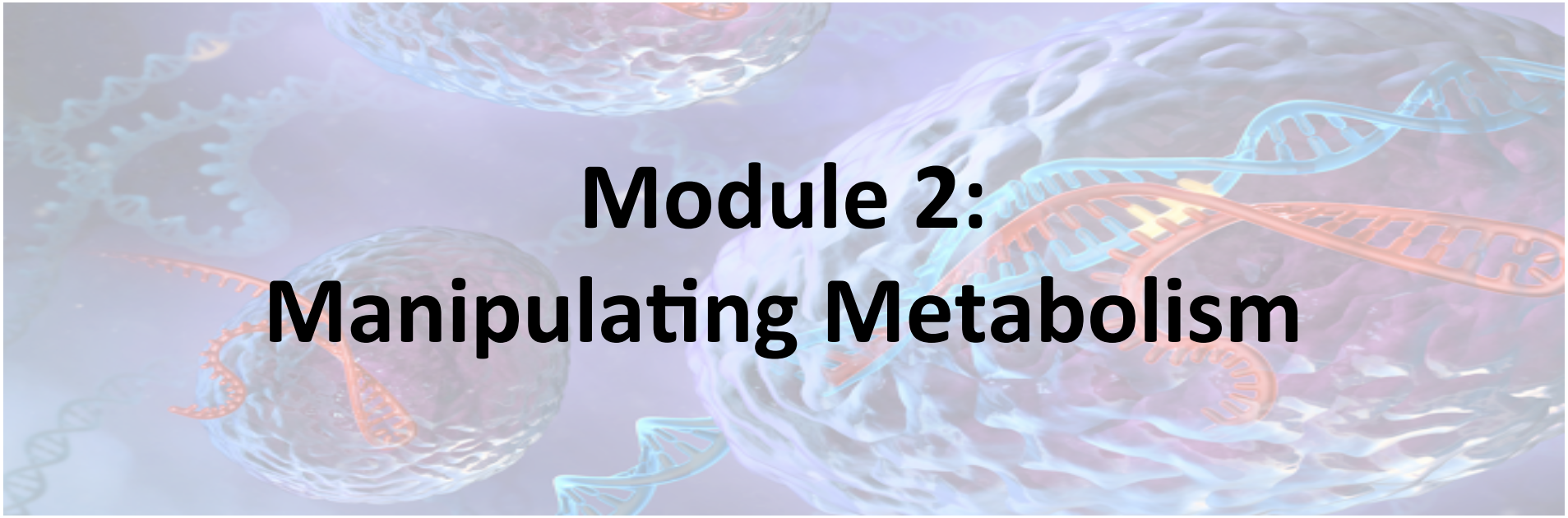
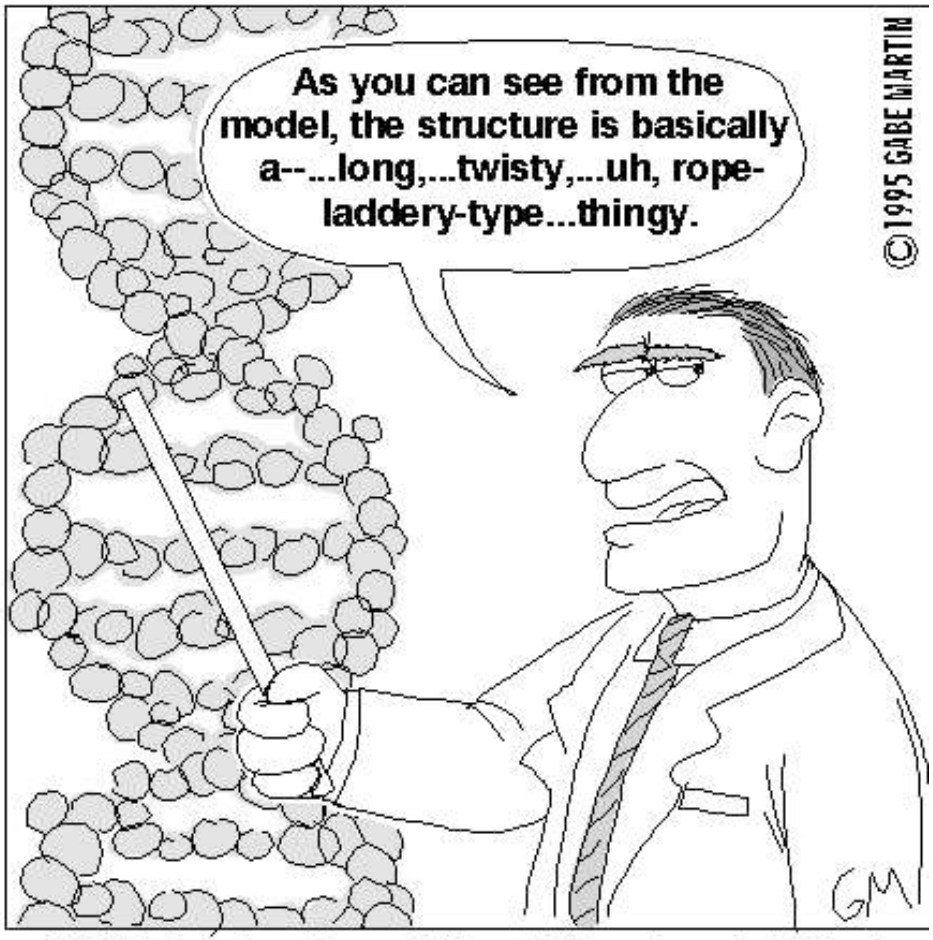


Module 2: Manipulating Metabolism

Metabolic engineering

10/17/17

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."

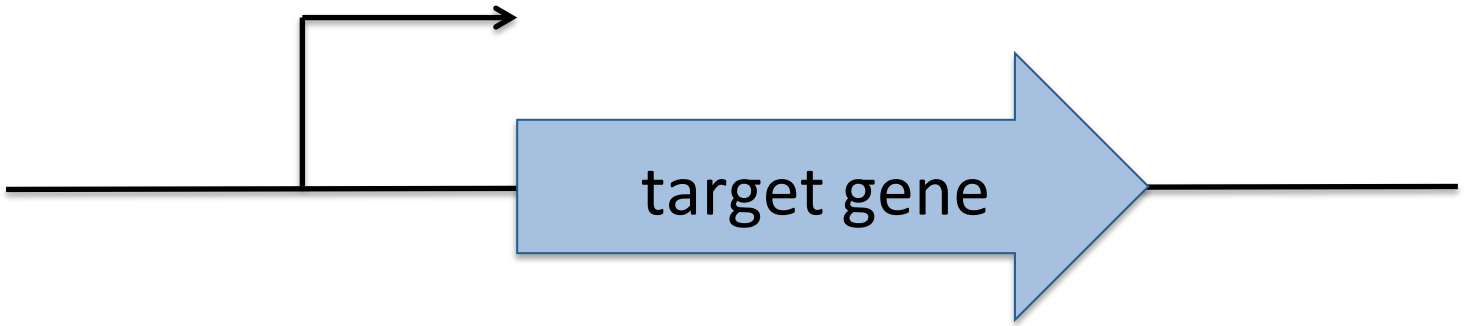
Metabolic engineering ‘toolkit’

- 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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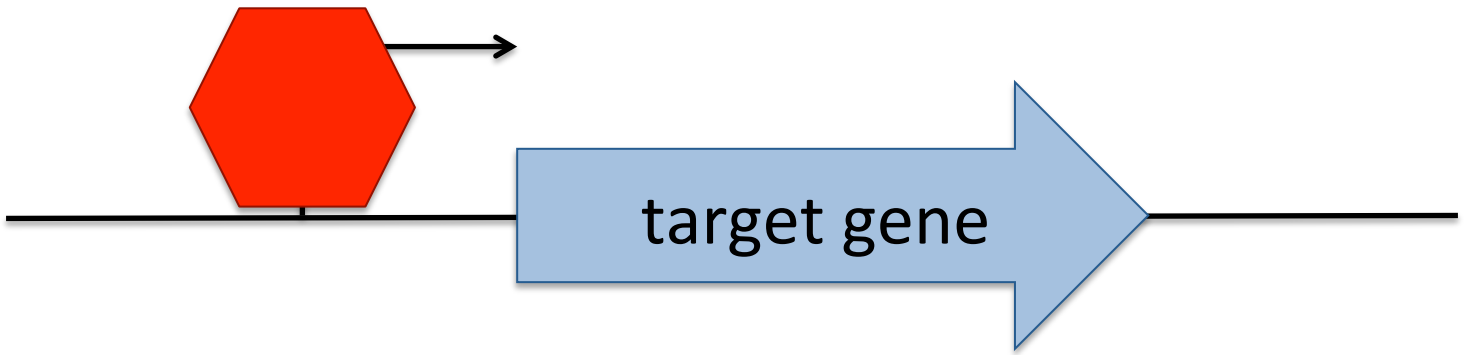

