Student Manual

Crime Scene Investigator PCR Basics Kit

You are about to conduct real world forensic DNA profiling. As a crime scene investigator, you will use the polymerase chain reaction (PCR) and agarose gel electrophoresis to analyze the DNA samples obtained from a hypothetical crime scene and four suspects. Your job is to identify the perpetrator. In this analysis, a genotype is the particular set of genetic markers, or alleles, in a DNA sample. Every person's genotype is their own uniquely personal genetic barcode. In this experiment, you'll be revealing the genetic barcodes of several individuals, and looking for a match.

How can DNA evidence solve crimes?

DNA profiling refers to the use of molecular genetic methods used to determine the genotype of a DNA sample. This powerful tool is routinely used around the world for investigations of crime scenes, missing persons, mass disasters, human rights violations, and paternity. Crime scenes often contain biological evidence (such as blood, semen, hairs, saliva, bones, pieces of skin) from which DNA can be extracted. If the DNA profile obtained from evidence discovered at the scene of a crime matches the DNA profile of a suspect, this person is included as a potentially guilty person; if the two DNA profiles do not match, the individual is excluded from the suspect pool.

A Brief History of Forensic Analysis

Forensic sciences describe the boundary between science and the law. Forensic science can as easily convict someone of a crime as free someone wrongly convicted. The earliest uses of forensic science for criminal investigations involved the use of photographs to document crime scenes. Fingerprint evidence has been in use for the past 100 or so years. The first genetic evidence to be collected for investigative work involved the use of blood group typing. The 1980's saw the first use of a DNA-based forensic test, restriction fragment length polymorphism analysis, or RFLP. Although RFLP analysis has its limitations, it has been the workhorse of forensic analysis for nearly 20 years. Only with the recent advent of PCR has this aspect of the criminal justice system become truly modernized. Modern forensic DNA profiling makes it possible to distinguish any two people on the planet (with the exception of identical twins), living or dead.

PCR is DNA replication gone crazy in a test tube

PCR produces large amounts of a specific piece of DNA from trace amounts of starting material (template). The template can be any form of double-stranded DNA. A researcher can take trace amounts of DNA from a drop of blood, a single hair follicle, or a cheek cell and use PCR to generate millions of copies of a desired DNA fragment. In theory, only a single template strand is needed to generate millions of new DNA molecules. Prior to PCR, it would have been impossible to do forensic or genetic studies with this small amount of DNA. The ability to amplify the precise sequence of DNA that a researcher wishes to study or manipulate is the true power of PCR.

One of the main reasons PCR is such a powerful tool is its simplicity and specificity. The specificity of PCR is its ability to target and amplify one specific segment of DNA a few hundred base pairs in length out of a complete genome of over 3 billion base pairs. In addition, all that is required for PCR is at least one DNA template strand, DNA polymerase, two DNA primers, and the four nucleotide building block subunits of DNA – A, G, T, and C – otherwise known as the deoxynucleotide triphosphates of adenine, guanine, thymine, cytosine, and reaction buffer.

PCR allows forensic scientists to reveal personal details about an individual's genetic makeup and to determine the most subtle differences in the DNA of individuals - from the tiniest amount

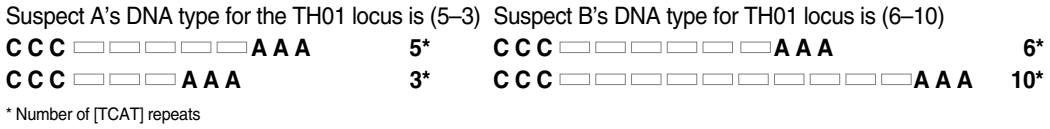
of biological material. The fact that millions of exact copies of a particular DNA sequence can be produced easily and quickly using PCR is the basis for modern forensic DNA testing.

