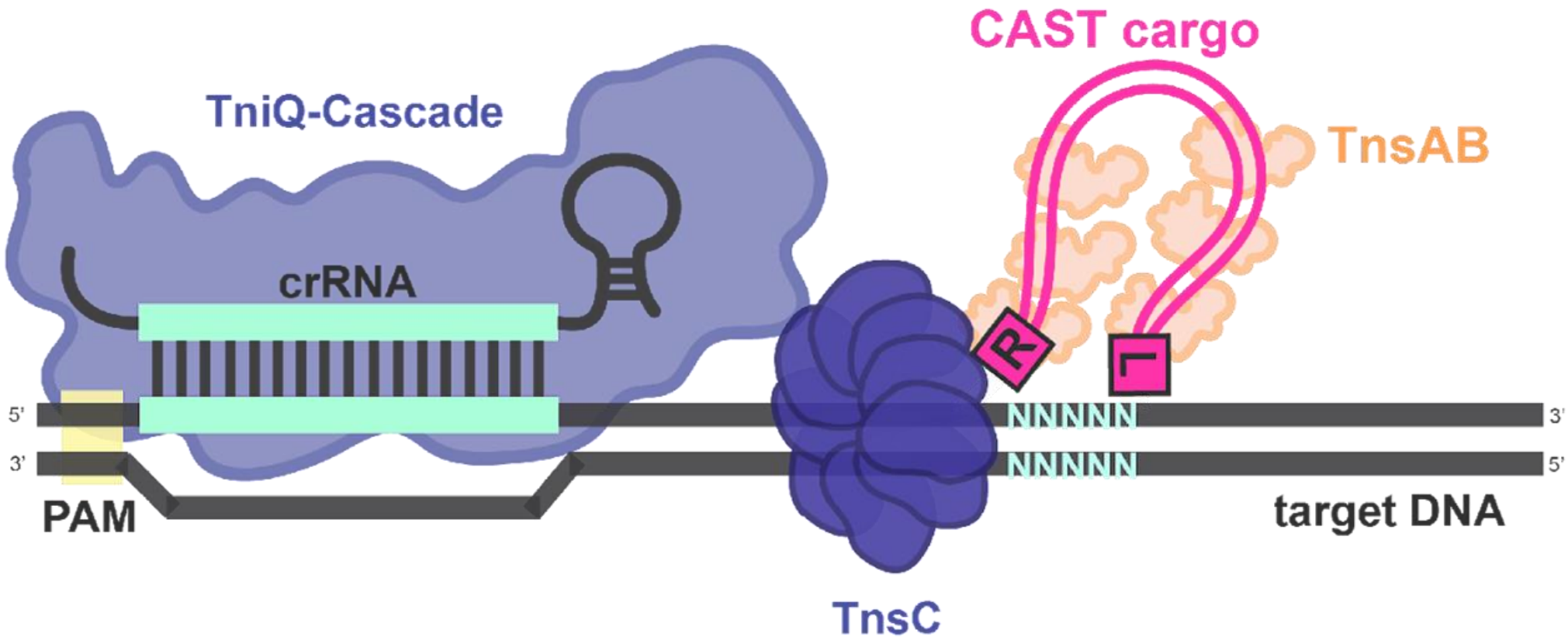
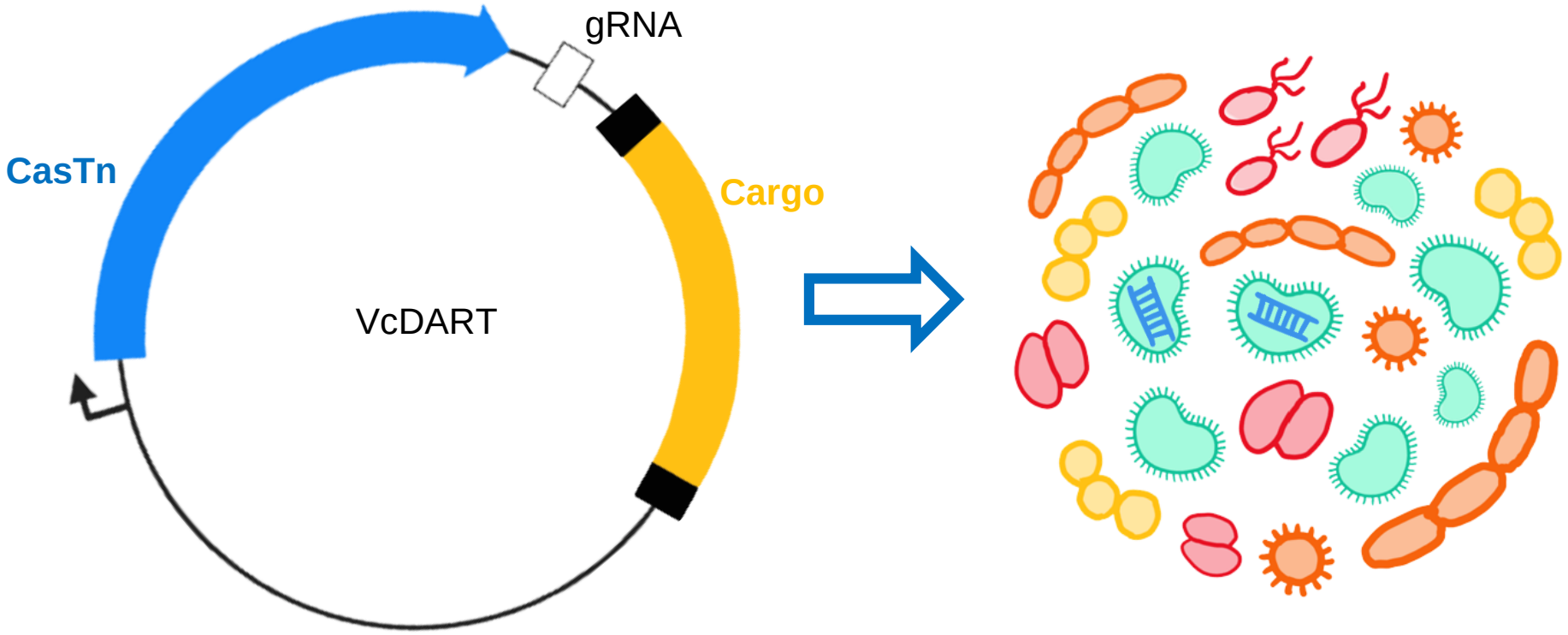
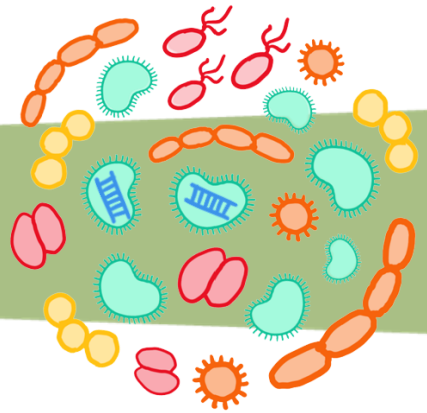


CASTs enable programmable community editing



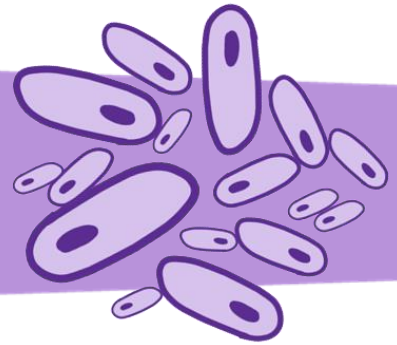
Rubin, Diamond, Cress et al. (2022), *Nature Microbiology*

