

- **Announcements** Memo due in 1 week
- **Lab Quiz** O H NK Sun. 3-5pm, lab
probably Mon ~5-6+
- **Pre-lab Lecture** <no lab Wed!>
 - ❖ Intro to lipofection, workflow
 - ❖ Samples for HR experiment
- **Optional Post-lab Lecture**
 - ❖ Statistics Review

Lipofection

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

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

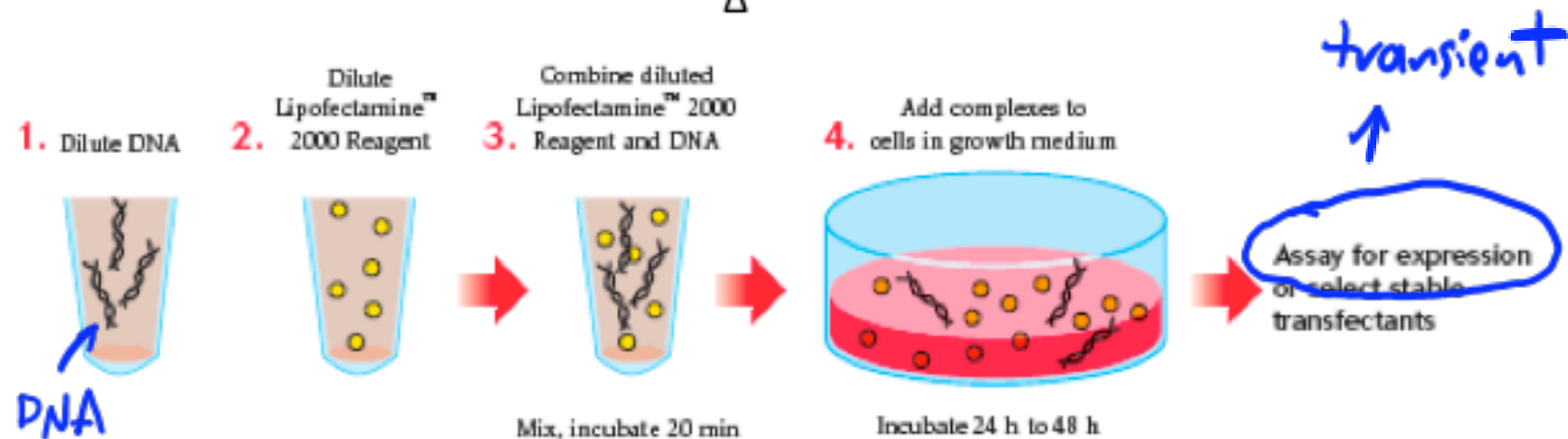


Figure from Invitrogen website

Lipofection Workflow

Wait 5-30 min



... then add to



Wait 20 min



... then add to

Lipofectomine
in Opti-MEM

50 μ L

L-2.5 μ L 0-47.5 μ L

16.5x Vol. prep

triplicate: 150 μ L + 150 μ L

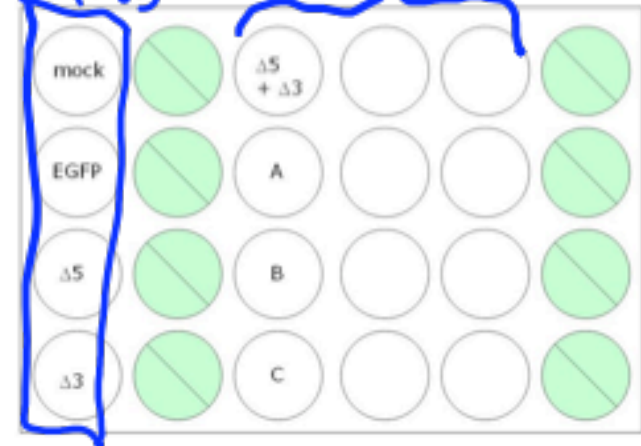
DNA/ in
Opti-MEM

50 μ L

$$0.1 \mu\text{g} \times \frac{\mu\text{L}}{0.05 \mu\text{g}} = 2 \mu\text{L}$$

Lipid/nucleic
acid complexes

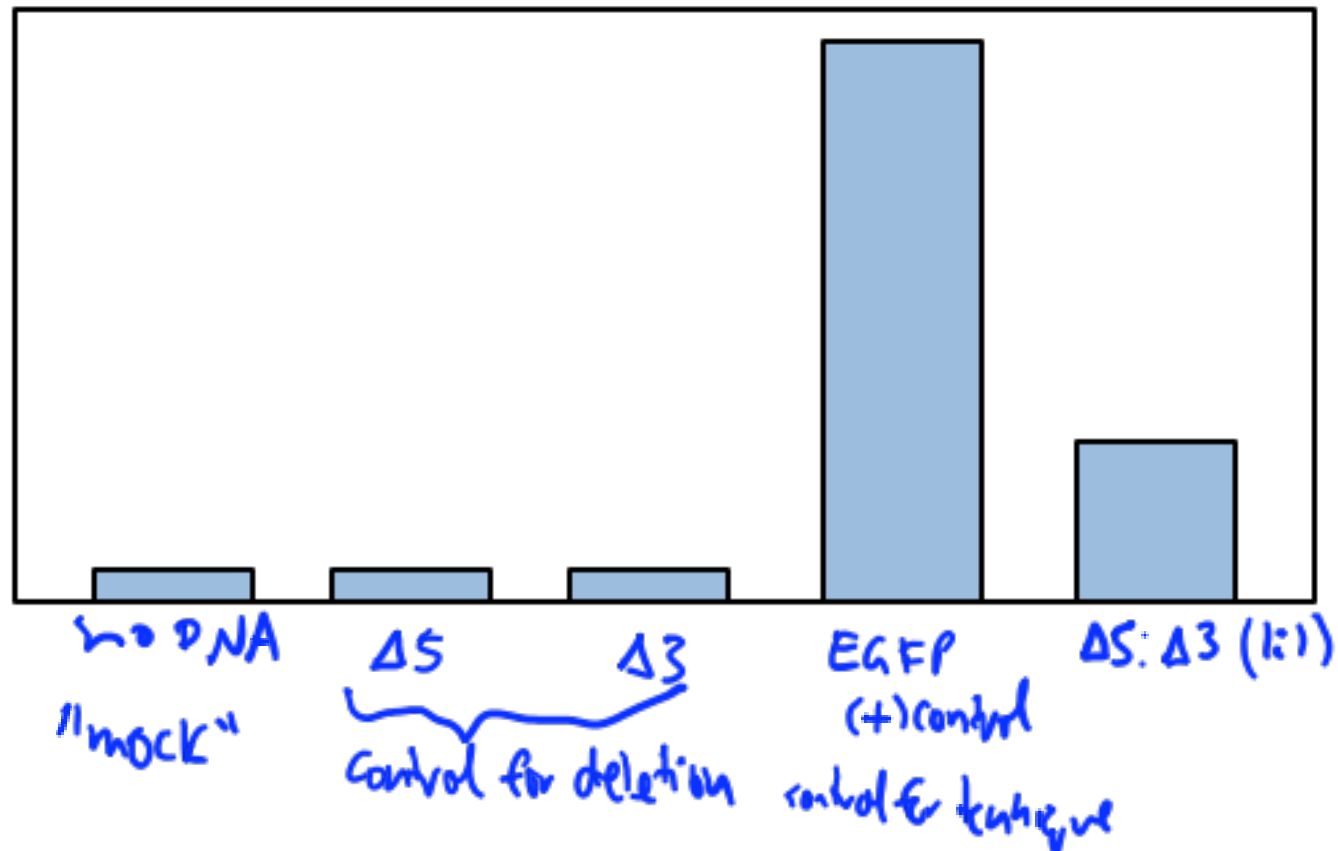
controls exp. - triplicate



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?

• break DNA before txn: UV, restriction digestion
 after txn: irradiation, chemicals
 if R.E. → location

• vary ratios } today
• vary [DNA] } /

- Plan for today

– Baseline: 1:1 Δ5: Δ3
– A: 1:0.5 "1" = 0.1 μg DNA
– B: 1:2
– C: 1:10

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 Lab

- Lipofection of MES in TC led by Michelle
- Return here to finish Sonada paper discussion 0
- Finally, complete statistics assignment
 - Dive right in, or listen to optional lecture
 - Hand in as part of your notebook
