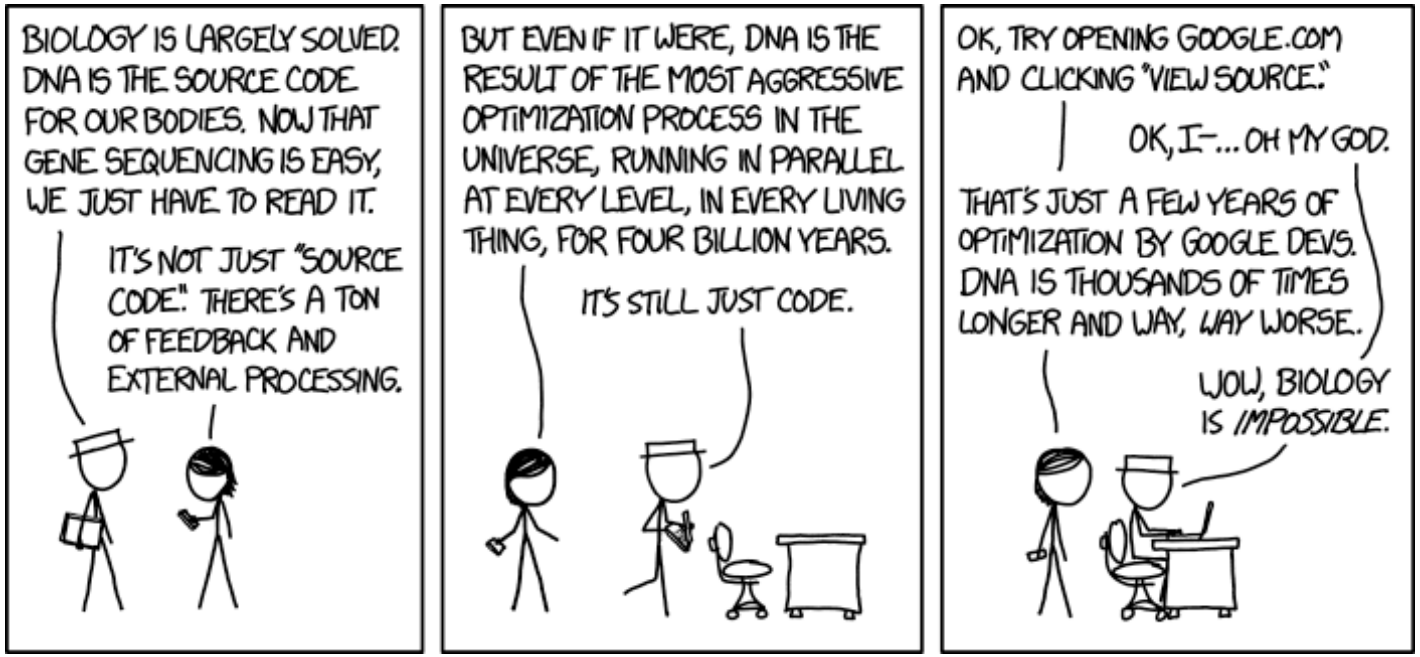
M2D6: Complete CRISPRi experiment and measure fermentation products

1. Pre-lab discussion
2. Measure OD of your bacteria
3. Measure fermentation products (ethanol/acetate) in media
4. Begin data analysis



Mod2 Overview

Research goal: Increase the yield of commercially valuable byproducts in *E.coli* using CRIPSRi technology to target genes involved in mixed-acid fermentation pathway.

Last Lab:

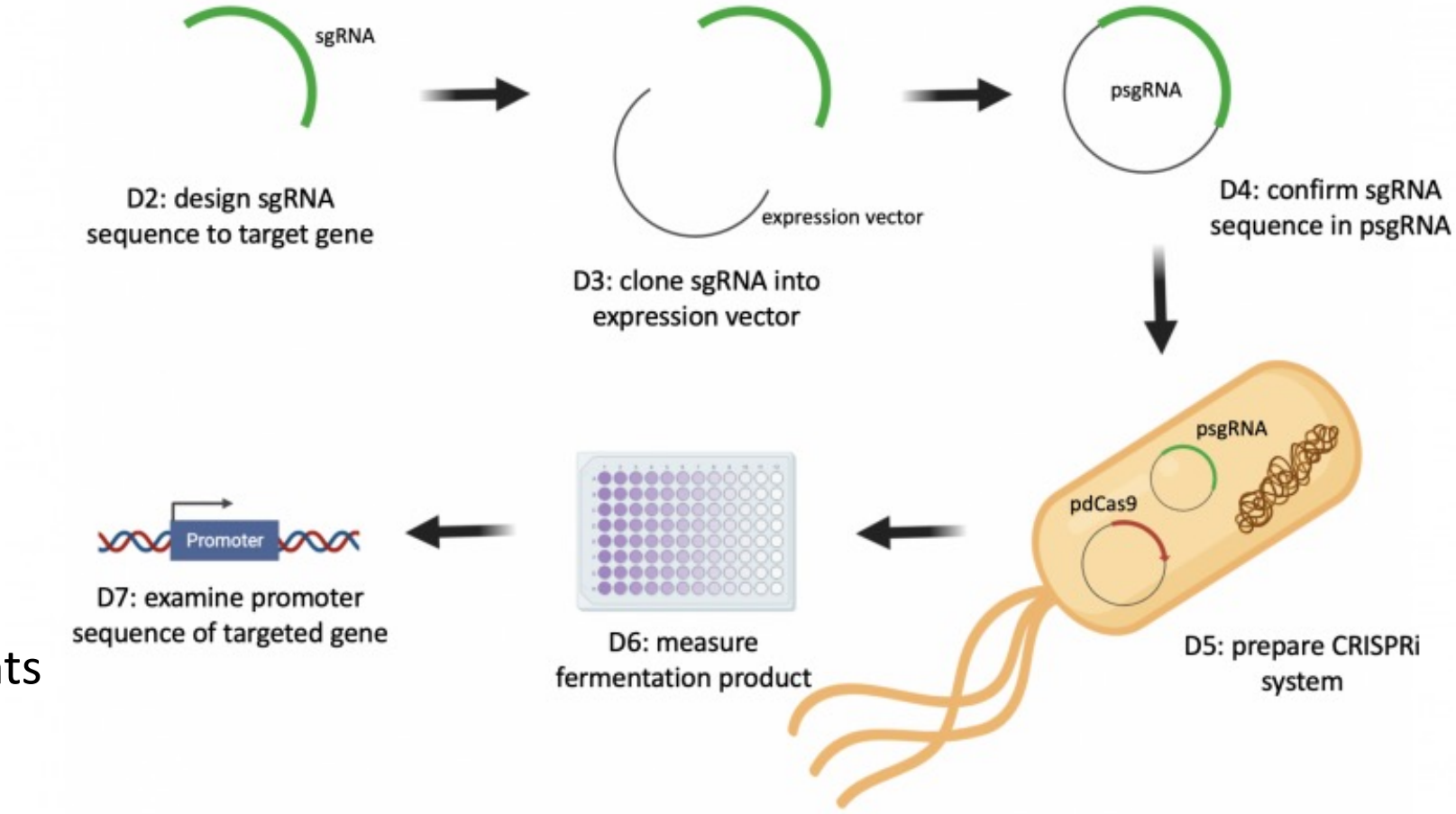
Confirm correct sgRNA cloning and do preliminary CRISPRi system preparations

This Lab:

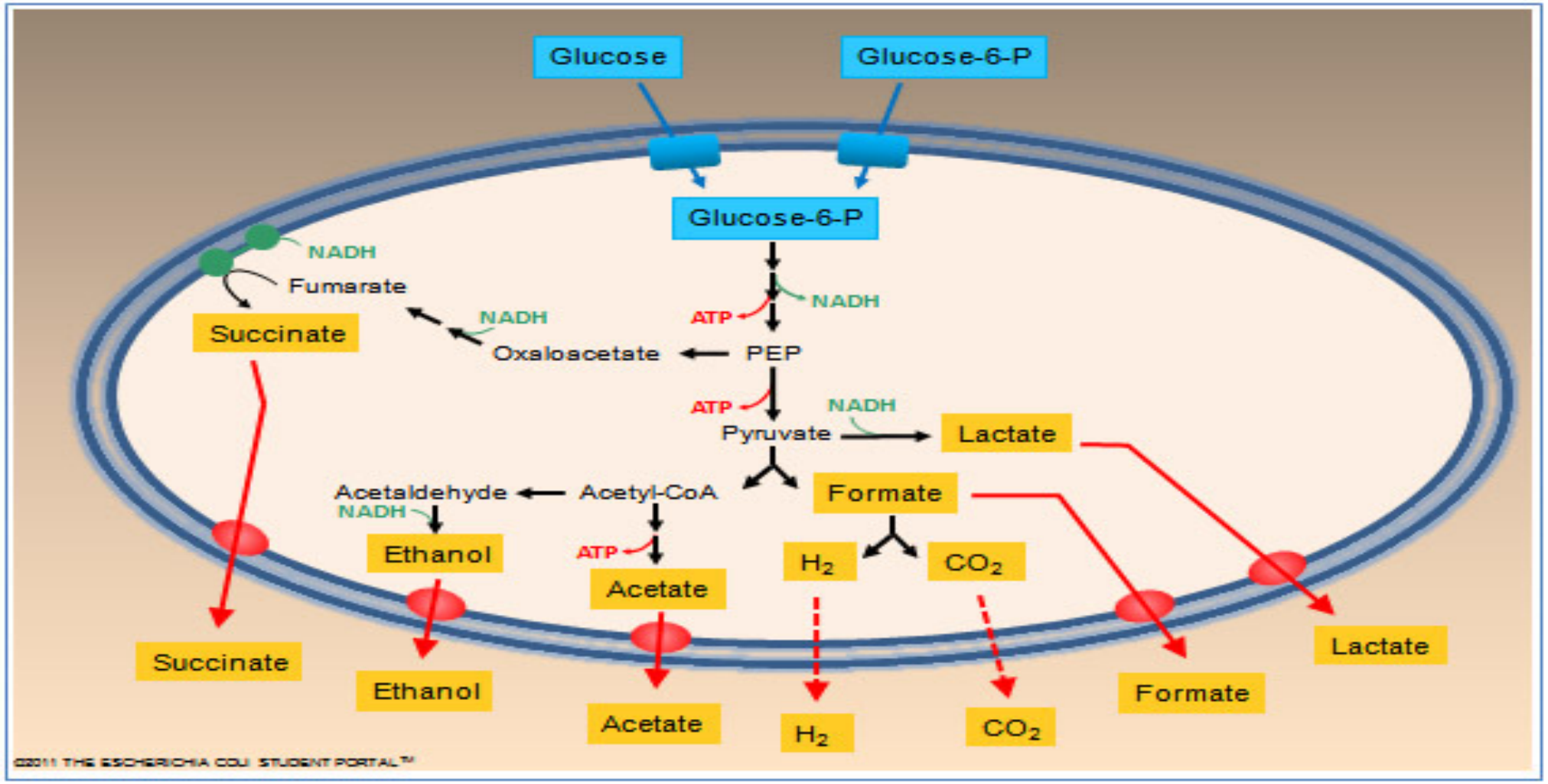
Measure bacteria O.D. and fermentation products

Next Lab:

Examine DNA regulatory elements that may impact the efficacy of your CRISPRi system



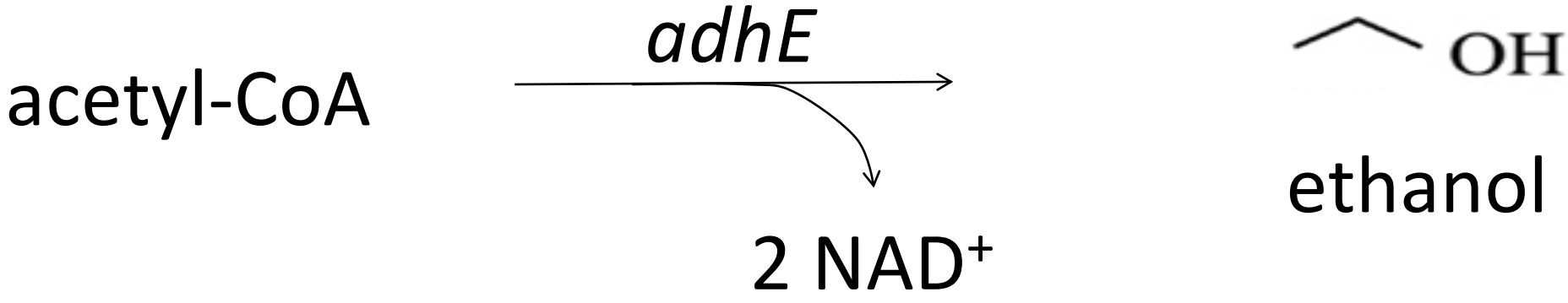
Manipulate the *E. coli* mixed-acid fermentation pathway to produce valuable products



