Mod 2 Day 6: Analysis & Planning II

10/30/2013

1. **Pre-lab Discussion, Mid-Term Evaluations**
2. Western Blot: Secondary antibody addition
3. Imaging the Western Blot (Licor Odyssey)
4. Tissue Culture: High Throughput Viability Assay

Notes on Introductions & Reports

1. Motivating sentence required! Why should I read your paper?
2. What is the goal? Let this be your guide.
3. Logical progression.
4. Preview of the rest of the manuscript – hypothesis/methods/results/conclusions
5. Class-wide data

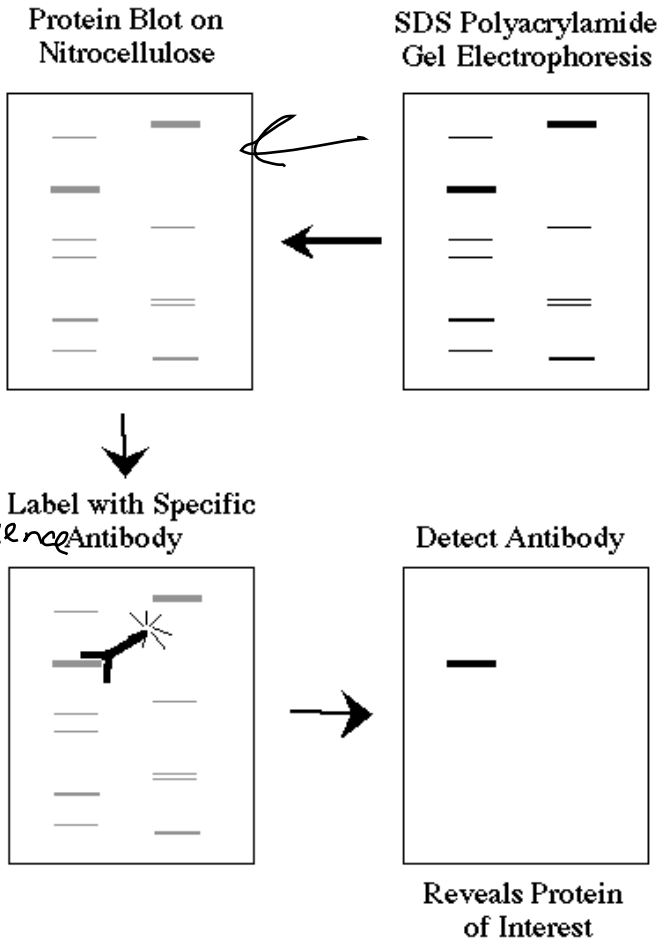
Throwback:
Mod 2 Day 4: Western Blot

4) Nitrocellulose transfer

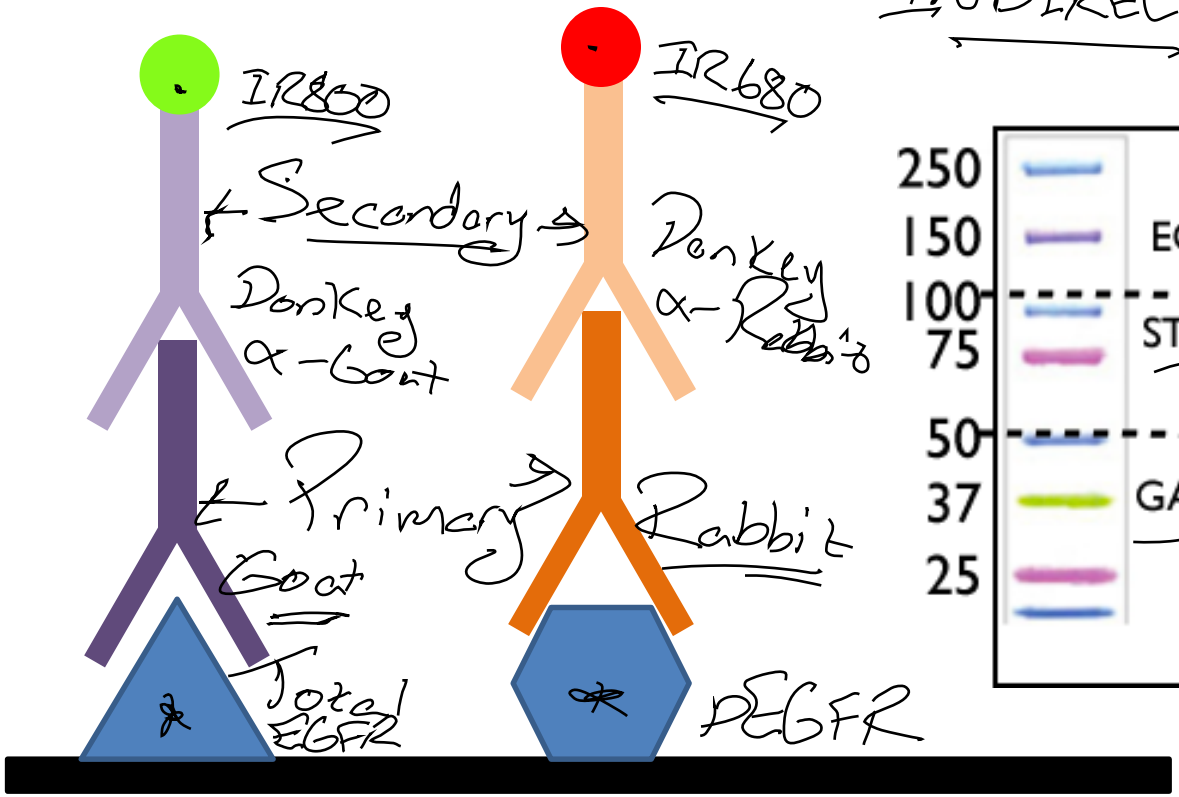
5) Probe w/ Ab's

Already done
- Blocking → low autofluorescence
- Primary Ab's

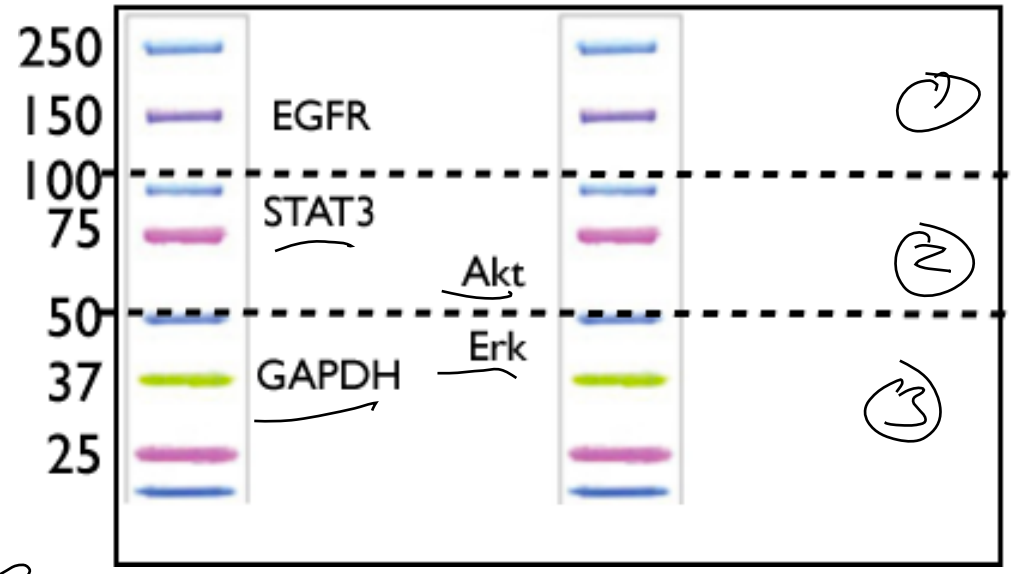
