



Module 2: Manipulating Metabolism

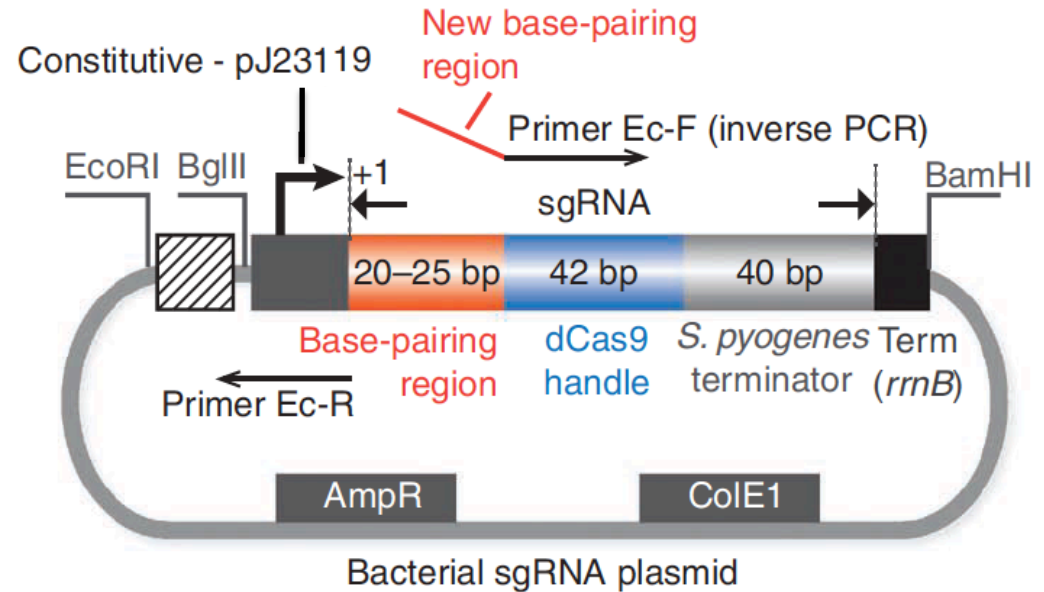
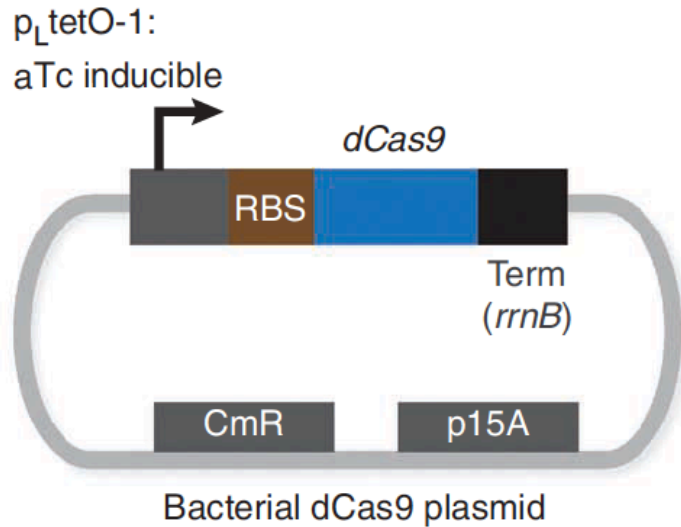
Measuring fermentation products

