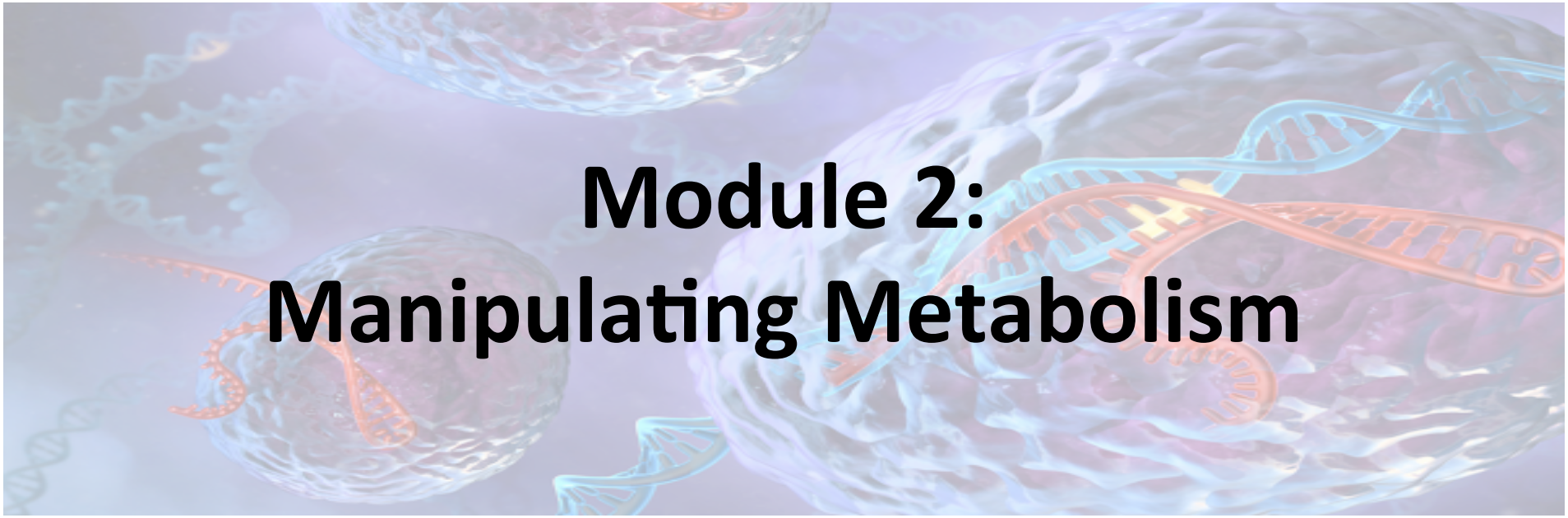
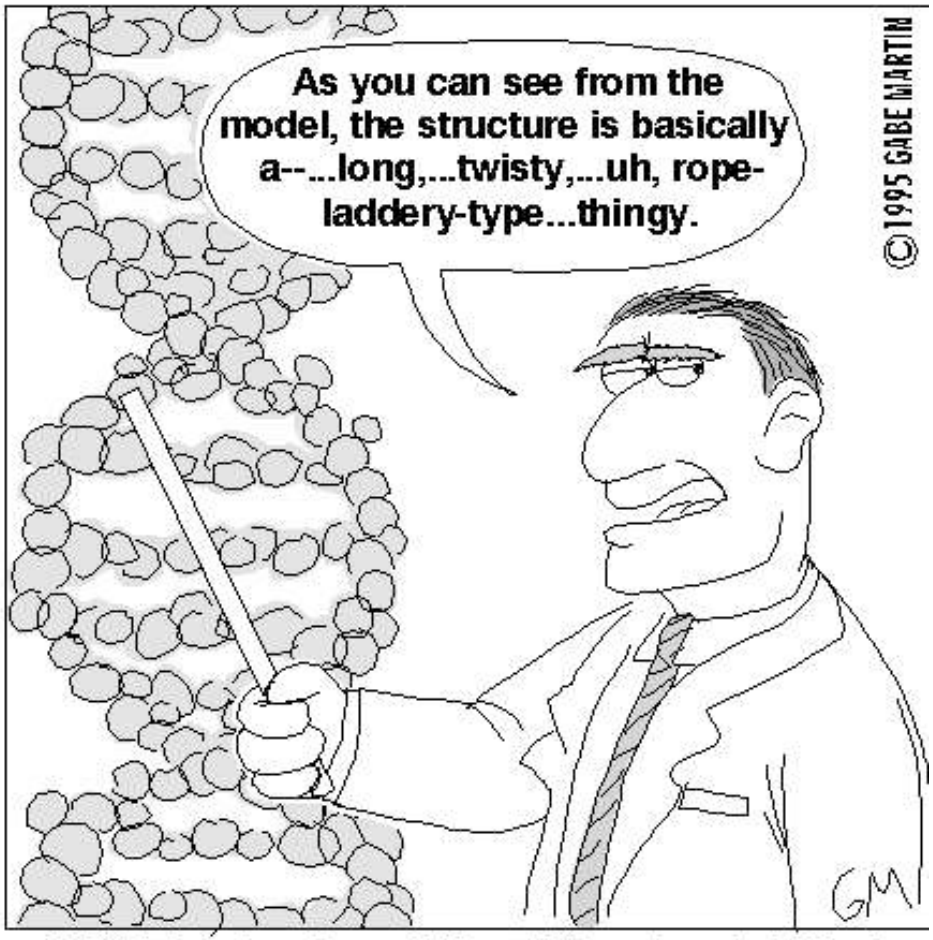


Module 2: Manipulating Metabolism

Metabolic engineering

10/18/15

What is metabolic engineering?



1953: The structure of the DNA molecule is first described.

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."

