M2D8: Measure fermentation products

11/7/17

1. Quiz
2. Pre-lab
3. Measure OD of your bacteria
4. Measure fermentation products (ethanol, acetate) in the bacterial supernatant
5. Start data analysis

We are grading M2D2 notebook page; due 10pm tonight.
Double check wiki rubric for all required sections!

There is candy!

MOD3 starts next week!!

Major assignments for M2

- **Research Article**
  - Due by 10pm on Mon., November 20th

Research Article content

1. Title
2. Abstract
3. Introduction
4. Materials and Methods
5. Figures and Results
6. Discussion
7. References

- **Blog post for M2** due by 10pm on Tues., Nov. 21st

Extra office hours

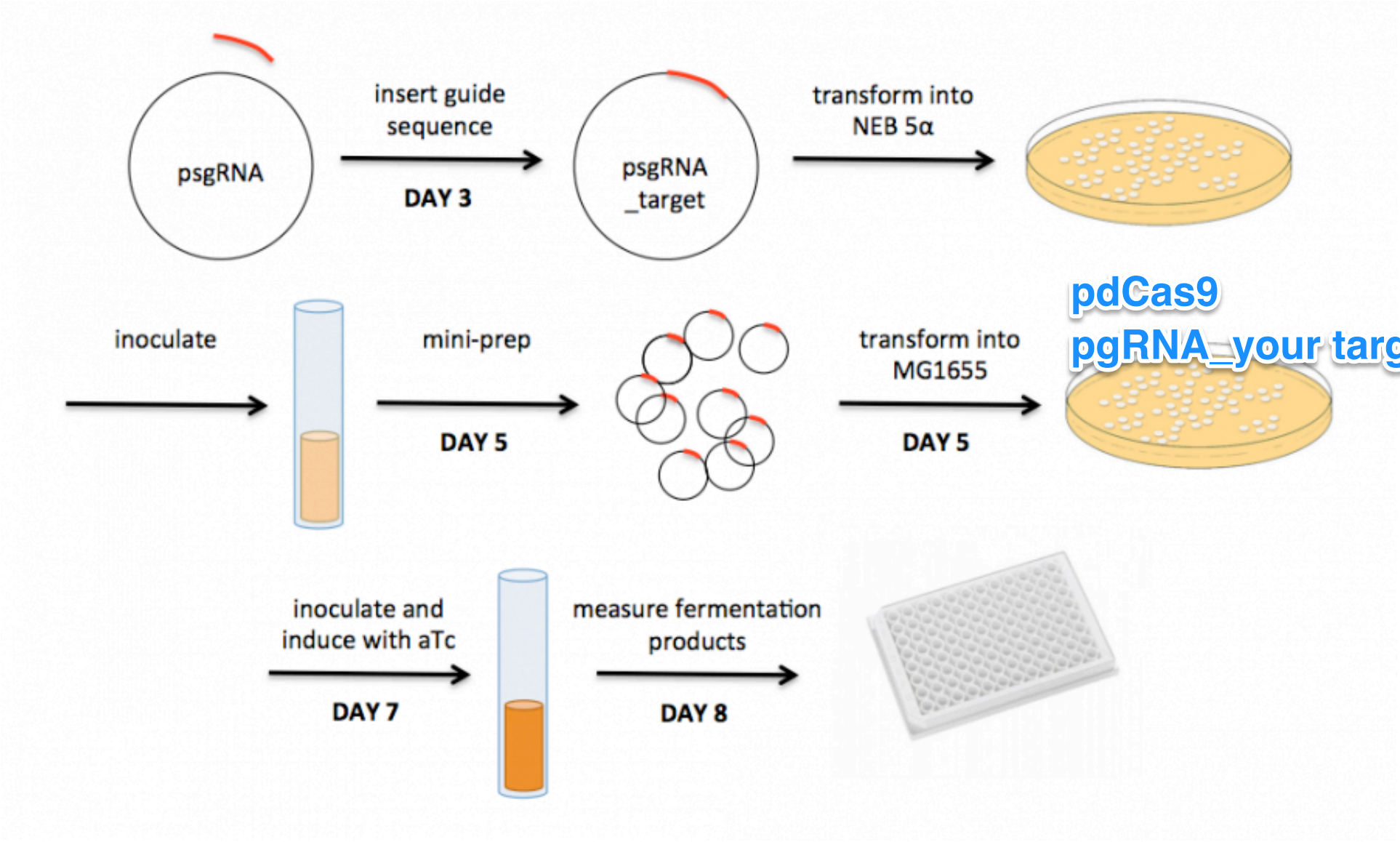
- Saturday Nov. 18th 12pm-5pm
- Monday Nov. 20th 11am-5pm

Regular office hours

- Noreen: Mon. 2-5pm (16-317)
- Leslie: Fri. 9am and 3pm (56-341c)
- Josephine: Mon. 1pm, Thurs. 2pm (56-341c)

The research article is your most formal writing assignment. Use proper formatting for references, don't include hand drawn images and pay attention to guidelines on the wiki.

