- Announcements
- Pre-lab Lecture
  - Your Colony Results
  - Tissue Culture
  - Lipofection Workflow
  - Samples for HR experiment
  - Today in Lab: M1D6

Announcements

- Slide summary due in one week, Oct 12th
- No class Oct 11th or 12th
- OH change this coming week:
  - Monday 4-5 pm
  - Tuesday… 10-12? 1-3?

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

Interpreting your ligation results

Group Colour	pCX-EGFP (#)	bkb + lig (#)	bkb + ins, no lig (#)	bkb + ins, lig 1 (#)	bkb + ins, lig 2 (#)
Hypothetical Data	1000	0	2	100	100
Red	1380	5	1	8	2
Orange	1200	10	25	15	150
Yellow	10 000	10	0	80	100
Green	4800	15	3	4180	404
Blue	10^6	25	0	90	90
Pink/Purple	8000	1	40	97	188

Consider…

- Why might some groups not have gotten many exptl colonies?
- What does the *no ligase* vs. the *no insert* sample control for? Which one do you expect to have more colonies?
- How big is the variation for identical samples, and what are the possible sources of error causing the variation?

Tissue culture (TC) environment

- What will "feel" physiological to a cell?

pH ~7.2-7.4 ⇔ CO_2 (often 5%)

T=37°C

ambient O_2

humidity

[salts]

sterility*

Tissue culture (TC) medium

- What do cells need to survive?

C-source for energy - glucose, glutamine, (Na pyr)

essential amino acids
(non-essential a.a.)
vitamins, minerals, lipids
} building blocks or co-factors for rxns

Serum - cytokines (eg growth factor)

