M2D3: Analysis & Planning I

10/17/13

1. Pre-lab discussion

2. Seed cells for M2D4 -- Tissue Culture

3. Sequencing analysis -- exon 19 & 21 EGFR

4. Paper discussion & figure presentation

Announcements

1. M2D5 JC presenters **must** sign up for a paper today
2. Check out the wiki for guidance on the Mod2 Report
3. Quiz next time -- but no pre-lab discussion
4. Look for a video from me over the weekend

Tissue culture medium

1959

McCoys 5A

What do cells need to survive?

- vitamins
- amino acids
- Sodium Bicarbonates
- HEPES

constant PH

phenol red

Food(s):

NEAA - Nonessential amino acids

- glycine, alanine
- Aspartic acid
- glutamine ← V. unstable

Non-food(s):

Sodium pyruvate

carbon source

for glycolysis

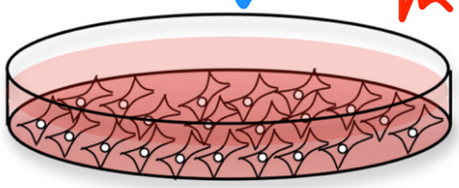
growth factors

Cytokines

Lipids

1xPBS
1.5mL
T/E

+8.5mL
media

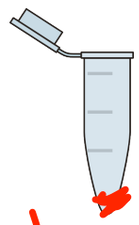


SKOV3

current
of
cells

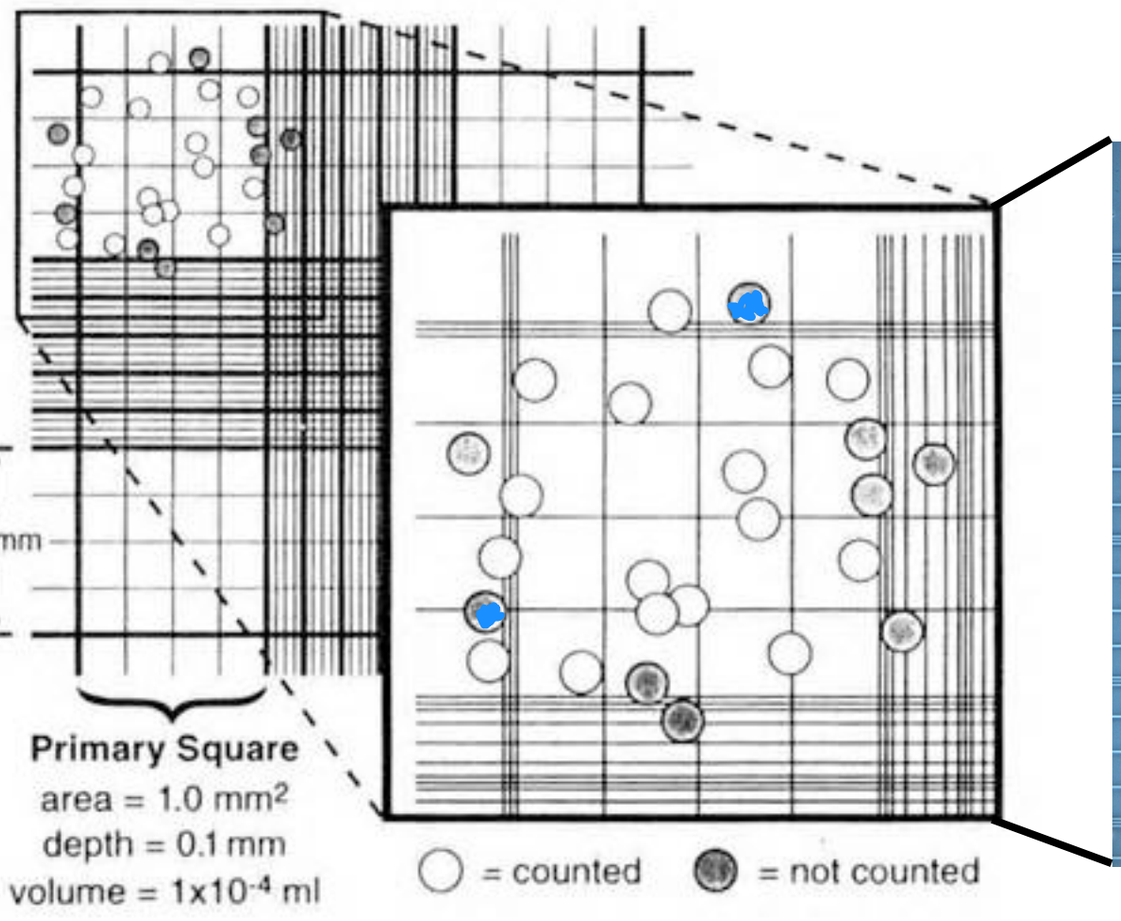
rp in
vol-spend

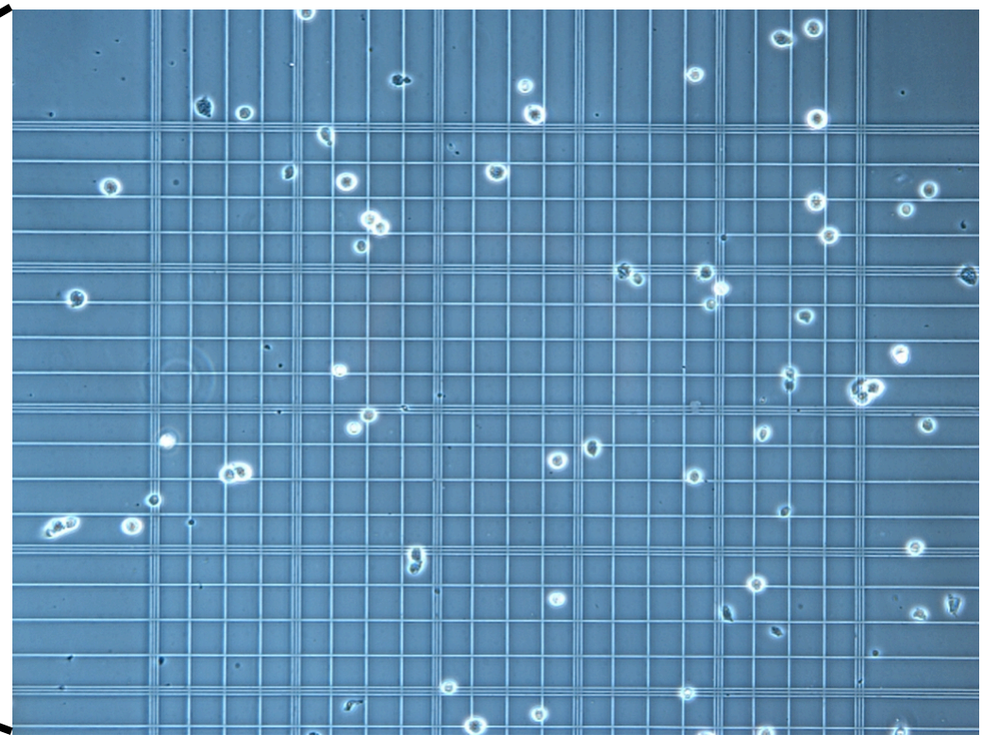
2mL



cells
mL

18μL
+2μL +Vam blue



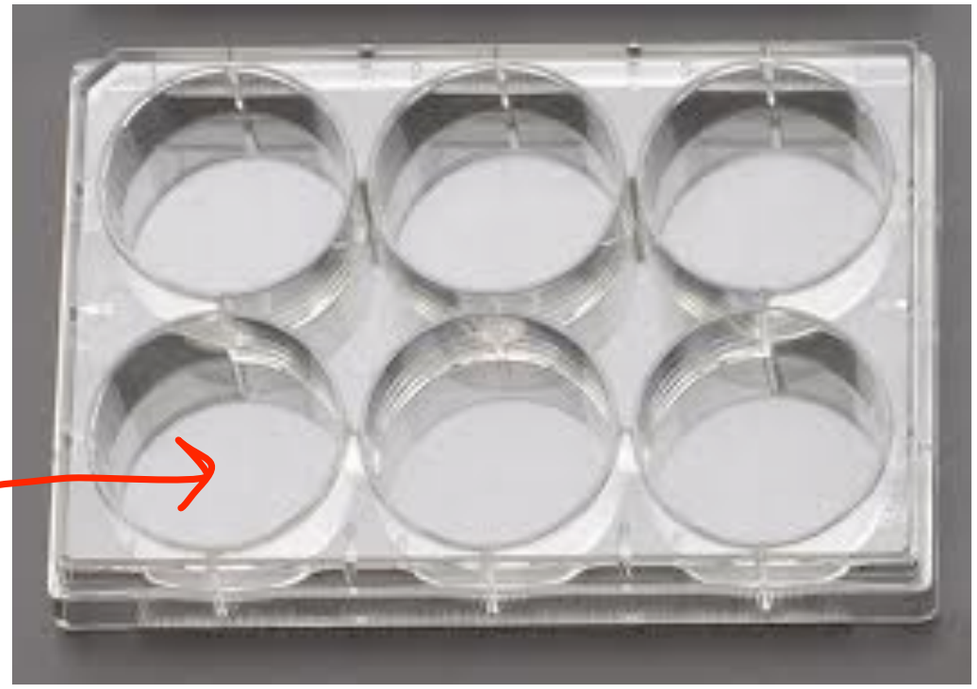


flexiblelearning.au.edu

Prepare 6-well plate for phosphotyrosine-analysis

"seeding density"

Want: 21,000 cells/cm^2
(9.5 cm^2 surface area)



Important:
(1) Well mixed cells
(2) Distribution within the wells
(3) > 4 hr serum starvation before experiment ✓

