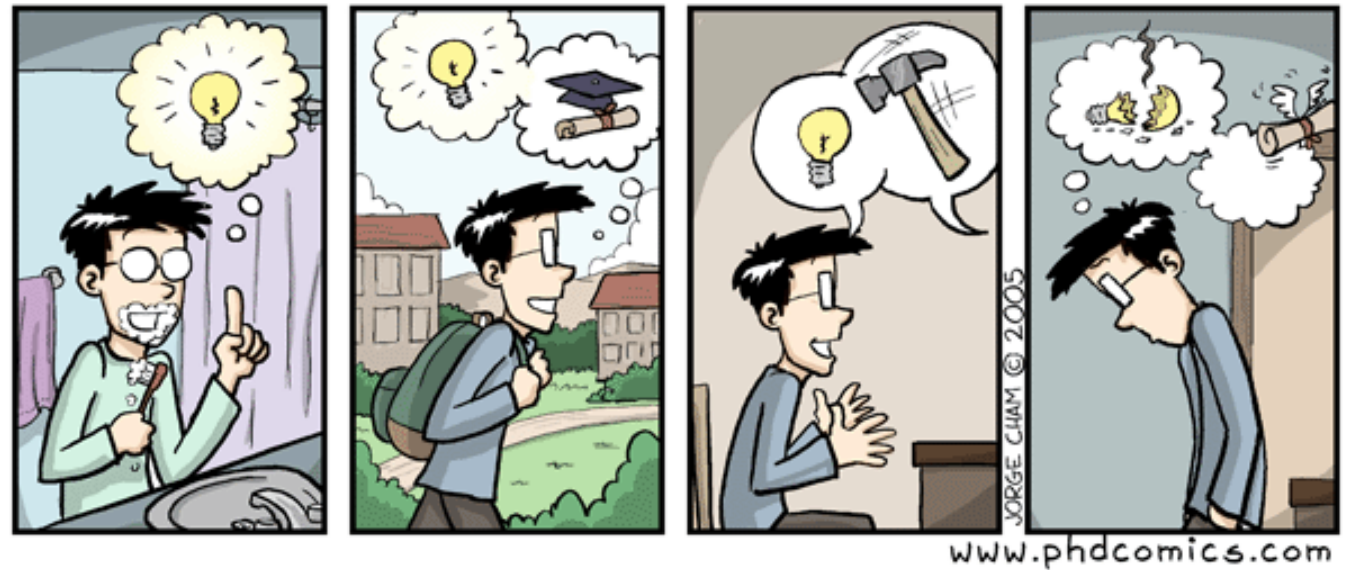
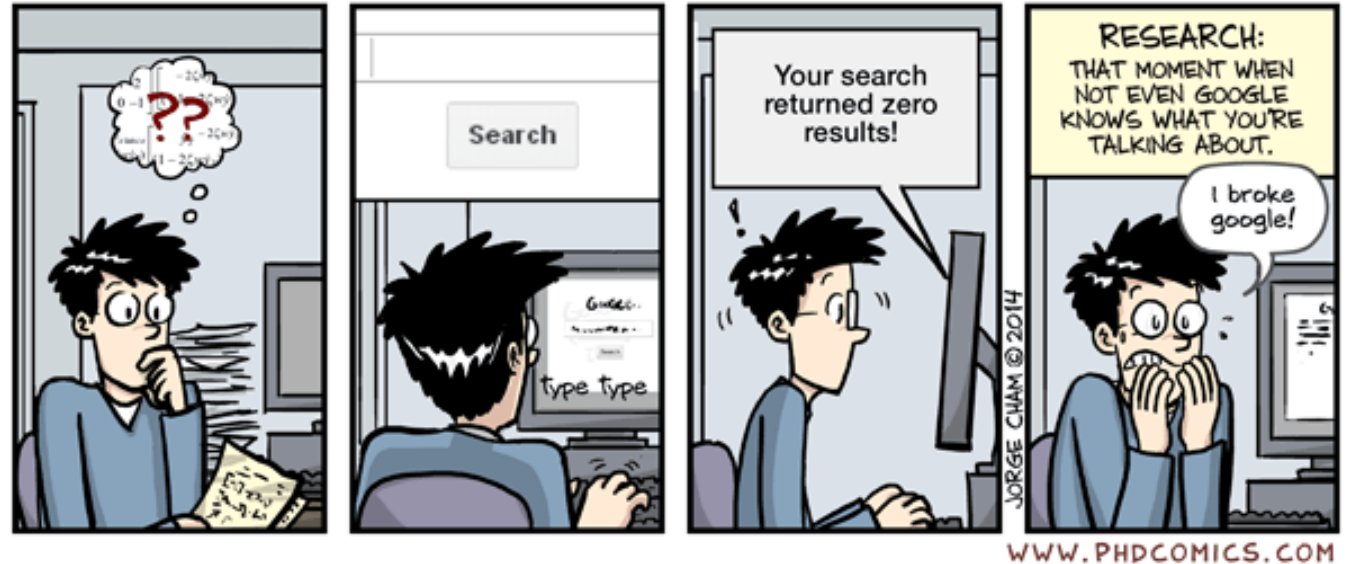


