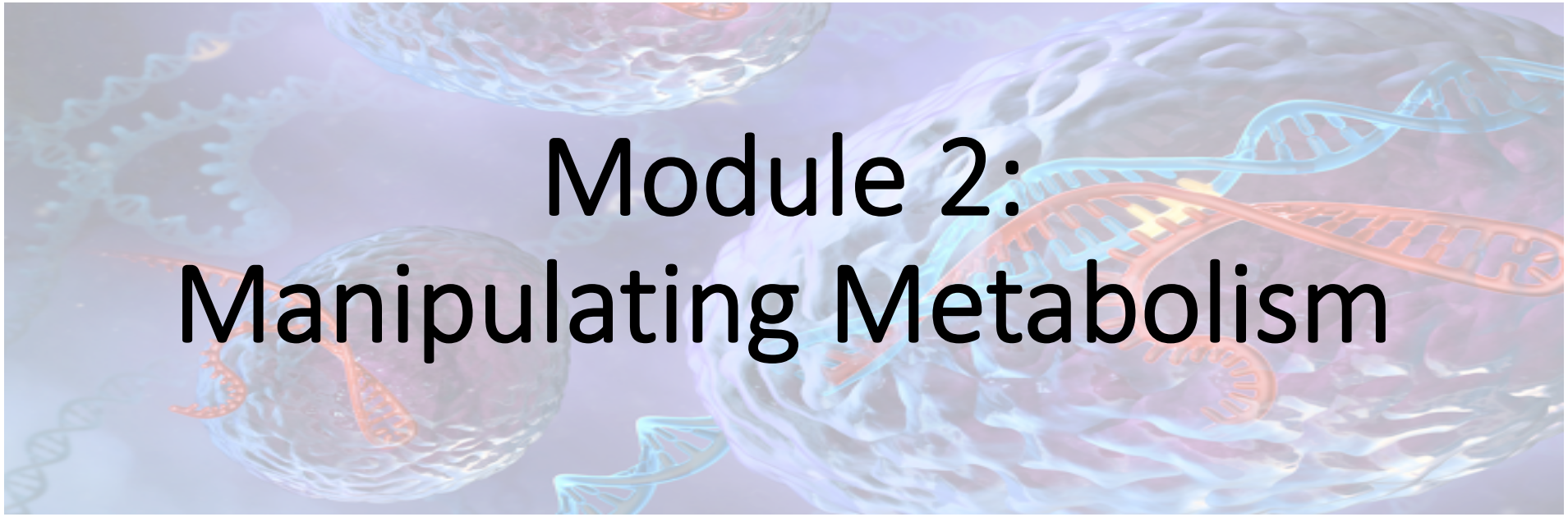


Module 2: Manipulating Metabolism

Engineering metabolism

10/10/19

What is metabolic engineering?

What is metabolic engineering?

nature.com

"…is the use of genetic engineering to modify the metabolism of an organism. It can involve the optimization of existing biochemical pathways or the introduction of pathway components…with the goal of high-yield production of specific metabolites for medicine or biotechnology."

Metabolic engineering ‘toolkit’

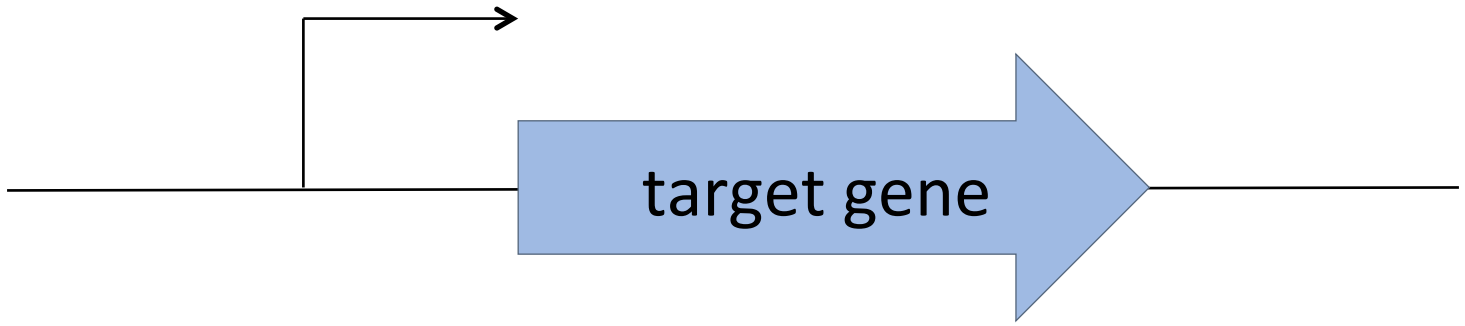
Genetic (DNA) engineering techniques:

1. Repress gene
2. Overexpress gene
3. Delete gene / function
4. Add gene
5. Mutate gene

1. Repress gene

- Inhibit polymerase or transcription factor binding at promoter

- Inhibit transcript elongation through gene by blocking polymerase



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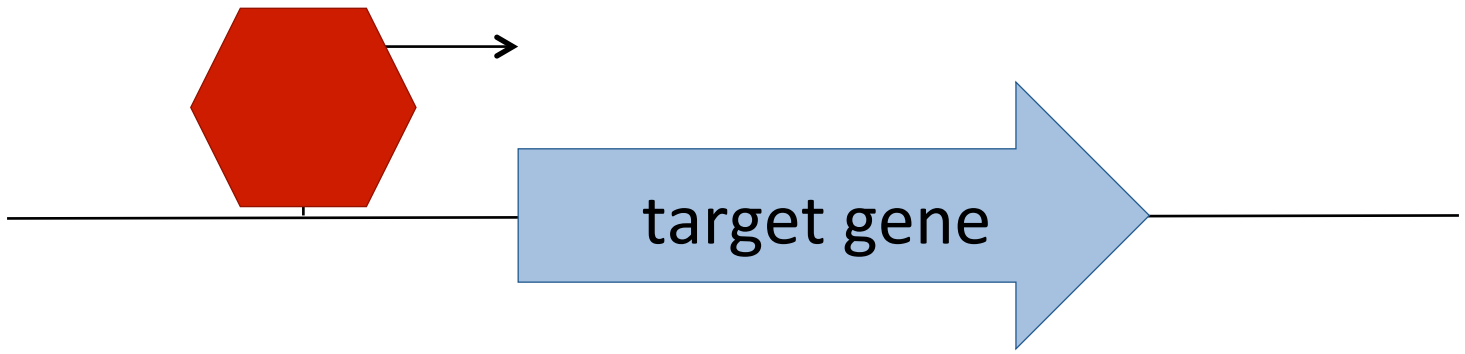

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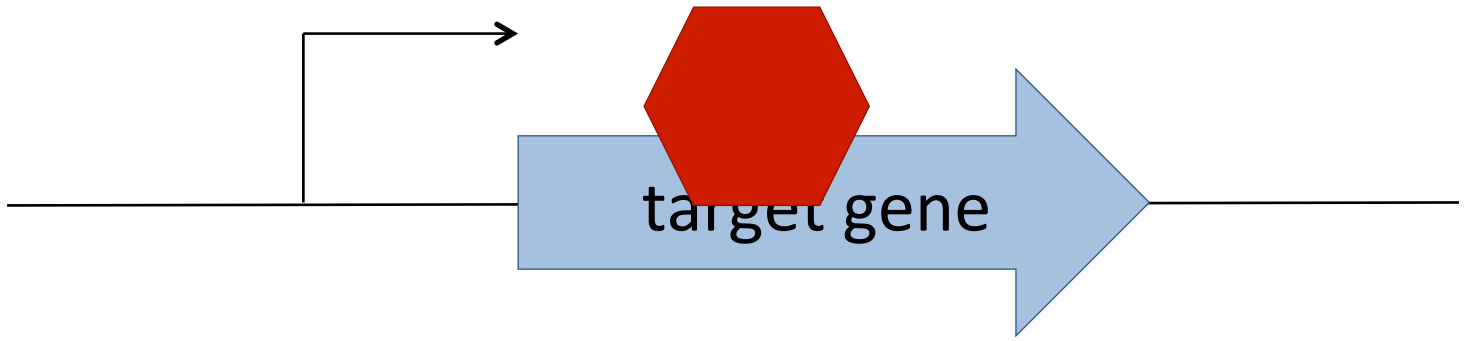
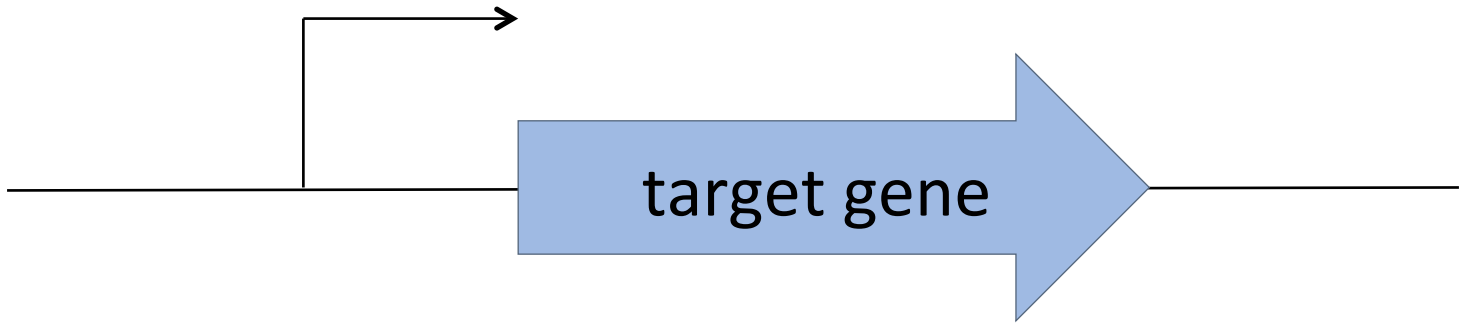


