M2D8: Measure fermentation products

11/05/19

- 1. Quiz
- 2. Pre-lab
- 3. Measure OD of your bacteria
- 4. Measure fermentation products
- 5. Start data analysis
- We will grade notebook M2D2



Sketchina-Science

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: You must compare team data vs. class data, 2-3 experiments
- 6. Discussion
- 7. References
- Lab notebook, specifically M2D2 due 10pm, 11/6
- Blog post for Mod 2 due 10pm, 11/12

Extra office hours

11/7 (Thurs): 5-7p pm, 16-469 11/9 (Sat): 12-2p, 56-302 11/10 (Sun): 12-2p, 56-302 **Regular office hours**

• Noreen: W, F 10-12p, 16-317

- Leslie: W 9:30-10:30a, 16-469
 **F 4-5p canceled
- Becky: T, R 12-1p, 16-469
- 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





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

acetyl-CoA
$$\xrightarrow{pta-ack}$$
 H_3C OH
ATP acetate

0

Noreen's M2D2 lecture

Experimental conditions: mixed-acid fermentation and pdCas9 induction



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 $\frac{1}{1}$ dilution of your culture—remember this for your analysis!

The ethanol colorimetric assay



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

The acetate colorimetric assay



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



Today in lab...

- 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. Post data in the formatted excel to the wiki class data page