

# MID6: Lipofection & Paper Discussion

10/1/13

Lab Treat 

1. Pre-lab discussion
2. 1/2 lab in TC to set up HR experiment  
  
\*White, Blue, Yellow & Orange in TC
3. 1/2 lab meet with Prof. Engelward to discuss paper  
  
\*Red, Green, Pink, Purple, Platinum to 56-711
4. Switch! Thank you Lizzie!!!

# Review MID3 FNT: Figures & Data Interpretation

★ Appreciate class-wide view ★

• Data Summary - Mod 1 11am  
- due Thurs, Oct 10 Stellar

• Concise statements  
(think poster)

- Options - order of importance  
- figure friendly

• No band in 'Neg Control' suggests ...

• Band @ approx 1400bp suggests positive PCR amp of 16sRNA

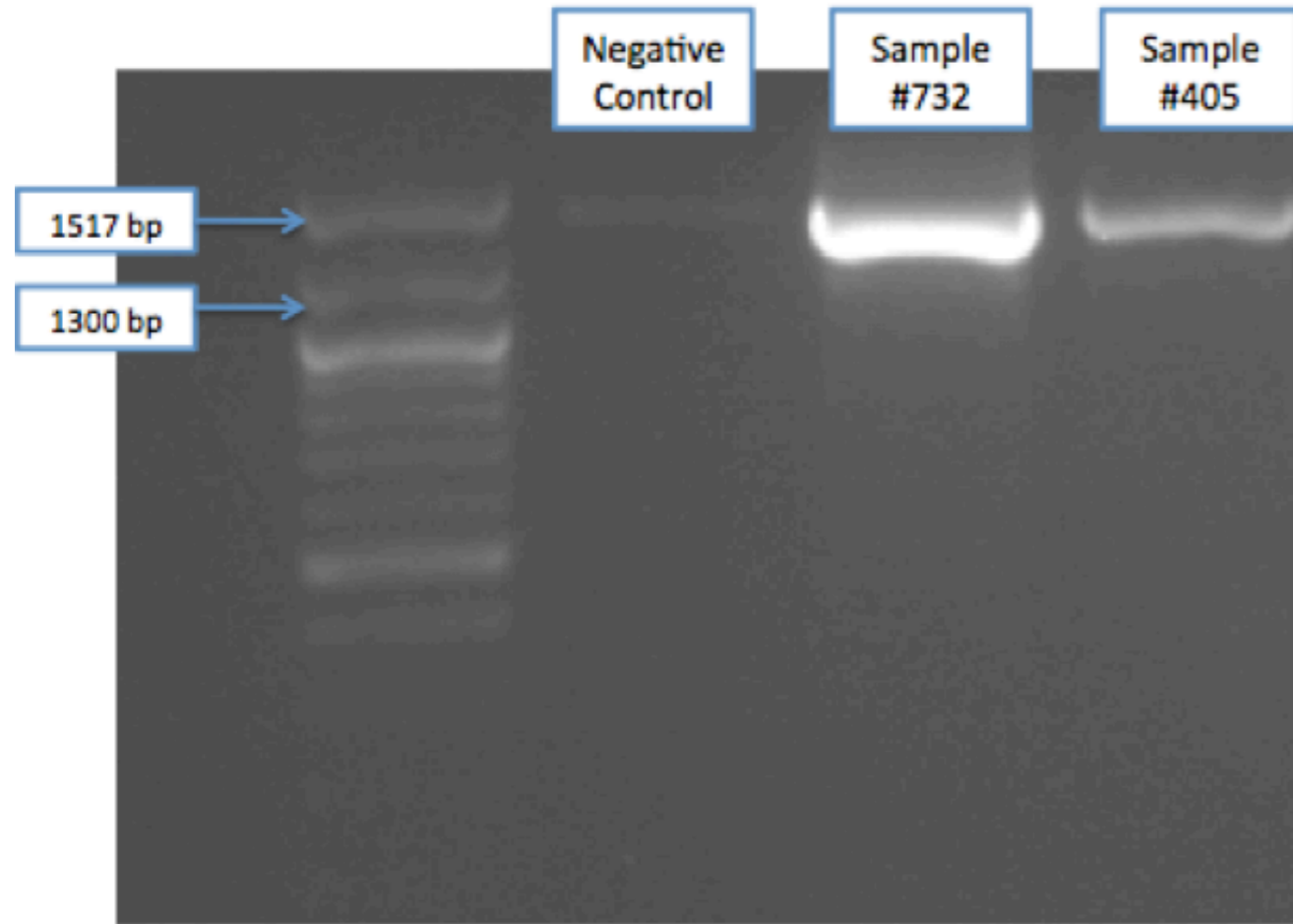
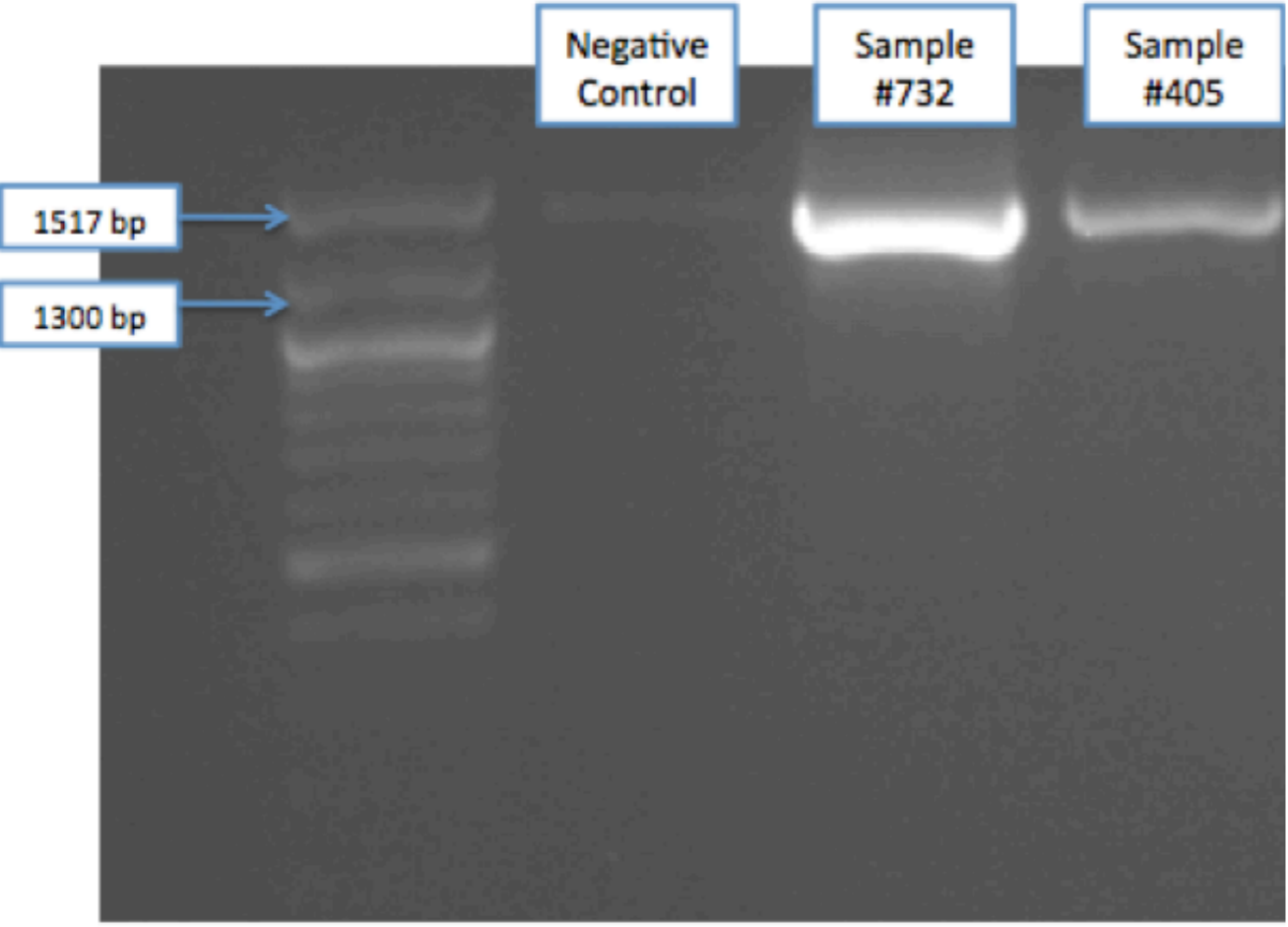


