

M1D1: Prepare CometChip microwell array and practice cell culture

09/11/17

1. Lab Orientation Quiz
2. Pre-lab Discussion
3. $\frac{1}{2}$ class goes to the Tissue Culture Room
4. $\frac{1}{2}$ class prepares a CometChip

Office Hours

Noreen

Monday 2pm-5pm
in 16-317

Leslie

Thursday 2-3pm
Friday 12-1pm
in 56-341c

Josephine

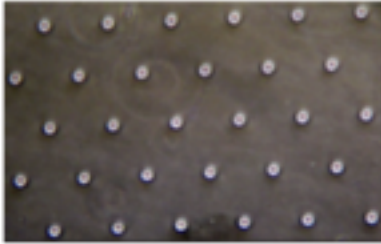
Wednesday 12-1pm
Friday 2-3pm

by appointment: nlyell@, lesliemm@, joshaw@

M1 major assignments

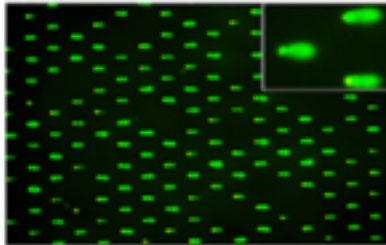
- **Data summary** (15%)
 - In teams, submit on Stellar
 - Draft due 10/8, final revision due 10/20
 - Bullet points, .PPTX
- **Mini-presentation** (5%)
 - Individual, submit video via Gmail
 - Due 10/13
- Lab quizzes –be on time!
 - M1D4 and M1D7
- Notebook (part of 10% Homework and Notebook)
 - Due **10/5** at 10pm, graded by Corban
- Blog: <https://be20109f18.blogspot.com> (part of 5% Participation)

