M2D5: Cell prep for DNA repair assays

Announcements

- Module 1 Data Summary re-write due on **Friday (4/4) at 1pm** to Stellar.
- Make sure to read the section on the wiki about revisions (comments and late policy)

- Extra office hour: Wednesday up front

Methods sections: common mistakes

1) Sub-section titles

2) Topic sentences → *must have one for each sub-section

3) Define CHO + define source → ATCC

4) Manufacturer location → (Biorad, Hercules, CA)

5) Flexible units! cell seeding density ⇒ cells/cm^2

6) Final concentration *most of the time*

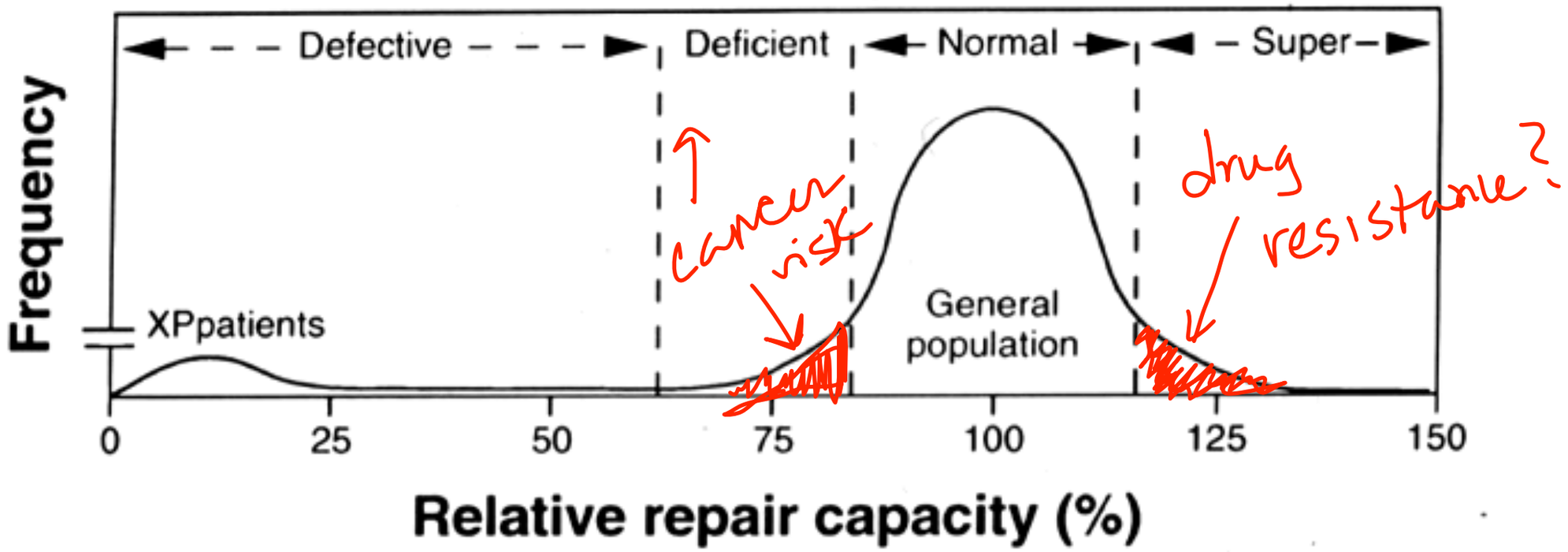
After removing from incubator, 3 mL of PBS was added to the cells. After removal, trypsin and EDTA was added for 5 min at 37C to dislodge cells.

After rinsing with PBS, cells were detached w/ X% trypsin/ XmM EDTA and plated at XX,XXX cells/cm^2.

8) "blotted to nitrocellulose and transfered at 100V for 60min"

9) *optional to cite the 109 wiki*

Why do we care about DNA repair capacity?



