

# PRELIMINARIES

This course is not without hazards. It is important that you know what dangers exist in the lab so that you can take the necessary precautions.

**--CHEMICALS--** This being a lab where we work with DNA, many of the chemicals used form associations with DNA. Most of these associations are indiscriminate, meaning that they will just as easily associate with your DNA as with the DNA in the test tubes. Many are mutagens, some are carcinogens and teratogens. We also use chemicals that destroy proteins and other cellular constituents. Again, most are indiscriminate and proper handling procedures should be observed.

-Acrylamide: Gel matrix material. The unpolymerized solutions are toxic and can be absorbed through the skin. The powder can be inhaled and absorbed in the lungs. Acrylamide accumulates in nerve cells producing irreversible nerve damage. Wear gloves at all times when handling solutions. Wear mask and gloves when handling powders. Do not open containers under drafty conditions.

-Chloroform: Used to separate proteins from DNA. Toxic, possible carcinogen. May cause headaches at low doses in some people. Use in the fume hood as much as possible.

-Ethanol (EtOH): Toxic, flammable. Do not use near flame (except in small amounts to flame clean cell transfer instruments).

-Ethidium bromide: Dye which intercalates between stacked bases in DNA. Mutagen. Use gloves when handling solutions of any concentration. Clean up all spills immediately! Use particle mask when weighing powder.

-Formamide: Used to aid in the denaturation of DNA during Southern hybridization. Toxic, teratogenic. Pregnant women should not be exposed to this chemical at all. Women of childbearing age should limit their contact with formamide.

-Hexadecyltrimethylammonium bromide (Cetyltrimethyl-ammonium bromide, or simply CTAB, pronounced see'tab): A detergent that forms soluble complexes with DNA in high salt conditions. Since it forms complexes with DNA and can easily pass through skin and cell membranes, gloves should be used when handling this chemical, although it has never been listed as a mutagen or carcinogen.

-Phosphorus-32 ( $^{32}\text{P}$ ): Used to label DNA for use as probes. Energetic beta decay can penetrate skin, eyes and when ingested accumulates in bones. Decay causes single and double strand breaks of DNA (i.e., possible mutagen, carcinogen).

-Phosphorus-33 ( $^{33}\text{P}$ ): Used to label DNA for sequencing. Less energetic beta decay than  $^{32}\text{P}$ . When ingested accumulates in bones. Decay may cause single and double strand breaks of DNA (i.e., possible mutagen, carcinogen).

-Phenol: Used to separate proteins from DNA. Toxic, can cause severe burns, especially to mucous membranes. Do not mouth pipette and keep away from eyes. Use in fume hood as

much as possible.

-Sodium dodecyl sulfate (sodium lauryl sulfate, SDS): This is found in most hair shampoos, and is not generally hazardous, but it can cause other substances to be absorbed through the skin.

-Sodium hydroxide: Used to denature nucleic acids. Can cause severe burns to skin and eyes. This is a probable carcinogen at high doses.

-Sulfur-35 ( $^{35}\text{S}$ ): Used to label DNA for sequencing. Less energetic beta decay than  $^{32}\text{P}$  or  $^{33}\text{P}$ . Decay may cause single and double strand breaks of DNA (i.e., possible mutagen, carcinogen).

**-EQUIPMENT-** Some of the equipment can be hazardous if not operated in a safe manner.

-Ultraviolet (UV) light sources: Used to visualize ethidium bromide-stained DNA and used to kill microorganisms. UV-irradiation can cause "sunburn." This type of burn can cause blindness or cataracts of the eye and can also cause damage of DNA in the skin, including formation of thymidine dimers. Repeated exposure can lead to skin cancer. Wear protective eyewear and a face shield. If exposure is longer than 5 minutes, wear long sleeves.

-Gel electrophoresis: Used to separate molecules on the bases of size and/or charge. The voltages and amperages used in agarose and polyacrylamide gel electrophoresis can be extremely dangerous, and sometimes fatal. Never handle the wires when the power unit is plugged in.