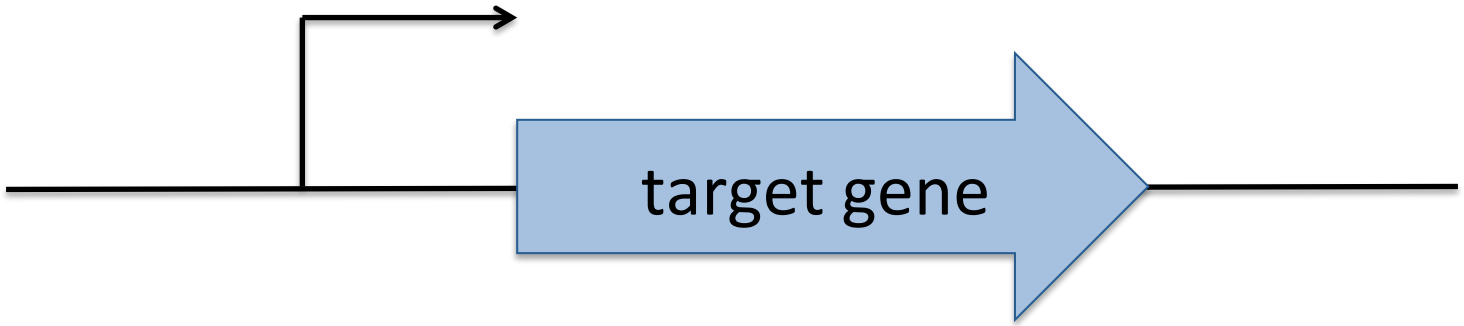
Metabolic engineering ‘toolkit’

- Genetic (DNA) engineering techniques
  1. Repress gene
  2. Overexpress gene
  3. Delete gene
  4. Add gene
  5. Mutate gene

1. Repress gene

- Inhibit binding to promoter

- Inhibit transcript elongation through gene



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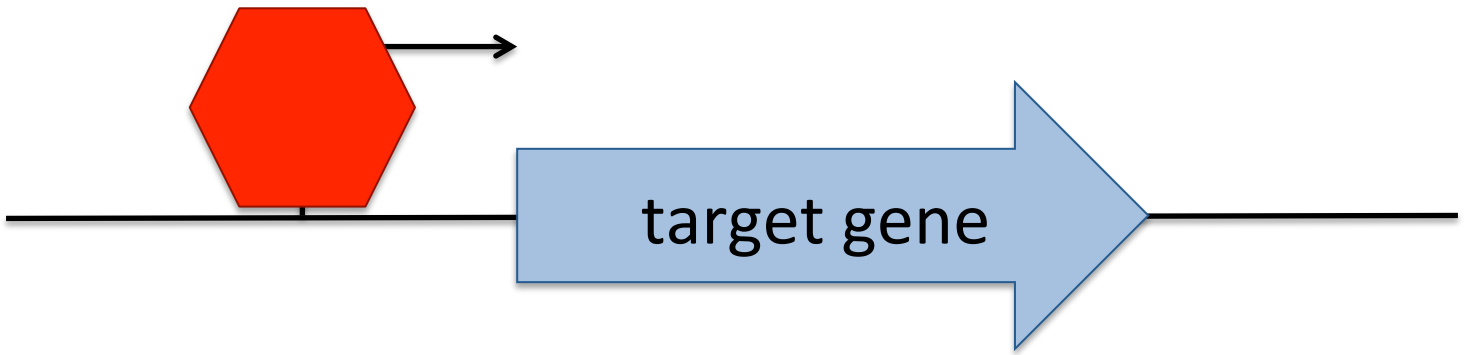

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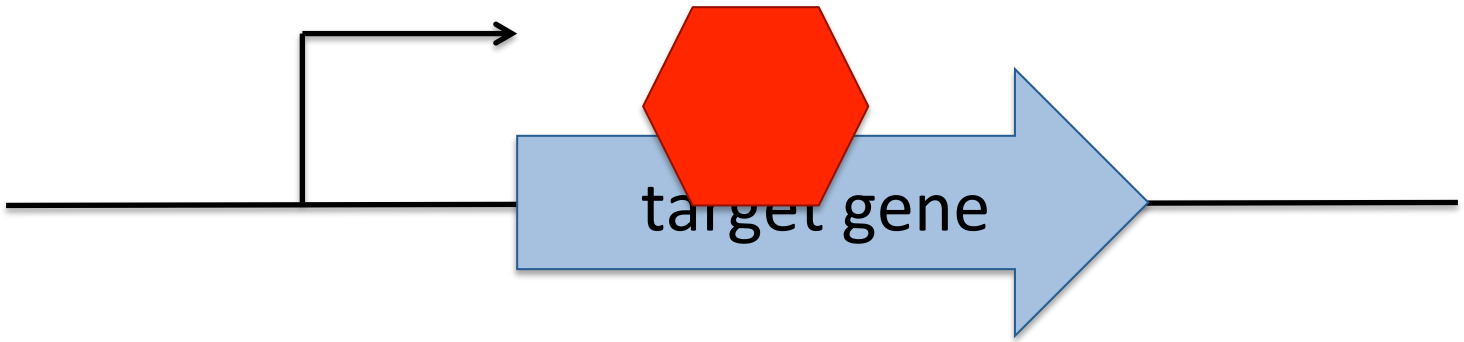
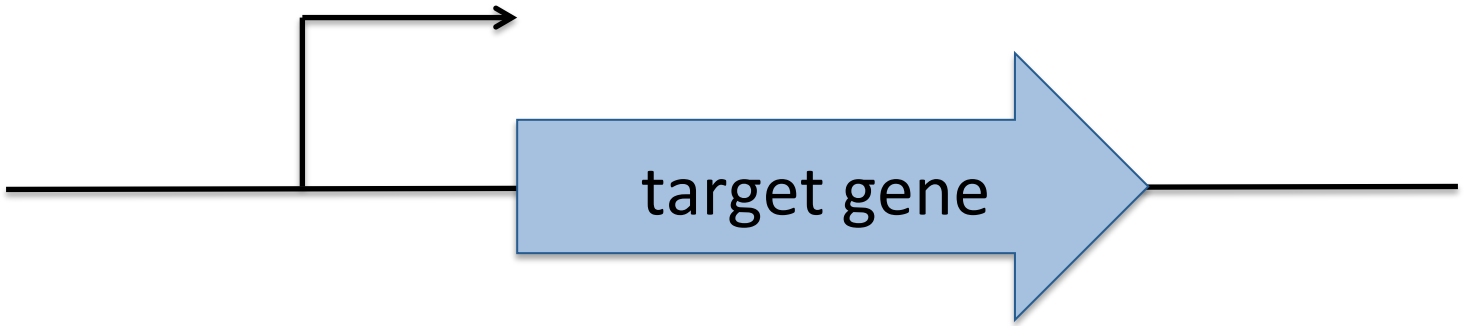


