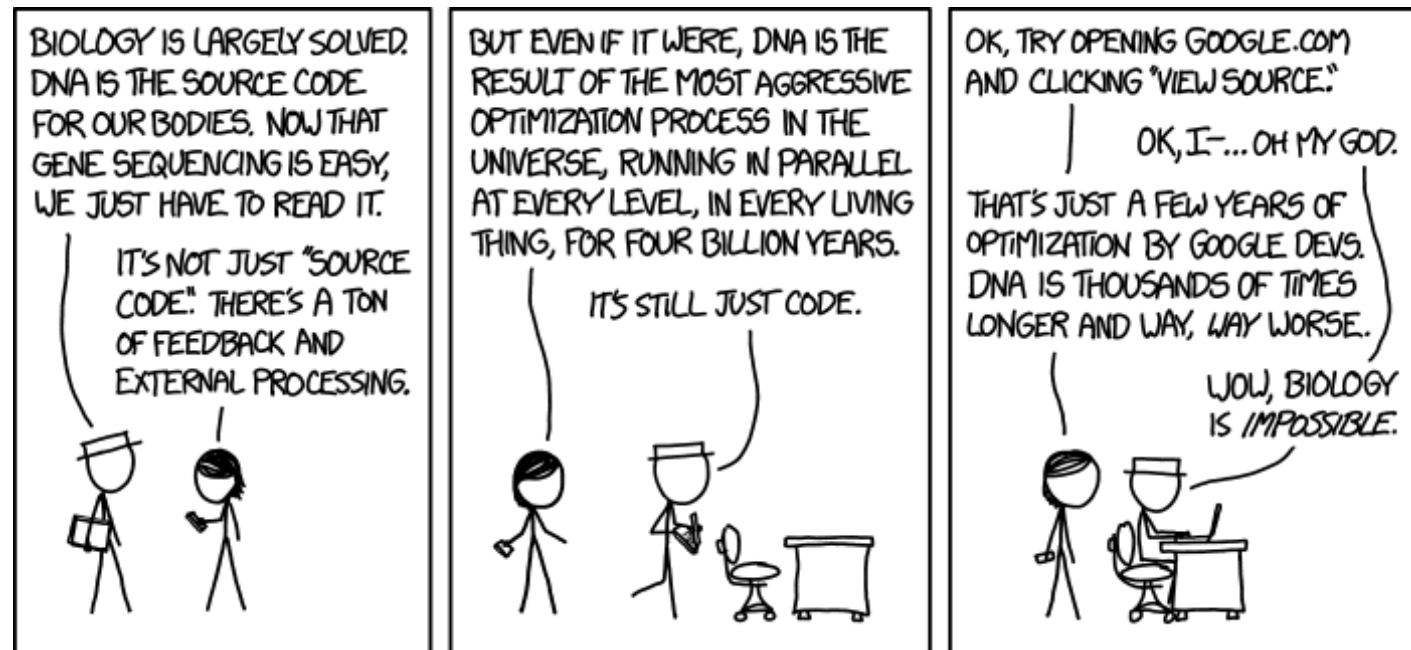


M2D6: Complete CRISPRi experiment and measure fermentation products

1. Pre-lab discussion
2. Measure OD of your bacteria
3. Measure fermentation products (ethanol/acetate) in media
4. Begin data analysis



Mod2 Overview

Research goal: Increase the yield of commercially valuable byproducts in *E.coli* using CRISPRi technology to target genes involved in mixed-acid fermentation pathway.

Last Lab:

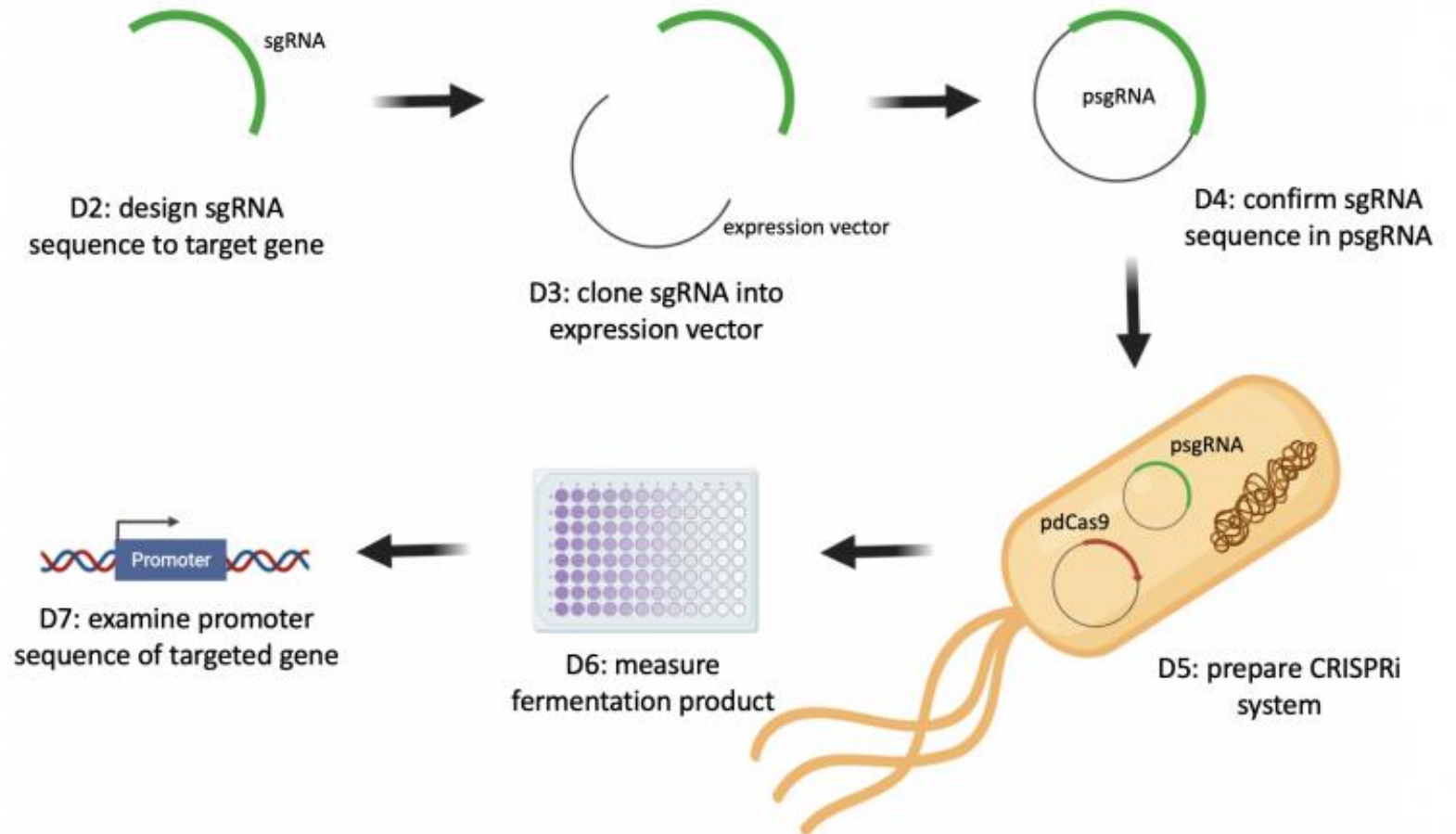
Confirm correct sgRNA cloning and do preliminary CRISPRi system preparations

This Lab:

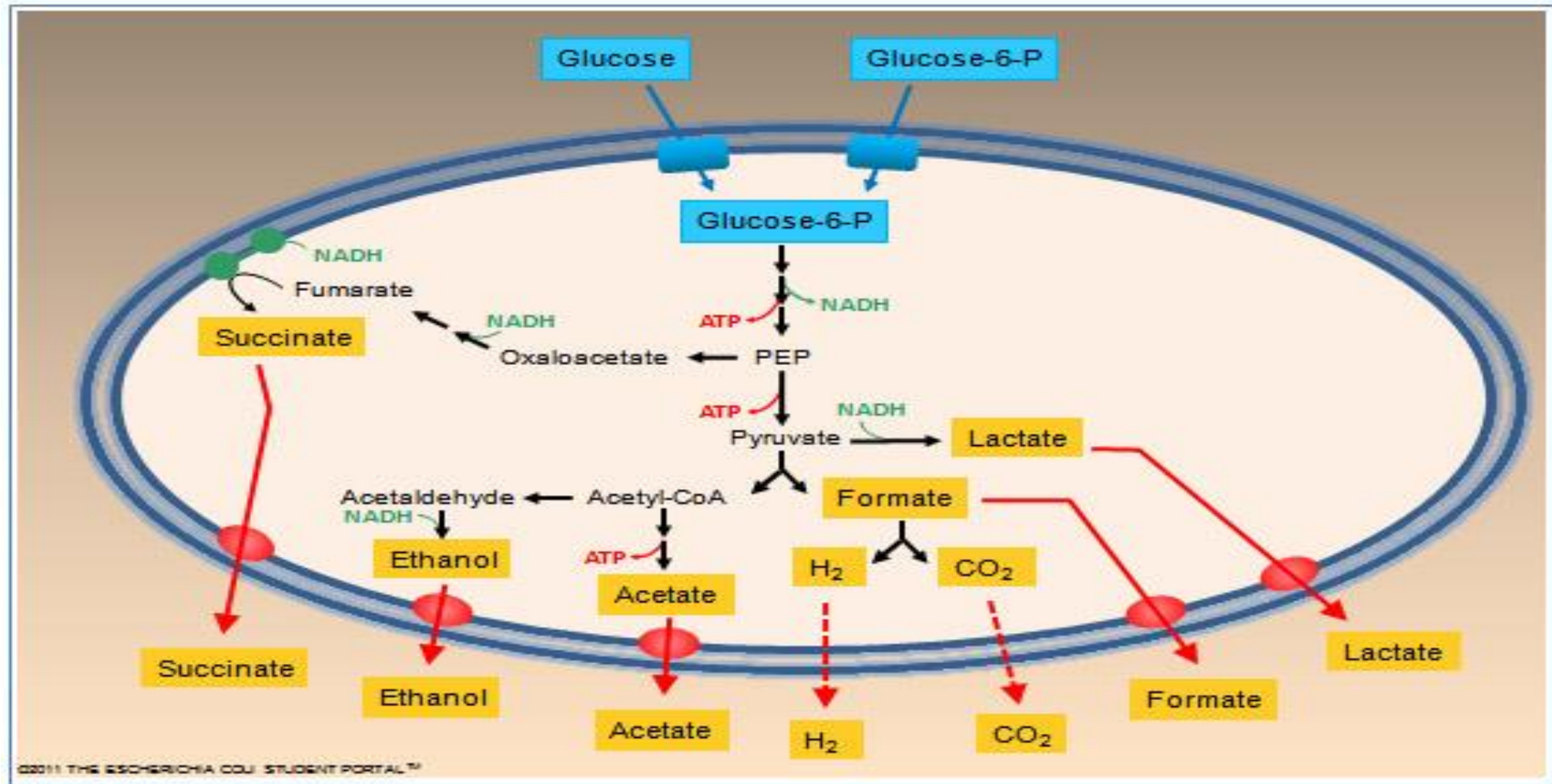
Measure bacteria O.D. and fermentation products