Figure 2: Agarose Gel of 16s rRNA PCR product amplified from gull samples.

Current title: Agarose gel of 16s rRNA PCR product amplified from gull samples.

# Review MID3 FNT: Figures & Data Interpretation



Alternate titles:

Amplification of bacterial 16s rRNA gene from Alaskan gull stool samples.

Electrophoresis of amplified bacterial 16s rRNA genes.

Successful amplification of bacterial 16s rRNA gene from two Alaskan gulls.

Figure 2: Agarose Gel of 16s rRNA PCR product amplified from gull samples.

# Revisit Methods section: What experiments fit together?

PCR

Transformation

XbaI/EcoRI Digest

PCR product purification

Diagnostic Digest GE

XbaI/EcoRI Digest  
Purification (GE)

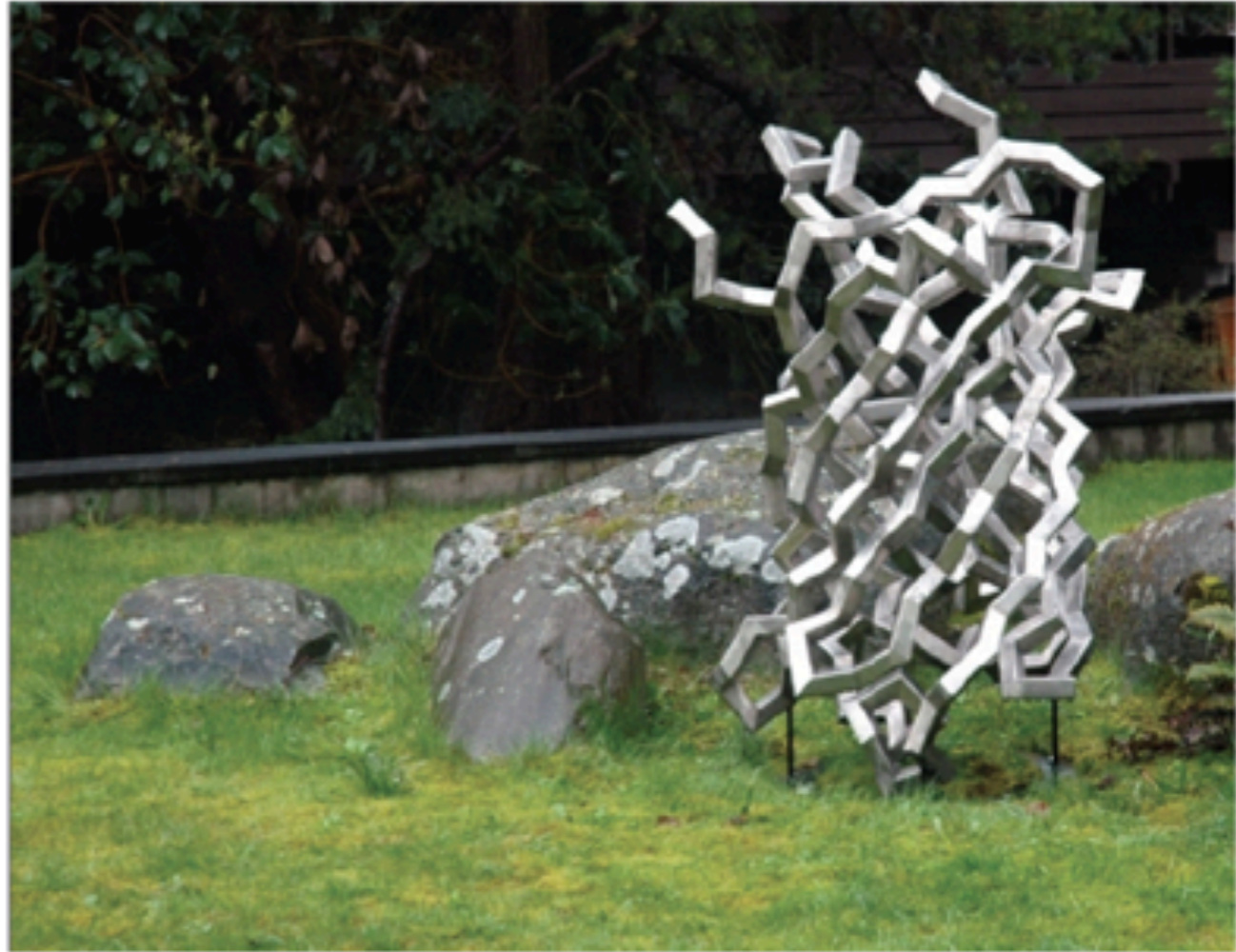
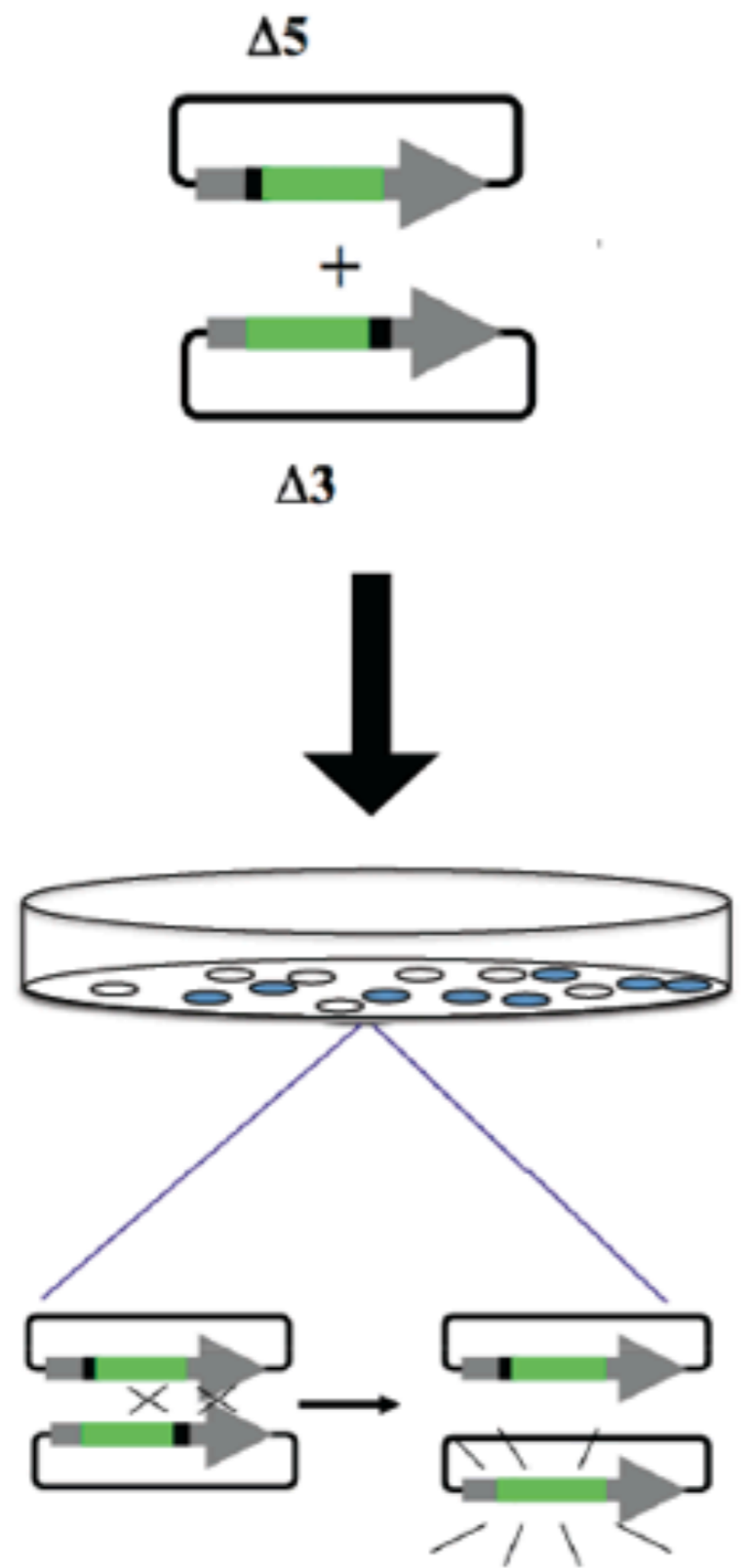
O/N e.coli cultures

