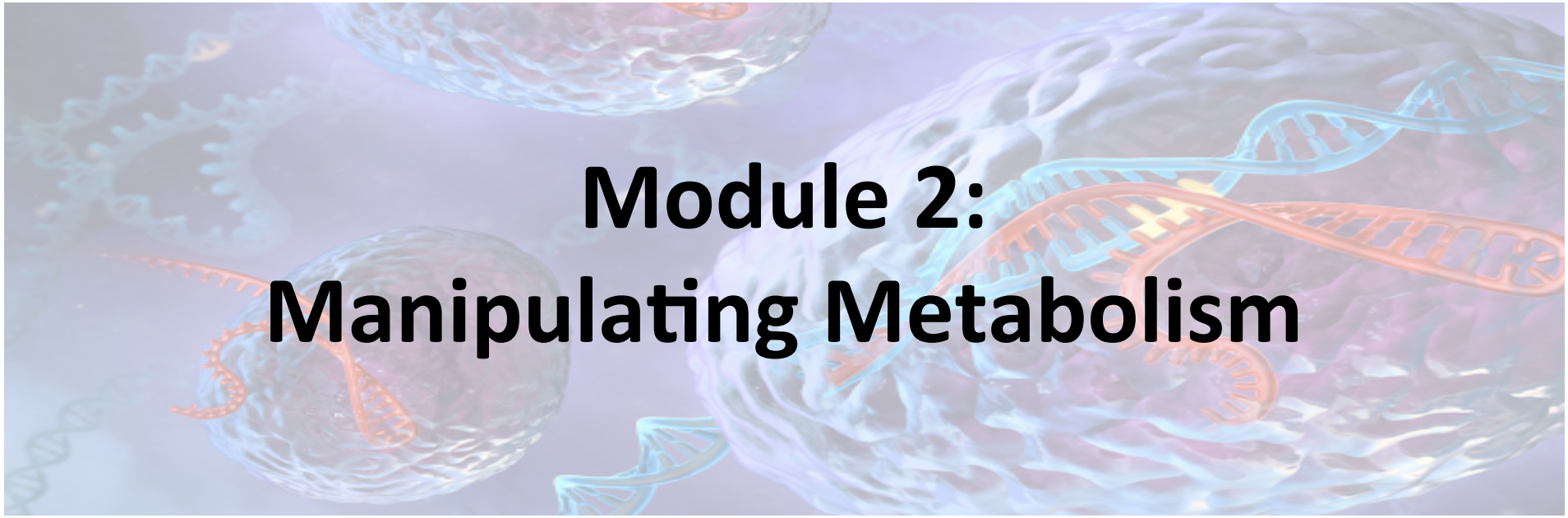


Module 2: Manipulating Metabolism

Measuring fermentation products

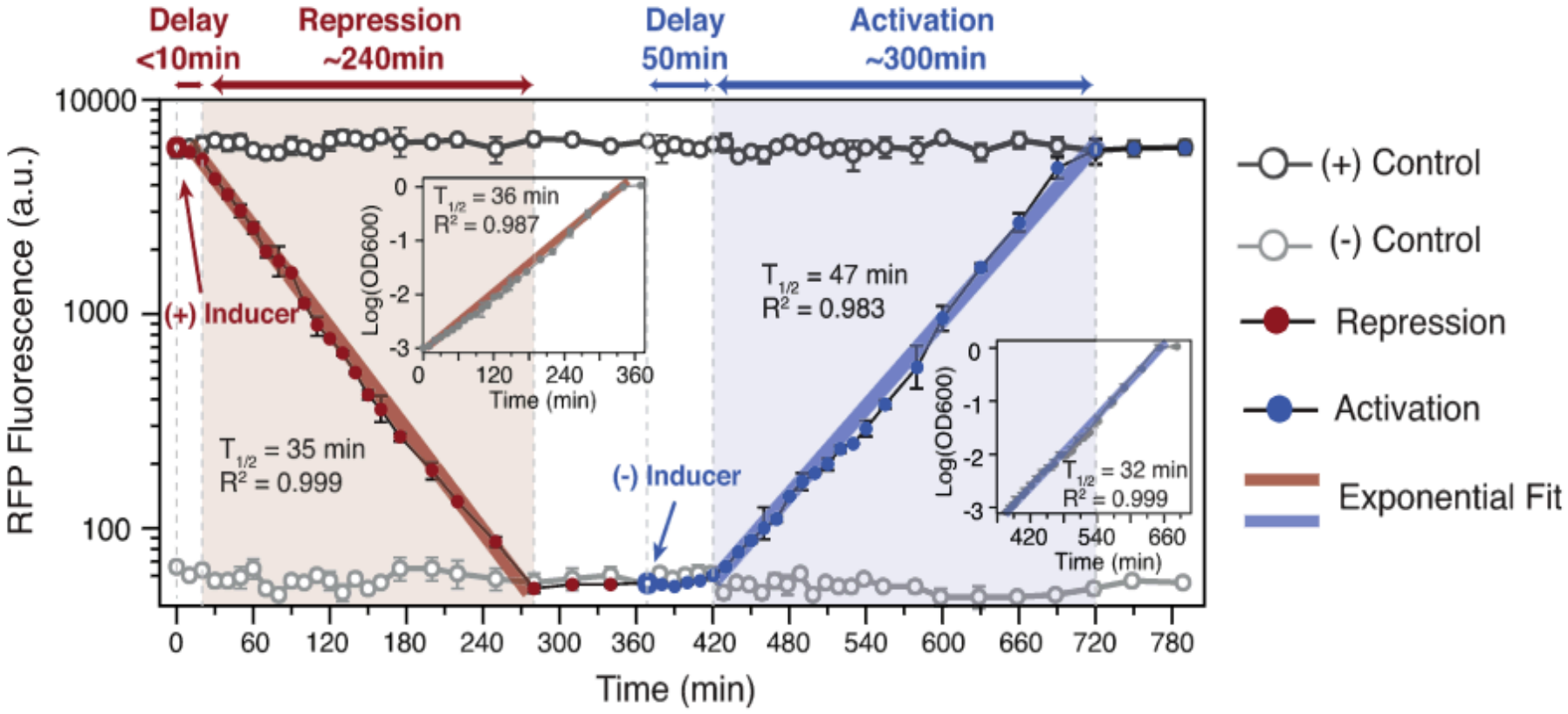
11/2/17


TIRED.
JUST TIRED.

Reminder for Mod 2 due dates

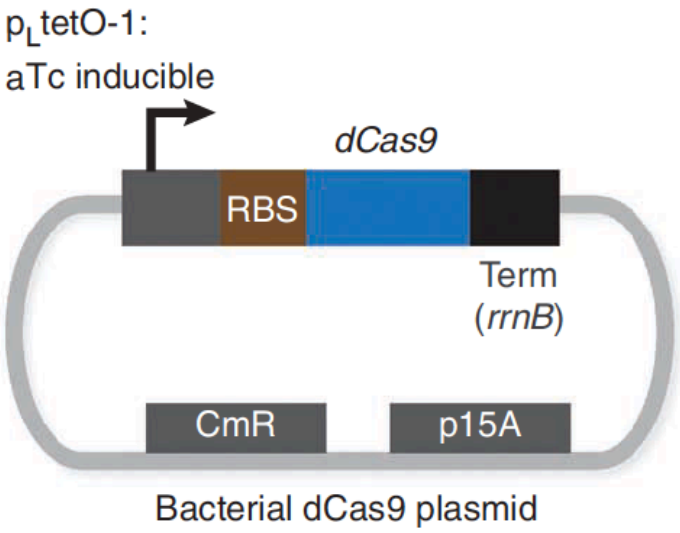
- Research article due Monday, Nov 20 by 10 pm
- Open office hours on Saturday, Nov 19 in 56-302
