

Exploring Genetic Variation in a Caffeine Metabolism Gene

Part One: DNA Isolation and PCR

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, while others can gulp down a Venti Americano without feeling a thing. 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).

Single Nucleotide Polymorphisms (SNPs) are single base pair mutations in a particular region of DNA. In the human genome, SNPs appear approximately every 300 bases on average. If the human genome is 3.1 billion bases, that means there are approximately 10 million SNPs! Because SNPs can occur anywhere in the genome, they can have dramatic effects on protein expression and function or no effect at all. Today we will start a multiple week lab exercise to look for a SNP in an intron of your DNA for CYP1A2. This SNP (rs762551) has been linked to how fast CYP1A2 metabolizes caffeine in those of European descent.

In today's lab we will be isolating your DNA from cheek cells. Hopefully, we will have isolated enough of your DNA to amplify the caffeine intron using **PCR**. While in theory we could try to cut the gene directly from your genomic DNA, your DNA is huge, with millions of base pairs per chromosome. This is extremely unwieldy and very difficult to distinguish. However, by amplifying the region using PCR first, we target only the DNA region of interest. Furthermore, because PCR results in thousands of copies of this region, we will have much more DNA of that region to examine. Finally, we will use DNA cutting enzymes, called **restriction enzymes**, to see which SNP version you have; there are two common ones in the general population.

adapted from https://www.23andme.com/you/journal/pre_caffeine_metabolism/overview/

Protocols

DNA Isolation

1. You will be given about 20mL of sterile PBS (phosphate buffered saline), 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 do this any time during the day and store the sample in the refrigerator until our lab period. Make sure to label your tube.

2. Centrifuge your cells at approximately 4000xg for 5 minutes.
3. Remove the supernatant, and resuspend 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 new tube.
4. Pellet your cells in a microfuge at 4000xg for 5 minutes.
5. Carefully remove the supernatant, and add 1mL DNAzol. Using a cut tip, pipet your cells up and down until they are resuspended in the DNAzol.
6. Add 0.5mL 100% ethanol to the cell resuspension. Invert the tube to mix. Incubate at room temperature for 3 minutes.
7. The DNA should appear as white strings in the clear ethanol solution. Using a small pipet tip, spool the DNA out of the solution and into a new microfuge tube, transferring as little liquid as possible.
8. Wash the DNA by adding 0.8mL of 75% ethanol. Allow the DNA to sink to the bottom, and carefully remove the ethanol using a pipet.
9. Repeat step 8.
10. Air dry the DNA for about 15 seconds.
11. Resuspend the DNA in 200 μ L 8mM NaOH by gently pipetting up and down slowly.
12. Add 6.4 μ L 1 M HEPES to neutralize the NaOH.
13. Use 2 μ L to take an absorbance 260nm nanodrop reading of your DNA: _____
ng/ μ L
14. The nanodrop will also tell you how much protein is left in your sample by reading the wavelength at 280nm. The best DNA to protein ratio is to have a 260:280 ratio of 1.8. Write the 260:280 ratio of your DNA here: _____.

Set up PCR

15. Dilute your DNA to 10ng/ μ L in 10mM Tris pH 8 if necessary. You will use this as your template.

16. Each reaction will contain the following. Fill in column 3.

Reagent and concentration	Final Concentration	Amount Needed in 50 μ L	Amount for a MasterMix
Template DNA (10ng/ μ L)	50ng		
10mM Forward primer	0.2mM		
10mM Reverse primer	0.2mM		
25mM MgCl ₂	1.5mM		
10X Taq Buffer	1X		
10mM dNTPs	0.2mM		
Water			
Taq DNA polymerase (5 units/ μ L)	1.25 units		

17. In order to simplify this process, we should make a MasterMix so that the amounts added are more consistent. In this case, everything but the unique DNA template will be mixed into one tube. We will make 2 MasterMixes for the class so take the volumes for each reagent in the table and multiply by 8. Fill in the column on the far right. This will give us enough to use for half of the class plus some extra to account for pipetting errors. Put all these components in a tube, flick the tube several times to mix, and store the MasterMix on ice.

18. Each student should combine _____ μ L of the MasterMix with 50ng of template DNA in a well-labeled PCR tube. The reactions will be cycled using the parameters indicated below and stored in the freezer.

19. Next week, we will analyze your sequences using restriction enzymes and agarose gel electrophoresis.

Stage	Temp	Time	# of Cycles
Initial Denaturation	94°C	5 min	1
Denaturation	94°C	30 sec	35
Annealing	58°C	30 sec	
Extension	68°C	1 min	
Final Extension	68°C	5 min	1
Hold	4°C	Indefinitely	

Concept Check:

1. The sequences of your primers are as follows:

h.caffeine.Forward: 5'- GAGAGCGATGGGGAGGGC -3'

h.caffeine.Reverse: 5'- CCCTTGAGCACCCAGAATACC -3'

What is the T_m for each?

2. You can find the sequence of the SNP by going here:

http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=rs762551

Copy the sequence below (you may copy/paste on your computer or print it out), and highlight the sequences that will be amplified by the stated primers. (Hint: remember that your reverse primer is always the reverse and complement of the gene sequence you are reading.) How big is the total piece that will be amplified?