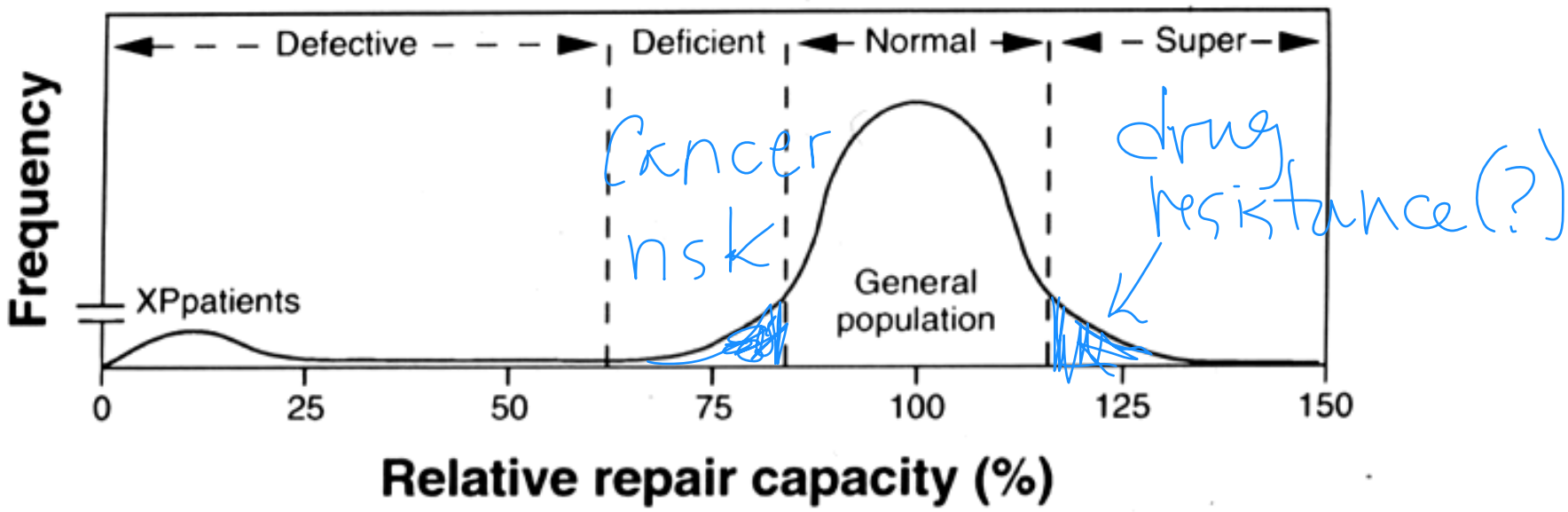
3/31/15

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
- Quantifying DNA repair is impt.