2. Overexpress gene

- Replace native promoter with one that is constitutively active
- Express additional gene copies exogenously using plasmids



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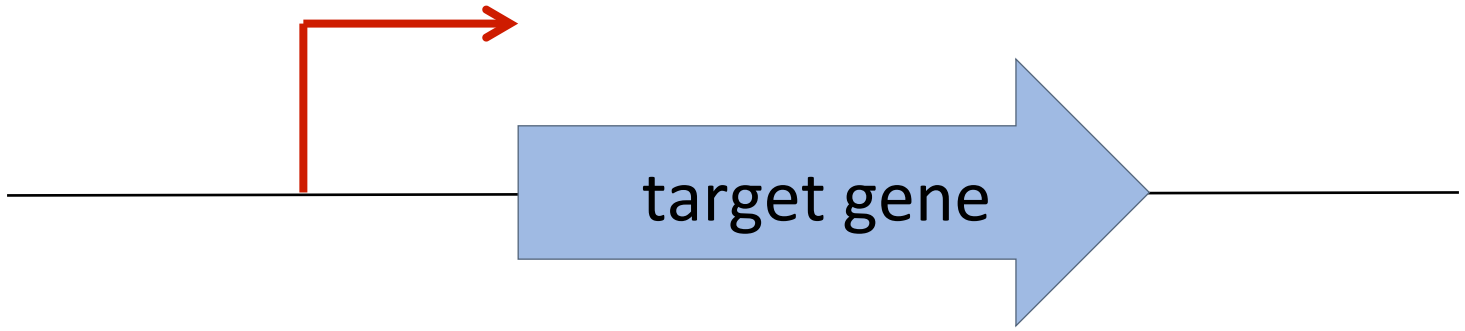

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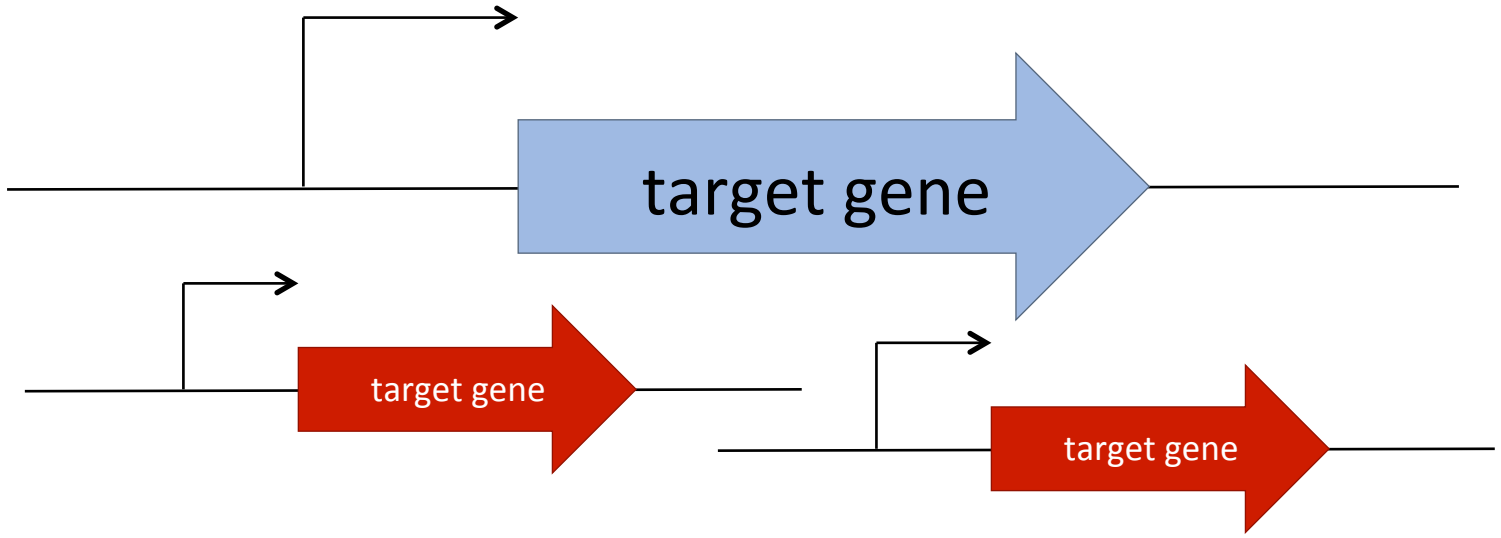
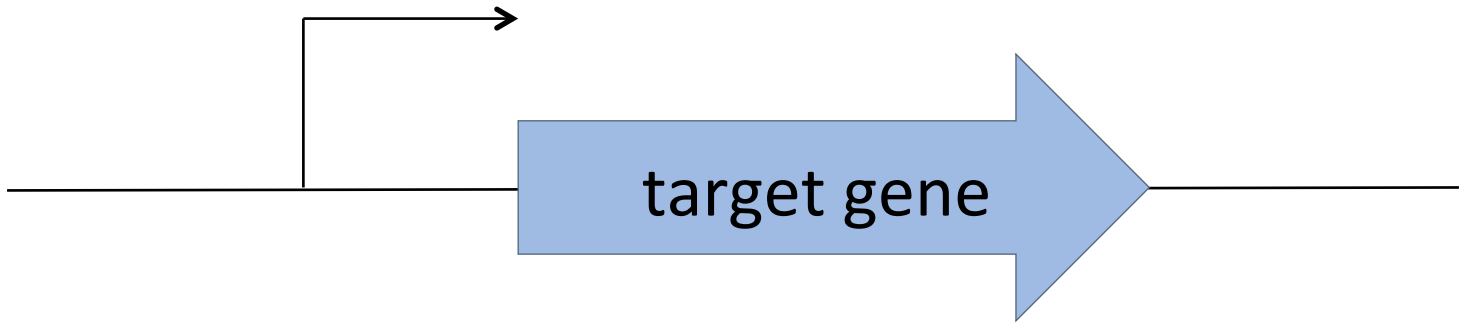


3. Delete gene / function

- Remove gene from genome

- Replace gene (either entirely or in part) with an antibiotic cassette



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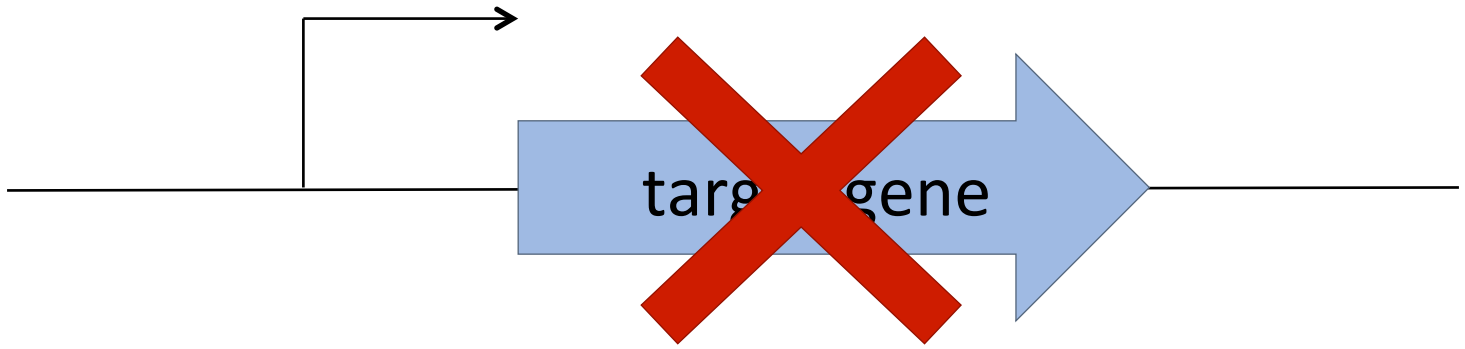