  - Leslie: 12 pm – 2 pm
  - Noreen: 2 pm – 5 pm
- Last minute office hours on Monday, Nov 20
  - Josephine: 11 am – 2 pm
  - Noreen: 2 pm – 5 pm
- Blog post due Tuesday, Nov 21 by 10 pm

Inducible promoter can be used to control dCas9-mediated gene expression

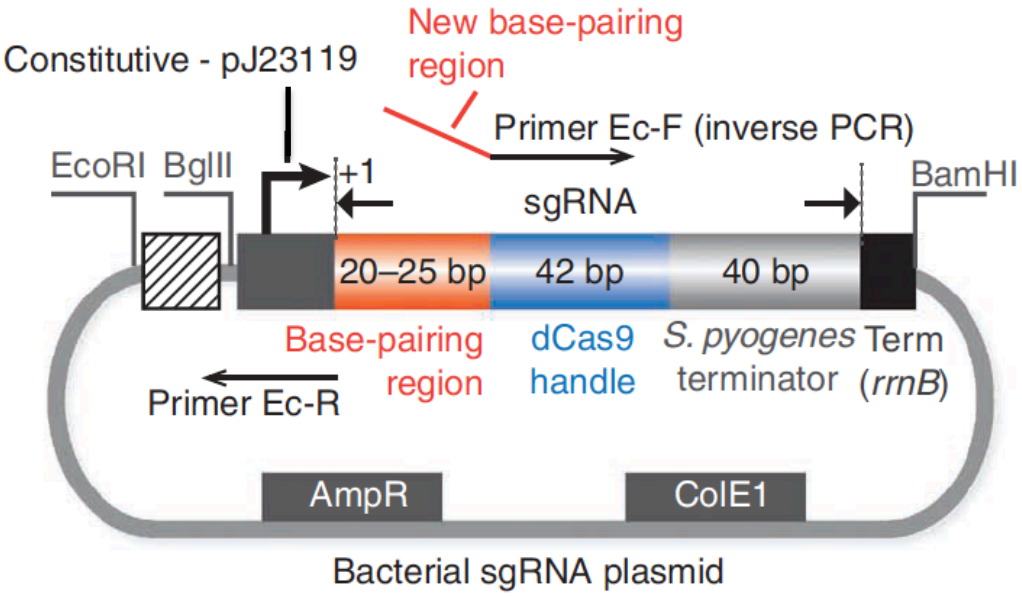


Lei et al. (2013) *Cell*. 152:1173-1183.

