

M2D8:
Measure fermentation products
and other uplifting news...

11/09/2016

No lecture tomorrow, no lab Friday !

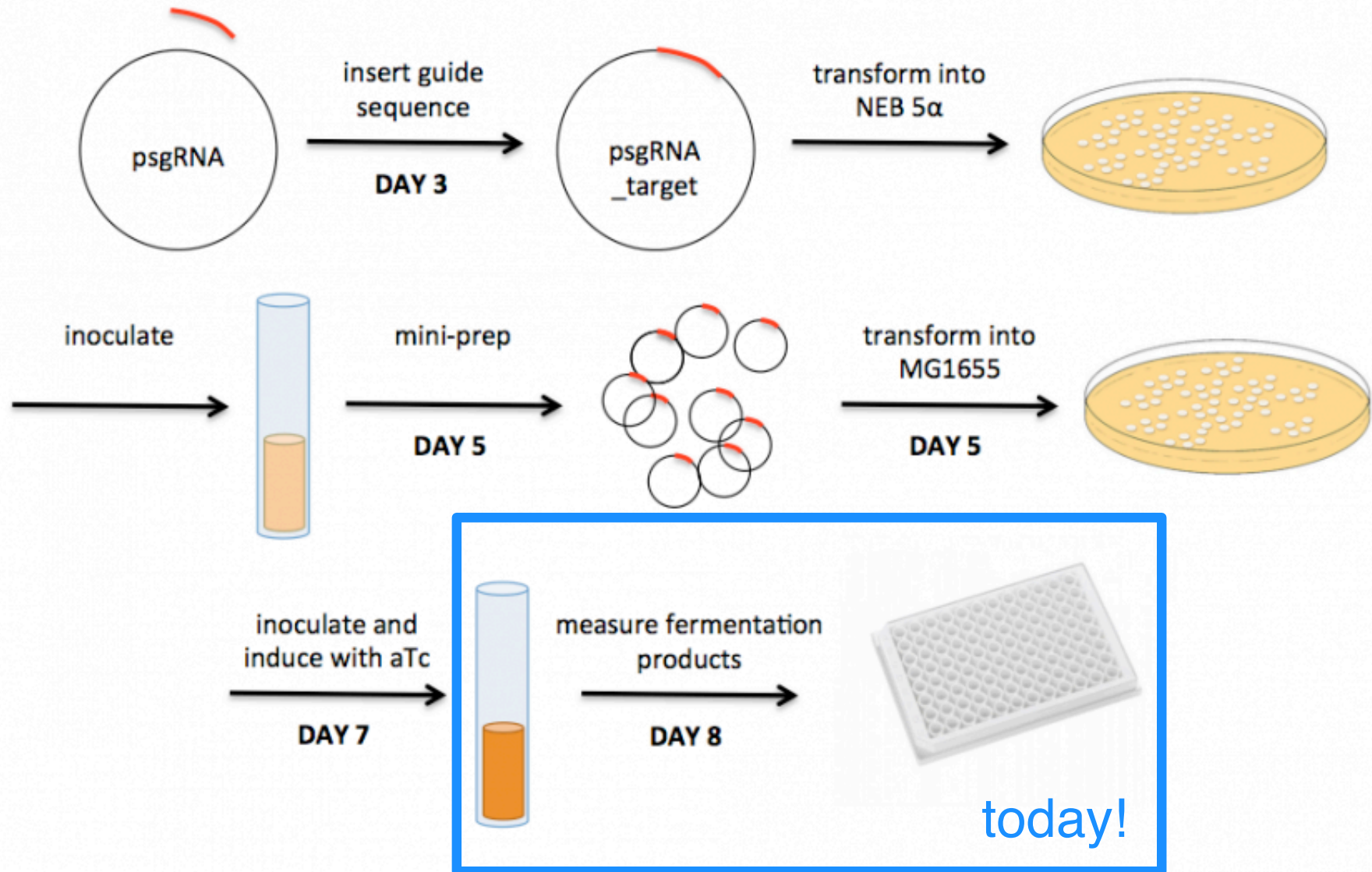


Key assignments of M2

- Journal club presentation
 - participation points for asking questions
- Lab notebook
 - at 10pm tonight, Emily will grade **M2D2**
- Research article
 - due 5pm on Sunday, November 20
 - regular office hours MWF
 - office hours Sat. 11/19 in 56-302 10am-5pm
- Blog post(s)
 - due 5pm on Monday, November 21



