M1D1: Practice cell culture and begin sub-nuclear foci assay

9/11/19

1. Orientation Quiz
2. Prelab discussion
3. Learn about cometchip assay
4. Tissue culture practice
    a) Seed for gamma-H2AX assay

Office Hours

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

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

Becky
Tues 12-1pm
Thurs 12-1pm
in 16-220

by appointment: nllyell@, lesliemm@, rcmeyer@

Mod 1: Major Assignments

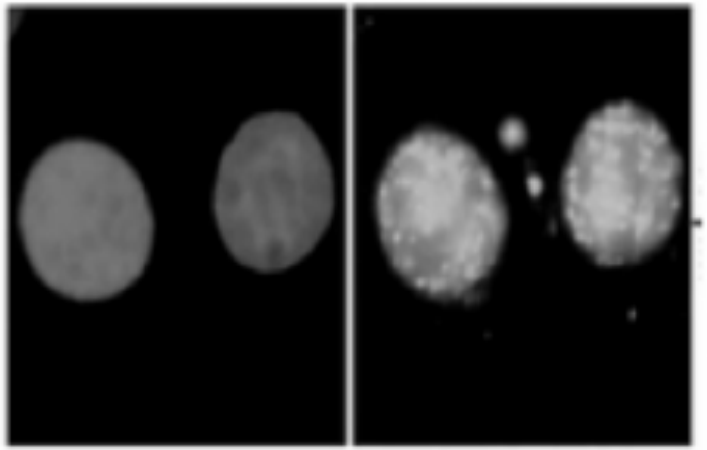
- Data summary (15%)
  - In a team, submit on Stellar
  - Draft due 10/14, final revision due 10/26
  - Format: Bullet points, .PPTX
- Mini-presentation (5%)
  - Individual, submit video via Gmail
  - Due 10/19 by 10pm
- Lab quizzes –be on time!
  - M1D4 and M1D7
- Notebook (part of 10% Homework and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

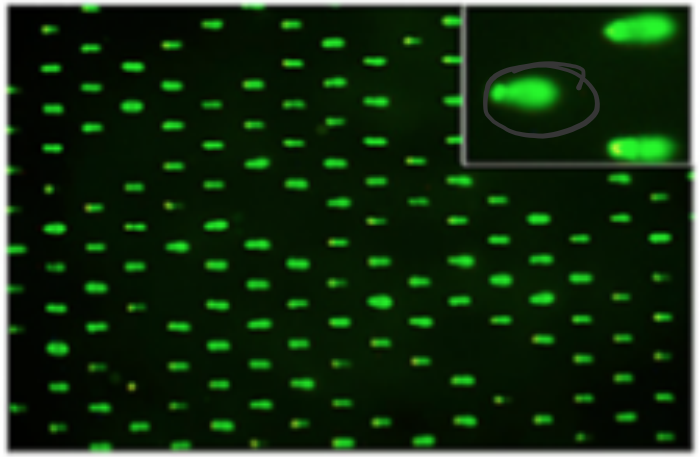
Elevator pitch

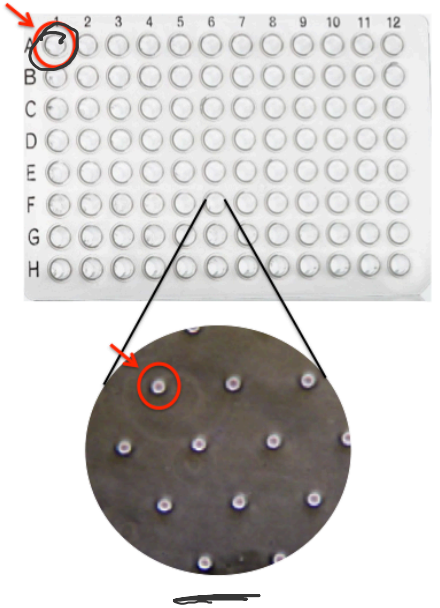
Overview of Module 1: Measuring Genomic Instability

Quantify DNA damage in mammalian cells following exposure to methylating agent and arsenite

Why these chemicals?







