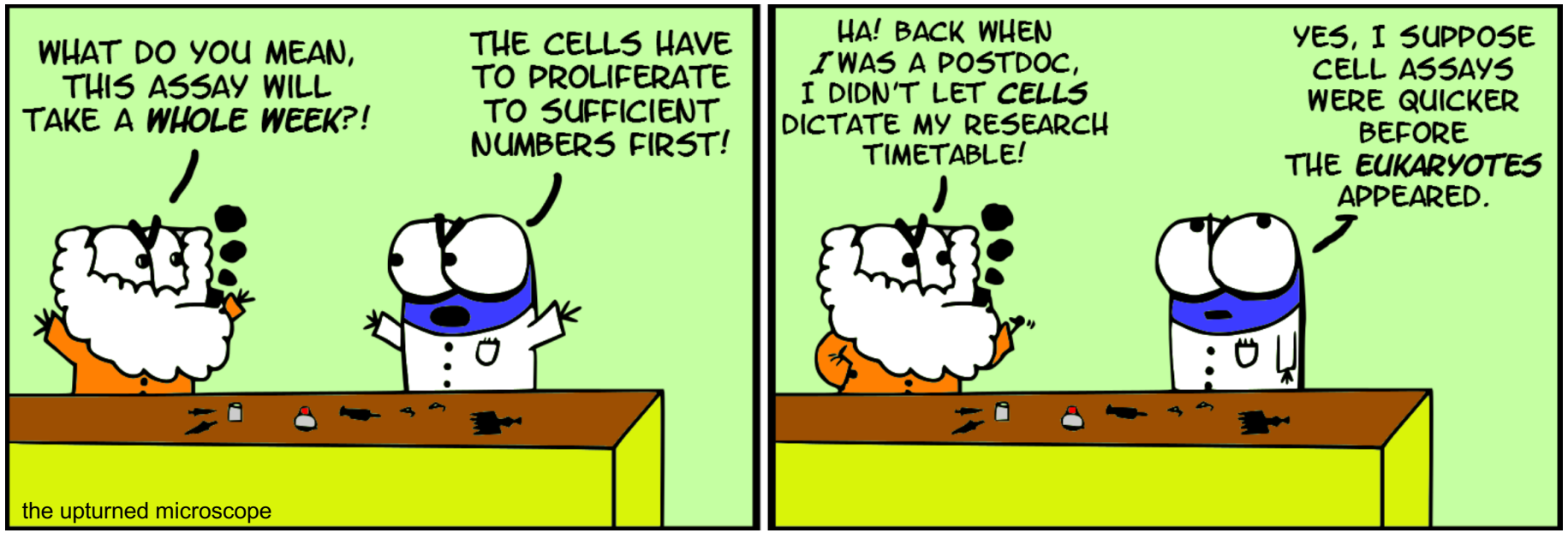
M1D1: Learn best practices for mammalian cell culture

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

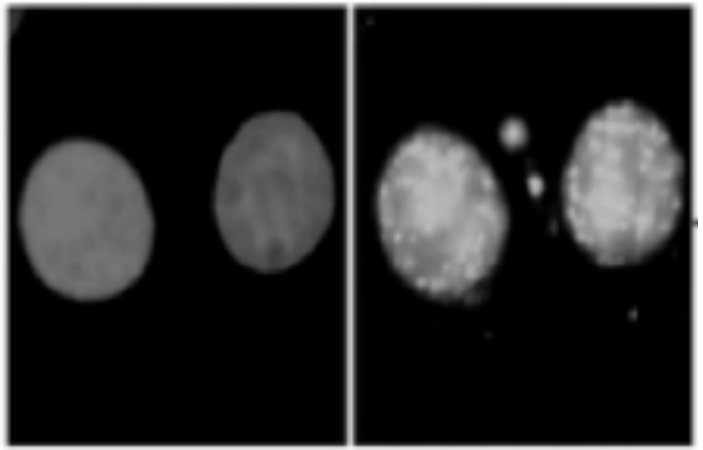


Mod 1: Major Assignments

- 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

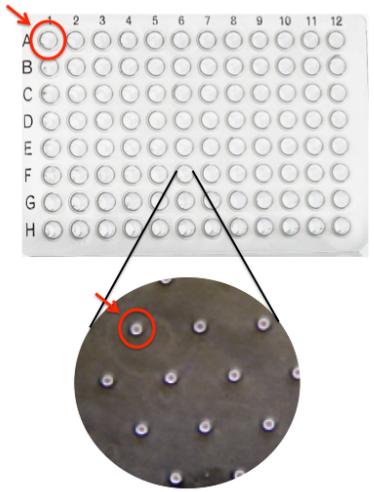
Overview of Module 1: Measuring Genomic Instability

