USING A DIGITAL MICROPIPETTER TO MEASURE VERY SMALL VOLUMES

The list of instruments used in molecular biology grows every day and it is impossible to make you familiar with all of them in this course. However, some of these instruments are essential for even the simplest of experiments in molecular biology. Your TA is going to demonstrate some of the more common ones that you will be using later. Thus far you have been using pipets to measure and dispense a specific volume of a liquid. Pipets are good for quantities in the range of 0.1 ml and up. However, in molecular biology, we may need much smaller volumes of a liquid, say in the order of a thousandth of a milliliter. Digital micropipettes are specifically designed for such small and accurate amounts and will be discussed in more detail below.

Digital Micropipettes

Before you start any experiment in the molecular level, it is necessary to get familiar with a useful tool without which molecular biology could not have gone far. In our lab, we use the “Pipetman continuously adjustable digital microliter pipets” or Pipetmans, for short, to measure and transfer volumes of liquid in the range of 0.1 to 1000 µl. Each microliter (µl) is a thousandth of a milliliter (ml). [Pipetman is pluralized into Pipetmans or Pipetmen].

![Diagram of a digital micropipette]
Let us first familiarize ourselves with different parts of a Pipetman. Each Pipetman is composed of a body that houses a Digital Volume Indicator (DVI). The numeric value of DVI changes with turning the Volume Adjustment Knob. This knob should not be turned below or over the volume capacity of the Pipetman. Attached to the body are the Plunger and Tip Ejector Buttons on one side and Plastic Shaft and stainless steel Ejector Arm on the other side.

Each group of students will receive a set of 3 Pipetmans, namely, P-20, P-200 and P-1000.

- P-20 is adjustable from 0.1 to 20 µl in 0.1 µl increments. The fractional volumes (0.1-0.9) are shown in red as the lowermost tier of numbers while the whole numbers are shown in black in the DVI.

- P-200 is adjustable from 1 to 200 µl in 1 µl increments and is recommended for volumes of 20-200 µl.

- P-1000 is used for volumes from 10 µl to 1000 µl (1 ml) in 2 µl increments. For this Pipetman, the uppermost tier of numbers is in red and can be either 0 or 1. The figures should be multiplied by 10 to obtain the actual volume dispensed. For example, if the DVI shows 041, the volume of the liquid withdrawn will be 410 µl and if DVI shows 100, you are transferring exactly 1.000 ml.

To use a Pipetman, follows these steps:

1. Set the desired volume by holding the Pipetman body in one hand and turning the volume adjustment knob until the correct volume shows on the digital indicator.

2. Attach a new disposable tip to the shaft of the pipet. Press on firmly with a slight twisting motion to ensure an airtight seal.

3. Depress the plunger to the first positive stop. This part of the stroke is the calibrated volume displayed on the micrometer.

4. Holding the Pipetman vertically, immerse the disposable tip into the sample liquid to a depth of 1-2 mm.

5. Allow the push-button to return slowly to the up position. Never permit it to snap up.

6. Wait 1 to 2 seconds to ensure that the full volume of the sample is drawn into the tip. Then withdraw the tip from the sample liquid.

7. To dispense sample, place the tip against the sidewall of the receiving vessel and depress the plunger slowly to the first stop. Wait about 1-2 seconds and then depress the plunger to the second stop (bottom stroke), expelling any residual liquid in the tip.

8. With the plunger fully depressed, withdraw the Pipetman from the vessel carefully with the tip sliding along the wall of the vessel.
9. Let the plunger return to the up position.

10. Discard the tip by depressing the tip ejector button smartly. A fresh tip should be fitted for each sample to prevent carryover between samples.

   As a test, use the 3 Pipetmans as you fill in the blanks below. What values would you see in the DVI slot if:

   1. You wanted to transfer 2.0 µl with a P20? Ans:_______

   2. You wanted to transfer 20 µl with a P200? Ans:_______

   3. You wanted to transfer 200 µl with a P1000? Ans:_______

If you answered 020 in all cases, you are correct. Do you see why?

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