γH2AX assay:

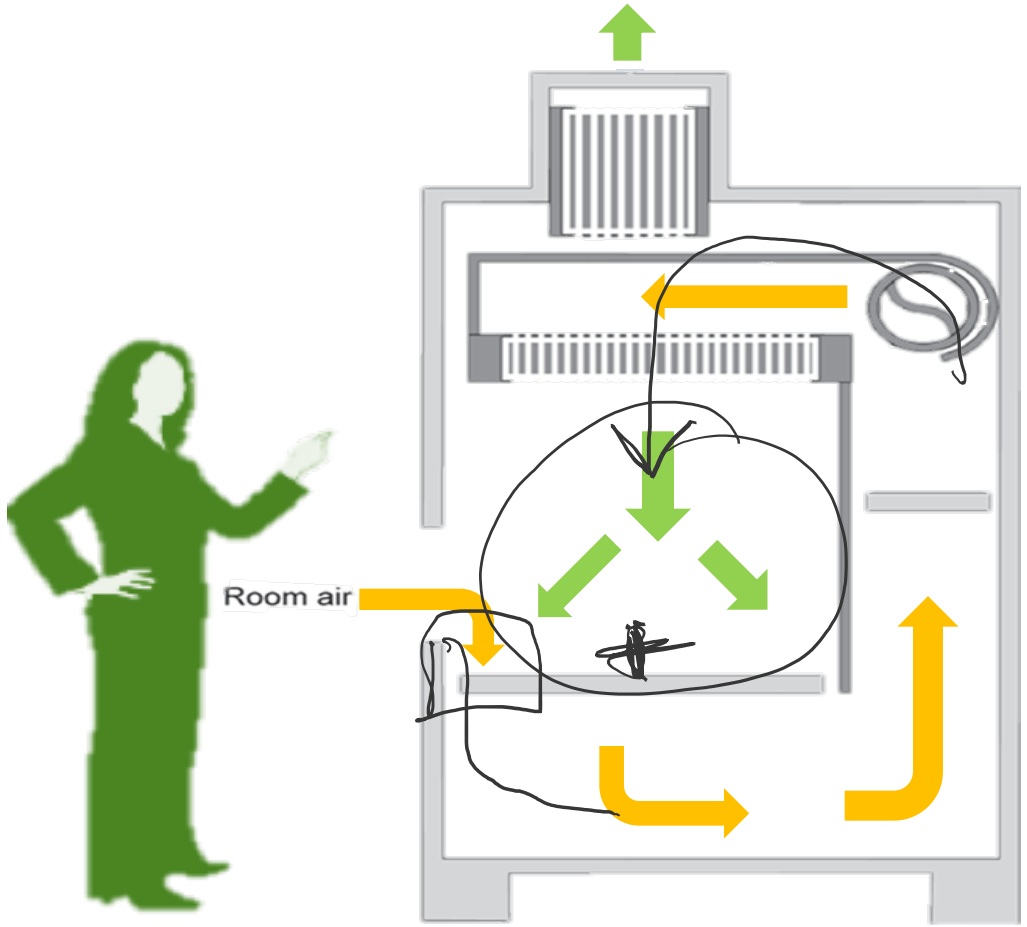
- Immunofluorescence
- Cellular response to DNA damage

CometChip assay:

- single cell gel electrophoresis
- 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



Mammalian Cell Culture Medium

We are using CHO cells

Food:

- DMEM (Dulbecco's Modified Eagle's medium)
  - Defined

sugar, amino acids, vitamins, salts, phenol red = pH indicator

- FBS (fetal bovine serum)
  - Undefined

growth factors, lipids, cholesterol

Non-food:

- antibiotics:
  - penicillin
  - streptomycin

] bacterial growth

Mammalian Cell Culture Terminology

80% confluent
↳ split them

- Confluence

Density

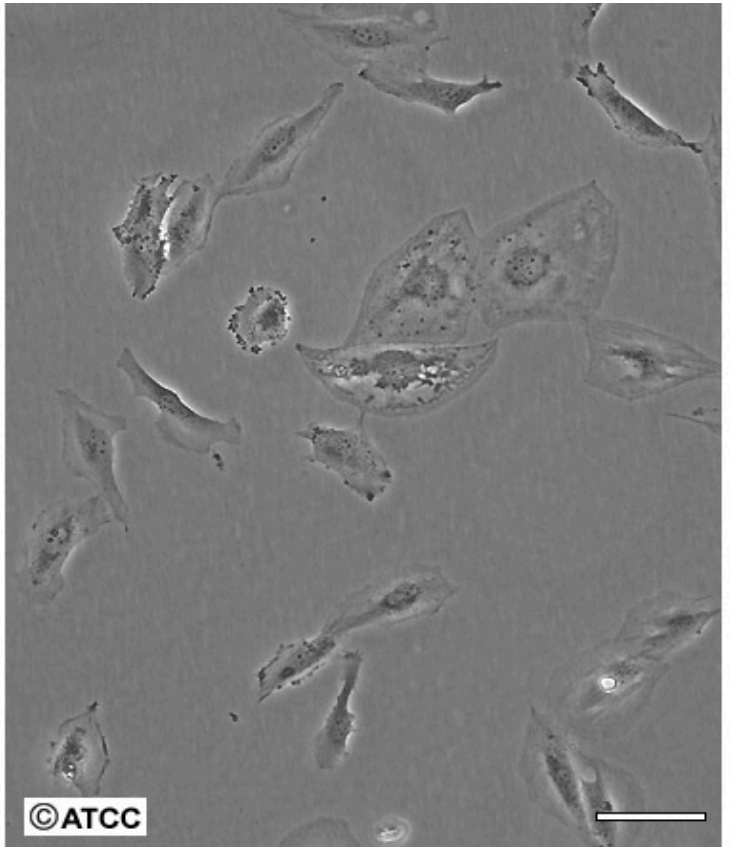
- Splitting

Subculturing

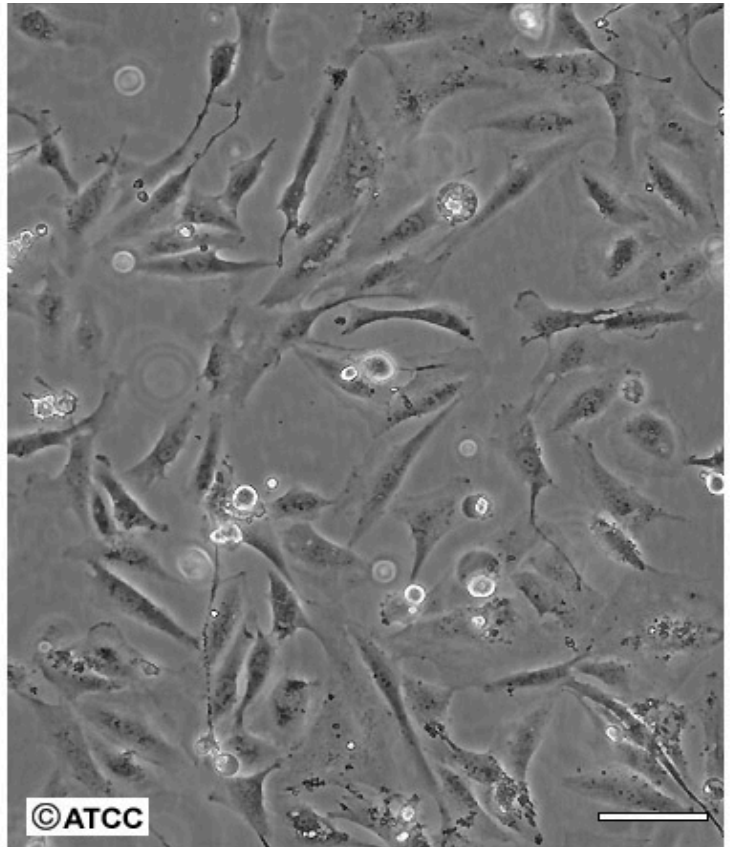
- Seeding

Moving cells to a new container

Low Density



High Density



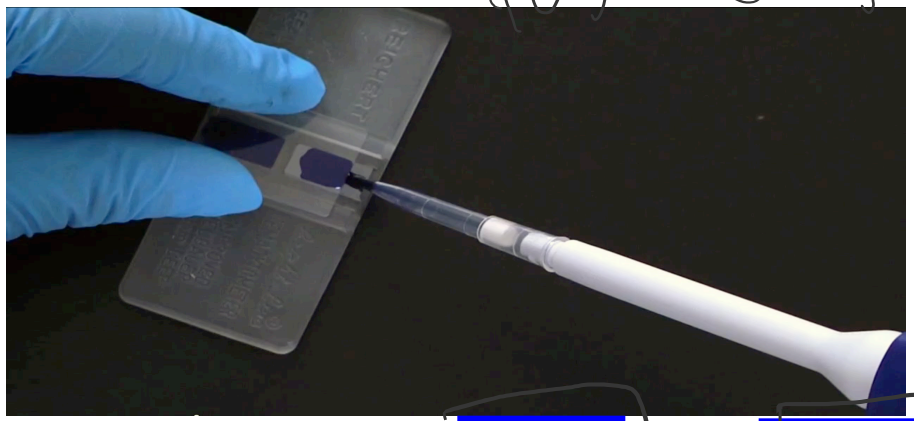
General steps for splitting cells

