

Exploring Genetic Variation in a Caffeine Metabolism gene

LAB THREE: RESTRICTION ENZYME DIGESTS

Purpose: In this laboratory, we will use restriction enzymes to literally cut the amplified DNA in your PCR.

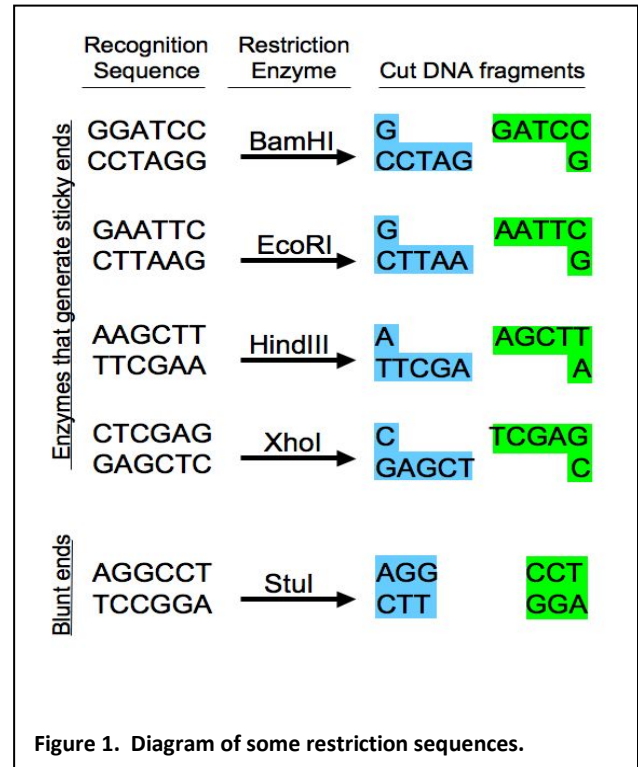
Background

Restriction enzymes are some of the most powerful tools in modern molecular biology. These enzymes, produced by bacteria, interact with **recognition sequences**, highly specific nucleotide sequences in DNA molecules (Figure 1). They then cut both strands of the double-stranded DNA, creating DNA restriction fragments. Because the recognition sequence is very specific and different DNAs will have different numbers of these sequences, digestion of different DNAs with the same enzyme may yield differently sized restriction fragments. This type of restriction analysis is called **restriction fragment length polymorphism (RFLP)** and can be used as a fingerprint to distinguish DNA molecules from each other. To date, more than 2500 restriction enzymes have been identified, and many are commercially available, offering a wide variety of options for cutting DNA molecules at different sites.

In this lab, we will digest your caffeine PCRs using certain restriction enzymes. This will generate DNA fragments of a certain length, and we can analyze this data to determine your **genotype** (genetic composition) and therefore make predictions about your **phenotype** (physical characteristics). You may also be able to determine whether your data set represents a **homozygote** (two copies of the same gene) or a **heterozygote** (two different genes) and whether this indicates that you will metabolize caffeine quickly or slowly.

Procedure: Set up the restriction digest reaction

1. In the interest of time, we will assume that your PCR worked and proceed with the next step of setting up a restriction digest. Label three 0.65mL tubes 'S', 'A', and 'O' on the lid of the tube and label your initials on the side of the tube. Tube S will contain the positive control of a digest with the enzyme **SacI**; tube A will contain the experimental condition of a digest with the enzyme **Apal**; and tube O will not contain any enzyme and will serve as a negative control.
2. Put 10uL of the appropriate restriction enzyme, buffer, and water mix into the appropriate tube, i.e. A goes into A. Make sure to use a new pipette tip every time.
3. Add 15uL of your PCR to each of the three tubes, making sure to pipette your PCR directly to the bottom of the tube. Make sure to use a new pipet tip every time. Gently flick the tube to mix.
4. Incubate the S digest at 37°C for 1 hr and the A digest and O tube at 25 °C for 1hr.



5. These reactions will be stored in the freezer until tomorrow's laboratory.

Concept Check and Challenge Question

Below is the CYP1A2 DNA sequence. The numbers of the bases are indicated at the right, but the sequence should be read from the first G, as base number 1, to the last G, as base number 743. Recall that there are two CYP1A2 **alleles** (variations of a single gene) that differ in sequence as the result of a Single Nucleotide Polymorphism (**SNP**). The underlined and bolded base at position 494 indicates the SNP in this allele. http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=rs762551

