M2D8: Measure fermentation products

11/8/17

No lecture tomorrow
Mod 3 starts next week!

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

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

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

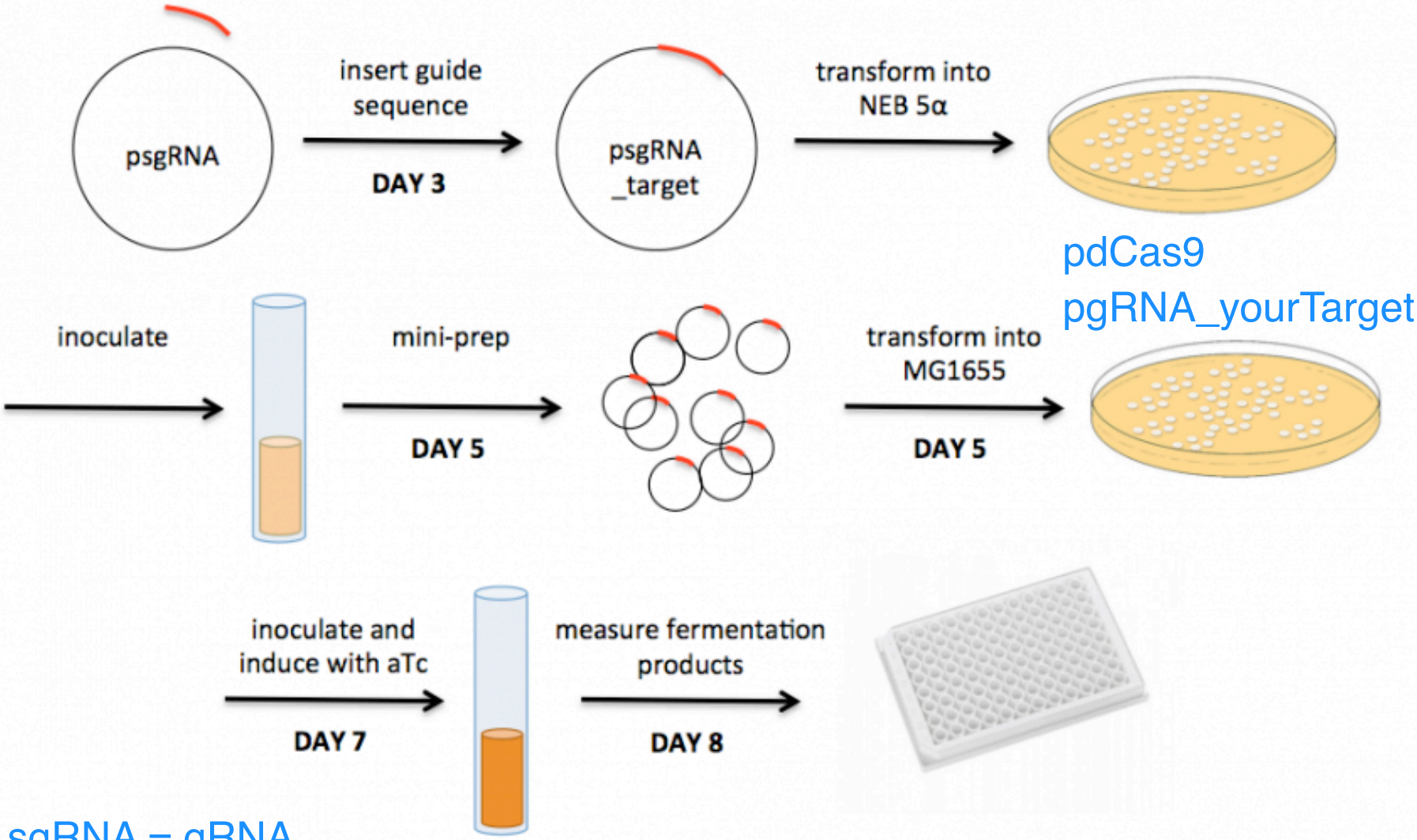
- Saturday Nov. 18th
  12pm-5pm (56-302)
- Monday Nov. 20th
  11am-2pm (56-341c)
  2-5pm (16-317)

