Welcome to UMass

Summer College Research Intensives.

Lab Boot Camp Manual 2017

(a work in progress, feel free to point out errors)

Goals for this short (2-morning) Lab Boot Camp

- Become familiar with lab culture
- Brush up on some basic lab techniques
    a. Practice Pipetting
    b. Extract DNA
    c. Run a gel
    d. Do some Pubmed searches and bioinformatics
- Get to know each other and where other Summer College students are working.

Overview

Day 1 (Tuesday, 9:00-12:00):

Introductions, Boot Camp over view, Discuss lab notebooks and lab culture

Pipette exercise/competition (color and weighing)

DNA isolation: Which cells have the most DNA? (be quantitative from the start)

Day 2 (Wednesday, 9:00-12:00):

DNA quantification and quality assessment: Nanodrop method

Serial dilutions! How accurate can you be?

DNA Quality: Run gels (+/- RNase): take cellphone pix & send to Rolf and Pat

Bioinformatics Exercise (as time permits): Pubmed search

Instructors:

Rolf Karlstrom, Biology, Karlstrom@bio.umass.edu

Pat Wadsworth, Biology, patw@bio.umass.edu
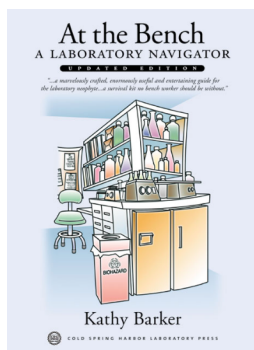
Kate Dorfman, Biology, kdorfman@bio.umass.edu

TAs:

Matt Loring (Karlstrom Lab), mloring@umass.edu

Shelby Mitchell (Karlstrom Lab), semitchell@umass.edu

Table of Contents

A useful resource:

At the Bench ; A Laboratory Manual Paperback – 2005

by Kathy Barker (Author)

Be the first to review this item

▸ See all formats and editions

Paperback
from $17.70

4 New from $17.70

Notes On Laboratory Safety

Personal protection

We do very little in this course that could result in a fire or an explosion. We will, however, be using a few reagents (in very small amounts and low concentrations – be reassured) that have the potential to cause harm. We will alert you to whatever dangers we see in the lab. Your best bet for protecting yourself is not to get anything in your mouth, eyes, or breaks in your skin. Do not eat or drink or apply lip balm or fix your contacts in the lab. ***Wash your hands:*** after you handle reagents, before you touch your face, before you leave the room, before you have a snack. ***Wear gloves:*** while you handle anything that might hurt you, or that you might contaminate. ***Take your gloves off:*** whenever you move from wet bench activities to computer keyboard, or whenever you have touched something you don't want to spread around the room. ***Wear your safety goggles:*** whenever you handle liquids that could splash or anything that might shatter.

Move slowly to reduce spills and breakage.

Cleaning up

We will alert you to the proper disposal techniques for the reagents and materials we use in this lab. Some are quite benign and can go right in the trash or down the drain, and others require special handling. Check the **Clean up** section for each lab for specific instructions.

Discarding Waste

There are many different types of waste in this lab, each disposed of separately:

- **recyclable paper or containers**
- **broken glass** (unrecyclable, dangerous to handle)
- **sharps** (small sharp objects like razor blades)
- **biohazard waste** (biological specimens that might be infective or invasive)
- **hazardous waste** (toxic, corrosive, or flammable waste)
- **regular trash** (solid waste that is not any of the above)
- **benign liquid waste** (liquid waste that is not hazardous or infective)

Always ask whenever you have any doubt about anything, including equipment use, techniques, and disposing of something.

LAB DAY 1

EXERCISE #1: INTRODUCTION TO MICRO-PIPETTING
TESTING ACCURACY AND REPRODUCIBILITY IN 2 WAYS

Goal for this exercise:

Master micropipetting technique.

Introduction

One of the most important skills you will need in this course is your ability to use a micropipettor. Micropipettors are used to make accurate measurements of extremely small volumes—from one milliliter down to one microliter (1 mL to 1 μL). Most of what we do in molecular biology involves manipulating volumes of liquid in this range. If you learn to do it accurately now, your experiments will go much more smoothly later.

Micropipettor Basics

These are precision scientific instruments, and must be treated with respect. The pipettor is used to draw liquid up into a cheap disposable tip. The three pipettors you will use take up and deliver liquids in the volume range from ~0.5 μL to 1.0 mL. Your instructor will show you how to use this device. Read and follow these guidelines to maintain the accuracy and precision of your pipettors.

Rotate the volume adjustor to the desired setting. Note the change in plunger length as the volume changes. Be sure to properly locate the decimal point when reading the volume setting. (Your instructors will demonstrate.)

You have three sizes of pipets in this lab: LTS20s, which can measure between 1 μL and 20 μL; LTS200s, which can measure between 21 μL and 200 μL; and LTS1000s, which can measure between 200 μL and 1000 μL (1 mL).

There are three numbers on the display of each pipettor. Look at the top of the pipet to see which one you are holding, then look at the display. The numbers represent volumes as shown below. The color change represents crossing the decimal place or changing units.

L 20		L 200		L 1000	
1	10μL	**1**	100μL	1	1000μL = 1.0 mL
0	1μL	**0**	10μL	**0**	100μL
0	0.1μL = 100 nL	**0**	1μL	**0**	1μL

Firmly seat a proper-sized tip on the end of the micropipettor. The tips boxes are color-coded to match the label on the plunger.

When withdrawing or expelling fluid, always hold the tube firmly between your thumb and forefinger, keeping it nearly at eye level to observe the change in the fluid level in the pipet tip. Do not pipet with the tube in the test tube rack or have another person hold the tube while you are pipetting.

Hold the tube in your hand during each manipulation. Open the top of the tube by flipping up the tab with your thumb. During manipulations, grasp the tube body (rather than the lid), to provide greater control and to avoid contamination of the mouth of the tube.

For best control, grasp the micropipettor in your palm and wrap your fingers around the barrel; work the plunger (piston) with the thumb. Hold the micropipettor almost vertical when filling it.

Notice the friction "stops" on the two-position plunger with your thumb. Depressing to the first stop measures the desired volume. Depressing to the second stop introduces an additional volume of air to blow out any solution remaining in the tip.

To withdraw the sample from a reagent tube:

1. Depress the plunger to **first** stop and hold it in this position. Dip the tip into the solution to be pipetted, and draw fluid into the tip by gradually releasing the plunger. Be sure that the tip remains in the solution while you are releasing the plunger.

2. Slide the pipet tip out along the inside of the reagent tube to dislodge any excess droplets adhering to the outside of the tip. To avoid future pipetting errors, learn to recognize the approximate levels to which particular volumes fill the pipet tip.

3. If you notice air space at the end of the tip or air bubbles within the sample in the tip, carefully expel the sample back into its supply tube. Collect the sample at the bottom of the tube by pulsing it in a microcentrifuge.

To expel the sample into a reaction tube:

1. Touch the tip of the pipet to the inside wall near the bottom of the reaction tube into which the sample will be emptied. This creates a capillary effect that helps draw fluid out of the tip.

2. Slowly depress the plunger to the first stop to expel the sample. Depress to second stop to blow out the last bit of fluid. Hold the plunger in the depressed position.

3. Slide the pipet out of the reagent tube with the measurement plunger depressed, to avoid sucking any liquid back into the tip.

Use the ejector button (located at the back and different from the plunger) to remove the tip into a waste container.

Important pipettor don'ts:

- Never rotate the volume adjustor beyond the upper or lower range of the pipet.

- Never use the micropipettor without the tip in place; this could ruin the piston. Pipettors use disposable plastic tips. Every molecular biology lab circulates its own version of the story of the not-too-bright grad student who did not use a tip. Do not be this student!

- Never invert or lay the micropipettor down with a filled tip; fluid will run back into the piston.

- Never let the plunger snap back after withdrawing or expelling fluid; smooth motions are the key to success.

- Never immerse the barrel of the micropipettor in fluid. Only the disposable tip touches the liquid.

- Never reuse a tip. Tips are pretty cheap (about $0.59 per rack). The risk of cross contaminating your solutions is too great to get tricky with tips. Just use a new one every time unless there is no possibility of cross contamination—like if you are pipetting the same solution into multiple empty tubes.

Exercise #1: Pipetting

Overview: An essential property of good science is that an experiment gives the same results even in different hands. Repeated measurements of the same thing should give the same value, no matter who makes the measurement. Precision and accuracy are different; ideally we will get you to achieve both in this exercise.

You will use 2 methods to examine pipettor accuracy and repeatability (precision?). Since we know how much a given volume of water weighs, it is relatively simple to test pipettors by weighing different amounts of water. For this exercise you will weigh 2 volumes of water for each pipettor (e.g. 2µl and 10µl for the p20) and do each measurement 10 times to see how reproducible you can become. You will also use a colorimetric method to check for accuracy.

