Module 2: Metabolic Engineering

Genetic engineering as a tool for manipulating systems

What is metabolic engineering?

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"…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."

How do we use genetic engineering to manipulate metabolisms?

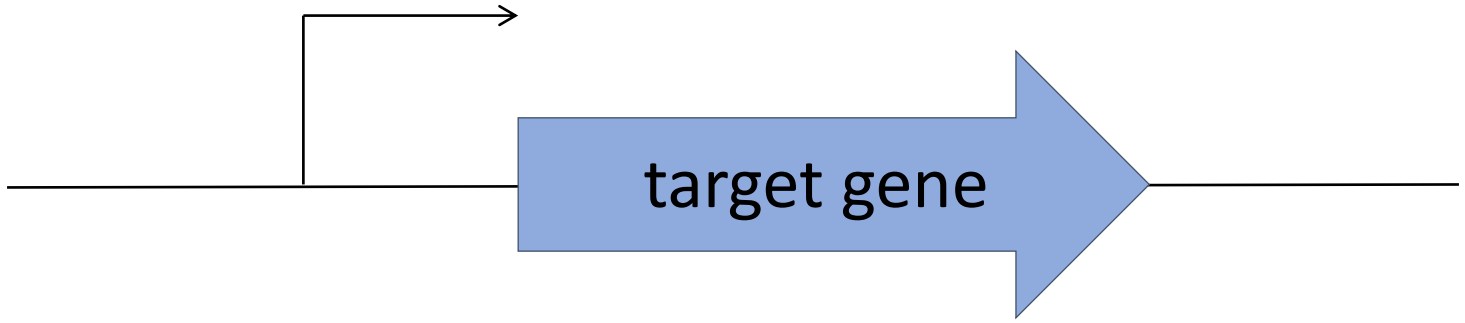
Genetic (DNA) engineering techniques:

1. Repress genes
2. Overexpress genes
3. Delete genes / functions
4. Add genes
5. Mutate genes

1. Repress genes

- Inhibit polymerase or transcription factor binding at promoter

- Inhibit transcript elongation through gene by blocking polymerase



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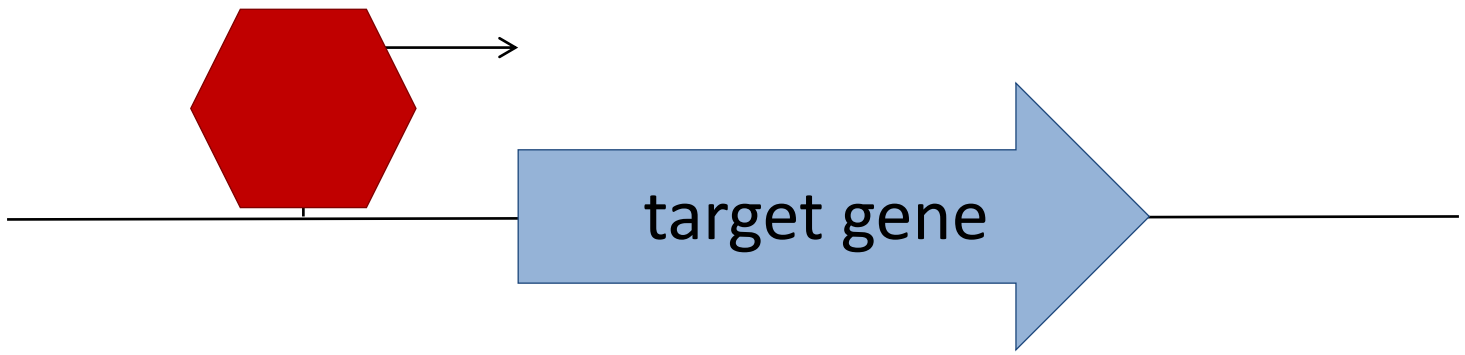

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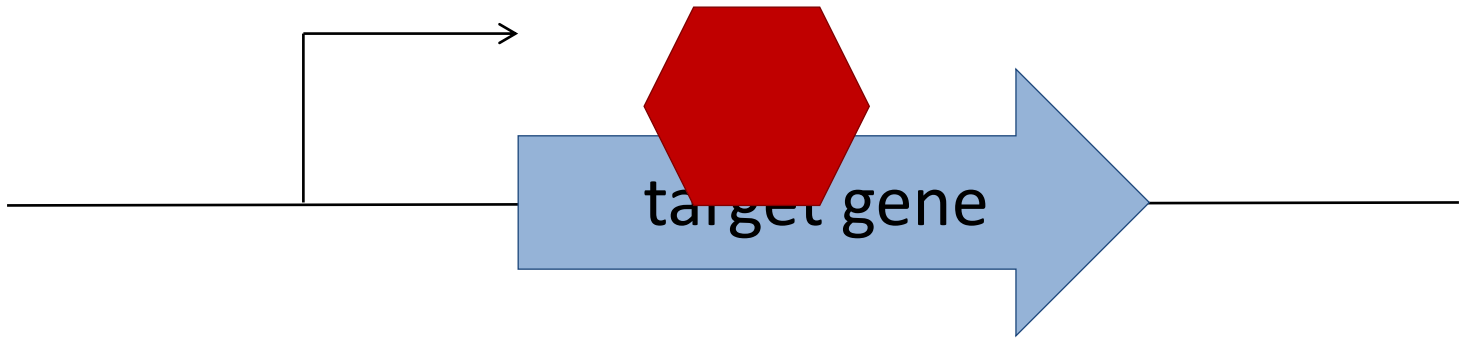
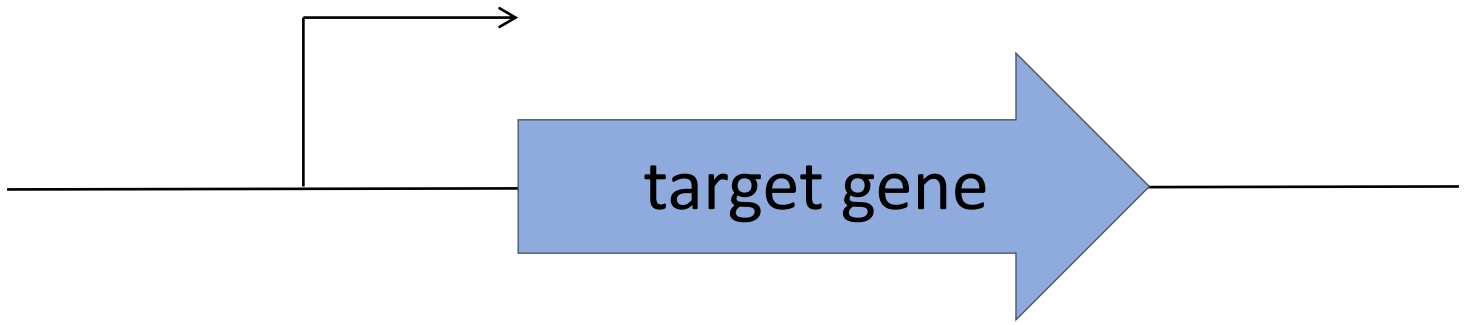