http://ecolistudentportal.org/article_fermentation#_

Production of ethanol

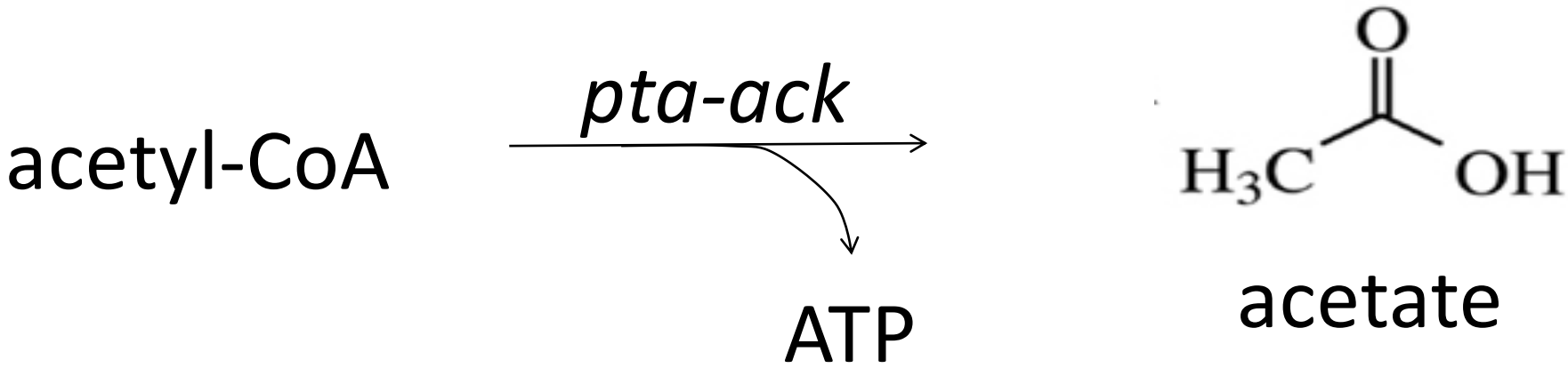
- Bioethanol is most important biotechnological commodity
- *adhE* only transcribed in anaerobic conditions



