

- Announcements
- Lab Quiz
- Pre-lab Lecture
 - ❖ Major assessment prep
 - ❖ Lipofection workflow
 - ❖ Samples for HR experiment
 - ❖ Tissue culture tips
 - ❖ Today in Lab: M1D6

Announcements

- Next time: flow cytometry in shifts
 - sign up on M1D7 “Talk” page
- Next time: notebooks due!
- Methods due by 5 pm Monday
 - individual, no revision
- Data summary due by 11 am Friday
 - with partner, may be revised
- OH tentatively Sunday 6th from 2-3:30 pm
 - + me T 4-5
 - Bevin M (2-3)?
 - SKH Sunday(?)

+ Bye and
thanks
to Lizzie!!

→ way shift

SKH eg. 513

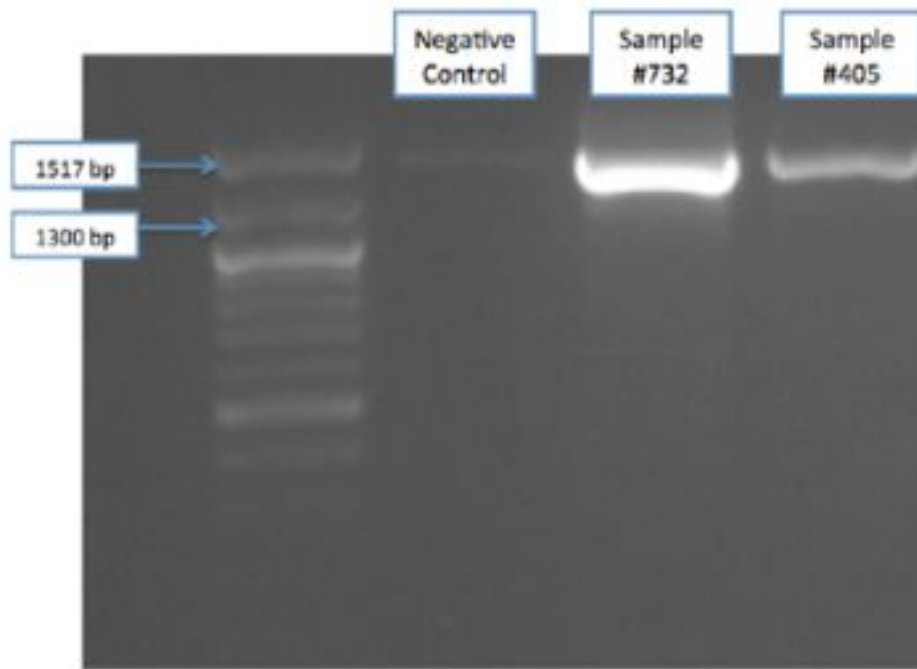


Figure 2: Agarose Gel of 16s rRNA PCR product amplified from gull samples.

interpretation (vs caption)

- concise statements
- options $\left\{ \begin{array}{l} \text{importance} \\ \text{"figure friendly"} \end{array} \right.$

"no band in NTC suggests ..."
draw conclusion each time

"band @ 1400bp suggests
ampl. of 16S rRNA ..."

Current title: Agarose gel of 16s rRNA PCR product amplified from gull samples.

