EDVOTEK & PLTW Experiment #445

Pipetting By Numbers: STEAM Pipetting Practice

Experiment Objective:

In this experiment, students will master micropipetting - an essential biotechnology technique. They will then use this skill to create dot art on a Pipetting By Numbers canvas.

See page 3 for storage instructions.

445.200430

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Experiment Components and Requirements

3

Components	Check (✓)
· Red Dye	❏
· Blue Dye	❏
· Purple Dye	❏
· Yellow Dye	❏
· Pipetting by Numbers Canvas	❏
· Microcentrifuge tubes	❏

Experiment #445 is designed for 10 groups of students.

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

Requirements

· Automatic micropipettes* with tips
· Small containers for discarding used tips
· Scissors
· Tape

* *The provided pipetting templates require students to pipette between 5-60 µL. Several pipette combinations can accommodate these different volumes. The Pre-Lab Preparations will also require a 100-1000 µL micropipette.*

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Background Information

Biotechnology is used to produce food, solve crimes, treat diseases, and protect the environment. To accomplish these goals, scientists use biological tools like enzymes that can quickly break down a compound, fluorescent proteins that can highlight specific tissues, and bacteria that can produce specialized chemicals. Scientists also rely on more traditional mechanical tools such as microscopes and thermal cyclers. Perhaps some of the most important biotech machines are pipettes (Figure 1). These devices allow scientists to accurately and safely work with experimental solutions. Consequently, they are used daily in almost all biotech labs.
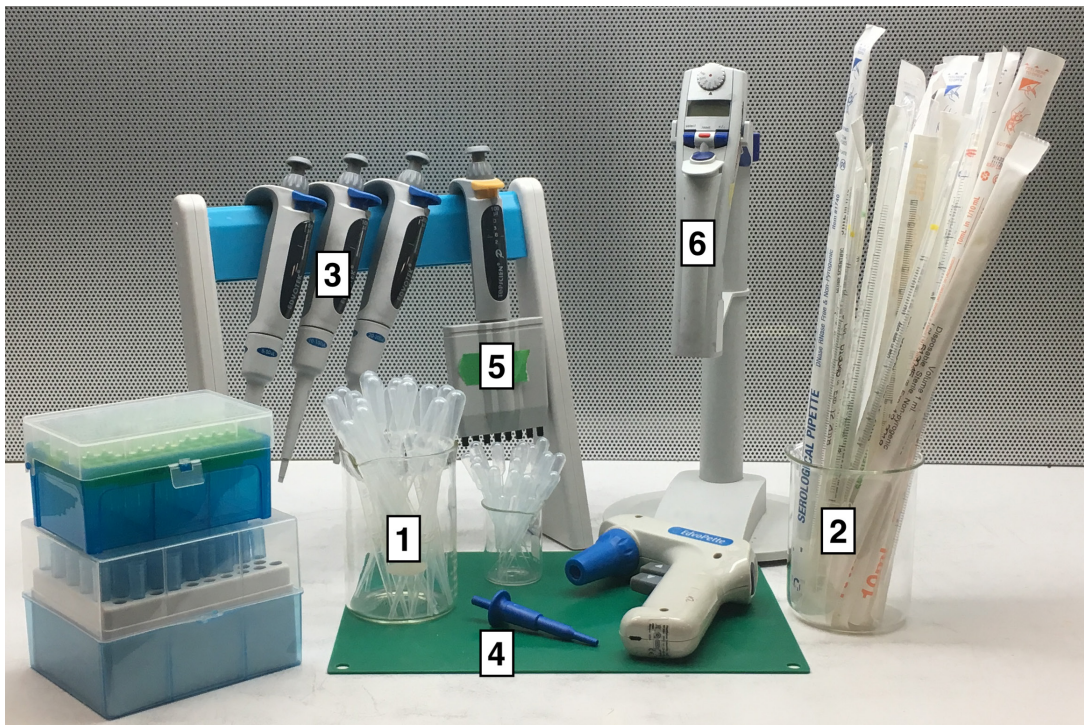

Figure 1: Common pipettes used in the lab. (1) Pasture pipettes, (2) Graduated pipettes, (3) Adjustable Micropipettes, (4) Fixed Micropipettes, (5) Multichannel Pipette, (6) Robotic Pipette.

WHAT IS A PIPETTE?

Pipettes are a large group of laboratory tools that scientists use to measure and manipulate liquids. The simplest pipettes closely resemble eye droppers with squeezable bulbs that can suction liquid up a narrow cylinder. These are known as *Pasteur pipettes* after their inventor Louis Pasteur. While Pasteur's primary goal was to protect his liquid samples from contamination during transfer, Pasteur pipettes can also be used to make approximate measurements.

DO NOT MOUTH PIPETTE!

Well into the 1960s, scientists would transfer solutions by treating pipettes like straws and sucking the liquid up to a specified volume. As you can imagine, the practice (called mouth pipetting) was somewhat hazardous even with precautionary filters and safety bulbs! In 1893, a doctor accidentally sucked Typhoid bacteria into his mouth. Much later, Manhattan Project scientist Lawrence Bartell accidentally ingested plutonium using this method. Luckily both lived to tell the tale!

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Graduated pipettes are similar to Pasteur pipettes but designed for higher volumes. Consequently, graduated pipettes tend to be used when preparing larger amounts of general use buffers, mass producing products, performing analytical chemistry experiments, and working with cells. In order to draw in large volumes of liquids, most graduated pipettes are attached to either a very large silicone bulb or an electronic pump.