2. Overexpress gene

- Replace native promoter with one that is constitutively active

- Express additional gene copies exogenously



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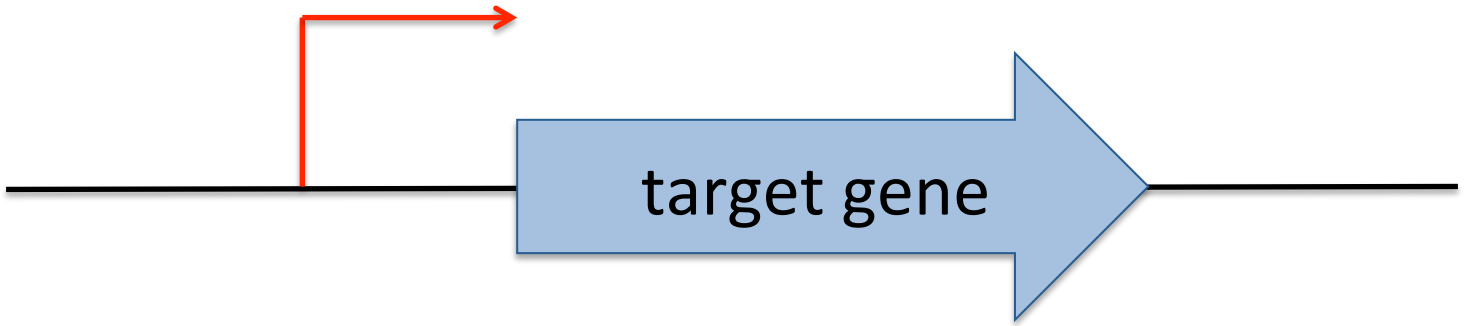

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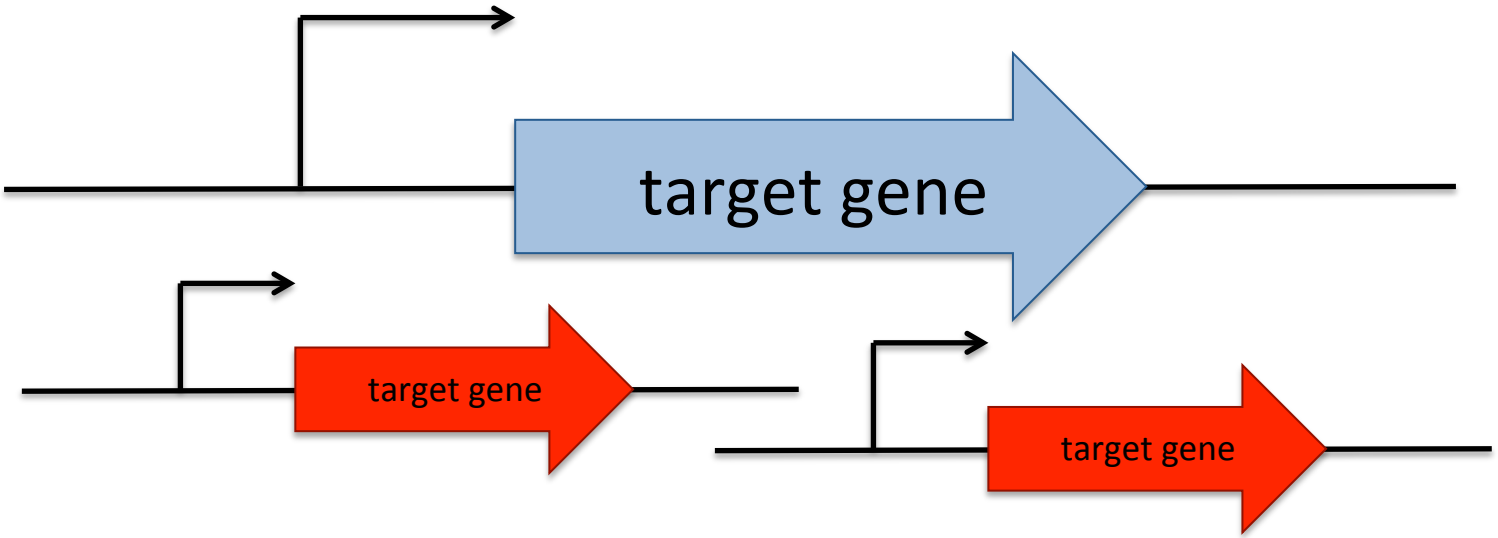
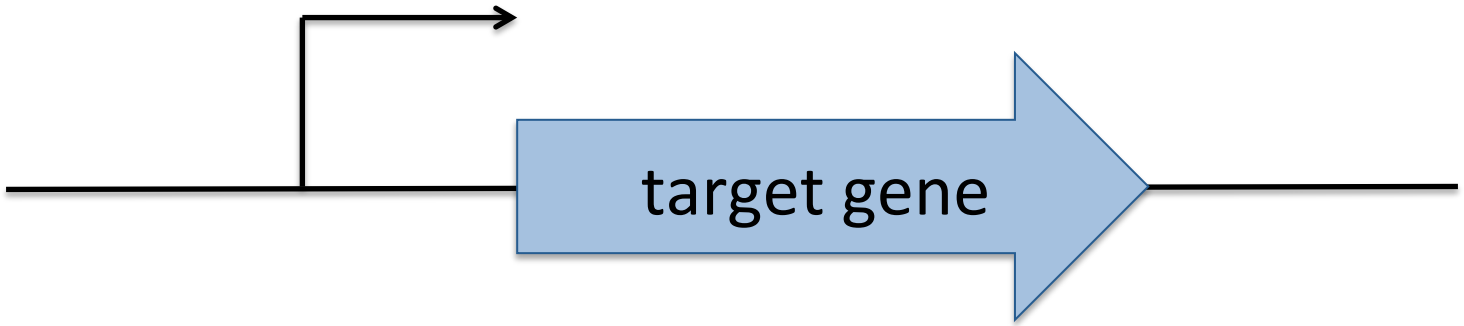


3. Delete gene

- Remove gene from genome

- Insert DNA fragment into gene



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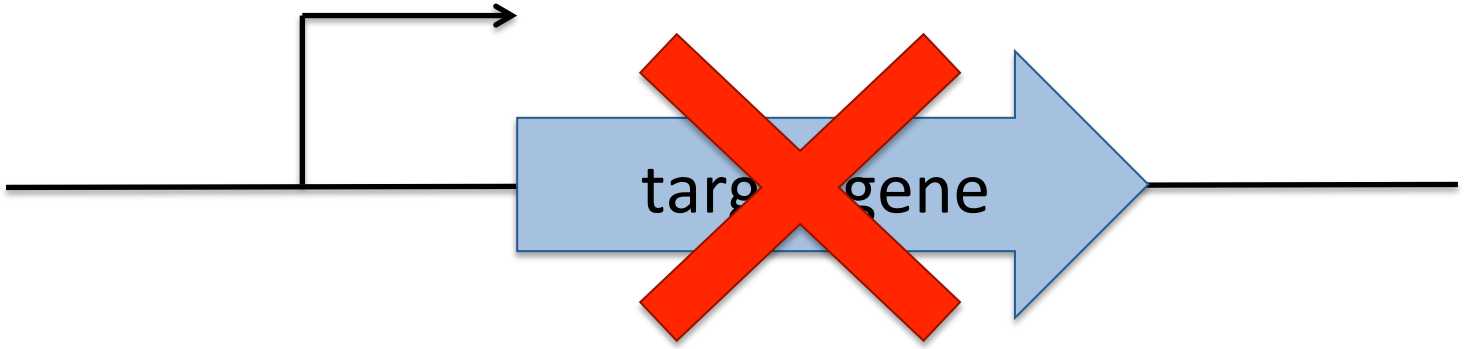

