M1D1: Prepare CometChip microwell array and practice cell culture

09/12/18

1. Lab Orientation Quiz
2. Pre-lab Discussion
3. ½ class goes to the Tissue Culture Room
4. ½ class prepares a CometChip

Office Hours

Noreen
Monday 2pm-5pm
in 16-317

Leslie
Thursday 2-3pm
Friday 12-1pm
in 56-341c

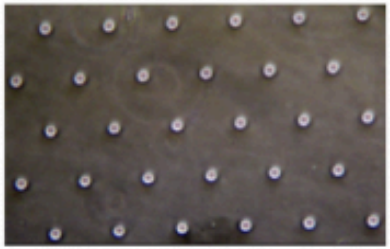
Josephine
Wednesday 12-1pm
Friday 2-3pm

by appointment: nllyell@, lesliemm@, joshaw@

M1 major assignments

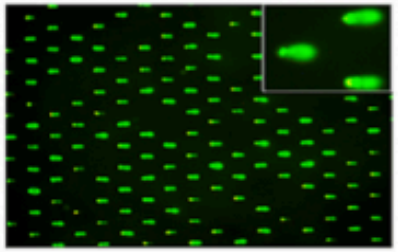
- Data summary (15%)
  - In teams, submit on Stellar
  - Draft due 10/8, final revision due 10/20
  - Bullet points, .PPTX
- Mini-presentation (5%)
  - Individual, submit video via Gmail
  - Due 10/13
- Lab quizzes –be on time!
  - M1D4 and M1D7
- Notebook (part of 10% Homework and Notebook)
  - Due 10/5 at 10pm, graded by Jai
- Blog: https://be20109f18.blogspot.com (part of 5% Participation)
  - by 10/9