Overview of Module 1: Measuring Genomic Instability



1. Optimize comet chip assay

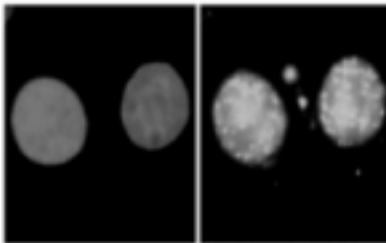
- Test loading variables



2. Use comet chip assay to measure DNA damage

- Measure effects of H_2O_2 on +/- DNA-PK cell lines

Protein involved in
DNA repair

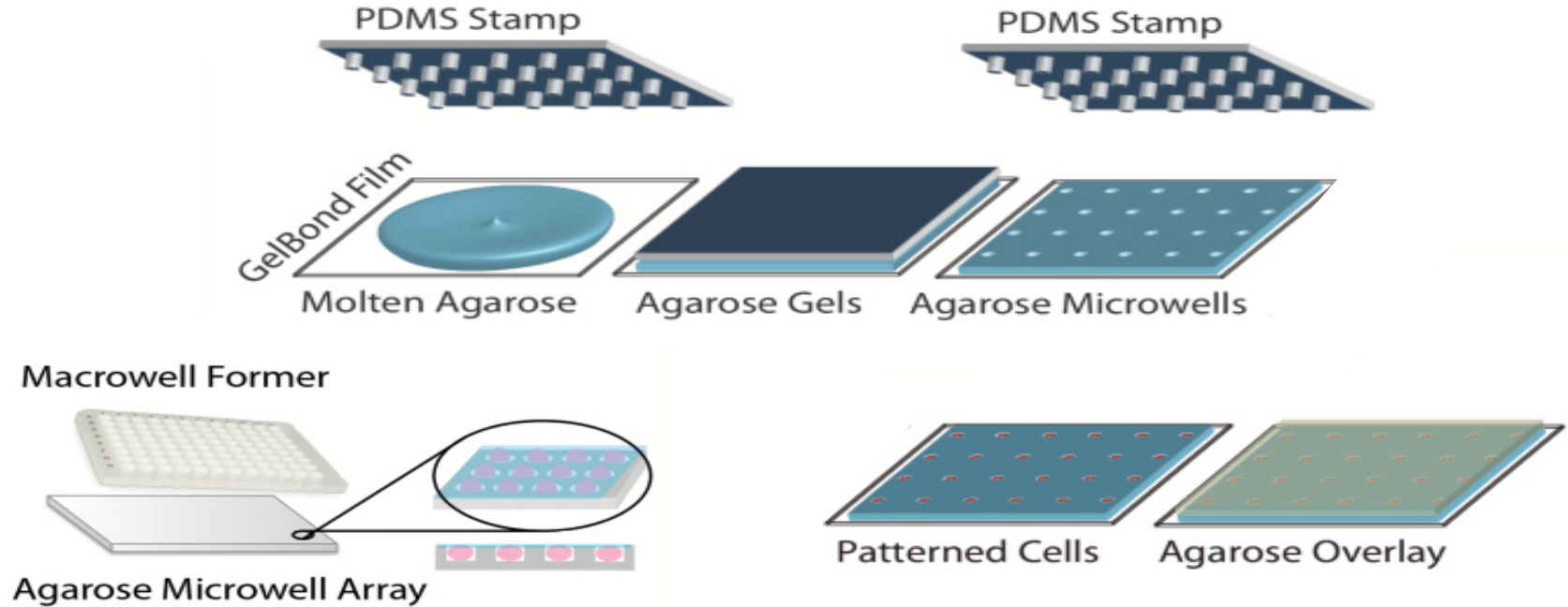


3. Use immuno-fluorescence assay to measure DNA damage

- Examine effect of H_2O_2 on $\gamma H2AX$ foci formation

Overview of this week:

Create a CometChip & optimize cell loading

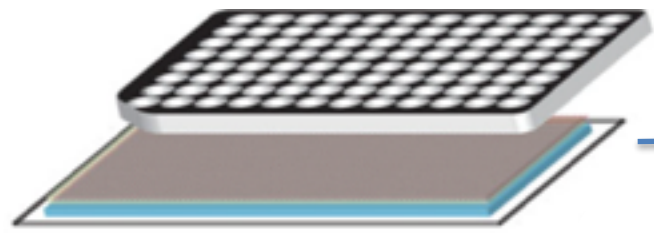


What is the minimum number of cells needed in each macrowell to obtain efficient loading?

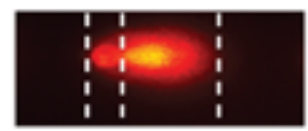
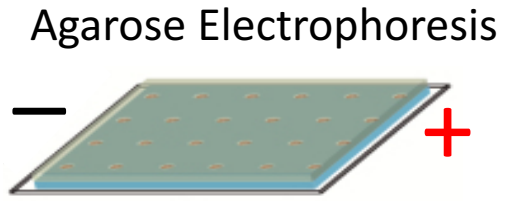
Overview of next week:

Assess DNA damage in tumor cells with & without DNAPKcs

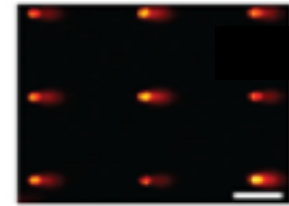
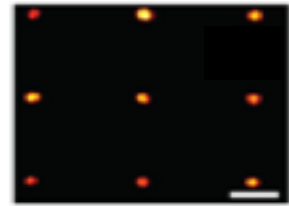
Treat captured cells in comet chip with H_2O_2 (oxidative damage)



Lyse cells & unwind DNA
(DNA still captured
agarose in overlay)