2. Overexpress genes

- Replace native promoter with one that is constitutively active

- Express additional gene copies exogenously using plasmids



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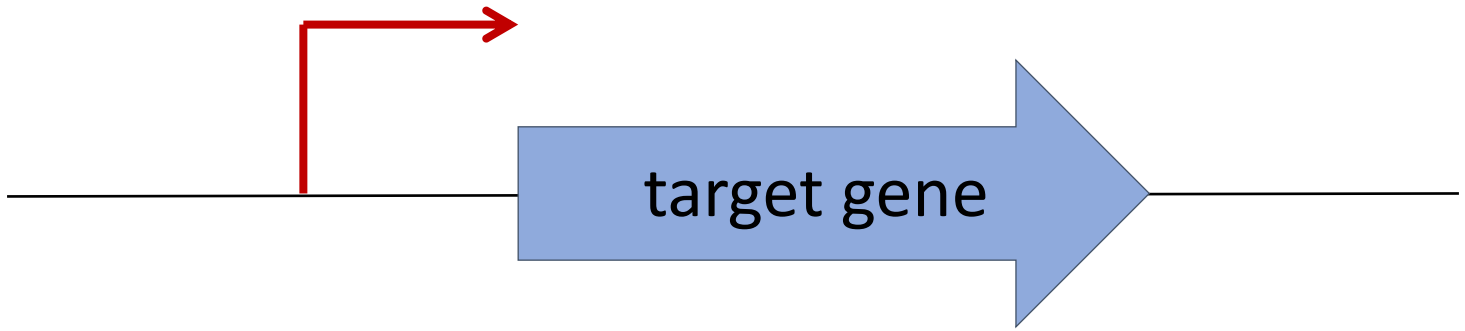

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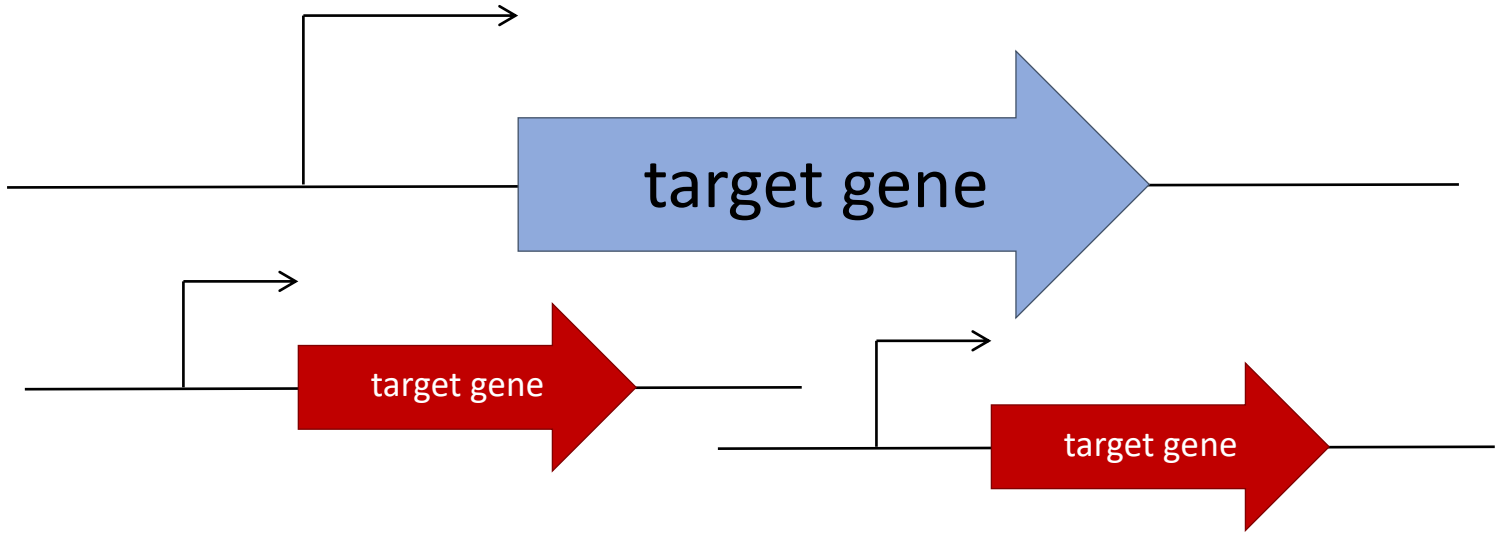

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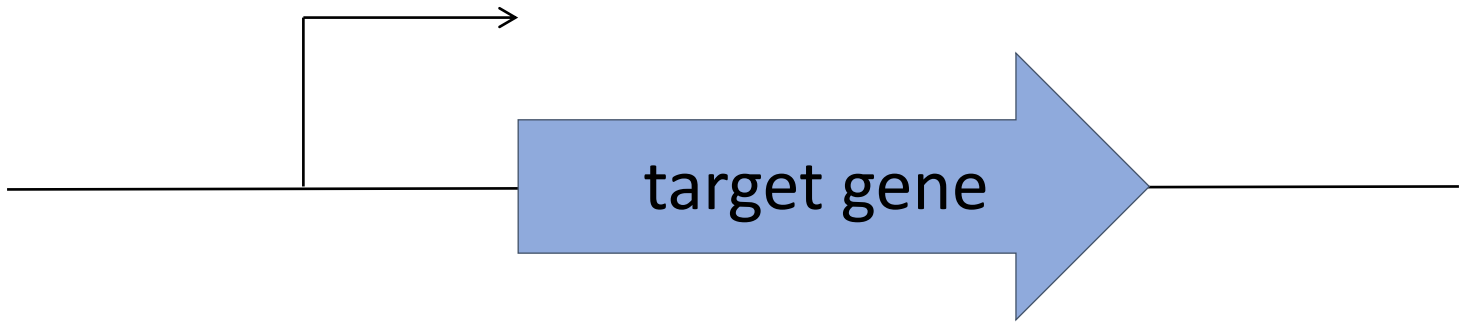
- Remove gene from genome

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



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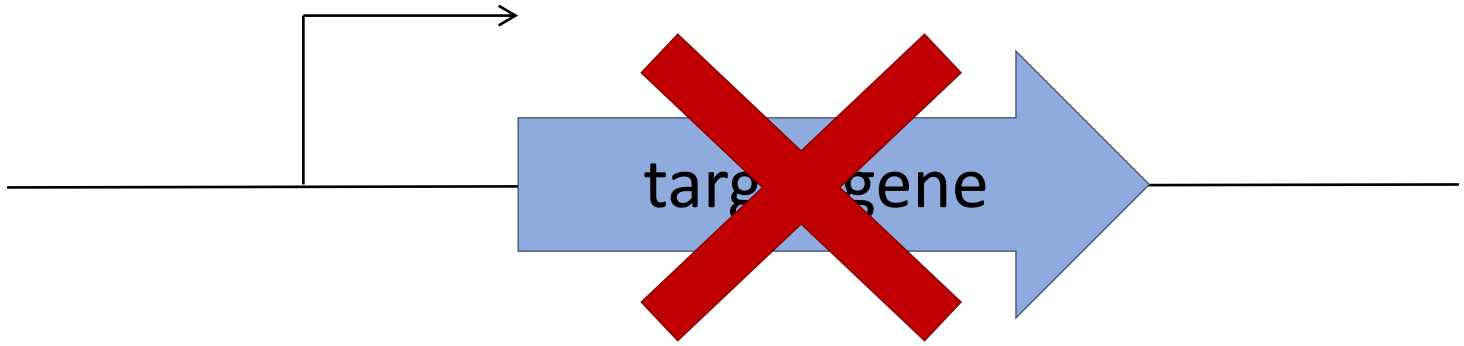