Next Lab:

Examine DNA regulatory elements that may impact the efficacy of your CRISPRi system

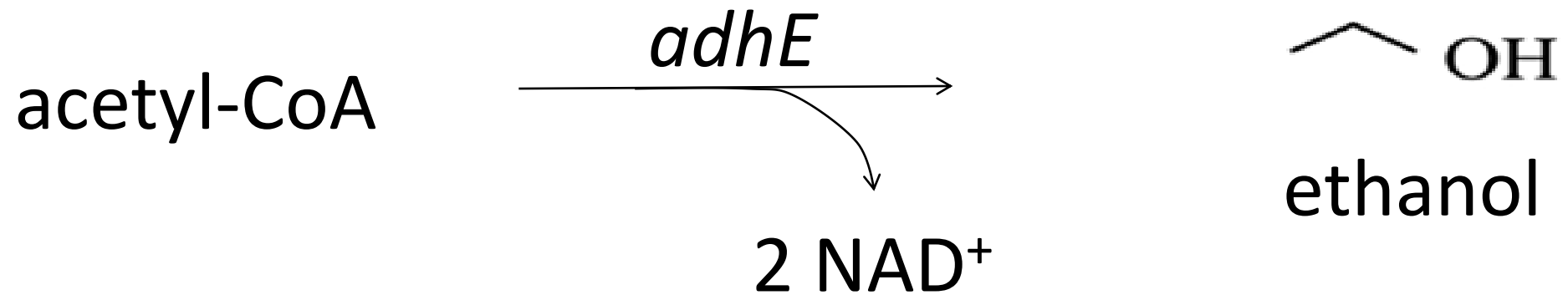


Manipulate the *E. coli* mixed-acid fermentation pathway to produce valuable products