M2 experimental overview (a few methods tips)

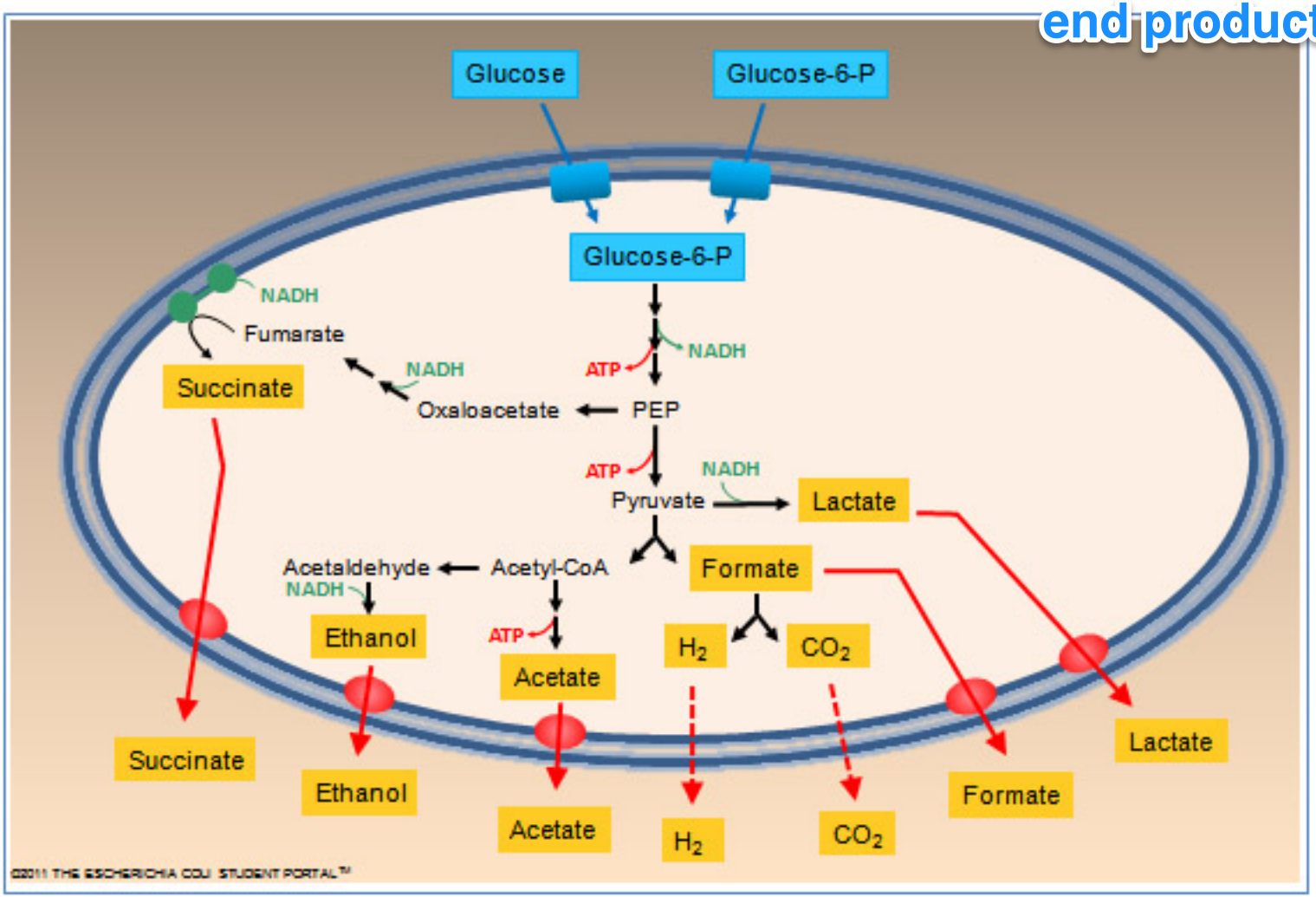


only under anaerobic growth

E. coli fermentation pathway

What does mixed-acid mean?

