M2D8: Measure fermentation products

11/8/16

1. Quiz
2. Pre-lab
3. Prep 8 samples and prepare D-lactate assay
4. Take samples to spectrophotometer and measure lactate
5. VOTE! (if you haven't already)

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

There are snacks!

MOD3 starts next week!!

***I'm available all day Thursday 11/10!**

Major assignments for M2

- Research Article (20%)
  - due by 5pm on Sun., 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 M1 due by 10pm on Mon., Nov. 21st

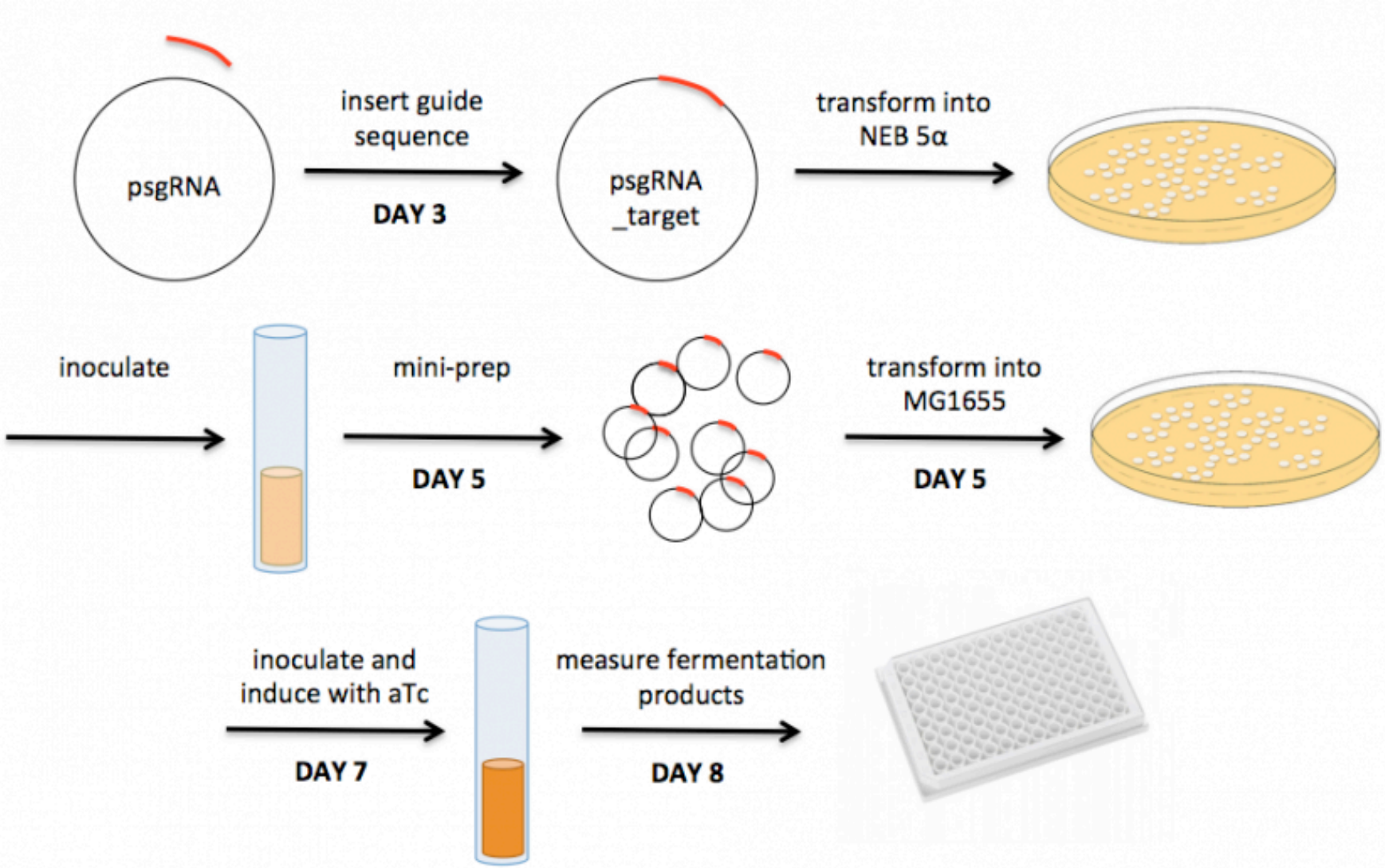
Extra office hours

- Saturday Nov. 19th 10am-5pm

Regular office hours

- Noreen: Monday 1pm and 5pm in 16-317
- Leslie: Monday 4pm, Wednesday 9am in 16-429b
- Maxine: Monday 2pm, Friday 9am in 16-239

