M1D6: Lipofection & Stats Practice

9/30/14

1. Pre-lab discussion

*Next time lab Nbks are due:

2. 1/2 lab in TC to set up HR experiment

3. 1/2 in lab to work on statistics practice — today is also a good notebook 'catch up' day. (or nap)

4. Switch!

Thank you Isaak!!!

Review M1D3 FNT: Figures & Data Interpretation

When you are writing your R&D, consider the following:

a. What was the overall goal of these data/figure?

b. What was your expected result?

c. What was the result?

d. What evidence do you have that your result is correct or incorrect?

e. In sum, what does this data suggest or indicate? What does this motivate you to do next?

Review M1D3 FNT: Figures & Data Interpretation

- Each R+D section needs a desriptive title
- Limit each figure (experiment) 1-2 pages max
- Dont include Methods

Revisit Methods section: What experiments fit together?

Due Monday 10/6 @ 5pm

PCR

Transformation

PCR product purification

XbaI/EcoR1 Digest

Diagnostic Digest GE

XbaI/EcoR1 Digest
Purification (GE)

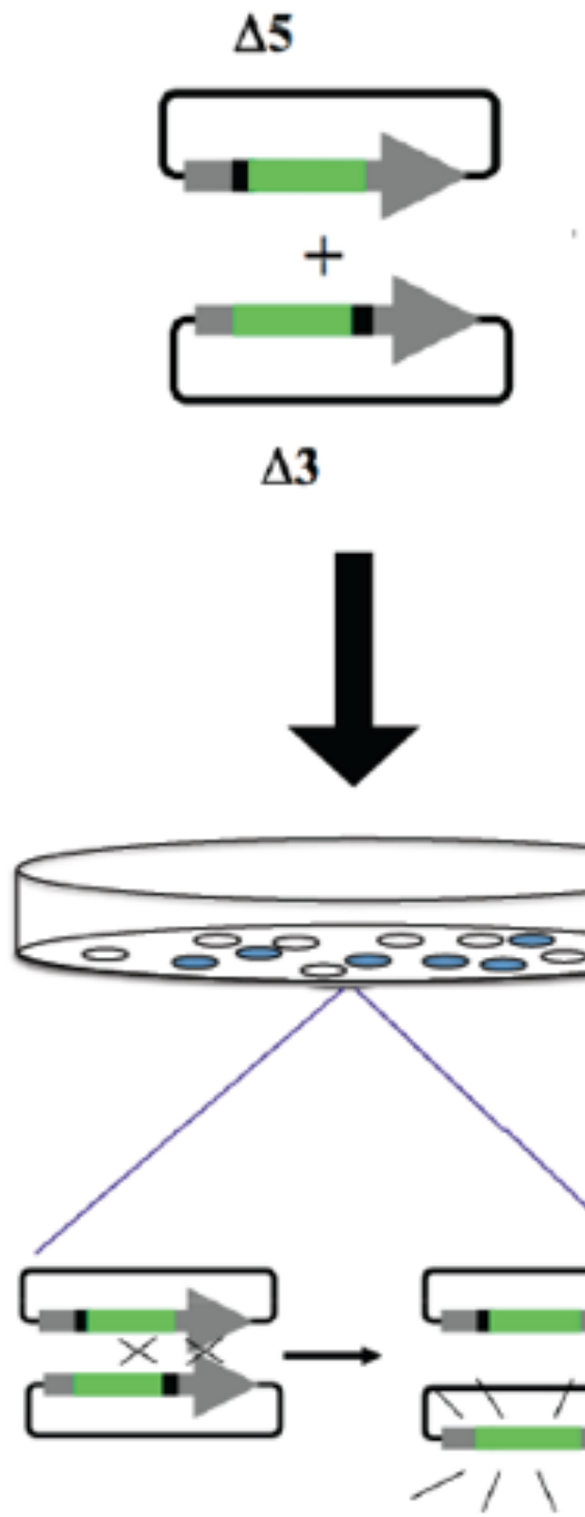
O/N e.coli cultures

Plasmid purification

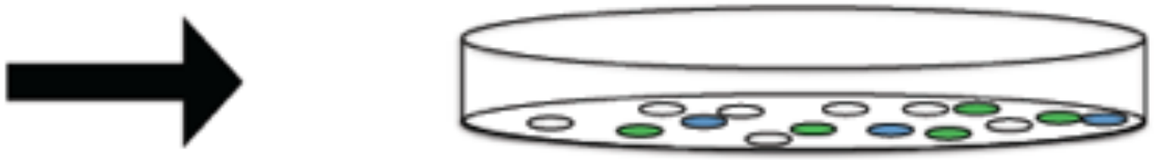
Ligation/Precipitation

Diagnostic Digest

Step 2: Test the system!

