Cell Biology Lab –Course Schedule

The instructor reserves the right to alter the following schedule. Students are responsible for being aware of any changes in assignments or exam dates announced during lectures missed by the student. Students are also responsible for being aware of university cancellations.

LABORATORY

Date		Lab Topic	Lab Skill or Tool	Assignment Due
Week	1	Introduction	Self-Assessment; Notebook; Safety; Inventory; Liquid transfer	
Week	2	Solutions and Cells	Microscopy; Solutions; Culture (media prep, seed cells);	Pre-self assessment
Week	3	Membrane Transport	Cell Culture (split cells); phase contrast microscopy	
Week	4	Cell Viability and Apoptosis	Cell Culture (cell counts)	WS1
Week	5	Organelles (Mitochondria, ER and/or Golgi)	Live stains; Spec Sheets	
Week	6	Cytoskeleton	Fluorescence Microscope	WS2
Week	7	Whole cell lysis	Protein Quantification, Standard Curves, Spectrophotometer	
Week	8	Lab Practical 1		Notebook
Week	9	Cell Fractionation	Differential Centrifugation	WS3
Week	10	Nucleus Cryopreservation	Cell Staining; Bar Graphs and Pre-lab planning & calculations	
Week	11	Protein Separation & Blot	Electrophoresis	
Week	12	Immunodetection	Image Analysis	WS4
Week	**13**	**THANKSGIVING BREAK (no lab)**		
Week	14	Lab Practical 2		Notebook
Week	15	Project Documentation	Science Communication	WS5; post-self assessment

2019

BIOL380 Cell Biology Lab Manual

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Table of Contents

Lab Assessment

The objective of this lab is to develop skills commonly needed by a cell biologist. Some laboratory modules supplement topics being learned in lecture, but most labs focus on the tools/hands-on work and scientific method commonly used by a cell biologists. In this regard, lab performance is assessed by:

1) performing laboratory tasks commonly used by cell biologists
2) naming and explaining the use of specific materials
3) presenting data
4) interpreting results

The laboratory portion of BIOL380 is worth 150 points, which is 25% of the final grade. The points earned in lab will be added to the points accumulated in lecture to determine the final grade. Performance in the lab is assessed with the following:

Lab Notebook (50 points). The student laboratory notebook is used for recording ideas and experimental work. Its purpose is to keep data and notes in a form that can be used at a later time. The notebook should have a sewn binding and stitched in pages (example, a composition notebook). The notebook can be used as a resource during the lab practicals. It will be collected twice during the semester at the conclusion of each lab practical (25 pts each time). Grading will be based on completeness (all entries present), formatting (all notebook rules followed) and a 'wow' factor.

Worksheets (50 points). Portions of each lab module worksheet provided in this packet will be assigned during the lab period in which the module is performed. The remainder of the worksheet is optional and is provided as a means to ask additional questions and seek independent answers. It can also be used as a resource for how to document results in the lab notebook. The assigned worksheet assignments are due at the start of the lab period on the due date listed in the syllabus. Each of the five worksheet sets is worth 10 points.

Sets	*Worksheets to turn in on the due date (portions assigned by instructor)**
WS1	Introduction, Solutions and Seed/Split Cells, Membrane Transport
WS2	Cell Count, Mitochondria and ER
WS3	Cytoskeleton, Nucleus
WS4	Whole Cell Lysis
WS5	Protein Separation, Immunoblot

* *see lecture syllabus for lab schedule*

Practicals (50 points). Two lab practicals (25 pts each) will be given during the semester. *Study guides will be provided for each practical*. These are open lab notebook. The entire lab period will be given to complete the practical. Each practical is composed of short answer questions asking the student to perform a task, name a material, perform a specific calculation, and/or answer questions presenting or interpreting a specific set of data.

Introduction

Notebook

We will start today by discussing how to generate proper notebook entries. A student laboratory notebook is used for recording ideas, methods and experimental work. Its purpose is to keep data in a form that can be used to record what was done. **The notebook should have a sewn binding and stitched in pages** (example, a composition notebook).

***** We will begin by labelling the cover of your notebook with your name, course number and title, semester and year and the professor's name.*****

The next page of the notebook will be a table of contents. It will list a general title for each entry and the page number where it starts.

The following is a list of rules for making an entry in the laboratory notebook:

1) Begin entries on the right hand page even if it means the left page is left blank.
2) Mark any blank pages with a line, "left blank intentionally" (LIB), date and initialed.
3) Assign each entry a **title** (e.g. making solutions)
4) Number the pages and list the title and start page in the table of contents
5) Write the **date** (month, day, year)
6) Indicate the **purpose** of the activity: A brief statement of the objectives for the day or experiment. Example, what skills were being learned/practiced?
7) **Introduce** the important aspects of what will be done. If necessary, define important words, provide formulas, define abbreviations.
8) If the entry concerns an experiment, write out the **hypothesis** or expected outcomes.
9) List **materials** needed. This list does not have to be exhaustive (e.g. does not need to include paper towels, etc.). It should list important supplies and equipment (things that are needed to complete the activity). The first time an experiment is run, it is good practice to be very specific about the equipment used. For example, a microscope won't help anyone reading the notebook. Olympus inverted microscope outfitted with a XC30 camera would be more helpful.
10) **Procedure**: describe what was done, include work for calculations

Note: Complete pages from lab manual can be taped into notebook, but need to be in sequence with written notes and author's initials signed over the tape.

11) **Data (observations)** and Analysis: write observations (e.g. solution turned yellow), write down raw data, summarize any collected quantitative data.
12) **Conclusion:** in narrative form write about the major outcomes, feelings about it and what it indicates. Write down any new ideas or things that were interesting. Include any problems encountered and a plan to solve them.

The Cell Biology laboratory notebook can be used during the lab practicals. After the practical, it will be turned into the instructor for grading. Grading guidelines include completeness (~15 pts), format (~7 pts) and 'wow' factor [beyond expectations] (~3 pts). Lab notebook grading rubric is included at the end of this manual.

General Lab Safety

Please note that Biosafety Level 1 lab practices should be used in this laboratory. The instructor and students alike are responsible for ensuring the use of proper safety practices and laboratory techniques.

Biosafety Level 1 (BSL1) is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. The laboratory is not necessarily separated from the general traffic patterns in the building. Work is generally conducted on open bench tops using standard practices. Special containment equipment or facility design is neither required nor generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in laboratory science.

The following standard and special practices, safety equipment, and facilities apply to agents assigned to Biosafety Level 1 (BSL1):

A. Standard Practices

1. Access to the laboratory is limited or restricted at the discretion of the instructor when experiments or work with cultures and specimens are in progress.
2. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in the work areas. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food, even if in sealed containers (e.g. water bottles) is kept outside laboratory.
4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. All procedures are performed carefully to minimize the creation of splashes or aerosols.
6. Work surfaces are decontaminated after the lab session and after any spill of viable material.
7. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as disposing in sanitizing agent. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leakproof container and closed for transport from the laboratory.

