Accelerated Engineering of Non-model Microbes

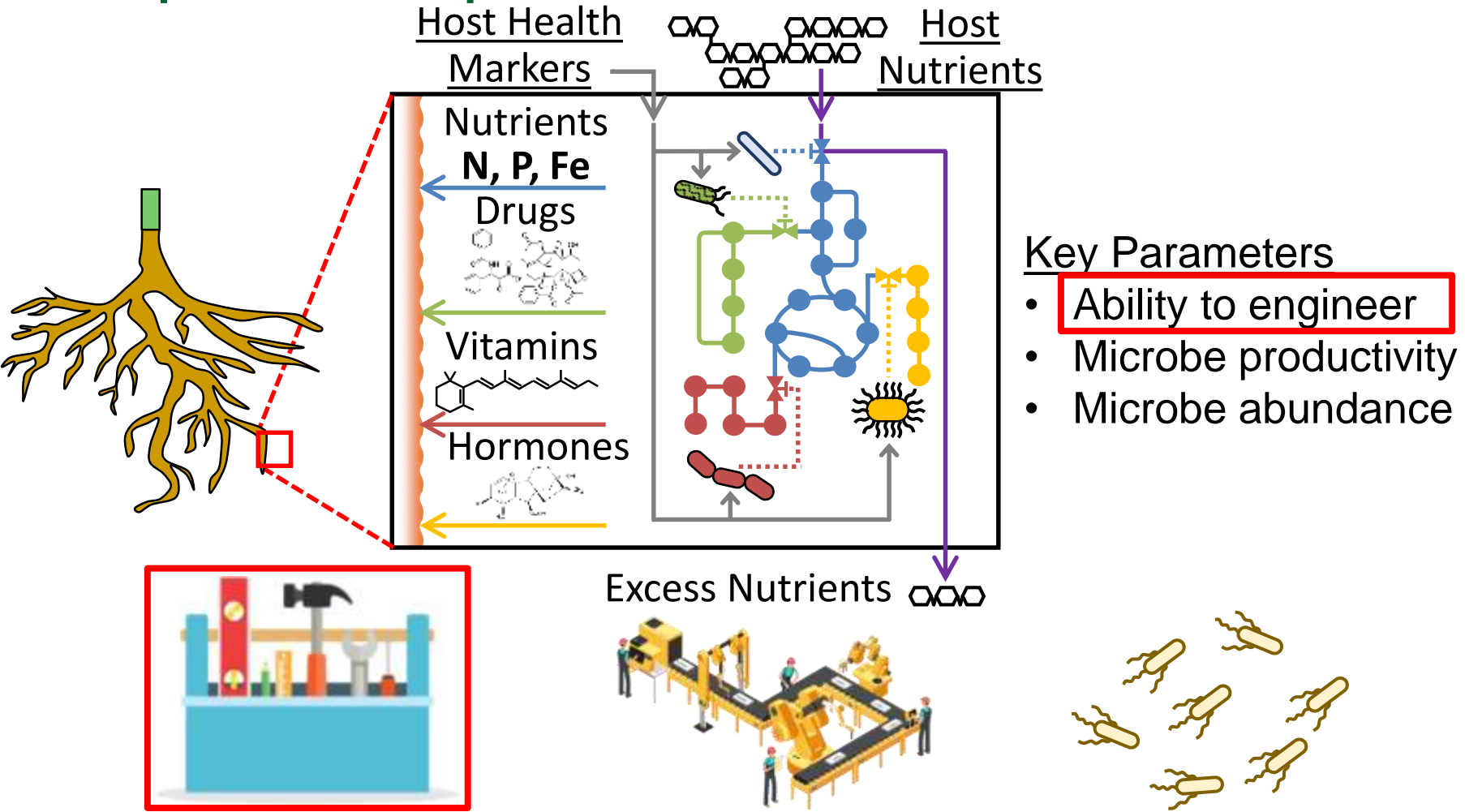**Nathan Crook**

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