Overview of
Module 1: Measuring Genomic Instability



1. Optimize comet chip assay

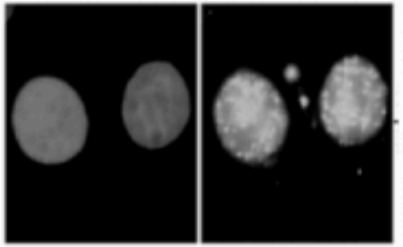
- Test loading variables



2. Use comet chip assay to measure DNA damage

- Measure effects of H_2O_2 on +/- DNA-PK cell lines

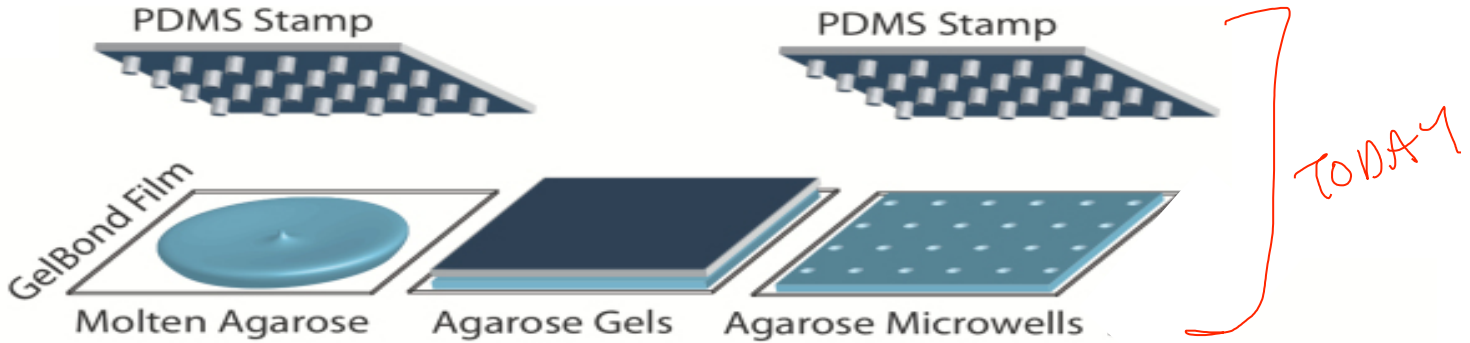
Protein involved in DNA repair

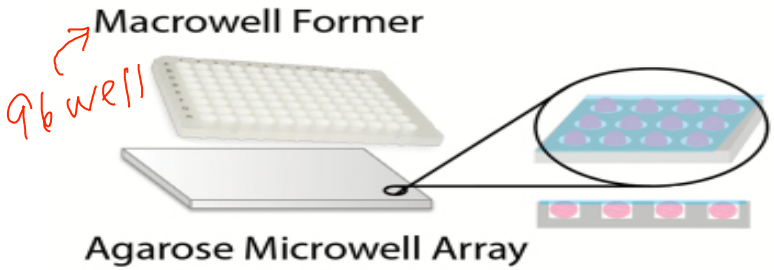


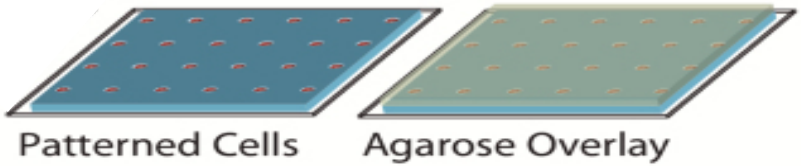
3. Use immuno-fluorescence assay to measure DNA damage

- Examine effect of H_2O_2 on γH2AX foci formation

Overview of this week:
Create a CometChip & optimize cell loading





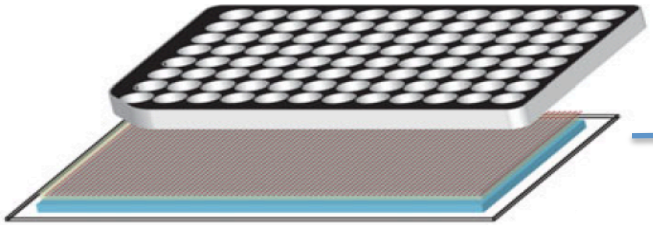


What is the minimum number of cells needed in each macrowell to obtain efficient loading?

Overview of next week:

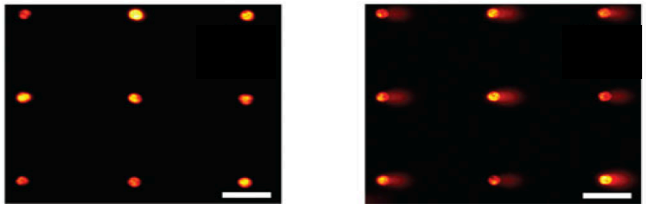
Assess DNA damage in tumor cells with & without DNAPKcs

Treat captured cells in comet chip with H_2O_2 (oxidative damage)

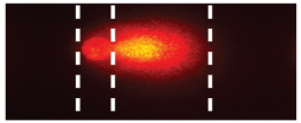


Lyse cells & unwind DNA (DNA still captured agarose in overlay)