B. Safety Equipment (Primary Barriers)

8. Special containment devices or equipment such as a biological safety cabinet are generally not required for manipulations of agents assigned to Biosafety Level 1.
9. Optional use of laboratory coats to prevent contamination or soiling of street clothes.
10. Gloves should be worn if the skin on the hands is broken or if a rash is present.
11. Protective eyewear should be worn for conduct of procedures in which splashes of microorganisms or other hazardous materials is anticipated.

C. Laboratory Facilities (Secondary Barriers)

12. Laboratories should have doors for access control.
13. Each laboratory contains a sink for handwashing.
14. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
15. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surface and equipment.

Printed name: _____ Course Info: BIOL380-L (cell bio)

Lab Safety Rules and Guidelines

Exercises in this lab may involve unfamiliar procedures using hazardous chemicals and a variety of lab equipment. Therefore, it is important you follow these safety rules. You will be required to sign a version of this sheet for every biology lab course.

1. Come to class well prepared for the lab. Read the required lab to understand the background material and procedures
2. At all times, follow the directions of your instructor. These directions may differ from the procedures listed in the lab manual or packet.
3. Keep your counter clean and uncluttered. Unnecessary items should be placed as directed by your lab instructor. Cubbies have been provided in most labs for storage of your personal items such as bags and coats.
4. Closed-toed shoes are mandatory. No shoes revealing the tips of the toes are allowed. Students wearing improper footwear will be sent to get proper foot covering. This is a Federal lab requirement.
5. Wear clothing that covers the body. Shirts that expose the abdomen are not allowed. Long pants are recommended. Lab coats are not required for most lab courses but may be worn (instructor can advise). When working with Biosafety Level 2, long pants and lab coats are mandatory.
6. Long hair should be tied back so it does not fall into chemicals or experiments.
7. Wear safety glasses when working with chemicals, liquid cultures of organisms, or when instructed.
8. Always use mechanical pipetting devices when pipetting fluids.
9. Keep water away from electrical cords and electronic equipment. Electricity and water do not mix.
10. Food and drink are forbidden in the lab. This includes gum, candy, throat drops, and all beverages. If you wish to eat or drink, you may step outside the doorway of the lab to do so. Drink bottles are never to be placed on the lab bench. Bacteria and other organisms are grown in the lab. This is another Federal lab requirement.
11. No cell phones are to be used in the lab. Keep it in your bag, not on you, when in the lab, unless instructed otherwise.
12. Inform your instructor immediately of any breakage, spill, or injuries, even minor ones so proper protocols can be followed.
13. Be sure you know how to use a piece of equipment before using it. When in doubt ask your instructor.
14. At the end of the period, wash your glassware and other materials and then return them to their original location. All equipment should be cleaned and returned to its original location also. If cleaner is provided, wash down your desktop. **Wash your hands before you leave the lab.**
15. Wear safety gloves when indicated by your instructor. If working with Biosafety Level 2, gloves are mandatory.

I have read the laboratory practices, procedures, and safety rules listed above. I understand these contents and agree to abide by all laboratory rules set forth by the laboratory instructor. I understand that my safety is entirely my own responsibility and that I may be putting myself and others in danger if I do not abide by all the rules set forth by the instructor.

Signature:_____ Date: _____

SAFETY WORKSHEET:

1) Which BSL level safety practices should be used in this lab?

2) Walk around the room and identify four objects in the lab utilized for safety. Write the names of the safety objects here:

i. _____

ii. _____

iii. _____

iv. _____

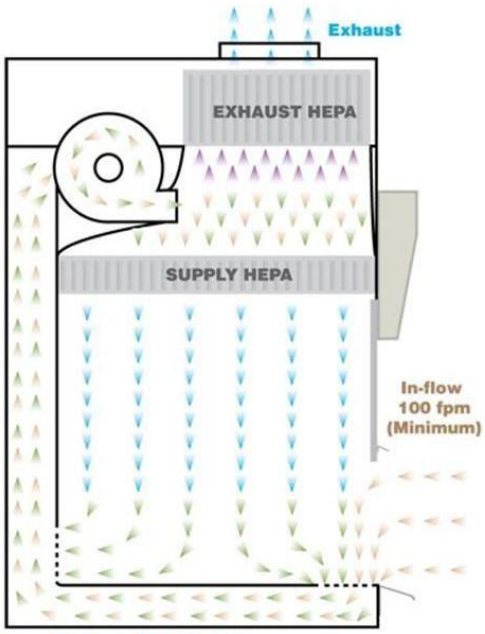
Equipment and Supply Inventory

Purpose

The main purpose is to identify (name) major laboratory equipment and supplies and to recognize their function. Also, a student should be able to determine where supplies are ordered and how much they cost.

Equipment

A major task of the cell biologist is to perform cell culture, a process of growing cells under controlled conditions. Several pieces of equipment are used in cell culture: a 'hood', a CO_2 incubator, a bucket centrifuge and an inverted microscope.



A 'hood' allows a cell biologist to keep a 'pure' culture of cells (not contaminated by other cell types). The 'hood' used for cell culture is a **Biological Safety Cabinet – Class II**. It is a laminar flow hood that intakes air from the room through a front grating system. This air runs behind the unit into a high-efficiency particulate absorption (HEPA) air filter that filters out any particulate bigger than 200 nm. The air from this supply HEPA is now allowed to flow toward the work space where the product (e.g. cell line) is being manipulated. The air then flows from the work space through a rear grating system into the back of the unit (away from the workspace). This air run through an exhaust HEPA and allowed to exit back into the room. Therefore, the BSC-Class II protects the environment, person and product. It can be identified from other hoods in the laboratory by a label.

A **CO_2 incubator** provides the environment where the cells grow and are maintained. <u>To keep cells alive, we must mimic their original environment.</u>

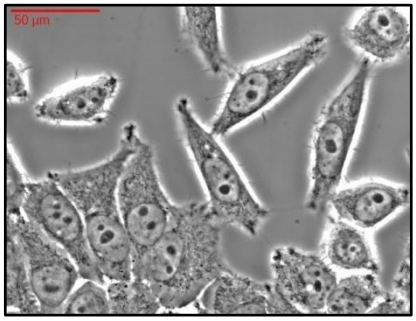
For example, if we are growing eukaryotic mammalian cells, they will need a temperature that mimics body temperature (37˚C (~98˚F)), 95% humidity (water source) and a system to control pH at neutrality such as 5% CO_2 in combination with bicarbonate.

A CO_2 incubator can be identified by the tube that leads from the incubator to a nearby CO_2 gas tank. It is usually set to 37˚C and 5% CO_2. We should be able to hear the clicking sound associated with the CO_2 being pumped into the incubator.

