M2D8: Measure fermentation products

1. Quiz
2. Pre-lab
3. Measure OD of your bacteria
4. Measure fermentation products (ethanol, acetate) in the supernatant
5. Start data analysis
   a) Graphpad Prism software available for a 30 day trial

https://www.graphpad.com/demos/

Major assignments for M2

- **Research Article**
  - Due by 10pm on Mon., November 11th

Research Article content

1. Title
2. Abstract
3. Introduction
4. Materials and Methods
5. *Figures and Results: Compare your data to 2-3 other experiments for class data*
6. Discussion
7. References

- Lab notebook, specifically **M2D2** due 10pm, 11/7
- Blog post for Mod 2 due 10pm, 11/12

Pdf → Stellar

Extra office hours

11/7 (Thurs): 5-7pm, 16-469

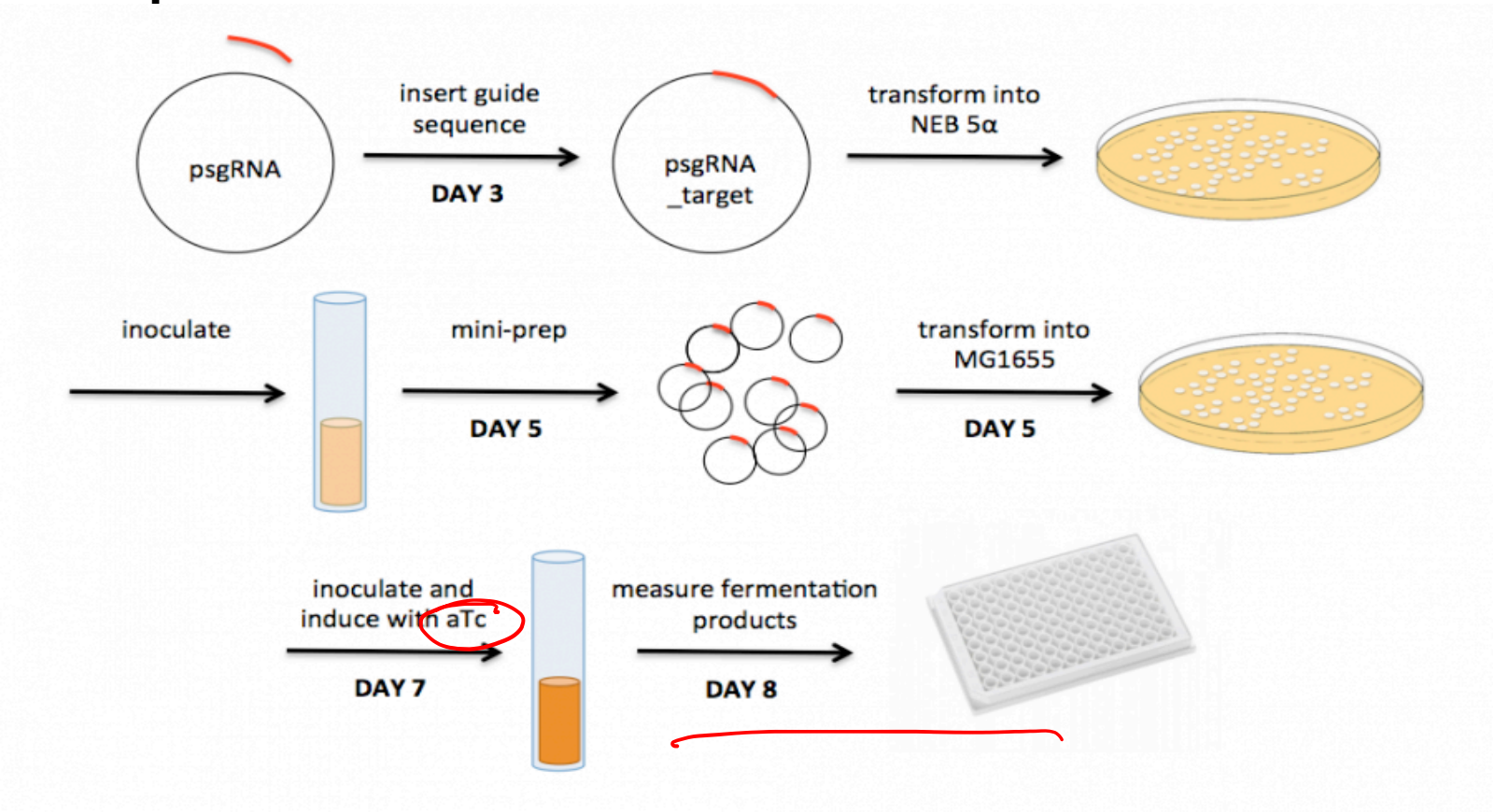
11/9 (Sat): 12-2 pm, 56-302

11/10 (Sun): 12-2 pm, 56-302

The research article is your most formal writing assignment.