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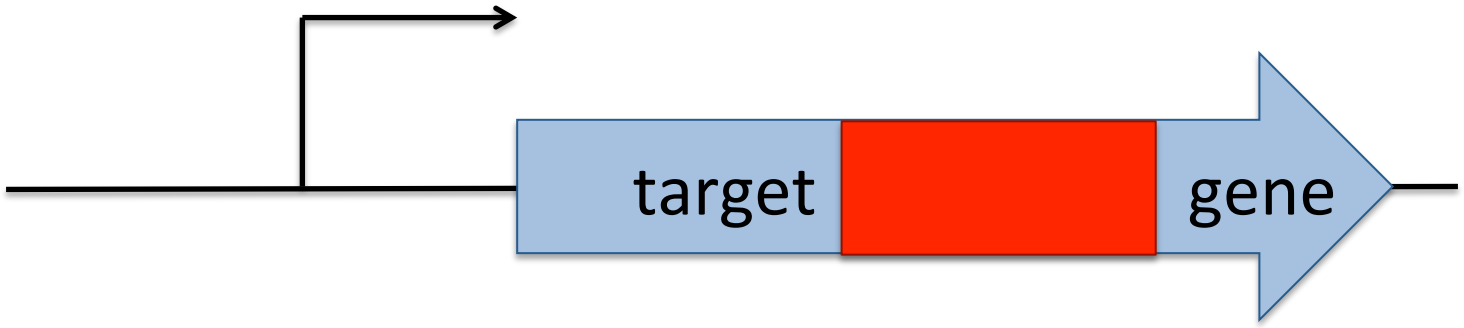
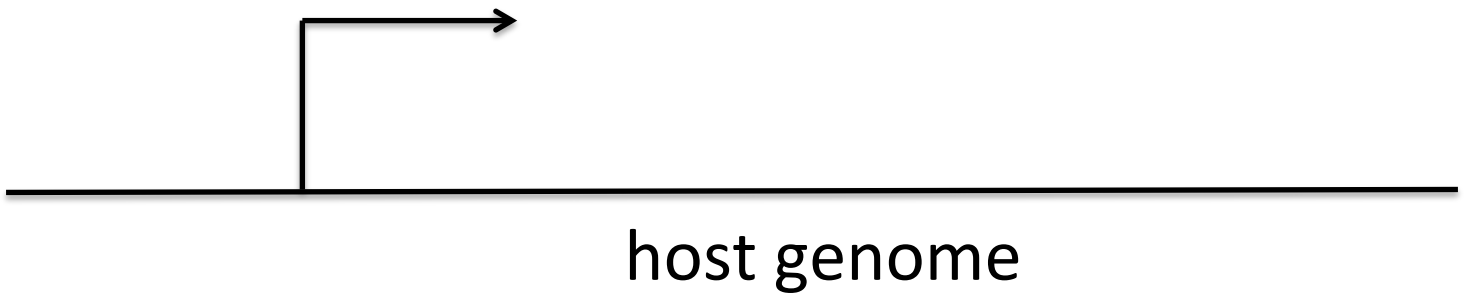


4. Add gene

- Insert non-native gene into host genome
- Express non-native gene exogenously



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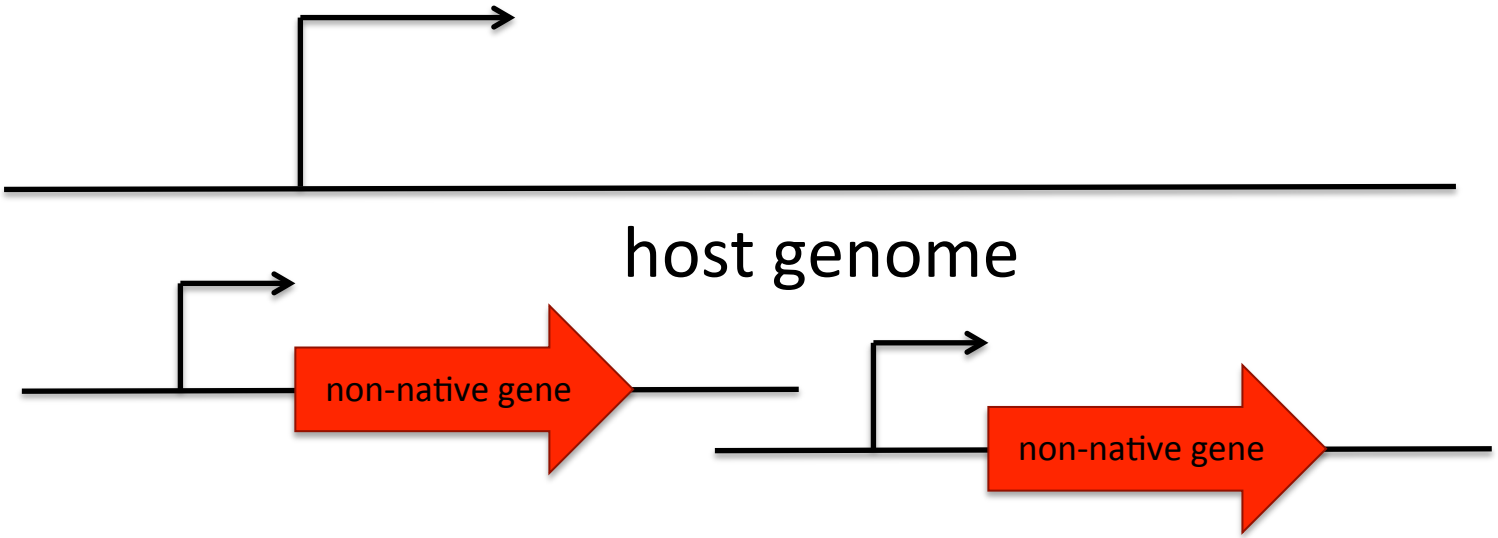
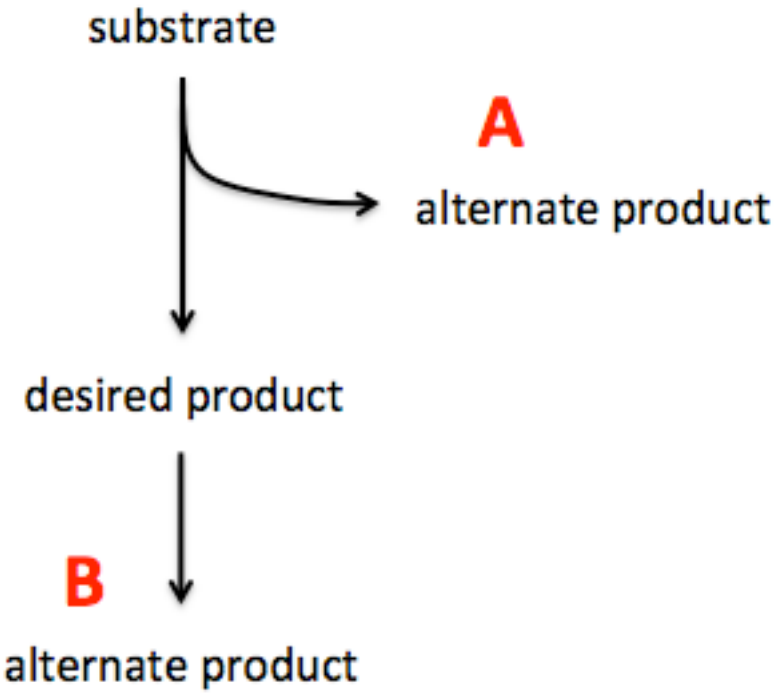


