- Lab Quiz
- Announcements
- Pre-lab Lecture
  - Review, review, review!
  - Lipofection
  - Today in Lab (M2D5)

Announcements

- Welcome back!
  - second half of M2, the real fun begins
- Mod 1 revision due Friday at 1 PM, to Stellar
  - mark/note your changes! also read lateness policy
- Let's talk grades
  - letters → numbers
  - 109 philosophy

+ OH R 9-10am

+ Cell doubling calc. "fun" ≠ optional

Methods FNT common issues

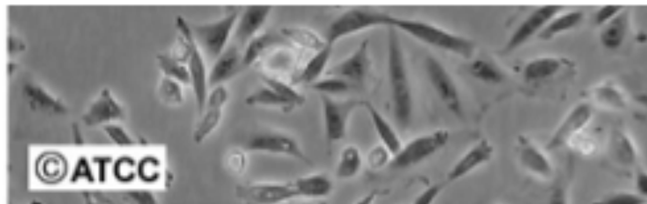
- Structure: sub-section groupings
  informative titles + topic sentences
- Sources and/or compositions: manufacturer – e.g. Laemlli, RIPA
  also composition – e.g., inhibitors (2 kinds)
  antibiotics
- Concentrations! usually final. NOT "1% of 100x stock" → final
  don't mislead. NOT " 100x (10mg/mL)"
  YES " 10mg/mL"
- Revision exercise from Shannon: "After removing from incubator, 3 mL of PBS was added to the cells. After removal, trypsin and EDTA was addded for 5 min at 37 C to dislodge cells."

After rinsing with PBS, cells were detached with [1mL] X % trypsin/Y mM EDTA and plated at xxx cells/cm².

Where were we?

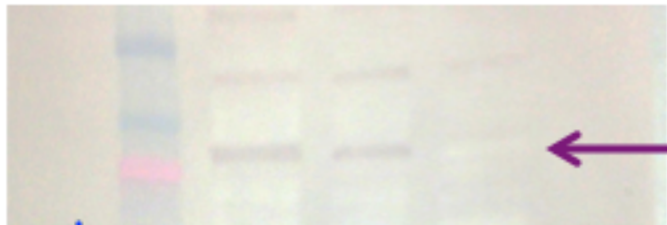
DAY 1

Plate K1 and xrs6



DAY 2 + 4

Measure Ku80 levels



why? validate cell lines

DAY 3

Reverse engineer plasmid construct

DAY 4

Prepare and assess damaged DNA

how?

