Module 2: Metabolic Engineering

Using CRISPRi to increase ethanol and acetate yield

What is your research goal?

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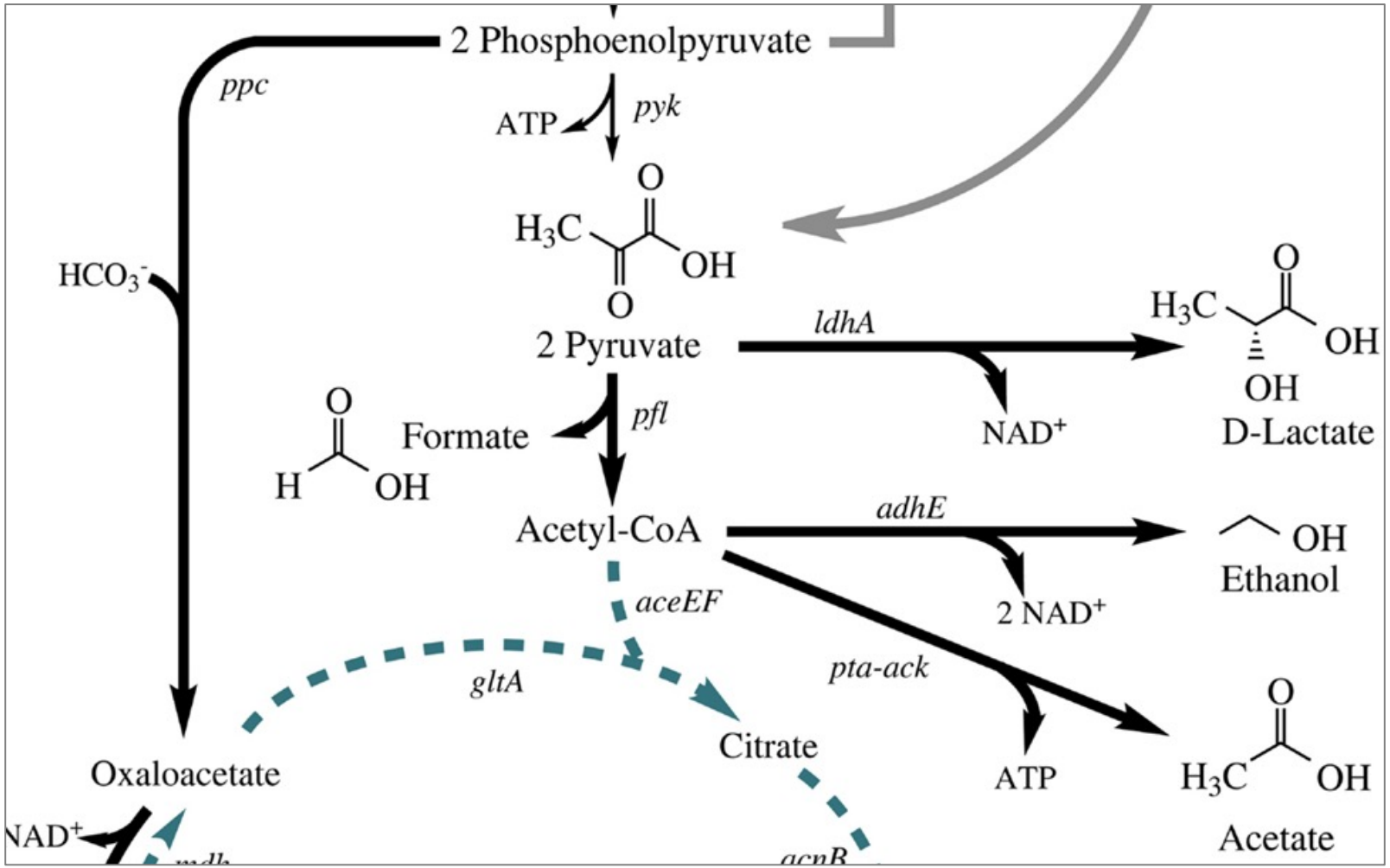
"Metabolic engineering is the use of genetic engineering to modify the metabolism of an organism. It can involve the optimization of existing biochemical pathways or the introduction of pathway components…with the goal of high-yield production of specific metabolites for medicine or biotechnology."

Why are we using this approach?

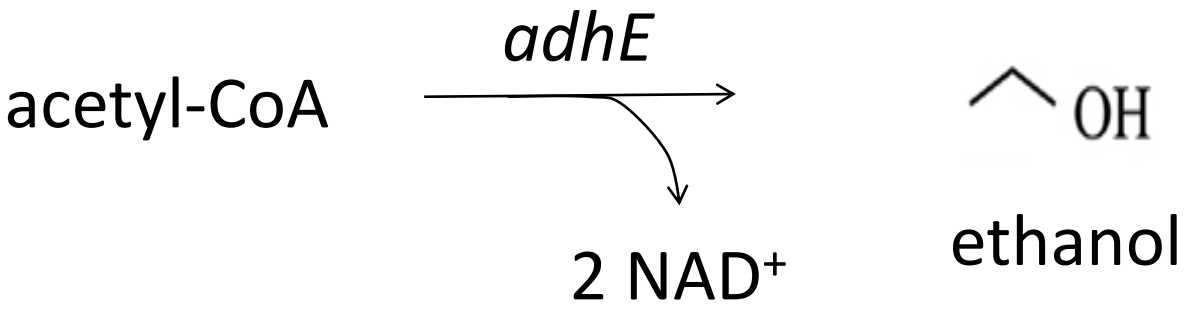
- Why ethanol and acetate?
- Why *E. coli*?
- Why CRISPRi?

