- Announcements
- Quiz
- Reporter plasmids
- Lipofection
- Luc and mouse expt'l scheme
- Workflow (Mod 2 Day 5)
- Later… optional stats lecture

Announcements

- iGEM reminder:
  - Info session Mar 31 (4:30-5:15 pm in 56-614)
  - Apps due Apr 3
- Handout re: previous homework
- Lab care: plug in laptops; delete jobs on non-responsive printer
- Next 2 weeks: 3 labs, 1 analysis session → Mod2 draft report due on Mod3D1

Reporter plasmid utility

- Mod 1 fusion protein
- Fusion mRNA: gene of interest (GOI) + luciferase
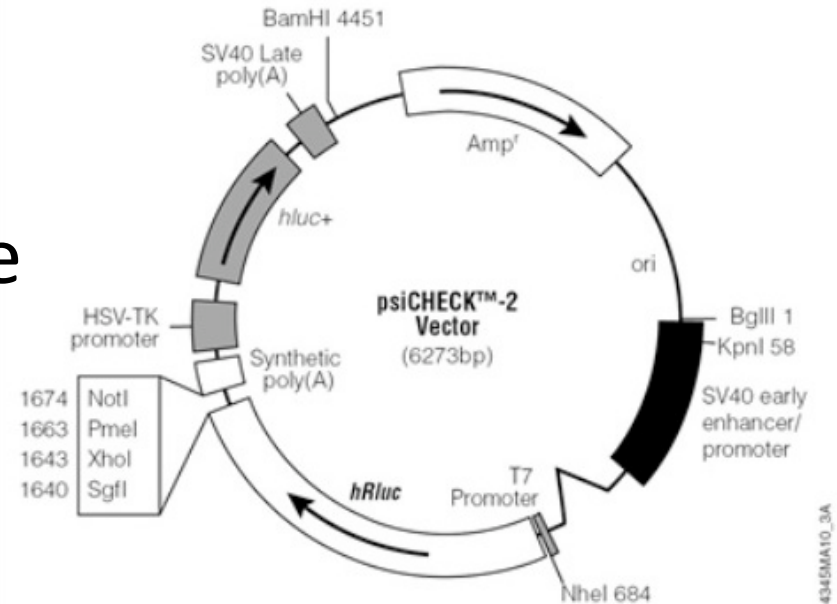- Easy assay for GOI knockdown
- Why target reporter?

Figure from Promega website

Lipofection

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

Figure 6 - Outline of transfection procedure for Lipofectamine™ 2000 Reagent
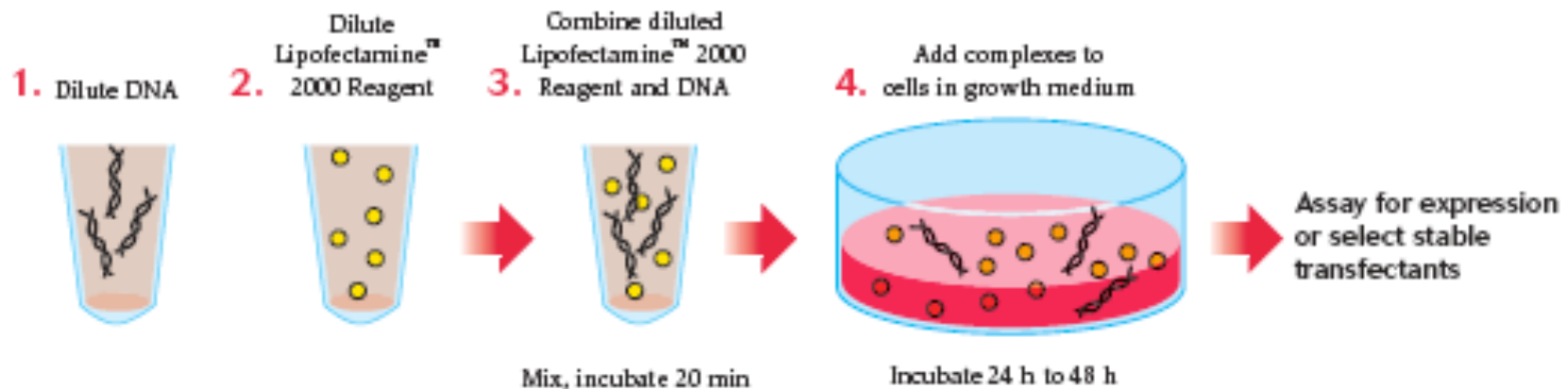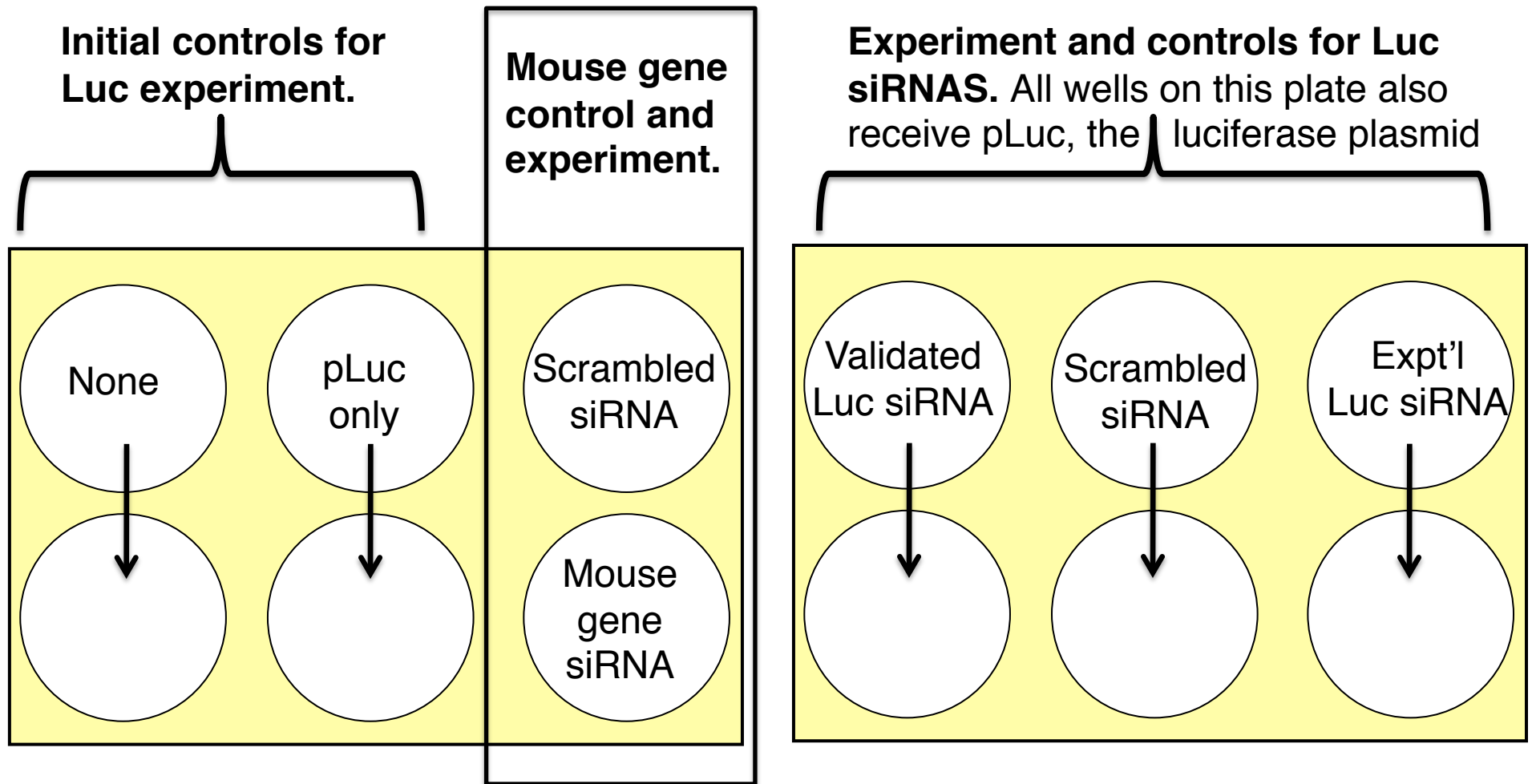
1. Dilute DNA
2. Dilute Lipofectamine™ 2000 Reagent
3. Combine diluted Lipofectamine™ 2000 Reagent and DNA
4. Add complexes to cells in growth medium