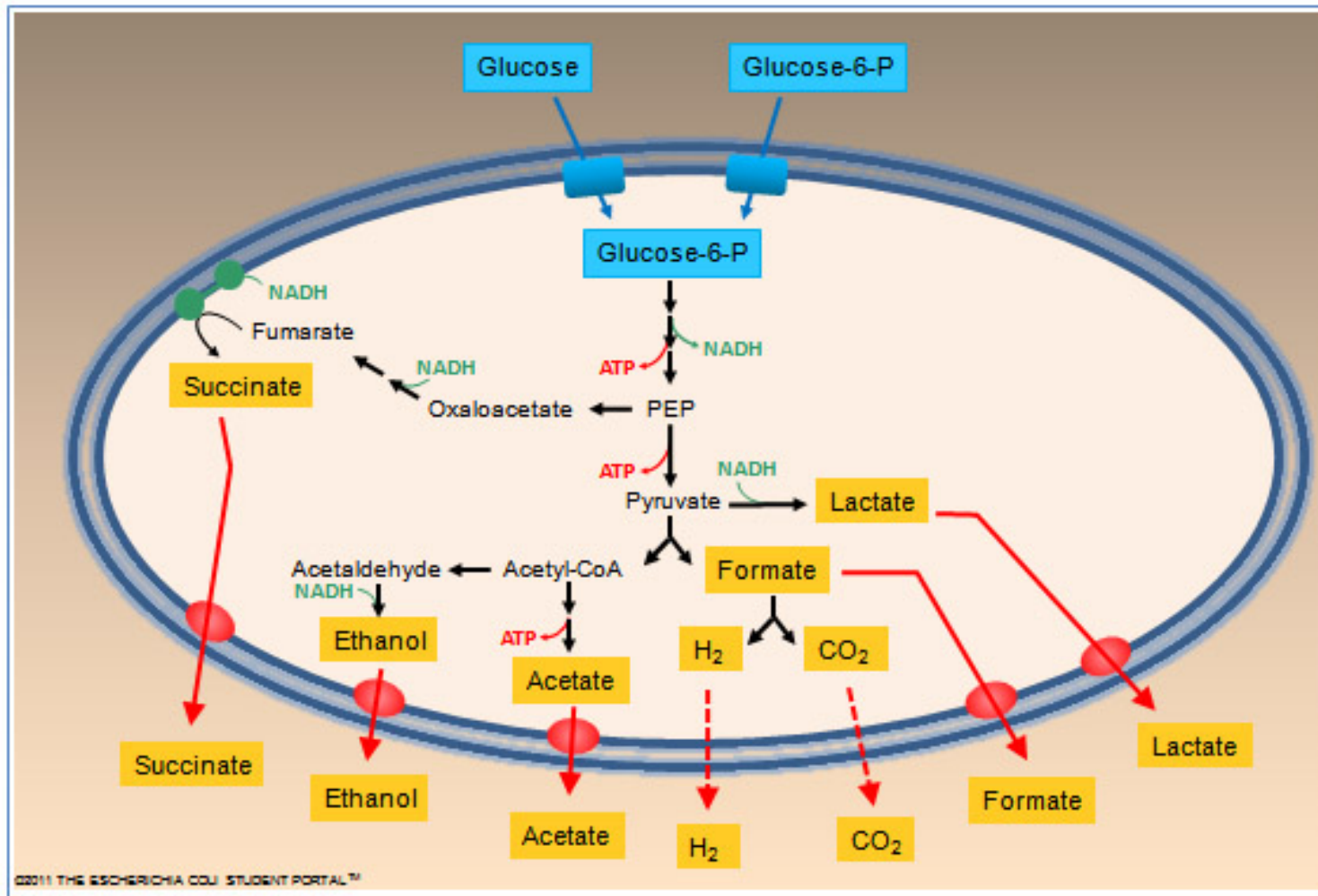
M2 experimental overview



nicotinamide adenine dinucleotide (NAD)

