

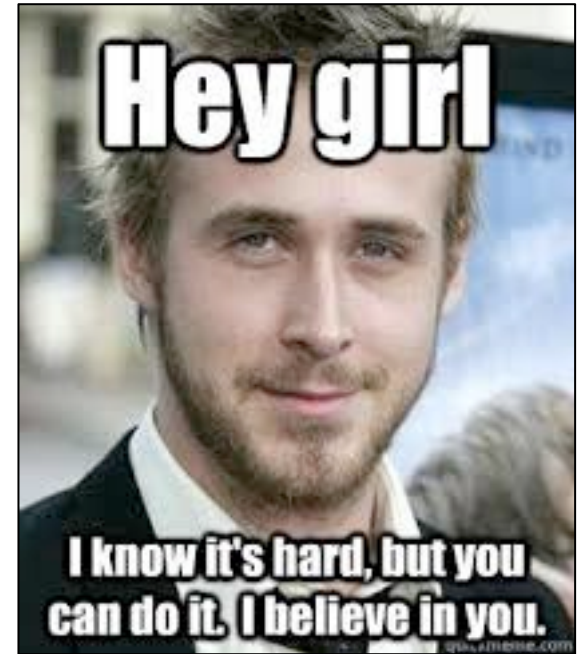
# M2D1: Introduction to cell strains & plating

03/09/2016



# A busy week

- Protein engineering summary
  - due 5pm on Saturday, March 12
- Mini-presentation
  - due 10pm on Wednesday, March 16
- Sign up for journal club
  - 4-7 students at 1pm on Friday, March 18
- Blog post
  - due 5pm on Tuesday, March 29



# Additional office hours

- to work on the protein engineering summary:
  - Noreen on W 03/09 and R 03/10, 6pm-9pm in 16-317
  - Leslie on W 03/09 and F 03/11, 2pm-5pm in 56-302
  - Maxine on R 03/10 and F 03/11, 9am-11am in 16-239
- to work on mini-presentation and journal club
  - Noreen & Maxine on S 03/13, 12pm-5pm in 56-302
  - Leslie on M 03/14, 9am-12pm in 16-429b
- and as usual
  - Noreen on M, 2pm-5pm in 16-317
  - Leslie on T, 10am-11am in 16-429b
  - Maxine on R, 10am-11am in 16-239

