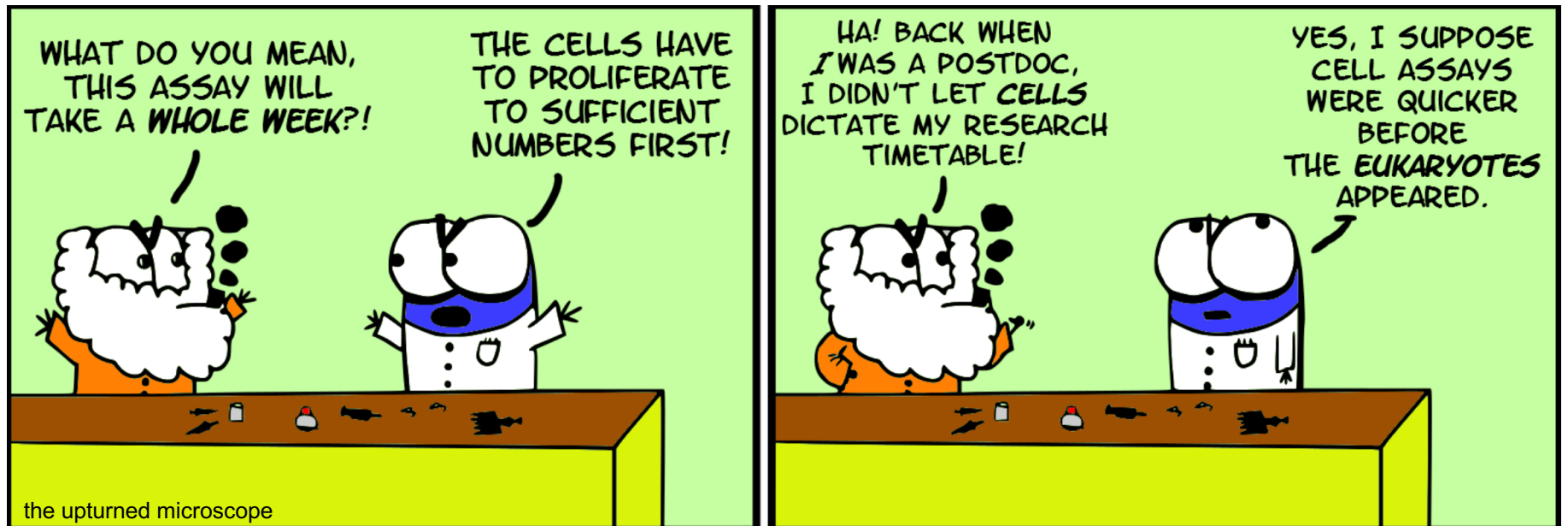


M1D1: Learn best practices for mammalian cell culture

1. Orientation Quiz
2. Prelab discussion
3. Learn about cell culture in the lab

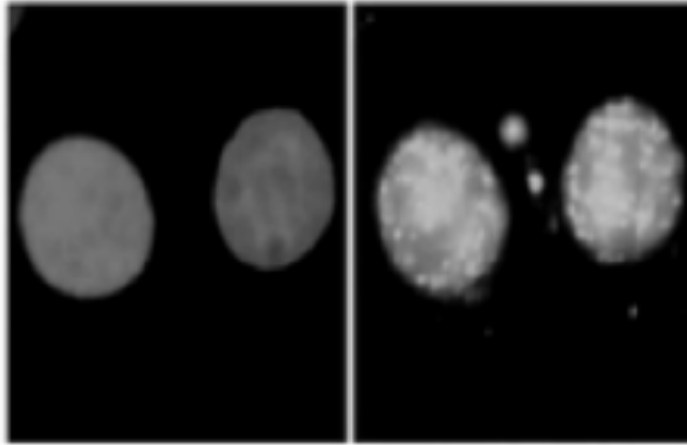


Mod 1: Major Assignments

- **Data summary** (15%)
 - In a team
 - Draft due 10/4, final revision due 10/14
 - Format: Bullet points, .PPTX
- **Mini-presentation** (5%)
 - Individual, submit video via gmail
 - Due 10/11 by 10pm
- **Lab quizzes** (5% collectively)
 - Individual (orientation quiz is exception)
 - Due 10pm day of lab, submit on Stellar
- **Notebook** (5% collectively)
 - Due 9/25 at 10pm, graded by Aimee
- **Blog** (part of 5% Participation)
 - Due 10/5 at 10pm