Overview of preparation experiments

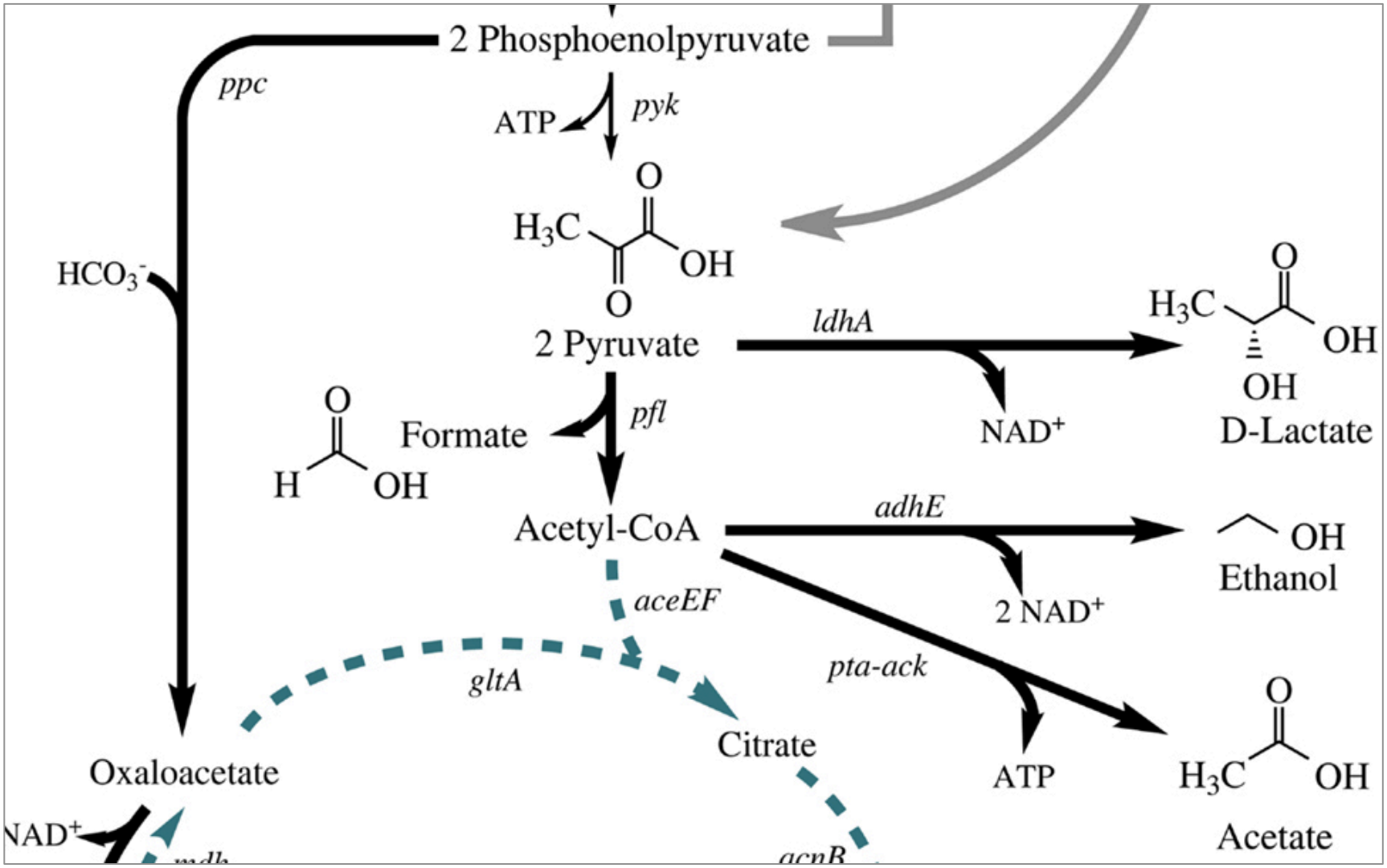


Bacterial dCas9 plasmid



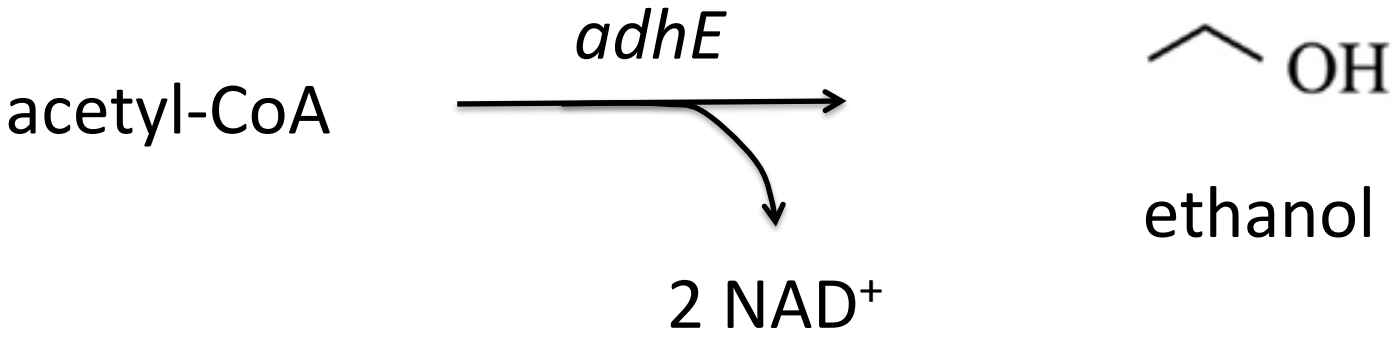
Bacterial sgRNA plasmid

A review of the fermentation pathway



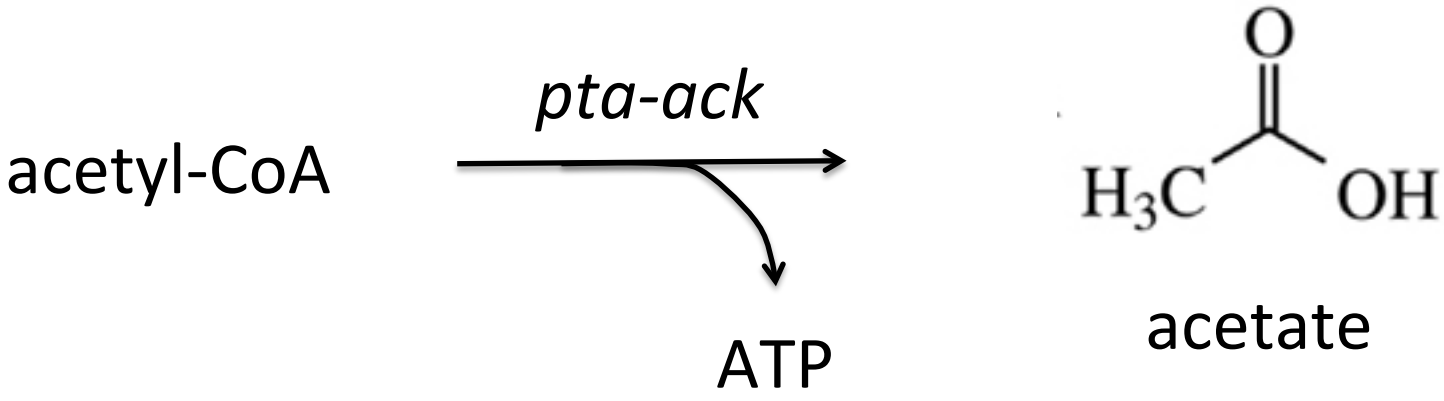
Production of ethanol

