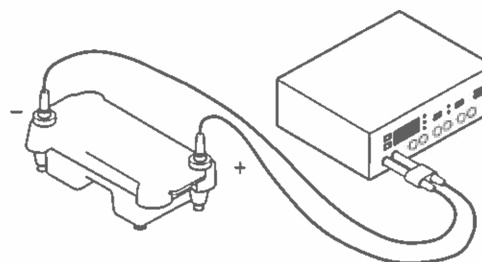
7. Secure the lid on the gel box. The lid will attach to the base in only one orientation: red to red and black to black. Connect the electrical leads to the power supply.



8. Turn on the power supply and electrophorese your samples at 100 V for 30 minutes.

9. Stain in Fast Blast DNA stain. Refer to the Student Manual for specific instructions.

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 ***STRs***. 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.

Suspect A's DNA type for the TH01 locus is (5–3)		Suspect B's DNA type for TH01 locus is (6–10)	
C C C ▭▭▭▭▭ A A A	5*	C C C ▭▭▭▭▭▭ A A A	6*
C C C ▭▭▭ A A A	3*	C C C ▭▭▭▭▭▭▭▭▭▭ A A A	10*

* 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.

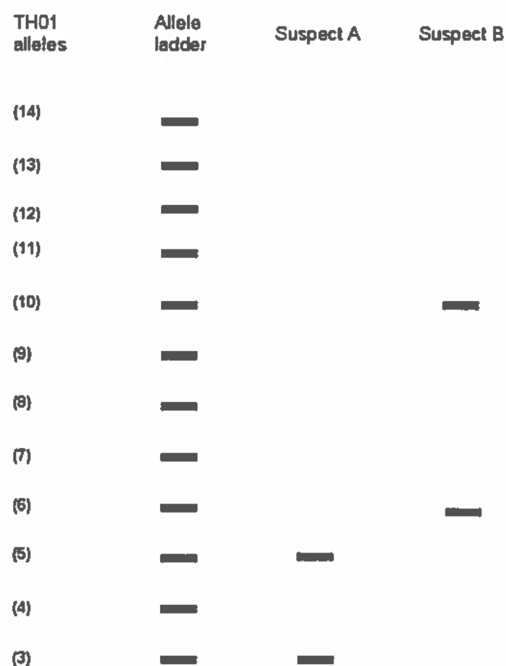


Fig. 10. Illustration of sample TH01 genotypes following gel electrophoresis.

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.

Student Questions – Introduction

1. What kinds of materials obtained from a crime scene might contain DNA?

2. Why do you need to perform PCR on DNA obtained from a Crime Scene?

3. What might you see if you ran a DNA sample extracted from evidence on a gel before PCR?

4. What is a genotype?

5. What is the difference between an allele and a locus?

6. Why do forensic labs analyse non-coding DNA and not genes?

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.

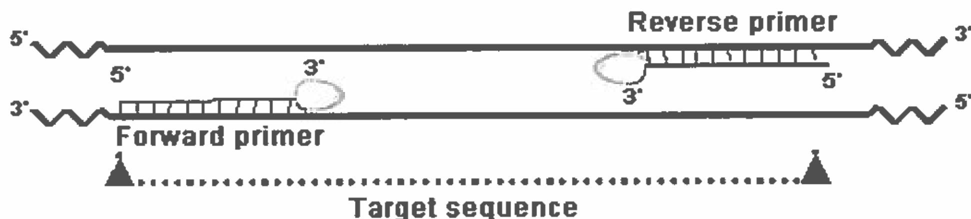


Fig. 11. Primers annealed to a target DNA sequence during PCR.

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?

Student Protocol – Lesson One

Student Workstations

Material	Quantity
Ice bath containing tubes of DNA (as below)	1
Master Mix + primers (MMP, blue liquid)	1
Crime Scene and Suspect A - D DNAs	5 (one of each tube indicated)
PCR tubes	5
PCR adaptors	5
Marking pen	1
2–20 µl adjustable micropipet or fixed volume 20 µl micropipet	1
2–20 µl pipet tips, aerosol barrier	1 rack

1. You will have 6 tubes on ice, and 5 x 0.2 ml PCR tubes in a rack at your workstation.

   In the ice, you should have -
   One yellow tube labeled MMP containing blue liquid.
   5 tubes labeled CS (purple tube), A (green tube), B (blue tube), C (orange tube), and D (pink tube).