**-COSTS-**

While some of the pieces of equipment and reagents in Molecular Biology are relatively inexpensive, there are many that are very expensive. Please take extreme care when operating any of the pieces of equipment. **DO NOT CONTAMINATE ANY REAGENTS BOTTLES OR VIALS. NEVER RETURN ANY CHEMICAL TO ANY REAGENT BOTTLE AFTER YOU HAVE WITHDRAWN IT FROM THAT REAGENT BOTTLE.** When withdrawing chemicals from bottles or reagents and enzymes from vials recently sterilized spatulas and pipette tips must be used. The pipette tips go into a reagent bottle ONCE and then are thrown away immediately. A disposable pipette tip costs pennies, while a vial of enzyme may cost over \$100. Below are the approximate costs (in US dollars) of some pieces of equipment and reagents:

Gel Electrophoresis Unit	400
Pipetman	380
Polaroid Camera	500
Polaroid Camera System	3500
UV Transilluminator	1000
Microfuge	2500
Waterbath	1000
Waterbath (Shaking)	2500
Densitometer	2000 - 10,000
Power Supply (for gels)	250 - 2500
Forceps	3 - 20
Coffee Mill	25

Balances	1000 - 2000
Thermometer	20 - 40
Photographic Film (per print)	1 - 2
UV Face Shield	100
Filters for Camera (each)	25
Blotting Membranes (for DNA)	200
DNA Labeling/Detection Kit	400
PCR Amplification Kit	300
Cycle Sequencing Kit	500
<i>Taq</i> DNA Polymerase (per vial)	60 - 200
Restriction Enzymes (per vial)	25 - 200
Competent <i>E. coli</i> Cells	100 - 200

### -GENERAL RULES-

1. No food or drinks in the lab. Wash your hands well after leaving the lab, especially if you are going to eat or smoke.
2. No open flames near volatile chemicals (e.g., alcohols).
3. Clean up all of your messes as they occur.
4. When working with hazardous chemicals, use the fume hood.
5. No smoking in the lab.
6. When in doubt, ask the instructor or T.A. for assistance.
7. Some of the chemicals we will be using degrade rapidly at room temperature (e.g., enzymes, nucleotides, nucleic acids, etc.) and replacement is generally expensive (e.g., one vial of restriction enzyme costs from \$30 to \$100). All of these items should be kept **on ice constantly** when out of the freezer (or refrigerator). Immediately after use, they should be returned to where you found them (freezer or refrigerator). The stock solutions (enzymes or other solutions) should **NEVER** be withdrawn from the container with a used pipette tip!! **ALWAYS USE A NEW AUTOCLAVED DISPOSABLE PIPETTE TIP WHEN WITHDRAWING AN ALIQUOT FROM A STOCK SOLUTION.** This is especially important for enzymes, reaction mixes, sterile solutions, etc. Disposable pipette tips cost about 2-3 cents each, so they are certainly more expendable than the solutions.

# GENERAL EQUIPMENT USAGE

You are expected to come into this course with some knowledge of some of the equipment used routinely in biochemistry, molecular biology and general biology laboratories. Below is a list of some of those pieces of equipment, their location and instructions on their use. You will be expected to learn how to operate them on your own time. However, if you need help with any of the equipment please ask the TA or the instructor.

## Autoclave (AMSCO 2022):

1. Make sure that the autoclave is not in the middle of a cycle. Chamber pressure should be at zero and the temperature indicator should be below 80. If they are not, come back later.
2. If the autoclave chamber door is closed, rotate the door wheel counterclockwise until the bars allow the door to open. Pull the chamber door open. Stand back a bit in case there is steam inside that might burn you. Place items to be autoclaved into the chamber (inside a container to catch any spills or broken glass) and close the door. Spin the wheel on the door clockwise until it is firm, but not severely tight.
3. Open the door above the chamber and check to see that the top switch is set to manual, the PRESSURE valve is turned to LOW, the STEAM valve is turned all the way counterclockwise (fully open) and the WATER valve is turned all the way counterclockwise (fully open).
4. If the POWER and CONTROL switches are not on, turn them on.
5. Set the STERILIZE time to 20 minutes. Set the STEAM DRY time only if you do not have any liquids to be autoclaved. If you have liquids in the autoclave, the dry cycle will probably cause boiling off of much of the water from the solution.
6. To start the autoclave, press the GRAVITY button (if you are autoclaving dry items) or LIQUIDS (if you have any liquid items in the chamber. [Note: The "GRAVITY" button is missing from this particular machine, but the button is just to the left of the "LIQUIDS" button. You can see the worn spot where people have pressed the button. Do not use the ISOTHERMAL setting without checking with the TA or instructor.] The autoclave will now begin letting steam into the chamber. The clock will not begin counting down until the sterilization pressure and temperature are reached (this takes a few minutes).
7. When the cycle is complete (pressure and temperature are down), rotate the door wheel and carefully open the door. You can be burned by the steam if you are not careful.
8. Remove the items from the chamber with heat-protective gloves. Turn off the CONTROL and POWER switches if it is after 4:00 pm.