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



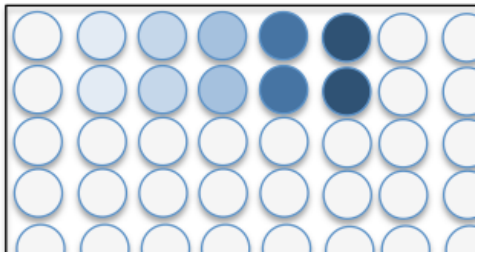
Production of acetate

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

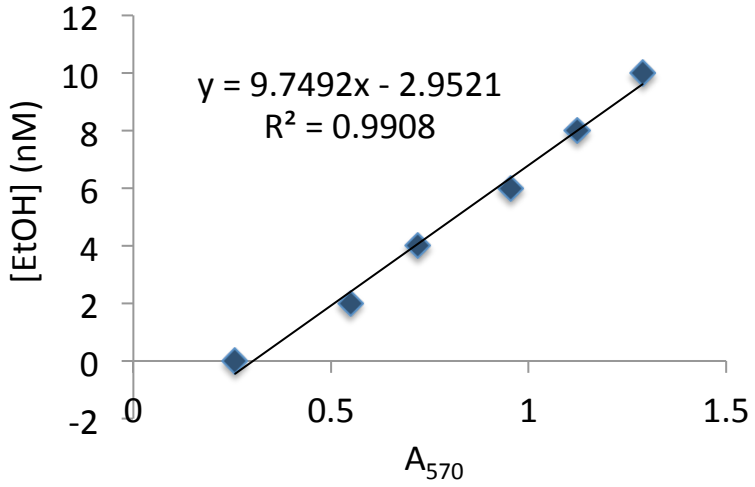


Our culminating experiment…finally!