11/3/15

Update to Mod 2 due dates

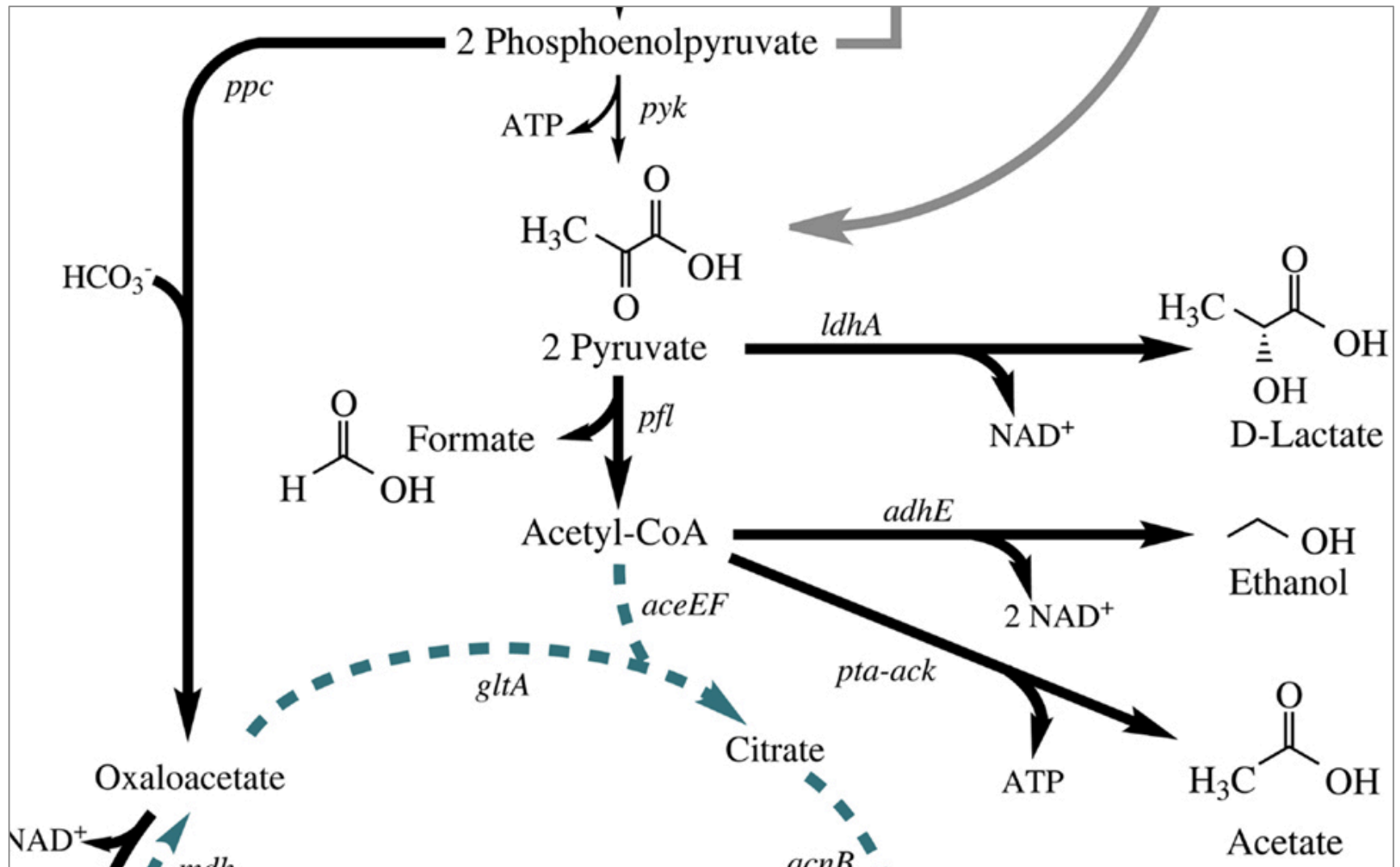
- Research article due **Sunday, Nov 20 by 5 pm**
- Open office hours on **Saturday, Nov 19**
 - Leslie: 10 am – 12 pm
 - Maxine: 12 pm – 2 pm
 - Noreen: 2 pm – 5 pm
- Blog post due **Monday, Nov 21 by 5 pm**

