- Announcements
- Pre-lab Lecture
  - Major assessment prep
  - Lipofection workflow
  - Samples for HR experiment
  - Tissue culture tips
  - Today in Lab: M1D6

# Announcements

- Thank you Isaak!
- Daily work next time:
  - notebooks due by 5P email note to Isaak
  - final M1 quiz
- Lab next time: flow cytometry in shifts
  - sign up on M1D7 "Talk" page
  - I won't be here
- Methods due by 5 pm Mon 10/6 → on wiki homepage
  - remember: weekend OH by Skype/phone/email
- Data summary due by 5 pm Sat 10/11
  - extra OH R pm or F am? 4-5 in 16-336

# Colony count data

| Group Colour | pCX-EGFP (#) | bkb + ins, no lig (#) | bkb + lig, no ins (#) | bkb + ins, lig 1 (#) | bkb + ins, lig 2 (#) |
|---|---|---|---|---|---|
| Hypothetical Data | 1000 | 2 | 20 | 100 | 100 |
| Blue | 1600 | 0 | n/a | 0 | 0 |
| Green | Lawn | 0 | 4 | 13 | 14 |
| Pinkle | Lawn | 2 | 2 | 210 | 305 |

**When you are writing your R&D, consider the following:** (+) title each sub-section

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

intro bullet  prepare for DNA cloning

b. What was your expected result?

bands at 4237 + 663 bp

c. What was the result? (1 of 2)

cons. – bands near 4kb marker, b/w 0.5–1 kb marker

interp. – bands ~4200, 650 bp. (even more: measure w/ ruler, log plot → calc.)

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

single digests controls for RE functionality

"suggests" or "consistent with" successful preparation NOT "confirms"

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

suitable/ready for ligation/cloning

via Shannon H

# Revisit Methods section: What experiments fit together?

PCR

Transformation

Xbal/EcoR1 Digest

PCR product purification

Diagnostic Digest GE

Xbal/EcoR1 Digest
Purification (GE)

O/N e.coli cultures

"usable product points"

Plasmid purification

* intro sentences implicitly indicate logic of grouping

Ligation/Precipitation
via Shannon H

Diagnostic Digest

# Lipofection method

- DNA carrier is cationic lipid
  - binds DNA and fuses/enters cell membrane
- Efficient transfection (can be >95%)
- Delayed expression – nuclear entry on division