observe digested products

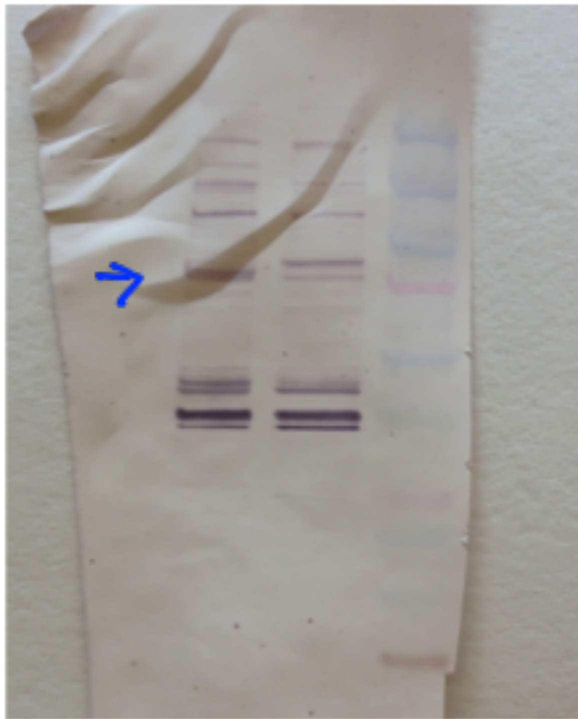
mcs1 junk 600 bp mcs2

C

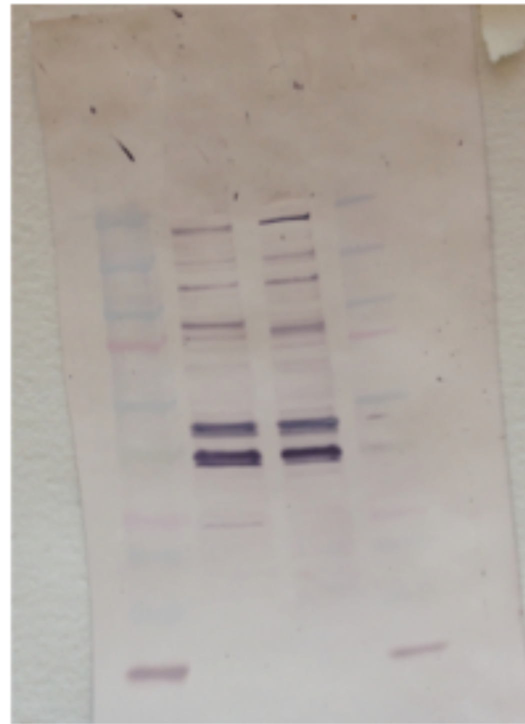
Sample Western results

reality – most looked like this

"ideal"



WF Pink



WF White

TR Blue

still see A

What validation remains?

validate inhibitor

DAY 6

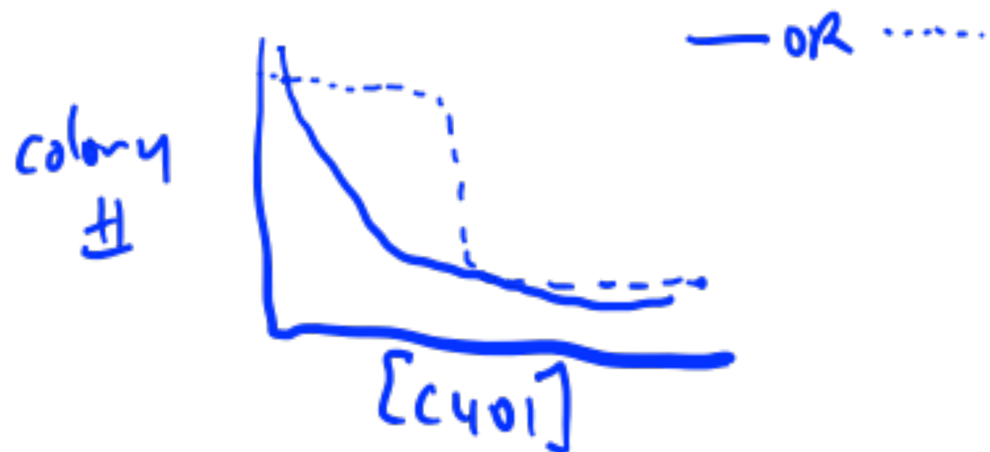
Plate irradiated K1 with varying [C401]

DAY 7

Stain for colonies

1) C401 advance treatment
2) irradiation
3) low [cell] plating
4) colonies! grow over 5d

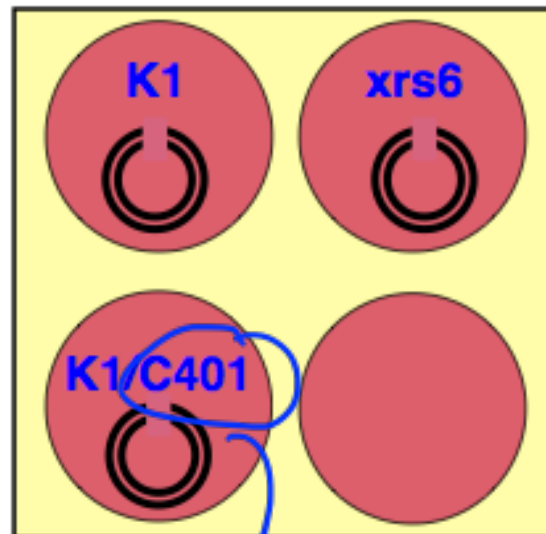
endresult:



The core/investigative experiment

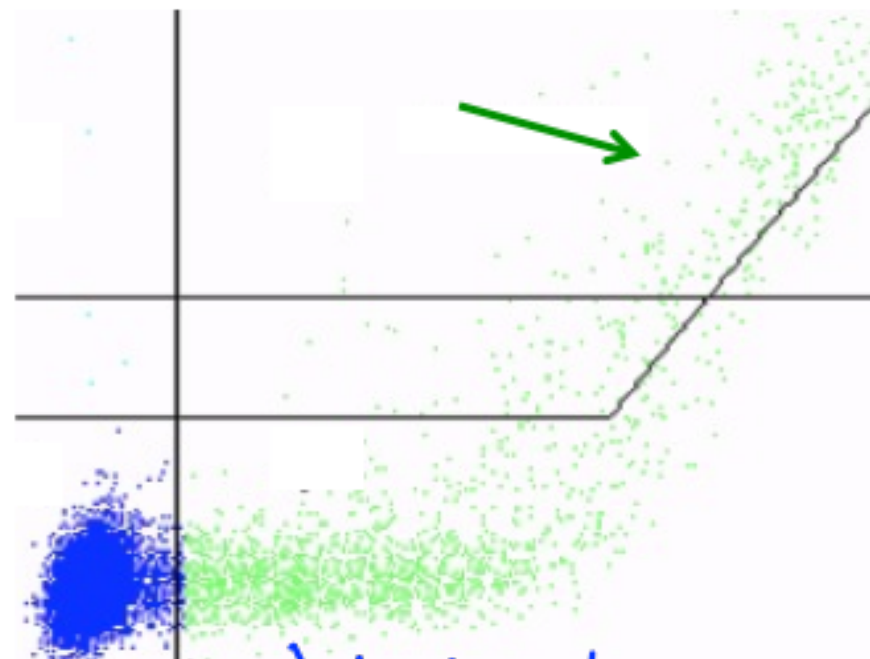
DAY 5

Transfect cells with damaged DNA



DAY 6

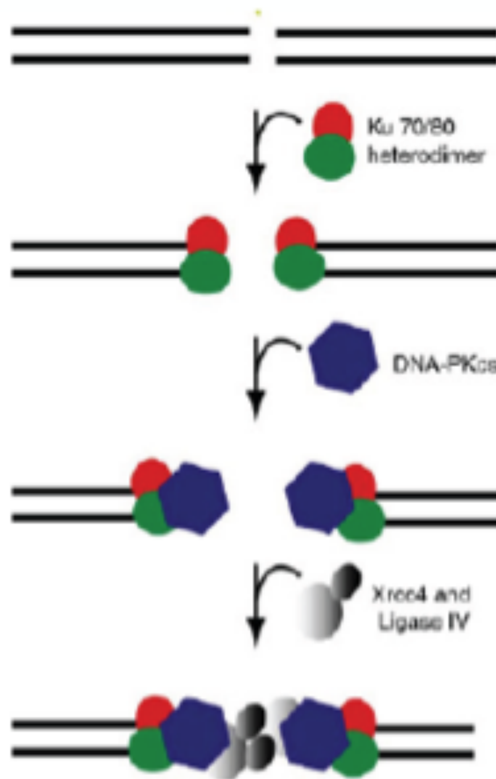
Measure repair via fluorescence of plasmid reporters



in advance: cell entry, DNA-PK(cs) binding, etc.

Canonical NHEJ Pathway:

How many experiments are we performing?



Ku70
Ku80 X xrs6 vs. WT

DNA-PKcs
C401-K1 vs. K1

Xrcc4
Ligase IV
(C401-K1 vs. xrs6)

Slide from Shannon H

Canonical NHEJ Pathway:

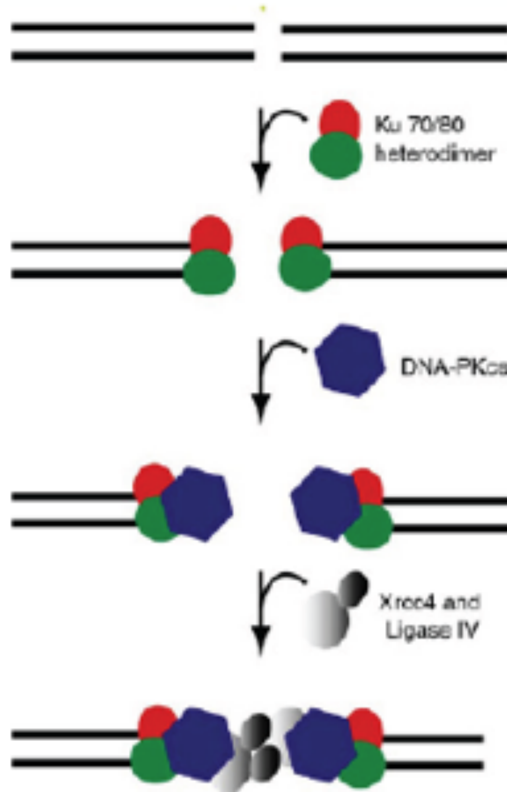
How many questions can we ask with our data?