Ligation/Precipitation

Plasmid purification

Diagnostic Digest

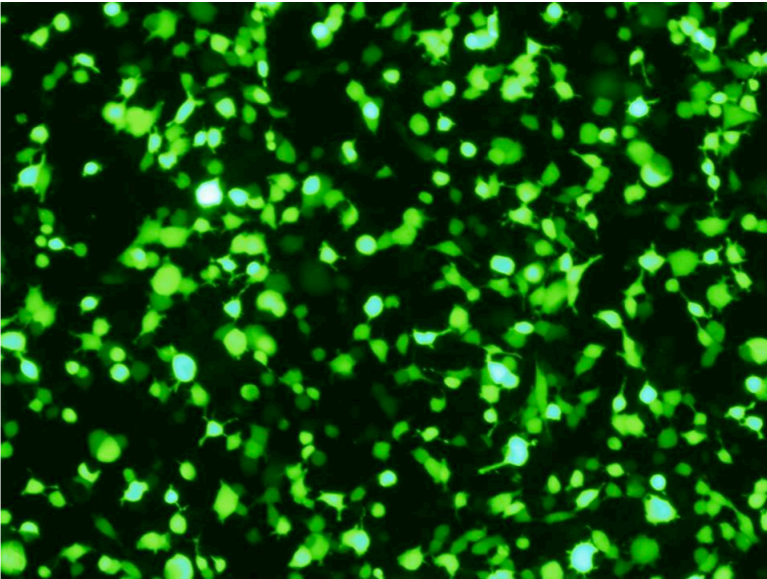
# Step 2: Test the system!