Pipette calibration

First figure out what each of these volumes of water should weigh, and which pipet should be used to dispense that volume:

µL		mL	weight (g)		weight (mg)	pipet
1000	=			=		
100	=			=		
10	=			=		

Weighing Method Procedure:

1) Put a weigh boat or piece of weigh paper on the balance pan, and zero the balance.
2) Dispense the lesser volume of water for each pipettor (e.g. 2µl for the p20) onto the boat/paper. Record the result.
3) Re-zero the balance, and dispense the same volume onto the same paper. Record your result.
4) Repeat for a total of 10 measurements (no need to change the weigh paper in between)
5) Repeat for the higher volume for the same pipettor
6) Repeat for the other two pipettors.
7) Check discrepancies with your instructor.

Record all results in a table in your lab notebook and make a hand-drawn graph showing the weights you got with each trial. **Advanced**: Enter the same data into an excel spreadsheet. Make a line graph, a bar graph, and dot plot of your data. Use excel to calculate and plot standard deviation.

LAB TIP: You should do a scaled down version of this when you get to your new lab!!! It is better to pay to have your pipette calibrated now than to get inaccurate experimental results all summer long.

Color Method Procedure:

1) Cut a sheet of parafilm long enough to cover the table provided.
2) In each space, pipette 5µL of bromothymol staining solution onto the parafilm. Think about whether you need to change tips between droplets.
3) Check to make sure that all drops are the same size. You can easily remove a drop and re-do it if necessary.
4) Using a new tip each time, add the requisite volume (in µL) of sodium phosphate dibasic to each droplet of stain. **(Why do you suppose you must change tips?)**
5) Add the requisite volume of sodium phosphate monobasic to each stain-dibasic droplet.
6) If you do not have a color gradient from yellow, through green, to blue, you have made an error somewhere.

7) Check that each mixed drop is the same as the others.
8) Take a picture of your results and send it to the instructor.

Discussion/thought questions:

1) **What is the difference between precision and accuracy?**
2) **Are your pipettors accurate?**
3) **Are your pipettors precise?**
4) **Did your technique improve with time?**
5) **What could be sources of variability?**
6) **Could/Would/Should you save plastic tips/the environment by re-using tips? (try it!)**

The chemistry behind the color method: why/how does the color system work?

Bromothymol blue is a pH indicator. It has a multiple conjugated ring structures (6-carbon rings with alternating single and double bonds) that give it a plane of electrons that interact readily with light. Slight changes in acidity alter the configuration of these rings and change the particular wavelengths it absorbs.
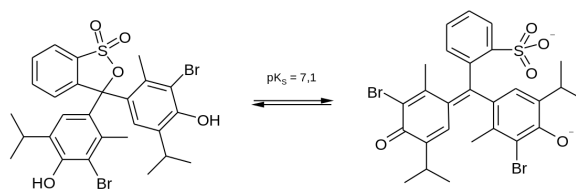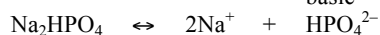

By Steffen 962 - Own work, Public Domain,
https://commons.wikimedia.org/w/index.php?cur
id=15179368

Sodium phosphate buffer works like this:

Sodium phosphate monobasic
(one univalent metal)

Dihydrogen phosphate ion
Hydrogen donor
acidic

$$NaH_2PO_4 \leftrightarrow Na^+ + H_2PO_4^-$$
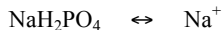
Sodium phosphate dibasic
(two univalent metal)

Hydrogen phosphate ion
Hydrogen acceptor
basic

$$Na_2HPO_4 \leftrightarrow 2Na^+ + HPO_4^{2-}$$

Dihydrogen phosphate ion
Hydrogen donor
acidic

Hydrogen phosphate ion
Hydrogen acceptor
basic

$$H_2PO_4^- \leftrightarrow H^+ + HPO_4^{2-}$$

If you add H^+, the equilibrium shifts toward dihydrogen phosphate
If you add OH^-, the equilibrium shifts toward hydrogen phosphate
But the concentration of H^+ stays the same. ($\therefore$ a buffer!)

				H_3PO_4		
				$\uparrow\downarrow$	pKa′	2.12
monobasic	NaH_2PO_4	$\leftrightarrow$	Na^+ +	$H_2PO_4^-$		
				$\uparrow\downarrow$	pKa″	7.21
dibasic	Na_2HPO_4	$\leftrightarrow$	$2Na^+$ +	HPO_4^{2-}		
				$\uparrow\downarrow$	pKa‴	12.32
tribasic	Na_3PO_4	$\leftrightarrow$	$3Na^+$	PO_4^{3-}		
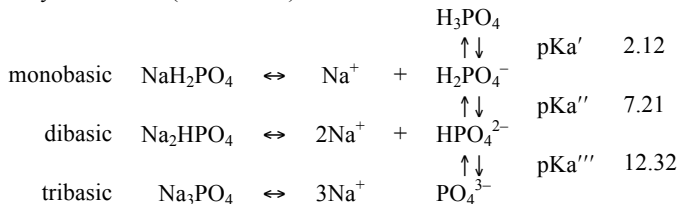
Day 1, Exercise #2:
DNA EXTRACTION

Goal:

Extract DNA from various leafy vegetables for use tomorrow (running on a gel and quantifying). We'll try to see if different plants have different DNA content, so make sure you weigh your starting sample well.

Introduction

In this lab, we will extract DNA from a variety of plants. Tomorrow we will examine the DNA we make today and try to make some conclusions about DNA quality, nucleic acid content in cells, and see if we can learn anything about the relative sizes of plant genomes.

In any DNA purification (or extraction) protocol, there are three important considerations:

1.   We must break up the tissues and cells to release the DNA.

2.   We must prevent the DNA from being degraded by cellular enzymes during the purification.

3.   We must get rid of cellular components that are not desired—in this case, proteins, lipids, carbohydrates (cell walls), and RNA.

Break up tissues and cells.

We will use a mortar and pestle to grind frozen leaf tissue into a fine powder. Freezing (in liquid nitrogen) makes the leaf tissue brittle and easy to grind. This breaks up the extracellular matrix in which the cells reside and allows better access of our chemicals to the cells themselves. In a lab you would probably use chemical disruption (buffers with detergent, sodium dodecyl sulfate (SDS), but that's not as much fun.

Prevent DNA degradation.

We prevent DNA degradation by inhibiting the enzymes (deoxyribonucleases, or 'DNases') that degrade DNA. Conveniently, DNases require Mg^{++} as a co-factor, so, by chelating the Mg^{++} with EDTA, we inhibit the activity of DNases. Prior to the addition of EDTA, we control DNase activity using low temperature (freezing in liquid nitrogen). If we were to break up the cells at room temperature, cellular DNases released during grinding would immediately start to degrade the DNA. Thus, it is imperative to get the EDTA containing DNA extraction buffer well mixed with the ground tissue before the temperature is raised.

Get rid of "undesirable" molecules.

Proteins and complex carbohydrates (such as those found in cell walls) are insoluble in solutions containing high concentrations of potassium acetate (KOAc). By adding KOAc, we force the proteins and carbohydrates to precipitate (become solid and fall out of solution) while the nucleic acids will remain in solution. Nucleic acids are insoluble in solutions containing high levels of sodium plus the alcohols ethanol (EtOH) or isopropanol (ISOP), while lipids are quite soluble in these conditions. Proteins and carbohydrates are somewhat soluble in alcohol. By adding alcohols to a salty solution, we can force the nucleic acids to precipitate, while leaving the lipids, and the remaining carbohydrates and proteins, in solution. The main problem with alcohol precipitation of nucleic acids is that the sodium precipitates along with the nucleic acids. This is undesirable, and so we always rinse nucleic acid pellets with 70% EtOH (containing no salts) to remove the sodium from the pellets prior to dissolving the nucleic acids in the next solvent.

Step By Step DNA Extraction Procedure:

SAFETY Note: LN$_2$ is very, very cold, –320° F (–196° C), and will severely damage tissue that it contacts! Use extreme caution while handling to avoid freezing injuries.

1) Pre-cool your mortar and pestle by pouring some liquid nitrogen (LN$_2$) into the mortar and letting the pestle sit in it. Allow the LN$_2$ to evaporate.

2) Choose 1 or 2 types of plants from the front of the room.

3) Now, add some more LN$_2$, and ~1 g of leaves (about half a flat of plants). ***Do not let the tissue thaw*** once it is frozen. Grind slowly at first, so you won't slop the LN$_2$ and leaves everywhere. Then, when the LN$_2$ has mostly evaporated (but while the tissue is still frozen) grind hard to get the tissue into a fine powder. The more you grind, the more you'll get. You can carefully add additional LN$_2$ to keep your tissue frozen if necessary.