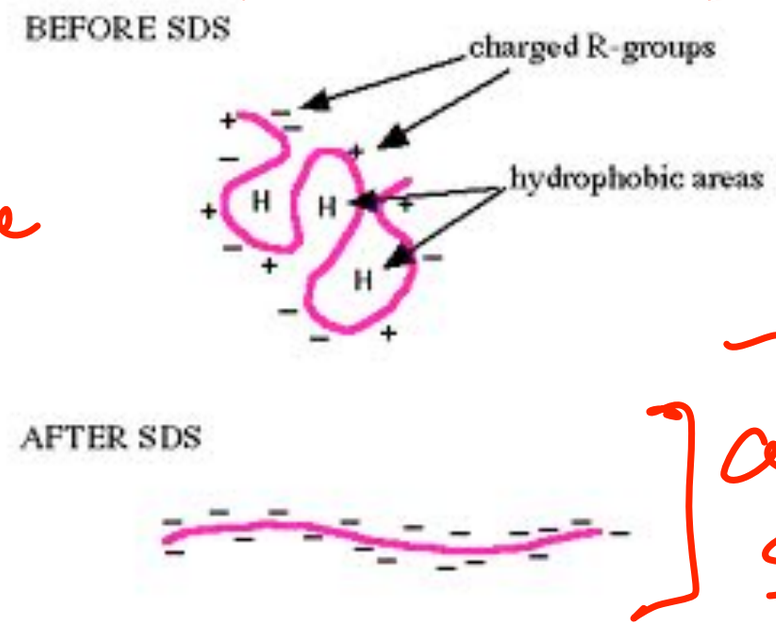
Next time: Western blot analysis

① "Lyse" ② protein assay - FNT

③ SDS-PAGE

