



Module 2: Manipulating Metabolism

Measuring fermentation products

11/1/18

TIRED.

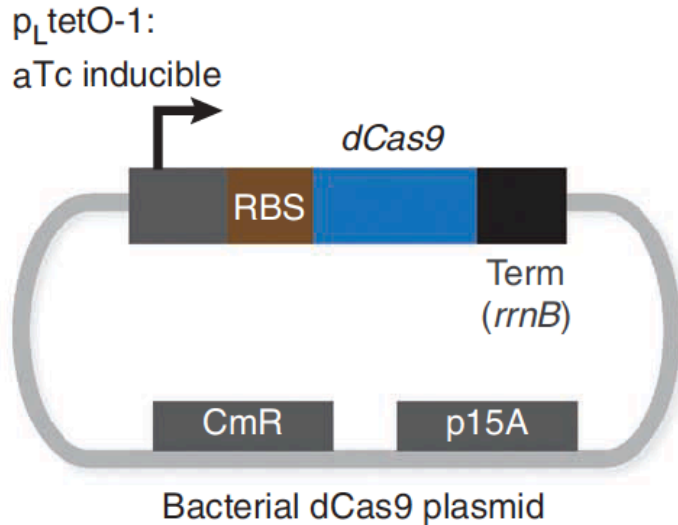


JUST TIRED.

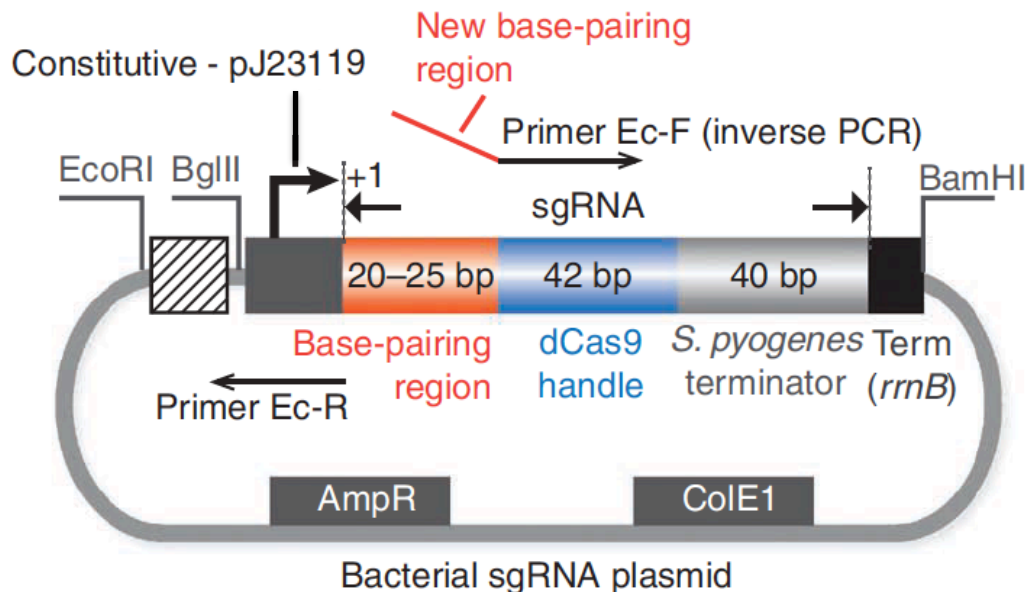
Important Mod 2 dates

- Research article due **Monday, Nov 12 by 10 pm**
- Additional office hours **TBA**
- Normal Office hours
 - Noreen: Monday 2-5p
 - Josephine: Wednesday 12-1p, Friday 2-3p
 - Leslie: Thursday 2-3p, Friday 12-1p
- Blog post due **Tuesday, Nov 13 by 10 pm**