For smaller volume reactions - such as those required for most molecular and microbial biology experiments - scientists use *micropipettes*. These were originally miniature versions of a graduated pipette attached to a bulb or even a suction piece (see box "**Do not mouth pipettes**"). However, today, most labs are equipped with *piston displacement micropipettes*. Inside these pipettes are a solid disk and tightly fitted tube (Figure 2a). When the user depresses a plunger the disk moves down which pushes air out of the tube and the connected tip. Next, the user immerses the tip into a solution and releases the plunger which creates a vacuum. This vacuum causes the liquid in the sample to flow up into the tip. The volume of this liquid (sometimes called the load volume) is equivalent to the volume of air displaced by the downward moving piston. Therefore, load volumes can be set to a specific value by calibrating the piston's height. Early displacement pipettes had pistons that were *fixed* at a single height and so could measure one specific volume. However, later displacement pipettes were redesigned so that the user could repeatedly *adjust* the piston height in order to measure out a range of volumes (Figure 3).
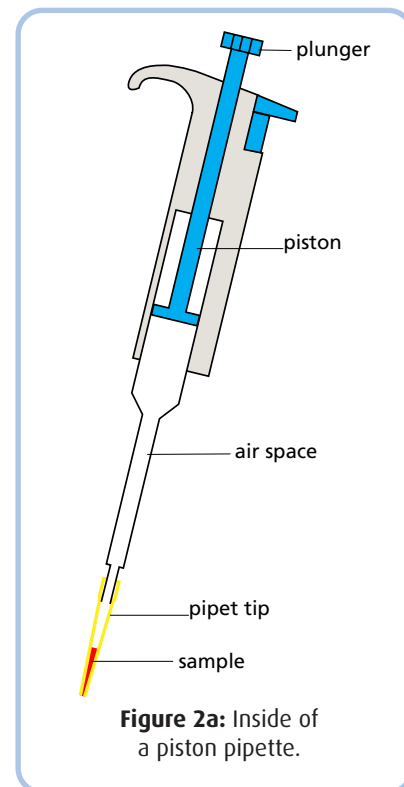

Figure 2a: Inside of a piston pipette.

Continued innovation has created several additional pipettes. For example, *multichannel pipettes* can hold a whole row of tips which allows scientist to prepare multiple samples at once and *positive displacement pipettes* have disposable pistons for when scientists are working with hot, hazardous, thick, or corrosive solutions. *Robotic pipettes* also exist. At their simplest, robotic pipettes repeatedly release a specified volume to multiple tubes or wells (a process known as aliquoting) in response to a light tap or time interval. More advanced robot pipettes take advantage of artificial intelligence to carry out whole experiments - measuring and mixing multiple volumes, examining the results, and then revising the volumes based on their interpretation!

HOW TO USE AN ADJUSTABLE MICROPIPETTE

Because pipettes are so frequently used in the lab, pipetting quickly becomes an almost instinctive action. However, the pipetting process can be far from intuitive and fluid at first. The major steps of pipetting are: prepare, aspirate, dispense, re-aspirate and re-dispense, and finally purge. These are briefly described below. In addition, Module I of the experiment will walk you through this process in detail.

Figure 3: Parts of an adjustable micropipette.

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1. **PREPARE:** Set the volume by dialing the pipette to the appropriate value and adding a tip.

2. **ASPIRATE:** Create a vacuum by pushing the plunger down and placing the tip into the sample. Then slowly release the plunger. Finally, pause to make sure the liquid has moved into the tip.

   *This step is slightly complicated by the fact that most micropipettes have two plunger positions (Figure 4). Depressing the button to the first "soft" stop (red brackets in Fig. 4) creates a vacuum that matches the set volume. This will result in the correct amount being aspirated. However, depressing the button to the second "hard" stop (red + blue brackets in Fig. 4) will expel additional air and create a larger vacuum than desired. While a great feature for purging, using the second stop when aspirating will create an inaccurate measurement.
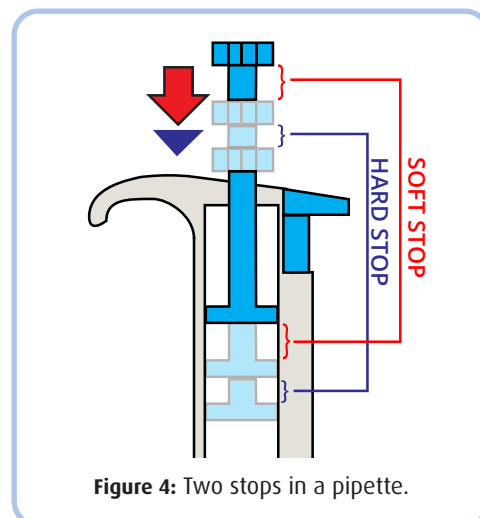

Figure 4: Two stops in a pipette.

3. **DISPENSE:** Place the tip where you want to add the sample and push the plunger down. This increases the pressure inside the pipette which causes the liquid to flow out. However, it also creates a new vacuum so make sure to lift the tip out of the solution before releasing the plunger.

4. **RE-ASPIRATE and RE-DISPENSE:** Repeat steps 2 and 3 if you are pipetting the same amount from the same start solution. Change tips as needed.

