Tips for Biotechnology

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Equipment

Gel Chambers
A variety of companies are now making smaller, more user friendly electrophoresis units. In some cases these are totally self-contained units with the power supply, gel chamber and viewing illumination all in one units. Others may have some combination of these but since they are smaller, they use less buffer and agarose. Possible venders:
Accuris Instruments
MiniPCR
MiniOne
There are others but these all have a price point around $375

Thermocyclers
In the past few years there have been a couple of miniature thermocyclers that have come on the market. While these do not necessarily work better than the conventional bench top versions they are often cheaper.
Possible Venders
MiniPCR
Teracore

Camera
A wireless camera, such as the Motic X, that connects to a tablet or computer is an incredible tool for documenting results and demonstrating techniques. It can also provide a permanent record of the techniques to use with students who have missed the class.
Venders:
Swift Optical
National Microscope
Carolina Biological
Fisher Scientific

Reagents

Agarose
Tablets-premeasured tablets for preparing gels
Fisher Scientific
Denville Scientific

DNA and enzymes
New England BioLabs provides free DNA and enzymes to high schools across the country. In the next year, there may be links to high school labs that use their products. Look for the "education" resource tab on their website.

Miscellaneous

Labels…can save you time!! Yes, it takes time to set up the templates but students are often looking for this type of work.
Venders:
Avery (standard address and smaller labels)
Diversified Biotech, Inc (Laser Tough-Spots come in a variety of sizes for different tubes)

Measuring Up: The Importance of Good Pipetting Technique

The success or failure of biotechnology labs often hinges on one factor—the ability of students to correctly use micropipettes. This fundamental skill is critical in measuring reagents and setting up reactions. Given that measurements are often less that 5 μL even a small mistake can result in twice as much reagent being added.

Micropipettes can accurately measure volumes as small as 0.5 μL and up to 1000 μL or 1.0 mL. Micropipettes come in many different sizes but in this lab we are going to focus on the three most common sizes.

The p20 micropipette is usually used to measure amounts between 1.0 μL and 20 μL. Although the instrument can be set for amounts below 1.0 μL these tiny amounts are difficult to measure accurately. The p20 micropipette can also be used to accurately transfer amounts over 20 μL but under 40 μL by pipetting twice. The p20 is generally considered the "workhorse" of the lab.