Review of pgRNA and pdCas9

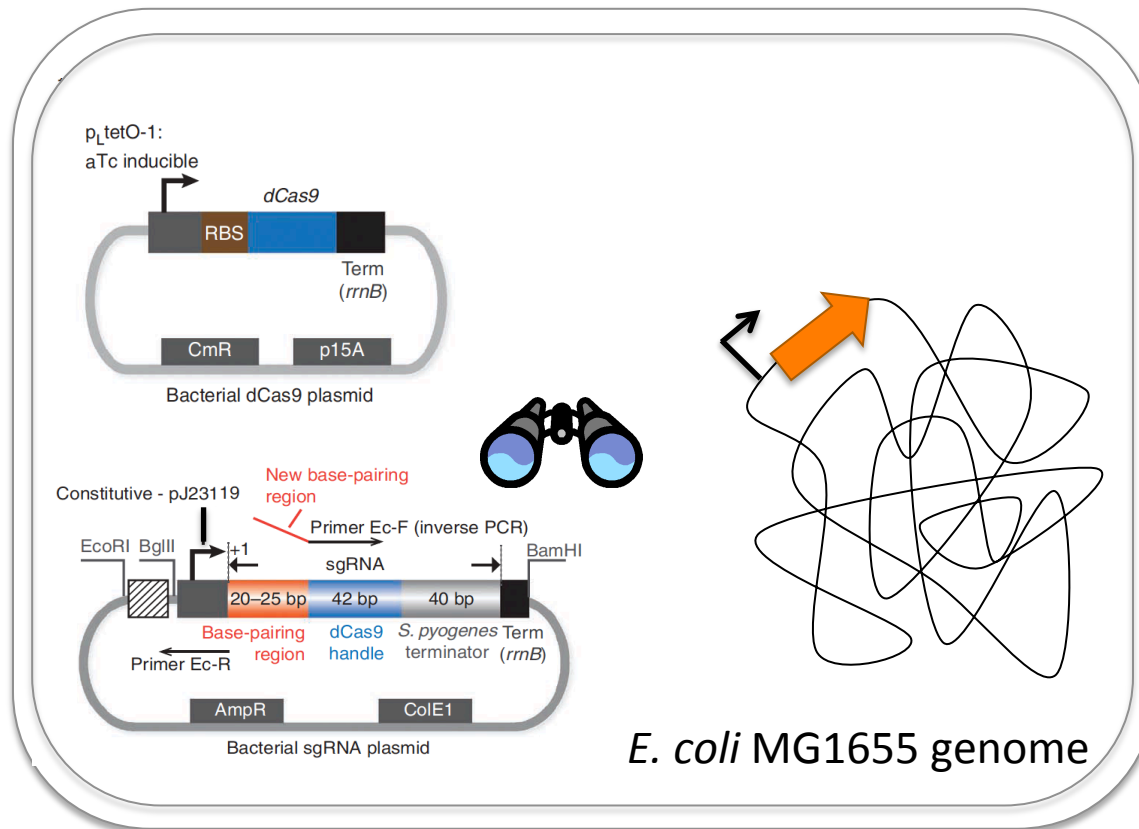


- Confirmation digest prepared on D1



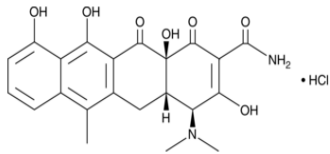
- Insert (gRNA target sequence) designed on D2