To move cells from place to place and/or change their concentration, we will have to pellet the cells away from the liquid in which they are living (media). To accomplish this goal, we will get the cells into suspension (different methods will be present during splitting cells lab) and transfer them into a tube (example, 15 mL tube – see supplies inventory). We will use a **bucket centrifuge** that will use centrifugal force (1000-1200 rpm, 5 minutes) to move the cells into a pellet. Now we can remove the supernatant and are left with a pellet of concentrated cells.

In cell culture, we usually use a dish or flask for growing cells. These vials do not fit in the working distance of an upright microscope. Most cell biologists have inverted **microscopes** where the objectives have been moved to below rather than above the stage. An inverted microscope is similar to an upright microscope having oculars, course focus knob, different magnifying objective, a stage and an iris diaphragm to adjust the light.

In addition, an inverted microscope usually has a **<u>phase contrast filter</u>** to convert phases of light that pass through a transparent object into difference in object brightness. Phase contrast allows a cell biologist to easily distinguish the different parts of a cell without the need for staining like methylene blue.

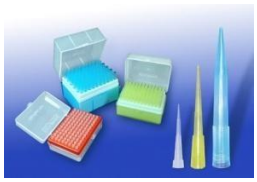


SUPPLIES

Certain supplies are common to all labs. Each group will put together a supply bin with these supplies so they are readily available. Laboratory supplies are classified as **nonconsumable** (used over and over) or **consumable** (used up, each group will be responsible for the inventory of these supplies and replenishing supplies when low).

Consumable supplies need replacing because they are used once and then thrown away. In a cell biology lab, most of the consumable supplies are sterile (all living organisms have been removed). Sterile supplies are labeled with autoclave tape and/or individually wrapped. They are no longer sterile once opened or used.

Consumable supplies needed for the supply bin include serological pipettes, pipetman tips, conical tubes, cell culture T25 flasks and C60 dishes. **Several sizes of each of these** are needed to complete the bin.



Nonconsumable supplies needed for the supply bin include a plastic beaker (for disposing of liquid), tube racks to hold tubes, markers and a spray bottle. Several nonconsumable supplies will be provided in the room during the lab and do not need to be stored in bins, such as scissors, transfer pipets, lens paper, paper towels, parafilm and biohazard bins.

TASK to PERFORM

1. The room has six table stations. Each table station will have no more than 3 people. Choose a particular table station. If all the table stations already have 3 people, see instructor.

2. Exchange contact information and agree upon a group name (shorter name, less labeling).

3. Each group choose a bin **to use all semester**. The group's supplies will be in this bin. When acquired, use tape to label bin with group name (best if tape is on sides not top).

4. Send one person to gather general supplies that will be used throughout the semester (use inventory on next page as a guide). The supplies are on a table in the lab room.

5. **Make sure to get one of everything.** Store the supplies in the bin.

6. Get acquainted with the lab, equipment and supplies.

7. Identify the name of each item. Look up the catalog number, unit and price for one of the serological pipets and one of the dishes/flasks that were provided (fishersci.com or other). **Record this information in the worksheet.** Note: Naming common consumable supplies is a learning objective for the lab.

INVENTORY from lab coordinator

Supplies that are housed in the lab room

- ✓ Bottles of liquid disinfecting solution (example, Lysol)
- ✓ Pipet Aids
- ✓ Pipetmen
- ✓ Carboy of distilled water

List of supplies for each group's supply bin

Nonconsumables

- □ Bin (56qt Tupperware)
- □ Plastic beaker (>500mL) for Lysol
- □ Empty Styrofoam 50mL tube rack
- □ Empty Styrofoam 15mL tube rack
- □ 1.5mL tube rack
- □ Sharpie – fine/thick tip
- □ Spray bottle
- □ Roll Lab Tape (any color)

Consumables

*** It is the group's responsibility to request consumables as they run out.*

- □ 60 mm tissue culture treated dishes (one bag)
- □ T25 tissue culture treated flasks (one bag)
- □ Serological pipettes , 5 mL (two sleeves)
- □ Serological pipettes, 10 mL (one sleeve)
- □ Serological pipettes, 25mL (banded pack)
- □ 15mL tubes (one bag)
- □ 50mL tubes (one bag)
- □ Container with sterile 1.5 mL tubes
- □ sterile yellow tips (one box)
- □ sterile blue tips (one box)

Microscopy

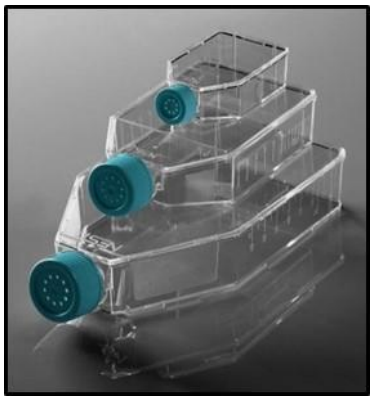
Purpose

The main purpose of this laboratory are to use of an inverted microscope and identify its parts

Background

Conventionally, cells grown in cell culture are in suspension or adherent to flat, plastic vessels. If the cells are adherent, the plastic is treated to enhance cell attachment. For example, the vessel can be coated with gelatin, a protein obtained from skin that contains positively charged amino acids, such as arginine and lysine. The cells are more likely to stick to cell culture treated plastic than regular plastic. As such, these special vessels are marked to indicate that they are treated for cell culture, such as "C" for culture-treated or "T" for tissue-culture treated.

Additionally, cell culture vessels are defined by their approximate dimensions or format. For example, a **C60** dish is a culture treated dish with approximately a 60 mm diameter and a **T25** flask is a culture treated flask with 2500 mm^2 of surface area. We are going to use either a 60 mm dish (C60) or a vented to grow our HeLa cells.



To view cells in culture with an upright microscope, we could float a coverslip in the vessel and then transfer it to a microscope slide, buy expensive vessels with removable sides or even saw off the sides of the vessels. Or, we could use an inverted microscope, which was invented by Dr. J. Lawrence Smith in 1850.

An **inverted microscope** has its objective underneath the stage and pointing upward to the bottom of the sample. The light is directed to the sample from a condenser lens above the sample. Cells in culture are colorless, transparent and difficult to distinguish from their surroundings by conventional bright field microscopy. So we are going to use **phase contrast** microscopy, developed by Dr. Frits Zernike in the 1930s, which applies rings to the condenser and objective to create interference light patterns in the sample. This results in a phase shift in the wavelength of light reaching the eye and enhances our ability to distinguish differences in object brightness or give the cells more contrast.