* for more detailed * mechanism, SKM

Figure 6 - Outline of transfection procedure for Lipofectamine™ 2000 Reagent

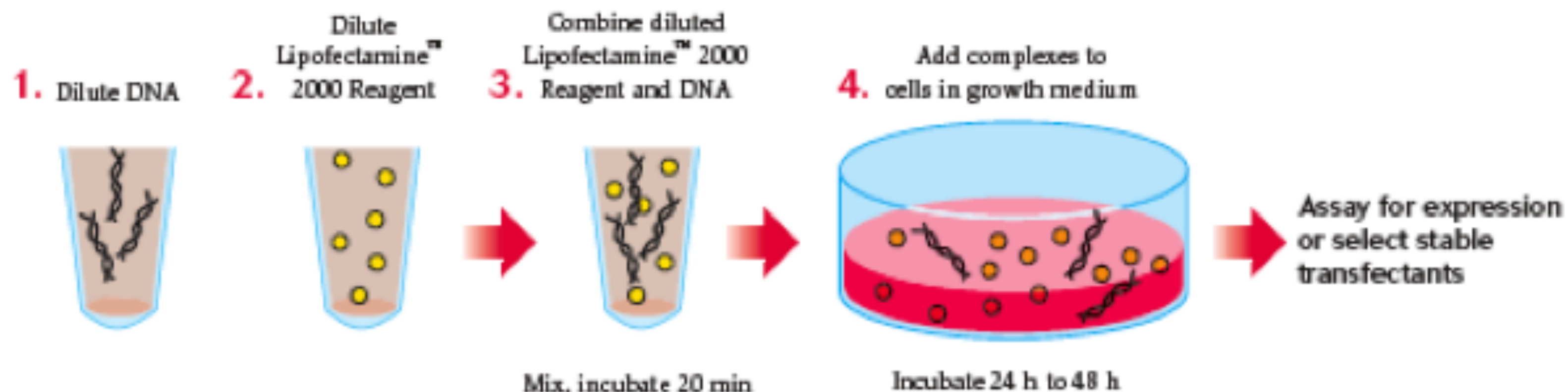



Figure from Invitrogen website

# Lipofection workflow

**Wait 5-30 min**

Lipofectamine in Opti-MEM

… then add to

DNA in Opti-MEM

various samples/volumes (1x, 3x)

9 tubes (not 7)

