

M2D5:  
Cell preparation for  
DNA repair assay

03/30/2016



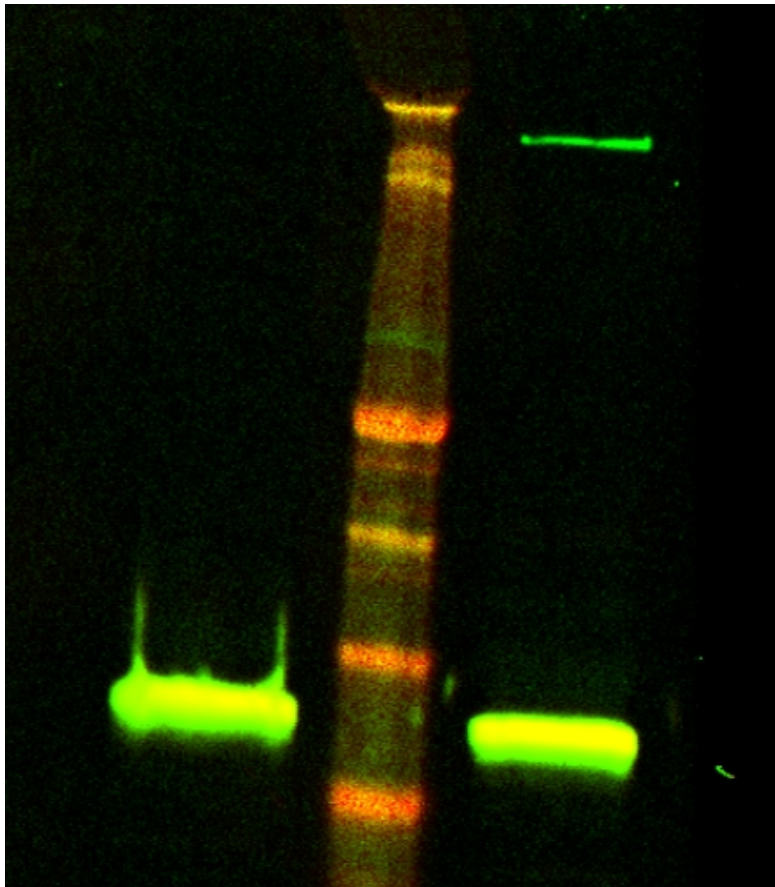
# Welcome back!

- This week
  - Methods + Methods peer review
  - Tissue culture work
  - Time to make progress on M2 research article in lab
- Next week
  - BE Communication Lab workshop on research articles
  - Journal club presentations on April 8<sup>th</sup>  
Sign up for paper before 12pm noon on Friday
  - Data analysis