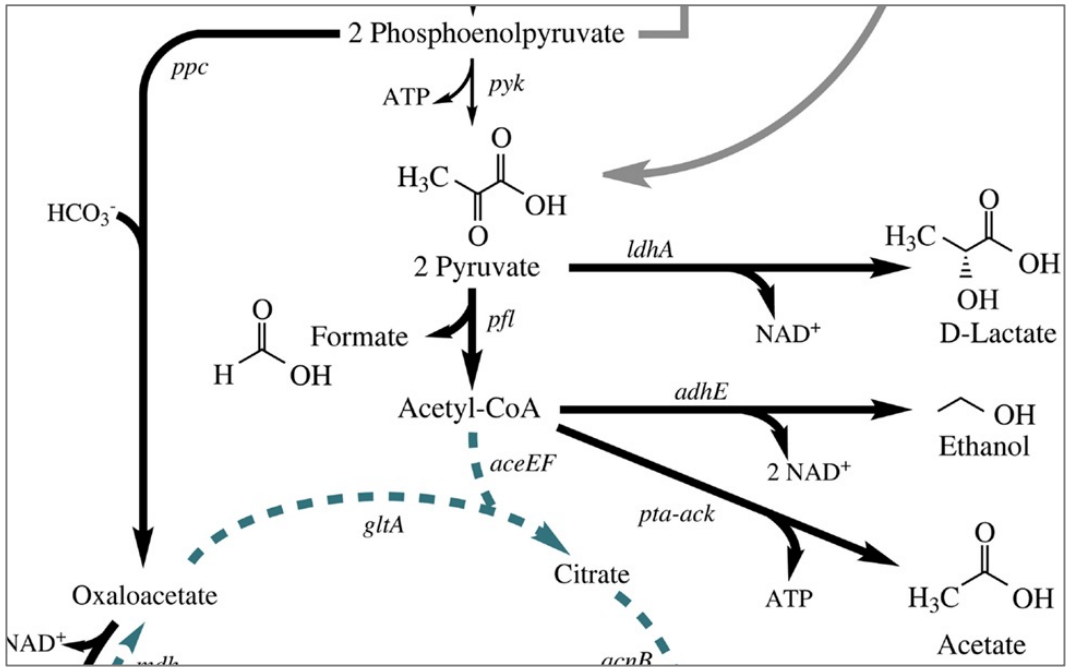
Review of *E. coli* fermentation pathway



Production of ethanol

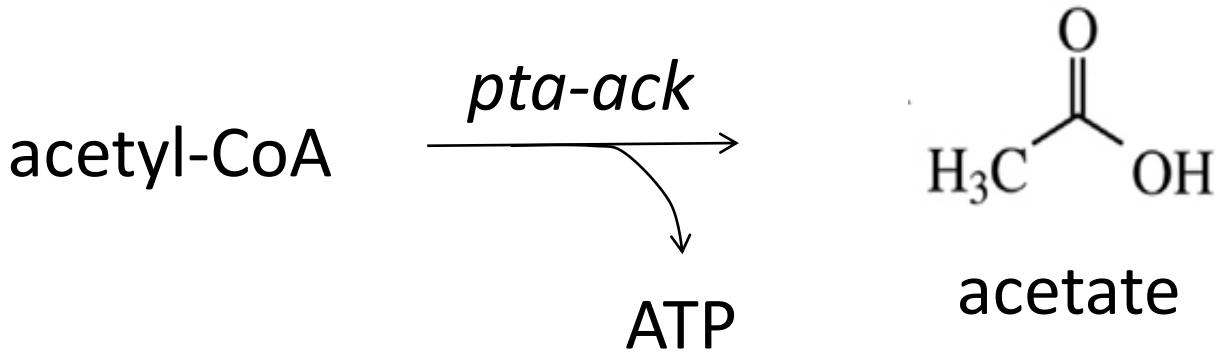
- Bioethanol is most important biotechnological commodity
- *adhE* only transcribed in anaerobic conditions