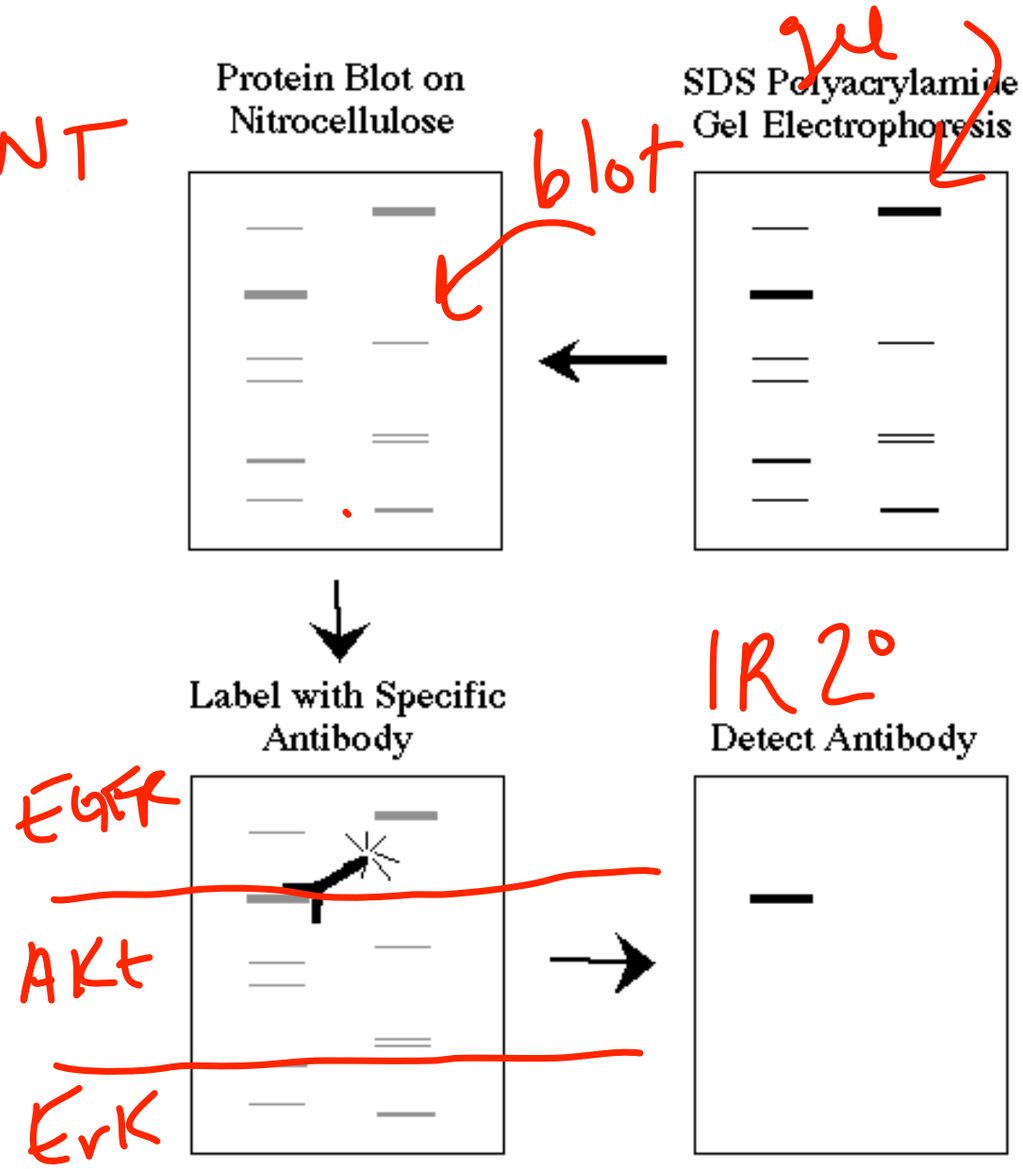
↓

Separate by size

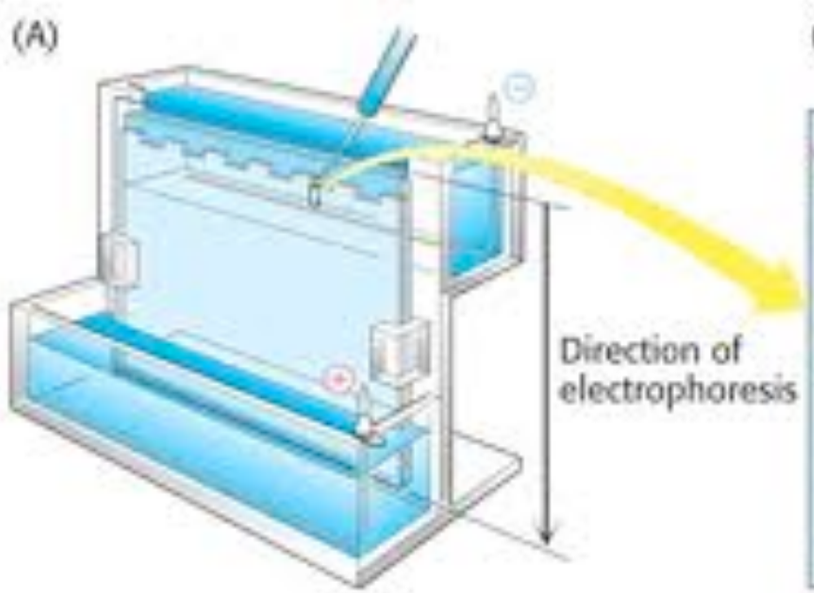


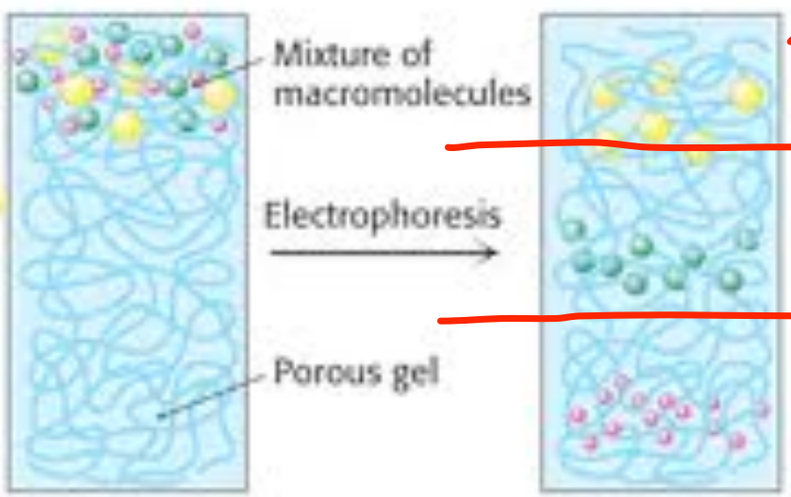