starting point

A) pMax-BFP + pMax-GFP] • max "repair" (intact)

B) pMax-cut MCS + pMax GFP • ratio of A/B expression

NHEJ readout — transfection control



Ku70

Ku80

DNA-PKcs

Xrcc4

Ligase IV

specific Q

1) compare topologies for NHEJ repair efficiency in WT cells

2) does xrs6 or C401 shift things?

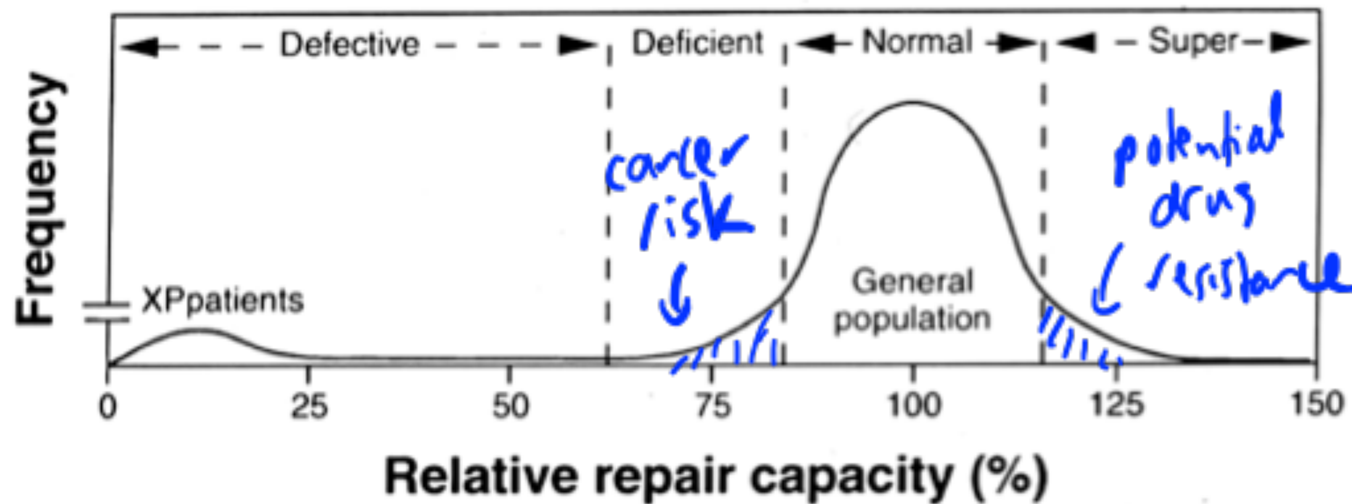
broader Q/impact

what pathways are important for different topologies? (competition, etc.)