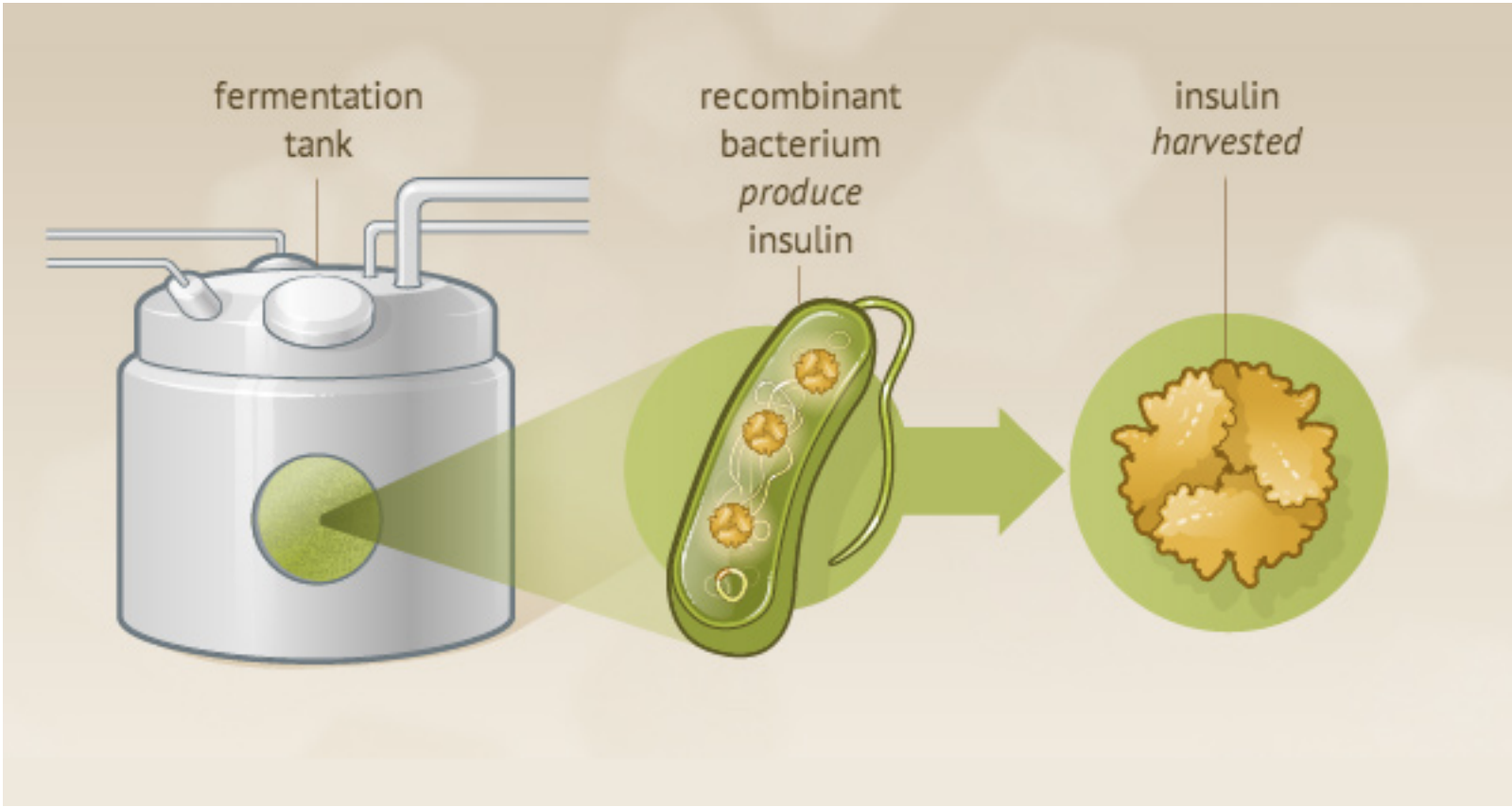
5. Mutate gene

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

How would you increase yield of the desired product?