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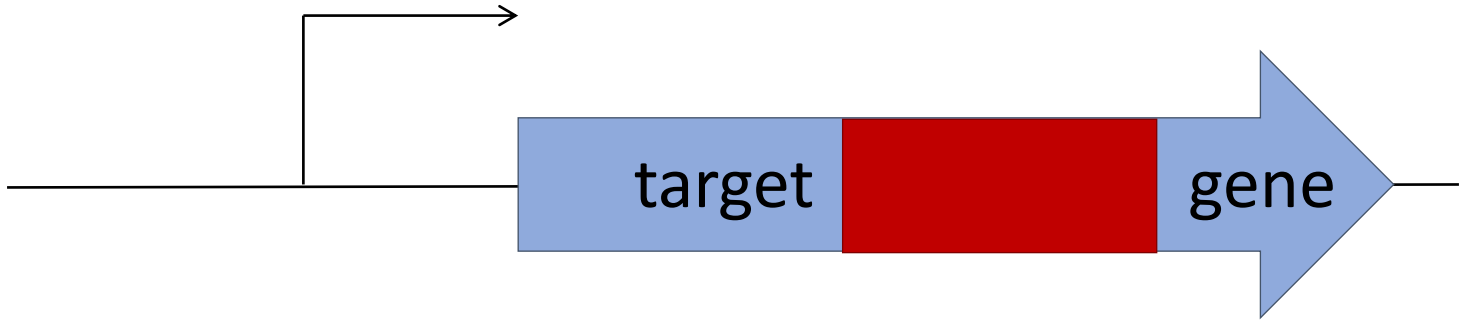

4. Add genes

- Insert non-native gene into host genome

- Express non-native gene exogenously using plasmids

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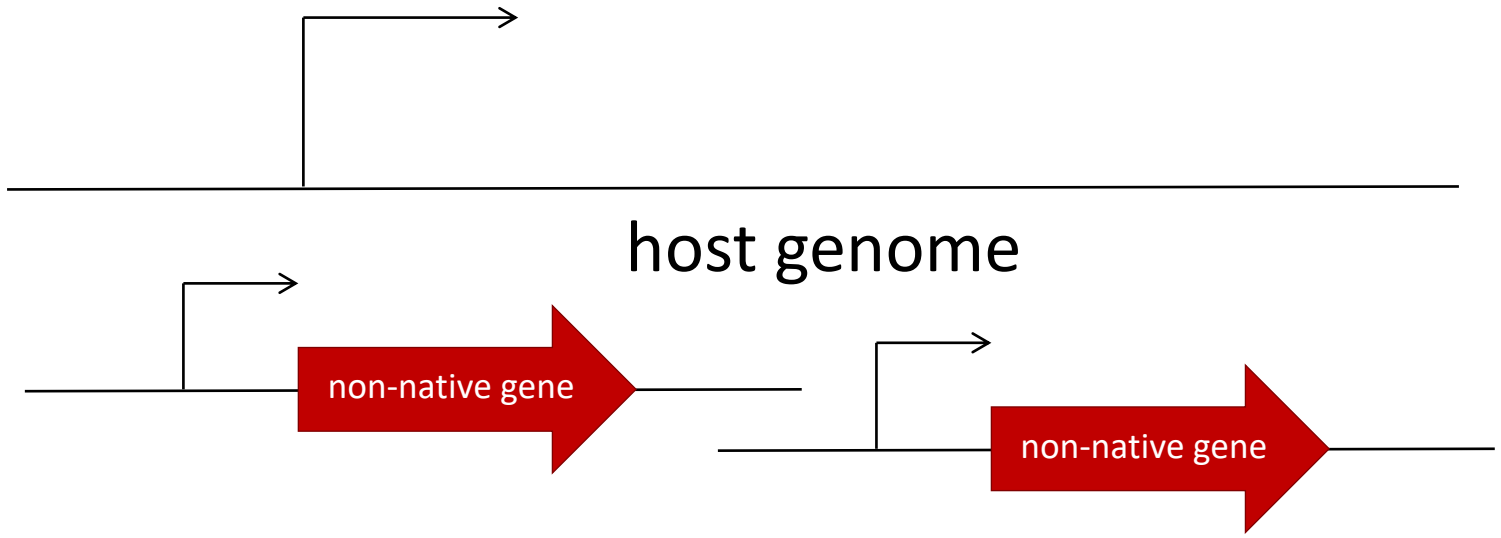
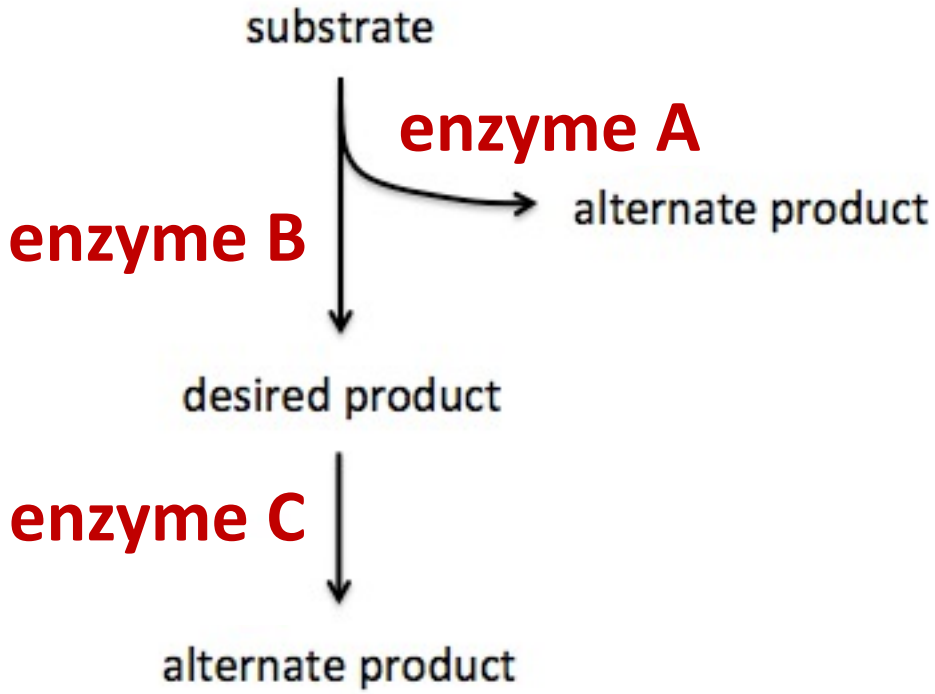