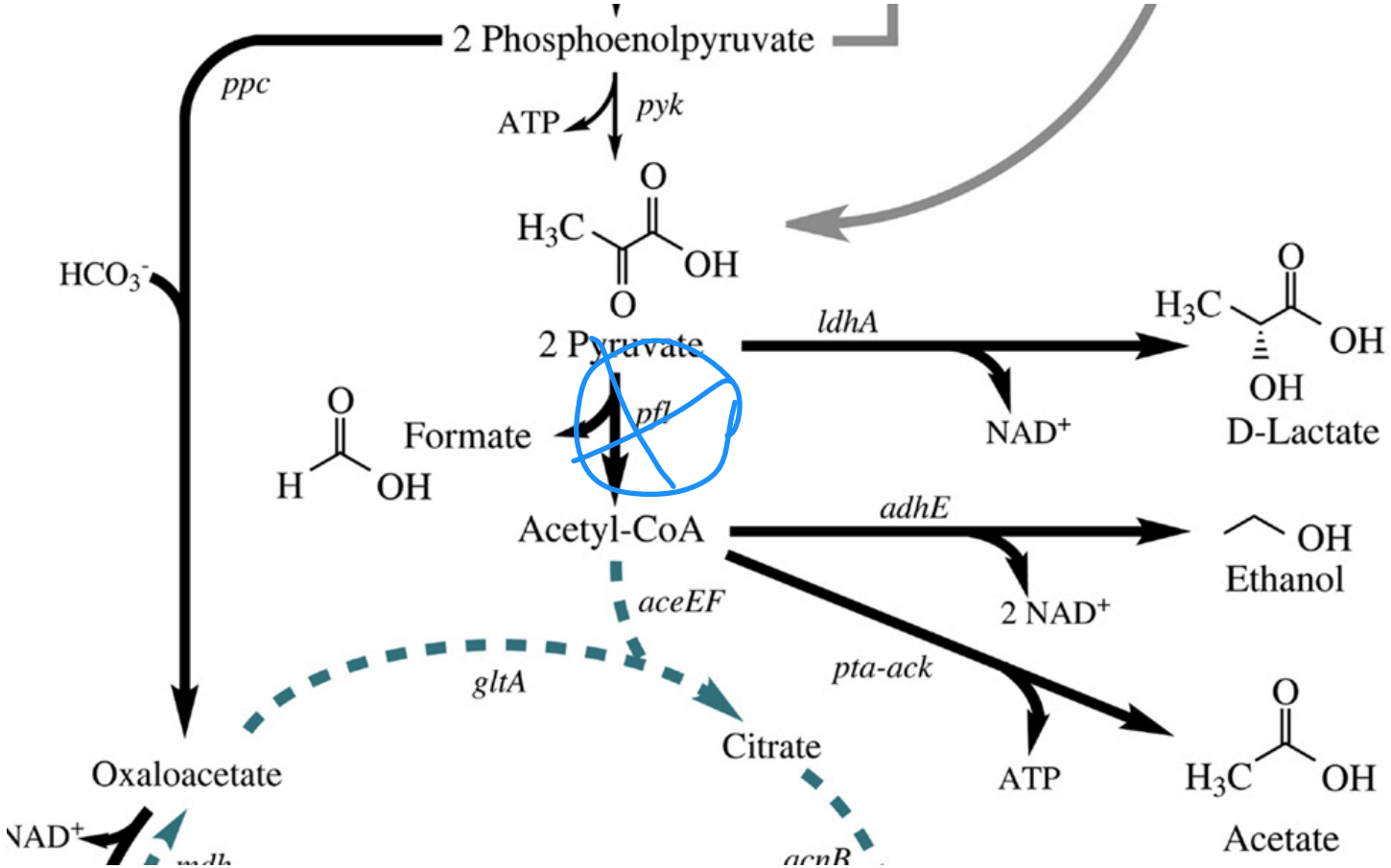
M2 experimental overview (a few methods tips)