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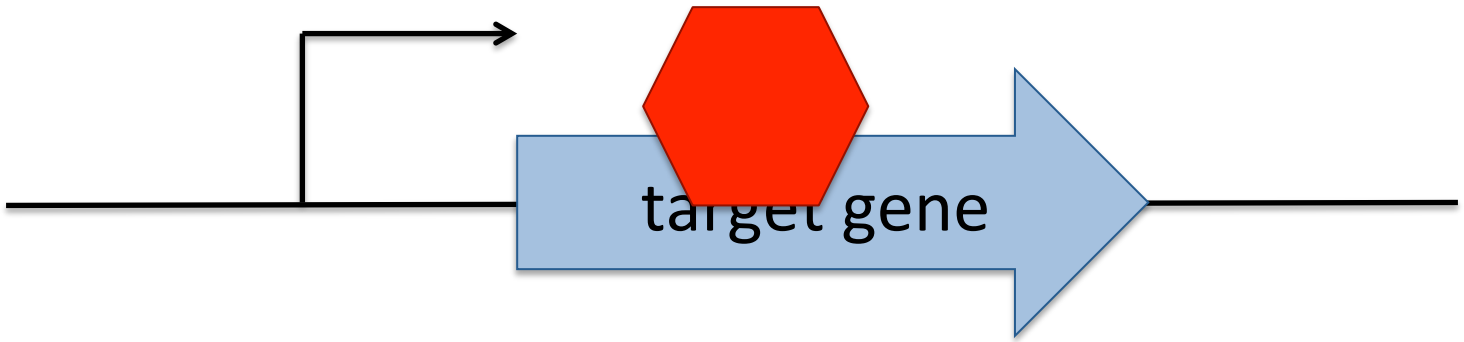
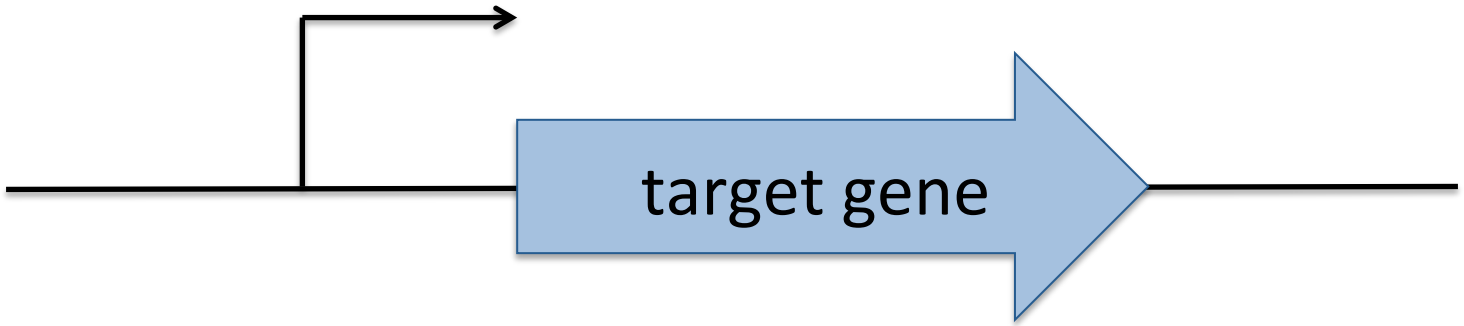


2. Overexpress gene

- Replace native promoter with one that is constitutively active

- Express additional gene copies exogenously



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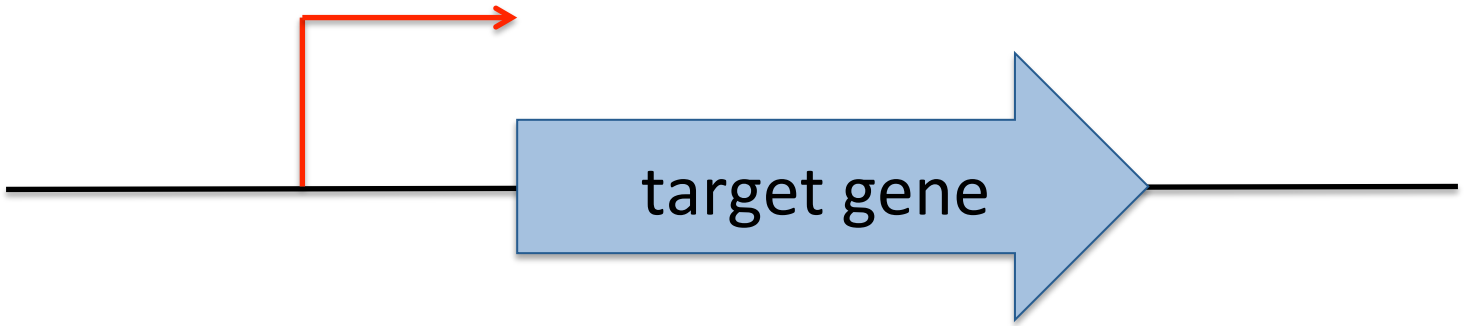

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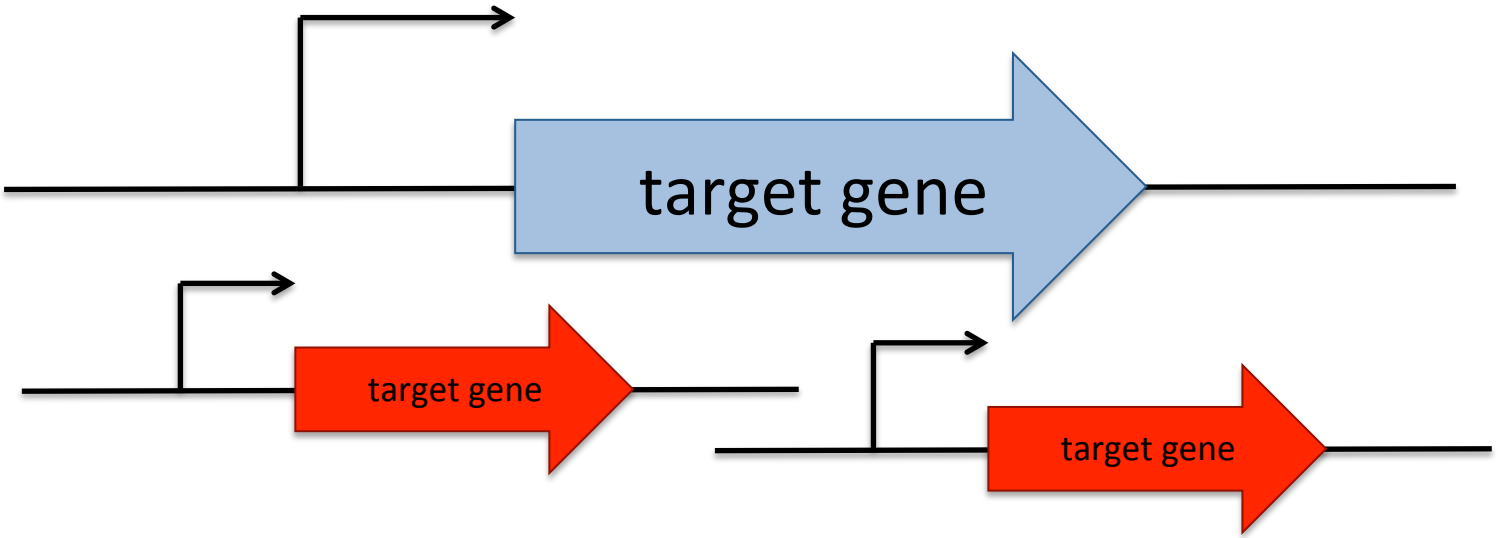
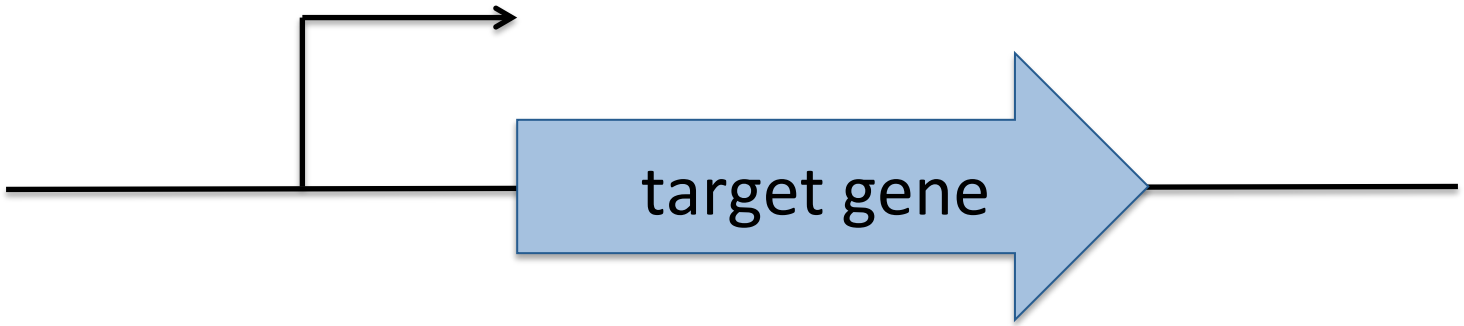


