Module 4: Introduction to Cell Culture

BMES Cell Team

Fall 2020

Outline

- Purpose of Cell Culture
- Sterilization Technique
- Passaging Procedure
    - Gauging confluency
    - Trypsinization
    - Centrifugation

Introduction to Cell Culture

- **Definition:** Cell culture is the growth of microorganisms (bacteria, yeast) or cells (plant, animal, human) in a laboratory.

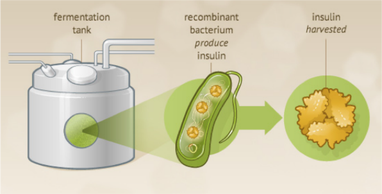
- Cells are handled inside of a Biological Safety Cabinet (BSC)

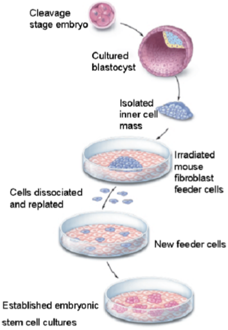
- Cells are stored inside of an incubator
  - The incubator mimics the cell's biological environment
    - Temperature = 37 ℃
    - Concentration CO_2 = 5%
    - Humidity = 90%

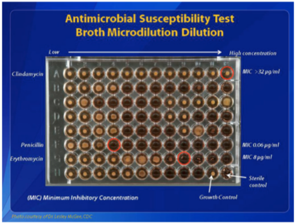
https://www.medgadget.com/2019/03/2023-biological-safety-cabinet-market-will-grow-at-7-4-of-cagr-globally-meticulous-study-by-top-industry-leaders-as-labconco-esco-micro-kewaunee-scientific-nuaire-market-research-future.html

Purpose of Cell Culture

- **Research** Study natural processes via *in vitro* experiments
- **Therapeutics** Produce drugs, tissue grafts, cell-based therapies
- **Diagnosis** Use patient sample to determine best treatment



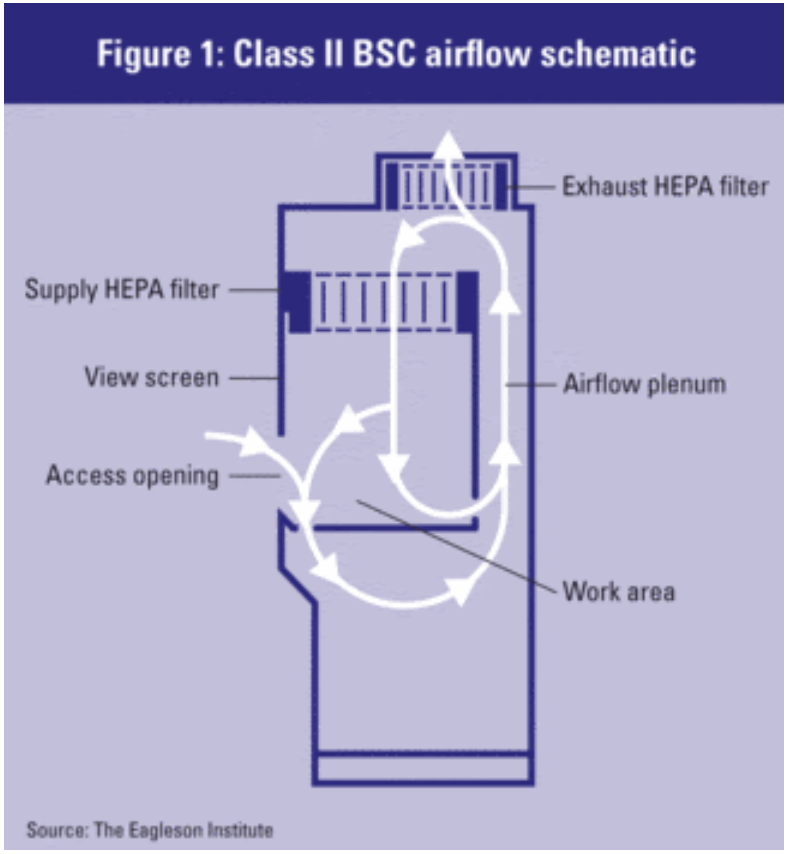




https://www.nlm.nih.gov/exhibition/fromdnatobeer/exhibition-interactive/recombinant-DNA/recombinant-dna-technology-alternative.html
https://www.researchgate.net/figure/Techniques-for-generating-embryonic-stem-cell-cultures-Illustration-is-reprinted-from_fig2_8551939
http://www.micromyx.com/invitro.html