glucose --> various end products (acids)

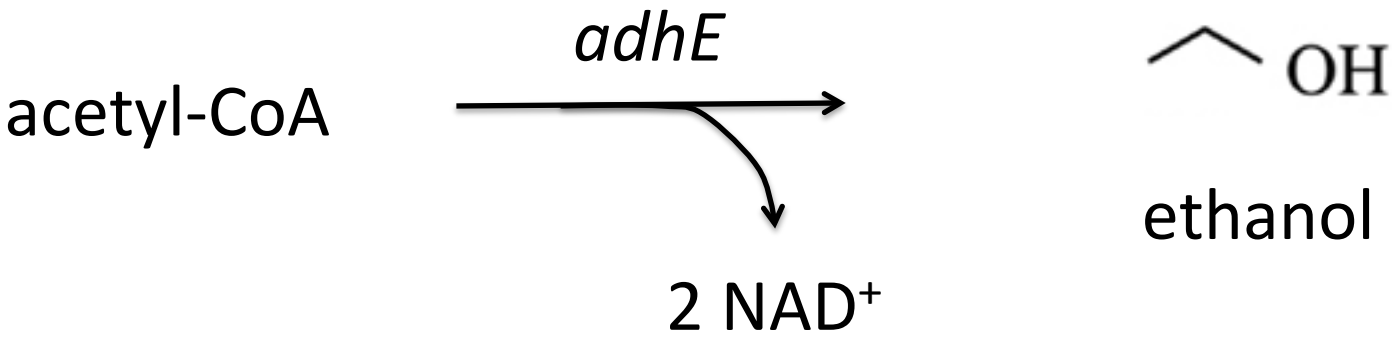


cell growth reduced under anaerobic conditions

http://ecolistudentportal.org/article_fermentation#_

Production of ethanol

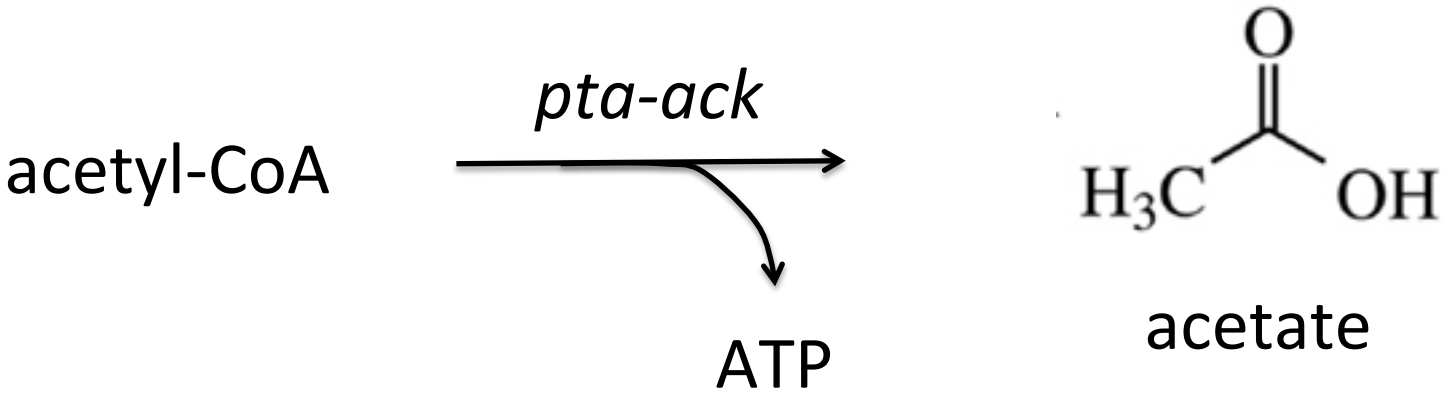
- Bioethanol is most important biotechnological commodity
- *adhE* only transcribed in anaerobic conditions