# What could lead to “bad” western blots?

M059J

M059K



cell lysate concentration

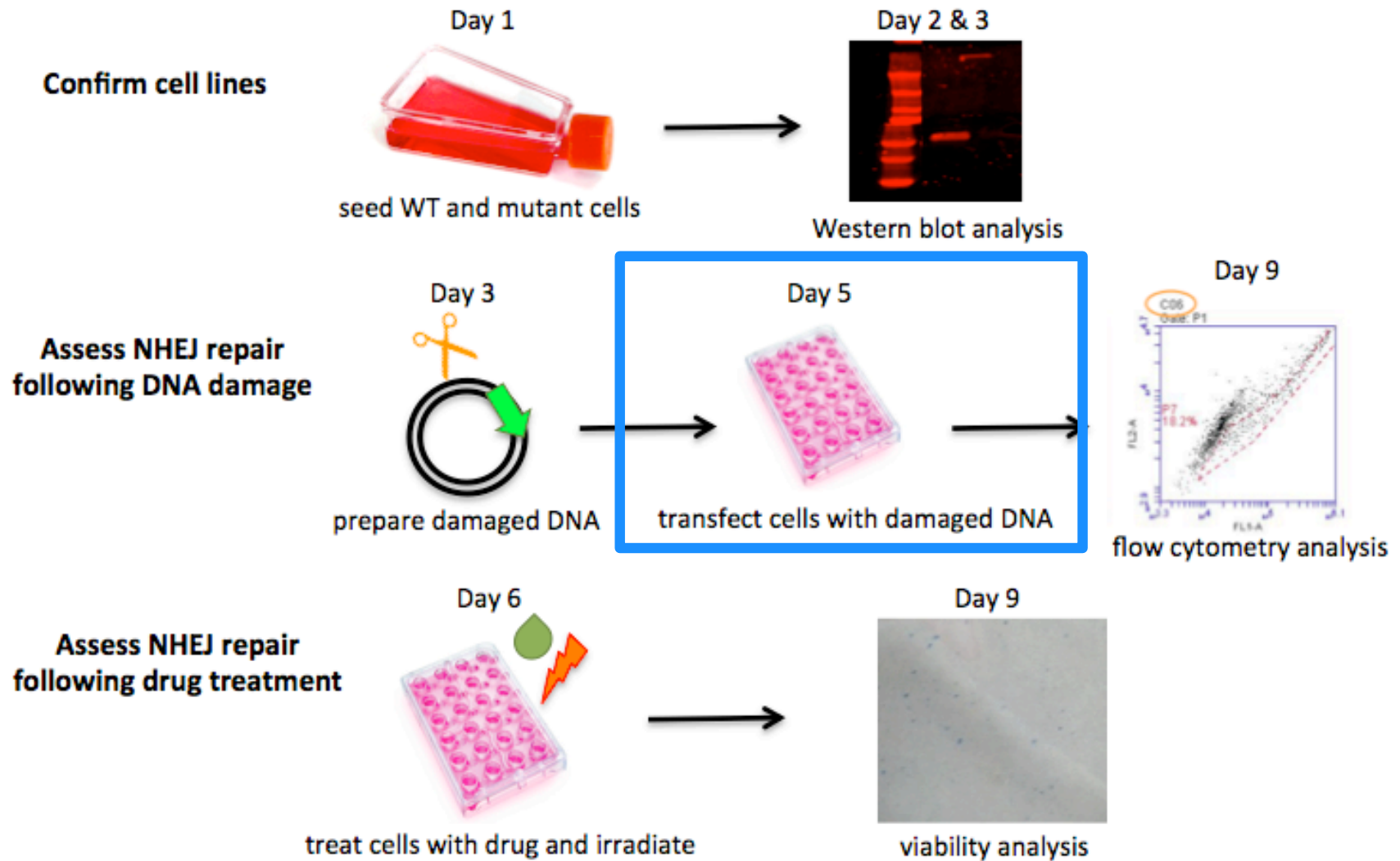
loading (but tubulin control)  
pipetting error (2x J instead of J + K)

large size of protein makes it travel little

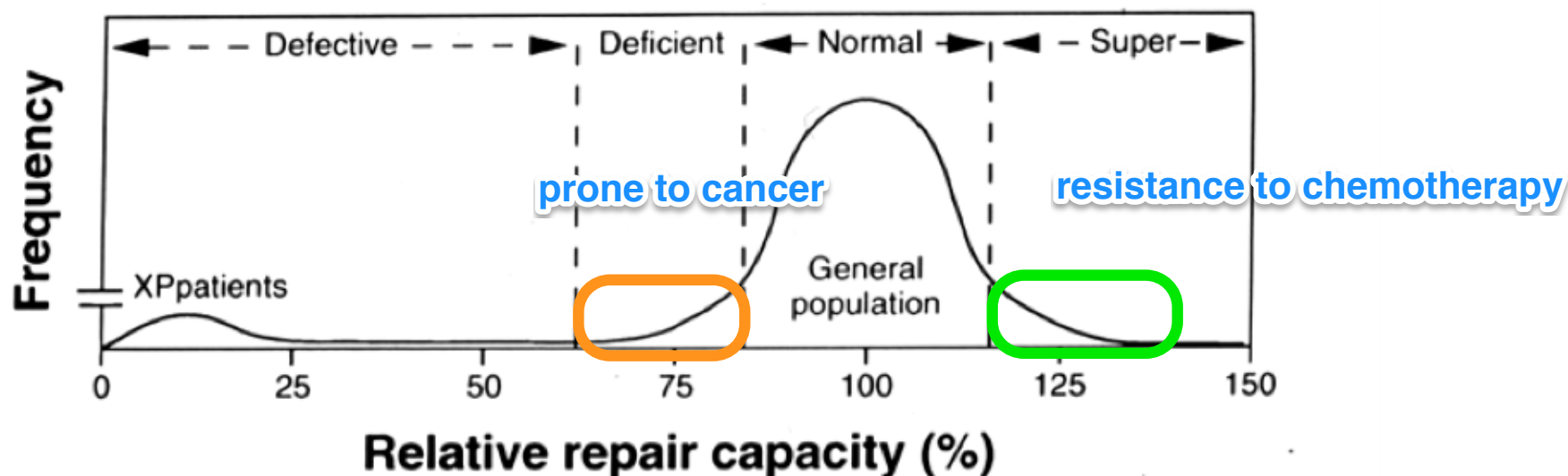
transfer: voltage non-optimal, dirty pads,  
handling of the membrane, bubbles

