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

Major assignments for M2

- **Research Article**
  - Due by 10pm on Mon., November 12th

Research Article content

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

- Lab notebook, specifically M2D2 due 10pm, 11/7
- Blog post for Mod 2 due 10pm, 11/13

Extra office hours

11/10 (Sat): 1-3 pm, 56-302

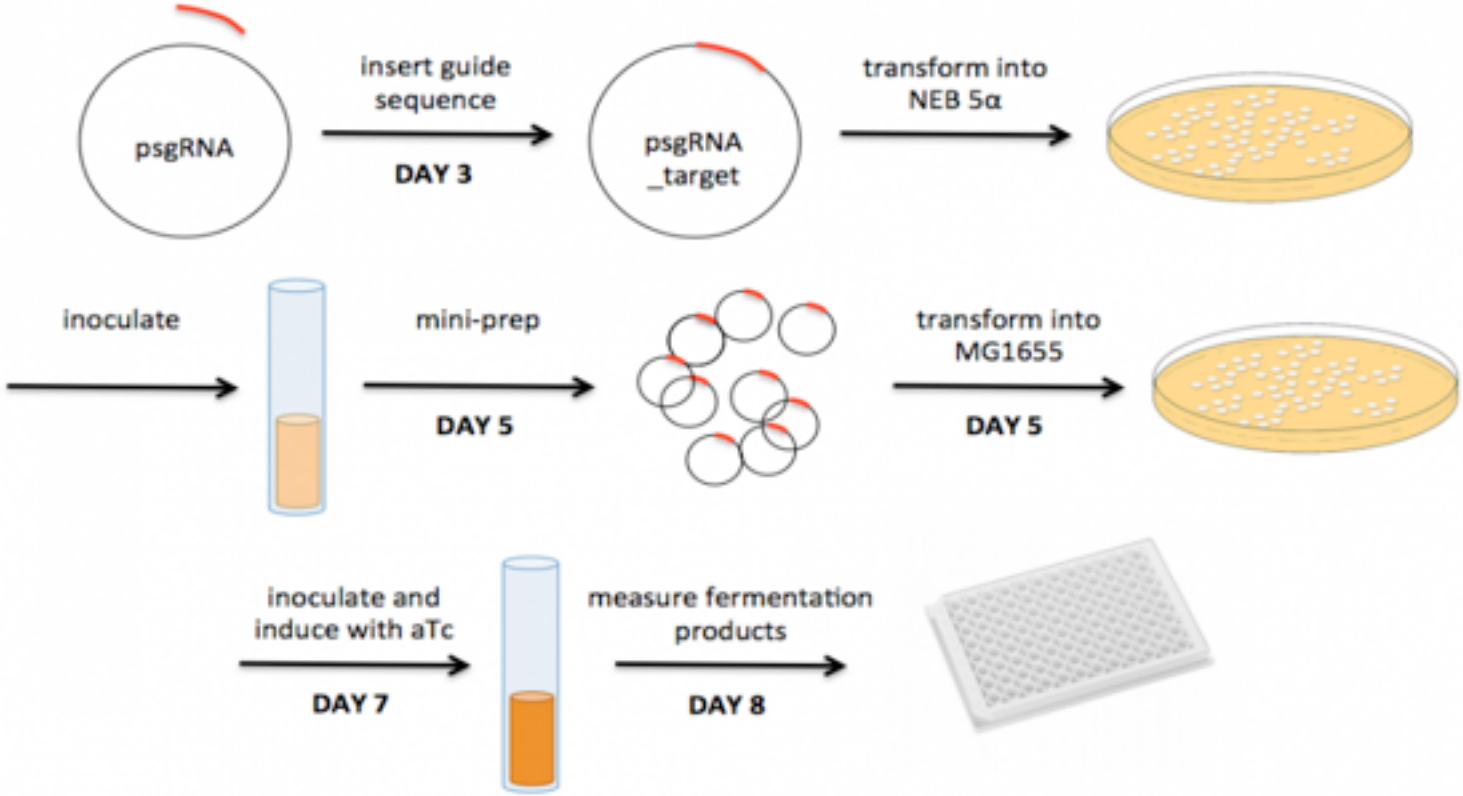
11/11 (Sun): 2-7 pm, 56-302

Regular office hours

- Noreen: Mon. 2-5pm (16-317)
- Leslie: Th 2-3pm, Fr 12-1pm (56-341c)
- Josephine: W 12-1pm, Fr 2-3pm (56-341c)
- Email us to schedule a different time

The research article is your most formal writing assignment. Use proper formatting for references, make neat figures, don't include images from lecture/prelab slides or wiki, and pay attention to guidelines on the wiki.