4) Transfer the powder into a 15 mL ROUND BOTTOMED plastic tube that contains 6 mL of DNA extraction buffer (DEB, see recipe below). (A paper cone made from a sheet of printer paper will make this much easier.) ***The amount of tissue used should fit easily on top of the extraction buffer in your tube. Do not overload the tubes!*** Overloading with too much tissue will result in poor quality DNA.

5) **Use a spatula to shove the powder down into the tube and mix well**, then cover the tube and **shake vigorously** to mix. Wait (mixing occasionally) until the mixture has completely thawed before going on to the next step.

6) When your sample is ready, transfer the tube to a 65°C water bath for 10 minutes.

7) Place the tube on ICE and add 2.5 mL 5M KOAc. Mix well by inverting the tube repeatedly. Incubate ON ICE for 20 minutes.

8) Centrifuge 5000 rpm 10 minutes at 4°C.

9) Remove your tube from the centrifuge and notice the very large pellet consisting of tissue debris and precipitated protein. REMEMBER, you are trying to GET RID of the pellet in this step!

10) Transfer the supernatant (the liquid on top of the pellet) through Miracloth filter into a fresh round-bottomed 15 mL tube containing 6 mL of 100% isopropanol. Mix well by inverting the tube gently but repeatedly. **Precipitating nucleic acids may be clearly visible at this point**.

11) Spin in the centrifuge at 5000 rpm for 10 minutes at 4°C.

12) Remove your tube from the centrifuge. REMEMBER, this time, YOU WANT YOUR PELLET—the pellet is the nucleic acids—including your DNA. Pour off the supernate.

13) Rinse your pellet carefully with 2-3 mL 70% EtOH. Aspirate as much liquid as humanly possible, then let the pellet air dry for a few minutes. Getting rid of the EtOH is essential before proceeding to the next step.

14) Add 450 μL T$_{10}$E$_5$ to your pellet. It is important to get your DNA pellets to dissolve completely into the T$_{10}$E$_5$. We still use a high concentration of EDTA at this step, because not all the proteins are gone yet, so nucleases could still attack our DNA. Transfer the liquid into a labeled 1.5 mL microtube.

15) Add 50 μL 3M NaOAc pH = 5.2. Mix. Add 1 mL 95% EtOH. Mix gently by inversion until phases are completely mixed. Fluffy whitish clouds of DNA should almost fill the tube. If you do not see DNA (or can't lift it out of the tube in the next step), consult an instructor. You may have to spin it down and rinse it with ethanol, and let it air dry before going on.

16) Use a 1 mL pipette tip like a spatula to lift out the nucleic acid fluff and transfer it into a clean, dry, labeled 1.5 mL microtube. Add 1 mL of 70% EtOH to rinse. If it cannot be scooped, spin it down, rinse with ethanol and air dry before going on.

17) Use a pipette tip to transfer the nucleic acid fluff into a clean dry labeled 1.5 mL microtube.

18) Spin briefly in the microfuge—15 seconds is plenty of time. Remove as much EtOH as humanly possible.

19) Gently dissolve[*] the pellets in 100 μL $T_{10}E_1$. We use a low concentration of EDTA now, because our nucleic acid preparation is fairly pure—protein contamination is minimal now. However, we keep some EDTA around 'just in case'. If we had too much EDTA in our final solution, we could never do subsequent enzymatic manipulation of our DNA.   Let the pellets dissolve COMPLETELY-- leaving LABELED tubes **in the refrigerator overnight if necessary**.

20) Label your tube carefully with your group initials, type of DNA (plant) and the date). Put it in a rack in a freezer box with your group name on it in label tape.

21) Write in your lab notebook what you did, either "exactly as in manual", or "as in manual, except that …" Give the date, and what label you put on the tube.

22) Clean-up:

Alcohols are safe to go down the drain ***only when they are less than 10%. Run plenty of water while discarding alcohols so that this dilution will be achieved.***

Put the tubes that contained any β-mercaptoethanol in the marked bucket in the hood.

Put plant debris and used potting soil in the bucket labeled "Compost".

Dispose of glass pipettes in the glass trash box.

Set your mortar and pestle to soak as directed by your instructor.

Reagents

	conc			pH	also known as
DEB	100	mM	NaCl	8.0	Sodium chloride
(DNA Extraction Buffer)	50	mM	Tris		trishydroxymethylaminomethane
	25	mM	EDTA		Ethylene diamine tetra-acetid acid
	1	%	SDS		Sodium dodecyl sulfate
	10	mM	BME		β-mercaptoethanol
$T_{10}E_5$	10	mM	Tris	8.0	
	5	mM	EDTA		
$T_{10}E_1$	10	mM	Tris	8.0	
	1	mM	EDTA		
EtOH	95	%		-	Ethanol
EtOH	70	%		-	Ethanol
ISOP	100	%		-	Isopropanol
KOAc	5	M			Potassium acetate (CH_3COOK)
NaOAc	3	M		5.2	Sodium acetate (CH_3COONa)

---

[*]   If it doesn't go into solution, add more $T_{10}E_1$ and heat to 65°C for a few minutes. Do not "rack" more than once.

Lab Day 2:
DNA Quantification, Agarose Gels, Bioinformatics

Goals for this lab:
- Quantify DNA with the Nanodrop
- Quantify and Examine Nucleic Acids on a gel.
- Practice some basic bioinformatics

Introduction:

Reliable measurement of DNA concentration is important for many applications in molecular biology. DNA quantification is generally performed by spectrophotometric measurement of the absorption at 260 nm, or by agarose gel analysis. In this exercise you will try both.

DNA quantification by molecular absorbance spectroscopy.

DNA concentration can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer using a UV-transparent cuvette (usually quartz). In molecular absorbance spectroscopy, a beam of ultraviolet or visible light (P_o) is directed through a sample. Some of the light may be transmitted through the sample (P). Light that was not transmitted through the sample was absorbed. Transmittance (T) is defined as the ratio of P/P_o. Absorbance (A) is defined as -log(T). The light that was absorbed is what we care about: the absorbance at 260 nm, or A_{260}.

Incident Light (P_o)   Transmitted light (P)
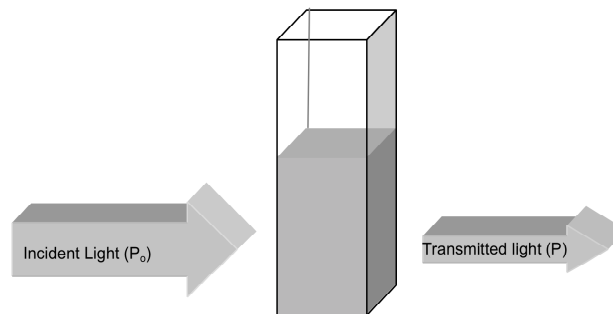
Unfortunately, spectrophotometric measurement does not differentiate between the two nucleic acids commonly found in a cell (DNA and RNA). The OD260 measurement thus tells you something about the total amount of nucleic acid in a preparation, but can't let you measure the concentration of DNA in a preparation that also contains RNA. RNA contamination of a DNA preparation can lead to overestimation of DNA concentration, if spectroscopy is the only method used to determine concentration. You will thus also run your samples on agarose gels with and without RNAse to see what the nucleic acids "look" like.

Procedure for Nanodrop[*] DNA Quantification

Module Startup

When the software starts, you should see this message:
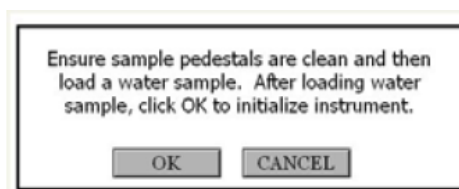
For best results, ensure measurement pedestal surfaces are clean, load a water sample onto the lower measurement pedestal and then click 'OK'. The message "Initializing Spectrometer- please wait" will appear. When this message disappears, the instrument will be ready for use. All data taken will automatically be logged in the appropriate archive file.
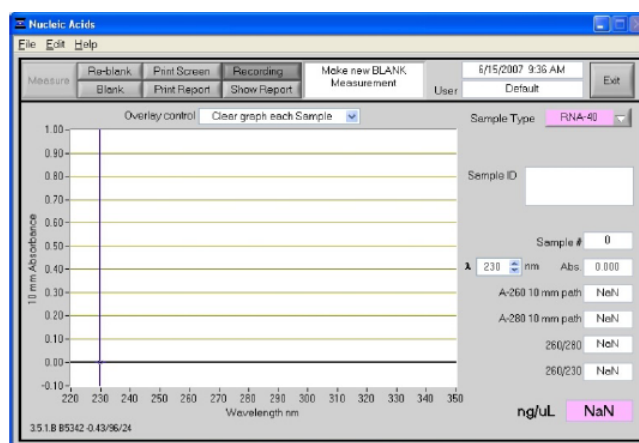
Measure (F1)

Each time a software module is opened (initiated), the Measure button is inactive as noted by its "grayed-out" appearance. A blank must first be measured before the Measure button will become active.

