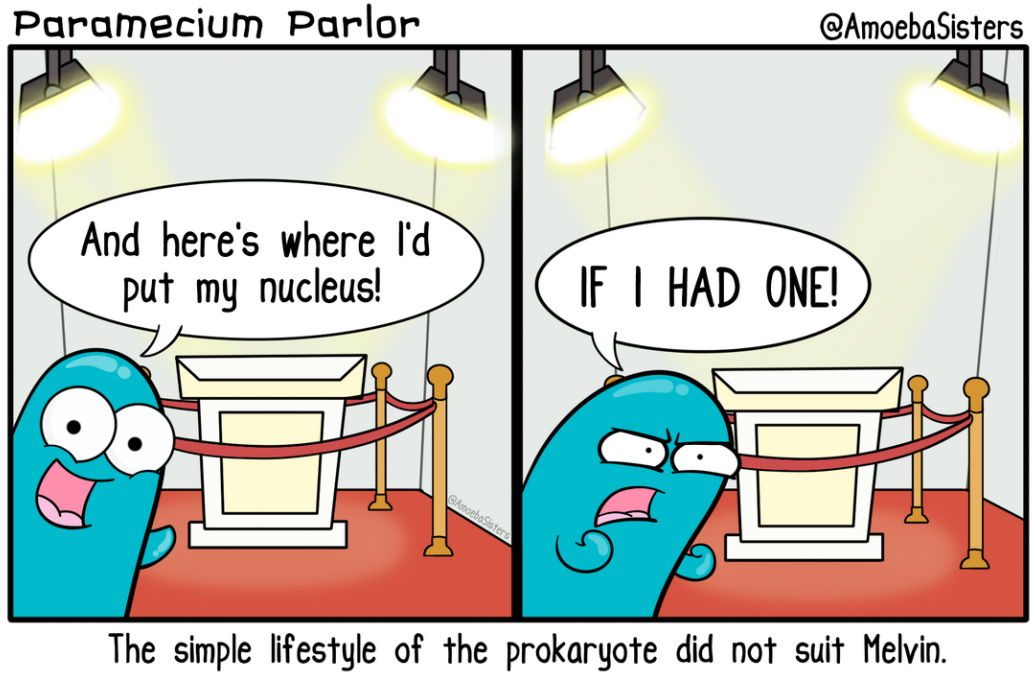
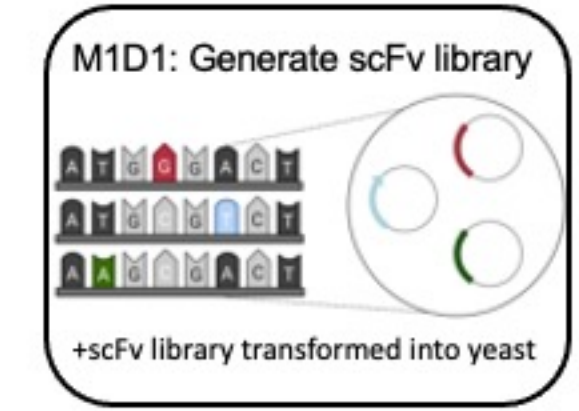
M1D4: Purify and sequence clone plasmids

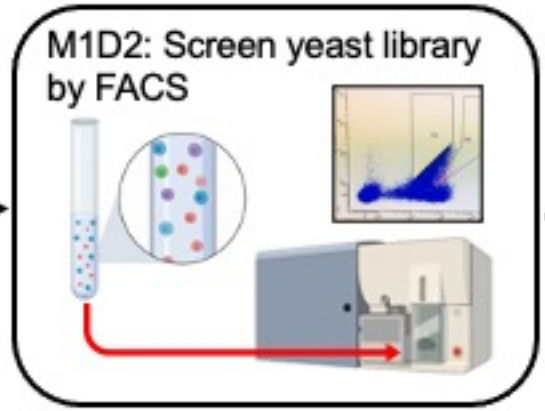
- Prelab discussion
- 2pm
  - Begin ethics case study
- Miniprep
- Prepare DNA for sequencing
- 3:30pm
  - Quiz!