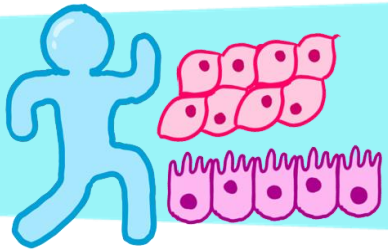
Limitations of CAST editing tools



Low editing efficiency in complex community

Inability to edit non-model, intractable bacteria

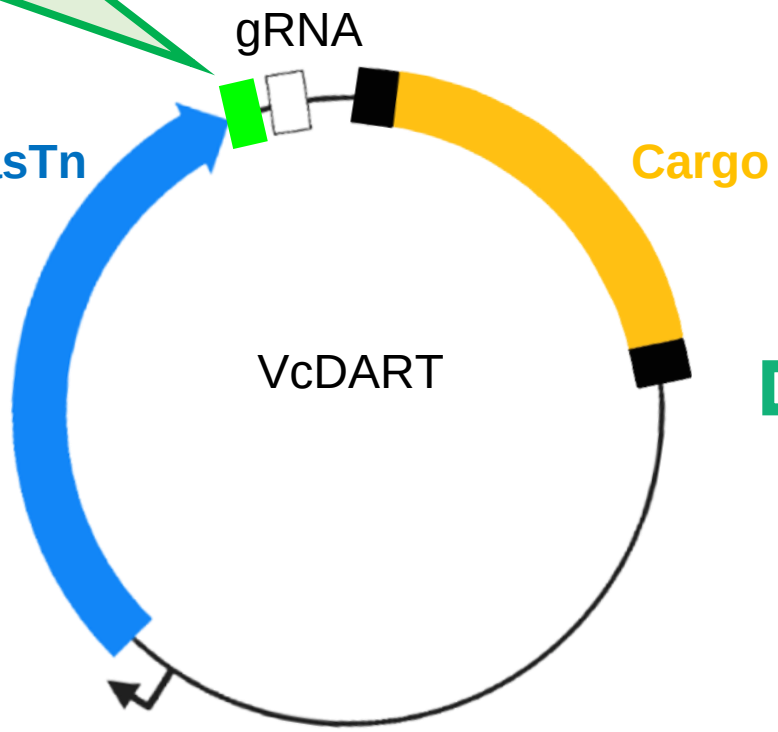




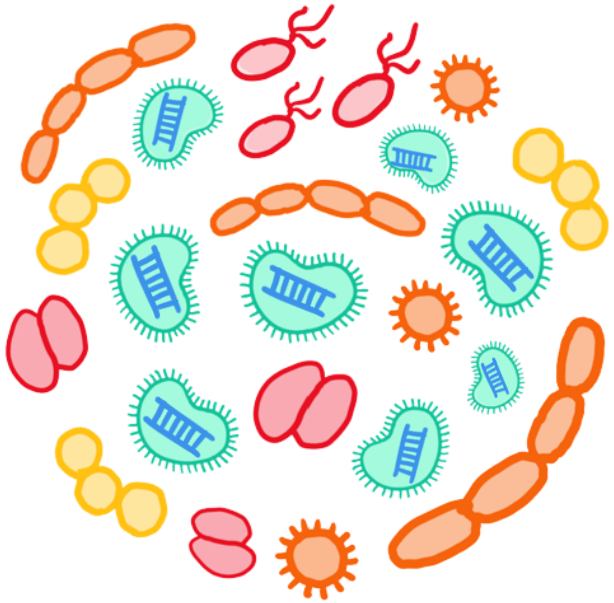
Extremely low editing efficiency in human cells

Project objective

Enhancer of Positive Regulator/
Inhibitor of Negative Regulator



High-Efficiency Community Editing



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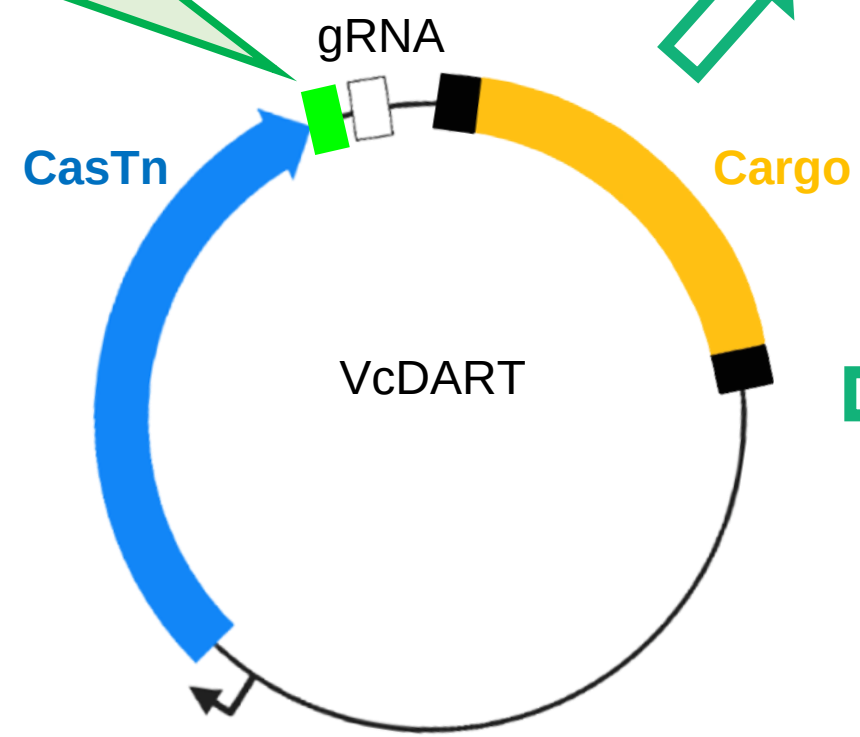
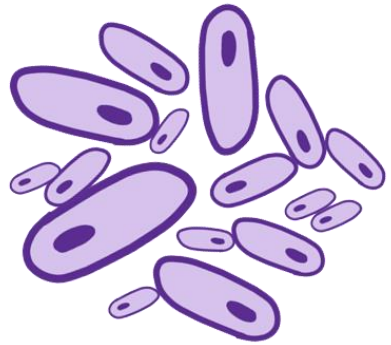


Hard-to-Edit Bacteria



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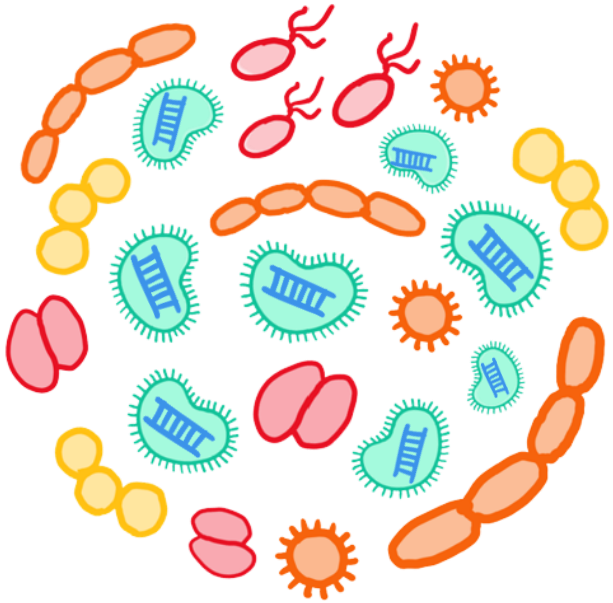


Project objective

Enhancer of Positive Regulator/ Inhibitor of Negative Regulator

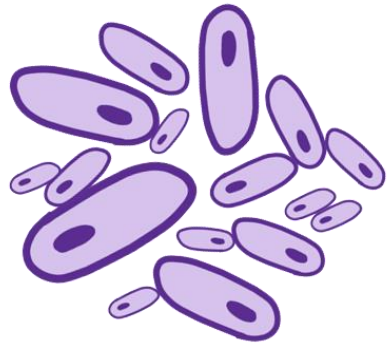
gRNA

CasTn

Cargo

VcDART

Hard-to-Edit Bacteria



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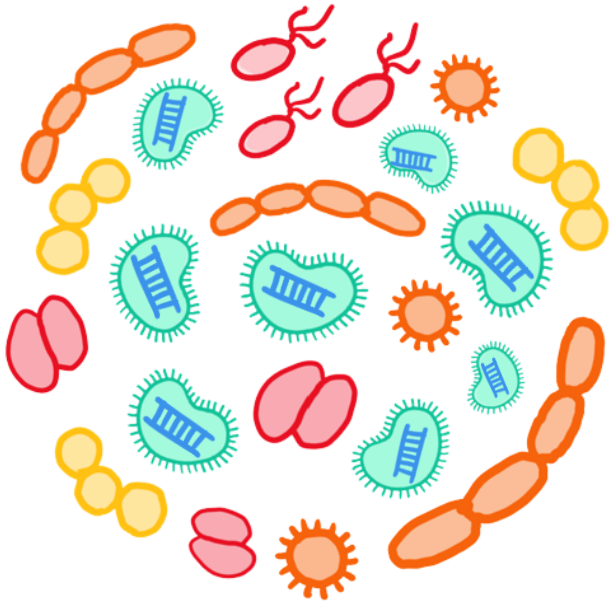
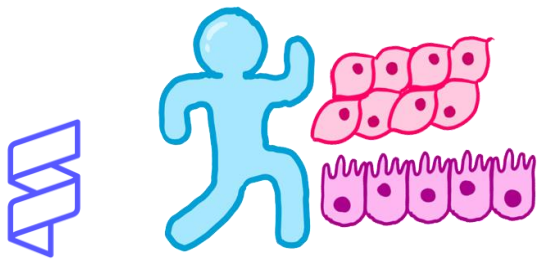