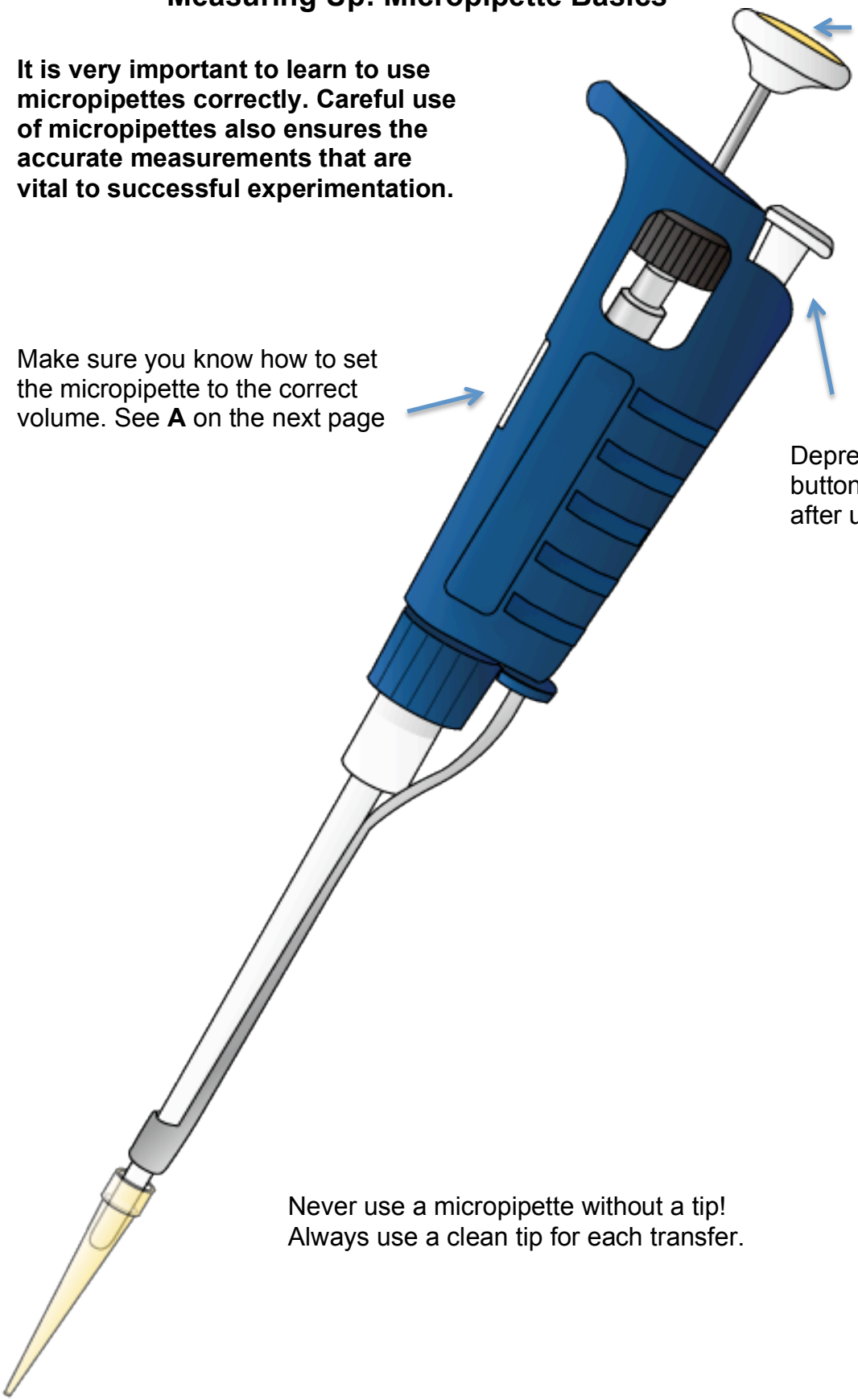
For amounts between 20 and 200 μL the p200 is generally used. The p200 can be set below 20 μL but it should not be used in this range if accuracy is critical. Loading 15 μL into a gel is fine but measuring 15 μL for a reaction is not a good idea.

The p1000 micropipette is generally used to measure amounts between 200 and 1000 μL. The p1000 can be set for amounts below 200 μL but accuracy will be sacrificed.

Make sure your students have an opportunity to practice with the micropipettes before doing labs. It is recommended that you set aside 30-40 minutes for students to work with the pipettes before starting a lab. It is crucial that all students hold and manipulate the pipettes so they really understand what is meant by the soft and hard stop. Going past the soft stop to the hard stop is probably the most common pipetting error that students make. Giving students several tasks that require that they transfer a variety of volumes will help ensure the success of future labs. Make sure that all students get to practice—don't let one eager beaver do all the pipetting

Measuring Up: Micropipette Basics

It is very important to learn to use micropipettes correctly. Careful use of micropipettes also ensures the accurate measurements that are vital to successful experimentation.



The numbers on the top of the plunger indicate the size of pipette.

The plunger has two “stops” that control the drawing up and dispensing of the liquid. See **B** on the next page.

Make sure you know how to set the micropipette to the correct volume. See **A** on the next page

Depressing the ejector button will push off the tip after use.

Never use a micropipette without a tip!
Always use a clean tip for each transfer.

A. Measuring Up: The Micropipettes

p20 Micropipette

The number on the top indicates that 20 μL is the maximum volume.