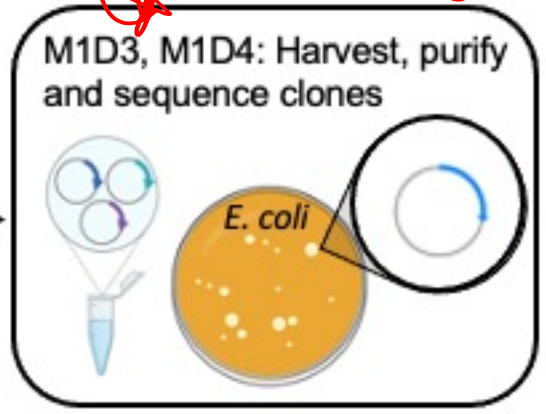


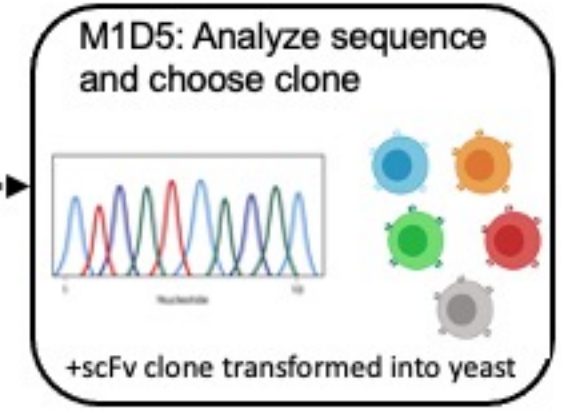
Mod1 Overview

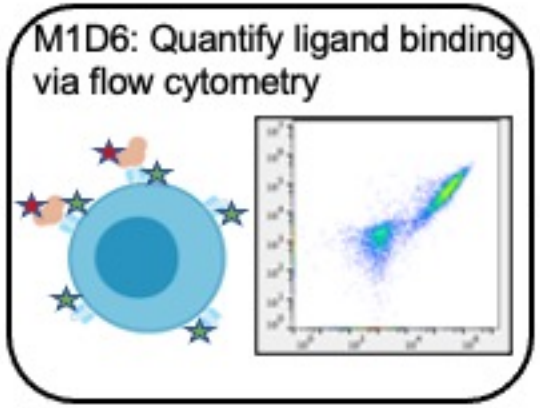
Research goal: Identify and characterize an antibody fragment (scFv) that shows improved binding to the antigen, lysozyme.