Biological Safety Cabinet (BSC)

- Enclosed, ventilated laboratory workspace for safely working with materials potentially contaminated with pathogens
- Filtered air circulates through the BSC
    - Must avoid obstructing the vents or moving around quickly inside of the BSC to retain good airflow

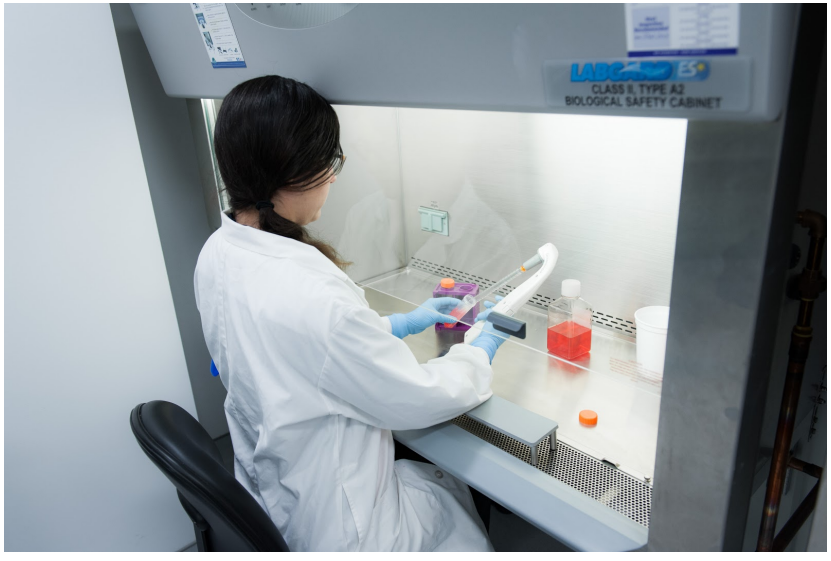


https://electroiq.com/2008/04/certification-of-class-ii-biological-safety-cabinets/

Sterilization Technique

DO:

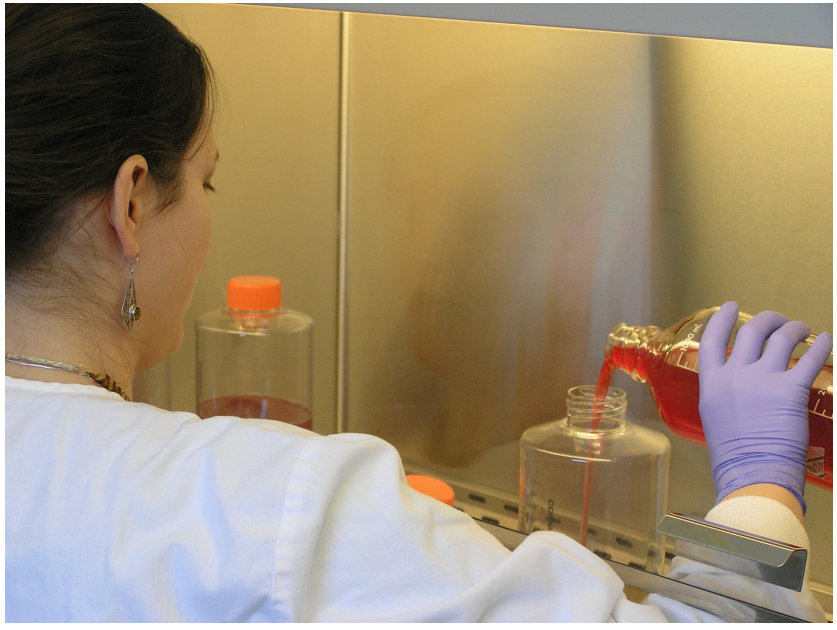
- Spray everything with ethanol before it enters the BSC or incubator
    - Includes your hands!
- Cover every container right after you use it
- Use a new pipette tip for each new step
- Leave lids facedown
- Keep shield low



Sterilization Technique

DON'T:

- Pass your hand over open containers
- Move around quickly in the BSC
    - Disrupts airflow inside the BSC
- Use a pipette tip if it has touched anything but the inside of the flask
- Directly pour media into the flask
- Keep the shield high