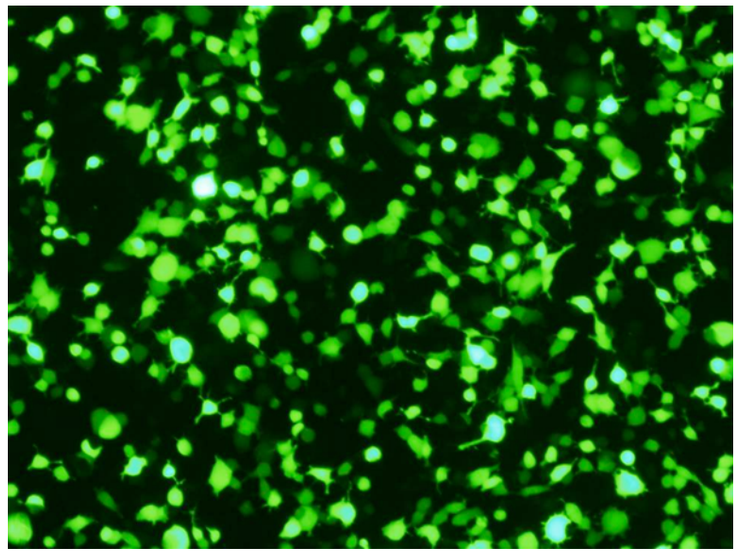


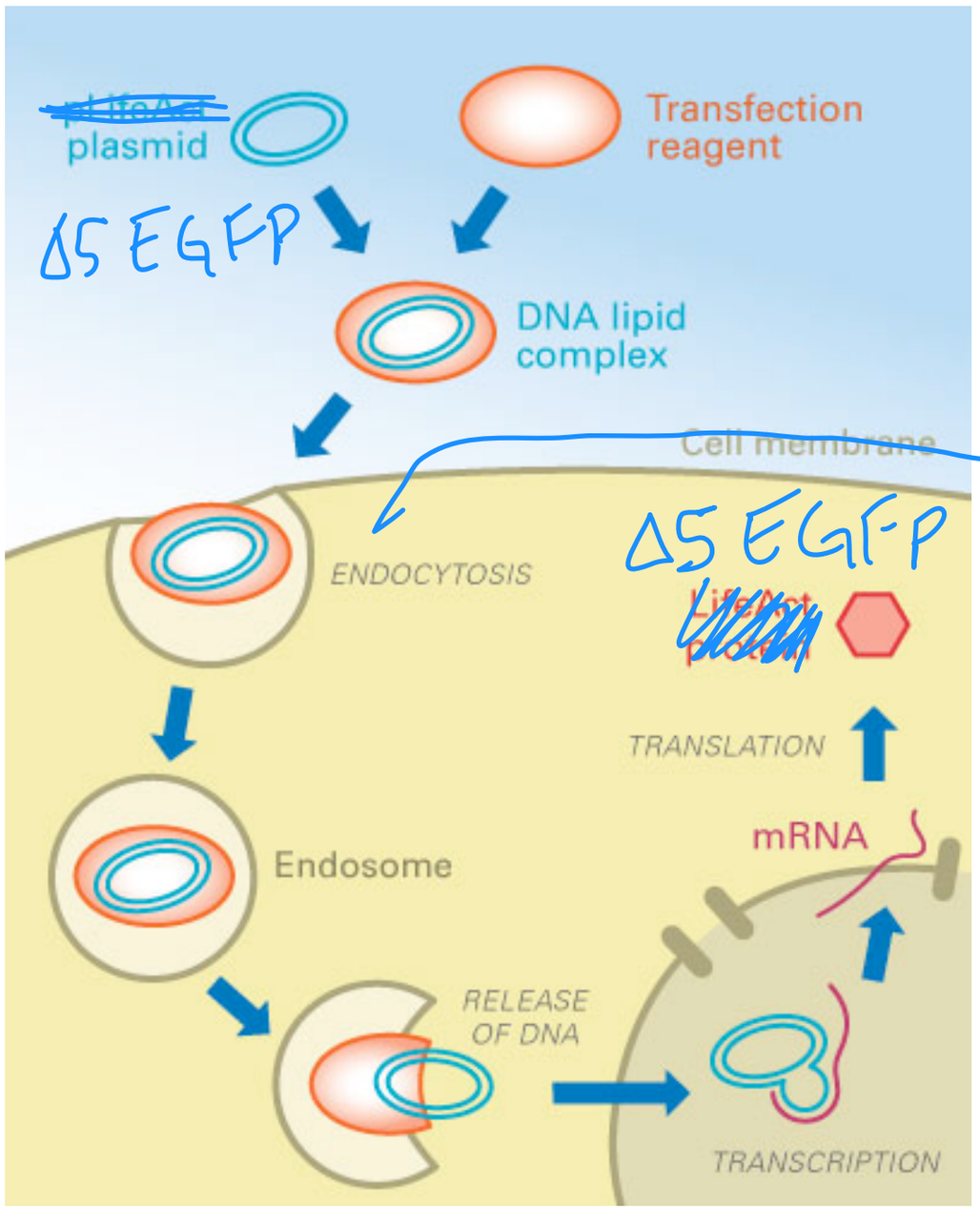
Julian Voss-Andreae
Steel Jellyfish (Green Fluorescent Protein), 2006
