

M2D5: Transfection for DNA repair assay

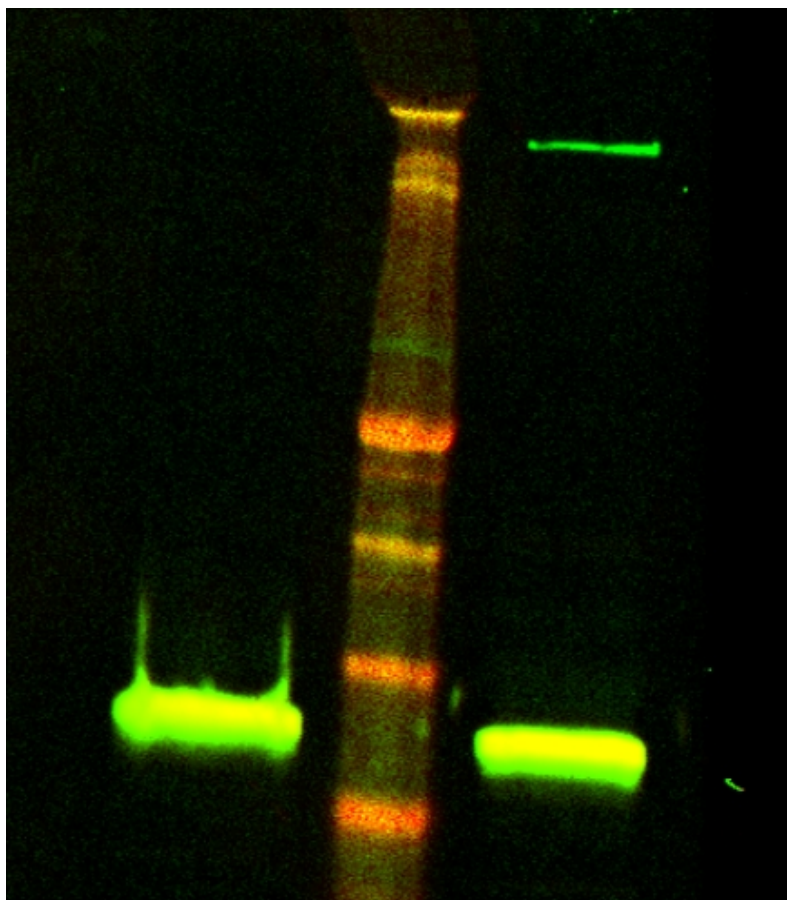
03/29/16

1. Quiz!
 2. Pre-lab discussion
 3. ½ group: Tissue Culture
 4. ½ group: Peer review Methods homework
- Mod1 Blog post due by 10pm tonight (3/29)
 - M2D6 HW: To receive full credit address all of the peer review HW prompts and write (or type) clearly!
 - Journal Club II Presentations April 8th

Good Westerns Gone Bad

M059J

M059K



What could have caused some bad blots?