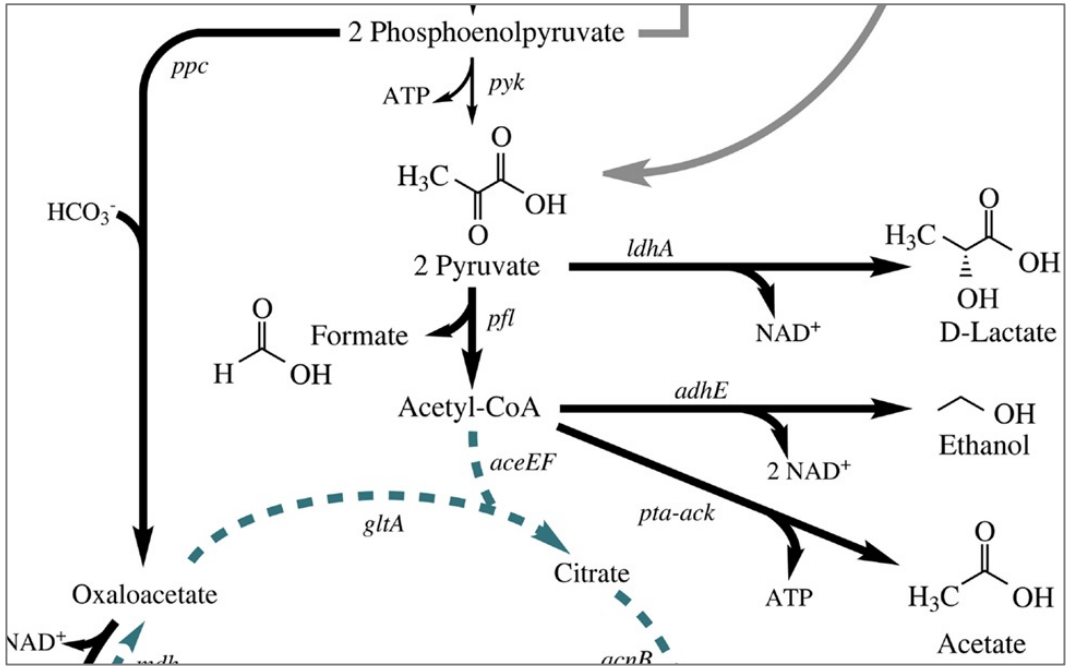




Production of acetate

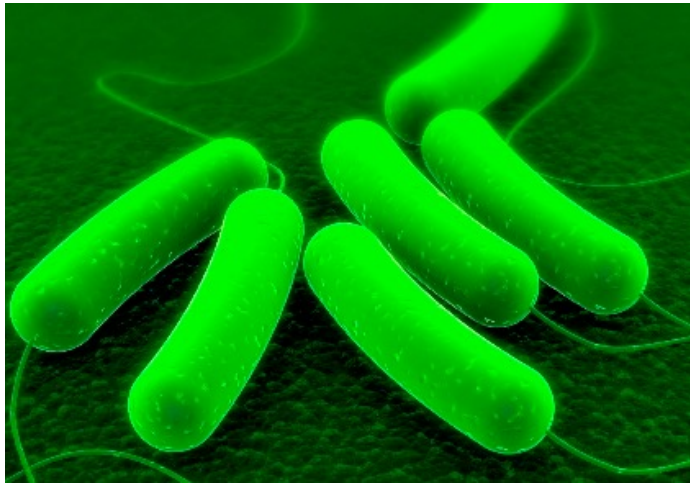
- Acetates used in production of polymers and as a solvent in paints
- *pta-ack* expressed constitutively
  - Aerobically grown cells produce negligible amounts of other fermentation products