Overview of preparation experiments



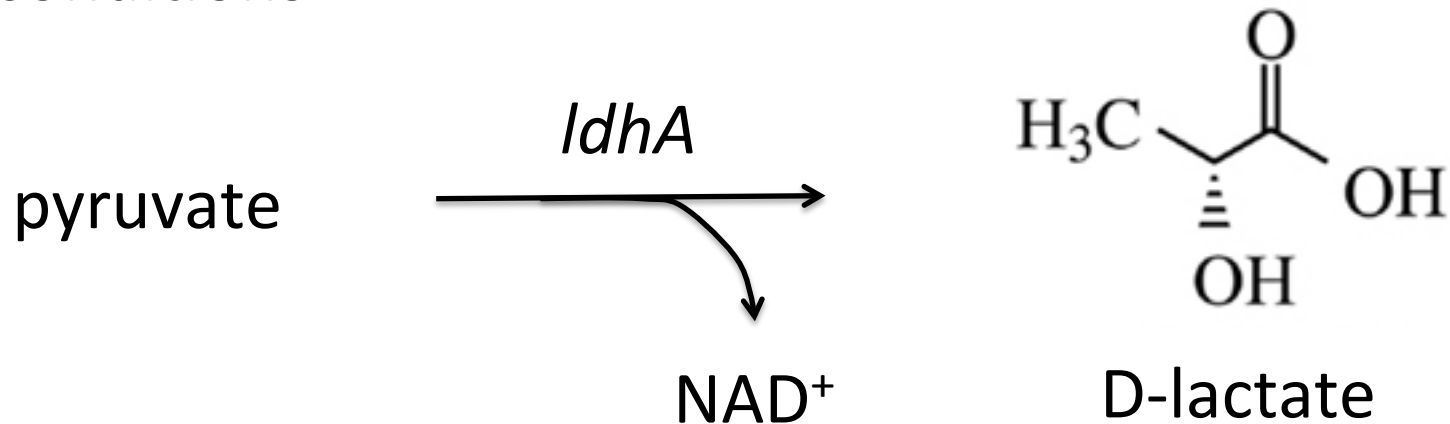
So what. Now what?