Hint: Motivation for report

Slide from M2D1 Lecture — Prof. Samson

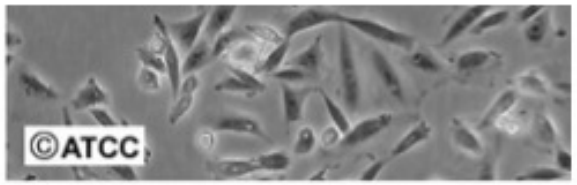
Remember *way* back…

M2 OVERVIEW: VALIDATE SYSTEM

CELLS

DAY 1

Plate K1 and xrs6

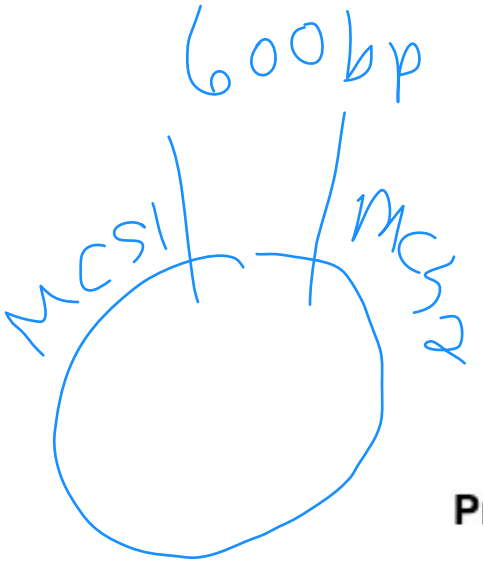


DAY 2 + 3

Measure Ku80 levels

xrs6 K1

Why? Validate cell lines



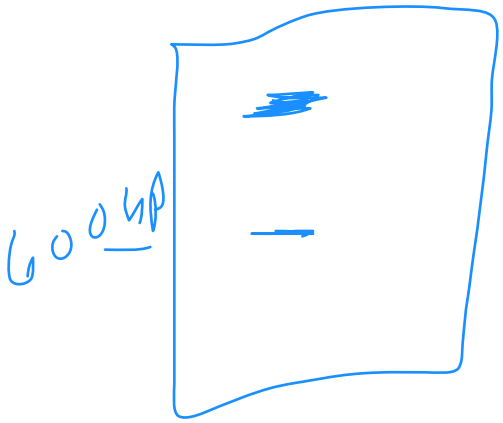
DNA

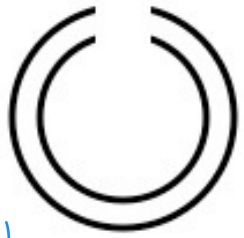
Day 2

Reverse engineer plasmid construct

Day 3

Prepare and assess damaged DNA

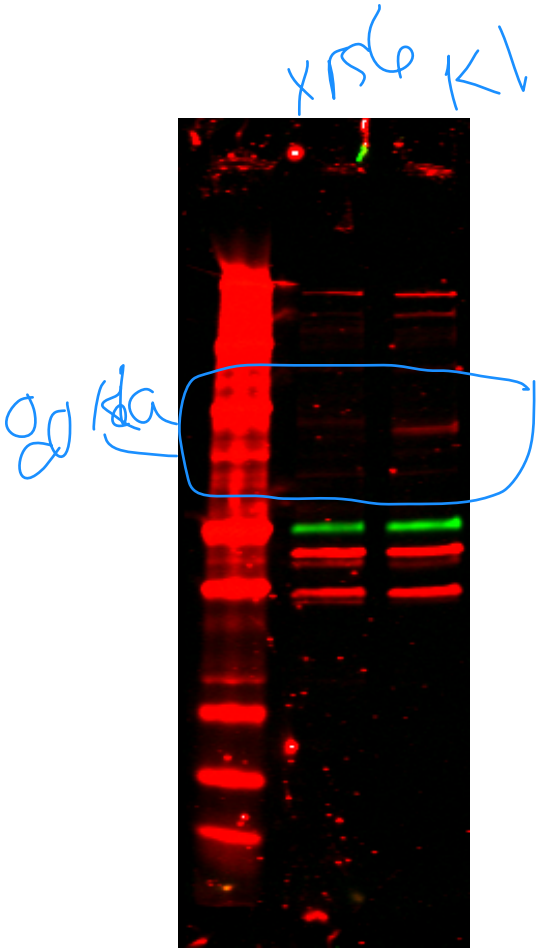




How? cut RE

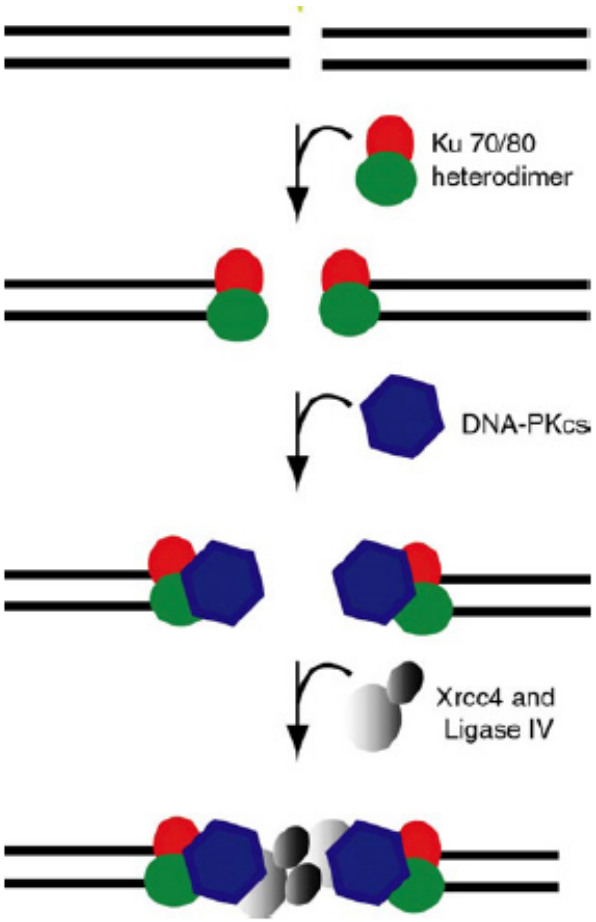
assess? digest efficency

Western blot analysis



1) Cto -1<1
2) Xrs6
3) K1 + inhib

Canonical NHEJ Pathway:



Ku70
Ku80

DNA-PKcs

Xrcc4
Ligase IV

How many experiments are we performing?

