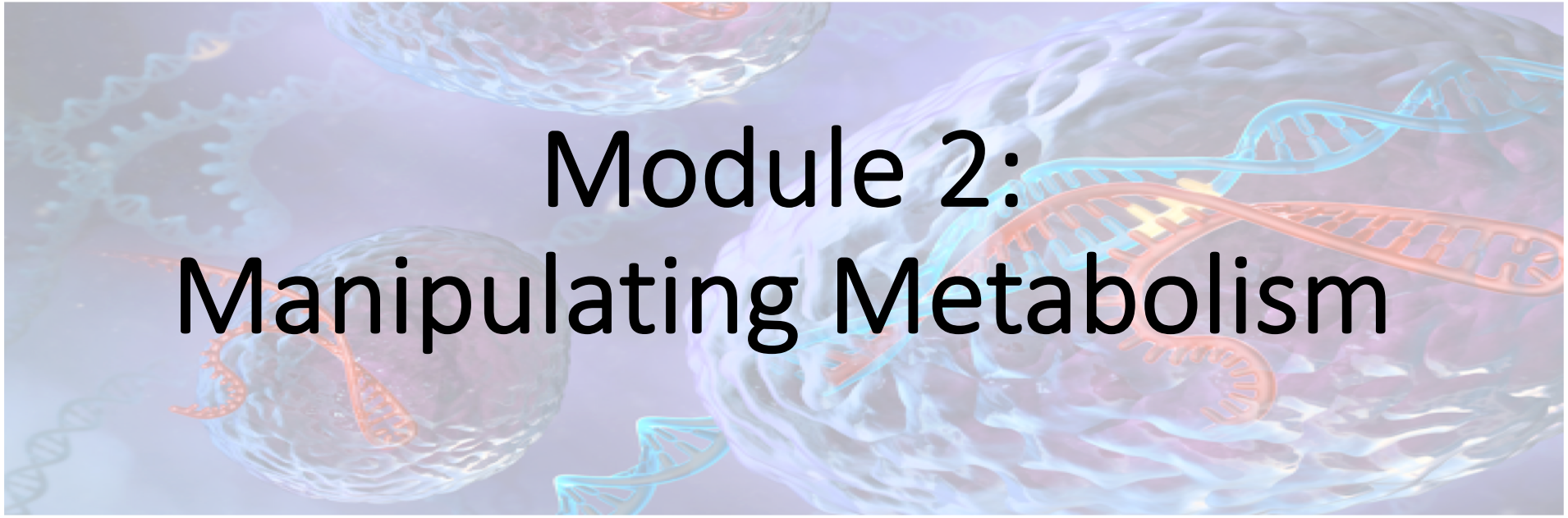


Module 2: Manipulating Metabolism

Measuring fermentation products

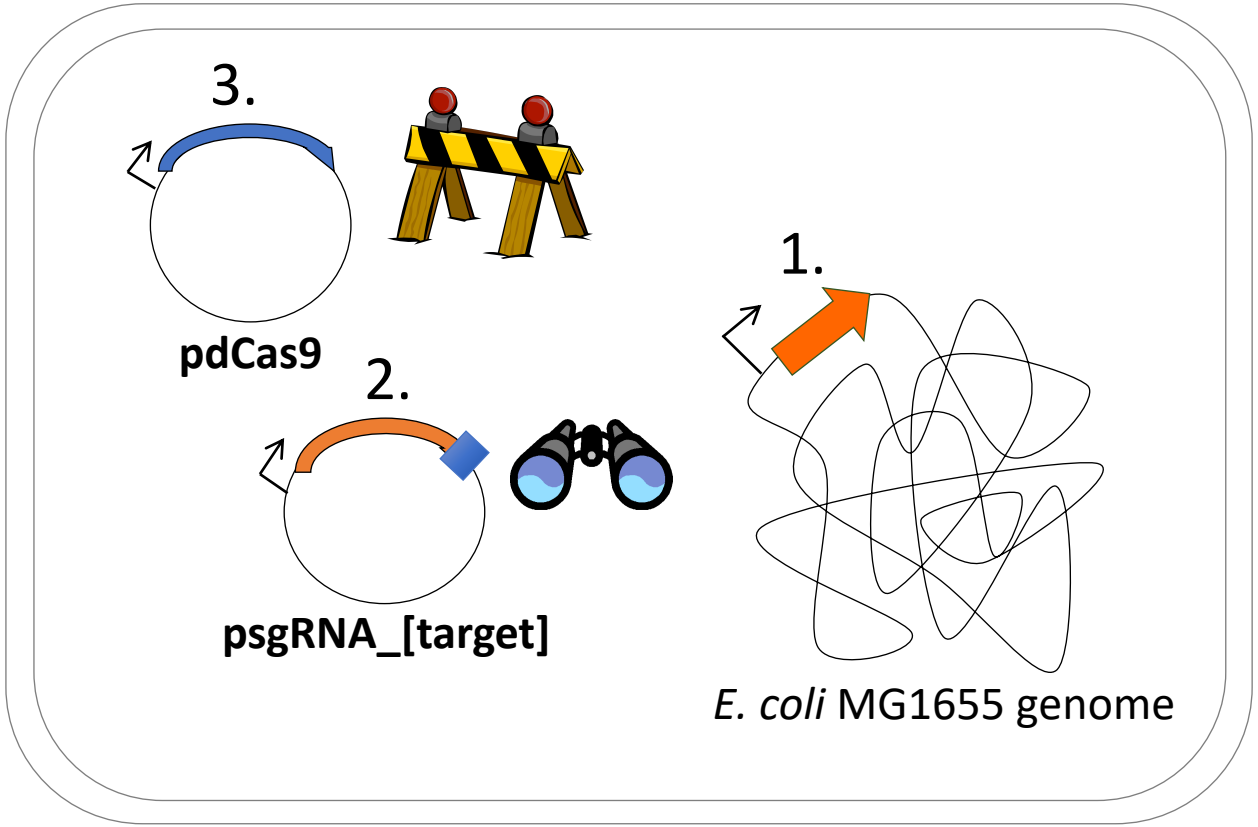
10/31/19


TIRED.
JUST TIRED.

Important (fast approaching!) Mod 2 dates

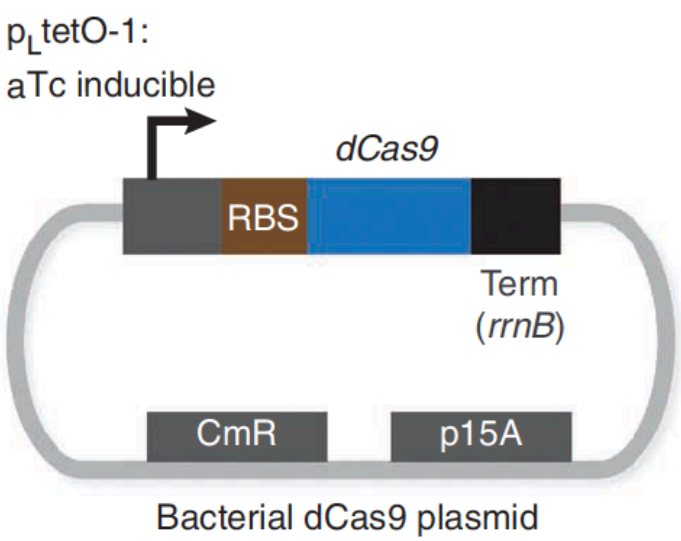
- Research article due Monday, Nov 11 by 10 pm
- Additional Office hours:
  - Thursday, Nov 7 from 5-7p
  - Saturday, Nov 9 TBA
- Normal Office hours: see wiki
- Blog post due Tuesday, Nov 12 by 10 pm