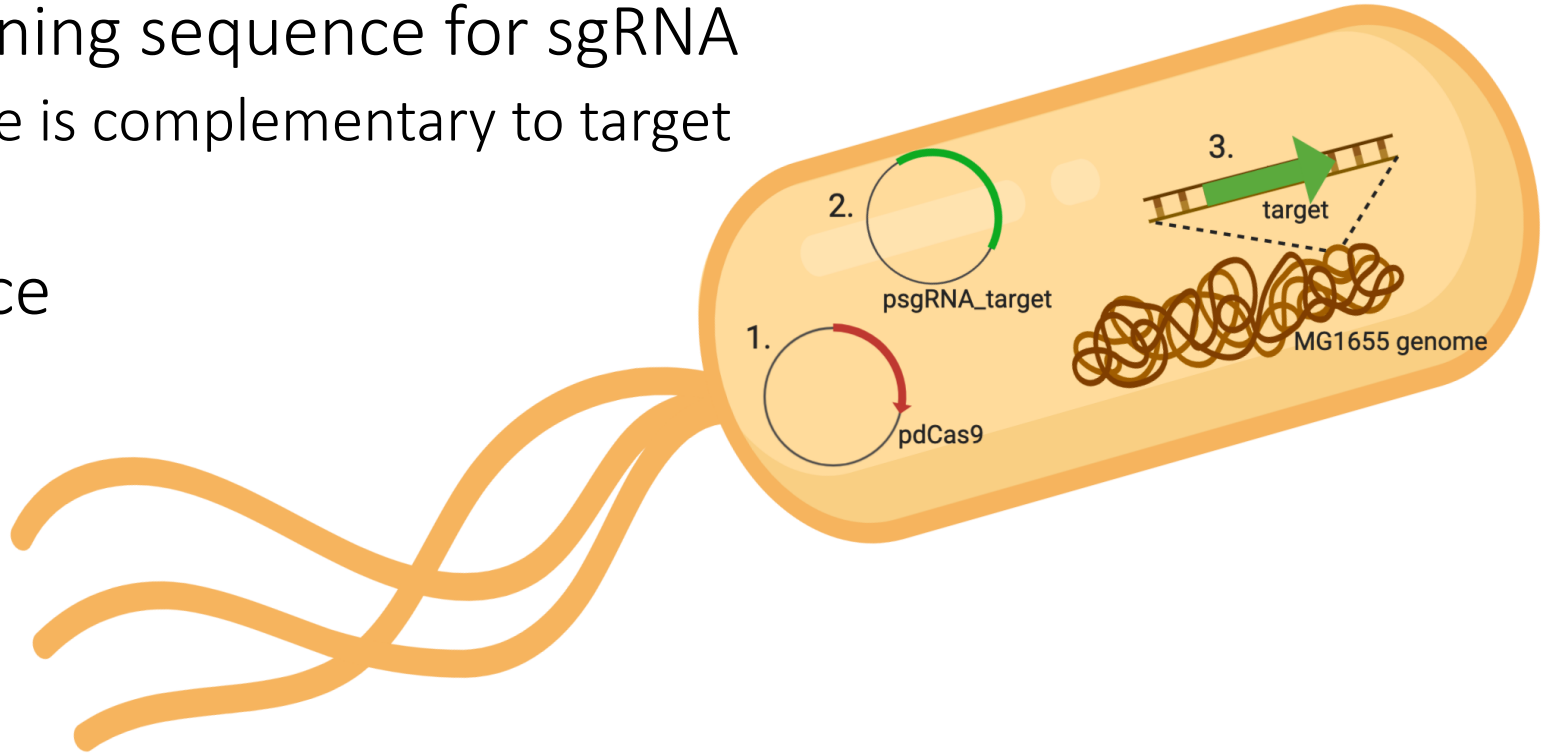
Review of *E. coli* biology

- 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

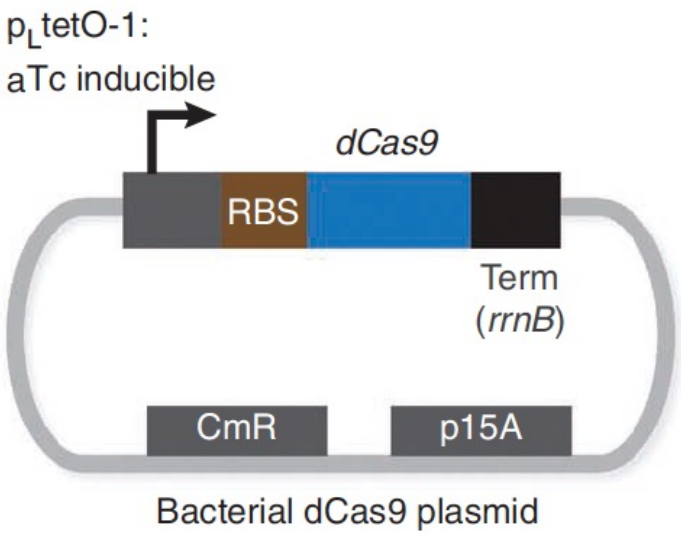


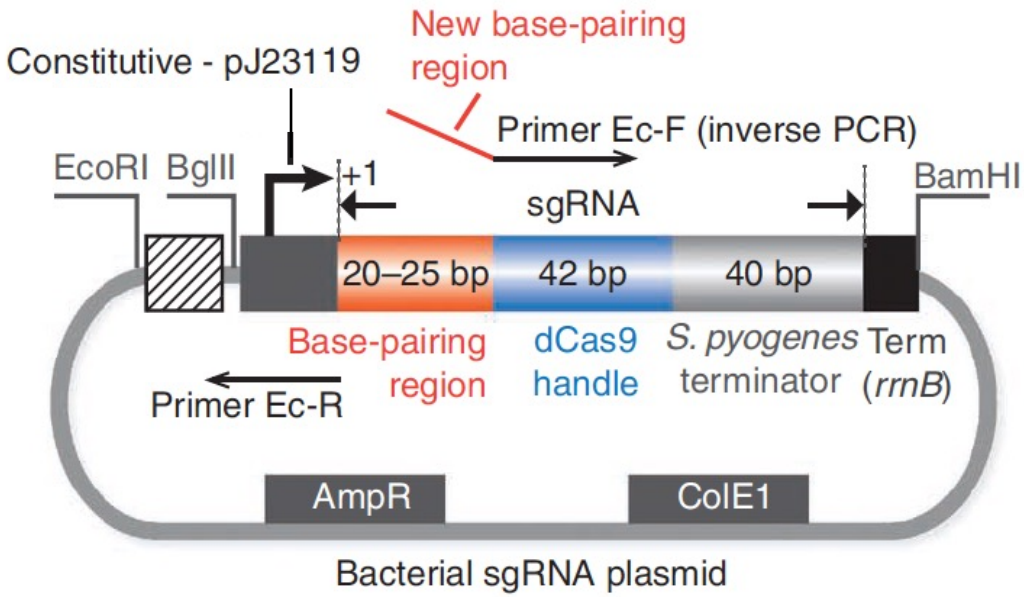
Review of CRISPRi system

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



Closer look at pdCas9 and psgRNA





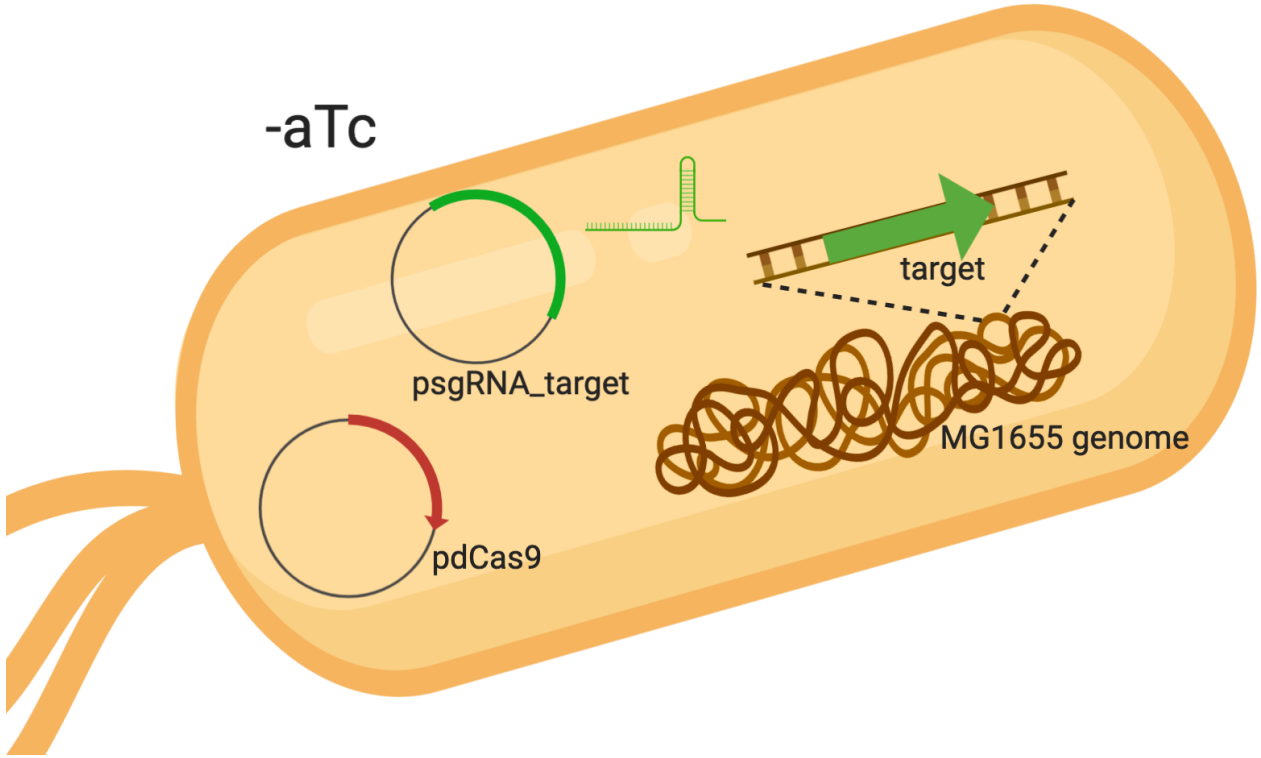
Prepare confirmation digest to check pdCas9 construct on M2D1