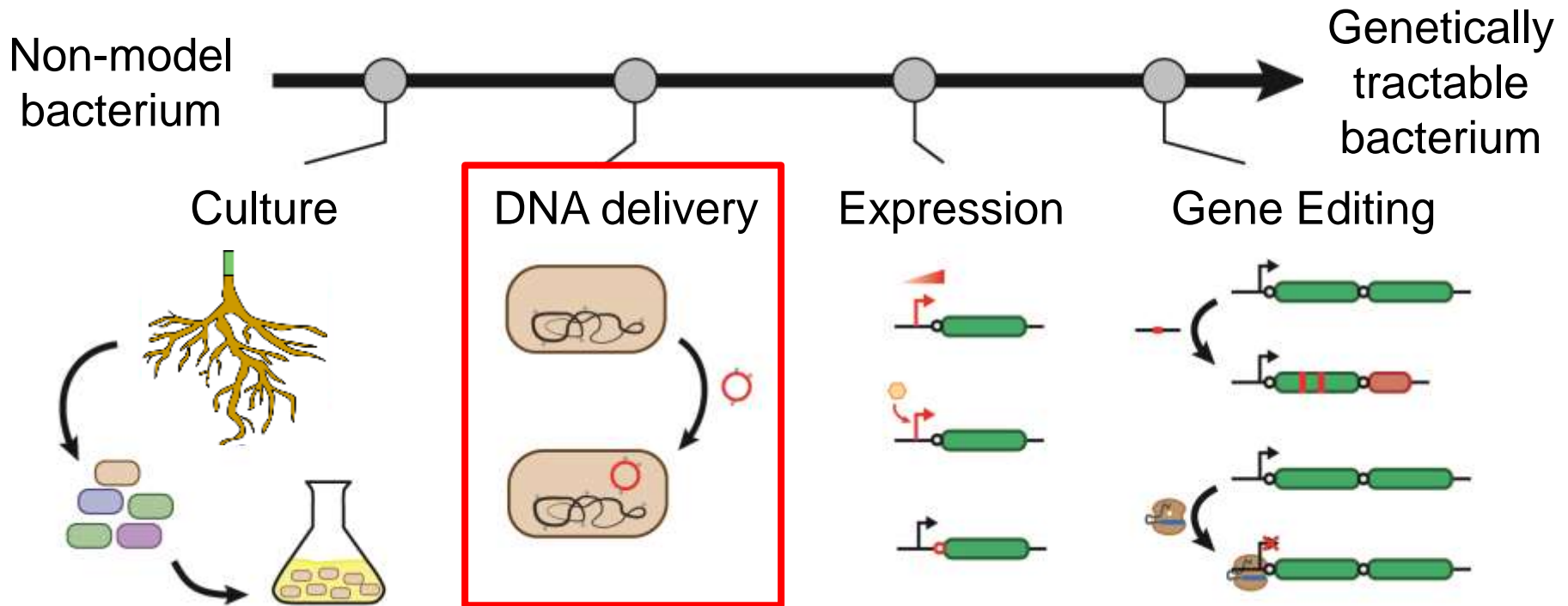www.crooklab.net
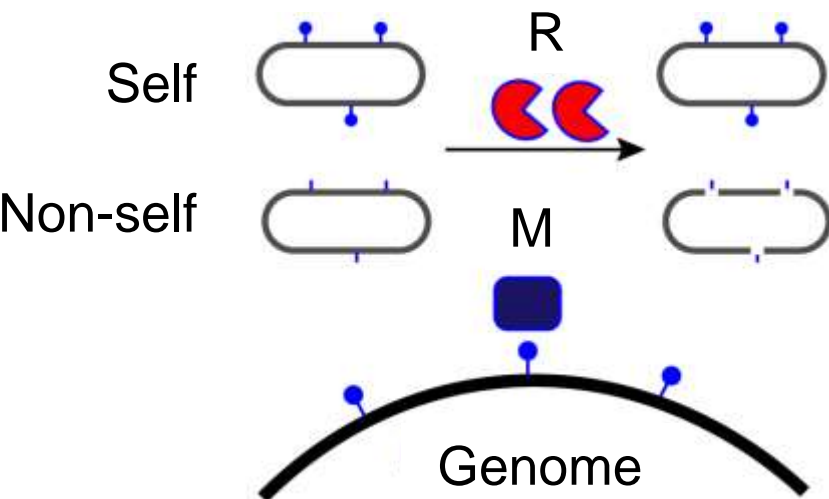
Engineering the microbiota to improve crop health