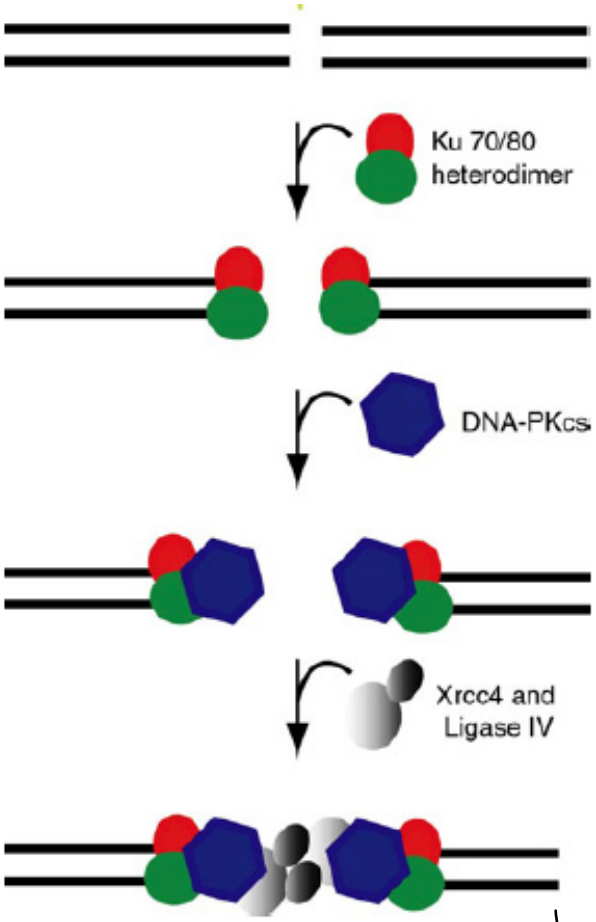
A) pMaxBFP-MCS + pMAX GFP
cut
B) NHEJ repair
transf control

B) pMaxBFP + pMax GFP
not cut
readout maximum repair

① compare cut typologies of RE
② +/- KuC0
+/- NHEJ inhib
} effects plasmid repair

Canonical NHEJ Pathway:

How many questions can we ask with our data?



① KI vs. XRS6 cells

Ku70
Ku80 X

② KI vs. KI + inhib

DNA-PKcs
inhib.

③ XRS6 vs KI + inhib

Xrcc4
Ligase IV

inhib added → inhib get into cell
morning → bind DNA PKcs

effective before PNA add

How will we know that the inhibitor works?

