

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)

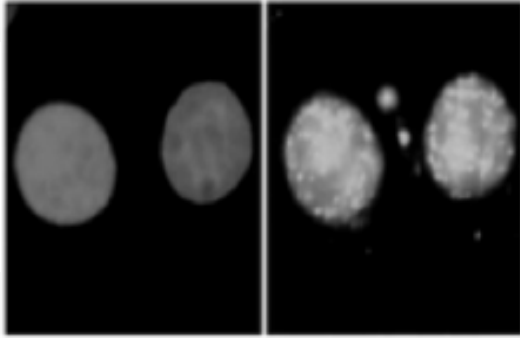
by appointment: nlllyell@, lesliemm@, rcmeyer@

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

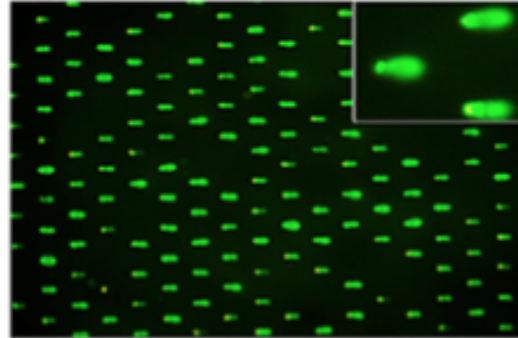
Overview of Module 1: Measuring Genomic Instability

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



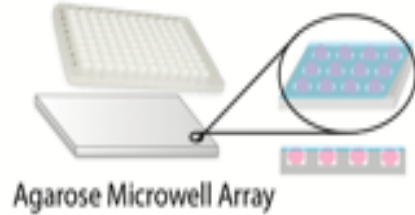
γ H2AX assay:

Immuno fluorescence of
cellular response to DNA
damage



CometChip assay:

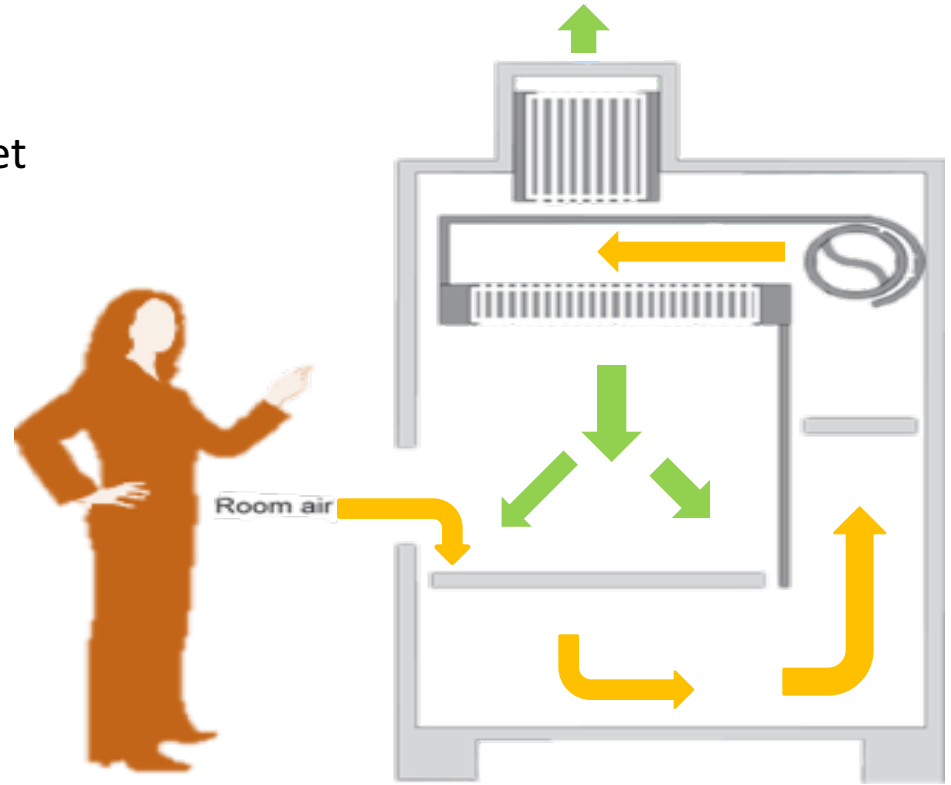
single cell gel electrophoresis
measuring single strand breaks



glass

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

phenol red - pH indicator

salts

amino acids

pH buffer ~ 7.5

vitamins

- FBS (fetal bovine serum) *undefined*

cholesterol

lipids

cytokines

growth factors

Non-food:

- antibiotics: } *prevent bacterial growth*
 - penicillin
 - streptomycin

Mammalian cell culture terminology

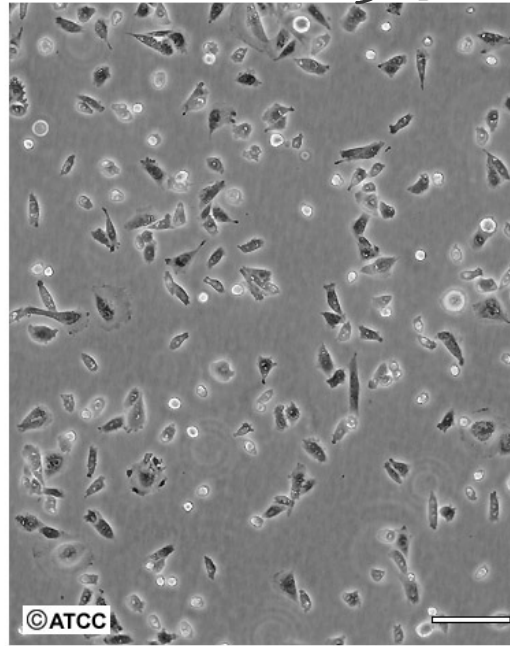
USE ~20-40%
Low Density culture

Seed new
when ~80%
conf.
High Density

- Confluence
density

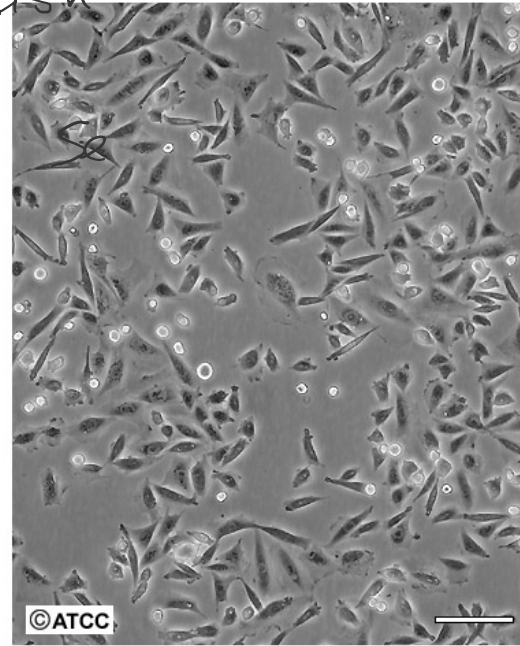
- Splitting
subculturing

- Seeding
moving cells to
new vessel



Low Density

Scale Bar = 100µm



High Density

Scale Bar = 100µm

General steps for splitting cells +WHY?