CRISPRi system involves three key players

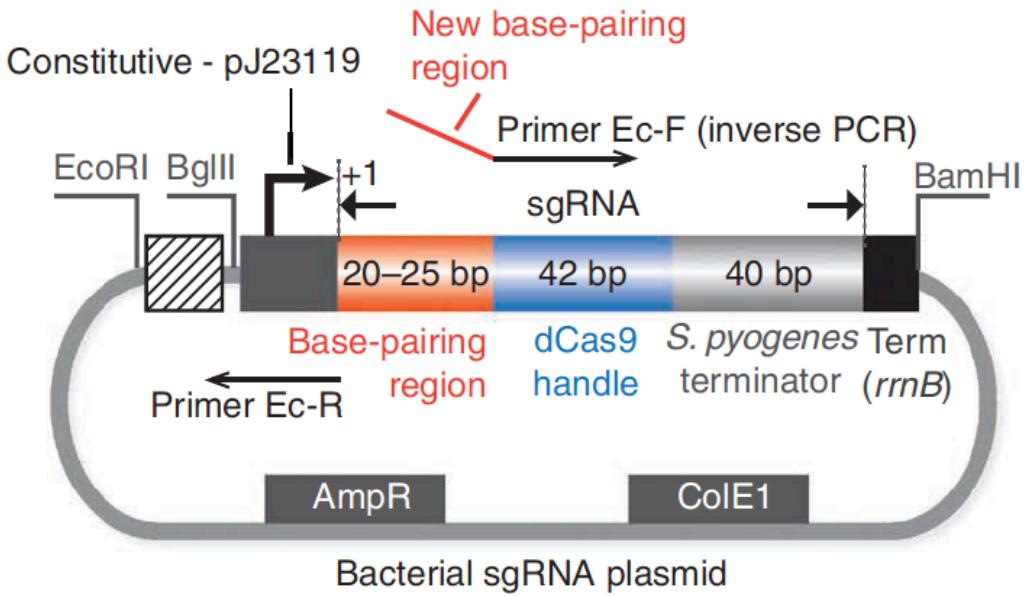


1. Target gene
2. psgRNA_[target]
3. pdCas9

Closer look at psgRNA and pdCas9

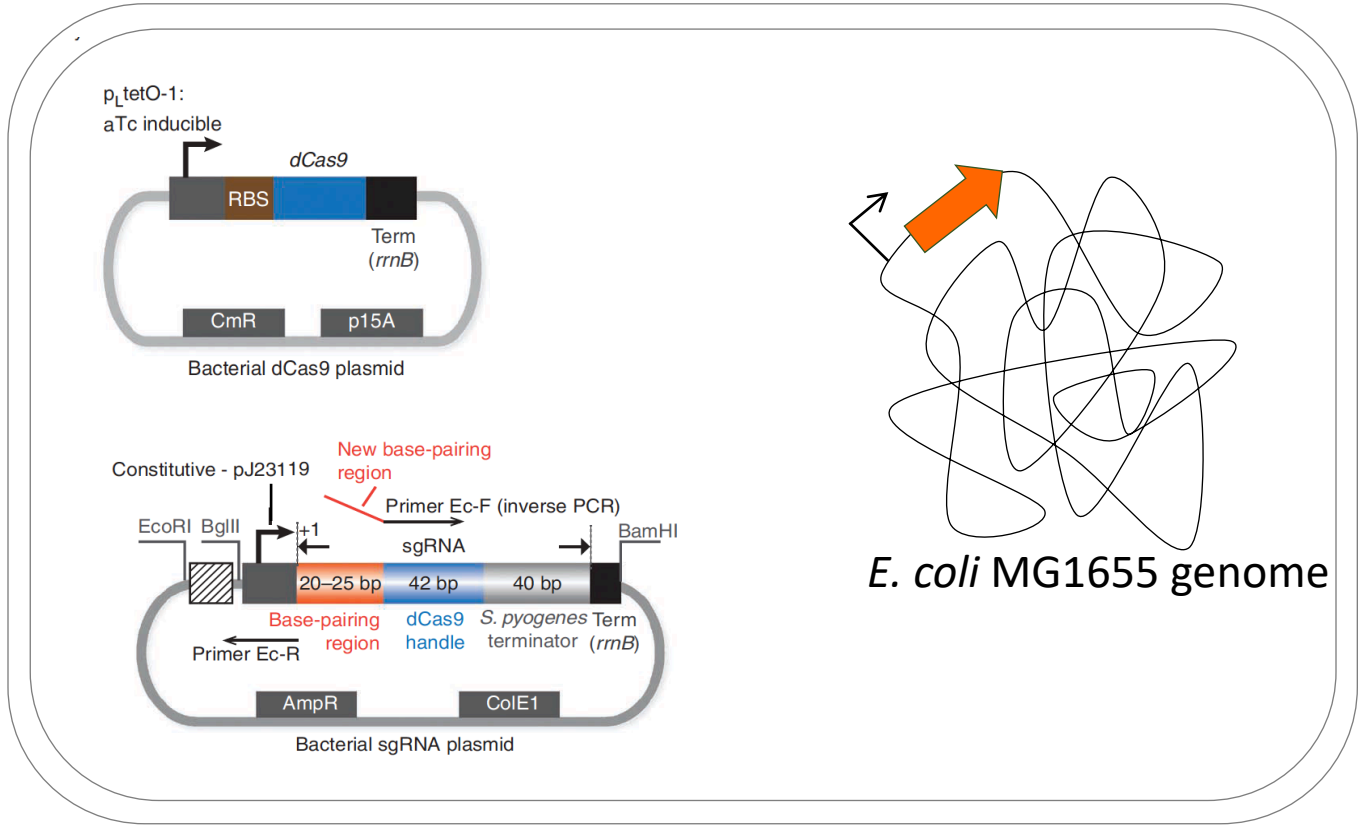


Prepared confirmation digest to check pdCas9 construct on M2D1



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

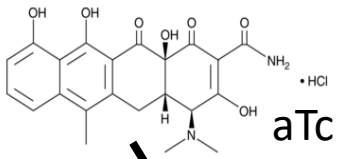
CRISPRi ‘inactive’ in absence of inducer