E. coli mixed-acid fermentation pathway

- The 2 NADH formed by the glycolysis pathway and recycled back to NAD⁺

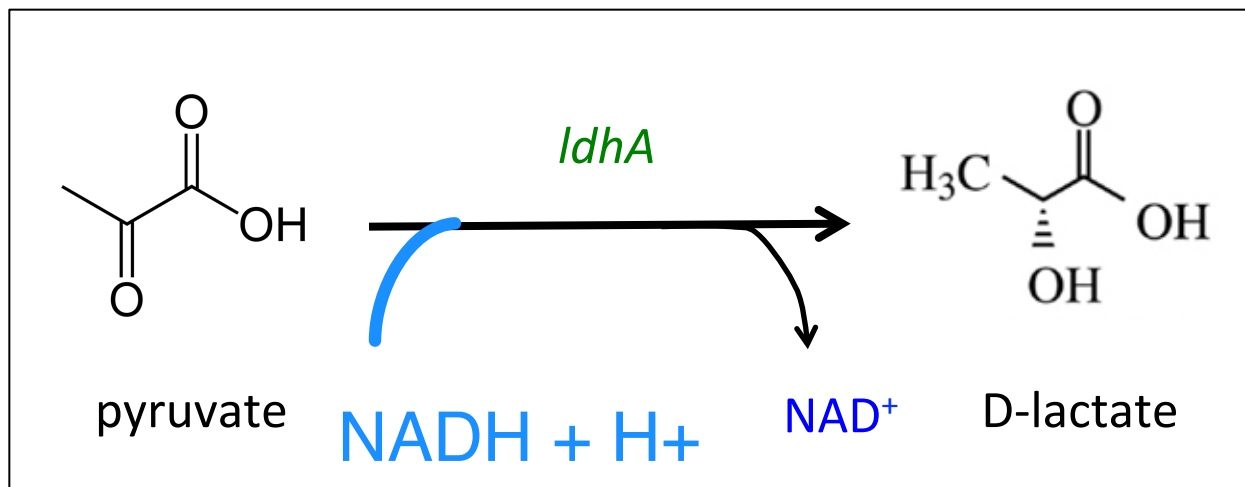


http://ecolistudentportal.org/article_fermentation#_

Example 1:

Production of lactate

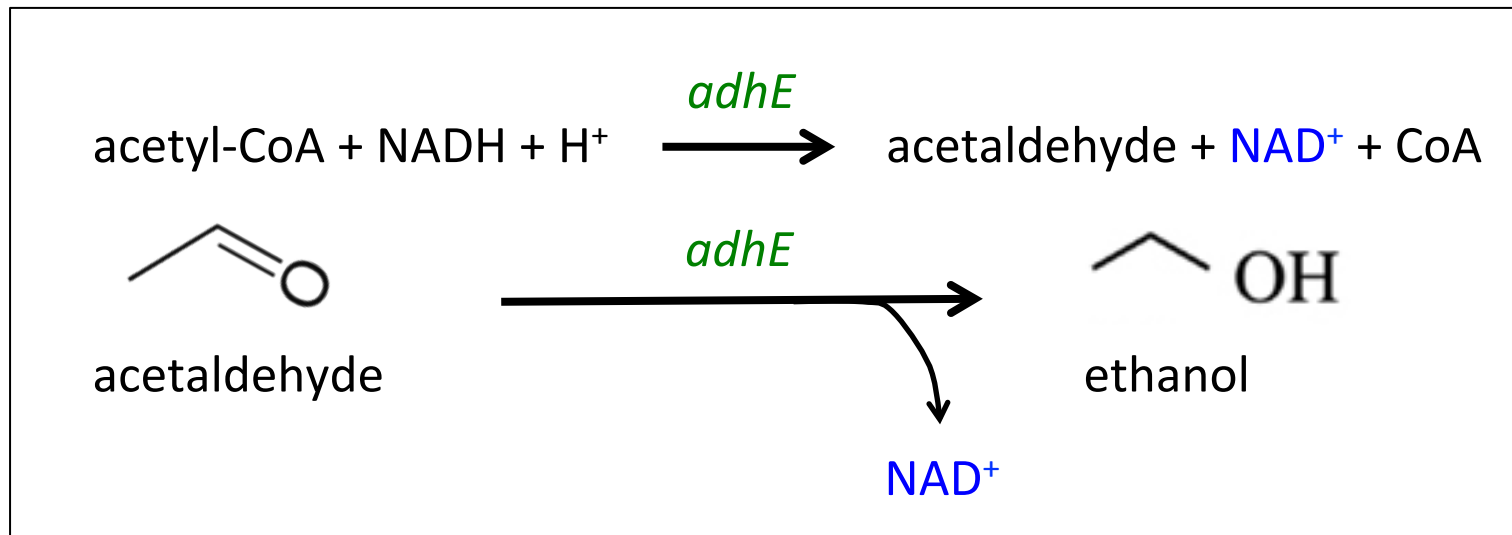
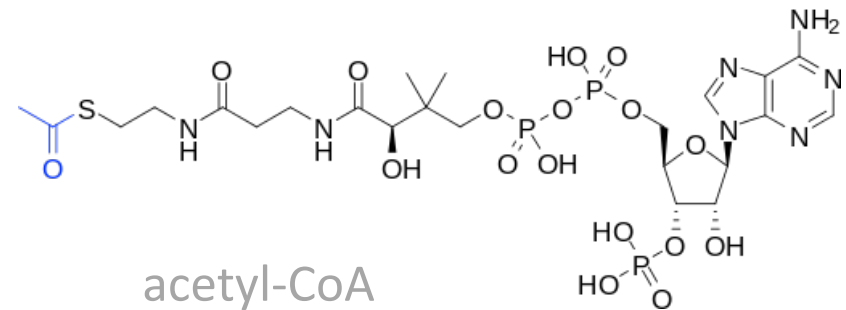
- Lactate is used in food (85%), production of polymers, pharmaceuticals, cosmetics
- *ldhA* : lactate dehydrogenase
 - expressed constitutively
 - increased 5 to 10-fold in *anaerobic* conditions