b4 SDS

after SDS



1R 2°





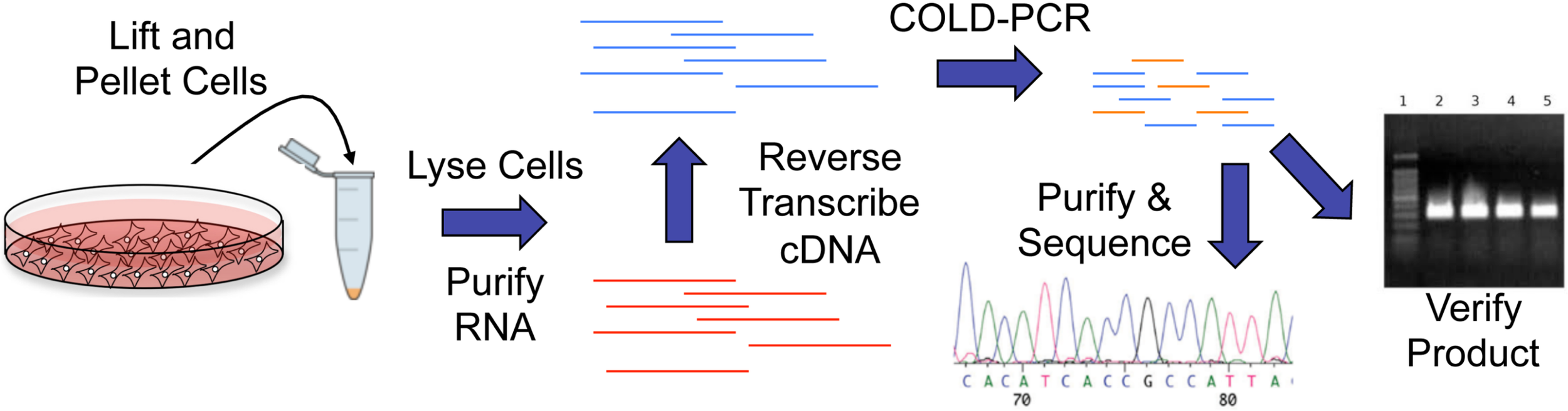
EGFR MW ~150KDa

Stat MW ~?