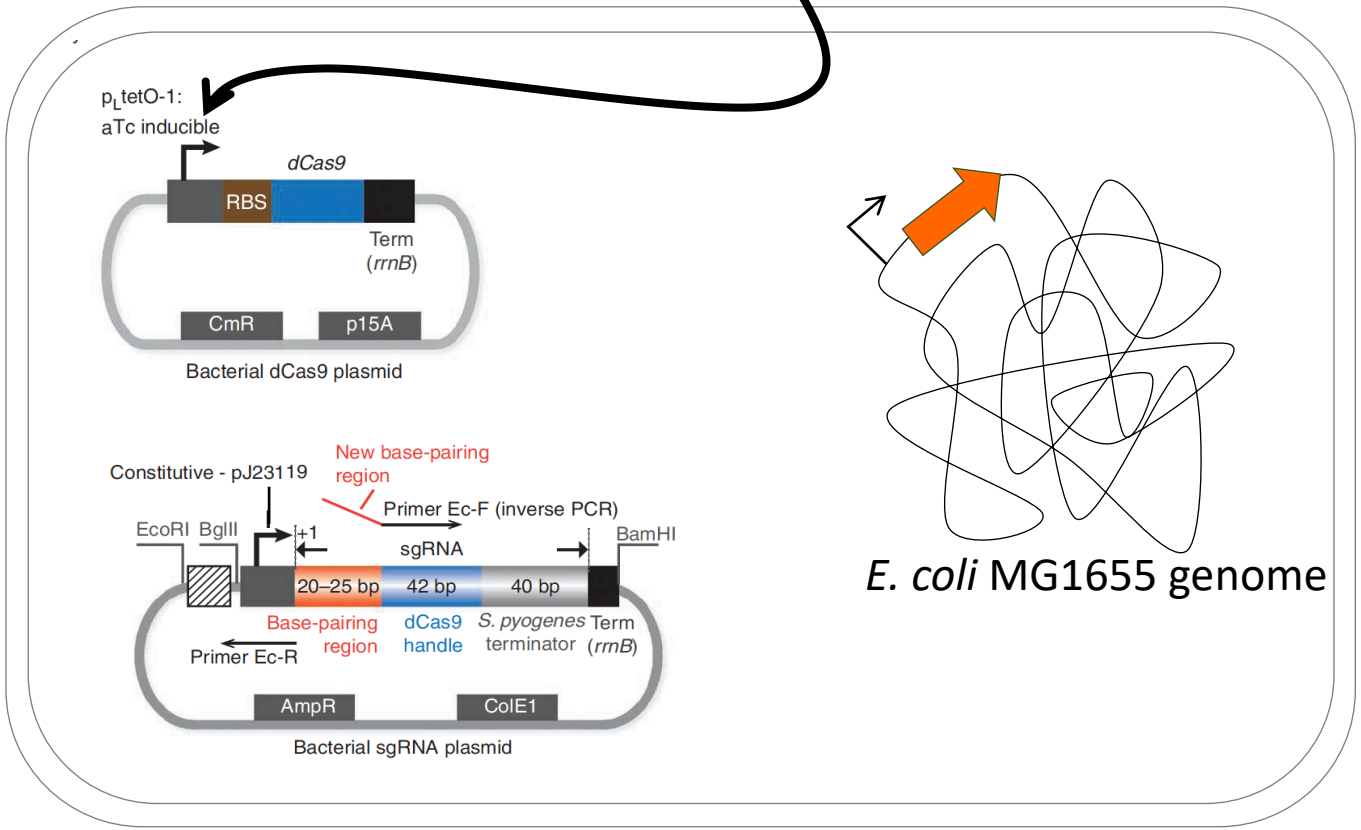


psgRNA_[target] is expressed constitutively

- sgRNA is continually transcribed

CRISPRi ‘blocks’ gene expression in presence of inducer





pdCas9 is expressed when inducer aTc added

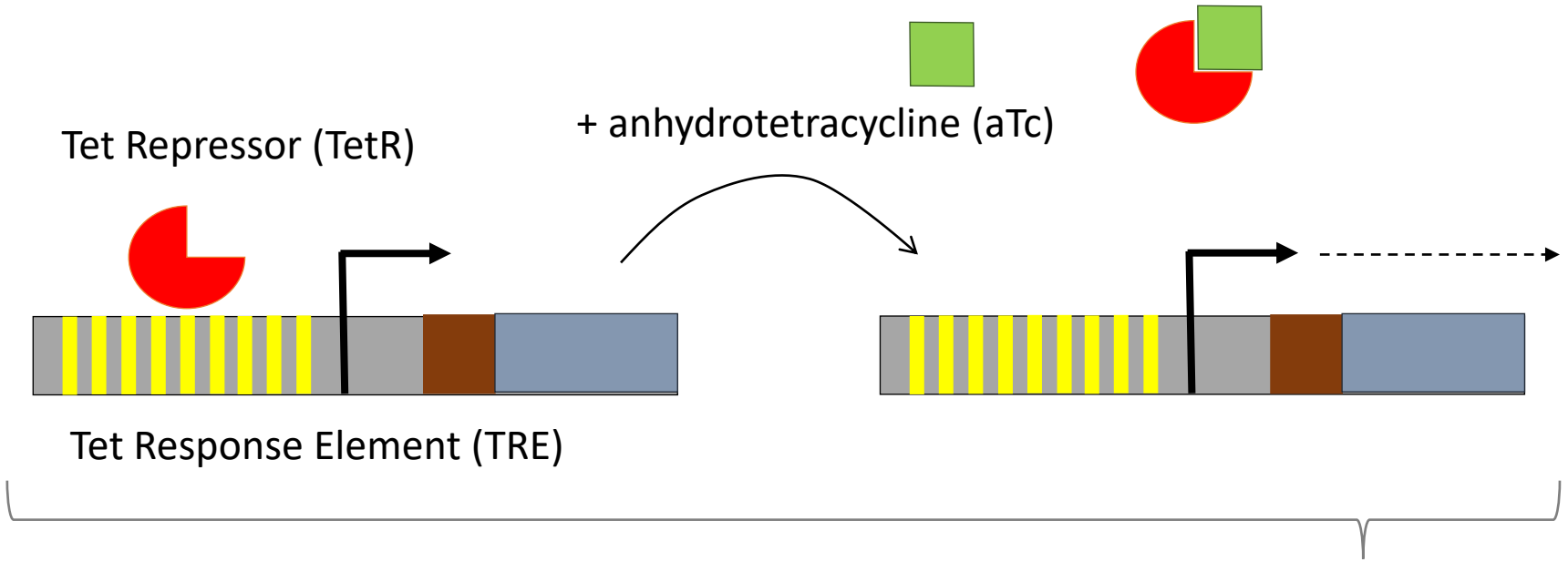
- when transcribed, associates with psgRNA_[target]
- Cas9 / psgRNA_[target] complex scans DNA for target gene

a(nhydro)Tc is a derivative of tetracycline

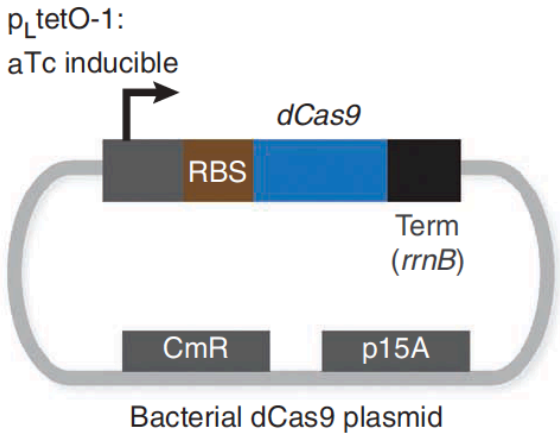
- Why is aTC is a more effective inducer than the antibiotic tetracyline?

- Why doesn’t aTc exhibit antibacterial activity?