Today
- Secondary Ab
- Read



Western Blot – Antibodies



INDIRECT → multiple targets

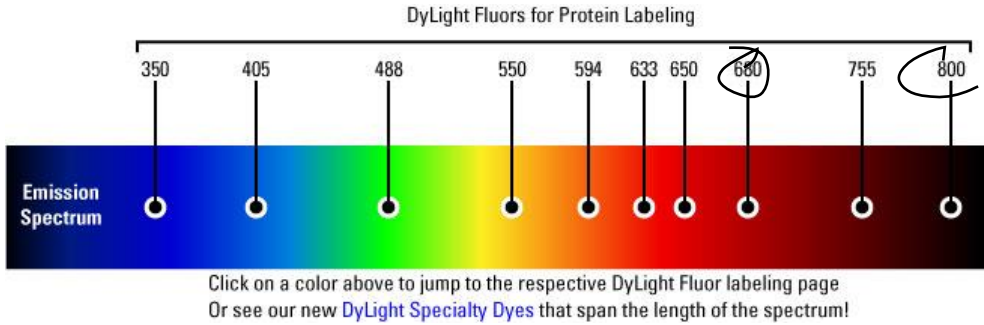


FAKE

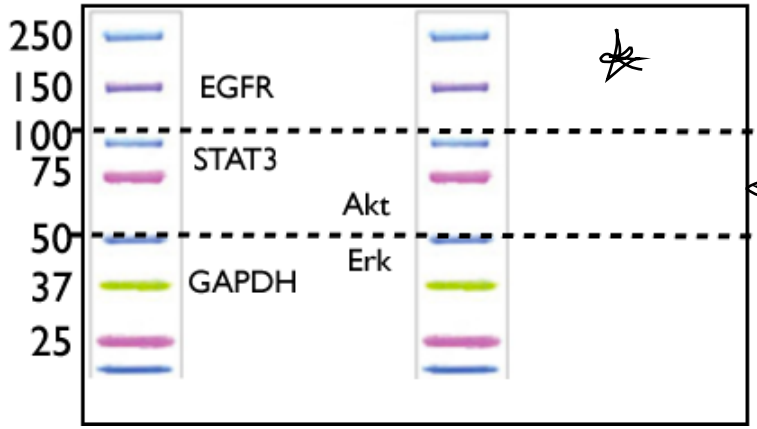
Host-specific antibodies:

Primary – goat anti-EGFR
rabbit anti-pY1068-EGFR

Secondary – donkey anti-goat IR800
donkey anti-rabbit IR680



Western Blot – Antibodies



Only one stripped!

Primary Ab	Species	Secondary Ab	Akt?	Erk?	STAT3?
EGFR	Goat	anti-Goat IR800	★	★	★
p-EGFR	Rabbit	anti-Rabbit IR680	★	★	★
GAPDH	Rabbit	anti-Rabbit IR800	★		★
p-Akt	Rabbit	anti-Rabbit IR800	★		
Total Akt	Mouse	anti-Mouse IR680	★		
p-Erk	Rabbit	anti-Rabbit IR800		★	
Total Erk	Mouse	anti-Mouse IR680		★	
p-STAT3	Rabbit	anti-Rabbit IR800			★
STAT3	Mouse	anti-Mouse IR680			★