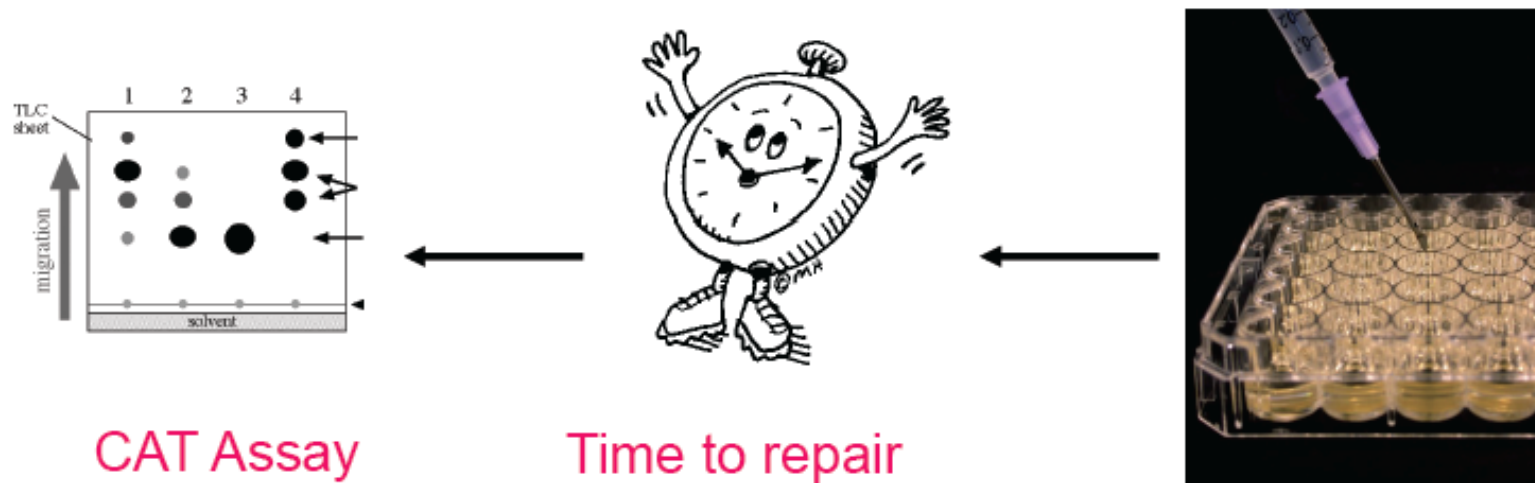
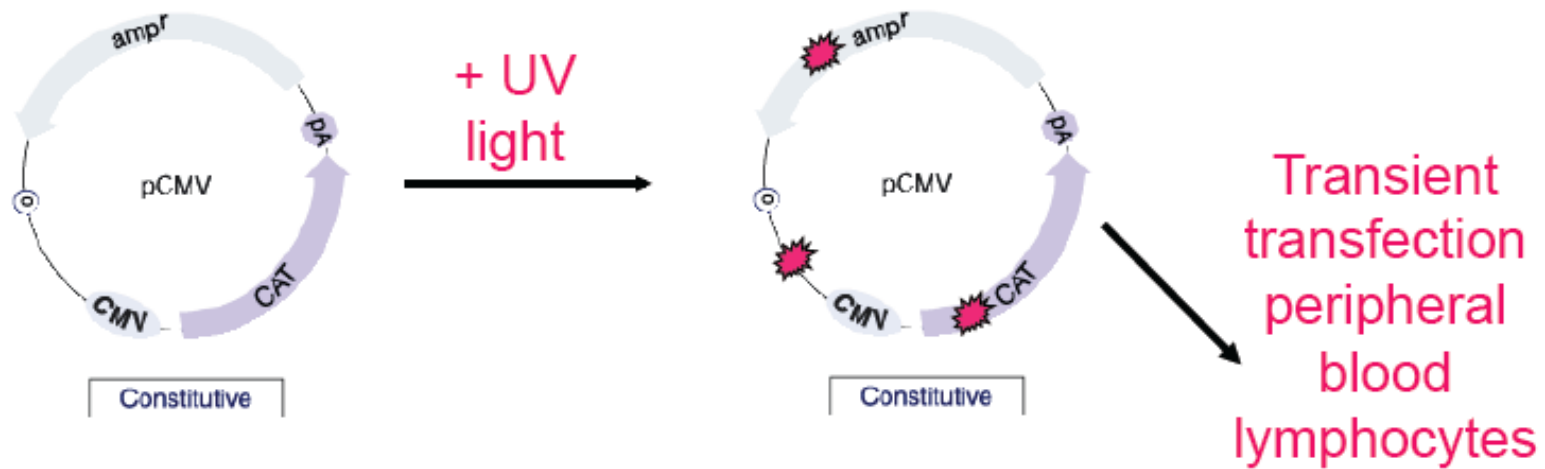
# Sign up for journal club

- Pick 1 of 19 papers, or suggest your own
- Present M2D4 (March 18) or M2D8 (April 8)
- Sign up by adding your name next to paper [MJ/WF/Rainbow]
  - first come first serve!
  - only one T/R and one W/F per article

Slot	Day 4 (T/R)	Day 8 (T/R)	Day 4 (W/F)	Day 8 (W/F)
1				
2				
3				
4				
5				
6				
7				

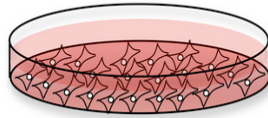
# Reactivation of UV damaged DNA by Host cell Reactivation (HCR)