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

CRISPRi inactive in absence of inducer

pgRNA_target expressed constitutively

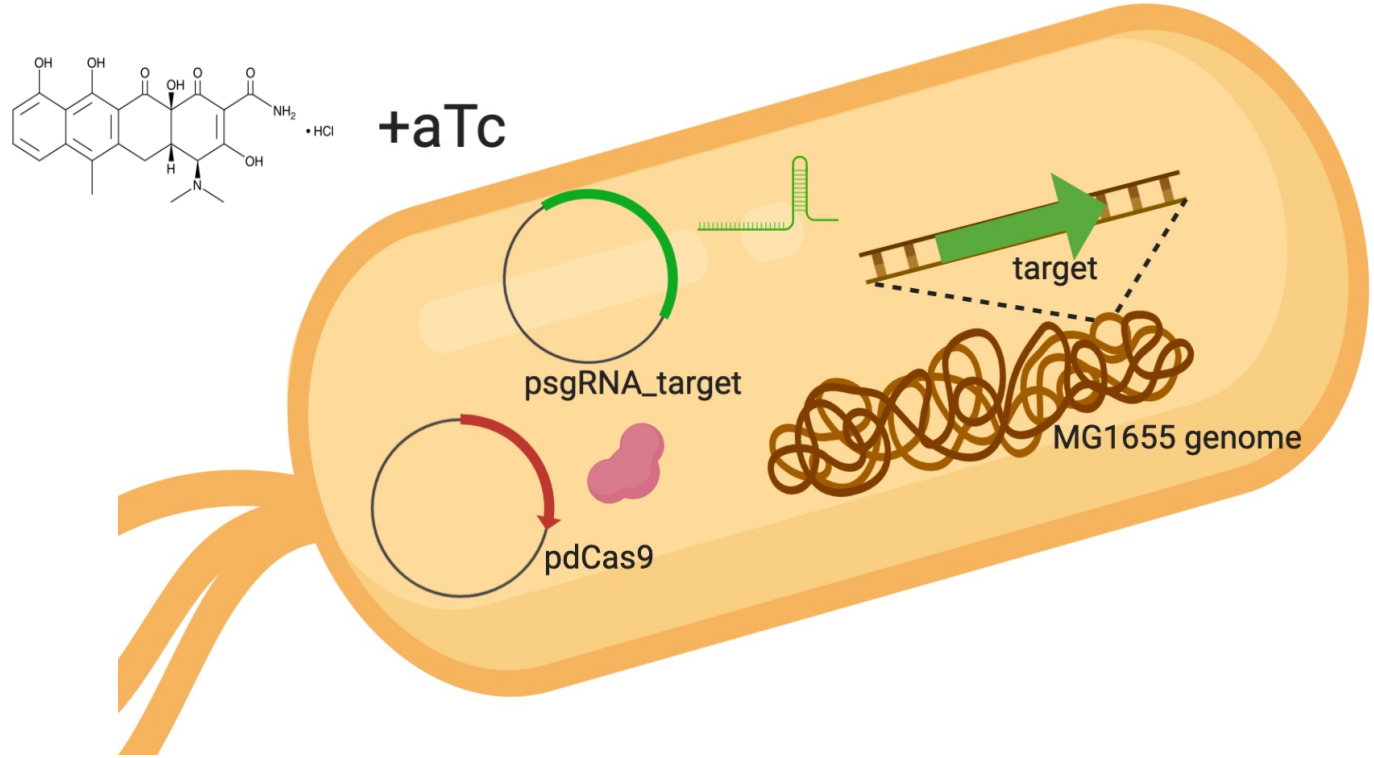
- Always transcribed and binding to target gene



CRISPRi inhibits gene expression in presence of inducer

pdCas9 expressed when aTc added

- When transcribed associates with pgRNA_target / target gene

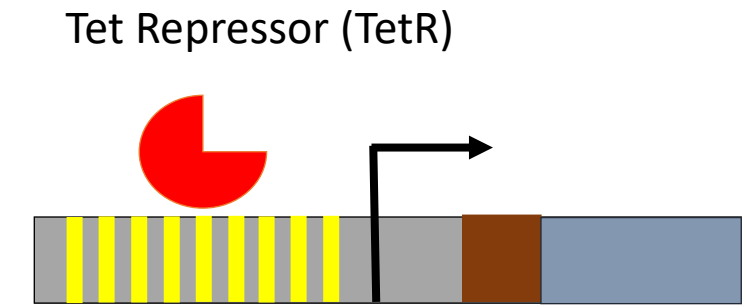


a(nhydro)Tc is a derivative of tetracycline

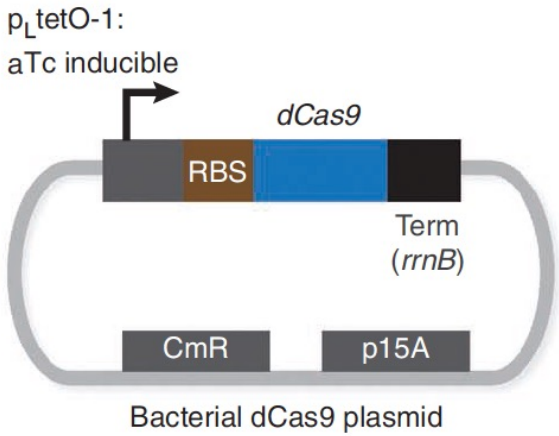
- Why is aTC is a more effective inducer than the antibiotic tetracyline?

- Why doesn’t aTc exhibit antibacterial activity?