Host Health Markers

Host Nutrients

Nutrients
N, P, Fe
Drugs
Vitamins
Hormones

Key Parameters
- Ability to engineer
- Microbe productivity
- Microbe abundance

Excess Nutrients

Nathan Crook
Engineering the Microbiota

Microbial Engineering Pipeline

Need to quickly and efficiently overcome barriers to foreign DNA
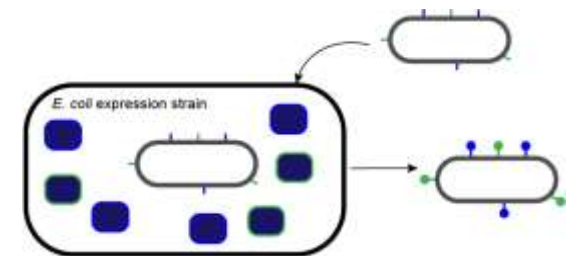
Restriction-Modification defense systems

R

Self

Non-self

M

Genome

Standard approaches too slow and limited

Purified M proteins

E. coli expressing M proteins

- Multiple MTases per bacterium
- MTases vary within species
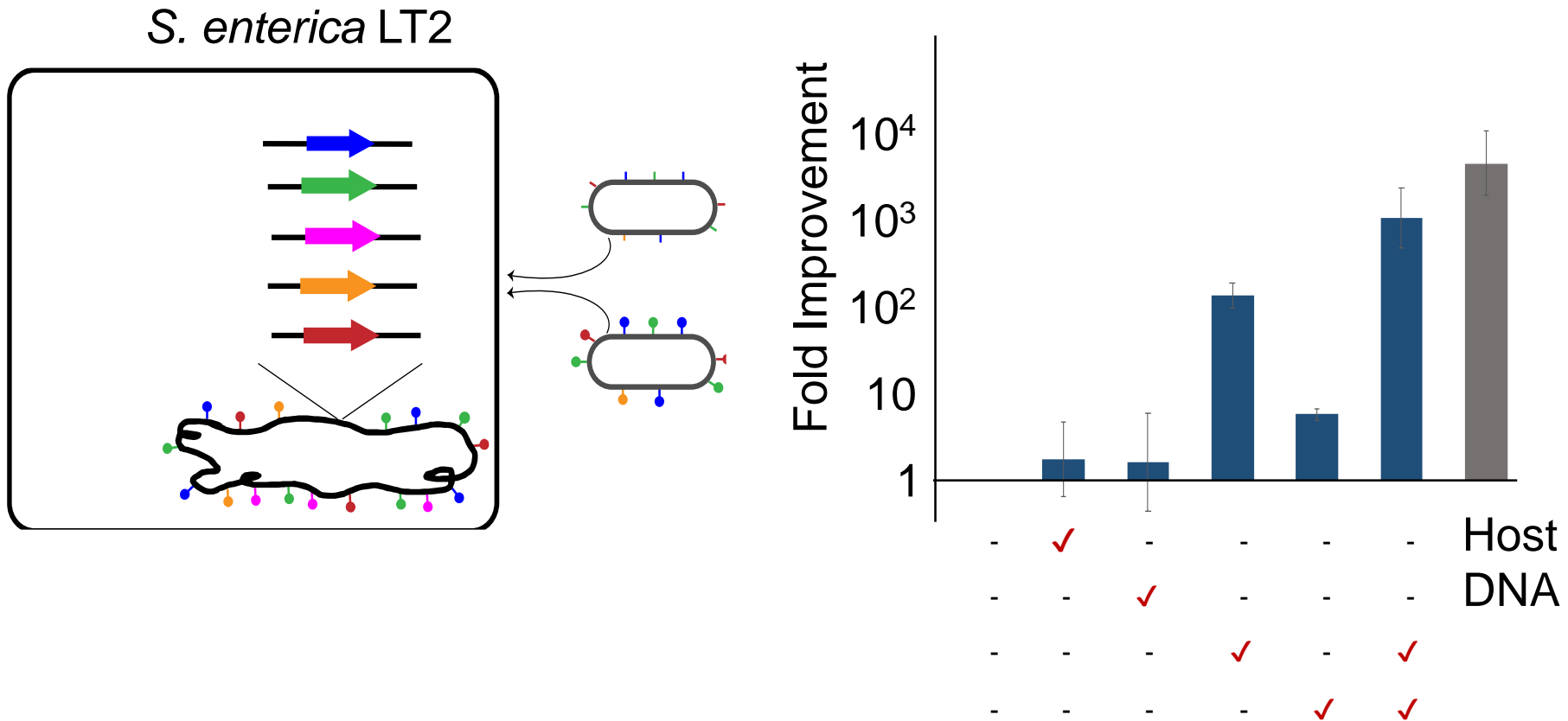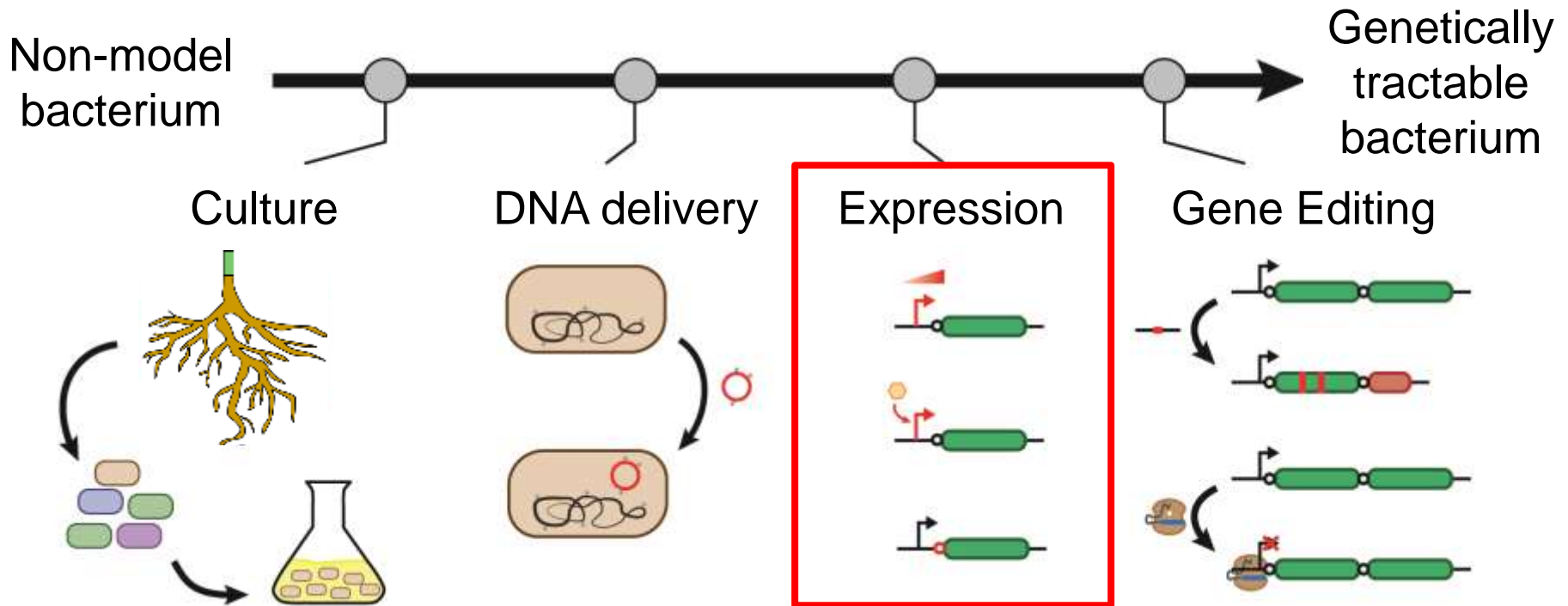
- M expression can be toxic
- Limited to 1 – 2 simple M proteins

Enhanced plasmid transformation in *S. enterica*

S. enterica LT2

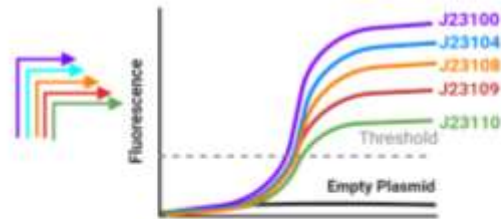Nathan Crook
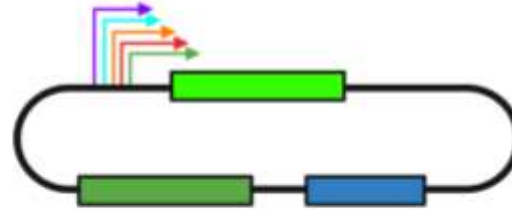Plant Microbiome Tools