3. Delete gene

- Remove gene from genome

- Insert DNA fragment into gene



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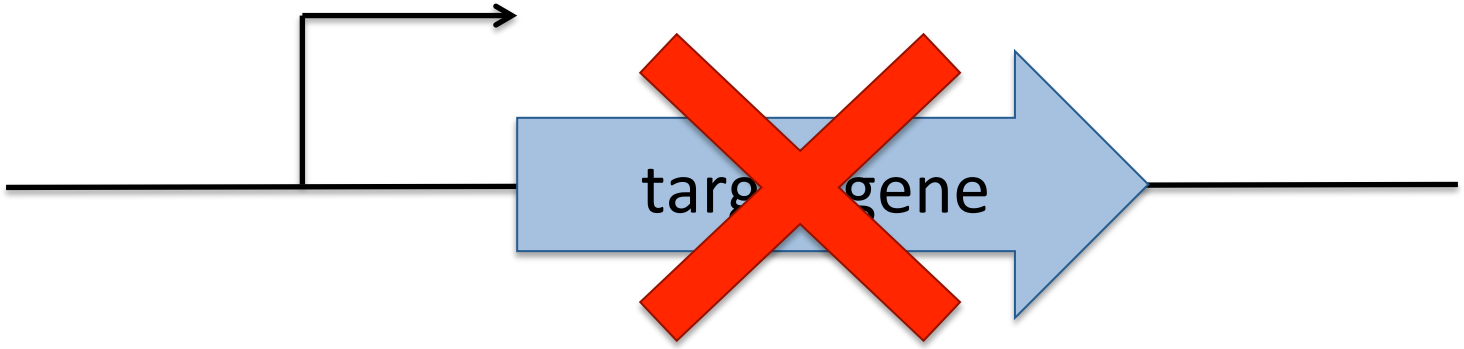

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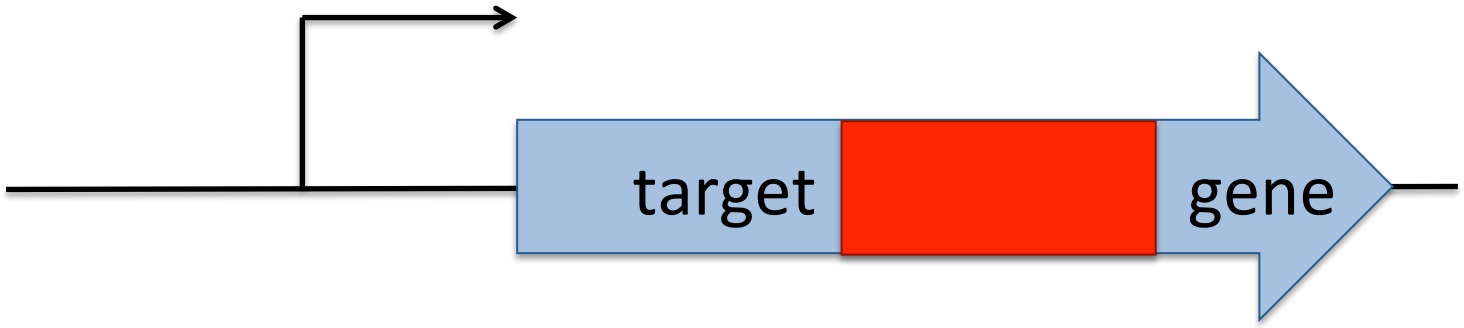
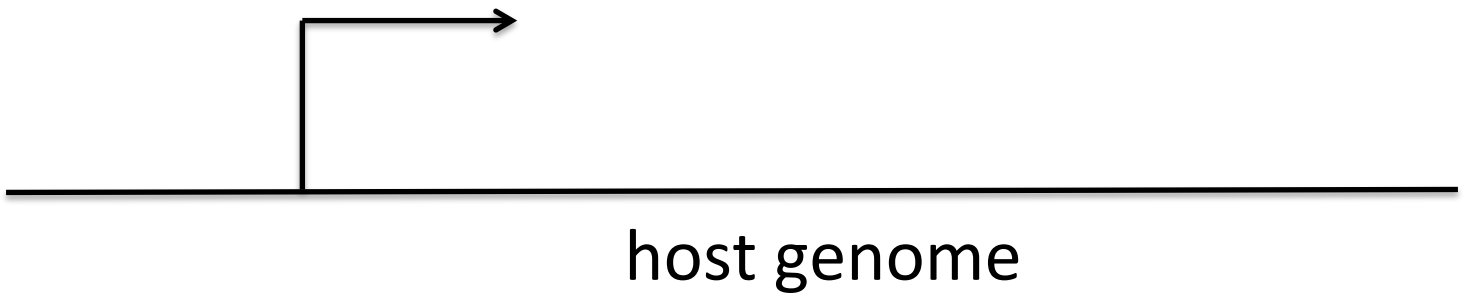