Closer look at aTc induction at TRE constructs



- Tet promoter used to regulate the expression of dCas9 gene



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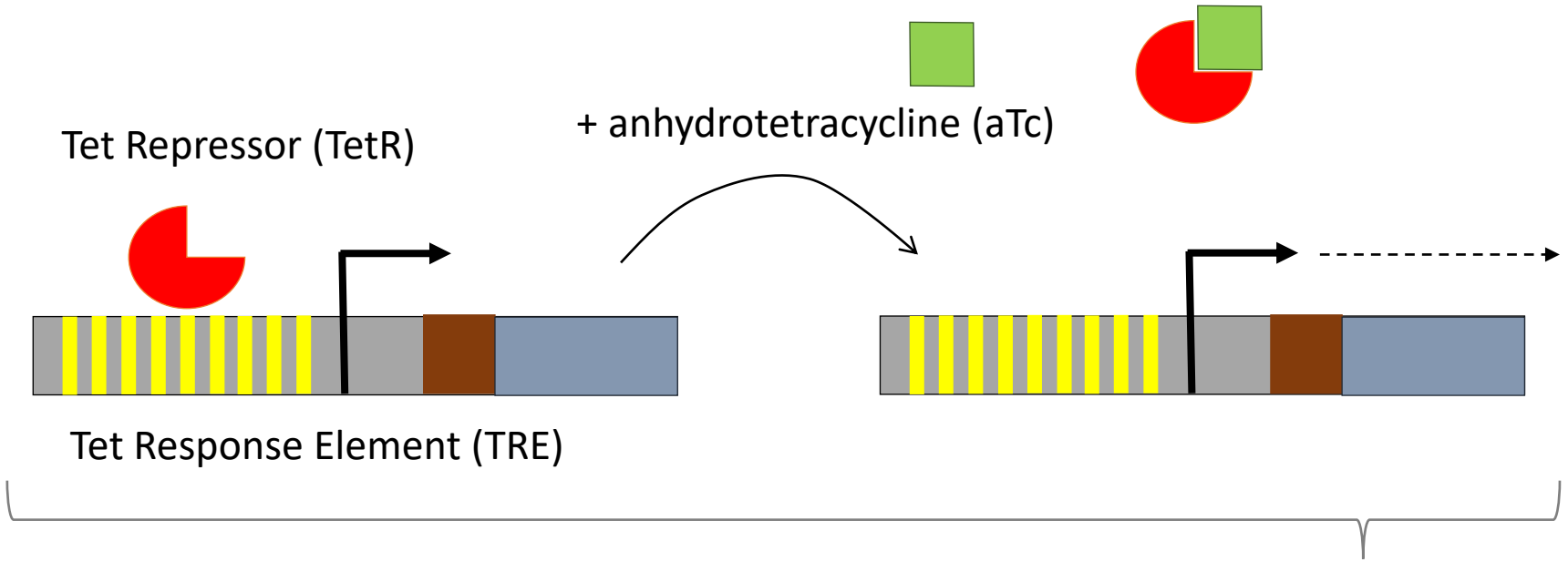

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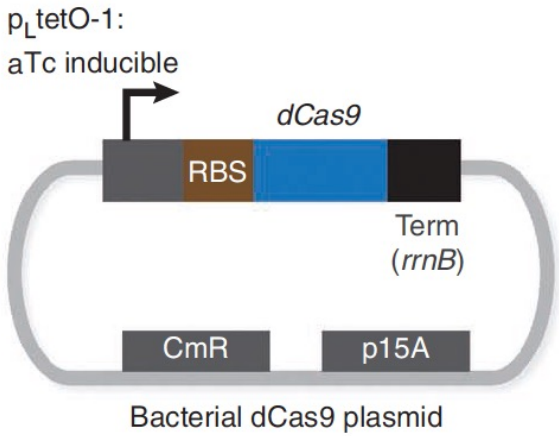
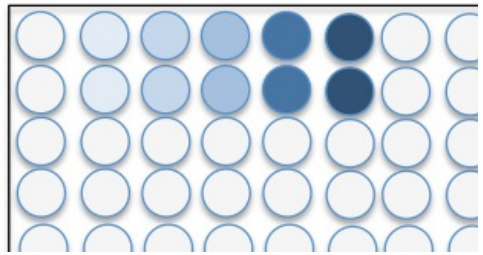


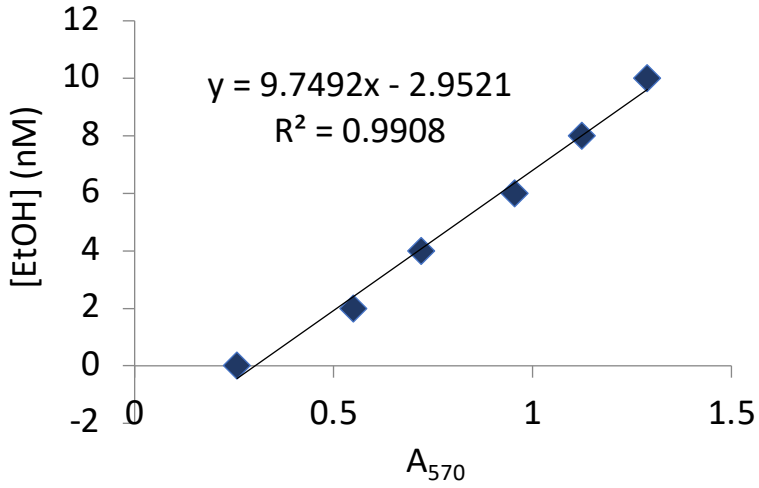
What is your experimental plan?

Our culminating experiment…finally!

- Commercially available kits will be used to measure ethanol / acetate
  - Indirect assays that couple enzymatic reactions, which result in colorimetric output