Human Cell Editing



Project roadmap

1 Genome wide mutant screen

Project roadmap

1. Genome wide mutant screen

2. Validate candidate screen hits

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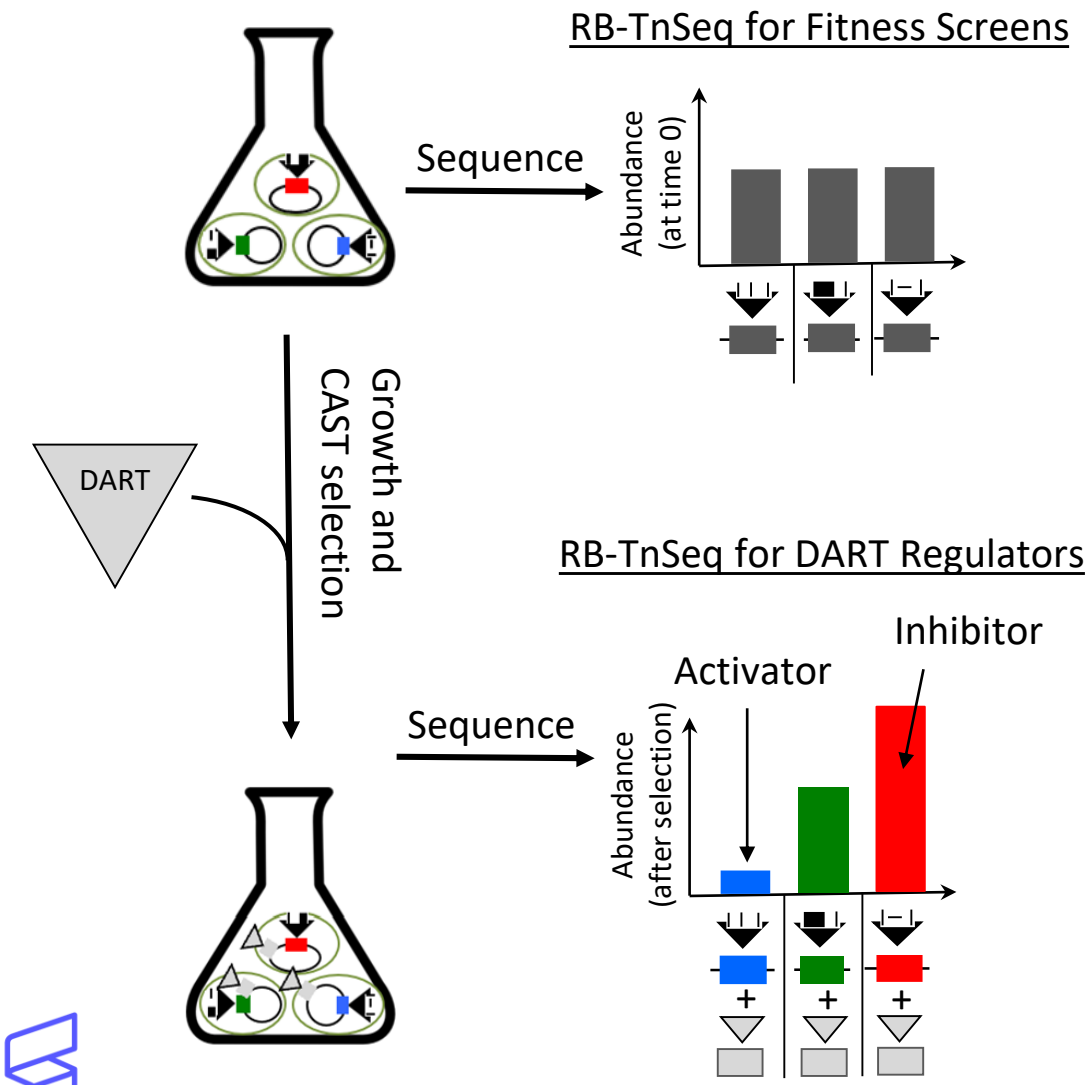
3. Incorporate regulators onto VcDART vector

Project roadmap

1. Genome wide mutant screen
2. Validate candidate screen hits
3. Incorporate regulators onto VcDART vector
4. Improve VcDART function

Genome wide mutant screen for identifying regulators

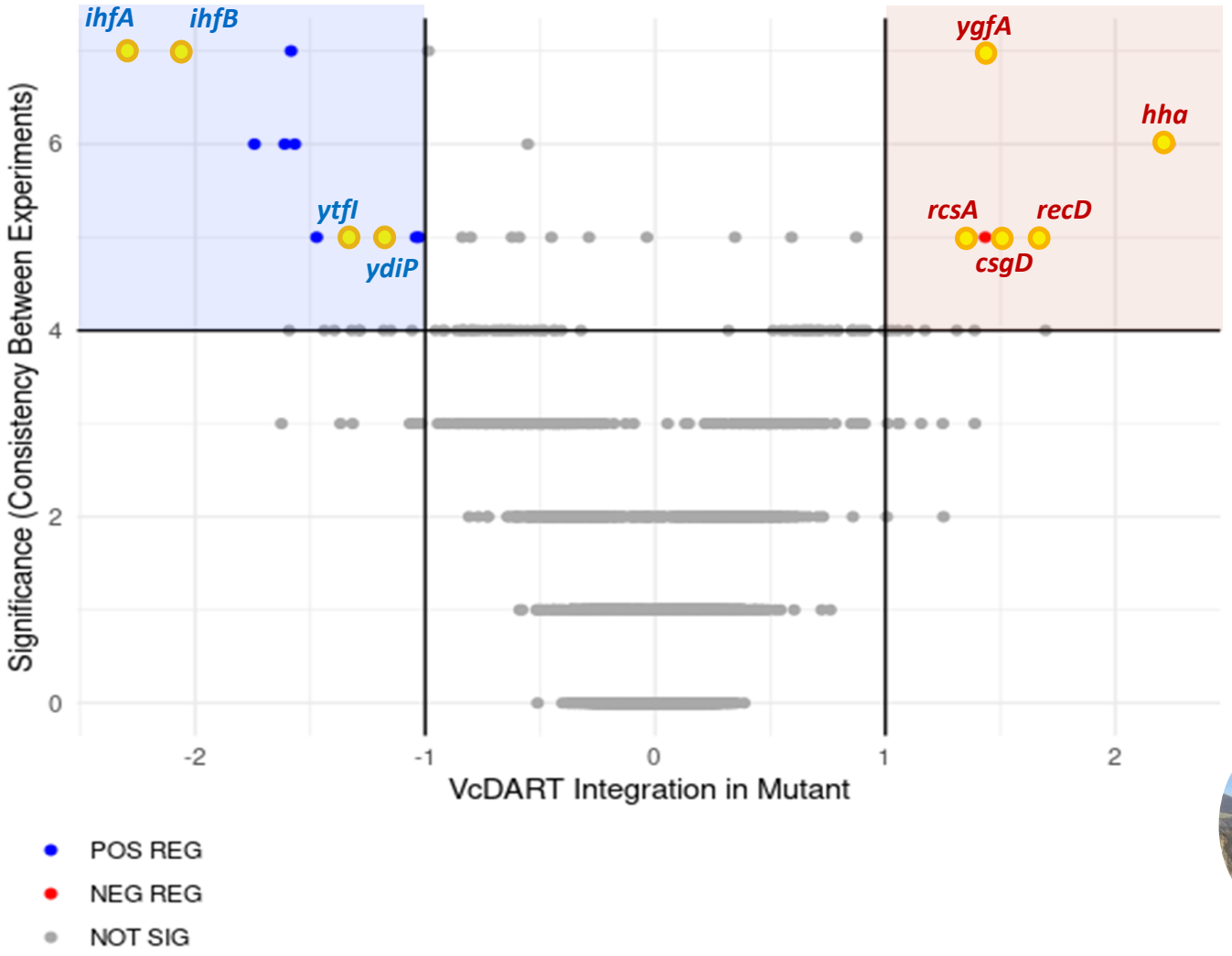


Decreased abundance –> **activator** gene knocked out

No change in abundance –> neutral gene knocked out

Increased abundance –> **inhibitor** gene knocked out

Combined genome wide mutant screen and validation



Functions of Interest

- DNA-interacting
- RNA-interacting
- Protein-interacting
- Unknown (hypothetical proteins)

