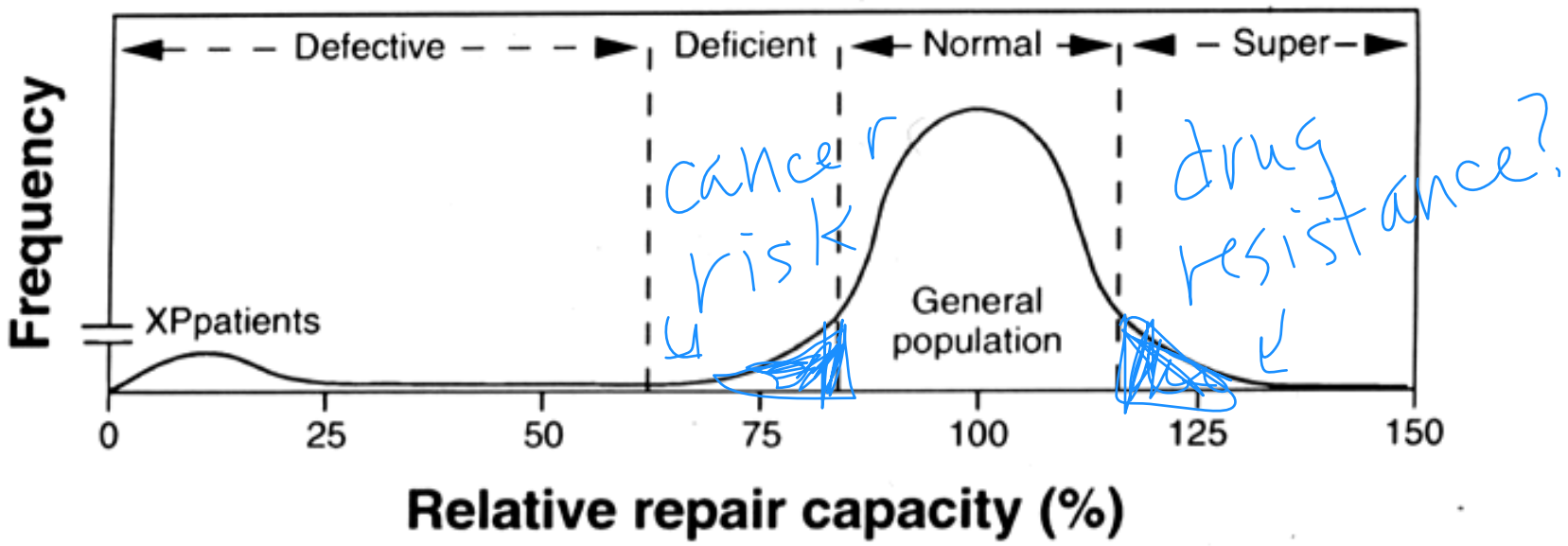
4/1/15

M2D4: Cell prep for DNA repair assays

Announcements

- Module 1 Data Summary re-write due on **Saturday (4/4) at 5pm** to Stellar.
- Make sure to read the section on the wiki about revisions (comments and late policy)

Why do we care about DNA repair capacity?



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

- DNA repair is variable
- Quahifying DNA repair is impt.

