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PCR for Genetic Screening

Student Scenario

Helpful Terms

Genome – complete collection of an organism's DNA
Gene – a section of DNA encoding a protein or RNA
Pharmacogenetics – examining a person's DNA to determine how their body will interact with a drug.
Allele – variant of a gene
Polymerase – a bioactive protein (**enzyme**) that creates strings (**polymers**) of DNA from individual base pairs (**nucleotides**) in a biochemical reaction.

Background

It is probable that at some time in the future, you will have a genetic analysis of your genome conducted. Most likely, this will be to screen for a disease. Genetic tests might determine if you have a disease or the potential to develop a disease, or if you carry a gene for a disease trait that you might pass on to your offspring. In addition, through an aspect of personalized medicine called pharmacogenetics, a doctor might screen your DNA to determine how well a particular drug might work for you. You will provide a blood sample and DNA from the white blood cells in the blood sample will be collected for analysis. A small amount of your DNA will be subjected to Polymerase Chain Reaction (PCR) amplification with primers specific for the region of DNA where an allele-of-interest is located.

Alleles are different versions of a gene, like the same piece of clothing in different sizes or colors. They come in a variety of forms, some with differences as small as a single base change, others with deletions (bases missing), insertions (extra bases) and inversions (sections of flipped DNA). The size of the mutation does not necessarily determine the severity of the effect. For example, sickle cell anemia is caused by a single point mutation, which creates a mutant form of hemoglobin that cannot fold or function properly. In some cases, a gene will naturally carry a repeat region. Alleles of these genes often carry varying numbers of repeats. The repeated areas are referred to as variable nuclear tandem repeats (VNTRs) and can be used to identify individuals. Some diseases have been linked to the number of repeats that exist within a gene. For example, in Huntington's disease the severity and age-of-onset of the disease has been linked to the number of repeats of a certain short sequence.

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The Scenario

In our example today we will be looking for alleles of the gene which influences the phenotype "cool/hip/groovy" – that unknown factor that makes one person more socially dominant than another. The situation is as follows: You are interested in three potential dates. In order to scientifically determine which of them is most worthy of a date, you decide to conduct a genetic analysis of each of the candidates. You are working in Dr. Who's lab where you have access to the molecular biology tools you need to conduct genetic analyses. In the interest of completing this experiment in the time allotted, you will start your PCR reaction now and study the scenario more completely while the PCR reaction is running. At this point, go to page one of the protocol and follow the directions.

Disclaimer

This scenario is made up. There is no gene for "coolness". Social popularity is a complex, unstable phenomenon based on multiple genetic and environmental factors with mixed selective results. This means that what's cool in Wisconsin today, may not be tomorrow, and may never have been cool in Tokyo. However, all of the molecular biology techniques are real, and the overall genetic analysis procedure follows the same steps as a real analysis would.

Back to the scenario…

Unbeknownst to the candidates, you begin collecting their DNA samples. Candidate A's younger brother is bribed into bringing you his sibling's toothbrush. Candidate B has a couple hairs tugged out during wrestling in PE by your assistant. You snag candidate C's buccal (cheek cell) swab in biology class.

1) Is it ethical to collect and test DNA samples without the subject's permission or knowledge? Are there circumstances under which you might change your mind? For example, would DNA sampling without permission be acceptable to solve a serial murder case? What about a national health study investigating possible treatments for diabetes?

You take this motley collection of illicitly obtained body parts to the lab and isolate DNA from each sample.

2) What cell type will yield DNA from each sample? Which sample will yield the most DNA? Why?

As control samples, you collect DNA from people you know are very cool (the prom king and queen, hot local DJ and a rapper, or the head of the debate society), and people who rank low on the cool scale (your younger sibling or Great Aunt Ida).

3) Why is it important to use controls? What information do the controls provide? What is the difference between a positive and a negative control? In this scenario, which samples provide the positive control? And the negative control?