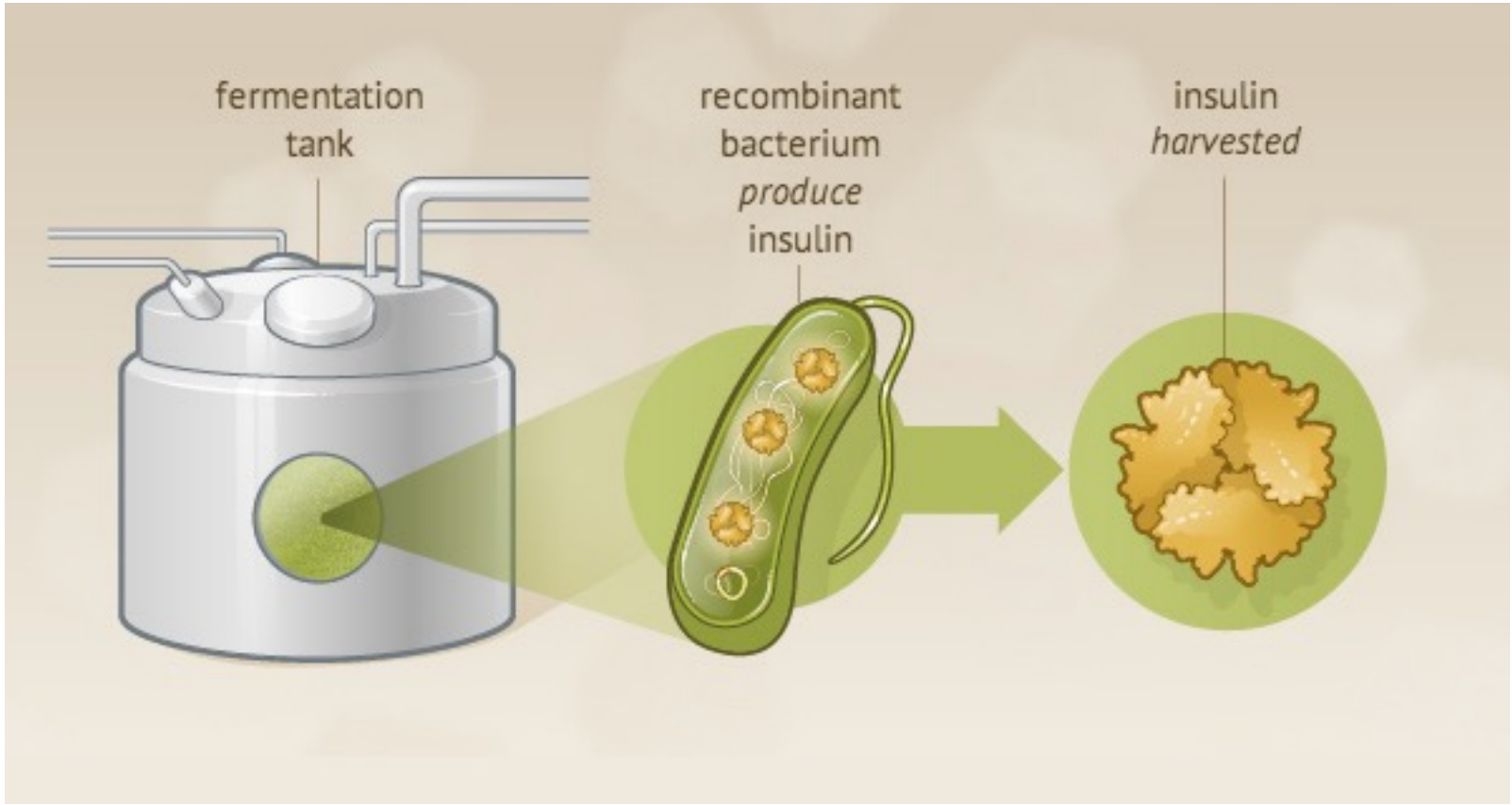
5. Mutate gene

- 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?

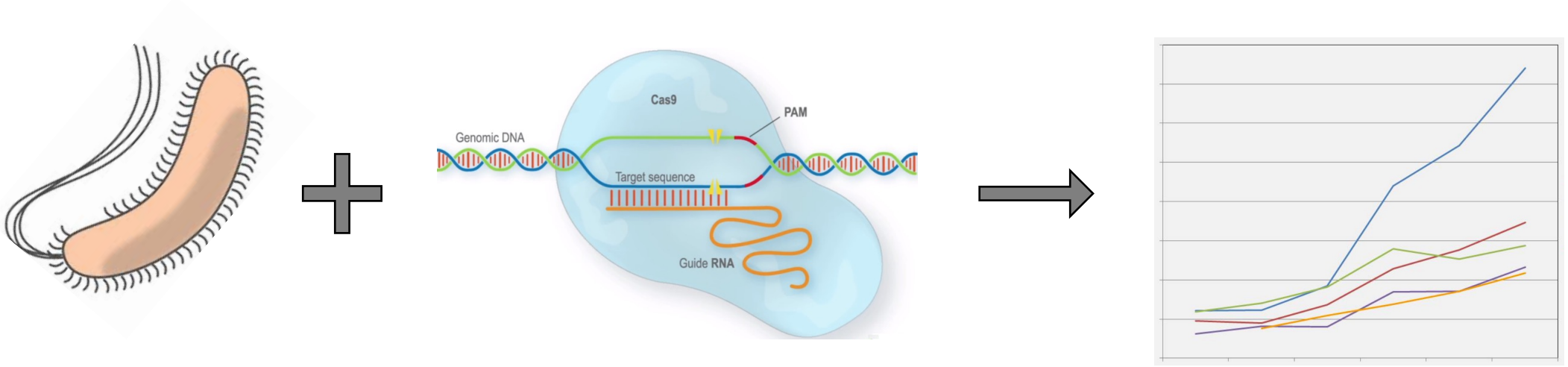


Metabolically engineered pathways can be expressed in host organisms



What is your biological engineering task in Mod 2?

Increase production of ethanol or acetate in *E. coli* MG1655 by manipulating the native fermentation pathway using CRISPR-based editing technology