What kinds of human DNA sequences are used in crime scene investigations? There are ~3 billion basepairs in the human genome – greater than 99.5% do not vary between different human beings. However, a small percentage of the human DNA sequence (<0.5%) does differ, and these are the special ***polymorphic*** ("many forms") sequences used in forensic applications. By universal agreement, DNA sequences used for forensic profiling are "anonymous"; that is, they come from regions of our chromosomes (also called ***loci***) that do not control any known traits and have no known functions. Loci are basically genetic addresses or locations. A single ***locus*** may have different forms or types; these different forms are called ***alleles***. A locus may be bi-allelic, having only two different forms, or it may be polymorphic, as described above.

The DNA sequences used in forensic labs are non-coding regions that contain segments of ***Short Tandem Repeats*** or ***STR*s**. STRs are very short DNA sequences that are repeated in direct head-to-tail fashion. The example below shows a locus (known as TH01) found on chromosome 11; its specific DNA sequence contains four repeats of [TCAT].

..C C C T C A T T C A T T C A T T C A T T C A..

For the TH01 STR locus, there are many alternate polymorphic alleles that differ from each other by the number of [TCAT] repeats present in the sequence. Although more than 20 different alleles of TH01 have been discovered in people worldwide, each of us still has only two of these, one inherited from our mother and one inherited from our father. For example as shown in figure 9, suspect A has one allele with 6 repeats, and one allele with 3 repeats, giving a DNA profile for the TH01 locus of 6-3.



* Number of [TCAT] repeats

Fig. 9. Two sample TH01 genotypes.

How are STR alleles detected?

The key to DNA profiling is amplification of the copies present in the small amounts of evidentiary DNA by ***polymerase chain reaction (PCR)***. Using primers specific to the DNA sequences on either side of the [TCAT] STR, billions of copies of each of the two original TH01 alleles in any one person's DNA type are synthesized in the reaction. These copies contain the same number of STRs present in the original DNA copies and can be visualized using agarose gel electrophoresis. By comparison with a DNA size standard, or allele ladder, that corresponds to the known sizes of TH01 alleles, the exact sizes of the PCR products from the sample DNAs can be determined and compared.

A diagram of the results for TH01 typing of Suspect A and Suspect B is shown in figure 10. In this cartoon example, PCR has been performed on DNA from 2 suspects using primers specific for the TH01 locus. Following gel electrophoresis which separates the PCR products according to their size, the pattern of bands is compared to the Allele Ladder to identify the alleles present in the original samples.

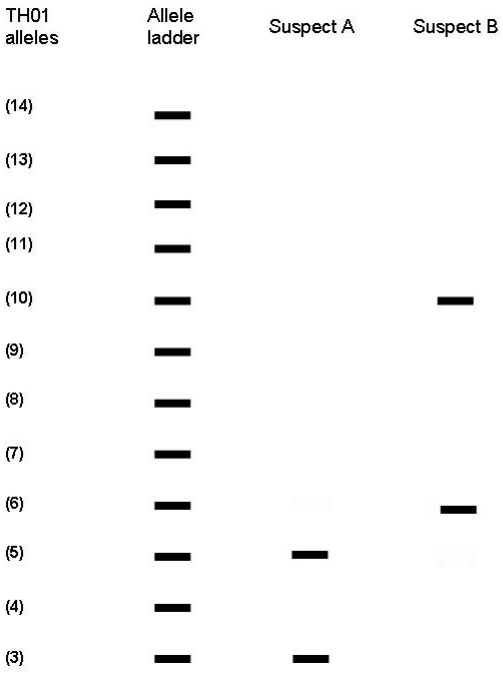


Fig. 10. Illustration of sample TH01 genotypes following gel electrophoresis.

Imagine a scenario in which Suspect A and Suspect B are accused of being involved in a love triangle and committing the murder of a third person in the Highway Motel; the person who actually pulled the trigger is unknown (for more information on this scenario, see the next page). In addition to DNA samples from the crime scene, the forensic specialist will isolate DNA from suspects, victims, and any others present to genotype as controls. Using PCR-based analysis, the samples will be examined at 13 different genetic locations, or loci, using software to interpret the results from the amplification products. In real crime scene analysis, DNA profiling is performed at many loci to improve the ***power of discrimination*** of the testing. In simple terms, the power of discrimination is the ability of the profiling to tell the genetic difference between different individuals. The larger the number of loci profiled, the more powerful the ability to discriminate.