Agarose Electrophoresis

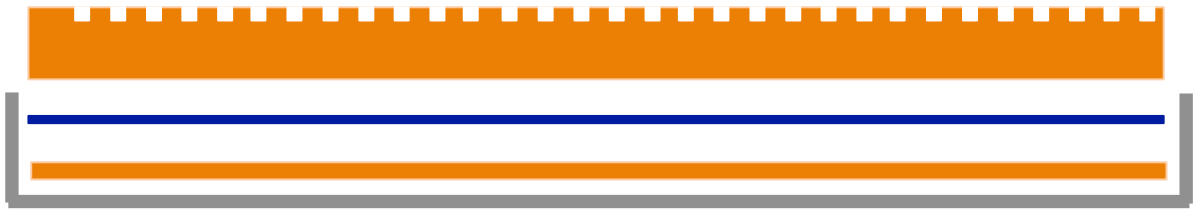


Stain DNA and image via fluorescence microscopy



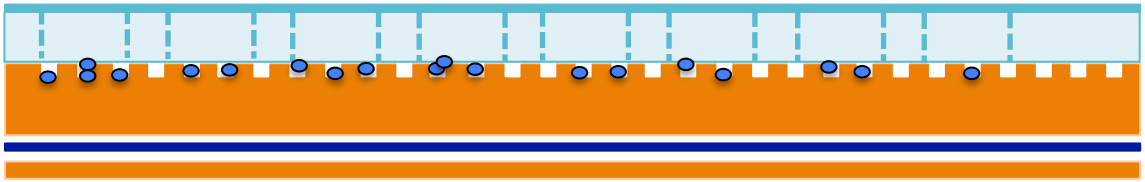
Analysis via Matlab

Today, make a CometChip



stamped
13ml 1% NMP agar
Gelbond
2.5ml 1% NMP agar
Plastic dish

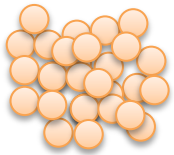
Next time, load cells



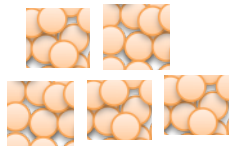
Bottomless
96-well plate

Background: Two glial cell lines— M059J (-DNAPKcs) and M059K (+DNAPKcs)

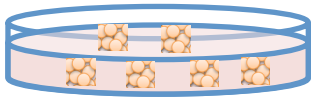
Tumor specimen from 33 year old male with untreated glioblastoma



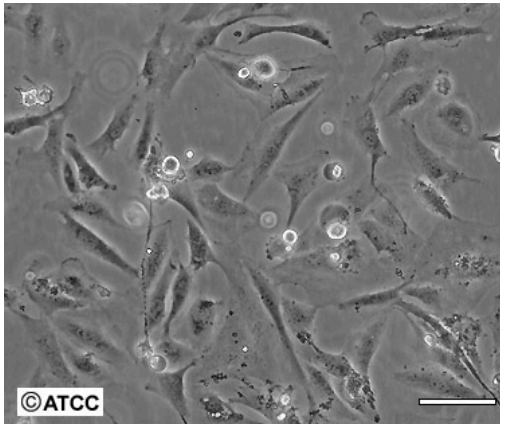
Miced into cubes



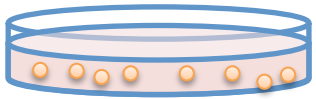
Maintain cultures in dish



M059 K



Dissociate with enzymes



Maintain cultures in dish

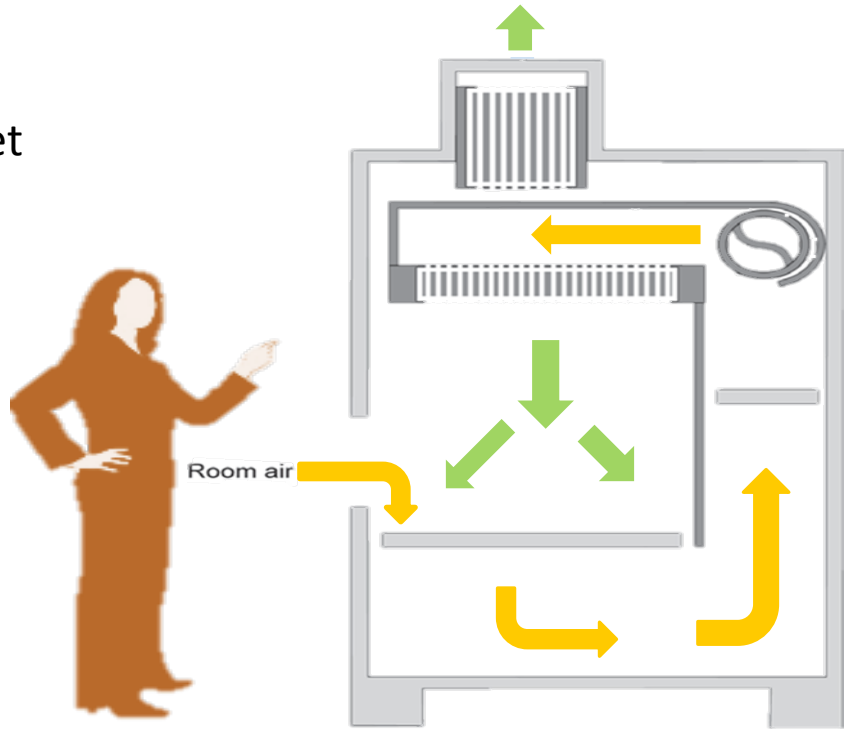
M059 J

deficient in DNApkcs

Allanlunis-Turner, et al. *Radiation Research*. 1993.