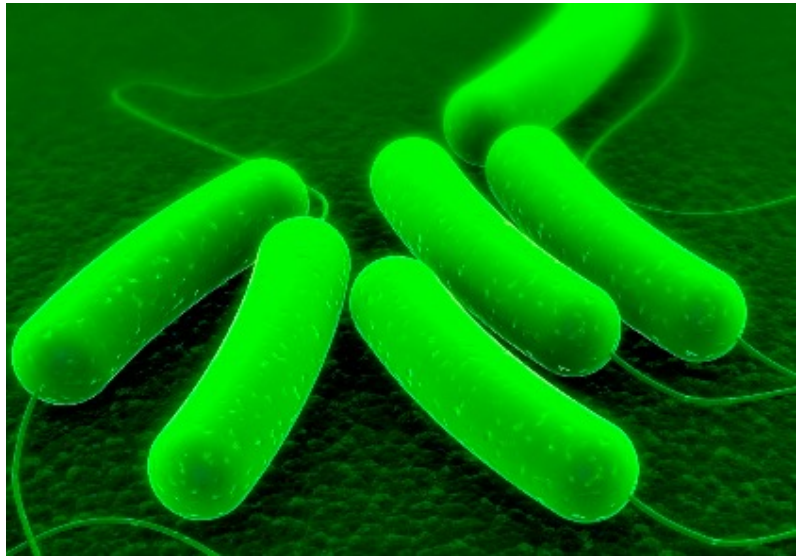


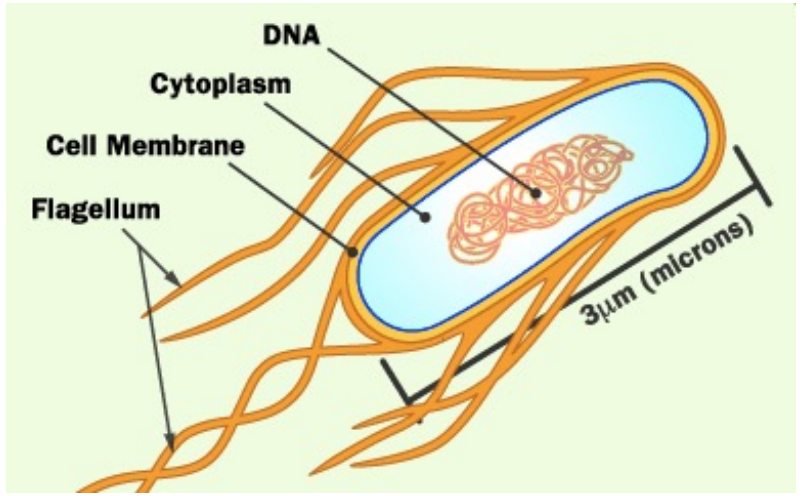
Why are we using this approach in Mod 2?

- Why ethanol and acetate?
- Why *E. coli*?
- Why CRISPRi?

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



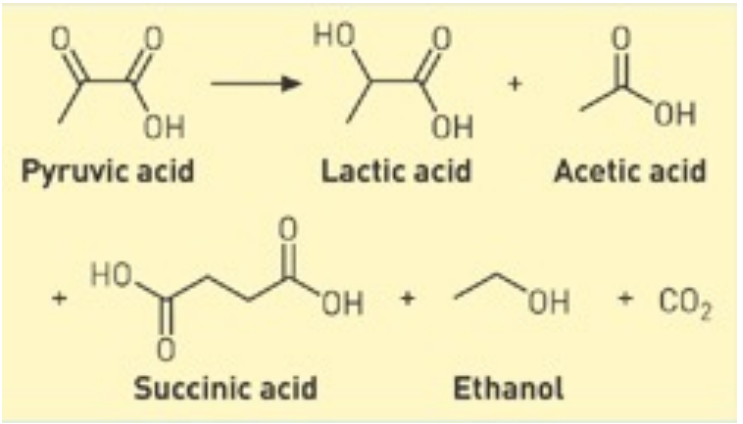


E. coli is a facultative anaerobe

- 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*

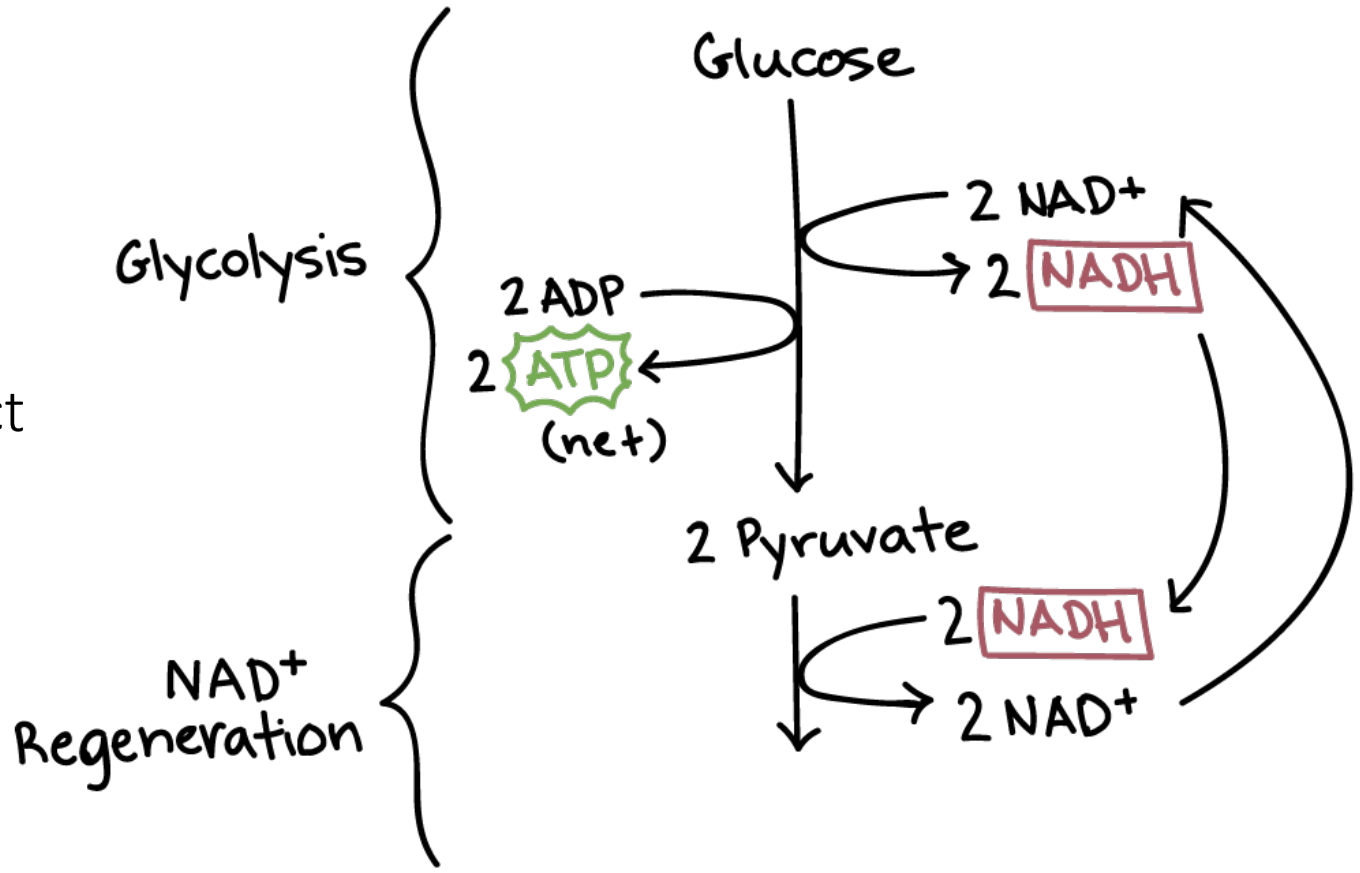
- Anaerobic respiration coupled to non-O_2 electron acceptor
  - Nitrate, trimethylamine oxide, and fumarate
  - Uses electrochemical gradient across a membrane (electron transport chain)
- Fermentation
  - Electron acceptor NAD+ is regenerated from NADH formed in oxidative steps by the reduction of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