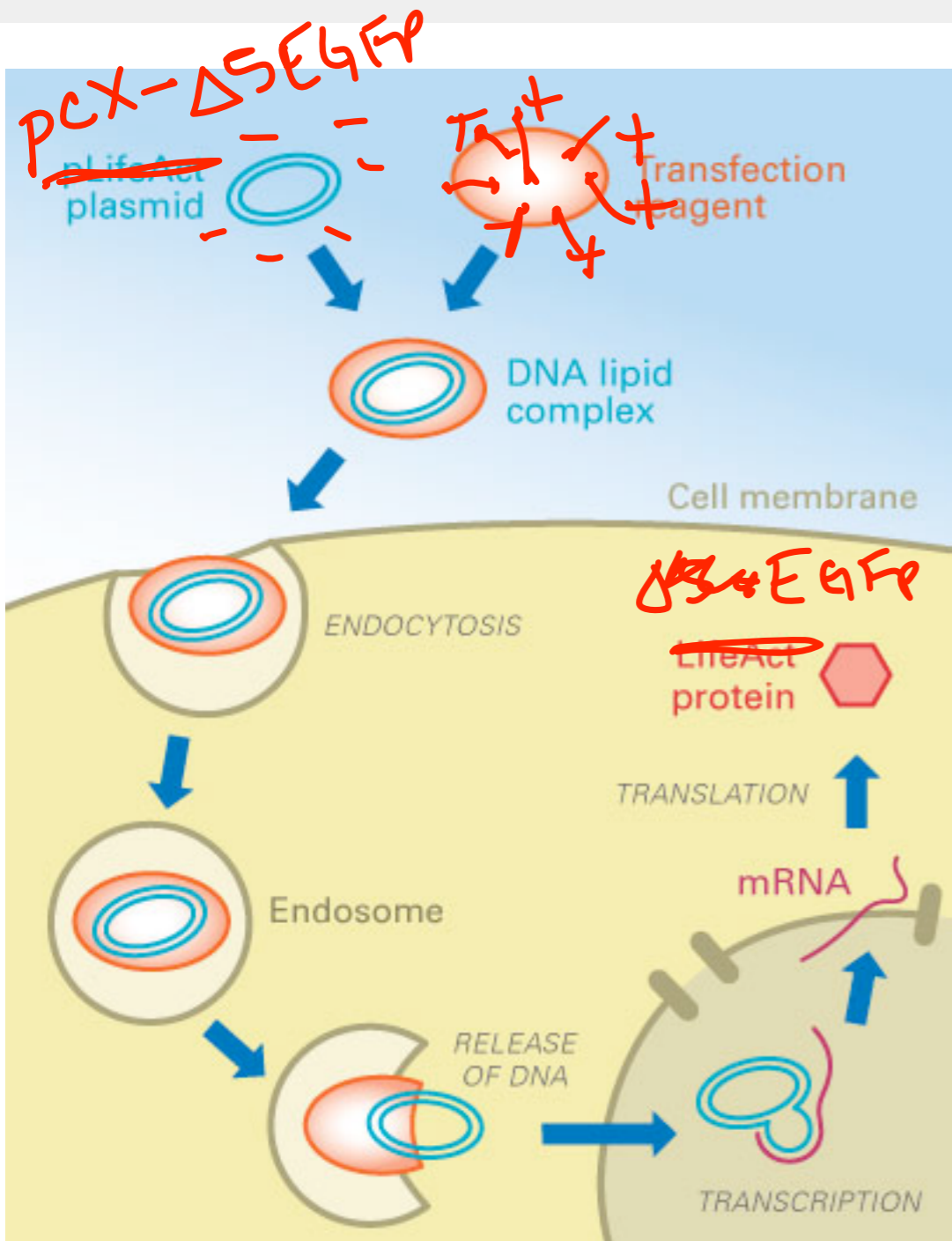
Julian Voss-Andreae  
Steel Jellyfish (Green Fluorescent Protein), 2006  
Stainless steel, 4' x 3' x 3' (1.20 x 0.90 x 0.90 m)  
Location: Friday Harbor Laboratories (San Juan Island, WA)



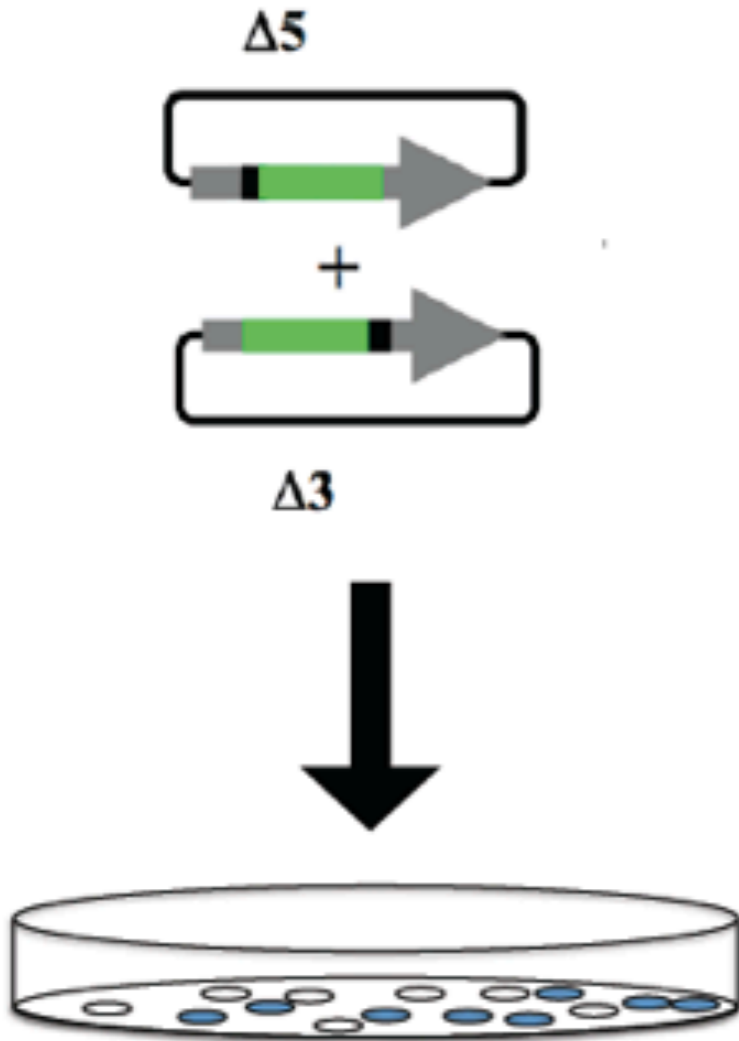
# Step 2: Test the system!