Stainless steel, 4' x 3' x 3' (1.20 x 0.90 x 0.90 m)
Location: Friday Harbor Laboratories (San Juan Island, WA)



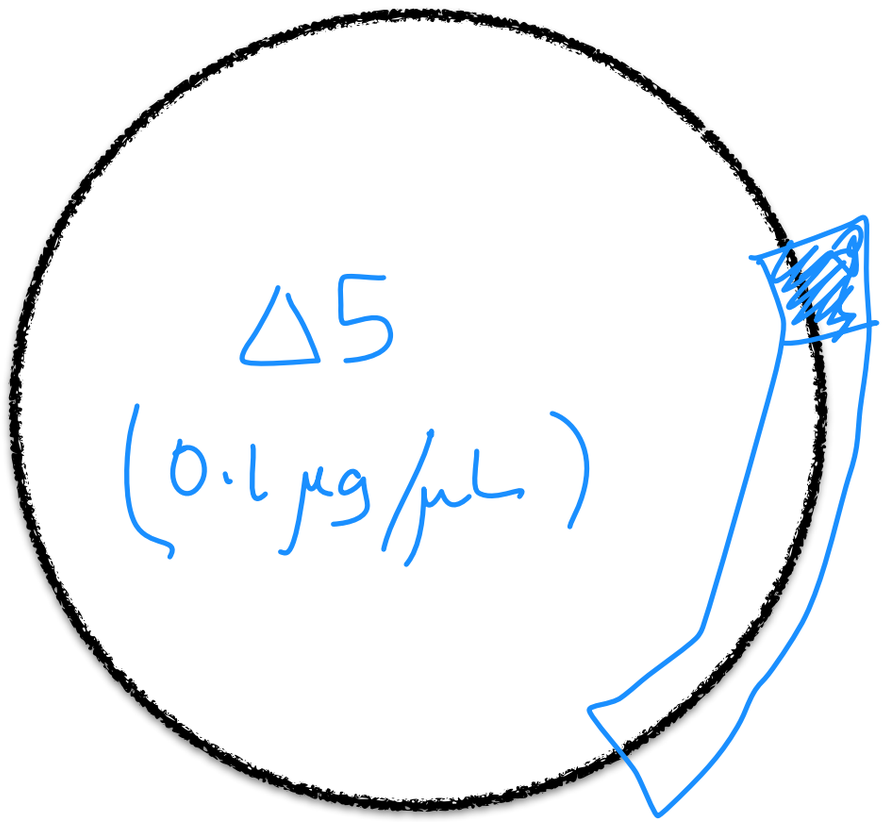
Step 2: Test the system!