5. **PURGE:** Ensure that all the sample is released by pushing the plunger down to the second hard stop. After all the liquid has been ejected, remove the pipette and tip from the solution, release the plunger, eject the tip, and finally store the pipette upright in an easy to access location.

Most biotechnology experiments involve preparing reactions by going through these steps multiple times for multiple reagents. Furthermore, most reactions get duplicated or even triplicated in order to confirm the reproducibility of the result. Such practice quickly builds pipetting familiarity!

In addition to the steps outlined above, there are several good laboratory practices to keep in mind when working with pipettes. These practices reduce the chance of cross contamination and increase experimental accuracy and reproducibility. They also help maintain the functionality of the equipment and reduce the risk of injury to you, the scientists. Below are seven "golden rules" of pipetting:

- Use the correct pipette for the volume you are pipetting. Micropipettes come in different sizes which have a specific range. Never adjust the volume beyond the upper or lower limit of this range as it can compromise the accuracy of the experiment and the integrity of the pipette.

- Use the correct tip for the pipette you are using. Micropipette tips come in different sizes and like their pipette counterparts each size has an optimal range. Using the correct tip helps to maintain a buffer between the solution and the internal pipette tubing and ensures a tight seal between the tip and pipette. (A poorly sealed pipette tip can affect accuracy by up to 50%!)

- Switch pipette tips between samples to avoid contamination. Also, switch pipette tips if the tip comes in contact with anything other than the samples or sample vessels.

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- Keep pipettes vertical or vertically angled with the tip or tip cone facing down. This is especially important when there is liquid in the tip. Laying a pipette down that has a liquid filled tip can contaminate both the sample and pipette. In some cases, it can also damage the pipette.

- For maximum accuracy and to avoid contamination, depress and release the plunger using smooth and slow movements.

- Apply a consistent technique between samples. Small differences in pipetting (the depth that a tip is immersed, the angle that the pipette is held at, the force used to lower the plunger, the speed used to raise the plunger, etc.) can quickly add up to larger differences in volume.

- Remember that you are a key component of the pipetting operation! Practice good ergonomics especially when pipetting multiple times - sit or stand so that you are comfortable and so that you can see your samples, hold the pipette safely but loosely, and when possible keep both wrists in a neutral position. Also wear gloves, a lab coat, and goggles when needed to protect yourself and avoid contamination.

WHAT DO PIPETTES PIPETTE?

Solutions! Common chemical and biological reagents that get repeatedly used in most biotechnology labs are listed in Table 1 along with several popular reactions and their primary "ingredients". Depending on the procedure being performed, these reactions can range in size from hundreds of liters to a few microliters. For biological reagents such as DNA, RNA, and enzymes the volumes involved are often measured in the latter. There are 1,000,000 microliters - denoted by the Greek letter μ (μL) - in one liter of a solution. This is very small! To put it in perspective, a 50 μL sample is approximately equal in size to a single raindrop and a raindrop-sized sample is relatively large when compared to experimental samples that are often 5 to 25 microliters in volume! Precise pipetting is particularly important in this micro range as even small inconsistencies, like air bubbles inside the tip or an extra droplet of liquid on the outside of the tip, can change the proportions in the reaction.

ACCURACY AND PRECISION

Pipetting is such an important technology and technique because it enables scientists to accurately and precisely measure volumes, which in turn results in successful and repeatable experiments. What are these two traits? Accuracy describes how close a measurement is to the true value of a given quantity while precision describes the reproducibility of the measurement. One way to think of this is to imagine a basketball player shooting baskets. If the player has a high accuracy then the ball will always be close to or in the basket.

Common Reagents	Examples
Aqueous Solutions	TE Buffer, Water, Stop Solution, Electrophoresis Buffer, PBS Buffer
Viscous and Volatile Solutions	Glycerol, Oil, Ethanol, Formaldehyde
Corrosive and Hazardous Solution	Acids, Bases, Radioactive Isotopes
Biological Suspension (Non-hazardous)	DNA, RNA, Restriction Enzymes
Biological Suspension (Hazardous)	Infectious bacteria, Viruses, Patient and Environmental Samples
Reactions	DNA Extraction Reaction *(DNA + Buffer + ProK Enzyme)* DNA Amplification Reaction *(DNA + Buffer + Taq Enzyme + Nucleotides)* Bacterial Transformation *(Nutrient Media + Bacteria Cells + DNA + Calcium Chloride)*

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If the player is precise then the ball will always go to the same location - which may or may not be close to the basket. In the case of multiple pipette measurements, the set of pipetted volumes would be accurate if their average was close to the amount intended to be measured and would be precise if all the values were close together. Accuracy and precision are the ideal duo but they do not necessarily go hand in hand. Measurements can be either accurate, or precise, or both, or neither (Figure 5).

Accuracy in pipetting is best ensured by using the pipette correctly, keeping the pipette (and in particular the internal piston and tube) in good working order, and by periodically recalibrating the volume. During calibration, an outside measurement is made - in pipetting it is the volume of water based on weight - and then used to determine how far off the pipette measured is from this alternative or "true" value. Precision is also related to having a well functioning and well-maintained pipette. Precise pipetting also depends on using a correct and consistent technique. At the end of this experiment, you should feel confident about using a pipette to maximize the accuracy and precision of all your experiments.