AKt MW ~64KDa

ERK MW ~44 KDa

M2D2: Mutation Analysis



★White 19F HCC

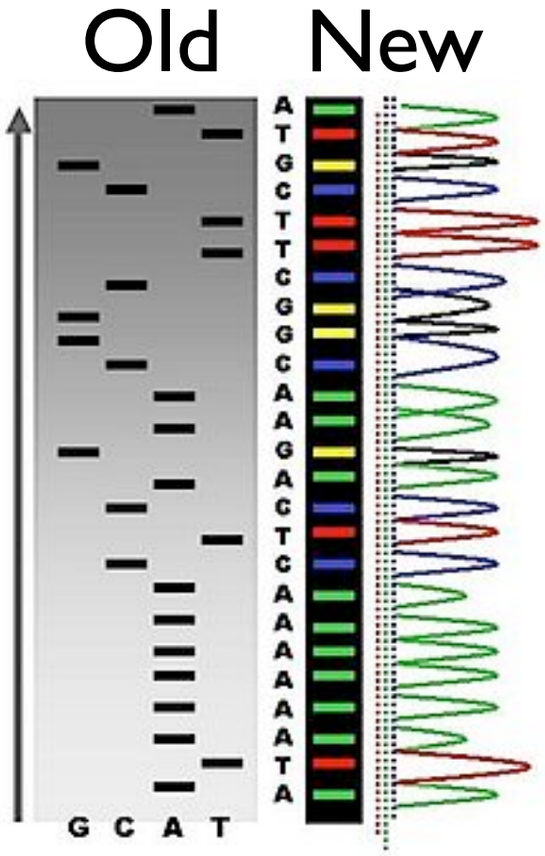
Positive Control: HCC-827 NSCLC +/− exon 19 del

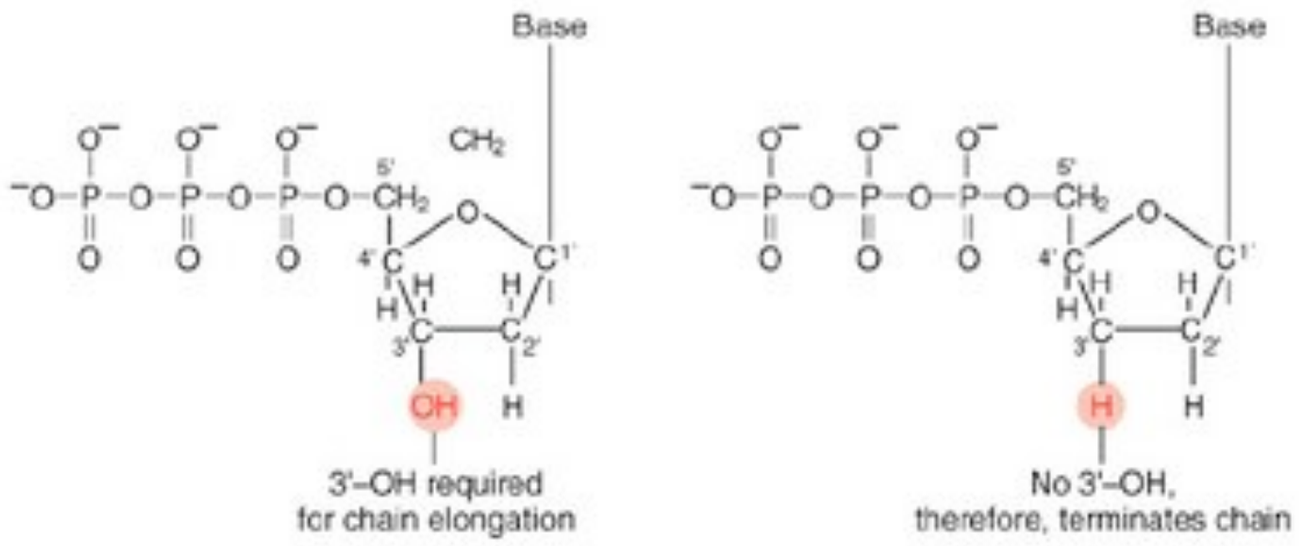
Negative Control: MDA-MB231 B.C.