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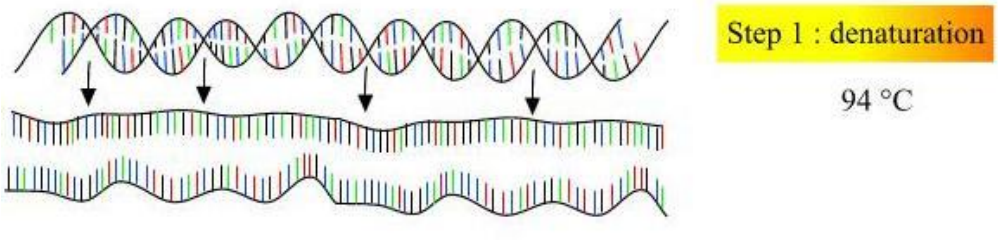
PCR:

Since the quantity of DNA obtained is so small, each sample must be amplified before it can be analyzed further. The samples will be amplified using the **Polymerase Chain Reaction** or **PCR**. PCR is a straightforward process which can generate a large quantity of DNA from a small sample of DNA or RNA in a short time.

As a process, PCR uses a series of important steps: Denaturation, Annealing, Extension and Cycling

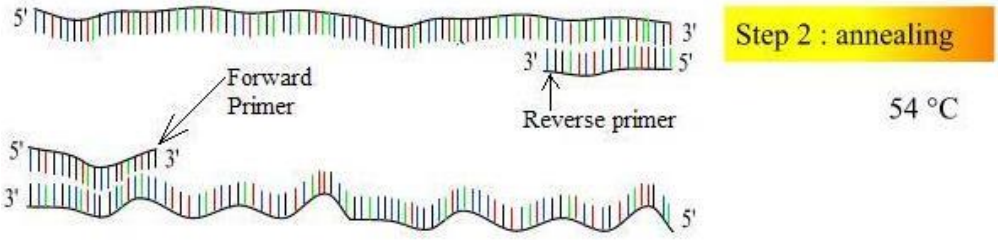
Denaturation:

The sample DNA is **denatured** (the two strands are separated), by heat.



Annealing:

After denaturing the template (original) DNA, the sample is cooled and tiny snippets of single-stranded DNA attaches to the template DNA. The basic concept is that a small piece of DNA called a primer is designed to anneal (stick) to a certain section of the DNA sample. For example, since you are investigating the cool gene, you would design a primer which anneals to a section just outside of the cool gene.



A second primer which **anneals** to the opposite strand downstream of the first primer is also designed.

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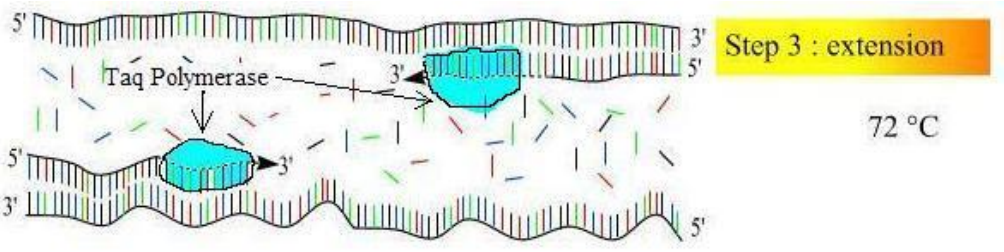
PCR (continued):

Extension:

DNA polymerases are a class of proteins which build a strand of DNA from nucleotides (G,A,T,C) by pairing them to the template DNA. In PCR, this is called **extension** or elongation. A DNA polymerase in the reaction mixture will extend a new strand of DNA starting from each primer by covalently attaching new nucleotides to the growing strand.

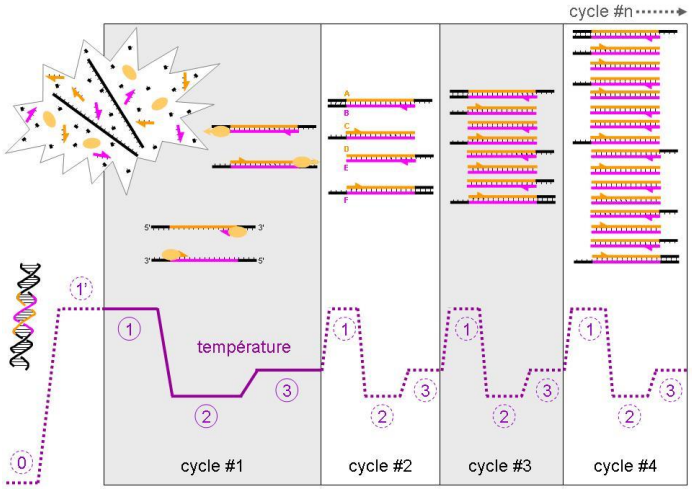


Polymerase Chain Reaction Graphics were derived from http://commons.wikimedia.org/wiki/File:PCR_Steps.JPG where it has been released by its copyright holder for unrestricted use.