-cell lysis: no completely harvested cells; over estimated protein concentration

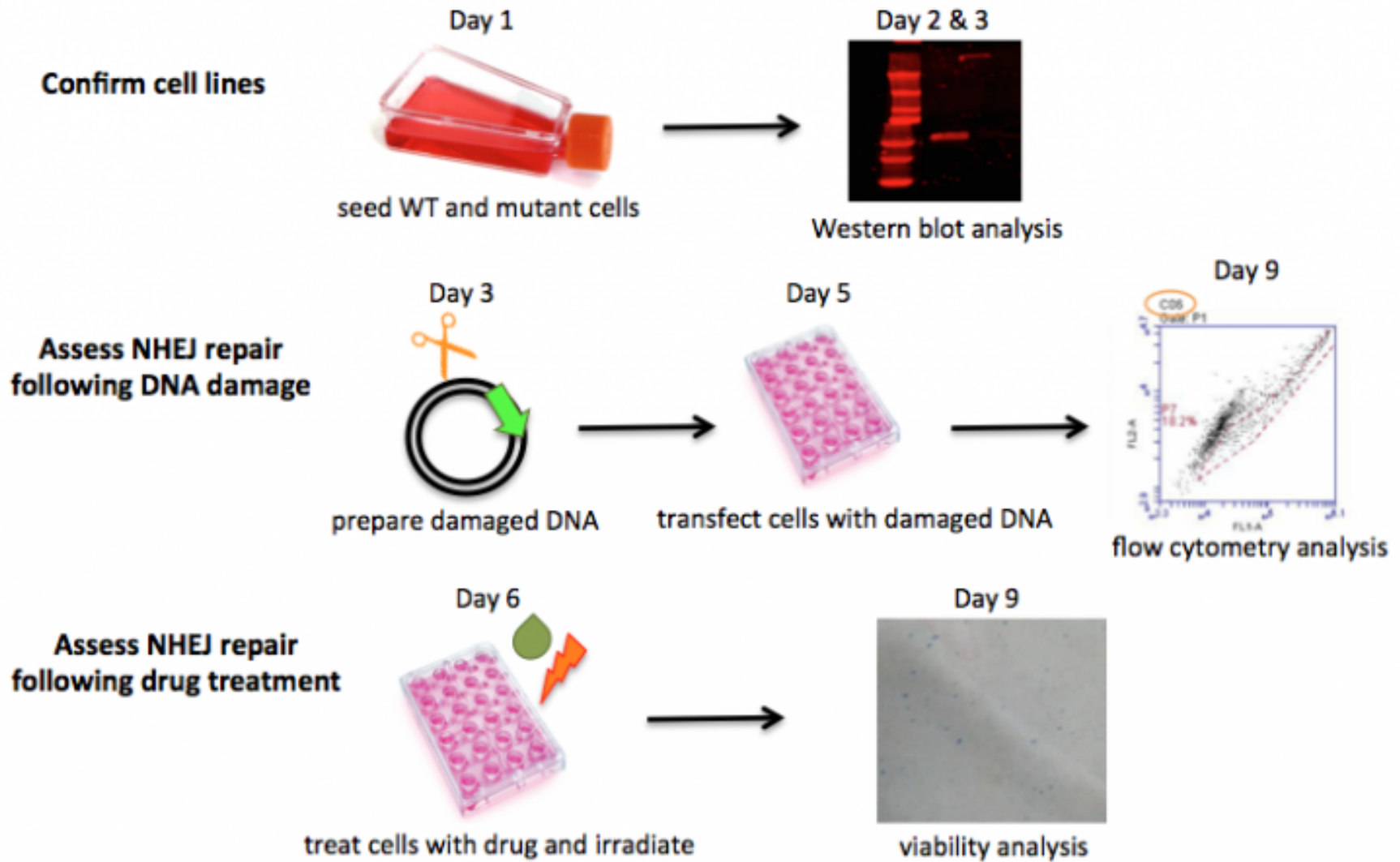
-loading lysate on SDS-PAGE

-Transfer: membrane dirty, not remove all bubbles, acrylamide gel stick to membrane, dirty sponges/ cassettes