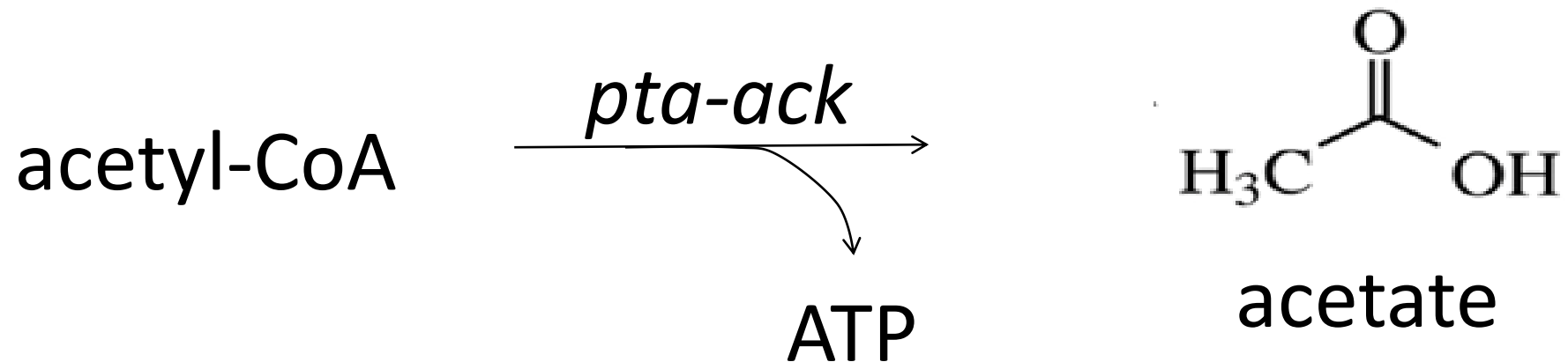
Production of ethanol

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

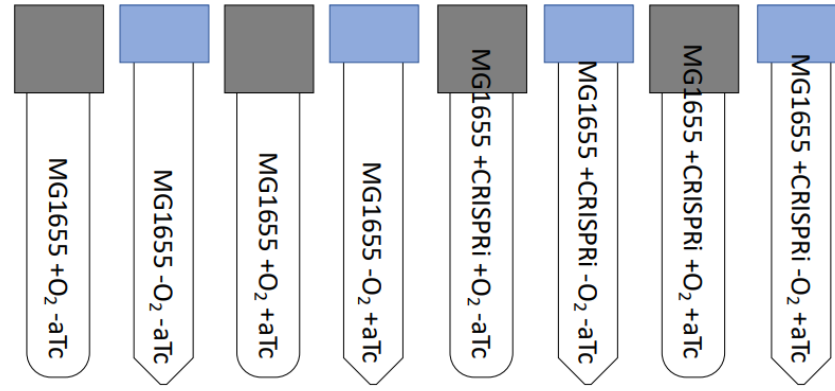


Production of acetate

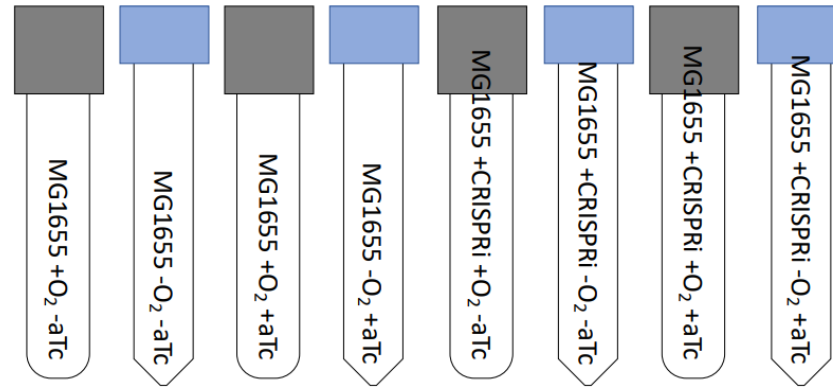
- Acetates used in production of polymers
- *pta-ack* expressed constitutively
 - Aerobically grown cells produce negligible amounts of fermentation products



Untangling Confounding Variables



Untangling Confounding Variables

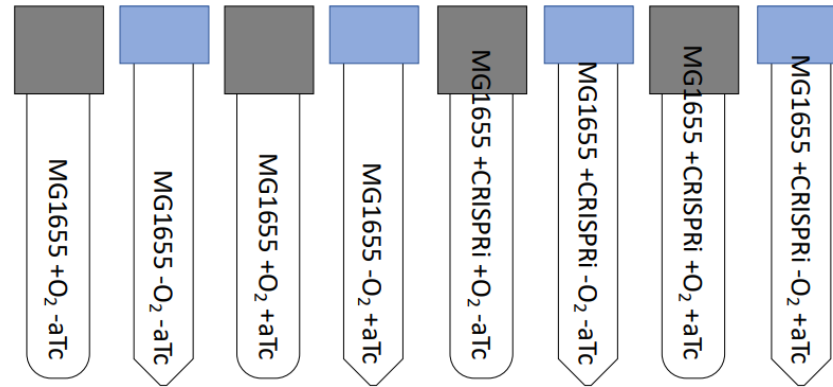


(Imaginary data)

Experimental Condition	Yield (Abs 570)
MG1655 + CRISPRi + O2 +aTc	0.845
MG1655 + CRISPRi – O2 + aTc	0.356

- 1) Interpretation 1: Incubation under anaerobic conditions using screw cap tube did not increase ethanol production
- 2) Interpretation 2:

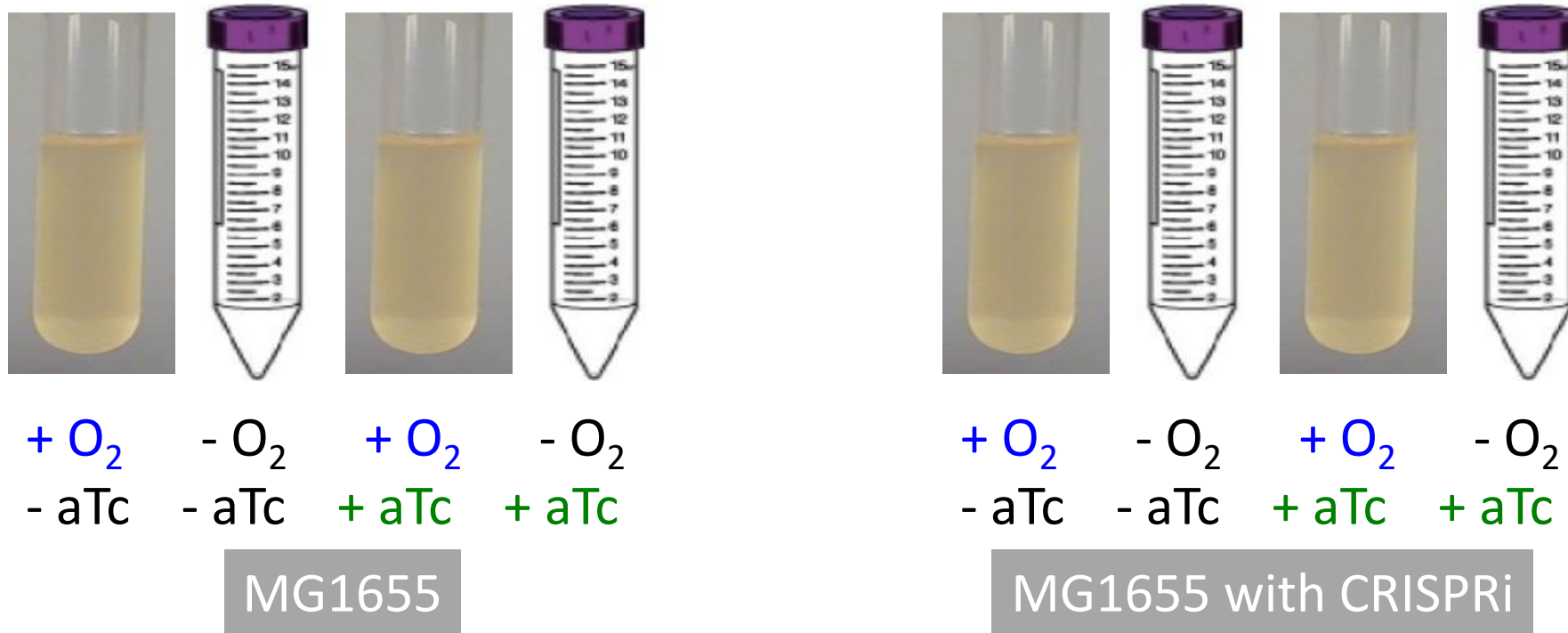
Untangling Confounding Variables



Experimental Condition	Yield (Abs 570)
MG1655 + CRISPRi + O ₂ +aTc	0.845
MG1655 + CRISPRi – O ₂ + aTc	0.356

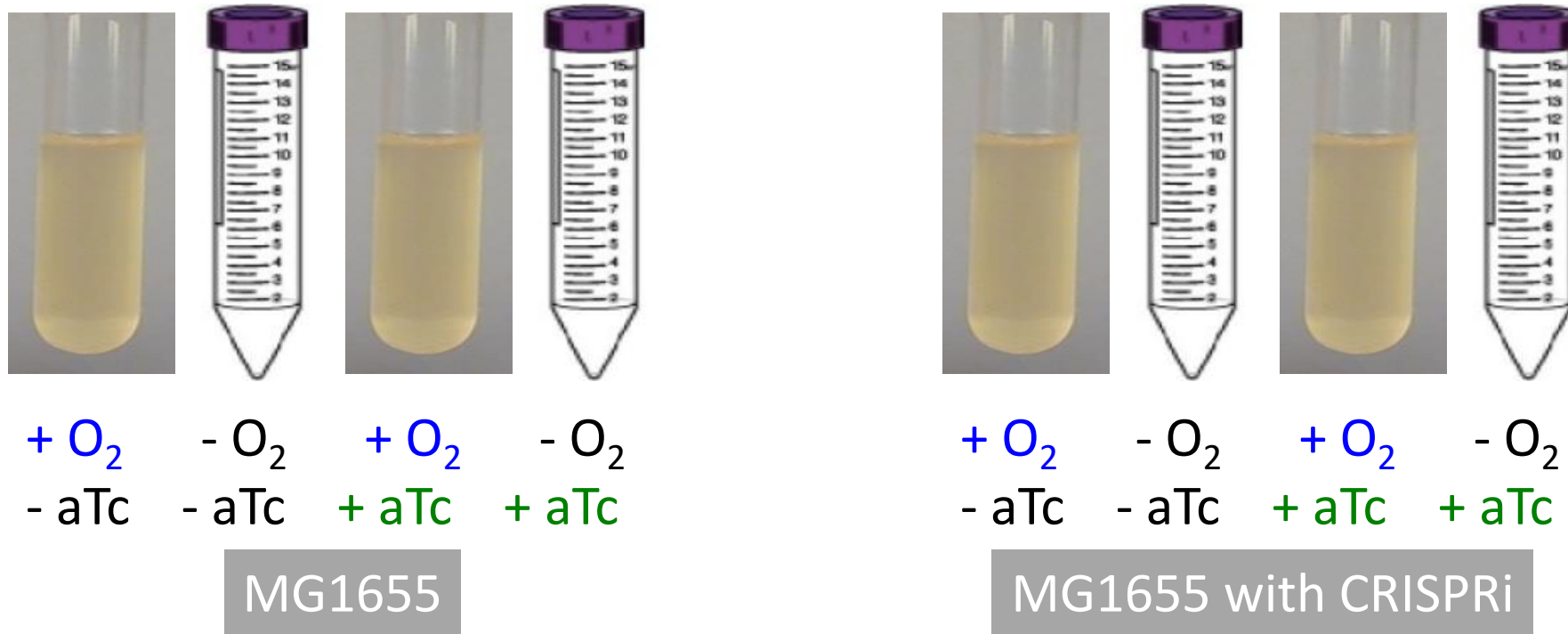
- 1) Interpretation 1: Incubation under anaerobic conditions using screw cap tube did not increase ethanol production
- 2) Interpretation 2: **Incubation under anaerobic conditions may affect bacterial growth kinetics**