A useful mnemonic:

A**C**curate is **C**orrect. (or **C**lose to real value)
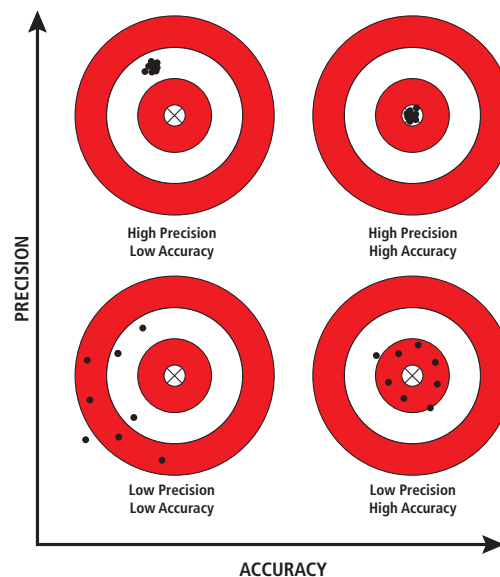P**R**ecise is **R**epeating. (or **R**epeatable)

Figure 5: Different accuracy and precision combinations.

Experiment Overview

EXPERIMENT OBJECTIVE:

In this experiment, students will master micropipetting - an essential biotechnology technique. They will then use this skill to create dot art on a Pipetting By Numbers canvas.

LABORATORY SAFETY

·   Gloves and goggles should be worn routinely as good laboratory practice.

·   Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

Wear gloves and safety goggles

LABORATORY NOTEBOOKS

Record the following in your laboratory notebook.

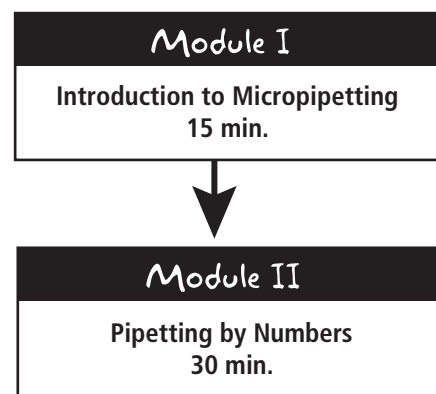### Before starting the Experiment:
·   Carefully read the introduction and the protocol. Then answer the study questions. Also write down any questions you might have.

### During the Experiment:
·   Record your observations and your experiences - particularly any events that may influence your results.

After the Experiment:
·   Take a picture of your results! You may also wish to write a list of tips to yourself for the next time you use a pipette.

Module I

**Introduction to Micropipetting
15 min.**

Module II

**Pipetting by Numbers
30 min.**

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Module I: Introduction to Micropipetting
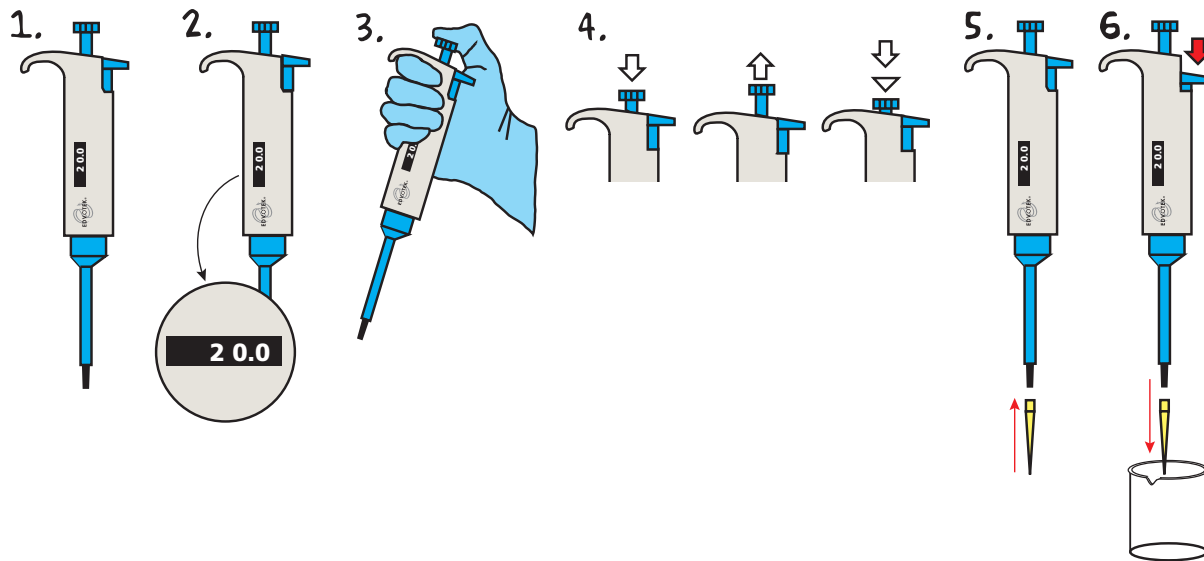
1. Examine the pipette(s) on your bench. Use Figure 3 on page 5 to **IDENTIFY** the following: pipetting button, volume setting wheel, tip ejection key, and tip cone.

2. Next, **IDENTIFY** the volume range and the volume display. In your lab notebook or below answer the following:
   - What is the maximum amount that you would pipette with this instrument?
   - What is the minimum amount that you would pipette with this instrument?
   - What volume is the pipette currently collecting?
   - What tip would you use with this pipette?