Microbial Engineering Pipeline

Non-model bacterium

Genetically tractable bacterium

Culture DNA delivery Expression Gene Editing

Need for species-specific promoters

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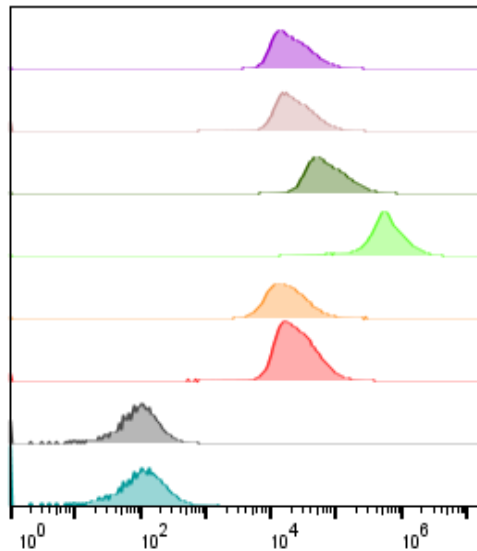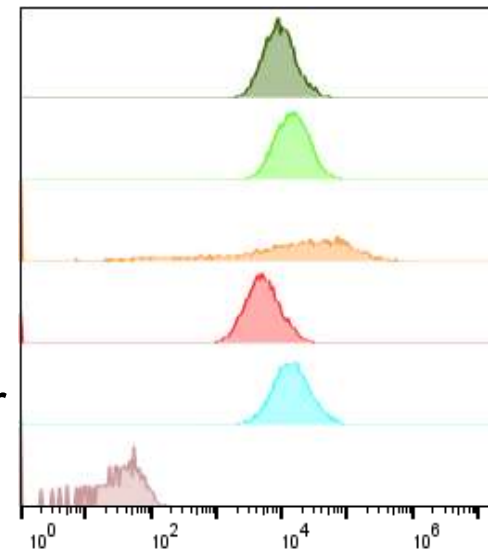
Mining the genome for promoters