Hint: Motivation for repair

Slide from M2D1 Lecture — Prof. Samson

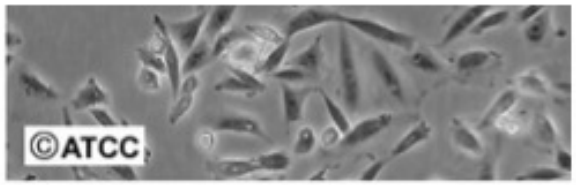
Remember *way* back…

M2 OVERVIEW: VALIDATE SYSTEM

CELLS

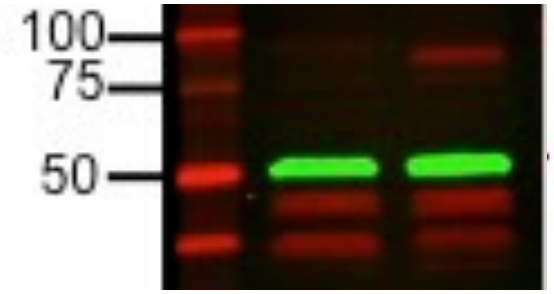
DAY 1

Plate K1 and xrs6



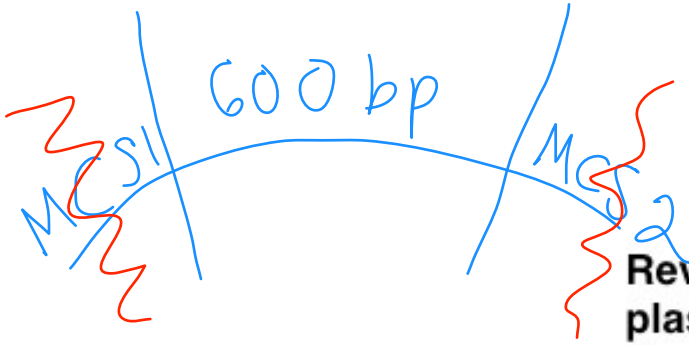
DAY 2 + 3

Measure Ku80 levels



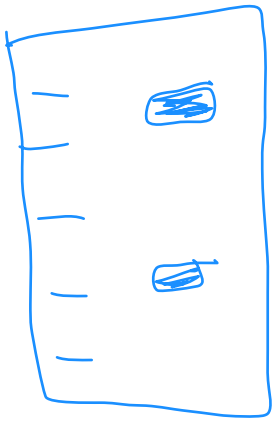
Why? Validate our cell lines

DNA



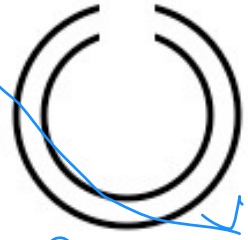
Day 2

Reverse engineer plasmid construct



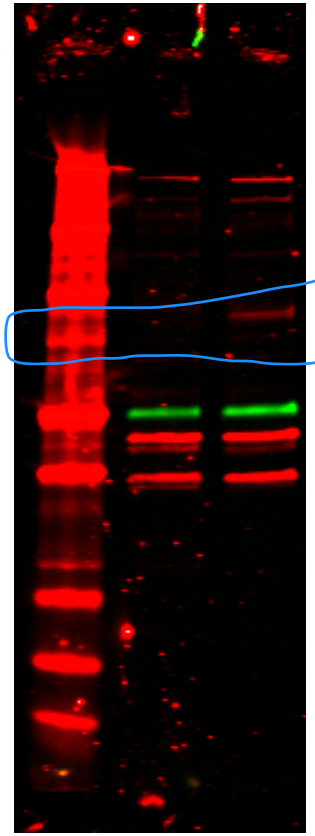
Day 3

Prepare and assess damaged DNA



How? Cut RE agarose gel

Western blot analysis

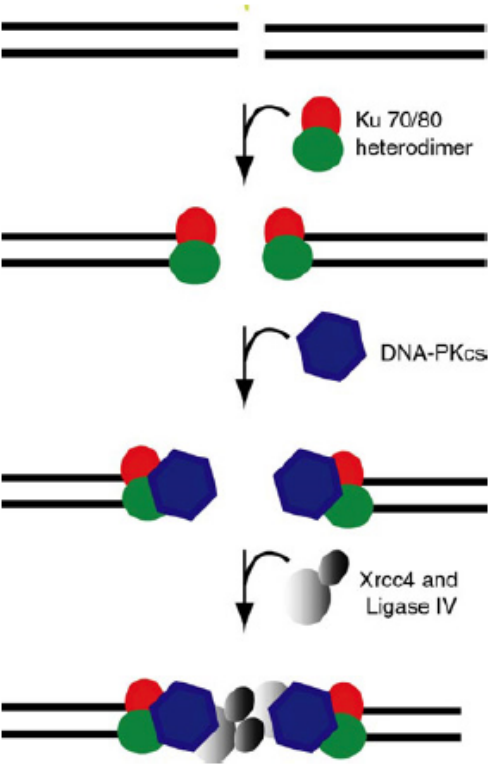


Ku80 (80 kDa)

← alpha tubulin
50 kDa

↑ ladder saturated

Canonical NHEJ Pathway:



Ku70
Ku80
DNA-PKcs
Xrcc4
Ligase IV

How many experiments are we preforming?

