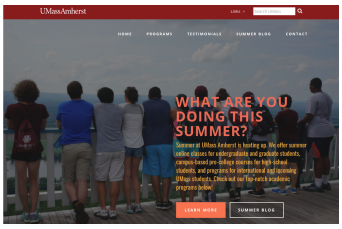
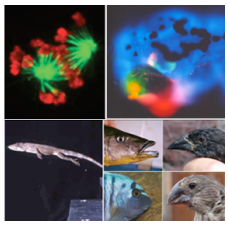
University of Massachusetts Amherst



UMASS Biology
Where the Study of Life Comes Together



Welcome to UMass Summer College Research Intensives

Lab Boot Camp Manual

(always a work in progress)

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

- Brush up on some basic molecular biology lab techniques
    a. Advanced pipetting skills and pipette testing
    b. PCR from zebrafish genomic DNA
    c. Restriction Enzyme Digestion to genotype uml/boc mutant zebrafish
    d. Agarose gel electrophoresis/identify heterozygous mutant zebrafish
    e. Basic Bioinformatics
- Get to know each other and where other Summer College students are working.

Overview

Day 1 (Monday, 9:00-12:30)

Introductions, Boot Camp overview, lab notebooks, and lab safety

Pipetting skills and pipette calibration

Set up PCR reactions

Day 2 (Tuesday, 9:00-12:30)

Run Agarose Gel

DNA BLAST search with PCR primer sequences

Nanodrop DNA quantification of genomic DNA and PCR product (as time permits):

Photograph gel and find any heterozygous fish (take cell phone pix & send to Rolf)

Field trip to zebrafish facility to find the identified heterozygotes (we hope)

Instructors:

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Anna Aristarkhova (Karlstrom Lab): aaristarkhov@umass.edu

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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.

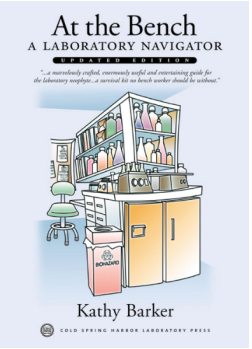
Discarding Waste

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.

A useful resource:



At the Bench ; A Laboratory Manual Paperback – 2005

by Kathy Barker (Author)

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LAB DAY 1

EXERCISE #1: PIPETTING

Goal for this exercise:

- Master your micropipetting technique
- Identify pippettors that are faulty ann need calibration.

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. **If you don't know how to use pipettors read appendix at the end of this lab manual, which has some good tips.**

You will use 2 methods to examine pipettor accuracy and repeatability (precision?). Since we know how much a given volume of water weighs (**1 ml = 1 gram**), 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.

Some pipettors may be inaccurate and need calibration. We will have you enter your data for each pipettor (each is individual labeled) in a common spreadsheet so we can identify any lemons.

ENTER YOUR DATA ON THIS GOOGLE SHEET
https://drive.google.com/file/d/1aKkA5NNv_3jWn3p5e2WyZPCDCPmfvoyy/view?ts=5d14e2ad

Pipette calibration

First figure out what each of these volumes of water should weigh, and which pipet should be used to dispense that volume. You will weigh the maximum volume for each pipettor.

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 maximum volume of water for each pipettor (e.g. 20µl for the L20) 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 5 measurements (no need to change the weigh paper in between)
5) Repeat for 1//2 the maximum volume for the same pipettor
6) Repeat for the other two pipettors.
7) Enter all data in the google sheet linked below.

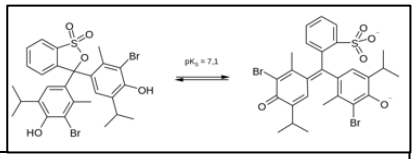
Record all results in a table in your lab notebook **and make a hand-drawn graph showing the weights you got with each trial**. Compare your data to the work of the class in the google sheet, looking at the standard deviation and significance as determined by a q-test (Kate will set up the sheet to do this automatically)

ENTER YOUR DATA ON THIS GOOGLE SHEET
https://drive.google.com/file/d/1aKkA5NNv_3jWn3p5e2WyZPCDCPmfvoyy/view?ts=5d14e2ad

LAB TIP: You should do a scaled down version of this when you get to your new lab!!! It is better to have your pipette calibrated (or find a pipette that works) at the beginning 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 size as the others.
8) Take a picture of your results and send it to the instructor.