- **Use proper reference formatting**
- **Make neat figures**
- **Don't include images from lecture/prelab slides or wiki,**
- **Pay attention to guidelines on the wiki.**

M2 experimental overview

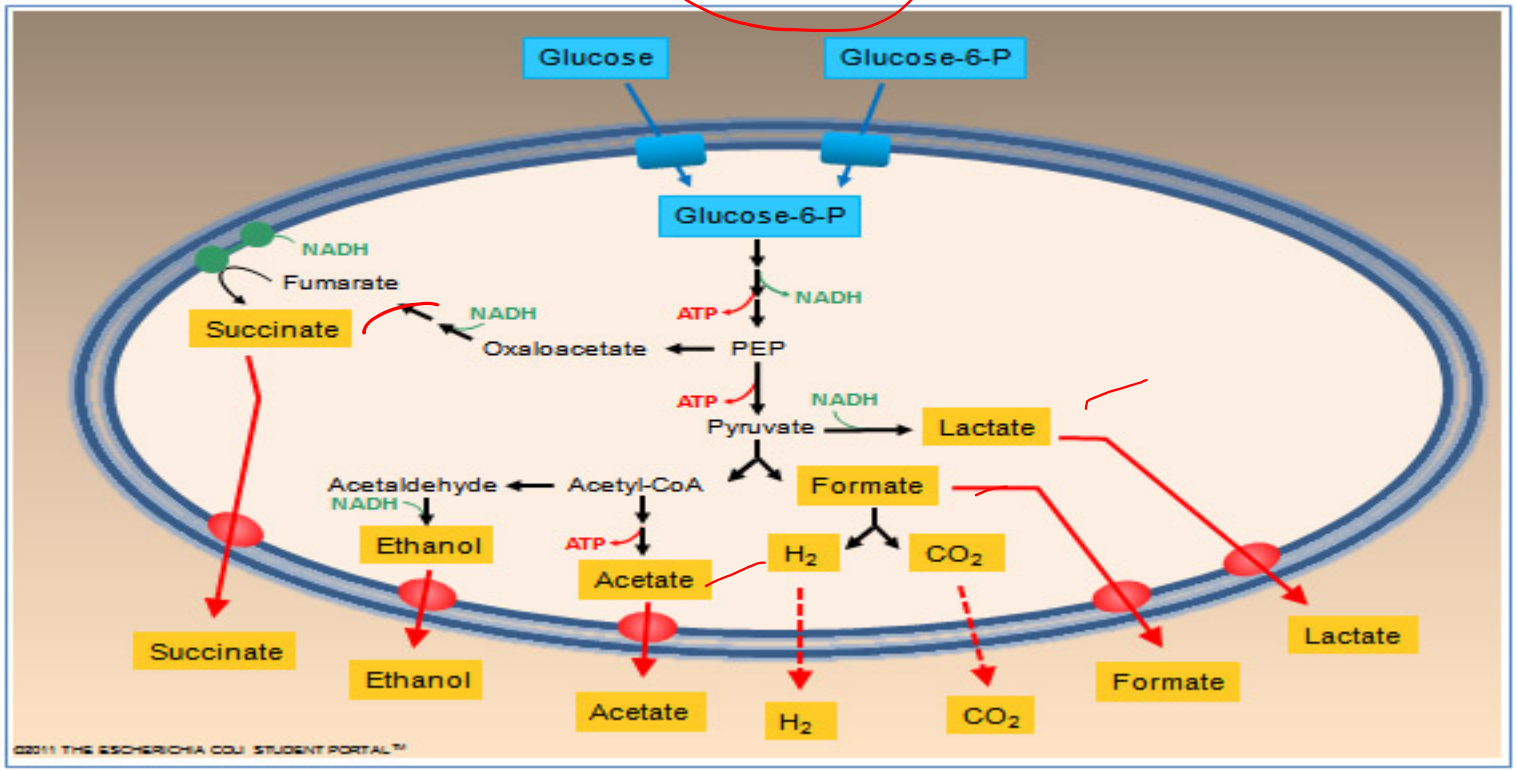


Name your target gRNA plasmid: pgRNA_ _____

E. coli fermentation pathway

What does mixed-acid mean?