MES ~ max 70%



# Step 2: Test the system!



Negative controls

~50% ↑ GFP



PmeI

→ INHEJ

% EGFP Expression



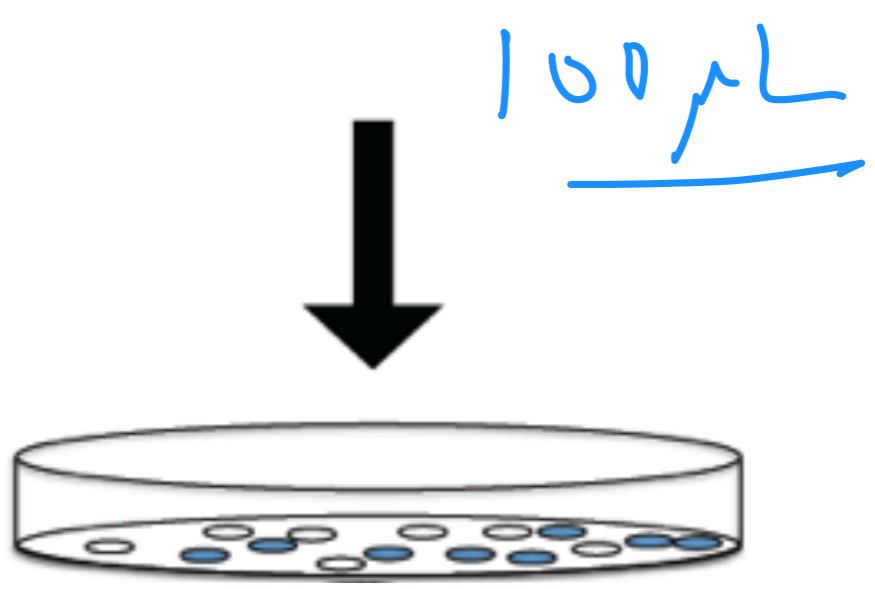
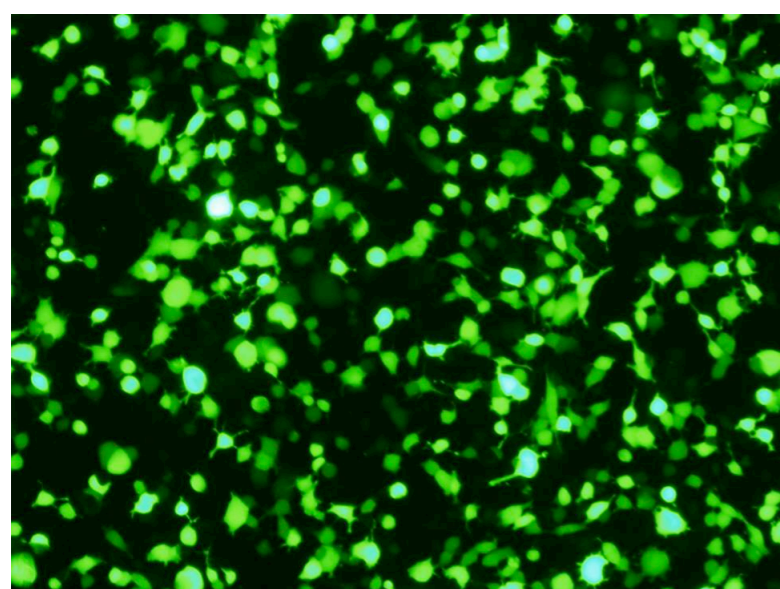
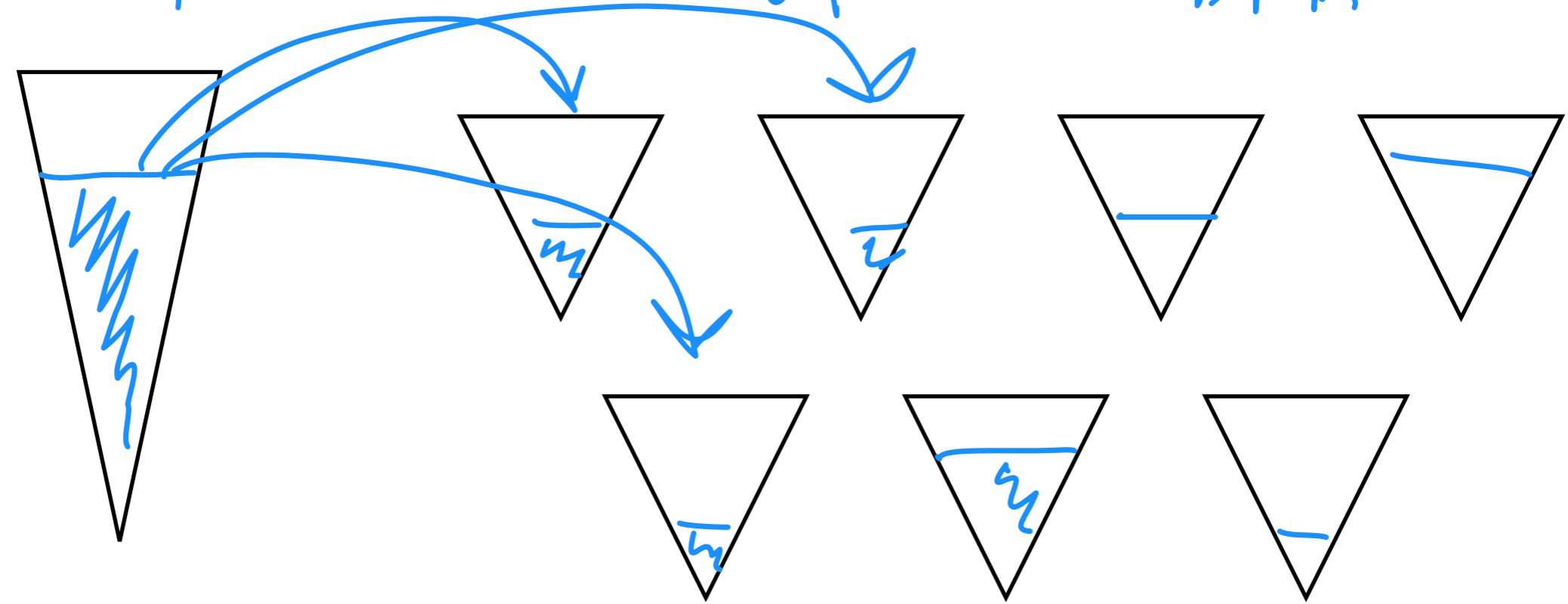
mock     $\Delta 5$      $\Delta 3$     GFP



# Step 2: Test the system!

L2000 + Optimum

Optimum + DNA



# 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 bonus condition
- Discuss paper with Prof. Engelward
- Look for feedback on Methods ASAP

## Next time in the lab:

- Sign-up on MID7 Talk Page
- FACS analysis to measure HR efficiency
- Post FACS data on MID7 Talk page promptly!!!