evaporated blocking buffers,  
acrylamide stuck to membrane  
old antibodies  
contamination

# M2 experimental overview



# Why do we care about DNA repair capacity?

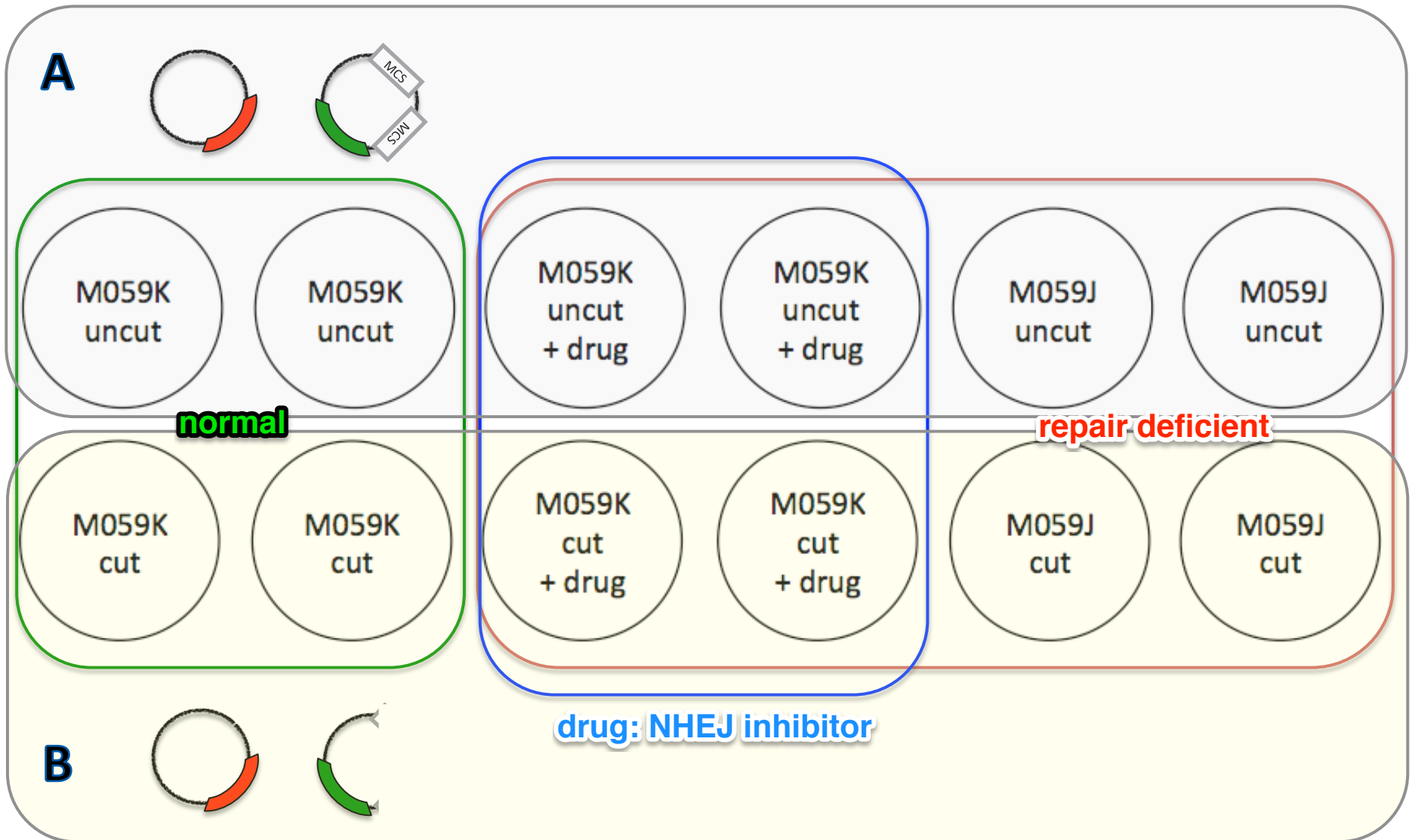


Adapted from **GROSSMAN and Wei (1995)** Clinical Chem 41: 1854-1863