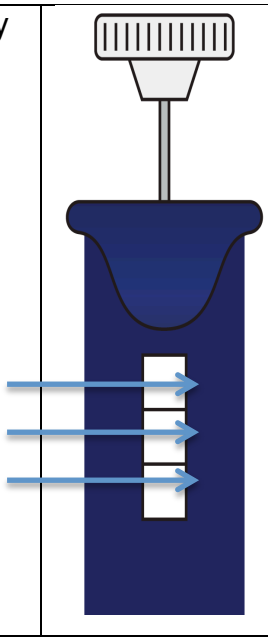
The p20 micropipette is one of the most commonly used pipettes. It is used for measuring small volumes and is often the pipette of choice for loading electrophoresis gels.
p20 micropipettes are used to measure volumes between 1.0 μL and 20 μL.

1
4
9

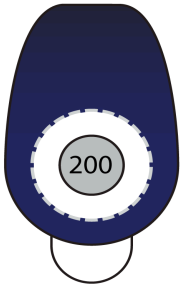
If the pipette were set like this it would measure 14.9 μL.

The top number should never be above 2.

Tens of μL
Single μL
1/10 μL



p200 Micropipette



The number on the top indicates that 200 μL is the maximum volume.

p200 micropipettes are used to measure volumes between 20.0 μL and 200 μL. The p200 is one of the easiest pipettes to set because "what you see is what you get." The numbers are read as they appear.

1
4
9

If the pipette were set like this it would measure 149 μL

The top number should never be above 2.

Hundreds of μL
Tens of μL
Single μL

P1000 Micropipette

The number on the top indicates that 1000 µL is the maximum volume.

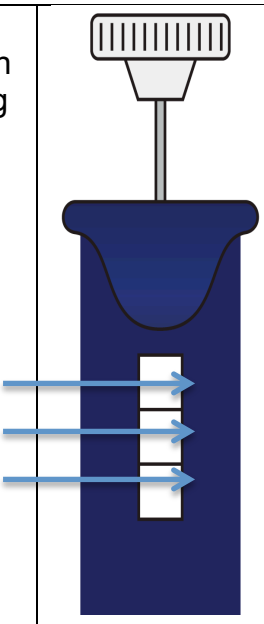
1000 micropipettes are used to measure volumes between 200 µL and 1000 µL. The numbers shown are multiplied by 10 to determine the amount being measured.

0
4
9

If the pipette were set like this it would measure 490 µL

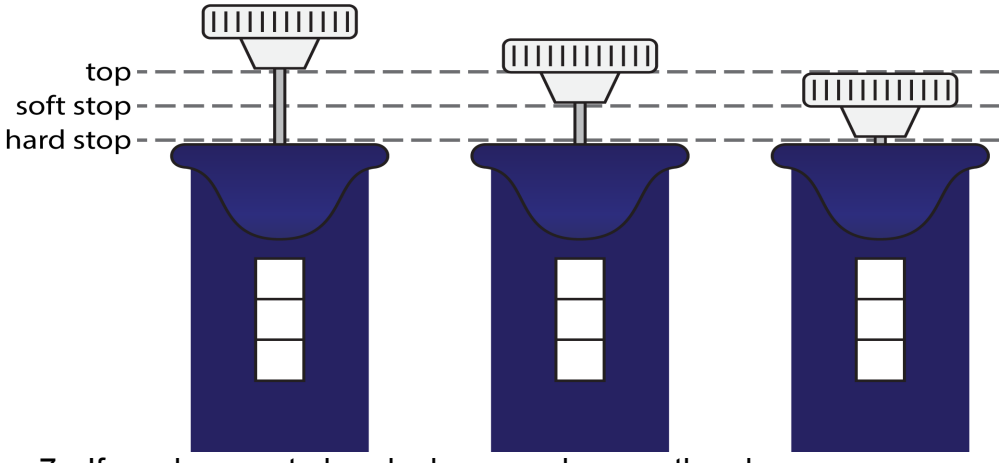
The top number should always be either 0 or 1.