Proof vs. implied

Sk H

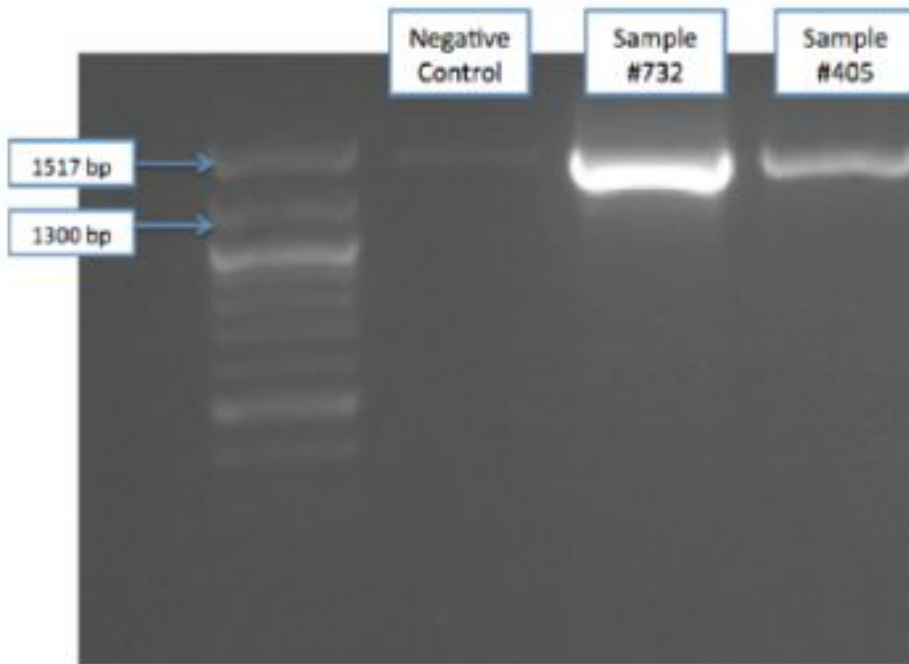


Figure 2: Agarose Gel of 16s rRNA PCR product amplified from gull samples.

- 1 page!
- bold titles!

Alternate titles:

Amplification of bacterial 16s rRNA gene from Alaskan gull stool samples.

Electrophoresis of amplified bacterial 16s rRNA genes.

Successful amplification of bacterial 16s rRNA gene from two Alaskan gulls.

action verby :)

* what is goal (s) of each gel? *

* opening sentence: expanding on title *

Review previous section. What experiments fit together:

PCR

Transformation

"usable product pts"

Xbal/EcoRI Digest

PCR product purification

Diagnostic Digest GE

Xbal/EcoRI Digest
Purification (GE)

O/N e.coli cultures

Plasmid purification

intro sentences
display grouping
logic purpose

Ligation/Precipitation

Diagnostic Digest
- ? ETC.

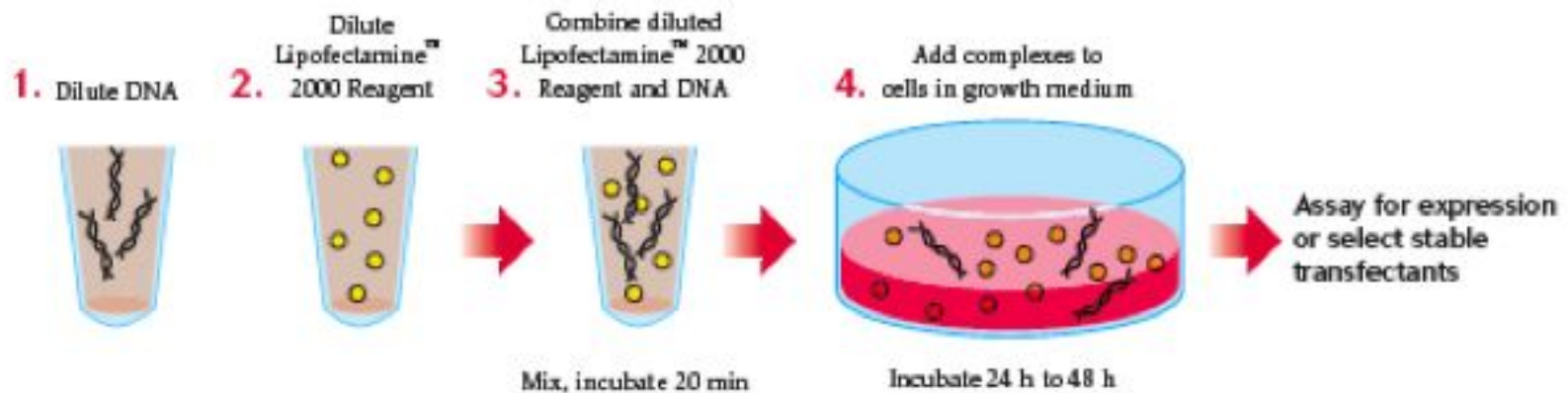
?

?