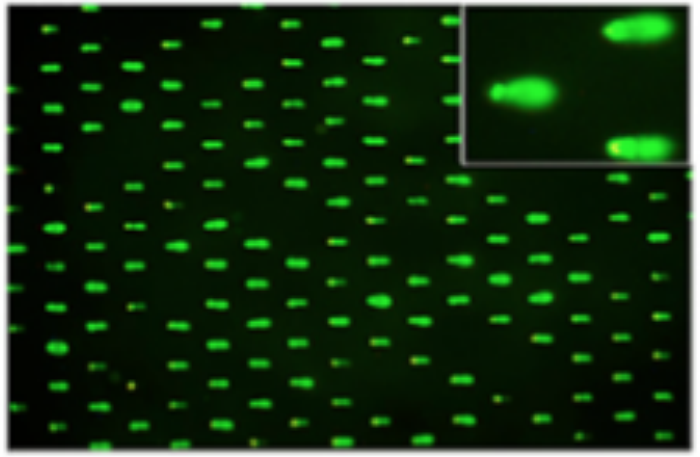
Quantify DNA damage in mammalian cells following exposure to hydrogen peroxide and arsenite



Examine effect of H_2O_2 +/- As on double strand DNA breaks by measuring γH2AX foci formation

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



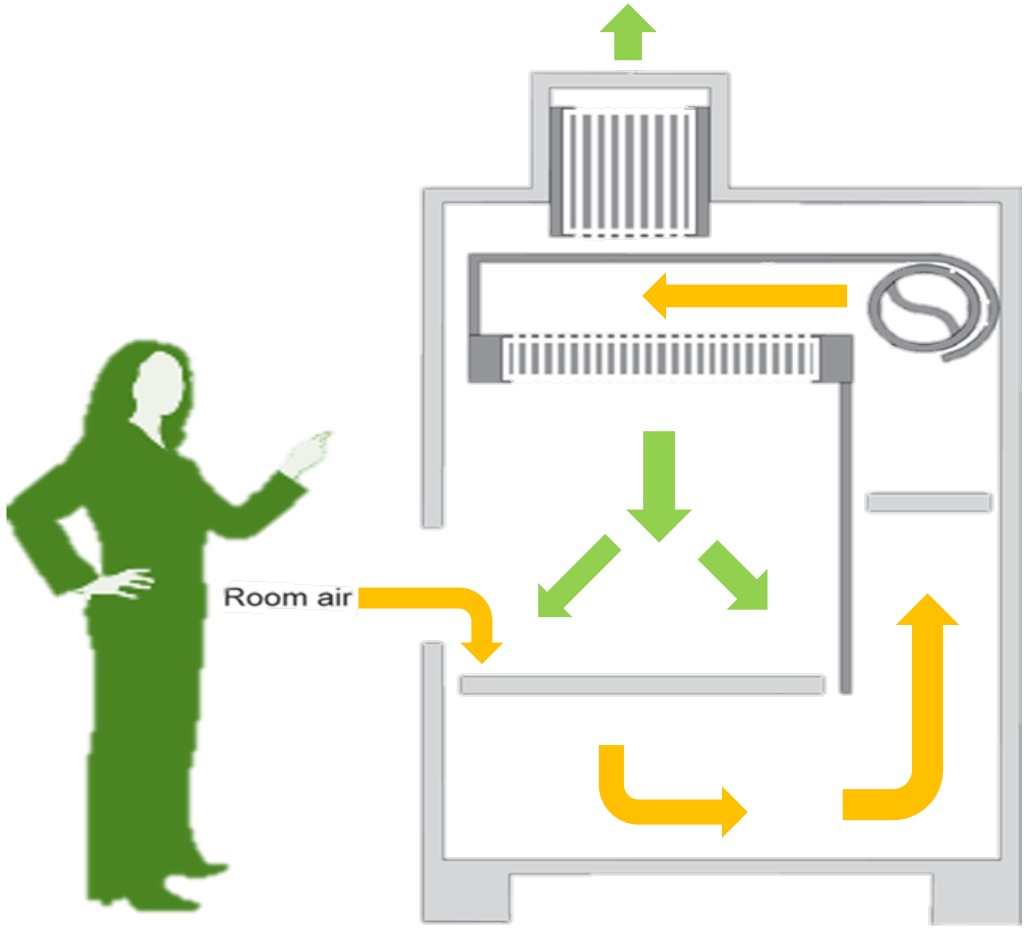


Measure the effects of H_2O_2 +/- As on DNA damage by measuring DNA migration in agarose matrix

- 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

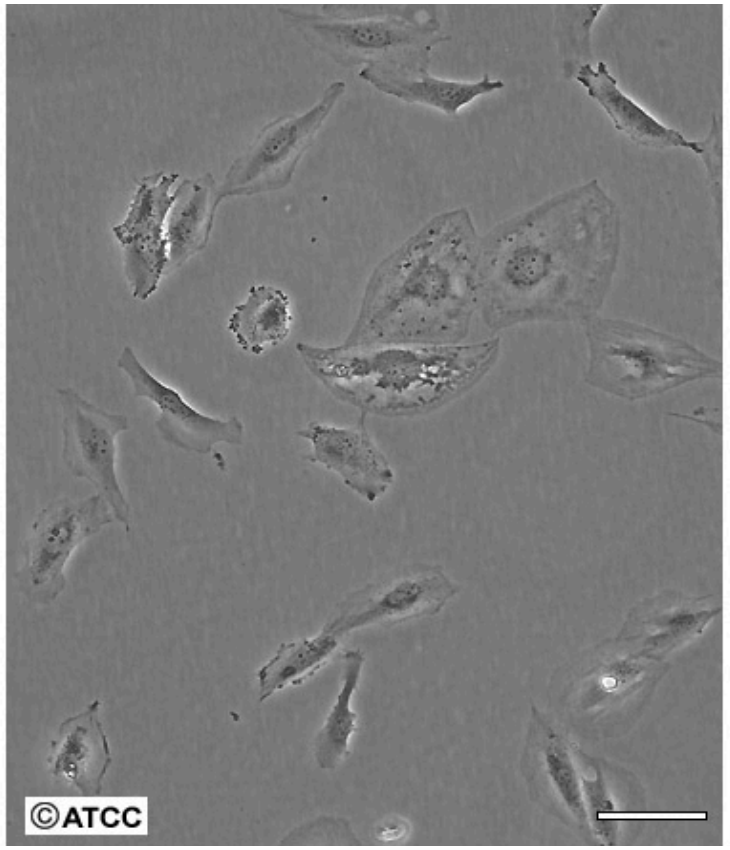
Non-food:

- antibiotics:
  - penicillin
  - streptomycin

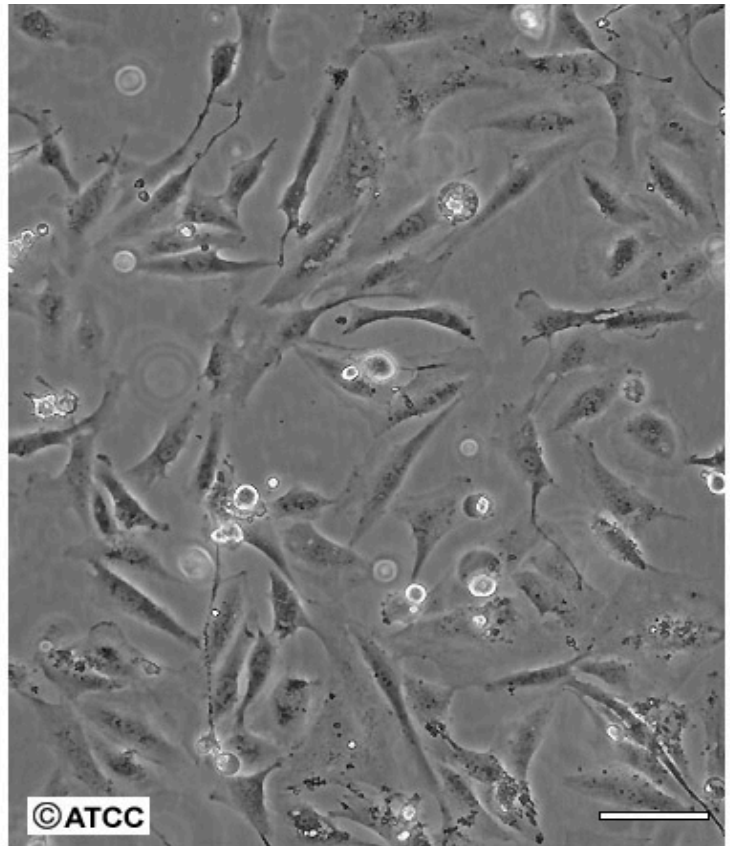
Mammalian Cell Culture Terminology

- Confluence
- Splitting
- Seeding

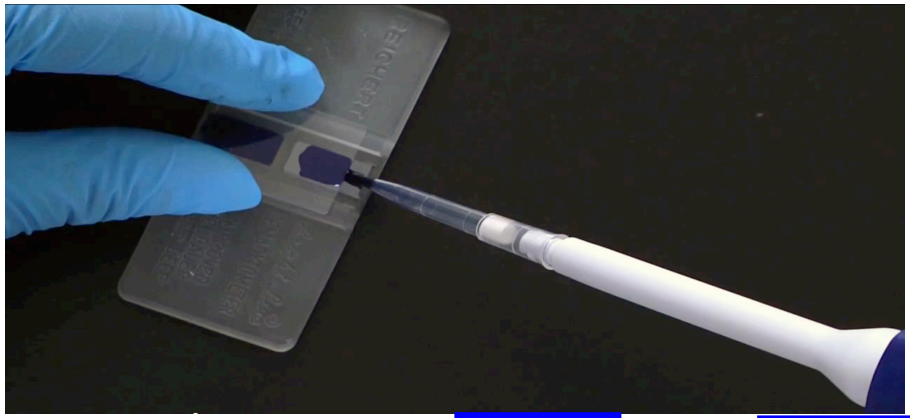
Low Density

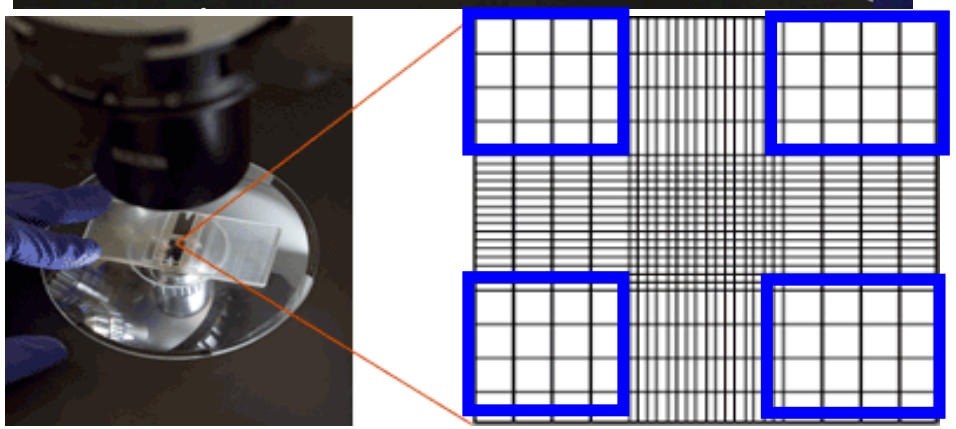


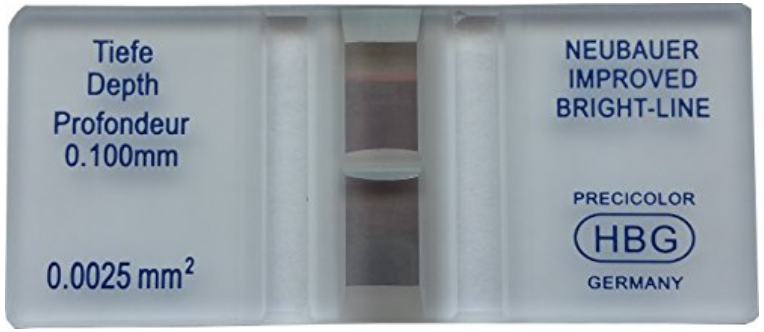
High Density



Counting cells