Inverted microscopes are expensive (example, the AccuScope EXI-300 inverted microscope in the lab costs ~$4,000) so please handle with great care. If something is wrong with a microscope, notify the instructor. Most of the lab work will be done using the inverted microscopes provided for each group. Review the general parts of a microscope and their function.

Today, we are going to become familiar with using an inverted microscope and identifying its parts, particularly the phase contrast filter.

TASK to PERFORM

Each group carry an inverted microscope to their station and complete microscope worksheet.

ADD PICTURE OF MICROSCOPE HERE

The following is a set of instructions for using the provided microscope.

Set up and place dish/flask for viewing

- Use both hands to set microscope on counter. Remove cover and store cover in desk slot
- Unwind cord and plug into socket, making sure cord does not dangle off the bench
- Turn on microscope with toggle switch. Adjust light with rotating dial on side
- Use nosepiece at base of microscope to rotate objectives to lowest objective
- Use course focus knob to move nosepiece up to stage

Observation of a specimen

- **Make sure to slide phase slider to middle position which is the phase contrast filter**
  If microscope has a "PH0" knob on side of arm, turn to activate phase contrast.
- Starting with lowest objective, use coarse adjustment to focus. *Hint: look through the eyepiece and move the sample. If you are actually focused on the sample than it should be moving*. Fine tune focus with fine adjustment knob (smaller knob).
- Place sample on stage. Move sample with hand until centered over light.
- Microscope is *parfocal*, so the objectives can be changed with minimal focusing needed between them; Switch to higher objective. Use fine focus knob to focus on higher objective.
- As increase magnification, adjust the light by adjusting the dial on the side

Clean up and put away

- Go back to lowest objective.
- Clean the stage and lenses with lens paper.
- Switch light off, coil cable, and cover microscope before putting away.

TASK to PERFORM

Each person should use inverted microscope to view one of provided samples and determine what is considered one cell and the total magnification.

In cell biology, the size of cells viewed under the microscope is an integral part of studying the cell. Therefore, we should always indicate the **total magnification** used to view the cell [ocular lens magnification power (10x) multiplied by objective lens magnification power].

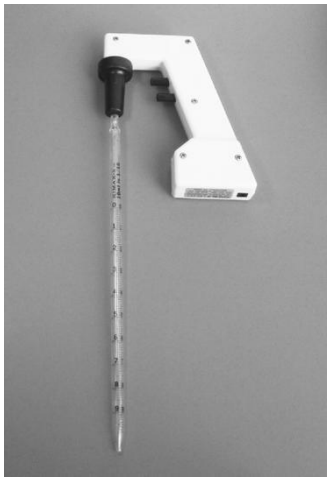
START LAB NOTEBOOK with liquid transfer entry

Liquid Transfer

Purpose

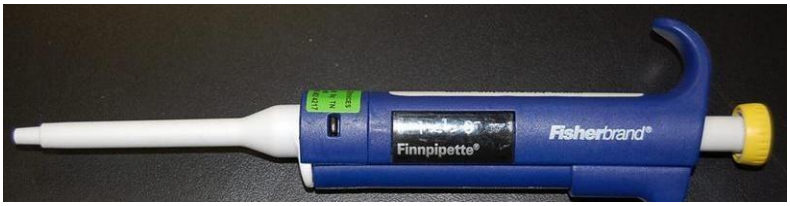
The main purpose of this laboratory is to learn how to transfer liquid from one vial to another. In cell biology, we maintain cells at a particular concentration by moving them from their current flask/dish into another flask/dish using liquid transfer techniques.

Background

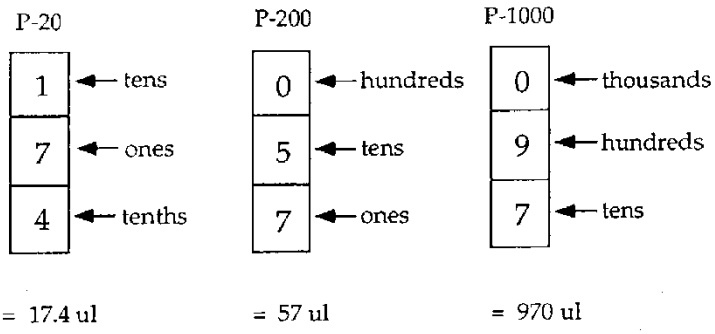


In order to transfer liquid in the lab, use two different tools: a pipet-aid (also called a pipet filler) and a pipetman (example Finnpipette®).

A **pipet-aid** is used to draw up <u>greater than 1 mL</u> of liquid and transfer it to another location. It fits different volume **serological pipettes** (**have 5 mL, 10 mL and 25 mL**). The serological pipettes have a cotton plug to catch liquid that goes beyond the volume of the pipette. However, any additional liquid will enter the mechanical part of the pipet-aid and **hamper its function**. Usage of the appropriate serological pipet for the volume needed is essential.
The pipet-aid has two button on its stem. The top button is for sucking up liquid into the serological pipet once the tip of the serological pipet is in the liquid of choice. The bottom button is for releasing the liquid once the tip of the serological pipet has been moved to the appropriate tube. Sometimes these buttons have speed (slow, medium fast), slow speed (S) is recommended. ***To work the pipet-aid you will gently push serological pipet into the stem, put the serological pipet tip into the liquid, gently press top button until reach volume desired, release top button, move tip to other vial and gently press bottom button to release liquid.*** Remove serological pipet once done. Note: number marking are sometimes backwards where 1 mL is marked on the top of the pipet.



A **pipetman** is used to draw up <u>less than 1 mL</u> of liquid and transfer it to another location. There are several different types of pipetman in this lab and each pipetman has its own way of working, usually the volume is changed by turning the knob, but sometimes knob needs unlocking.



Each pipetman has its own way of displaying volumes. P-20 pipetman range in volumes up to 20 µL. They give the ability to pipet a tenth of a µL (example, 17.4 µL). P-200 pipetman range in volumes upto 200 µL. P-1000 pipetman range in volumes upto 1000 µL (note: 1000 µL is equal to 1 mL). Each pipetman has its range of volumes printed on the side. Check this before using it. If use a volume beyond the capacity of the pipetman**, it breaks**. Become familiar with each type and 'range of volume' pipetman.

Each pipetman fits a different tip type. In most cases, a pipetman that can pipet a volume of 1000 µL fits a **blue tip**. All other pipetman volumes usually fit a **yellow tip**. *The blue/yellow tips in the boxes have been autoclaved for sterility*. Open them for minimal amounts of time.

To work the pipetman, gently put the pipetman over a tip and push down to engage the tip onto the pipetman [do not use hands as the tips are sterile], gently press the top button down until reach the first stop, keep the button depressed, put the pipet tip well into the liquid, slowly release the top button, move tip to other vial and gently press top button to second stop to release liquid. The second stop is also used for repeat pipetting and gives extra working volume beyond the value set on the pipetman. Remove tip once done.