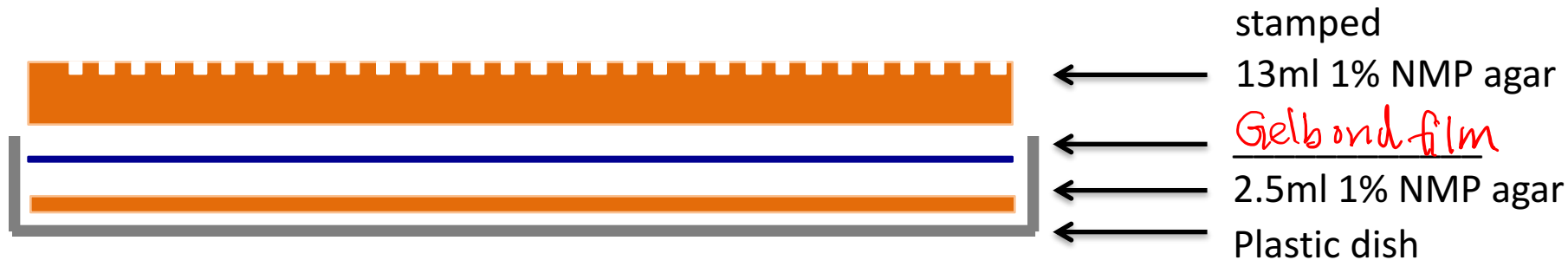


Analysis
via
Matlab

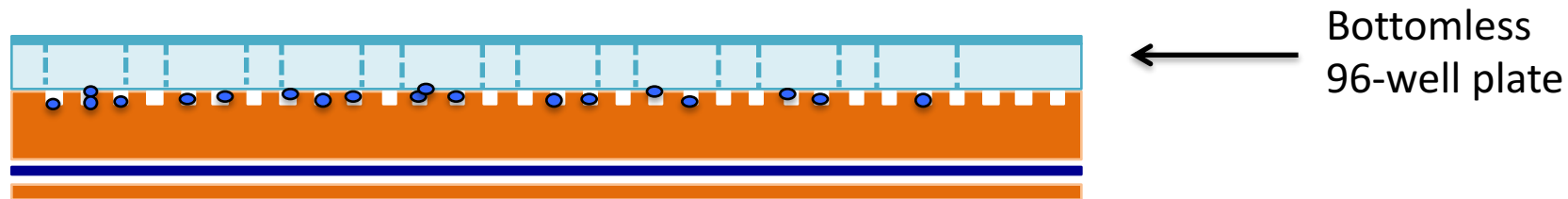


Stain DNA and image via
fluorescence microscopy

Today, make a CometChip

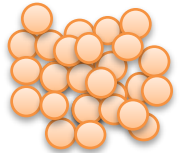


Next time, load cells

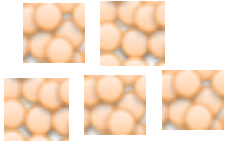


Background: Two glial cell lines— M059J (-DNAPKcs) and M059K (+DNAPKcs)

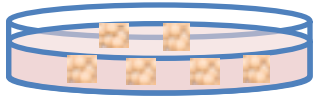
Tumor specimen from
33 year old male with
untreated glioblastoma