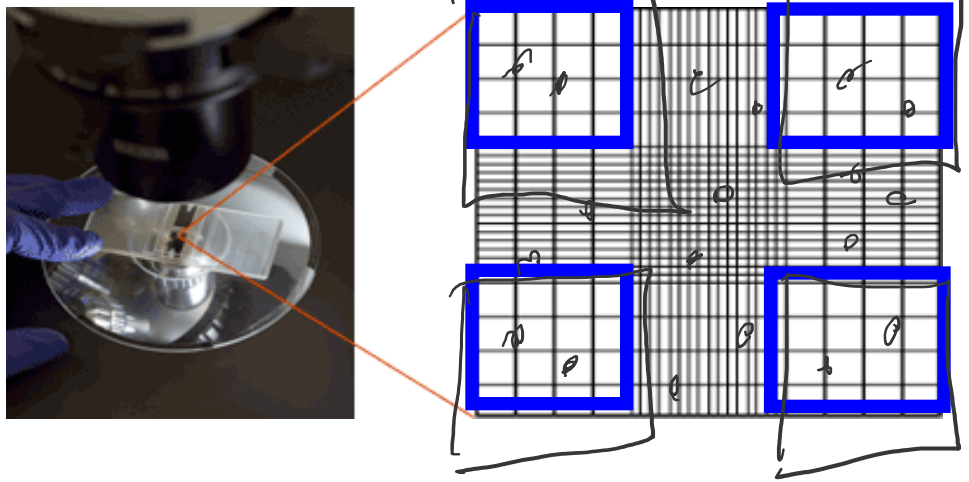
Why do we do each step?

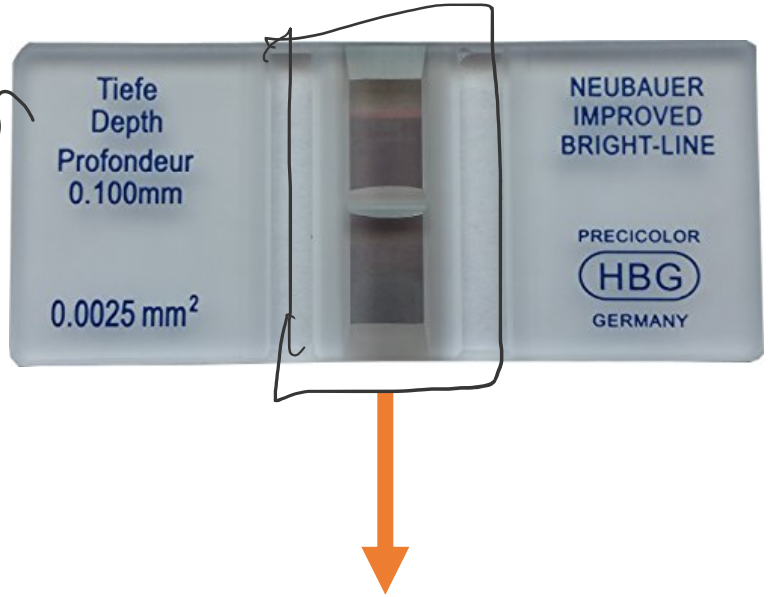
1. Look at cells, estimate confluence
   healthy, confluent
   media color
   1b. Remove media - aspirator
2. Rinse with PBS
   washing off debris
3. Detach cells with trypsin / anti-trypsin
   3b. Incubator for 2 min (enzyme)
4. Count cells
   know how many
5. "Seed" new culture vessel