Avoided

- Membrane proteins


Leo Song

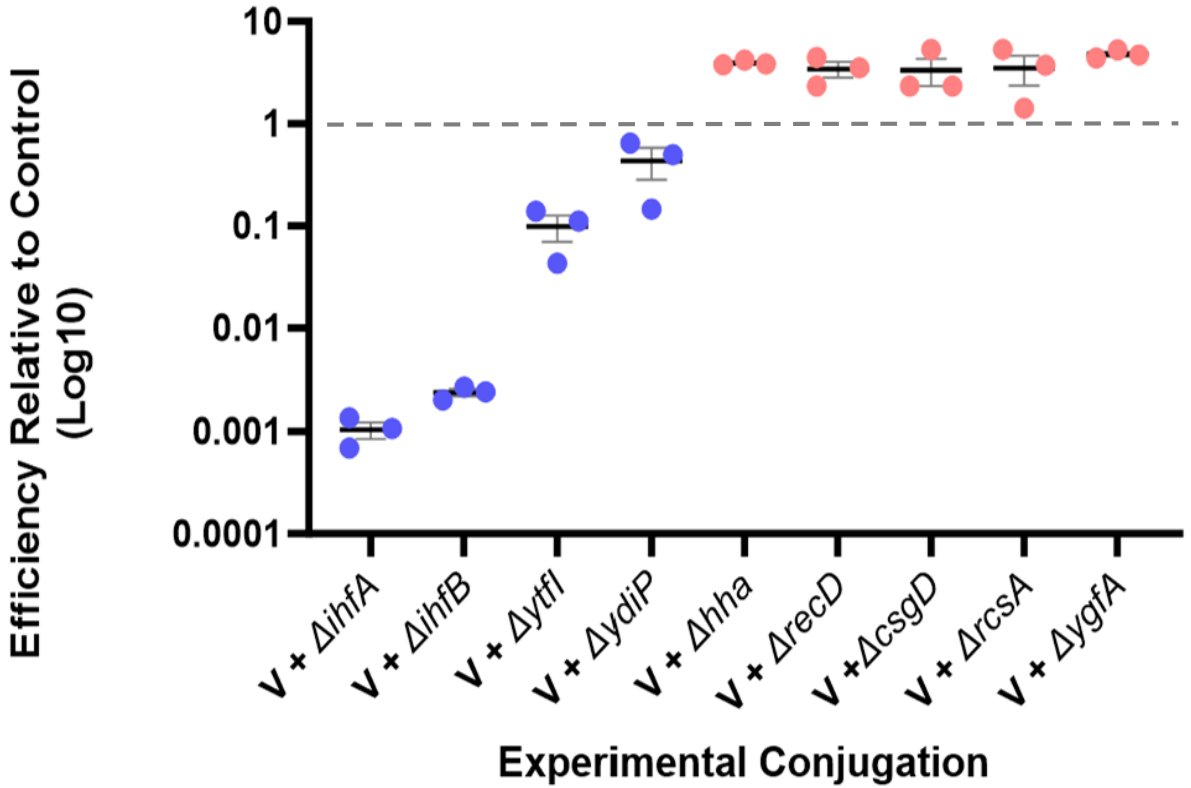

Rachel Rovinsky

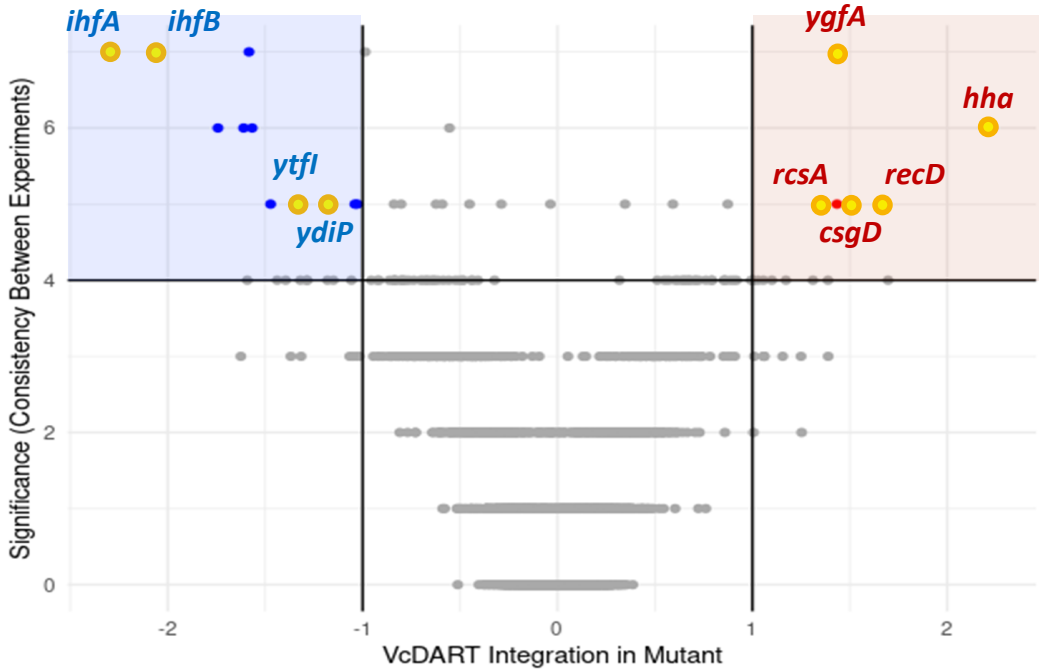

Emily Pierce (Arcadia)


Sara Smith

Shout out to Abby, Sophia and Zoë's contributions as well!

Combined genome wide mutant screen and validation



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Abby Wang


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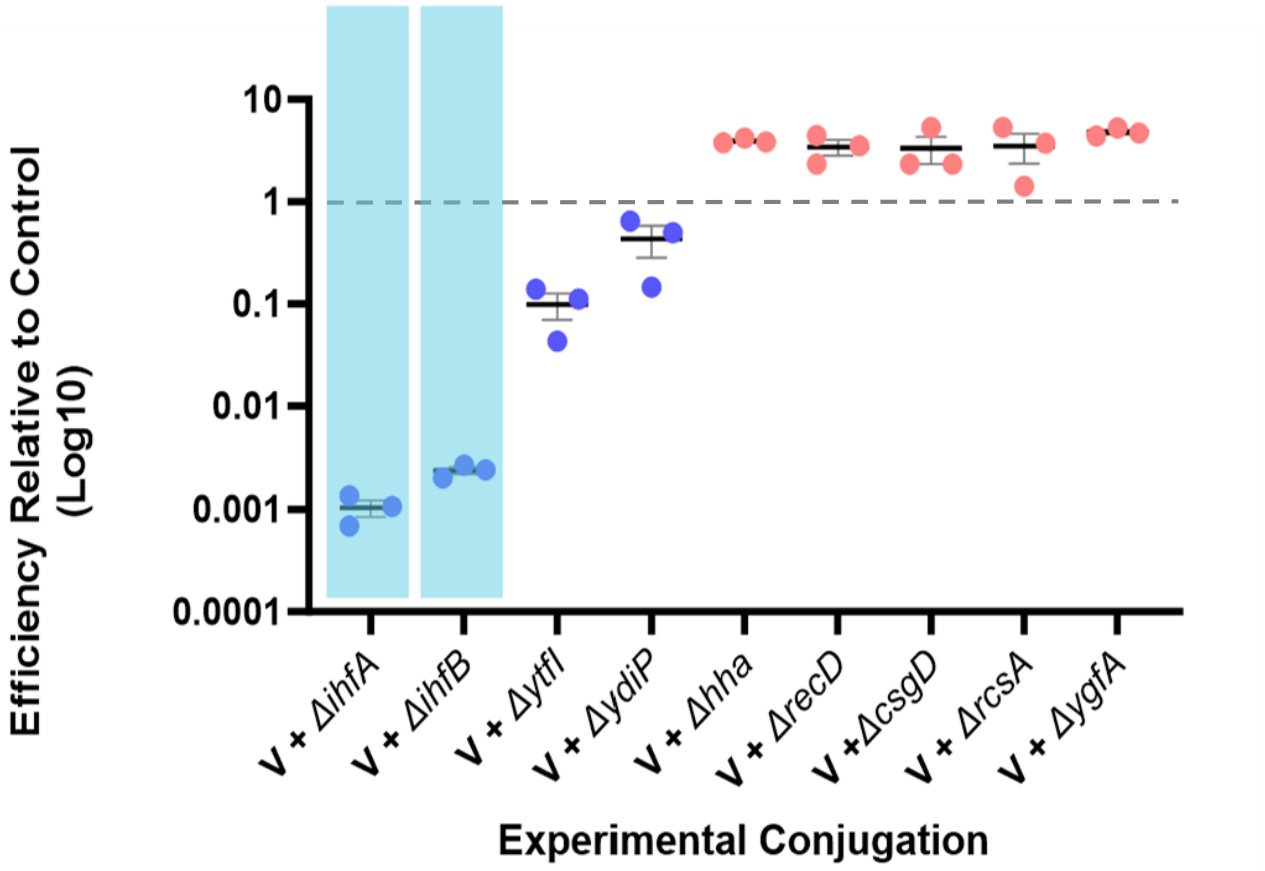