This is where the story would end if it were not for the discovery of a thermostable DNA polymerase. Bacteria called thermophiles (heat lovers) live in extremely hot environments, such as thermal vents and hot springs. Most organisms cannot live in extreme heat because their proteins denature (unfold) and cease to function. The proteins in a thermophile are naturally resistant to denaturation by heat.

Cycling:

The new DNA strands created in the extension step and the original DNA templates are denatured by heating the reaction, and another round, or **cycle**, of PCR begins. Cycling is essentially taking Denaturation, Annealing, and Extension and doing them over and over again.



Cycling graphic is from http://commons.wikimedia.org/wiki/File:PCR_basic_principle1.jpg where it is published by its author (Ygonaar) for sharing under both GNU Free Documentation License and creative commons by-sa licenses with some rights reserved.

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PCR (continued):

Cycling (continued):

The DNA polymerase from the thermophile *T. aquaticus* is used to build new DNA strands in PCR because the denaturation step used to separate the DNA strands does not affect the polymerase. Therefore, the steps can be repeated many times, each time with more template DNA available for primer annealing and extension by polymerase. The quantity of DNA increases exponentially, generating usable quantities of high quality DNA in a relatively short time.

4) Could PCR be conducted with DNA polymerase from another organism? If so, which organism(s) would be good candidates? If not, why not?

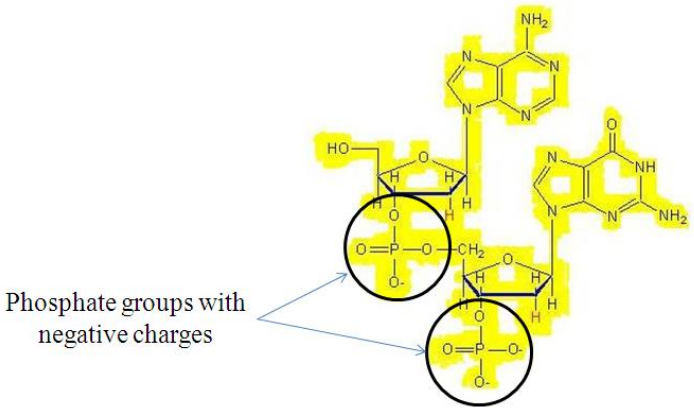
5) What does PCR allow us to do that we previously could not? Why is this important?

Electrophoresis

The DNA generated by your PCR experiments will contain the amplified fragment of the cool gene. There are several alleles of this gene which vary in the number of repeats of a certain area. (Go back and read that again until you are VERY clear about it. If you are having trouble seeing exactly what this means, ask for help.) In order to determine which allele each person has, you must be able to see the DNA and determine the size of each amplified fragment.

To do this, you will use electrophoresis. DNA has an overall **negative** charge from the phosphate groups; therefore DNA will move toward the positive pole of an electrical field.

Two DNA nucleotides bound together:



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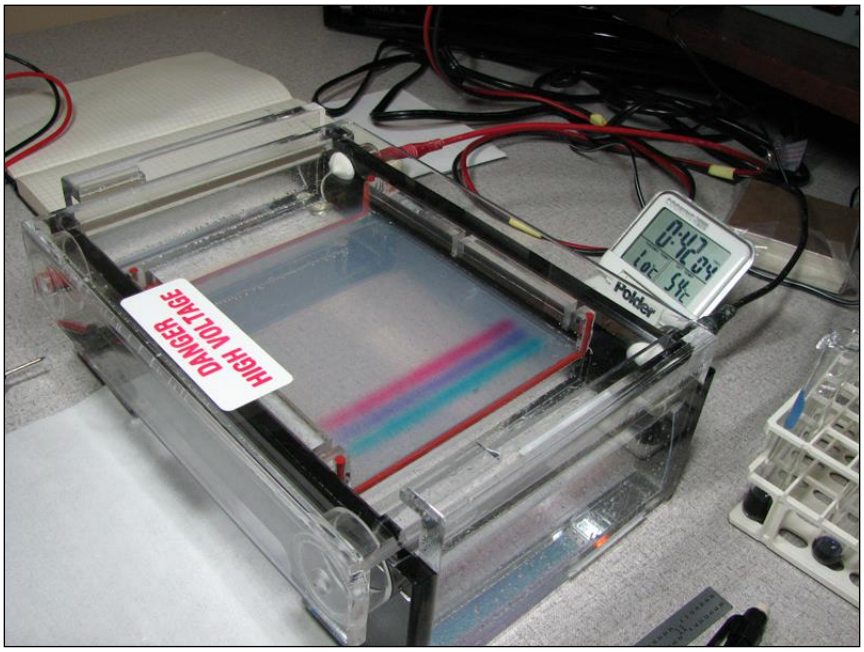
Electrophoresis (cont.)