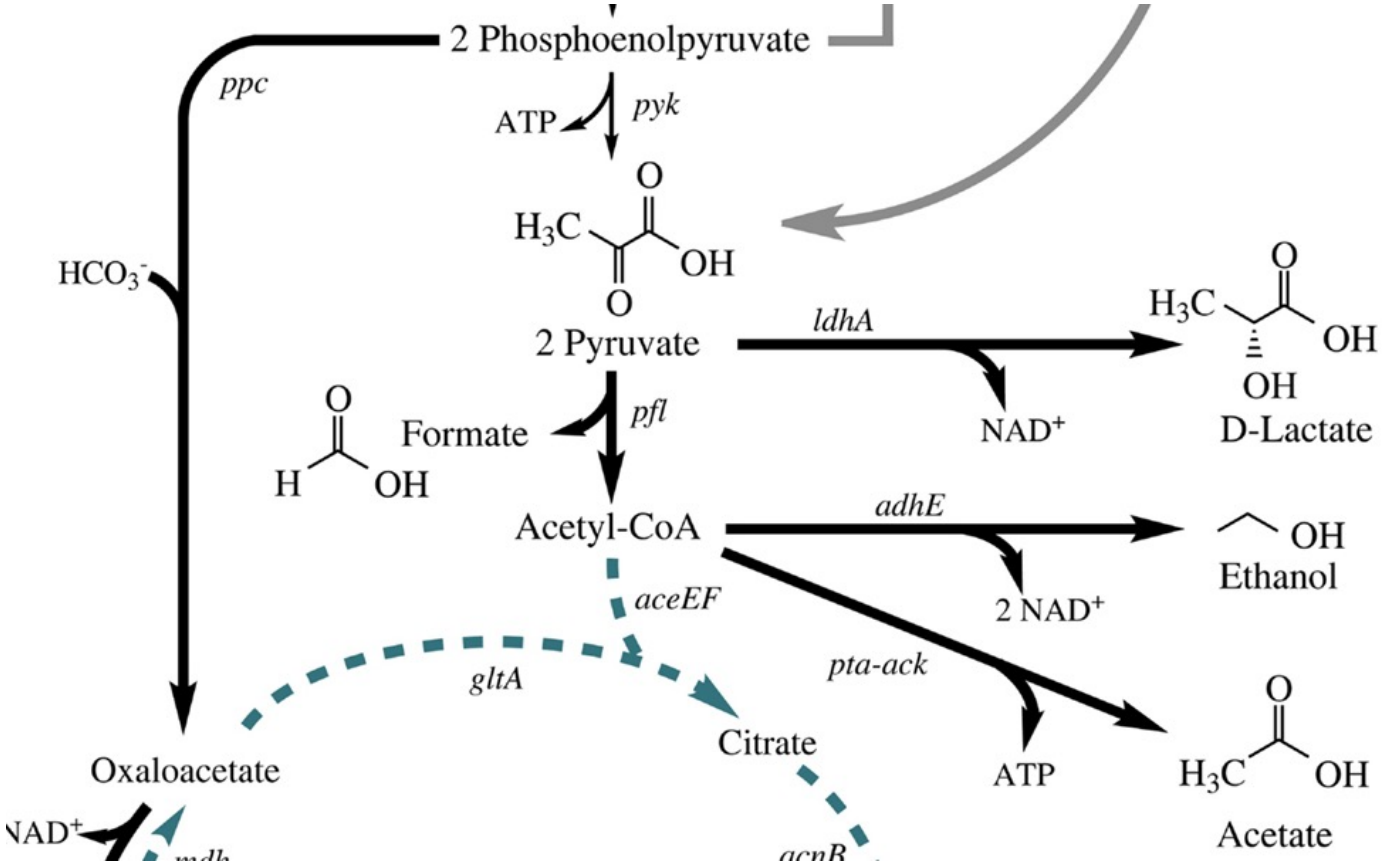
Fermentation uses enzymes to breakdown organic substrates