Counting cells

10 µl cell suspension







- Hemocytometer
- Trypan blue → cells dead 10%

cells / mL = 10,000 x average of 4 corners

2x4 = 8/4 = 2x10,000 = 20,000 cells/mL

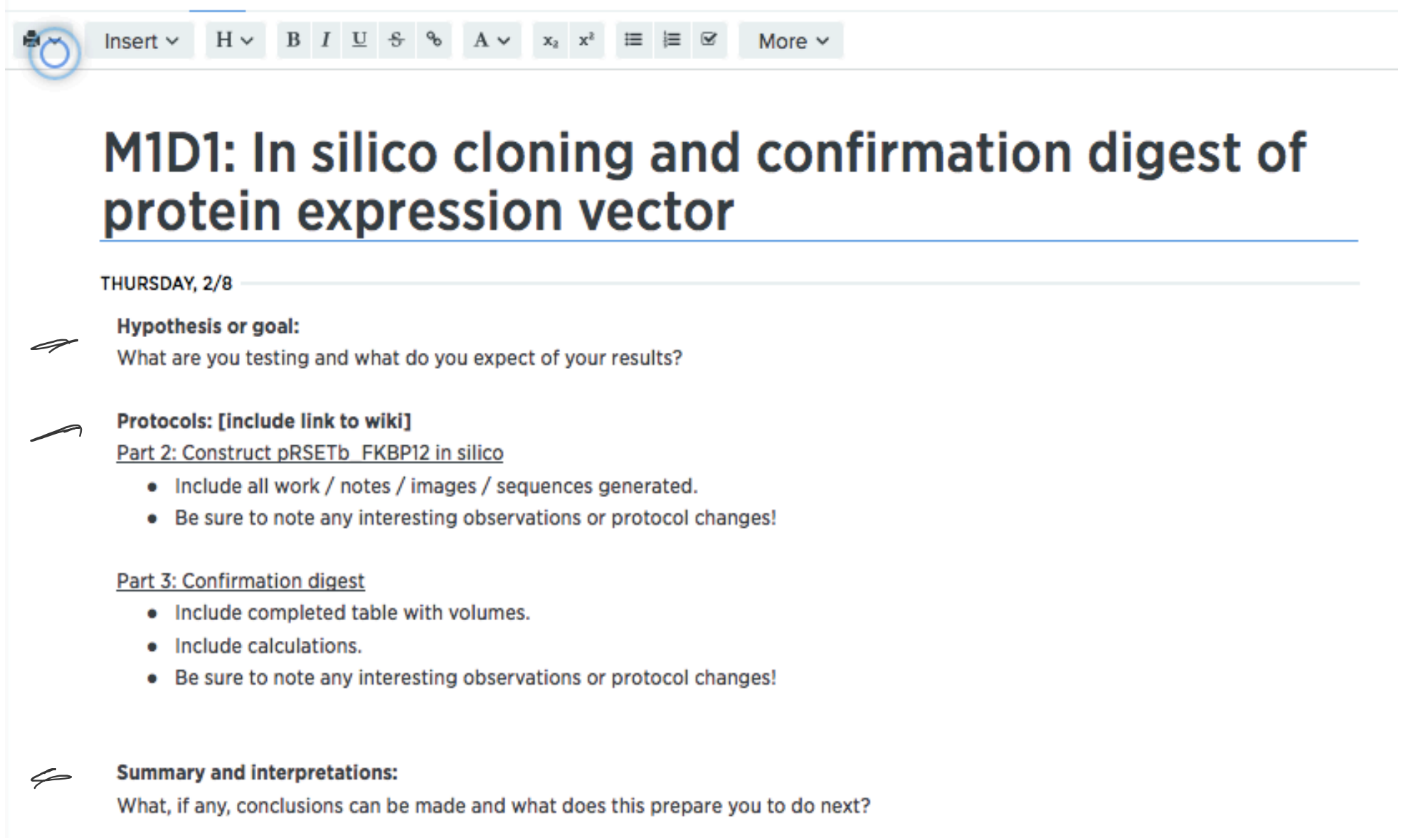
Upcoming assignments: What should go in your notebook?

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(F19)_YourName”
  - Make each module a new folder
  - Make each day a new entry within module folder

- Share the project with Becky and Colin (if you have not already)
  - Right-click and choose ‘settings’
  - Add collaborators by email address
    - rcmeyer@mit.edu
    - cyhkim@wi.mit.edu

Today in Lab

1. TC practice
   - Estimate cell size
   - Split and seed CHO cells
2. Make sure to keep notes in Benchling!
3. Review Cometchip assay and watch Engleward lab JOVE video

(https://www.jove.com/video/50607/cometchip-high-throughput-96-well-platform-for-measuring-dna-damage)

M1D2 HW: 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				