M2 experimental overview

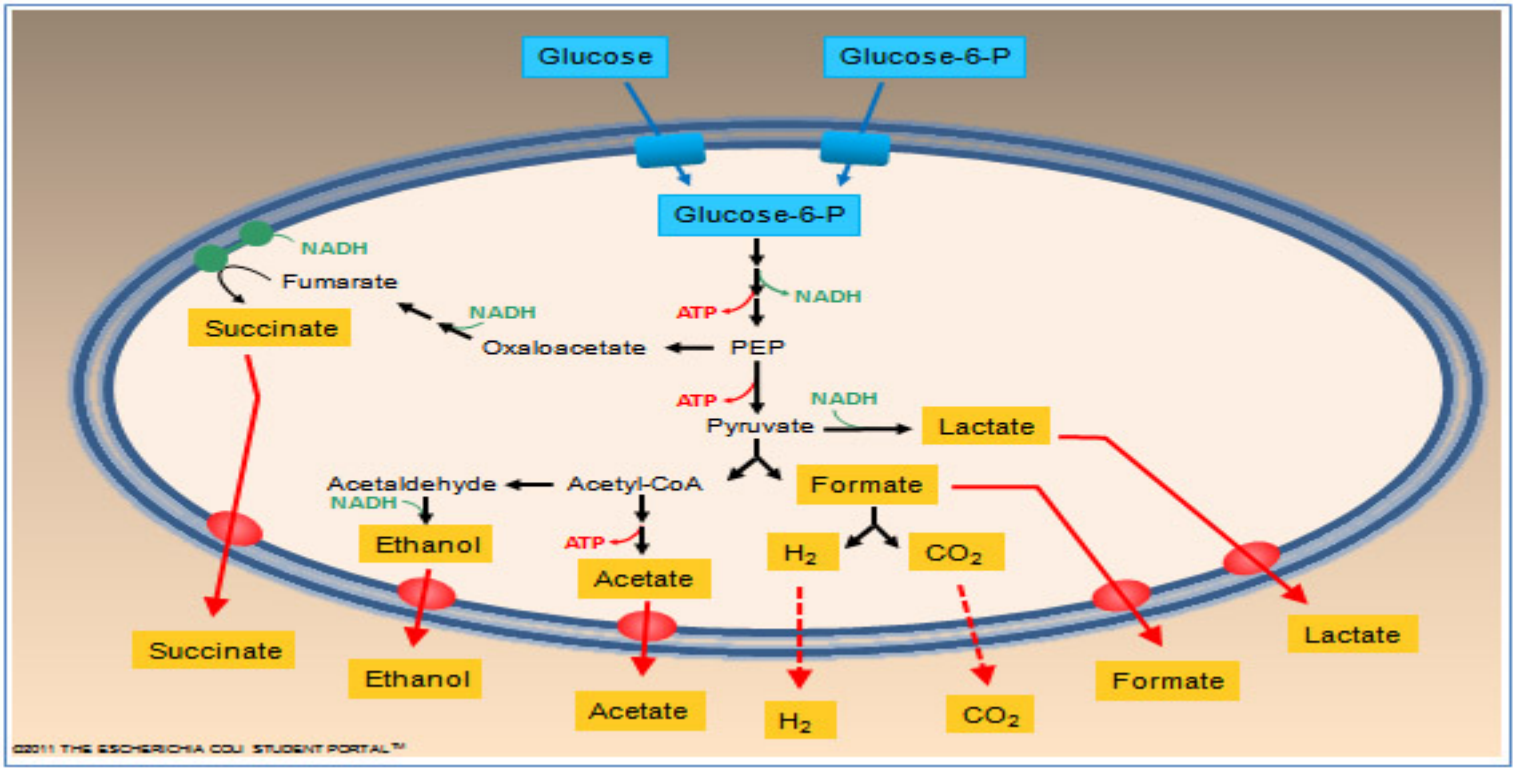


Name your target gRNA plasmid: pgRNA_ _____

anaerobic

E. coli fermentation pathway

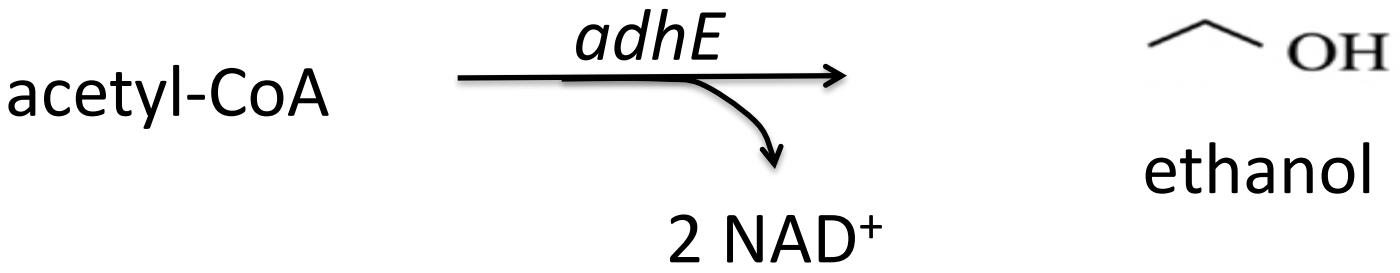
What does mixed-acid mean?



