M1D1: Practice cell culture

09/10/19

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

Ch

Office Hours

Noreen
Wed 10a-12pm
Fri 10a-12pm
in 16-317

Leslie
Wed 9a-10am
Fri 4p-5pm
in 16-469

Becky
Tues 12p-1pm
Thurs 12p-1pm
in 16-469 (16-220)

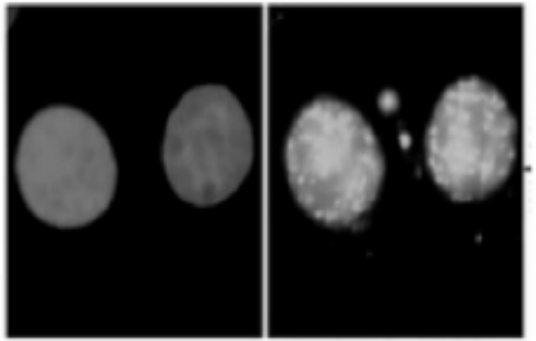
by appointment: nllyell@, lesliemm@, rcmeyer@

Module 1 assignments

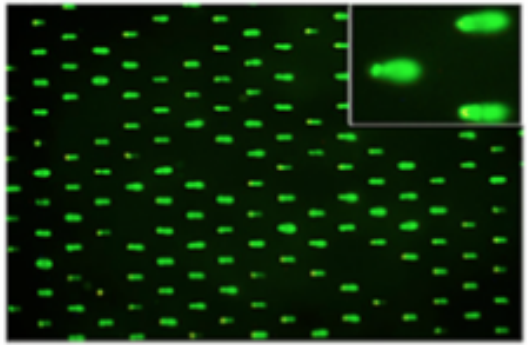
- Data summary (15%)
  - **In teams**, submit on Stellar
  - Draft due 10/14, final revision due 10/26
  - Format: Bullet points, .PPTX
- Mini-presentation (5%)
  - Individual, submit video via Gmail
  - Due 10/19 at 10pm
- Lab quizzes –be on time!
  - M1D4 and M1D7
- Notebook (part of 10% Homework and Notebook)
  - Due 10/4 at 10pm, graded by Shelbi
- Blog (part of 5% Participation)
  - by 10/15 at 10pm

Overview of Module 1: Measuring Genomic Instability

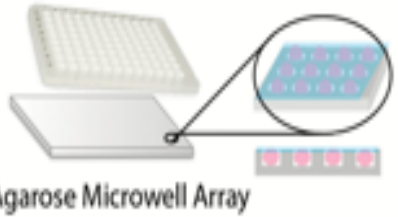
We will quantify methylation DNA damage in mammalian cells in the presence of Arsenite.