The Measure button is used to initiate the measurement sequence for all samples (non-blanks). It is actuated by depressing the F1 key or clicking the Measure button. The entire measurement cycle takes approximately 10 seconds.

Blank (F3)

Before making a sample measurement, a blank must be measured and stored (see "Nanodrop Blanking and Absorbance Calculations" on page 29 for more details on absorbance calculations). After making an initial blank measurement, a straight line will appear on the screen; subsequent blanks will clear any sample spectrum and display a straight line, as shown in the following image:

For the most consistent results, it is best to begin any measurement session with a blanking cycle. This will assure the user that the instrument is working properly and that the pedestal is clean.
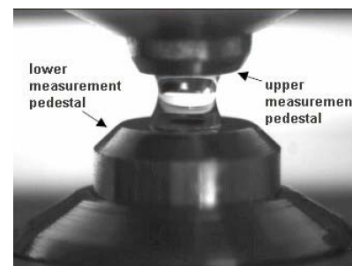

Illustrated Procedure:

1. With the sampling arm open, load a 1 µL blank sample (the buffer, solvent, or carrier liquid used with your samples) onto the lower measurement pedestal. Make sure there are no bubbles!

2. Close the sampling arm. The sample column is automatically drawn between the upper and lower measurement pedestals. Click the "Blank" button (F3).

3. When the measurement is complete, open the sampling arm and wipe the sample from both the upper and lower pedestals using a soft laboratory wipe. Simple wiping prevents sample carryover in successive measurements for samples varying by more than 1000-fold in concentration. See the Nanodrop website (www.nanodrop.com) for performance data on sample carryover.

Wiping the sample from both the upper and lower pedestals (as shown to the right) upon completion of each sample measurement is usually sufficient to prevent sample carryover and avoid residue buildup. Although generally not necessary, 2 µL water aliquots can be used to clean the measurement surfaces after particularly high concentration samples to ensure no residual sample is retained on either pedestal.

4. Analyze an aliquot of the blanking solution as though it were a sample. This is done using the "Measure" button (F1). The result should be a spectrum with a relatively flat baseline. Wipe the blank from both measurement and pedestal surfaces and repeat the process until the spectrum is flat.

5. Analyze 1 µL of your sample with the "Measure" button. Do a second measurement to be sure, following the steps above.

Note: Absorbance data shown in archive files are represented as displayed on the software screen. For Nucleic Acid, Protein A280 and Proteins and Labels modules, data are normalized to a 1.0 cm (10.0 mm) path. For MicroArray, UVVis, Protein BCA, Protein Bradford, Protein Lowry and Cell Culture modules the data are normalized to a 0.1 cm (1.0 mm) path. For high absorbance UV-Vis samples, data are normalized to a 0.1 mm path.

Lab Day 2:

Agarose Gel Electrophoresis

Overview: We examine the purified nucleic acids by your running samples on 2 agarose gels, one with and one without the enzyme RNAse (which degrades the RNA) to answer the question: **Which nucleic acid is the more prevalent in a cell?**

Quantification of DNA can also be done using agarose gel analysis. This method is only effective with small amounts of DNA, since high concentrations of DNA cannot be accurately measured this way. As little as 5 ng DNA can be detected by agarose gel electrophoresis with ethidium bromide staining. In this method, the DNA sample is run on an agarose gel alongside known amounts of DNA standards. The amount of sample DNA loaded can be estimated by comparison of the band intensity with the standards using a scanner or imaging system.

Quantification using agarose gel electrophoresis is also extremely useful when DNA samples are contaminated with RNA, since RNA is separated from DNA in agarose gels by virtue of the difference in length (size) of an average genomic DNA fragment versus the average size of a mRNA, tRNA or rRNA molecule. RNA is small: tRNA is only ~100 nucleotides (nt) long, and mRNA averages ~1.5 kilobases (kb), depending on the size of the encoded protein. Genomic DNA is theoretically the size of a whole chromosome (1000s of kb), although breakage during handling causes the size of an extracted DNA sample to be smaller (~34-50 kb).   By analyzing DNA that has been *physically* separated from RNA on agarose gels, the concentration of DNA can be measured in the absence of contaminating RNA.

Procedure:

Kate has nicely provided pre-measured and melted agarose that is +/- RNAse for each group.

1) Assemble your gel boxes with the help of your instructors (don't forget the combs!),
2) Pour in the melted agarose (2 gels, one with RNAse, one without)
3) Let the agarose harden for 20' or so
4) While your agarose hardens, Make serial dilutions of your DNA as instructed
5) Remove the combs, add running buffer
6) Mix DNA with loading buffer
7) Add identical samples to each of the two gels (Markers, DNA1, DNA2, DNA3 etc)
8) Run the gels as instructed.
9) Photograph the gels with your cell phones
10) Send the photo to Rolf (we'll compare results at the end of the lab.)

Clean-up

Pour the used running buffer into the specially marked waste container.

Put your used gel, as well as the 50 mL tube that held the agarose in the specially marked containers in the hood.

The rest of your trash, unless you think it is particularly contaminated with ethidium bromide (e.g., a paper towel that held the gel), can go in the regular trash containers.

Rinse the living daylights out of all your gel equipment: casting trays, electrophoresis apparatus, etc. Are you willing to touch it barehanded? Is it free of all traces of RNase? If not, rinse it some more! Let everything air dry.

Rinse your Erlenmeyer flasks thoroughly, first in tap water, then once with distilled water from the big carboy. Hang them up to air dry.

See your instructor about cleaning up any spills.

Unused running buffer can go down the drain.

Save your DNA standards and your loading dye.

Discard your diluted DNA, but save your genomic DNA!

Post-Lab Questions: Lab write up

Answer the following questions in your notebook:

- **Report both the yield (total amount in µg) and the concentration (in µg/µL) of DNA that you purified.**

- **Assess the quality of your genomic DNA preparation.**

- **If you had problems with your DNA purification, where do you reckon the problems arose? Be specific – "experimental error" explains nothing.**

- **What differences did you notice between the gel with RNase and the one without RNase? (Explain possible reasons that account for the differences.)**

- **Did different plants have different amounts of DNA?**

    o **What could explain this?**

    o **Which plant had the most DNA/mg of starting material?**

Serial Dilutions

It is often very important to know the precise concentration of some chemical you are using in your experiment. Various units of concentration are used in biology and chemistry:

Molarity (M)   moles/Liter

mg/mL   g/L

% w/vol   g/100 mL (because 1 mL of water weighs 1 g)

% vol/vol   mL/100 mL

Sometimes you can start from scratch, that is, weigh out the substance and dissolve it in the appropriate amount of solvent (usually water for biological applications).

What if I need 10 mL of 5M NaCl (MW = 58)? You can calculate how much NaCl to dissolve in 10 mL water this way (notice how all the units but g cancel out):

$$\frac{5mol}{L} \times \frac{58g}{mol} \times 10mL \times \frac{1L}{1000mL} = 2.9g$$

What if I need 1 mL of 5mM NaCl?

$$\frac{5mmol}{L} \times \frac{1mol}{1000mmol} \times \frac{58g}{mol} \times 1mL \times \frac{1L}{1000mL} = 0.00029g$$

Unfortunately, 0.00029 g is an almost imaginary amount of sodium chloride. Even if you made 10 times what you needed, you'd have to weigh out 0.0029 g, and most of our prep room balances only report three decimal places. You could make a liter of 10mM NaCl with 0.29 g, because sodium chloride is cheap to buy and legal to pour down the drain, but many reagents are much harder to come by and dispose of, so we need other ways of making solutions.

If you already had your 10 mL of 5 M NaCl, you could make your 1 mL of 5 mM NaCl by dilution, that is, by taking a small volume of your 5 M NaCl and diluting it in water. To calculate how, we use the lab preparer's best friend:

$$c_1 v_1 = c_2 v_2$$

c_1 = the concentration of the initial solution used to make the more dilute solution

v_1 = the initial small volume of the first solution used to make the dilute solution

c_2 = the concentration of the second, more dilute solution

v_2 = the final volume of the second solution after the dilution is carried out

So we need to figure out what volume (v_1) of the 5 M (c_1) solution to use to make 1 mL (v_2) of a 10 mM NaCl (c_2) solution.

$$v_1 = \frac{c_2 v_2}{c_1} = \frac{5mM \times 1mL}{5M} \times \frac{1M}{1000mM} = 0.001mL$$

Another way to look at this is to calculate the dilution factor, that is, the ratio between the initial and final concentrations. Diluting a 5M solution to a 5mM solution is a 1000-fold dilution:

$$\frac{c_2}{c_1} = \frac{5mM}{5M} \times \frac{1M}{1000mM} = \frac{1}{1000}$$

Therefore, you need 1 part stock solution to make 1000 parts of your final solution.

$$v_1 = \frac{c_2}{c_1} \times v_2 = \frac{1}{1000} \times 1mL = 0.001mL$$

Fortunately, I have a micropipettor that can deliver 0.001mL (=1µL). So I can make the dilute solution by mixing 0.001mL of my concentrated solution with 0.999 mL of water.