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

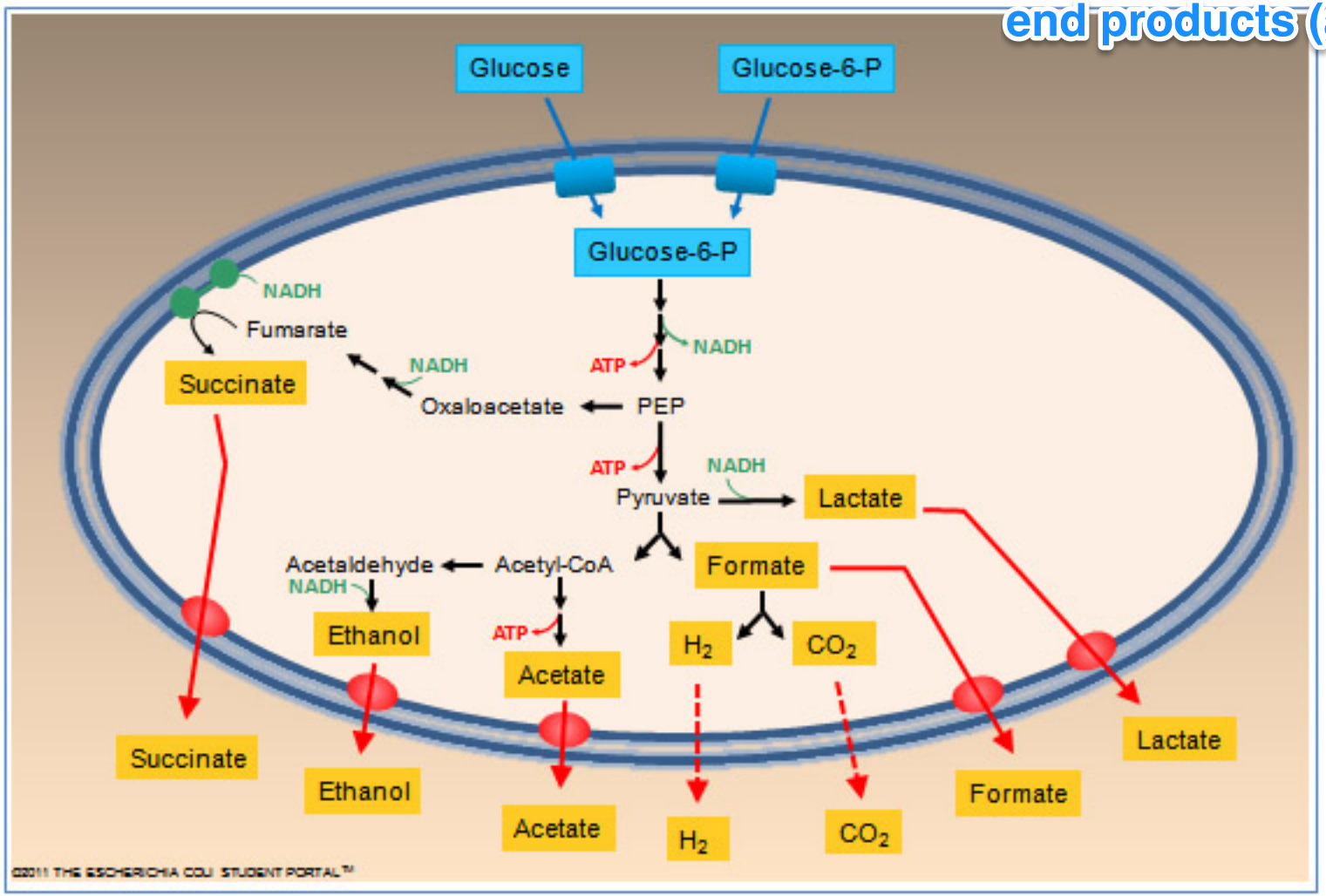


sgRNA = gRNA

only under anaerobic conditions

E. coli fermentation pathway

What does mixed-acid mean? glucose--> various end products (acids)