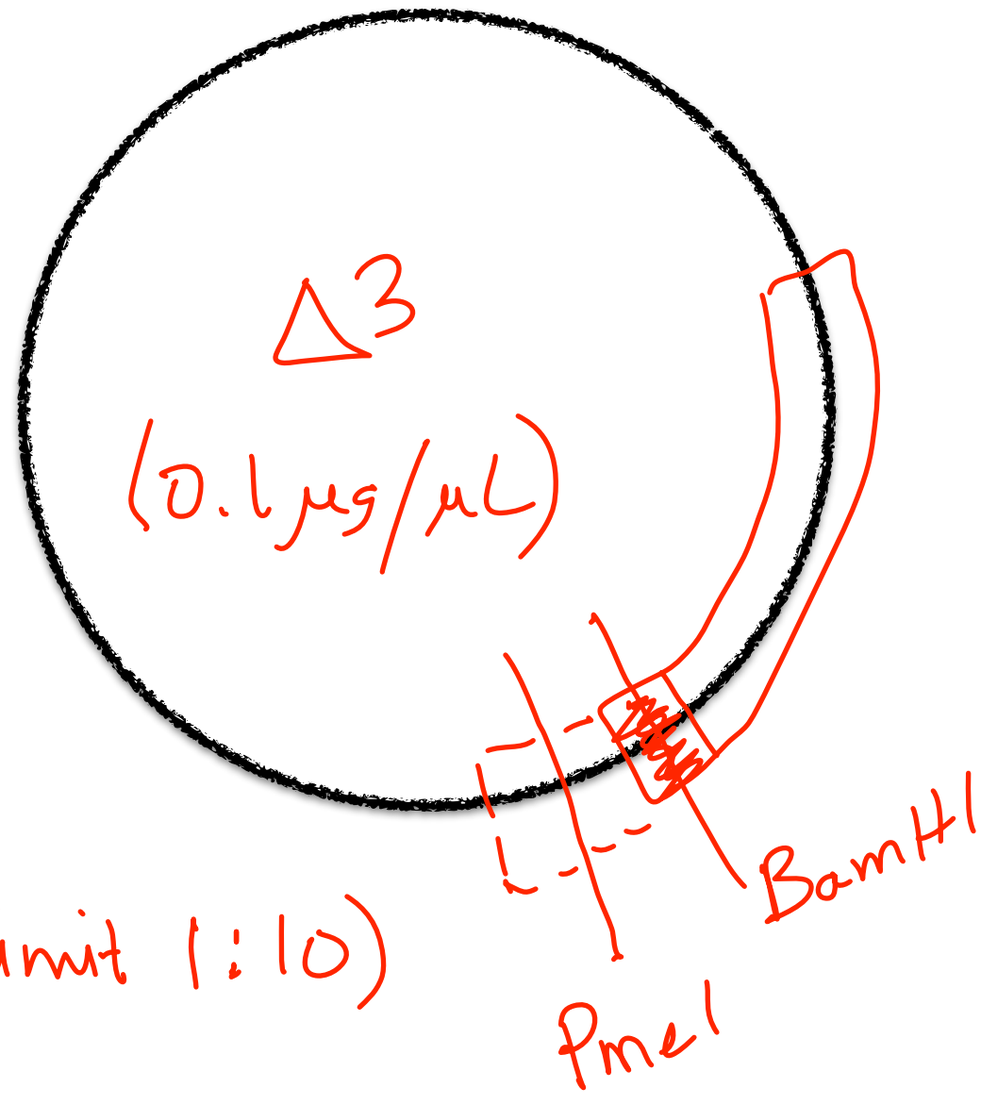
LIPOFECTAMINE®
20-YEAR ANNIVERSARY





Today you have