## Micropipetors (Pipetman, Microman, etc.):

These will be located in the teaching lab. You will be using three sizes of Pipetman

(manufactured by Rainin) pipetters: a P1000 (to measure volumes of 100  $\mu\text{l}$  to 1000  $\mu\text{l}$ , or 1 ml) which uses the blue disposable tips, a P200 (for volumes of 20  $\mu\text{l}$  to 200  $\mu\text{l}$ ) which uses the yellow tips, and a P20 (for volumes of 1  $\mu\text{l}$  to 20  $\mu\text{l}$ , which also uses the yellow tips. Each one costs over \$200, so please handle them with care!

Each has a scale in a window near the top consisting of three numbers. The top place on the P1000 has red numbers and indicates 1 milliliter, the middle position (black numbers) indicates hundreds of microliters and the bottom position (black numbers) indicates tens of microliters. On the P200 (all of the numbers are black), the top position indicates hundreds of microliters, the middle position indicates tens of microliters and the bottom position indicates single microliters. On the P20, the top position (black numbers) indicates tens of microliters, the middle position (black numbers) indicates single microliters, and the bottom scale (red numbers) indicates tenths of microliters.

### **To Use Pipetman:**

1. Set scale to desired volume by turning the black rubber adjustment knob at the top opening of the Pipetman.
2. Push the tip of the Pipetman into a disposable pipette tip in the rack (rack with tips should be autoclaved before use). Use enough force so that the tip stays on the end of the Pipetman.
3. Depress the white plunger button on the top of the Pipetman (this button has the yellow or blue circle on it) before putting the tip into the solution.
4. Put the tip (and only the tip, not any part of the Pipetman itself) into the solution to be withdrawn and SLOWLY let the plunger up so that the liquid is drawn up into the disposable tip. If the plunger is released too rapidly, the volume may be incorrect and/or some of the solution may be drawn into the Pipetman itself, in which case the Pipetman will need immediate disassembly and cleaning (by the TA or instructor only)!
5. To transfer the liquid in the tip to another container, simply depress the white plunger all the way while the tip is in the new container (or solution).
6. To dispose of tip, hold the Pipetman over a garbage can and press on the smaller white button on the side of the Pipetman. This is connected to the stainless-steel piece that will eject the tip off of the end of the Pipetman. **ALWAYS USE A NEW TIP WHEN GOING INTO ANY SOLUTION. IN OTHER WORDS, NEVER REUSE A DISPOSABLE PIPETTE TIP!!** If you go into a stock solution or into an enzyme solution with a used pipette tip, the solution or the expensive enzyme may be ruined. Please think before inserting a pipette tip into any solution!!

### **Microwave oven:**

1. Open oven door and place item(s) to be heated into the oven.
2. Close door.

3. Set power and time settings.
4. Press "Start" button.
5. Monitor liquids to make sure they do not boil over inside the oven. If they do, clean up the mess yourself! Please do not place solutions containing hazardous chemicals (e.g., ethidium bromide, beta-mercaptoethanol, etc.) into the oven.
6. Open door to stop and remove the items from the oven.