4. Add gene

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



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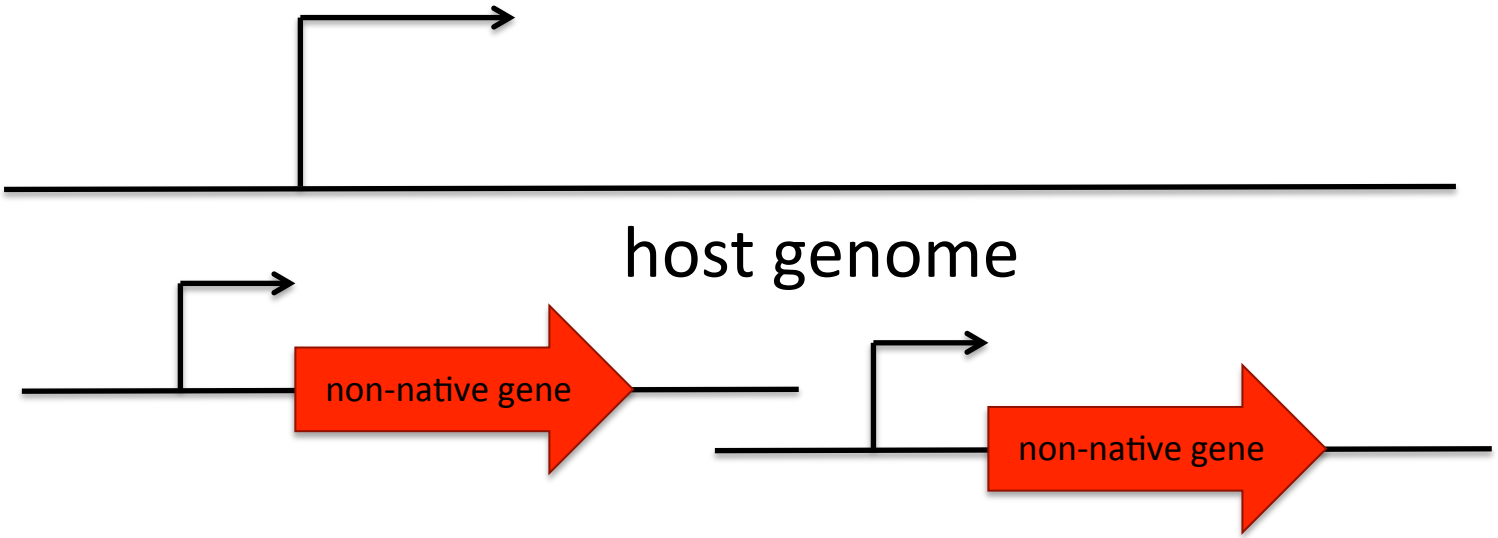
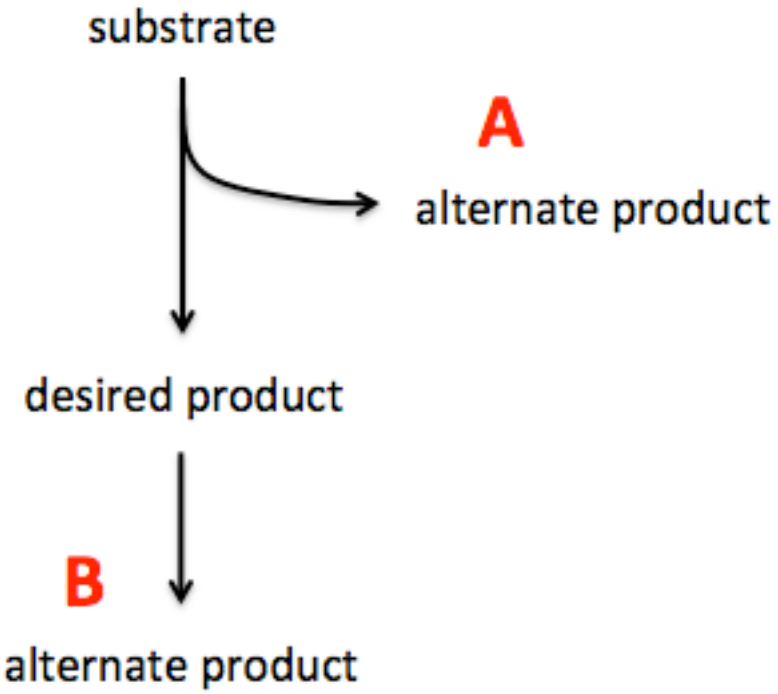


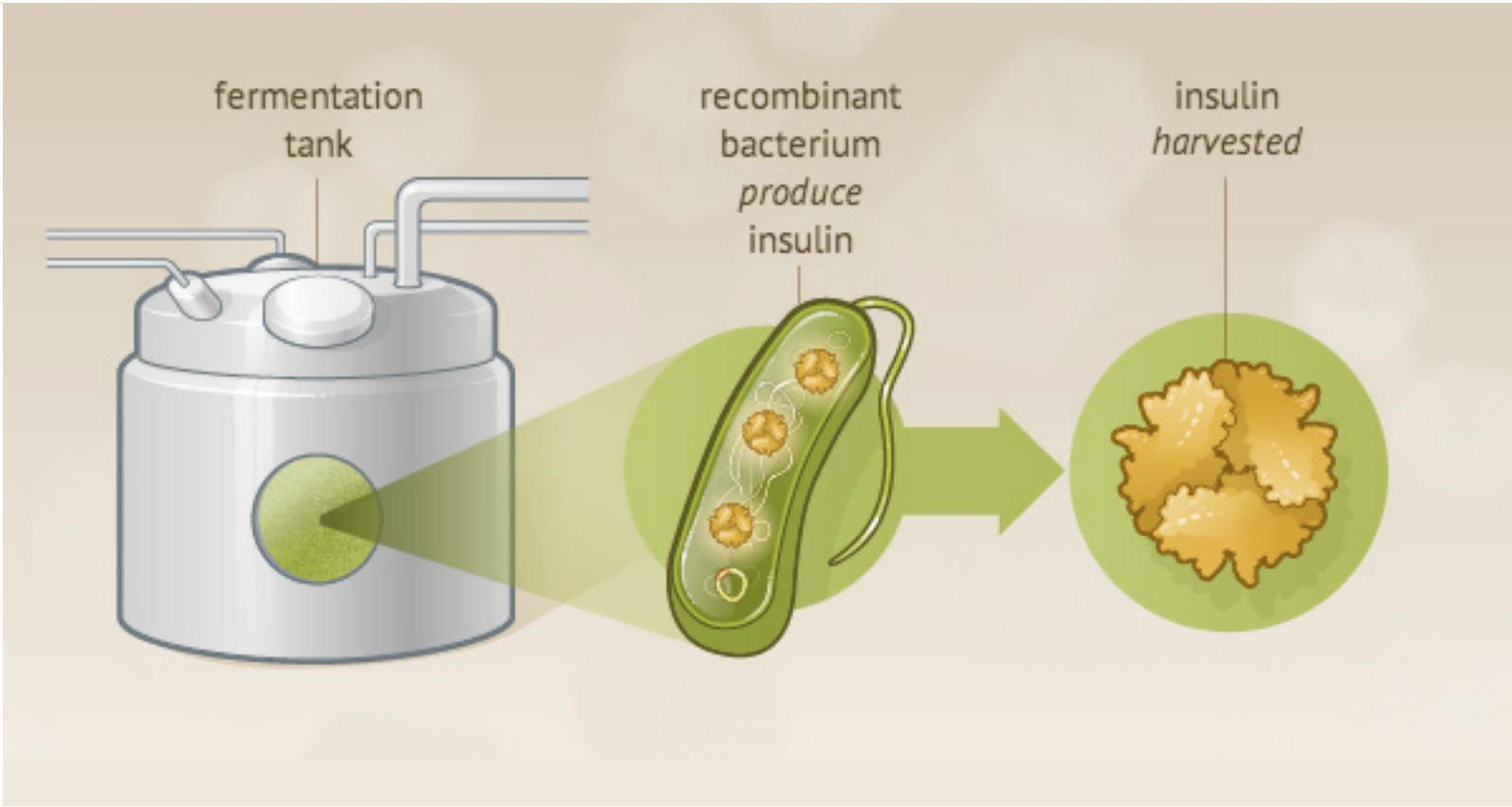
5. Mutate gene

- 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?