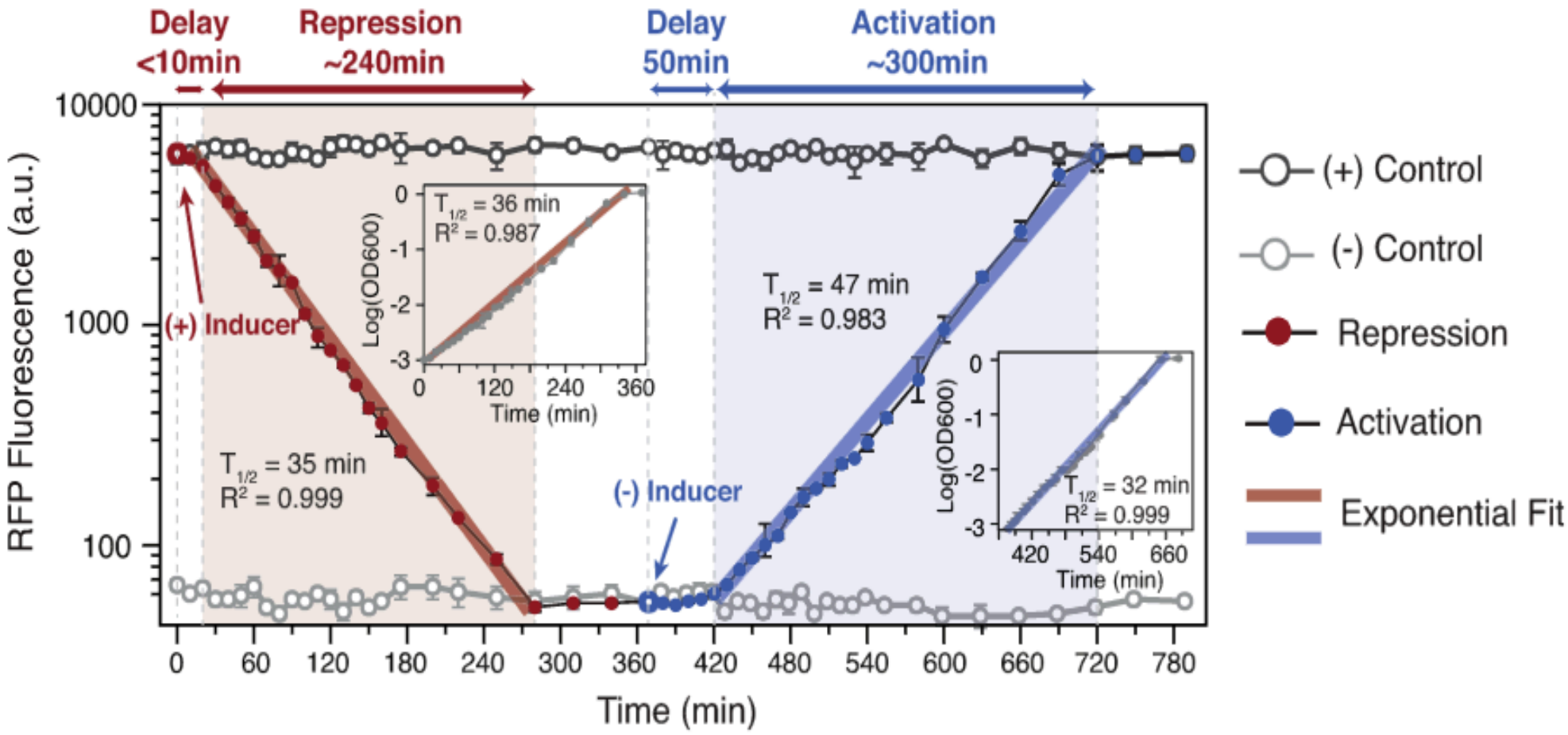
Closer look at aTc induction at TRE constructs



- Tet promoter used to regulate the expression of dCas9 gene



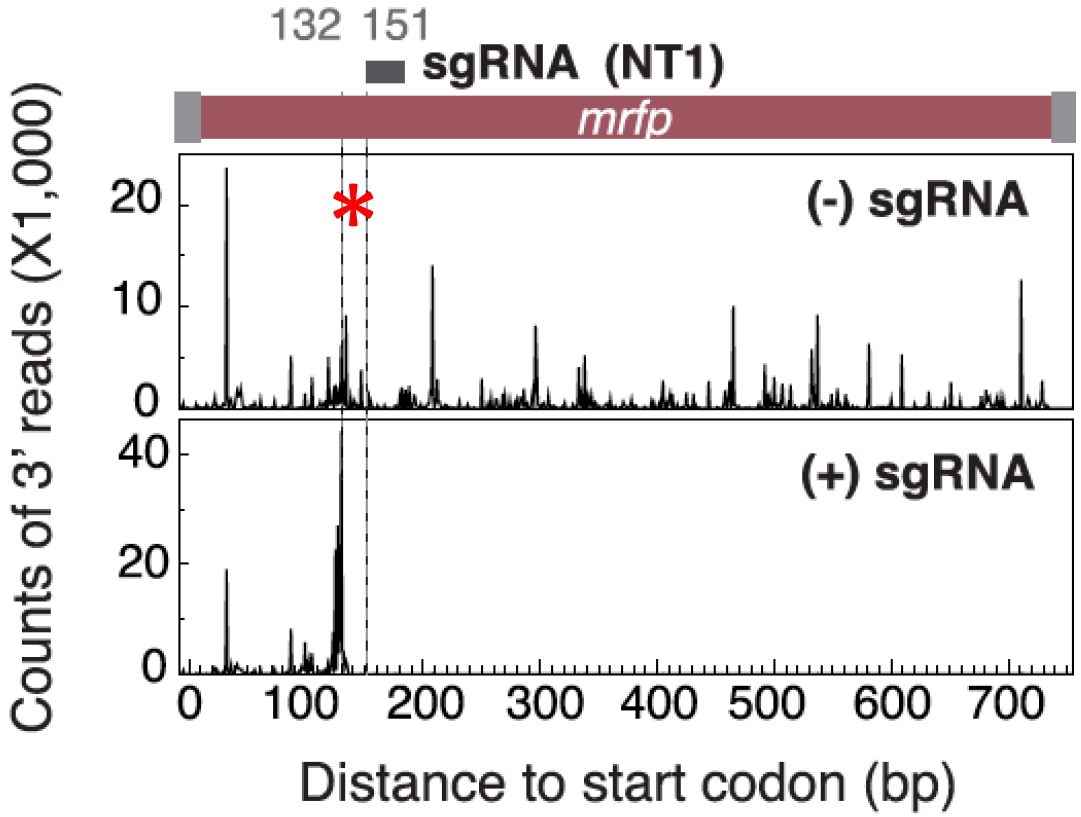
aTc-inducible promoter used to control CRISPRi inhibition of targeted gene