How This Works: 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.

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. (∴ a buffer!)

				H_3PO_4		
				↑↓	pKa′	2.12
monobasic	NaH_2PO_4	↔	Na^+ +	$H_2PO_4^-$		
				↑↓	pKa″	7.21
dibasic	Na_2HPO_4	↔	$2Na^+$ +	HPO_4^{2-}		
				↑↓	pKa‴	12.32
tribasic	Na_3PO_4	↔	$3Na^+$	PO_4^{3-}		

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!)**

Day 1, Exercise #2:
PCR FROM GENOMIC ZEBRAFISH DNA

Goal:

PCR amplify a ~250 bp sequence from genomic DNA that was prepared from individual zebrafish (fin clip DNA). These fish may not be carriers for a mutation in a gene that affects early brain development. The point mutation leads to a loss of a ClaI restriction enzyme recognition sequence, thus the PCR product that is amplified from a mutant chormosome will **not** be cut by the enzyme. You will set up the PCR and we will digest the PCR products overnight for you. Tomorrow you will run the DNA on a gel to see if we can identify individual fish that carry the mutation by looking at the DNA bands.

We will provide you with genomic DNA templates from 8 or so individual zebrafish. To get this DNA we clipped the end of their caudal fins and lysed the DNA with NaOH. Zebrafish fins regenerate quickly, so these fish are now swimming happily in our fish facility while we determine which of them are carrying a single copy of a particular recessive mutation that can be identified by PCR followed by restriction digestion of the resultant DNA fragment.

1) Add 20 µl PCR master mix (= everything but the DNA) to each labeled PCR tube

Calculate how much master mix to make = number of DNA samples + 1 positive control + 1 negative control + 2 extra. Table 1 shows an example for 18 reactions (= **16** DNA samples + **1** positive control + **1** negative control + **2** extra= 20).

Table 1: Master Mix Recipes

	Per reaction	20 rxn master mix	Master Mix for ___8___ Rxns
10X PCR buffer	2.5µl	50	
10mM dnTP	0.5 µl	10	
5µM Fw + Rv primer mix (0.4 µM final conc.)	2 µl	40	
Taq	0.15 µl	3	
Diluted DNA	5 µl	NA	
H2O	14.85µl	297	
Total	25 µl	400	

Note: 5µM Fw+Rv Primer Mix = 90µl H2O + 5µl 100µM Fw primer stock + 5µl 100µM Rv primer stock.
Forward primer sequence: GGGTCAACTTCTGAAGCGGCTCGTATCATCGA
Reverse primer sequence: GAGCAGAGCTTGTGGAGCCGATG

2) Add 5µl diluted genomic DNA to labeled PCR tubes. EACH TUBE = A DIFFERENT FISH.

Note 1: The DNA we are giving you has already been diluted 1:10 in water.
Note 2: These genomic DNA samples were made by clipping fins from individual zebrafish and lysing the tissue using a simple NaOH lysis method (Meeker et al. 2007 *BioTechniques 43:610-614).*

3) Run PCR reaction: (94° 2', then 38 cycles of 94° 30s, 55° 30s, 72° 30s, 72° 5min, 15° hold)

4) Add 10 µl ClaI Master Mix Digestion to each PCR tube, digest Overnight at 37°

Note: Cla1 master mix = per tube: 3.5µl NEB buffer #4, 0.35µl BSA, 0.3 µl ClaI, 10.85 µl H_2O

5) **Next Day: Load Samples onto a 4% Agarose Gel (see below)**

LAB DAY 2:

Agarose Gels, Bioinformatics, DNA Quantification

Goals for today:

- Run an agarose gel to separate DNA fragments (PCR amplified yesterday and digested overnight)
- Quantify DNA with the Nanodrop (if time)
- Photograph the gel and identify which (if any) fish carry the mutation
- Figure out what gene you are amplifying (Blast Search with PCR primer sequences below)

Agarose Gel Electrophoresis

We will run the digested PCR fragments on a 4% agarose gel to try to separate the bands of different sizes and identify which fish carried the mutation that eliminated a Cla1 restriction site. The high percentage gel is being used to allow us to distinguish DNA fragments that are only about 30 base pairs different in length.

Procedure:

Kate has nicely provided pre-measured and melted agarose 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
3) Let the agarose harden for 20' or so
4) While your agarose hardens, remove 5µl from each reaction to another tube for Nano Drop exercise (Below)
5) The, add loading buffer to each PCR tube
6) Remove the combs, add running buffer
7) Add 5µl DNA Markers to lane 1
8) Add 20µl of each sample to lanes 2-8. KEEP track of which is which so we can go find the fish.
9) Run the gels as instructed.
10) Photograph the gels with your cell phones
11) Send the photo to Rolf (we'll compare results at the end of the lab.) Karlstrom@bio.umass.edu
12) **Clean-up as directed**

Expected Results:

Undigested PCR product = 268 bp
Cla1 digested PCR product = 236 bp + 32 bp

Wild Type Fish = Single band at **236 bp** + a 32 bp fragment (won't see at dye front?)
Heterozygous Fish = Doublet at **268bp and 236bp** + the 32bp fragment

Wild Type sequence: GGGTCAACTTCTGAAGCGGCTCGTATCATCTATCCTCCGGCATCACGCTCCA

Mutant sequence (T->A): GGGTCAACTTCTGAAGCGGCTCGTATCATCTAACCTCCGGCATCACGCTCCA

ClaI recognition site: ATCGAT

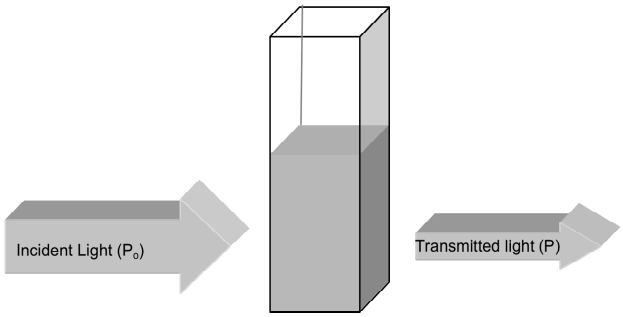
Record the following in your notebook:

- **Did you get a PCR product of expected size from all Genomic DNA samples?**
- **Do any lanes have 2 bands between the 200 bp and 300 bp? What does this mean?**
- **If you had problems with your PCR, where do you reckon problems could arise?**

DNA quantification by molecular absorbance spectroscopy.

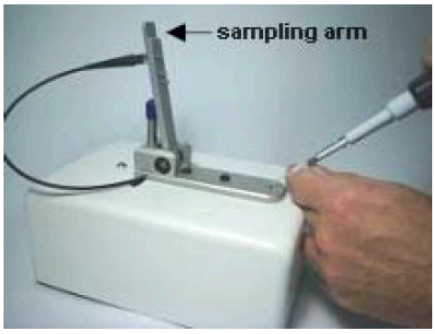
Reliable measurement of DNA concentration is important for many applications in molecular biology. DNA quantification is generally performed by spectrophotometric measurement of the absorption at 260 nm, or by agarose gel analysis. We are running a gel anyway, so let's see how much DNA you PCR amplified and whether this corresponds to what you see on gel.