What if I don't really trust[*] my pipettor down in the single µL range?

Worse, what if my concentrated sodium chloride stock solution is 1M, rather than 5M? The initial volume would be 0.0002 mL (=0.2µL), and I **really** don't have a pipettor I trust to deliver that small a volume.

I can solve the problem of a v_1 too small to deliver by using a *series* of dilutions, each transferring a volume I *can* accurately deliver, to achieve my intended volume and concentration. For this procedure, I use a modification of $c_1v_1=c_2v_2$:

$$c_1 v_t = \left(\frac{c_1}{d}\right)\left(v_f + v_t\right)$$

c_1	=	the concentration of the initial solution used to make the next dilution in the series
v_t	=	the transfer volume
d	=	the dilution factor (*e.g.,* 2 for a 2-fold dilution series, where the concentration of each solution in the series is half that of the previous one)
$c_2 = \frac{c_1}{d}$	=	the concentration of the second solution in the series
v_f	=	the desired final volume of each solution
$v_f + v_t$	=	the initial volume of a diluted solution, until the transfer volume is removed to make the next dilution in the series

This procedure is illustrated in Figure 1.

---

[*] When I say I don't trust the pipettor, I mean that I worry about tiny errors in delivery representing a large percentage of the volume I'm transferring.
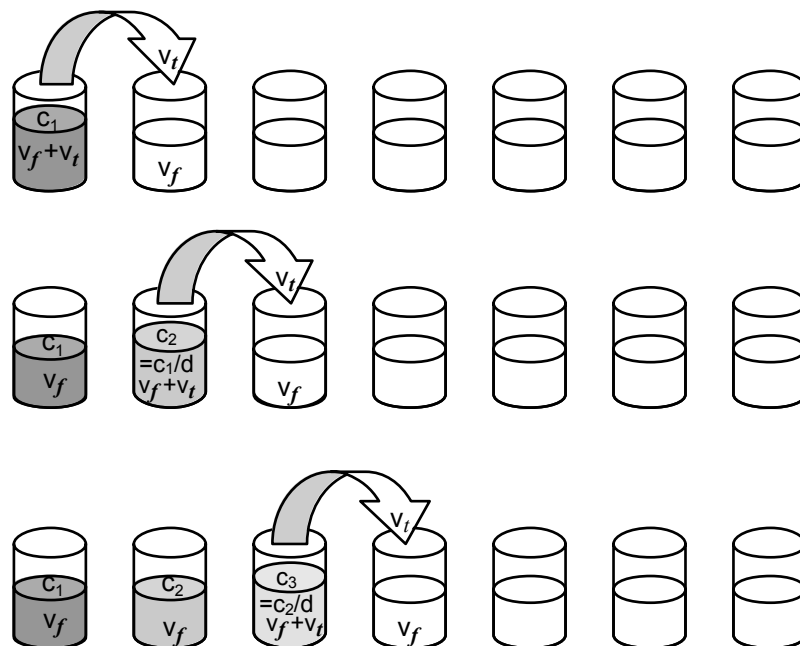
Figure 1. A serial dilution. A small volume (v_t) of solution is transferred to a vessel containing a volume (v_f) of solvent. After these are mixed, v_t of the second solution is transferred to the third vessel, also containing v_f of solvent. The concentration of each solution is 1/d the concentration of the previous one in the series.

So if I didn't think I could trust a 1 µL transfer volume, I might try a 10-fold serial dilution ($d = 10$) (both because calculations with 10 are easy, and because I want the last dilution in my series to be one one-thousandth of the initial concentration). Suppose that the 1 mL volume in the example above was more than enough for my experiment. An easy 10-fold dilution series could be done with a 0.1mL transfer volume, as follows:

$$\text{Starting with the general equation:} \qquad c_1 v_t = \left(\frac{c_1}{d}\right)\left(v_f + v_t\right)$$

$$\text{Substituting the initial concentration, the dilution factor, and the transfer volume:} \qquad 1M \times 0.1mL = \left(\frac{1M}{10}\right)\left(v_f + 0.1mL\right)$$

$$\text{Solving for } v_f: \qquad \left(1M \times 0.1mL \times \frac{10}{1M}\right) - 0.1mL = v_f = 0.9mL$$

So by starting with 1 mL ($v_f + v_t$) of my 1M (c_1) solution, and transferring 0.1 mL (v_t) of it to 0.9 mL (v_f) of solvent gives me 1 mL ($v_f + v_t$) of 0.1M $\left(c_2 = \dfrac{c_1}{d} = \dfrac{c_1}{10}\right)$ solution.

Repeating this process will give me a series of solutions, each one-tenth the concentration of the one before it.

What if I don't know what concentration range of a particular substance is suitable for use in my experiment or in my measuring device? You need to test a wide range of concentrations. Serial dilution is the best way to achieve such a range. With a 2-fold dilution series of 10, you can take a solution down to less than a 1000th of its original value:

$$\frac{1}{2^{10}} = \frac{1}{1024} = 0.000977$$

With a 10-step10-fold dilution series, you can get down to 10^{-10} (less than a billionth!) of the original concentration.

What if the manufacturer gives me exactly 1 mg of the chemical? You can take advantage of the manufacturer's precision by dissolving the chemical, still in its original package to prevent loss of material, in a small, known volume of suitable solvent (which the package insert should identify for you), thereby making a stock solution of known concentration (*e.g.*, 1 mg/mL). From that, you can make whatever dilute concentrations you need.

LAB DAY 2:
USING PUBMED

PubMed provides free access to a database of journal articles in the national library of medicine (MEDLINE) and additional life science journals. This resource is very useful for retrieving abstracts and articles for research.

[The Web of Science is another database, and includes some articles not found in PubMed. Access to the web of science is via the Umass library, and you need to sign in to gain access.]

An online tutorial for PubMed can be found here:
https://www.nlm.nih.gov/bsd/disted/pubmedtutorial/cover.html

To get started try these exercises:

Go to PubMed.   https://www.ncbi.nlm.nih.gov/pubmed

1. Search by topic. Use the search bar to add some key words.

   Do a topic search on "mitosis". How many articles did you get?   _____

   Limit your search. You can limit by year, ['Date limits' button at the top of the page] or by adding more specific search terms [mitosis AND kinesin, for example]. (Note that once you change limits, it's permanent until your change it back).

   How many articles mention mitosis in 2010? _____

   How many articles mention mitosis AND kinesin?_____

   You can search for review articles.   Use the 'Set search limits' menu at the bottom of the general search page.

   Did you turn up any reviews on mitosis? How many_____

2. Search for a particular article:

   Search for an article by typing in the search bar some identifying information – Author, topic, or some combination. Try finding a review on Kinesin 5 that was written by N. P. Ferenz.
   Can you find it? How? What is the citation?_____

   One of the easiest ways to move *forward in time* through the literature is to find all the papers that have cited a piece of work. **How many times has this article been cited since it was published? _____**

   What is the most recent citation?_____ Can you find that article? Has it been cited? _____Any ideas why or why not?_____

Look at the citations in the article you just found (Ferenz, Gable and Wadsworth). A different approach to find out more information on the topic of interest would be to look at the references in the

article you read. You can see if these other researchers have published any additional papers. Tip: the last author is (usually) the "corresponding author" and typically the person who is in charge of the project. Using their name to look for additional resources can be very helpful (as compared to using the first author, who may be a junior person with few or no other works).   From the Ferenz article, you want to know more about kinesin 5 binding to microtubules so you go to the article by Cahu et al. Search on Cahu and then on Surrey (first and last authors). What did you find? Is is relevant to your interests?

Reading what you find on PubMed:
Reading scientific articles is challenging, even for those who do it frequently. Most scientific articles are too dense to comprehend with a simple reading. To make sense of the article you may need to look up terms in the article in textbooks or online sources; you may need to read cited articles to get the necessary background information; and you may need to read the article several times to make sense of it.

Some ideas for reading scientific articles:

1. Scan the article: Read the title – is it pertinent to your work? When was it published—is this an old 'classic' or hot off the press? Both types of articles are important! Is the work in a refereed journal? Has it been cited?