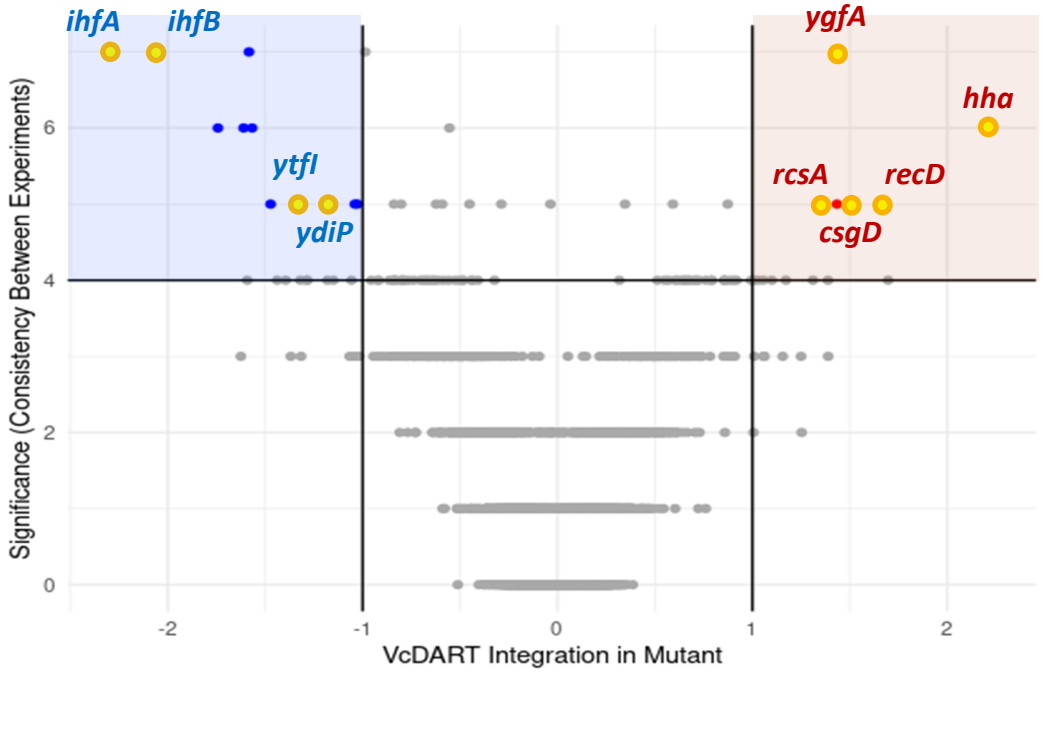


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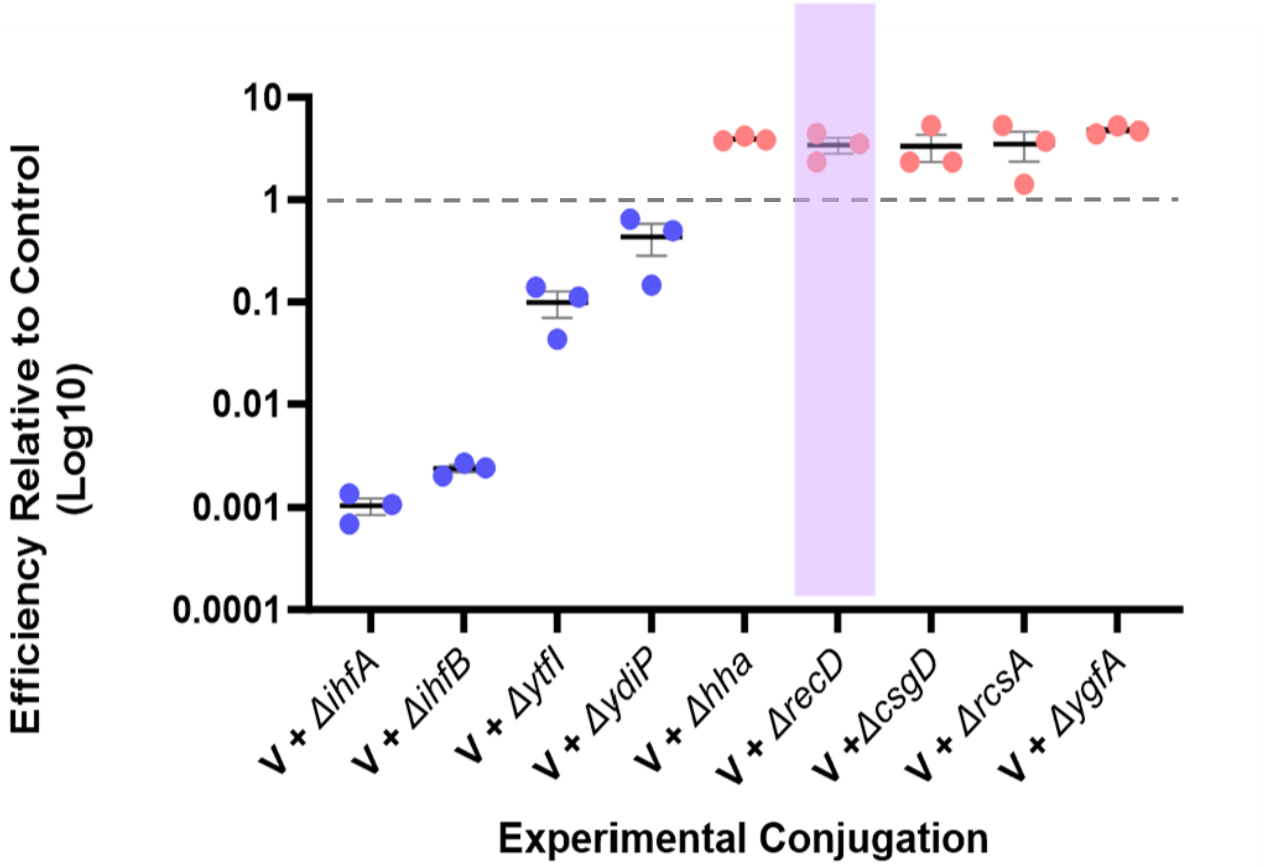

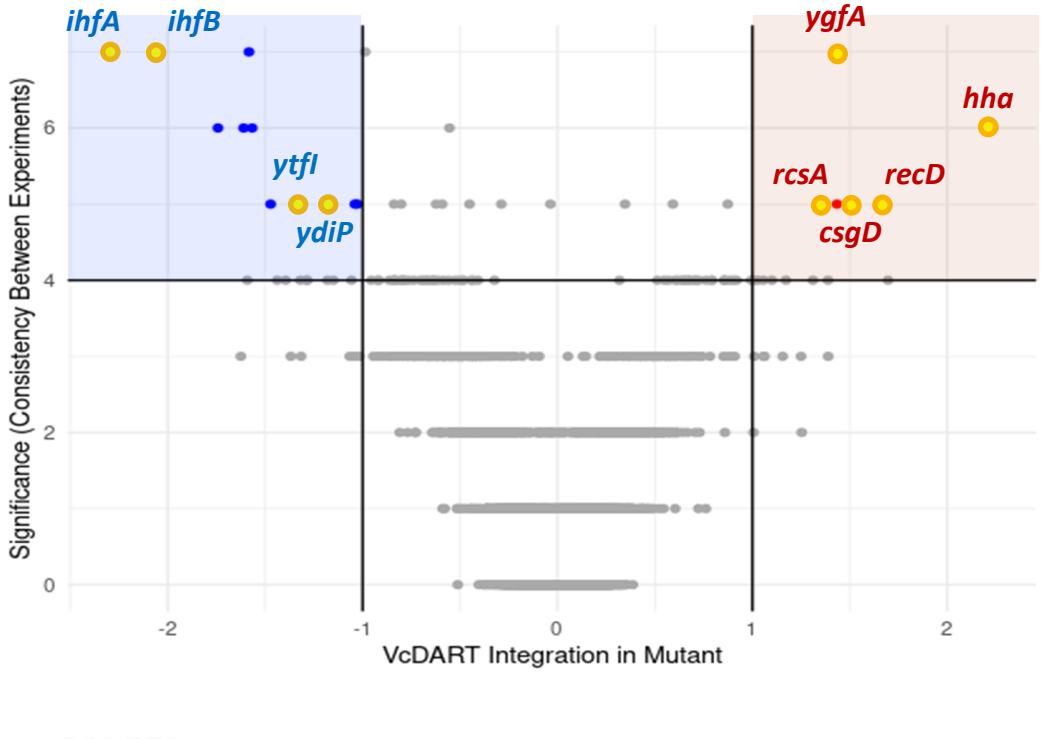


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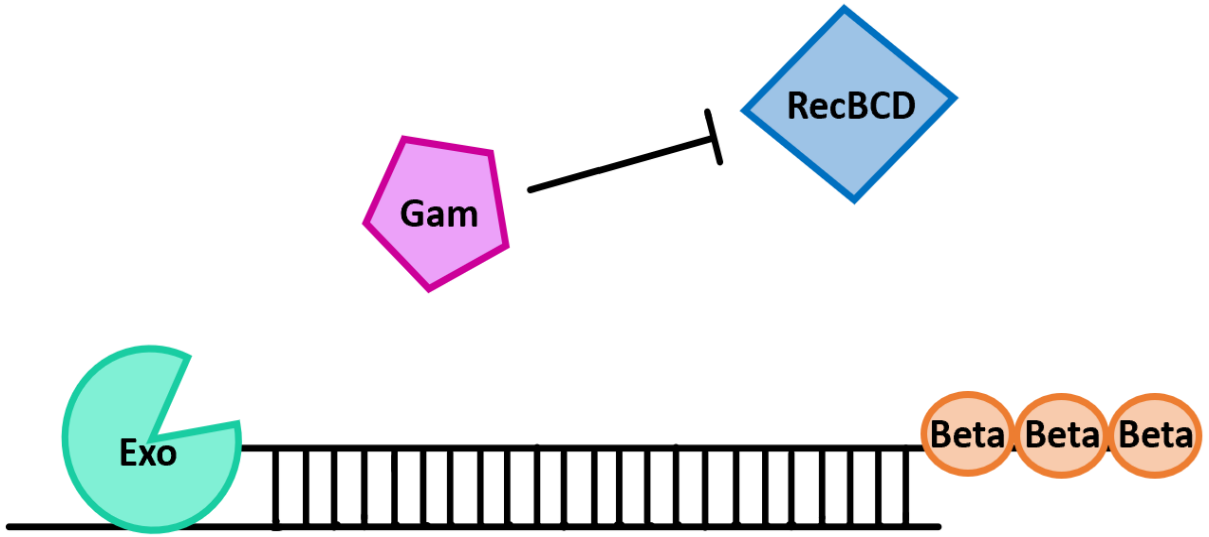
Lambda red hypothesis