Imagine the following scenario:

Scene: The Highway Motel, #1 Dark Highway, Nowhere
Setting: Room #13.

The motel manager hears loud voices, a woman screams, and a shot rings out. The manager runs to the window in time to see the receding lights of a car leaving in a hurry. The door to room # 13 hangs open. The manager runs to the open door, to see a man lying face down in a pool of blood. He calls 911. The police arrive, and begin to examine the crime scene. An apparent homicide, but with no obvious clues as to who committed the crime. Or…?

A forensic specialist is called in to examine the crime scene and collect evidence. Even though it looks like the people involved left no evidence behind, the specialist can use laboratory tests that can tell who was at the crime scene from a single drop of blood or a lone hair. Is this a science fiction story, or reality?

Very much a reality. Testing is routinely done in forensic testing labs across the US and in many other parts of the world from only a single cell, and sometimes from samples that are decades old. The reason this is possible is because of DNA. To be able to perform laboratory tests, the specialist needs biological material to work with. Often, there is very little material left at the scene of a crime, and not in quantities that will allow analysis. To get around this problem, the specialist takes advantage of a process that each and every cell in your body uses to divide.

The most important part of any cell's life is when it commits to reproducing itself and dividing. The basic result of any cell division is the creation of two identical daughter cells from one original cell. To ensure that this happens, DNA replication must have a high degree of specificity and accuracy, that is, it must copy DNA exactly. To do so, the enzymes involved in DNA replication use the information already contained in the existing strands to make new DNA copies. This basic idea - the exact copying of DNA from a template - is the basis for a new technology that has revolutionized many areas of science, medicine, and the courts.

PCR allows the forensic specialist to specifically amplify, or copy, any region of DNA that he or she is interested in. PCR is the basis for DNA testing that is currently used in nearly all forensic analysis.

In this experiment, you will perform PCR analysis on a single locus, the BXP007 locus, using template DNAs obtained from a simulated crime scene and a victim. Following PCR, you will run an agarose gel to separate the PCR products, visualize the PCR products, compare them to a simulated ladder of possible alleles for this locus, and assign a genotype for the templates. You will then look to see if any of the suspects' genotype match the crime scene, and see whether you can determine whodunit!

Let's examine the DNA evidence and find out who pulled the trigger.

Lesson 1 PCR Amplification

PCR amplification is DNA replication in a test tube. The portion of the DNA you want to make copies of is called the target sequence. The sample of DNA obtained at a crime scene and the suspect's DNA samples contain the target sequence.

PCR relies on three principles of molecular biology

1. Denaturation - melting double stranded DNA template into single stands
2. Annealing - complementary DNA strand hybridization via DNA primers
3. Extension - DNA strand synthesis via DNA polymerase

Denaturation. Before new DNA synthesis can begin the double stranded DNA template must be unwound and separated into single strands. In cells this is carried out by a family of enzymes. In PCR, heat is used to melt apart – or **denature** – the double stranded DNA template.

Annealing. Before a target region of DNA can be amplified, one must determine short sequences of DNA upstream (at the 5' end) and downstream (at the 3' end) of the target loci region of interest. These areas are then used to make short pieces of DNA, called primers or oligonucleotides, which are complementary to regions upstream and downstream of the target loci region (Figure 11). Primers serve as start and stop points for amplifying the target region of the DNA to be copied.