http://ecolistudentportal.org/article_fermentation#_

Production of ethanol

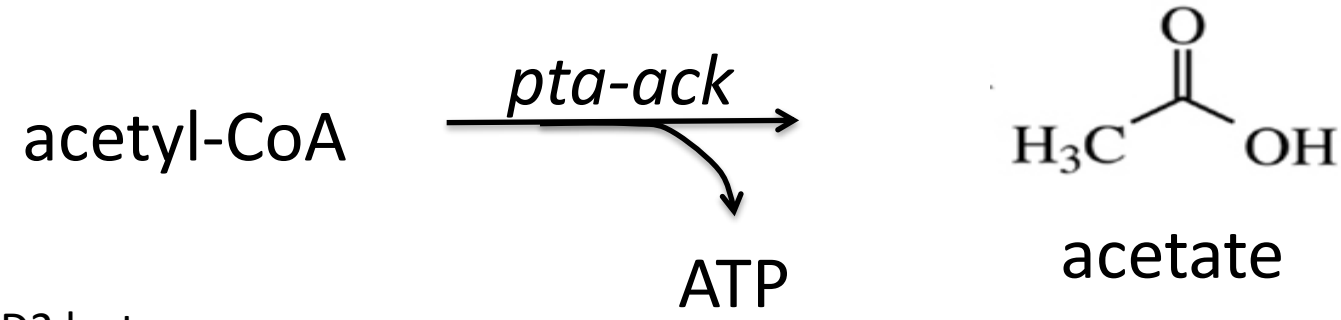
- Bioethanol is most important biotechnological commodity
- *adhE* only transcribed in anaerobic conditions