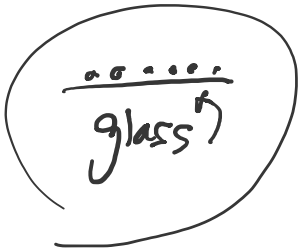


γH2AX assay:
Immuno fluorescence of cellular response to DNA damage



CometChip assay:
single cell gel electrophoresis measuring single strand breaks



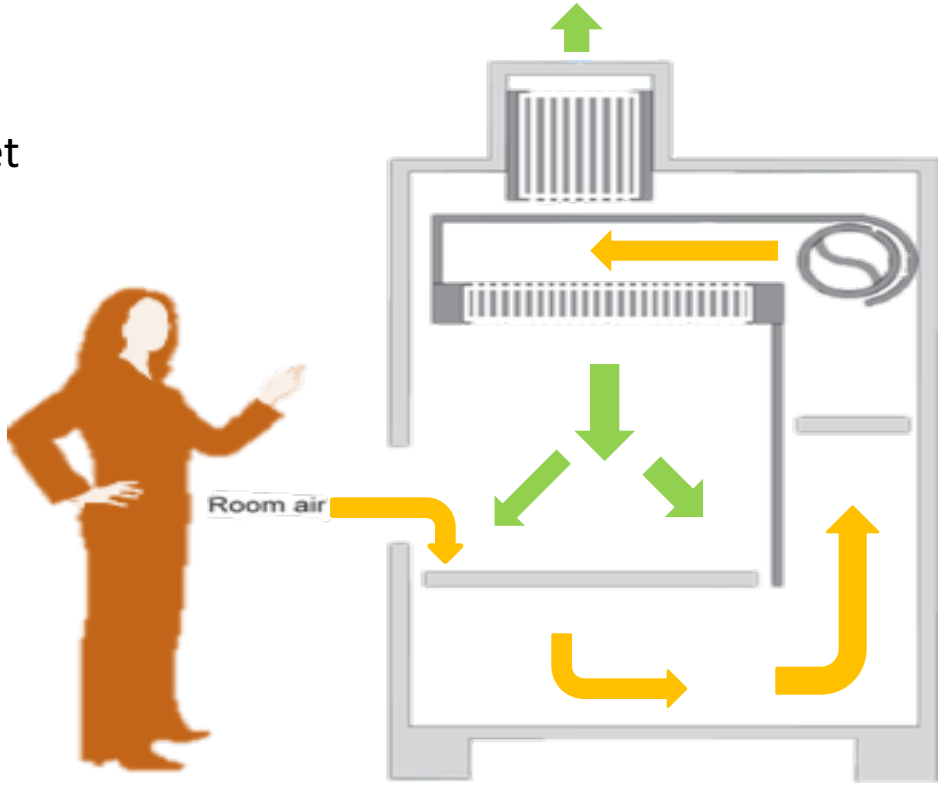


Tissue culture sterile technique

- **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



Chinese hamster ovary cells (CHO) are a commonly used lab cell line, What do CHO cells need to survive?

Food: Defined

- DMEM (Dulbecco's Modified Eagle's Medium)
  - sugar
  - salts
  - amino acids
  - pH buffer ~7.5
  - vitamins
  - phenol red – pH indicator
- FBS (fetal bovine serum) undefined
  - cholesterol
  - lipids
  - cytokines
  - growth factors

Non-food:

- antibiotics: prevent bacterial growth
  - penicillin
  - streptomycin

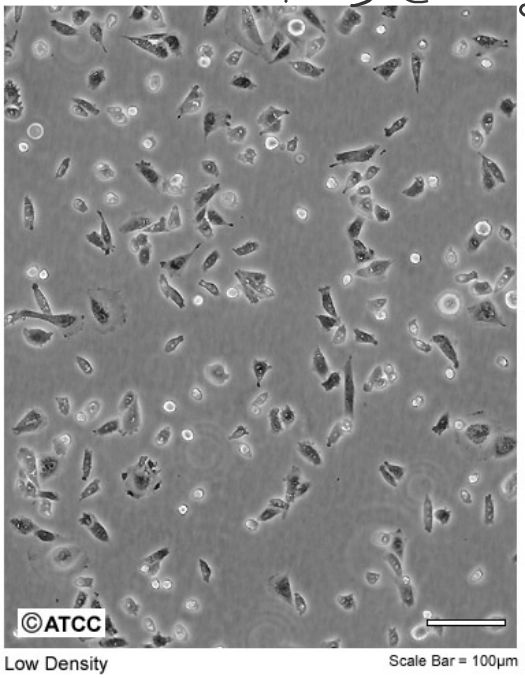
Mammalian cell culture terminology

Seed new when ~80% conf.

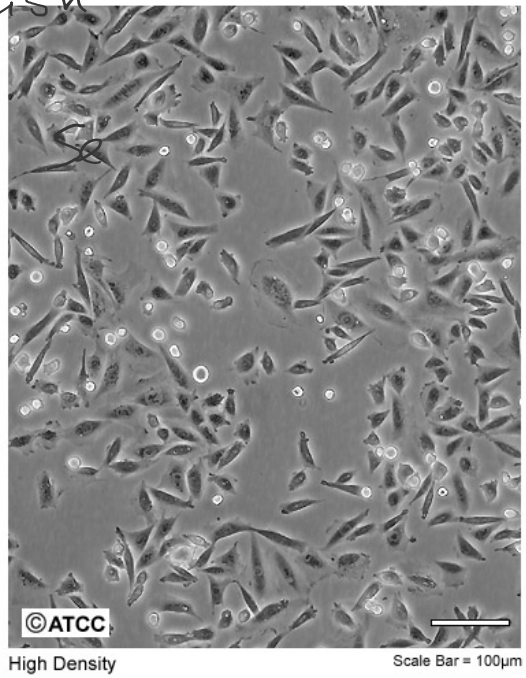
- Confluence
  density
- Splitting
  subculturing
- Seeding
  moving cells to new vessel