CRISPRi 'inactive' in absence of inducer

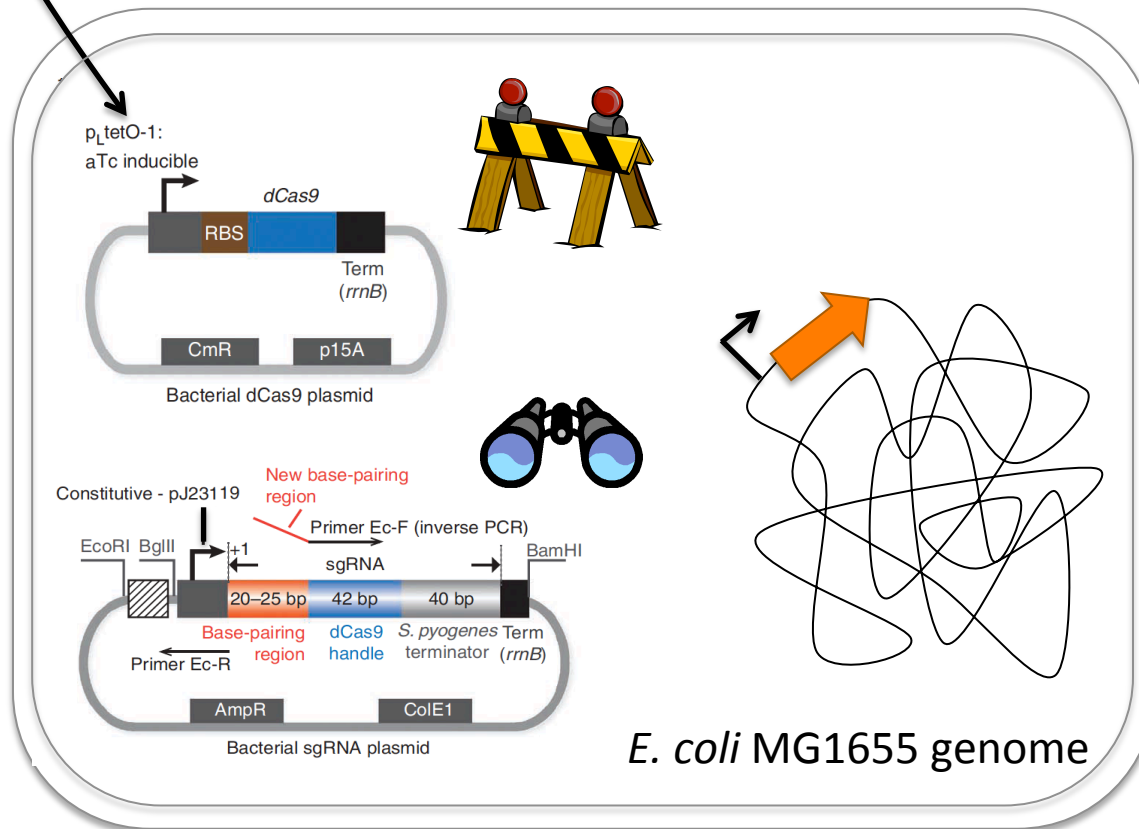


- *pgRNA_target* expressed constitutively
 - Always transcribed

CRISPRi 'blocks' gene expression in presence of inducer



aTc = anhydrotetracycline

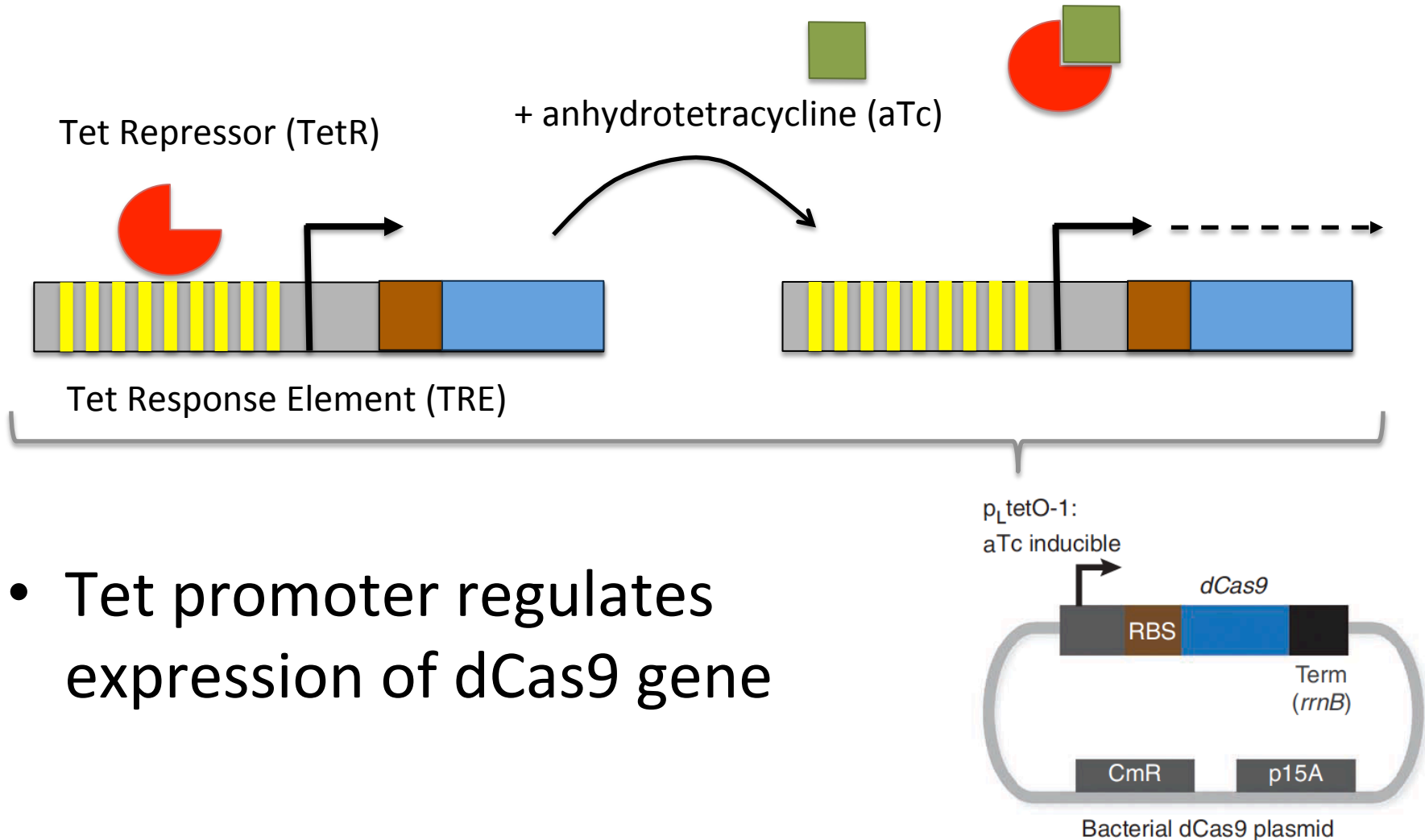


- pdCas9 expressed when aTc added
 - When transcribed associates with pgRNA_target, then target gene