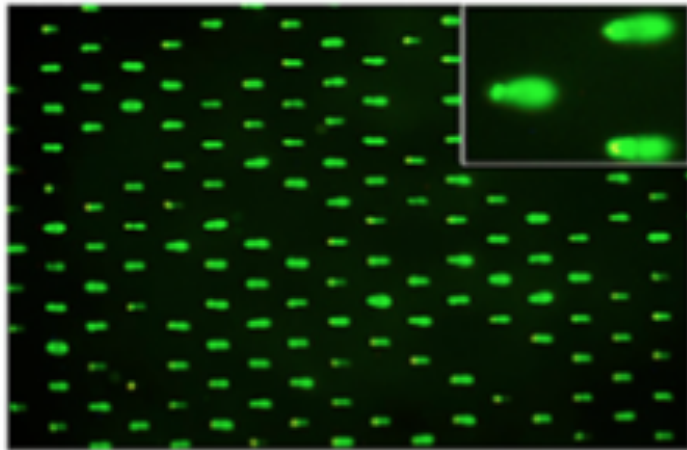
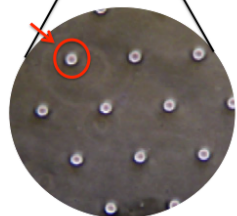
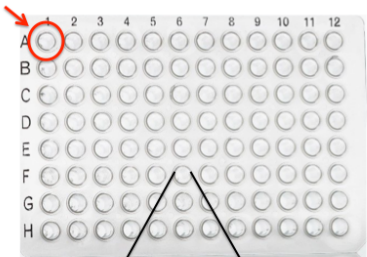
Overview of Module 1: Measuring Genomic Instability

Quantify DNA damage in mammalian cells following exposure to hydrogen peroxide and arsenite



Examine effect of H_2O_2 +/- As on double strand DNA breaks by measuring $\gamma H2AX$ foci formation

- Immunofluorescence (IF)
 - Cells attached to glass coverslips
- Cellular response to DNA damage