some options:



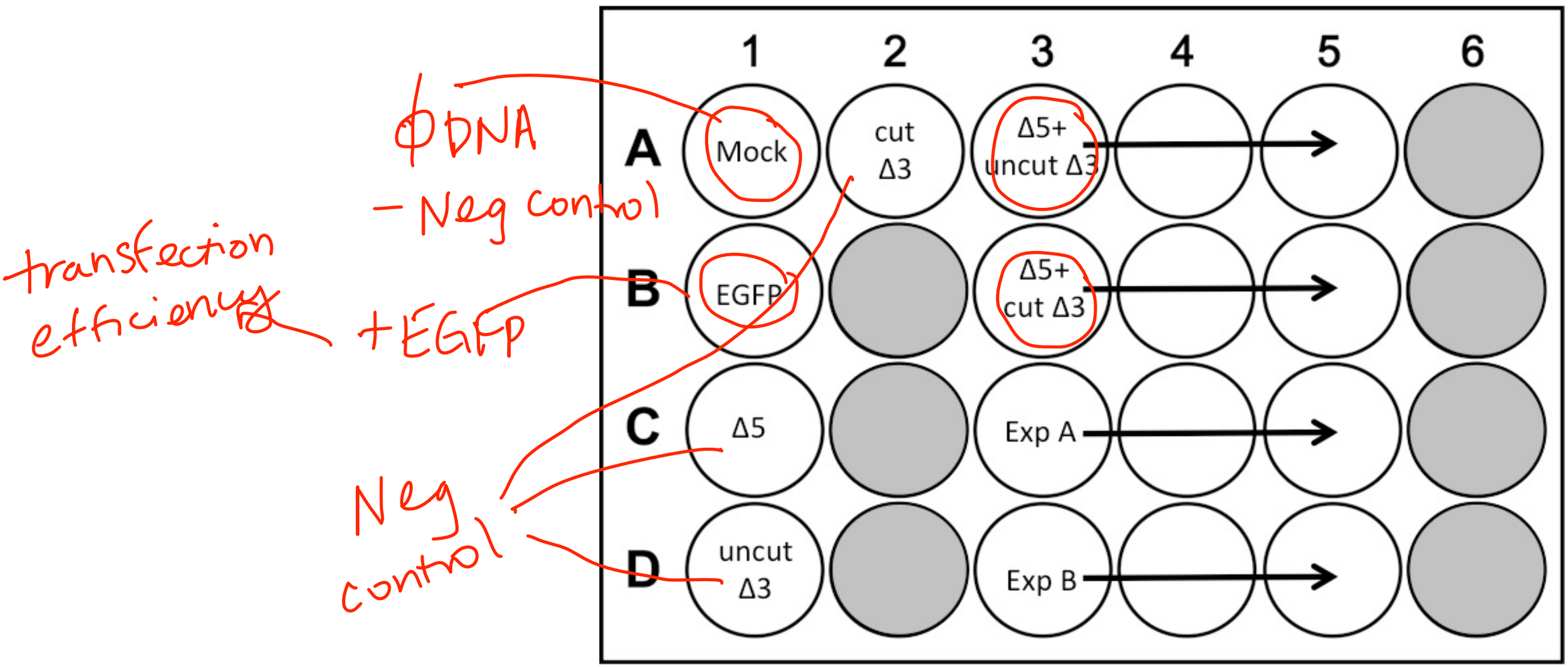


1) Ratio of Δ5 : Δ3 ⟹ 1:1 (limit 1:10)