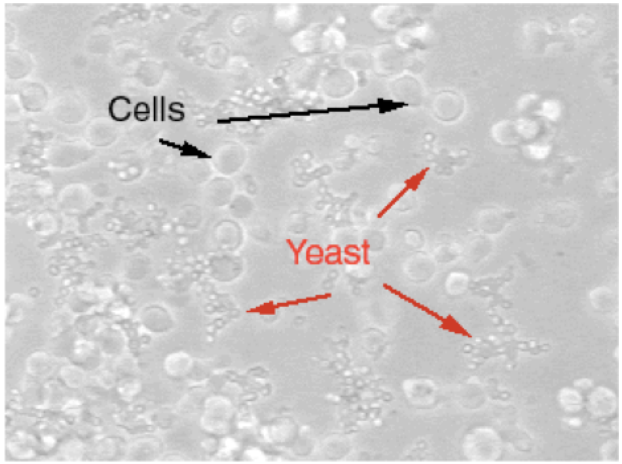
https://www.bionique.com/mycoplasma-resources/technical-articles/better-aseptic-technique.html

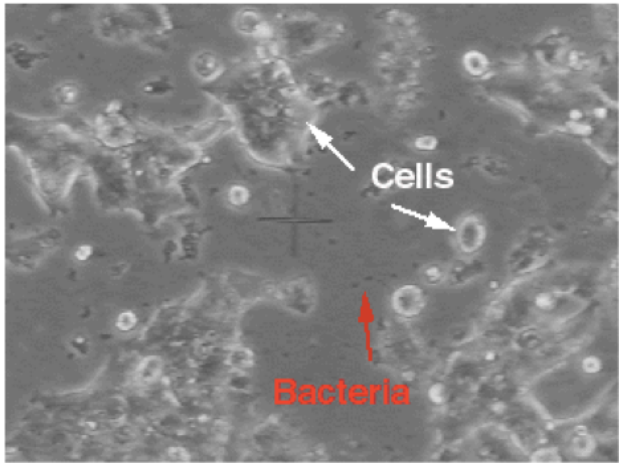
Sterilization Technique

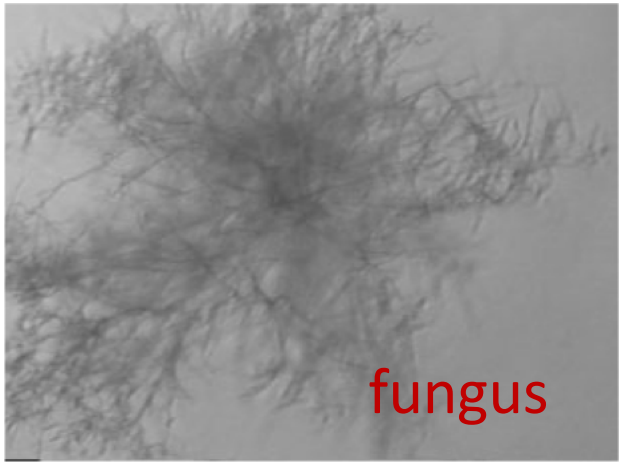
TAKEAWAYS

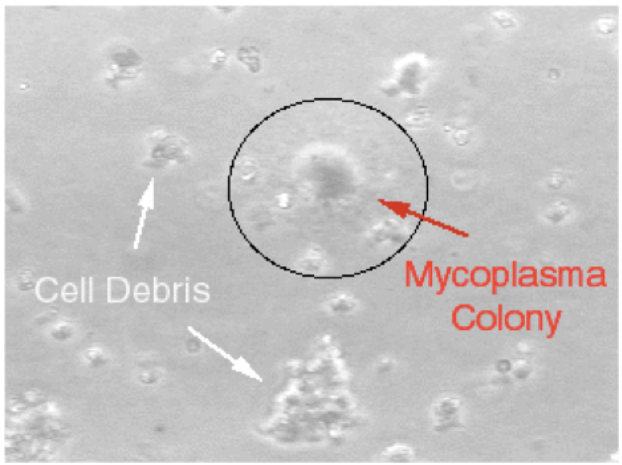
Cells are VERY easily contaminated

Better safe than sorry







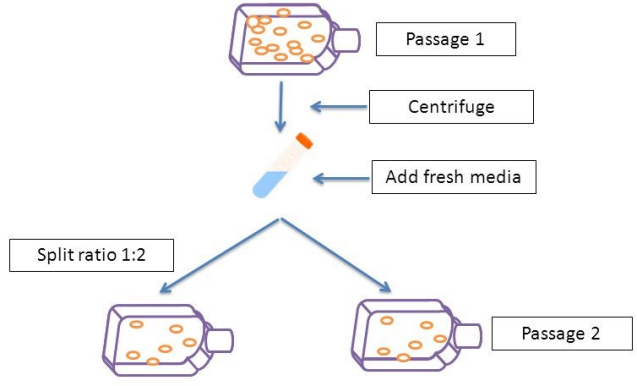


https://unclineberger.org/tissueculture/contaminant/

Cell Passaging

- **Definition:** Cell passaging removes cells from one container and seeds them in a new container

- Why would you passage?
    - Prevent overcrowding
    - Maintain ideal cell confluency
- When should you stop passaging a cell line?
    - When you reach the maximum passage number of the cell type
    - Keep track of your passage number on the flask



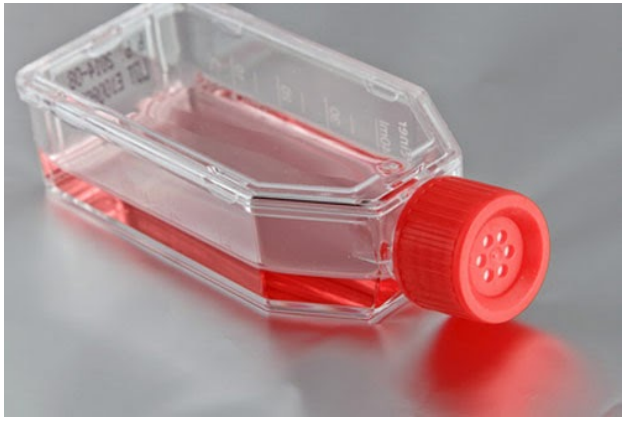
Cell Passaging Protocol