Leo Song

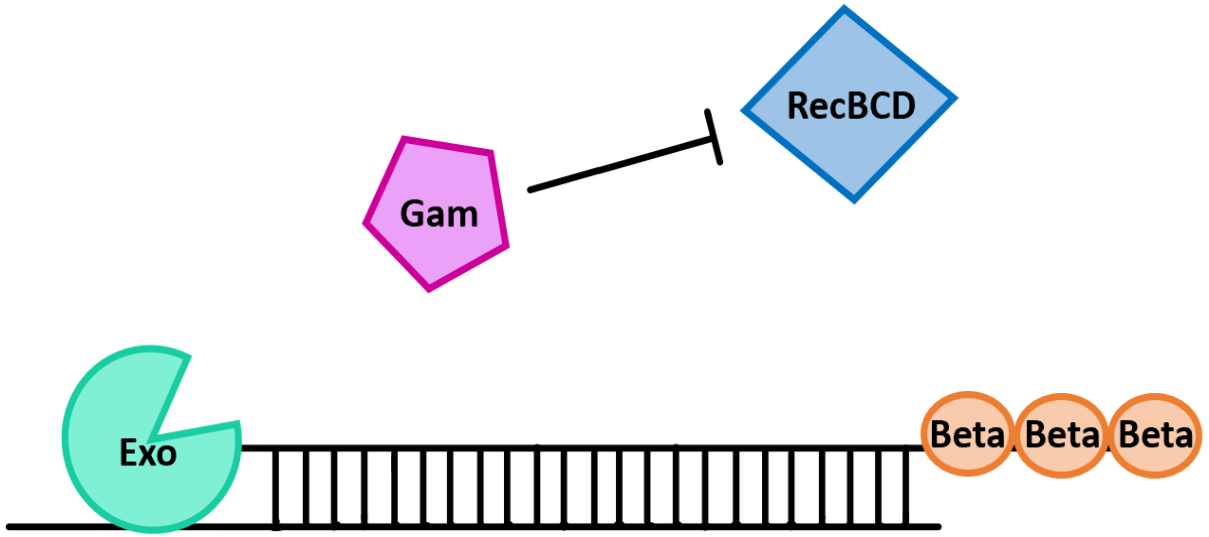


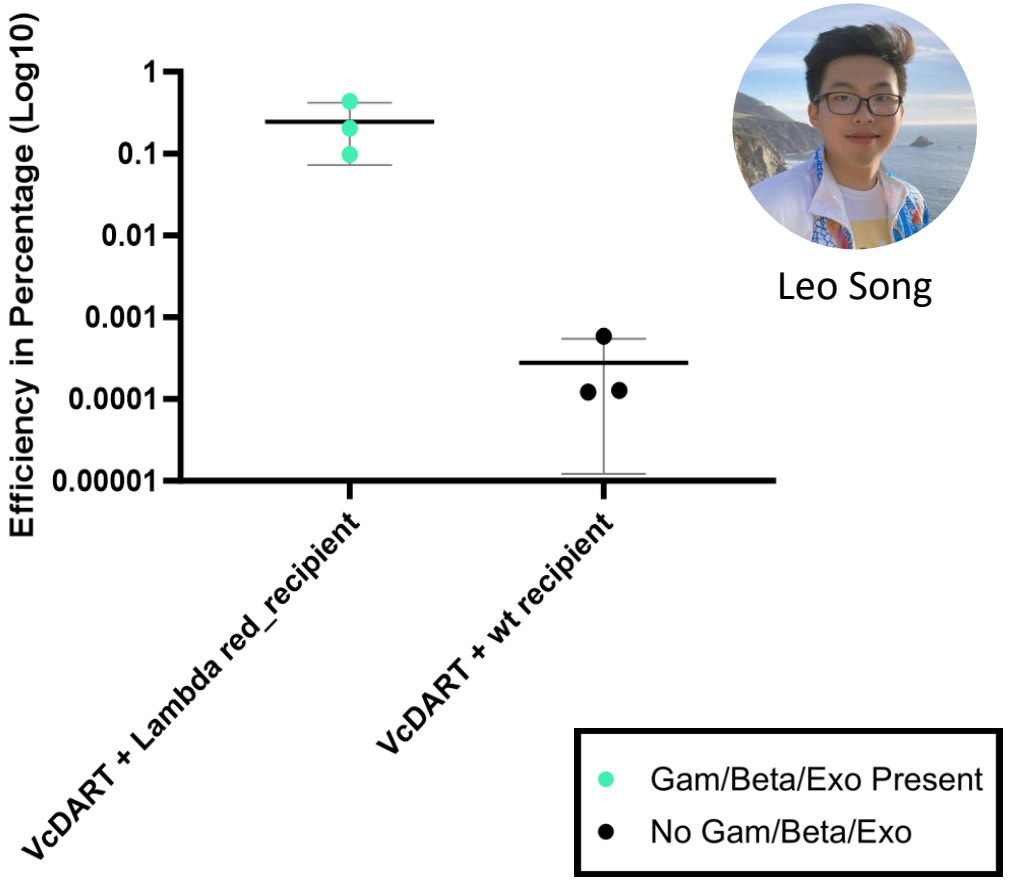
Hypothesis

Recombination plays a key role in VcDART integration.

We can leverage the Lambda red recombineering system to improve VcDART editing efficiency.

Lambda red hypothesis





Leo Song

Hypothesis

Recombination plays a key role in VcDART integration.

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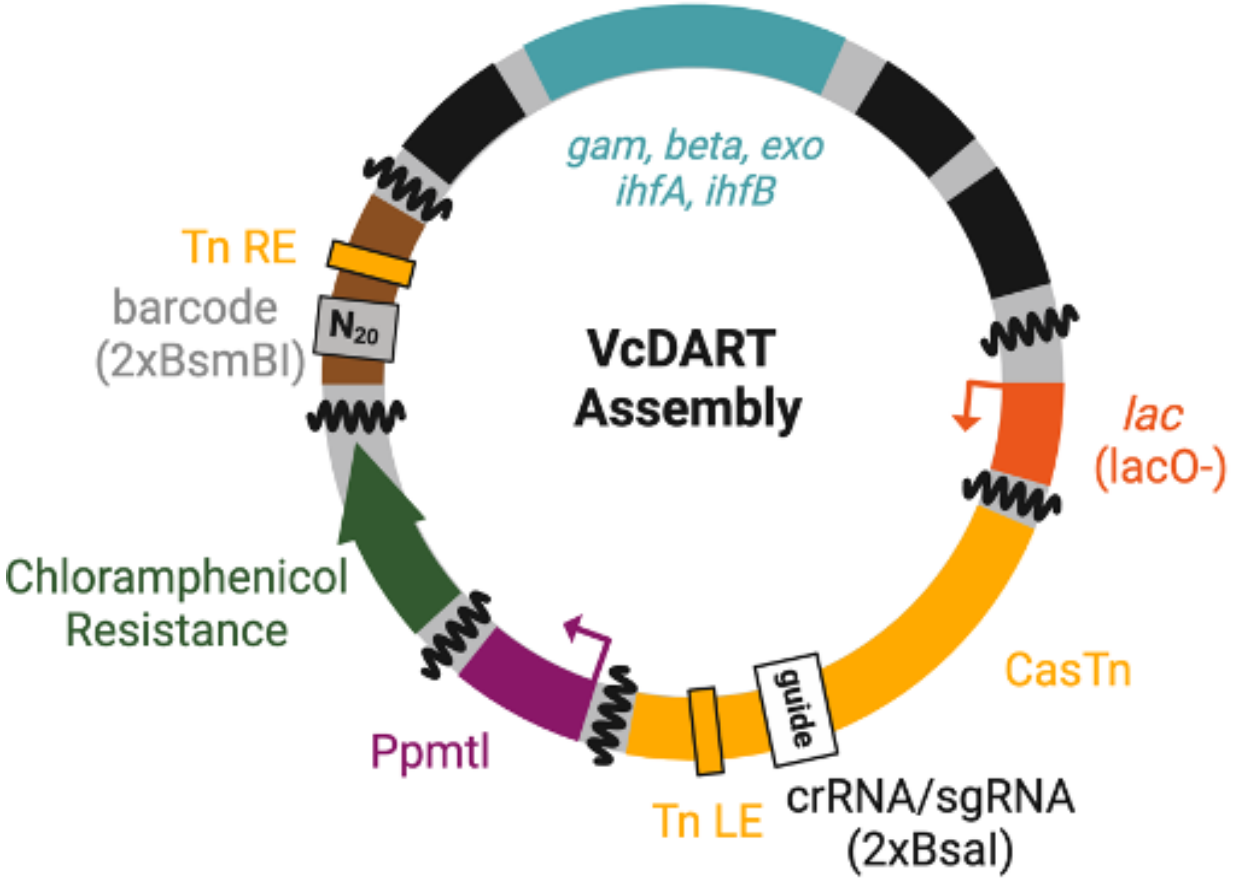
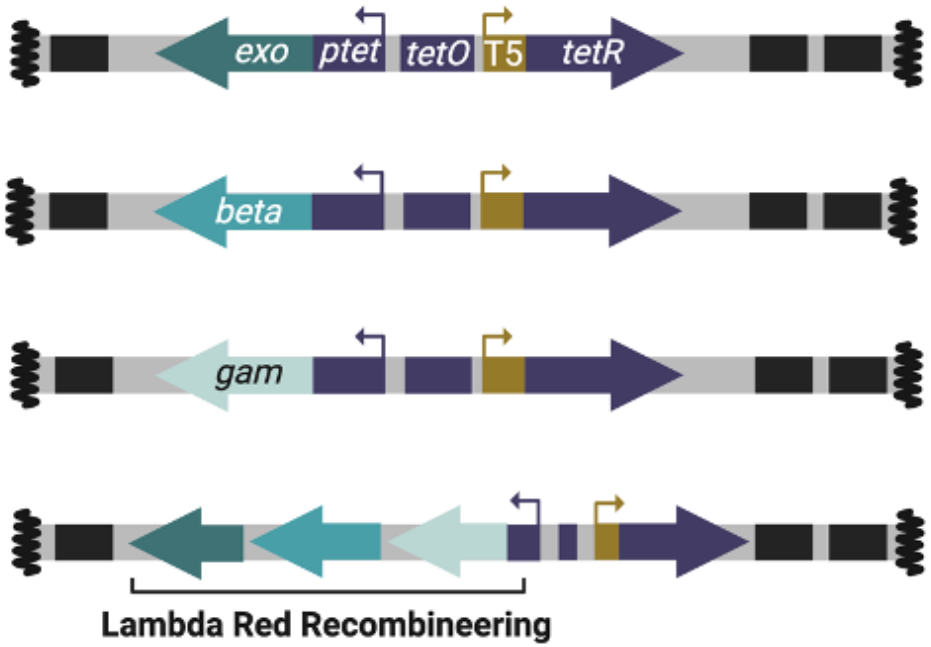
Lambda red VcDART vectors