Noreen's lecture

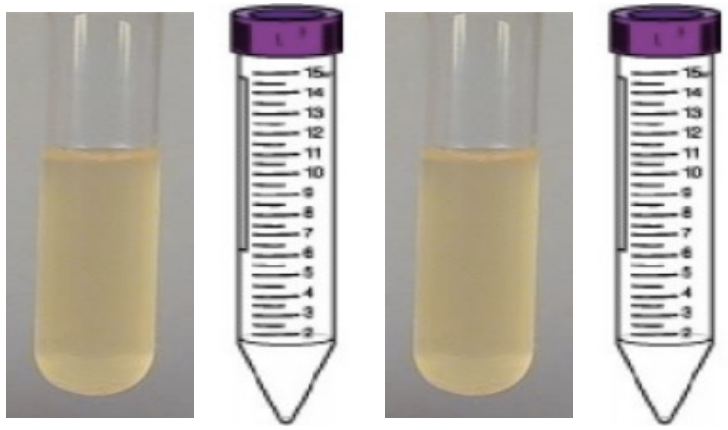
Production of acetate

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



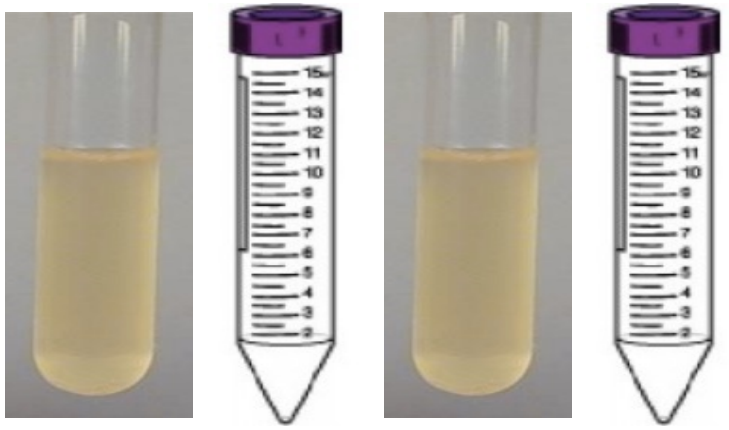
Noreen's lecture

Experimental conditions: mixed-acid fermentation and pdCas9 induction



+ O_2 - aTc | - O_2 - aTc | + O_2 + aTc | - O_2 + aTc

MG1655



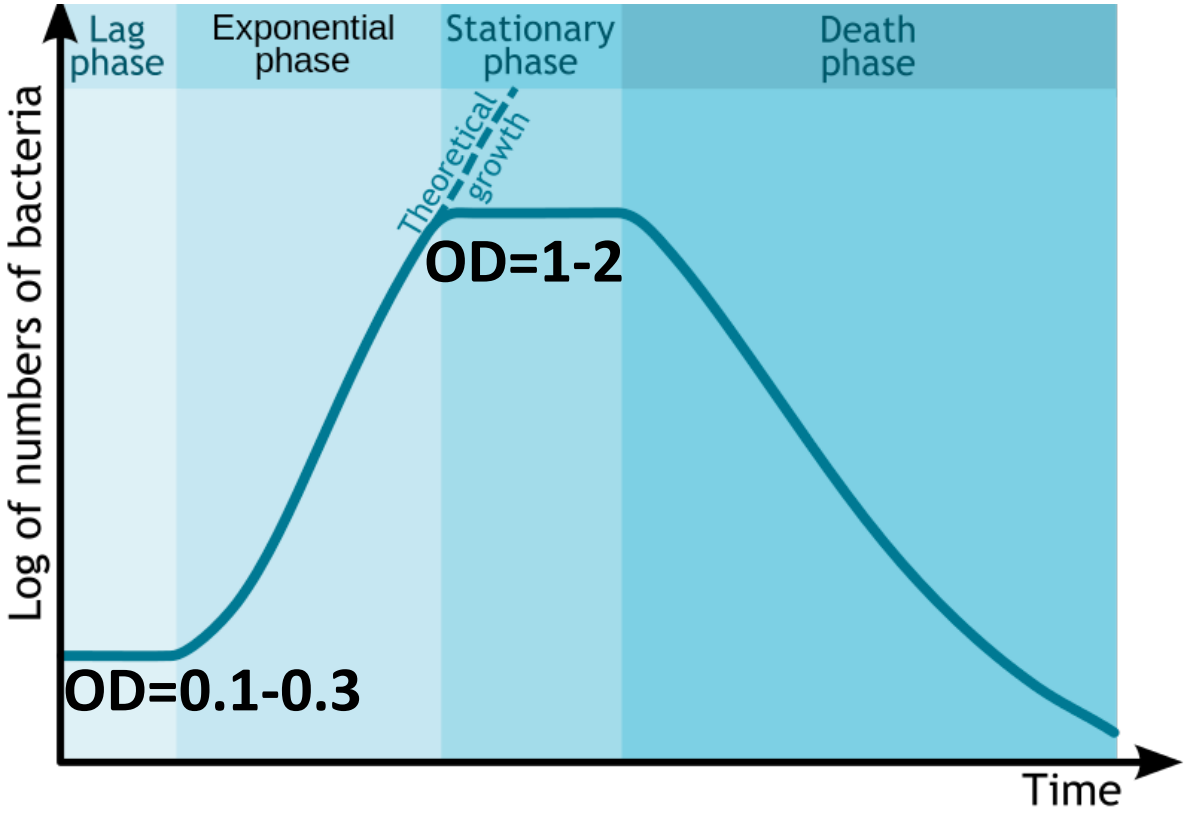
+ O_2 - aTc | - O_2 - aTc | + O_2 + aTc | - O_2 + aTc

MG1655 with CRISPRi

Normalize for _____ by measuring _____

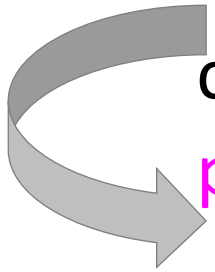
Measure *E. coli* (MG1655) concentration by optical density

- Optical Density (O.D.) ≠ absorbance
- Measuring turbidity rather than absorption (relates to number of cells)



*You will measure a _____ dilution of your culture—remember this for your analysis!