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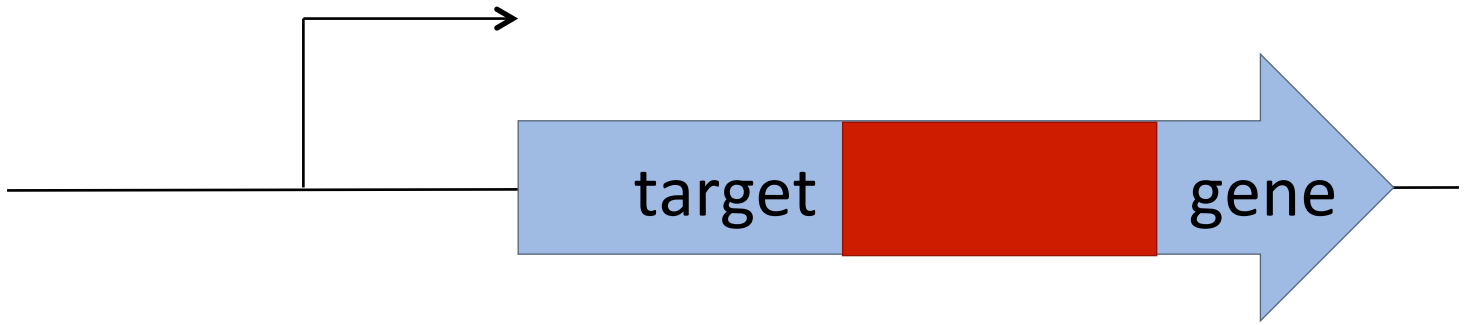

4. Add gene

- Insert non-native gene into host genome

- Express non-native gene exogenously using plasmids

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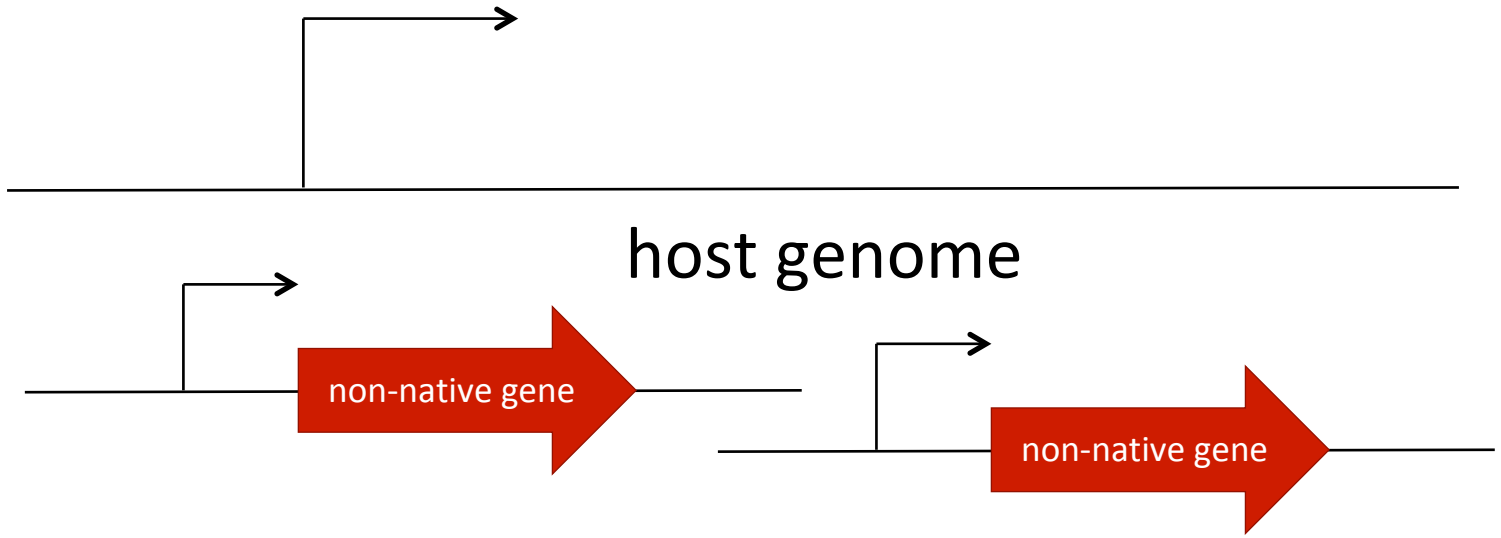
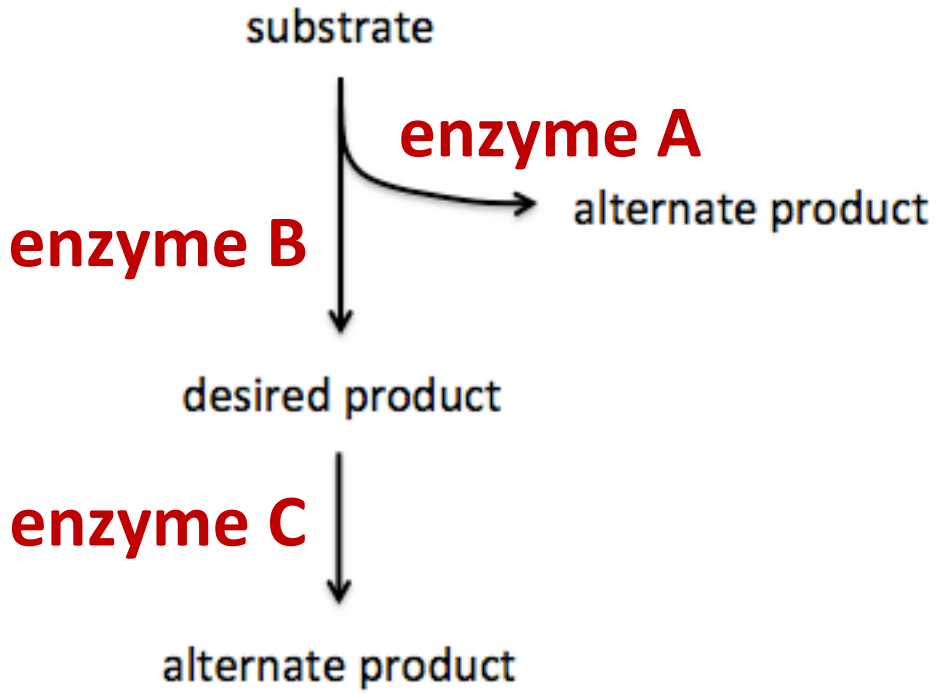