3. **PICK UP** your pipette. For best control, grasp the pipette in your palm and **WRAP** your fingers around the barrel. Rest your thumb on the pipetting button. Remember to always hold the micropipette so that the tip cone is facing down.

4. **PRACTICE** the motions for **ASPIRATING**, **DISPENSING**, and **PURGING** liquids. Push the pipetting button down to the first (friction) stop and then the second (hard) stop. Also practice *slowly* raising the button back to the original height.

5. **PRACTICE ATTACHING** a disposable tip. First, **BRING** the pipette above the tip. Next, gently **LOWER** the pipette so that the tip fits around the tip cone. Slightly **TWIST** the pipette to ensure that the tip is sealed. Finally, **LIFT** the pipette. The tip should come out of the rack and stay on the pipette. Remember not to let the tip touch objects other than the sample and its container.

6. **PRACTICE EJECTING** the tip. First, **HOVER** the pipette over a beaker or similar waste container. Next, use your thumb to **PRESS DOWN** on the tip ejector button. This will propel the tip off of the pipette and into the beaker - likely with some force!

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Module I: Introduction to Micropipetting, continued
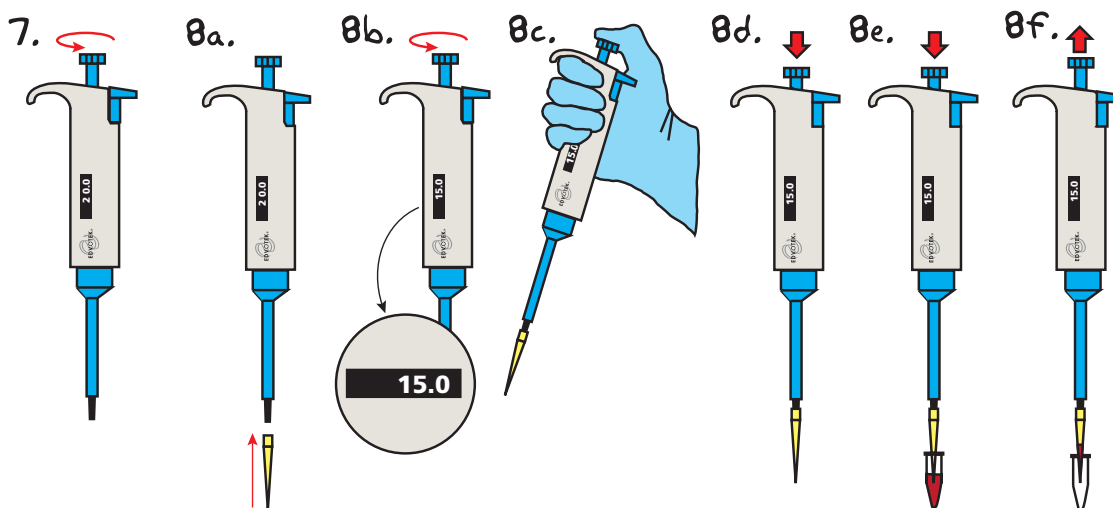
7. **PRACTICE ADJUSTING** the volume. Use your free hand to **TURN** the volume-setting wheel clockwise to increase the volume and counter clockwise to decrease the volume (see Box 1). **OBSERVE** the volume display (see Box 2). Remember to always stay within the volume range of the pipette.

8. **PRACTICE** using the pipette! (For this step, you will need a test strip and red dye which you will collect from your teacher before starting.)

   a. **PLACE** a new tip on your pipette.

   b. **SET** the volume to 15 µL.

   c. With your free hand **PICK UP** the test tube. **HOLD** the tube between your thumb and forefinger and at or near eye level to best observe the liquid moving into the pipette during the next few steps.

   d. **PRESS** the plunger down to the first soft stop and **HOLD** it in this position.

   e. **DIP** the tip into the solution. Immerse the tip enough to cover the end but not so deep that it obscure your view of the liquid ~ 5 mm.

   f. Keeping the tip in the solution, slowly **RELEASE** the plunger until it is in its original position. You should see the liquid flowing into the pipette tip (see Box 3).

   continued

> **Box 1:**
> If the volume-setting wheel is not moving, it may be that your pipette is locked at a certain volume. In many pipettes the volume lock is a small button that is located just below the tip ejector. This button can be disengaged by pushing upwards and relocked by pushing downwards.

> **Box 2:**
> Most pipettes have volume displays that are read top down. Some volume displays will contain a white dash line representing the decimal point. Account for this decimal place when setting your volume!

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Module I: Introduction to Micropipetting, continued