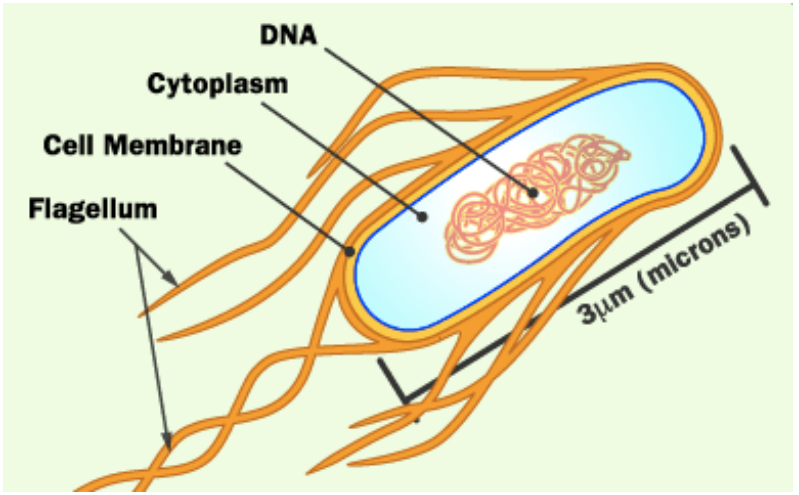


Metabolically engineered pathways are expressed in host organisms



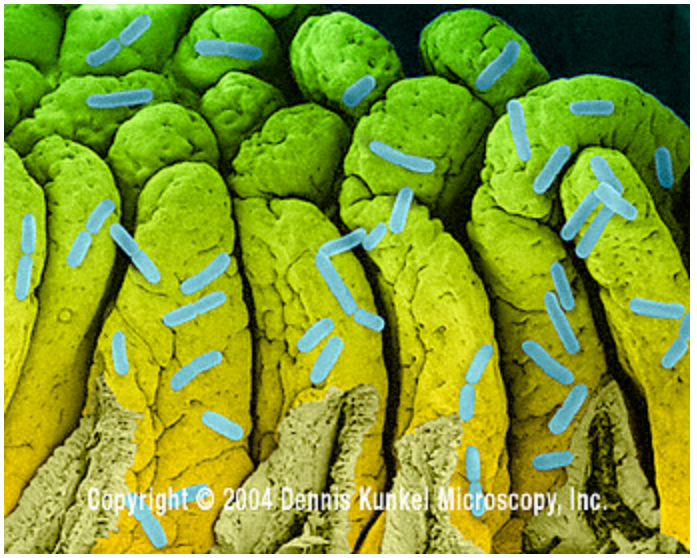
E. coli overview

- Gram negative
- Rod-shaped
- Native inhabitant of lower intestine in warm-blooded mammals
  - Certain serotypes cause disease



E. coli is a facultative anaerobe

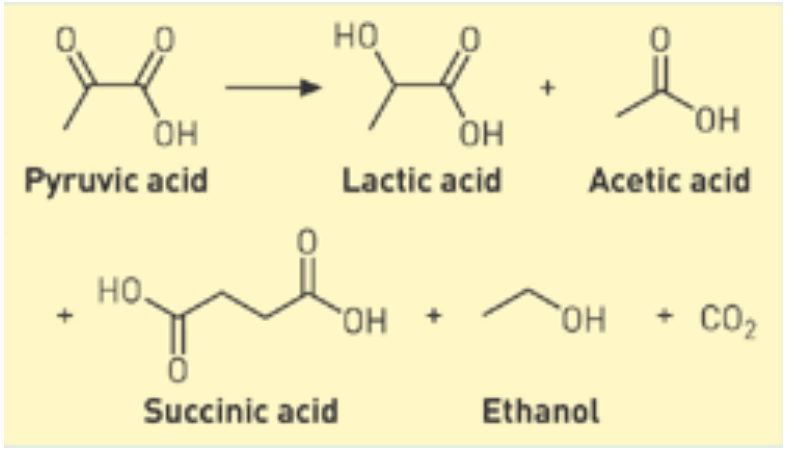
- Growth 'in nature' occurs in absence of oxygen
  - Adheres to mucous and epithelium of intestinal wall
  - Accounts for up to 1% of bacteria in the GI tract
  - Prevents colonization by pathogenic organisms