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

* DNA repair is variable
* Quantifying DNA repair is important
* Hint: Motivation

Slide from M2D1 Lecture — Prof. Samson

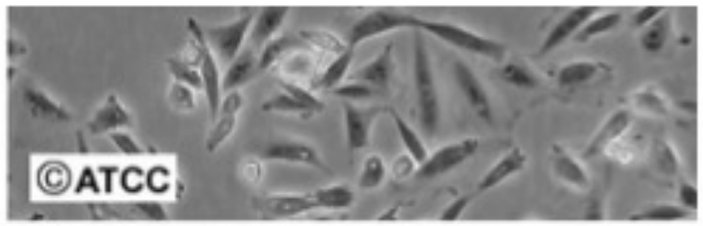
Remember *way* back…

M2 OVERVIEW: VALIDATE SYSTEM

CELLS

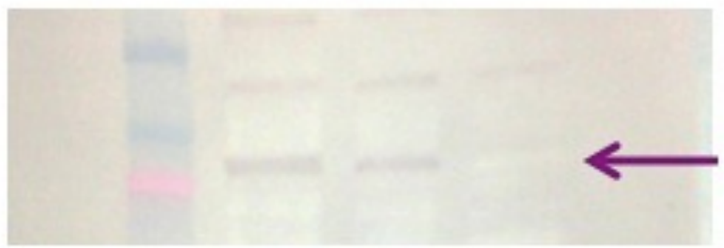
DAY 1

