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

Announcements

- Next time: flow cytometry in shifts
  - sign up on M1D7 "Talk" page
- First blog post due Oct 5th by midnight
- No class Oct 9th or 10th
- Slide summary due Oct 11th by 11 AM
- Oral defenses Oct 12th, 4-5 pm in 56-202
- OH tentatively Oct 9th 4-5 pm, only

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

Lipofection method

- DNA carrier is similar to the cell membrane
- Efficient transfection (can be >95%)

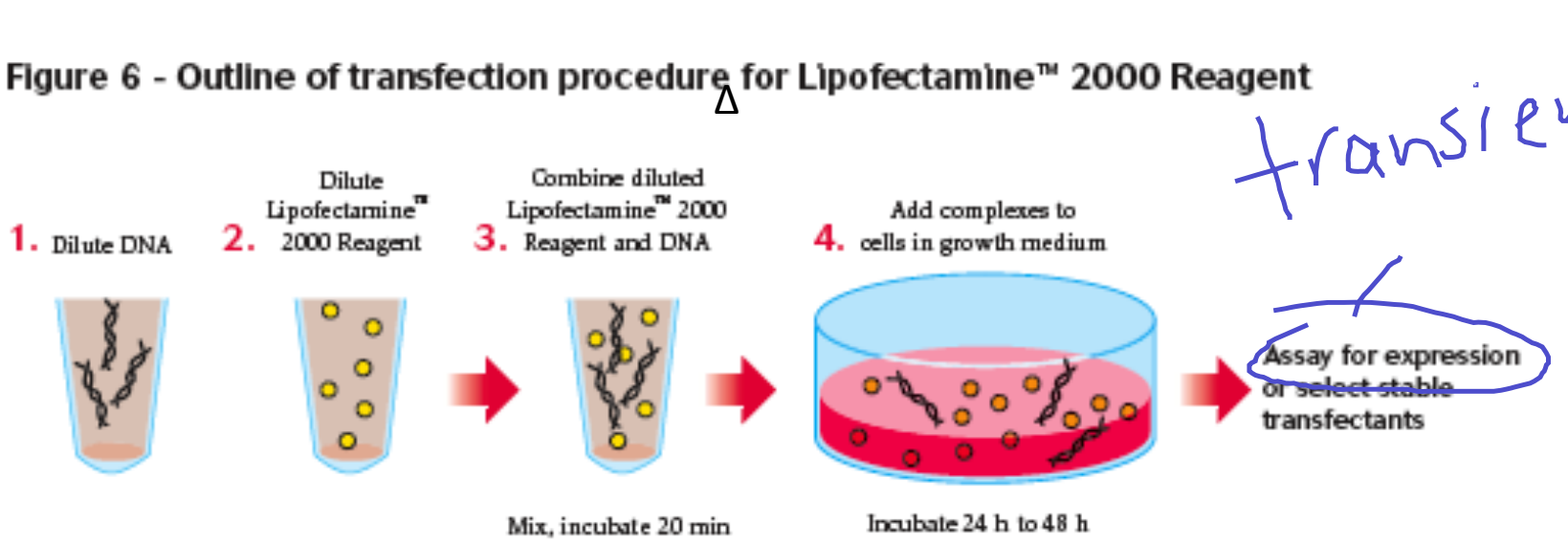
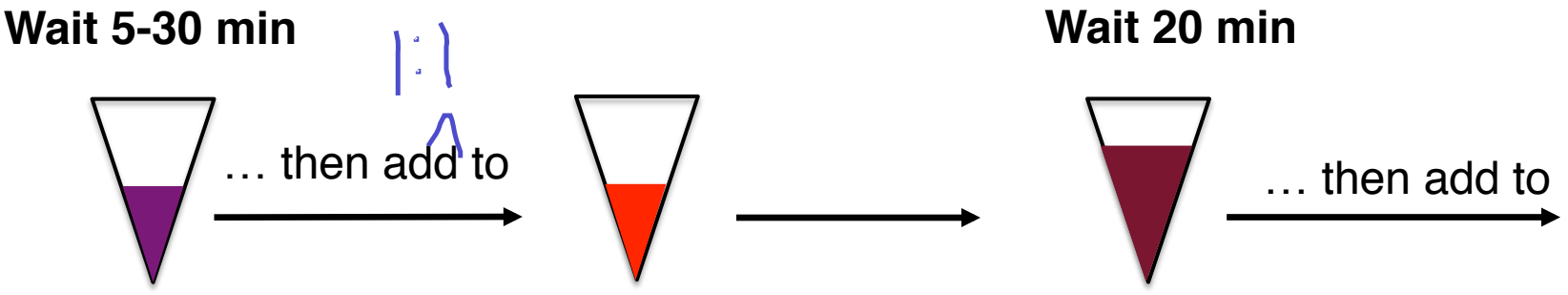