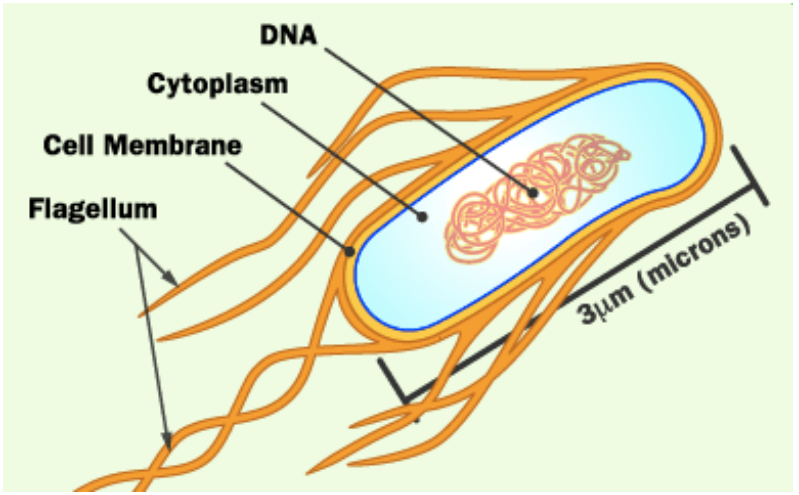
Metabolically engineered pathways are expressed in host organisms



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

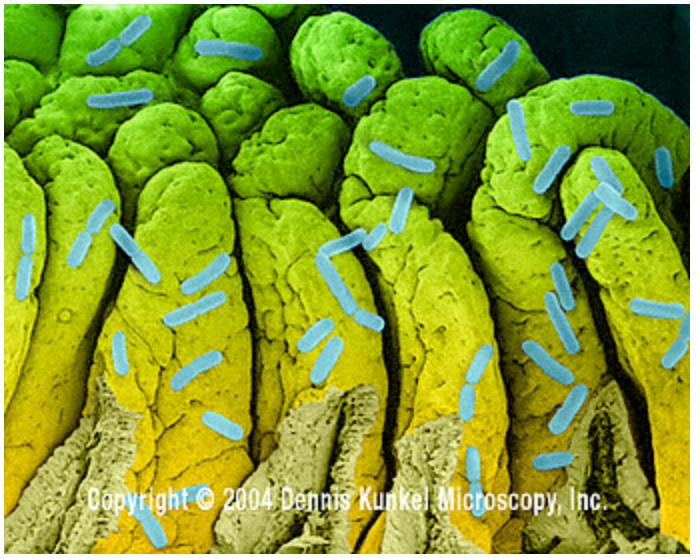
E. coli overview

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



E. coli is a facultative anaerobe

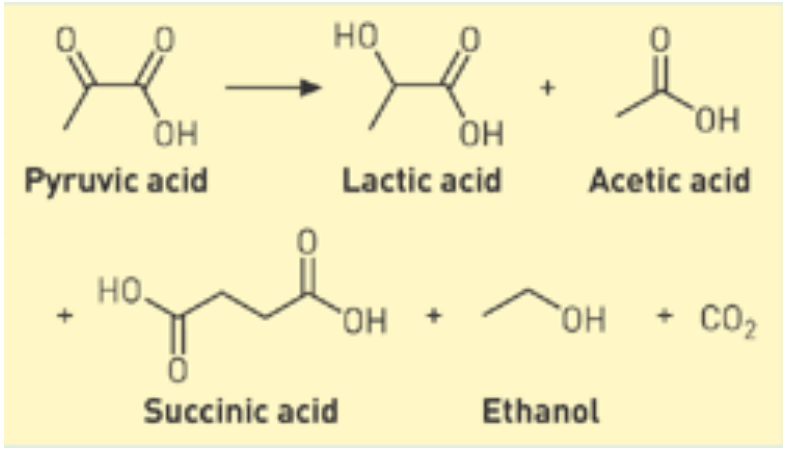
- Growth 'in nature' occurs in absence of oxygen
  - Adheres to mucous and epithelium of intestinal wall
  - Accounts for up to 1% of bacteria in the GI tract
  - Prevents colonization by pathogenic organisms



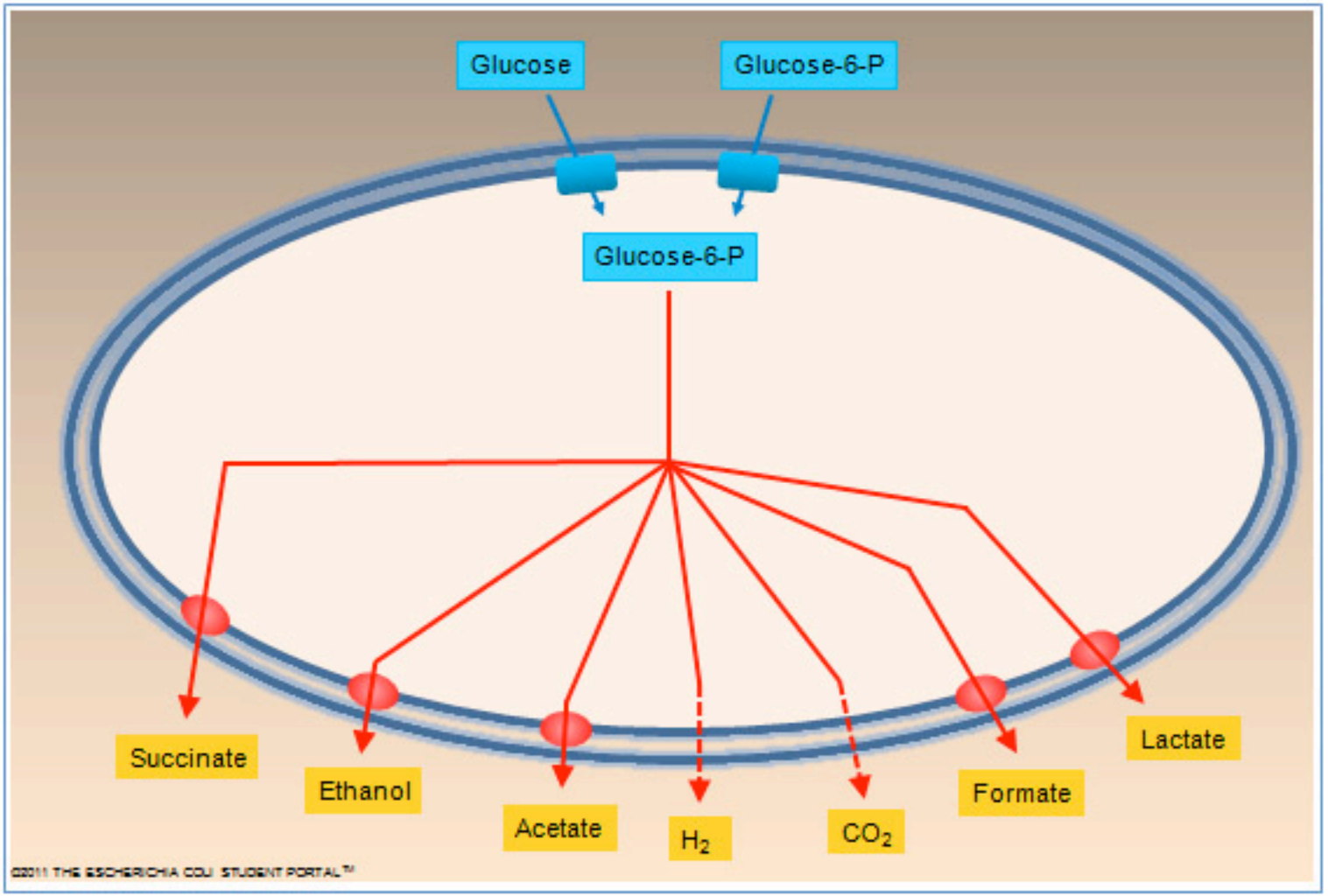
- In absence of oxygen, completes anaerobic respiration or fermentation