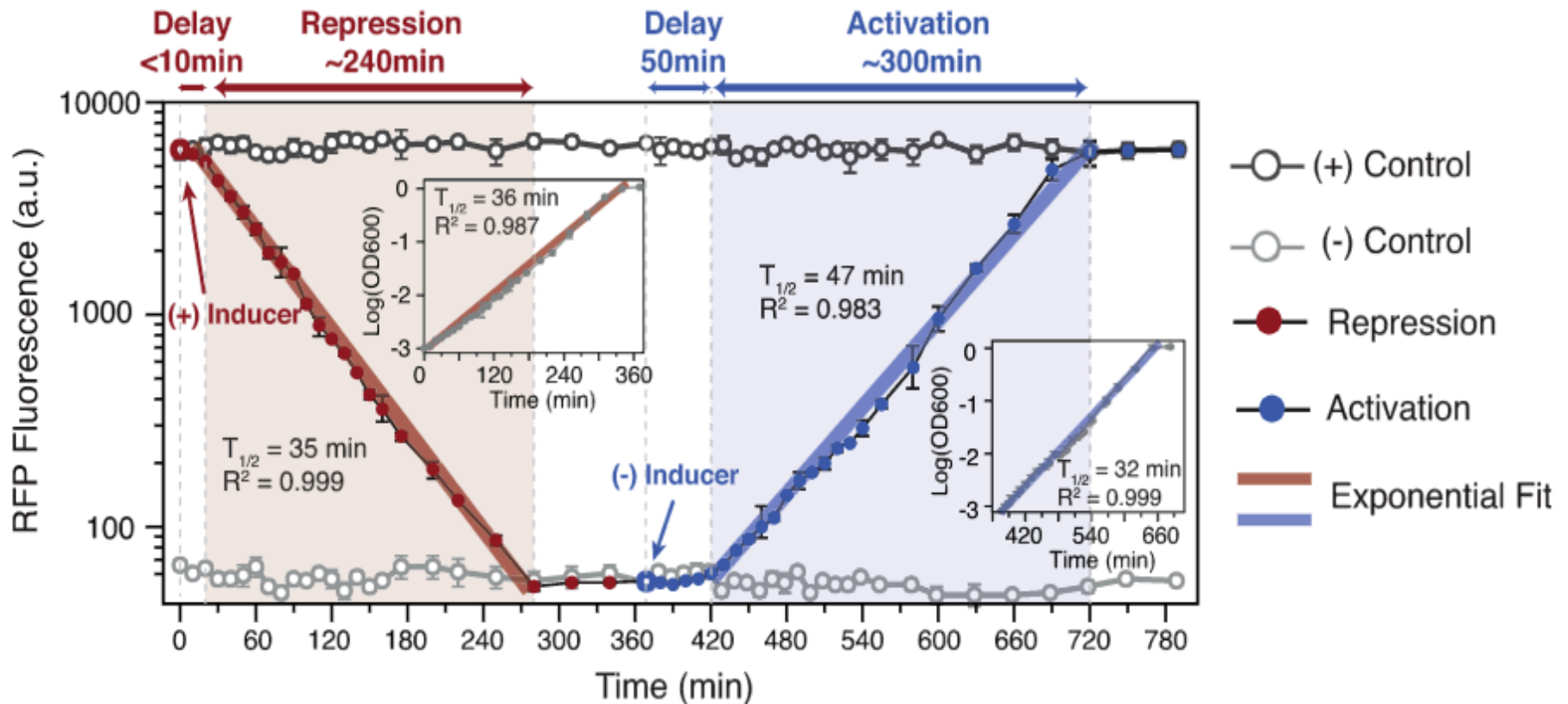
aTc is a derivative of tetracycline

- Why is aTC is a more effective inducer than the antibiotic tetracycline?
- Why doesn't aTc exhibit antibacterial activity?