Expt conditions:

cell (KI) (KI inhib) (xrs6)

transf.

A) pMaxBFP + pMaxGFP

maximum repair

B) pMaxBFP NCS + pMaxGFP

NHEJ repair sensor

transf control

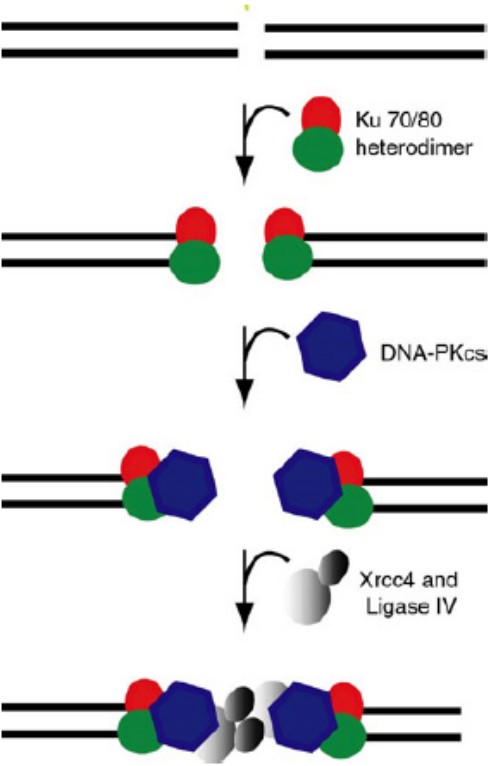
1) +/- Ku80 (effect on NHEJ)

2) +/- DNA-PKcs inhib

3) cut topologies

Canonical NHEJ Pathway:

How many questions can we ask with our data?



Ku70
Ku80 X

DNA-PKcs X

Xrcc4
Ligase IV

① CHO K1 vs. XR56

② CHO K1 vs CHO K1 inhib.

③ XR56 vs. CHOK1 inhib
(absence vs inhib.)

inhibitor added morning → NHEJ machinery shut down
① get into cell ② bind DNA-PK

How will we know that the inhibitor works?

Seperate, important control expt

Seed cells at low density

↓

+inhib dose response

expose to radiation

↓ 5 days

↓

colony formation assay

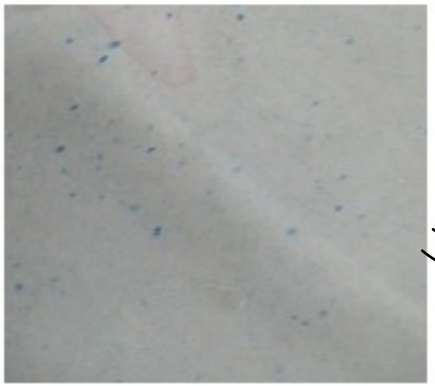
INHIBITOR

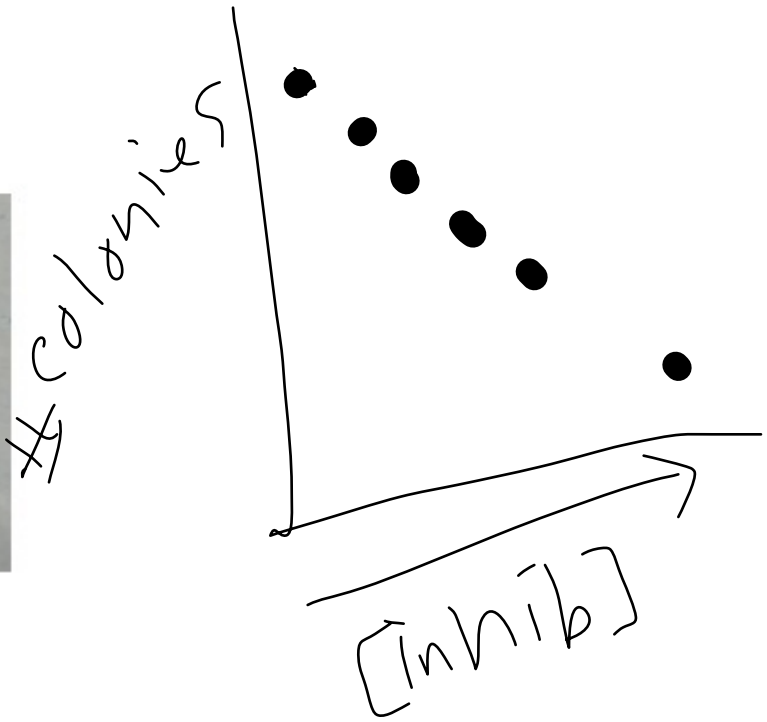
Day 5

Plate irradiated K1 with varying [Drug]