Slide from Shannon H

Putting our exp't back in context – L. Samson

Interindividual Variation in DNA Repair Capacity

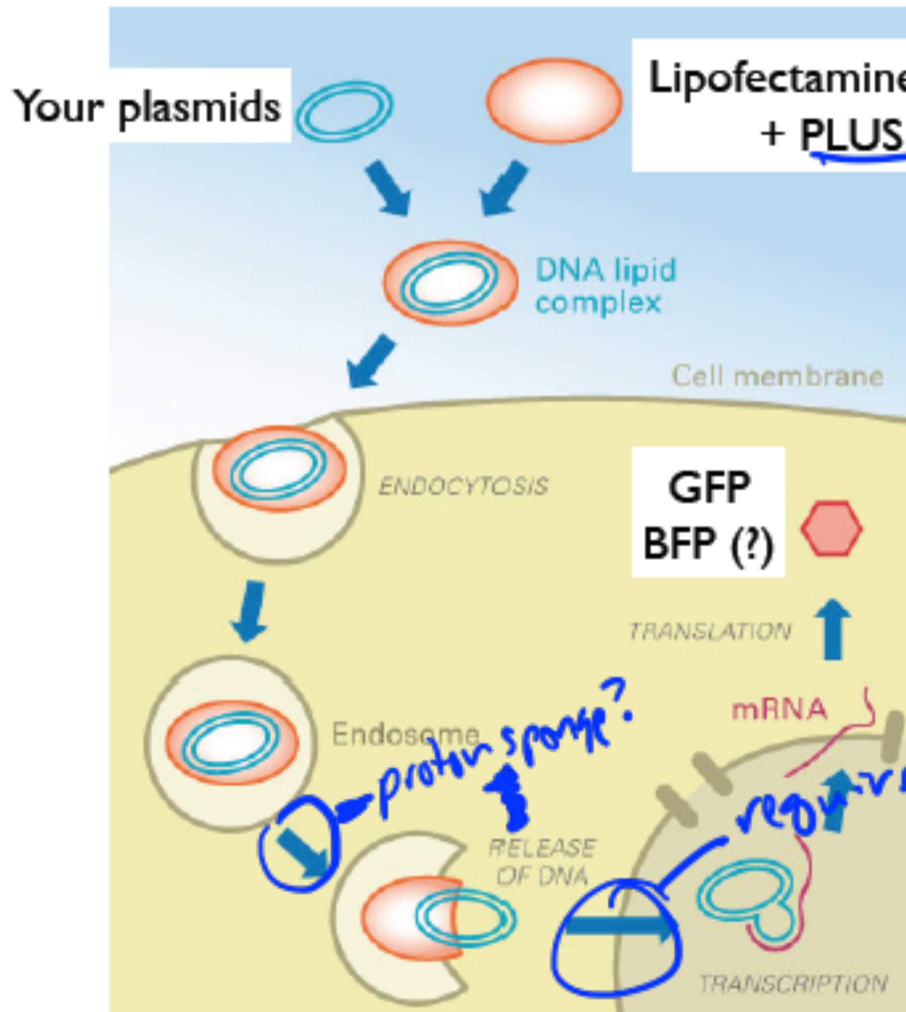


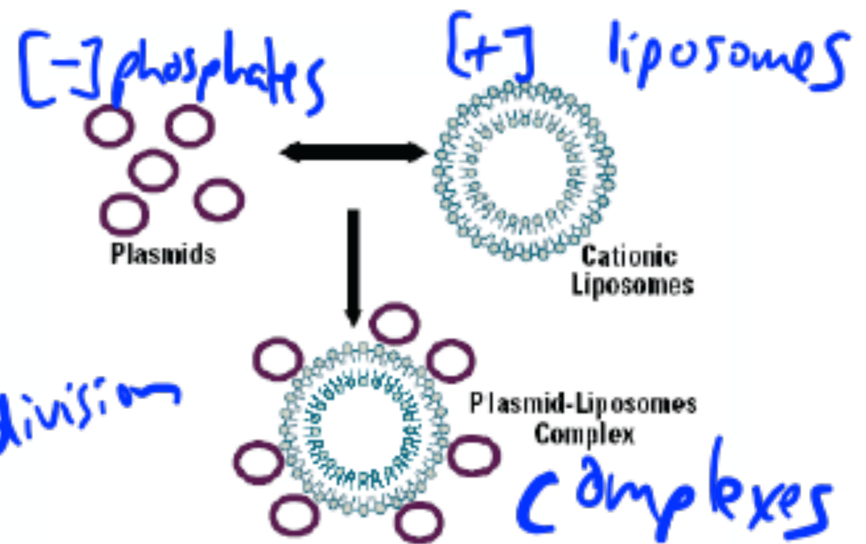
Adapted from **GROSSMAN and Wei (1995)** Clinical Chem 41: 1854-1863

- DNA repair is variable – in both healthy and tumor cells
- Quantifying DNA repair is interesting important
  - ★ Big picture motivation for intro ★

Lipofection background

* high efficiency (cf electroporation)
* can be toxic to cells





http://www.azonano.com/article.aspx?ArticleID=1233

Slide after Shannon H

Today in Lab: M2D5