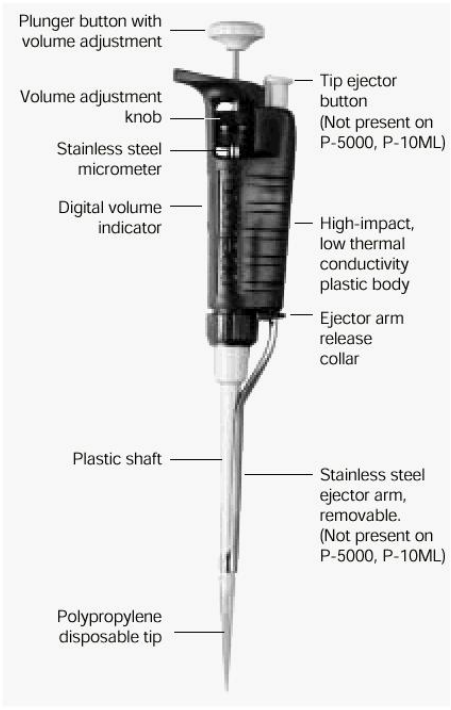
Pipetting Excercise

Proficient pipetting is probably the most crucial part of this lab, for three reasons:

- Accurate pipetting is crucial to the success of molecular projects. Mistakes during pipetting may cause your experiments (e.g. PCR) to fail or to be irreproducible, and thus cause long delays and considerable expense
- Pipettes are delicate pieces of equipment with high accuracy, which can easily be knocked off their calibration. Furthermore, they are expensive – the five pipettes in front of you represent about $ 1,500.
- Pipettes are the main source of contamination in PCRs, and thus can cause a lot of problems.

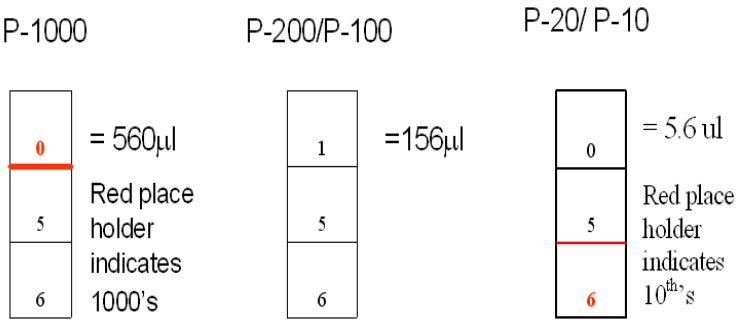
You may have some experience with pipettes, but a refresher on pipetting is probably quite useful.



Rainin Pipetman

Pipette	Top	Volumes (µl)	Tips
P-1000	Blue	200 – 1000	Blue Box
P-200	Green	20 – 200	Green Box
P-100	Green	10 – 100	Green Box
P-20	Red	2 – 20	Red Box
P-10	Red	0.5 – 10	Red Box

Volume Adjustment



Red/black indicates the decimal point.

Never

- **Never rotate volume adjustor beyond the upper limit**
  - Changes calibration
- **Never use without a tip.**
  - Liquid will get into piston – damage and contamination.
- **Never lay down or turn upside down a pipette with filled tip.**
  - Liquid could run back into the piston, and damage/contaminate it.
  - Use pipette holders provided
- **Never let plunger snap back after withdrawing or ejecting fluid**
  - Damages piston

Use

- Use the correct pipette. Never adjust the volume beyond the maximum setting, but also do not use pipettes that are too large. Accuracy and precision drop rapidly towards the lower limit of settings.
- Adjust volume by turning the volume adjustment knob or the plunger. Be sure to locate the decimal point correctly when reading the volume setting (see above). Always dial DOWN to the desired volume to avoid mechanical backlash affecting accuracy.
- Firmly seat the proper-sized tip on end of the pipette. If the fit is lose, you will draw up less volume than intended and the liquid will drip from the tip during use.
- When withdrawing or expelling fluid, always hold the tube firmly between thumb and forefinger. Hold close to eye level to observe change in fluid level in tip. Do not pipet with the tube in rack or when someone else is holding the tube.
- The pipettes have a two-stop position plunger. Depressing to the first stop measures the desired volume. Depressing to the second stop introduces an additional volume of air to blow out any remaining fluid from the tip.
- To withdraw fluid from tube.
  - Hold the pipette almost vertically ($< 20^{o}$ from vertical)
  - Depress plunger to first stop and hold. Dip tip 2-4 mm into the fluid. Do not push down to the bottom of the vial, otherwise the tip is blocked and you draw less liquid than intended.
  - **Gently** release thumb. If you release quickly, you will create aerosols (small droplets) which will contaminate the pipette.
  - Wait for a second or so, to confirm that all the liquid has been taken up.
  - Slide pipette tip out along wall of tube to dislodge remaining droplets adhering to the outside of the tip.
  - Check that there is no air space at the very end of the tip.
  - Learn the approximate levels that particular volumes fill the tip – this will allow you to check your pipetting visually.
- To expel sample into tube
  - Touch tip to wall of tube
  - Slowly depress plunger to first, and then to the second stop to expel fluid.
  - While keeping the plunger at the second stop, slide the tip out of the fluid, along the tube wall and out of the tube.
- Eject the tip into trash, by pressing the tip ejector button.

Prevention of cross contamination

- Use a fresh tip each time
- Do not touch the tube with the pipette, only with the tip
- If you suspect pipette contamination, wipe with ethanol on the outside.
- Draw up liquid slowly to prevent the formation of aerosols
- For specific applications, use pipette tips with filters

Pipetting exercise

Be careful to use the correct pipette. Use distilled water. You don't have to change tips for these exercises.

Large volume:

1. Add 179 μl to a tube
2. Add 465 μl to the same tube
3. Remove 602 μl from the tube
4. Remove 42 μl from the tube

Small volume:

1. Add 8.3 μl to a tube
2. Add 13.5 μl to the same tube
3. Remove 15.6 μl from the tube
4. Remove 6.2 μl from the tube

Very small volume (as used for PCR):

1. Add 2.3 μl
2. Add 1.8 μl
3. Remove 3.2 μl
4. Remove 0.9

To make the last experiment more interesting, repeat using glycerol (has similar viscosity to *Taq*I polymerase).

How accurate were you?

- Is some liquid left in the tube? Measure it by drawing it up, and adjusting the pipette volume until all air below the liquid is expelled from the tip. Remember to hold the pipette vertically while doing so.
- Did you draw air up with the last removal of liquid? Measure the volume of step 4 by adjusting the volume downward. Remember to hold the pipette vertically while doing so.

The pipettes have an accuracy and precision of about 1% - any more than that is human pipetting error! If you got it very wrong, try again.