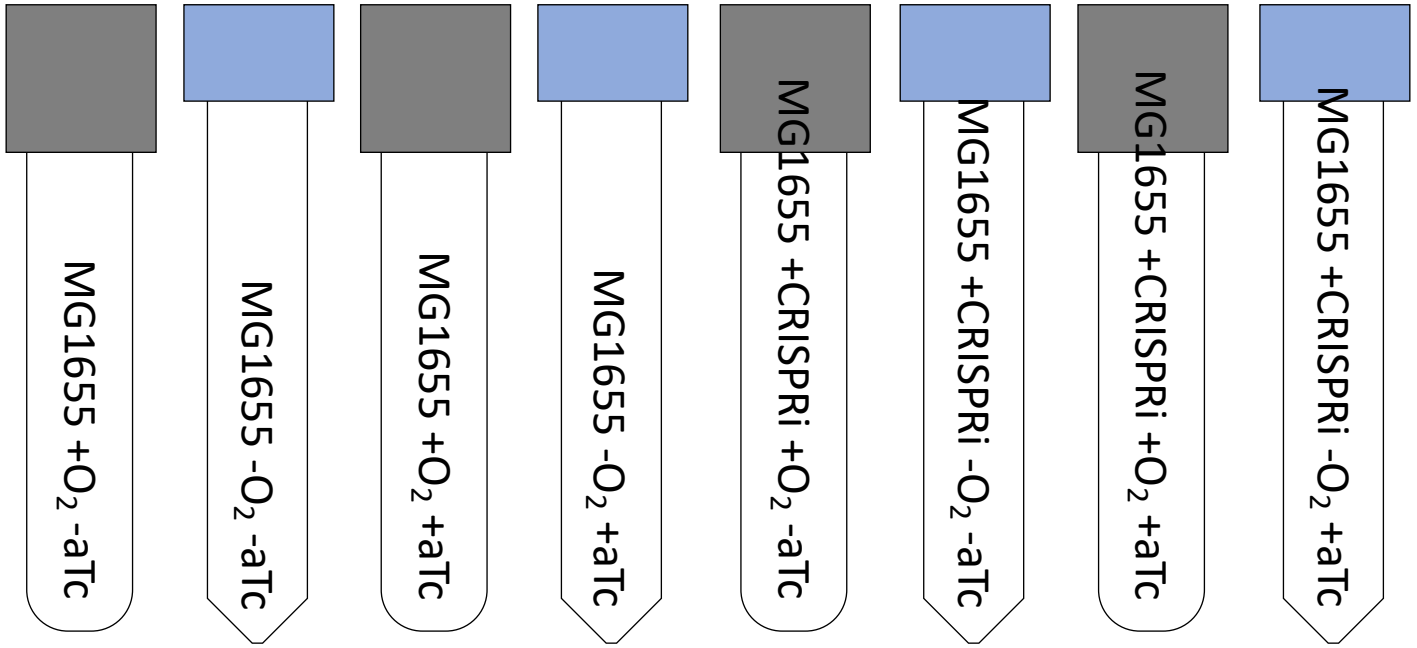
use samples of known concentrations to plot standard curve



How will we prepare our samples?

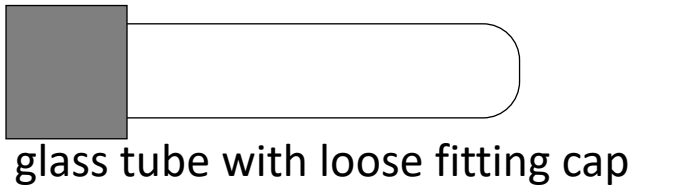
Conditions for testing hypothesis / validating experimental approach:

1. Aerobic vs anaerobic cultures
2. aTc induced vs uninduced
3. MG1655 vs +CRISPRi strains

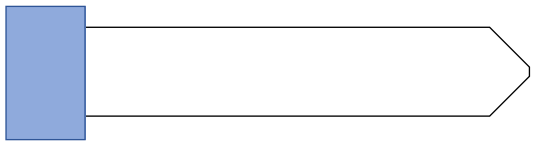


2. Aerobic vs anaerobic cultures

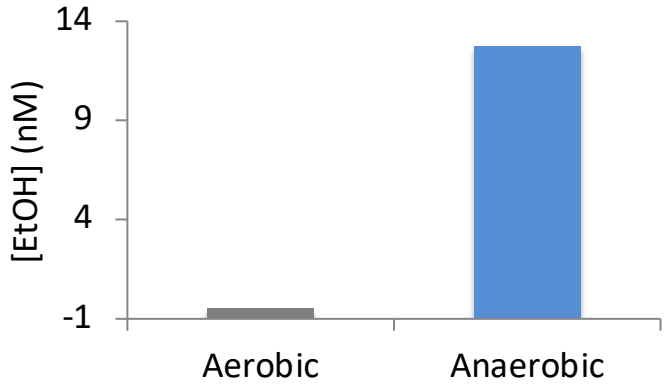
What are the two conditions?



glass tube with loose fitting cap



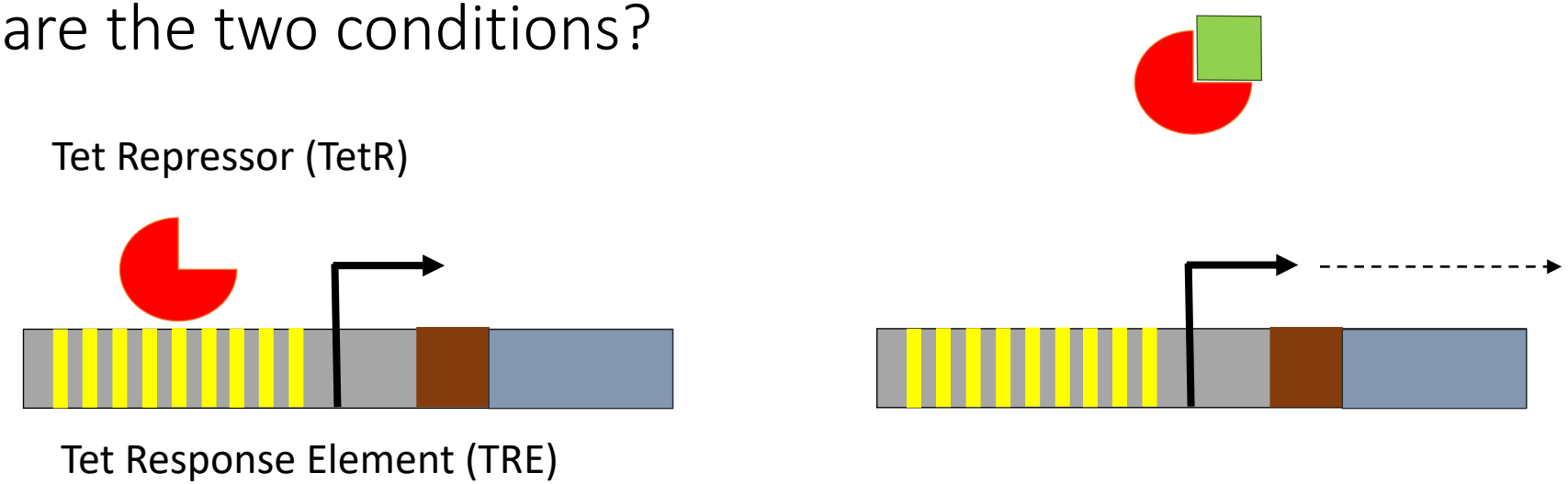
plastic conical tube with screw cap



For what does this control / check?