Mix, incubate 20 min

Incubate 24 h to 48 h

Assay for expression or select stable transfectants

Figure from Invitrogen website

Today's experiment(s)

Initial controls for Luc experiment.

Mouse gene control and experiment.

Experiment and controls for Luc siRNAS. All wells on this plate also receive pLuc, the luciferase plasmid

M2D5 Workflow

First, sign up for a mouse gene

Transfection

Wait 5-30 min
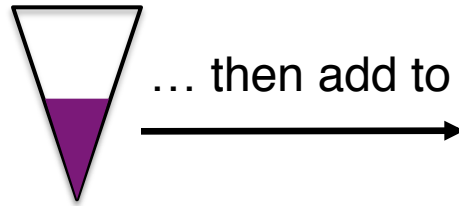
Wait 20 min

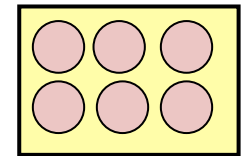Lipofectomine
in Opti-MEM

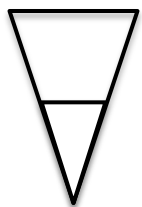… then add to
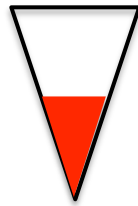
DNA/siRNA
in Opti-MEM
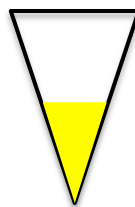
Lipid/nucleic
acid complexes

… then add to

Wells with
MES cells
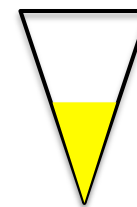
Luminescence assay practice + stats

LAR II

+ cell lysate

Measure
firefly light

+ Stop+Glo

Measure
Renilla light

Statistics Review: Basics

- Need-to-know concepts: standard deviation, mean, sample size $n \neq$ degrees of freedom *DOF*

- Normal (Gaussian) distribution
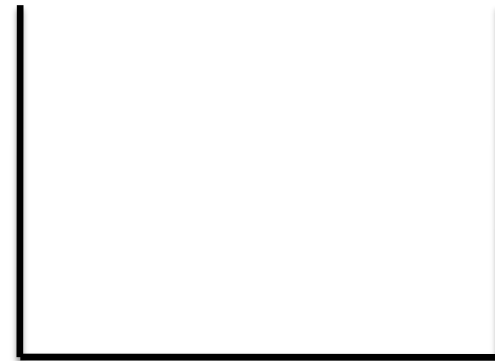
Confidence intervals (CI) Principle

- 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, us *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

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

DOF =

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

Assignment Today

- Get *Renilla* and firefly luminescence ratios: your own and 2 other groups, 3 samples each
- Calculate 95% CI for all three means
- Plot means on bar graph with CI error bars
- Try t-test to compare each pair of means
  - In Excel, and using a table if you have time
  - Note: for multiple comparisons, ANOVA is better; to compare many pairs of means need correction