**EQUIPMENT LOCATIONS:**

Most equipment and supplies will be located in the teaching lab (room 425 Illick). However, some of these materials are located in different rooms, as listed below:

<u>Equipment/Supplies</u>	<u>Room (Illick)</u>
Autoclaves.....	434
Centrifuges	
High speed (Sorvall RC-5B).....	444
Ultra (Beckman L8-60M, L2-65B).....	436
Distilled water.....	425
Dry ice.....	Loading dock (Baker)
Gel photography.....	453
Ice machine.....	323
Microwave oven.....	425
Spectrophotometers.....	329, 444, 425
Teaching lab.....	425
Laminar flow transfer hood.....	432

# USEFUL INFORMATION FOR MOLECULE MANIPULATORS

1.  $1 \mu\text{m}$  (contour length in electron microscope) = 2 Md (molecular weight, megadaltons) = 3 kb (number of kilobase pairs).
2. In the electron microscope, the limit of resolution is approximately 30-50 base pairs for DNA and RNA (although RNA forms much more secondary structure, so that its length is more difficult to determine accurately).
3. In solution, DNA-RNA hybrids form stronger duplexes than do DNA-DNA hybrids (of the same base sequence).
4.  $10^9$  bp (base pairs) =  $10^6$  kb (kilobase pairs) = 1 pg (weight in picograms)
5. Melting temperature,  $T_m$ , increases  $16.3^\circ$  for a 10-fold increase in monovalent cation (e.g.,  $\text{Na}^+$ ,  $\text{K}^+$ , etc.) concentration over the range of about 0.001 M to 0.4 M.
6. Base composition affects the  $T_m$  such that an increase in the G + C content of 1.99% will cause an increase of the  $T_m$  by  $1^\circ\text{C}$ . (Mandel, et al., 1970, J. Bacteriol. 101, 333).
7. Methylated DNA (in eukaryotes, 5-methylcytosine) causes an increase in  $T_m$  and a decrease in density of the DNA. (Erlich et al., 1975, BBA 395, 109; Kemp and Sutton, 1976, BBA 425, 148).
8. Because of the phosphate backbone of DNA, it has a net negative charge which causes it to form salts with cations. These cations tend to stabilize the DNA. In solution, the DNA is surrounded by an ordered shell of water molecules. The shell of water molecules, the cation concentration and any DNA modifications (e.g., methylation, etc.) can greatly affect the conformation of the DNA. Under physiological conditions, much of the DNA is in the "B" form, but other conformations exist at various locations along the DNA.
9. When formamide is used as a solvent, the  $T_m$  is decreased by  $0.60^\circ\text{C}$  per percent of formamide used. (Hutton, 1977, Nucleic Acids Res. 4, 3537)
10. The  $T_m$  decreases by approximately  $1^\circ\text{C}$  for each 1% base pair mismatch. (Wetmer, 1976, Ann. Rev. Biophys Bioeng. 5, 337)
11. A minimum of about a dozen hydrogen bonds are required to form a stable duplex of DNA at room temperature.
12. A 1 mg/cc density change equals a 0.98% change in G + C content. (Mandel et al., 1968, Meth. Enz. 128, 185)

13. *E. coli* DNA characteristics: 50.5% G + C,  $T_m = 90.5$  °C in 1X SSC, density = 1.710 g/cc.

14. DNA desorbs from nitrocellulose at high temperature, but not from most nylon-based membranes.

15. Basic units of measure:

1  $\mu$ g (microgram) =  $10^{-6}$  g

1 ng (nanogram) =  $10^{-9}$  g

1 pg (picogram) =  $10^{-12}$  g

1 fg (femtogram) =  $10^{-15}$  g

16. Other nucleic acid characteristics:

1 kb =  $6.6 \times 10^5$  daltons = 0.66 Md (megadaltons)

1 kb of DNA = 333 amino acid coding capacity

270 bp DNA = 10,000 dalton protein

1  $\mu$ g/ml of a 1 kb nucleic acid = 3 nM end concentration

An O.D. of 1.0 @  $A_{260}$  = 50  $\mu$ g/ml of DNA (double-stranded)

An O.D. of 1.0 @  $A_{260}$  = 40  $\mu$ g/ml of DNA (single-stranded)

The O.D. increases when the nucleotides are liberated as single bases.