There are a variety of different tubes that hold a variety of different volumes; **50 mL tube, 15 mL tubes and 1.5 mL tubes**. Although these tubes are packaged in bulk, they are sterile on the inside.

TASK to PERFORM

One person from each group fill a 50 mL tube to ~25 mL with the distilled water in the carboy in the lab room. Use the group's 50 mL tube rack to hold the tube upright. The whole group will use this for the liquid transfer task.

Look for the racks, tips and tubes in groups' personal bin.

A. **Each person in the group** transfer the following three volumes of distilled water.

1) 3 ml (using pipet-aid and serological pipet)
2) 500 µl (using 'large' pipetman and tip)
3) 50 µl (using 'small' pipetman and tip)

The choice of pipet/tip (5 mL, 10 mL, 25 mL serological pipet OR blue/yellow tip) and tube (1.5 mL, 15 mL or 50 mL) are chosen by each person.

B. Once the tubes are chosen, **use a sharpie** to write your first name on the plastic of each of the three tubes (do not use tape) [Note: if a group has 3 people that group turns in 9 tubes].

C. Once complete send one person to bring all of the group's tubes to the instructor.

Instructor will check transfer and note completion on master list. Liquid transfer skill level depends on ability to choose the appropriate supplies (e.g. pipet and tube size) and have the right amount of liquid in the tube chosen.

** Mark off completion on your worksheet.

A liquid transfer skill will also be performed during the lab practical.

Worksheet - Introduction

Name:_____

Supplies Inventory Table

	Name of Item	Catalog #	Unit	Price
1)				
2)				

Microscope

1) What is the function of a phase contrast filter? (hint: read 'equipment inventory' intro)

2) Write the name of the microscope part at the tip of the arrow (A-E) on the diagram of an inverted microscope. (hints: B is the lever above the condenser; C is holding a filter)

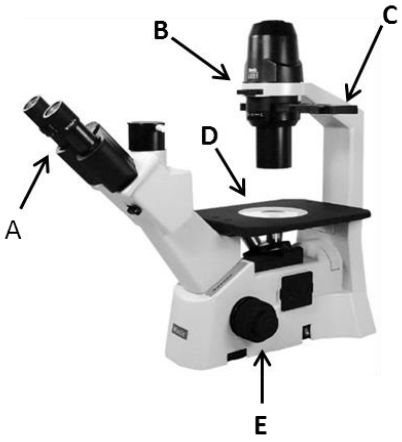
A. _____

B. _____

C. _____

D. _____

E. _____



_____ Three Liquid Transfer Tubes submitted to instructor (check off once turned in)

Solutions

Purpose

The main purposes of this laboratory are 1) to review how to calculate solution concentrations and use basic laboratory tools to make solutions, 2) review how to utilize sterile technique to avoid contamination and how to detect contamination in eukaryotic cell culture

Background

Much of the work in a cell biology lab involves making and diluting solutions. Therefore, we will review/learn how to calculate stock and working solution concentrations in various units (examples are %, m/v, M). We also need to know how to convert one unit of concentration to another and how to make or dilute a stock solution into a desired working concentration.

Solutions are solutes dissolved in solvent. We will use distilled water (dH_2O), phosphate buffer saline (PBS), media and DMSO as solvent [make sure to check procedure for appropriate solvent]. The expression of solution concentrations that we will be using are percentage (%), mass/volume (such as g/L) and molarity (M).

Percentage (%) is the amount of solute, by weight (g) or volume (mL), in a total solution volume of 100 mL. As an example, a 1% solution is made by weighing out 1 g of solute and dissolving it up to 100 mL of water. So, a 10% solution has 10 g of solute dissolved up to 100 mL of solvent (like water, PBS etc.). Percentage can also be volume to volume so a 20% solution of ethanol has 20 mL of pure ethanol with solvent (like 80 mL water) added to get to 100 mL of total solution. *Practice: 3% H_2O_2 is how many g/L?*

Mass/Volume (g/L or mg/ml) is an expression of concentration in which the amount of solute measured by its mass (g) is dissolved in total amount of solution measured by its volume (L). If the same prefix is put before both the mass and the volume it is equal to the original. (conversions of these nature do not have to be shown)

$$\frac{\boldsymbol{g}}{\boldsymbol{L}} = \frac{\boldsymbol{mg}}{\boldsymbol{mL}} = \frac{\mu\boldsymbol{g}}{\mu\boldsymbol{L}} \ \boldsymbol{and\ so\ on} \ldots$$

Molarity is an expression of concentration in which the amount of solute measured by the number of moles is dissolved in one liter of solution (mol/L). Molarity assumes that the molecular weight (mw), which is g/mol, of the solute is known.

As an example, to make a 1M solution multiply the molecular weight of the solute by 1M and this will reveal how many grams of solute to dissolve upto 1 L solvent. Many times 1L of solution will be overkill (too much), so remember 1 g/L is equal to 1 **m**g/**m**L and so on. *Practice: How would we prepare 45 mL of a 0.5 M sucrose solution?*

$$\boldsymbol{mw}\left(\frac{\boldsymbol{g}}{\boldsymbol{mol}}\right) \times \boldsymbol{1M}\left(\frac{\boldsymbol{mol}}{\boldsymbol{L}}\right) = \frac{\boldsymbol{g}}{\boldsymbol{L}} = \frac{\boldsymbol{mg}}{\boldsymbol{mL}} = \frac{\mu\boldsymbol{g}}{\mu\boldsymbol{L}} \ \boldsymbol{and\ so\ on} \ldots$$

Alternatively, we can rearrange this formula *to determine the Molarity (M)* from a given mg/mL. For example, the Molarity of a 10 mg/ml (1%) solution of glucose with a molecular weight of 180.2 g/mol would be “10 divided by 180.2”, or a 0.055 M (55 mM) solution. *Practice: How many mg of insulin (mw 5778) to make 50 mL of a 30 µM solution?*

$$M\left(\frac{mol}{L}\right) = \frac{10\ mg}{mL} \times \frac{g}{1000\ mg} \times \frac{mol}{180.2\ g} \times \frac{1000\ mL}{L} =$$

If we are unable to accurately weigh out the solute (example 50 µg of solute), it is pre-weighed at the company and provided in a vial. We will calculate g/L from the mw and use part:part ratios to determine how much solvent to add to the vial of solute to get desired M.

$$\frac{calculated\ amount\ of\ solute\ (g, mg, \mu g, etc)}{calculated\ amount\ of\ solvent\ (L, mL, \mu L\ etc)} = \frac{how\ much\ solute\ in\ vial}{?\ how\ much\ solvent\ to\ add\ to\ vial}$$

Practice: Describe how to make a 1mM stock of MitoTrack (mw 500) given a 100 µg vial?