- Will use commercially available kits to measure ethanol / acetate
  - Indirect assays that couple enzymatic reactions, which result in colorimetric output



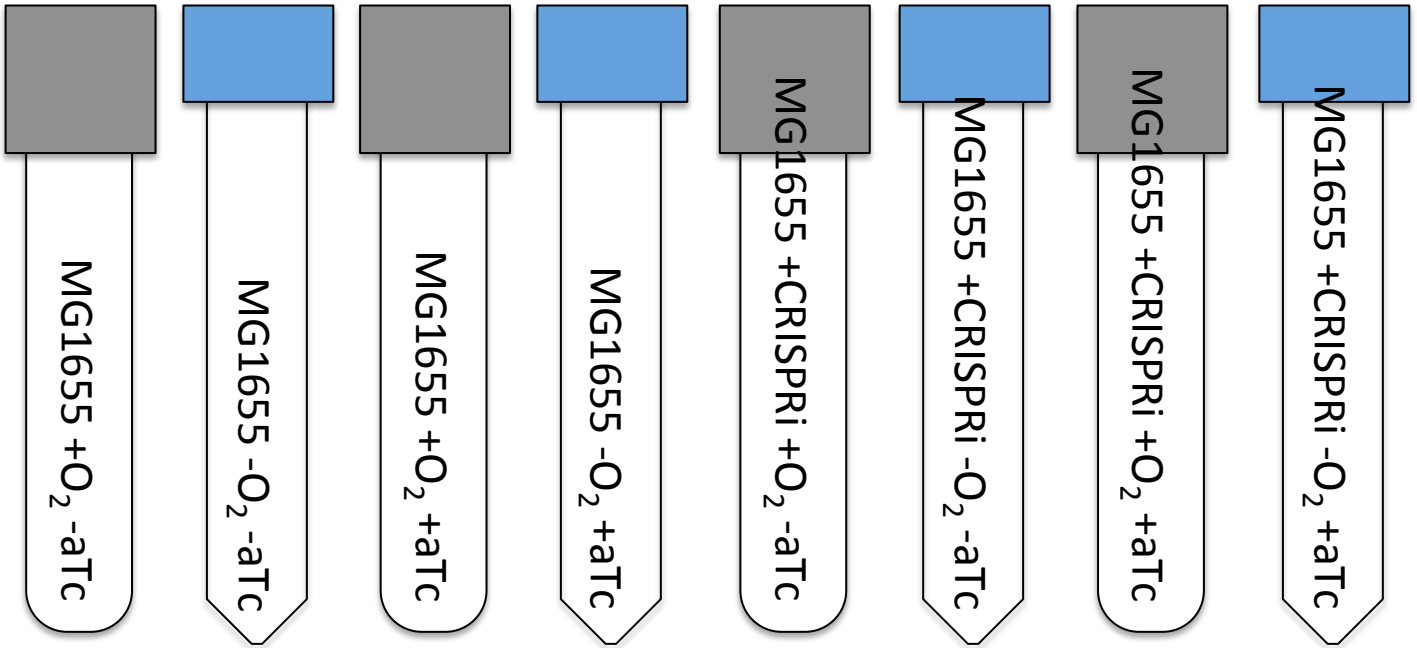
use samples of known concentrations to plot standard curve



So what. Now what?

How will we prepare our samples?

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



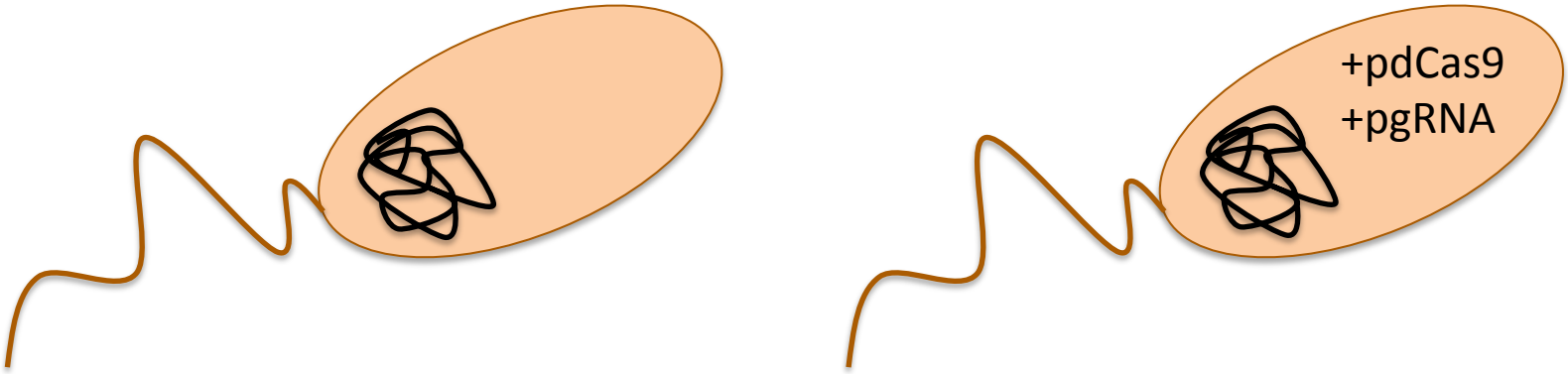
1. MG1655 vs +CRISPRi strains

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?