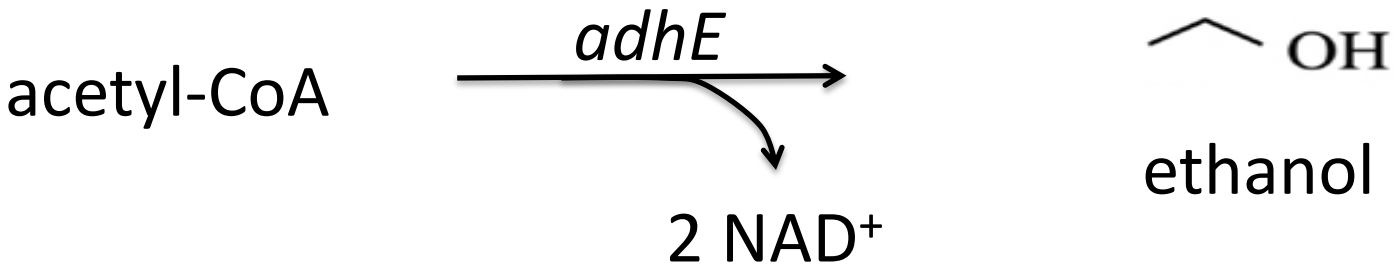
Produce energy w/ a lack of O2



http://ecolistudentportal.org/article_fermentation#_

Production of ethanol

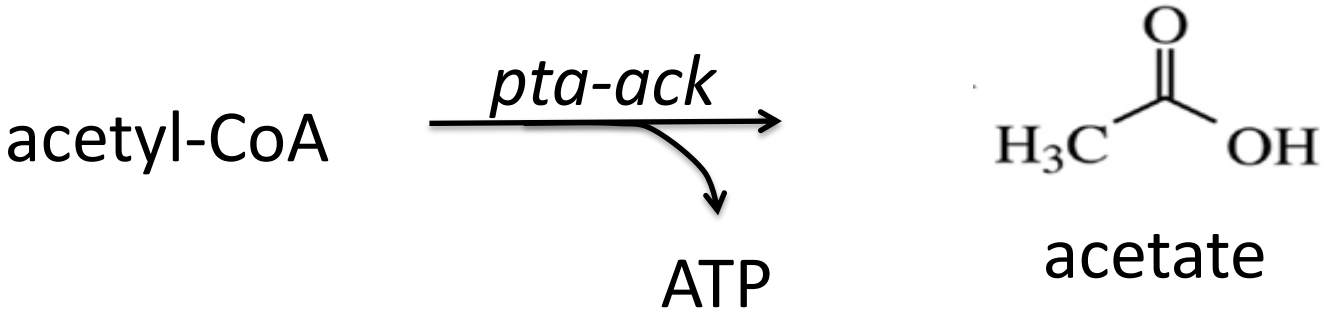
- Bioethanol is most important biotechnological commodity
- *adhE* only transcribed in anaerobic conditions