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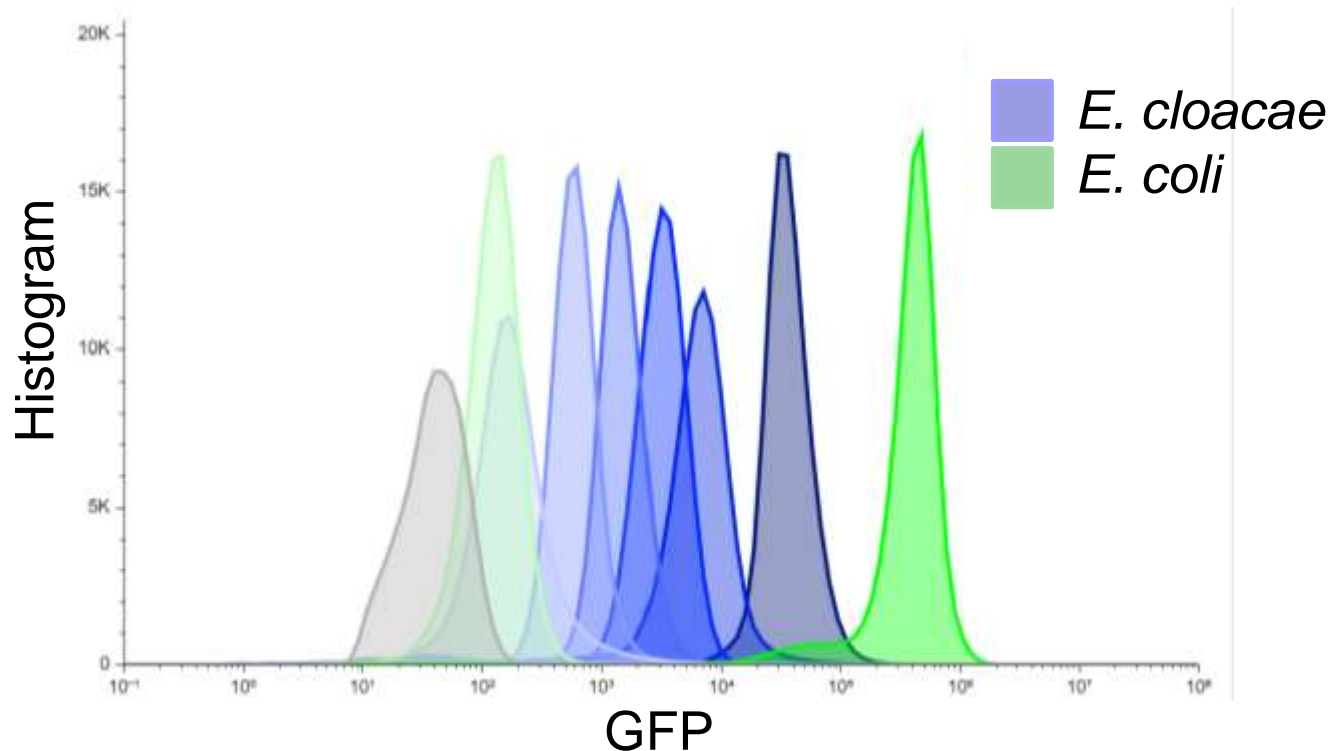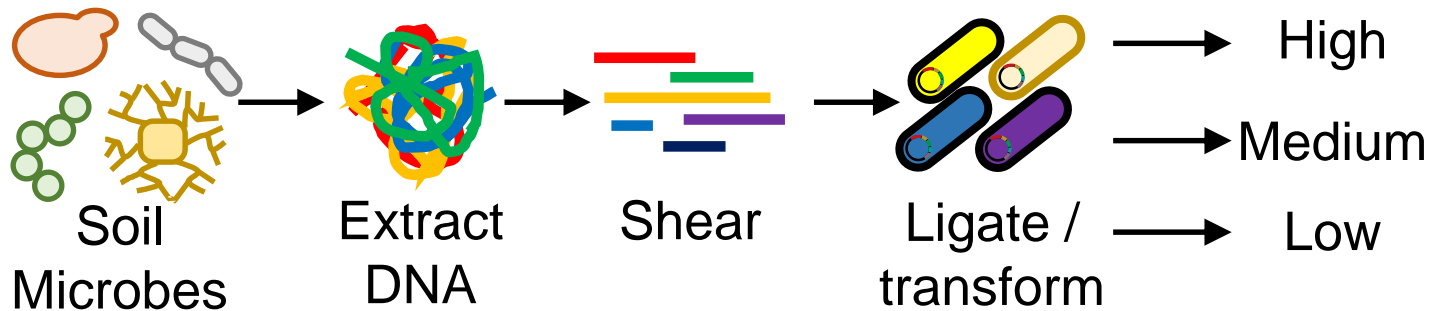
A modular engineering toolkit
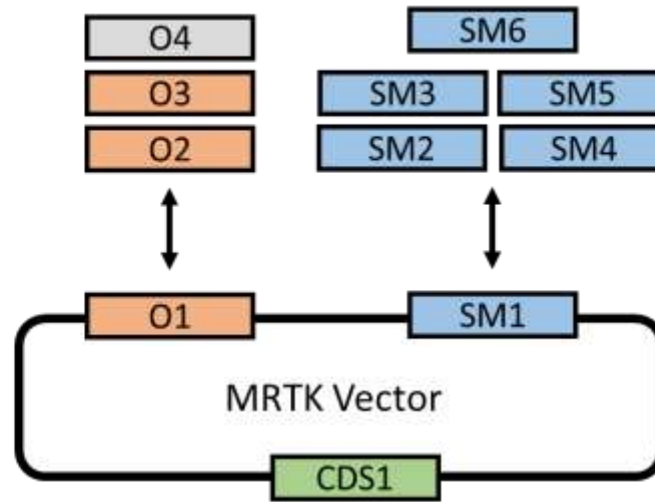
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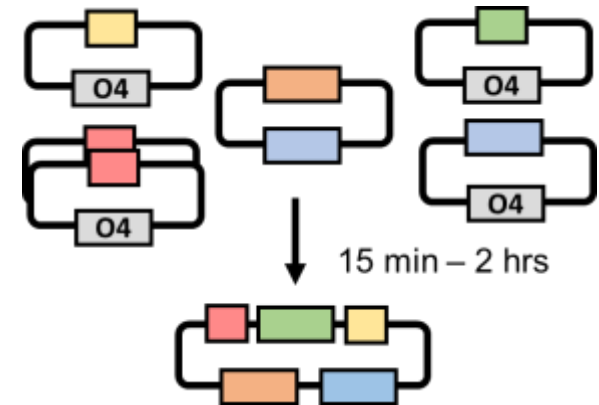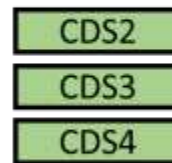
Partnership opportunities