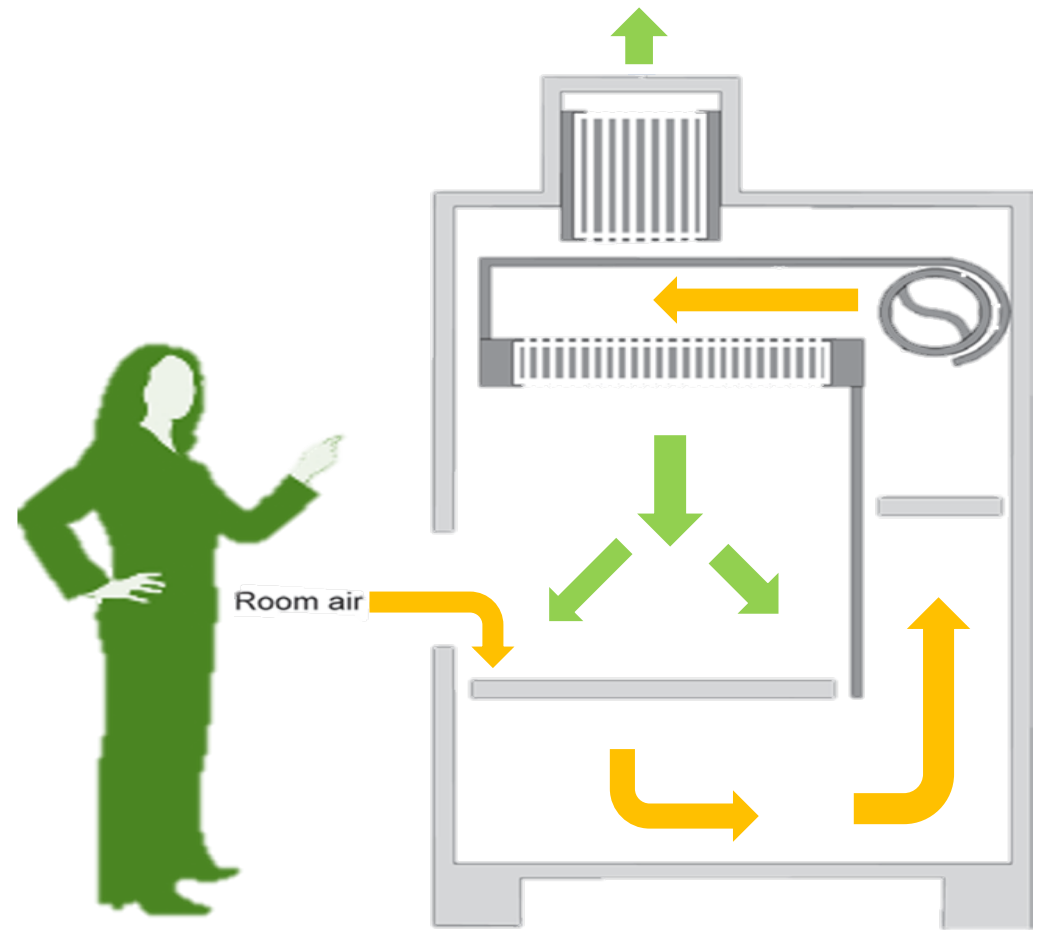


Measure the effects of H_2O_2 +/- As on DNA damage by measuring DNA migration in agarose matrix

- CometChip assay
 - single cell gel electrophoresis in 96 well format
- Single strand DNA 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 _____ cells

Food:

- RPMI 1640 (Roswell Park Memorial Institute)
 - Defined



- FBS (fetal bovine serum)
 - Undefined



Non-food:

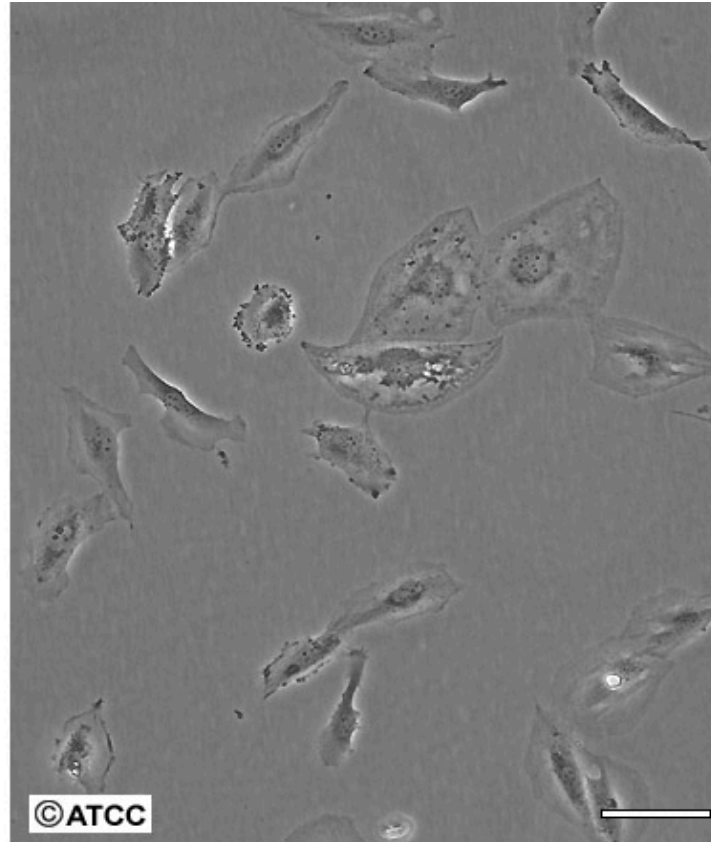
- antibiotics:
 - penicillin
 - streptomycin