2. What is the main message of the paper? Read the Abstract to get a sense of what was done. The abstract should state the rationale for the work, the basic method used, the results and the conclusion(s).

3. Read the Introduction. This section should provide the background needed to understand what questions are being addressed (what is *already known* about the subject and *what is not known*). Introductions typically contain many references to the literature – so if you are feeling lost, you can check out these older papers first.

3. Read the Results section and look at the figures and tables. Does it make sense? If not, be sure to read the Methods so you can understand out what was done. The Results is the meat of the work and usually reports the results of a series of experiments that build a story. Read the text and look at the Figures – can you follow the author?

4. Finally, read the Discussion. At this point the authors should put the data in context of the field. Are their results consistent with other work?   Did they discover something novel?   Do you understand how they reached these conclusions?

After you read the article think about:

What is the specific question that is addressed?
Are the findings persuasive?
Are the methods appropriate? Are there alternative approaches?
Do the results relate to what I'm interested in?
What experiments can be done to answer outstanding questions?
Did I understand the terminology?
Do I need to go back and read a review to understand the background?

APPENDICES

Example Lab Notebook Entry

2/10/11

Lab X.Y: Microscopy of rice tissue samples

Purpose/Goal:
To prepare samples of rice flowers for sectioning and microscopy; to take images of rice flowers using a microscope.

Methods:
We followed the methods on pages A-F of the lab manual. In summary, tissue was fixed in standard fixative, dehydrated in an ethanol series (10% - 95%), and then acetone. We embedded the tissue in plastic resin and sectioned it 1mm thick using microtome. Sections were placed on glass slides, stained with methylene blue dye and viewed under the microscope.

Calculations: $v_i = \dfrac{v_f \times c_f}{c_f} = \dfrac{10mL \times 1\%}{5\%} = 2mL$

— Need this much to stain slide
— Need this concentration
— Use this much stock, plus H_2O to 10 mL
— Stock solution concentration (See Errors, below.)

Observations:
- Tissue was floating on top of the fixative at first, but after 2 hours had sunk to the bottom of the tube.
- Tissue was green to start but by the end of the fixing and dehydrating was much paler.

Errors:
Stock solution was 2.5%, not 5%. Should have used 4mL stock. Needed to leave slide in dye longer than time given in manual.

Results:
labeled image of my rice flower:

Vascular Bundle
Flower
Pedicel
Abscission layer
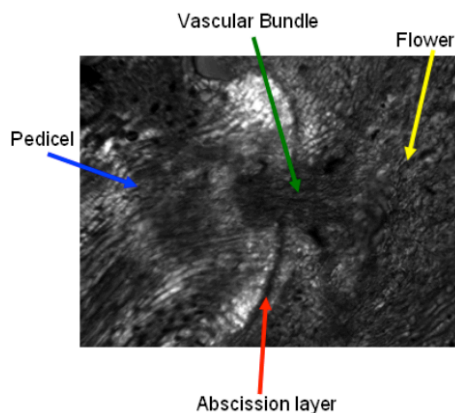
Conclusions:
The presence of a dark band (abscission layer) between the flower and pedicle suggests that this flower can easily be shed from the panicle. Easy shedding flowers are a hallmark of weedy or wild rice relatives thus I believe my flower comes from either weedy or wild rice.

Laboratory Report Format

Scientific writing

For additional reading, there is a very nice web site "How to Write Lab Reports for Biology" at the Union College Biology Department web site:

http://www.union.edu/academic_depts/biology/ResearchReports.php.

Pay special attention to the sample lab report on Jumpamine that is given there. It is an excellent example to use both in terms of content and length.

The basics:

- Double space all text

- Use a 12 point font

- Run the spell checker before printing

- Print lab reports—e-copies will NOT be accepted

Plagiarism

DO NOT PLAGIARIZE! Even the use of a ***phrase*** from a publication or the internet without quotation marks and citing the reference is considered plagiarism. Be especially careful in cases where you write your passages immediately after reading information on the topic in a book or on the Internet. Also, be careful when you and your lab partner are working together. While you both have the same data, and will have identical 'Results' sections, the ideas presented in the rest of the lab report must be your own.

Cite your sources. It is imperative to acknowledge where ideas or knowledge not originally your own come from, ***even though*** you have stated your understanding of the idea in your own words. This is done by putting the first author's last name and the date of the paper, or the page of a textbook, in parentheses at the end of the sentence containing the idea (as shown on page **Error! Bookmark not defined.**). Then, all sources (books or articles or web sites) you mention in the text of your paper are included in a list of references in a separate section at the end of the paper, as illustrated on page **Error! Bookmark not defined.**.

Although it is technically permissible to use brief quotations (a phrase or one sentence at most), these are very rarely used by practicing biologists. It is ***never*** appropriate to borrow extensive passages (more than two sentences) from a text or web site. The only reason to use a direct quotation is that the bizarre or amazing wording in the source must be shared with your reader. If you ever did have to use a quotation, you would use quotation marks and cite a reference at the end of the sentence. However, it is very unlikely that that it would be appropriate to make a direct quotation in a lab report.

Clarity, grammar, syntax and spelling: the mechanics of writing

The actual mechanics of your writing ***do*** matter. This includes spelling, grammar, logic, the flow of the writing and general understandability of the paper. Therefore, you should make sure you proofread your reports, and edit them as necessary. There are two 'tricks' that almost always give marked improvement in the clarity of your writing. The first is to ***make an outline before beginning to write***. You learned to do this in grammar school (sometimes they called it a web), then again in middle school, and again in high school. Even though you may have learned the technique in English class, you should use it for all of your writing. Another excellent way to be certain that your writing is clear is to ***read it out loud to yourself***. Students always think this is stupid/corny/something I made up to embarrass them. In fact, I always read my own writing out loud in order to catch the unclear bits.

There is help available from the University Writing Center (http://www.umass.edu/writingcenter/). You paid for it (with your tuition and fees); you should take advantage of it. Your grades will thank you.

Scientific terminology

It is worth learning the correct singular and plural forms of scientific terms. A selection of the most common follows:

one	two
bacterium	bacteria
chiasma	chiasmata
cilium	cilia
criterion	criteria
datum	data
flagellum	flagella
genus	genera
helix	helices
index	indices
larva	larvae
medium	media
mitochondrion	mitochondria
nucleus	nuclei
phenomenon	phenomena
species	species (not a typo! – singular and plural are the same)
stigma	stigmata

Learn the correct format for abbreviations, as well:

- Many scientific acronyms are capitalized, but the phrases they stand for are not. For example, **DNA**, **OD**, and **GFP** stand for **deoxyribonucleic acid**, **optical density**, and **green fluorescent protein**, respectively. The names of the elements are capitalized when abbreviated, but lower case when written out*: NaCl* stands for **sodium chloride**.

- Scientific names of organisms take this form: *Genus species*. The genus name is capitalized, and the species name is lower case. Both are italicized. For very common species, or after you have first written out the entire name, you may abbreviate the genus name with just its first initial. E.g., *Escherichia coli, Drosophila melanogaster*, *Homo sapiens*, *Mus musculus*, can be abbreviated *E. coli*, *D. melanogaster*, *H. sapiens*, *M. musculus*. (You do have to be careful, however, that the reader wouldn't think you were referring to the intestinal ameba *Entamoeba coli* instead of the bacterium *Escherichia coli*.)

Use the most specific term you can, even if it is repetitious and your English teacher told you not to repeat the same word too often. For example, "hydrochloric acid" is more specific than "acid", which is more specific than "chemical". Write "lactose" when you mean lactose in particular, and "sugar" when the reader couldn't possibly think you mean any other sugar in that sentence, or when you do mean any sugar.

Miscellaneous good writing

Not scientific exactly, but important nonetheless, clear writing represents clear thinking (and makes a favorable impression on the reader). Watch out for common errors; if you're not sure, look it up. The OWL at Purdue has a good selection of brief explanations of English grammar and mechanics: http://owl.english.purdue.edu/owl/ Some of my favorites:

- *it's* is short for *it is* or *it has*. There is no apostrophe in *its* (meaning belonging to it) any more than there is in *his* or *hers*.

- The whole *comprises* its parts; the parts *compose* the whole.

- To decide whether to use *me* or *I*, think about what you would write if only one person were involved. For example, since you would never write "Me did an experiment," (or at least I *hope* you wouldn't), you shouldn't write "Me and my partner did an experiment." In addition, for the sake of politeness, put yourself second: "My partner and I did an experiment." Do the same mental analysis for she/her, he/him, they/them, we/us.

- If you count them, you have a *number* of them. If you weigh it, or measure its volume, you have an *amount*.

