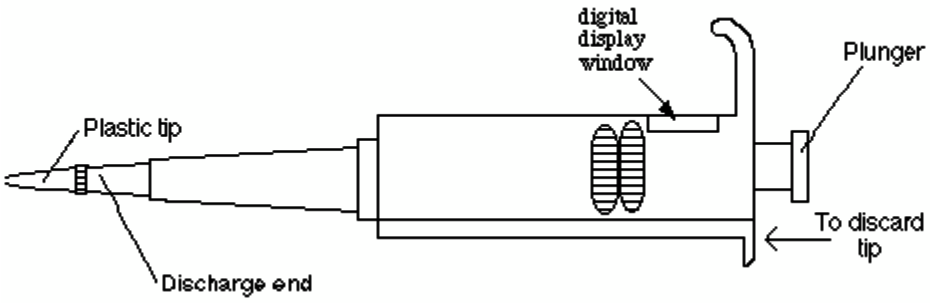
How to Use a Micropipette

Sample Delivery with Variable Automatic Micropipettes:

The micropipette is used to transfer small amounts (< 1 ml) of liquids. The scales on micropipettes are in microliters (1000 µl = 1 ml). The brand of micropipettes we will be using is made by Rainin and called a "Pipetman". These are very expensive, delicate instruments costing $250-300 apiece. We have four sizes identified by the number on the round button on the plunger. The value is the maximum volume in microliters that can be transferred with that size pipette. They are used in conjunction with disposable sterile plastic tips. The following is an illustration of a micropipette:



The Pipetman

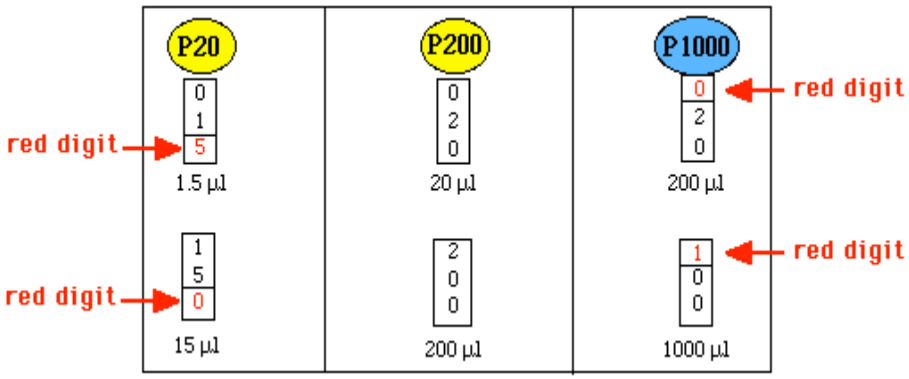
Using a Micropipette:

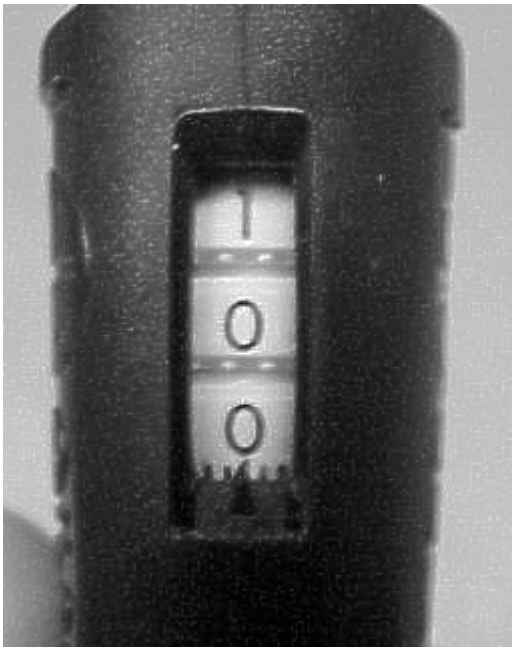
1. **NEVER exceed the upper or lower limits of these pipettes.**

The limits are:
P10: 1.0 - 10.0 µl
P20: 2.0 - 20.0 µl
P200: 20 - 200 µl
P1000: 200 - 1000 µl

Look at the front face of the pipet and you will see a window with three digits inside. The diagram below shows the MAXIMUM value that can or should be dialed in on each size pipet. To exceed these values will put the pipet out of calibration. Beside each "window" below is the numbers place it represents. Please take the time to learn how to read them so as to avoid damaging them by dialing values out of their range.

The examples below show how to read the volume on the micropipette:





http://abacus.bates.edu/~ganderso/biology/resources/pipet.html

P1000		P200 or P100		P20	
1	1000's	**2, 1**	100's	**2**	10's
0	100's	**0**	10's	**0**	1's
0	10's	**0**	1's	**0**	1/10ths

2. What size of micropipet is right for the job?

RULE OF THUMB: Always select the SMALLEST size pipet that will handle the volume you wish to move to achieve the greatest accuracy. Accuracy *decreases* as you use unnecessarily large pipets for small volumes.

3. Set the desired volume by turning the centrally located rings clockwise to increase volume or counterclockwise to decrease volume.

P10: Maximum volume 10 µl. Accurate between 1 µl and 10 µl. Numbers on the micropipette (typically black-black-red) are read as XX.X µl. The change in color indicates the position of the decimal point.

P20: Maximum volume 20 µl. Accurate between 2 µl and 20 µl. Numbers on the micropipette (typically black-black-red) are read as XX.X µl. The change in color indicates the position of the decimal point.