Noreen's M2D2 lecture

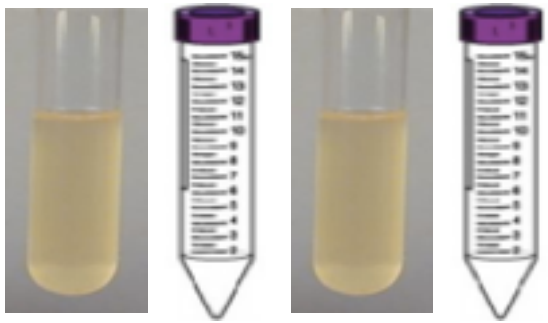
Production of acetate

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



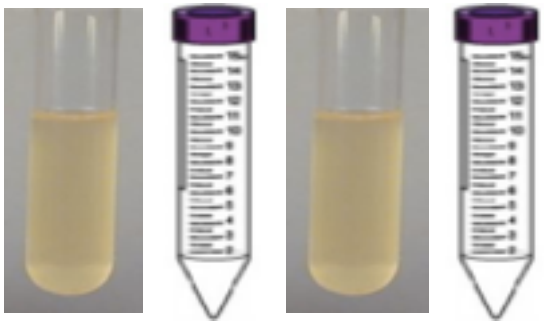
Noreen's M2D2 lecture

Experimental conditions: mixed-acid fermentation and pdCas9 induction



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

MG1655



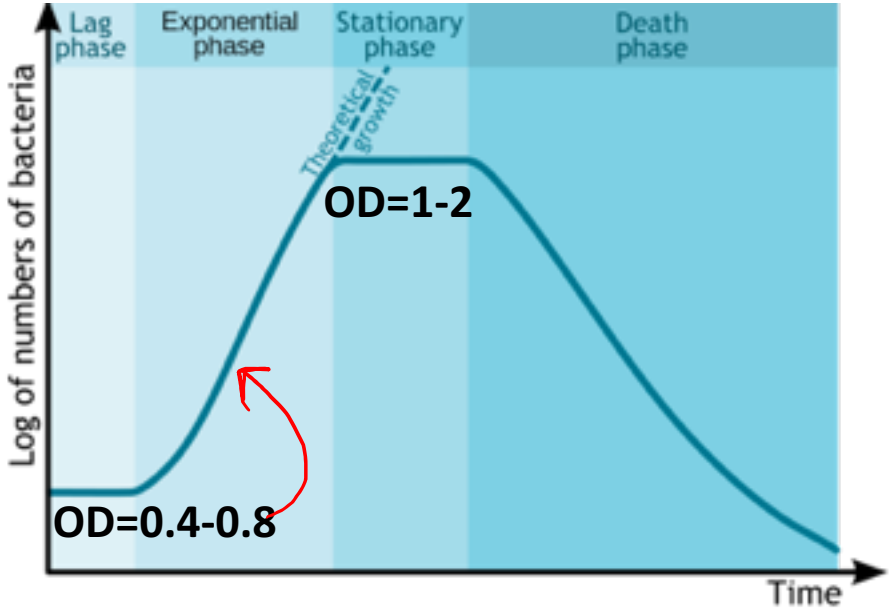
+ O_2 - aTc | - O_2 - aTc | + O_2 + aTc | - O_2 + aTc

MG1655 with CRISPRi

Normalize for number of cells by measuring OD

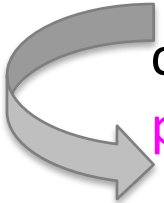
Measure *E. coli* (MG1655) concentration by optical density

- 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)



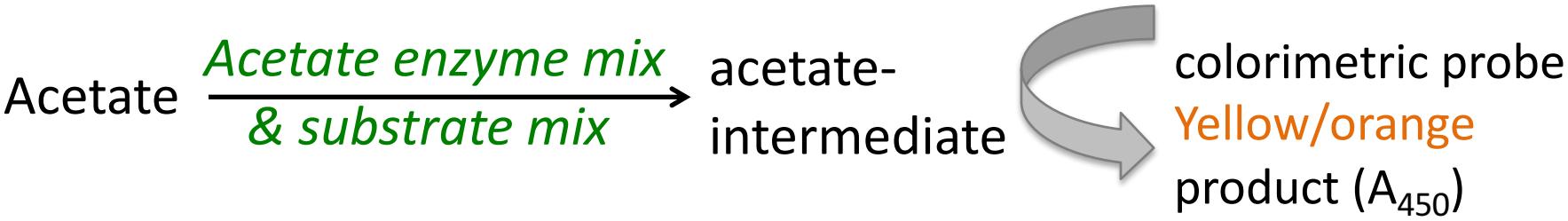
*You will measure a 1:10 dilution of your culture—remember this for your analysis!

The ethanol colorimetric assay is (very!) proprietary

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

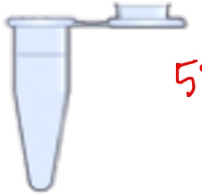
The acetate colorimetric assay is also (very!) proprietary