**Wait 20 min**

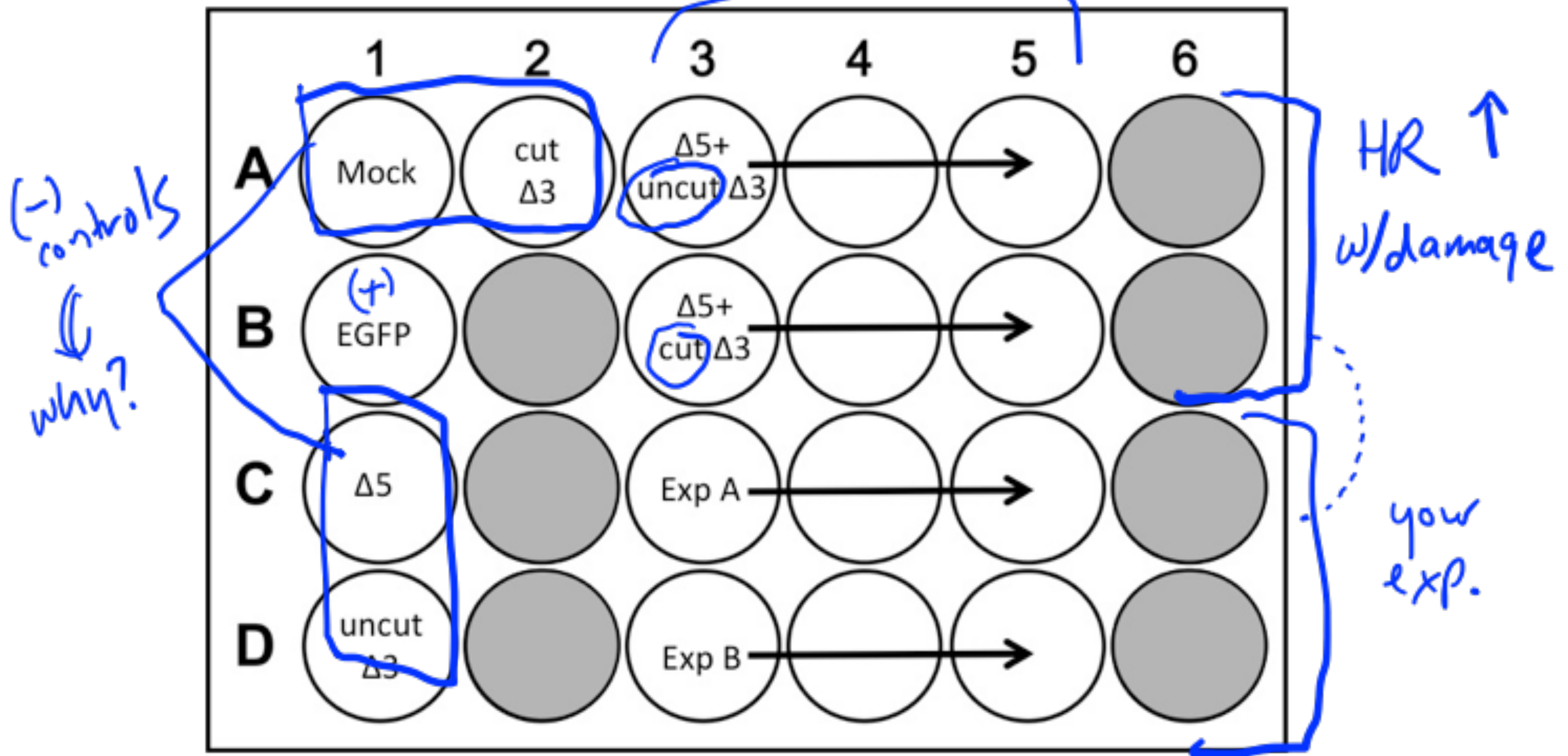
Lipid/nucleic acid complexes

… then add to

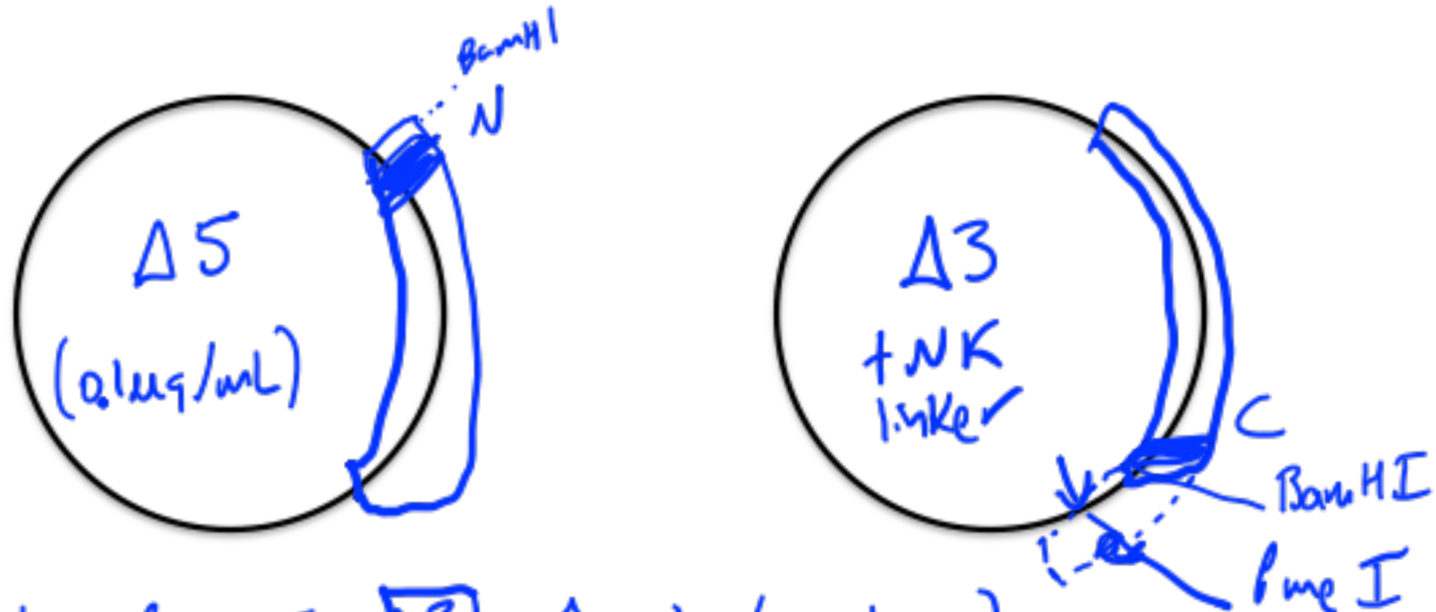
Wells with MES cells, freshly rinsed with PBS and fresh media added. (Start in pre-transfection media – no P/S!)