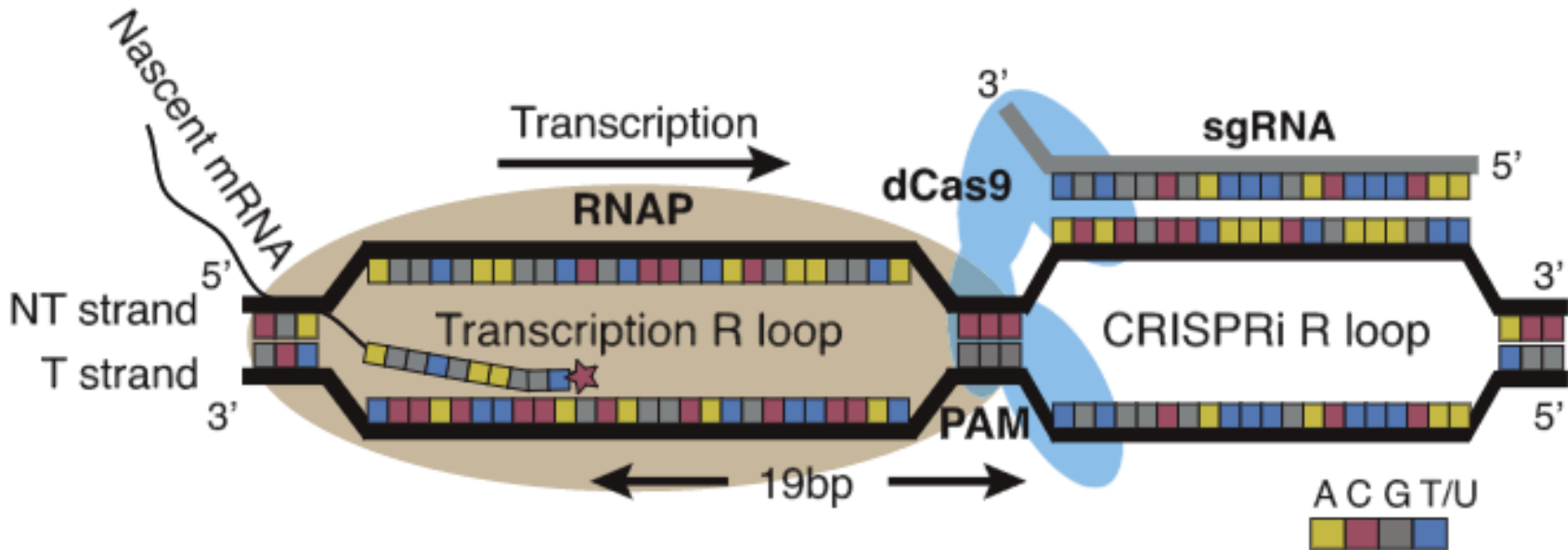
Closer look at aTc induction of pdCas9



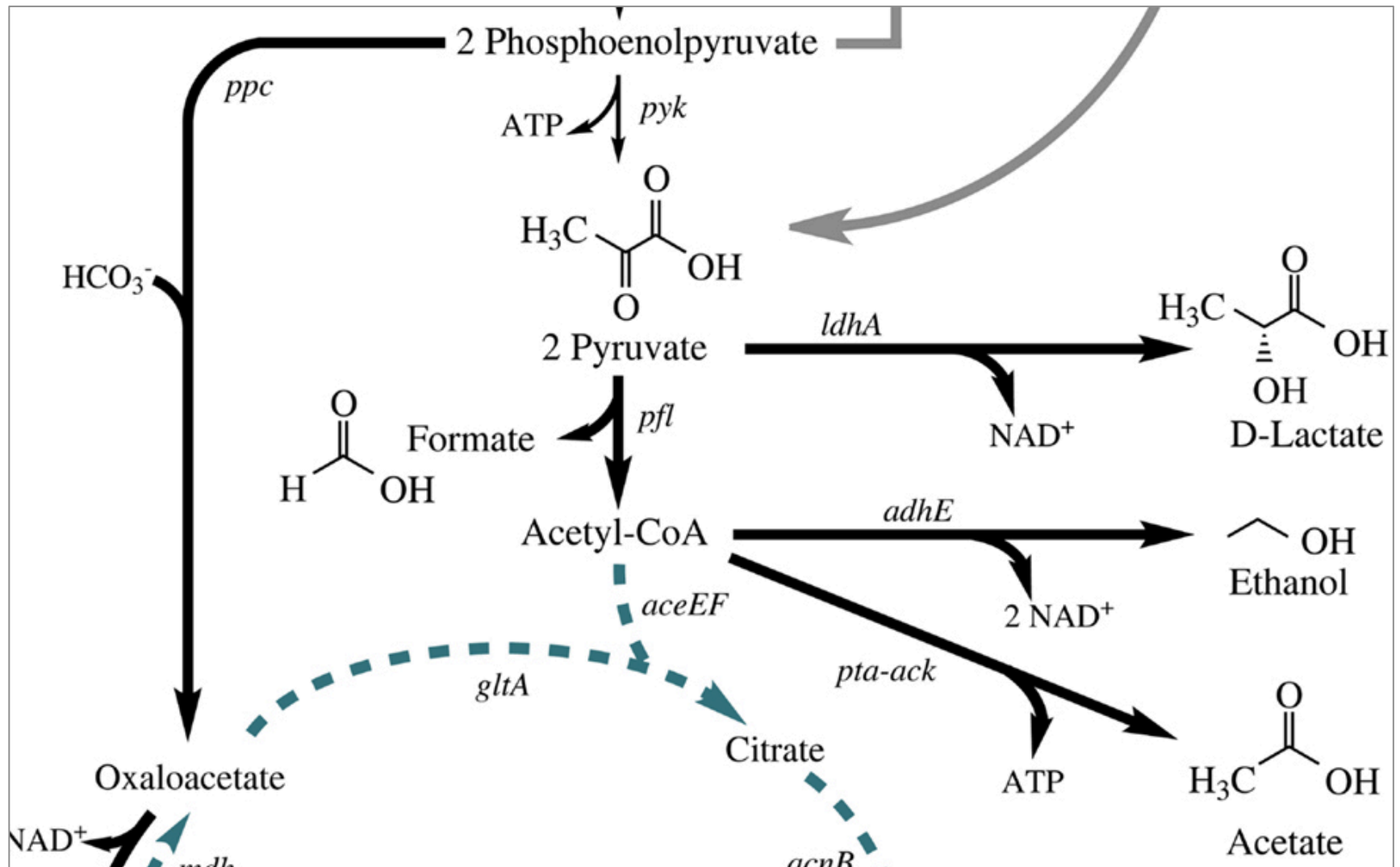
Inducible promoter can be used to control dCas9-mediated gene expression