- 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



50 µL/well

E. coli Samples 1-8

· dilute
· 50 µL/well

Reaction mix for ALL standard and sample wells

· Calculate 20% extra
· 50 µL/well

	1	2	3	4	5	6	7	8
A	0 nmole	2 nmole	4 nmole	6 nmole	8 nmole	10 nmole		
B	0 nmole	2 nmole	4 nmole	6 nmole	8 nmole	10 nmole		
C								
D								
E								

standard curve samples

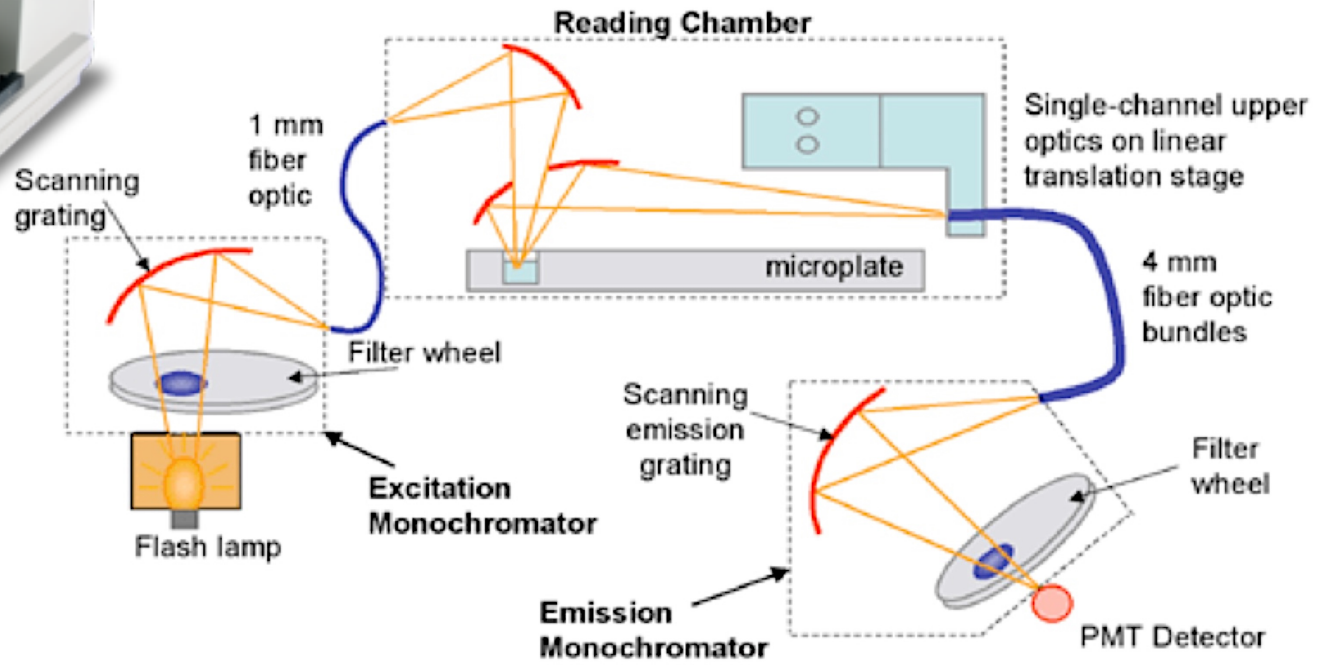
duplicate

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 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 red	E	pta	TGCGCCCGATCAGACTACGACTATC	Middle of the operon	Non-template	Raw data
TR orange	E	ldhA	gttgcaggtacttcttgtcgt	32 bps downstream from start of gene	Template Strand	Raw data
TR green	A	adhE	TACTAAAAAAGTTTAACATTATCA	locus targeted: -50 upstream (promoter)	Template strand	Raw data
TR pink	A	Citrate Synthase (gltA)	tgagttttgcttttgtatcagccat	Beginning of gene	Non-template Strand	Raw data
TR purple	A	ldhA	TAGTAGCTTAAATGTGATTCAACAT	Locus targeted: -40 upstream region (promoter)	Non-template strand	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 yellow	A	adhE	ATTCGAGCAGATGATTTACTAAAAA			Raw data
WF green	Acetate	adhE	TTACTAAAAAAGTTTAACATTATCA	locus targeted: -35 upstream (promoter)	template strand	Raw data
WF blue	A	adhE	TTCGAGCAGATGATTTACTAAA	locus targeted: -65 upstream (promoter)	Template Strand	Raw data

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. Calculate fermentation product concentration from assay results
7. Upload an Excel spreadsheet with your ODs (x10) and absorbance readings on Wiki