Seperate, important control expt

Seed cells @ low density

↓

Add inhib dose response,

expose radiation

↓ 5 days

colony formation assay

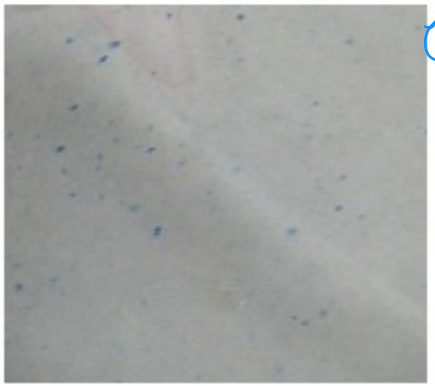
INHIBITOR

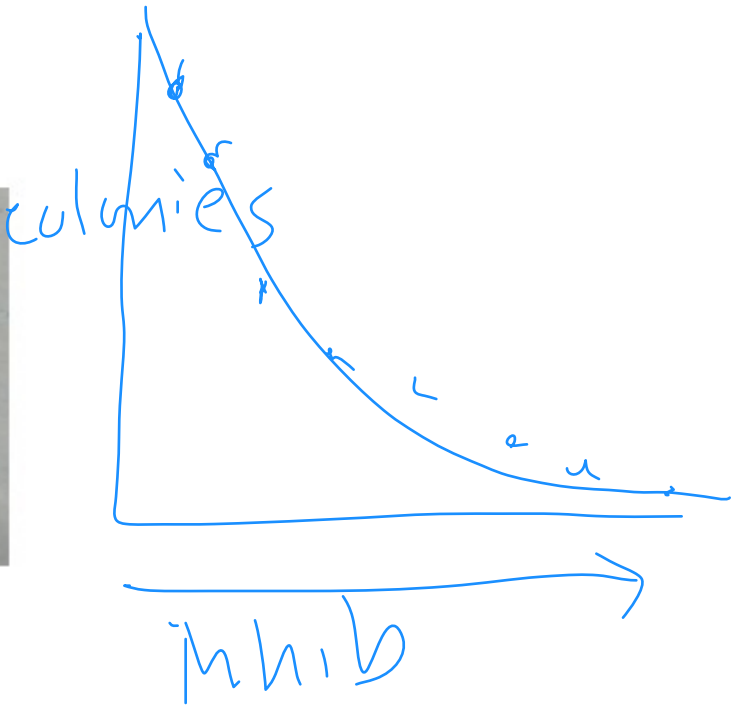
Day 5

Plate irradiated K1 with varying [Drug]

DAY 7

Stain for colonies





Mammalian Cell Transfection:

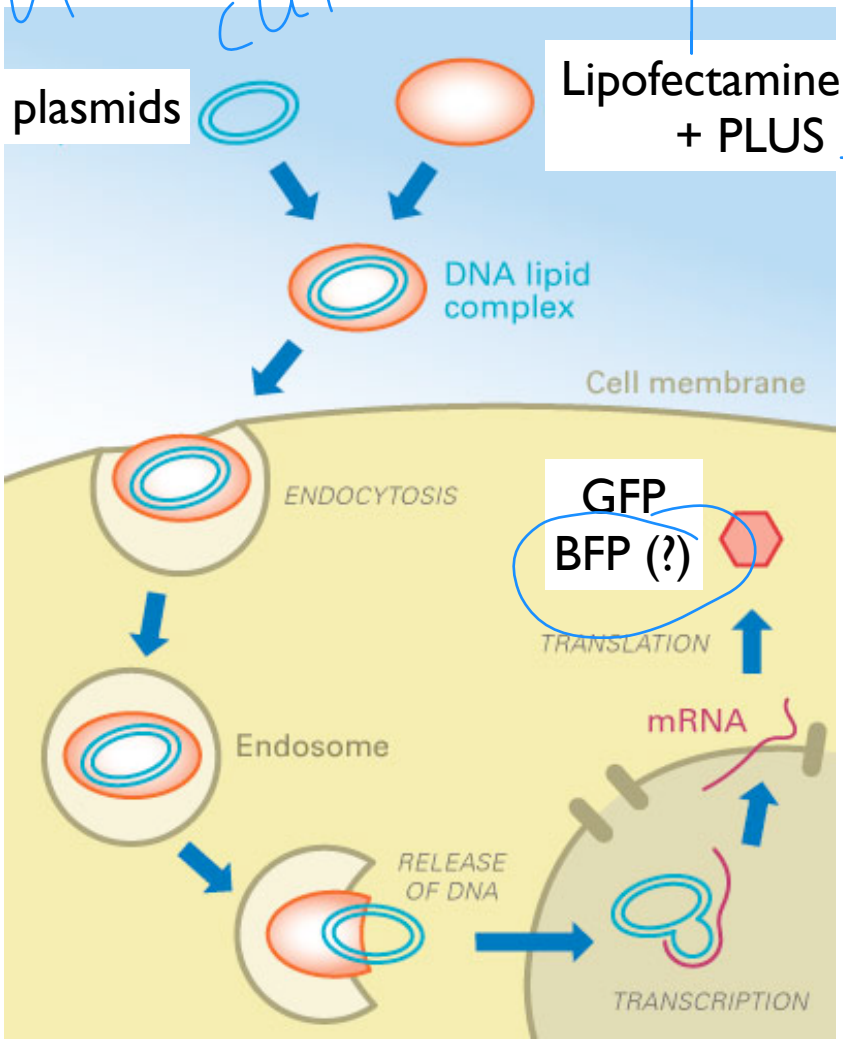
cationic liposome → (+) charged lipid

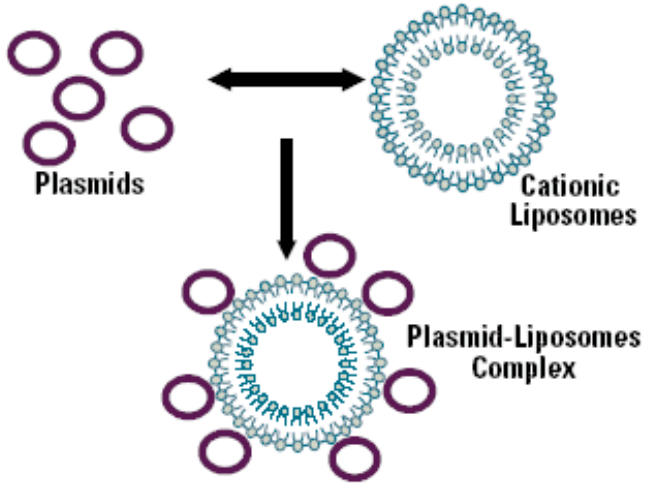
cut + not cut

Your plasmids

Lipofectamine LTX
+ PLUS

→ bundle, compact DNA

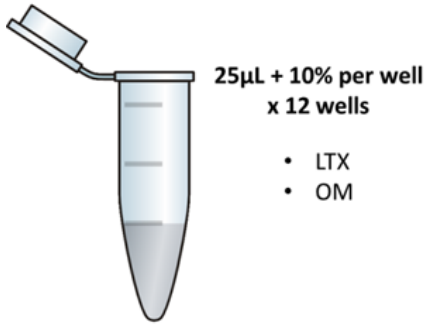




http://www.azonano.com/article.aspx?ArticleID=1233

Today in lab:

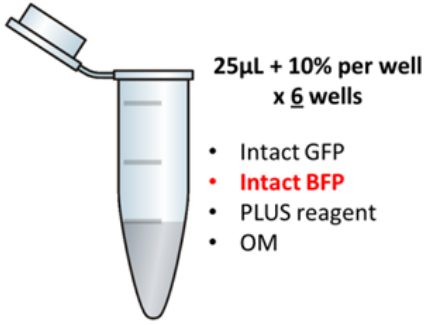
1. LTX solution



25µL + 10% per well
x 12 wells

- LTX
- OM

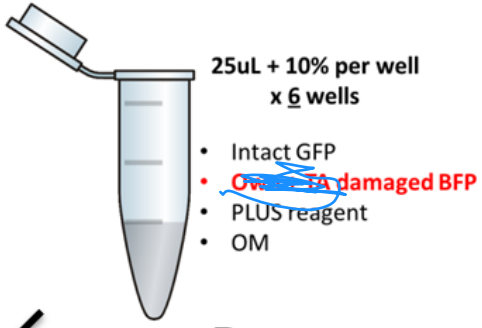
2. Prep DNA solutions



25µL + 10% per well
x 6 wells

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

A
[Control]

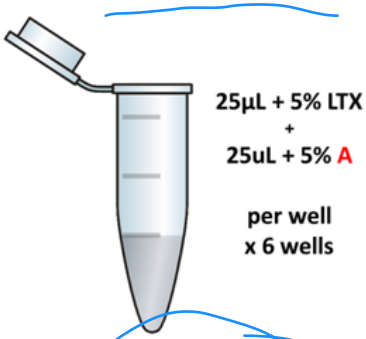


25uL + 10% per well
x 6 wells

- Intact GFP
- **[illegible] damaged BFP**
- PLUS reagent
- OM

B
[Experimental]

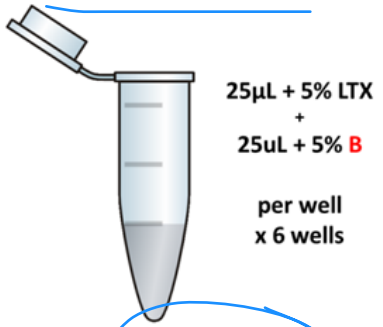
3. Distribute LTX **then** add DNA solution



