- Announcements, Review HW
- Lab Quiz
- Pre-lab Lecture
  - ❖ Samples for HR experiment
  - ❖ Safety + Technical Tips
- Optional Post-lab Lecture
  - ❖ Statistics Review

Announcements, old HW

- Powerpoint Pitch due in one week
  - OH: 1 session Sun Mon Tue

2:30pm

- Careful with calculations
  - For transformation, needed to take into account cell fraction plated to get DNA amount
  - In some cases precision will *matter*

200mL cells+DNA
550mL

Lipofection

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

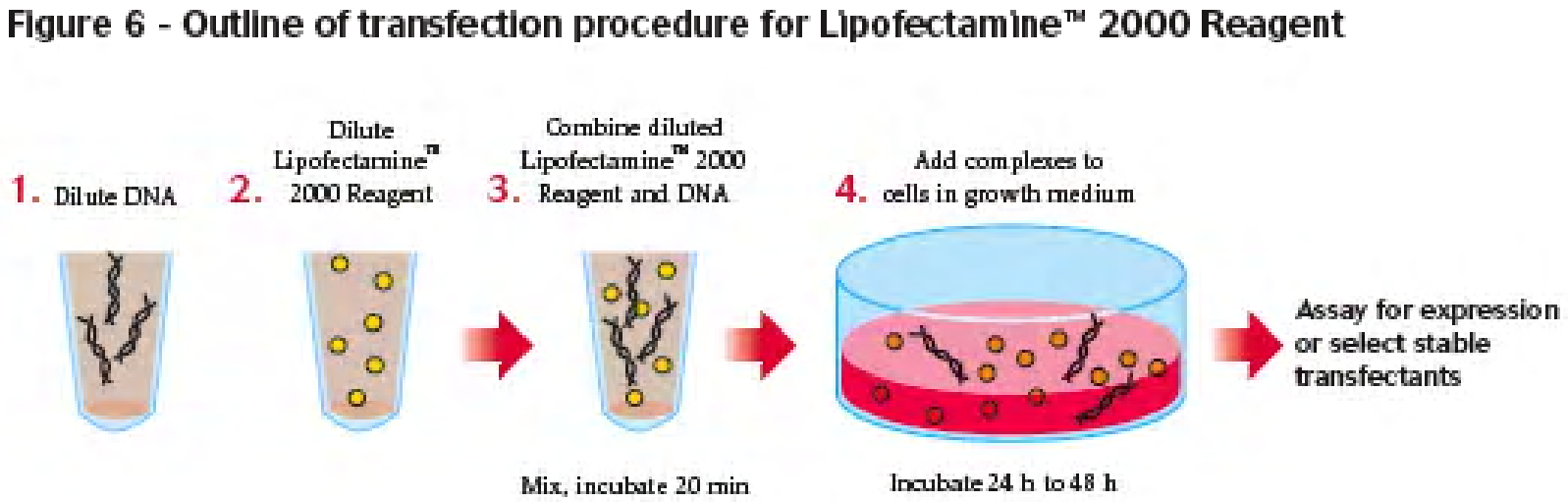
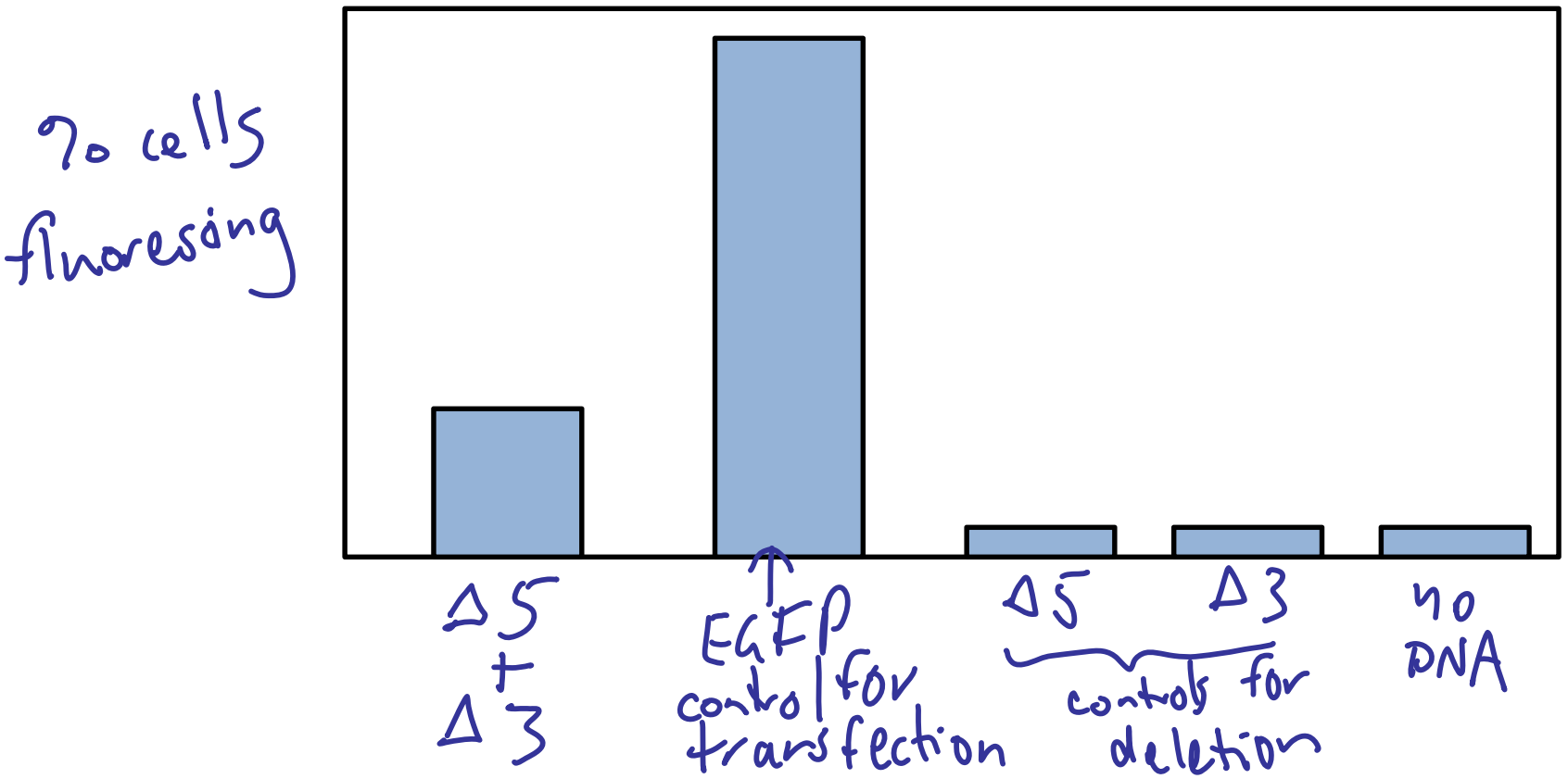