Tissue culture sterile technique

- **70% ethanol** everything:
  - Wipe cabinet before and after use
  - Wipe everything that enters the cabinet
  - Do not spray cells with EtOH
- **Do not disturb air flow:**
  - Do not block grille or slots
  - Minimize side-to-side arm movements
  - Work > 6” away from sash
  - Leave blower *on always*
- Do not talk into incubator!
- Only open sterile media in hood



Mammalian cell culture medium

What do cells need to survive? growth/divison/viability

Food:

- DMEM/F12(Dulbecco's Modified Eagle's Medium and Ham's F12 medium)
  Defined
  sugar
  AA(amino acids)
  salts
  vitamins
  phenol red - pH indicator
- FBS (fetal bovine serum) undefined
  growth factors
  cytokines
  cholesterols
  lipids

Non-food:

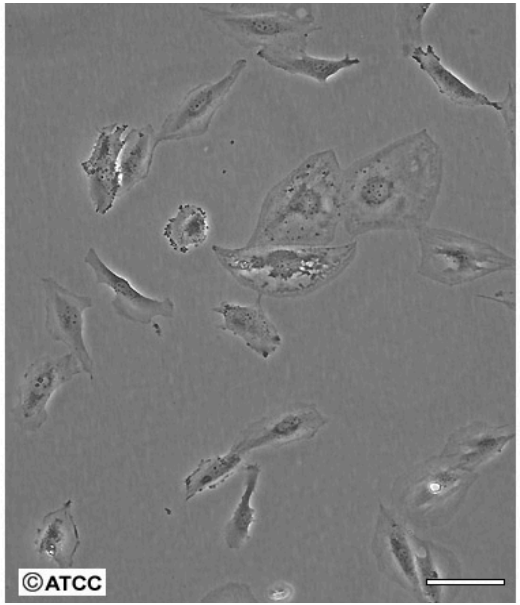
- antibiotics:
  - penicillin
  - streptomycin

prevent bacterial growth

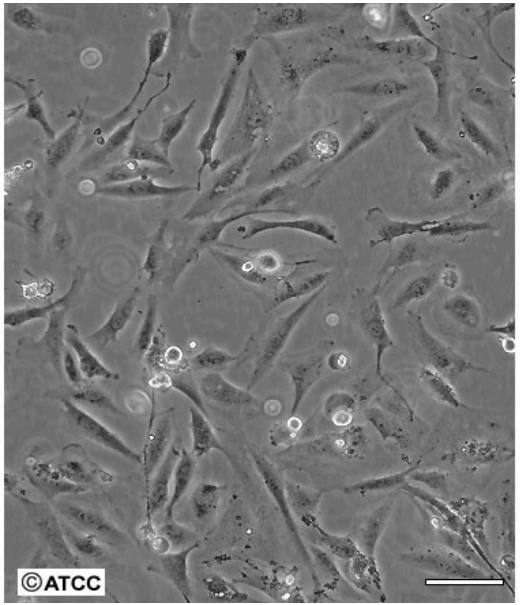
Mammalian cell culture terminology

- Confluence = density
- Splitting = subculturing
  put cells on new dish
  ~80% confluent, split
- Seeding
  ~20-40% of confluent culture

Low Density



High Density



General steps for splitting cells +WHY?

Be sure not to mix the two cell lines!