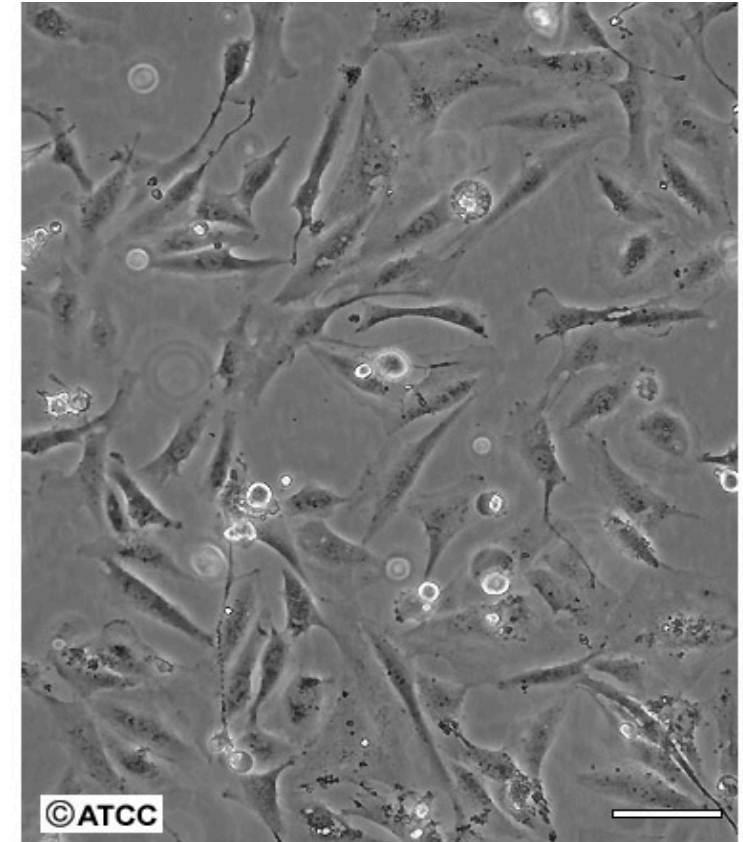
Mammalian Cell Culture Terminology

- Confluence
- Splitting
- Seeding

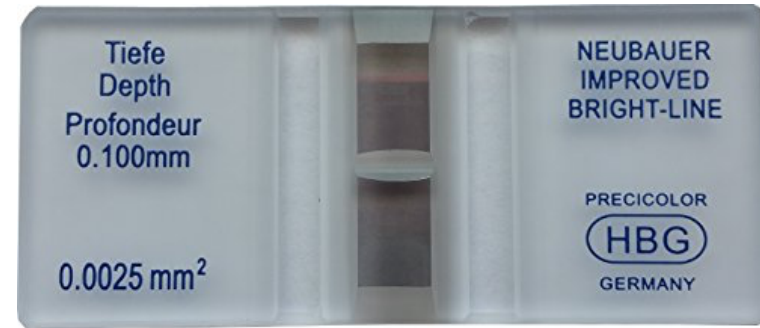
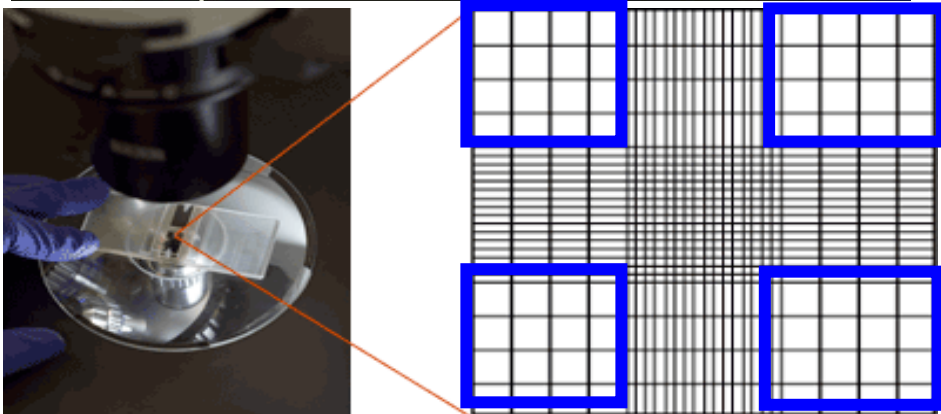
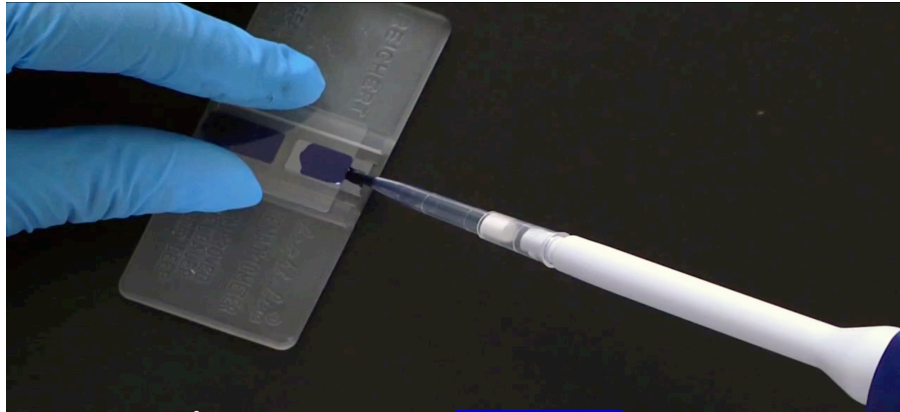
Low Density



High Density



Counting cells



- Hemocytometer
- Trypan blue

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

Upcoming assignments: What should go in your notebook?

Laboratory notebook entry component:

Points:

Complete

Partial

Missing

Date of experiment (include Module#/Day#) and Title for experiment

1

0.5

0

Hypothesis or goal / purpose

2

1

0

Protocols (link to appropriate wiki sections)

