M1D1:
Learn best practices for mammalian cell culture

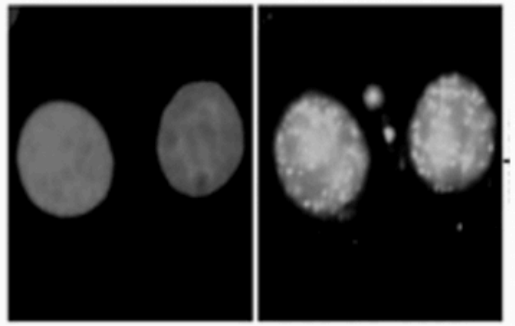
1. Prelab discussion
2. Orientation quiz
3. Cell culture exercises

Mark your calendar!

- Data summary (15%)
  - completed in teams and submitted via Stellar
  - draft due 10/4, final revision due 10/14
- Mini-presentation (5%)
  - completed individually and submitted via Gmail
  - due 10/11
- Laboratory quizzes (collectively 5%)
  - scheduled for M1D4 and M1D7
- Notebook (collectively 5%)
  - one entry will be graded by Aimee 24 hr after M1D7
- Blog (part of 5% Participation)
  - due 10/5 via Blogspot

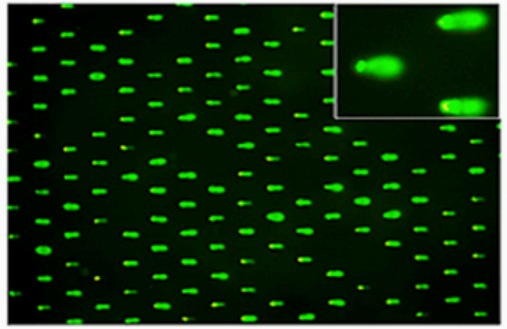
Overview of M1: genomic instability

Research question: Does exposure to As inhibit, or decrease, repair of H_2O_2-induced DNA damage, raising the possibility that combined exposure is an important risk to public health?



1. Use repair foci experiment to measure DNA breaks

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

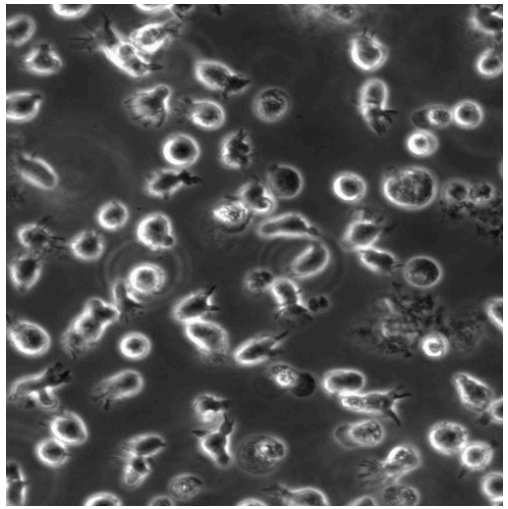


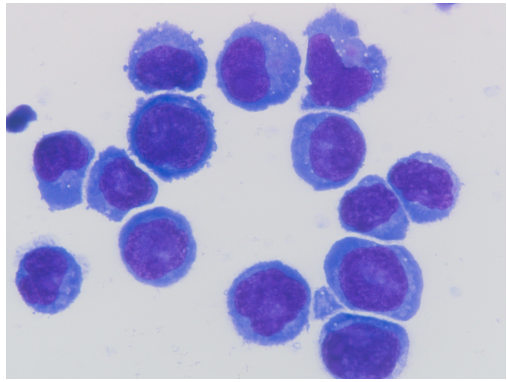
2. Use high-throughput genome damage assay to measure DNA damage