E. coli fermentation pathway

-under anaerobic conditions when cells can not perform cellular respiration

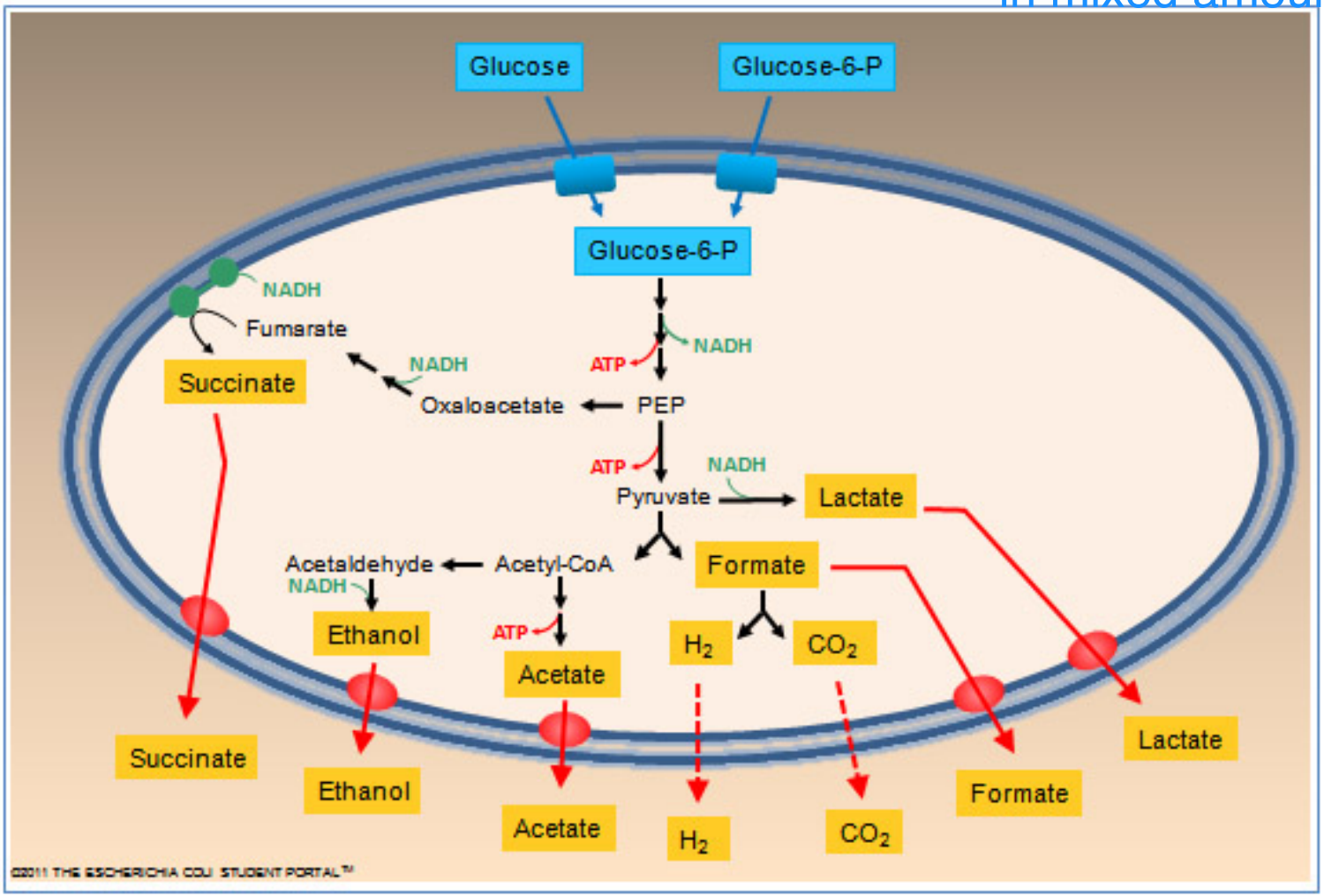
-energy yields are low and cells are slow to grow



E. coli fermentation pathway

What does mixed-acid mean?