A review of the fermentation pathway



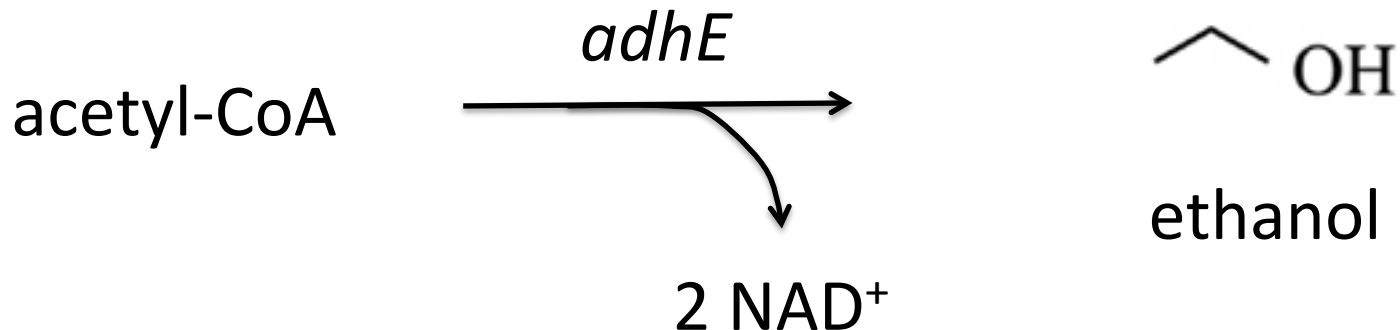
Production of lactate

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



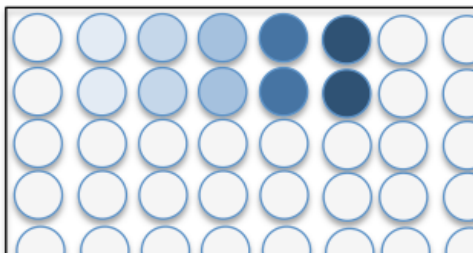
Production of ethanol

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

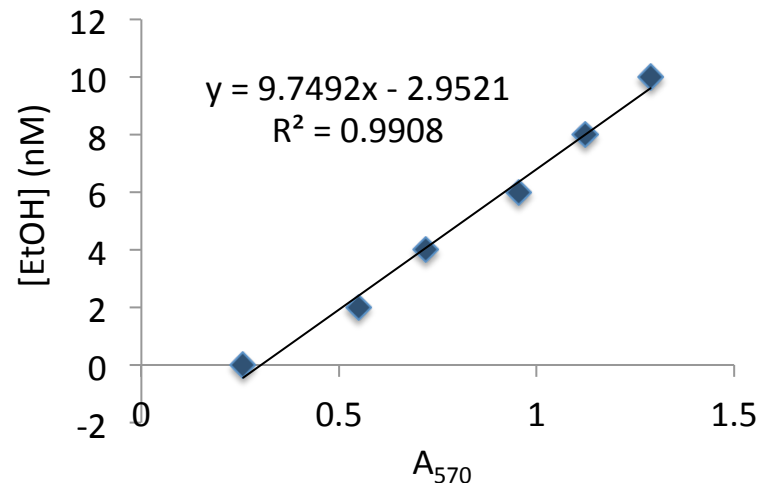


Our culminating experiment...finally!

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

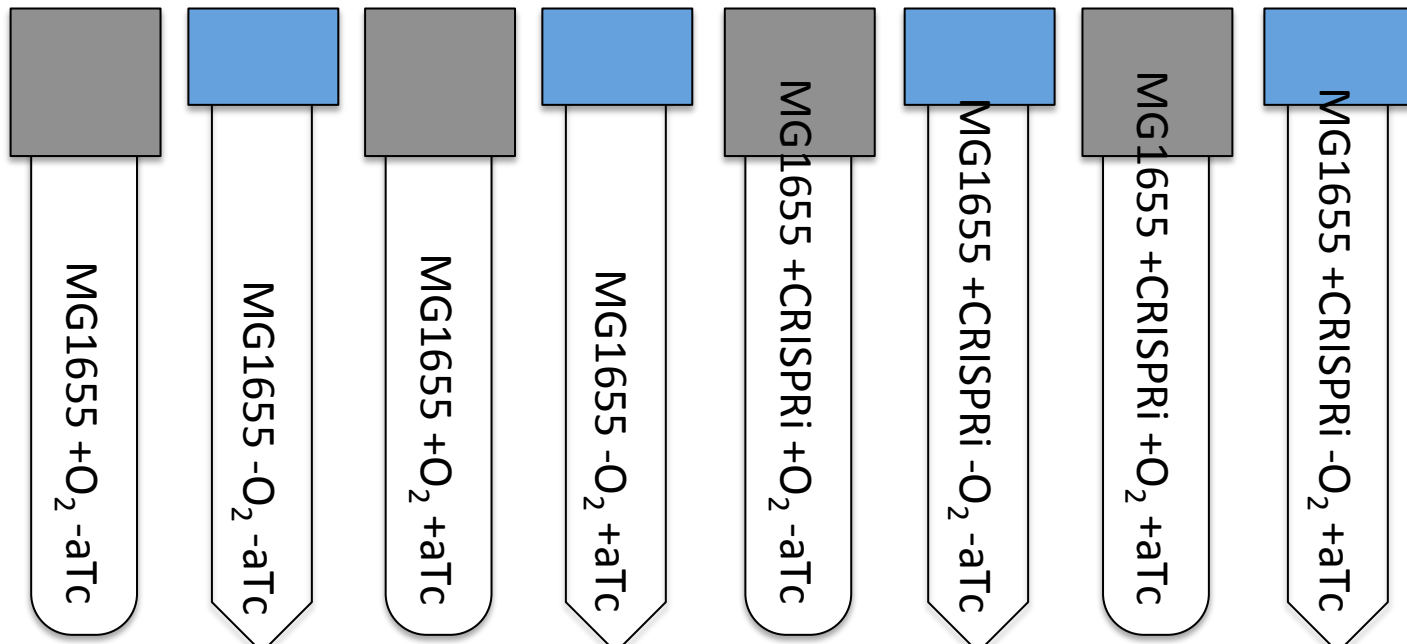


use samples of known concentrations to plot standard curve



How will we prepare our samples?