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

We will use human lymphoblastoid cells

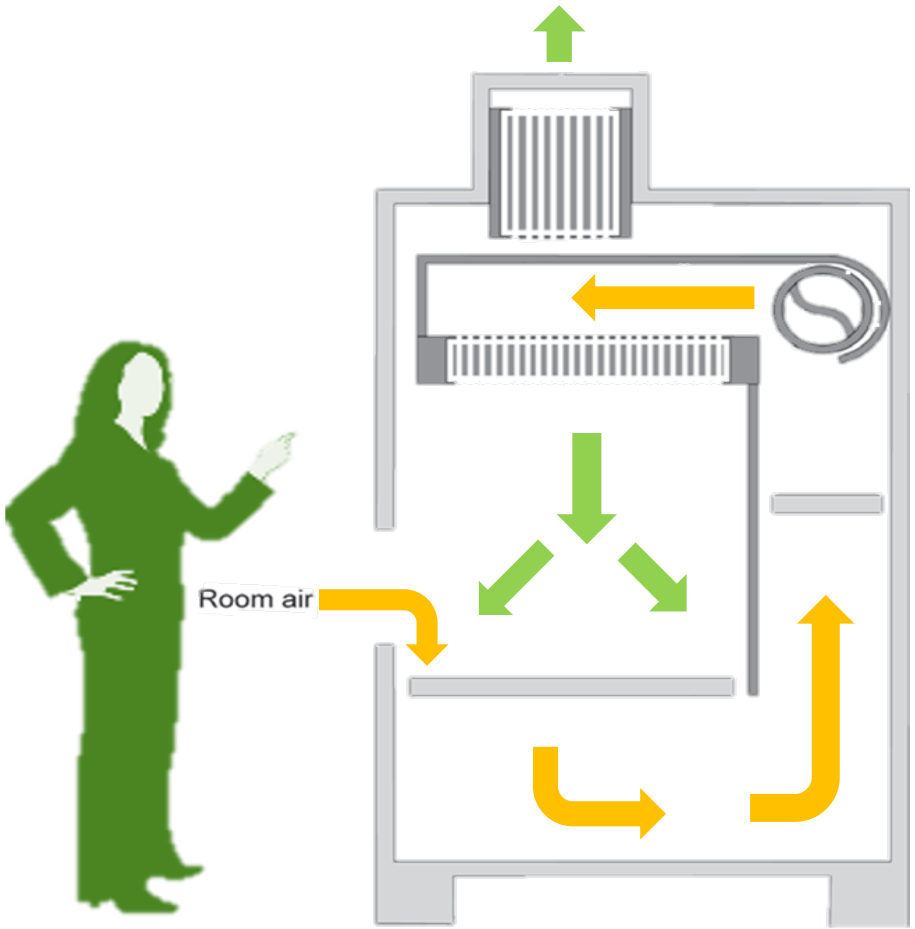
- Specifically, what cell line are we using in M1?
- What are primary cells? Why are they difficult to use in experiments?
- Why are cancer cells easier to use in experiments?
- What growth conditions are important when culturing mammalian cells?





Biosafety cabinets are used to maintain sterility

- Spray everything with 70% ethanol
  - 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
- Do not talk into cabinet or incubator!
- Only open sterile media in the cabinet