8g.   8h.   8i.   8j.   8k.   8l.   9.
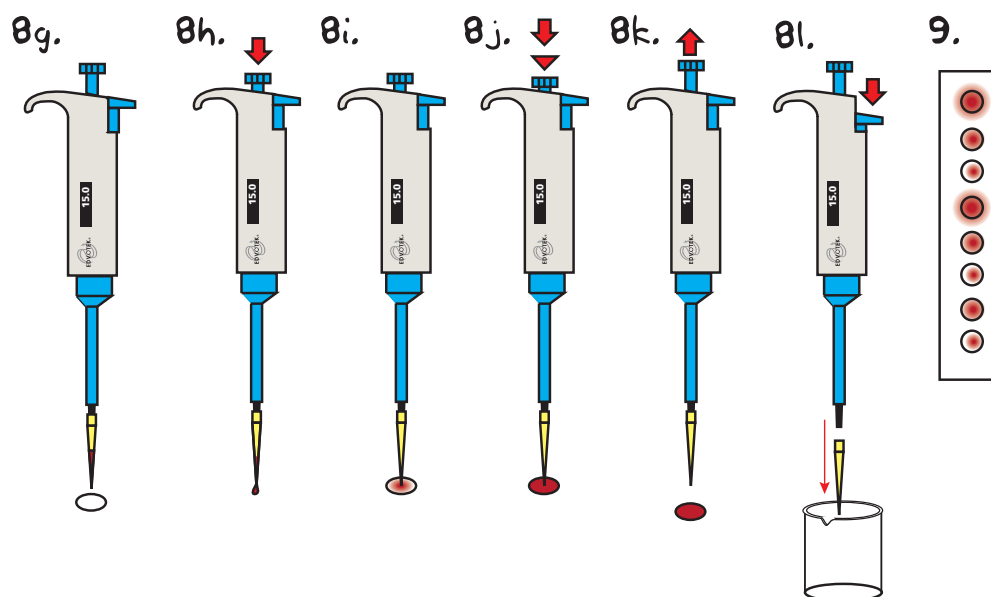
g.   **SLIDE** the pipette and tip out of the tube using the inside wall to dislodge any excess droplets that may be adhering to the outside of the tip. Then **MOVE** your pipette to just above the first practice circle.

h.   Slowly **DEPRESS** the plunger to the first stop. You should see the liquid flowing out of the pipette tip.

i.   Gently **TOUCH** the tip to the paper to create a capillary effect that will help draw any remaining fluid out of the tip.

j.   Keeping the tip on the paper, **DEPRESS** the pipette plunger to the second hard stop to ensure all liquid has been ejected.

k.   **RAISE** the pipette so that the tip is no longer on the paper and then **RELEASE** the plunger to its original position. (This is more important when pipetting from one liquid solution into another but also good practice here.)

l.   **EJECT** the tip into a beaker for used tips.

9.   **REPEAT** Step 8 for the remaining circles. As this is practice, you may want to experiment and observe what happens when common micropipetting errors are made. For instance, you could intentionally press down to the second stop while sucking up the liquid or intentionally skip pressing down to second stop when ejecting the liquid to see how this affects circle size.

Box 3:
Watch out for bubbles in the tip or air space at the end of the tip. These can significantly offset the measurement! If you observe either, expel the liquid back into the tube (Steps 8h through 8k) and then repeat the process starting at Step 8c. Often air is introduced when pipetting quickly so perform these steps slowly.

Module II: Pipetting By Numbers

1. Your teacher will provide you with a Pipetting By Numbers canvas. There are different patterns available.

2. **COMBINE** dyes to make Green (G) and Orange (O) dyes.
   a. **MIX** 350 µL of blue and 350 µL of yellow dye into a fresh tube. **LABEL** this tube "Green".
   b. **MIX** 100 µL of red and 400 µL of yellow dye into a fresh tube. **LABEL** this tube "Orange".

   NOTE: If you run out of dye you can always make more!

3. Colors and pipetting volumes are indicated inside each circle. Color abbreviations are Red (R), Blue (B), Purple (P), Yellow (Y), Green (G), and Orange (O). Volumes are given in microliters. So a circle marked "R50" would get 50 µL of the red dye.

3. Use the techniques described in Module I Step 8 to pipette the specified color and volume.

   <u>For Best Results:</u>
   - Use a small amount of tape to secure the top and bottom corners of the canvas to your lab bench. Fasten the tape to the back side (bench facing side) by wrapping the tape into a cylinder.
   - Pipette into the center of each circle and hold the pipette perpendicular to the paper.
   - When possible, start with the largest circles / highest volumes.
   - To save time, pipette all the same colored dots at once using the same tip. Then switch tips and move to the next color.
   - Dyes vary in color and in viscosity (thickness). High viscosity dyes like the Blue and Red will require slower pipetting.

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Study Questions

PRELAB QUESTIONS

1. Define, draw or describe the difference between the terms below.
   - Pasteur Pipette, Graduated Pipette, Displacement Micropipette
   - Soft Stop, Hard Stop
   - Accuracy, Precision

2. Below each image of a piston and tube, write the appropriate pipetting step (prepare, aspirate/dispense, purge).

a. _____   b. _____   c. _____
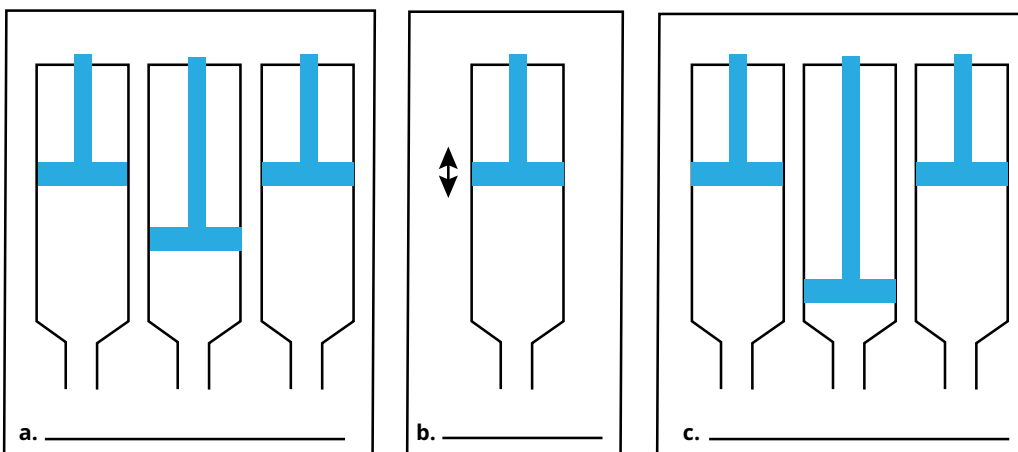
3. Why do you think that so many biotechnology experiments involve small volumes of reactions?