Noreen's M2D7 lecture

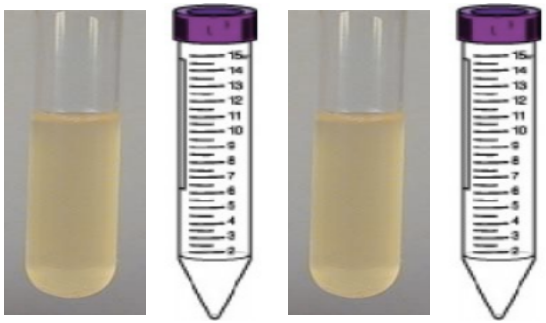
Production of acetate

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



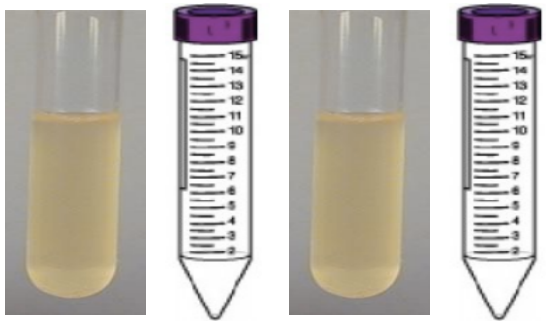
Noreen's M2D7 lecture

Experimental conditions: mixed-acid fermentation and pdCas9 induction



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

MG1655



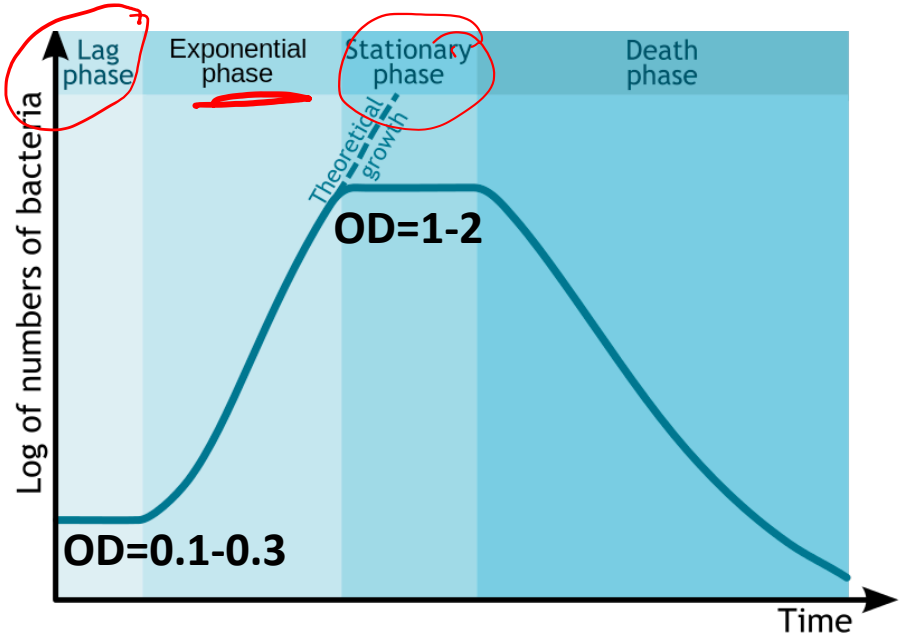
+ O_2 - aTc | - O_2 - aTc | + O_2 + aTc | - O_2 + aTc

MG1655 with CRISPRi

Normalize for bacterial cells by measuring optical density O.D.

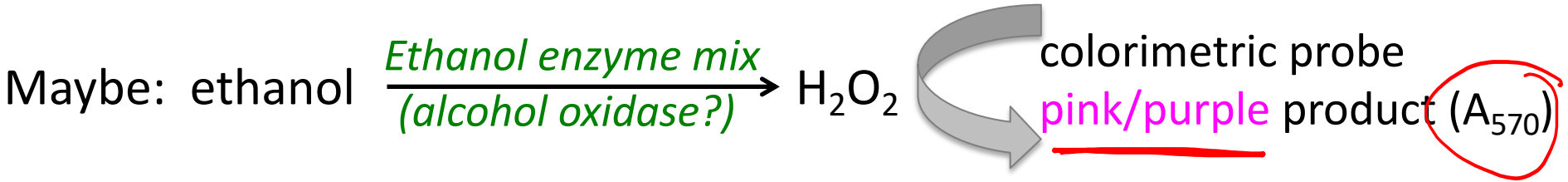
Measure *E. coli* (MG1655) concentration by optical density

- Optical Density (O.D.) ≠ absorbance
- Measure of light scattering
  - *E. coli* yellowish, 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)



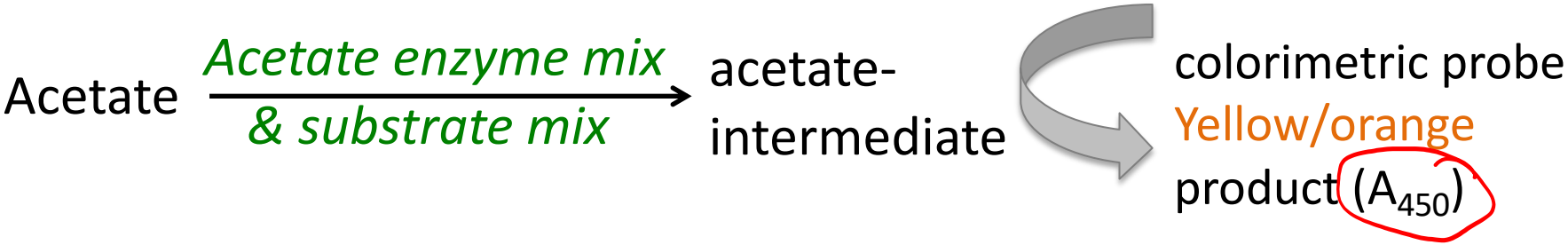
*You will measure a 1:10 dilution of your culture—remember this for your analysis!

