

Section 2: Working with DNA

Genomic DNA can be extracted from practically any cell type of any organism following very straightforward procedures. DNA extraction is a critical first step in the process of working with a particular gene or DNA sequence. The extraction protocol yields high-quality purified DNA molecules that are free from proteins and other cellular material.

Traditionally, DNA extraction involves several basic steps. First, cells are broken open, or lysed, to expose the cellular contents. Next, lipids and proteins are precipitated through a series of washes and pelleted via centrifugation. The supernatant (containing the DNA) is removed, and the DNA is precipitated with isopropanol, pelleted, washed, and dried. Then the DNA is re-suspended in water or other aqueous solution. Once back in solution the DNA is ready for use in a variety of “downstream” applications, including the Polymerase Chain Reaction (PCR).

PCR is a method that makes many copies of (or amplifies) a selected section of DNA. To target just a specified selection of nucleotides, two primers (or oligonucleotides), which are short, single strands of DNA. One of the primers is complementary to the 5' (upstream) flanking sequence of the target, and the other is complementary to the 3' (downstream) flanking sequence. The most important result of PCR is the amplification of your target DNA sequence and only your target with no ambiguity. Generally, 20 nucleotides per primer are sufficient to ensure amplification of the target sequence and only the target sequence, but only if the conditions of the reaction are optimal for those primers.

Each PCR reaction contains the primer pair, deoxynucleotide triphosphates (dNTPs), magnesium chloride ($MgCl_2$), Taq polymerase, reaction buffer, and DNA (called the template). The success of amplification is dependent upon manipulation of the various reagents (Fig. 5) and temperatures of the reaction (Fig. 6).

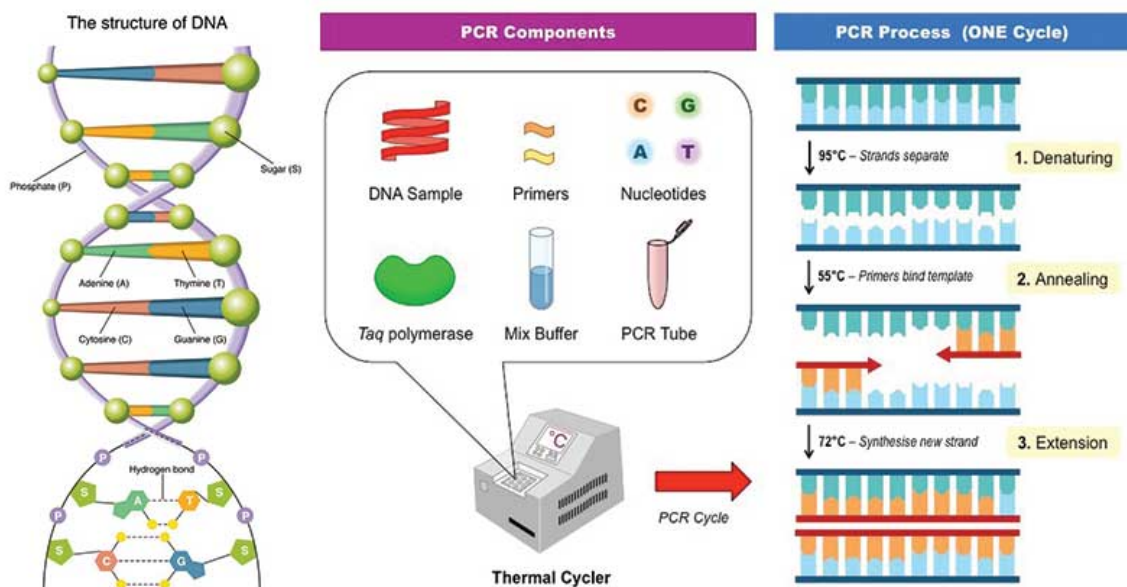


Figure 5. Overview of PCR. Double stranded DNA provides the template for producing many copies of a specific genetic region. High temperatures denature the DNA exposing the nucleotides to the primers and Taq polymerase. New strands are synthesized using the free DNA nucleotides, and the cycle repeats until sufficient DNA for the targeted region is produced.

LEARNING OBJECTIVES:

1. Learn the technique of DNA extraction
2. Understand PCR as a method of isolating and amplifying a particular gene among tens of thousands in the human genome

Procedures (Extracting DNA)

1. Go to YouTube.com and watch the DNA extraction videos [Part 1](#) and [Part 2](#).
2. Go to Learn.Genetics.edu and [perform the simulation](#) on DNA extraction and answer the corresponding questions on your worksheet.
3. Now that you have your virtual genomic DNA, you can use PCR to amplify the TAS2R38 gene.

Procedures (Amplifying DNA)

4. Go to YouTube.com and watch the PCR videos [Part 1](#) and [Part 2](#) and answer the corresponding questions in your worksheet.
5. Go to Learn.Genetics.edu and [perform the simulation](#) on PCR and answer the corresponding questions on your worksheet.
6. You have now amplified the TAS2R38 gene using the primers listed from Part 1. Both the taster and the non-taster alleles may be present in your sample. We will use a restriction digest to determine your genotype.