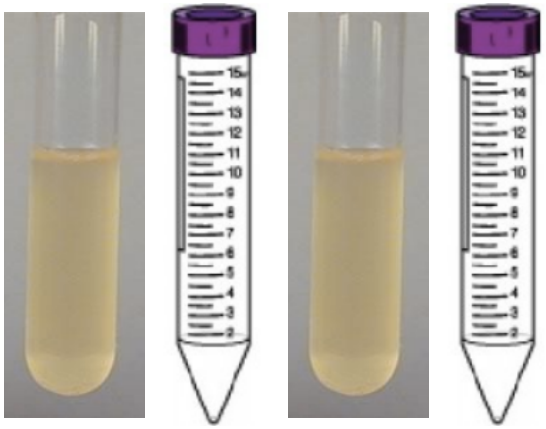
Noreen's M2D2 lecture

Production of acetate

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

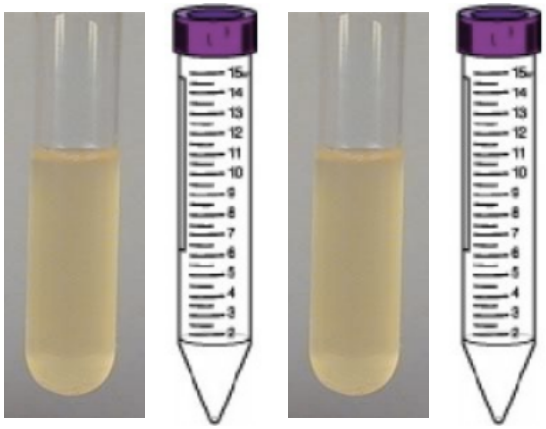


Experimental conditions: mixed-acid fermentation and pdCas9 induction



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

MG1655

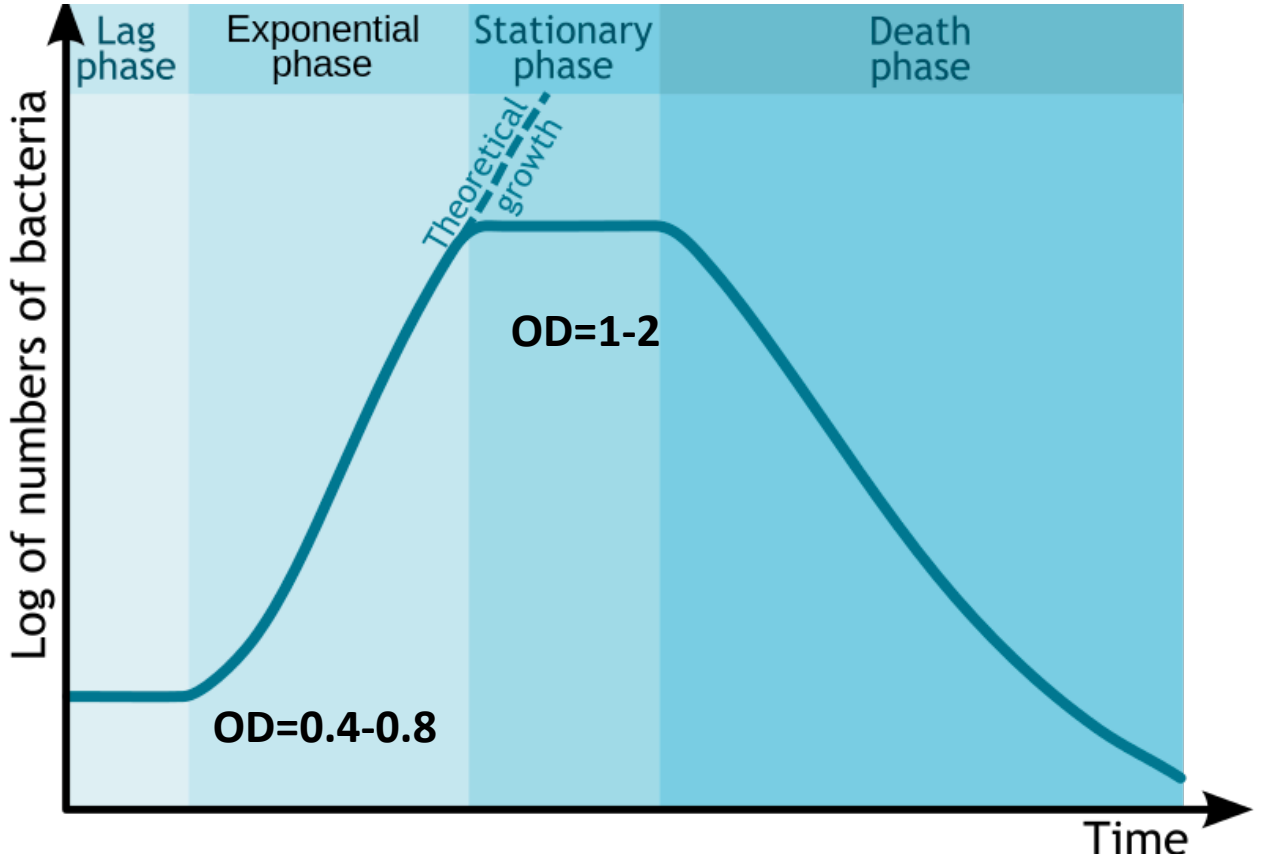


+ O_2 - aTc | - O_2 - aTc | + O_2 + aTc | - O_2 + aTc

MG1655 with CRISPRi

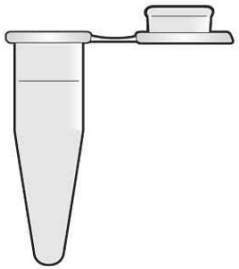
Measuring *E. coli* (MG1655) growth

- Optical Density (O.D.) ≠ absorbance
  - -measuring turbidity rather than absorption
    - -relates to the number of cells
  - -measure of light scattering
    - -*E. coli* yellowish, so they don't absorb 600nm (=orange)
    - -600nm is safer than UV (UV~300nm) for DNA in *E. coli*



