Pipetting skills

Focus question	How can we develop and practice micropipetting skills? Why is micropipetting important?
Vocabulary	Micropippette

Micropipettes are used to transfer small measured volumes of liquid in the lab. Micropipettes can be fixed rate or adjustable, and can vary in the increment measured as well—full microliters (µl) or fractions of a µl. On an adjustable micropipette, the volume can be changed by twisting the handle or top of the plunger, depending on the brand/type you have. The readout dial will show the volume the micropipette is adjusted to transfer. The micropipette is limited to the volume range that is pre-set on the instrument. The most common ranges include: 0.5–10µl, 2–20µl, 10–100µl and 100–1,000µl. A pipette tip must be used with the micropipettor. The tip is where the liquid is stored as it is being transferred. A new pipette tip should be used for each different liquid. The tip is ejected by using the ejector mechanism on the micropipette; some tips must be manually removed, depending on the micropipette.

Before pipetting, practice!

Practice feeling the “soft ” stop several times; taking the sample from the center of the container holding the liquid to be transferred; transferring the liquid while the pipette is upright; releasing the liquid into the appropriate microtube or cell well by touching the tip to the side and depressing the plunger, first to the “soft ” stop, next to the “hard” stop; changing tips for each liquid.

When ready to draw up a liquid, obtain a clean tip. Press the pipette into the open end of the tip and tap gently to “seat” the tip on the pipette. Place the tip in the liquid, just below the level of the liquid, in the center of the sample, but not to the base of the container. Depress the plunger on the pipette to the “soft” or first stop. The position of this stop will vary depending on the volume set to transfer (since the point of initial resistance is determined by the desired volume of solution being transferred). Release the plunger to draw up the liquid. Once the desired volume is drawn into the tip, remove the pipette, and take the liquid to its destination. Release the liquid along the side of the tube then depress the plunger, first to the “soft” stop, then one second later to the second or “hard” stop to remove all of the contents. Remove the tip from the liquid before the plunger is released. Discard the tip by ejecting into a proper waste receptacle. If you need additional direction, watch this pipetting video: **youtu.be/bex0itUMxmI**

Materials

- Micropipettes of various sizes
- 96-well plate 1 per group
- Colored water in 10mL amounts (color needed dependent upon which protocol)
- Micropipetting protocol colors:
  - red and blue water
  - yellow and green water
  - green and blue water
  - blue, red and green water
  - red and yellow water
  - yellow and green water
  - blue and red water

Procedure

1. Follow your assigned protocol to practice pipetting.
2. Mass your empty 96-cell well plate on a scale that measures to 0.001 g. What is the mass of your cell well plate?

3. Use the micropipette to measure the amounts for your protocol. Add up the total of the amounts you have added to the cell well plate. Measure periodically (after 3-5 additions) to see how accurate you are. Be sure to subtract the amount from step 2.
4. If the mass is equal to the pipetted amounts you have added (1000µl = 1g), then you are being accurate in your measurements. Take turns with others in your group, adding the amounts called for, and continue checking your accuracy as you go along.
5. What is your pattern?

Assessment

1. Check your accuracy by using a mass scale that measures to the thousandths.
   a. Add up the volume of the liquid added.

   b. Convert volume to mass. (1000µl = 1mL; 1mL = 1 g)

   c. Use the mass of your empty well plate. (from number 2 above)

   d. Add the mass of the liquid added to the well plate mass to calculate the expected mass of the completed design

2. Check your percent error by doing the following:
   a. What is your experimental mass? Mass your cell well with the completed design.

   b. Subtract your experimental mass from the expected mass.

   c. Take the absolute value of the answer.

   d. Divide the difference by the expected mass.

   e. Multiply by 100 to get the percent error.

   Example:
   Expected mass = 4789µL or 4.789g
   Experimental mass = 5 g
   4.789 – 5 = –0.211
   0.211/4.789 = 0.044 x 100 = 4.4%

   Because the amounts are so small, even a low percent error can result in major difficulties if completing a protocol in a lab. You want to be as close to 1% error as possible.

Rubric for self-assessment

Skill	Yes	No	Unsure
Our group pipetted the correct amounts into the cell well plate and we calculated our percent error.			
Our pattern helped me to ask questions and define problems related to biotechnology.			
We were able to connect our pattern to a biotechnology-related topic.			