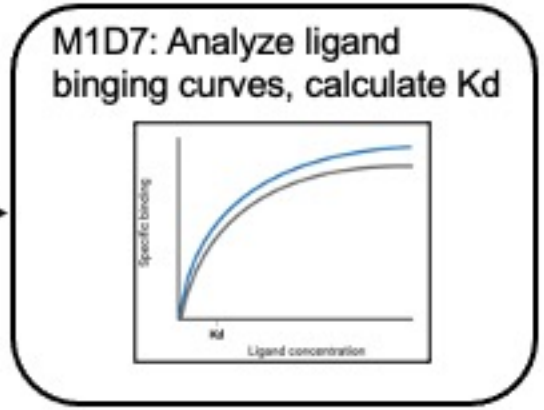








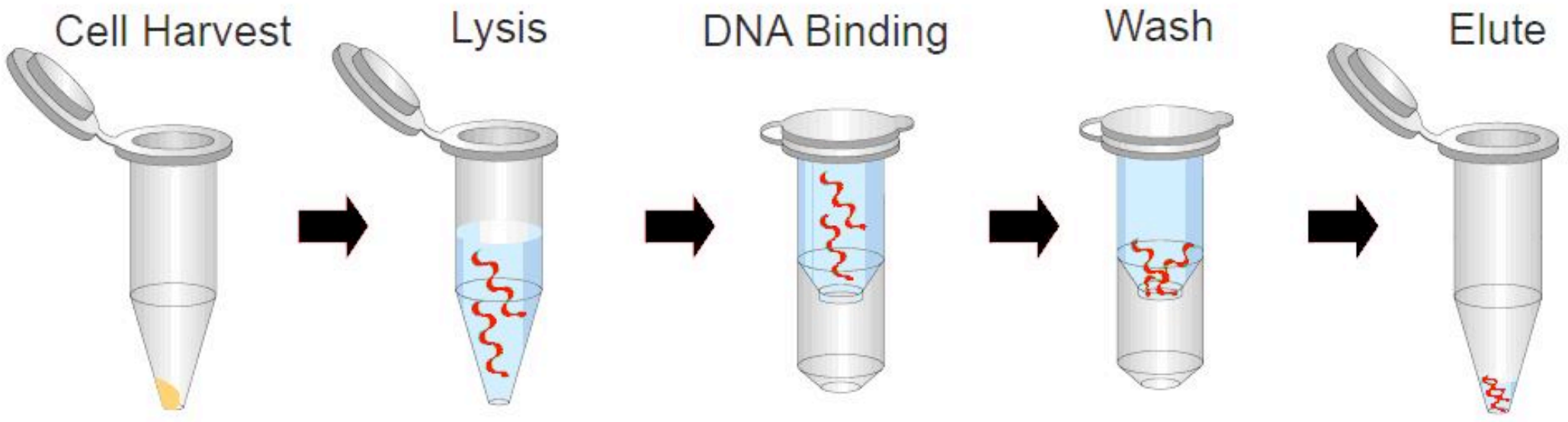




* - moved
clone plasmids from yeast to E. coli
- produce lots of plasmid fast!

The Miniprep: isolating DNA from bacteria cell lysate

Goal of mini-prep: purify plasmid



Strategy:

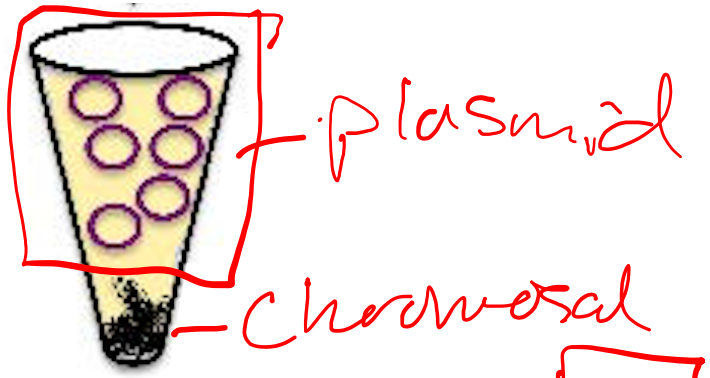
1. Lyse cells to extract DNA
2. Separate DNA from proteins and lipids
3. Separate plasmid DNA from chromosomal DNA
4. Purify and collect plasmid from other soluble factors

Miniprep: preparing and lysing cells

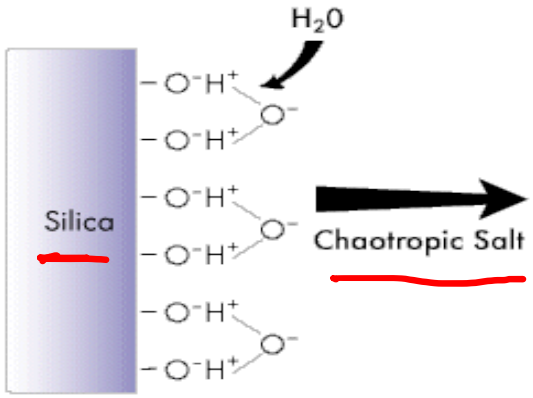
- Alkaline lysis

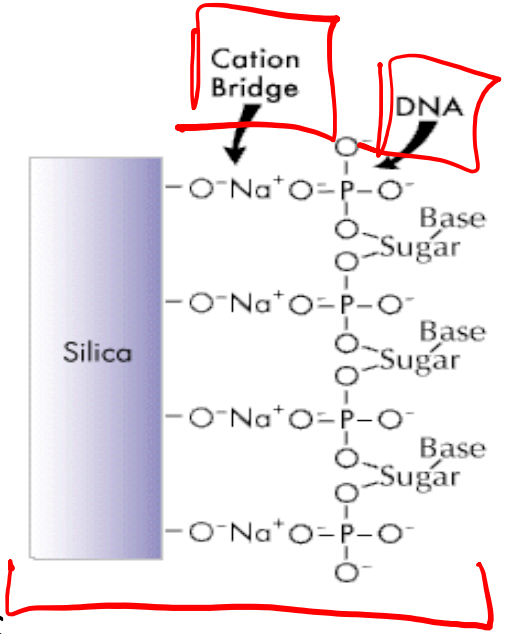
- Cells resuspended with Buffer P1
  - Tris / Ethylenediaminetetraacetic acid (EDTA)
  - RNAse
- Cells lysed with Buffer P2
  - Sodium dodecyl sulfate (SDS)
  - Sodium hydroxide (NaOH)