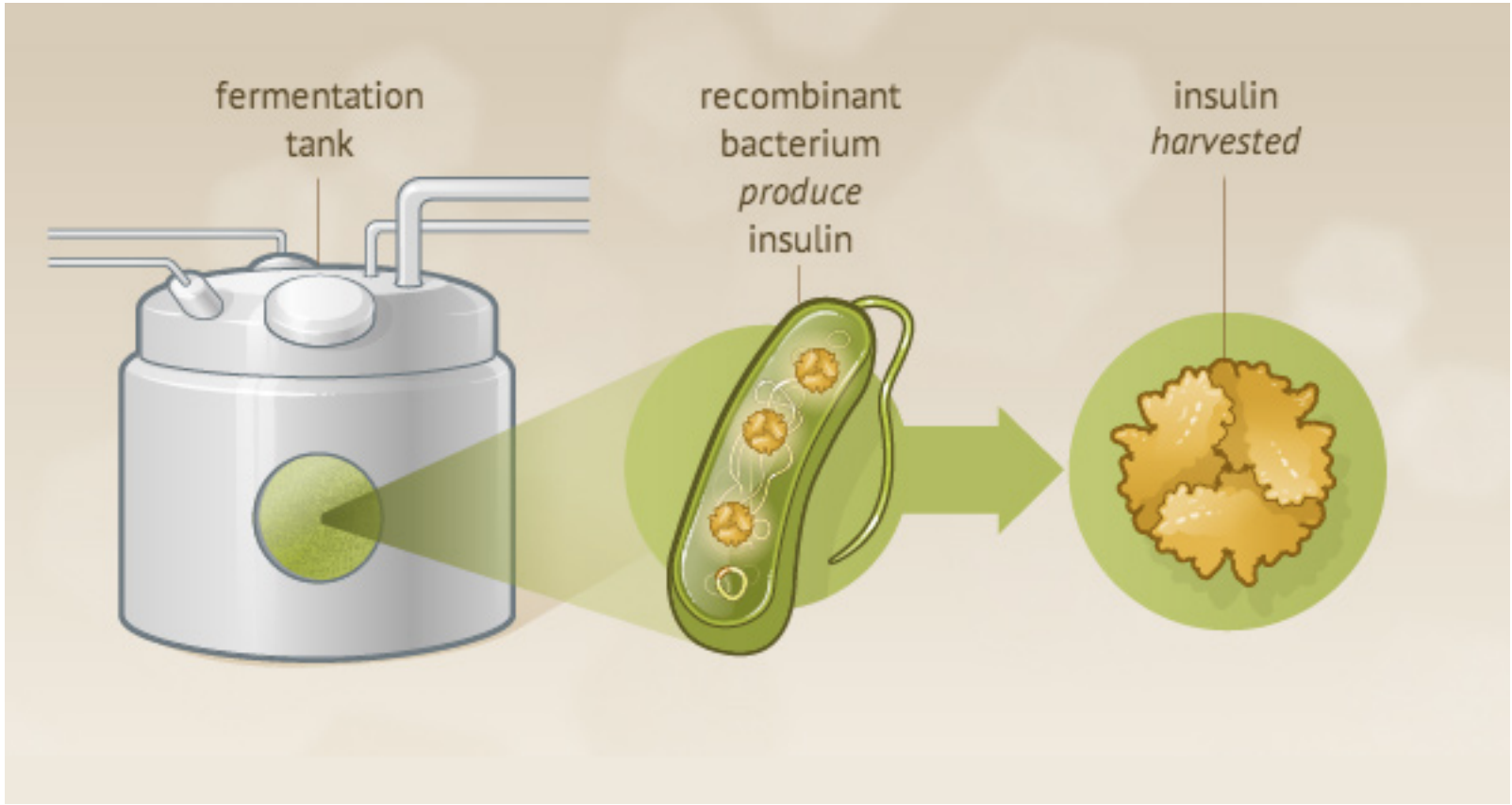
5. Mutate gene

- Alter gene sequence such that residues in encoded protein are modified
  - Enhance / eliminate substrate binding
  - Increase / decrease efficiency of reactions

How would you increase yield of the desired product?



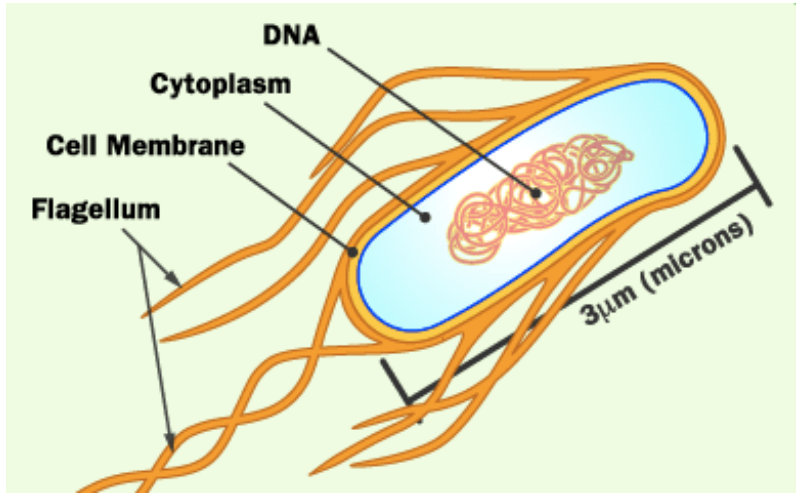
Metabolically engineered pathways can be expressed in host organisms



Why use *E. coli* to express products in metabolic engineering?

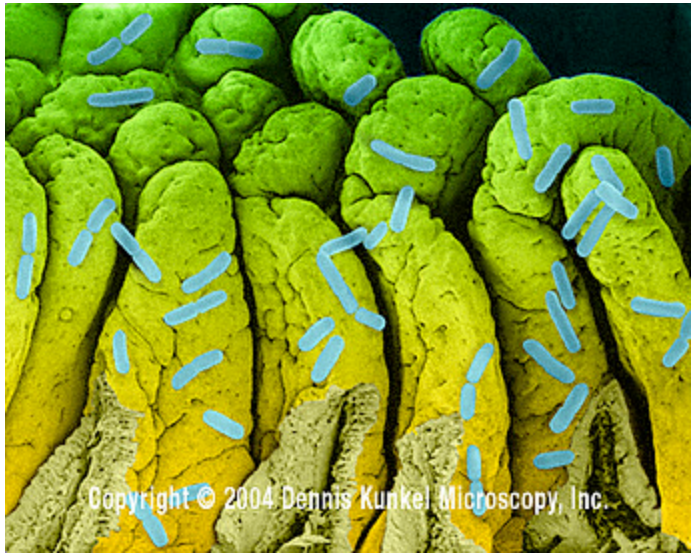
E. coli overview

- Gram negative
- Rod-shaped
- Motile; flagellated
- Native colonizer of lower intestine in warm-blooded mammals
  - Certain serotypes can cause disease
  - Used as an indicator for fecal contamination in water



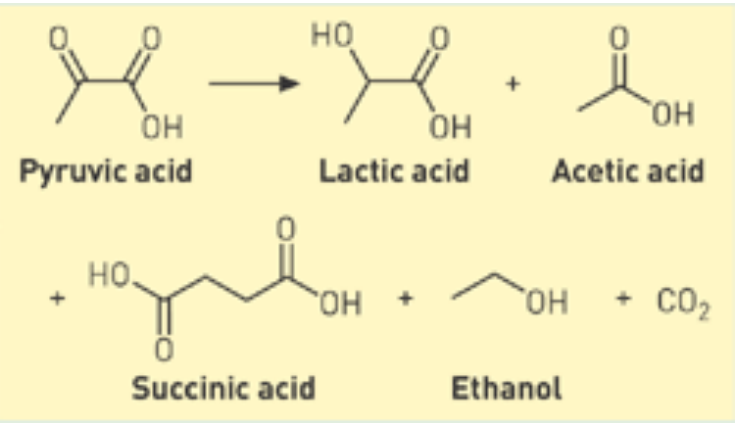
E. coli is a facultative anaerobe

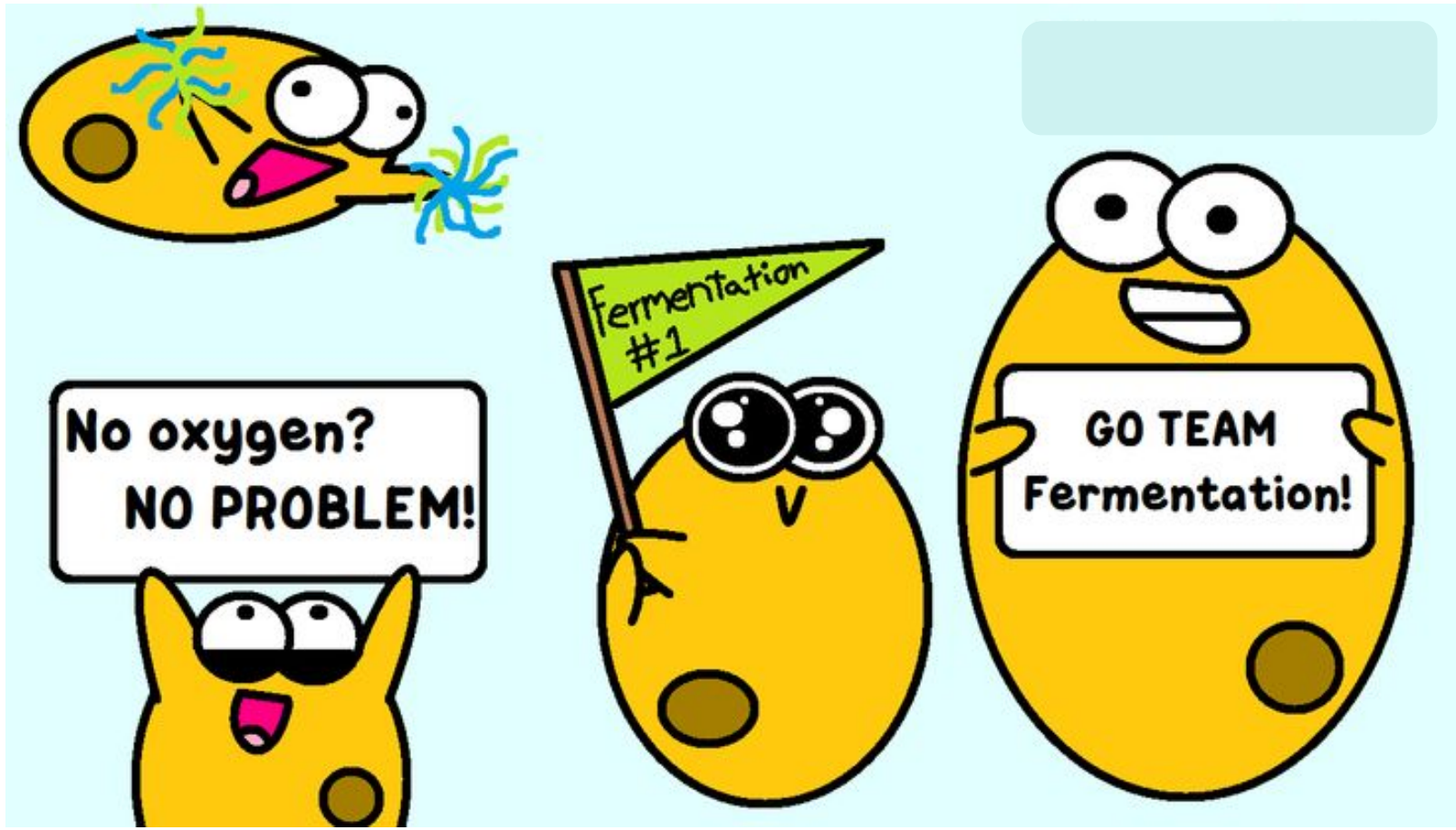
- Growth ‘in nature’ occurs in absence of oxygen
  - Adheres to mucous and epithelium of intestinal wall
  - Accounts for up to 1% of bacteria in the GI tract
  - Prevents colonization by pathogenic organisms
- In absence of oxygen, completes anaerobic respiration or fermentation