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



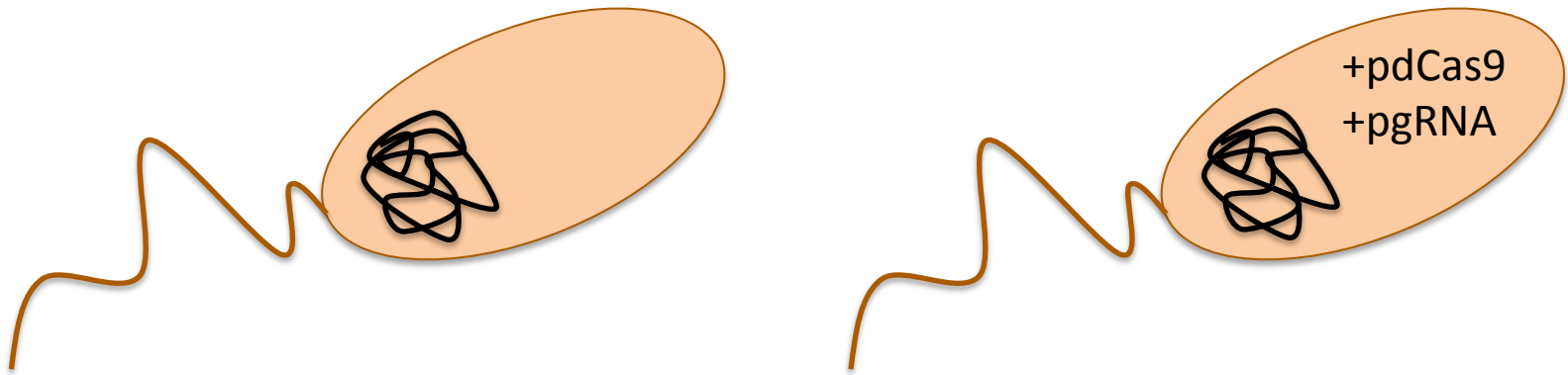
1. MG1655 vs +CRISPRi strains

What are the two conditions?

For what does this control / check?

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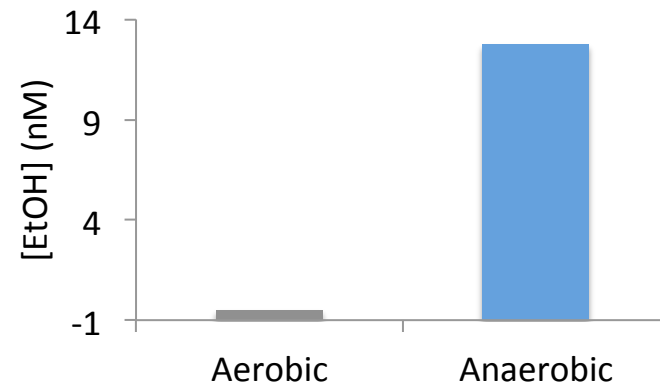
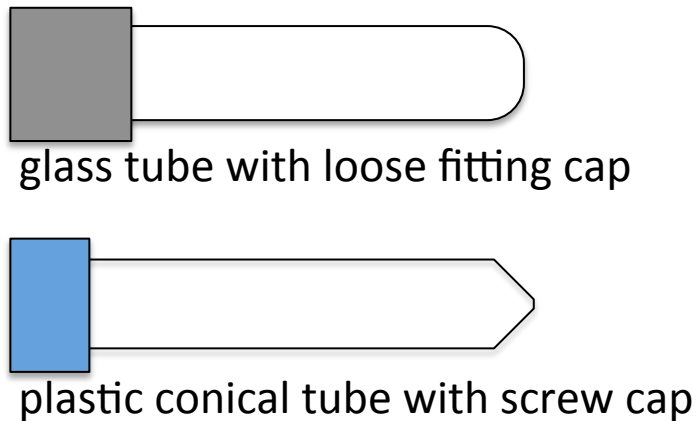
2. Aerobic vs anaerobic cultures