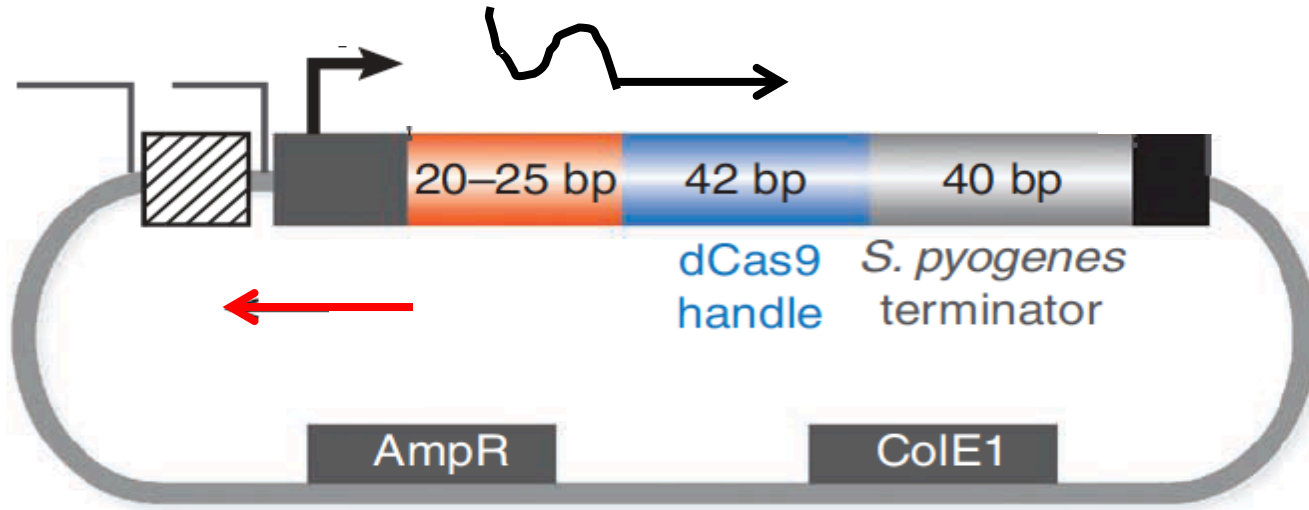
# M3D3: Analyze ethanol yield data

- Prelab discussion
- Quiz!
- Analyze ethanol yield data
- Work on proposals

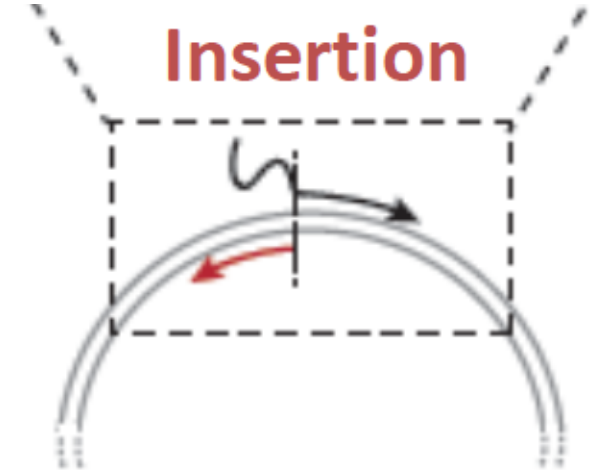




# Generate pgRNA\_target by SDM



pgRNA\_template



Insertion (q5 kit)