Plate K1 and xrs6



DAY 2 + 3

Measure Ku80 levels

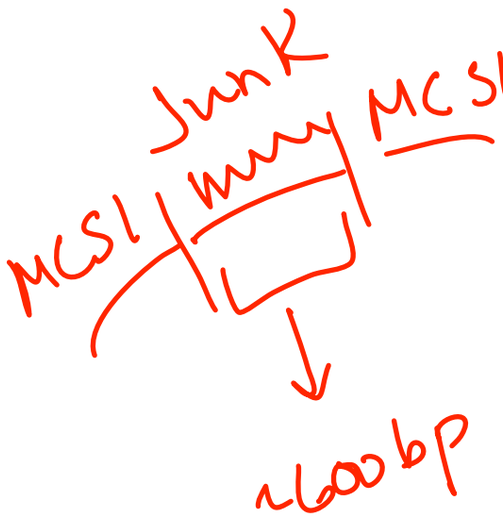


Why? Validate our cell lines

DNA

DAY 3

Reverse engineer plasmid construct

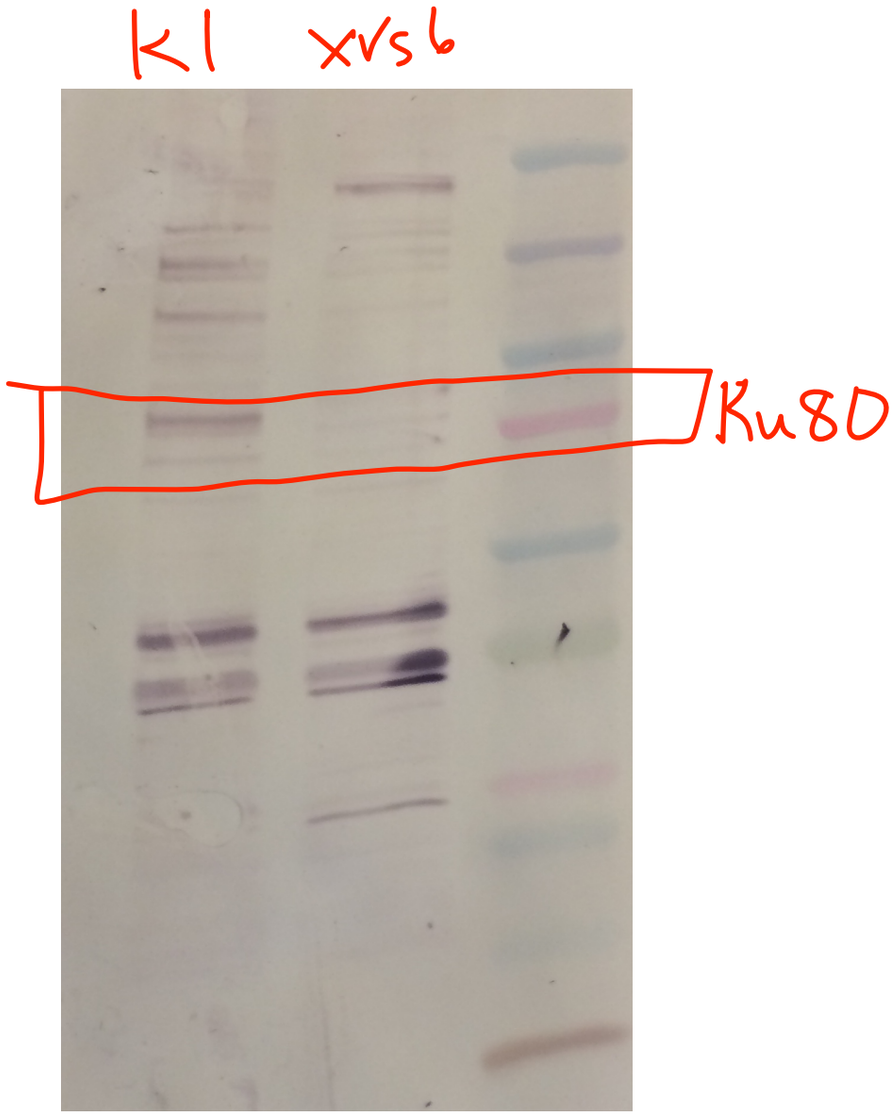


DAY 4

Prepare and assess damaged DNA

How? Evaluated digest efficiency

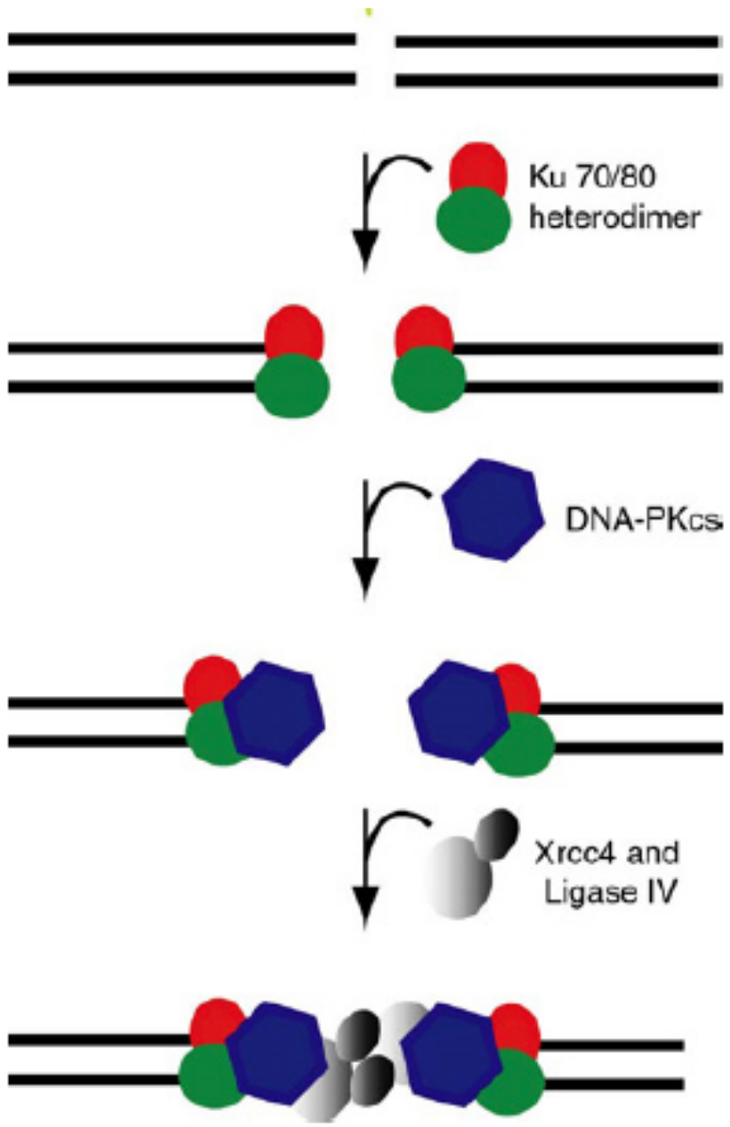