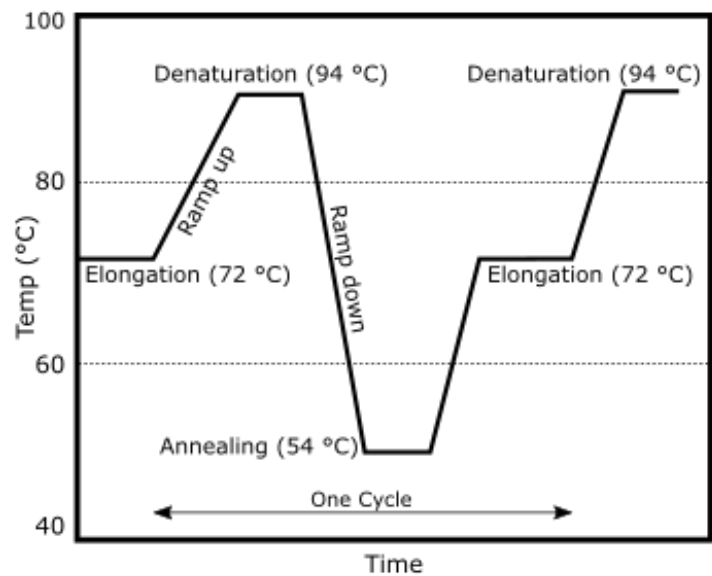


Figure 6. Thermal profile of a PCR reaction. DNA is denatured at 94°C prior to annealing of primers at 50°C and action of polymerase at 72°C. The cycle is repeated 30+ times.

Section 3: Collecting DNA Data

Restriction endonucleases are enzymes that cleave the sugar-phosphate backbone of DNA. A given enzyme cuts both strands of DNA within a stretch of 4-8 bases. Several thousand different restriction endonucleases have been isolated, which collectively exhibit a few hundred different sequence specificities (restriction sites). A large majority of restriction enzymes have been isolated from bacteria, where they appear to serve a host-defense role. Foreign DNA, for example from an infecting virus (bacteriophage), can be chopped up and inactivated by the restriction enzyme. By convention, restriction enzymes are named after their host of origin. For example, EcoRI was isolated from *Escherichia coli*. EcoRI, like all restriction enzymes recognizes a specific DNA sequence and cleaves the DNA where that sequence is encountered by the enzyme.

Because of the enzyme specificity in cleavage sites, restriction enzymes can be used to distinguish among different alleles of a gene by recognizing single base changes in DNA known as single nucleotide polymorphisms (SNPs) that result in alteration of the restriction site. Therefore, the restriction enzyme can be used to genotype a DNA sample without the need for sequencing. You will digest your virtual PCR samples with a restriction enzyme to generate DNA fragments. After separating and visualizing the

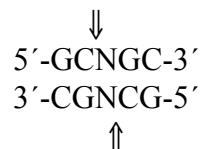
different sized fragments by gel electrophoresis, you will have determined the genotypes of the individuals from which the genomic DNA was extracted.

Polymorphism is common in nature, and functions to retain a multitude of phenotypes in a population. Phenotypic diversity within populations is important for adapting to changing environments (evolution). Polymorphism results from mutation, alteration of the sequence of DNA nucleotides that encode for particular phenotypes. The most common type of mutation involves the substitution of one nucleotide (A, G, C, or T) for another. These small changes result in a common type of genetic polymorphism called a single nucleotide polymorphism (SNP, Fig. 7). Most of the genes used to illustrate the principles of genetics in textbooks have two alleles: one common “wildtype” and one rare “mutant”. However, with polymorphisms, many alleles exist in wild populations, many at high frequencies. Therefore, with polymorphic genes, the traditional use of the terms “wildtype” and “mutant” do not apply.

You will set up a virtual restriction enzyme digestion that will allow us to take advantage of the molecular basis of polymorphism to genotype the individuals in our study. Recall that restriction endonucleases cleave DNA based on the identification of a particular restriction site (4-8 base sequence of nucleotides). We can use this to our advantage to reveal the presence of SNPs in the PCR-amplified gene. In other words, we can expose our amplified PCR product to the activity of a particular restriction enzyme to determine which allele (or alleles for heterozygotes) of the genes occurs in your samples.

You will digest your sample with the restriction enzyme *Fnu4HI* to generate DNA fragments based on your genotype at the TAS2R38 gene. Within the PCR amplicon, ‘tasters’ have a genetic sequence difference (a SNP) that contains an *Fnu4HI* restriction site, whereas the genetic sequence of non-tasters does not contain the restriction site.