DNA repair is variable among individuals

Quantifying it can inform choices

# What experiments are we performing today?





Smart study:

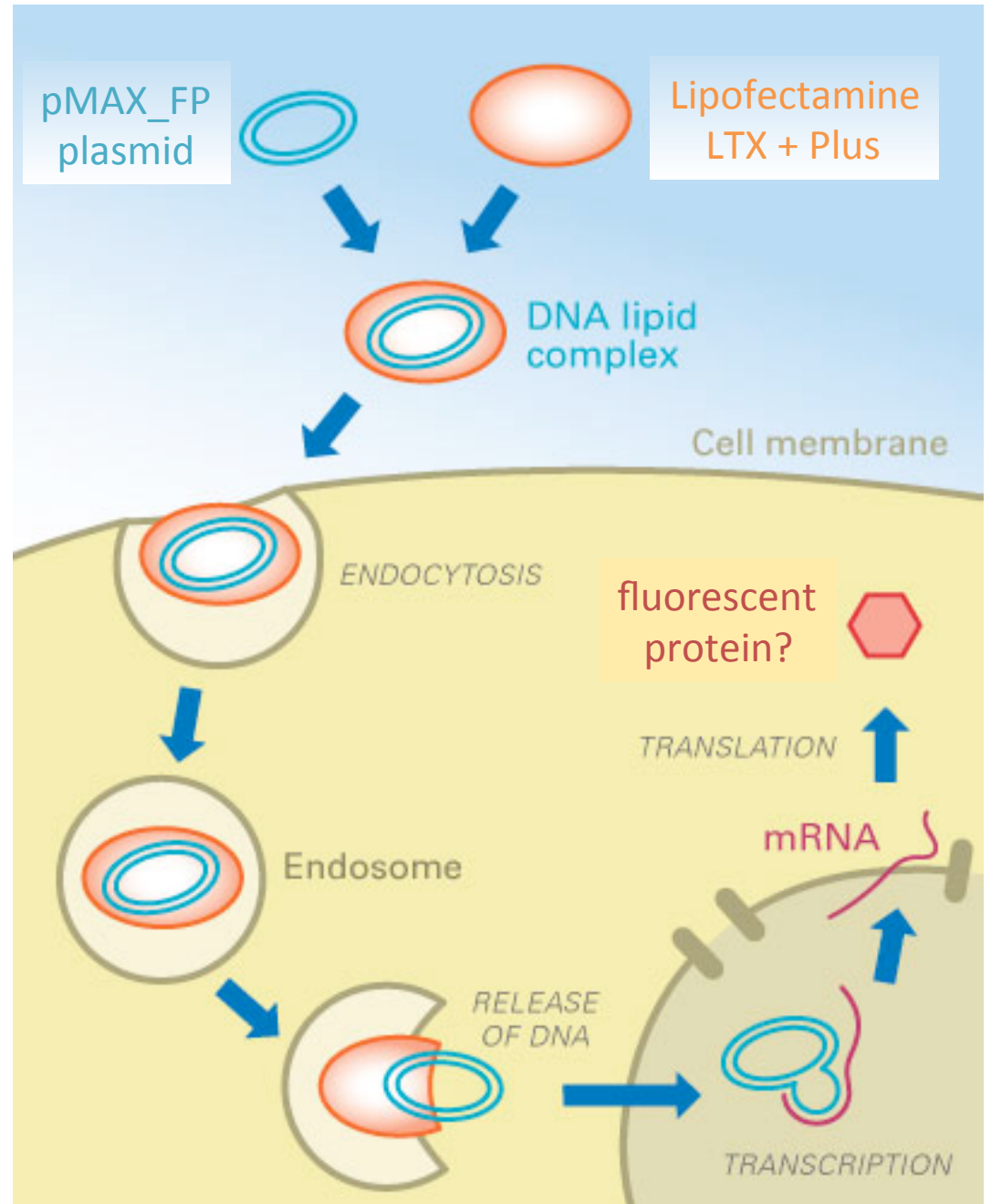
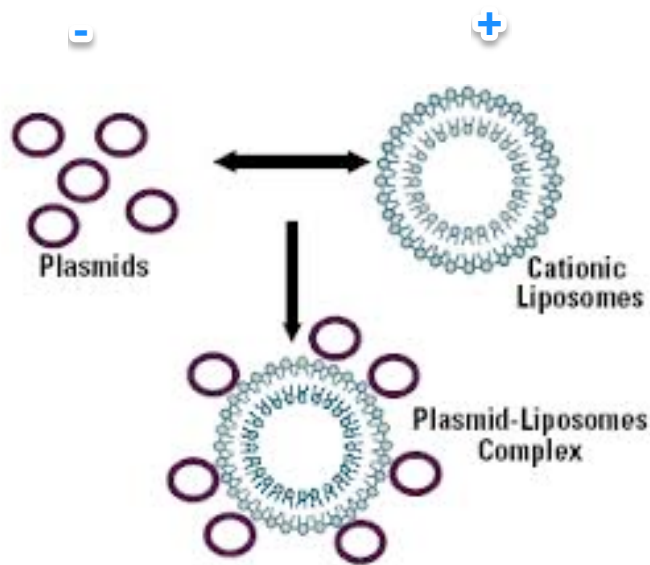
Same cut end, different drugs?  
Different cut ends, same drug?