4. Even with the help of pipettes, scientists can still introduce measurement errors into their experiments with improper technique. What are some user related sources of pipetting variability?

5. A standard practice in many biotechnology experiments is to have replicates - multiple copies of the sample or mixture being analyzed. What can replicates tell you about the precision of the experiment? The accuracy of the experiment?

POSTLAB QUESTIONS

6. Examine the replicate dots you created in Module I or any same sized dots in the Pipetting By Numbers canvas. Do they match exactly? If not what could be the source of variability? Next, compare same sized dots from your canvas with those of another group.

7. Go back to the list of golden rules presented in the introduction. Which of these came intuitively or were easy to remember? Which were harder to remember or implement?

Instructor's Guide

Notes to the Instructor

This experiment is set up for 10 students groups. In Module I, students will become familiar with pipetting and in Module II, each group will have the chance to create their own piece of STEAM artwork using a Pipetting by Numbers canvas.

OVERVIEW OF INSTRUCTORS PRELAB PREPARATION

Preparation for:	What to do:	When:	Time Required:
Modules I & II	Cut practice stips and canvases.	Anytime before Module I.	5 min.
	Label tubes and aliquot dyes.	Anytime before Module I.	15 min.

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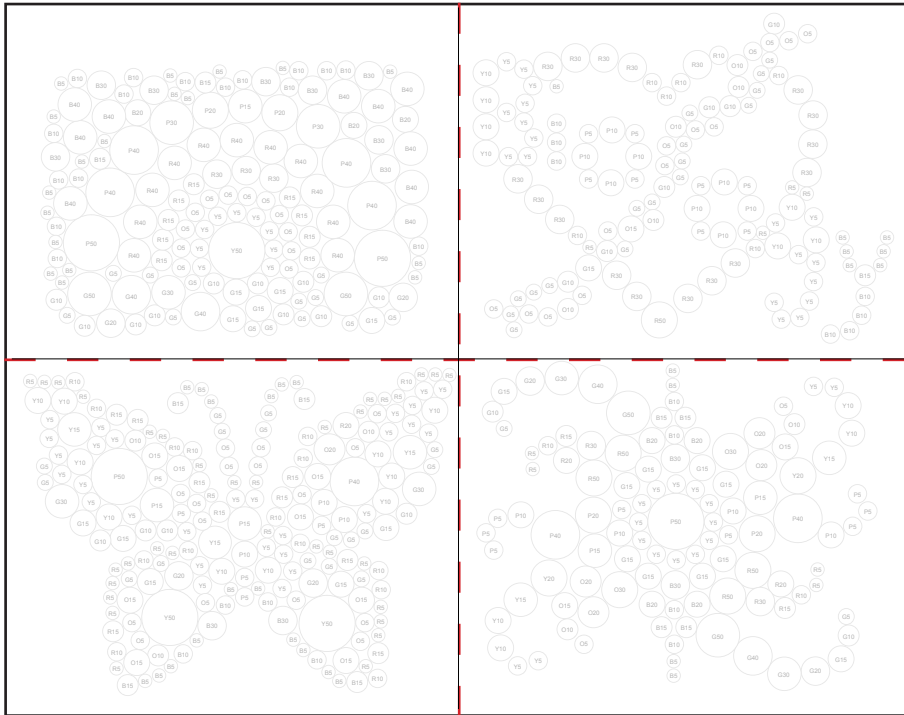· Experiment Protocols
· Tech Support
· Resources!

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Pre-Lab Preparations

1. Pipetting by Numbers canvases are provided. Cut each sheet along the lines (as shown in red, below) to divide the canvases. Test strips are also provided on one of the canvases.

FOR MODULE I
Groups will need:
· Pipette and tips
· Red dye
· Practice strip
· Small container for used tips

FOR MODULE II
Groups will need:
· Pipette and tips
· All colored dyes
· Small container for used tips
· Clear tape
· Pipetting by Numbers canvas
· Two empty snaptop tubes

2. Label ten microcentrifuge tubes as "Red". Repeat for "Blue", "Purple", and "Yellow".

3. Aliquot 1 mL of the Red and Purple dyes into the appropriate tubes. Next, aliquot 1.4 mL of the Yellow and Blue dyes. Finally, provide 2 empty tubes for each group to mix green and orange dyes.

4. For both modules, students will also need pipettes, tips, and a container to dispose of used tips.

Results and Analysis

MODULE II

Below is a rendering of the canvases.