Fnu4HI cuts DNA at the following sequence:



Restriction digestions of PCR products can be visualized by gel electrophoresis. During electrophoresis, DNA is loaded into a gel (often made of agarose) where the DNA fragments are separated based on size. Powdered agarose comes from algal seaweed and when solidified into a gel provides a matrix through which DNA migrates when exposed to an electric field. The gel is constructed with small wells in it, into which the DNA is loaded. DNA has a slightly negative charge and will therefore migrate toward the positive electrode (anode) when placed within the electric field. The gel is placed in a gel rig containing a 1X buffer with electrodes on either end. Post-PCR DNA is combined with a dye that contains glycerol and is heavier than the buffer in the gel rig, so that it

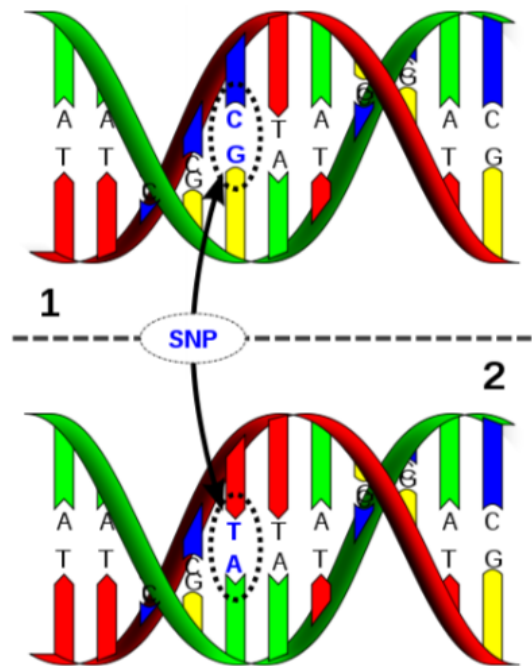


Figure 7. Graphical representation of a single nucleotide polymorphism (SNP). Sequence 1 has a ‘C’ where sequence 2 has a ‘T’.

will sink down into the wells. The electrodes are plugged in, with the one at the bottom of your gel being plugged into the positive (red) end, and the DNA migrates through the gel. To actually see the DNA fragments, gels are stained with chemicals that bind to the crevices of the DNA double helix. When the stained DNA is exposed to ultraviolet light, the dye is excited and glows, allowing the DNA fragments to become visible. By comparing the DNA fragments with markers of known size (DNA ladder), you can determine the size(s) of DNA in your sample.

LEARNING OBJECTIVES:

1. Learn how to observe the results of PCR
2. Understand the molecular nature of DNA migration
3. Use electrophoresis to reveal polymorphisms

Procedures (Determine your phenotype)

1. As explained in Part 1, bitter foods such as broccoli contain compounds that stimulate the receptors produced by *TAS2R38*. Therefore, homozygous “*strong tasters*” who have two copies of the ‘taster’ allele should find broccoli and related vegetables quite bitter tasting and be less likely to eat them. Heterozygous, “*weak tasters*”, having only one ‘taster’ allele and one ‘nontaster’ allele should find broccoli less distasteful, and homozygous “*non-tasters*” should not have an aversion to broccoli (this is, of course a grand simplification). Please consider your affinity for broccoli, and assign yourself to one the three phenotypic categories described above. Record your decision on the worksheet.

Procedures (Restriction Digest)

2. Go to YouTube.com and [watch the video](#) on restriction digest.
3. Go to restrictionmapper.org to [perform a virtual restriction digest](#) of the PCR-amplified *TAS2R38* gene.
4. Enter the amplicon sequence for the allele you want to digest in the ‘Paste Sequence Here’ box, and select the appropriate enzyme from the ‘Select Individual Enzymes’ list. Click the “Virtual Digest” button and record your results on your worksheet.

Welcome to RestrictionMapper - on line restriction mapping the easy way.

Maps sites for restriction enzymes, a.k.a. restriction endonucleases, in DNA sequences. Also does virtual digestion.

Conformation	Include	Sequence Info	Menu
Circular ☐ Linear ☒	Select Individual Enzymes All Enzymes AarI AasI AatI AatII AccI AccII	No non-base letters. Numbers and spaces OK. Paste Sequence Here	Map Sites Virtual Digest Reset Form
Sort By 1. frequency 2. overhang 3. name	All Commercial ☒ NEB only ☐ 5' overhang ☒ 3' overhang ☒ blunt ☒ Prototypes Only ☒ All Isoschizomers ☐		
Filter By Maximum Cuts: all Minimum Site Length: 5		Name your sequence Untitled	

Important: you must perform this step twice: once for the non-taster allele and once for the taster allele.

Procedures (Gel Electrophoresis)

5. Go to YouTube.com and [watch the video](#) on restriction digest.
6. Go to Learn.Genetics.edu and [perform the simulation](#) on electrophoresis and answer the corresponding questions on your worksheet.
7. Using what you know about gel electrophoresis, draw the banding pattern you expect for *your* sample on the worksheet.