

M2D1: Intro to M059J/K cell lines & Tissue culture

03/08/16

1. BE communication lab workshop: Journal Clubs
Workshop in 26-328 (down the hall)
2. Prelab Discussion
3. Half the class: Tissue Culture
4. Half the class: Research the M059K and M059J cell
lines

Assessments for Mod1:

- Protein engineering summary:
due at **5pm on Saturday, March 12th**
feedback on 03/17 from Noreen & Diana
revision due **5pm on Monday, March 28th**
- Protein engineering presentation:
due at **10pm on Tuesday, March 15th**
- blog post due **03/29**

- **Extra office hours in 56-302:**
 - Noreen W 03/09 and R 03/10, 6pm-9pm
 - Maxine ThT 03/10 and F 03/11, 9am-11am
 - Leslie W 03/09 and F 03/11 2pm-5pm
- **Extra office hours for Mini-Presentation**
 - Noreen Su 03/13 2pm-5pm
 - Maxine Su 03/13 12pm-2pm
 - Leslie M 03/14 9am-12pm

Sign up for journal club

1. Pick 1 of 19 papers, or suggest your own
2. Sign up by adding your name next to paper[LMM/TR/Rainbow]
 - **first come first serve!**
 - **one T/R and one W/F per article**
3. Sign up for a time slot: M2D4 (Mar. 17) or M2D8 (Apr. 7)

Journal article options [\[edit\]](#)

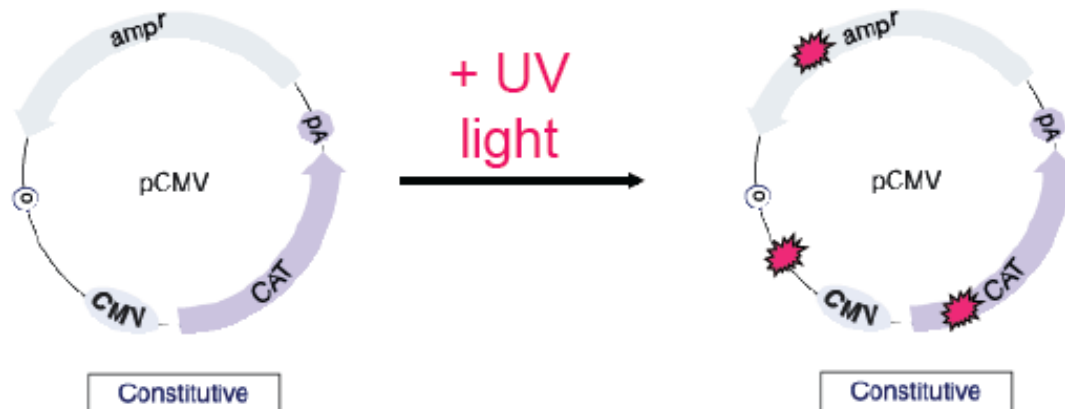
If the links below do not work, the easiest way to locate each paper is to type the "PMID" (PubMed identifier) in at the [PubMed website](#). If that approach gives you an error for some reason, or in future cases where you might not know the PMID, you can try typing the title of your article into PubMed to find it. If you have trouble accessing your article directly from there, go to <http://libraries.mit.edu/vera>, which is MIT's collection of journals online. Try selecting "exact title" from the search pulldown menu if the name of your journal is a common word such as *Science*. For older articles, you need to choose the JSTOR rather than Highwire interface.

1. Ahmed, E. A. et al. *DNA double strand break response and limited repair capacity in mouse elonga*. Mol Sci. PMID:26694360 [↗](#)
2. Banerjee, R. et al. *TRIP13 promotes error-prone nonhomologous end joining and induces chemore cancer*. (2014) Nat Comm. PMID:25078033 [↗](#)
3. Chang, H-Y. et al. *RON nuclear translocation under hypoxia potentiates chemoresistance to DNA d anticancer drugs*. (2016) Mol Cancer Ther. PMID:26772202 [↗](#)
4. Chang, C-F. et al. *PHRF1 promotes genome integrity by modulating non-homologous end-joining*. (2016) [↗](#)
5. Chen, P. et al. *Thrombospondin-1 might be a therapeutic target to suppress RB cells by regulating t repair*. (2016) PMID:26756218 [↗](#)
6. Gelot, C. et al. *The cohesin complex prevents the end joining of distant DNA double-strand ends*. (2016) [↗](#)