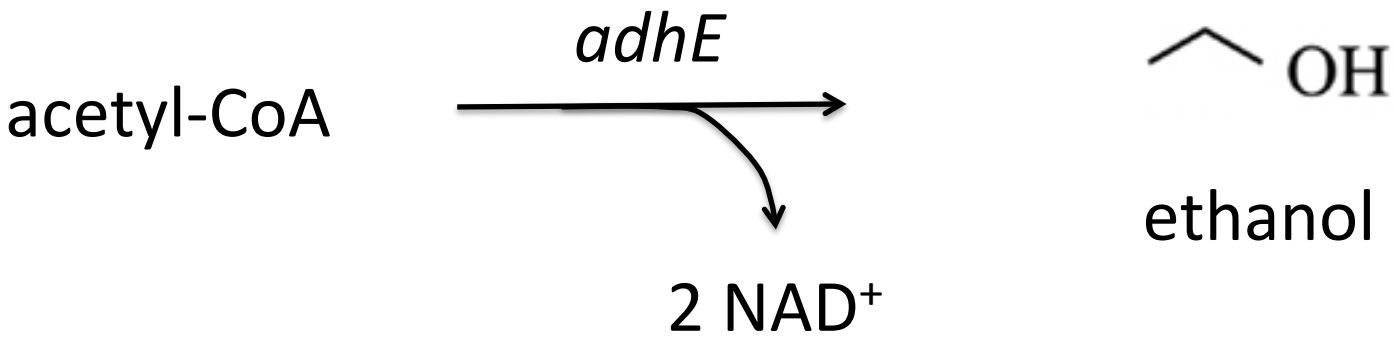


Cell growth is reduced under anaerobic conditions

http://ecolistudentportal.org/article_fermentation#_

Production of ethanol

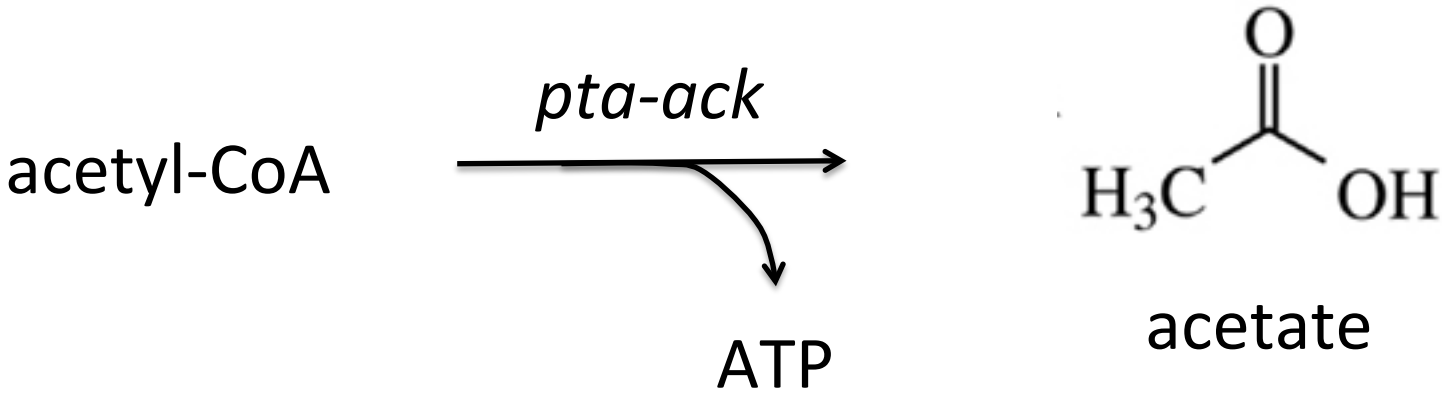
- Bioethanol is most important biotechnological commodity
- *adhE* only transcribed in anaerobic conditions