Reporting numbers

Decimals. Write the zero in front of a decimal, e.g., 0.05.

Significant figures. Don't report more significant figures than are found in the least precise of your measurements. Put your least precise measurement into scientific notation to find the appropriate precision. If you report DNA concentration as 250.139652 µg/mL, you are suggesting that you can distinguish between 250.139653 µg/mL and 250.139652 µg/mL; it is more honest to give the concentration as 250 µg/mL or 2.50×10^2 µg/mL.

Just because your calculator or Excel calculated a number to 9 decimal places doesn't justify reporting all those significant digits. In Excel, use the Format/Cells/Number menu to adjust the number of decimal places. Do it yourself if you are copying a number from your calculator display.

It is very hard to read a list of numbers embedded in a sentence. If you want to report more than two numerical values, put them in a table so that the reader can carefully compare them. In the written part of your paper that refers to the table, you need only point out the most important values, or the range, or how many times larger the maximum is than the second largest, or whatever else you think is important.

Choose your units in a way that will make it easiest for your reader to understand your measurements. In your introductory chemistry and biology labs, you probably used only a few of the most common metric prefixes, such as milli-, centi-, and kilo-. Various textbooks and lab manuals contain longer lists of prefixes, but not many contain a complete list. There is no point in memorizing all of them, but it is nice to be able to look them up when you encounter them as you read the scientific literature. The smaller prefixes, such as nano-, pico- and femto-, are becoming increasingly common as analytical chemistry and biotechnology develop more sensitive methods. The larger prefixes, such as mega-, giga-, and tera-, are becoming more common in computing as engineers find ways to pack more and more memory into tiny devices. **Error! Reference source not found.** on page 31 presents prefixes ranging from 10^{24} to 10^{-24}. To help you visualize the effect of these prefixes, the column labeled "a sense of scale" gives some examples of the magnitudes represented.

Presenting your Findings

Prepare a final presentation. Give a broad overview of your gene: what it might do, how you discovered this function, etc.

Follow the guidelines below for making an engaging and informative presentation.

Your talk will be evaluated on

- Content

- Communication (whether it was engaging and clear)

- Organization

- Slide quality

- Professionalism (accuracy and reference to sources)

- Length (did it fit into the allotted time?)
- Presentation style (audibility, enthusiasm, gestures, posture)
- Handling of audience questions

How to make a great presentation:

The tldr version:

Pictures! Short labels! Big font! No reading from the slide!

The longer version:

Show pictures:

The main advantage to using slides over simply giving a speech is the power of pictures. The best talks are spontaneous explanations of *pictures* you show your audience.

Make the picture fill the screen! Don't waste valuable slide real estate with a written explanation of the image – it detracts from the power of the image and your ability to tell the story.

If you have a complicated experimental design, draw a diagram and walk your audience through it. Harness the power of your presentation software to bring one element up at a time, as you get to it in your explanation.

Tables full of multi-digit numbers are hard enough to read on the page right in front of you. They are nearly impossible to make out during a presentation, especially if the audience has to listen to you while simultaneously trying to decipher how many times bigger 431078.009652 is than 3967.031458. Make a figure!

Use only enough text to make your image understandable:

Short, well-placed, easy-to-read labels make an image comprehensible.

Make the labels short and legible. Most of the audience will be a lot farther away from the screen than you are. If your label has to wrap around to the next line, shorten it.

Short, title-like phrases (or even single words!) are sufficient to help the audience follow your reasoning.

Avoid taking up a huge portion of your slide with a not-so-specific word like "RESULTS".

The detailed figure titles necessary in print are *completely inappropriate* in a presentation – the audience can't read one and listen to you at the same time.

Make it big enough to read from the back of the room:

Project your slide, walk to the back of the room, and check legibility. If you can't do a trial projection, maximize the image on your computer screen and step back from it about 6 feet.

Don't write out your talk on your slide:

First of all, if you can't give your talk without reading from your slides, *you aren't ready!* What does this mean? It means you need to know the material inside and out, and be able to tell the audience about it (while facing them instead of the screen).

Second, if your whole talk is written out on your slides, either you read the slides out loud to the audience, which is boring, or you say different words while they are trying to read the ones on the slide, which is distracting. Don't force your audience to choose between paying attention to you and reading the text.
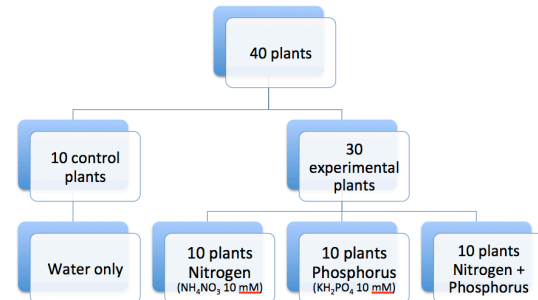
Some Examples:

Compare the following pairs of slides: The left slide in each pair has too small a picture (or none at all) and too much text. The slides on the right, however, contain an image large enough to be seen

from a distance, and text (including headings) that enhances audience understanding of the experiment.

| **EXPERIMENTAL DESIGN**	
• We obtained 40 plants, all at the early seedling stage, where the cotyledons were first visible above the soil. • We used 10 of these as controls. They got only water. • 30 plants were experimental plants. 10 of these got nitrogen supplementation in their water (as NH_4NO_3 – ammonium nitrate – at a concentration of 10 mM). • Another 10 got phosphorus supplementation in their water (as potassium phosphate monobasic- KH_2PO_4 – at a concentration of 10 mM). • The last 10 got nitrogen *and* potassium supplementation. • We harvested all the plants (the above-ground structures) when they were 20 days old, ground them to a powder in liquid nitrogen, and then weighed that powder to get the dry weight in grams. • We recorded the weight of each plant, and calculated the averages and standard deviations, and ran a t-test.	Fertilizer Experiment Harvest at 20 days, freeze dry, weigh

RESULTS:

The control plants had an average dry weight of 4.4967841 ± 1.801024; the plants supplemented with nitrogen had an average dry weight of 9.402568 ± 1.899573; the plants supplemented with phosphorus had an average dry weight of 9.720315 ± 1.19730487. There was a significant difference between the dry weight of the control plants and the dry weight of the supplemented plants (p = 0.019953 and 0.020396, respectively). The nitrogen supplemented plants were 2.090954 times heavier than the control plants and the phosphorus supplemented plants were 2.161615 times bigger.
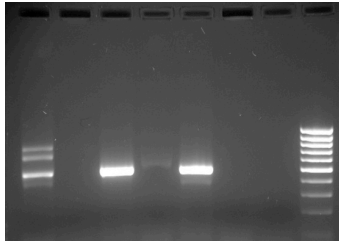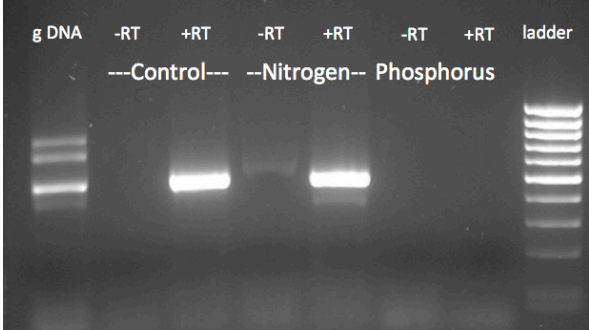
dry weight (g)	control	nitrogen	phosphorus
average	4.4967841	9.402568	9.720315
standard deviation	1.801024	1.89957	1.19730487
	fold change =	**2.090954**	**2.1616151**

Fertilizer increases plant growth
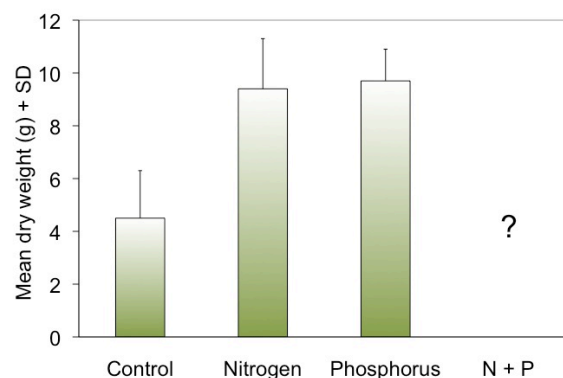
		p =	0.019 953	0.0203 96

Unfortunately, we were unable to test the simultaneous application of nitrogen and phosphorus due to a growth chamber mix-up. We would have predicted, based on the same papers, that such an application would have produced even greater increases in plant dry weight.