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

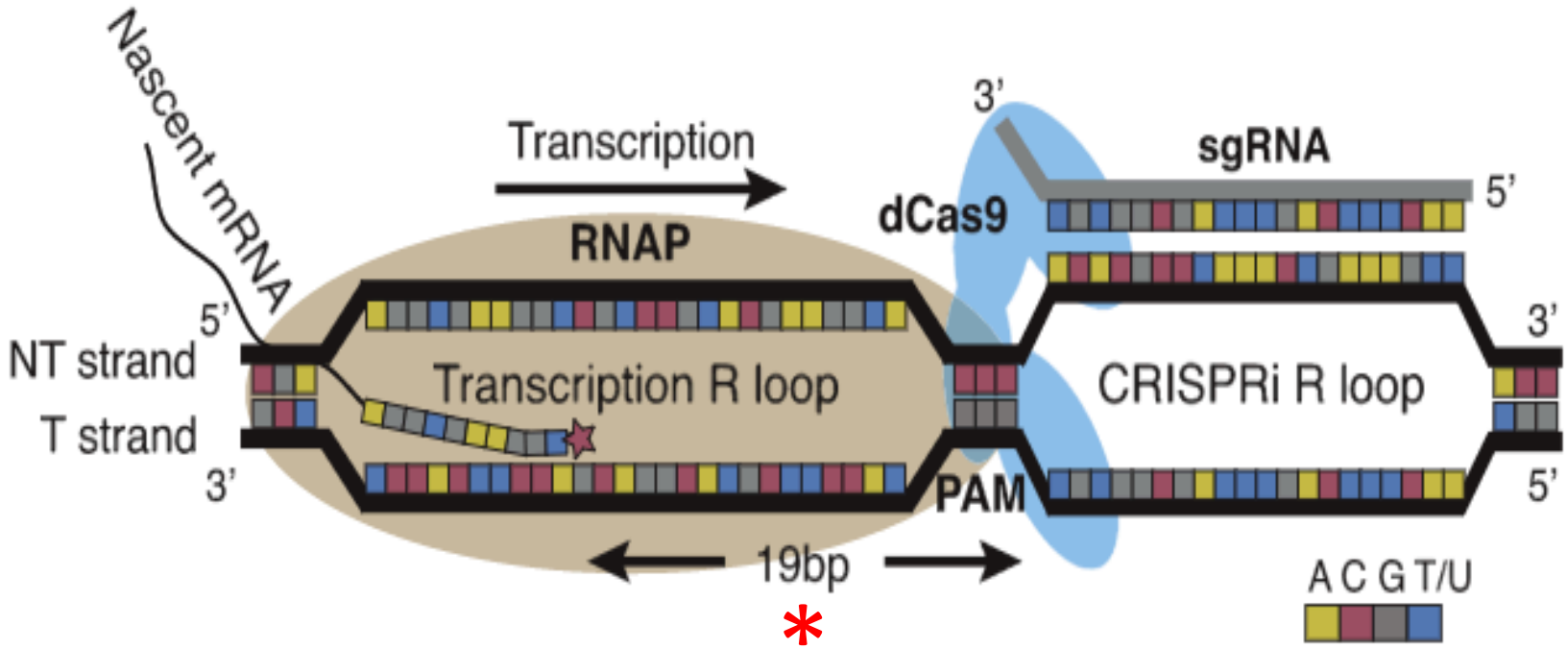
CRISPRi blocks transcription

- FLAG-tagged RNAP separated from cellular components
- Associated / bound mRNA were sequenced
- mRNA specific to RFP counted and graphed according to read length



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

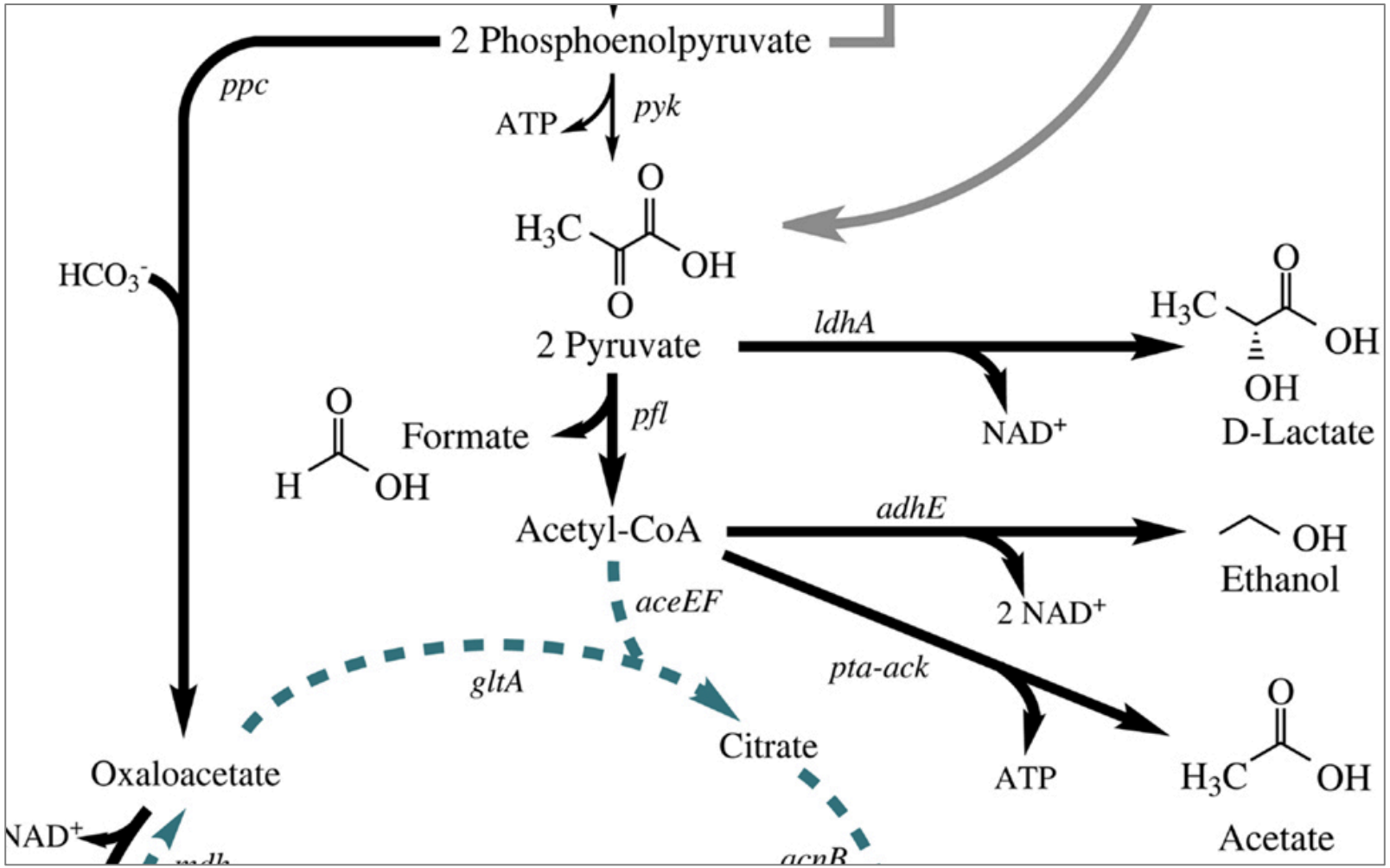
CRISPRi collision model



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

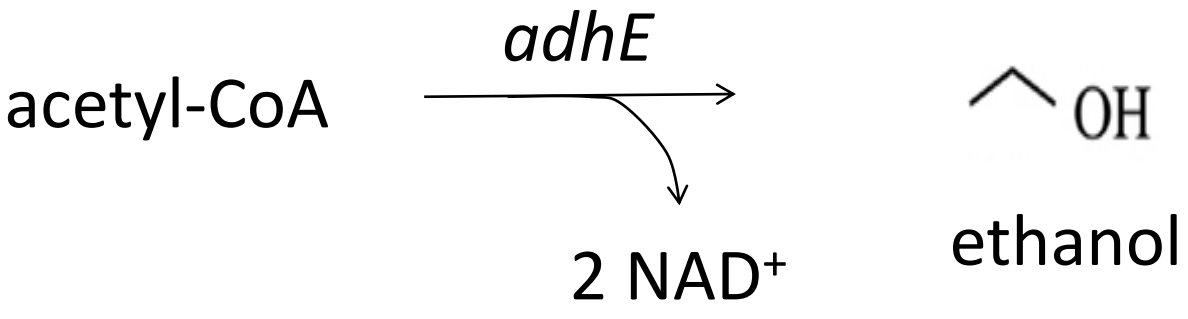
What is our experimental goal?