Noreen's M2D2 lecture

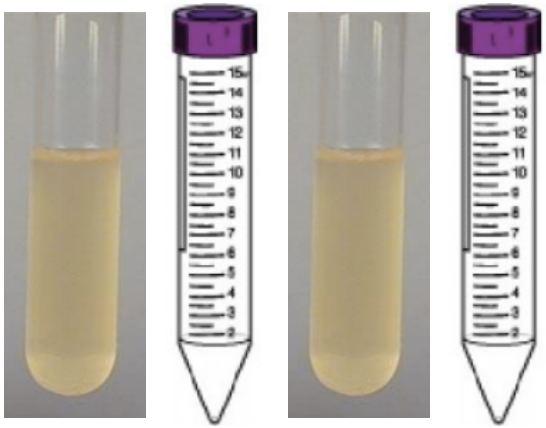
Production of acetate

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



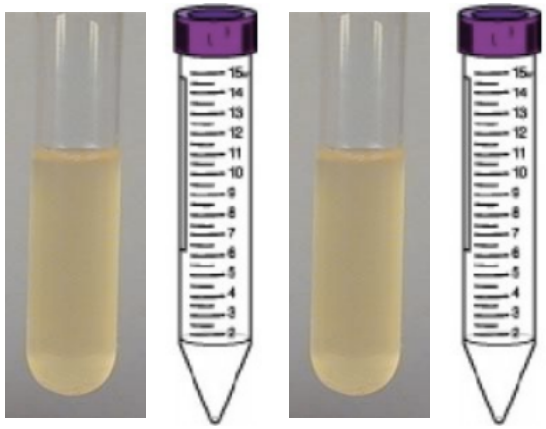
Noreen's M2D2 lecture

Experimental conditions: mixed-acid fermentation and pdCas9 induction



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

MG1655

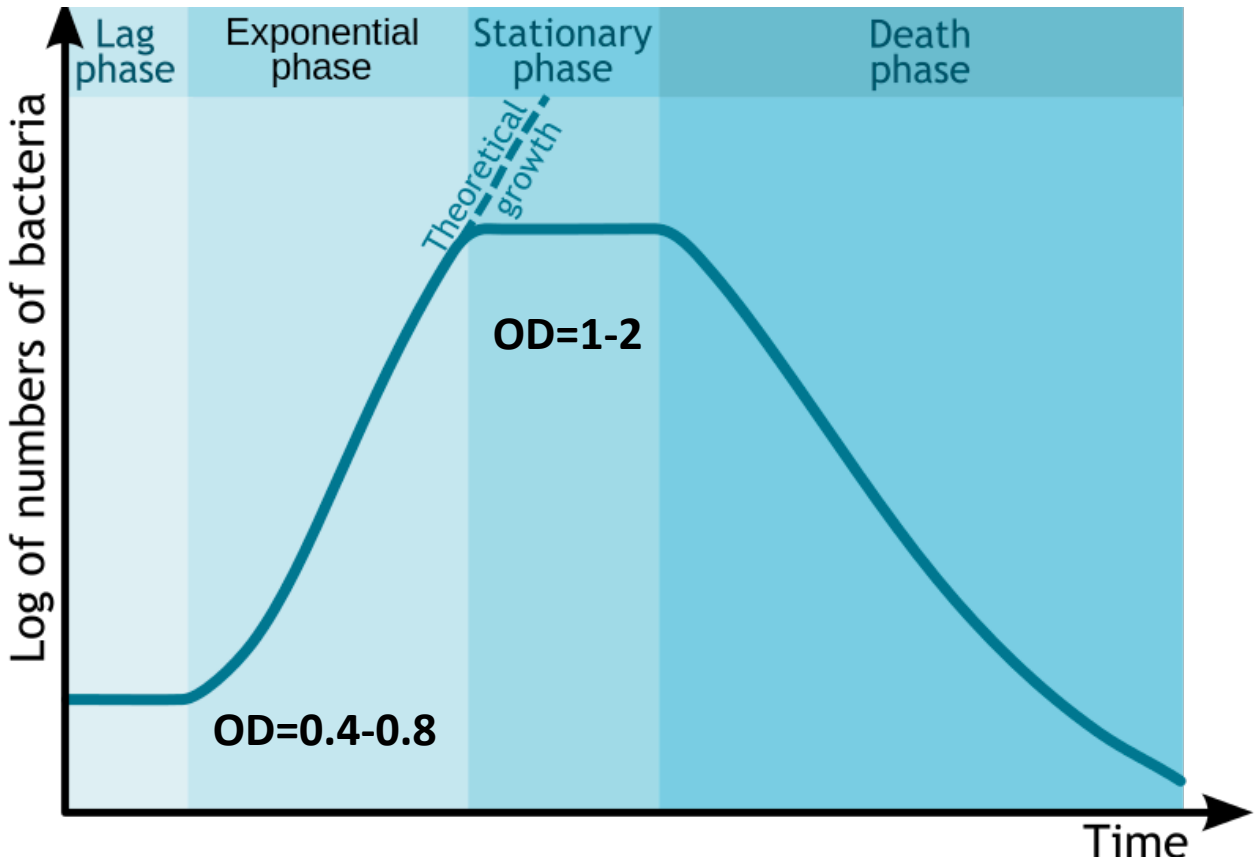


+ O_2	- O_2	+ O_2	- O_2
- aTc	- aTc	+ aTc	+ aTc

MG1655 with CRISPRi

Measuring *E. coli* (MG1655) growth

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



The ethanol colorimetric assay is (very!) proprietary

- Maybe: ethanol $\xrightarrow[\text{oxidase}]{\text{alcohol}}$ H_2O_2 $\xrightarrow[\text{ethanol enzyme mix}]{\text{ethanol probe}}$ pink/purple product (A_{570})