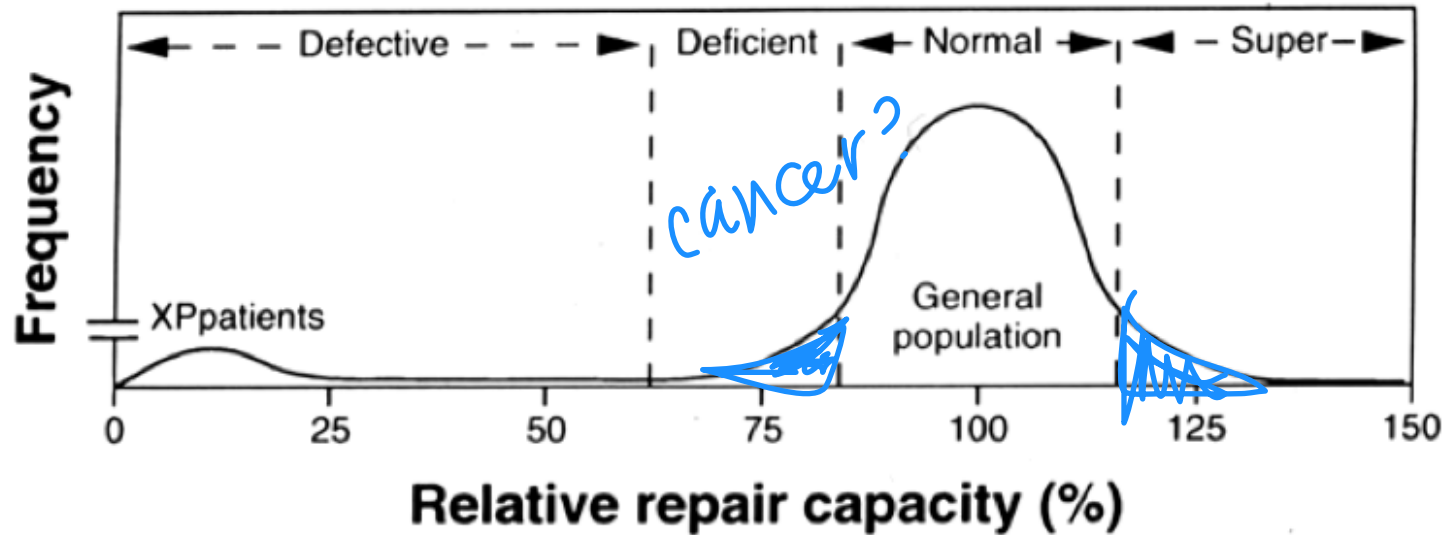
-Block: incomplete blocking, dried out at block step

