

## Exploring Genetic Variation in a Caffeine Metabolism gene

### LAB ONE, PART ONE: MICROPIPETTE PRACTICE

**Purpose:** Micropipettes are the main tools of any molecular biologist. The purpose of this exercise is to practice your pipetting technique for precision and reproducibility. You will work with a partner, but make sure that each person can do all of the exercises in this lab.

#### **Procedure**

Scientists work only in a metric scale. You are probably familiar with a 2-liter (L) bottle of soda. Half of that volume is 1L. A volume that is 1000 times smaller than a liter is a milliliter (mL). A volume that is 1000 times smaller than a milliliter is a microliter ( $\mu\text{L}$ ). Because DNA is so small, we will spend most of our time dealing with volumes that range from  $1\mu\text{L}$  to 1mL. In order to move such a small volume from place to place, we must learn to use micropipette (sometimes referred to as pipetmen).

Compare the three sizes of micropipettes: P1000, P200, and P20. The volume limits of each pipet are different and can be found on the round top part of the micropipette. A P1000 pipets from  $100\mu\text{L}$  to 1mL, a P200 from  $20\mu\text{L}$  to  $200\mu\text{L}$ , and a P20 from  $1\mu\text{L}$  to  $20\mu\text{L}$ . (NB: Although the pipets can be set higher or lower than these volumes, it is not recommended for accurate pipetting.) To set a micropipette to a specific volume, it should read like this:

	P1000	P200	P20
Top:	0	2	2
	2	0	0
Bottom:	0	0	0

In this case, the P1000 and the P200 are set to  $200\mu\text{L}$ . The P20, however, is set to  $20\mu\text{L}$ . For the P20, the bottom number is the decimal place.

To change the settings on a pipet, rotate the wheel at the top until the numbers read the desired amount. Before pipetting, the micropipette requires a disposable plastic tip. A P1000 micropipette uses a blue tip, and P200 and P20 micropipette use a yellow tip. Use the micropipette to tap the appropriate tip onto the end; **do not apply the tip with your hand**. Your tips are sterile so once a tip is on the micropipette, do not allow it to touch *anything* but the liquid you would like to pipet and the container you are pipetting into.

To use a micropipette, hold the pipet upright in your dominant hand. Put a tip on the micropipette. Depress the plunger to the first stop to pick up the solution and then depress the plunger gently to the second stop to release the liquid. Make sure that once you have liquid in the pipet that you keep it upright, i.e. **the tip should NEVER be level with or above the handle**.

**Practice**

1. The limits of each micropipette are listed above in  $\mu\text{L}$  amounts. What are the volumes in mL for each micropipette? To determine this, divide the number in  $\mu\text{L}$  by 1000, as done in row 1 for the P1000. Fill in the chart below, including units in your answer.

	low limit/smallest volume	high limit/largest volume
<b>P1000</b>	0.1mL	1mL
<b>P200</b>		
<b>P20</b>		

2. To change the settings on a pipet, rotate the wheel at the top until the numbers read the desired amount. With your group, set your P1000 to  $500\mu\text{L}$ , your P200 to  $50\mu\text{L}$ , and your P20 to  $5\mu\text{L}$ . Raise your hand to check with your teacher before proceeding.

3. Practice pipetting with the micropipettes. Hold the pipet upright in your dominant hand. Put a tip on the micropipette. Depress the plunger to the first stop to pick up water and then depress the plunger gently to the second stop to release the liquid into a beaker. Pop the tip off into the waste bag by pressing the other button at the top of the pipet.

4. Using a P1000 micropipette, fill an Eppendorf tube with  $500\mu\text{L}$  of water. Look at the markings on the tube to approximate your accuracy. Add another  $500\mu\text{L}$  of water to the same tube and check your accuracy again.

5. Each person should obtain a tube of blue dye. Before starting, make sure all of the dye is at the bottom of the tube and not on the sides; if not, tell your instructor. Set the P20 to  $10\mu\text{L}$ . Lay a rectangle of parafilm flat on the bench. How many  $10\mu\text{L}$  dots can you pipet onto the parafilm? Check with your instructor.

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### LAB ONE, PART TWO: ISOLATION OF YOUR OWN DNA

**Purpose:** In this laboratory, we will isolate your genomic DNA in preparation for the polymerase chain reaction tomorrow.