Allele 1

GGA GAGCGATGGG GAGGGCTTCC CCCTTAGCAC AAGGGCAGCC CTGGCCCTGG	53
CTGAAGCCCA ACCCCAACCT CCAAGACTGT GAGAGGATGG GGAATCATCC CTGGAGGAGG	113
TGCCCCTCCT GGTATTGATA AAGAATGCC TGGGGAGGGG GCATCACAGG CTATTTGAAC	173
CAGCCCTGGG ACCTTGCCA CCTCAGTGTC ACTGGGTAGG GGGAACCTCT GGTCCCTTGG	233
GTATATGGAA GGTATCAGCA GAAAGCCAGC ACTGGCAGGG ACTCTTTGGT ACAATACCCA	293
GCATGCATGC TGTGCCAGGG GCTGACAAGG GTGCTGTCTT TGGCTTCCCC ATTTTGGAGT	353
GGTCACTTGC CTCTACTCCA GCCCCAGAAG TGGAAACTGA GATGATGTGT GGAGGAGAGA	413
GCCAGCGTTC ATGTTGGGAA TCTTGAGGCT CCTTTCCAGC TCTCAGATTC TGTGATGCTC	473
AAAGGGTGAG CTCTGTGGGC <u>A</u>	494
CAGGACGCAT GGTAGATGGA GCTTAGTCTT TCTGGTATCC AGCTGGGAGC CAAGCACAGA	554
ACACGCATCA GTGTTTATCA AATGACTGAG GAAATGAATG AATGAATGTC TCCATCTCAA	614
CCCTCAGCCT GGTCCCTCCT TTTTCCCTG CAGTTGGTAC AGATGGCATT GTCCCAGTCT	674
GTTCCCTTCT CGGCCACAGA GCTTCTCCTG GCCTCTGCCA TCTTCTGCCT GGTATTCTGG	734
GTGCTCAAG	743

The SNP site is variable, of course, and the alternate allele of CYP1A2 has a C nucleotide in place of an A. This alternate allele sequence is shown below.

Allele 2

GGA GAGCGATGGG GAGGGCTTCC CCCTTAGCAC AAGGGCAGCC CTGGCCCTGG	53
CTGAAGCCCA ACCCCAACCT CCAAGACTGT GAGAGGATGG GGAATCATCC CTGGAGGAGG	113
TGCCCCTCCT GGTATTGATA AAGAATGCC TGGGGAGGGG GCATCACAGG CTATTTGAAC	173
CAGCCCTGGG ACCTTGCCA CCTCAGTGTC ACTGGGTAGG GGGAACCTCT GGTCCCTTGG	233
GTATATGGAA GGTATCAGCA GAAAGCCAGC ACTGGCAGGG ACTCTTTGGT ACAATACCCA	293
GCATGCATGC TGTGCCAGGG GCTGACAAGG GTGCTGTCTT TGGCTTCCCC ATTTTGGAGT	353
GGTCACTTGC CTCTACTCCA GCCCCAGAAG TGGAAACTGA GATGATGTGT GGAGGAGAGA	413
GCCAGCGTTC ATGTTGGGAA TCTTGAGGCT CCTTTCCAGC TCTCAGATTC TGTGATGCTC	473
AAAGGGTGAG CTCTGTGGGC <u>C</u>	494
CAGGACGCAT GGTAGATGGA GCTTAGTCTT TCTGGTATCC AGCTGGGAGC CAAGCACAGA	554
ACACGCATCA GTGTTTATCA AATGACTGAG GAAATGAATG AATGAATGTC TCCATCTCAA	614
CCCTCAGCCT GGTCCCTCCT TTTTCCCTG CAGTTGGTAC AGATGGCATT GTCCCAGTCT	674
GTTCCCTTCT CGGCCACAGA GCTTCTCCTG GCCTCTGCCA TCTTCTGCCT GGTATTCTGG	734
GTGCTCAAG	743

1. The restriction enzyme *SacI* has the recognition sequence GAGCTC. Find this site in both allele sequences above and highlight both. (Hint: this is not far from the SNP.)
2. *SacI* cuts between the T and the last C of the recognition sequence.
 - A. If *SacI* cuts at this site, how many pieces of DNA do you get for each allele? _____
 - B. How big is each piece? _____ bp & _____ bp
3. The restriction enzyme *Apal* cuts at the sequence GGGCCC. Look for this sequence at the SNP site in Allele 2 and highlight it with a different color. Notice that this site does NOT occur in Allele 1 due to the SNP variation.
 - A. If *Apal* **only** is used to digest allele 2, how many pieces of DNA do you get? _____
 - B. *Apal* cuts between the penultimate and final C of its restriction site. How big will each piece be?
_____ bp & _____ bp
4. Define the terms positive and negative control. Which samples serve as a positive and negative control in this experiment? Why is each important, and what do you expect to determine from these samples?