Anaerobic metabolism in *E. coli*

- Anaerobic respiration coupled to non-O_2 electron acceptor
  - Nitrate, trimethylamine oxide, and fumarate
  - Uses electrochemical gradient across a membrane (electron transport chain)
- Fermentation
  - Electron acceptor NAD+ is regenerated from NADH formed in oxidative steps by the reduction of oxidized compounds
  - Uses substrate level phosphorylation

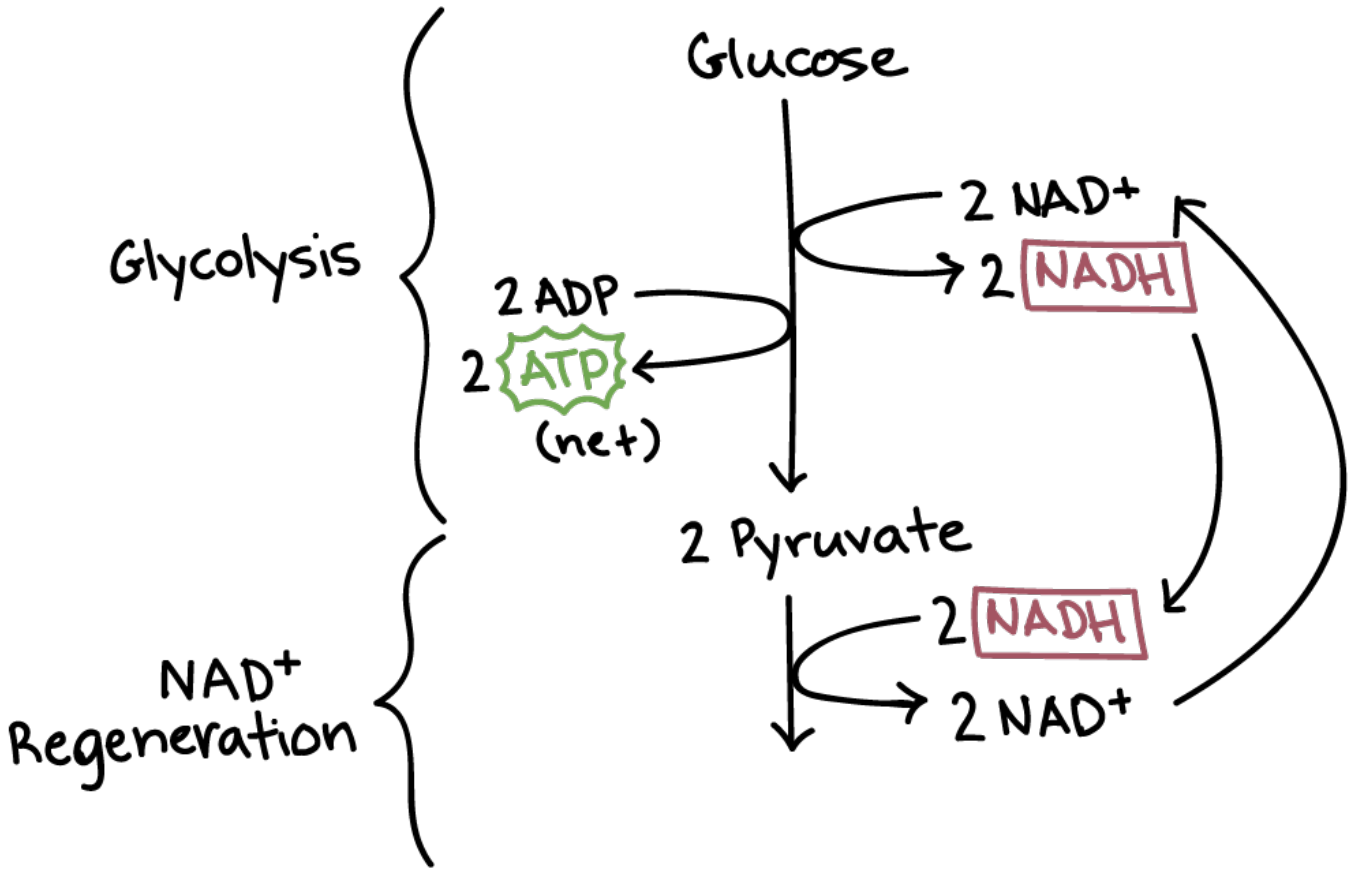



No oxygen?
NO PROBLEM!
Fermentation
#1
GO TEAM
Fermentation!

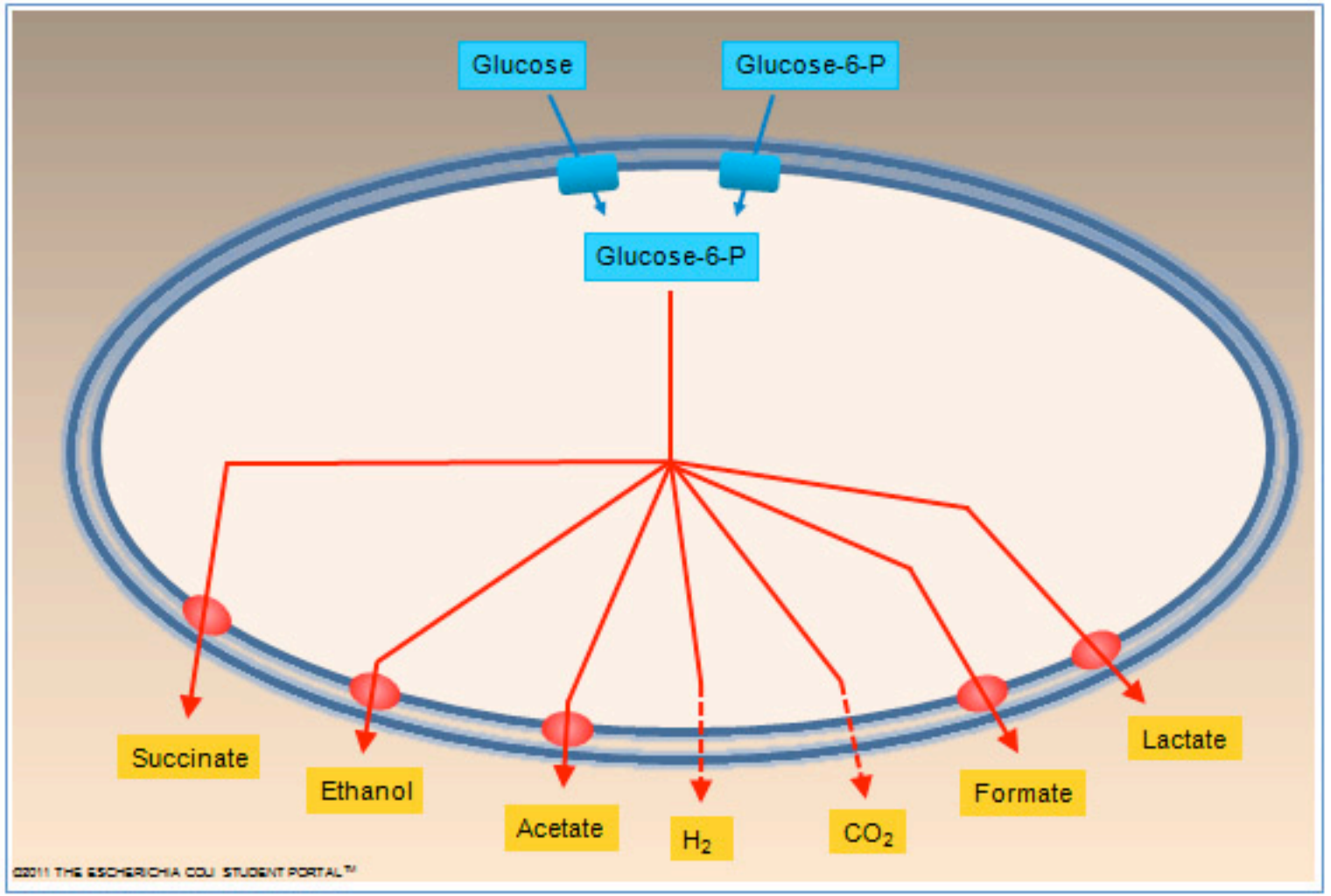
Fermentation uses enzymes to breakdown organic substrates