#### **Background**

Ninety percent of people consume caffeine on a daily basis worldwide, making it the most commonly used stimulant. Tea is the most popular worldwide, but coffee is more commonly consumed in developed countries with 150 million regular coffee drinkers in the United States alone. Besides giving us a kickstart in the morning, coffee consumption has been linked to a decreased risk of type 2 diabetes, Parkinson's disease, and Alzheimer's disease, and tea drinking has been linked to a lower risk for some cancers. However, too much caffeine can also have negative effects. With regard to how caffeine affects us, some people get jumpy after drinking a single cup of coffee (slow metabolizers), while others can gulp down a Venti Americano without feeling a thing (fast metabolizers). Part of that variability is due to the development of tolerance by regular coffee drinkers (an environmental factor), but there are genetic differences in how people metabolize caffeine as well. Caffeine is primarily metabolized by the liver enzyme cytochrome P450 1A2 (CYP1A2). If you slowly metabolize the drug caffeine, you could be wide awake for hours after drinking a cup of coffee while those of us who are fast metabolizers will fall right to sleep.

Food or Beverage	Approximate Amount of Caffeine
1 cup of coffee	137mg
1 cup of tea	47mg
1 can of soda	46mg
1 ounce of chocolate	7mg

**Single Nucleotide Polymorphisms (SNPs)** are single base pair mutations in a particular region of DNA. In a previous biology class, you may have learned about the SNP in the hemoglobin gene that causes sickle cell disorder. Today we will begin an experiment to look for a SNP in your CYP1A2 gene. This SNP (rs762551) has been linked to how fast CYP1A2 metabolizes caffeine in some populations.

In today's lab we will be isolating your DNA from cheek cells. Your cells and the DNA within them are surrounded by oily membranes. **Detergent**, by dissolving these oils in water, destroys the membranes and exposes the contents of the cell and the **nucleus**. **Salt** makes the water solution more dense and heavy. This increased density will allow the separation of the DNA strands into the **alcohol**. Salt also neutralizes the charge of the DNA, making it insoluble in alcohol. Hopefully, we will isolate enough of your DNA to amplify the caffeine gene using a procedure called polymerase chain reaction, or **PCR**. Finally, we will use special DNA cutting enzymes, called **restriction enzymes**, to see which SNP version you have; there are two common ones in the general population.

#### **Procedure: DNA Isolation**

1. You will be given about 20mL of sterile PBS (saltwater), and you should put this in your mouth and swish vigorously for at least 60 seconds while chewing on your cheeks to isolate as many cells as possible. Spit back into the same container. ***Ideally, do this before eating, rather than immediately after a meal.*** You may store the sample in the refrigerator until class. Make sure to label your tube with your initials.
2. Centrifuge your cells at approximately 4000xg for 10 minutes at 4°C.

3. Remove the liquid and save the pellet. Add 25mL of fresh PBS to wash the cells.
4. Spin again as in step 2.
5. Remove the liquid, and dissolve the cell pellet in 1mL of PBS. Transfer the cell/PBS mix to a 2mL tube and store it on ice. Make sure to label your tube with your initials.
6. Pellet your cells again in a microfuge at 4000xg for 10 minutes.
7. Carefully remove the liquid, and dissolve your cells in 0.5mL Lysis Buffer. This buffer dissolves the cell membranes using a **detergent** and contains a **protease** to degrade cellular proteins.
8. Put your tube of cells in the 50°C water bath. I will finish this DNA preparation for you by removing the protease, adding alcohol to pull your DNA out of solution, and then dissolving your DNA in water for tomorrow's class.

### **Challenge Questions**

Think about where you got your DNA sample. Do you think that the only DNA in the tube is from your cells? What else might contribute DNA to this sample?

Adapted from: [https://www.23andme.com/you/journal/pre\\_caffeine\\_metabolism/overview/](https://www.23andme.com/you/journal/pre_caffeine_metabolism/overview/)

Other sources: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3071630/>  
<http://www.ncbi.nlm.nih.gov/pubmed/22854411>  
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3080612/>  
<http://www.ncbi.nlm.nih.gov/pubmed/21876539>