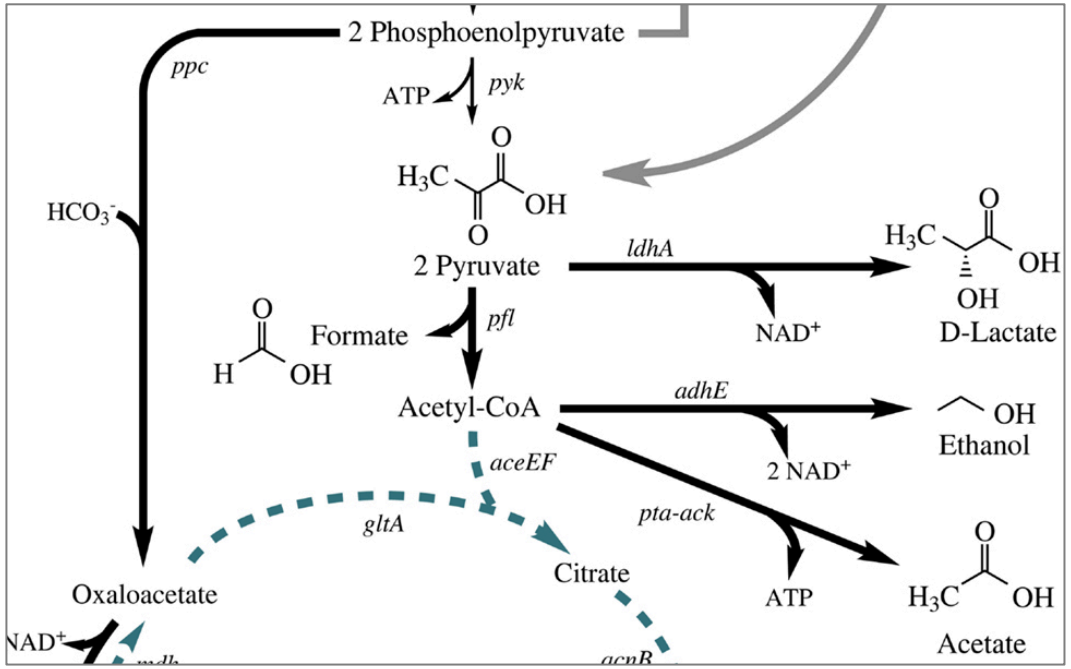
A review of the fermentation pathway



Production of ethanol

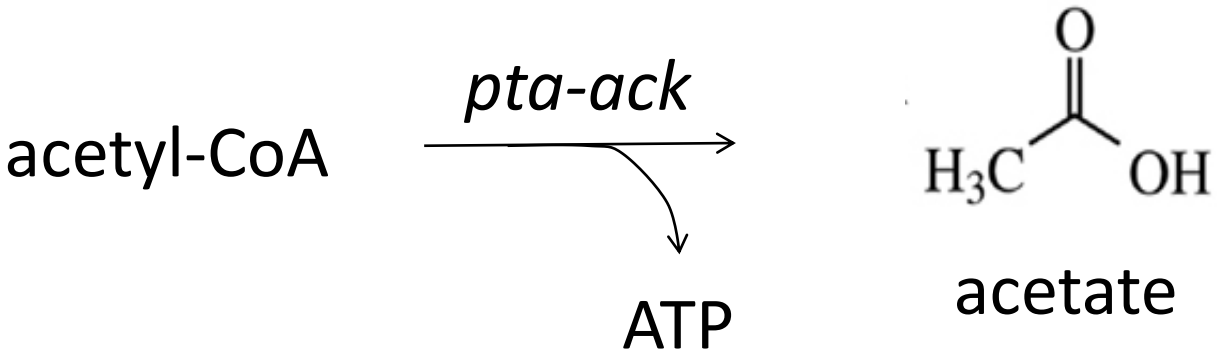
- Bioethanol is most important biotechnological commodity
- *adhE* only transcribed in anaerobic conditions

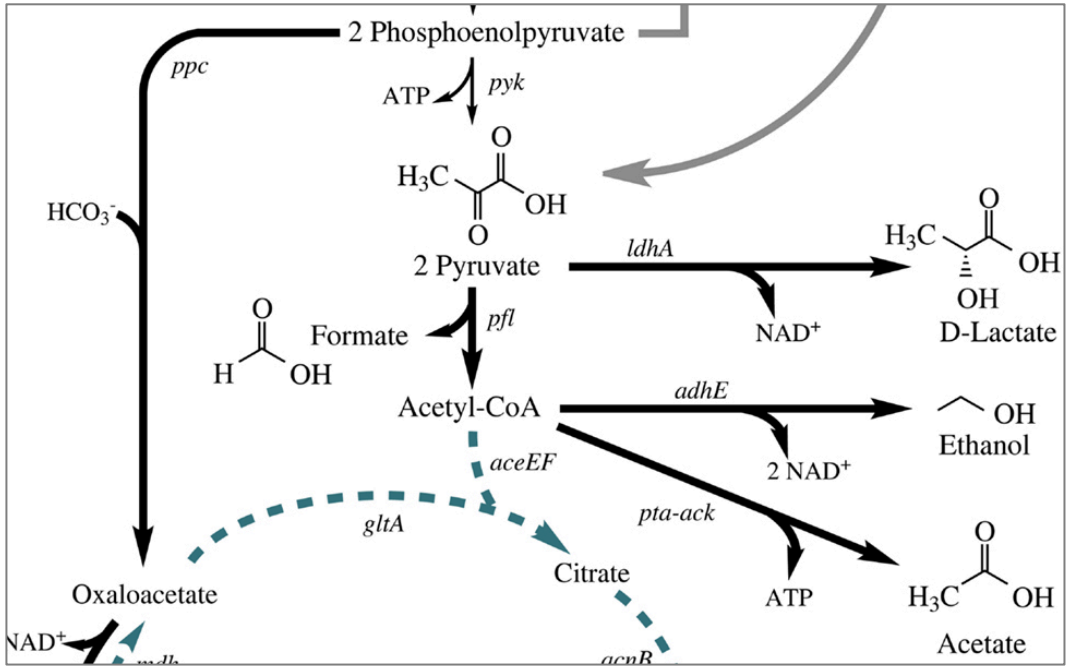




Production of acetate

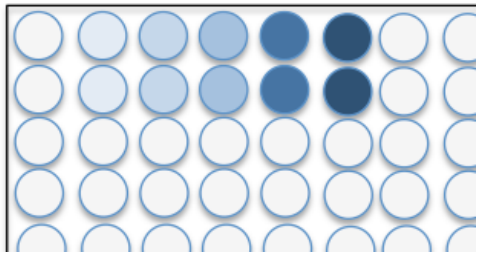
- Acetates used in production of polymers
- *pta-ack* expressed constitutively
  - Aerobically grown cells produce negligible amounts of other fermentation products