CRISPRi collision model

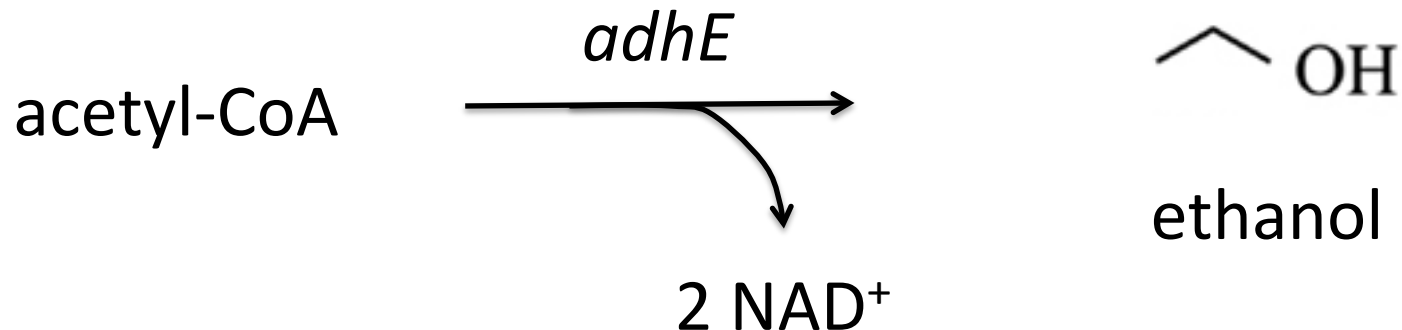


A review of the fermentation pathway



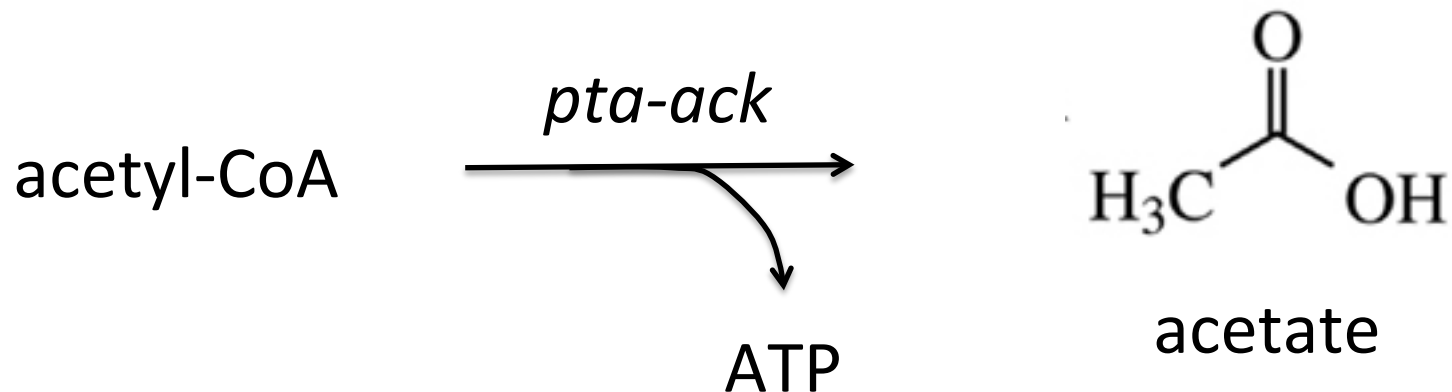
Production of ethanol

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



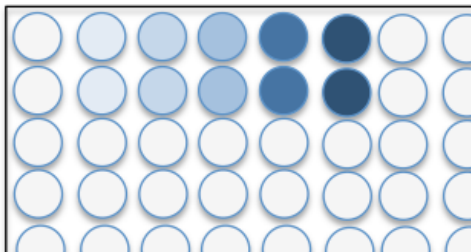
Production of acetate

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

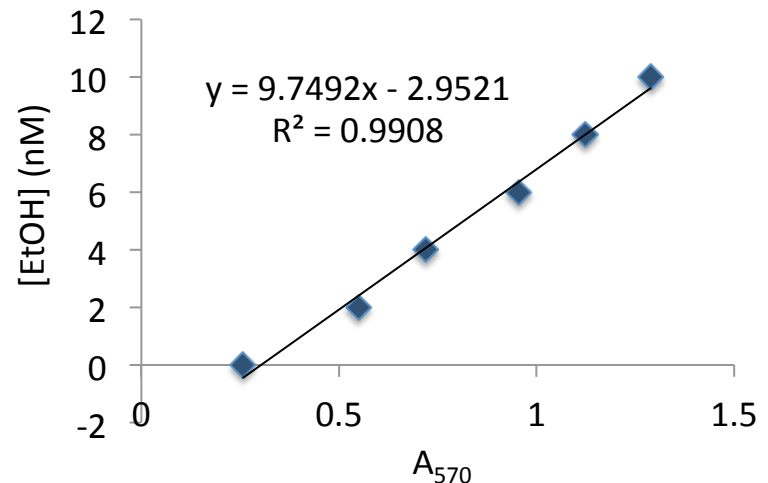


Our culminating experiment...finally!