-1*, 2*: membrane handling, contamination in plasticware

Mod 2 experimental overview



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 can inform risk/choices

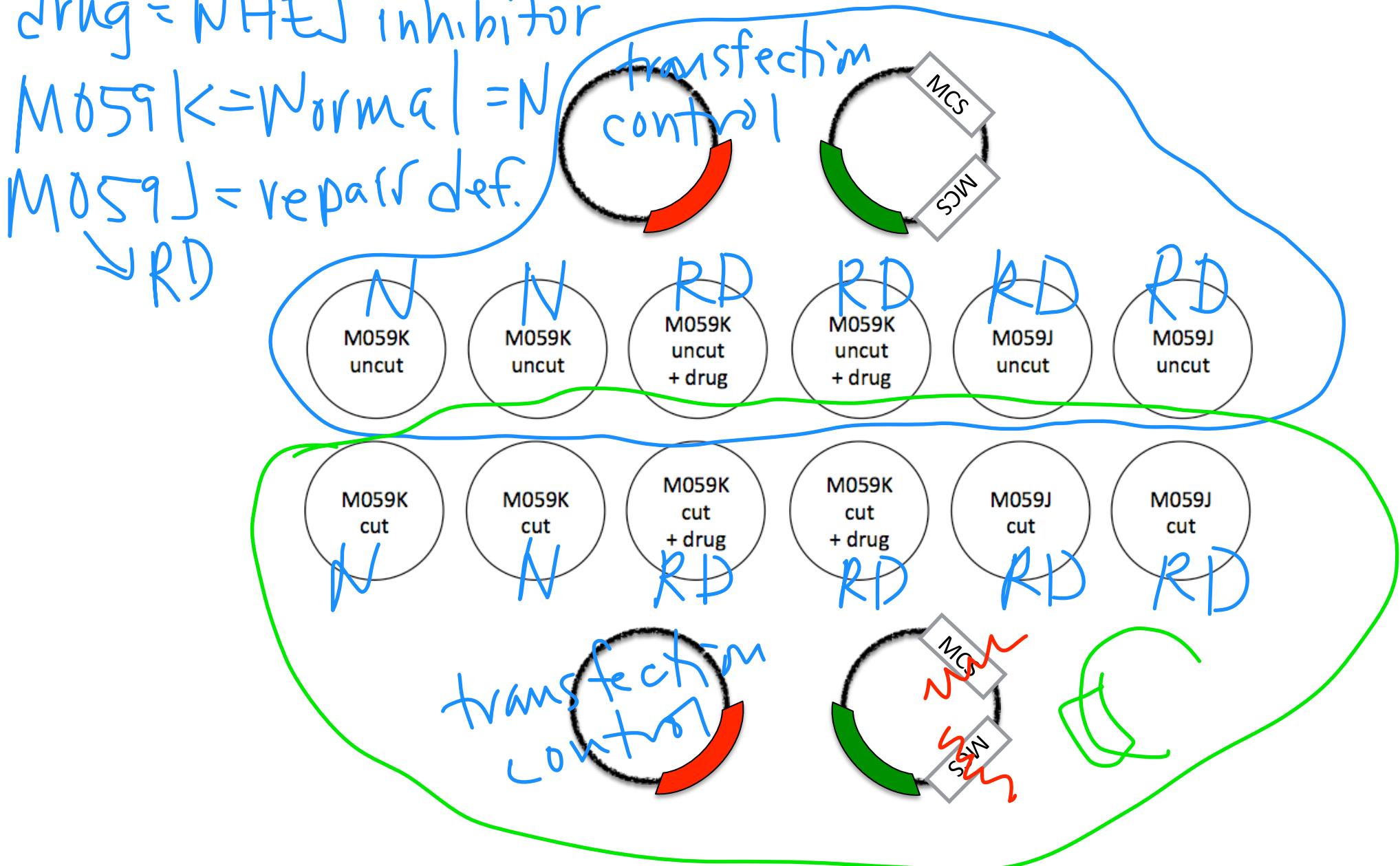
What experiments are we performing today?