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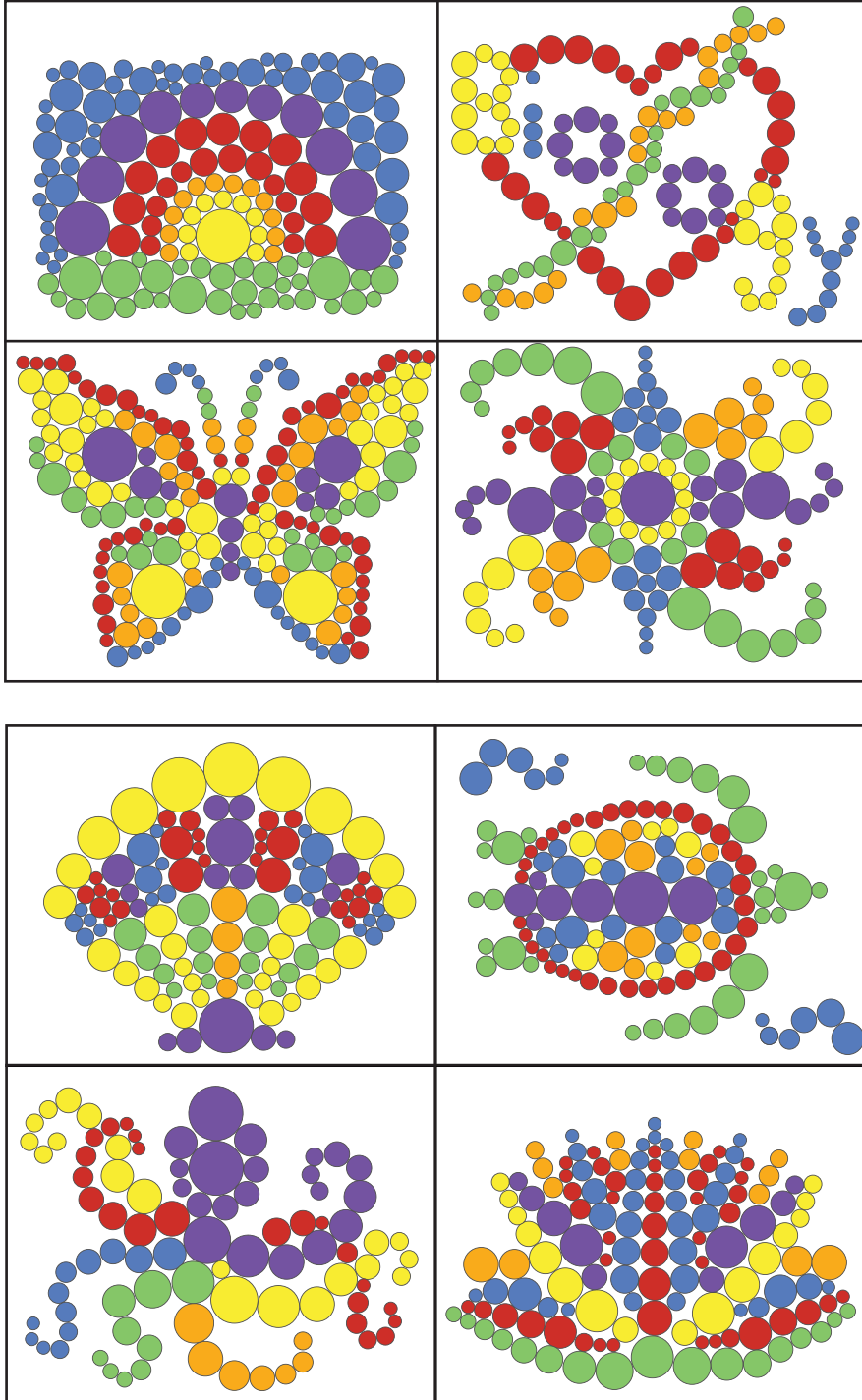
**Please refer to the kit
insert for the Answers to
Study Questions**

Please refer to the kit insert for the Answers to Study Questions

Appendix A
Volumetric Applications of the Metric System

The metric system is used in micropipetting. The milliliter (mL) and microliter (µL) are two very useful units of measure in molecular biology. "Milli" means one-thousandth and "Micro" means one-millionth. The symbol "µ" means micro, the prefix for 1×10^{-6} (expressed in scientific notation) or 0.000001 (expressed in decimals). One microliter is abbreviated as "µL". The two ways that this would be expressed is:
1 µL = .000001 or 1 µL = 1×10^{-6}. There are 1,000 µL in 1 milliliter, and 1,000 mL in one liter.

1.   Perform the following conversions:

	In decimals			In scientific notation	
1 mL =	_____ liter	1 mL =	_____ liter
1 liter =	_____ mL	1 liter =	_____ mL
1 mL =	_____ µL	1 mL =	_____ µL
1 µL =	_____ mL	1 µL =	_____ mL
10 µL =	_____ mL	10 µL =	_____ mL
20 µL =	_____ mL	20 µL =	_____ mL
50 µL =	_____ mL	50 µL =	_____ mL
100 µL =	_____ mL	100 µL =	_____ mL

2.   How many times greater is a mL than a µL?   _____

3.   How many times greater is a liter than a mL?   _____

4.   How many times greater is a liter than a µL?   _____

Metric System Prefixes		
Prefix	**Abbreviation**	**Meaning**
Giga-	G	10^{9}
Mega-	M	10^{6}
Kilo-	k	10^{3}
Deci-	d	10^{-1}
Centi-	c	10^{-2}
Milli-	m	10^{-3}
Micro-	µ	10^{-6}
Nano-	n	10^{-9}
Pico-	p	10^{-12}
Femto-	f	10^{-15}

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