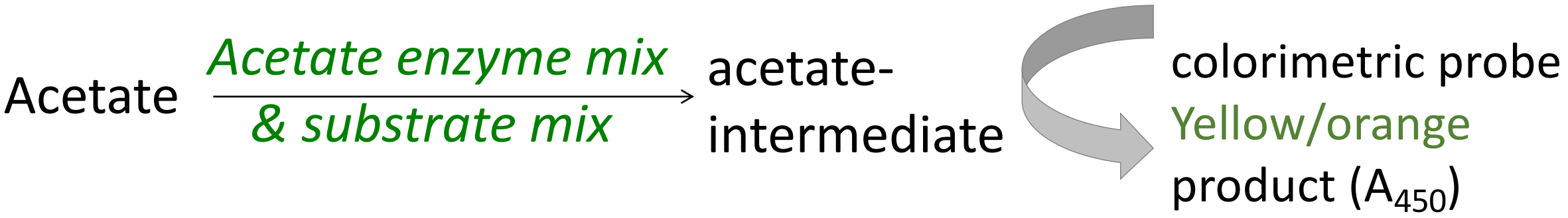
The ethanol colorimetric assay is (very!) proprietary

Maybe: ethanol $\xrightarrow[\textit{(alcohol oxidase?)}]{\textit{Ethanol enzyme mix}}$ H_2O_2  colorimetric probe

pink/purple product (A_{570})

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

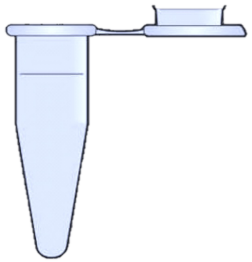
The acetate colorimetric assay is also (very!) proprietary



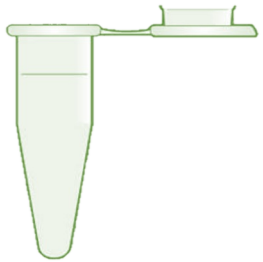
- Abcam ab204719 colorimetric acetate assay kit:
  - Acetate assay buffer
  - Acetate enzyme mix
  - Acetate substrate mix
  - Acetate probe
  - Acetate standard