# IMAGINING CELLULAR AND MOLECULAR DIMENSIONS

In cellular and molecular biology we use measurements that cannot easily be imagined. Try to visualize in your mind a micron ( $10^{-6}$  meter), a nanometer ( $10^{-9}$  meter) or an Angstrom ( $10^{-10}$  meter). This is very difficult at best, after all a micron is one one-thousandth of a millimeter, well below the resolving power of your eyes. A nanometer is one thousand times smaller than that. You can barely see about 25 micrograms of DNA when it is pelleted with a centrifuge. Try to imagine what a microgram ( $10^{-6}$  gram), nanogram ( $10^{-9}$  gram), picogram ( $10^{-12}$  gram) or femtogram ( $10^{-15}$  gram) of DNA looks like. Again, this is very difficult since a microgram of DNA would be smaller than your eyes could resolve.

To help you to get a feel for cellular and subcellular dimensions imagine that we could expand a cell to become the size of a large classroom or a small auditorium, about one million times larger than an average cell (of course cells vary in size a great deal). The cell wall would then be about as thick as the walls of the room. Covering the inside surface of the walls, ceiling and floor would be the plasma membrane. It would be 8 mm thick.

The cell nucleus would be about 5 meters in diameter (2-3 times the height of an average person). The nucleolus would have a diameter about the height of a person. A typical metaphase chromosome would be the approximate size and shape of a person with arms stretched overhead (although most peoples' central regions are larger than the centromere region of the chromosome). When the chromosome is unwound the DNA would be 2 mm thick (the thickness of twine) and 20 kilometers long (about the distance between northern Syracuse and Lafayette). [We all have 46 chromosomes (23 pairs) in each of our somatic cells. Calculate the length of these chromosomes if their DNA were stretched out end to end!] The nuclear membrane enclosing the nucleus would have a thickness of 2 cm. It would be covered with 6 mm wide nuclear pores, spaced 15 mm apart.

Out in the cytoplasm, the chloroplasts would be about 5 m in length and 2-3 m in width. Mitochondria would be about 2 m x 1 m (the size of a tall heavy person). Bacteria would be the size of an average-sized person. Typical viruses infecting the cell would be 5 cm in diameter, the size of a racquet ball.

At the molecular level, a gene's worth of DNA would be around 0.5 m in length. If you add the controlling regions of the gene it would range from 1 to 2 m long. The RNA encoded by the gene would be only half as thick, about the thickness of heavy string. Since RNA typically has a great deal of secondary structure, it would be twisted and folded back on itself. Ribosomes the size of golf balls would be attached to mRNAs. A typical protein would have a diameter of about 5 mm (the size of a mung bean or soybean). An average atom would have a diameter of 0.2 nm, which might look like specks of dust floating around the cell.

Now imagine all of these organelles, molecules and atoms floating around the room. Imagine the nucleus, mitochondria, chloroplasts, ribosomes and membranes in the room. Now, in your mind, shrink the cell down. Shrink it down until it is barely visible in front of you. You will need to shrink it a bit further to achieve its actual size. In fact, you would need to put at least 20 to 30 cells together in order to see them as a very small speck.

Keep this exercise in mind when your are going through the lab work and whenever you see light or electron micrographs.

Here are some other interesting dimensions:

<u>TOTAL GENOMES:</u>	<u>LENGTHS</u>	
	<u>Expanded by <math>10^6</math></u>	<u>Actual</u>
mammals (incl. humans)	900 km	0.9 m
higher plants	5,000-10,000 km	5-10 m
fungi	3-30 km	3-30 mm
bacteria	0.3-3 km	0.3-3 mm

<u>AMOUNTS OF DNA</u>	<u>LENGTHS</u>	
	<u>Expanded by <math>10^6</math></u>	<u>Actual</u>
1 $\mu$ g (microgram)	$3 \times 10^8$ km	300 km
1 ng (nanogram)	$3 \times 10^5$ km	300 m
1 pg (picogram)	300 km	0.3 m
1 fg (femtogram)	300 m	0.3 mm