Figure from Invitrogen website

Controls for HR Assay

- In lecture 7 you were asked – how do you know that your experiment worked?

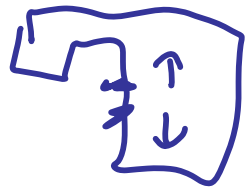


Experimental Samples for HR Assay

- You were asked to consider
  - How to increase HR frequency? introduce DSB
  - Within that, effects of
    - # of cuts → 1
    - cut location → near the truncation (↑ or ↓ stream?)
    - blunt vs. sticky ends @ DSB
    - ratio of the two plasmids
  - Your options/DNA available today:
    - change Δ5:Δ3 ratio, 1:5 or 5:1
    - digested Δ3 plasmid
    - optional: combine

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

available: digested Δ3

- Lipofection of MES in TC
  - Calculations: (DNA + O-MEM) + (Carrier + O-Mem)

16.5x

0.1mg DNA x mL / 0.05mg (or /0.5)

50mL + 50mL

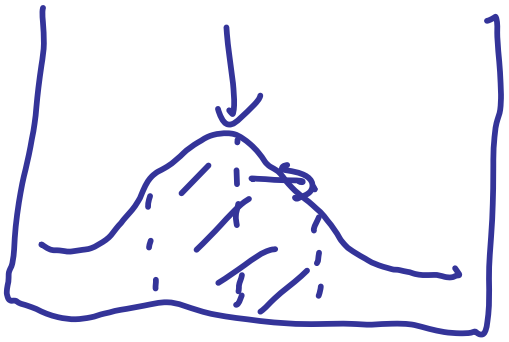
3x 150mL + 150mL

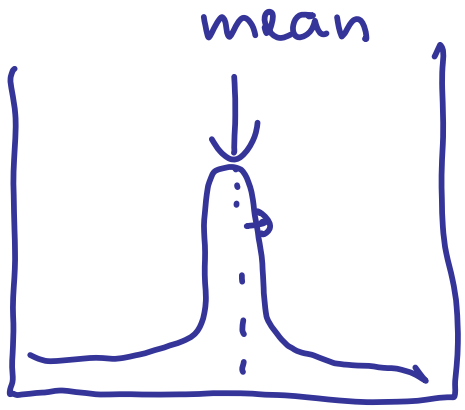
- Return here to complete statistics assignment
  - Dive right in, or listen to optional lecture
  - Hand in as part of your notebook
- Sign up for a Friday FACS time: you only have to come to lab at your time (no lab quiz)

Day 8 talk page

Statistics Review: Basics

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





S.d. = 5

1S = 68%

of your data

x → value (intensity)

y → # of objects having that value

Confidence intervals (CI) Principle

mean, observed in reality, finite sample #

- Sample problem, $\bar{x} = 60$
- 95 % CI: "I'm 95% sure that the true mean"
- $\mu = \bar{x} \pm 3 = 57 - 63$
- 90% CI: $\mu = \bar{x} \pm a$ where $a < 3$ $a > 3$ $a = 3$?

pop. mean ← ∞ samples

60 ± a

trade off b/w precision & confidence

as n ↑, precision increases

Calculating Confidence Intervals (CI)

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

std. dev.

t-value tabulated as DOF vs. CI %

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

7 heights DOF = 6

(why? $\sum$ (errors $= x_n - \bar{x}$) $= 0$ limitation)

CI = 95%, $p = 0.05$

Introduction to t-test

- Every statistical test
  - Has assumptions
  - Asks question
  - Requires human interpretation

- Some t-test assumptions
  - normal distribution
  - equal variances (Type 2 in Excel)
    unequal – use Type 3
- Question

Are male heights greater than female on average at a confidence level of 95%?

Calculating t-test Significance

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

s → pooled

If t_{calc} > t_{table}

difference is significant

DOF = $n_1 + n_2 - 2$

- In Excel, us *TTEST* function
- Excel returns a *p*-value → confidence level

e.g. $p = 0.1$ C.L% = 90

- 1-tailed vs. 2-tailed test

1- have hypothesis in advance
2- unknown effect, more stringent (default)
* Type 3 (unequal variance)

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