3. aTc induced vs uninduced

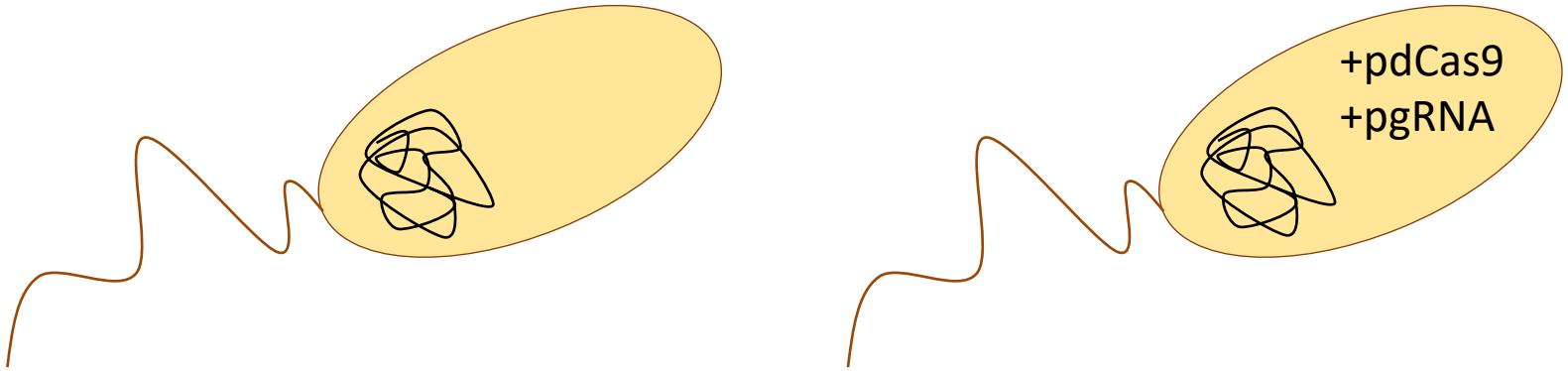
What are the two conditions?



For what does this control / check?

1. MG1655 vs +CRISPRi strains

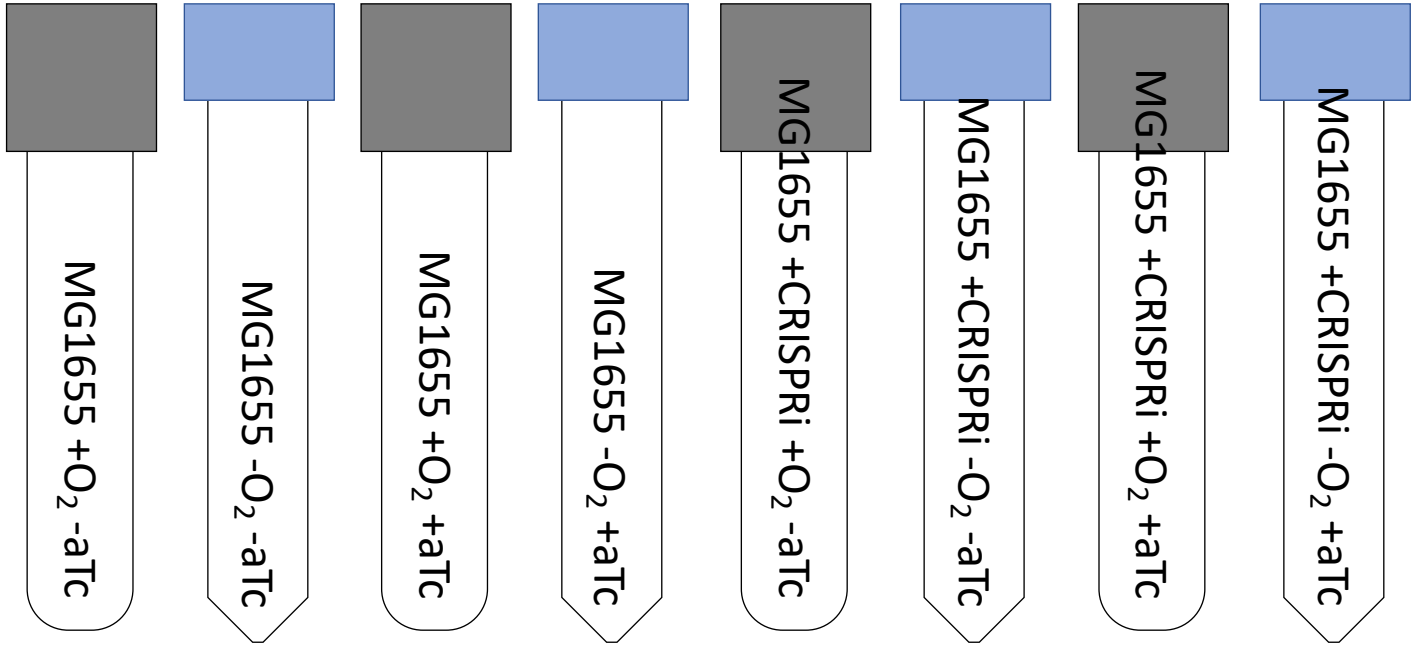
What are the two conditions?



For what does this control / check?

What questions will *your* data address?

Specific to your experimental setup



How will you represent your data?

- Important to normalize fermentation product amount to the OD of the culture

- Consider how best to represent / highlight the data
  - Graphs
  - Tables
  - Text

What questions can *class* data address?

What is the take-home message?

- Know how the TetR-based system is used to control gene expression
- Know the purposes of the controls that are used to evaluate the effectiveness of the sgRNA_target in your experiment
- Know why it is important to normalize your product yield