25µL + 5% LTX
+
25uL + 5% A

per well
x 6 wells

LTX + A



25µL + 5% LTX
+
25uL + 5% B

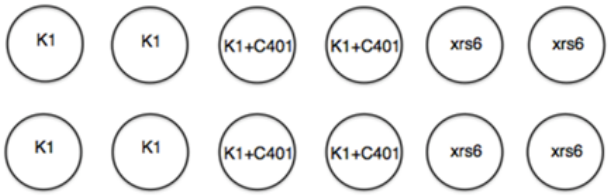
per well
x 6 wells

LTX + B

20 minute incubation

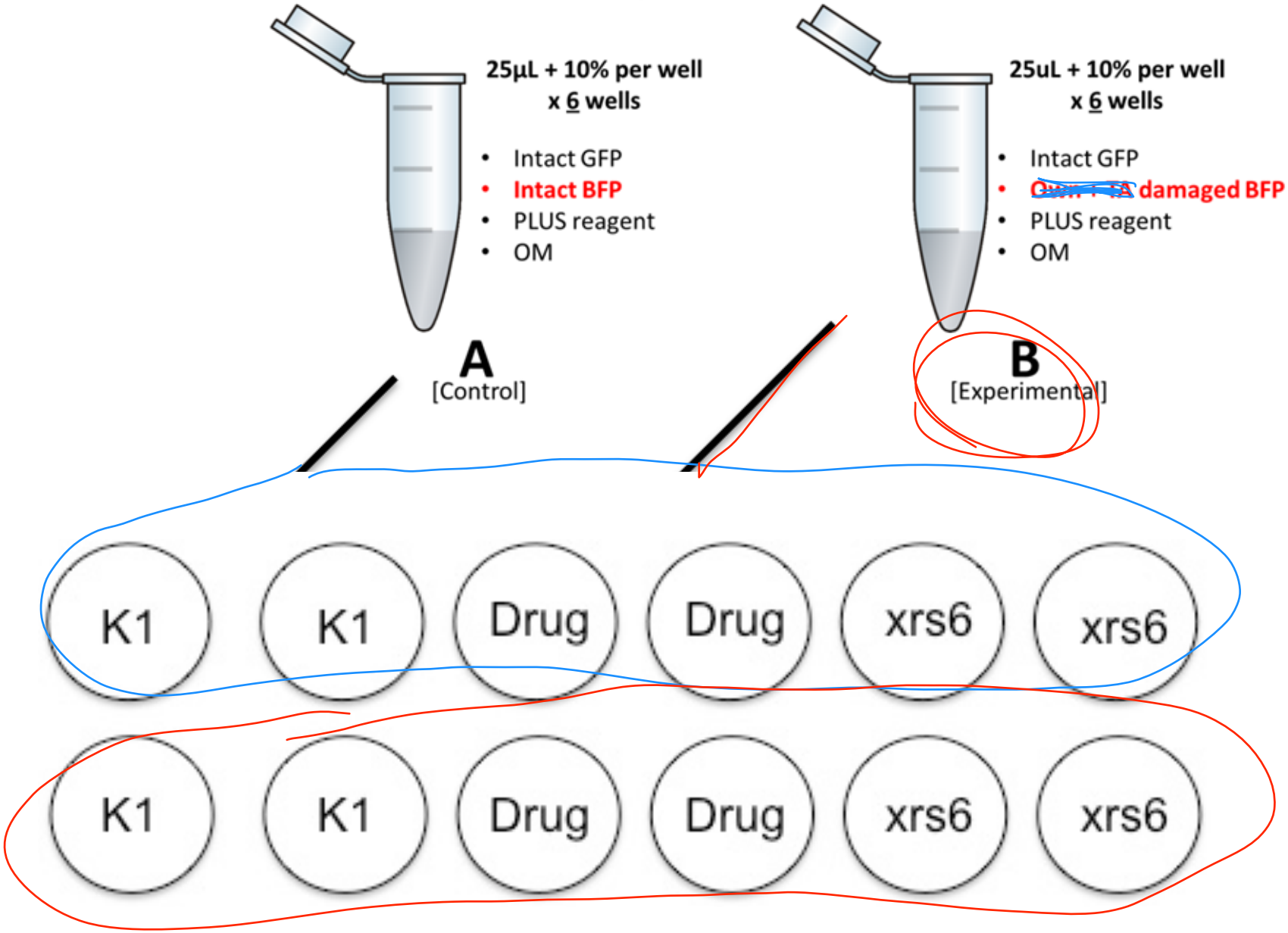
4. Add 50uL **LTX + DNA** to each well

LTX + A → (50 uL)



LTX + B → (50 uL)

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-tie	2 (~ 3)
(2)		3A	3A ?
(3)		3B	3B ?
(4)		2-tie	3C (A)
(5)		1	1
(6)		1	1
(7)		4	4