- Announcements
  - Memo due in 1 week
  - H NK Sun, 3-5pm, b5
  - probably Mon 5-6+
  - <no lab Wed!>

- Lab Quiz

- Pre-lab Lecture
  - 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

1. Dilute DNA
2. Dilute Lipofectamine™ 2000 Reagent
3. Combine diluted Lipofectamine™ 2000 Reagent and DNA
4. Add complexes to cells in growth medium

Assay for expression of select stable transfecants

Figure from Invitrogen website
Lipofection Workflow

Wait 5-30 min

... then add to

Lipofectamine in Opti-MEM

$50 \text{ mL}$

$62.5 \text{ mL}$ $0.475 \text{ mL}$

$16.5 \times \text{ Vol. prep}$

triplicate: $150 \text{ mL} + 150 \text{ mL}$

Wait 20 min

... then add to

DNA in Opti-MEM

$50 \text{ mL}$

$0.1 \text{ mg} \times \frac{\text{ mL}}{0.05 \text{ mg}} = 2 \text{ mL}$

Wait 20 min

... then add to

Lipid/nucleic acid complexes

Wells with MES cells

Controls, exp. - triplicate
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 
    - UV, restriction digestion
  - vary ratios after 
    - irradiation, chemicals
  - vary [DNA] if RIE → location

- Plan for today
  - Baseline: 1:1 \( \Delta 5: \Delta 3 \)
  - A: 1:0.5 "1" = 0.1 \( \mu \)g 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
- Return here to finish Sonada paper discussion
- 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 \( a < 3 \quad a > 3 \quad a = 3 \) ?
• Consider betting example
• What about \( n \)?
Calculating Confidence Intervals (CI)

\[ \mu = \bar{x} \pm \frac{t \cdot s}{\sqrt{n}} \]

- \( t \) is tabulated by DOF vs CI%
  - DOF = \( n - 1 \)
- In Excel, use \( TINV \) function
  - Input \( p \)-value = \( (100 - \text{CI})/100 \)
Introduction to t-test

• Every statistical test
  – Has
  – Asks
  – Requires

• Some t-test assumptions

• Question
Calculating t-test Significance

\[ t_{\text{calc}} = \frac{x_1 - x_2}{S} \sqrt{\frac{n_1 n_2}{n_1 + n_2}} \]

DOF =

- If \( t_{\text{calc}} > t_{\text{table}} \) difference is significant
- In Excel, use \( TTEST \) function
- Excel returns \( p \)-value \( \rightarrow \) 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