Dilutions are often used when a stock solution is made at a high concentration and subsequently needs to be diluted to a working concentration for it to be useful. Depending on what information we have, we will use either part:part ratios as above or C1V1=C2V2.

Note: Do not confuse this part:part dilution that is used for cell counts and other calculations with the part:whole dilution factor (1:10, 1:4, etc.) that is used when splitting cell cultures.

TASK to PERFORM
Individually or as a group, complete the practice problems above.

C1V1=C2V2 is used to prepare a volume of solution (V2) of a certain concentration (C2) by using a stock solution of higher concentration (C1). Where C1 and V1 are the concentration and volume before dilution and C2 and V2 are those after dilution. Keeping track of the units associated with the concentrations and volumes are extremely important.

The equation C1V1=C2V2 is rearranged to suit our purpose. Example: To prepare 100 mL (V2) of a 0.1 M (C2) solution from a stock solution of 1 M (C1), we need to determine the volume of stock to use (V1). $1M \times V1 = 100\ mL \times 0.1M$

Therefore, $V1 = \frac{100\ mL \times 0.1M}{1M} = 10mL.$

We would dilute 10 mL of the 1 M stock solution and mix that with enough water (or other solvent) to make 100 mL of the 0.1M final solution.
C1V1=C2V2 can also be used to calculate percentages (100%)(V1)=(70%)(100mL).

TASK to PERFORM (per group)
We are going to calculate concentrations of solutions and perform dilution of these solutions throughout the semester. The formulas discussed are needed to calculate how to make a variety of solutions. Show all calculations and units in answers. For now:

Prepare 250 mL of a 70% solution of ethanol from the ethanol provided.
Use C1V1=C2V2 to calculate the volume of ethanol that will be measured to prepare this solution. [Example, (110% EtOH stock) (V1) = (80% EtOH working solution) (500 mL)]

Use the graduated cylinders to measure out this volume of ethanol and fill up to 250 mL with distilled water from lab carboy. Transfer to the squirt bottle in the bin, cap and mix. **Use tape** to label with the group name, 70% EtOH and the date. 70% EtOH can be stored in the bin.

SKILL: Solutions stored in the laboratory need appropriate labels. Every solution needs to identify the solution (including concentrations), the person/group responsible and the date.

Cell Culture
Sterile Technique

Background. When culturing cells, a scientist usually works in a Biological Safety Cabinet Class II ("hood"). As we do not have a BSC Class II for regular usage, we will be working on our laboratory bench tops following sterile technique on sanitized table tops.

Sanitized versus Sterilized.

Sanitization is making the item free of MOST live bacteria. A sanitization agent such as 70% Ethanol or hydrogen peroxide (H_2O_2) is sprayed onto the surface. To sanitize, allow the agent to sit on the surface for 30 seconds and then wipe off usually moving in a direction farthest from you toward you so you do not run your arm over the sanitized surface. If sanitization agent sits on the item for 10 minutes, it is considered disinfected.

Sterilization is making the item free of ALL live bacteria. This process can not be accomplished in a standard lab room. Special equipment such as an autoclave or a gas sterilizer (similar to what is used in hospitals) is needed. To show that the item has been sterilized, it will have some type of indicator. An example of an indicator is autoclave tape. It has white striped when it has not been sterilized and black striped when it has been sterilized. A similar color changing indicator is used when the item has been processed with gas sterilization.

Therefore, something that is sterilized can also be considered sanitized, but something that is sanitized is not sterile. We are unable to sterilize the laboratory table top or 'hood' work space as it can not fit into an autoclave. We will only be able to sanitize it.

Sterile technique (aseptic technique) is the execution of eukaryotic cell culture procedures without introducing contaminating microorganisms (e.g. bacteria) or other cell types. Sterile technique ensures that all cell culture procedures are performed to a standard that will prevent contamination from bacteria, fungi, mycoplasma and contamination with other cell lines.

Before starting: spray surfaces with 70% ethanol (or similar) and wipe surfaces dry from back to front; avoid rapid movements that will draw particulate to the area; all equipment that will be placed on the table top such as media bottles, pipette tip boxes, and hands/gloves should be sprayed with 70% ethanol.

The following is a step by step procedure for using sterile technique to transfer liquid:
Begin by loosening the cap of the tube before you open the pipette or the tip box. Open the cover of the individually wrapped serological pipet from the bottom of the pipette (where the cotton tip is) or open the lid to the pipet box only to remove a tip. Make sure the tip of the pipette does not touch areas that are not sterile. Put the pipette-aid on the serological pipette or the pipet tip firmly on the pipetman. Aspirate ('suck up') the appropriate amount of liquid. Again, be very careful that the tip of your pipette does not touch the outside of the tube or any other area that is not sterile. Release the liquid. Do not reuse the serological pipette or pipet tip unless making multiple plates that are identical and/or certain that it is not contaminated. Use a fresh pipette if use different cells or different media. When work is done spray the work surface with 70% ethanol and dispose of all waste appropriately.

Liquid (fluid) waste that is not biohazardous will be transferred into a beaker containing ~25-50 mL of Lysol. Allow the liquid to mix with the Lysol for 10 minutes. After disinfection, the liquid can be disposed of down the sink. If biohazardous, dispose in proper container as advised by instructor. Solid Waste will be disposed of in proper trash receptacle (trash or biohazardous container) as advised by instructor.

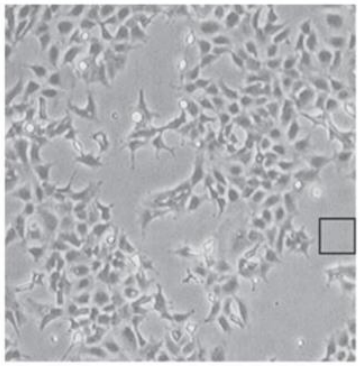
Sterile Technique and Contamination

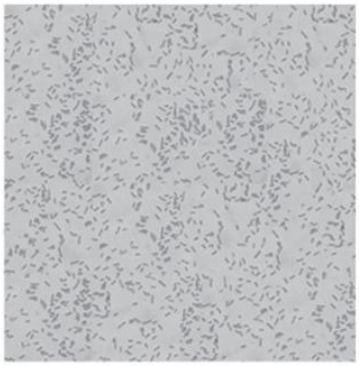
Detecting contamination while culturing a cells is imperative. Contaminants can alter the behavior of the cells and lead to false data reporting. Because of their ubiquity, size, and fast growth rates, bacteria, along with yeasts and molds (fungus), are the most commonly encountered biological contaminants in cell culture.

Bacterial/Fungal contamination is easily detected by visual inspection of the culture;

- Infected cultures usually appear cloudy (i.e., turbid)
- Sudden drops in the pH of the culture medium is also frequently encountered leading to a change in media color if it contains phenol red
- Under a low-power microscope, the contaminants appear as tiny, moving granules

The images below show the media of a cell culture flask (T25 flask) and the adherent cultured cells. The left side is of cultures that are not contaminated (media is red). The right side is of a contaminated culture. The media is yellow and the cells are covered with bacteria.