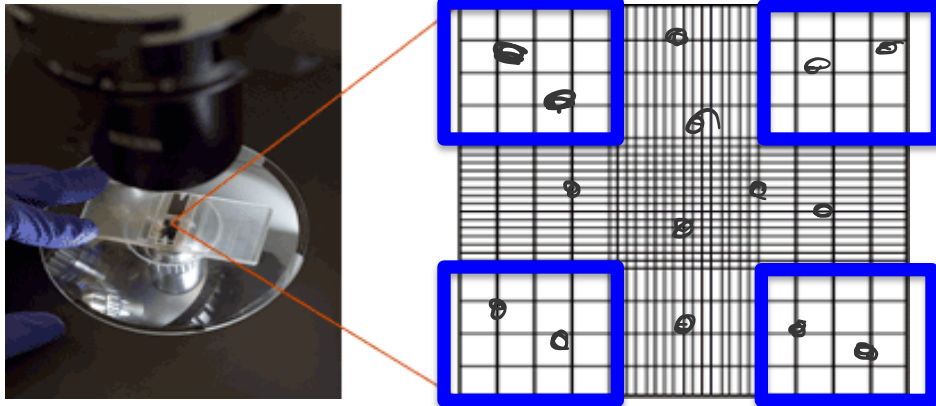
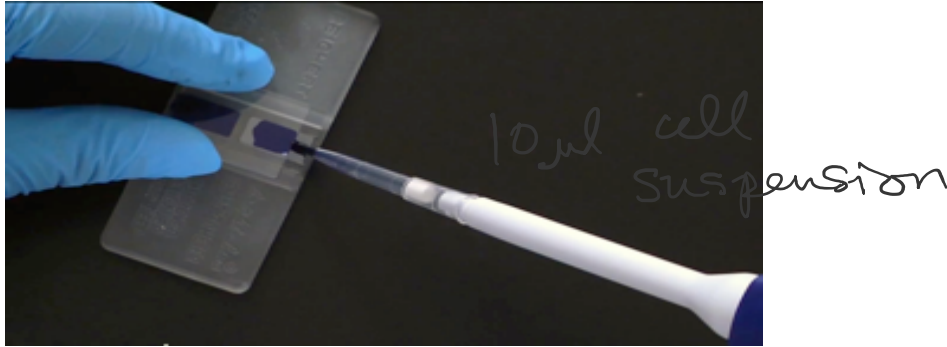
T75



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

↙

$$\frac{8}{4} = 2 \times 10,000 = 20,000 \text{ cells/mL}$$

What should go in your notebook?

Laboratory notebook entry component:

Points:

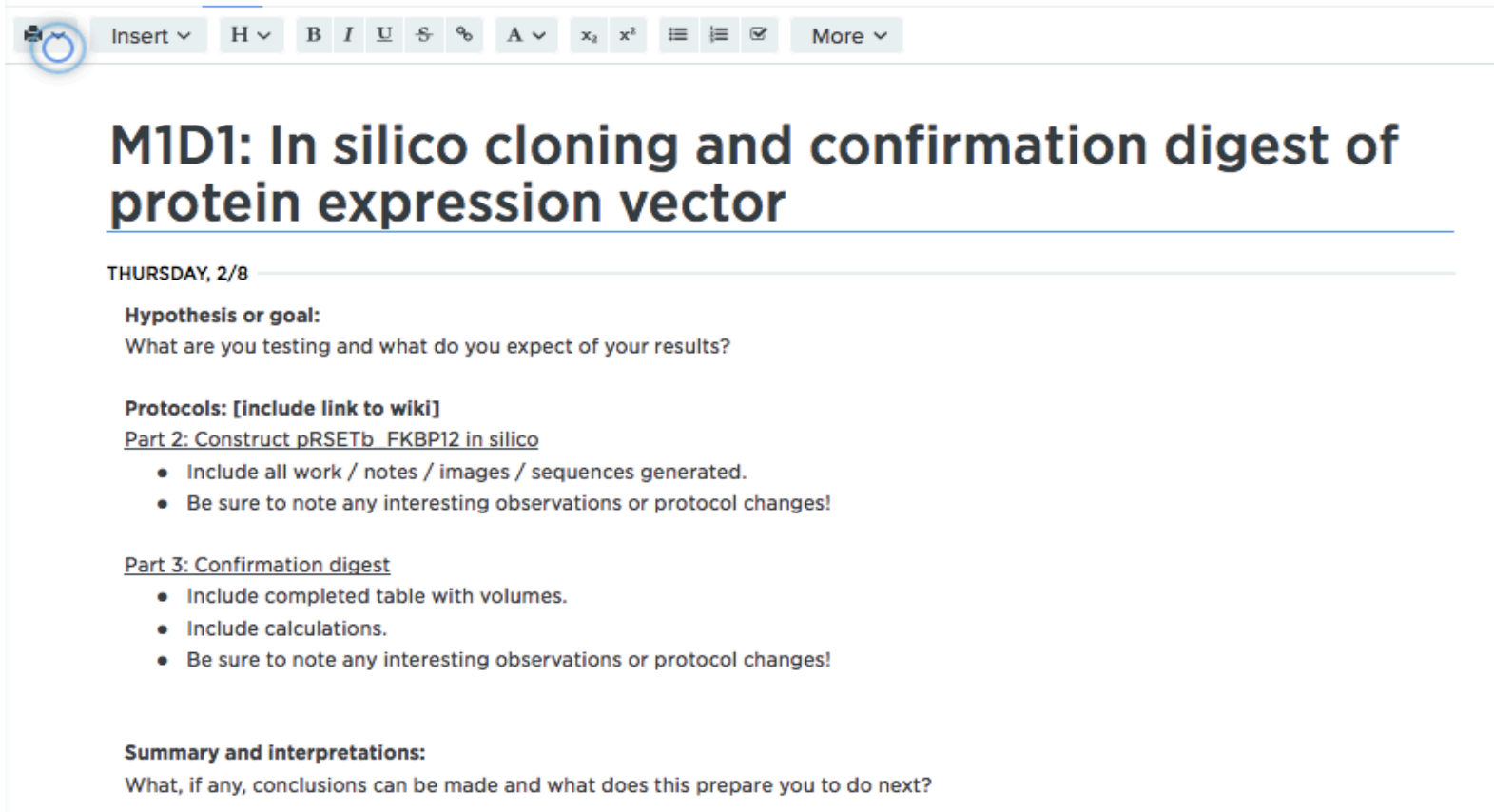
	Complete	Partial	Incomplete
Date of experiment (include Module#/Day#) and Title for experiment	1	0.5	0
Hypothesis or goal / purpose	1	0.5	0
Protocols (link to appropriate wiki sections)	1	0.5	0
Notes on protocol changes / clarifications	1	0.5	0
Observations	2	1	0
*Visual details			
*Qualitative information			
*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](http://engineerbiology.org/wiki/20.109(F19):_Assignments)

How should you format your notebook?



The screenshot shows a digital notebook interface. At the top is a toolbar with icons for undo, redo, insert, bold, italic, underline, strikethrough, link, text color, background color, list, indent, and a 'More' dropdown. Below the toolbar is a large heading 'M1D1: In silico cloning and confirmation digest of protein expression vector' followed by a horizontal line. Below this is the date 'THURSDAY, 2/8' followed by another horizontal line. The main content area contains three sections: 'Hypothesis or goal:' with the text 'What are you testing and what do you expect of your results?'; 'Protocols: [include link to wiki]' with two sub-sections, 'Part 2: Construct pRSETb_FKBP12 in silico' and 'Part 3: Confirmation digest', each followed by a bulleted list of instructions; and 'Summary and interpretations:' with the text 'What, if any, conclusions can be made and what does this prepare you to do next?'.

Insert ▾ H ▾ B I U ~~ABC~~ % A ▾ x₂ x² ☰ ☷ ☑ More ▾

M1D1: In silico cloning and confirmation digest of protein expression vector

THURSDAY, 2/8

Hypothesis or goal:
What are you testing and what do you expect of your results?

Protocols: [include link to wiki]
Part 2: Construct pRSETb_FKBP12 in silico

- Include all work / notes / images / sequences generated.
- Be sure to note any interesting observations or protocol changes!

Part 3: Confirmation digest

- Include completed table with volumes.
- Include calculations.
- Be sure to note any interesting observations or protocol changes!

Summary and interpretations:
What, if any, conclusions can be made and what does this prepare you to do next?

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 μ M)	As (final concentration = 80 μ M)	Media	Final volume (1 mL / well)
No treatment control (no MMS, no As)	1 μ L	0	2 mL	2 mL
MMS, no As				
no MMS, As				
MMS, As				