DAY 7

Stain for colonies





Mammalian Cell Transfection:

cationic liposome →

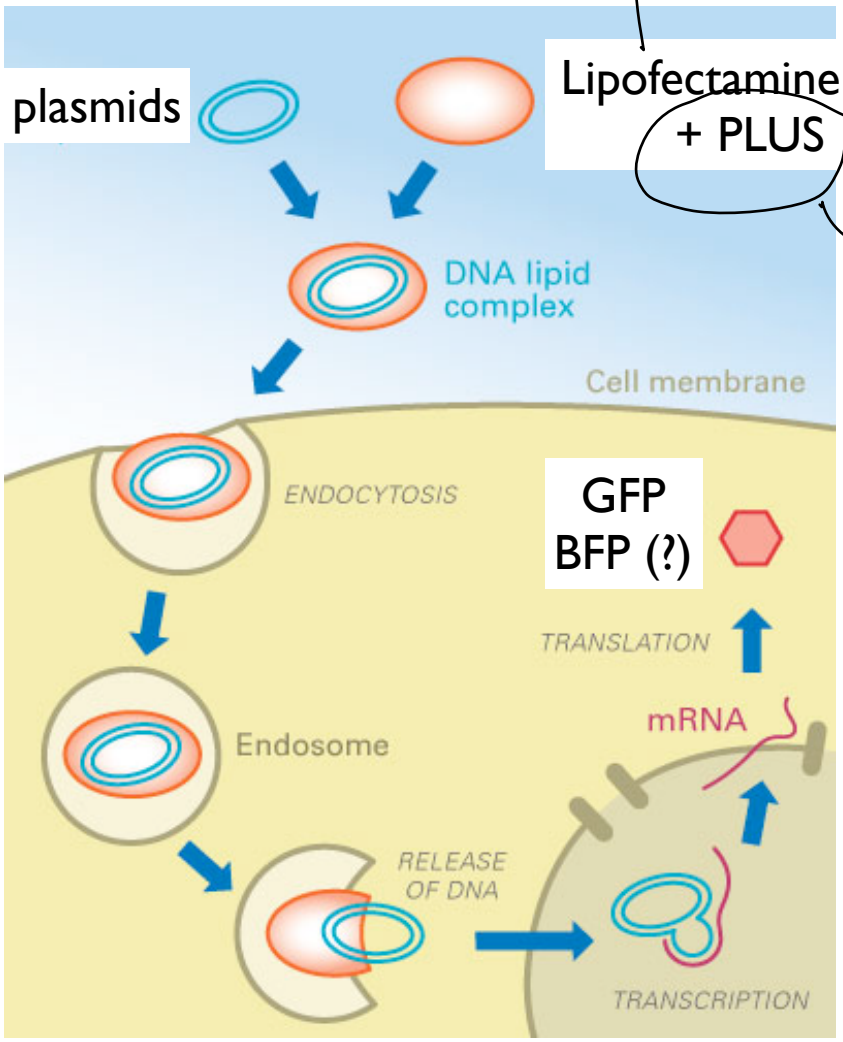
(+) charged lipid

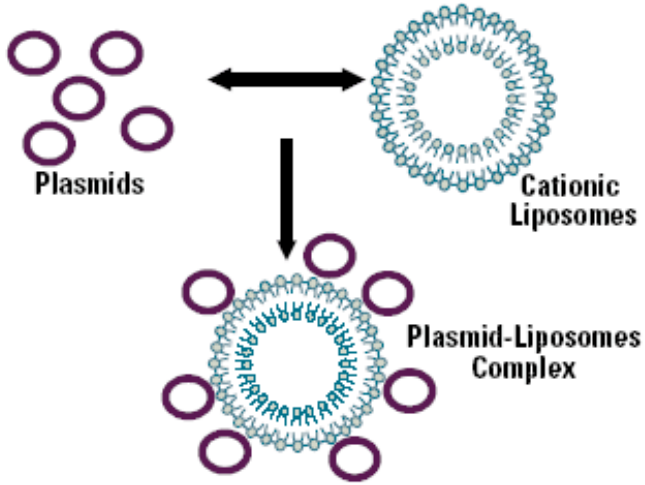
(attracts (−) DNA)