- In absence of oxygen, completes anaerobic respiration or fermentation

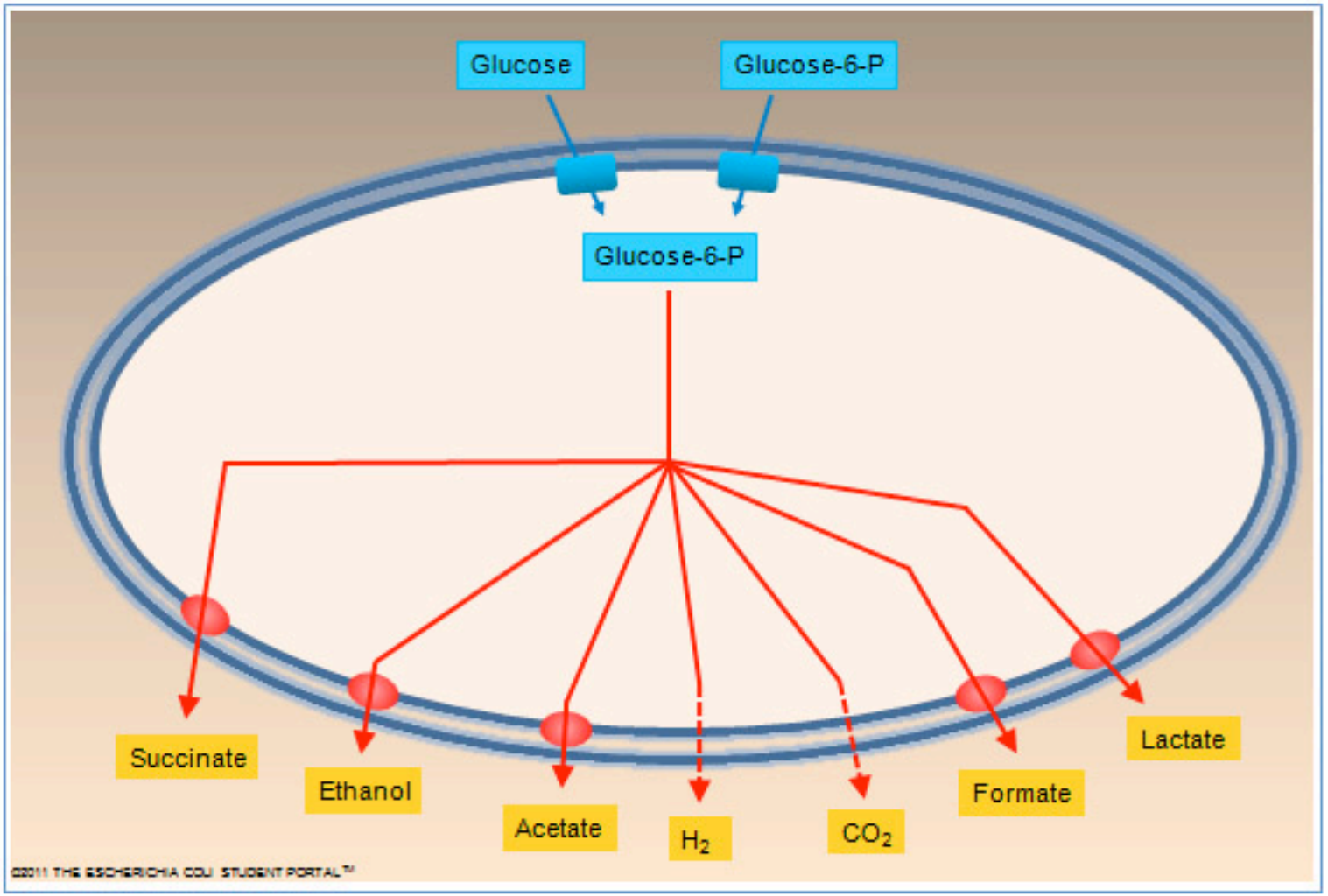
Anaerobic metabolism in *E. coli*

- Anaerobic respiration coupled to non-O_2 electron acceptor
  - Nitrate, trimethylamine oxide, and fumarate
- Fermentation



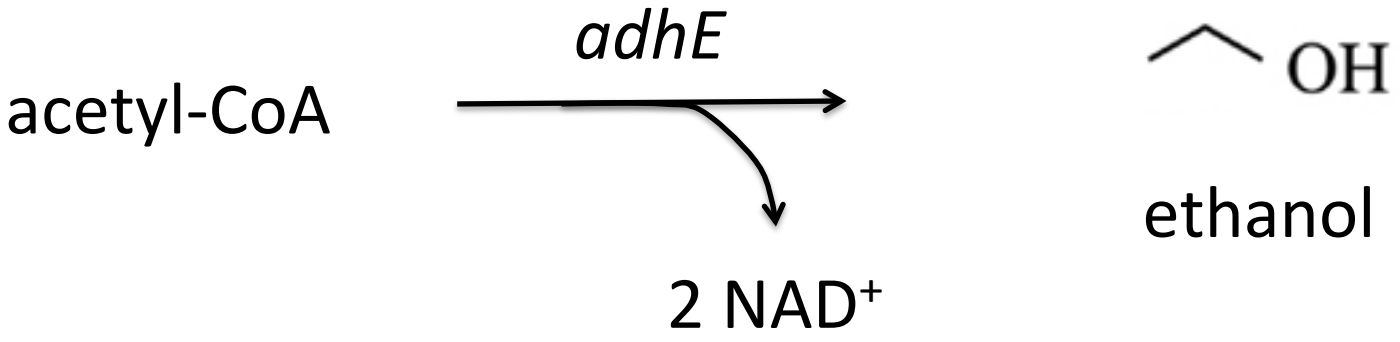
Why use *E. coli* to express products in metabolic engineering?