Anaerobic metabolism in *E. coli*

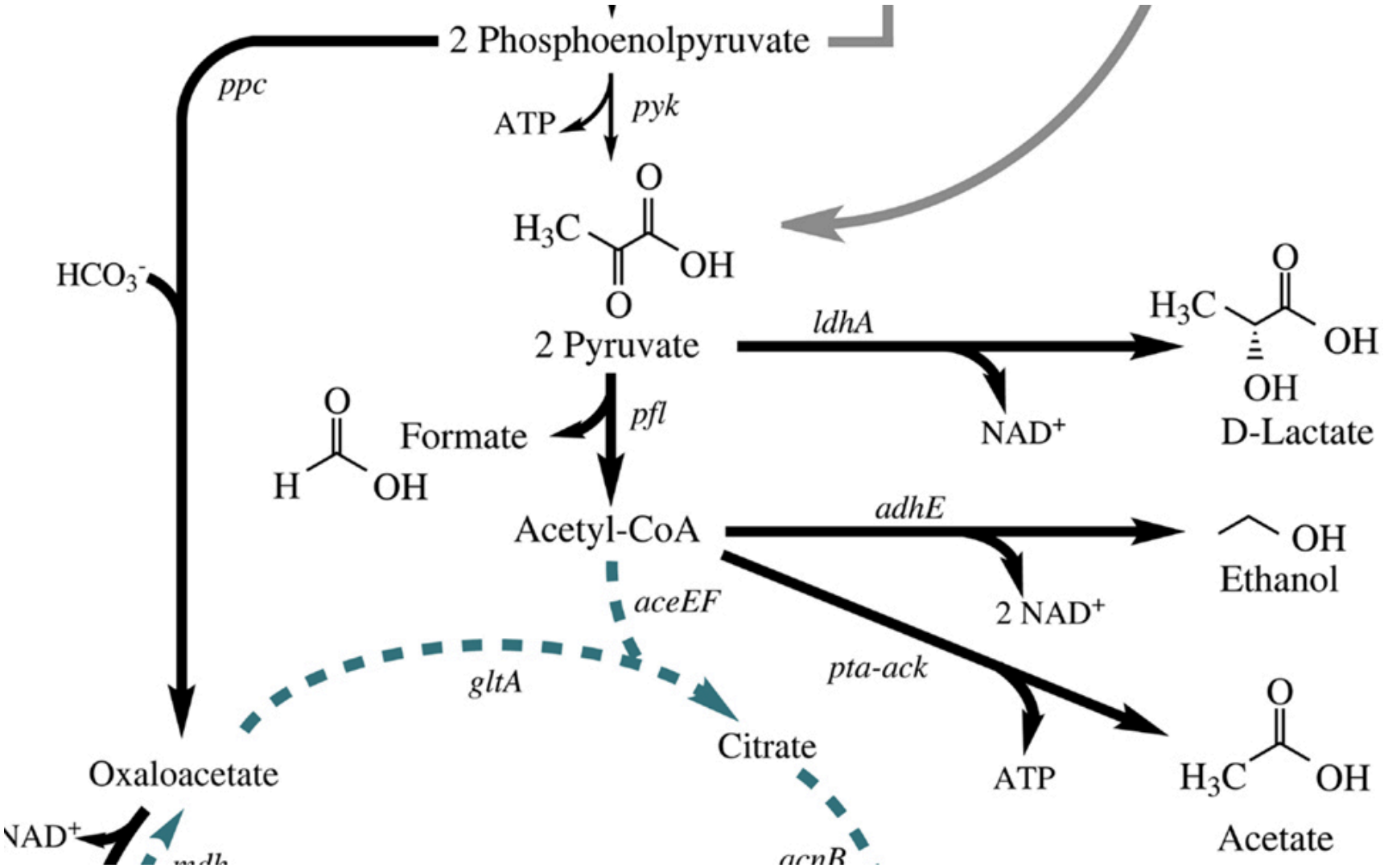
- Anaerobic respiration coupled to non-O_2 electron acceptor
  - Nitrate, trimethylamine oxide, and fumarate
- Fermentation