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| A | Mock | cut Δ3 | Δ5+ uncut Δ3 → | → | → | |
| B | EGFP | | Δ5+ cut Δ3 → | → | → | |
| C | Δ5 | | Exp A → | → | → | |
| D | uncut Δ3 | | Exp B → | → | → | |

# Lipofection samples = HR exp(s)!



# Planning exps A + B




1. ratio of Δ5 : Δ3 ↑ or ↓ (limit 1:10)
2. cut w/ PmeI (blunt) versus BamHI (sticky ends)

☆ hypotheses ☆

3. cut vs. intact Δ3 → background level of damage → stimulates HR

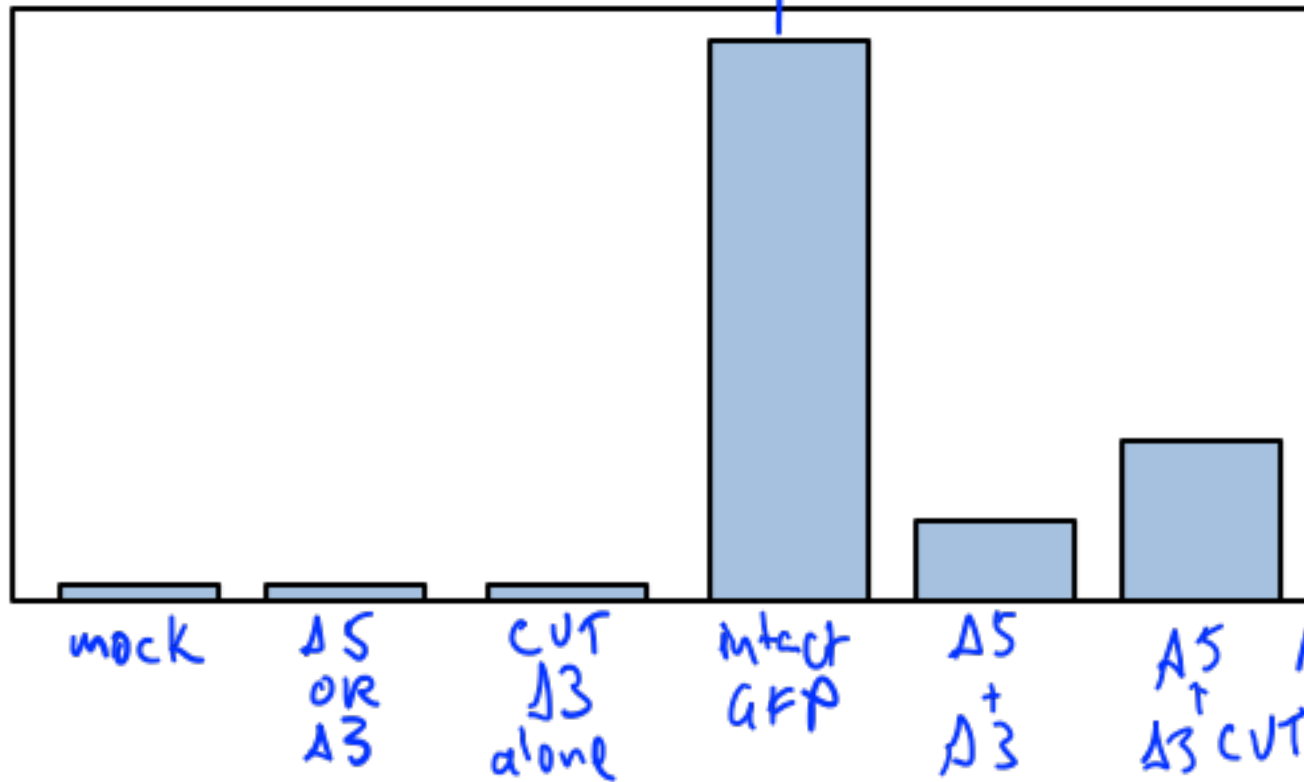
# Controls for HR assay

☆ which stats comparisons to make? ☆

- How do you know if your experiment worked?

class-wide?

50-80% ⇒ think: what if varies across class?



# Tissue culture tips

and don't block!!

- 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 Lab: M1D6

- Start by designing Exps A+B
- Then we'll head to TC <= 1.5 hrs
- Afterward, at own pace
  - stats practice → in lab notebook
  - catch up on notebook entries
  - ?? sleep/leave/eat/etc :)
- Will announce methods pick-up when ready