E. coli naturally produces commercially relevant products



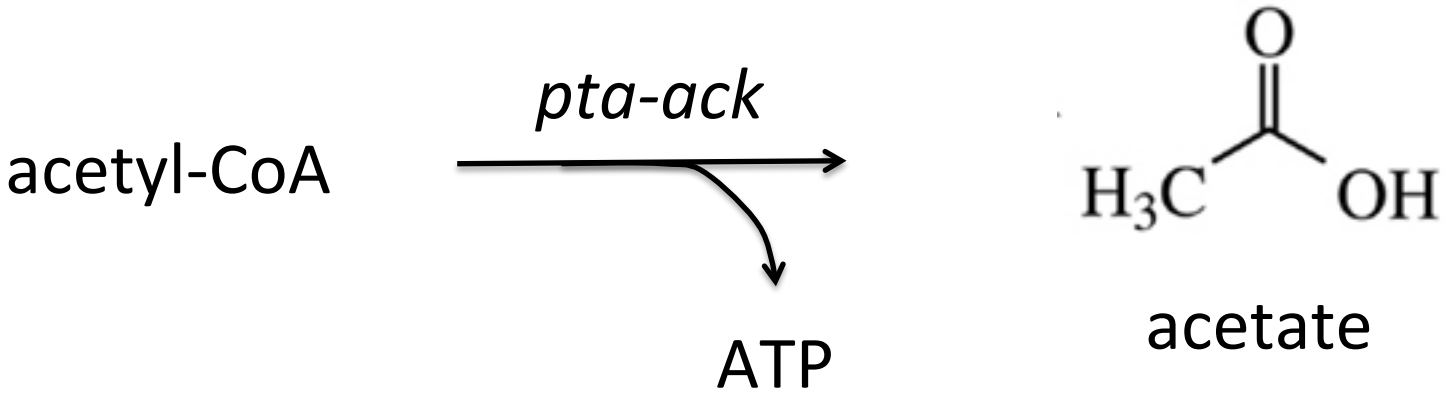
Production of ethanol

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



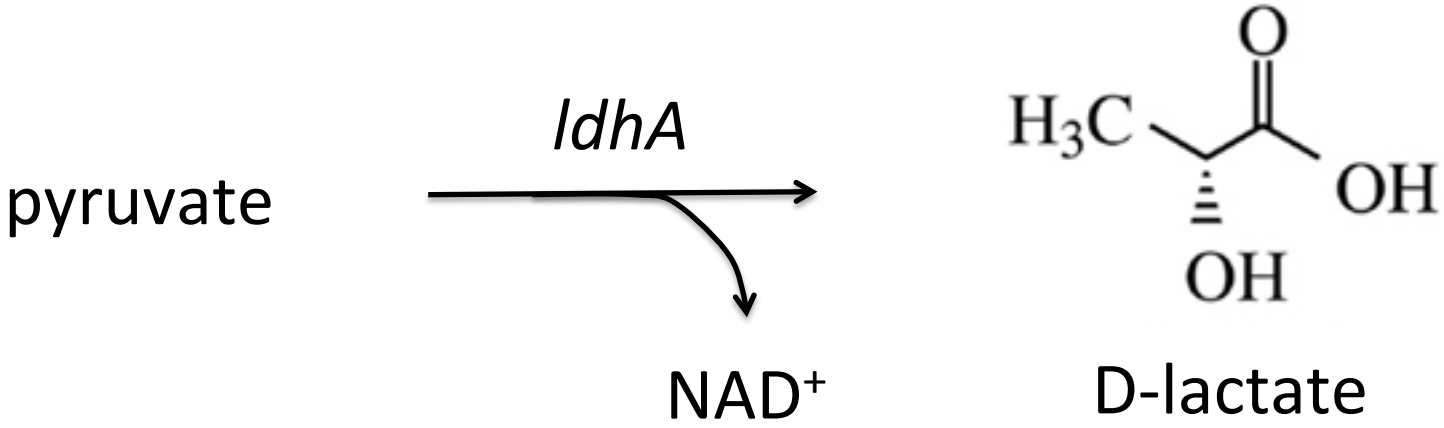
Production of acetate

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

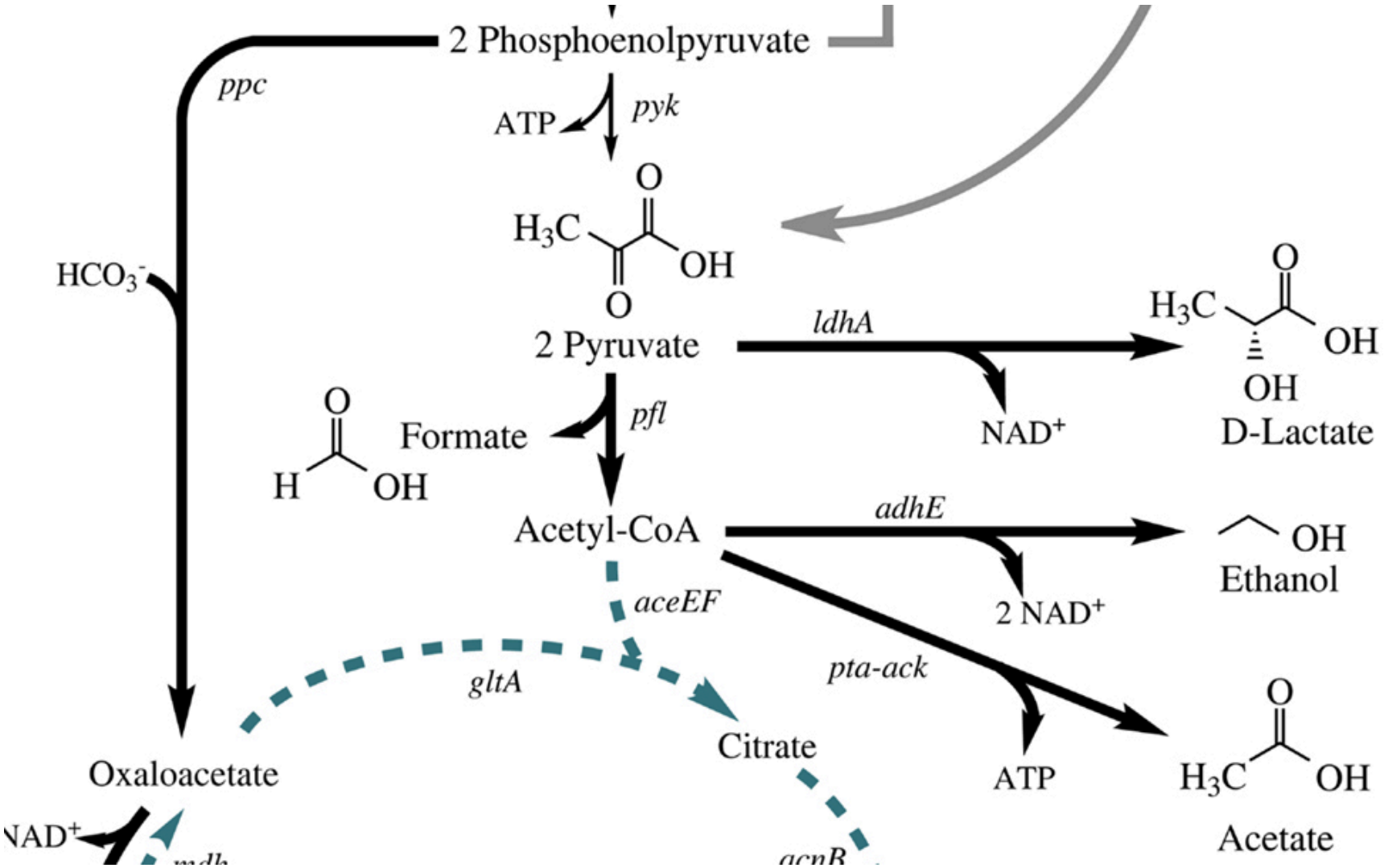


Production of lactate

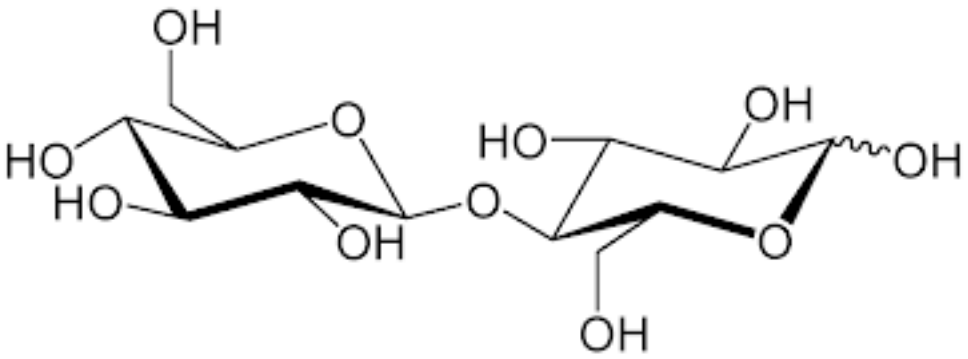
- Lactate is used in production of polymers, pharmaceuticals, and cosmetics
- *ldhA* expressed constitutively
  - Level increased 5 to 10-fold in anaerobic conditions



A closer look at fermentation pathway



The next step?



cellobiose, formed during cellulose hydrolysis

molasses, formed during cane sugar and beet sugar refining

Conversion of byproducts into commercially valuable commodities

In the laboratory…

1. Research *E. coli* fermentation pathway
   - Select a gene such that ethanol or lactate production are increased when expression of gene is decreased
2. Design gRNA target sequence
   - Target selected gene such that transcription is decreased using CRISPRi system
3. For M2D3:

CRISPR Perturbation of Gene Expression Alters Bacterial Fitness under Stress and Reveals Underlying Epistatic Constraints

Peter B. Otoupal,[†] Keesha E. Erickson,[†] Antoni Escalas-Bordoy,[†] and Anushree Chatterjee[*,†,‡]