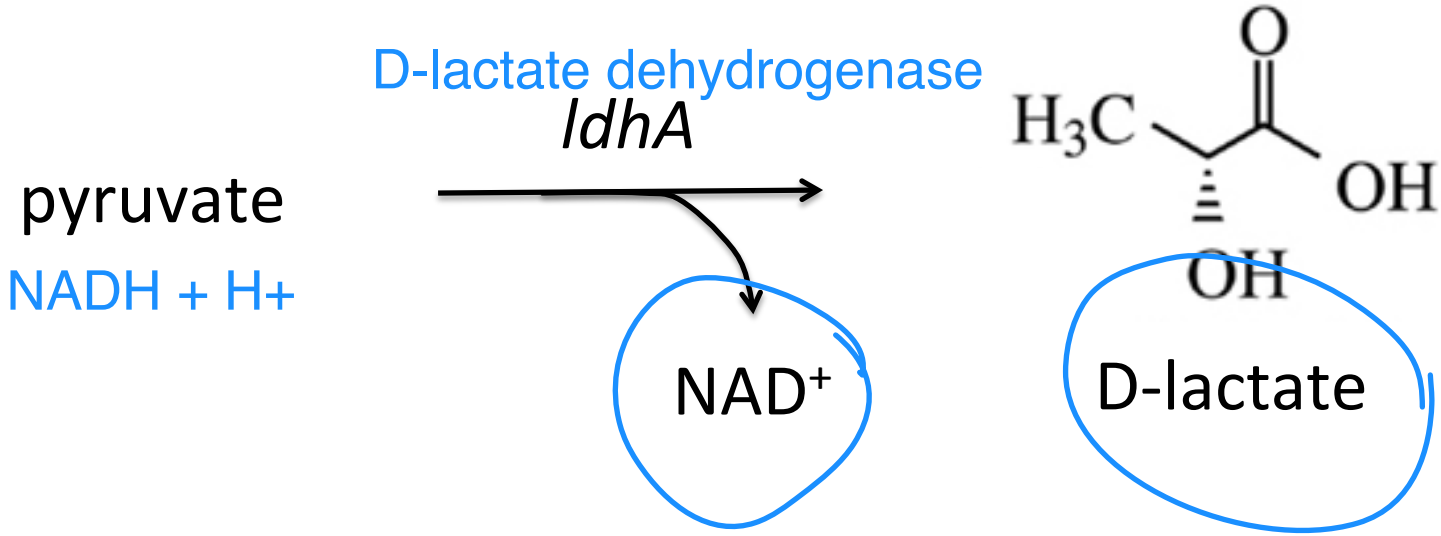
alternative end products in mixed amounts