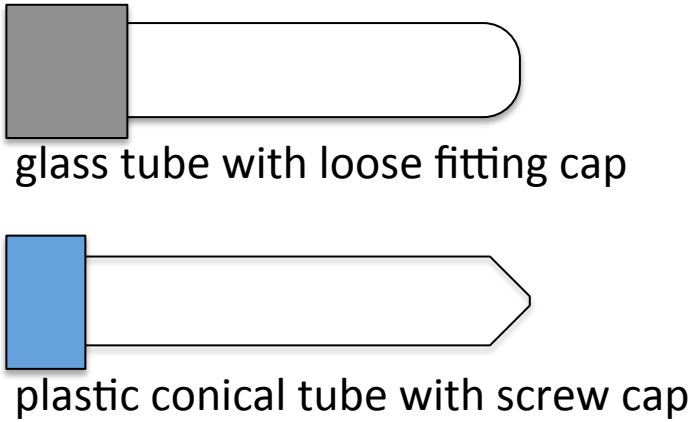
2. Aerobic vs anaerobic cultures

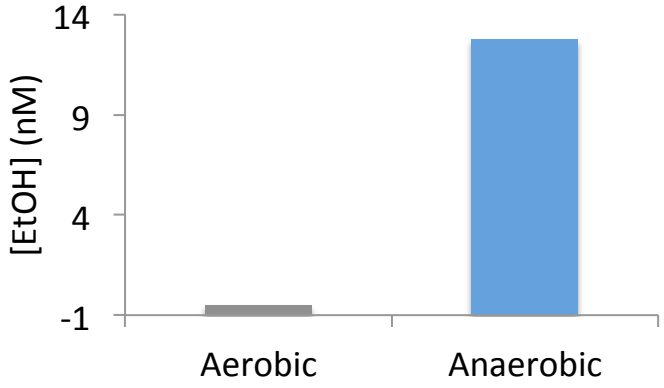
What are the two conditions?

For what does this control / check?

2. Aerobic vs anaerobic cultures

What are the two conditions?





For what does this control / check?

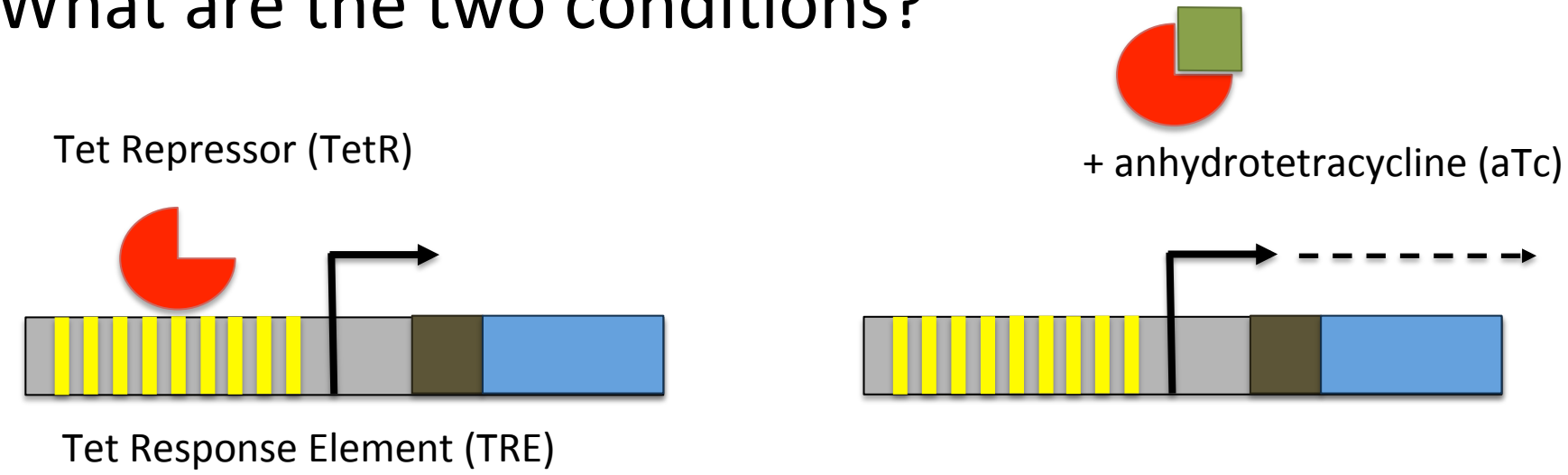
3. aTc induced vs uninduced

What are the two conditions?

For what does this control / check?