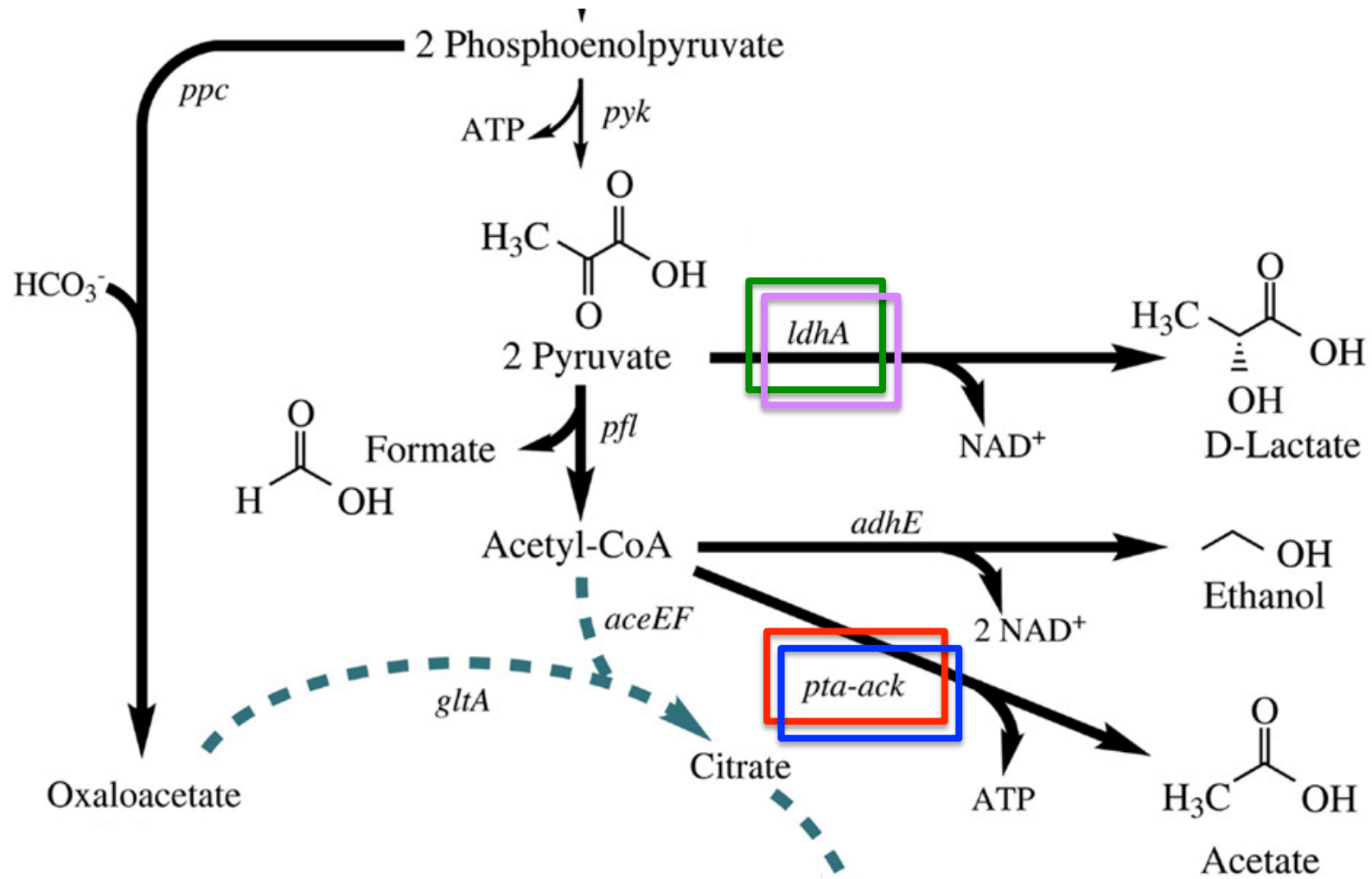
Example 2:

Production of ethanol

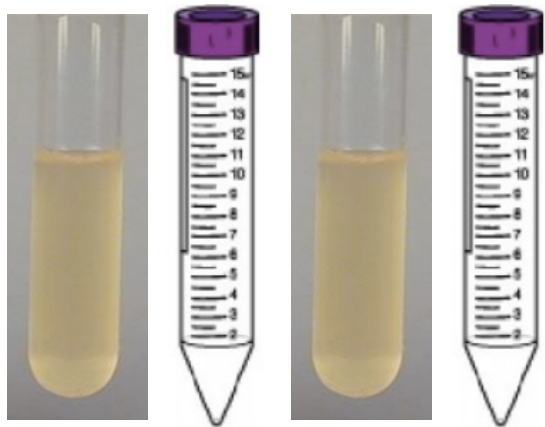
- Bioethanol is important biotechnological commodity
- *adhE* : alcohol dehydrogenase
 - only transcribed in *anaerobic* conditions



E. coli fermentation pathway

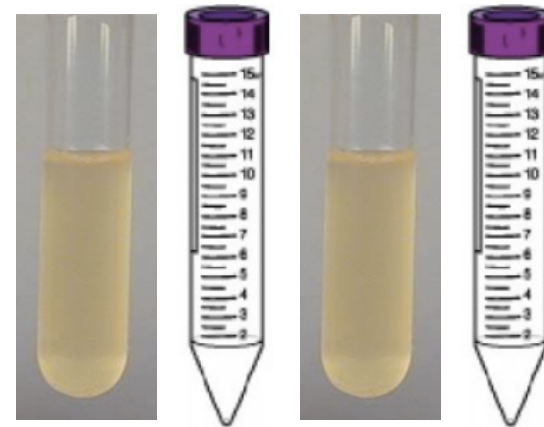


Experimental conditions: mixed-acid fermentation and pdCas9 induction



+ O₂ - O₂ + O₂ - O₂