← CRISPRi universal *amplification* reverse primer

→ forward primer including sgRNA + dCas9 handle to be inserted (wavy line)



# Analyzing Sequence Information

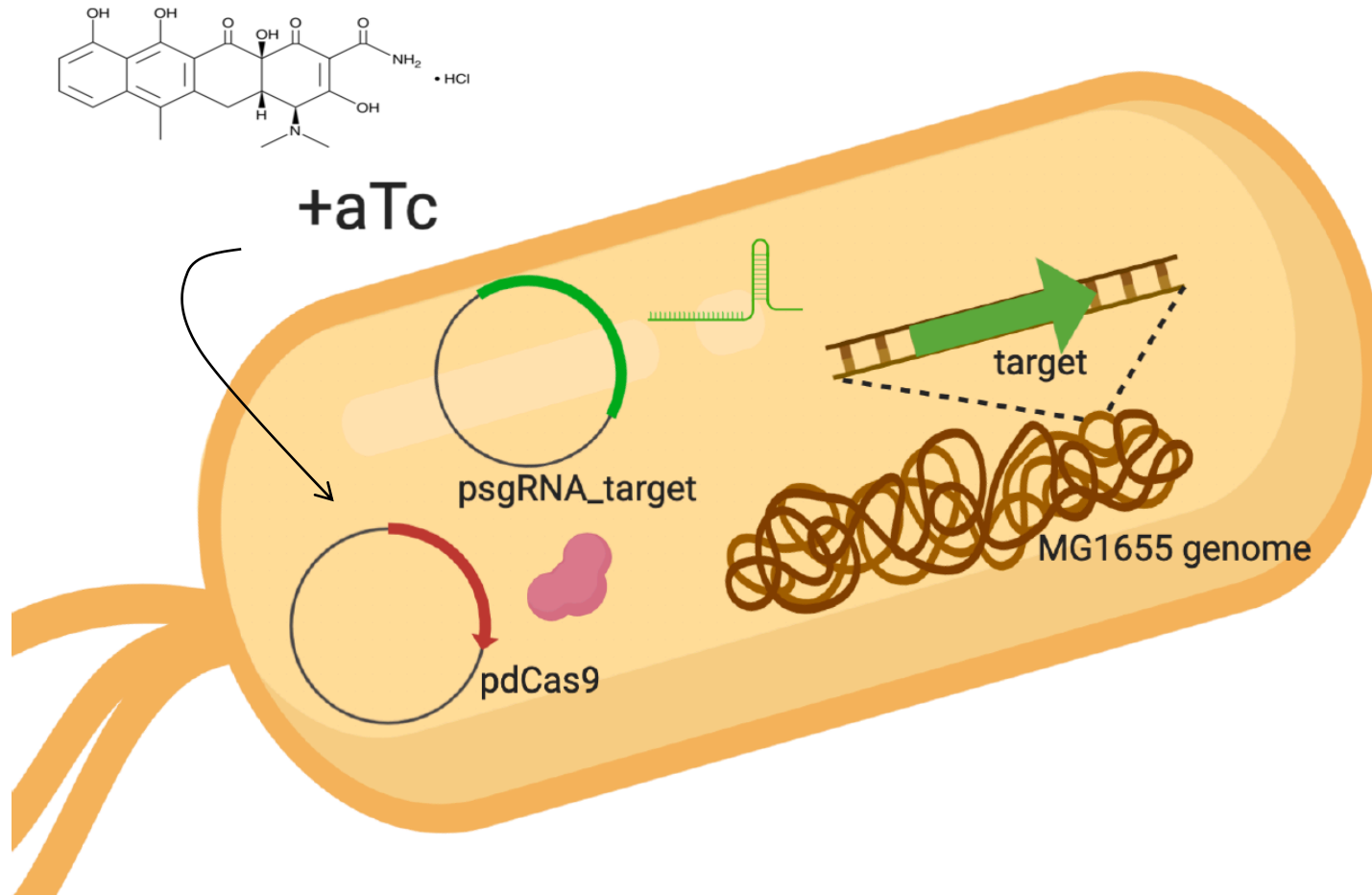
- Aligned sequences to identify where previously designed sgRNAs target *ldhA* and *pta-ack* genes in MG1655 E. coli

```
1>~~~gaattctaaagatctttgacagctagctcagtcctaggtataatactagt-----gttttagagctagaaatagcaag>73
73>---GAATTCTAAAGATCTTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTAAATCCACTTAAGAAGGTAGGTGTGTTTTAGAGCTAGAAATAGCAAG>269
01>CTGGAATTCTAAAGATCTTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTAAATCCACTTAAGAAGGTAGGTGTGTTTTAGAGCTAGAAATAGCAAG>500
1>~~~gaattctaaagatctttgacagctagctcagtcctaggtataatactagt-----gttttagagctagaaatagcaag>73
```