Miniprep: neutralizing cell lysate



- Cell lysate neutralized with Buffer N3
  - Acetic acid / Potassium acetate
    - Lower pH → back to neutral
    - DNA renatures ⇒ plasmid first
  - Guanidine hydrochloride (chaotropic salt)
    - disrupt hydrogen bonds
    - create salt bridge b/w DNA & column
      - pulling DNA out of solution



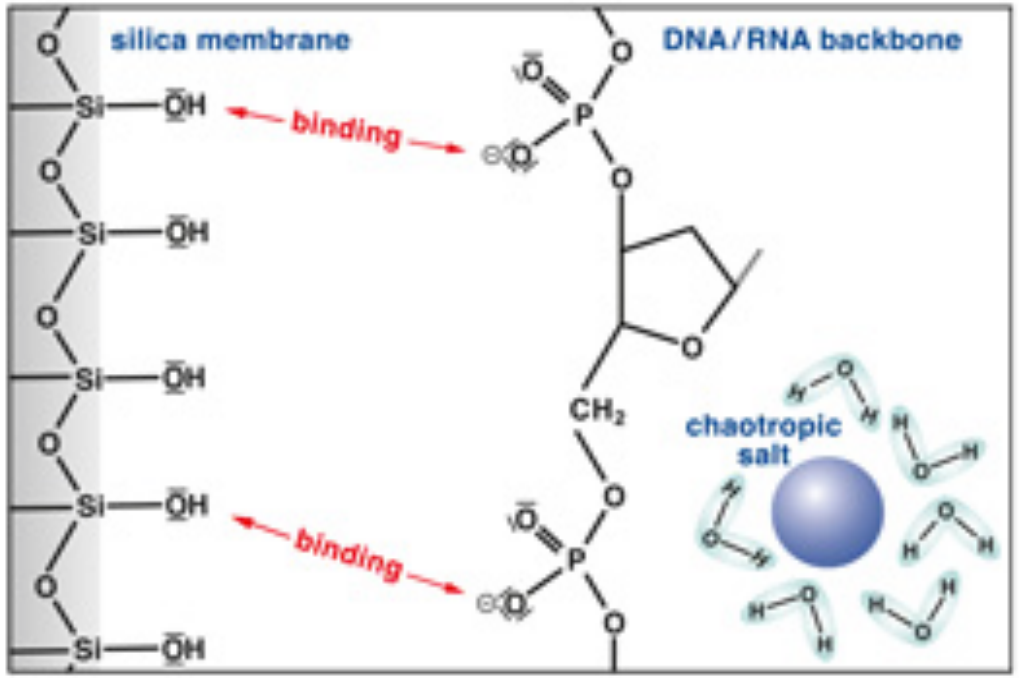


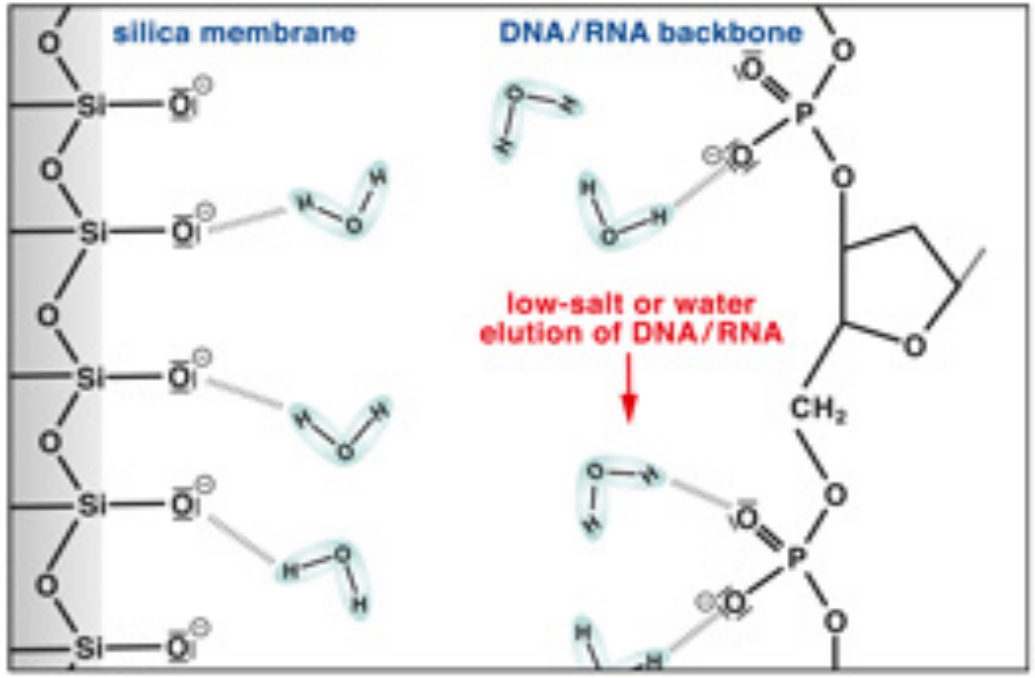
- After DNA bound to column, wash steps remove contaminants
  - Buffer PB: isopropanol and Guanidine hydrochloride
  - Buffer PE: ethanol and Tris-HCl

Miniprep: eluting DNA

Reverse → different than the company protocol

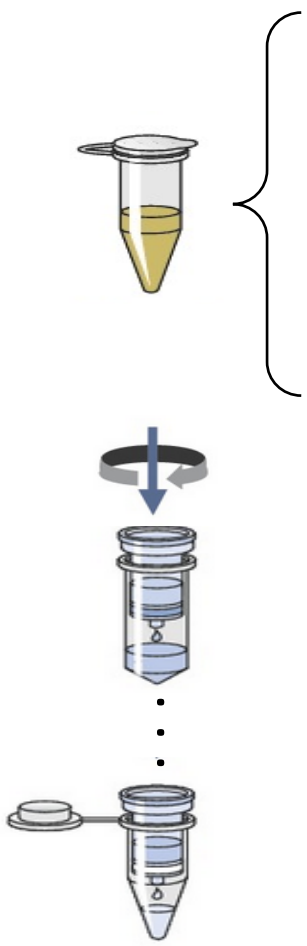
- DNA eluted from column with H_20, pH = 8





= water molecule

Summary of miniprep technique to isolate DNA



Steps	Contents	Purpose
Prepare (P1)	Tris/EDTA buffer RNase	Resuspend cells, weaken membrane, EDTA blocks DNase, RNase degrades RNA
Lyse (P2)	SDS (detergent) NaOH (alkaline lysis)	solubilize proteins, denature DNA
Neutralize (N3)	Acetic acid, chaotropic salt, potassium acetate	Renature short DNA, precipitate long DNA and protein
Spin		
Bind	Silica column	Concentrate and isolate DNA
Wash (PB, PE)	Isopropanol, ethanol	Remove contaminants
Elute	Water, pH 8.0	Elute all DNA off column

*Note: All liquid waste should be collected in conical tube, never aspirated

Notes on data pages in the Data Summary

Data Summary to be completed using **PowerPoint**

- easy for figures
- easy for bullet points

→ 8.5x11" portrait

Each figure should relay one message

- Subpanels should be related to single conclusion
- Remember the title and caption
- Figure, Title/caption, bullet points must be contained to one slide

Text should be related to results in the figure

- See guidelines in homework description
- **Write in bullets!!**

Results slide example

- Image **should not** be the entire page
  - Only needs to be large enough to be clear / visible
- Title **should** be conclusive
  - Don't include what you did, rather state what you found (take home message)
- Caption **should not** detail the methods or interpret the data
  - Define abbreviations, symbols, etc.
  - Info needed to "read" figure
- Bullet points **should** present and interpret the data