2) cut Δ3 vs. intact Δ3 → endogenous GFP levels (some HR)
↳ ↑ HR

3) cut Δ3 – PmeI vs. cut Δ3 – BamHI

Today you have some options:



You get to pick

1) What is your cut Δ3 — PmeI / BamH

2) Ratio of Δ5 : cut Δ3 (or uncut Δ3)

yay statistics!!

(how many cells underwent HR)

% EGFP Expression

68%

N=3 wells

mock

GFP

Δ5

Δ3

cutΔ3

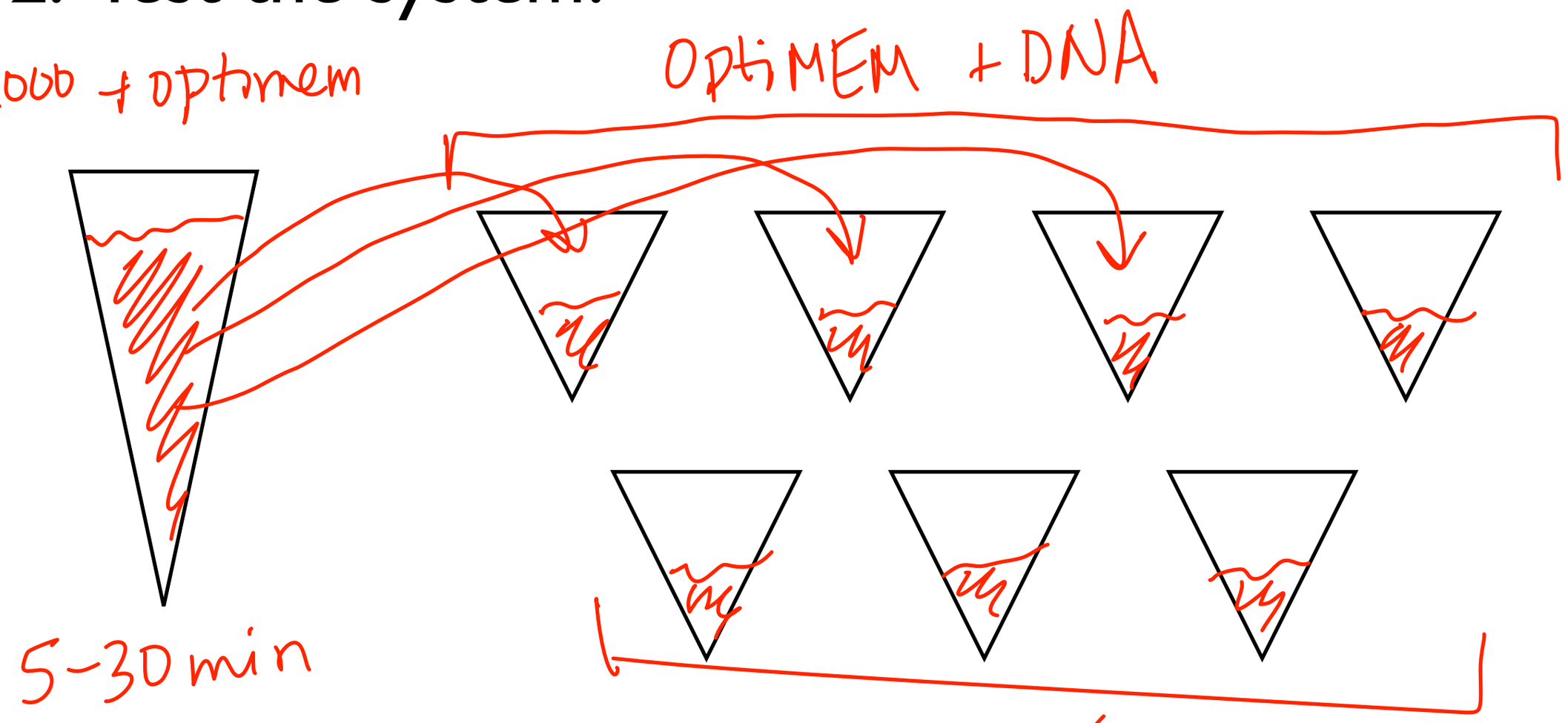
cutΔ3 + Δ5

class-wide data

Step 2: Test the system!