- **Apply technologies to your favorite bacterium:**
  - ❑ 6 mo – 1 yr projects, $50k-$100k
  - ❑ Improve transformation efficiency
  - ❑ Identify promoters at defined strength/conditions
  - ❑ Established IP process with NCSU ORC
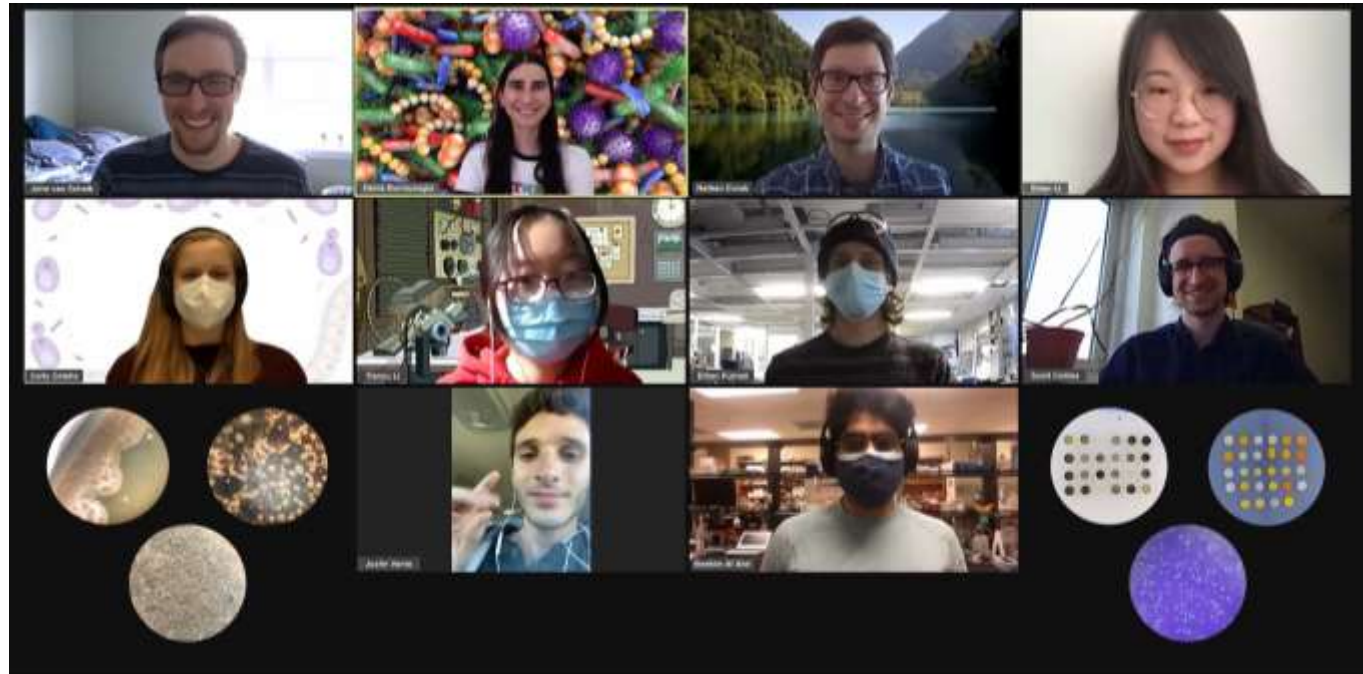
- **Longer-term collaborative interests**
  - ❑ Bioprospect for colonization-enhancing genes
  - ❑ Directed evolution of catabolic or biosynthetic pathways

Thank you!

Deniz Durmusoglu
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Justin Vento
John Van Schaik
Zidan Li
Tianyu Li

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