1. Use a microscope to gauge flask confluency
2. Remove old media
3. Wash cells with Phosphate Buffered Saline (PBS)
4. Add trypsin to detach cells from flask
5. Add media (DMEM) to deactivate trypsin

6. Remove cells from flask and transfer to a conical tube
7. Centrifuge conical tube to form cell pellet at bottom of tube
8. Aspirate supernatant from conical tube to remove liquid and debris
9. Resuspend cell pellet in fresh media
10. Plate cells into a new flask
11. Place flask back into the incubator

The Starting T-25 Flask

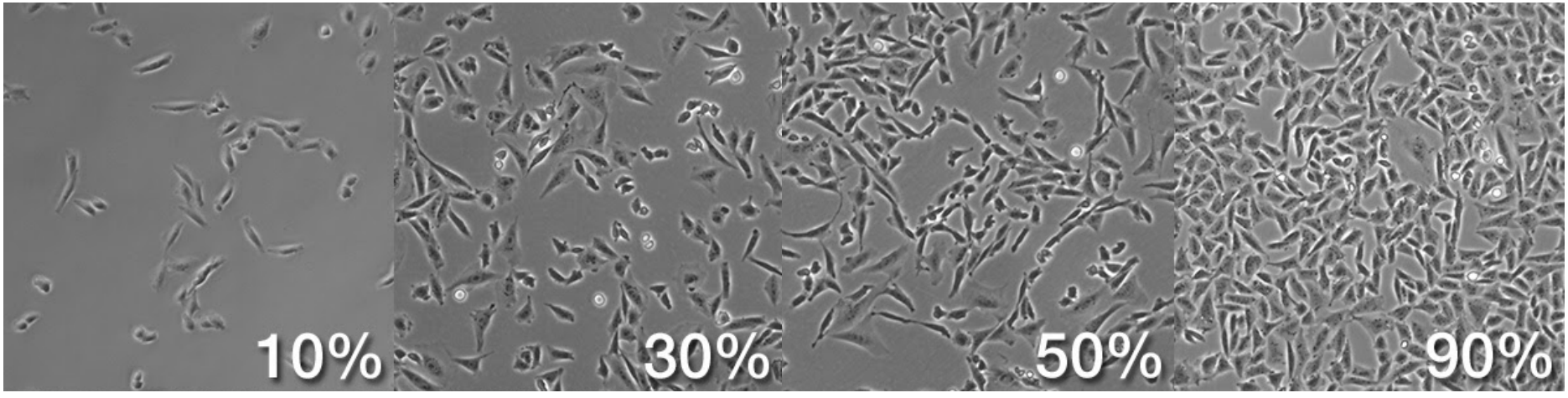
- Why the name?
  - Surface area of a T-25 flask is 25 cm^2
- Why the color?
  - Cell culture media is Dulbecco's Modified Eagle Medium (DMEM)
    - 10% Fetal Bovine Serum (FBS) provides growth factors and proteins for cells
    - 1% Antibiotics helps protect against contamination
    - Phenol red provides a visual indication of pH