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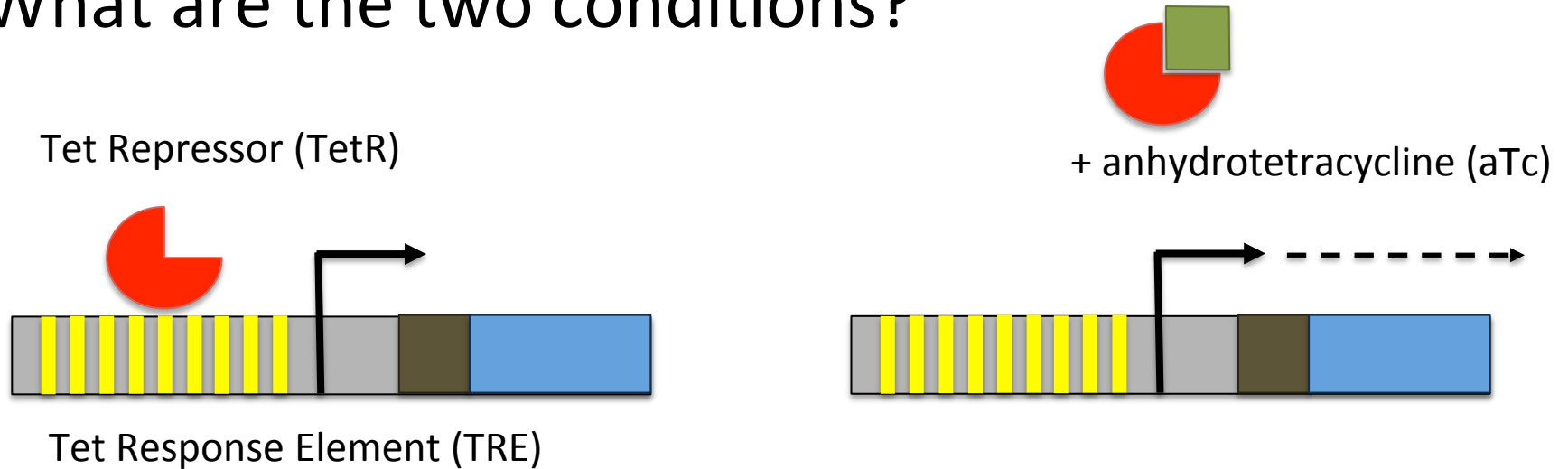
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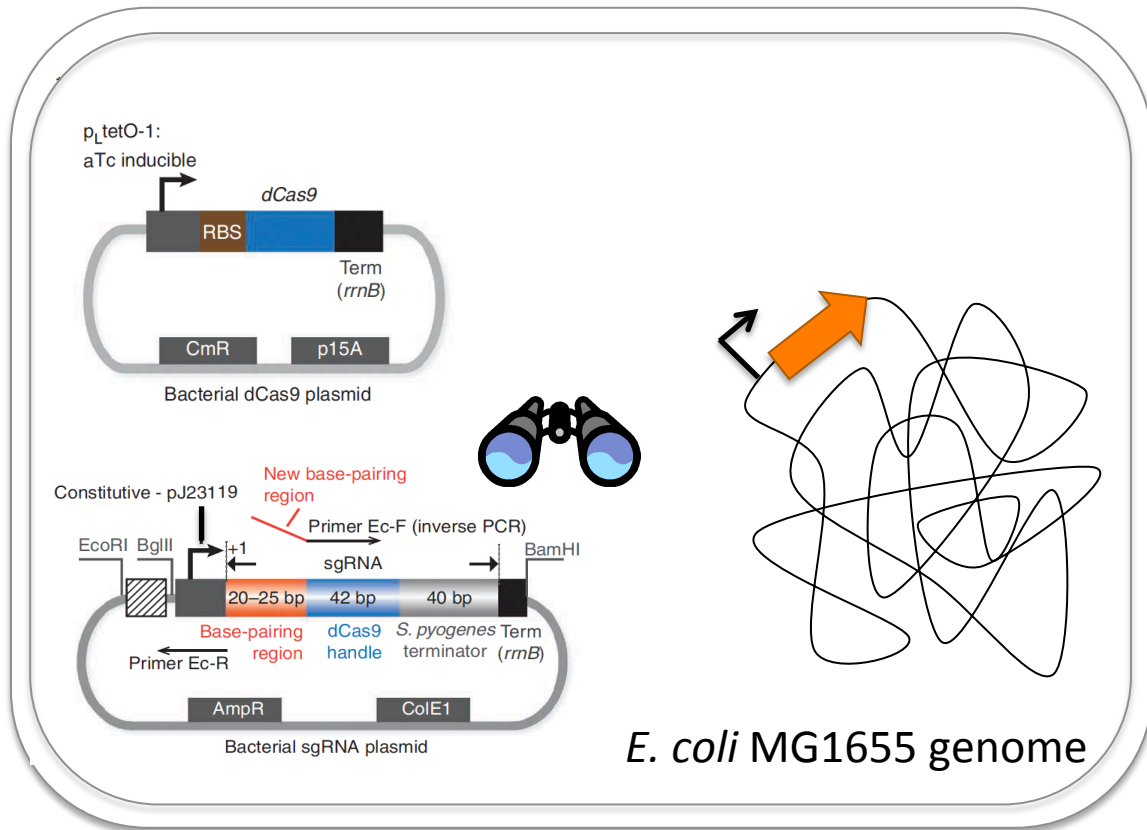