Ethanol/acetate colorimetric assay procedure

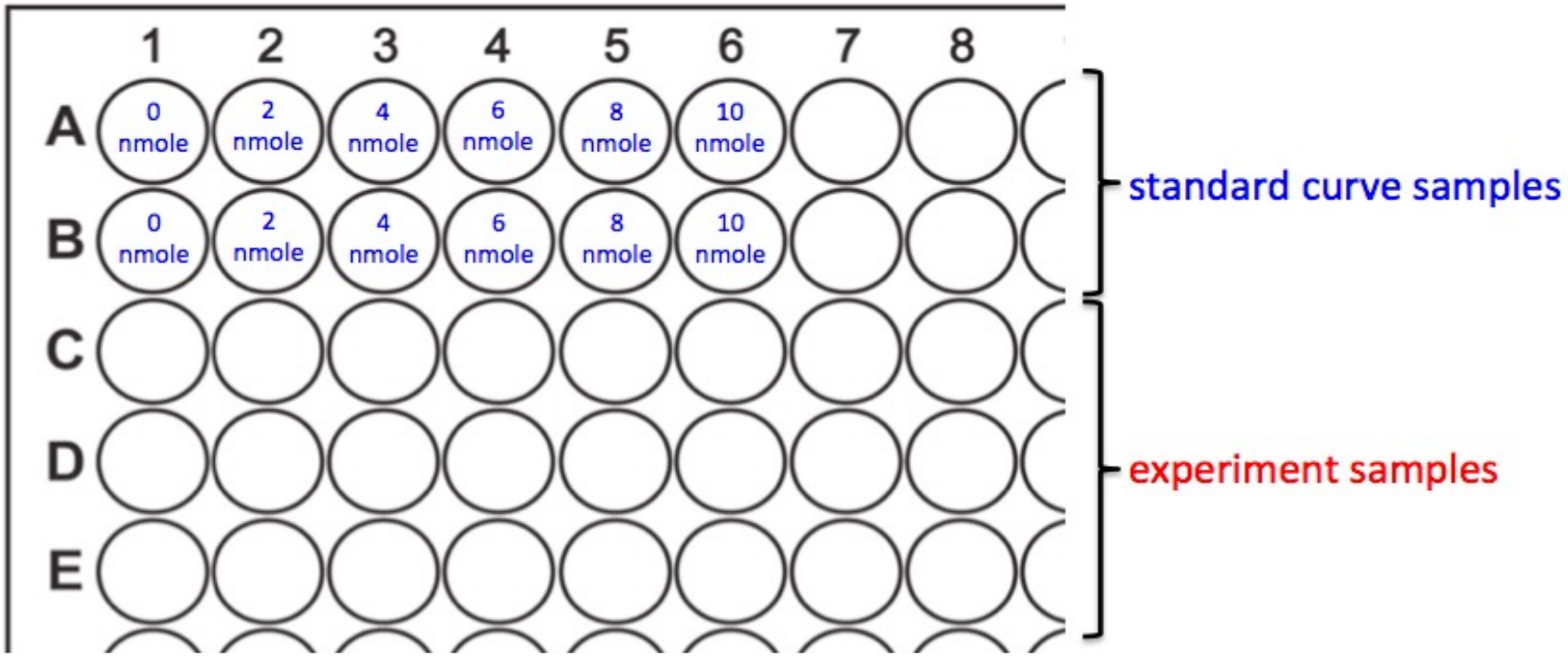
Standard 1-6



E. coli Samples 1-8



Reaction mix for ALL standard and sample wells



Cover with foil during final incubation!

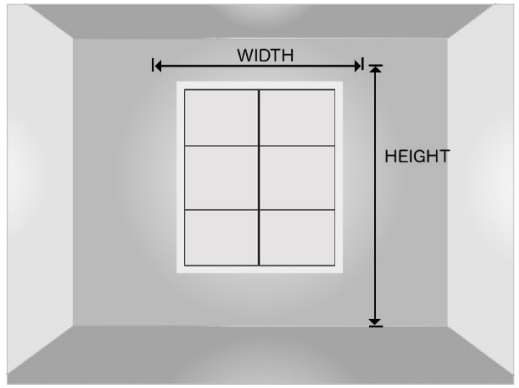
You must compare team data vs. class data

Please upload Excel spreadsheet with your ODs and raw absorbance readings to Class Data page today

T/R [edit]

Team	Ethanol (E) or Acetate (A)	Gene targeted by CRISPRi gRNA	gRNA (DNA) sequence (without tag at 3' end)	Locus targeted (eg. beginning of gene, putative promoter, -35 region)	Target coding or non-coding strand	Ethanol / Acetate Assay Results
TR Red	Acetate (A)	aceE	gagtttcgatcggatccacgtcatt	beginning of gene	target coding strand	
TR Orange	Ethanol (E)	*gltA*	gaacacacctttgaaccgagagta	beginning of gene	target coding strand	
TR Yellow	Ethanol (E)	*pta-ack*	GTTTTTTTAGCCACGTATCAATTAT	-35 region	noncoding strand	
TR Green	Ethanol (E)	*aceE*	TTATTCCTTATCTATCTAATAACGT	-30 region	coding strand	
TR Blue	Acetate	*aceE*	GTCGCGAGTTTCGATCGGATCCACG	beginning of gene	coding strand	
TR Teal	Acetate	*aceE*	CGTCATTTGGGAAACGTTCT	beginning of gene	coding strand	
TR Pink						
TR Purple	Ethanol	*pta*	GTAGGGATCAGCATAATAATAC	beginning of gene	non-template strand	
TR Grey	Ethanol	*aceE*	CGTCATTTGGGAAACGTTCTGACAT	beginning of gene	noncoding strand	
TR White	Ethanol	*aceE*	AGTTTCGATCGGATCCACGTCATTT	beginning of gene	targeting the coding strand (Non template)	

Overview Schematics



Measure windows

Drive to fabric store

Shop for fabric

Buy all of the fabric

Drive home

Cut fabric

Sew curtains

Hang curtains

For Today

1. Retrieve cultures from front bench and measure optical density (O.D.)
2. Prepare samples and kit reagents
    1. Centrifuge = large tabletop centrifuge in lab and cold room
    2. Ethanol/acetate kits are at front bench and need to be aliquoted there
3. Measure absorbance on plate reader (4th floor)
4. Calculate fermentation product concentration from assay results
5. Upload Excel spreadsheet with ODs (x10) and absorbance readings to Class Data Page

For M2D7:

- Create Overview Schematic
    - With title and figure caption…

- Answer questions on wiki to brainstorm discussion outline