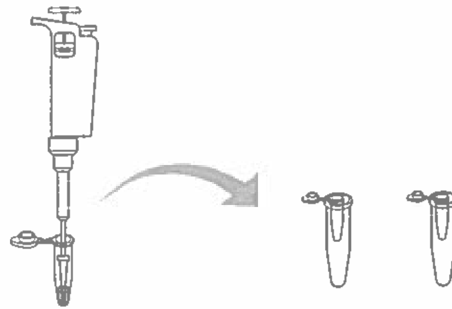
   Label PCR tubes CS, A, B, C, and D and include your group name or initials as well. The labels correspond to the following tube contents:

PCR tubes labelled	DNA templates	Master mix + primers (blue liquid)
CS + your initials	20 µl Crime Scene DNA (purple tube)	20 µl MMP (yellow tube)
A + your initials	20 µl Suspect A DNA (green tube)	20 µl MMP (yellow tube)
B + your initials	20 µl Suspect B DNA (blue tube)	20 µl MMP (yellow tube)
C + your initials	20 µl Suspect C DNA (orange tube)	20 µl MMP (yellow tube)
D + your initials	20 µl Suspect D DNA (pink tube)	20 µl MMP (yellow tube)

PCR tube

Capless tube

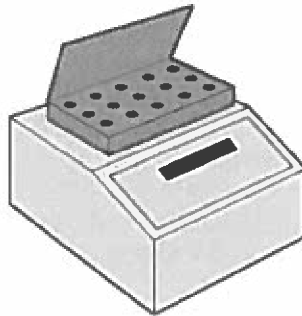
2. Keep tubes on ice during the procedure.
3. Using aerosol barrier pipet tips and either an adjustable micropipet set to 20 µl, or a fixed-volume 20 µl micropipet, add 20 µl DNA to each tube as indicated in the table above. For example, for the Crime Scene DNA, transfer 20 µl of the template into your 'CS' labeled PCR tube. **Important: use a fresh pipet tip for each DNA.**
4. Using aerosol barrier pipet tips and either an adjustable micropipet set to 20 µl, or a fixed-volume 20 µl micropipet, add 20 µl of the Master Mix + primers to each tube as indicated in the table above. Mix the contents of your PCR tubes by gently pipetting up and down. **Important: use a fresh pipet tip each time.** Once you've added MMP to a tube, close the cap. The solution in your PCR tubes should be blue. If it's not blue, talk to your instructor.



Master mix + primers (MMP)

5. Place your capped PCR tubes in their adaptors on ice.

6. When instructed to do so, place your tubes in the thermal cycler.



Lesson Two Electrophoresis of PCR Products

You have completed your PCR amplification. However, at this point, you can't actually tell whether or not you have PCR products. To do this, you must sort your PCR products using gel electrophoresis and then visualize them using a DNA stain. Since DNA is negatively charged, it can be separated using an electric current. In fact, electrophoresis means "carry with current". In agarose gel electrophoresis, DNA is placed in solidified agarose, which forms sieves containing pores that vary in size depending on the concentration of the agarose. The higher the concentration of agarose, the smaller the pore size, and the longer it takes for larger molecules to move through. This is particularly useful when you want to compare DNA molecules of different sizes contained in the same sample. Movement through the gel occurs when an electric current is applied across the gel. Since the gel is immersed in buffer, the current will travel through the buffer and gel, carrying the negatively charged DNA with it toward the positive anode.

In addition to your PCR products, you will also be running a DNA Allele Ladder that represents all of the possible alleles at the BXP007 locus. This is a reference, or marker, that you can compare your PCR reactions to so you can judge their relative sizes and their identities. In the following drawing of a gel, the samples, or bands, seen in the first track, or lane, all come from the BXP007 Allele Ladder. These are the standard sizes of all the alleles know to occur at this locus. There are 8 possible alleles, with the largest at the top of the gel and the smallest at the bottom. The sizes are, from top to bottom, 1500, 1000, 700, 500, 400, 300, 200, and 100 base pairs (bps). Allele names are indicated in the figure. In the next several lanes, we see PCR products that come from DNA samples that have been tested for what alleles they carry at this particular locus. As shown in figure 12, the sample in the lane next to the Allele Ladder, the Crime Scene Sample (CS) has a genotype that corresponds to alleles 5 and 2 on the allele ladder. We would say that the genotype for this sample is 5-2. For the next sample, the genotype would be 7-4, and so on.