Experimental conditions: mixed-acid fermentation and pdCas9 induction



Normalize for _____ by measuring _____

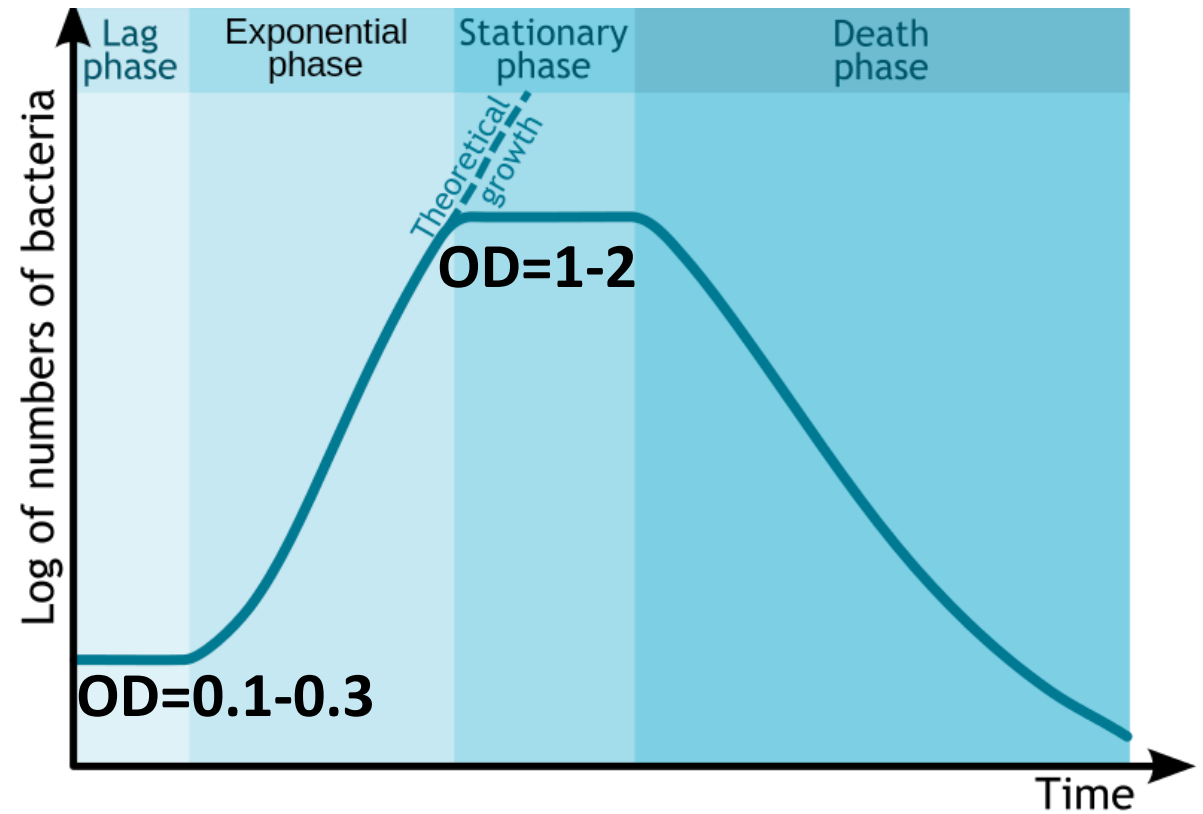
Experimental conditions: mixed-acid fermentation and pdCas9 induction



Normalize for Growth by measuring OD

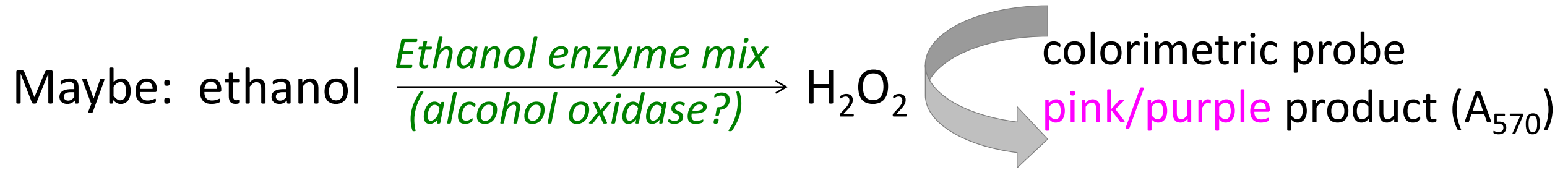
Measure *E. coli* (MG1655) concentration by optical density

- Optical Density (O.D.) \neq absorbance
- Measuring turbidity rather than absorption (relates to number of cells)