http://ecolistudentportal.org/article_fermentation#_

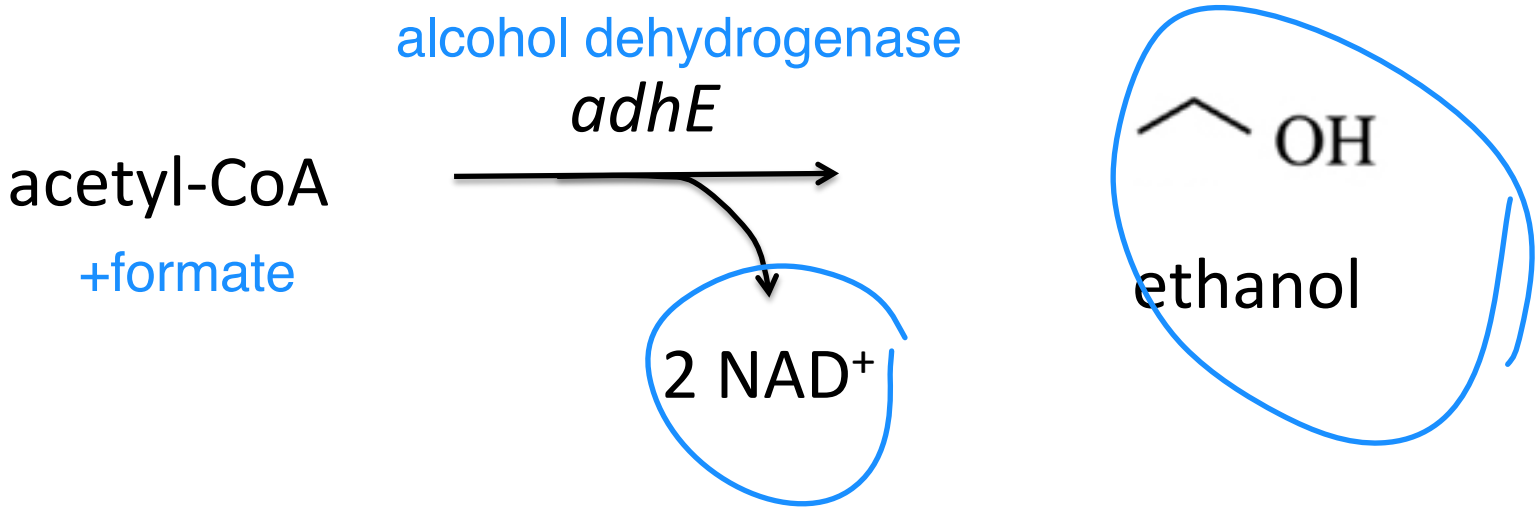
Production of lactate

- Lactate is used in food and production of polymers, pharmaceuticals, and cosmetics
- *ldhA* expressed constitutively
  - Level increased 5 to 10-fold in anaerobic conditions

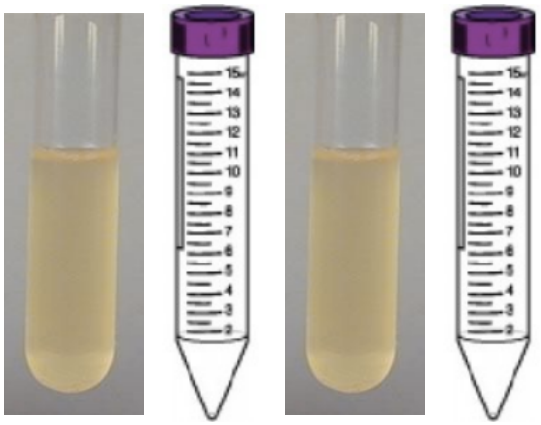


Production of ethanol

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

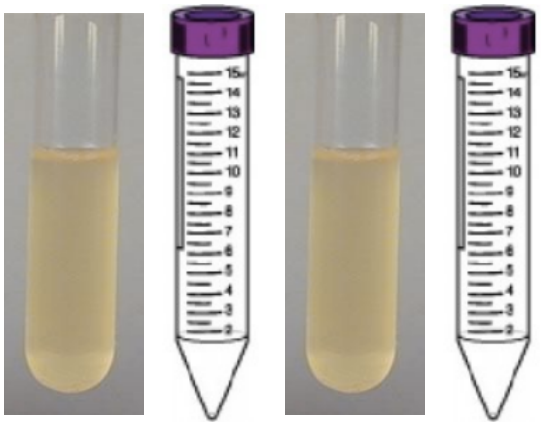


Experimental conditions: mixed-acid fermentation and pdCas9 induction



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

MG1655



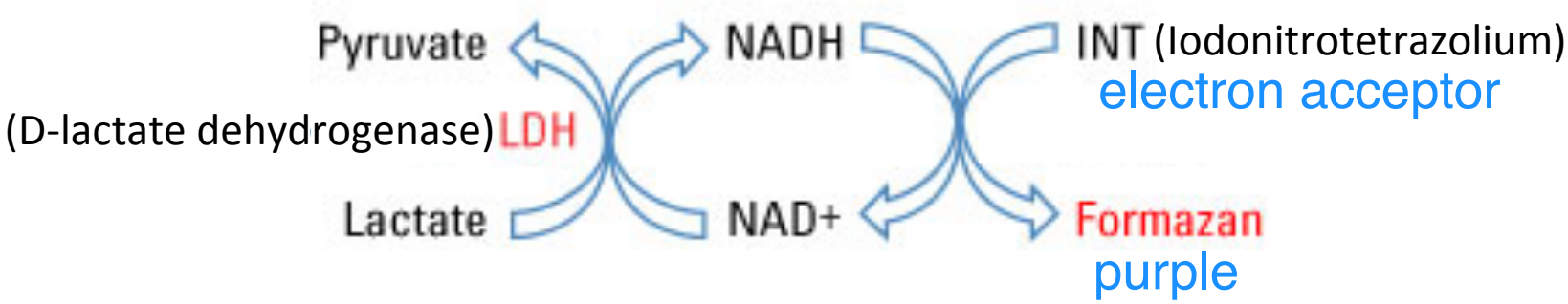
+ O_2 - aTc | - O_2 - aTc | + O_2 + aTc | - O_2 + aTc