Media Preparation

Purpose

The main purpose of this lab is to prepare media to support the growth of our cells.

Background

Media is liquid designed to support growth of cells outside of the organism. The media we will make today is made up of media base plus 3 additives as follows:

Dulbecco's Modified Eagle Media (DMEM) is the base for the media. In addition to water, it contains 1) amino acids and vitamins needed for cell growth, 2) bicarbonate as part of the buffer system to maintain a neutral pH, and 3) phenol red which is a pH indicator and changes color according to the pH. Phenol red is red in neutral pH (which is optimal), dark pink (fuschia) in basic conditions and yellow in acidic conditions. DMEM spiked with phenol red turns dark pink upon exposure to room air and yellow when too many cells are present in the culture as would occur if the culture was contaminated with quickly dividing bacteria.

DMEM is supplemented with the following additives.

- **Fetal Bovine Serum (FBS)** is a mitogen that stimulates proliferation (mitosis) to increase cell numbers
- **L-Glutamine ('L-glu')** is an amino acid that feeds into citric acid cycle. L-glutamine is light sensitive and breaks down over time. Adding extra L-glutamine assures that cells have access to this amino acid that would be made by the organism (nonessential).
- **Antibiotics/Antimycotic (A/A)** prevents contamination and containe penicillin & streptomycin to inhibit bacterial growth and amphotericin to inhibit fungal growth.

TASK to PERFORM (per group)

Make 250 mL media. Use C1V1=C2V2 to calculate how much volume (V1) of FBS, L-glutamine and antimicrobial to add to DMEM.

Media Supplement	Stock concentration (C1)	Volume of stock to add to media (V1)	Final concentration of supplement (C2)	Volume of media to prepare (V2)
FBS	100%		10%	250 mL
L-glu	200 mM		2 mM	250 mL
A/A	100%		1%	250 mL

Hint: To find out how much DMEM (base) to add subtract the volume of the supplements from 250 mL (250 mL total – ____ mL FBS – ___ mL L-glutamine – ___ mL A/A = _____ mL DMEM).

Note: If the FBS, glutamine or antimicrobial are still frozen, please warm them in your hands to thaw. Also, L-glutamine tends to settle upon freezing so bring it back into solution by vortexing the tube.

Use sterile technique to liquid transfer (no pouring) calculated volumes of supplements and DMEM into sterile bottle. *Reminder: bin has 25 mL serological pipets.* Use tape wrapped all around upper part of bottle and sharpie to label with "DMEM+", the group name and the date. Store in the refrigerator (4°C) for later use.

Seeding cells

Background

Cell lines in continuous culture are prone to genetic drift, finite (primary) cell lines are fated for senescence, all cell cultures are susceptible to microbial contamination, and even the best-run laboratories can experience equipment failure. Because an established cell line is a valuable resource and its replacement is expensive (one cryovial of cells cost ~$450) and time consuming, it is vitally important that they are frozen down and preserved for long-term storage. In the USA, cell lines can be obtained from a repository called the American Tissue Type Collection (ATCC), **www.atcc.org**.

As soon as a small surplus of cells becomes available, they should be frozen (cryopreserved) as a **seed stock** and protected. **Working stocks** can be prepared and replenished from frozen seed stocks. Frozen cells are at ≥ -80C (e.g. liquid nitrogen) in tubes called **cryovials** that can withstand extreme cold temperatures and suspended in cryopreservation media such as DMSO in media.

DMSO (dimethyl sulfoxide) is a cryoprotectant and is used in the cryopreservation media to protect the cells from lysis ('bursting') during freezing. DMSO accomplishes this task by interrupting the formation of ice crystals that disrupts cell membranes and partially solubilizing membrane (increasing fluidity) so the cell membrane is less prone to rupture. However, DMSO is toxic to cells at temperatures >°4. So as the cells thaw, the DMSO causes cell death. The thawing procedure is stressful to frozen cells, and using good technique and working quickly ensures that a high proportion of the cells survive the procedure. As with all cell culture procedures, instructions specific to the cell should be followed.

TASK to PERFORM

A. Each person should go to the atcc.org website and search for catalog #CCL-2 to find out the name of the cell line and the tissue that the cell line originated from and the base culture media. Record this information in the worksheet. The website also provides other information and detailed protocols specific to the cell. **Finding information appropriate to a specific cell line is an important skill when culturing cells.**

B. Each group seed one vial of HeLa cells into one T-25 flask and maintain it in culture.

PROCEDURE

1) Use sterile technique.
2) Transfer 4 mL of pre-warmed **DMEM+** into a 15 mL tube.
3) Remove the cryovial containing the frozen cells from liquid nitrogen storage and **immediately** place it into a 37°C water bath.
4) Quickly thaw the cells (< 2 minutes) by gently swirling the vial in the 37°C water bath until there is just a small bit of ice left in the vial.
5) Before opening the cryovial, drench the outside of the vial with 70% ethanol.
6) Use a pipetman set to 1000 µL (1 mL) and sterile blue tip to transfer the entire contents of the vial slowly into the tube containing the pre-warmed DMEM+.
   ***Remember, unlike the tip, the pipetman is not sterile so do not allow it to touch the inside of the tube.*
7) Using the bucket centrifuge. Pellet cells at 1000 rpm, 5 min. [seek help if using the centrifuge for the first time]. After centrifugation, check for a pellet. These are cells.
8) Remove as much supernatant as possible (without disrupting the cell pellet) into liquid waste (beaker with lysol).
9) Use the pipet-aid and serological pipet, pouring (decanting) increases likelihood of contamination.
10) Finger flick the pellet to loosen it from the bottom of the tube.
11) Gently resuspend the cells in 5 ml DMEM+ and transfer entire cell suspension into one T-25 flask.
12) View cells under microscope. They should be floating or suspended in media.
13) Label with sharpie directly on plastic around outside of top. Incubate the flask correctly (cap orientation) on shelf in the 37°C, CO_2 incubator. Throw away cryovial in biohazard bin.

PROJECT DOCUMENTATION

Purpose
The purpose of this lab is to develop skills for communicating study results and answering the "what next?" question.

Background
One way to communicate our experimental results is to present the research to peers face-to-face, e.g. oral or poster presentations. Key points to the research are documented in writing and the 'take home message' is orally delivered by the presenter.

To communicate research to an audience, we must logically progress through the necessary background information that led to the problem/observation that eventually led to the testable hypothesis. From here we describe our experimental design and how we collected data. We then analyze the data, make conclusions, and indicate whether we supported/contradicted our hypothesis.