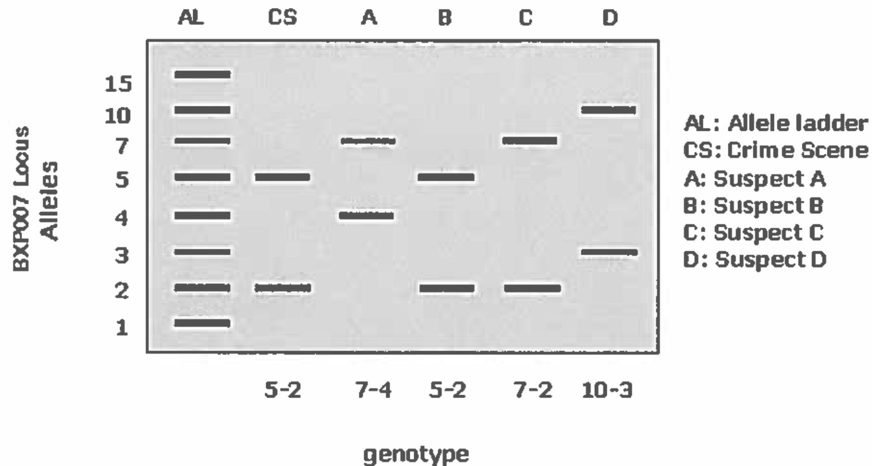


Fig. 12. A cartoon of potential Crime Scene Investigator PCR Basics kit results at the BXP007 locus.

Student Questions – Lesson Two

1. Why does DNA move through an agarose gel?

2. What are the two techniques used to create a DNA profile in this experiment? What function does each perform?

3. What is an Allele Ladder? What is its function in DNA profiling?

4. What is required to visualize DNA following electrophoresis?

Student Protocol – Lesson Two

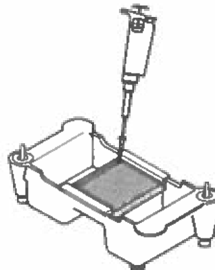
Student Workstation

Material	Quantity
3% agarose gel	1
PCR Samples from previous lab	5
1X TAE running buffer	300–350 ml
Orange G loading dye (LD; orange liquid)	60 µl
Crime Scene Investigator Allele Ladder (orange liquid)	25 µl
Note: Do not confuse these two tubes of orange liquid. They contain different compounds.	
2–20 µl adjustable volume pipet or fixed volume 20 µl micropipet	1
1–20 µl pipet tips, aerosol barrier	1 rack
Gel electrophoresis chamber (may be shared by 2 workstations)	1
Power supply (may be shared by multiple workstations)	1
Fast Blast DNA stain (at common workstation)	1
Gel staining tray	1

Protocol

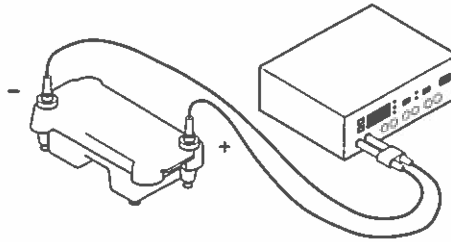
1. Set up your gel electrophoresis apparatus as instructed.
2. Obtain your 5 PCR reactions from the previous lesson, place them into a capless tube adaptor and into a rack.
3. Using aerosol barrier pipet tips and either an adjustable micropipet set to 10 µl, or a fixed-volume 10 µl micropipet, add 10 µl of Orange G loading dye (from the tube labeled 'LD') to each PCR reaction tube and mix well. **Important: use a fresh tip each time.**
4. Using the table below as a guide, load 20 µl of the allele ladder and 20 µl each sample into your gel in the order indicated below.

Lane	Sample	Load volume
1	Allele Ladder	20 µl
2	Crime Scene	20 µl
3	Suspect A	20 µl
4	Suspect B	20 µl
5	Suspect C	20 µl
6	Suspect D	20 µl



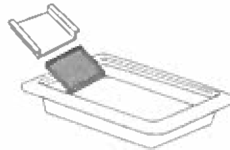
5. Run your gel at 100 V for 30 minutes. Do not let the orange dye front migrate out of the gel.

6. Stain in Fast Blast DNA stain as directed by your instructor and described below.



Staining of Agarose Gels

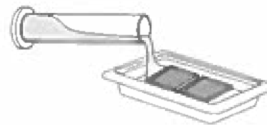
1. When electrophoresis is complete, turn off the power and remove the lid from the gel box. Carefully remove the gel tray and the gel from the gel box. Be careful, **the gel is very slippery**. Nudge the gel off the gel tray with your thumb and carefully slide it into a container for staining.



