Pipetting Technique Introduction

Please read this before doing the pipetting activity to learn about proper use of a pipette.

Accurate pipetting is essential to successful implementation of molecular genetics techniques. The reagents that we use are extremely expensive, so we use very small volumes. These techniques are extremely sensitive to having exactly the right amount of each enzyme and chemical. Many techniques fail in the hands of inexperienced individuals because of inaccurate pipetting.

Look on the disk at the top of each pipette to see the maximum volume that the pipette will hold. If it says 1000 µL, we call this a 1000 µL pipet. We also have 200 µL, 100 µL, 20 µL and 10 µL pipets. 10 µL is a tiny tiny amount of liquid.

All of the pipettes should have 3 digits that change as you rotate the disk on the top of the pipet. These digits tell you the volume that you will be picking up. But the digits have different meanings on the different-sized pipets, so it can be a bit confusing. When the 1000 µL pipette reads 100, this means you will pick up 1000 µL , and when they read 020, you will pick up 200 µL (Fig. 1). The 1000 µL pipette does not go below 200 µL. However, on the 200 µL pipet, 100 means that you will pick up 100 µL (Fig. 2). On the 20 µL pipets, 100 means 10.0 µL (Fig 3). Confusing, isn't it? Always check the top of the pipette to see the maximum value. Use this information to help you interpret the digits on the side. The colored digits can also be a clue.

a. 

b. 

c. 

Figure 1. 1000 µL pipet: (a) top of pipette showing maximum volume (1000 µL), (b) volume indicator showing that the pipette is set to pick up 1000 µL, (c) set to pick up 300 µL.

a. 

b. 

Figure 2. 200 µL pipet: (a) top of pipette showing maximum volume (200 µL), (b) volume indicator showing that the pipette is set to pick up 50 µL.

a. 

b. 

Figure 3. 20 µL pipet: (a) top of pipette showing maximum volume (20 µL), (b) volume indicator showing that the pipette is set to pick up 2.1 µL.

Figure 4. How to hold a pipette: The thumb is on the plunger button used to expel air and suck up liquid. pipette is straight up and down (not horizontal). Tipping the pipette can result in inaccurate measurements , and can allow liquid to contaminate the pipette.

RULES:

- Hold the pipette as shown in Figure 4.
- Never rotate the volume knob beyond the upper or lower range.
- Never use pipette without a disposable tip in place.
- Don’t touch the tip with your fingers (at least not the part that goes into the liquid)

    - If the tip touches your hand or the table, etc, it is no longer sterile. Get a new one
- Always put a fresh tip on the pipette before using and before changing solutions or changing samples to avoid contamination.
- Never lay a pipette down when there is fluid in the tip. The fluid can run back into the piston and damage the pipet.
- Move the plunger in a steady, relatively slow way. Never let the plunger snap back after withdrawing or ejecting fluid. It can damage the piston and allow liquid to get sucked into the pipette.
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- Use the smallest volume pipette that will hold the desired volume.
- Never place used tips back into a box of tips. Throw used tips away.

The plunger on the pipette has two stops – one gentle stop (first stop), and one further down that you can't go past (second stop). The first stop is for picking up liquid. The second is for ejecting all of the liquid from the tip. The second stop pushes a bit of air out after the liquid to make sure all of it is removed from the pipet.

When putting the tip onto the pipette, leave the tip in the box, so you don't need to touch the tip and potentially contaminate it.

When picking up a sample or solution, depress the plunger to the first stop, place the tip in the tube, and slowly release the plunger to draw up the liquid. Always watch the liquid as you pull it into the tip to be sure you are actually picking up the liquid. If you pick up air bubbles, expel the liquid and start over. Check to see that the volume looks correct.

When ejecting a sample, touch the tip to the side of the tube or place the tip into liquid already in the tube and push the plunger down slowly to the second stop. Lift the tip out of the tube and then slowly release the plunger. By touching the side of the tube or liquid, you ensure that all of your liquid goes into your tube, rather than forming a droplet on the outside of the tip.

As soon as you are done ejecting the liquid, eject the tip into a garbage can or disposable waste container so you don't accidentally reuse it.

When ejecting the tip, use the ejector button near the plunger and shoot it into the waste container rather than touching it with your hands and potentially getting chemicals on them.