- 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

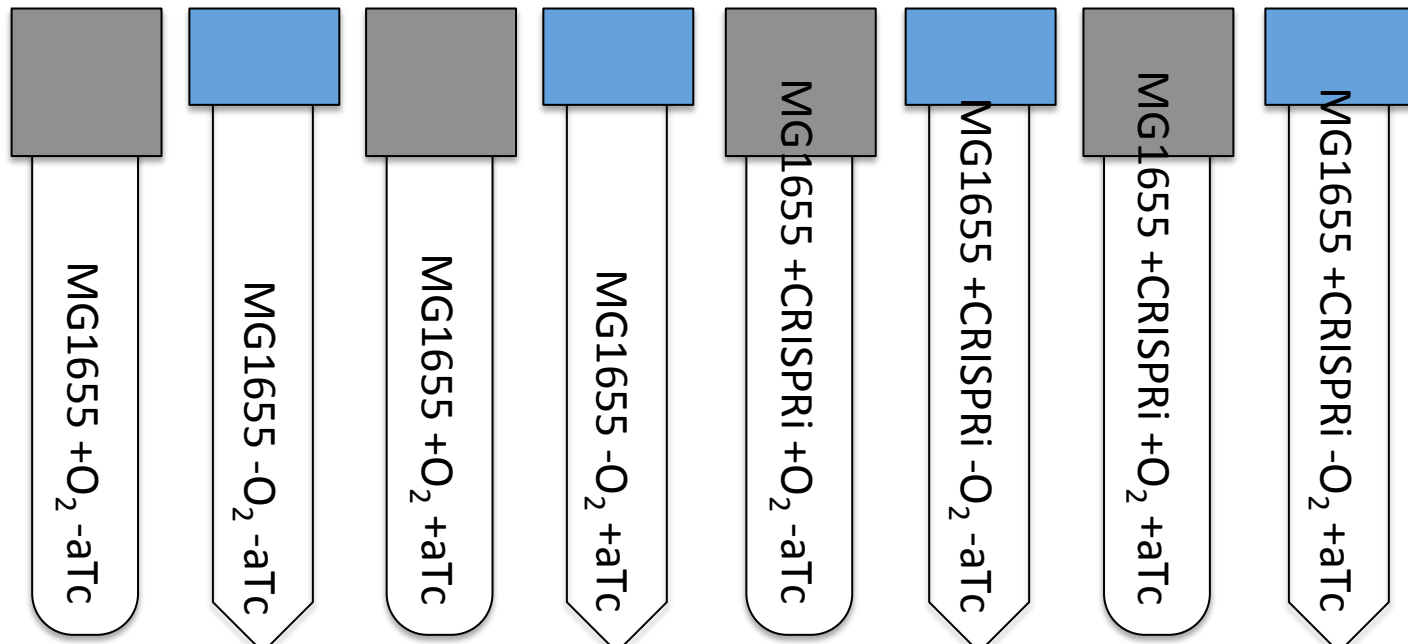


What is the experimental goal?



How will we prepare our samples?

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



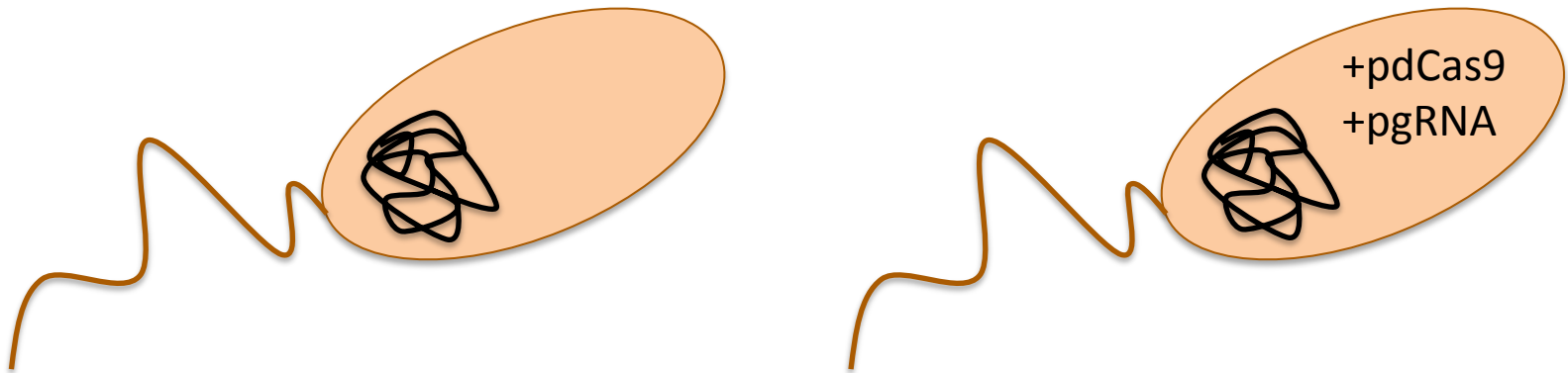
1. MG1655 vs +CRISPRi strains

What are the two conditions?

For what does this control / check?

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What are the two conditions?



For what does this control / check?

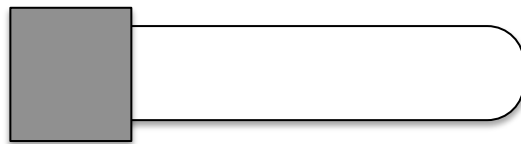
2. Aerobic vs anaerobic cultures

What are the two conditions?

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2. Aerobic vs anaerobic cultures

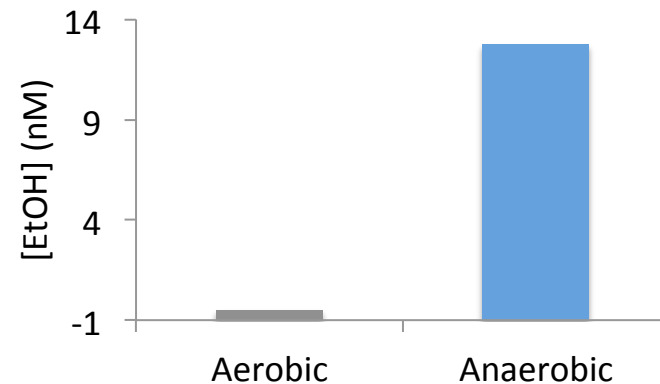
What are the two conditions?



glass tube with loose fitting cap



plastic conical tube with screw cap



For what does this control / check?