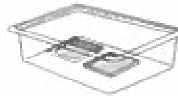
2. There are two protocols for staining your gel. Your instructor will inform you which one you will use.

Protocol 1: Overnight staining (Recommended)

a. Add 120 ml of 1x Fast Blast DNA stain to your staining tray (2 gels per tray).

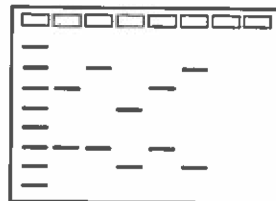


b. Let the gels stain for approximately 4–24 hours with gentle shaking for best results. No destaining is required.



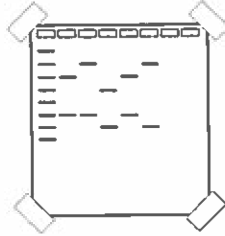
c. The next day, pour off the stain into a waste beaker.

d. Place the gel on a light background and record your result. With a permanent marker, trace the wells and band patterns onto a clear sheet of plastic or acetate sheet.



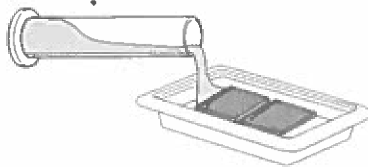
f. Trim away any empty lanes of the gel with a knife or razor blade.

g. To obtain a permanent record, air-dry the gel between cellophane sheets (your instructor will show you how). Tape the dried gel into your lab notebook. Avoid exposure of the stained gel to direct light, since it will cause the bands to fade.



Protocol 2: Quick staining (requires approximately 20 minutes) – This method will allow you to see bands quickly (within 15 minutes), but may require extensive destaining to obtain optimal band-to-background intensity. **Note**: it is important to use warm tap water for the destaining steps of this protocol.

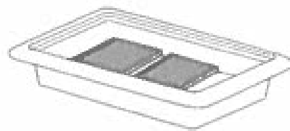
a. Immerse your gel in 100x Fast Blast.



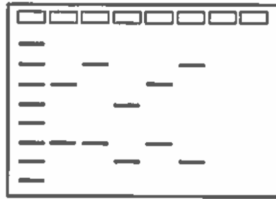
b. Stain the gel for 5 minutes with gentle agitation. Save the used stain for future use. Stain can be reused at least 7 times.

c. Transfer the gels into a large washing container and rinse with **warm** (40–55°) tap water for approximately 10 seconds.

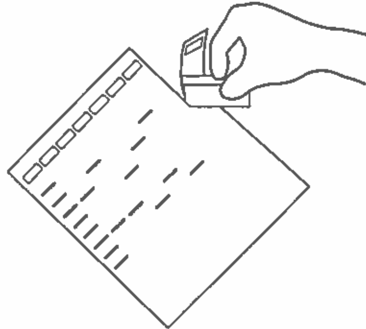
d. Destain by washing three times in **warm** tap water for 5 minutes each, with gentle shaking for best results. You should be able to see bands after 10 minutes with light coming up through the bottom of the staining tray. If necessary, keep destaining in warm water until the desired contrast is reached.



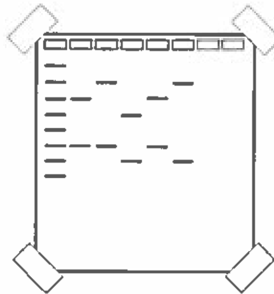
e. Place the gel on a light background and record your result. With a permanent marker, trace the wells and band patterns onto a clear sheet of plastic or acetate sheet.



g. Trim away any empty lanes of the gel with a knife or razor blade.



h. To obtain a permanent record, air-dry the gel on cellophane sheets (your instructor will show you how). Tape the dried gel onto your lab notebook. Avoid exposure of the stained gel to direct light, since it will cause the bands to fade.



Lesson 3: Drying Gels and Analysis of Results

For a permanent record of the experiment, gels can be dried between cellophane sheets and then inserted into lab notebooks. To analyze the wet gels, gels can be scanned, photocopied (a yellow backing improves contrast) or traced onto acetate film. There are 2 methods for drying gels. Please note that agarose gel support film does not work effectively for 3% gels.

GelAir drying frame method

Materials needed for drying gels using the GelAir dryer	Quantity
Cellophane sheets	4
GelAir assembly table	1
GelAir drying frame	2
GelAir clamps	16
GelAir Dryer (optional)	1
Distilled water	500 ml

Procedure

Step 1: Prewet 2 sheets of cellophane in a container of water for 15–20 seconds.