Overview of Acetate and Ethanol Assay Kits

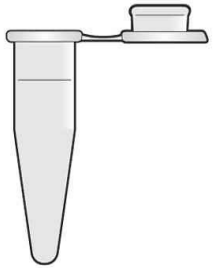
E. coli Samples 1-8



1. Pellet bacteria
2. Transfer supernatant
3. Dilute supernatant in LB or buffer
4. Aliquot to the appropriate wells, in triplicate

50 ul

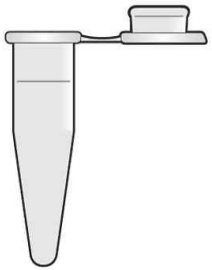
Standard 1-6 (or 1-7)



1. Find standard stock at front bench
2. Make dilution series
3. Don't forget 0 nmole!
4. Aliquot to the appropriate wells, in duplicate

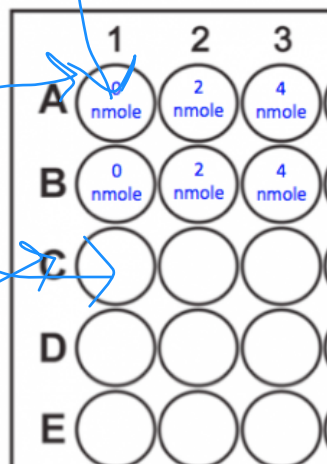
50 ul

Make 1 Reaction mix for all standards and samples



1. Retrieve reaction mix components from frozen block at front bench
2. Calculate volumes needed, account for pipetting error
3. Carefully aliquot 50ul to each well, mix and change pipette tips!
4. Incubate protected from light