Miced into cubes

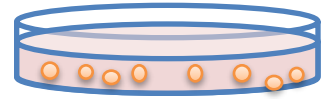


Maintain cultures
in dish



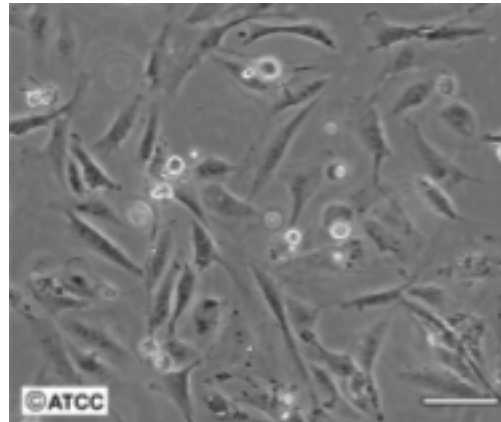
M059K

Dissociate
with enzymes



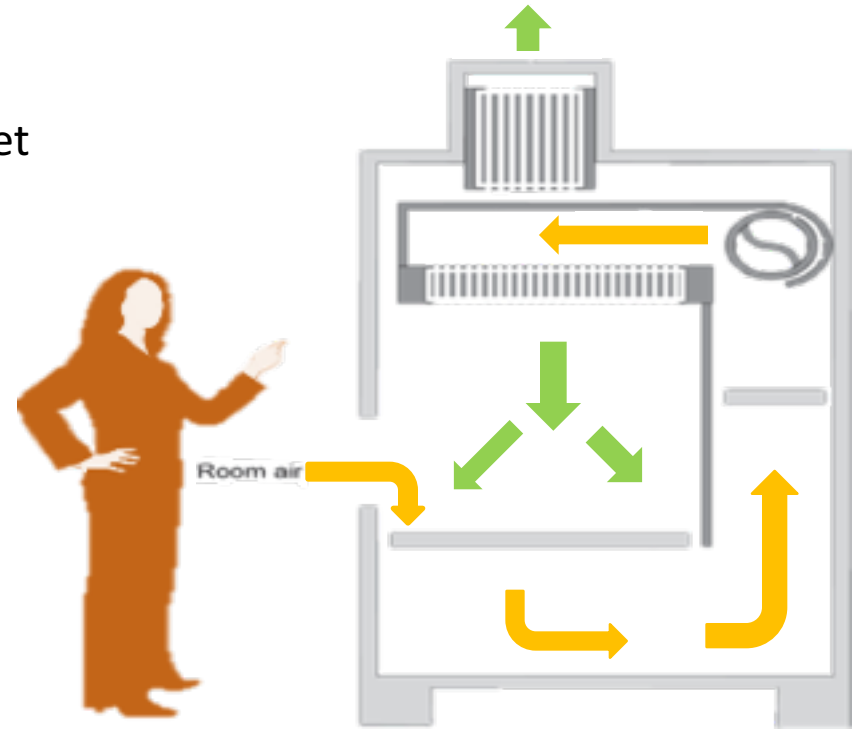
Maintain cultures
in dish

M059J



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

What do cells need to survive?



Food:

- DMEM (Dulbecco's Modified Eagle's medium) : F12 (defined)
 - amino acids
 - sugars
 - salts
 - phenol red - pH indicator
 - pH buffers
 - vitamins
- FBS (fetal bovine serum) (undefined)
 - growth factors
 - lipids
 - cholesterol
 - cytokines



Non-food:

- antibiotics:
 - penicillin
 - streptomycin
- prevent bacterial growth



Mammalian cell culture terminology

- Confluence

density

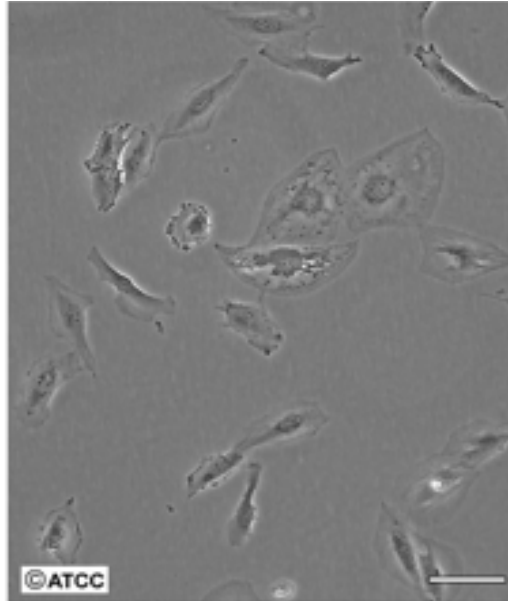
- Splitting (*subculturing*)

*~ 80% confluent,
put cells on new dish*

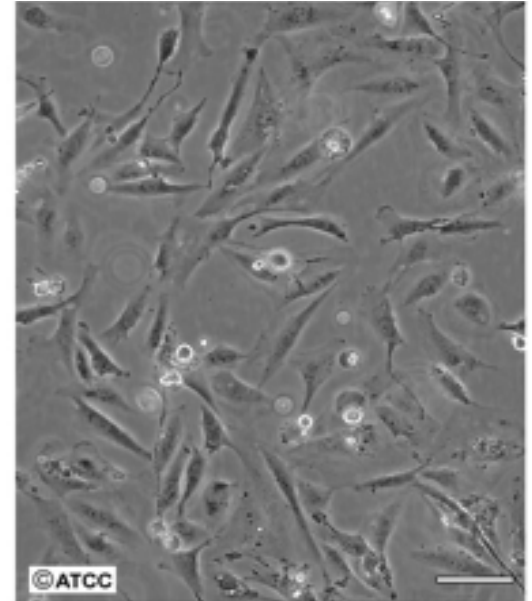
- Seeding

*~ 20-40% of confluent
culture on to new dish*

Low Density



High Density



General steps for splitting cells +WHY?