- Yellow = too acidic → needs to be passaged
- Purple = too basic → problem with incubator CO2

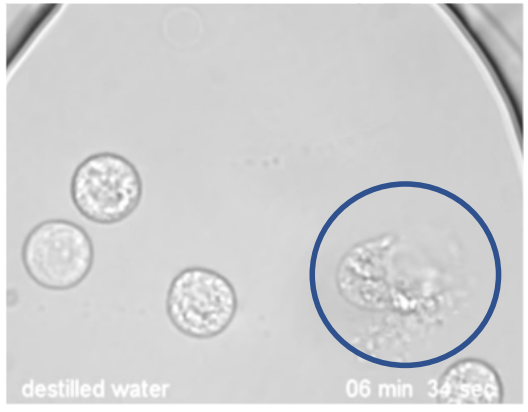
1. Gauging Confluency

- Cell confluency expresses the density of cultured cells
    - High confluency = too many cells = cell competition for resources → cell death
    - Low confluency = too few cells = cells are unable to signal with one another → cell death
- When a flask passes a certain level of confluency, you must passage the flask and reduce confluency



2 & 3: Prepare Cells for Removal

- Our cells adhere to the flask
- To prepare them for dissociation from the flask, we first remove the media
    - Media deactivates trypsin (the dissociation agent)
    - Fluids are removed from the flask via aspiration
- Then, we rinse with Phosphate Buffered Saline (PBS)
    - PBS is a saline solution designed for cell osmolarity and pH
    - removes any excess media and cell waste



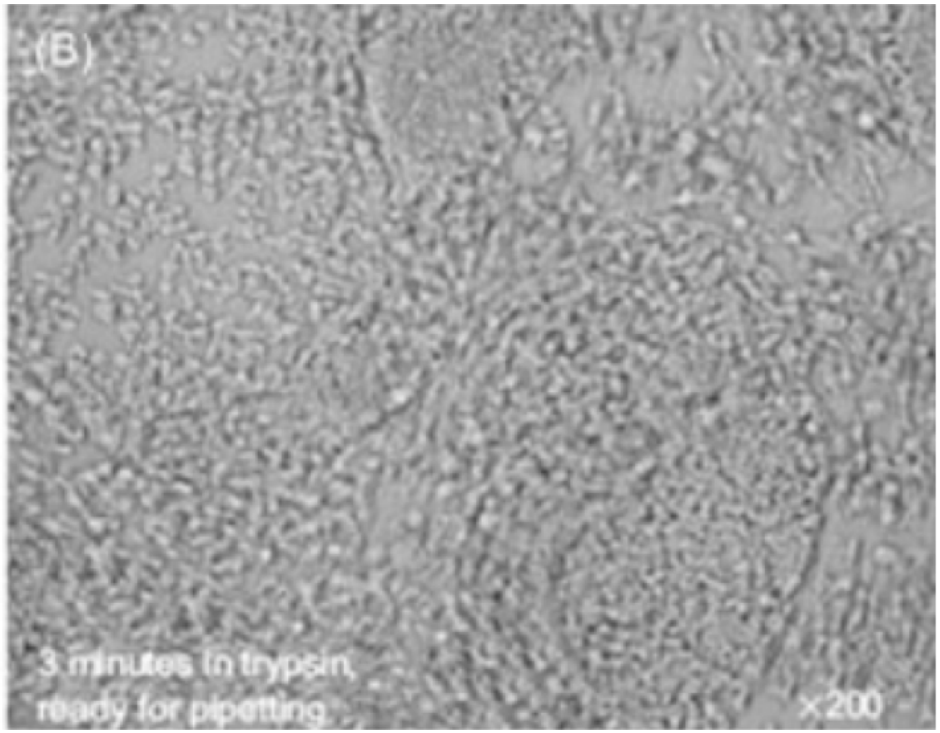
https://www.youtube.com/watch?v=QYoaLzobQmk