Primary method for producing ATP in microorganisms growing anaerobically

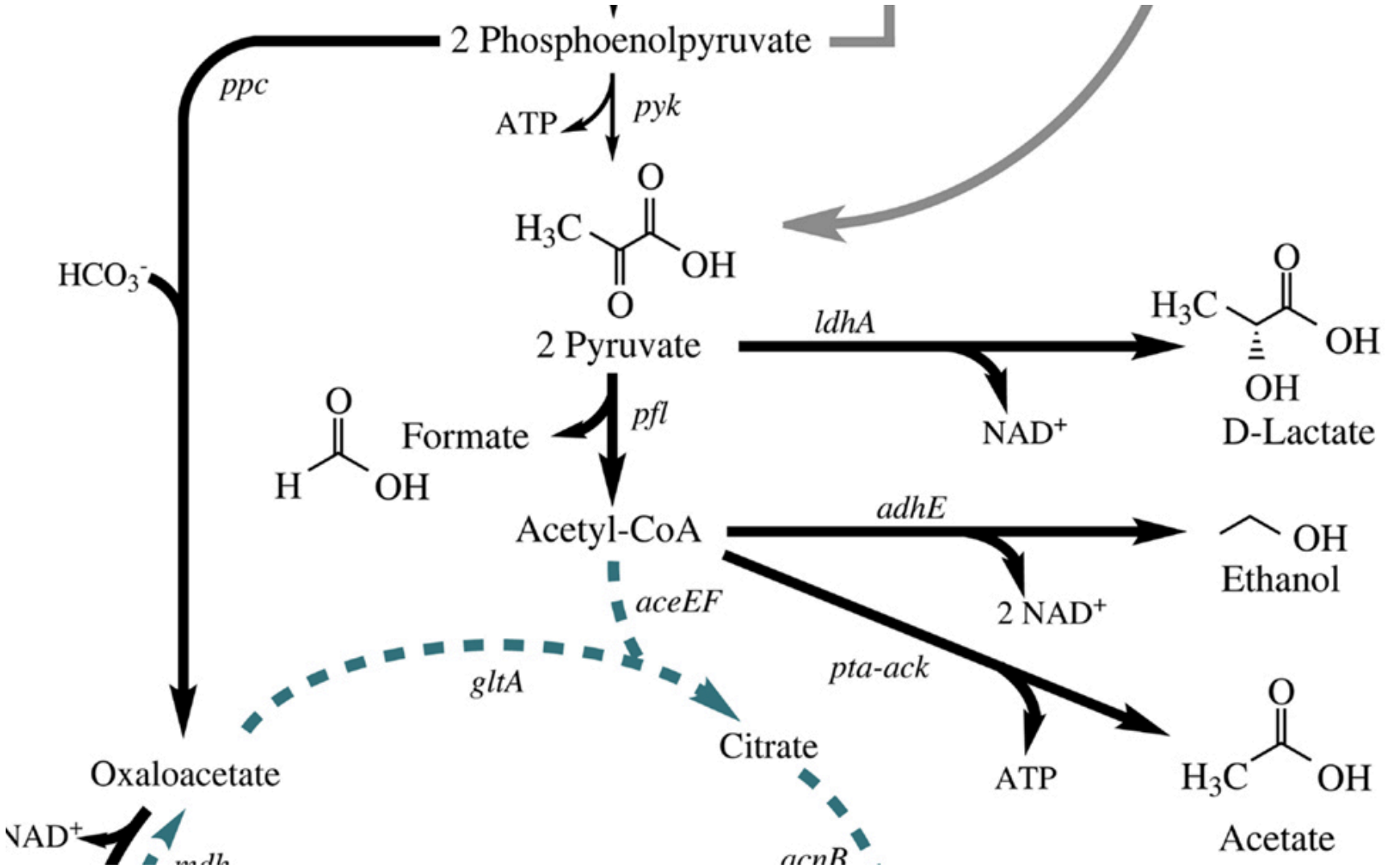
- NADH reacts with pyruvate (product of glycolysis)
- NAD+ and an organic product are generated



E. coli naturally produces commercially relevant products

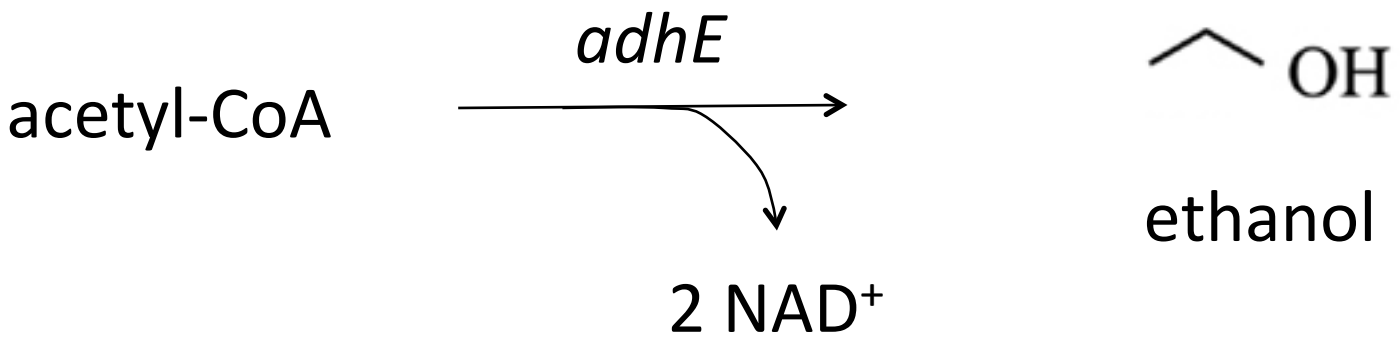


A closer look at the fermentation pathway



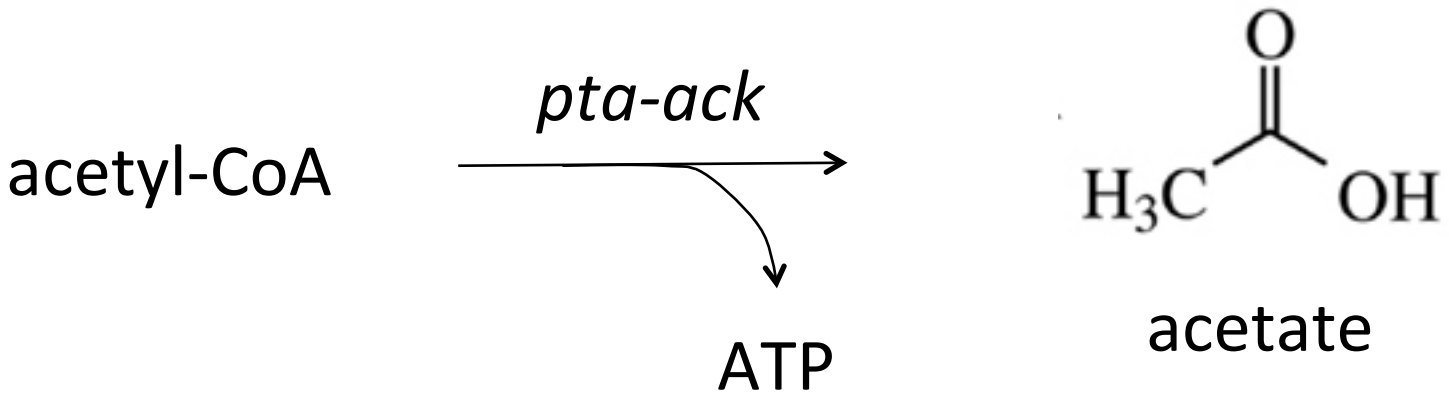
Production of ethanol

- Bioethanol is most important biotechnological commodity
- *adhE* only transcribed in anaerobic conditions