1. Look at cells, estimate confluence

2. Rinse with PBS

Wash away dead cells, debris, anti-trypsin agents

3. Detach cells with trypsin (enzyme)

break substrate adhesions

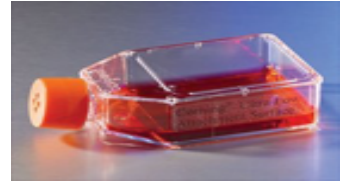
4. Count cells

5. "Seed" new culture vessel

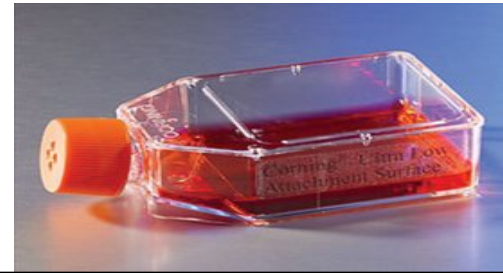
give room to grow

Be sure
not to mix
the two
cell lines!

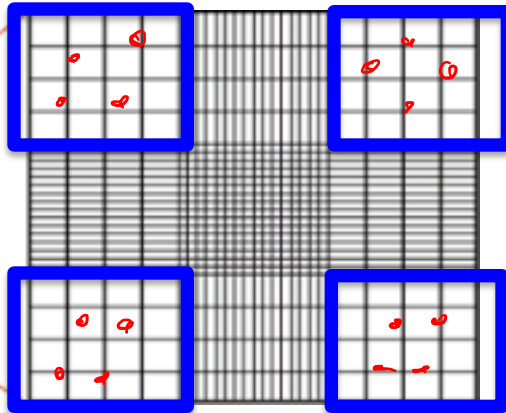
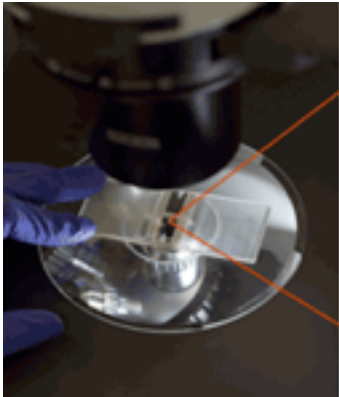
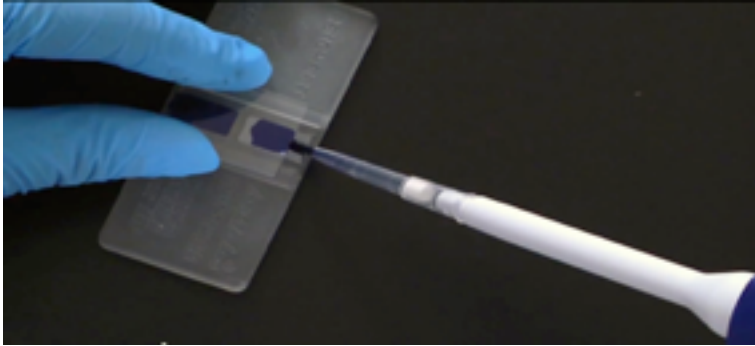
T25



T75



Counting Cells



- Hemocytometer:
- Trypan blue:
stains dead cells
- # cells / mL = 10,000 x average of 4 corners

$$16/4 = 4$$

$$4 \times 10,000 = 40 \text{ k cells/mL}$$

What should go in your notebook?