50 ul

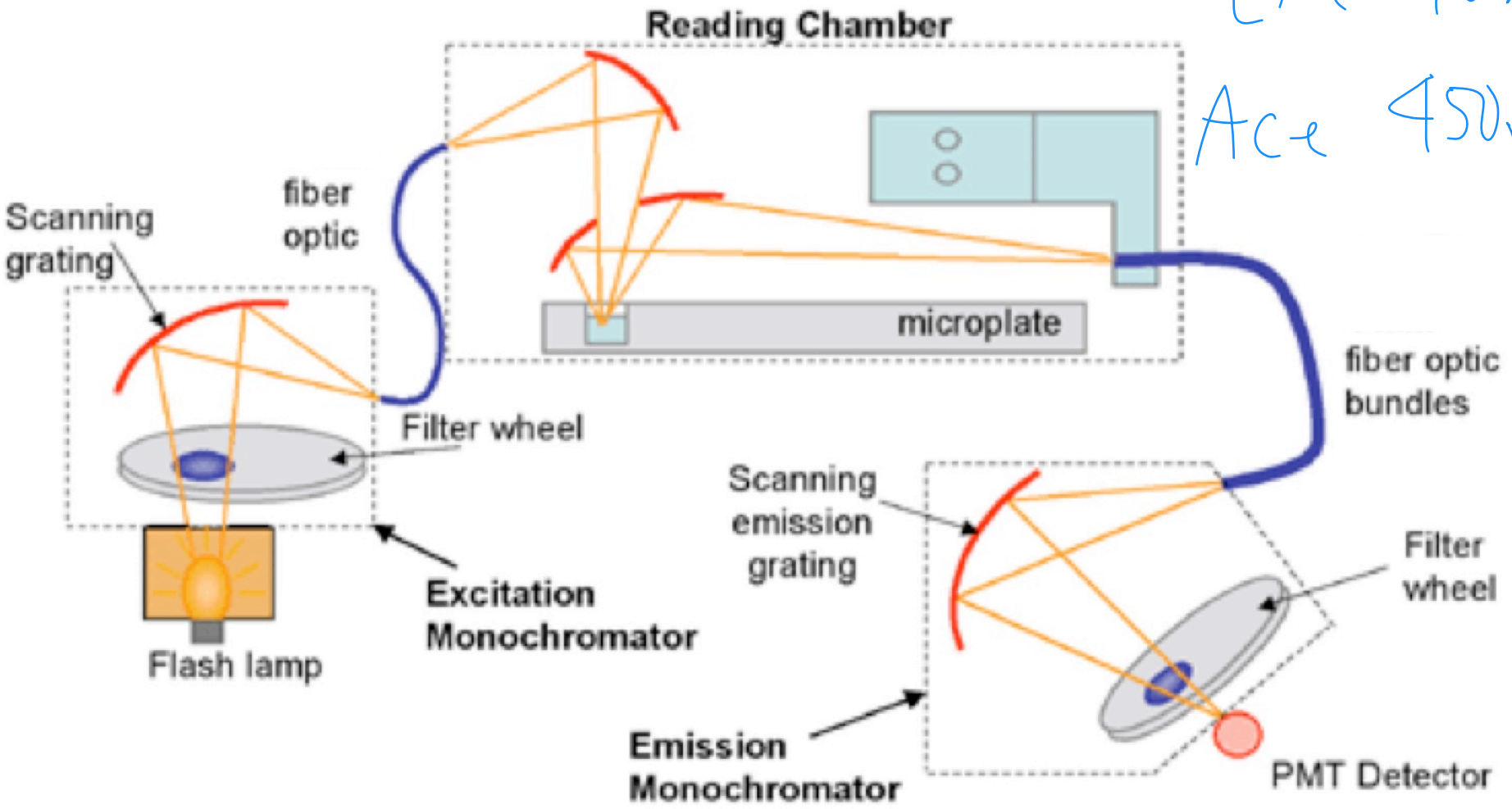


Cover with foil during final incubation!

Microplate reader measures absorbance of individual wells at a specific wavelength

Eth 570 nm

Ace 450nm



adapted from keck.med.yale.edu

You must compare team data vs. class data

Fermentation product and gene targeted: [edit]

T/R [edit]

Update you info on the wiki!

Team	Ethanol (E) or Acetate (A)	Gene targeted by CRISPRi gRNA	gRNA sequence (without tag at 3' end)	Locus targeted (eg. beginning of gene, putative promoter, -35 region)	Target template or nontemplate strand
red	Acetate	adhE	TTAACGCACTCGTAGAGCGTGTAAA	beginning of coding region	
orange	Ethanol	pta-ack	CTATGGCTCCCTGACGTTTT		
yellow	Ethanol	frdA	GCTGTGGGATAAAAACAATCTGGAG	minus 35 region	template strand
green	E	LdhA	ttgtgctataaacggcgagtttcat		
blue	E	pta	ttcacgacaacgttcaataatcat		
pink	Acetate	adhE	TTACTAAAAAAGTTTAACATTATCA	promoter region	template strand
purple	Acetate	adhE	TTTACTAAAAAAGTTTAACATTATC	' -35 region'	'template'
white	Acetate	adhE	CTGATAATGTTAAACTTTTT	promoter	non-template strand

Today in lab…

1. *(Refresh wiki page before starting)*
2. Retrieve cultures from 37°C incubator and measure optical density (O.D.)
3. Prepare supernatant samples
4. Prepare standard curve
5. Combine sample or standards and assay reaction mix 1:1, incubate
6. Measure absorbance on plate reader (4^{th} floor)
7. Calculate fermentation product concentration from assay results