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An Introduction to Microvolumetrics and Pipetting

The purpose of this laboratory exercise is to provide you with a hands-on experience using some of the important tools and techniques commonly used in molecular biology and introduce you to some of the volumetric measurements that are most often used in this field of science. The laboratory will provide you with an opportunity to practice some of the skills needed for fairly sophisticated labs such as a restriction digest and the building of a recombinant DNA molecule. The restriction digest lab requires you to pipet small volumes of DNA and restriction enzymes, enzymes which cut the DNA at specific locations. We then separate the DNA fragments by "running a gel." The recombinant DNA molecule, again, requires the use of the pipet to measure small volumes of two different circular pieces of DNA or plasmids, and mix each with its own restriction enzyme. Parts of each plasmid are then combined to make a "recombinant DNA molecule."

While the theoretical foundations upon which biotechnology and DNA sciences have been built extend back to the early 1900s, most of the laboratory techniques utilized are relatively recent. And though the techniques you will be learning over a series of labs have become routine in modern research laboratories, few high school and college students have an opportunity to do such sophisticated molecular biology. So if you have a desire to go into this or a related field, you are gaining some very good experience.

If you can recall your chemistry class, you may have performed some labs using beaker, flasks, and other large scale equipment. You may have also done some microscale chemistry using small plastic micropipets. In this lab you will be using even smaller volumes, microliters or one-thousandth of a milliliter. Volumes such as 10, 20 or 50 microliters (μl) are common measurements.

You might be wondering why molecular biologists use such small volumes and amounts of materials. The reason is related to the cost of these materials and the difficulty involved with obtaining them. For example, in an upcoming lab you will be given some specially engineered plasmids or circular DNA. If this DNA were sold "by the pound," it would cost around $360,000,000 per pound. That is even an unbelievable amount to me. So don't be surprised if we only give you a tiny amount of these DNA molecules and want you to take care of them in a certain way. The reason why these chemicals are so expensive is related to the difficulty in preparing them in pure form. Many of these chemicals are produced within living organisms, such as bacteria, and have to be purified and separated from all of the other thousands of substances in the cell. Molecular biology, however, really requires this level of purity and precision.

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Materials

Reagent	Equipment & Supplies
Solution I	Microtubes, labeled A,
Solution II	B and C
Solution III	P-20 micropipettor
$_dH_2O$ microtube	P-200 micropipettor
	Box of "Practice Tips"
	Foam rack
	Permanent Marker

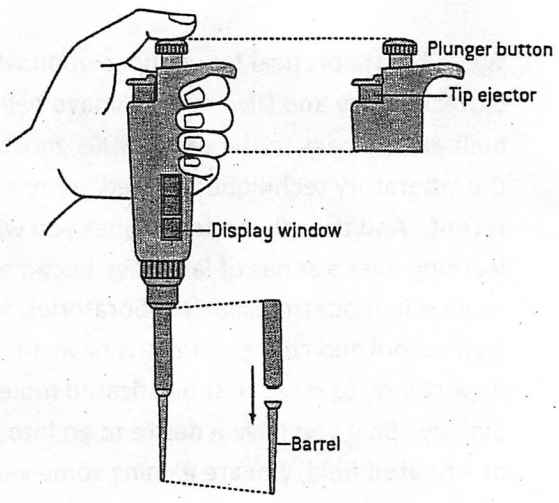
Methods

The Digital Micropipette

Molecular biology protocols require the use of adjustable micropipettes. Micropipettes are used to dispense volumes of liquids. While researchers will have several kinds of micropipettes at their lab bench, one lab partner will first use a P-20, while the other lab partner uses the P-200. Then lab partners will switch pipettors. The P-20, with the yellow tipped plunger, is engineered to dispense liquid volumes between 2 and 20 µl. Look at the top of top of the pipettor and you will see these two numbers, 2-20, indicating the range that can be dispensed. The P-200, the gold-tipped plunger, has the numbers 20-200 on the top and therefore measures between 20 and 200 µl. These high-quality, precision instruments are expensive, need to be cared for, and it is essential that you learn to use it properly.

Please read and follow these precautions:

- Regarding the P-20, do not set the adjustment below 2 µl or above 20 µl.
- The P-200 is not to be set below 20 µl or above 200 µl.
- Do not use the micropipette without a disposable tip on its end. Failure to use a pipette tip will contaminate the pipette barrel and result in a costly repair.
- Keep the micropipette vertical. Do not lay down the micropipette with fluid in the tip or hold it with the tip pointed upward. If the disposable tip is not firmly seated onto the barrel, fluid could leak back into the pipette. In any case, keep the micropipette vertical.
- Avoid letting the plunger "snap" back when withdrawing or ejecting fluid; it will eventually destroy the piston.