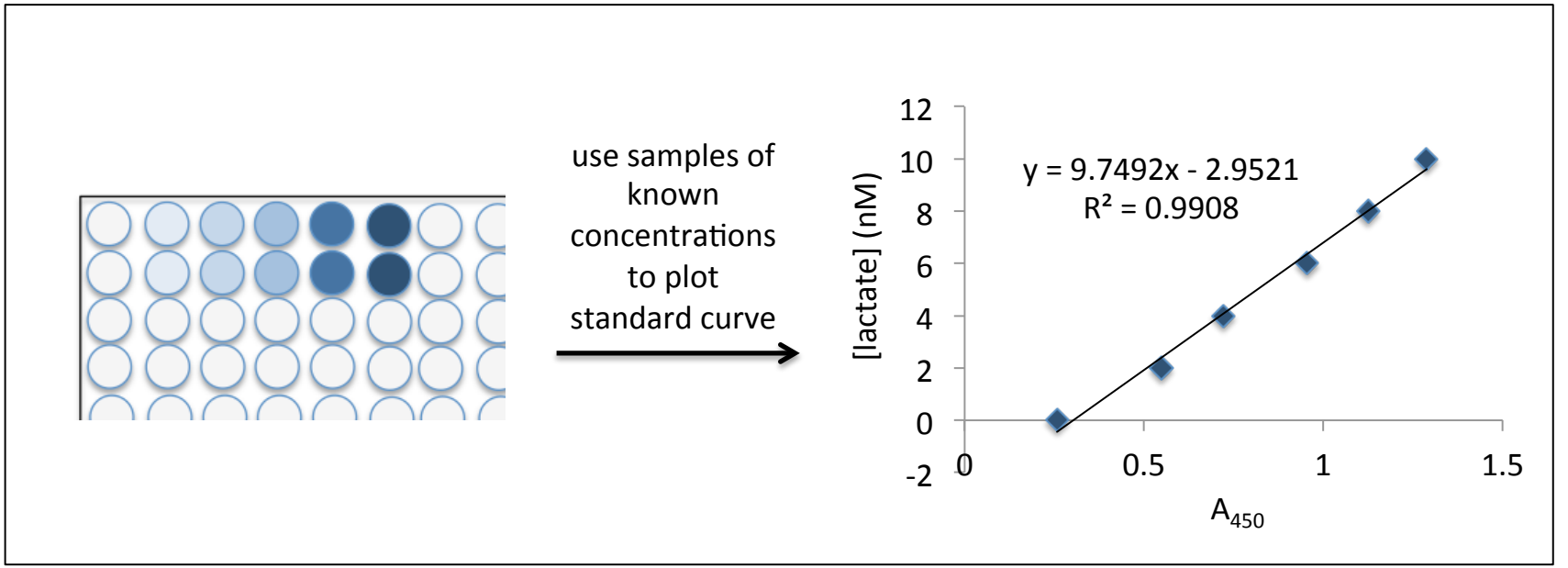
MG1655 with CRISPRi

hypothesis: we hypothesize that we can increase lactate production MG1655 using CRISPRi promoter site of the pflb gene to prevent gene expression. We predict that block of gene expression of pflb will reroute fermentation products to produce more lactate.

D-lactate colorimetric assay

enzyme works in both directions!