drug = NHEJ inhibitor

M059K = Normal = N

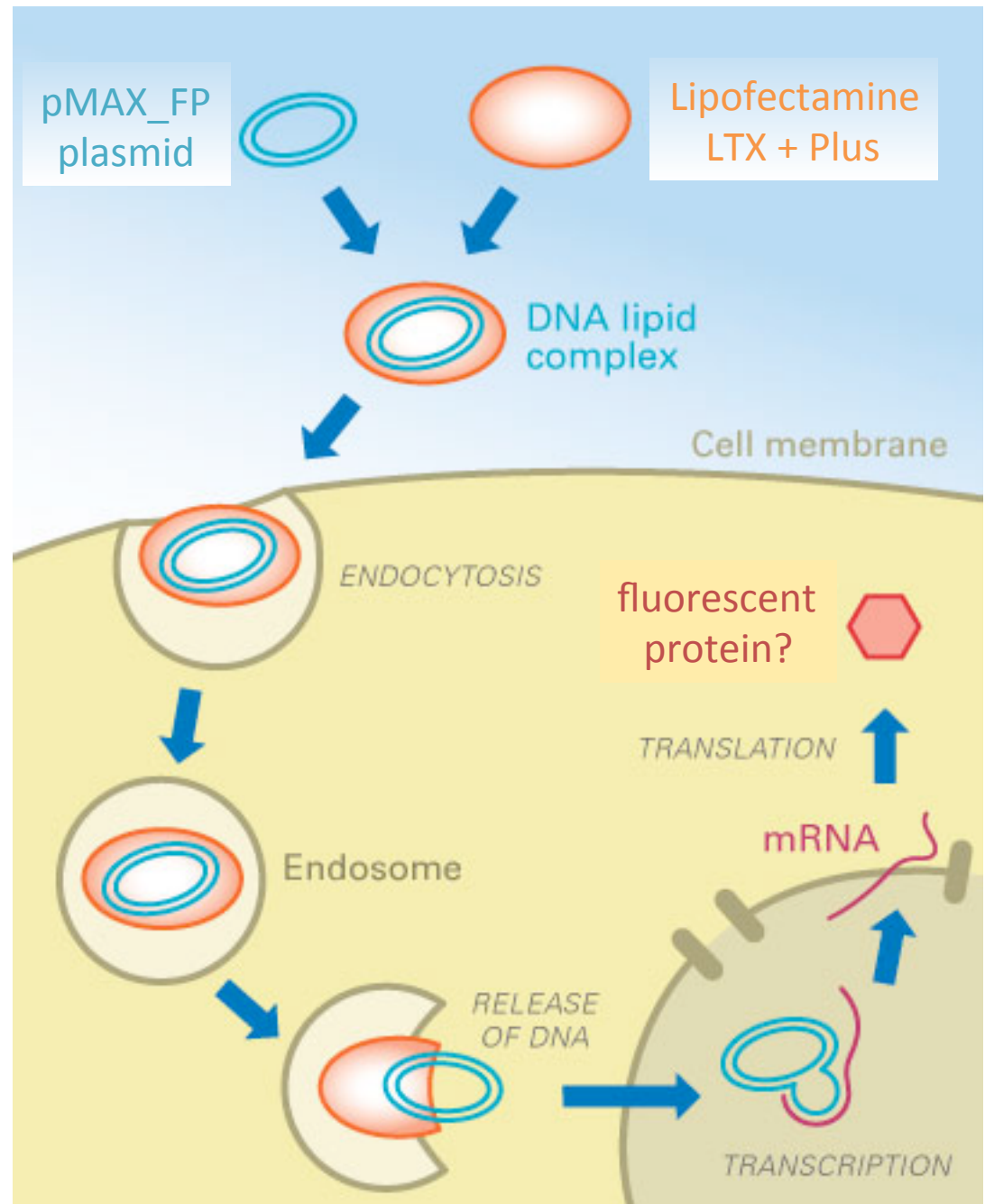
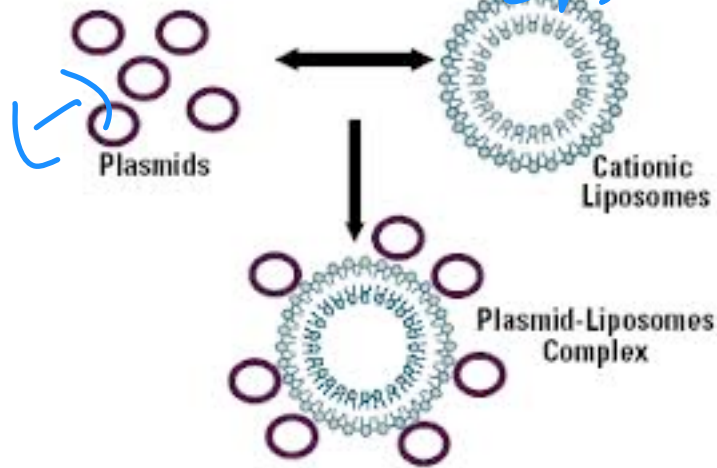
M059J = repair def.

↳ RD

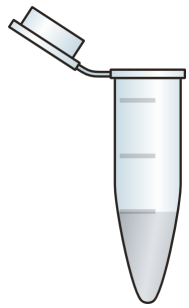


Mammalian cell transfection: lipofection

(+) charged lipid LTX

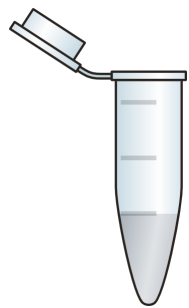


1. Prepare 'LTX', 'A', and 'B' solutions (at each at 10% excess volume), pipet to mix



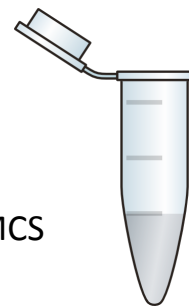
'LTX'