Athas & GROSSMAN  
Cancer Res. 1991

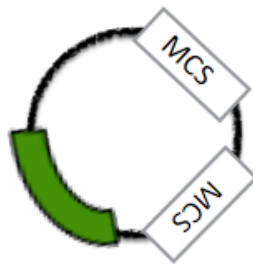


# M2: Quantify NHEJ in fibroblasts, DNA repair capacity by host cell reactivation

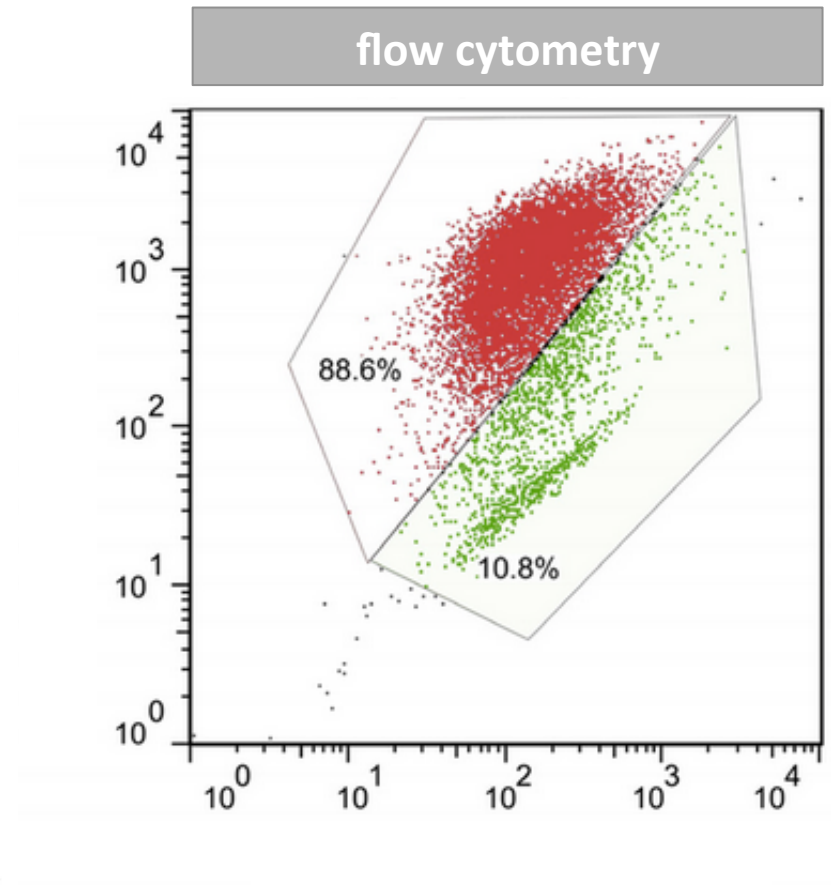
1. “normal cells” **M059K** or  
“DNA repair-deficient cells” **M059J**  
(= no p350 subunit)



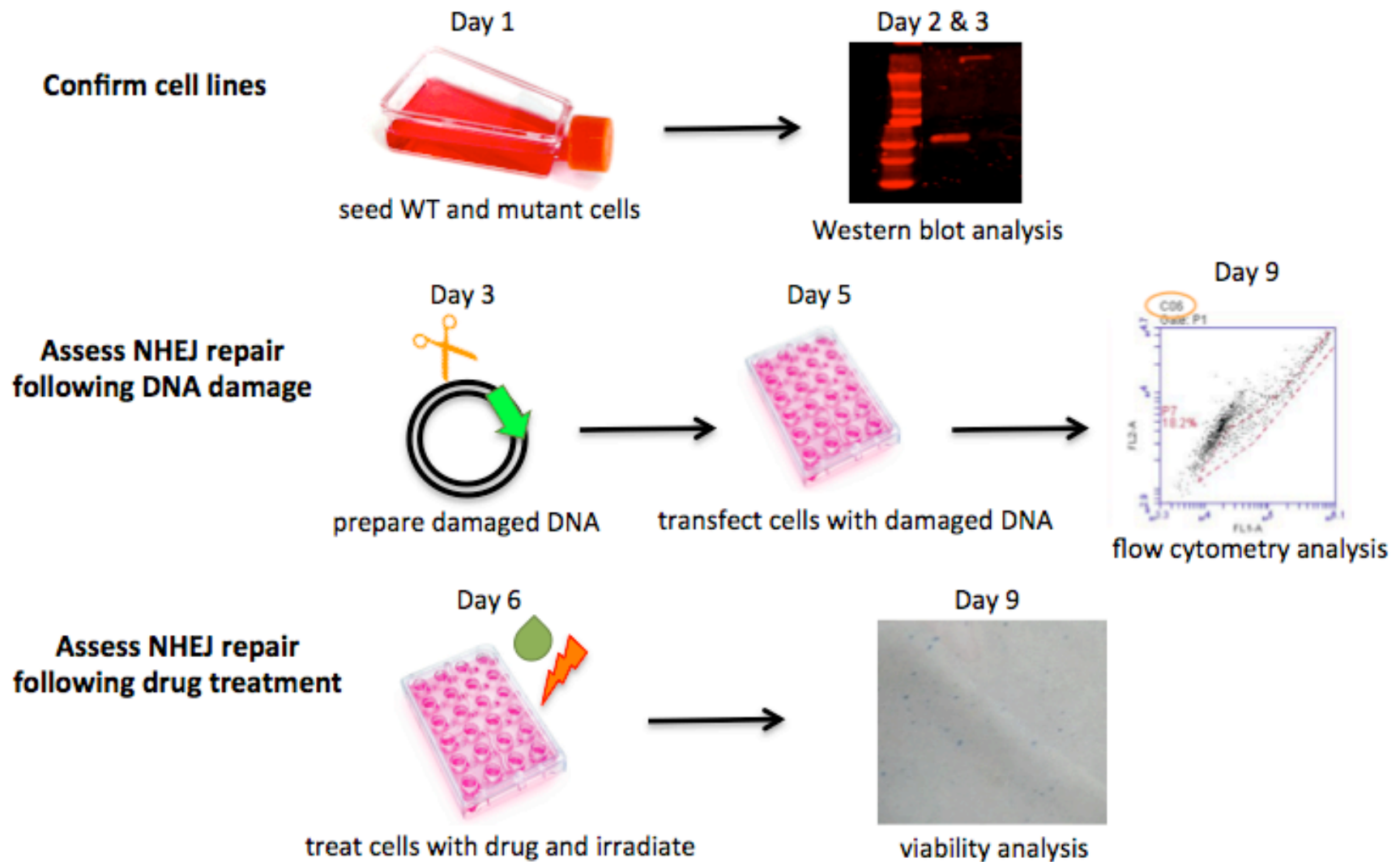
2. DNA damage  
cuts with blunt vs. overhang ends  
irradiation