Akt

Total → rabbit

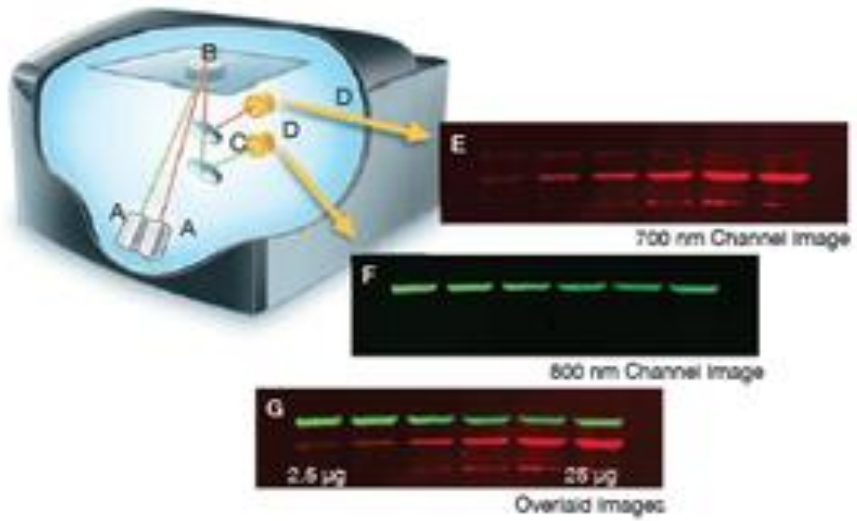
So Strip Ab's

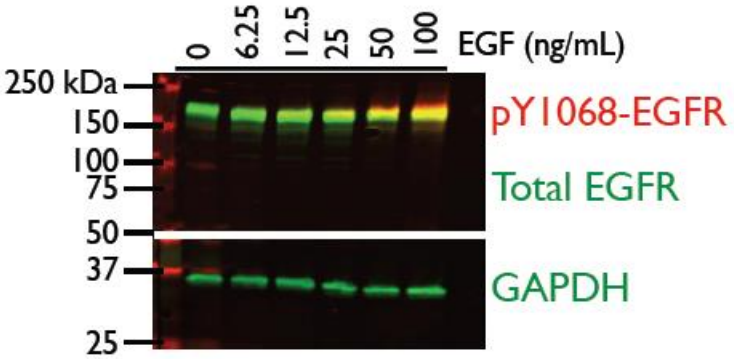
0.2m NaOH

THEN Total Akt

Read on Licor Odyssey

Inverted microscope/camera