- aTc - aTc + aTc + aTc

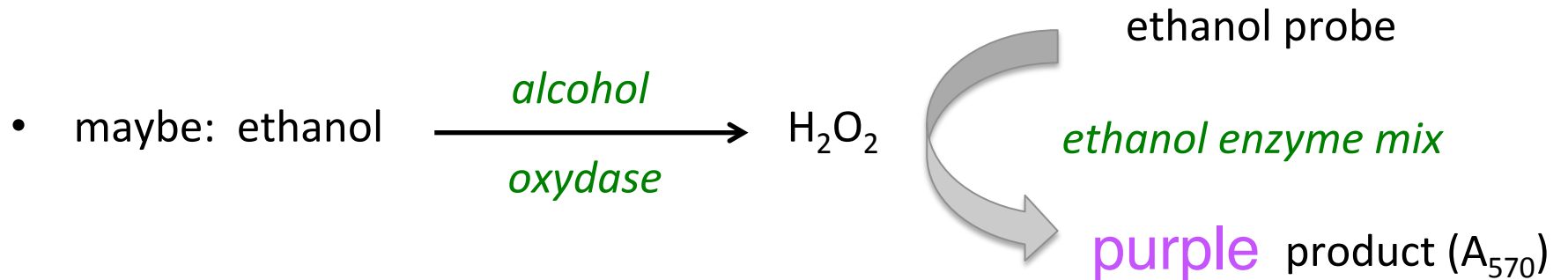
MG1655



+ O₂ - O₂ + O₂ - O₂
- aTc - aTc + aTc + aTc

MG1655 with CRISPRi

The ethanol colorimetric assay is (very!) proprietary



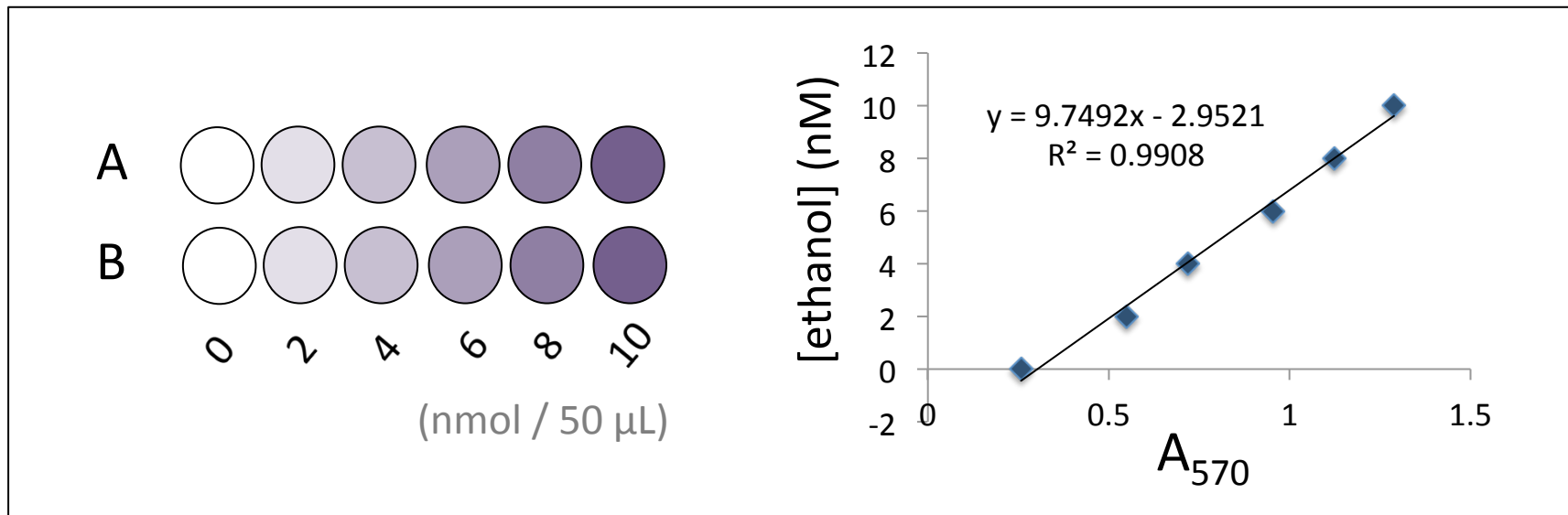
- Sigma-Aldrich MAK076 colorimetric ethanol assay kit:

- ethanol assay buffer (AB)
- ethanol probe
- ethanol enzyme mix
- ethanol standard