# CRISPRi 'blocks' gene expression in presence of inducer

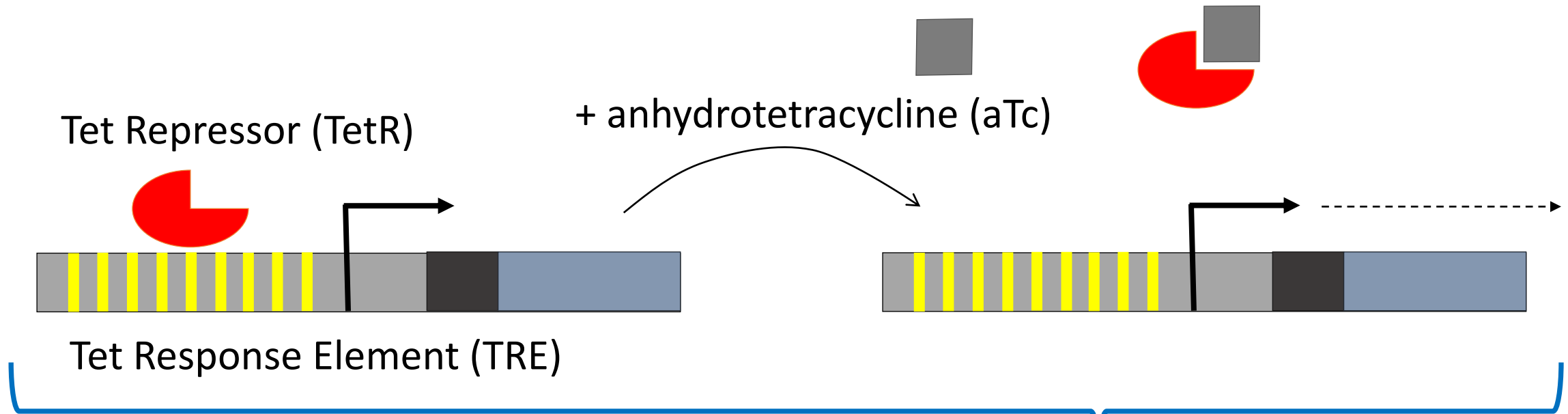
- Expressed constitutively:
- Expression induced with aTc:



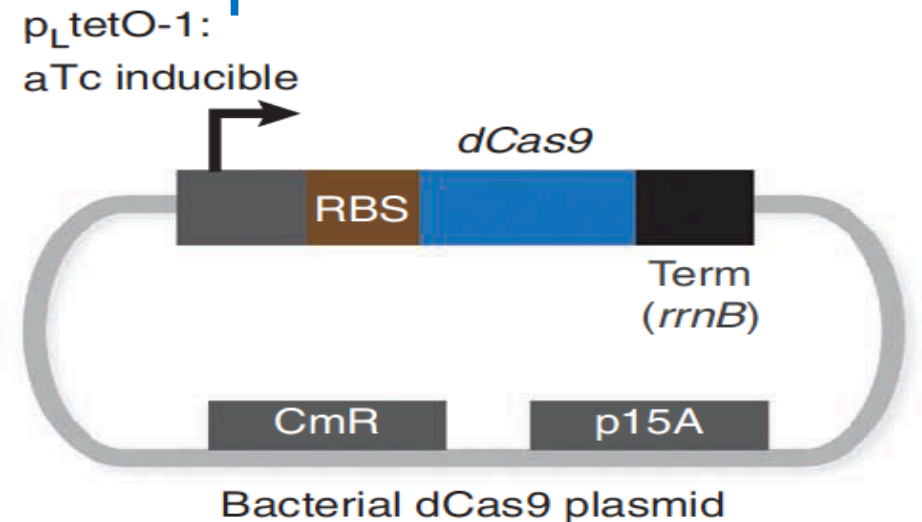
dCas9 protein associates with gRNA/target gene to repress target gene expression