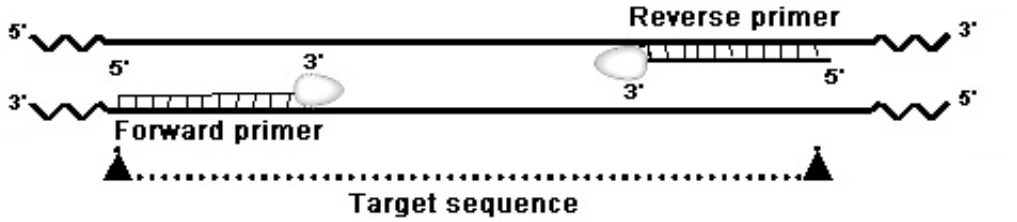


Fig. 11. Primers annealed to a target DNA sequence during PCR.

In PCR, complementary strand hybridization takes place when oligonucleotide primers anneal, or bind, to their respective complementary base pair sequences on the template. Hybridization is the process that describes the binding of the oligonucleotide primer to the template DNA. The two strands anneal to each other, forming a 'hybrid'. Like bookends, the two primers are designed and synthesized in the laboratory with a specific sequence of nucleotides so they will anneal at the opposite ends and on the opposite strands bracketing the target stretch of double-stranded DNA (template strand) to be amplified. Therefore, the target sequence is determined by the location that the primers anneal to.

Extension. Primers are needed because the **DNA polymerase** requires an already existing nucleotide chain to bind and add nucleotides to one at a time. Once the polymerase locates and binds to template DNA and the primer, it initiates the addition of nucleotides and synthesizes new copies of the double stranded template DNA by adding nucleotides onto the primer and extending it. Therefore, primers provide a starting point for the DNA polymerase.

These 3 steps – denaturation, annealing, and extension together make up one PCR cycle. A complete PCR reaction involves many repetitious of a single PCR cycle. In this experiment, your PCR reactions will cycle 35 times.

The enzyme used in PCR – **DNA polymerase** – must be thermally stable because PCR cycles between temperatures of 52°C and 94°C. The thermostable DNA polymerase that performs the polymerization was isolated from a thermophilic bacterium, *Thermus aquaticus* (*Taq*), which lives in high-temperature steam vents such as those found in Yellowstone National Park.

Two template strands are created from the original template after each complete cycle of the strand synthesis reaction – denaturation, annealing, and extension. It is called the polymerase chain reaction because exponential growth of the number of template molecules occurs after each cycle is complete, i.e., the number of DNA copies doubles at each cycle. Therefore, after 35 cycles there will be 2^{35} times more copies than at the beginning. After 35 cycles, the DNA of interest has been amplified sufficiently to be visualized using gel electrophoresis and DNA stains. This allows researchers to determine the presence or absence of the desired PCR products.

In order for PCR to happen efficiently, several components are needed. In addition to the template, the oligonucleotide primers, and the enzyme (*Taq* DNA polymerase), a special reaction buffer is also required, called a **master mix**. The master mix contains all of the components for PCR to occur, including the individual building blocks of DNA (nucleotides, or dNTPs), a special buffer to maintain optimum pH, salts, and $MgCl_2$. Salts and magnesium ions (also known as cofactors) are needed for the *Taq* DNA polymerase to perform optimally. In this experiment, your instructor will provide you with a master mix that comes prepared with all of the ingredients listed above, but also includes colored primers and *Taq* polymerase mixed in. For this reason, it's important to keep the master mix cold before use, so that the enzyme doesn't start to work before you add your DNA templates.

In this part of the experiment, you will obtain DNA samples which have been collected from a crime scene and four individuals suspected of being involved in the crime. Your task is to amplify the region of interest (the BXP007 locus, a polymorphic allele) from the DNA samples. Once complete, you will analyze your PCR products using gel electrophoresis to determine the genotypes of the samples at the BXP007 locus and match the crime scene DNA to one of the suspects.

Student Questions: Lesson 1

PCR Student Questions

1. What does PCR allow you to do with DNA?

2. What components do you need to perform PCR?

3. What is in the master mix and why do you need each component?

4. Why do you need to perform PCR on DNA evidence from a crime scene?

5. What steps make up a PCR cycle, and what happens at each step?