4. Trypsinization

- We use trypsin to detach our cells from the T-25 flask
- Trypsin is a proteolytic enzyme that breaks down proteins involved in cell adhesion
- Add enough trypsin to cover the flask and leave for 5 minutes
    - Avoid leaving trypsin in for too long, as it can damage surface proteins or lyse cells
- After five minutes, place the flask under the microscope and tap
    - If trypsinization was successful, you should see bead-like cells move around

5. Trypsin Deactivation

- Add DMEM to deactivate trypsin
    - Calcium and magnesium ions in the Fetal Bovine Serum (FBS) will deactivate the trypsin present in the flask



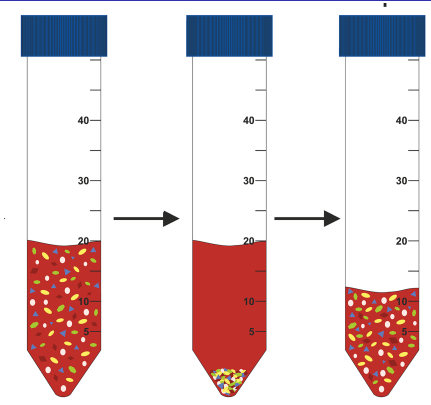
https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/trypsinization

6 & 7: Centrifugation

- After adding media, transfer the contents of the T-25 flask to a conical tube using a micropipette
- Next, centrifuge the conical tube
    - Make sure the centrifuge is balanced!
- Centrifugation separates the cells from liquid and debris
    - Centrifuge spins quickly → separation of particles by density
    - Cells form a pellet at the bottom of the conical tube

https://www.quora.com/What-does-20-tonnes-of-cancer-cells-look-like

8 & 9: Prepare for Seeding



- After centrifugation, aspirate the supernatant
    - Supernatant: the liquid and cell debris at the top of the conical tube
    - Decreases debris reseeding
    - Leaves behind the cell pellet
- Add media to the conical tube and pipette up and down to mix and resuspend the cells in media
    - Resuspend the cells in media as soon as possible
    - Without access to nutrients from media, the cells can die

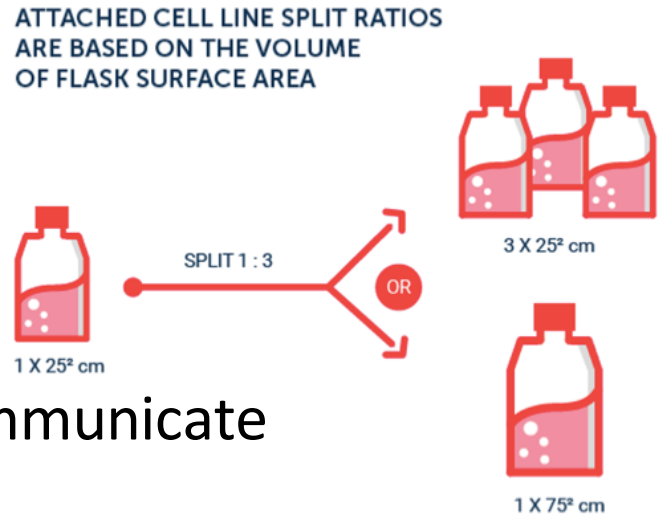
10: Reseeding Cells

- Add the media necessary to attain your desired cell density
- Reseed cells in a new T-25 flask
    - Properly label your T-25 flasks!
        - passage number
        - cell type
        - your initials / your group's initials
        - date of cell passaging
        - any treatment applied to the cells

Split Ratio

- **Definition:** The Split Ratio is the fraction of original cells that are reseeded in the new flask.

- Example: A 1:3 Split is when one third of the original cells are reseeded in each plate
- Two factors that impact Split Ratio
  - Suggested seeding density
    - Want to ensure cells can communicate
  - Time until next passage
    - Seed at the a lower density if time to next passage is longer



https://www.antibodygenie.com/cell-culture-guide-techniques-and-protocols/

Video: Cell Passaging in the BE Lab

https://drive.google.com/drive/u/0/folders/1fyCUsGUTKNscRyCzmMfNynDLq8dpZ7xY