Primary method for producing ATP in microorganisms growing anaerobically

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

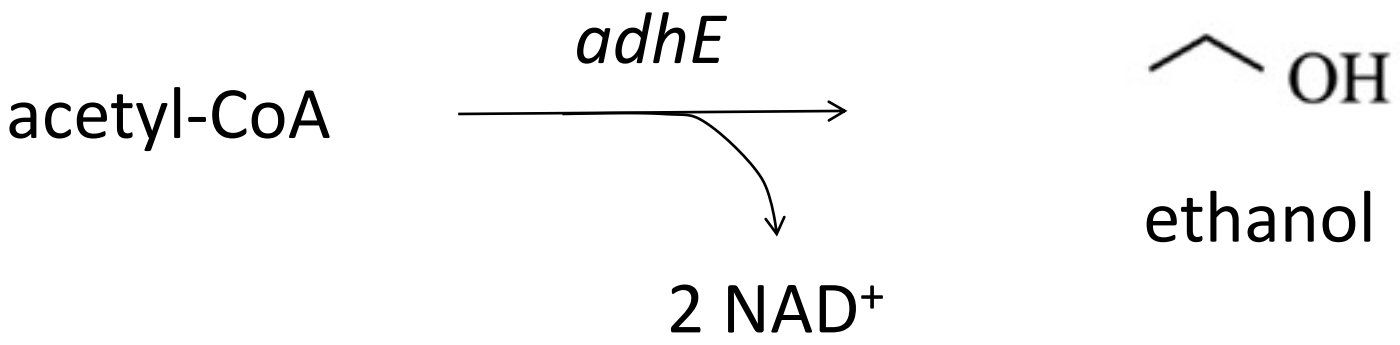


E. coli produces commercially relevant products



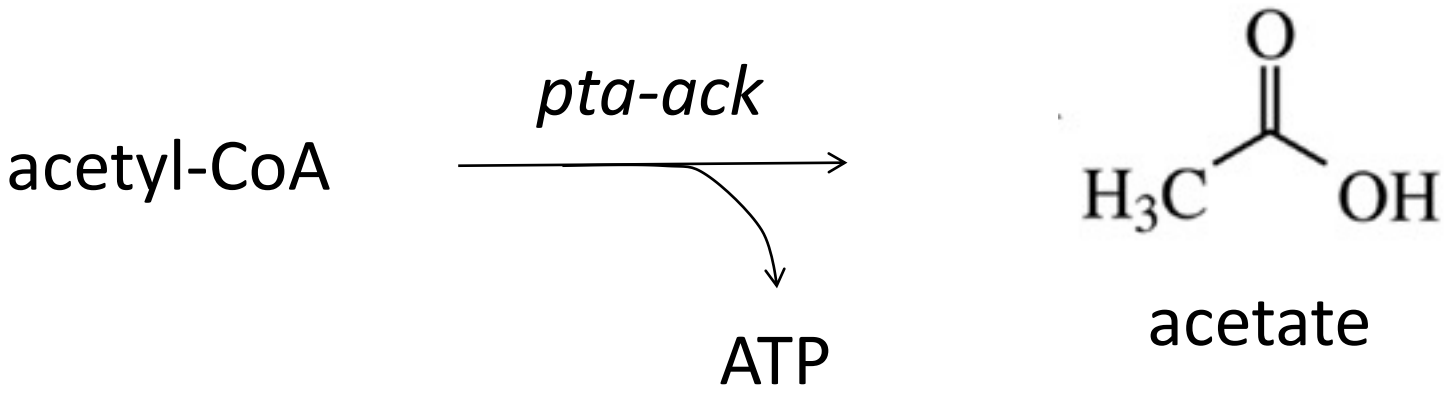
Production of ethanol

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



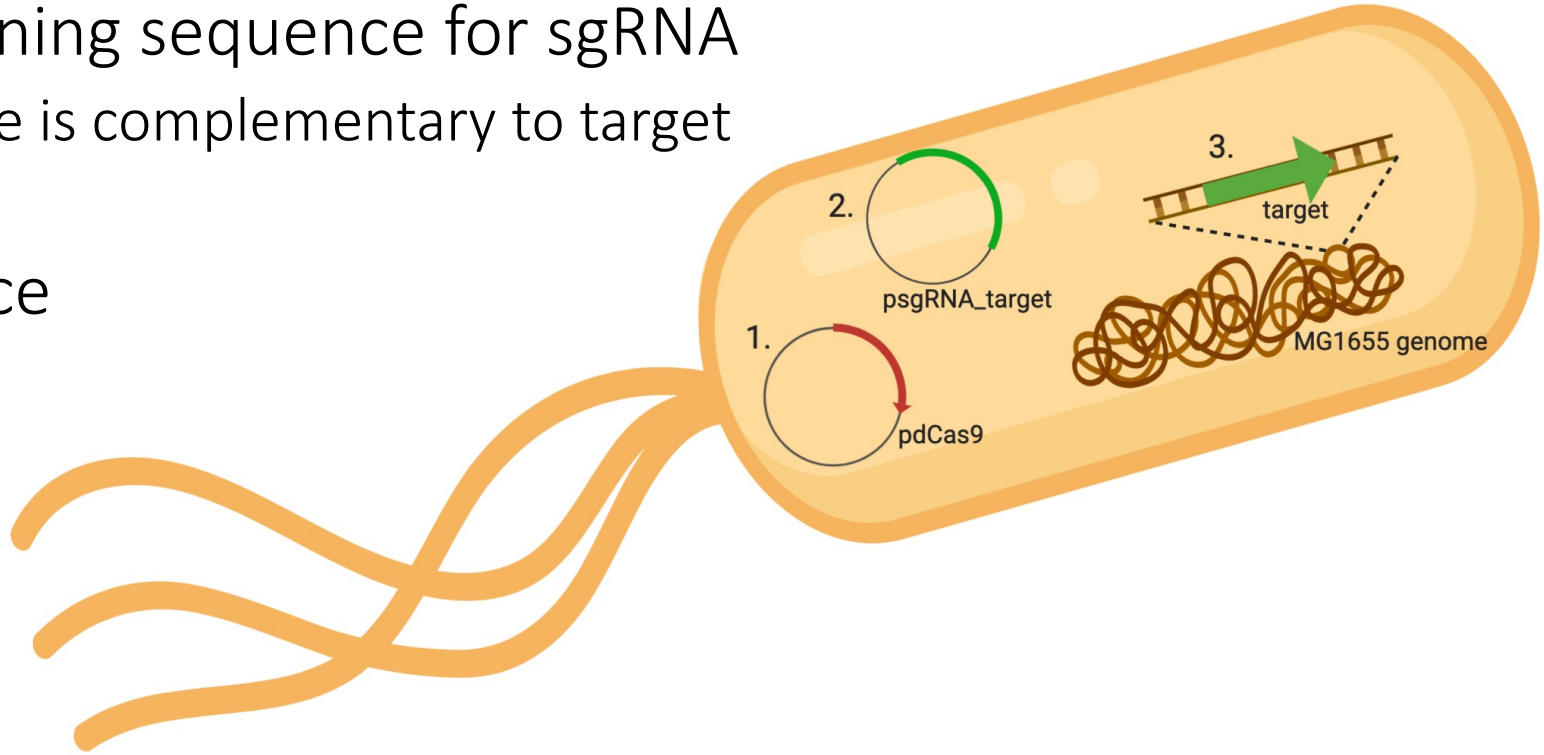
Production of acetate

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



How will we manipulation metabolism in *E. coli*?

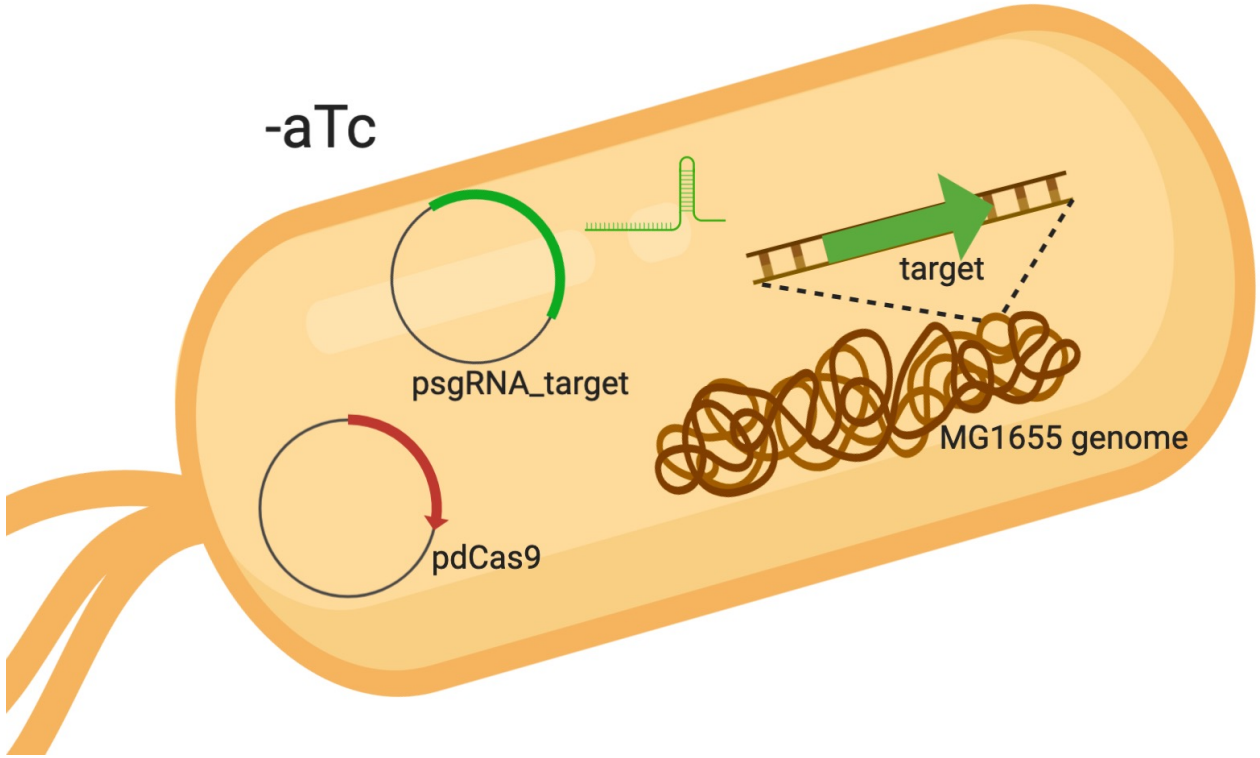
1. Plasmid containing gene that encodes dCas9
2. Plasmid containing sequence for sgRNA
   - sgRNA sequence is complementary to target sequence
3. Target sequence