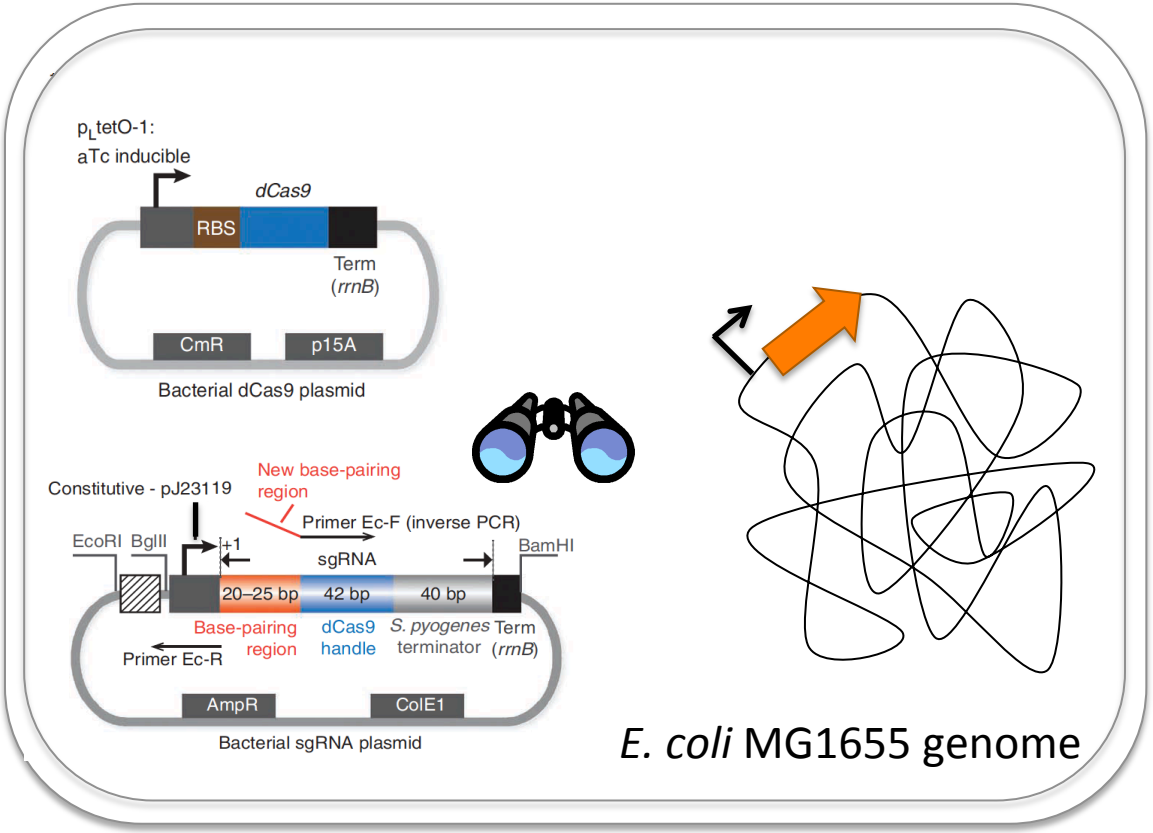
3. aTc induced vs uninduced

What are the two conditions?



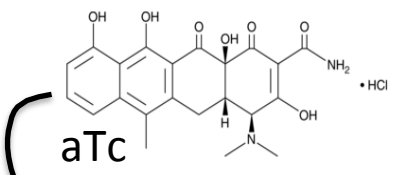
For what does this control / check?

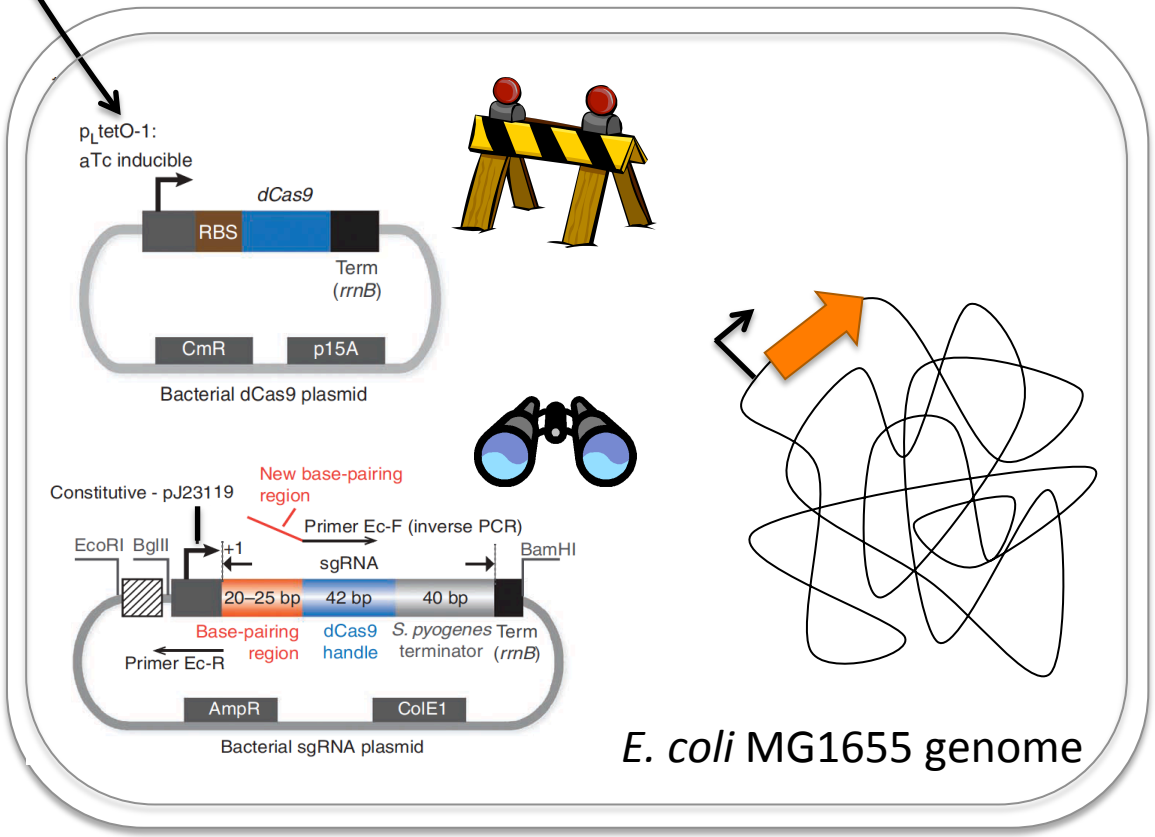
CRISPRi ‘inactive’ in absence of inducer