# aTc induction of pdCas9



- Tet promoter regulates expression of dCas9 gene



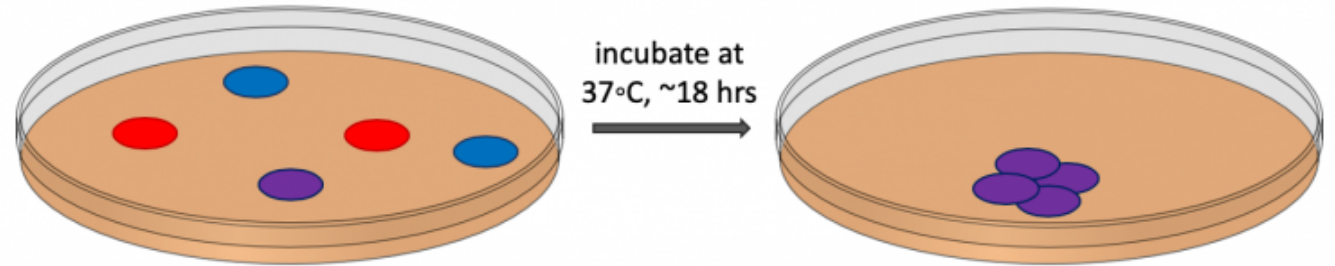


# Setting up the CRISPRi experiment to measure ethanol production

## 1. Transform MG1655 with pdCas9 and psgRNA

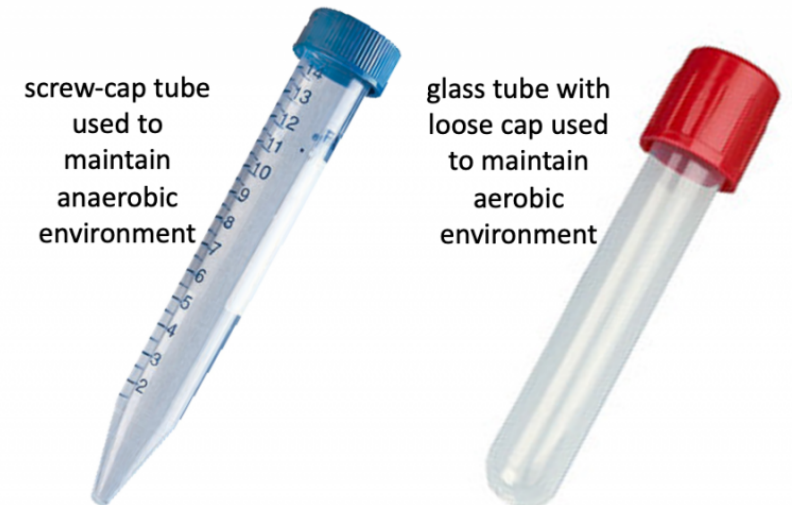
pdCas9 contains CmR cassette