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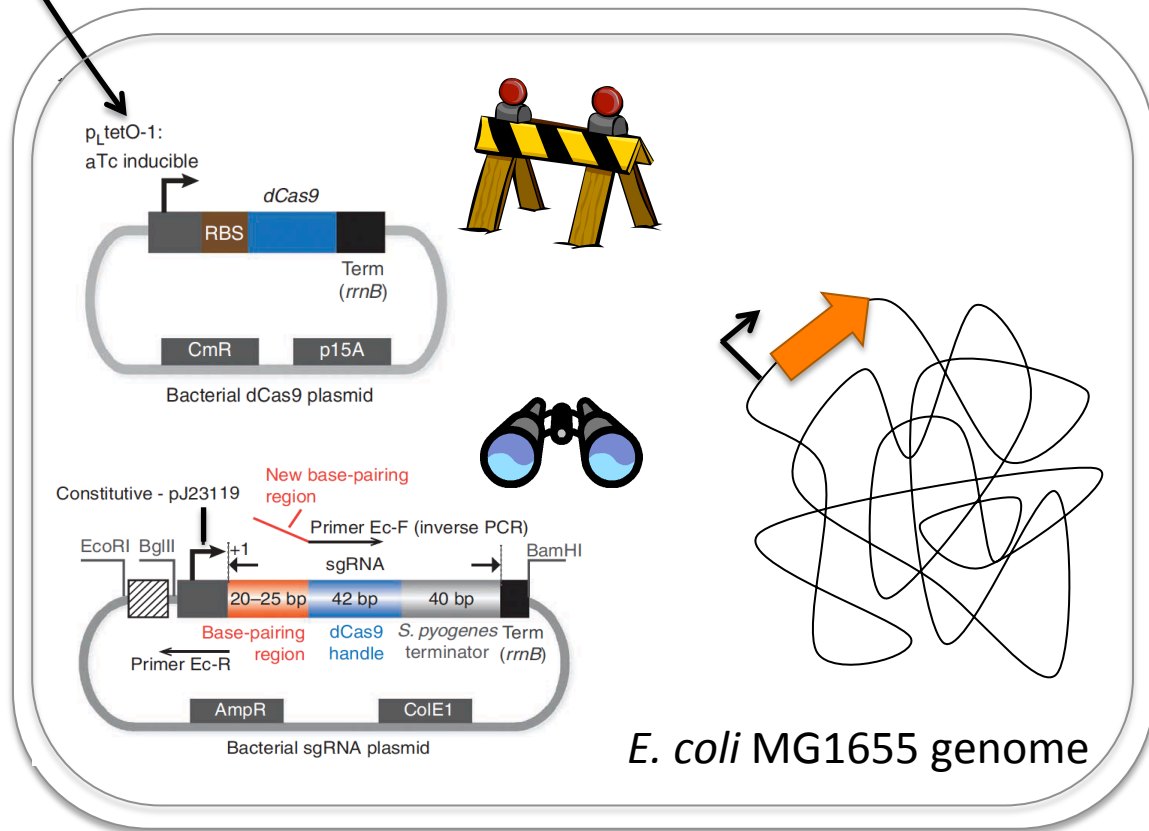
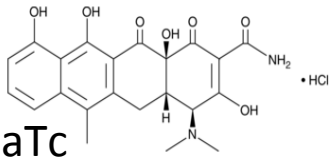
For what does this control / check?