Western blot analysis



Blue Team

Canonical NHEJ Pathway:

How many experiments are we performing?



CHOK1 vs XRS6 cells

Ku70

XKu80

DNA-PKcs

CHOK1 vs CHOK1-C401

C401

XRS6 vs CHOK1-C401

Xrcc4

Ligase IV

*Added last night ~5pm

C401

- get into the cell
- bind to DNA-PKcs

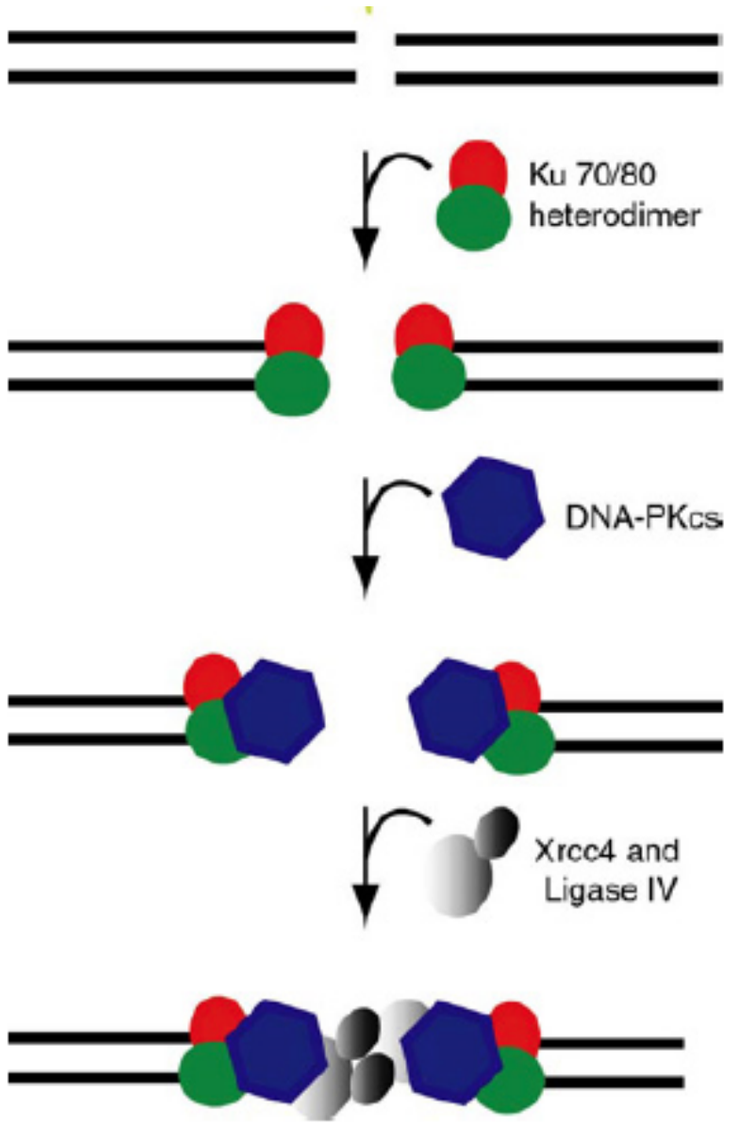
] why early?