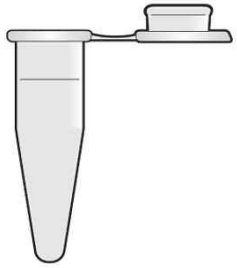
thermofisher.com, LDH Cytotoxicity Assay Kit

Abcam Colorimetric D-lactate Assay Kit

- D-lactate is specifically oxidized by D-lactate dehydrogenase and the product generates a proportional color (Abs= 450 nm)
- Major kit components:
  - D-Lactate Assay Buffer detergents, salts
  - D-Lactate Enzyme Mix D-LDH
  - D-Lactate Standard purified lactate
  - D-Lactate Substrate Mix INT

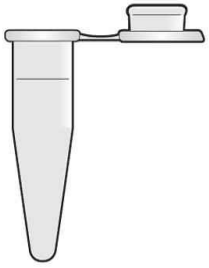
M2D8 Experimental Outline

E. coli Samples 1-8



1. Pellet
2. Wash with PBS
3. Lyse in assay buffer
4. Spin at 4°C
5. Transfer to fresh tube

lactate standard 1-6

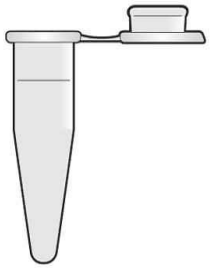


1. Retrieve lactate standard stock from front bench
2. Dilute stock
3. Make dilution series
4. Don't forget 0 nmole tube!

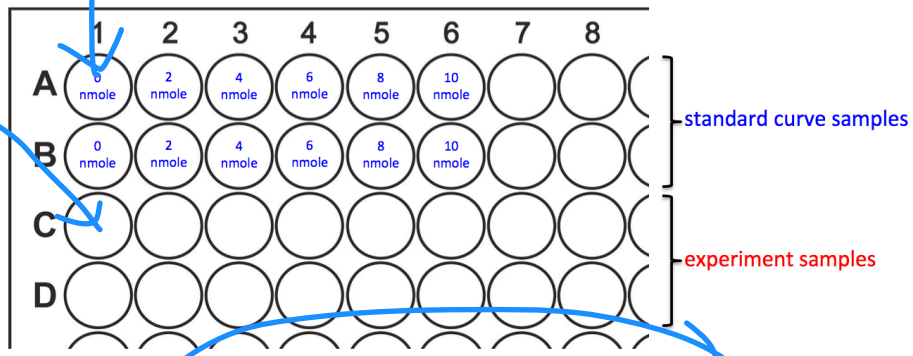
50 ul + 50 ul RXN MIX

50 ul + 50 ul RXN MIX

1 Reaction mix for all standards and samples



1. Retrieve substrate mix and enzyme mix from front bench
2. Mix buffer, substrate and enzyme.
3. Aliquot 50ul to each reaction well



Cover with foil!

Compare group data vs. class data

M2D2: gRNA design [edit]

T/R: lactate [edit]

Team	Gene targeted by CRISPRi gRNA	gRNA sequence (without tag at 3' end)	Locus targeted (*e.g.* beginning of gene, putative promoter, -35 region)
yellow	pflB	TGTCGAAGTACGCAGTAAAT	putative promoter
green	pflB	ATAAAAAATCCACTTAAGAAGGTA	Putative Promoter
blue	pflB	TTCATTAAGCTCGGACATGTAACA	End of Putative Promoter & beginning of pflB gene
pink	pflB	AAATAAAAAATCCACTTAAGAAGGT	Putative Promoter
purple	pflB	AAATCCACTTAAGAAGGTAGGTGT	putative promoter

W/F: ethanol [edit]

Team	Gene targeted by CRISPRi gRNA	gRNA sequence (without tag at 3' end)	Locus targeted (*e.g.* beginning of gene, putative promoter, -35 region)
red	pta-ack	GCC ACG TAT CAA TTA TAG GTA C	putative promoter
green	ldhA	GTAGCTTAAATGTGATTCAACATC	putative promoter
blue	fdrA	CAAGATCGGCTTGAAAGGTTTGCAC	Beginning of gene
purple	ldhA	TCGTACTGTTTTGTGCTATAAA	Beginning of coding sequence
instructors	ackB	AGTACCTATAATTGATACGTGGCTA	promoter, -30 region
instructors	ldhA	CTTAAATGTGATTCAACATCACTG	5'UTR

Today in lab…

- Retrieve samples from 37°C incubator and prepare bacterial lysate for assay
- Prepare lactate standard curve
- Combine sample or standards and assay reaction mix 1:1, incubate 30min
- Measure absorbance at 450nm
- Calculate lactate concentration from samples