Figure from Invitrogen website

Lipofection workflow



1:1

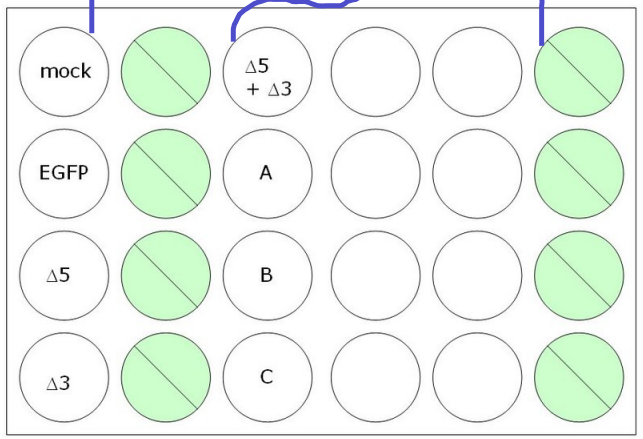
$V_{tot} = 50\mu L$

$V_L = 2.5\mu L$

16.5x prep aliquot

50μL OR 150μL

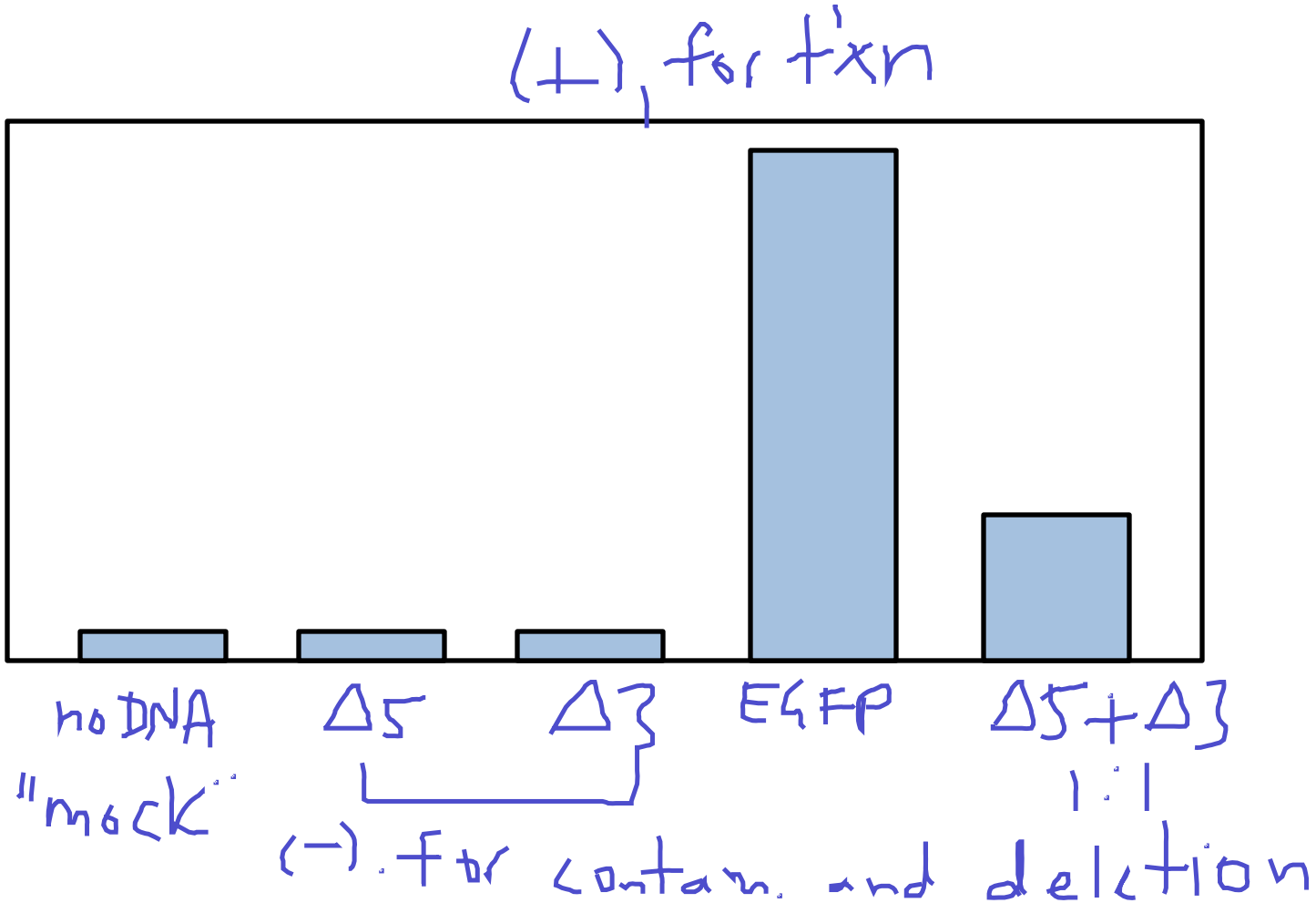
controls exp. triplicate



Wells with MES cells

Controls for HR assay

- How do you know if your experiment worked?



Experimental samples for HR assay

- How might we increase HR frequency?

vary Δ5:Δ3

break DNA { pre-txn : digest, UV, etc.
post-txn : γ-irr, chem.

- Plan for today
  - Baseline: 1:1
  - A: 1:2
  - B: 1:10
  - C: ? your idea

Δ5:Δ3

[DNA] 0.5
0.1 μg/mL
0.05

Today in Lab: M1D6

- One hour lab certification
- Lipofection of MES in TC
- Oral defenses with Jenny

+colony counts

+bye-bye Jenny :)