1

0.5

0

Answering questions embedded in wiki sections

5

3

0

Observations from demonstrations and video tutorials

3

2

0

*Visual details

*Qualitative information

*Raw data

Data analysis

3

2

0

*Calculations

*Graphs and Tables

Summary and interpretation of data

3

2

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

5

3

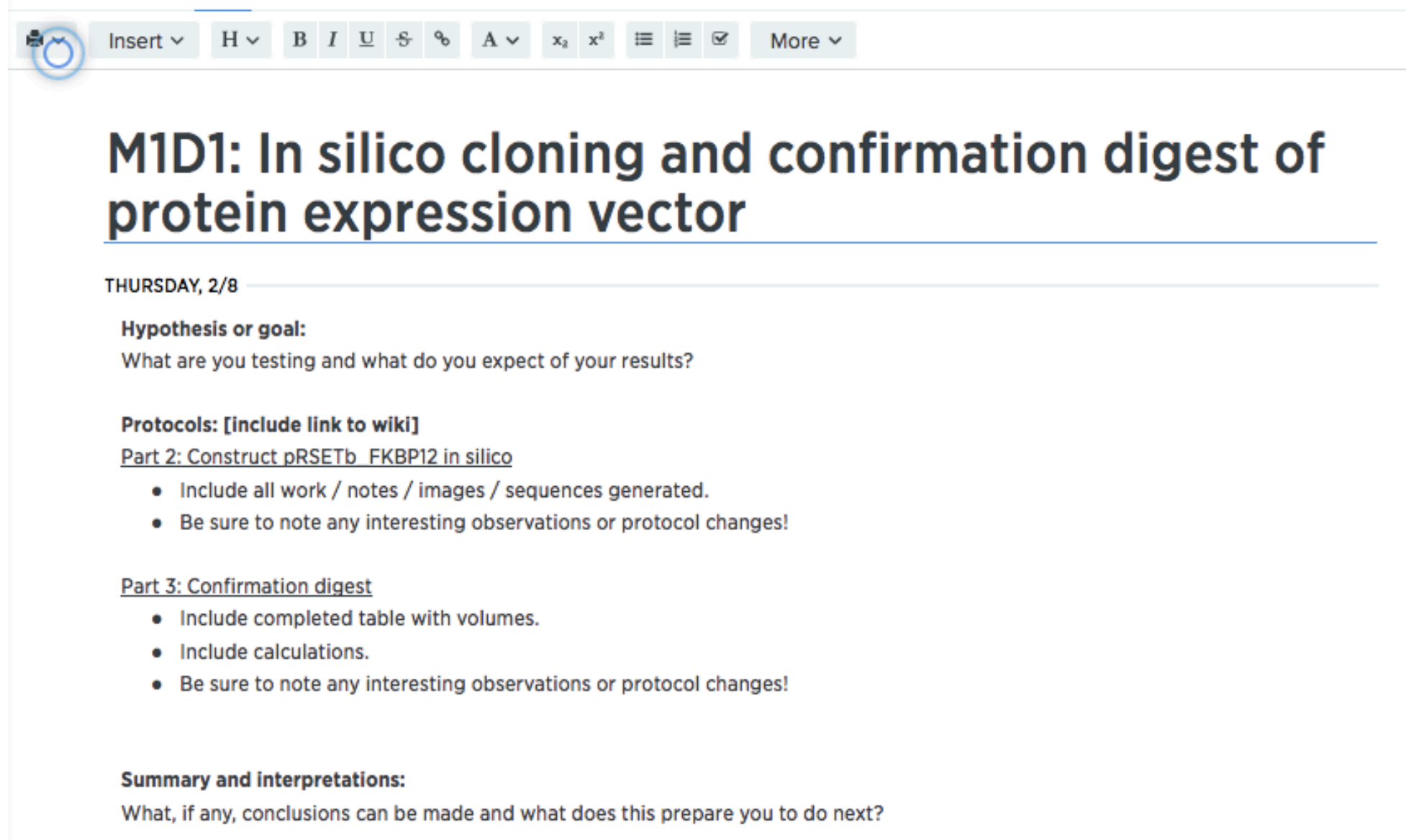
0

OVERALL /25

Due 10pm after each module, as posted on wiki

[http://engineerbiology.org/wiki/20.109\(F20\):_Assignments](http://engineerbiology.org/wiki/20.109(F20):_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, text color, background color, bold, italic, underline, strikethrough, link, unlink, text size, font family, list, indent, and a 'More' dropdown. The main content area has a title 'M1D1: In silico cloning and confirmation digest of protein expression vector' in a large, bold, black font, underlined. Below the title is a horizontal line, and then the date 'THURSDAY, 2/8'. The entry is organized into sections: 'Hypothesis or goal:' followed by the question 'What are you testing and what do you expect of your results?'; 'Protocols: [include link to wiki]' followed by a sub-section 'Part 2: Construct pRSETb FKBP12 in silico' with a bulleted list of instructions; 'Part 3: Confirmation digest' with another bulleted list of instructions; and 'Summary and interpretations:' followed by the question '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(F20)_YourName”
 - Make each module a new folder
 - Make each day a new entry within module folder
- Share the project with the instructors and Aimee (if you have not already)
 - Right-click and choose ‘settings’
 - Add collaborators by email address
 - amoise@mit.edu
 - rcmeyer@mit.edu
 - mebane@mit.edu
 - nllyell@mit.edu

For today:

1. Complete Orientation quiz with lab partner
2. Work through wiki to learn cell culture protocol
 1. Be sure to keep notes in Benchling!

For M1D2:

Create a template for your Benchling notebook and use it to create a M1D2 entry

** Make sure you have shared your notebook with me!