psgRNA contains Amp cassette



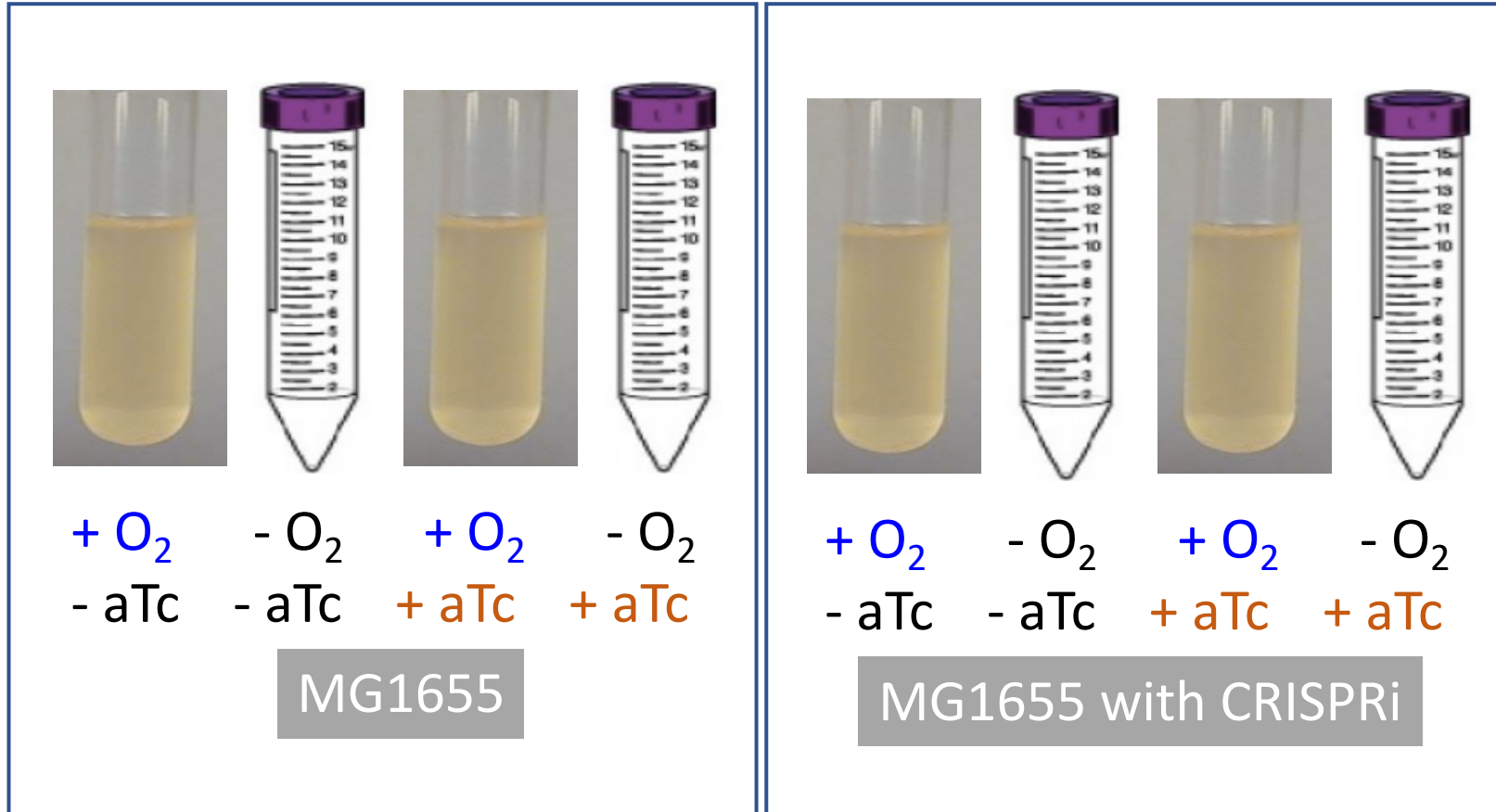
## 2. Prepare liquid culture conditions

1. 15ml conical tube vs glass tube with loose cap
2. Luria-Bertani (LB) broth
3. Antibiotics
4. -/+ aTC
5. -/+ CRISPRi plasmids





# Experimental conditions



What conditions do we want to test here?