RT-PCR

Lane 1: gDNA; Lane 2: Control -RT; Lane 3: Control +RT; Lane 4: Experimental (Nitrogen) -RT; Lane 5: Experimental (IAA) + RT; Lane 6: Experimental (Phosphorus) +RT; Lane 7 Experimental (Phosphorus) -RT; Lane 8: Fermentas MassRuler, LMW

As can be seen in this gel, our RT-PCR worked, because the lanes without RT have no bands, and the lane with nitrogen treatment and RT have cDNA bands. We did not get an RT band for the phosphorus treated plants. Also, there is approximately the same amount of cDNA in the two RT lanes, showing that our nitrogen treatment did not increase or decrease the amount of gene expression. This is a surprise, because we designed this experiment based on several papers that suggested an effect of nitrogen on the expression of our gene (Brown & Green, 2001, Farmer & Cook, 2009, Gardner & Chamber, 1999).

N does not affect gene expression

Nanodrop Blanking and Absorbance Calculations

When the NanoDrop 1000 Spectrophotometer is "blanked", a spectrum is taken of a reference material (blank) and stored in memory as an array of light intensities by wavelength. When a measurement of a sample is taken, the intensity of light that has transmitted through the sample is recorded. The sample intensities along with the blank intensities are used to calculate the sample absorbance according to the following equation:

$$Absorbance = -log \frac{Intensity\ sample}{Intensity\ blank}$$

Thus, the measured light intensity of both the sample and of the blank are required to calculate the absorbance at a given wavelength.

Concentration Calculation (Beer's Law)

General

The Beer-Lambert equation is used to correlate the calculated absorbance with concentration:

$$A = E \times b \times c$$

Where

A is the absorbance represented in absorbance units (A),

E is the wavelength-dependent molar absorptivity coefficient (or extinction coefficient) with units of liter/mol-cm,

b is the path length in cm, and

c is the analyte concentration in moles/liter or molarity (M).

Nucleic Acids

For nucleic acid quantification, the Beer-Lambert equation is modified to use an extinction coefficient with units of ngcm/mL. Using this extinction coefficient gives a manipulated equation:

$$c = \frac{A \times e}{b}$$

Where

c is the nucleic acid concentration in $\frac{ng}{\mu L}$

A is the absorbance in AU

e is the wavelength-dependent extinction coefficient in $\frac{ng \times cm}{\mu L}$

b is the path length in cm

The generally accepted extinction coefficients for nucleic acids are:

$$Double\text{-}stranded\ DNA:\quad 50\ \frac{ng \times cm}{\mu L}$$

$$Single\text{-}stranded\ DNA:\quad 33\ \frac{ng \times cm}{\mu L}$$

$$RNA:\quad 40\ \frac{ng \times cm}{\mu L}$$

For the NanoDrop 1000 Spectrophotometer, path lengths of 1.0 mm and 0.2 mm are used compared to a standard spectrophotometer using a 10.0 mm path. Thus, the NanoDrop 1000 Spectrophotometer is capable of measuring samples that are 50 times more concentrated than can be measured in a standard spectrophotometer.

DNA Molecular Weight Standard

Many times in this course you will need to know the length of a fragment of DNA that you have amplified by PCR or digested with restriction enzymes, or produced by reverse transcription. This is done by loading the DNA in an agarose gel, applying a voltage, and letting the DA migrate through the gel. Shorter fragments move faster than long ones, and you can use the distance traveled during the experiment as an indicator of fragment length. In order to calibrate DNA length and distance traveled, you compare the distance your fragment traveled with the distances traveled by a series of standard fragments of known length. The DNA standard used in this course is shown in Figure 2.
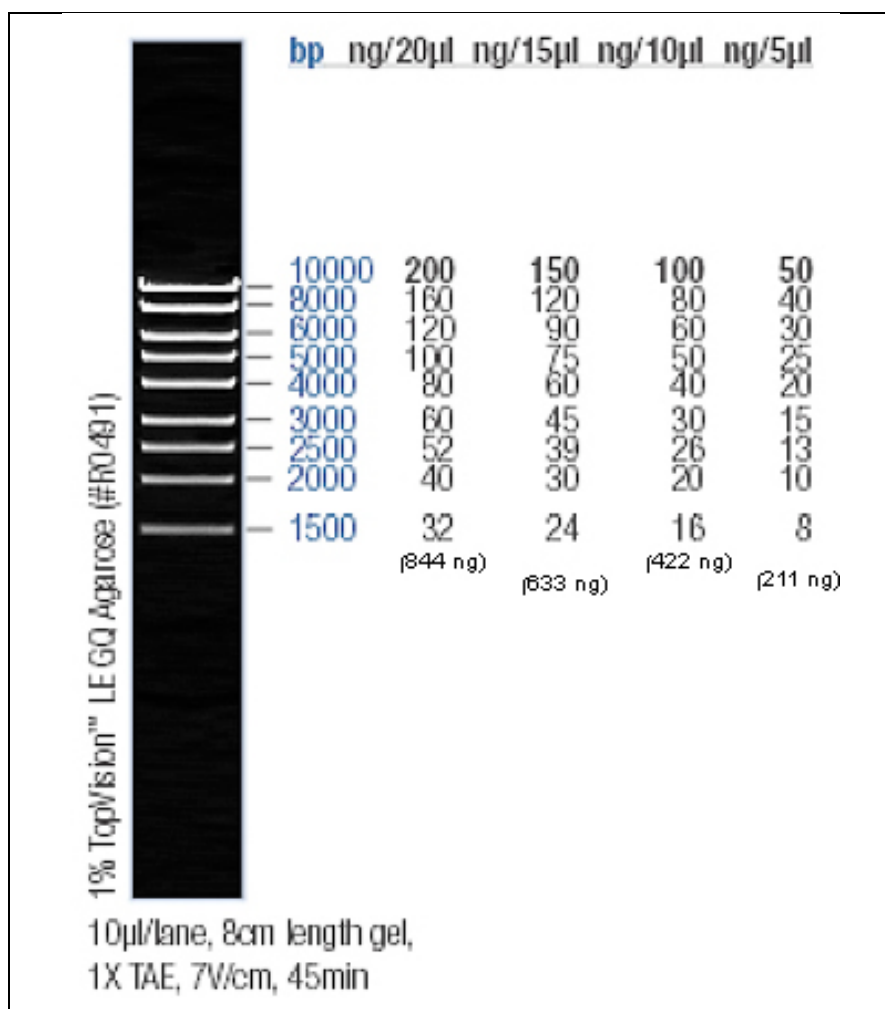

Figure 2. Fermentas #SM0393 MassRuler High Range DNA Ladder

Metric Prefixes

Prefix	Abbreviation (note upper and lower case)	Meaning	synonym	A sense of scale (approximate)	
yotta-	(Y-)	10^{24}	1 septillion	Mass of water in Pacific Ocean	~1 Yg
				Energy emitted by sun per second	~400 YJ
					~1 YL
				Volume of earth	~6000
				Mass of earth	Yg
zetta-	(Z-)	10^{21}	1 sextillion	Radius of Milky Way galaxy	~1 Zm
				Volume of Pacific Ocean	~1 ZL
				Annual world energy production	~0.4 ZJ
exa-	(E-)	10^{18}	1 quintillion	Age of universe (12 billion yr)	~0.4 Es
peta-	(P-)	10^{15}	1 quadrillion	1 light-year	~9.5 Pm
tera-	(T-)	10^{12}	1 trillion	Sun-to-Jupiter distance	~0.8 Tm
giga-	(G-)	10^{9}	1 billion	Human life expectancy	~3 Gs
				1 light-second	~0.3 Gs
mega-	(M-)	10^{6}	1 million	Two weeks	~1.2 Ms
kilo-	(k-)	10^{3}	1 thousand		
hecto-	(h-)	10^{2}	1 hundred		
deka-	(da-)	10	1 ten		
deci-	(d-)	10^{-1}	1 tenth		
centi-	(c-)	10^{-2}	1 hundredth		
milli-	(m-)	10^{-3}	1 thousandth		
micro-	(μ-)	10^{-6}	1 millionth	Diameter of human ovum	~1 μ
				Volume of mosquito blood meal	~2 μL
				Volume of wood frog egg	~3 μL
nano-	(n-)	10^{-9}	1 billionth	Radius of chlorine atom	~0.1 nm
pico-	(p-)	10^{-12}	1 trillionth	Mass of bacterial cell	~1 pg
femto-	(f-)	10^{-15}	1 quadrillionth	Radius of proton	~1 fm
				Volume of *E. coli* cell	~1 fL
				Volume of red blood cell	~100 fL
atto-	(a-)	10^{-18}	1 quintillionth	Time for light to cross an atom	~1 as
				Bond energy of C=C	~1 aJ
zepto-	(z-)	10^{-21}	1 sextillionth	600 atoms or molecules	~1 zmol
yocto-	(y-)	10^{-24}	1 septillionth	Mass of proton or neutron	~1.7 yg