The ethanol colorimetric assay is (very!) proprietary



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

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

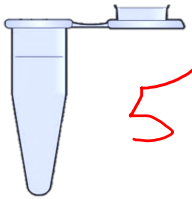
duplicate

Standard 1-6

triplicate

E. coli Samples 1-8

Reaction mix for ALL standard and sample wells



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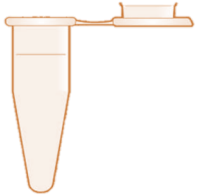

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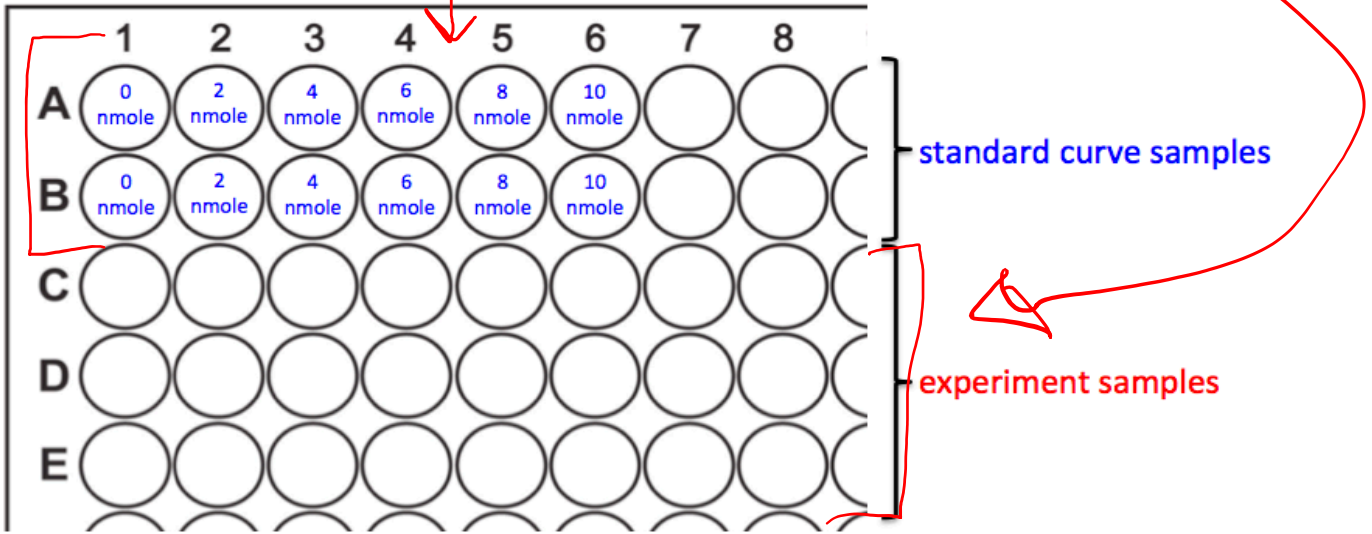
+20% pipettor error



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 template or nontemplate strand	Colorimetric Assay Results
TR orange	E	ldhA	GTACTGTTTTGTGCTATAAA	Coding region	Non-template	[[File: I Raw data]]
TR yellow	E	ldhA	AAATTCCAGCTCAAAGCCAAAGG	Coding region	Non-template	[[File: I Raw data]]
TR green	E	ack	TTAGCCACGTATCAATTATAGG	Promoter Region	Template	[[File: I Raw data]]
TR blue	A	adhE	ccagagcggcggcgcggaag	Coding region	Non-template	[[File: I Raw data]]
TR pink	E	ppc	AGCATACTGACATTACTACGCAATG	Coding region	Non-Template	[[File: I Raw data]]
TR purple	E	ldhA	CTTAAATGTGATTCAACATCACTGG	Promoter region	Template	[[File: I Raw data]]

W/F [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 template or nontemplate strand	Colorimetric Assay Results
WF Cyan	E	frd	GTGGGATAAAAACAATCTGG	promoter	Template	[[File: I Raw data]]
WF Cyan	E	ppc	ACTGACATTACTACGCAATG	beginning of gene	Non-template	[[File: I Raw data]]
WF Cyan	E	pta	TTTGTAACCCGCCAAATCGG	promoter	Template	[[File: I Raw data]]

2-3 other data sets for report

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 (4^{th} floor)
6. Upload an Excel spreadsheet with your ODs (x10) and absorbance readings to Class Data Page
7. Calculate fermentation product concentration from assay results