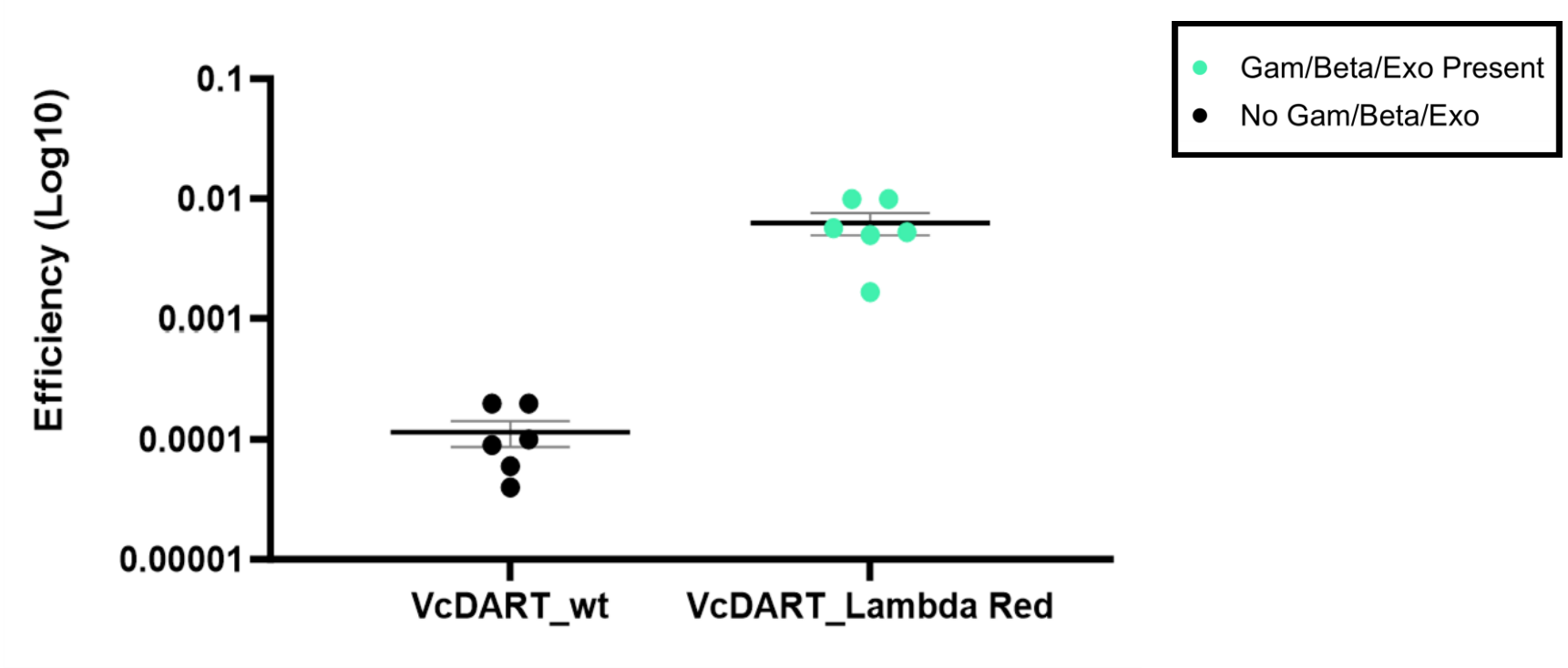
Amanda Alker

Abby Wang



Using Lambda red to improve VcDART function

Test efficiency (in model *E. coli* strain)



Leo Song

Agnès Oromí-Bosch

Amanda Alker

Conclusions

Identified Regulators

Conclusions

Identified Regulators

Validated Regulators

Conclusions

Identified Regulators

Recombination Plays a Key Role in CAST integration

Validated Regulators

Next steps

Test Lambda red-containing VcDART vector in **non-model microbes**

- Soil microbes

Explore **other regulators** of VcDART

Investigate the **molecular mechanism** and **kinetics** of VcDART integration


Amanda Alker


Jigyasa Arora

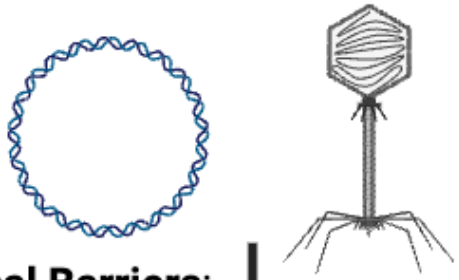

Agnès Oromí-Bosch

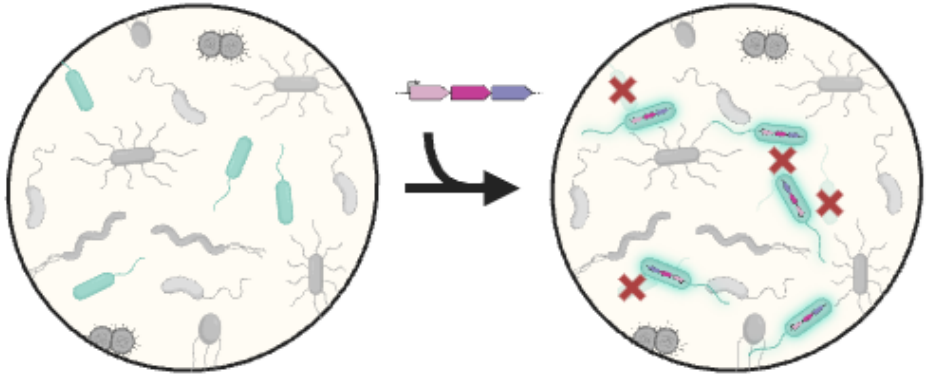

Sophia Swartz

CAST Editing Tool Efforts


Avery Roberts

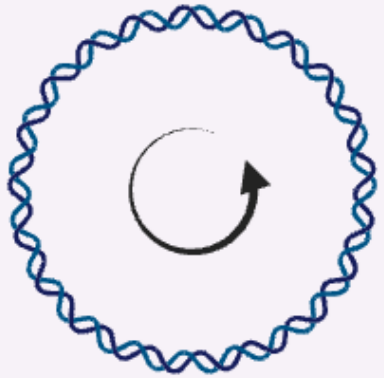
1. **Overcome Physical Barriers**: Plasmid/Phage Mining (conjugation systems, adhesins, depolymerases)