Lasers to excite fluor.
Store for a long time



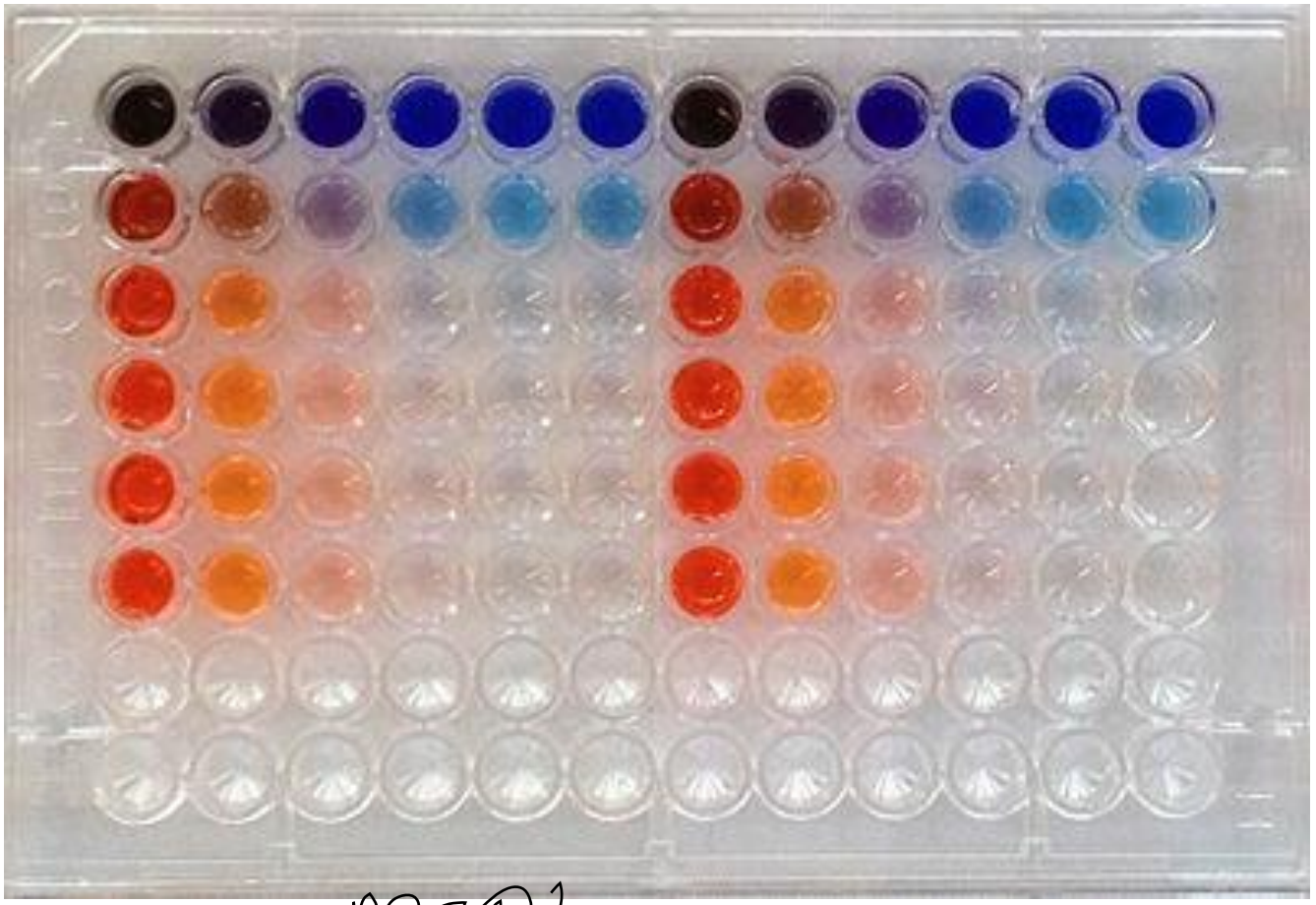


1. Remove primary
2. Wash 3x TBS-T (1 min)
   T = Tween 20
3. ~~B~~ Secondary Ab * (2nd wash 2 min)
4. Wash TBS-T 3x
5. PBS