Use ~20–40% culture to seed new dish

Low Density



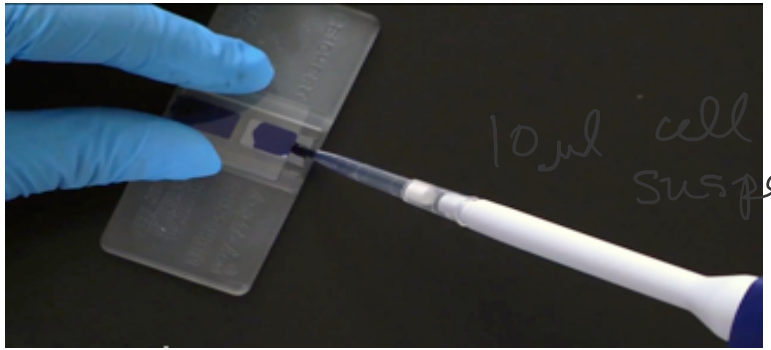
High Density

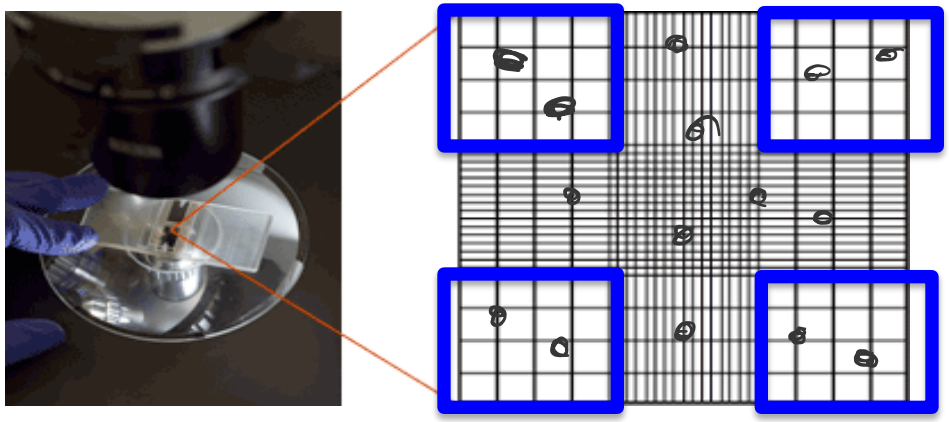


General steps for splitting cells +WHY?

1. Look at cells, estimate confluence
   note cell shape, media color
2. Rinse with PBS
   remove extra protein, debris, α-trypsin
3. Detach cells with trypsin (enzyme)
   break substrate adhesions
4. Count cells
   accurate # to seed in new vessel
5. "Seed" new culture vessel
   give them room to grow or carry out experiment

Counting Cells





- Hemocytometer: cell counting device
- Trypan blue: stain for dead cells
- # cells / mL = 10,000 x average of 4 corners