E. coli naturally produces commercially relevant products

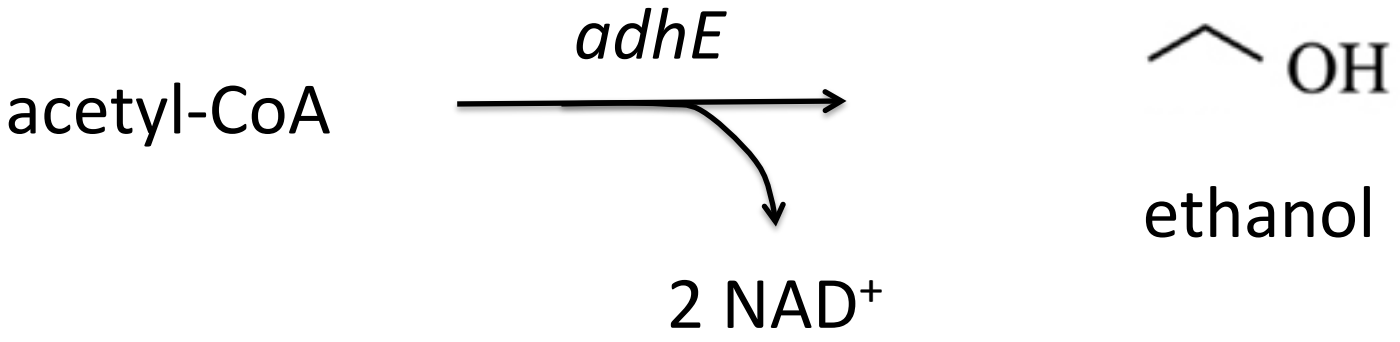


A closer look at fermentation pathway



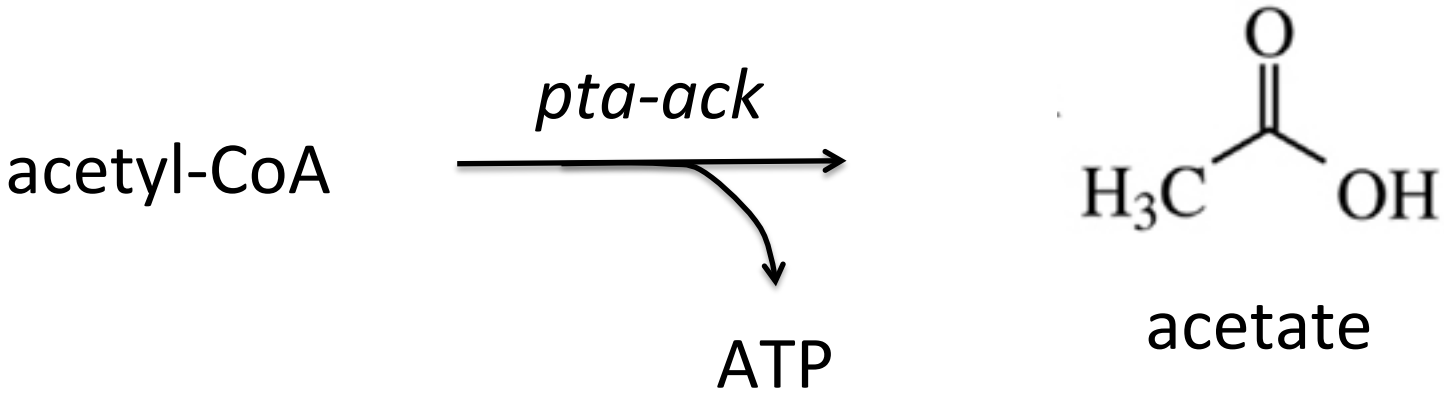
Production of ethanol

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

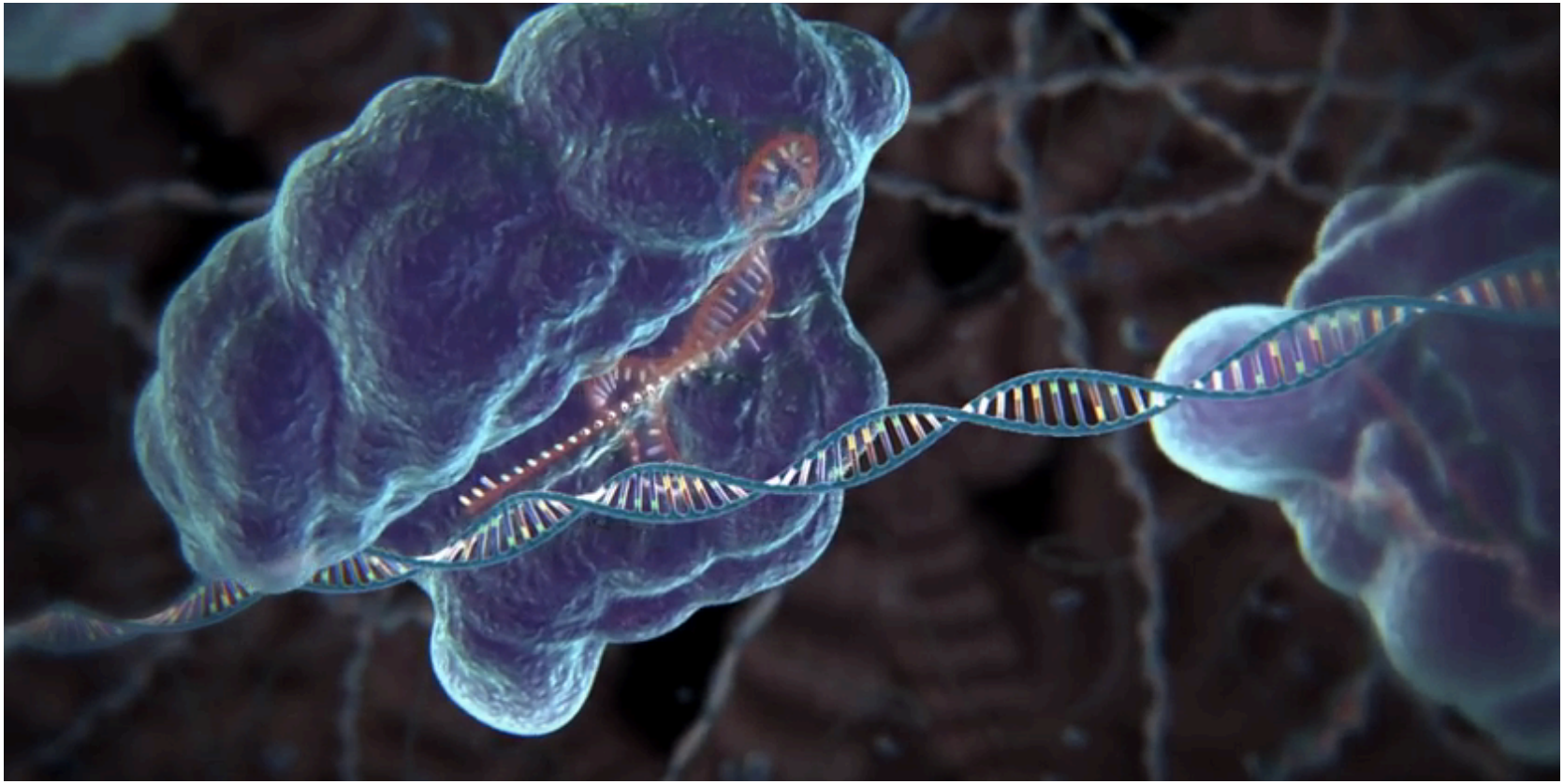


Production of acetate

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

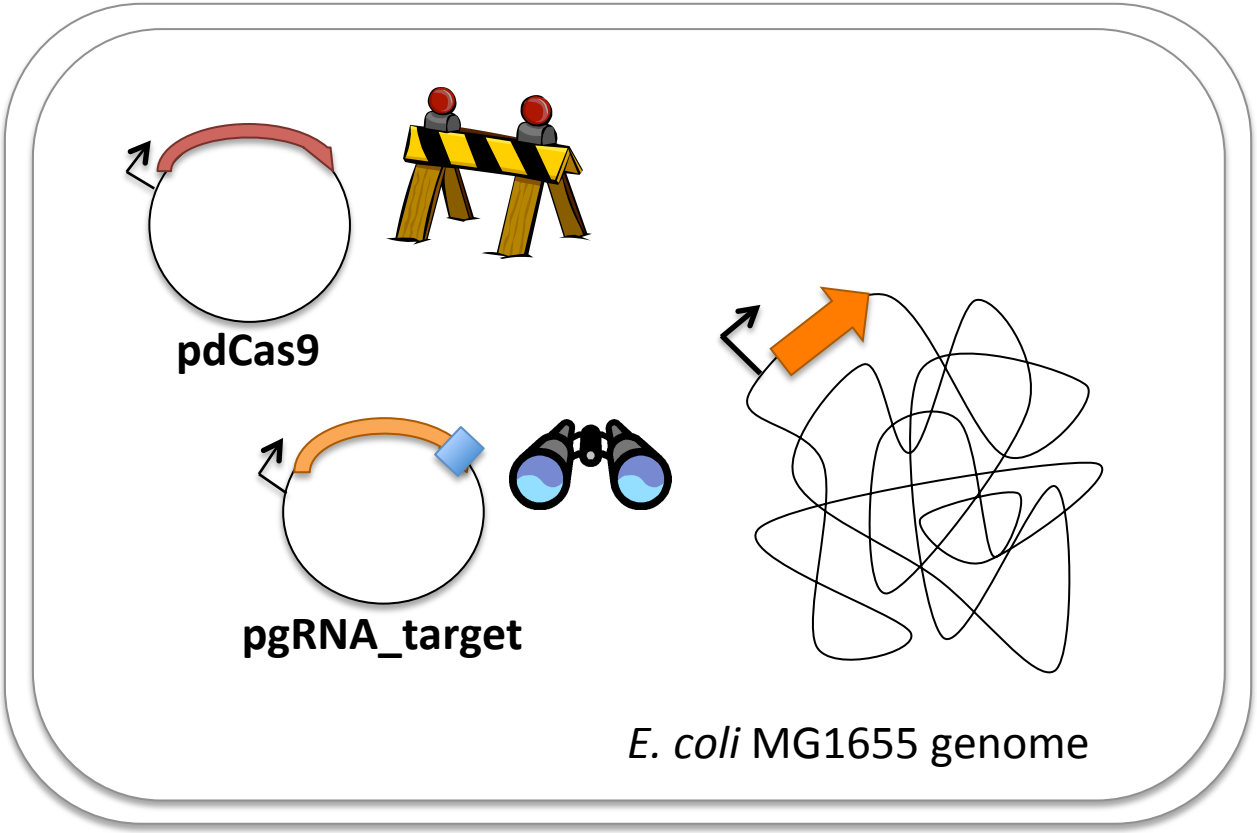


How will we alter fermentation?



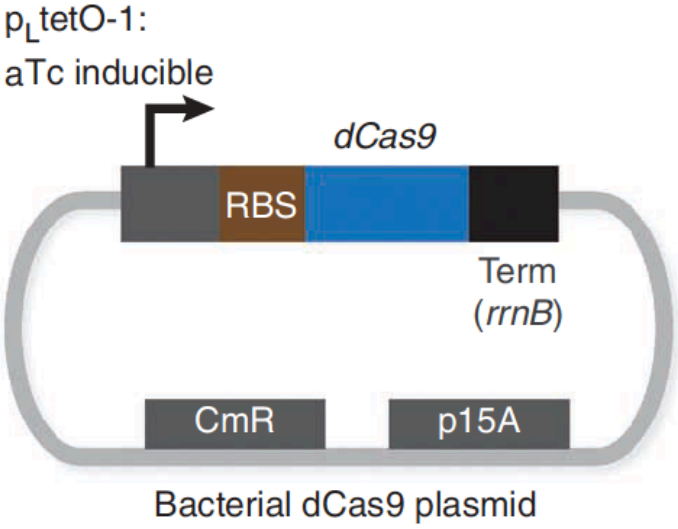
https://www.youtube.com/watch?v=2pp17E4E-O8