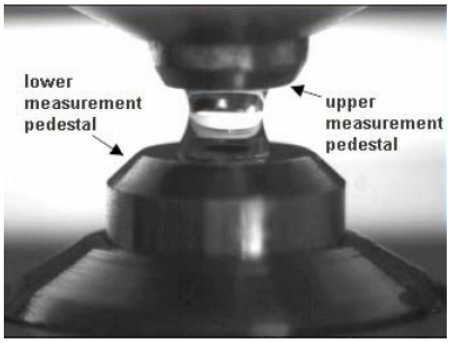
DNA concentration can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer using a UV-transparent cuvette (usually quartz). In molecular absorbance spectroscopy, a beam of ultraviolet or visible light (P_o) is directed through a sample. Some of the light may be transmitted 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}.



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.

Illustrated Procedure for Using the Nanodrop device:





While your gel is running let's see if we can quantify the DNA from our PCR reaction using the Nano-drop device.

1. With the sampling arm open, load a 1µL 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.
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. Add 1µl of your most dilute sample. Record the measurement.
6. Wipe and repeat with next higher concentration of DNA sample
7. Repeat.

Record the A280 and A260 measurements and calculate the ratio to get a feel for the purity of your sample. For pure DNA the ratio will be 1.8

As time permits, let's try to measure the concentration of the DNA in the original Fin Clip samples and measure the yield of your PCR reaction.

Fin Clip Genomic DNA analysis:

Fish #	Fin Clip A260	Fin Clip A280	Fin Clip A260/A280	DNA Concentration
1				
2				
3				
4				
5				
6				
7				
8				

PCR Product DNA Analysis

Fish #	PCR Prod. A260	PCR Prod. A280	PCR Prod. A260/A280	DNA Concentration
1				
2				
3				
4				
5				
6				
7				
8				

Blast Search To Figure Out What GENE you are PCR-amplifying

1. Go to ZFIN.org (Zebrafish Information Network)
2. Click “Blast at ZFIN”
3. Copy in the wild type sequence used to make the forward PCR primers
    a. GGGTCAACTTCTGAAGCGGCTCGTATCATCTATCCTCCGGCATCACGCTCCA
4. Click the box that says “Search for short, nearly exact matches”
5. Click “begin search”
6. Scroll down to the words and click on the top “associated gene”
7. See what you can learn about this gene/mutation.
    a. What type of gene is this?
    b. What signaling pathway is affected? (see Bergeron et al paper)
    c. What is the name of the mutation?

Literature Search 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 "neurogenesis". How many articles did you get? _____

Limit your search. You can limit by year, ['Date limits' link at the side (or maybe 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 neurogenesis in 2015? _____

How many articles mention neurogenesis AND aging in 2015?_____

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 neurogenesis in old brains (link on side of page)**? 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 title?_____

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?_____

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?

5. Tip: the last author is (usually) the "corresponding author" and typically the person who is in charge of the project. The first author is (usually) the person who did the lab work and probably wrote at least the first draft of the paper.

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

Micropipettor Basics

Micropipettors 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.

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:

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

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

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

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.

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: $V_i = \frac{V_f \times c_f}{c_f} = \frac{10mL \times 1\%}{5\%} = 2mL$

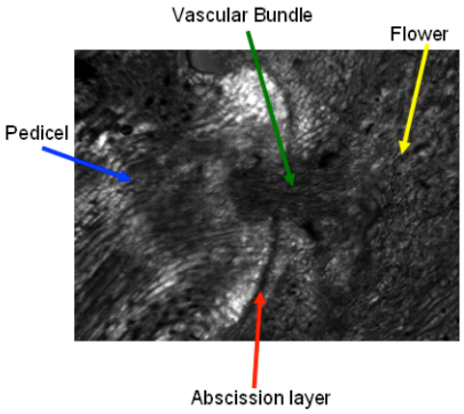
Need this much to stain slide
Need this concentration
Use this much stock, plus H_2O to 10 mL
Stock solution concentration (See Errors, below.)

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:



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.

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
				Volume of earth	~1 YL
				Mass of earth	~6000 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