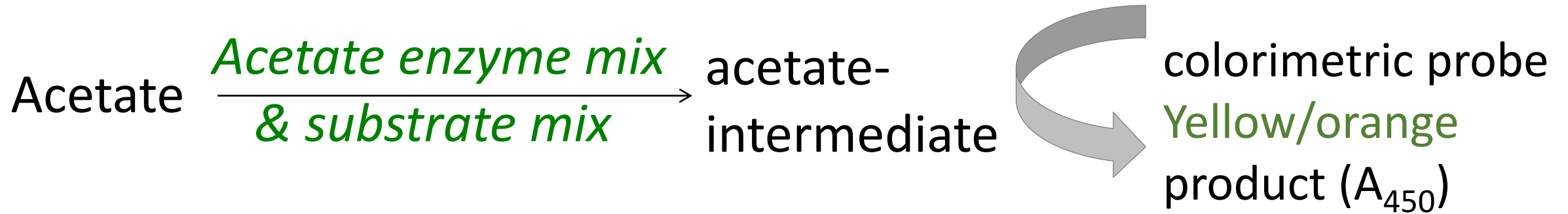
*You will measure a 1:10 dilution of your culture—remember this for your analysis!

The ethanol colorimetric assay is (very!) proprietary



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

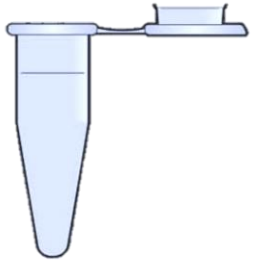
The acetate colorimetric assay is also (very!) proprietary



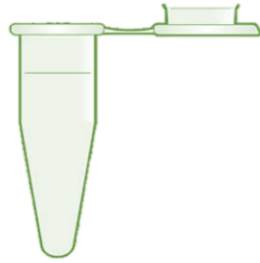
- Abcam ab204719 colorimetric acetate assay kit:
 - Acetate assay buffer
 - Acetate enzyme mix
 - Acetate substrate mix
 - Acetate probe
 - Acetate standard

Ethanol/acetate colorimetric assay procedure

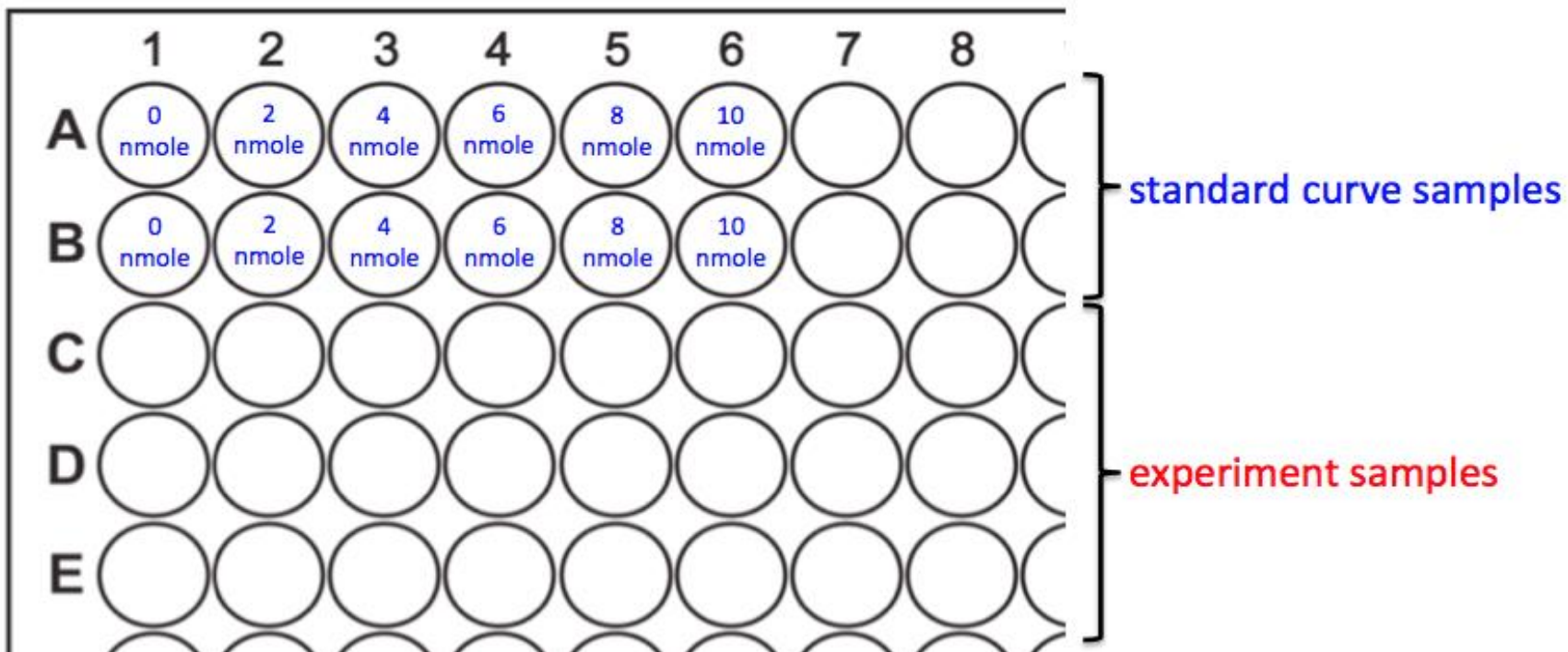
Standard 1-6



E. coli Samples 1-8



Reaction mix for ALL
standard and sample wells



Cover with foil during final incubation!