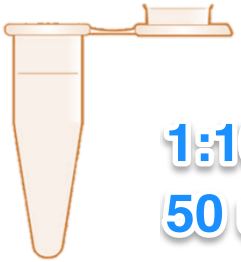
- Sigma-Aldrich MAK076 colorimetric ethanol assay kit:
  - ethanol assay buffer
  - ethanol enzyme mix
  - ethanol probe **(color)**
  - ethanol standard **(known concentration of ethanol, nmol)**

Ethanol colorimetric assay

Standard 1-6

50 uL/well

E. coli Samples 1-8

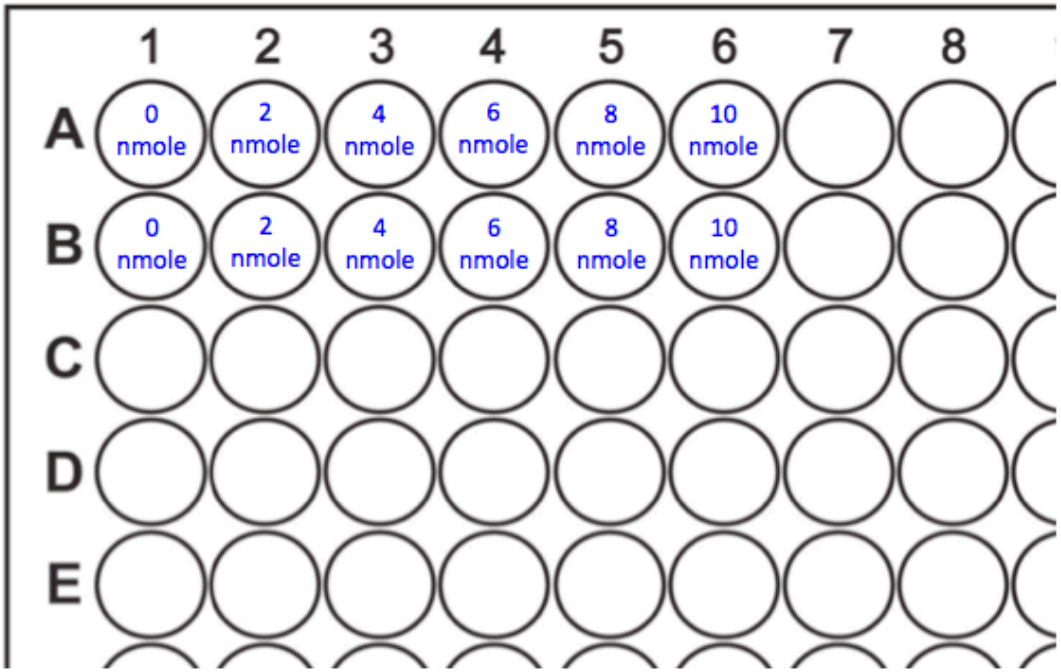


1:10 dilution
50 uL/well

Reaction mix for ALL standard and sample wells

-calculate 10% extra

-50 uL/ well

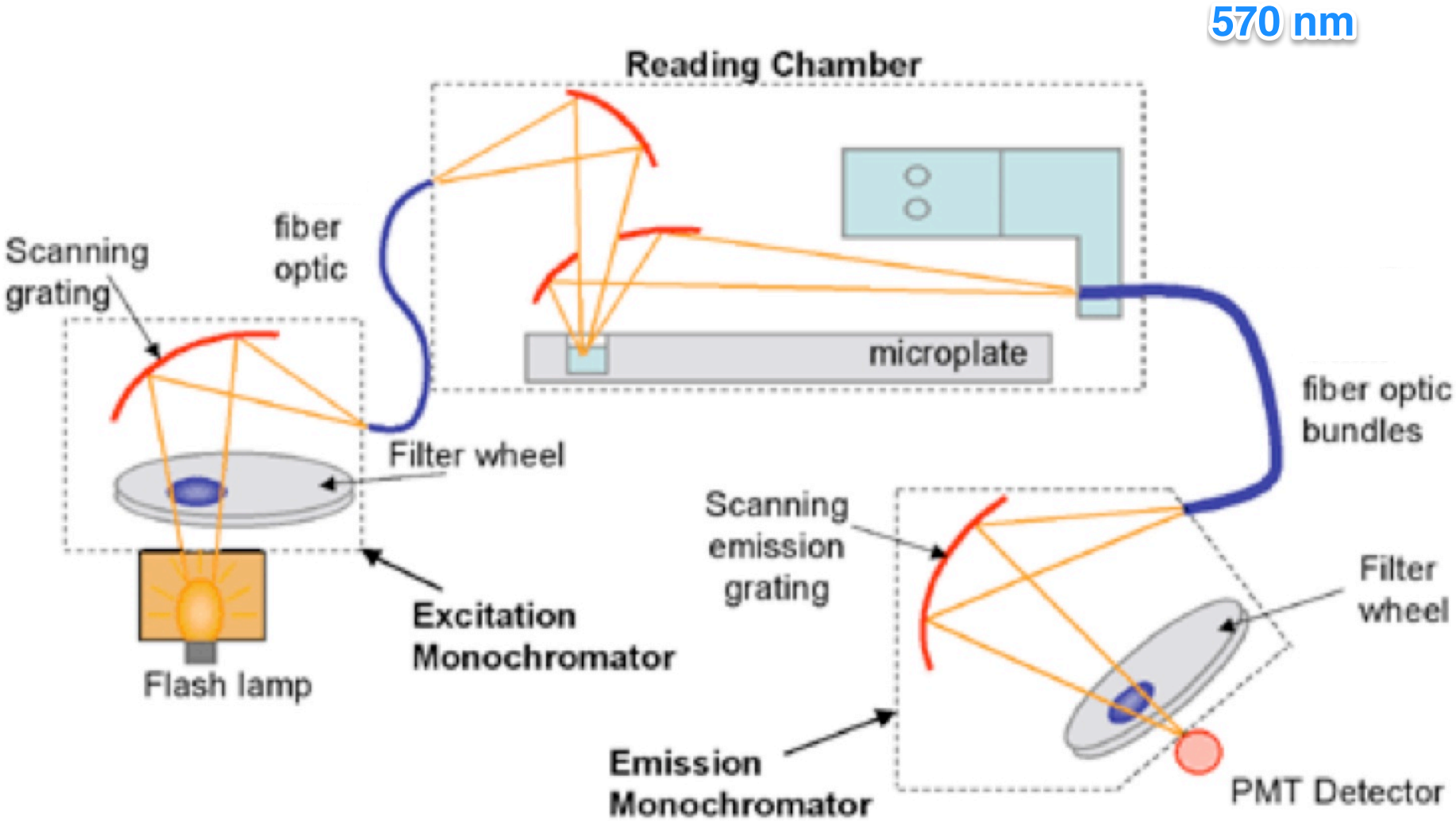


standard curve samples
duplicate

experiment samples
triplicate

Cover with foil during final incubation!

Microplate reader measures absorbance of individual wells at a specific wavelength



adapted from keck.med.yale.edu

You must compare team data vs. class data

Please email me an Excel spreadsheet with your ODs and raw absorbance readings today

T/R

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	non-template
orange	Ethanol	pta-ack	CTATGGCTCCCTGACGTTTT	promoter region	template strand
yellow	Ethanol	frdA	GCTGTGGGATAAAAACAATCTGGAG	minus 35 region	template strand