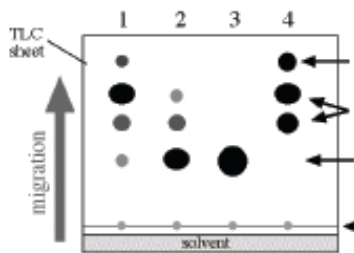
Slot	Day 4 (T/R)	Day 8 (T/R)	Day 4 (W/F)	Day 8 (W/F)
1				
2				
3				
4				
5				
6				
7				

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

Athas & GROSSMAN
Cancer Res. 1991



Transient transfection peripheral blood lymphocytes



CAT Assay

enzyme activity=repair
no enzyme activity=no repair

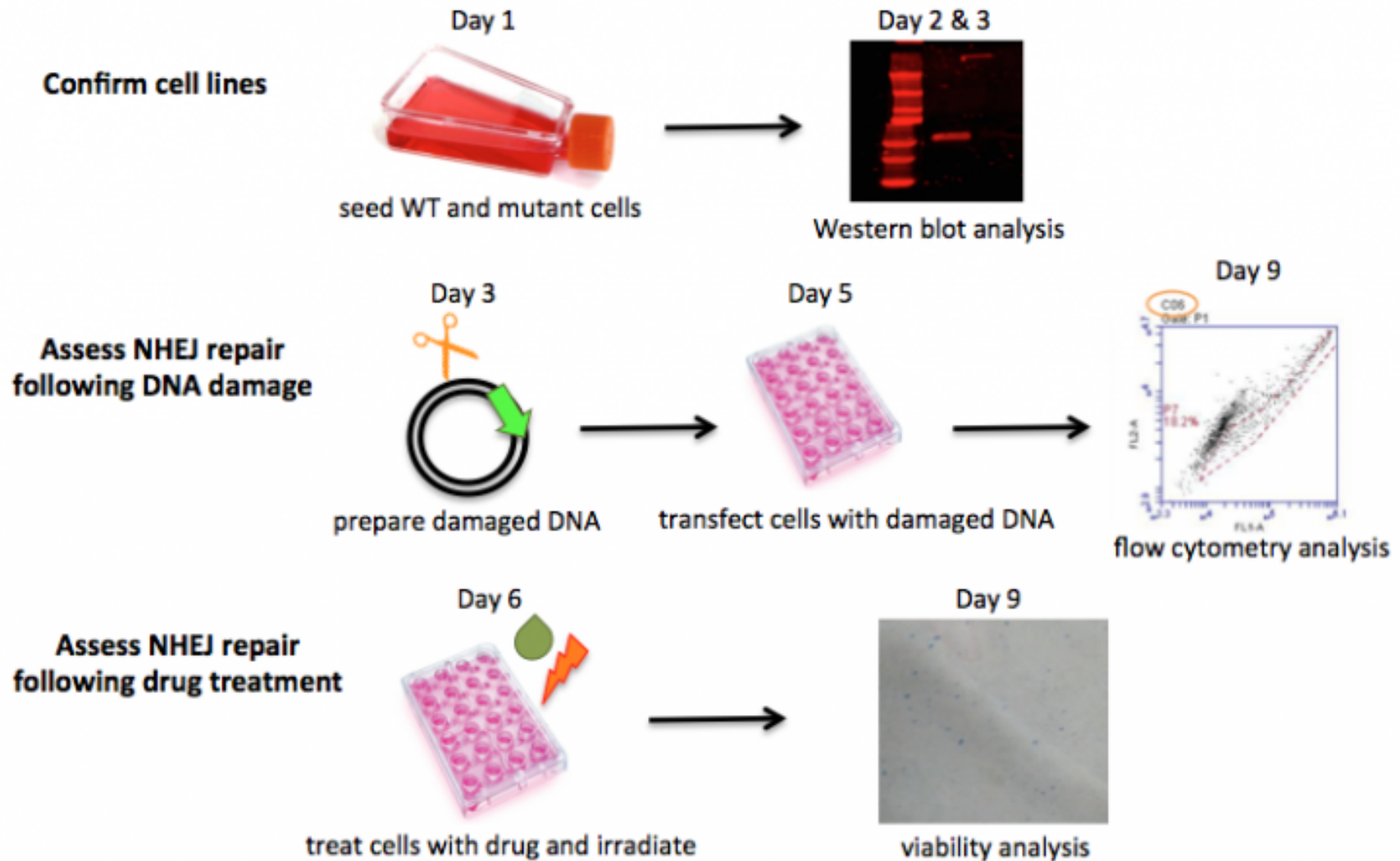


Time to repair



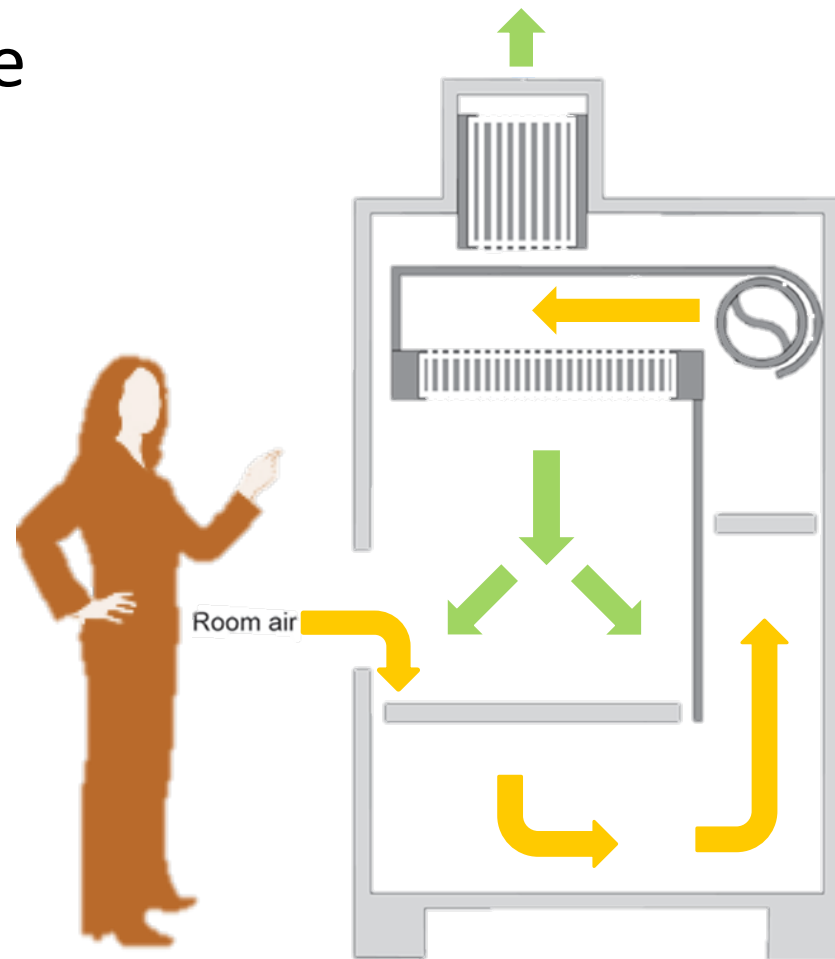
Slide from M2D1 Lecture — Prof. Samson

Mod 2 experimental overview



Mammalian cell culture: Biosafety Cabinet or “hood”

- Wipe cabinet with 70% EtOH before and after use
- Wipe everything that enters the cabinet with 70% EtOH
- Do not block grille or airflow slots
- Minimize side-to-side arm movements



Tissue Culture Medium

What do cells need to survive? (Food)

DMEM/F12: -Dulbecco's Modified Eagle Media
-Ham's F12

-amino acids, glucose, salts, vitamin's, buffer



FBS: **Fetal Bovine Serum**

-growth factors
-cytokines
-lipids
-cholesterol



(Non-food)

Antibiotics: penicillin
streptomycin

-prevent growth of all bacteria media



NEAA: **Non Essential Amino Acids**

-increases growth rate to supplement NEAA



"Splitting" cells

(lots of jargon!)

(why?)