CRISPRi system overview



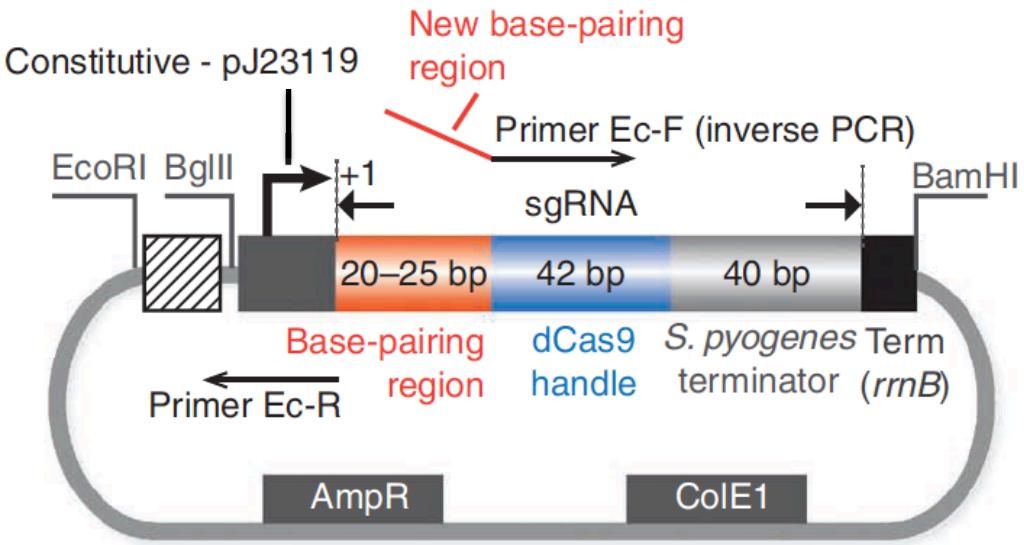
- Target gene
- pgRNA_target
- pdCas9

Closer look at pgRNA and pdCas9



Bacterial dCas9 plasmid

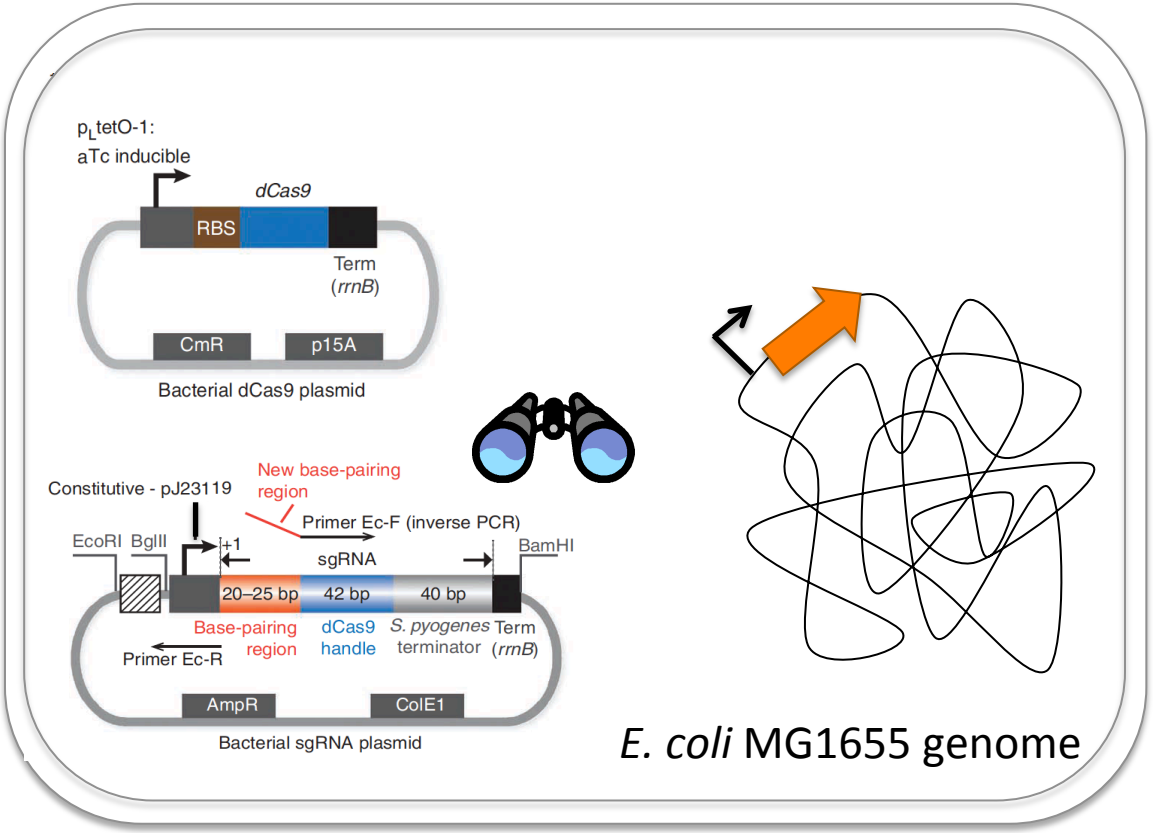
- Confirmation digest prepared on M2D1



Bacterial sgRNA plasmid

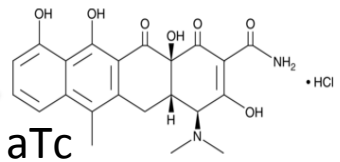
- Insert (gRNA target sequence) designed on M2D2

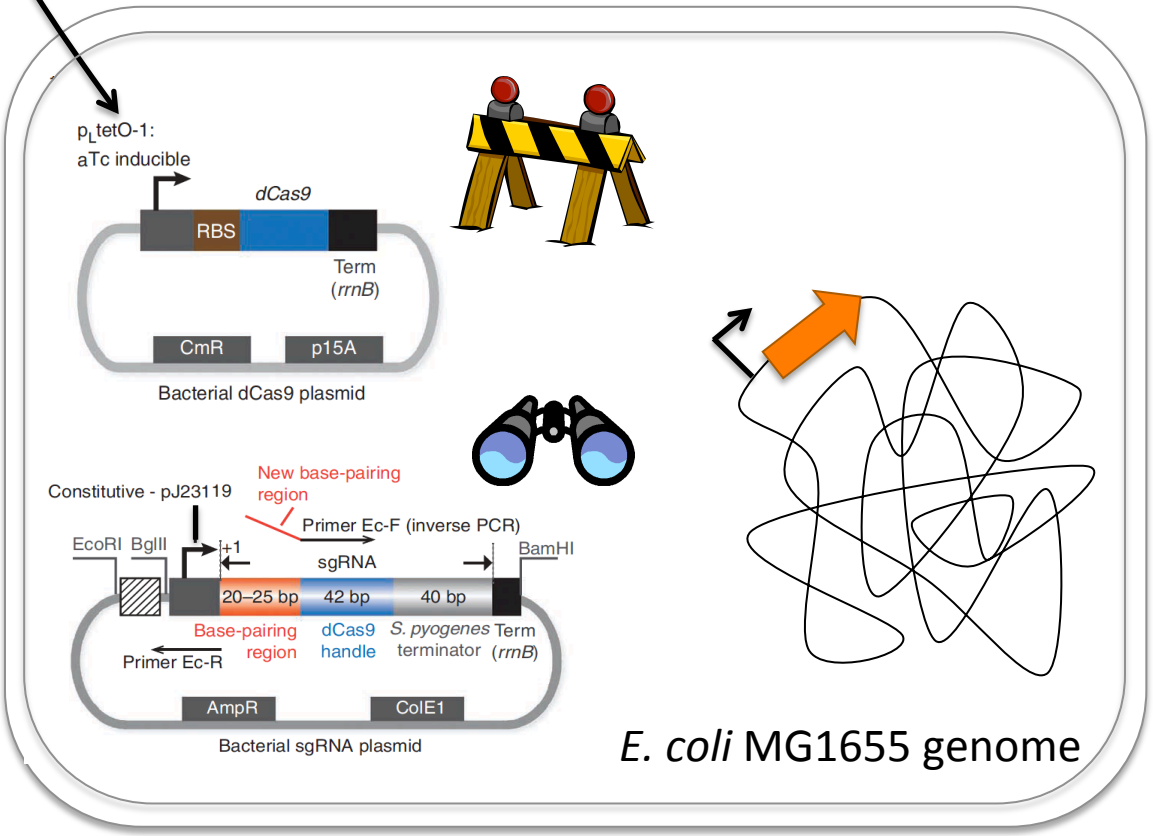
CRISPRi 'inactive' in absence of inducer



- pgRNA_target expressed constitutively
  - Always transcribed and binding to target gene

CRISPRi ‘blocks’ gene expression in presence of inducer





- pdCas9 expressed when aTc added
  - When transcribed associates with pgRNA_target / target gene

Stupidity in research

"…we don't do a good enough job of teaching our students how to be productively stupid… Productive stupidity means being ignorant by choice."

"One of the beautiful things about science is that is allows us to bumble along, getting it wrong time after time, and feel perfectly fine as long as we learn something each time."

In the laboratory…

1. Research *E. coli* fermentation pathway
   - Select a gene such that ethanol or acetate 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:

Multiple Gene Repression in Cyanobacteria Using CRISPRi

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