Experiment: SKOV3 < exon 19 / exon 21

Overview: Sanger Sequencing

Four dye labeled dideoxynucleotides added instead of dNTPs.





'Chain terminating reaction'

Examples of PCR-Sequencing results:

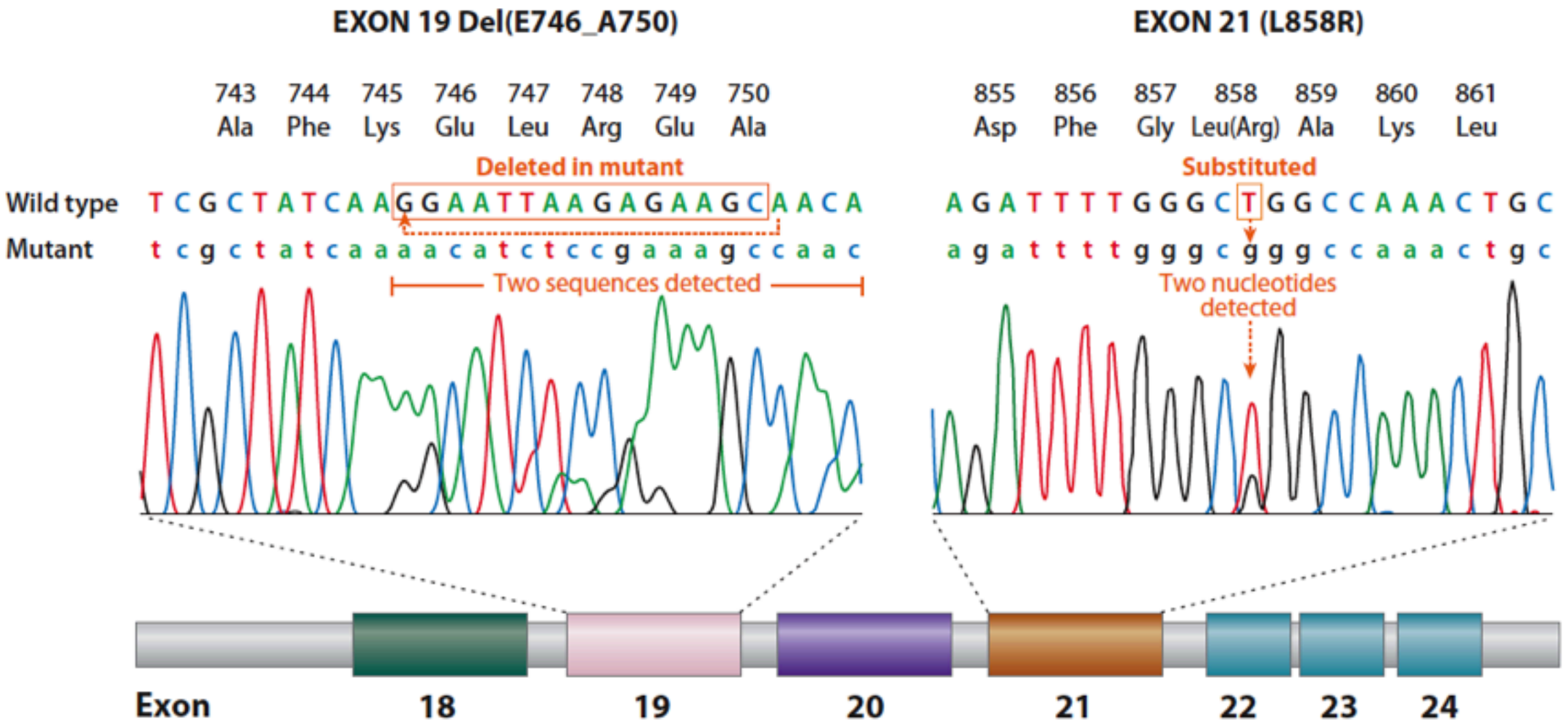
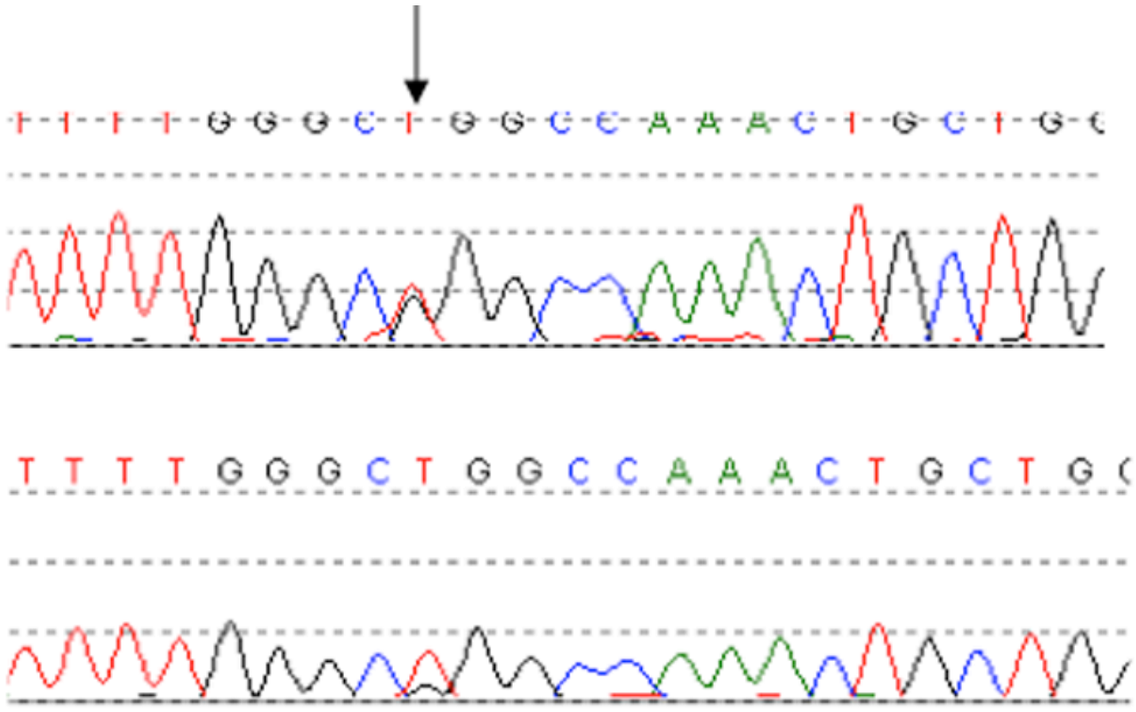


Figure 2

Amino acid and nucleotide sequence changes in exon 19 deletion and exon 21 L858R mutations involving the tyrosine kinase domain of epidermal growth factor receptor.

Santos et al. Annu. Rev. Pathol. Mech. Dis. 2011. 6:49–69

COLD-PCR



From Santis et al. (our protocol today)

COLD-PCR

EGFR L858R

Standard- PCR

. The reactions will undergo the following PCR cycle:

1. 95° 10 min
2. 94° 30 sec
3. 56° 30 sec
4. 72° 30 sec
5. repeat steps 2-4 10 times
6. 94° 20 sec
7. 71° 3.5 min
8. 87° 20 sec
9. 56° 30 sec
10. 72° 30 sec
11. repeat steps 5-9 40 times
12. 72° 5 min
13. 4° hold

Today in the lab:

1. Yellow, Blue, Platinum, White -- to TC
2. Red, Orange, Green, Pink, Purple -- sequencing
3. 3:30pm -- paper discussion / slide presentation

Next time in the lab:

- *Very busy day -- stimulate and lyse cells, measure protein concentration & run an SDS-PAGE gel*
- Today's FNT will help you significantly with time.
- Watch for a pre-lab video via email -- and then watch the pre-lab video! :-)