- pgRNA_target expressed constitutively
  - Always transcribed

CRISPRi ‘blocks’ gene expression in presence of inducer





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

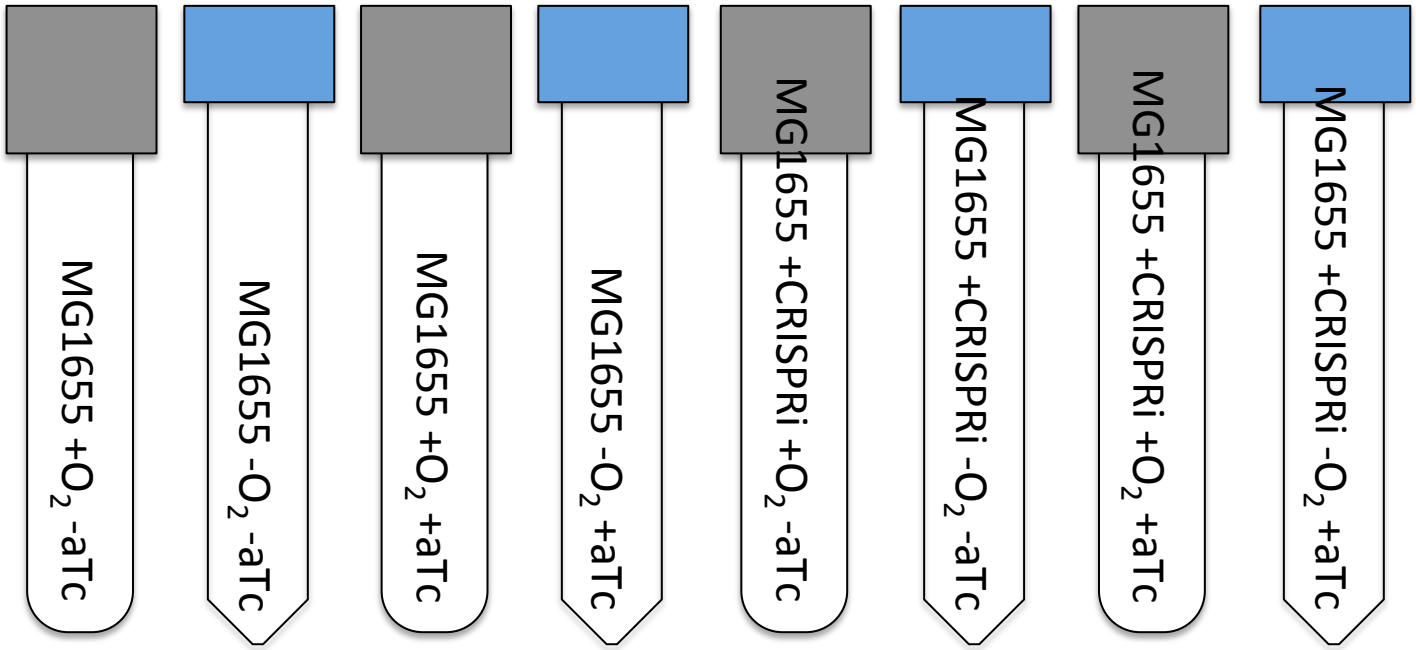
So what. Now what?

How will we represent our data?

- Need to normalize fermentation product amounts
- Consider how best to show the data
  - Graphs
  - Tables
  - Text

What questions will our data address?

Specific to your experimental setup



What questions can our data address?

In the laboratory…

1. BE Communication Lab workshop
   - Manuscript architecture
2. Confirm sgRNA_target insertion
   - Analyze sequencing results
3. Prepare culture tubes for fermentation product assay
4. Use in-class ‘free time’ to work on your research article!