- Hemocytometer
- Trypan blue

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

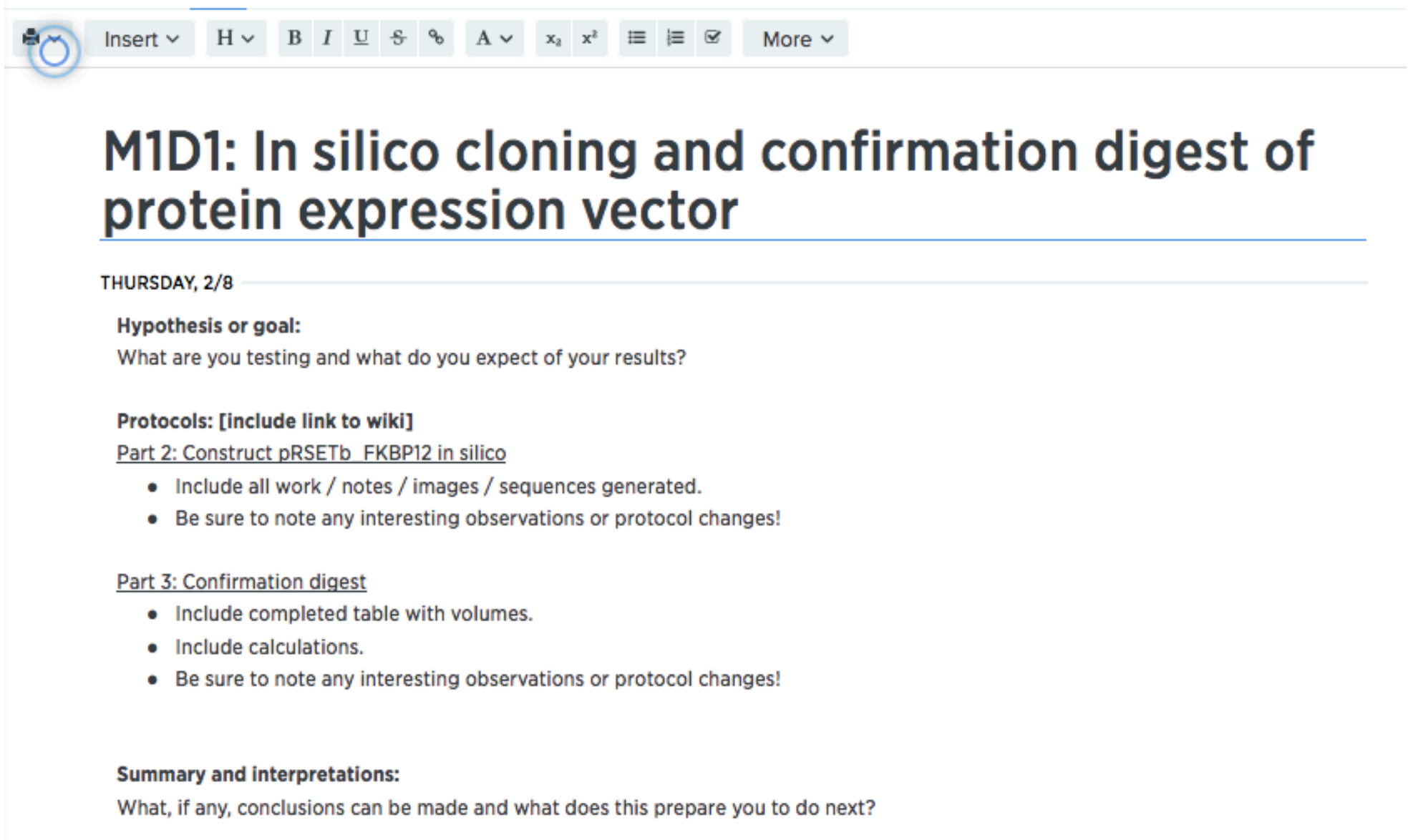
Upcoming assignments: What should go in your notebook?

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

How should you format your notebook?



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!