Step 2: Place a plastic drying frame on the GelAir assembly table. Center one sheet of cellophane on the assembly table.

Step 3: Carefully lay your gel on the cellophane, positioning it to accommodate other gels (up to 6 total). If there are bubbles between the gel and the cellophane, gently push them out with your gloved finger.

Step 4: Flood the gels with water and lay the second sheet of cellophane on top of them. If you are drying polyacrylamide gels, try not to trap any bubbles in the sandwich since bubbles will cause cracks in the gel during drying. If there are any bubbles, gently push them out with a gloved finger. You cannot avoid bubbles at the edges of agarose gels since they are so thick, but avoid bubbles between the cellophane and the face of the gel.

Step 5: Place the square metal frame on top of the cellophane sandwich. Secure the eight clamps onto the frame, two on each side. If you are not using a GelAir dryer oven, place the frames upright in a well-ventilated area for 12–36 hours. If you have a GelAir dryer, place up to four drying frames into the oven, turn the heater switch on and set the dial to 3 hours. The dryer will shut off automatically.

Step 6: When the gels are completely dry, they will be flat. Remove the clamps and take the gel/cellophane sandwich from the frame. Trim the excess cellophane surrounding your dried gel with scissors.

Cellophane sandwich and plastic container method

Materials needed for drying gels using plastic containers	Quantity per student workstation
Cellophane sheets	2
Plastic container–Tupperware-type (minimum 6 x 6" opening)	1
Rubber bands	2
Distilled water	500 ml

Procedure

Step 1: Wet two pieces of cellophane in a large volume of water, approximately 500 ml.

Step 2: Place one sheet of cellophane over a plastic container. Pull the cellophane taut so that it makes a flat surface over the top of the container, and use a rubber band to secure the sheet in place.

Step 3: Place a gel onto the cellophane. Remove any air bubbles that are under or around the gel. Flooding the surface of the cellophane around the gel with water will aid in the removal of bubbles.

Step 4: Place the second sheet of wetted cellophane over the gel, being careful not to trap any bubbles. Secure the second sheet of cellophane to the box with a second rubber band.

Step 5: Allow gel to dry for several days in a well-ventilated area.

Analysis of results

Although it is possible to analyze the dried gels, be aware that higher percentage agarose gels may be difficult to dry flat. In addition, exposure of the stained gel to light will cause the stain to fade. It is therefore recommended that analysis and determination of genotypes be done on wet gels.

Once the gels have been stained with Fast Blast DNA stain, it is time to determine the alleles present in each sample, and assign a DNA profile (genotype). For each PCR reaction, compare the bands obtained in each lane to the Allele Ladder run in lane #1. See page 40 for representative results, sizes of the ladder bands, and labeling of the alleles in the ladder. Assign each band in each PCR reaction with an allele assignment according to the band of corresponding size in the allele ladder. The bands in the allele ladder are numbered from top to bottom starting with the largest allele, #15, at the top. The sizes of the bands are indicated in the table below. In the example shown in figure 8 (page 26), the allele assignment for the sample in lane 2 is 3-7, since there is one allele 7 and one allele 3 in that lane. Write down the genotype for each of your samples in the chart below.

Lane	**Sample**	**Number of Bands?**	**What BXP007 alleles are present?**
1	BXP007 Allele Ladder		
2	Crime Scene DNA		
3	Suspect A DNA		
4	Suspect B DNA		
5	Suspect C DNA		
6	Suspect D DNA		

1. Did your samples all generate PCR products? If not, give reasons to explain why.

2. What is the genotype of each of your samples?

3. Does the Crime Scene DNA sample have a genotype that matches any of the suspects? If so, which one matches?

4. What does this result tell you about which suspects are included in the investigation? excluded? Explain your answer.

5. Imagine that each allele at the BXP007 locus is found at exactly the same frequency in a population. Since there are 8 possible alleles at the BXP007 locus, what is the frequency of any one allele from this locus in this population?

6. Given Mendel's Law of Independent Assortment and the assumption above, what is the frequency of the genotype of the Crime Scene sample?

7. If you had a pool of 13 suspects, and only one suspect had a genotype that matched the BXP007 locus found at the crime scene, would you be satisfied that you had identified the perpetrator based only on the genotype frequency calculated for the BXP007 locus? Why or why not? Explain your answer.