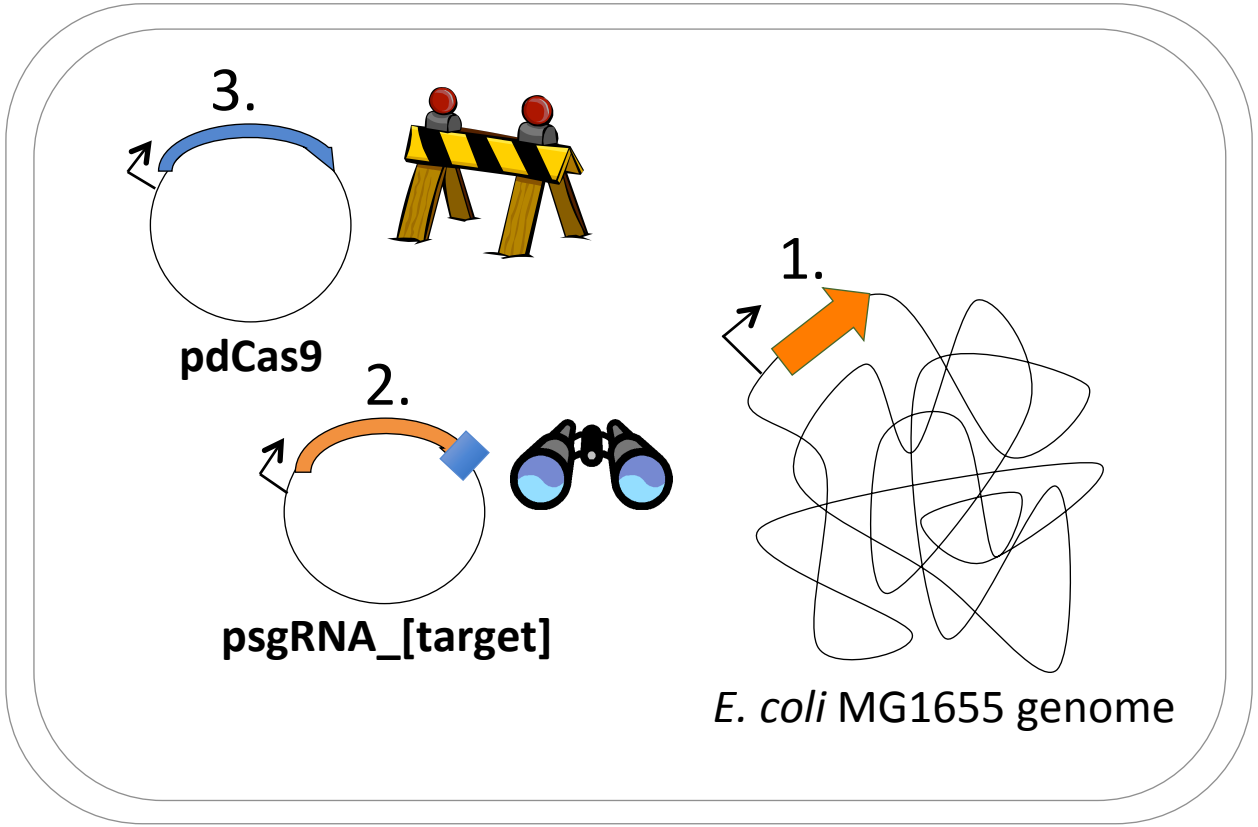


Production of acetate

- Acetates used in production of polymers
- *pta-ack* expressed constitutively
  - Aerobically grown cells produce negligible amounts of other fermentation products

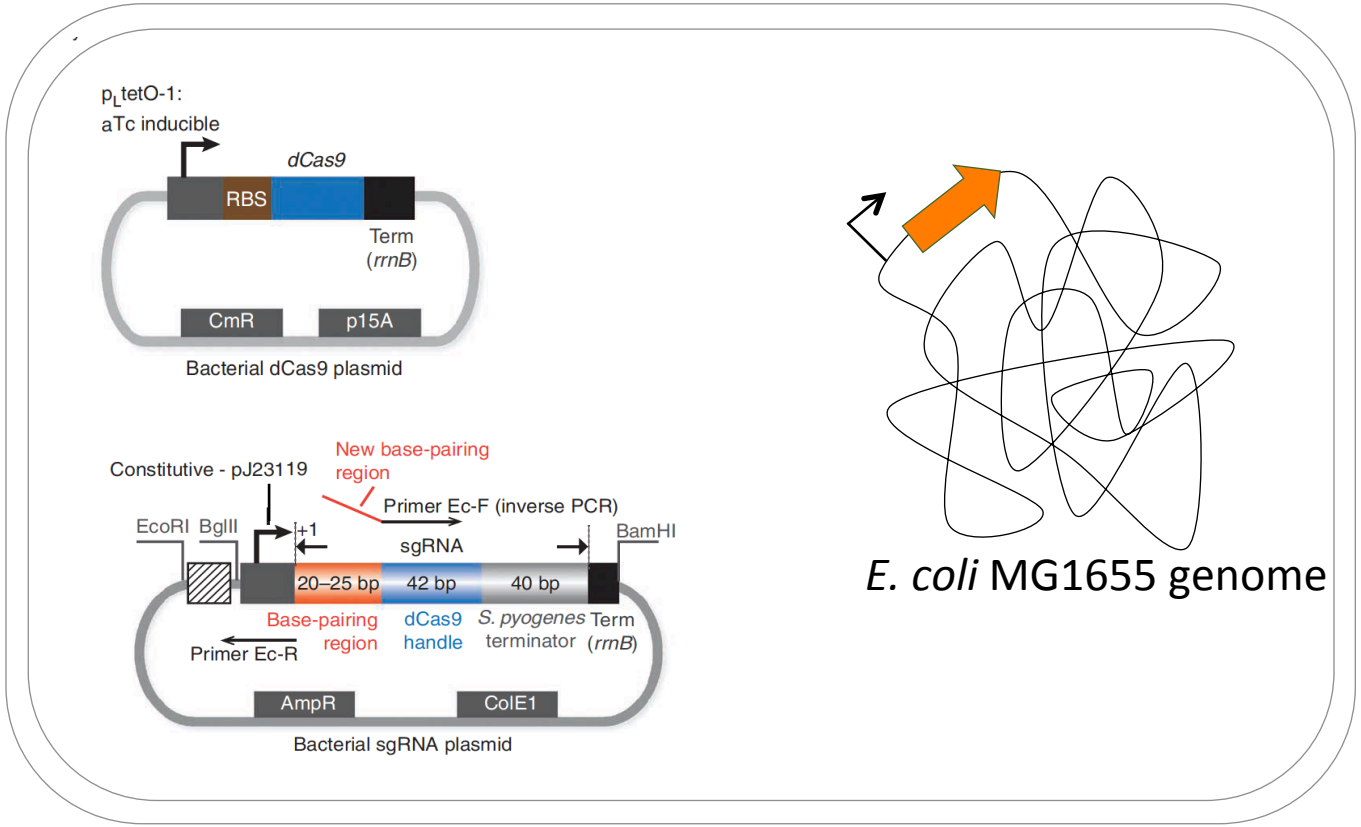


How will we manipulate fermentation in *E. coli*?



1. Target gene
2. psgRNA_[target]
3. pdCas9

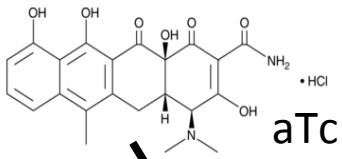
CRISPRi ‘inactive’ in absence of inducer