1. Look at cells, estimate confluence

estimate the growth rate, cell count, health

2. Rinse with PBS

wash away anti-trypsin agents, extra serum

3. Detach cells with trypsin

break substrate/cell adhesions

4. Count cells

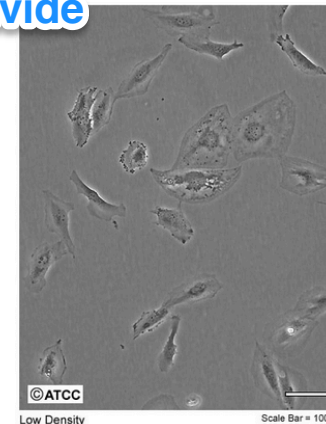
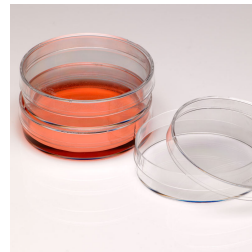
seed a specific # in a new vessel

5. "Seed" new culture vessel room to divide

Flask

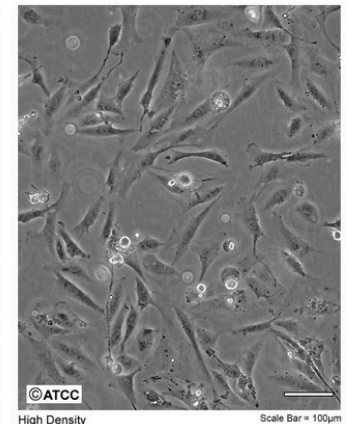


Dish



Low Density

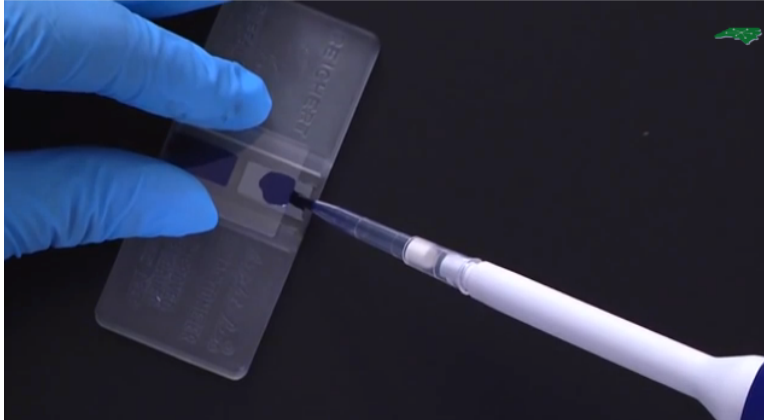
Scale Bar = 100µm



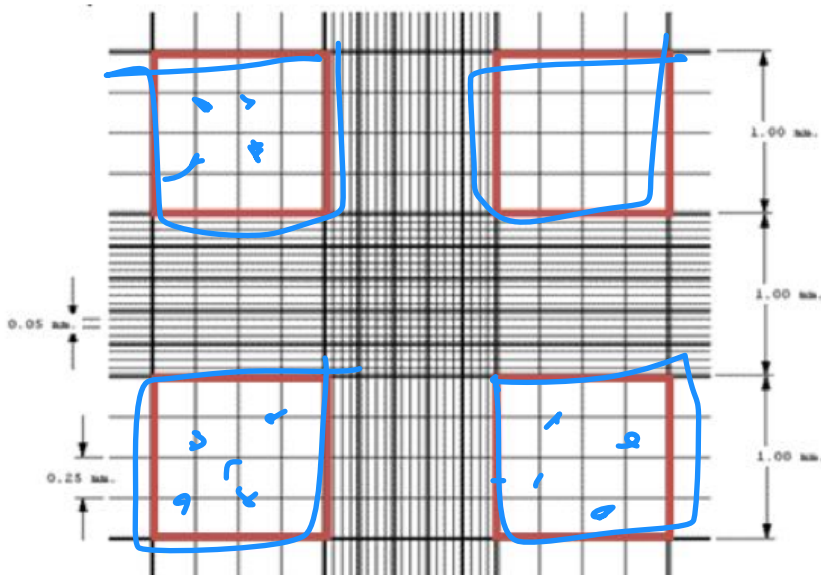
High Density

Scale Bar = 100µm

Counting Cells



- pipette 10ul onto hemocytometer
- count cells in 4 corners
- average the cell # in 4 corners
- average #x10,000=#cells/ml



Today in lab

- Tissue Culture (TC)
 - Protocols printed for TC use, no need to move laptops etc.
 - Do not wear PPE in or out of TC room!
 - Group order to TC:
 - 1st: Red, Orange, Pink, Purple
 - 2nd: Yellow, Green, Blue