Research is dynamic and ongoing. If data is conclusive and supports the hypothesis, we can then further test the hypothesis or modify the hypothesis to make it more detailed. If the data contradicts the hypothesis, we can modify the hypothesis to incorporate alternate ideas. If the data is deemed inconclusive, we can change or improve the experimental design.

MATERIALS
- Individual notebook generated during cell biology lab
- Immunoblots generated by the class
- Computers

EXERCISE

Introduction
Cell biology laboratory students in previous sections noticed that cells exposed to magnetic and vibrational forces (at levels beyond environmental norms) moved closer to one another or changed shape. This led them to ask why do forces induce cell shape changes?

Our current hypothesis is that forces induce changes in cell shape by changing expression of cytoskeleton-associated proteins.

Task
Each group will analyze their generated immunoblot. In their own words, each student will complete questions #1-6 on the worksheet. Once worksheet is completed join other group with same protein.

Groups responsible for same proteins will join forces to analyze the generated immunoblot(s) and determine how to progress with the research project. Each person write their own interpretation of this discussion/plan as the answer for question #7.

Each "protein group" will develop a 30-60 second 'take home message' presentation to share with other groups responsible for other proteins.

Two groups with different proteins of interest will join and present to each other their "take home message" presentation.

Worksheet – Project Documentation **Name: _____**

Names of additional members in group: _____

Note: Points earned here will be added to notebook grade.

1) Force used: _____

   Name of protein:_____
   Brief description about how protein ties into hypothesis (google: protein and cytoskeleton)

2) Assess Data (circle one)

   Data inconclusive progress to question 3 Data conclusive progress to question 4

3) Why data was deemed inconclusive? (hint: discuss control). How can we specifically modify/improve so we can get conclusive data next time?

4) Was the hypothesis supported or contradicted? (circle one)

   Contradicted (go to question 5) Supported (go to question 6)

5) Modify the hypothesis to include alternate idea from generated data.....Write a hypothesis that would be supported by the current data.

6) Zoom in on promising cytoskeleton-associated protein…. Rewrite hypothesis to include supportive, conclusive data.

7) As a large group, what would be the next experiment that you believe will progress the project?

Grade Sheet for Notebook 1

Student name: _____

Completeness (15)	
LIQUID TRANSFER [t p m&m] SOLUTIONS and STERILE TECHNIQUE [t p m&m] MEDIA PREPARATION [t p m&m c] SEED CELLS [t p m&m] SPLIT (SUBCULTURE) CELLS [t p m&m c] MEMBRANE TRANSPORT (tonicity) [t p m&m d (what are unknowns and how determined using controls)] COUNT CELLS [t p m&m c] MITOCHONDRIA / ER [t p m&m (ER) c (ER) d (ER)] m&m (mito) c (mito) d (mito)] CYTOSKELETON (phalloidin) [HeLa **and** cheek cells or hair] [t p HeLa m&m d (labeled pict stained HeLa) Cheek cell/hair m&m d(pict stained cheek/hair)] (t = title; p = purpose/intro; m&m = materials/methods; c = calculations; d = data/observations/conclusion)	
Format (7)	
Cover (name, course, semester, professor name) Table of Contents Dates and page numbers "Rules" (no staples or masking tape, right hand of page to start, sign taped pages, intentional blanks marked, etc.) Title of lab day Purpose/Introduction to lab day Materials/Method (if taped in referred to in text), check equipment details Order of sections (example data/observation not before procedure) No extra content (examples, methods not done, excessive notes)	
"Wow" (3)	
Pictures, Details, Comments, Creativity, Neatness, Autonomy, Ability for Others to Understand, Other	

Total (25)	

Comments (if needed)

Grade Sheet for Notebook 2

Student name: _____

Completeness (20)	
WHOLE CELL LYSIS [t p m&m (include speed used) c (raw data for std/unknowns, std curve, unknown calcs)] CRYOPRESERVE [t p m&m] NUCLEUS (methylene blue/Hoechst) [t p Methylene blue m&m c (micronuclei count/graph) d (picture/description cell) Hoechst m&m c (make solution) d (picture/description cell)] PROTEIN SEPARATION & BLOT [t p m&m c (lysate prep) d (total protein gel with labels) concl (total protein) IMMUNODETECTION [t p m&m d (PVDF with labels (ladder/wells)) conc (control, mag impact on actin level] PROJECT DOCUMENTATION [participated and worksheet completed] (t = title; p = purpose/intro; m&m = materials/methods; c = calculations; d = data/observations; conc = conclusion)	
Format (3)	
Table of Contents Dates and page numbers "Rules" (right hand of page to start, sign taped pages, intentional blanks marked and initialed, etc.) Materials/Method (if taped in referred to in text), check equipment details Order of contents(example data/observation not before procedure) No extra content (examples, methods not done, excessive notes)	
"Wow" (2)	
Pictures, Details, Comments, Creativity, Neatness, Autonomy, Other	

Total (25)	

Comments (if needed)

References

Sterile Technique: http://www.sigmaaldrich.com/life-science/cell-culture/learning-center/cell-culture-videos.html

http://en.wikipedia.org/wiki/HeLa

Image of chloroplasts from Lang et al., Plant Cell Rep (2011) 30:205–215

Method modified from UCI – Developmental and Cellular Biology lab manual, winter 1998

Background modified from Karp, “Cell and Molecular Biology”

Modified from original version written by Dr. Idit Hazan

http://en.wikipedia.org/wiki/Hemocytometer

Useful numbers chart
http://tools.lifetechnologies.com/downloads/Useful_Numbers_Y14472_Useful_Nmbrs.pdf

http://www.microscopyu.com/articles/fluorescence/fluorescenceintro.html

http://serc.carleton.edu/microbelife/research_methods/microscopy/cellstain.html
Methylene Bridge Image: Copyright 2000 by Today Enterprises, in Microscopy Today 00-1 pp. 8-12 (2000)

Biuret standard curve image: Experimental Biosciences at
http://www.ruf.rice.edu/~bioslabs/methods/protein/biuret.html

Erythrocyte Membrane Image: Wataru Nunomura and Yuichi Takakuwa. Regulation of Protein 4.1R Interactions with Membrane Proteins by Ca^{2+} and CalmodulinFrontiers in Bioscience 11, 1522-1539, May 1, 2006

http://en.wikipedia.org/wiki/In_silico

ImageJ tutorial modified from lukemiller.org; analyzing western blots with ImageJ and ImageJ gel submenu instructions (http://rsb.info.nih.gov/ij/docs/menus/analyze.html#gels)

Western Blot image provided by Edvotek, a biotechnology education company.

http://www.emdmillipore.com/US/en/life-science-research/protein-detection-quantification/western-blotting/protocols/q9ib.qB.710AAAFBRP0RRkww,nav?bd=1