1. Look at cells, estimate confluence

2. Rinse with PBS
   wash media/debris, remove trypsin agents
   extra protein

3. Detach cells with trypsin (enzyme cuts protein)
   break ~~substrate~~ cell adhesions

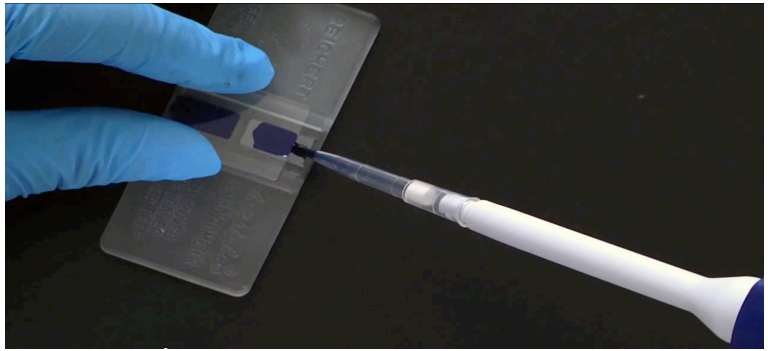
4. Count cells
   seed specific # in new vessel

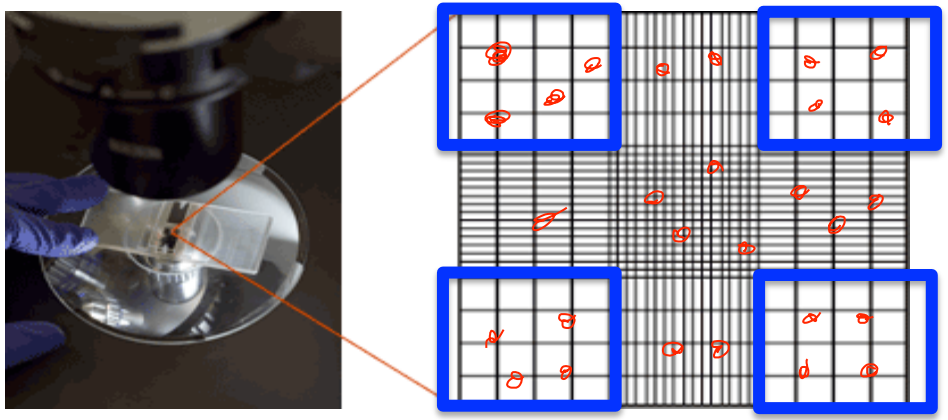
5. "Seed" new culture vessel
   room to divide + grow

T25 25cm^3

T75 75cm^3

Counting Cells





- Hemocytometer: Count cells
- Trypan blue: Stains dead cells
- # cells / mL = 10,000 x average of 4 corners