- Sign up for a Friday FACS time: you only have to come to lab at your time (no lab quiz)
Day 8 "talk" page

Statistics Review: Basics

- Need-to-know concepts: standard deviation, mean, sample size $n \neq$ degrees of freedom DOF
- Normal (Gaussian) distribution



Confidence intervals (CI) Principle

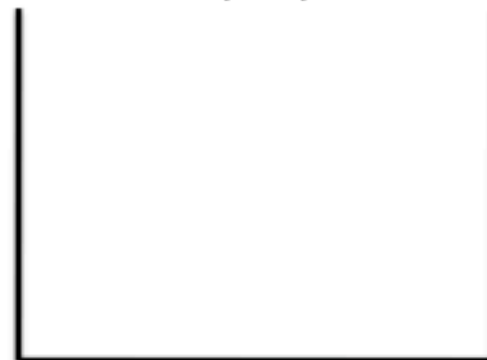
- Sample problem, $x = 60$
- 95 % CI: “I’m 95% sure that the true mean”
 $\mu = x \pm 3 = 60 \pm 3 = 57 - 63$ (shorthand, not exactly true)
- 90% CI: $\mu = x \pm a$ where $a < 3$ $a > 3$ $a = 3$?

- Consider betting example
- What about n ?

Calculating Confidence Intervals (CI)

$$\mu = \bar{x} \pm \frac{t s}{\sqrt{n}}$$

Sample plot:



- t is tabulated by DOF vs CI%
 - DOF = $n - 1$
- In Excel, use $TINV$ function
 - Input p -value = $(100 - CI) / 100$

Introduction to t-test

- Every statistical test
 - Has
 - Asks
 - Requires
- Some t-test assumptions
- Question

Calculating t-test Significance

$$t_{calc} = \frac{\bar{x}_1 - \bar{x}_2}{s} \sqrt{\frac{n_1 n_2}{n_1 + n_2}} \quad \text{DOF} =$$

- If $t_{calc} > t_{table}$ difference *is* significant
- In Excel, use *TTEST* function
- Excel returns *p*-value → confidence level
- 1-tailed vs. 2-tailed test

Assignment Today

- Get heights of men and women in class
- Calculate 95% CI for both means
- Plot means on bar graph with CI error bars
- Try t-test to compare the two means
 - In Excel, and using a table if you have time

Comparing HR Samples