High-Throughput Viability Assay

(your choice inhib.)
Inhibitor X → Directed → RED

Erlotinib → Blue



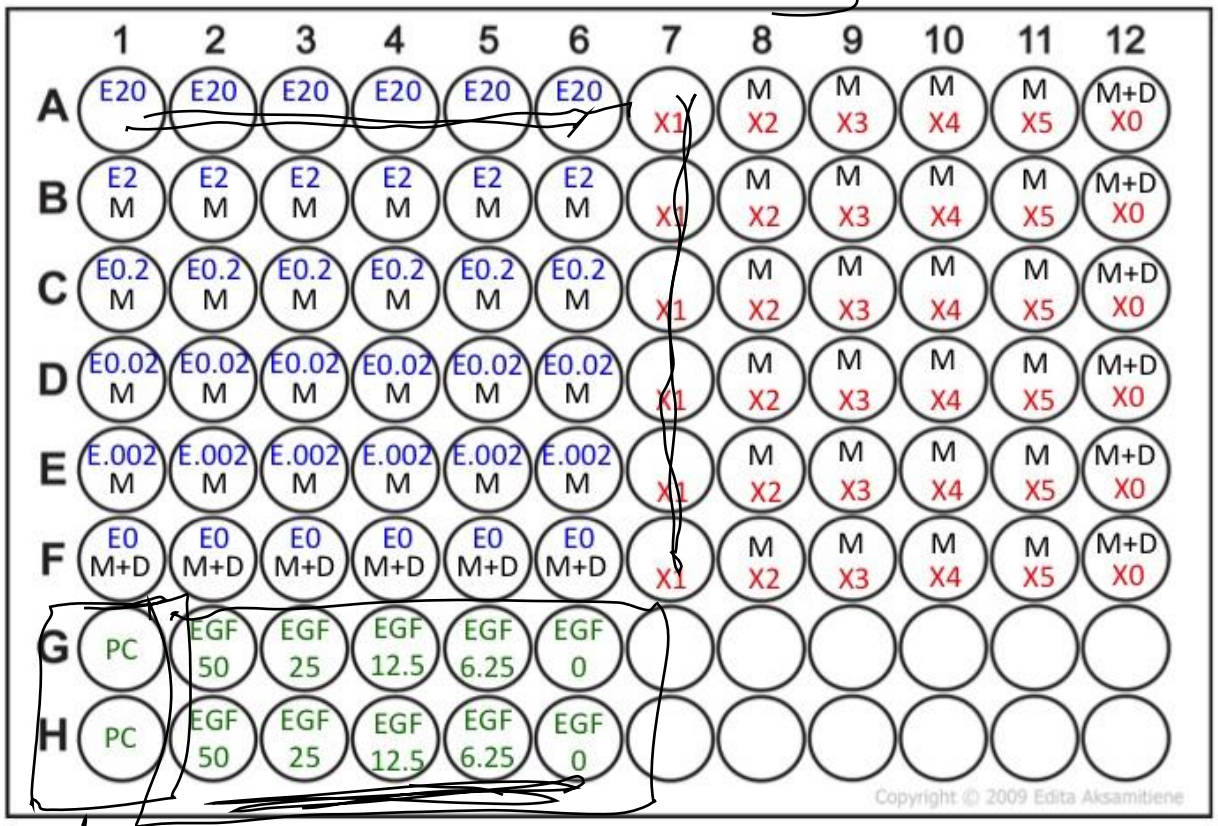
BUT, everything will be red this time (b/c media)