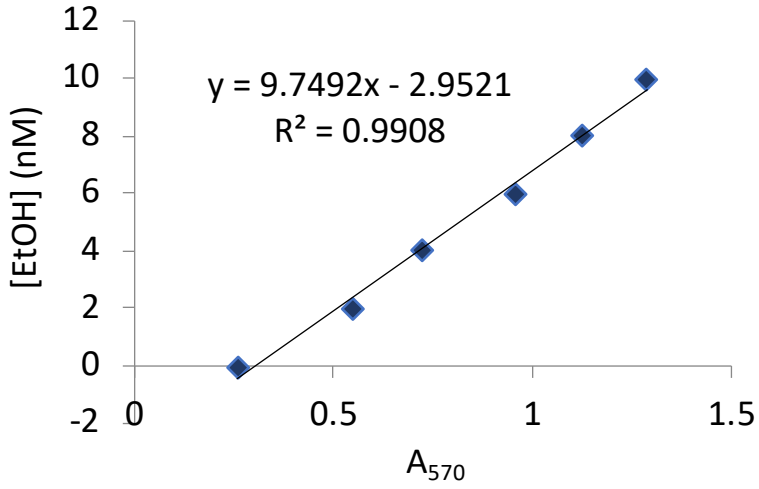


Our culminating experiment…finally!

- 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

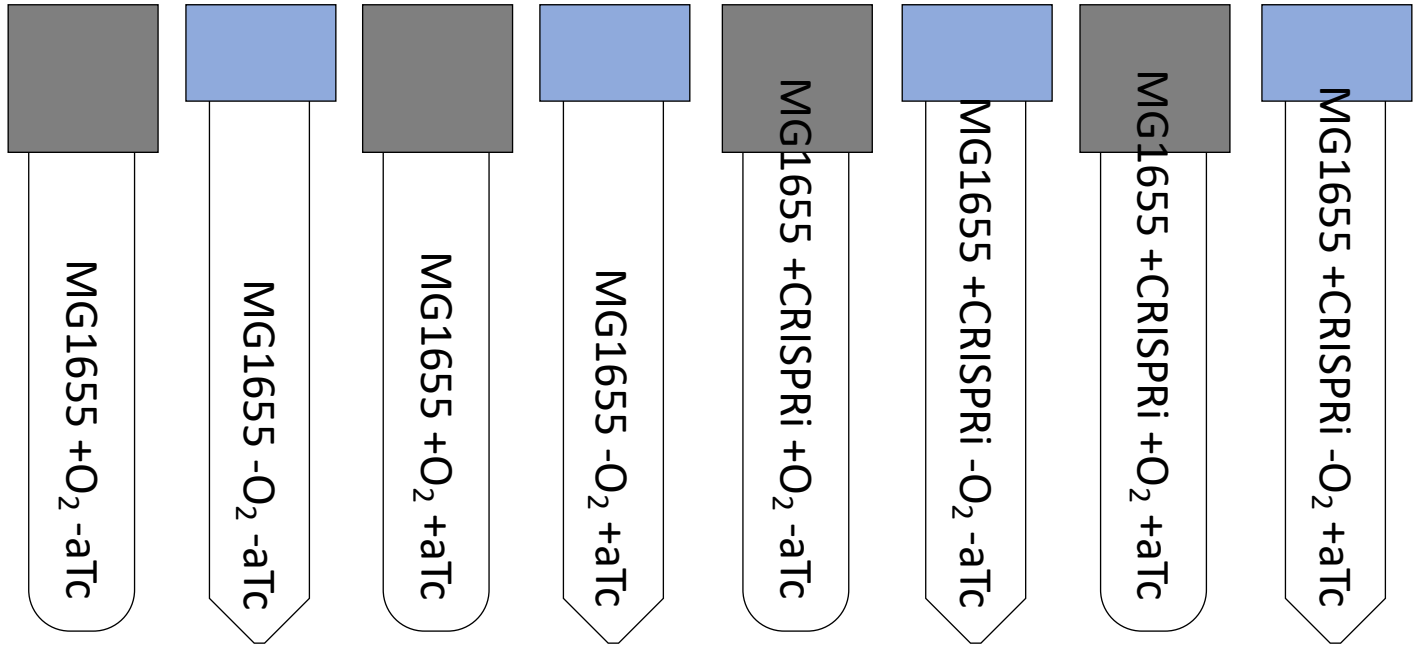


What is our experimental plan?

How will we prepare our samples?

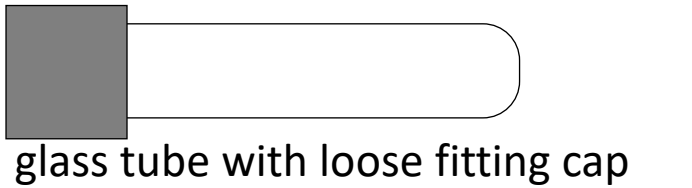
Conditions for testing hypothesis / validating experimental approach:

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

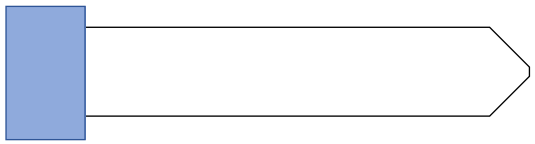


2. Aerobic vs anaerobic cultures

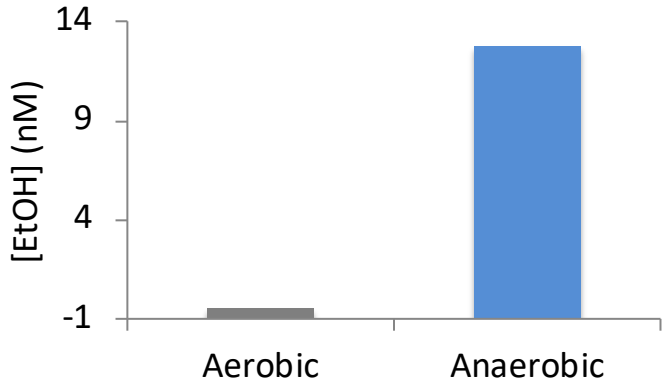
What are the two conditions?