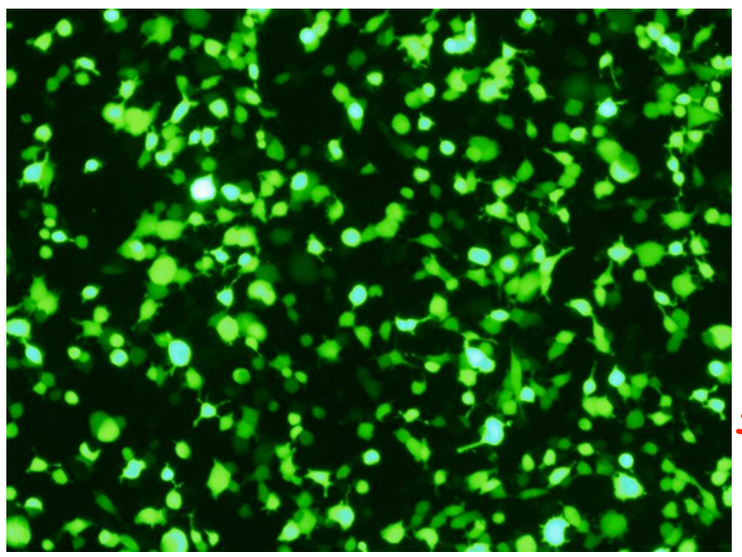
L2000 + optimem

OptiMEM + DNA



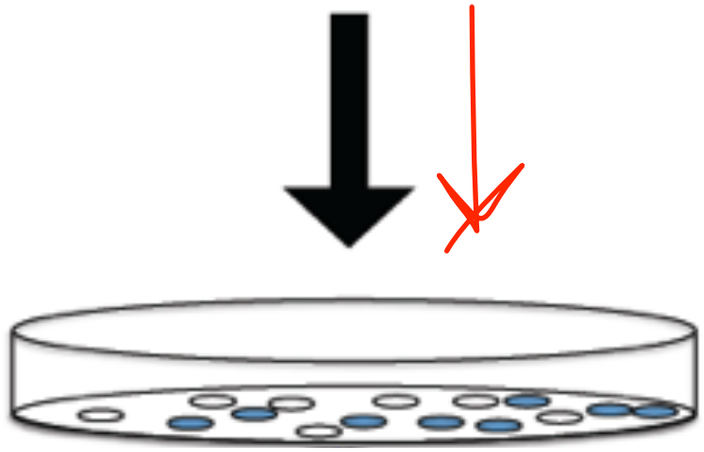
5-30 min

20 min (L2000 + DNA)



48 hrs

Flow cytometry



Tissue culture tips

- Set up a few inches *behind* the barrier/grate
- Minimize opportunities to bump or expose sterile equipment or your samples
  - Uncap bottles *before* opening pipet
  - Keep tips and dishes *closed* when not in use
  - Avoid passing your hands/arms over open dishes
  - Don't try to hold > 2 things at once! ☺
- Take care not to clog the pipet-aids

Today in the lab:

- Set-up lipofections -- pick your conditions and do your calculations BEFORE you start TC
- Look for feedback on Methods ASAP — for sure Thursday in lab, possibly tomorrow night.

Next time in the lab:

- Sign-up on M1D7 Talk Page
- FACS analysis to measure HR efficiency
- Post FACS data on M1D7 Talk page promptly!!!