MZDI

Set-up Plate

M = McCoy's media
+ 1% Serum
+ 12.5 ng/mL EGF

Multichannel
For dilutions



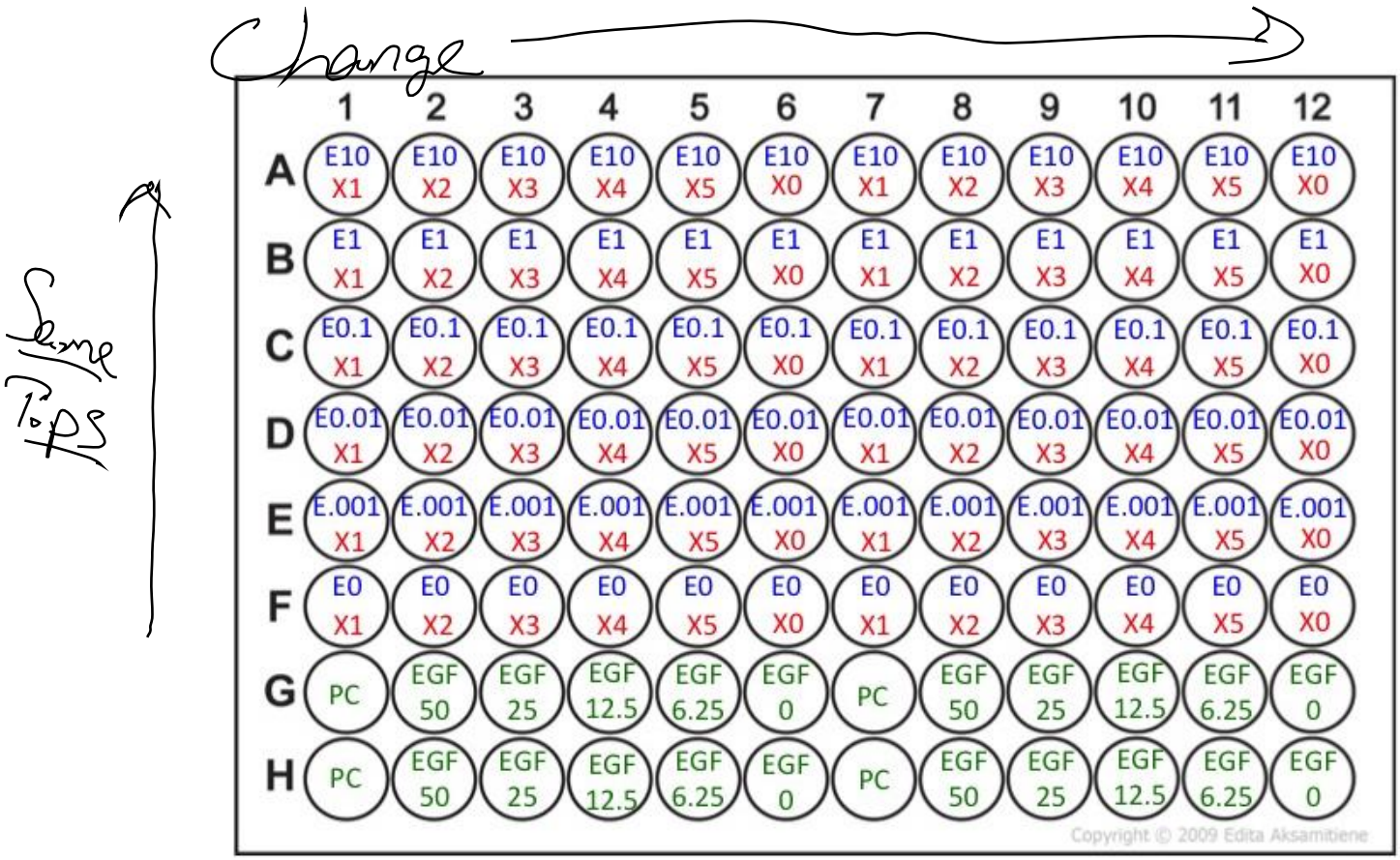
Pos Control
Kills cells!
Media + 1% DMSO

Reg cellular resp to how much we activate EGFR pathway

Final concentrations will be... HALF! 10nM Erlot

Regular
M+D → Media + 0.001% DMSO
E20 X1 → Erlotinib [X]↑
EGF dilutions

Experimental Plate



- **Cell seeding density: 31,250cells/cm^2, 16 hours on plate**

Multichannel pipette for everything here!

Hints

- Have everything ready before media is removed from cells

- Do not change aspirating pipette/wash with ethanol between wells. Do not touch cells.

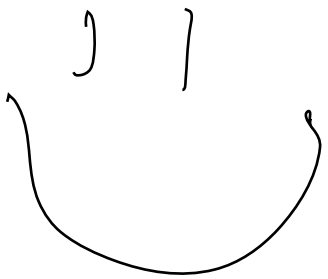
Leave a bit of media

- "Purposeful pace"!

Next time

- CellTiler-Glo
  - Add CTG reagent to lyse cells
  - Incubate for 20 minutes
  - Measure luminescence signal

Done!



Today!

- Tissue Culture:
  Orange, Yellow, Purple
- Western Blot:
  White, Pink, Green, Red, Blue

Mod 2 Day 7:

- CellTiler-Glo assay (here or in Koch)
- Analyze with Matlab/Excel on personal computer (can be done in lab or on your own time)
- **M2D6 FNT due Sunday, Nov 2nd at 5pm to Stellar!**