Lipofection method

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

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



See SKH for mechanism

Figure from Invitrogen website

Lipofection workflow

1.5 hrs

Wait 5-30 min



... then add to



DNA in
Opti-MEM

various

samples/volumes

7 tubes

Wait 20 min

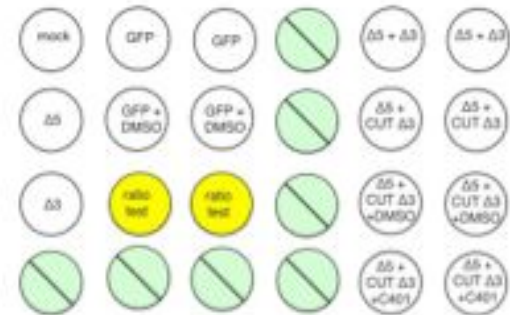


... then add to

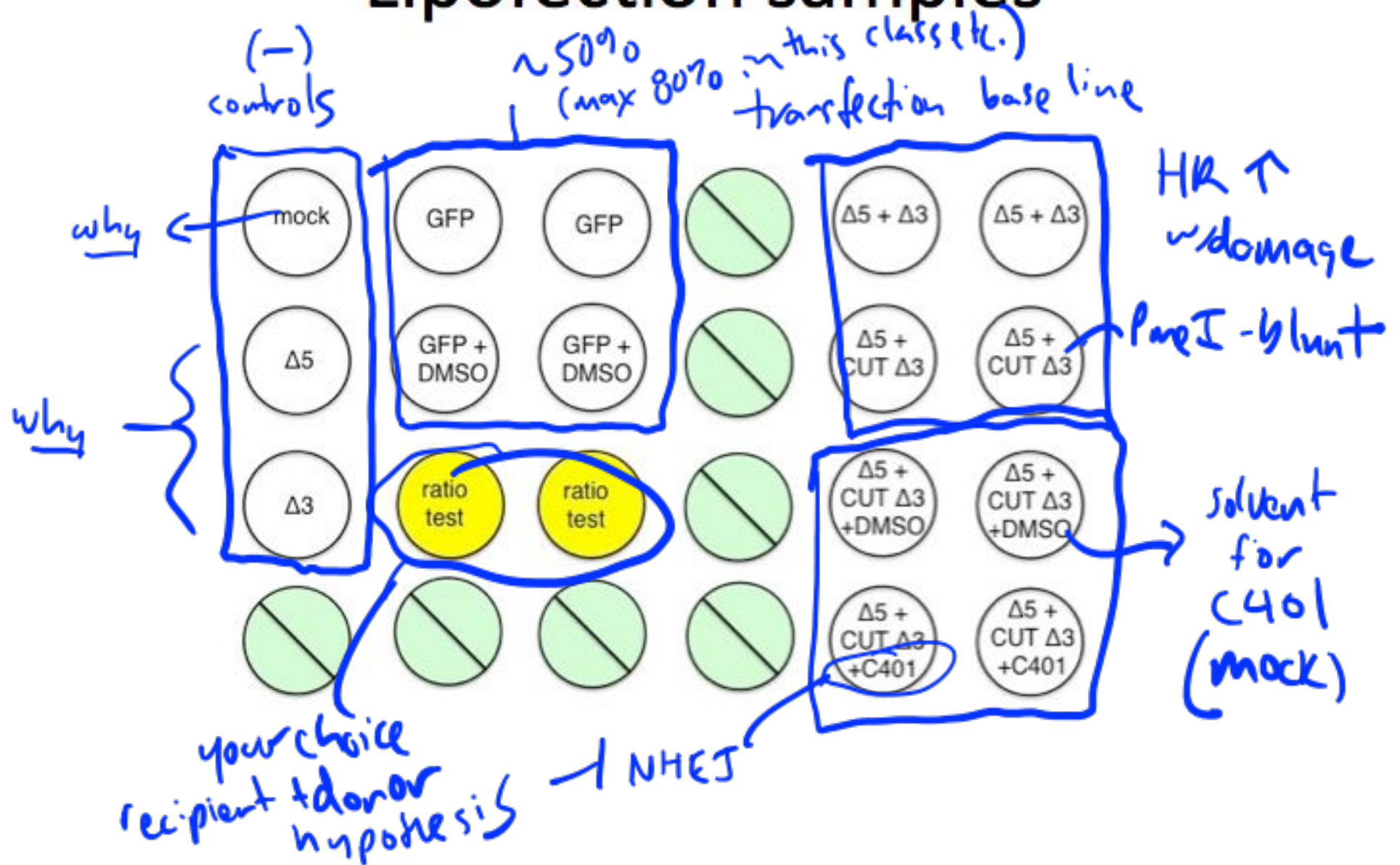
Lipid/nucleic
acid complexes

Lipofectamine
in Opti-MEM

Wells with MES cells, freshly rinsed with PBS and fresh media added. (Start in pre-transfection media – no P/S!)