1. OptiMEM
2. Lipofectamine



'A'

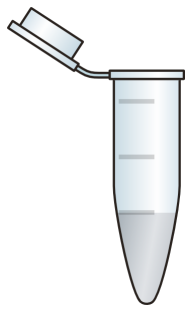
1. OptiMEM
2. pMAX_mCherry
3. intact pMAX_EGFP_MCS
4. PLUS reagent



'B'

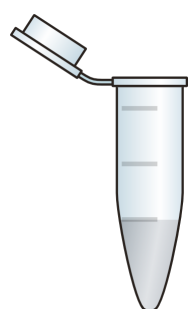
1. OptiMEM
2. pMAX_mCherry
3. damaged pMAX_EGFP_MCS
4. PLUS reagent

2. Combine 'LTX + A', 'LTX + B' mixtures (add each at 5% excess volume), pipet to mix



'LTX + A'

1. 'A'
2. 'LTX'

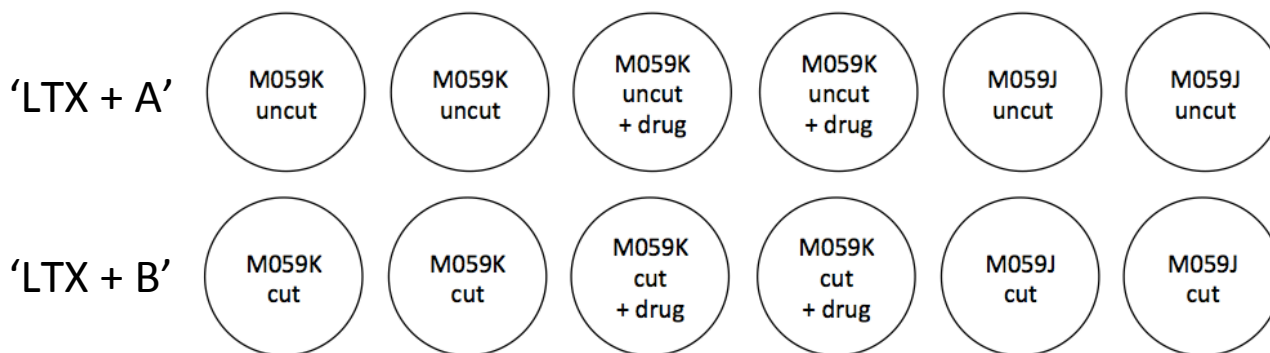


'LTX + B'

1. 'B'
2. 'LTX'

20 min

3. Add 'LTX + A', 'LTX + B' mixtures to appropriate wells, 'squeaky style' to mix



4. Add drug to appropriate wells, 'squeaky style' to mix

Same cut end, different drugs? Different cut ends, same drug?

Options:

1) Loperamide hydrochloride

2) DMNB (4,5-dimethoxy-2-nitrobenzaldehyde)

Team Color	DNA damage type	NHEJ inhibitor
T/R Red	compatible overhangs	DMNB
T/R Orange	blunt	DMNB
T/R Yellow	incompatible overhangs	DMNB
T/R Green	blunt	Lop
T/R Blue	compatible overhangs	Lop
T/R Pink	compatible overhangs	DMNB
T/R Purple	blunt	DMNB
W/F Red	incompatible overhangs	
W/F Orange	compatible overhangs	
W/F Blue	blunt	
W/F Pink	incompatible overhangs	
W/F Purple	incompatible overhangs	

Today in lab

- Tissue Culture (TC)
 - Make sure instructors check your transfection calculation before entering tissue culture
 - Protocols printed for TC use, please take written copy or print out of transfection calculations
 - Group order to TC:
 - 1st: Yellow, Green, Blue, Purple
 - 2nd: Red, Orange, Pink
- Peer Review Methods
 - Clearly write or type comments similarly to instructors homework feedback
 - Comments will be turned in as M2D6 homework