glass tube with loose fitting cap



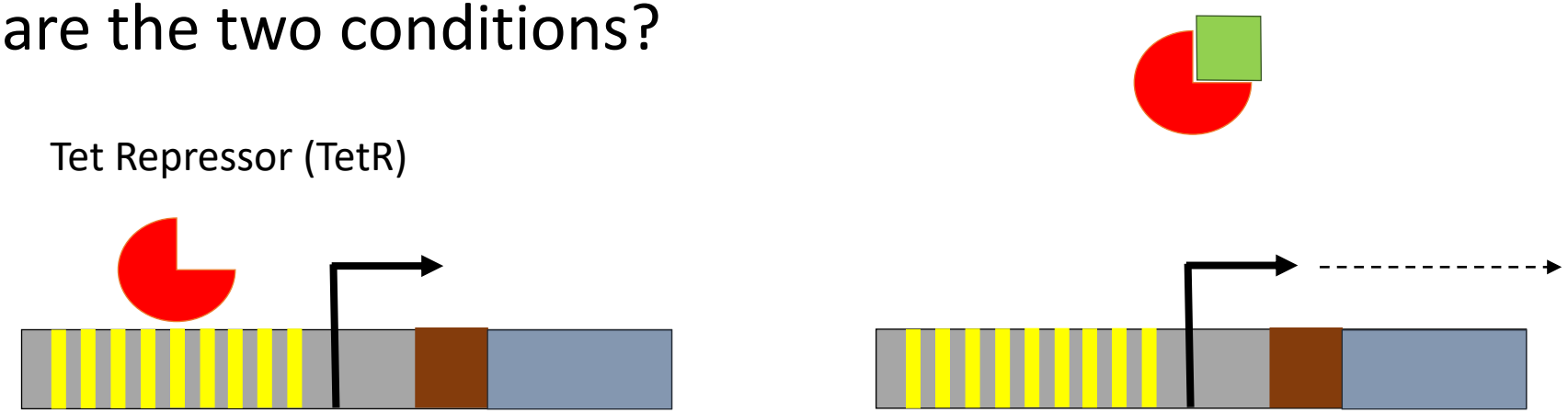
plastic conical tube with screw cap



For what does this control / check?

3. aTc induced vs uninduced

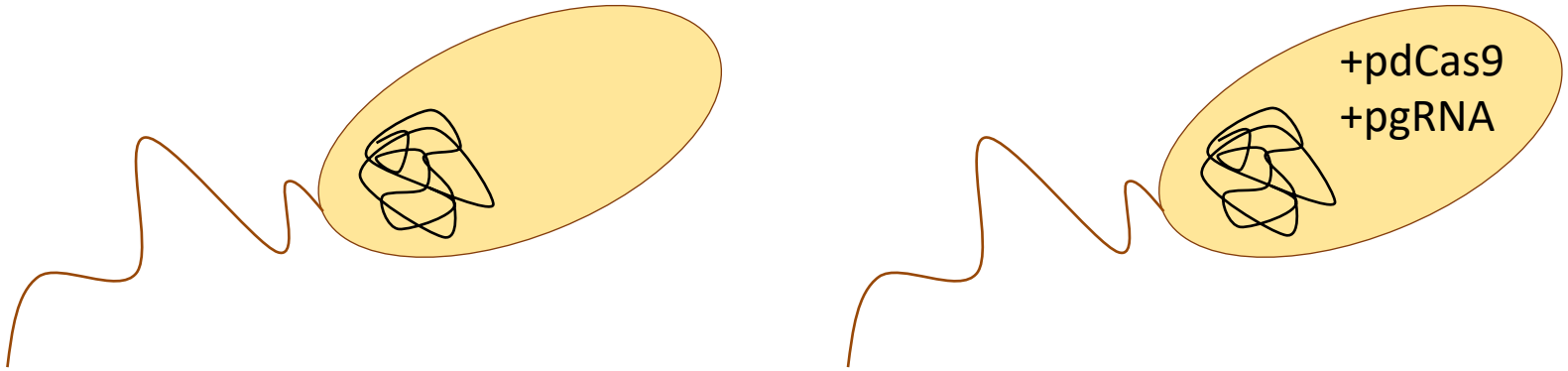
What are the two conditions?



For what does this control / check?

1. MG1655 vs +CRISPRi strains

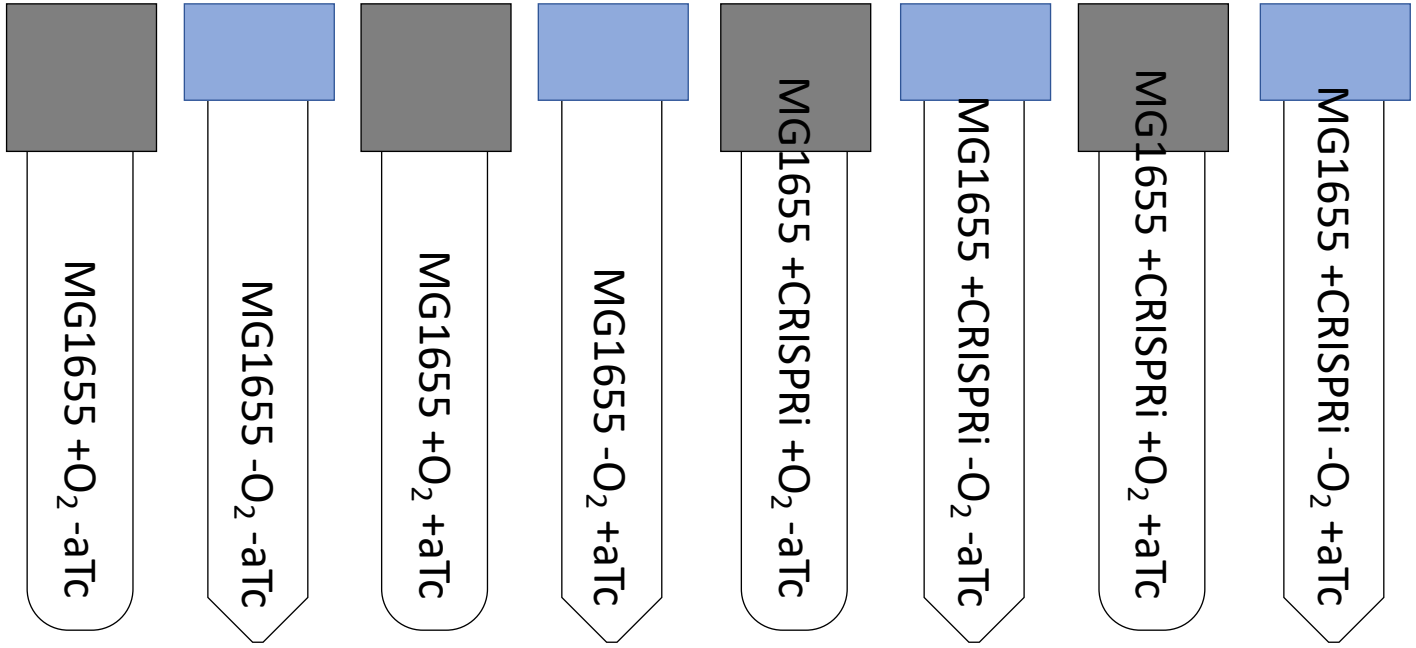
What are the two conditions?



For what does this control / check?

What questions will *your* data address?

Specific to your experimental setup



What questions can *class* data address?

How will we represent our data?

- Important to normalize fermentation product amounts

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

In the laboratory…

1. BE Communication Lab workshop
   - Manuscript architecture
2. Confirm sgRNA_[target] insertion
   - Analyze sequencing results
3. Prepare culture tubes for final experiment!
4. Use in-class ‘free time’ to work on your research article!