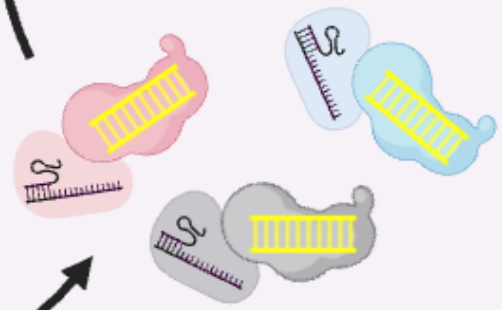


5. **Fix/Enrich Edited Mutants**: Community-Based Selections (metabolic niche, bacteriocins)

2. **Evade Immune Systems**: Repurposing Anti-Defense Genes and Deleting/Attenuating Immune Triggers



3. **Replication Compatibility**: Plasmid Replicon/Phage Mining



4. **Improved Editing**: Testing Alternative Editors

Figure credit: Brady Cress

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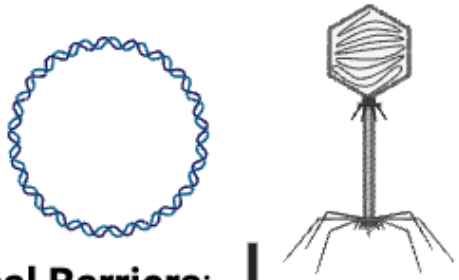

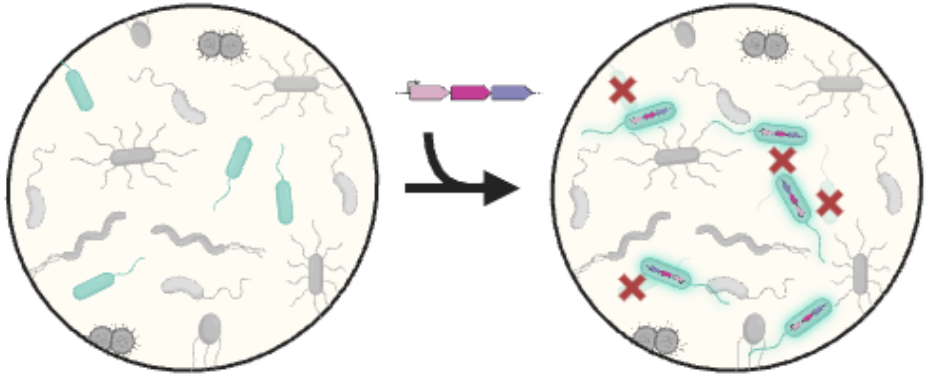

Niche Clearance

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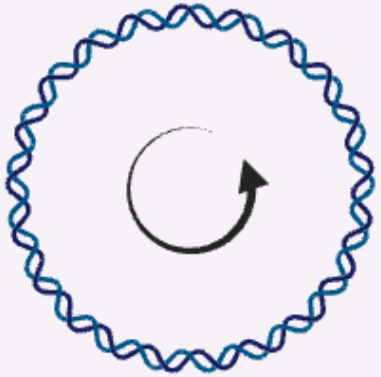
Dr. Jaymin Patel



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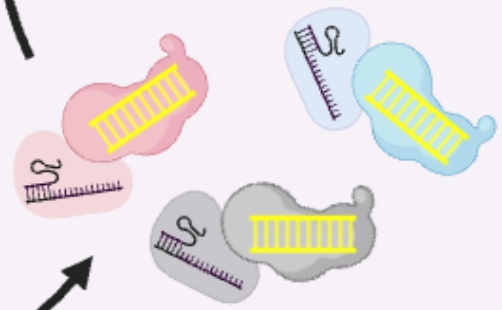

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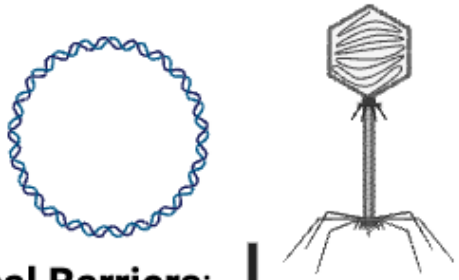

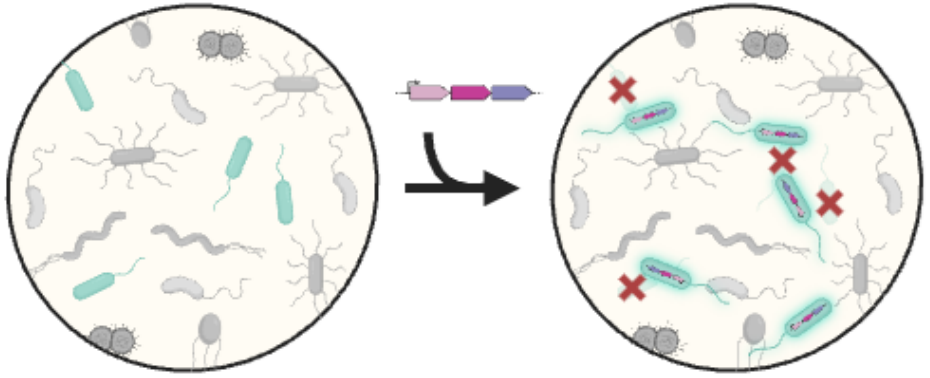

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Dr. Carlotta Ronda

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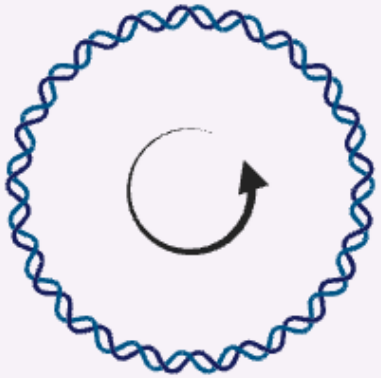
STERNBERG LAB

Dr. Diego Gelsinger

Dr. Jaymin Patel



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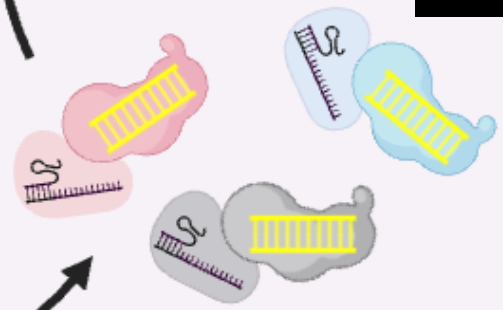

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Acknowledgments

Rubin Lab

Ben Rubin
Amanda Alker
Agnès Oromi-Bosch
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Jigyasa Arora
Abby Wang
Zoë Chan
Jon Martinson
Lindsey Pieper
Bingliang Xie

Former Members

Sara Smith
Rachel Rovinsky

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Trent Northern
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Arushi Lahiri
Owen Tuck
Kate Miller

Lab website: **https://www.therubinlab.org/** Lab twitter: **@therubinlab**

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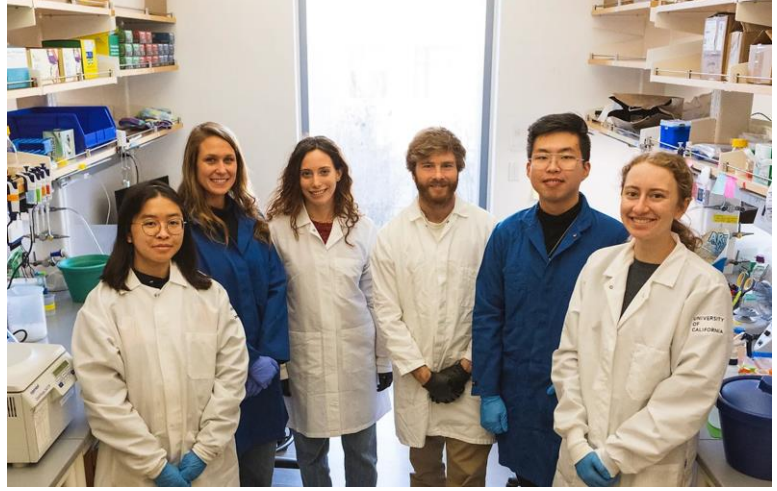
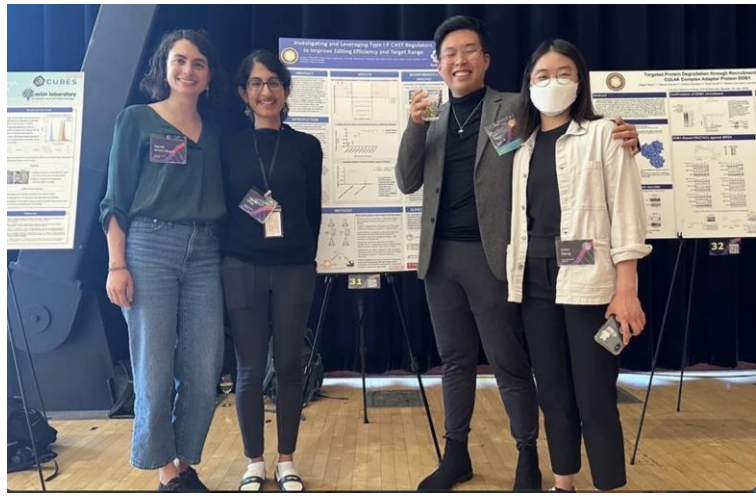


HAPP





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