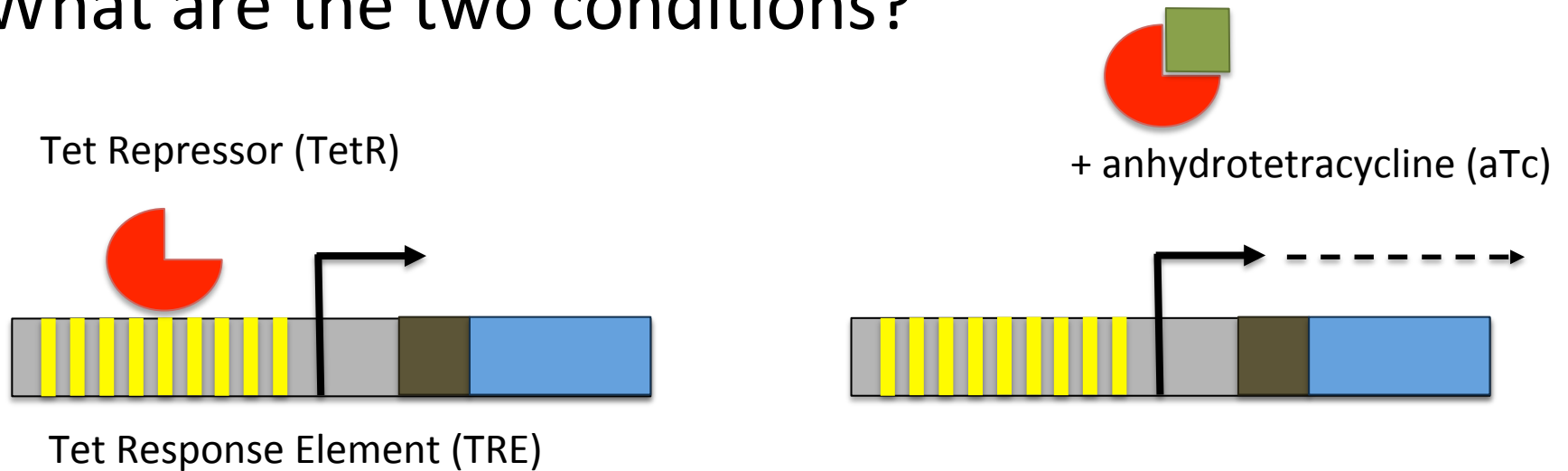
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What are the two conditions?



For what does this control / check?

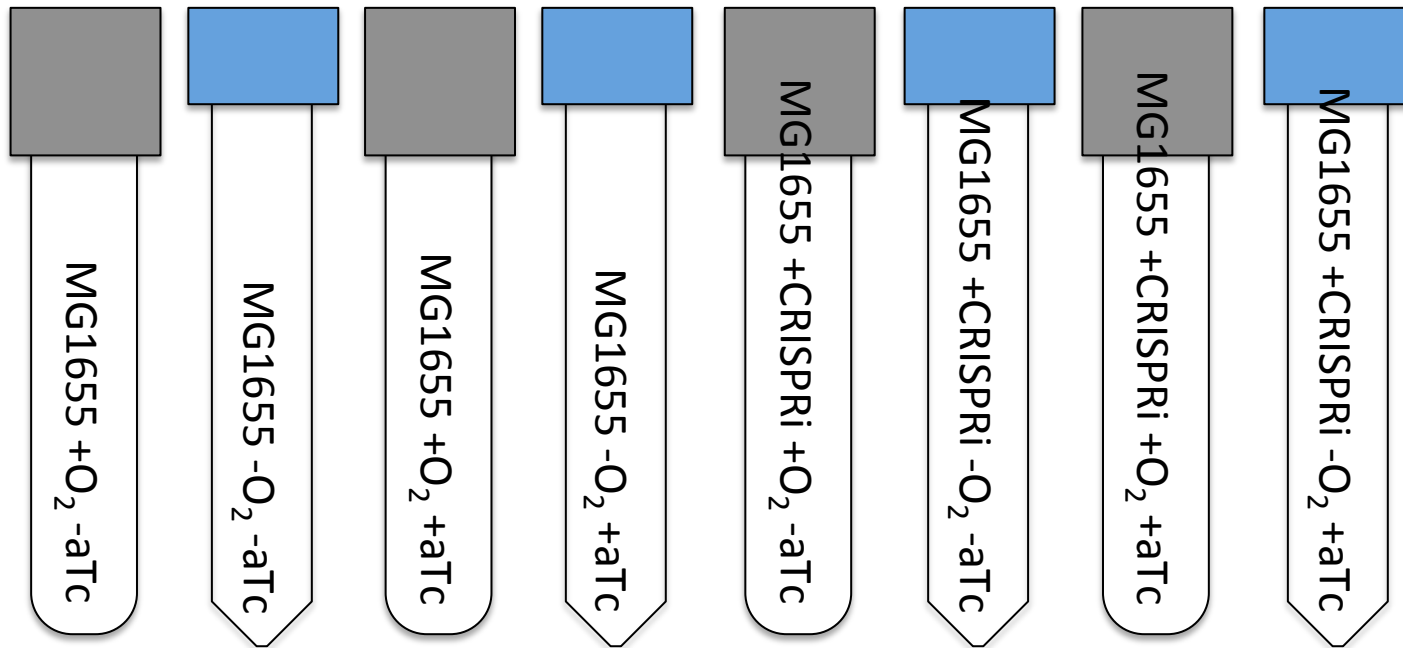
How will we represent our data?

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



What questions will *your* data address?

Specific to your experimental setup



What questions can *class* 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!