Growth medium is used to culture cells

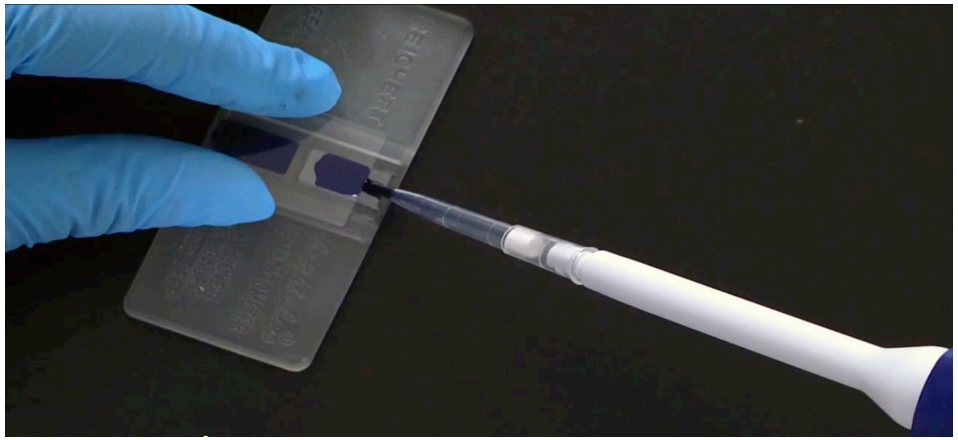
<u>Food</u>

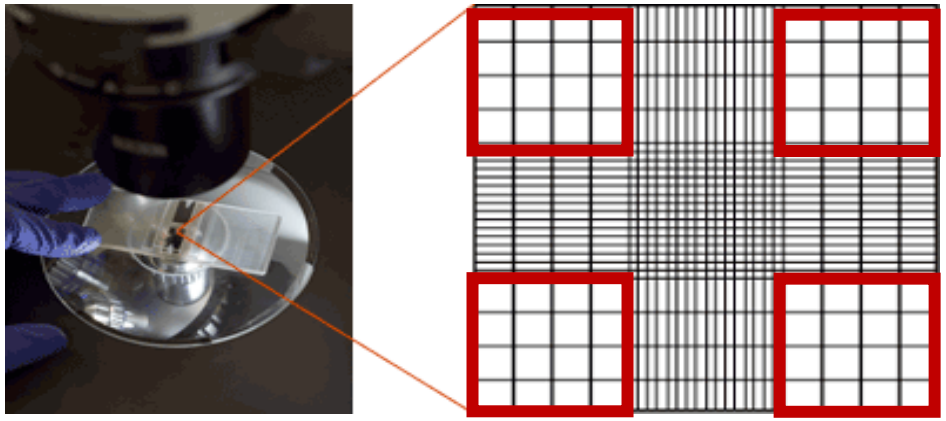
- RPMI 1640 (Roswell Park Memorial Institute)
- FBS (fetal bovine serum)

<u>Non-food</u>

- Antibiotic solution: penicillin and streptomycin

Hemocytometers are used to count cells





- Trypan blue mixed with cell suspension at 1:10 ratio, then 10 µL added to hemocytometer
- Cells within highlighted sections of the hemocytometer grid are counted

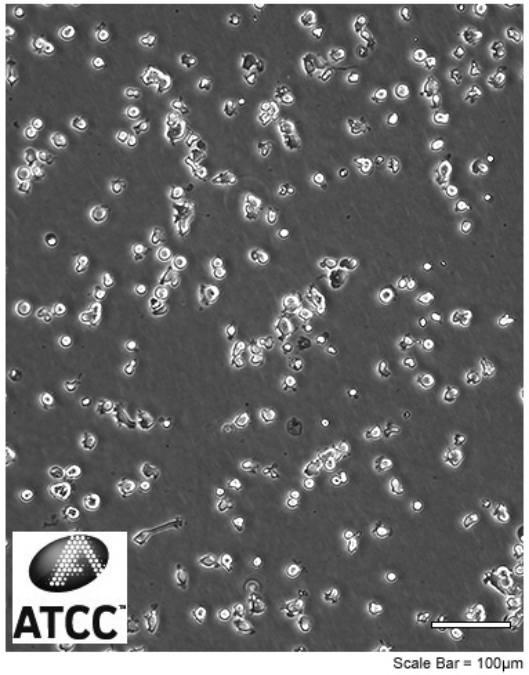
cells / mL = average # of cells in the 4 highlighted boxes * 10,000

The language of cell culture

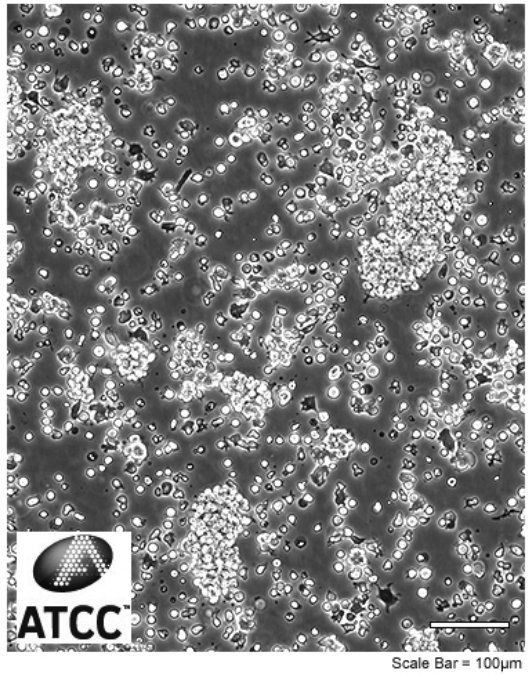
- Confluence

- Splitting / Sub-culturing

- Seeding



Low density



High density

For today…

- Choose a team name!
- Complete Orientation quiz
  - Submit to Stellar by 10 pm
- Work through cell culture exercises
  - Be sure to record your notes in your laboratory notebook

For M1D2…

- Prepare a template for Benchling laboratory notebook entries
- Be sure to share your Benchling laboratory notebook

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			

Be sure to include your responses to the prompts within the laboratory exercises!

How should you format your notebook?

Insert H B I U S A x_2 x^2 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?

- Title 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 Instructors and Aimee
  - Right-click and choose ‘settings’
  - Add collaborators by email
    - nllyell@mit.edu
    - amoise@mit.edu
    - rcmeyer@mit.edu
    - mebane@mit.edu