3. drug inhibitor of NHEJ



# M2: Experimental overview







# Mammalian cell culture medium

**M059K/J**



## Food:

- DMEM : F12 Ham's
  - Dulbecco's Modified Eagle's Medium
  - nutrient mixture F12

**glucose, salts, amino acids, vitamins**

**phenol red is a pH indicator**



- FBS: fetal bovine serum

**BSA and other proteins**

**growth factors, cytokines, lipids, cholesterol**

- non-essential amino acids

**glucose primarily used for growth**

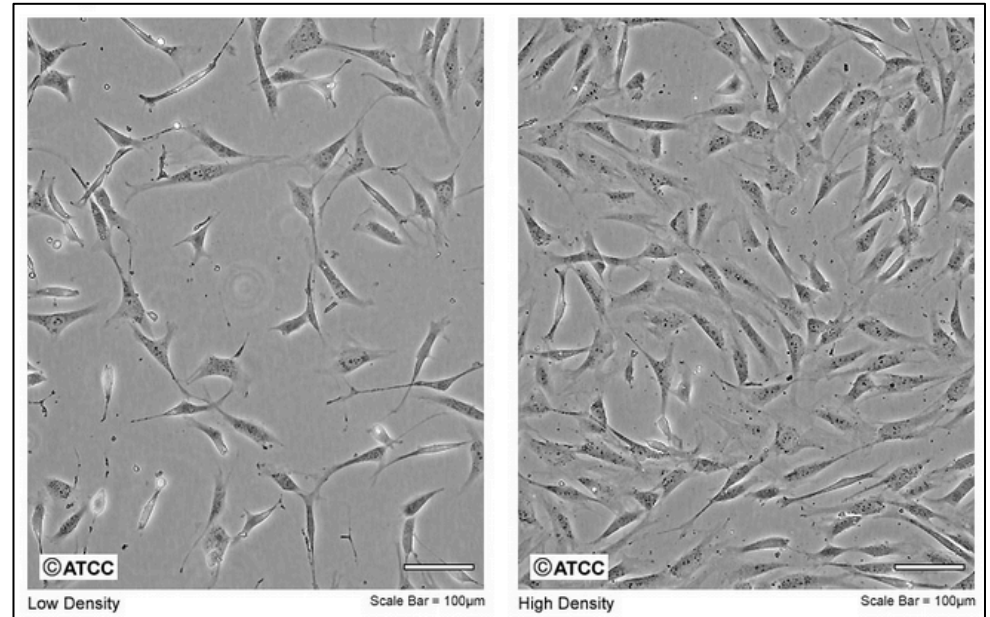
## Non-food:

- antibiotics:
  - penicillin
  - streptomycin



# Mammalian cell culture terminology

- confluence  
~ density around 80%  
when split
- splitting  
sub-culturing
- seeding



# Seeding your M059J/K cells

why?