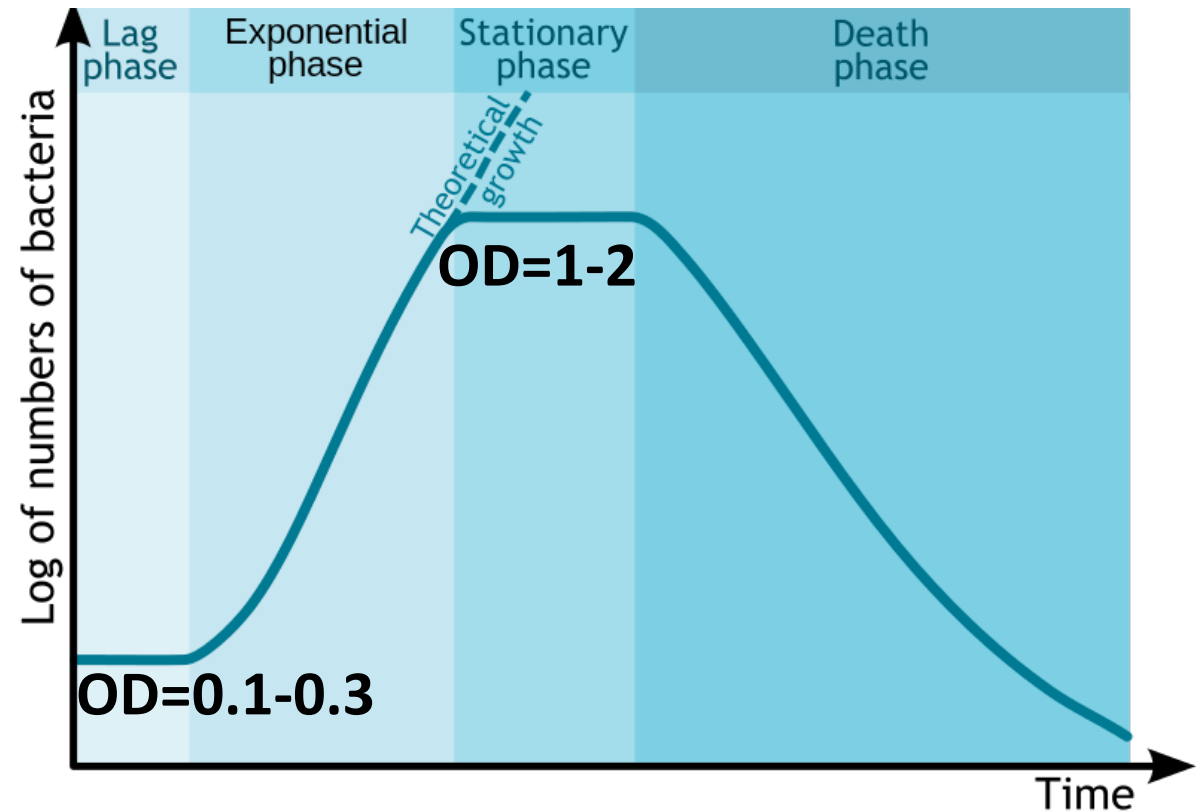
\* Comparisons are made with t-test rules in mind...

Where do we expect most ethanol if our hypothesis is confirmed?



# Measure *E. coli* (MG1655) concentration by optical density

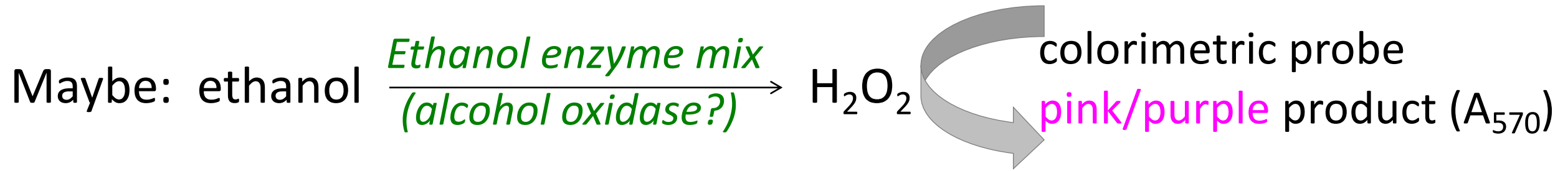
- Optical Density (O.D.)  $\neq$  absorbance
- Measure of light scattering
  - turbidity rather than absorption (relates to number of cells)
- Typically 600nm wavelength is used
  - *E. coli* yellowish, don't absorb 600nm (orange)
  - Can also use UV ( $\sim 300\text{nm}$ ) but that is more likely to cause damage



\*Students measured a \_\_\_\_\_ dilution of your culture—remember this for your analysis!