Example Data Summary Figure

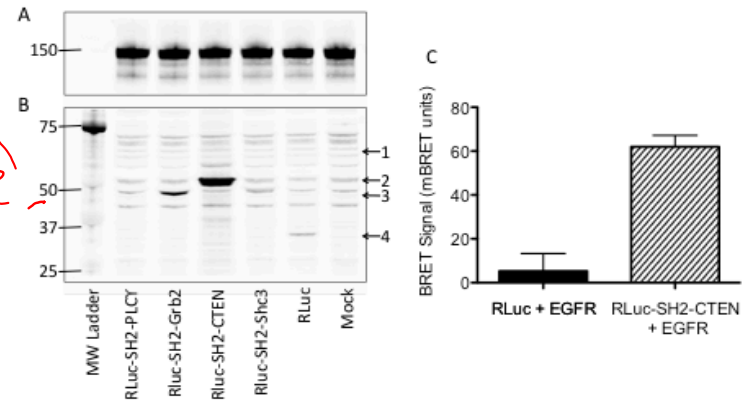


Figure 1: Development of BRET assay to monitor EGFR and SH2 domain interactions. CHO-K1 cells were transfected with Citrine-EGFR (A) and renilla luciferase (RLuc)-tagged SH2 domains from PLCg, Grb2, CTEN, and Shc3 (B). Western blots of CHO-K1 lysates were probed with anti-EGFR (A) or anti-RLuc (B) antibodies. Arrowheads indicate the expected molecular weight of the RLuc-tagged proteins; (1) RLuc-SH2-PLCg, (2) RLuc-SH2-CTEN, (3) RLuc-SH2-Grb2 and RLuc-SH2-Shc3, and (4) RLuc alone. Mock indicates no cDNA was utilized during transfection. (C) For CTEN only, BRET signal was quantified using a luminometer after stimulation of CHO-K1 with 100 ng/mL EGF for 15 min.

BRET system effectively measures EGFR activation:

- To determine if the BRET system could be used to monitor EGFR activation, CHO-K1 cells were transfected with fluorescent EGFR and luciferase-tagged SH2 domains and a BRET assay was performed after growth factor stimulation.
- CHO-K1 were transfected with Citrine-EGFR in all conditions as indicated by correct molecular weight band at 150 kDa (Figure 1A).
- Several protein bands are present in Mock transfection lane suggesting off-target binding of the RLuc antibody (Figure 1B).
- RLuc alone, RLuc-SH2-Grb2, and RLuc-SH2-CTEN were successfully transfected as indicated by correct molecular weight bands (Figure 1B).
- RLuc-SH2-PLCg and RLuc-SH2-Shc3 did not appear by Western blot analysis -- bands different from those in the Mock lane are not identifiable. This outcome could be due to protein expression levels below the detection limit by Western blot or to unsuccessful transfection of cDNA.
- BRET signal increased in cells transfected with Citrine-EGFR and RLuc-SH2-CTEN versus Citrine-EGFR and RLuc alone after EGF stimulation. This difference suggests that the BRET signal is specific for an SH2-EGFR interaction versus randomly localized RLuc.
- In sum, these data suggest that the RLuc-SH2 constructs can be utilized to monitor EGFR phosphorylation, as SH2 domain-EGFR association occurs only at sites of EGFR tyrosine phosphorylation. Next, we determined the dynamic range of the BRET assay.

Ethics Case Study: Herd Immunity (details on wiki)

- Round 1: Individual Research
  - Read articles about your assigned topic (based on group color)
  - 20 minutes
- Round 2: Debrief Groups
  - Assigned into debrief groups
  - At least one person from each topic in the group
  - Take group notes on Google Doc
  - 20-30 minutes
- Round 3: Group discussion
  - Meet back in main room for discussion on MA state policies based on the information learned in first rounds

Reminder, discussion guidelines:

- Listen respectfully and actively. Avoid judgment and criticism of people.
- Any thoughtful answer is a good answer.
- When sharing your thoughts, use any ideas, sources, or evidence to support your opinions.
- Take the message out of the classroom, not the messenger. Respect confidentiality of your peers.

For today…

- ~~2pm~~: Begin Ethics discussion
- Read through wiki experiments
- 3:30pm: Quiz sent out

Participation points will be assigned for participating in lab discussion
- No need to turn in lab notebook check in today

For M1D5…

- Write bullet points for FACS figure for Data Summary
  - Place in powerpoint document set to be a 8.5x11" portrait paper
  - Write bullet points using wiki notes as initial guidance
- Write paragraph about Comm Lab appointment