Your plasmids

Lipofectamine LTX
+ PLUS

DNA } compact DNA
plus } bundles

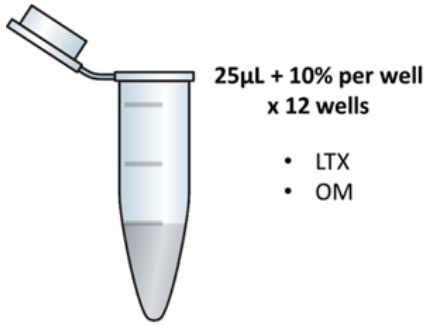




http://www.azonano.com/article.aspx?ArticleID=1233

Today in lab:

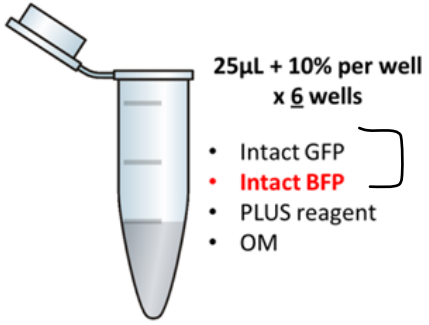
1. LTX solution



25µL + 10% per well
x 12 wells

- LTX
- OM

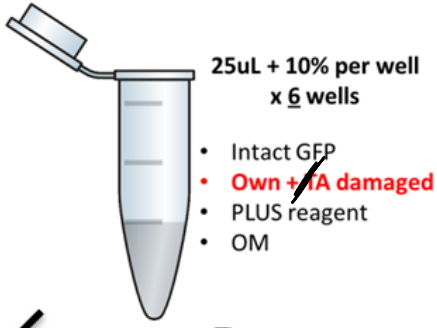
2. Prep DNA solutions



25µL + 10% per well
x 6 wells

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

A
[Control]



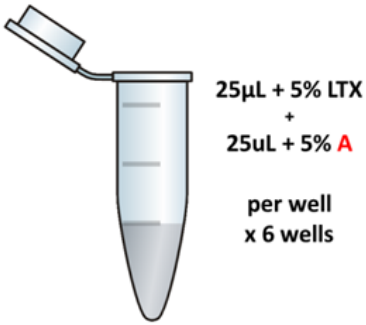
25uL + 10% per well
x 6 wells

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

either

B
[Experimental]

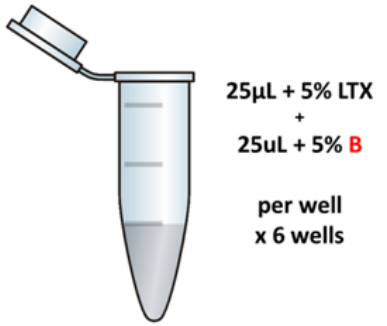
3. Distribute LTX **then** add DNA solution