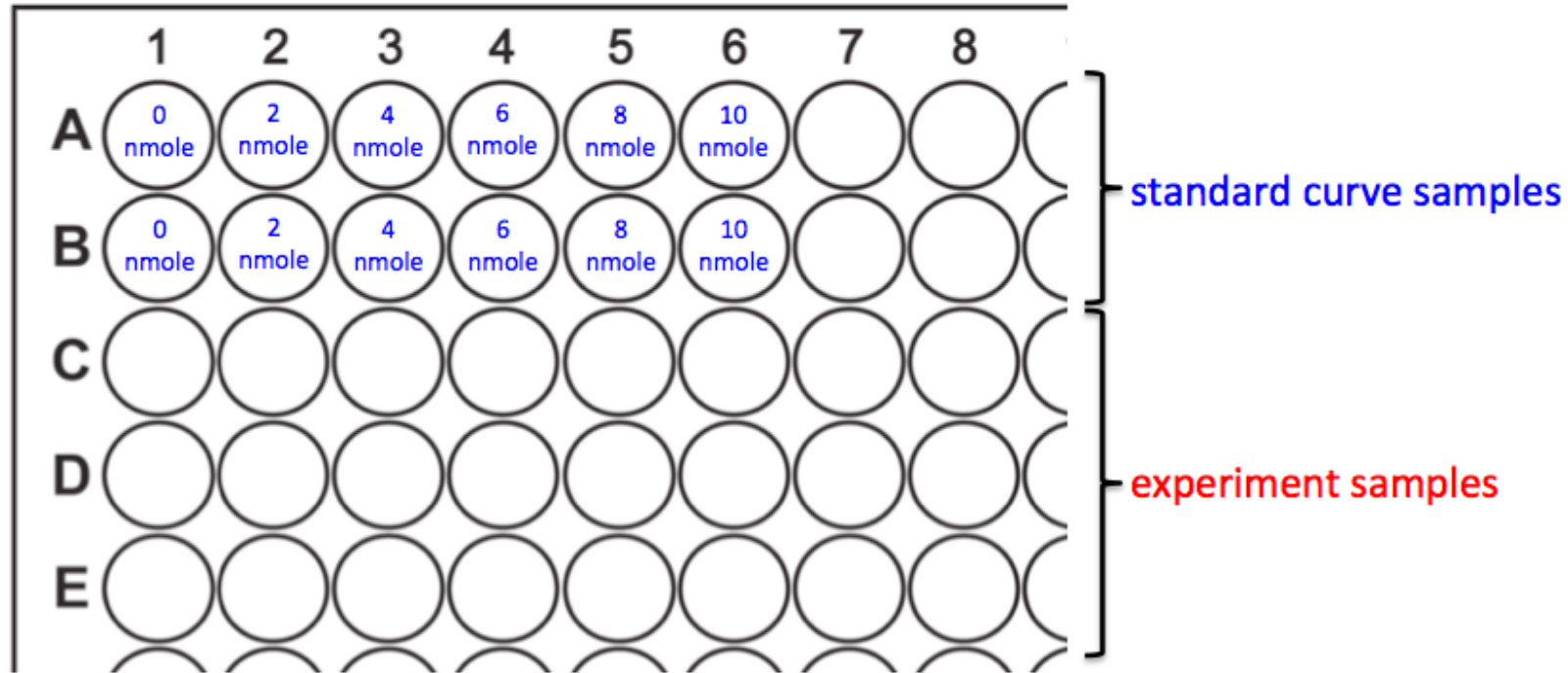
The ethanol colorimetric assay is (very!) proprietary



- Sigma-Aldrich MAK076 colorimetric ethanol assay kit:
  - ethanol assay buffer
  - ethanol enzyme mix
  - ethanol probe
  - ethanol standard



# Ethanol assay plate set up



- Refer to the wiki for sample well placement

\*Media samples were diluted \_\_\_\_\_ prior to ethanol assay—remember this for your analysis!



## For today

- Analyze ethanol production data
- Work on research proposal ideas for **pitch day (lecture 11/17)**

**Lab next Tuesday (11/17) is office hours/workshop time.**  
**Attendance is not mandatory**

## For M3D4 (Thursday 11/19)

- Refine research proposal plan based on additional work and feedback during pitch
  - Guidelines on wiki
  - Submit as a team