NHEJ inhibitor  
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	DNMB
T/R Yellow	incompatible overhangs	DNMB
T/R Green	blunt	Loperamide
T/R Blue	compatible overhangs	Loperamide
T/R Pink	compatible overhangs	DMNB
T/R Purple	blunt	DNMB
W/F Red	incompatible overhangs	loperamide
W/F Orange	compatible overhangs	loperamide
W/F Blue	blunt	loperamide
W/F Pink	incompatible overhangs	loperamide
W/F Purple	incompatible overhangs	DMNB

# Mammalian cell transfection: lipofection





### 1. Prepare 'LTX', 'A', and 'B' solutions

Add reagents to tubes in this order!



- '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



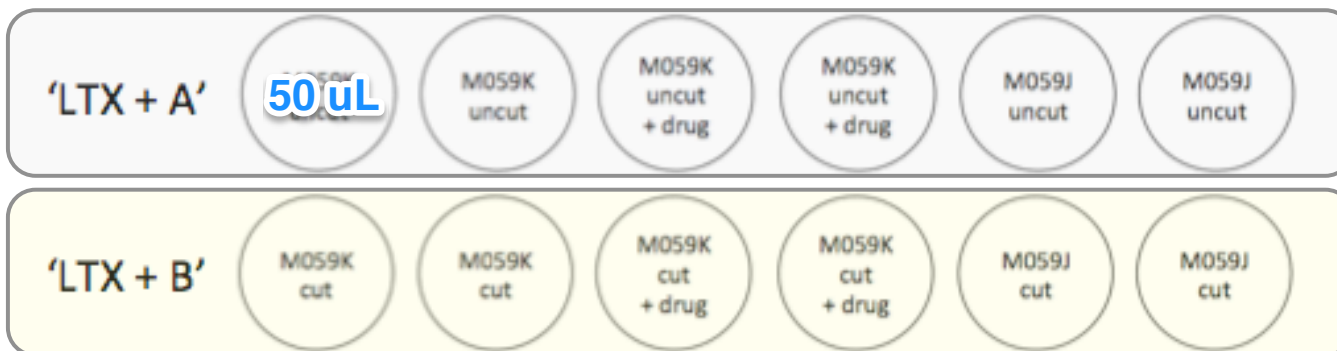
- 'LTX + A'**
1. 'A'
  2. 'LTX'



- '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

## In lab today, split up:



- Tissue culture room
  - Orange, Pink, Purple
  - Check calculations with Leslie & Maxine
- Methods peer review
  - ✓ Homework due M2D6 😊