25µL + 5% LTX
+
25uL + 5% A

per well
x 6 wells

LTX + A



25µL + 5% LTX
+
25uL + 5% B

per well
x 6 wells

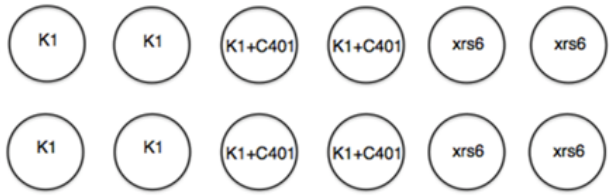
LTX + B

20 minute incubation

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

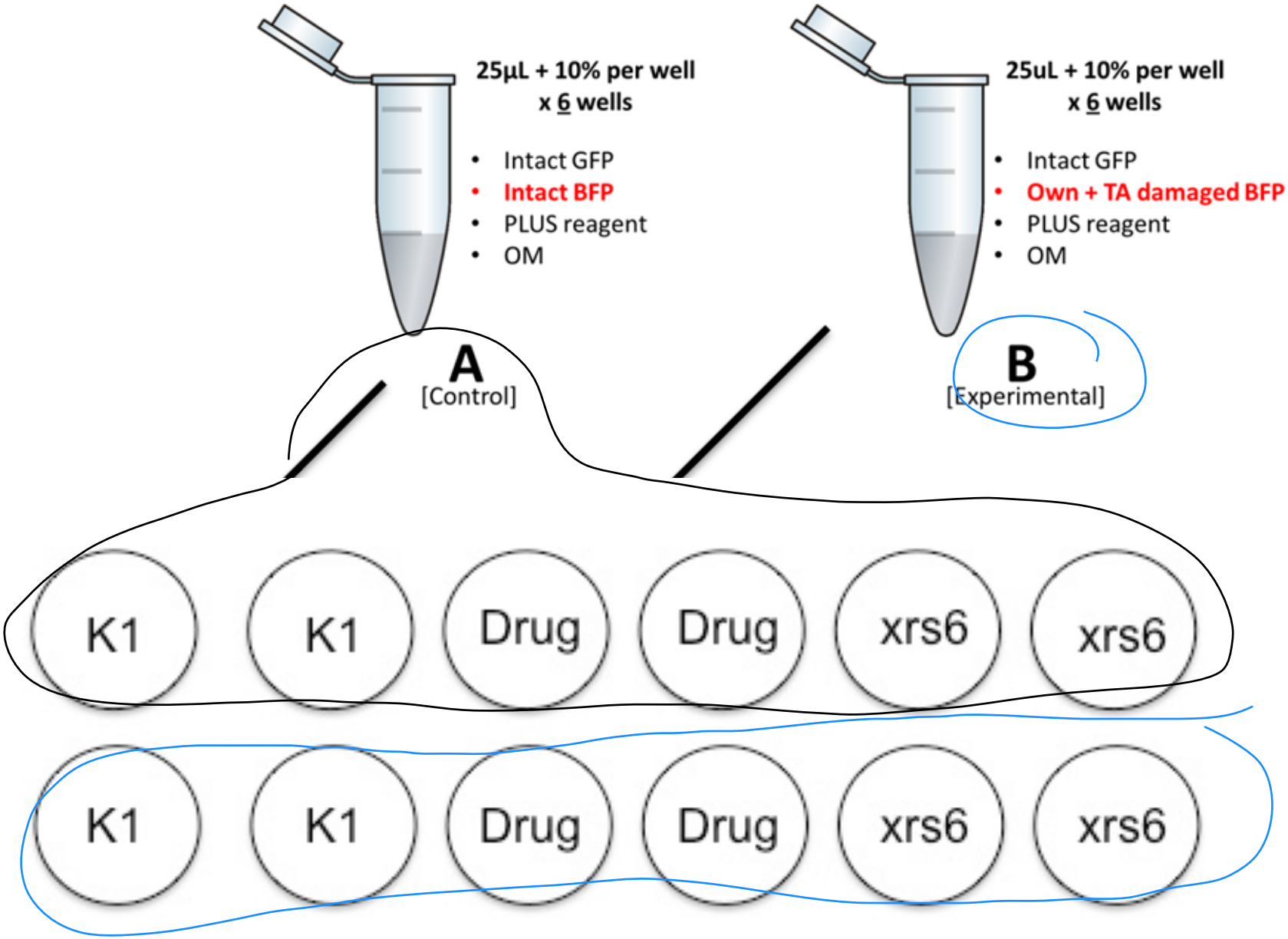
LTX + A → (50 uL)

LTX + B → (50 uL)



inhib

Your DNA damage repair assay:



Today in lab:

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

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

★While you wait — complete the peer Methods review.

Our System:

Possible cut topologies:

NHEJ Hypotheses:

		M2D2	M2D4
(1)		2	
(2)		3-tie	
(3)		3-tie	
(4)		4	
(5)		1-tie	
(6)		1-tie	
(7)		5	