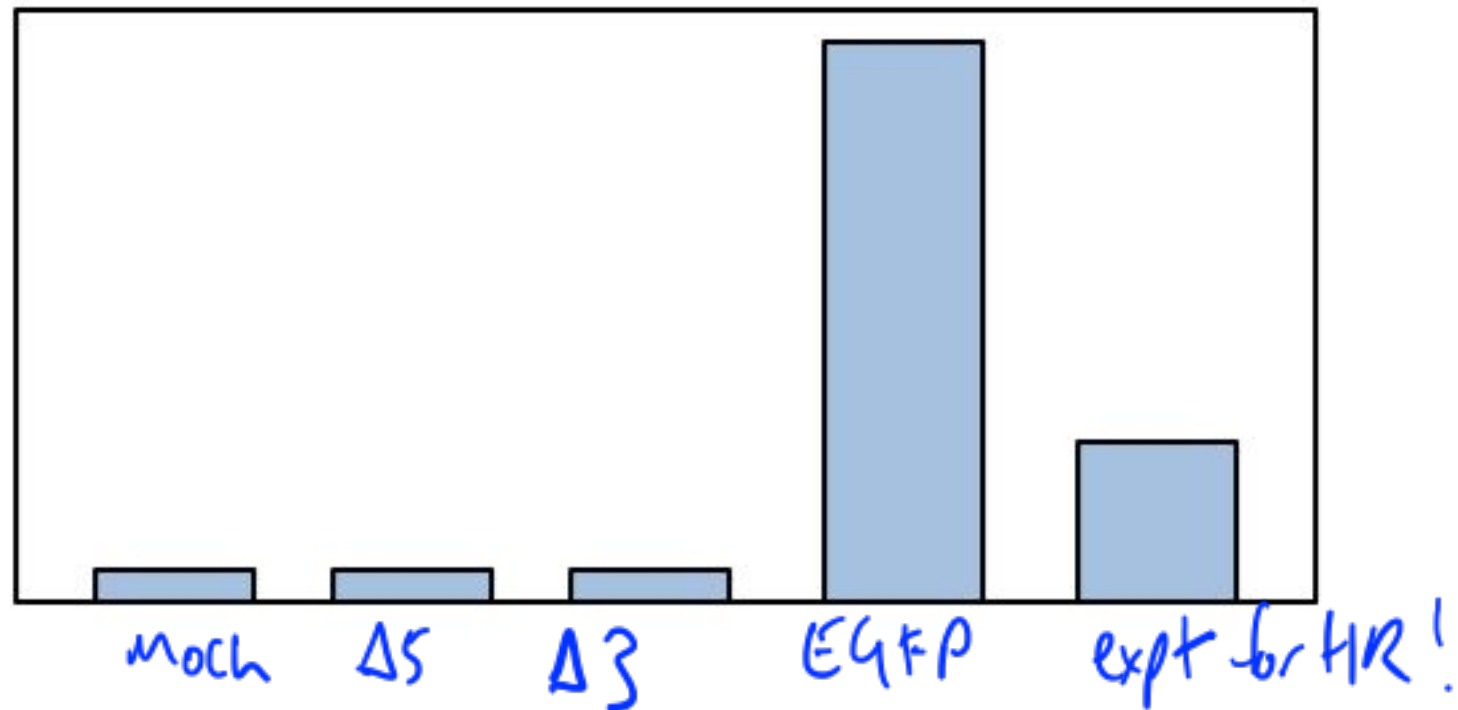
Lipofection samples



Controls for HR assay

- How do you know if your experiment worked?

☆ which stats comparisons to make?? ☆



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: M1D6

- Half of class in TC
 - Same four groups go first as last time
- Half in 56-711 to discuss paper w/Bevin
 - **Session 1:** 1:45-2:45
 - **Session 2:** 3:15-4:15
- Will announce methods pick-up when ready