Additional things to keep in mind

- 1) There are no ethanol cleaners today (*Assay very sensitive*)
- 2) Pop major bubbles in your plate before reading
- 3) Vortex bacteria before aliquoting for OD measurement
- 4) Upload data from template to the wiki ***before leaving class today***

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 coding or non-coding strand	Colorimetric Assay Results
WF Red	Ethanol (E)	pta	gaccgacgctggttccgta	Beginning of coding sequence of gene	Coding	
WF Orange	Ethanol (E)	pta	ttcgtagttcagagactgggcaaac	beginning of gene	coding	
WF Yellow	Ethanol (E)	ppc	CATTGCGTAGTAATGTCAGTATGC	beginning of gene	coding strand (non template)	
WF Green	Acetate (A)	ppc	CCCCAGACACCCCATCTTATCGTTT	promoter	coding strand (non template)	
WF Blue	Acetate (A)	adhE	ttcagcgacattagtaacagcc	beginning of the gene	coding strand (non-template)	
WF Teal						
WF Pink	Ethanol (E)	ldhA	GTGATGTTGAATCACATTTAAGC	-35	conding strand (non-template)	
WF Purple	Acetate (A)	adhE	gttcagcgacattagtaacagccat	beginning of gene	coding strand (non-template)	
WF Grey						
WF White	Acetate (A)	adhE	ACAATTTATTAAGTGTAGCTATAA	promoter (-10)	non-coding strand (template)	

For Today

1. Retrieve cultures from front bench and measure optical density (O.D.)
2. Prepare samples and kit reagents
 1. Centrifuge = large tabletop centrifuge in lab and cold room
 2. Ethanol/acetate kits are at front bench and need to be aliquoted there
3. Measure absorbance on plate reader (4th floor)
4. Calculate fermentation product concentration from assay results
5. Upload Excel spreadsheet with ODs (x10) and absorbance readings to Class Data Page

For M2D7:

- Create Overview Schematic
 - With title and figure caption...
- Answer questions on wiki to brainstorm discussion outline

Notes on overview schematics...

What does Noreen do all day?



Wake up at 6a and brush teeth



Workout at ACF in Arlington, MA



Eat breakfast and drink coffee while watching the local news



Iron clothes and get ready for the day



shutterstock.com · 64246879

Drive to work



Check, respond to emails and review lecture slides



Teach 20.109!



Brush teeth and go to bed at 10p



Make and eat dinner



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Drive home

What should be in the Title and Caption?

Title: State what is shown / represented in the schematic

Caption:

- Explain the flow of information using concise / clear language
- Expand on text shown in figure labels to eliminate excess wordiness / clutter from the figure
- Define all abbreviations / jargon / labels / symbols

Revised example:

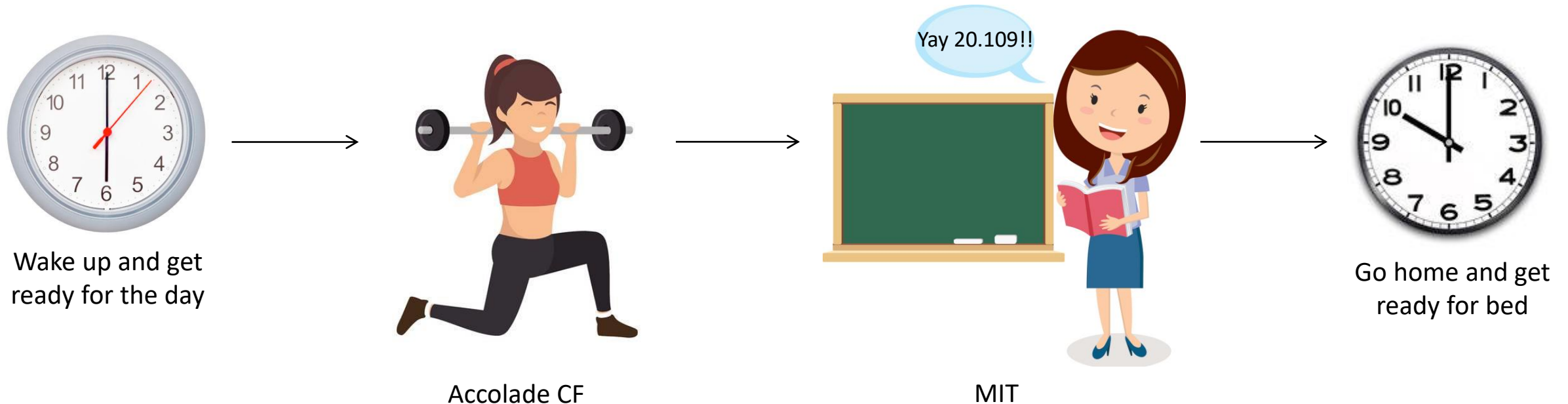


Figure 1: Average week day for Noreen. Over the course of a normal day Noreen is active from 6a until 10p. In the morning, she exercises at Accolade CF. During the day she works as a lecturer for the 20.109 class at MIT. CF = CrossFit, MIT = ...