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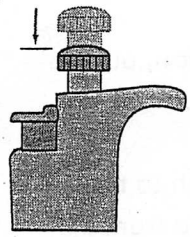
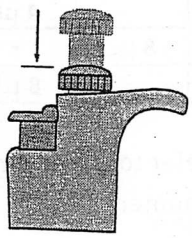
- When aspirating (drawing up) a solution, remove the microtube from its rack; hold it up at eye level, push the plunger to the first stop THEN lower the pipette tip below the level of the solution. Be sure to actually see the tip below the level of the liquid that you are sampling. It's important to actually see the solution enter the pipette tip. Do not push the plunger down once the tip has broken the liquid's surface. It will cause air to bubbling into the solution.



- ***Slowly*** release the plunger and allow the liquid to move into the pipette tip. Be certain that you're not aspirating air into the tip. Remove the tip from the solution and look to see if you have solution in the tip.

- When dispensing the liquid, hold the microtube that will be receiving the solution at eye level and place the pipette tip into the tube. Position the tip so that it touches the bottom of the tube. Slowly push down on the plunger to the ***first stop and then to the second stop.*** Be certain that you see the solution leaving the tip. Keeping your thumb depressed on the plunger, remove the tip from the tube into which you're dispensing the liquid.



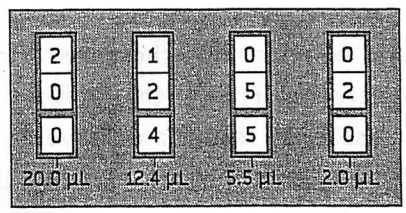
This will avoid reaspirating the liquid into the pipette tip.

- Locate the waste container at your lab station. It is probably a yogurt container. Remove the tip by ejecting it into the waste container; there is an eject button (white button) on the pipette. If you're dispensing the same reagent into separate tubes AND there is no danger of contaminating additional tubes with a reagent, you can use the same tip several times. Usually you can only use the same tip for several tubes if it is the very first reagent. After that I would recommend using different tips for different tubes.

- When dispensing a new reagent, always ***use a fresh tip*** to avoid contamination.

Pipetting Exercise 1

1. Find the display window on the handle of the micropipette and note its setting. In just a moment you will turn the knurled knob, clockwise to decease the volume or counterclockwise to increase the volume. Turning this knob changes the distance the plunger will travel. The figures below represent some P-20 pipette settings and the volumes of liquid dispensed.



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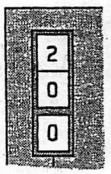
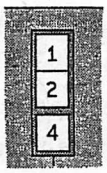
Remember the P-20 should never be dialed to a volume less than 2.0 µL and never greater than 20.0 µL.

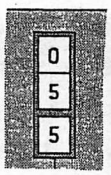
The figures below represent the P-200 pipettor, its settings and volumes dispensed.
Your lab partner should be currently holding and becoming prepared to use this pipettor.



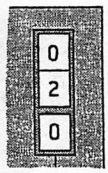
200.0 µL



124.0 µL



55.0 µL



20.0 µL

2. Place a disposable tip onto the end of the pipette barrel. Using your thumb and index finger in a twisting motion, check to see that the tip is firmly seated onto the barrel. Avoid touching the pointed end, as this may contaminate the tip and thus sample. **Remember that you must have a tip in place when using the pipette.**

3. Place your thumb on the button that activates the plunger. Push down on this button with your thumb and notice that it has a "stop" position. If you exert a little more pressure with your thumb, you can push the button of the plunger to a second stop. The second stop pushes a small volume of air into the tip to eject the solution.

   These two positions, these two "stops," are very important in that they ensure successful lab results and prevent mismanagement of the reagents, aka, being a hog and not leaving reagents for the other lab sections.

So remember:

a) To obtain or aspirate solution, push to the first stop, then place your tip into the solution.
b) To dispense a solution, push to the second stop, remove the tip from the solution and then release the plunger.

Pipetting Exercise 2

1. Each student in the lab group is to obtain three microtubes, A, B and C. If the letters are not clearly marked on the tubes, please see the instructor. Place the tubes into the foam container at your lab station.

   If you are using the P-20, continue with Step 2. If you have the P-200, go to Step 17, then return to step 2.

P20

2. The table below summarizes the contents for each tube, BUT FOLLOW THE DIRECTIONS that begin with step 3 to set up the samples.