green	E	LdhA	ttgtgctataaacggcgagtttcat	The middle of the gene	Non template strand
blue	E	pta	ttcacgacaacgttcaataatcat	coding region	non-template strand
pink	Acetate	adhE	TTACTAAAAAAGTTTAACATTATCA	promoter region	template strand
purple	Acetate	adhE	TTTACTAAAAAAGTTTAACATTATC	' -35 region'	'template'
white	Acetate	adhE	CTGATAATGTTAAACTTTTT	promoter	non-template strand

W/F

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	Ethanol	ack (indirectly, pta)	GTTTTTTTAGCCACGTATCAATTAT	promoter region of ack	Nontemplate strand
orange	Ethanol	ldhA	ATTCAACATCACTGGAGAAAGTCTT	promoter	template
blue	Ethanol	ackA	TTTTTAGCCACGTATCAATTAT	promoter region of ackA, starting at -32	nontemplate

Today in lab…

1. Retrieve cultures from 37°C incubator and measure optical density (O.D.)
2. Prepare supernatant samples
3. Prepare standard curve
4. Combine sample/standards with reaction mix, incubate
5. Measure absorbance on plate reader (4th floor)
6. Calculate fermentation product concentration from assay results
7. Email me (joshaw@mit.edu) an Excel spreadsheet with your ODs (x10) and absorbance readings