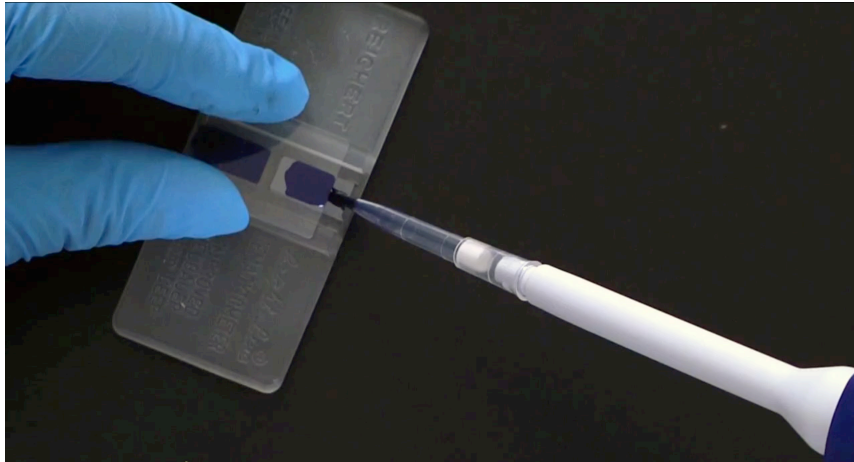
flask



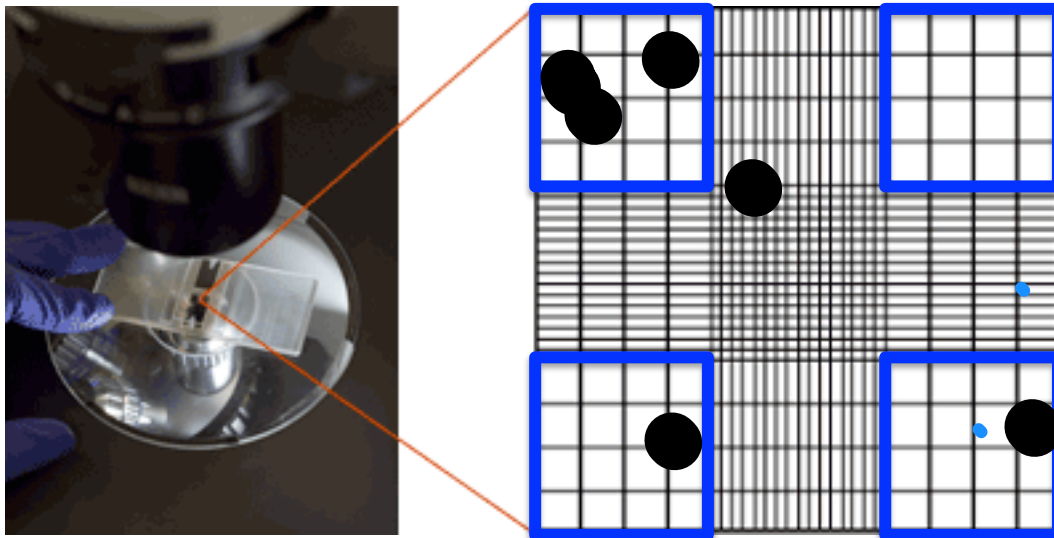
dish

1. Rinse with PBS  
**get rid of anti-trypsin agents**
2. Detach with trypsin  
**breaks substrate-cell adhesions, serine protease**
3. Calculate number of cells  
**consistency, reproducibility**
4. Seed new culture dish

# Calculating number of cells



- Hemacytometer
- Trypan blue **dyes dead cells**
- # cells / mL = 10,000 x average of 4 corners



# Today

- BE Communication Lab workshop on journal club presentations
- Prelab discussion

- Class split between

**(Part 3)** – learning about cell lines and

**(Part 2)** – working in the tissue culture (TC) room

➤ **Red**, **Orange**, and **Purple** Teams begin in TC