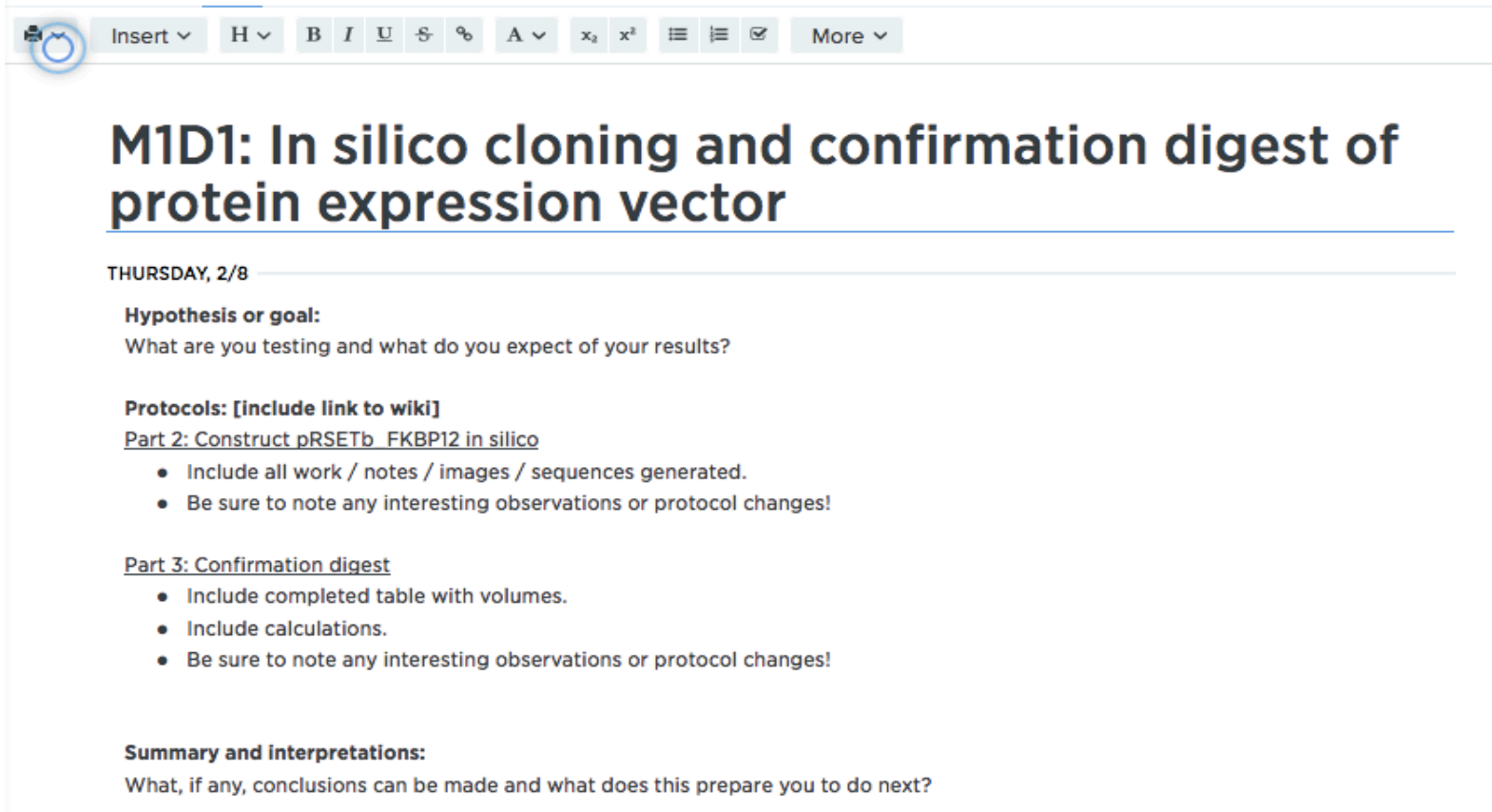
Laboratory notebook entry component:

	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\(F18\):_Assignments](http://engineerbiology.org/wiki/20.109(F18):_Assignments)

How should you format your notebook?



The image shows a screenshot of a digital notebook interface. At the top, there is a toolbar with various icons for text formatting (bold, italic, underline, strikethrough, link, unlink, text color, background color) and a 'More' dropdown menu. Below the toolbar, the notebook content is displayed. The title is 'M1D1: In silico cloning and confirmation digest of protein expression vector', which is underlined. Below the title is the date 'THURSDAY, 2/8'. The content is organized into sections with bold headings: 'Hypothesis or goal:', 'Protocols: [include link to wiki]', 'Part 2: Construct pRSETb_FKBP12 in silico', 'Part 3: Confirmation digest', and 'Summary and interpretations:'. Each section contains specific instructions or questions, with the 'Part 2' and 'Part 3' sections including bulleted lists of requirements.

Insert ▾ H ▾ B I U S % 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(F18)_YourName”
 - Make each module a new folder
 - Make each day a new entry within module folder
- Share the project with Josephine and Corban
 - Right-click and choose ‘settings’
 - Add collaborators by email address

Today in lab:

1. 3 teams into tissue culture room to split cells (Red, Orange, Green)
2. 2 teams start preparing CometChip (Yellow and Blue)
3. Make sure to keep notes in Benchling!
 - Watch Engleward lab JOVE video during downtime (<https://www.jove.com/video/50607/cometchip-high-throughput-96-well-platform-for-measuring-dna-damage>)
 - M1D2HW: Create a template for your benchling notebook and make a M1D2 entry from it.