

Displacement pipettes are valuable for manipulating tiny volumes of liquid. Their calibrated thumb-driven piston sucks up the desired volume of fluid into the pipet tip. The units of volume are in microliters ( $\mu\text{L}$ ), also termed lambdas ( $\lambda$ ). Since there are a million  $\mu\text{L}$ s in a liter, 100  $\mu\text{L}$  is 0.1 mL. (Learn how set, read the pipet and interconvert these volumes!) The plastic tips are usually discarded after a single use. The pipettes (and sometimes their tips) are color coded according to their capacity: white for 10  $\mu\text{L}$ , yellow for up to 200  $\mu\text{L}$ , blue for up to 1000  $\mu\text{L}$ .

The advantage of single-use disposable tips in genetic engineering is that there is no possibility of contamination with endonucleases which could destroy DNA samples. Also, the tips are much less expensive than single-use pipettes. However, the instruments are very expensive (about \$200 each), and must be handled and cared for properly. NEVER allow fluid up into the body of the pipetter. **Illustrate a displacement pipette and include all of the following features:**

body	lock ring	tip
thumb plunger	volume of aliquot (in window, <i>practice reading</i> )	tip ejector button
calibrated stop	thumb knob (to adjust volume)	ejection ring
blow out stop	shaft	capacity range indication

**USE OF THE DISPLACEMENT PIPETTER**

1. **Select the pipetter** whose range is appropriate for the volume you wish to measure out.
2. **Set the volume** as follows:
  - a. Loosen the lock ring at the base of the thumb plunger so that it turns easily.
  - b. Rotate the thumb knob until the desired number of  $\mu\text{L}$  appears in the window. [Do not go above the specified range of the pipetter or you may damage the instrument.]
  - c. Snug the lock ring. (Do not over-tighten.)
3. **Attach a fresh tip to the pipetter:**
  - a: Open the box of sterile tips.
  - b: Press the pipetter firmly down onto one of the tips, tap once or twice to firm the fitting.
  - c: Pull out the attached tip, immediately close the box to maintain sterility.
4. **Draw up the desired volume of fluid:**
  - a: *Before* you insert the tip into the sample solution, gently depress the plunger to the calibrated (first) stop and hold in place. **DO NOT GO PAST THE CALIBRATED STOP.**
  - b: Insert the pipetter into the sample solution so that the tip is just below the surface.
  - c: Allow the plunger to slowly and smoothly return to its original position. Note that fluid is drawn up into the tip (*no bubbles*). **NEVER LET FLUID ENTER PIPETTER BODY.**
  - d: Withdraw the pipetter from the sample vessel, flame and re-cap vessel to maintain sterility.
5. **Deliver the fluid into the receiving vessel:**
  - a: Insert the pipet into the receiving vessel so that the tip a: is 3 mm below the surface of the receiving fluid, or b: touches the bottom of the empty vessel.
  - b: Depress the plunger slowly and smoothly until the first stop is felt.
  - c: Press more firmly on the thumb plunger to blow out the remainder of fluid. You should see a bubble expelled. **KEEP THE PLUNGER HELD DOWN.**
  - d: Withdraw the pipetter from the vessel, dragging it along the side to leave any adhered fluid.
  - e: Allow the plunger to return to its normal position.
6. **Discard the used tip** (unless you are to deliver additional aliquots of the same fluid):
  - a: Hold the pipetter over a discard vessel.
  - b: Depress the tip eject button with the thumb until the tip is ejected.

Cut here-----

**PRACTICE USING THE PIPETTER'S FEATURES**

**Per desk** for practice by two students:

- 2 20-200 uL pipettors
  - 2+ non-sterile tips in a tray
- test tube rack with:
- 2 13 x 100 mm test tubes, half full of distilled water
  - 4 13 x 100 mm test tubes, empty

- 1) Loosen the lock ring, select the volume by rotating the thumb knob. **Do NOT exceed the range of the pipet as indicated on the handle top.** Relock (gently).
- 2) Gently press the plunger to the first *calibrated* stop, press more firmly to feel the *blow out* stop.
- 3) Pick up a tip from a non-sterile practice box by tapping the pipetter firmly once or twice into the tip, then discharge the tip to a different hole in the box by pressing the tip ejector button.
- 4) Set the pipetter to 100  $\mu\text{L}$ , and draw up this volume of  $\text{dH}_2\text{O}$ , deliver into a clean test tube. Change setting to 10  $\mu\text{L}$ , deliver to a second clean test tube. Note how small a volume this is.