Canonical NHEJ Pathway:

1) KI

2) Xrs6

3) KI + C401



Ku70

Ku80

DNA-PKcs

Xrcc4

Ligase IV

How many questions can we ask with our data?

A) pMAX-BFP + pMAX GFP MAX repa

B) pMAX-BFP-MCS + pMAX-GFP

NHEJ detector

transfection control

1) compare topologies

2) +/- Ku80 effect repair of these +/- C401 cuts

(What pathway is most important for a specific cut topology?)

How will we know that the inhibitor works?

** This is REALLY important *

~low cell density

↓

expose to radiation

+ ↑C401

'dose-response'

↓ 5 days

Colony formation assay

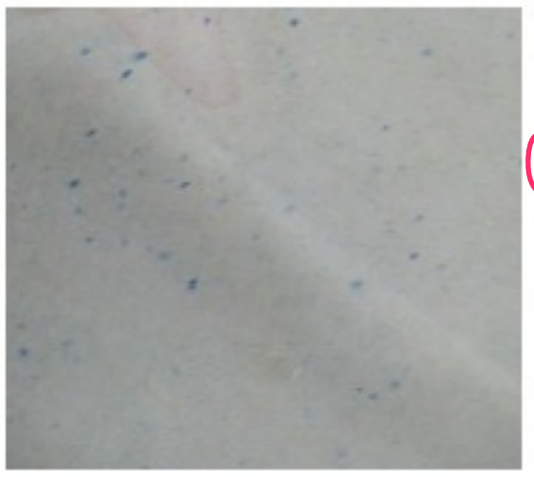
INHIBITOR

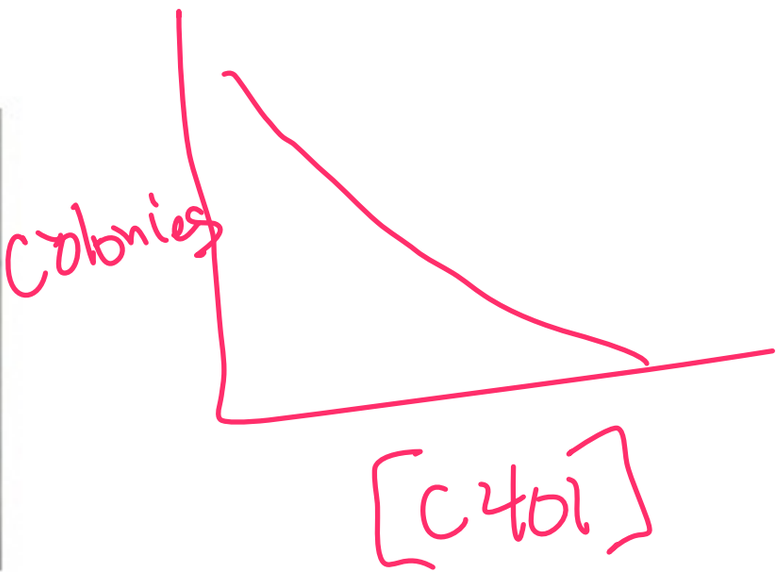
Day 6

Plate irradiated K1 with varying [C401]