Tube	dH_2O	Solution 1	Solution 2	Solution 3	Total volume
A	2 µL	4 µL	-	4 µL	10 µL
B	2 µL	-	8 µL	-	10 µL
C	2 µL	-	-	8 µL	10 µL

Solutions 1, 2 and 3 refer to any colored solution at your lab counter.

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3. Set the P-20 micropipette to 2 µL and, **holding each tube at eye level**, dispense dH_2O into tubes A, B and C. Yes, since it is the first reagent and it will go into each tube, you can use the same tip.

4. Eject the tip into the waste container and replace with a fresh tip.

5. Holding solution 1 at eye level, place 4 µL of solution 1 into tube A.

6. Eject the tip into waste container and replace with a fresh tip.

7. Holding solution 3 at eye level, use a fresh tip to dispense 4 µL of solution 3 into tube A.

8. Holding solution 2 at eye level, use a fresh tip to dispense 8 µL of solution 2 into tube B.

9. Holding solution 3 at eye level, use a fresh tip to dispense 8 µL of solution 3 into tube C.

10. Save all three tubes. Refer back to the table and realize that all three tubes should have equal volumes, 10 µL. Hold the 3 tubes at eye and observe if you have equal volumes. Now, microliters are very small and you may not be able to detect a difference of 1 or 2 µL but large discrepancies you could see.

    You will now use the micropipette to determine if you accurately measured a total volume of 10 µL.

11. Tubes A, B and C should each contain 10 µL solution.

12. Place each tube into the microcentrifuge such that the hinge of the tube is to the outside of the microcentrifuge. Be sure the tubes are balanced and pulse spin (10 seconds) the liquid to the bottom of the tubes.

13. Set your P-20 micropipette to 10 µL and place a fresh tip onto the barrel.

14. Carefully check the volume of each microtube. There should be 10 µL in each of these tubes. Look at the tube to see if any liquid remains. Look at the tip to see if there is an air space at the end of the tip.

15. This liquid can be dispensed into the sink (NOT THE WASTE CONTAINER).

16. Using your distilled water bottle, rinse tubes A, B and C. Dry them inside and out with paper towel as you will use them with the P-200 and you do not want interference from the residual water OR you are done and the tubes need to be dried for the next class. Thank you.
    Obtain the P-200 and continue below.

P200

The table below summarizes the contents for each tube, BUT FOLLOW THE DIRECTIONS that begin with step 17 to set up the samples.

Name _____

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Tube	dH_2O	Solution 1	Solution 2	Solution 3	Total volume
A	20 µL	40 µL	-	40 µL	100 µL
B	20 µL	-	80 µL	-	100 µL
C	20 µL	-	-	80 µL	100 µL

Solutions 1, 2 and 3 refer to any colored solution at your lab counter.

17. Set the P-200 micropipette to 20 µL and, **holding each tube at eye level**, dispense dH_2O into tubes A, B and C. Yes, since it is the first reagent and it will go into each tube, you can use the same tip.

18. Eject the tip into the waste container and replace with a fresh tip.

19. Holding solution 1 at eye level, place 40 µL of solution 1 into tube A.

20. Eject the tip into waste container and replace with a fresh tip.

21. Holding solution 3 at eye level, use a fresh tip to dispense 40 µL of solution 3 into tube A.

22. Holding solution 2 at eye level, use a fresh tip to dispense 80 µL of solution 2 into tube B.

23. Holding solution 3 at eye level, use a fresh tip to dispense 80 µL of solution 3 into tube C.

24. Save all three tubes. Refer back to the table and realize that all three tubes should have equal volumes, 100 µL. Hold the 3 tubes at eye and observe if you have equal volumes. Now, microliters are very small and you may not be able to detect a difference of 1 or 2 µL but large discrepancies you could see.

You will now use the micropipette to determine if you accurately measured a total volume of 100 µL.

25. Tubes A, B and C should each contain 100 µL solution.

26. Place each tube into the microcentrifuge such that the hinge of the tube is to the outside of the microcentrifuge. Be sure the tubes are balanced and pulse spin (10 seconds) the liquid to the bottom of the tubes.

27. Set your P-200 micropipette to 100 µL and place a fresh tip onto the barrel.

28. Carefully check the volume of each microtube. There should be 100 µL in each of these tubes. Look at the tube to see if any liquid remains. Look at the tip to see if there is an air space at the end of the tip.

29. This liquid can be dispensed into the sink (NOT THE WASTE CONTAINER).

30. Using your distilled water bottle, rinse tubes A, B and C. Dry them inside and out with paper towel as you will use them with the P-20 and you do not want interference from the residual water OR you are done and the tubes need to be dried for the next class. Thank you.

31. Now return to Step 2 to learn to use the P-20 or if you have already practiced with the P-20 you are done.