Thousands of µL
Hundreds of µL
Tens of µL



B. Measuring Up: Using the Micropipettes

1. Choose the correct size of micropipette (see part A for information)
2. Make sure you have the appropriate tips (sterile if necessary)
3. Set the micropipette for the correct volume (see part A for information)
4. Attach a tip by pushing the shaft down into the tip. Do NOT take the tip out of the box and put it on using your hand. Make sure it is firmly seated.
5. Now…drum roll…the most critical step… drawing up the liquid.
6. Micropipettes have two stops, the soft stop and the hard stop. (see figure below)



7. If you have not already done so, depress the plunger so you can feel the difference between these two stops.
8. Pick up the sample container so you can see the level of liquid in it.
9. Start by depressing the plunger to the SOFT (or first) stop.
10. Hold the plunger in this position as you put the tip into the liquid. You should watch the tip going into the liquid and make sure you are only inserting it 2-3 mm under the surface.

11. Slowly let the plunger come back to the top position.
12. **LOOK AT THE TIP**. Does the amount of liquid look appropriate? If not eject the tip and start over.
13. If the amount looks right, continue by picking up the container you are transferring the liquid into. Put the tip into the container. If there is liquid in the container, immerse the tip into the liquid so you can be sure you have dispensed the liquid. If there is no liquid in the container you can touch the end of the tip to the side of the container.
14. Slowly depress the plunger to the soft stop and continue applying gentle pressure until you feel the hard stop. CONTINUE to apply pressure as you remove the tip from the container. If you let the plunger come back up before you remove the tip the pipette will suck up some of the liquid in the tube.
15. Eject the tip.

Let's review…

- Start with plunger all the way up
- Depress to the soft stop
- Draw up the liquid
- Check the volume
- Dispense by slowly pushing to the soft stop and then all the way to the hard stop.
- Lift the tip out of the container before you release the plunger

It cannot be said enough times…proper micropipette technique is critical to the success of your biotechnology laboratory experience

It is also recommended that you read the "Helpful Hints for Micropipetting" document.

Measuring Up: Practice Pipetting

Accurate pipetting is key to success in biotechnology labs. Use these activities to make sure you are comfortable with setting and transferring the amounts

Task 1

Materials

- 1 x microcentrifuge tube of 500 μL blue water
- p20 micropipette with tips
- container for used tips
- 10 x 10 cm piece of Parafilm® or wax paper

Procedure

1. Following the instructions for use of the micropipette that your teacher provides, pipette 5 separate 10 μL drops of water onto the Parafilm®. Look closely. Are they the same size? If not, try again to see if you can make them all the same size.
2. Now, do the same thing but try aliquoting 5 separate drops that are 2 μL each. Compare the drops to see if they are the same size.
3. Check your partner's drops. Are they the same size as yours?
4. Use a paper towel to clean up

Task 2

This is to help you perfect your laboratory technique. It is important that you follow the directions carefully and use a clean tip for each measurement.

Materials

- 1 x microcentrifuge tube of 300 μL of Liquid W
- 1 x microcentrifuge tube of 300 μL of Liquid X
- 1 x microcentrifuge tube of 300 μL of Liquid Y
- p20 micropipette with tips
- container for used tips
- 15 x 3 grid
- Parafilm® or wax paper

Procedure

1. Set up the Parafilm or wax paper on top of the paper grid
2. The information in the table is a guide to tell you what liquid and how much of that liquid to use. You will **combine** two liquids to make each drop. Make sure you put the drops in the center of the boxes. Don't forget to change tips!

Grid	A	B	C	D	E
1	9 μL Y 1 μL X	8 μL Y 2 μL W	7 μL Y 3 μL W	6 μL Y 4 μL W	5 μL Y 5 μL X
2	18 μL Y 2 μL W	16 μL Y 4 μL X	12 μL Y 8 μL W	4 μL Y 16 μL X	2 μL Y 18 μL W
3	11 μL Y 19 μLW	13 μL Y 17 μL W	15 μL Y 15 μL X	17 μL Y 13 μL W	19 μL Y 11 μL W

- If you have completed this task correctly you should see a pattern in the grid. What is the pattern?

- Look carefully at the drops as you go right to left. Are they the same size?

- Look carefully at the drops as you go top to bottom. Are they the same size?

Grid	A	B	C	D	E
1					
2					
3					