ethanol at a known concentration

Prepare your 96-well plate

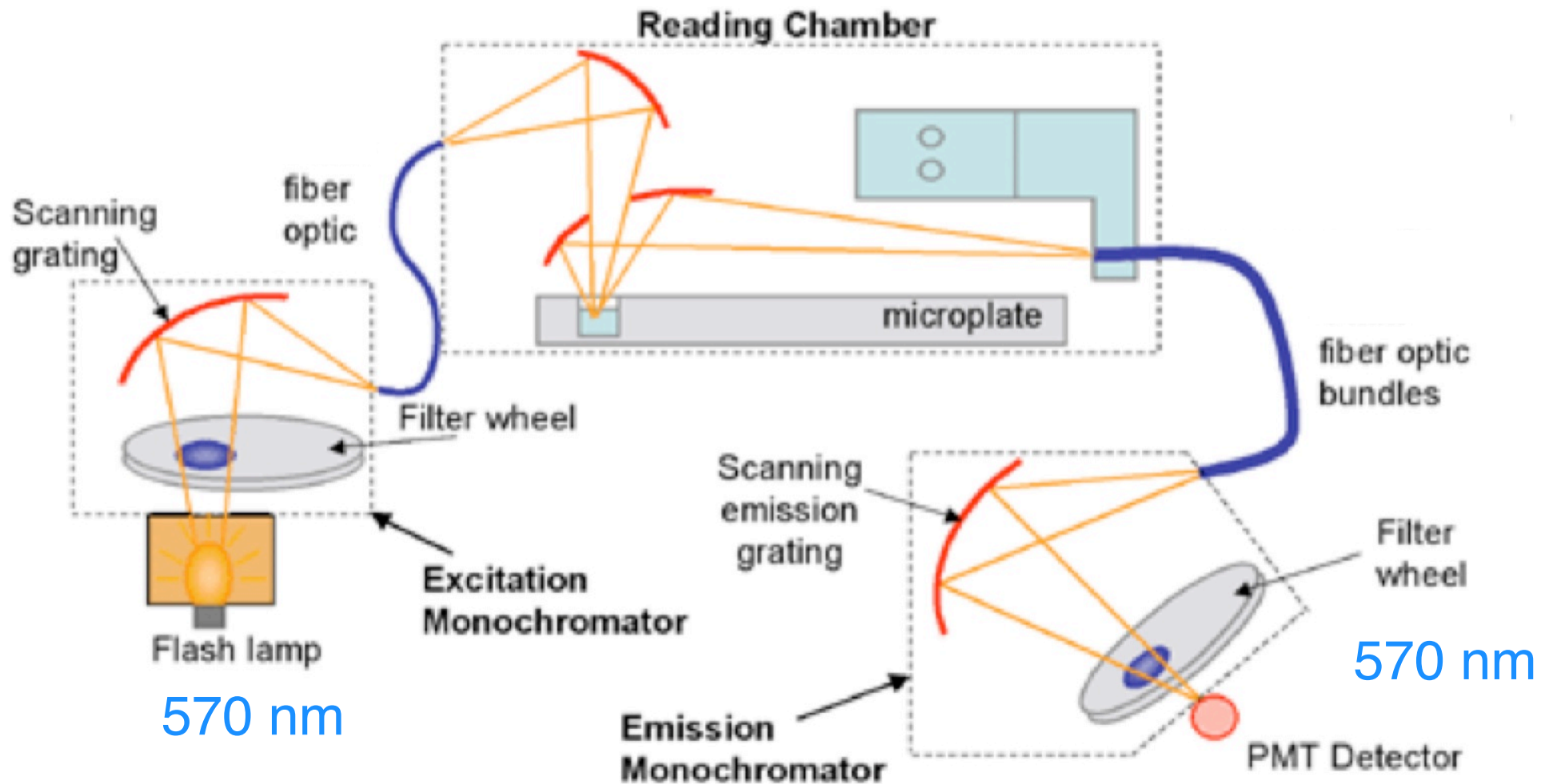
1. ethanol standards in rows A, B **6 x 2 = 12 wells**



2. *E. coli* samples in rows D,E **8 x 2 = 16 wells**
3. **Reaction mix** in all **28** wells (standards and samples)
 - 50 μL mix of buffer, probe and enzymes

➤ **Cover with foil !**

Microplate reader



Compare group data vs. class data

M2D2: gRNA design [\[edit\]](#)

[Discussion page of main M2 page](#)

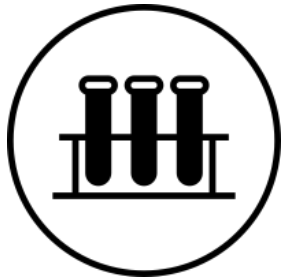
T/R: lactate [\[edit\]](#)

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	TCGTA CTGTTTTGTGCTATAAA	Beginning of coding sequence
instructors	ackB	AGTACCTATAATTGATACGTGGCTA	promoter, -30 region
instructors	ldhA	CTTAAATGTGATTCAACATCACTG	5'UTR

Today in lab... proportional to # of bacteria used to normalize [ethanol]



- Retrieve samples from 37°C incubator
 - Measure OD₆₀₀
 - Collect supernatant for assay
- Prepare ethanol standards
- Add reaction mix to all wells

- Measure absorbance at 570 nm
- Calculate ethanol concentration from samples

Nobel Prize winner (chemistry 2015)
Paul Modrich gives the BE seminar!



- Mechanisms of human DNA mismatch repair
 - 4pm on Thursday, 11/17 in NE46 (Ragon Institute)
 - Hosted by Bevin Engelward