In electrophoresis, the DNA is forced to move through a molecular sieve, which works exactly like your kitchen sieve only much smaller; small fragments move through the sieve more quickly than large fragments. Fragments of the same size will move at the same rate resulting in "bands" of DNA. The molecular sieve is provided by an agarose gel. Agarose is a large sugar polymer. More agarose makes a tighter sieve while less makes a looser sieve. Agarose is used as a thickener in many foods, and you will probably recognize the similarity to jello.

Do not attempt to eat your gel. It will not taste good and contains toxic elements.

To generate the electrical field that separates the DNA through the seive, the gel is submerged in a buffer which will conduct an electrical current.



Electrophoresis image from http://commons.wikimedia.org/wiki/File:Electrophoresis_-_Moving_along_gel.jpg where its author (michael) has published it for sharing under a creative commons-by license with some rights reserved.

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Electrophoresis (cont.)

To allow you to see the DNA, a dye that sticks to DNA is added to the gel. The dye glows green when illuminated by UV light allowing you to see where the DNA bands are in the gel.

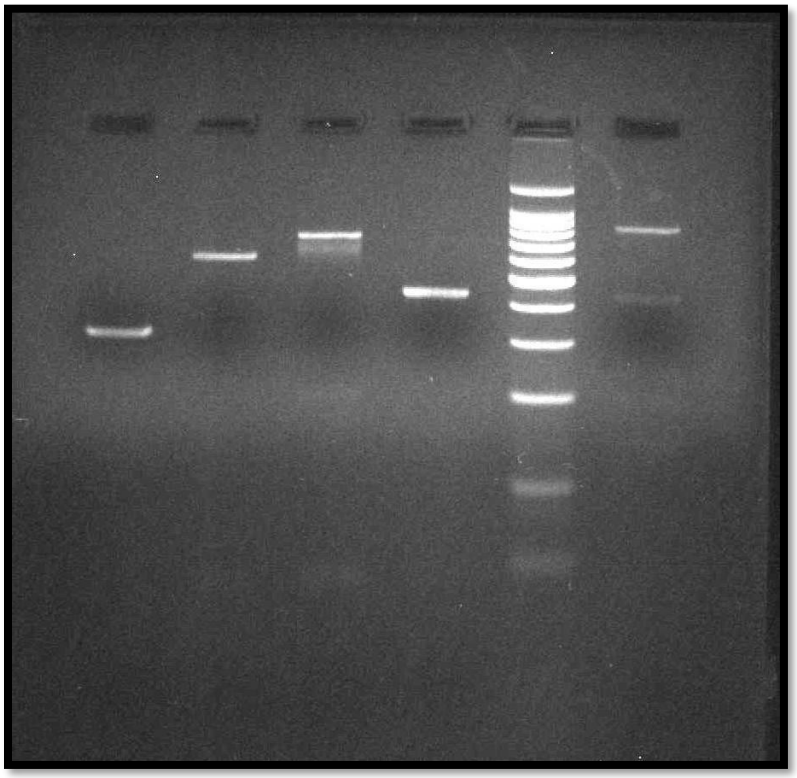

Image of a student DNA gel from Nicolet High School's 2009 Field Trip

Follow the protocol on pages 2 and 3 of your procedure for electrophoresis.

6) Why can't you see the DNA itself?
7) Which bands move the farthest down the gel, bands containing large fragments or bands of small fragments? Why?

Analysis:

Use the results to determine which person is the coolest. Compare the control DNA samples to the subjects' samples. Remember that "coolness" is directly related to the number of repeats in the "cool" gene, so more repeats means a cooler person.

8) Will the bands from the cool people or the not -cool people run farthest down the gel?