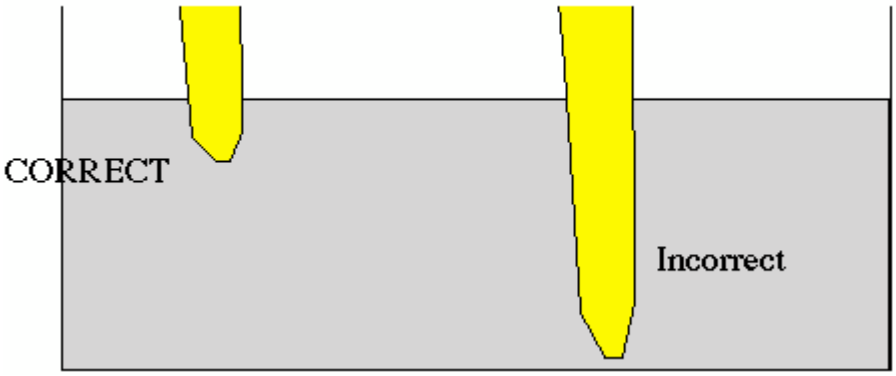
P200: Maximum volume 200 µl. Accurate between 20 µl and 200 µl. Numbers on the micropipette (one color) are read as XXX µl.

P1000: Maximum volume 1000 µl (= 1 ml). Accurate between 200 µl and 1000 µl. Numbers on the micropipette (typically red-black-black) are read X.XX ml. Note that this micropipette reads milliliters while the other two read microliters.

4. Load a sterile tip. Use **blue** tips for P1000 pipettes and **clear** tips for P200 and all smaller sizes. Use filter tips when performing PCR or working with RNA. Close the tip box to maintain sterility. NOTE: Do not allow the pipet tip to touch any object (including your gloves, clothes, hair, skin, bench).

5. Load the sample.

- The plunger will stop at two different positions when it is depressed. Push the plunger down slowly to the point of *first resistance*: this is the load volume. Because this first stopping point is dependent on the volume that is being transferred, the distance you have to push the plunger to reach the point of initial resistance will change depending on the volume being pipetted.
- While holding the plunger at the load volume set point, put the tip into the solution so that it is immersed just enough to cover the end (3-4 mm), not as deep as possible.
- **Slowly** release the plunger to draw up the liquid making sure to keep the tip immersed. NOTE: If the solution you are pipetting is viscous, allow the pipet tip to fill to final volume before removing it from solution to avoid the presence of bubbles in the plastic tip, which will result in an inaccurate volume.
- Visually inspect the load to make sure it is correct - there should be no air space in the distal end tip.



6. **Deliver the sample.** The second stopping point can be found when the plunger is depressed beyond the initial resistance until it is in contact with the body of the pipette. This second stopping point is used for the complete discharging of solutions from the plastic tip. You should not reach this second stop when drawing liquid into the pipette, only when expelling the last drop.

   To deliver the volume,
   - Place the tip into the receiving vessel.
   - Depress the plunger to the point of initial resistance
   - Wait 1 second
   - Continue to press the plunger *all the way to the bottom* - this expels all the liquid.
   - THEN, WITHOUT RELEASING THE PLUNGER, withdraw the tip.

7. **Discharge the tip**. While holding the tip over an appropriate waste receptacle, press the discharge slider on the back of the grip.

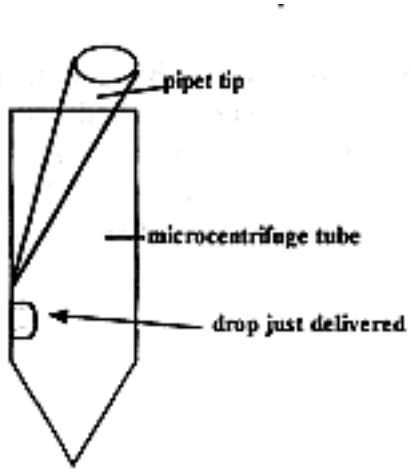
NOTES:

- Never point a pipette up. This may cause liquid to run down into the pipette destroying it.
- When withdrawing liquids with the pipette, always release the plunger slowly. This prevents liquid from rushing into the end of the pipette and clogging it up. This is especially important with large volume pipettes (200-1000 µl).
- Be sure you use the proper size tip for each pipette.
- Always use a new tip for each different liquid.
- Use the correct pipette for the volume that is to be dispensed. Never use the 200-1000 µl pipette to dispense volumes below 200 µl. going below or above the range of the micropipette may damage the instrument.

Small Volumes Technique

With small volumes, especially the 1-10 µl range, you must keep track of the droplets you pipet. Carefully expel the liquid droplet *on the side wall* of the tube so that you can see it, drawing the tip away/out carefully BEFORE releasing the plunger.

If adding to a larger volume, flush the tip with the solvent liquid after expelling the droplet to make sure you get all the delivery liquid. With small volumes you'll usually need to centrifuge and then vortex the tube to get a good mixing of the reagents.



A Simple Check for Proper Calibration

Check the calibration of your micropipet by using the fact that 1 ml of deionized (or distilled) water has a mass of 1 g. Pipet a range of volumes spanning the pipette's useable range and weigh them on a top loading balance having at least 3 decimal place accuracy. Pipets having greater than 5 % error should be recalibrated.

Adapted by Kelli Henry April 2011 from:
http://www.bio.davidson.edu/Courses/Bio111/Bio111LabMan/Preface%20D.html