CRISPRi inactive in absence of inducer

pgRNA_target expressed constitutively

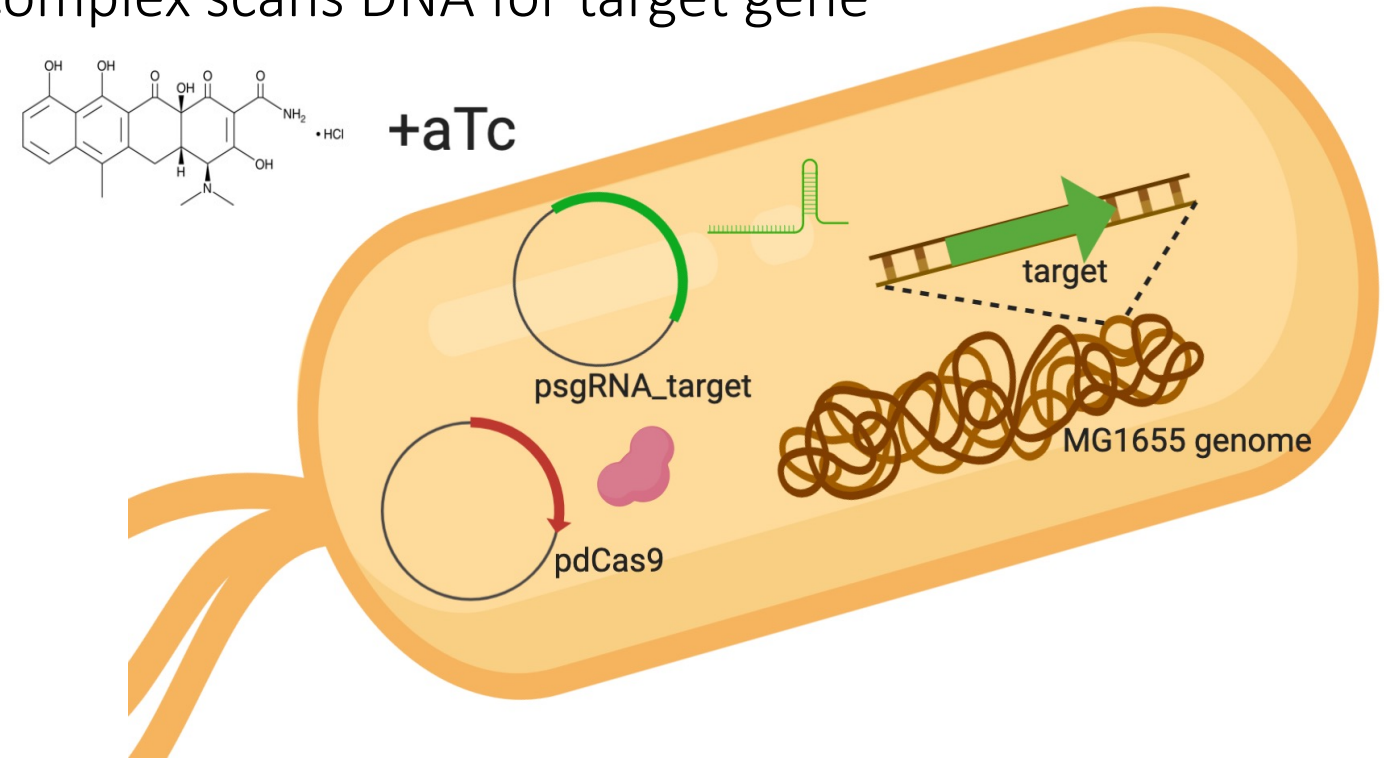
- Always transcribed and binding to target gene



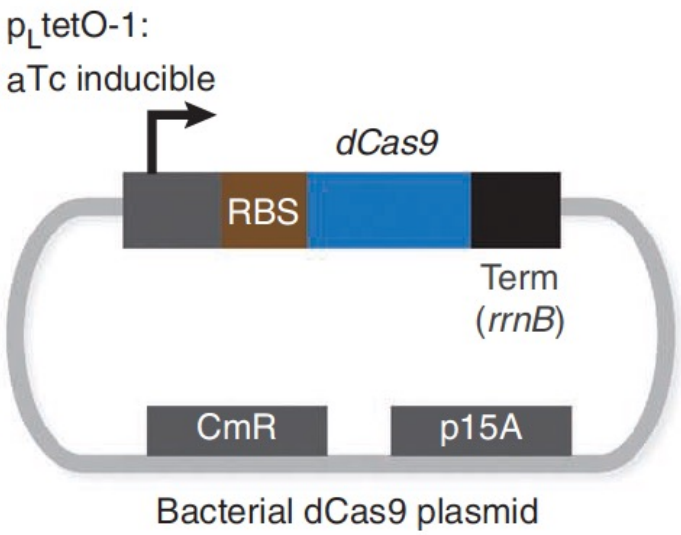
CRISPRi inhibits gene expression in presence of inducer

pdCas9 expressed when aTc added

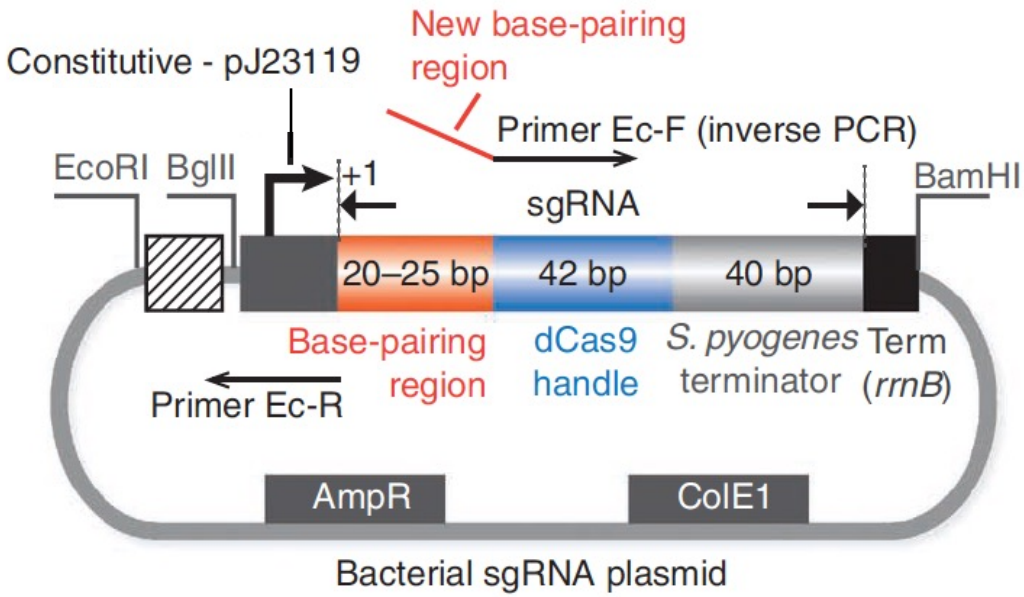
- When transcribed associates with pgRNA_target / target gene
- Cas9 / psgRNA_[target] complex scans DNA for target gene



Keep track of the plasmids used in CRISPRi



Bacterial dCas9 plasmid



Bacterial sgRNA plasmid

Prepare confirmation digest to check pdCas9 construct on M2D1

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

What is the take-home message?

- Genetic engineering tools and methods are key in manipulating systems.
- Several approaches can be used to engineer and regulate native systems.
- Bacterial cells can be employed as ‘natural factories’ to produce commercially valuable products.