8/4 = 2 x 10,000 = 20,000 cells/mL

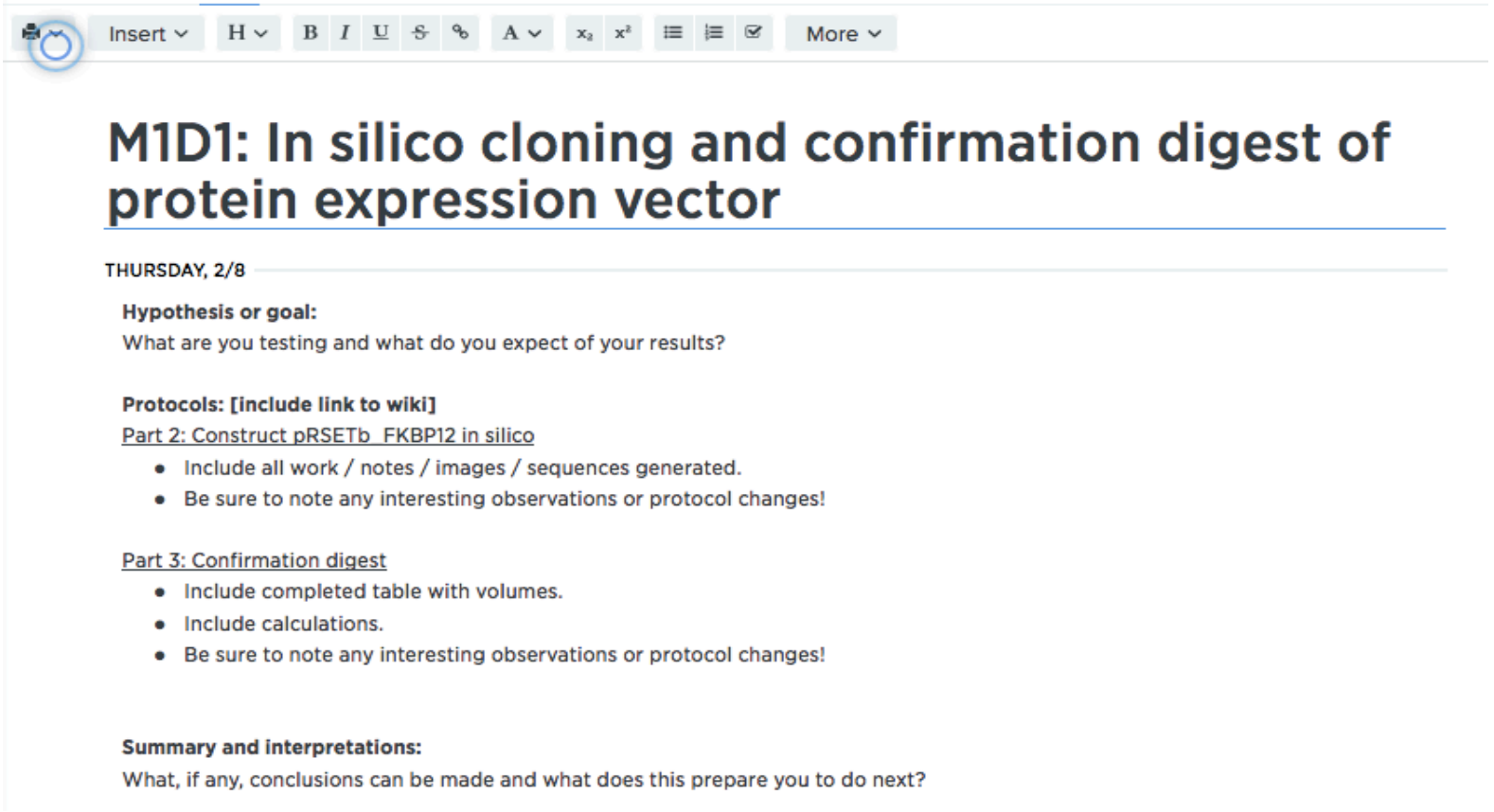
What should go in your notebook?

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(F19):_Assignments

How should you format your notebook?



How should you organize your notebook?

- Entitle your project “20.109(F19)_YourName”
  - Make each module a new folder
  - Make each day a new entry within module folder
- Share the project with Leslie and Shelbi
  - Right-click and choose ‘settings’
  - Add collaborators by email address

Today in lab:

1. 3 teams into tissue culture room to seed cells for H2AX expt. (Orange, Yellow, Green)
2. 3 teams review CometChip Assay
3. Make sure to keep notes in Benchling today

- HW due M1D2: Create a template for your benchling notebook and make a M1D2 entry from it. For full credit you must include calculations necessary to complete the table in Part 1 on M1D2.

Condition	EdU (final concentration = 5 µM)	As (final concentration = 80 µM)	Media	Final volume (1 mL / well)
No treatment control (no MMS, no As)	1 µL	0	2 mL	2 mL
MMS, no As				
no MMS, As				
MMS, As				