DAY 7

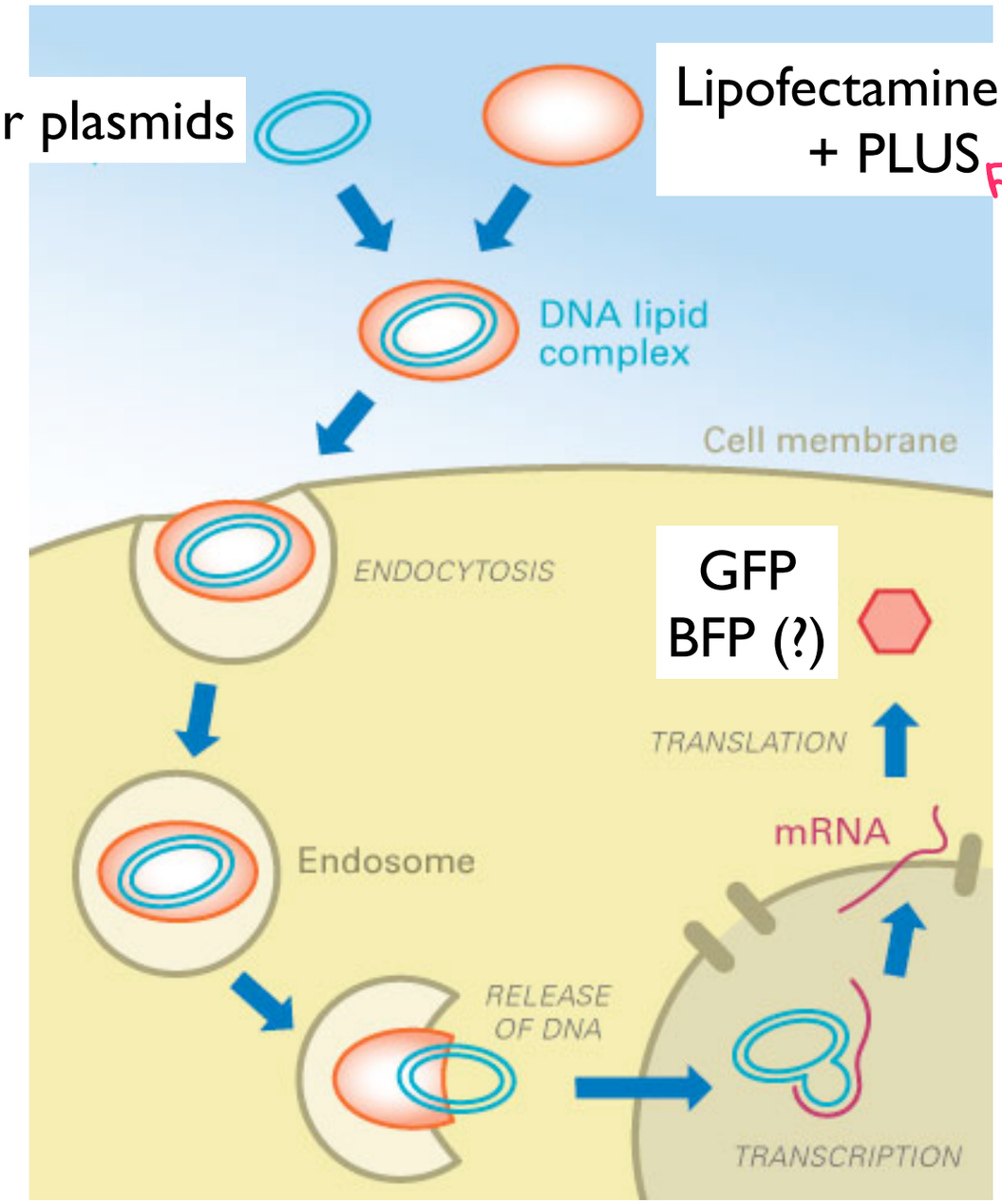
Stain for colonies



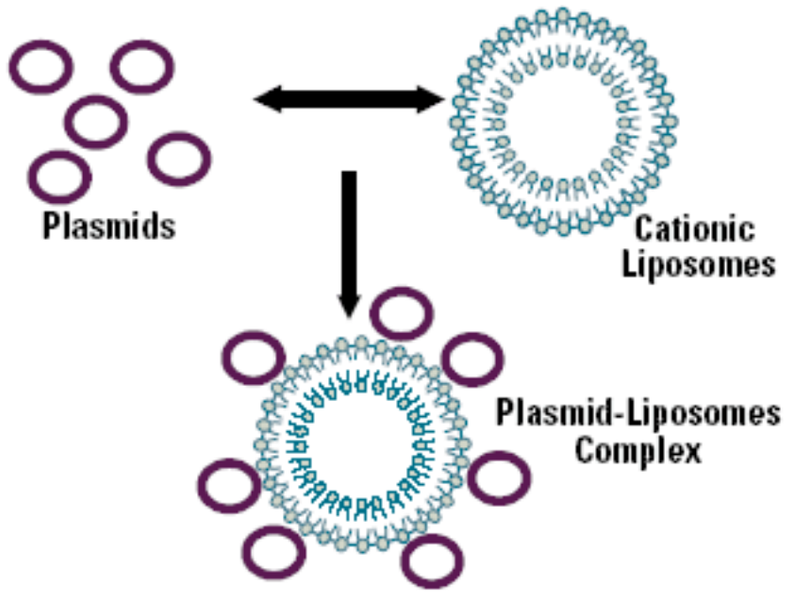


Mammalian Cell Transfection:

cationic liposome → + charged lipid



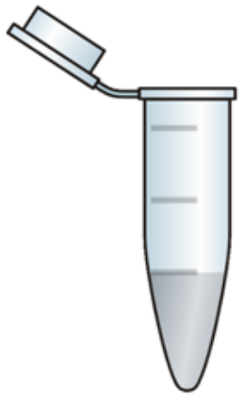
Guess: bundle DNA; compact



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

Today in lab:

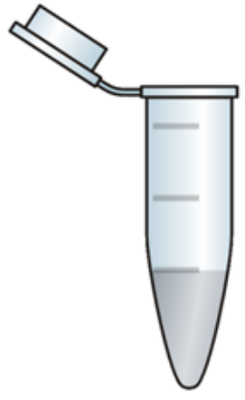
1. LTX solution



25µL + 10% per well
x 12 wells

- LTX
- OM

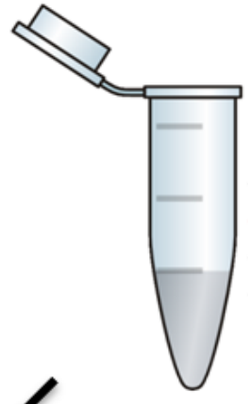
2. Prep DNA solutions



25µL + 10% per well
x 6 wells

- Intact GFP
- **Intact BFP**
- PLUS reagent
- OM

A
[Control]

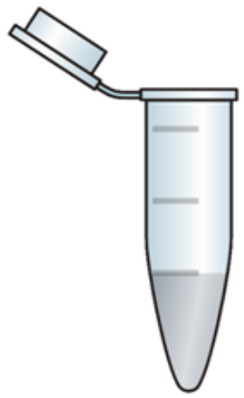


25uL + 10% per well
x 6 wells

- Intact GFP
- **Own + TA damaged BFP**
- PLUS reagent
- OM

B
[Experimental]

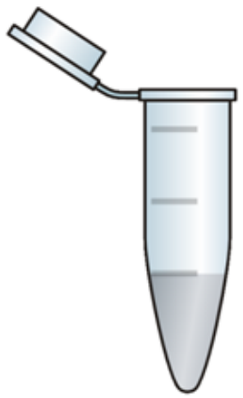
3. Distribute LTX **then** add DNA solution



25µL + 5% LTX
+
25uL + 5% A

per well
x 6 wells

LTX + A



25µL + 5% LTX
+
25uL + 5% B

per well
x 6 wells

LTX + B

20 minute incubation

4. Add 50uL **LTX + DNA** to each well

LTX + A → (50 uL)

LTX + B → (50 uL)

Today in lab:

★Do your transfection calculations FIRST — four groups max in TC at one time.

★Once you check off your calculations with me or Su, you can head into TC.

★While you wait — work on your Mod1 revision, ask questions about your Methods FNT, etc.