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

So what. Now what?

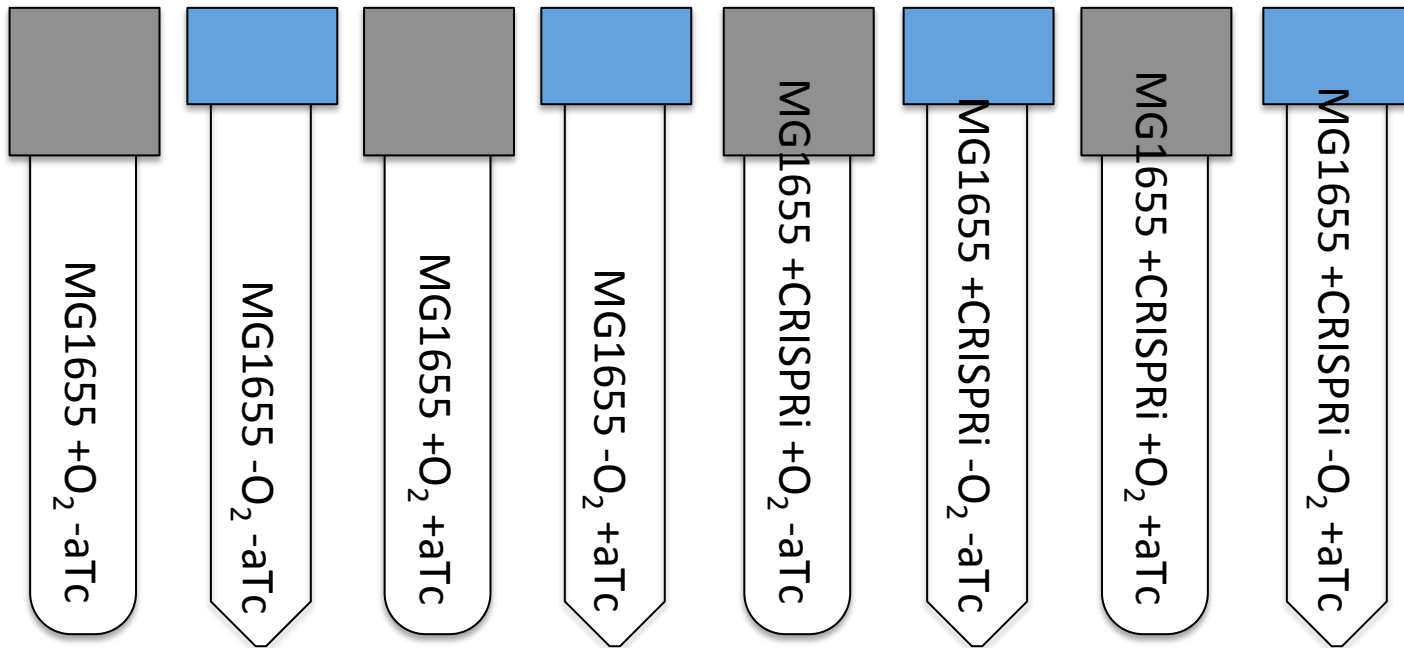
How will we represent our data?

- 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?

Comparisons using class data pool

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!