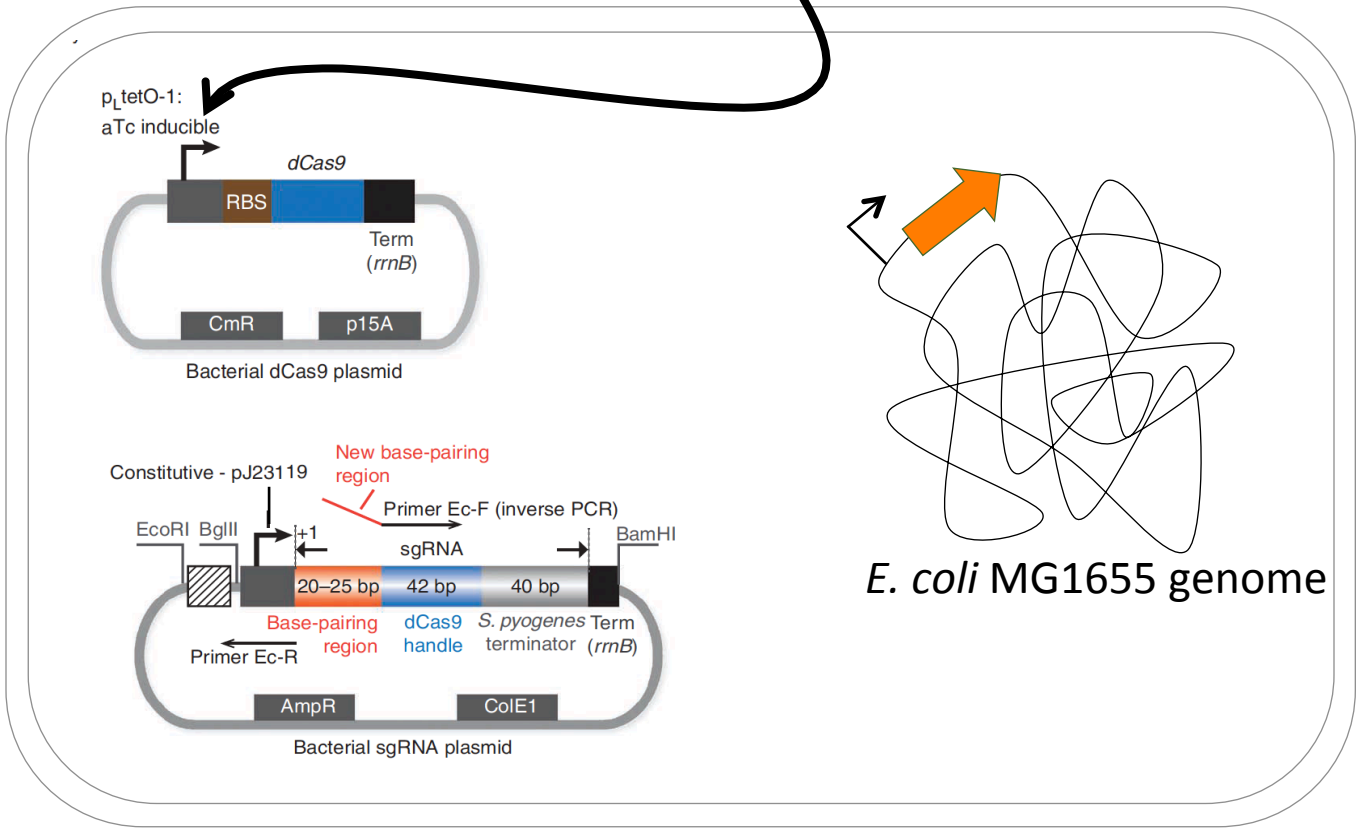


psgRNA_[target] is expressed constitutively

- sgRNA is continually transcribed

CRISPRi ‘blocks’ gene expression in presence of inducer

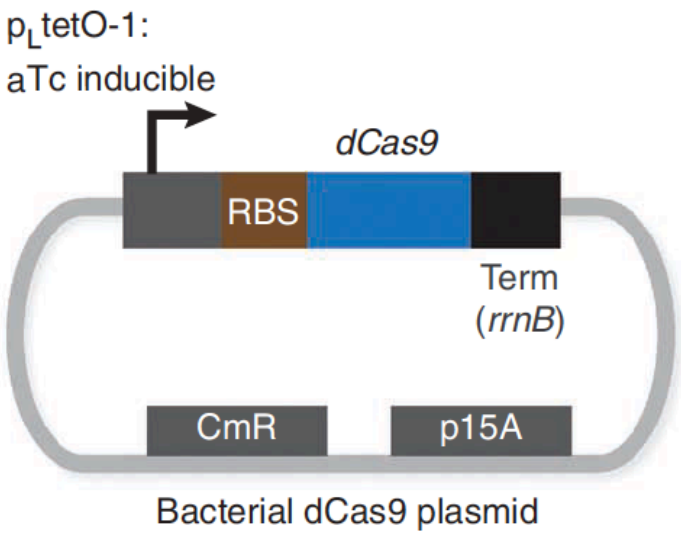




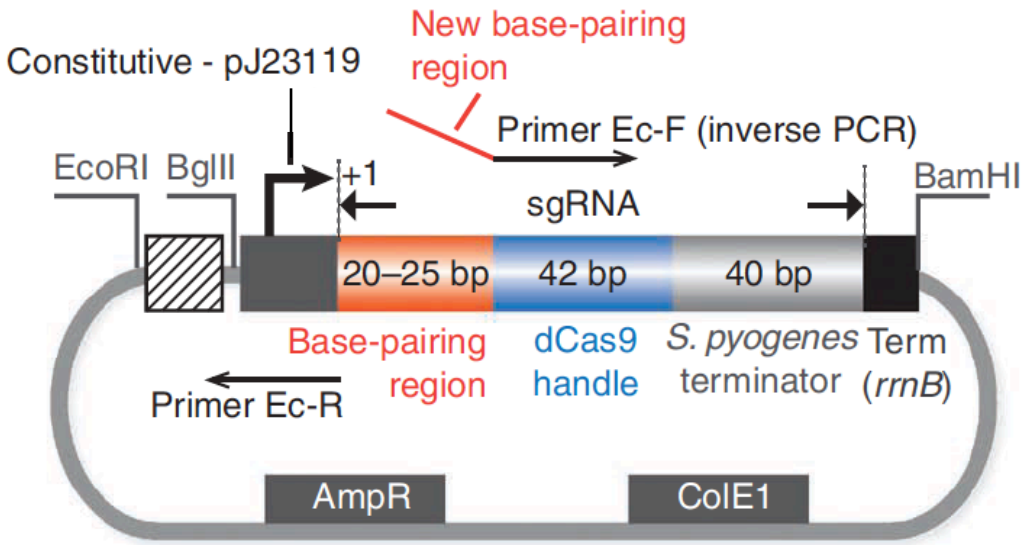
pdCas9 is expressed when inducer aTc added

- when transcribed, associates with psgRNA_[target]
- Cas9 / psgRNA_[target] complex scans DNA for target gene

Keep track of the plasmid constructs!



Bacterial dCas9 plasmid



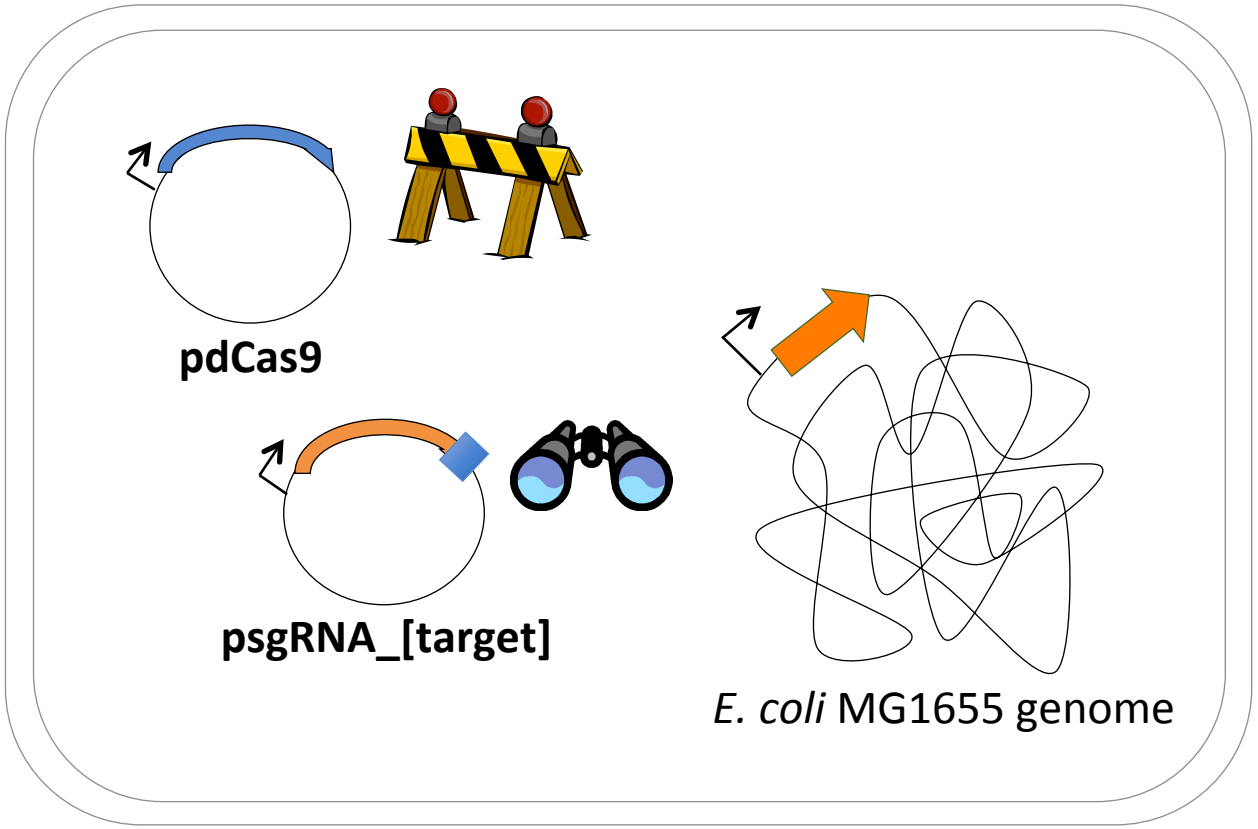
Bacterial sgRNA plasmid

Prepared confirmation digest to check pdCas9 construct on M2D1

Design gRNA target sequence for psgRNA_[target] construct on M2D2

In the laboratory…

1. Research *E. coli* fermentation pathway and select target gene
2. Design gRNA target sequence



For the next lecture…

Multiple Gene Repression in Cyanobacteria Using CRISPRi

Lun Yao, Ivana Cengic, Josefine Anfelt, and Elton P. Hudson*