non-food/optional
antibiotics
pH indicator

Lipofection method

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

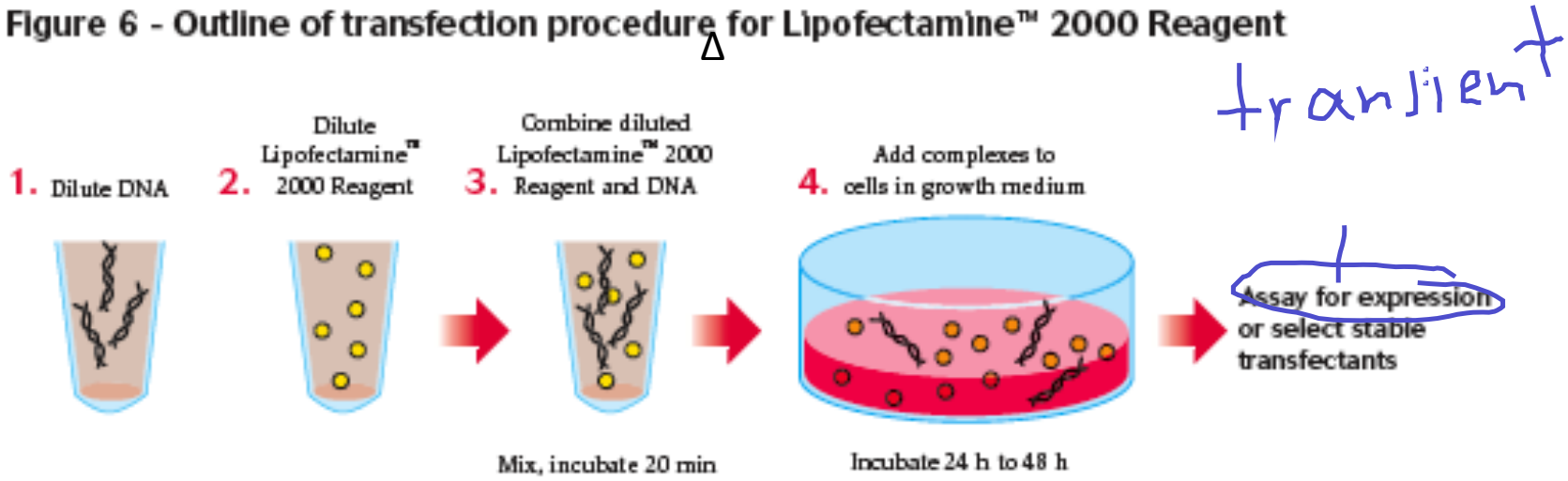


Figure from Invitrogen website

Lipofection workflow

Wait 5-30 min

… then add to

Lipofectomine
in Opti-MEM

DNA/ in
Opti-MEM

Wait 20 min

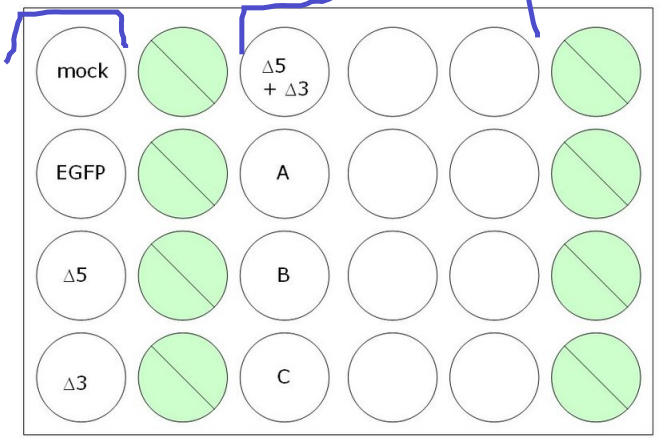
… then add to

Lipid/nucleic
acid complexes

triplicate

control

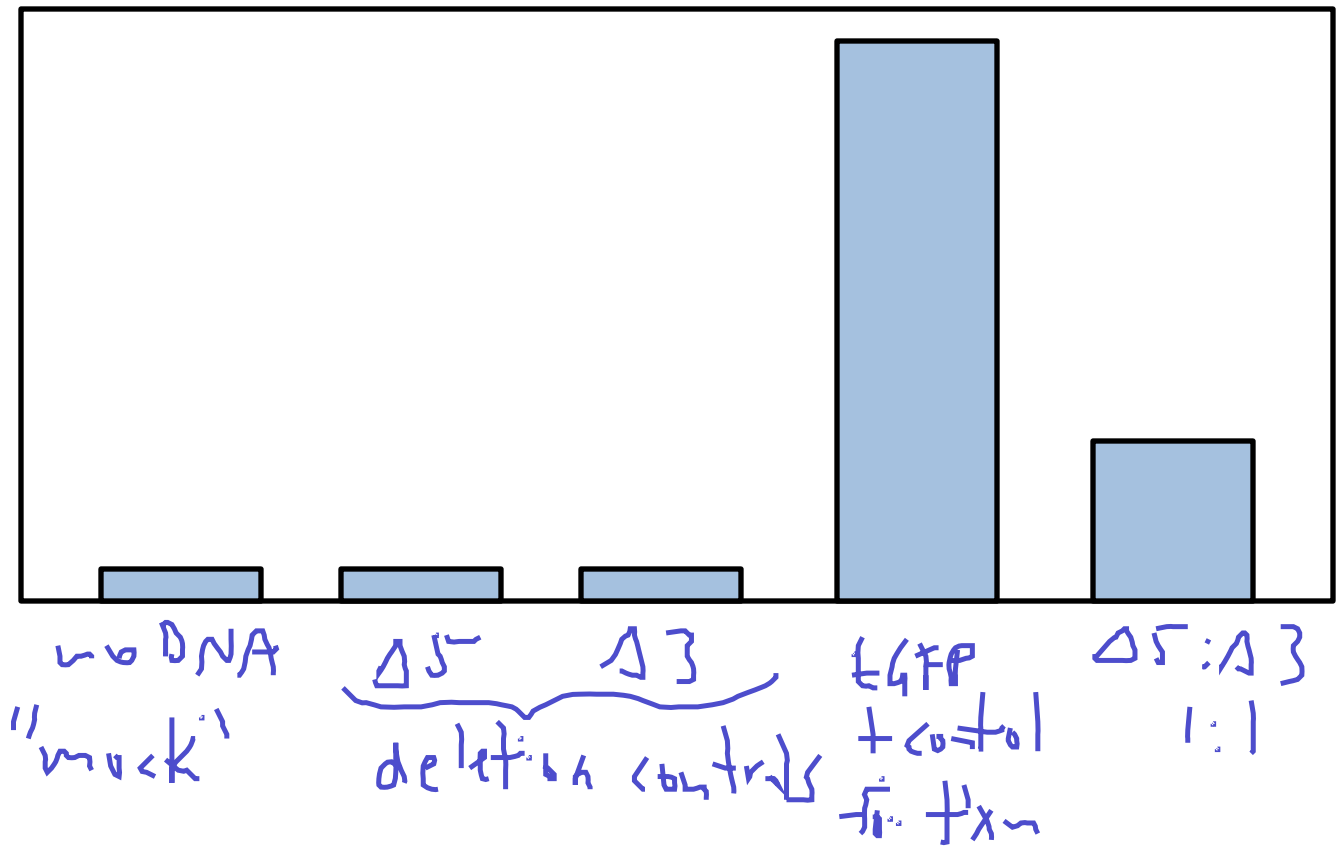
exp.



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 – chemical, digest, UV
    after txn – irradiate, chemical
  - vary 5':3'
  - vary [DNA]
  - restriction site location
- Plan for today
  - Baseline: 5':3' 1:1
  - A: 3' cut PmeI – blunt
  - B: 3' cut BamHI – sticky
  - C: talking on your design

Today in Lab: M1D6

- If you were in TC first on D5, go second today
- Half at a time: Lipofection of MES in TC
- Half at a time: Lab practical (**1.5 hours** exactly)
- No need to hand in lab notebooks this week
- Sign up for a Friday FACS time M1D8 "Talk" page
  - you only have to come to lab at your time
  - no lab quiz