16 / 4 = 4

4 x 10,000 = 40,000 / mL

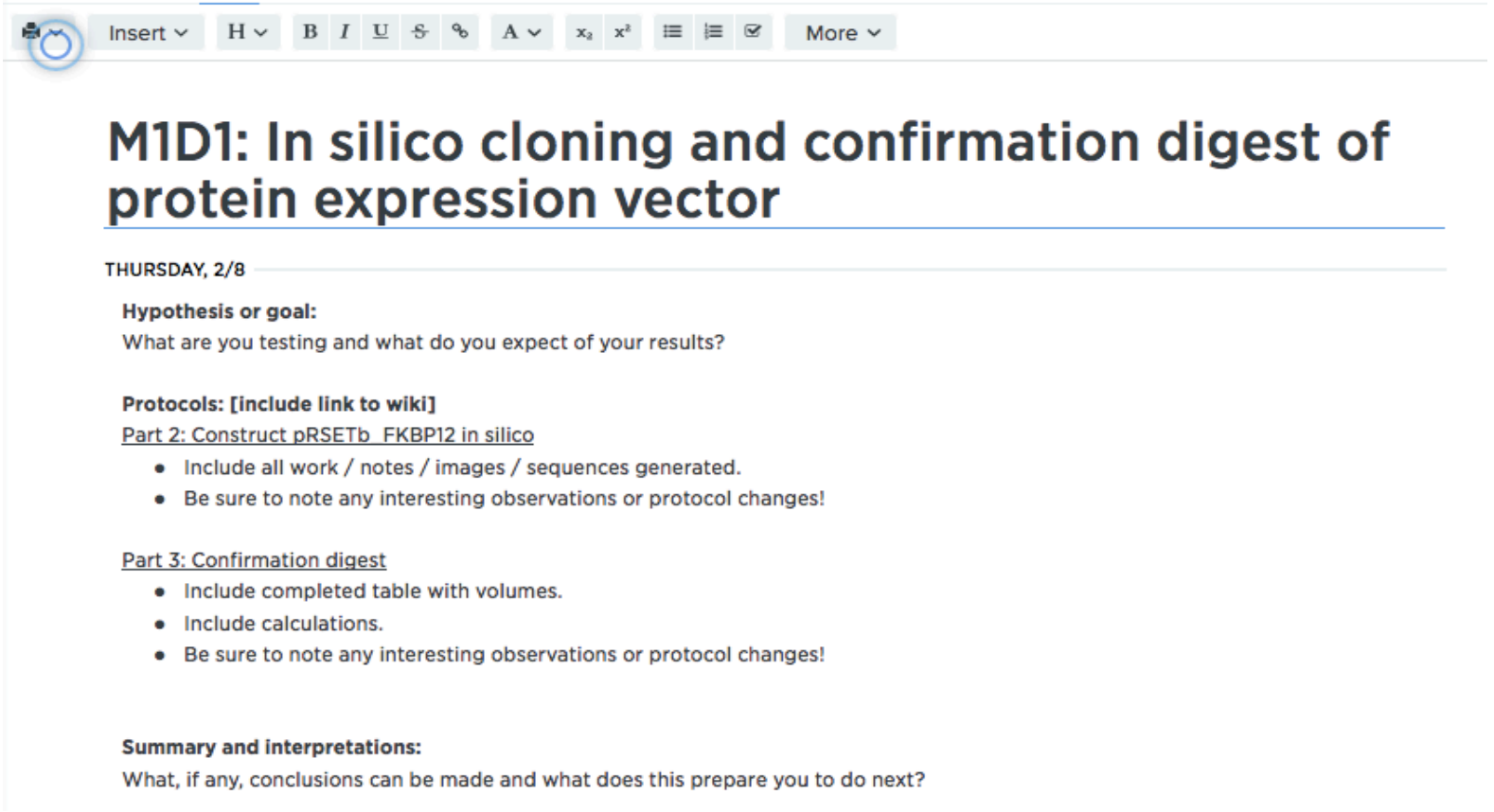
What should go in your notebook?

Laboratory notebook entry component:	Points: Complete	Partial	Incomplete
Date of experiment (include Module#/Day#) and Title for experiment	1	0.5	0
Hypothesis or goal / purpose	1	0.5	0
Protocols (link to appropriate wiki sections)	1	0.5	0
Notes on protocol changes / clarifications	1	0.5	0
Observations	2	1	0
*Visual details			
*Qualitative information			
*Raw data			
Data analysis	3	1.5	0
*Calculations			
*Graphs and Tables			
Summary and interpretation of data	3	1.5	0
*What did you learn?			
*How does this information fit into the larger scope of the project?			
Information is clear	2	1	0
All days represented	1	0.5	0
OVERALL /15			

Due 10pm after each module, as posted on wiki

http://engineerbiology.org/wiki/20.109(F18):_Assignments

How should you format your notebook?



How should you organize your notebook?

- Entitle your project “20.109(F18)_YourName”
  - Make each module a new folder
  - Make each day a new entry within module folder
- Share the project with Leslie and Jai
  - Right-click and choose ‘settings’
  - Add collaborators by email address

Today in lab:

1. 2 teams into tissue culture room to split cells (Yellow,Green)
2. 2 teams start preparing CometChip (Blue, Pink)
3. Make sure to keep notes in Benchling!

- Watch Engleward lab JOVE video during downtime https://www.jove.com/video/50607/cometchip-high-throughput-96-well-platform-for-measuring-dna-damage
- M1D2HW: Create a template for your benchling notebook and make a M1D2 entry from it.