DNA Fingerprinting

When first introduced, DNA fingerprinting (also called DNA profile analysis or DNA typing) involved the electrophoretic analysis of DNA fragment sizes generated by restriction enzymes. In contrast to more conventional methodologies, such as blood typing, which excludes suspects, traditional DNA fingerprinting provides accurate, unambiguous identification of the source of DNA samples.

Variations in DNA sequences between individuals as determined by differences in restriction enzyme cleavage patterns are known as restriction fragment length polymorphisms (RFLPs). RFLPs are a manifestation of the unique molecular genetic profile, or "fingerprint", of an individual's DNA.

RESTRICTION ENZYMES

Restriction enzymes are endonucleases that catalyze cleavage of phosphate bonds. They require Mg^{+2} for activity and generate a 5 prime (5') phosphate and a 3 prime (3') hydroxyl group at the point of cleavage. The distinguishing feature of restriction enzymes compared to other endonucleases is that they only cut at very specific sequences of bases. Restriction enzymes are produced by many different species of bacteria (including blue-green algae). Over 3,000 restriction enzymes have been discovered and catalogued.

Restriction Enzyme	Organism
Bgl I	*Bactillus globigii*
Bam HI	*Bacillus amyloliquefaciens* H
Eco RI	*Escherichia coli* RY13
Eco RII	*Escherichia coli* R 245
Hae III	*Haemophilus aegyptius*
Hind III	*Haemophilus influenzae* R_4

Figure 1: Examples of restriction enzymes and the organism of origin.

Restriction enzymes are named according to the organism from which they are isolated. The first letter of the genus followed by the first two letters of the species (Fig. 1). Only certain strains or sub-strains of a particular species may produce restriction enzymes. The type of strain or substrain sometimes follows the species designation in the name. Finally, a Roman numeral is used to designate one out of several restriction enzymes produced by the same organism.

A restriction enzyme requires a specific double-stranded recognition sequence of nucleotide bases to cut DNA. Recognition sites are generally 4 to 8 base pairs in length and cleavage occurs within or near that site. Recognition sites are frequently symmetrical, i.e., both DNA strands in the site have the same base sequence when read 5' to 3' and the cleavage positions are indicated by arrows. Such sequences are called palindromes.

Background Information

DNA Fingerprinting

Consider the recognition site and cleavage pattern of *Eco* RI as an example. *Eco* RI causes staggered cleavage of its site. The resulting ends of the DNA fragments are called "sticky" or "cohesive" ends.

```
        ↓
5'-GAATTC-3'          5'-G      AATTC-3'
3'-CTTAAG-5'          3'-CTTAA      G-5'
      ↑
```

In DNA forensics laboratories, the two most commonly used restriction enzymes were *Hae* III and *Hin*f I, which are 4-base and 5-base cutting enzymes.

Hae III

```
    ↓
5'-GGCC-3'
3'-CCGG-5'
    ↑
```

*Hin*f I

```
    ↓
5'-GANTC-3'
3'-CTNAG-5'
    ↑
```

The size of DNA fragments generated depends on distances between the recognition sites. In general, the longer the DNA molecule, the greater the probability that a given recognition site will occur. The DNA of an average human chromosome is very large, containing over 100 million base pairs. A restriction enzyme having a 6-base pair recognition site, such as *Eco* RI, would be expected to cut human DNA into approximately 750,000 different fragments.

No two individuals have exactly the same pattern of restriction enzyme recognition sites. There are several reasons for this fact that exists in a population. Alleles are alternate forms of a gene that result in alternative expressions of genetic traits that can be dominant or recessive. Chromosomes occur in matching pairs, one of maternal and the other of paternal origin. The two copies of a gene at a given chromosomal locus represent a composite of parental genes and constitute an individual's unique genotype. It follows that alleles have differences in their base sequences which consequently creates differences in the distribution and frequencies of restriction enzyme recognition sites. Other differences in base sequences between individuals can occur because of mutations and deletions. Such changes can also create or eliminate a restriction endonuclease palindromic site. The example in Figure 2 shows how a silent mutation can eliminate a recognition site but leave a protein product unchanged.

DNA Fingerprinting

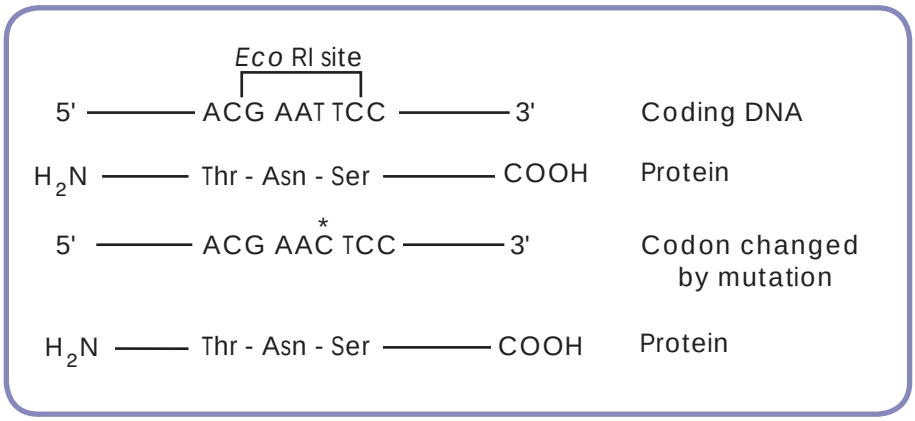


Figure 2:
Silent mutation (T → C) changes the *Eco* RI site.

Individual variations in the distances between recognition sites in chromosomal DNA are often caused by intervening repetitive base sequences. Repetitious sequences constitute a large fraction of the mammalian genome and have no known genetic function. These sequences can occur between genes or are adjacent to them. They are also found within introns. Ten to fifteen percent of mammalian DNA consists of sets of repeated, short sequences of bases that are tandemly arranged in arrays. The length of these arrays (the amount of repeated sets) varies between individuals at different chromosomal loci.

TGTTTA | TGTTTA | TGTTTA | .....variable number

When these arrays are flanked by recognition sites, the length of the repeat will determine the size of the restriction enzyme fragment generated. Several types of short, repetitive sequences have been cloned and sequenced.

AGAROSE GEL ELECTROPHORESIS

Agarose gel electrophoresis is used to analyze DNA fragments generated by restriction enzymes. Agarose gels consist of microscopic pores that act as a molecular sieve. DNA fragments are loaded into wells made in the gel during casting. Since DNA has a negative charge at neutral pH, it migrates through the gel towards the positive electrode during electrophoresis. DNA fragments are separated by the gel according to their size, charge and shape. DNA fragments are linear and the ratio of mass to charge is the same. Therefore, only the size of the fragment affects the mobility. The smaller the fragment the faster it migrates. After electrophoresis, DNA can be visualized by staining.

Restriction enzyme cleavage of relatively small DNA molecules, such as plasmids and viral DNAs, usually results in discrete banding patterns of DNA fragments after electrophoresis. However, cleavage of large and complex DNA, such as human chromosomal DNA, generates many differently sized fragments that the resolving capacity of the gel is exceeded.

The Experiment

DNA Fingerprinting

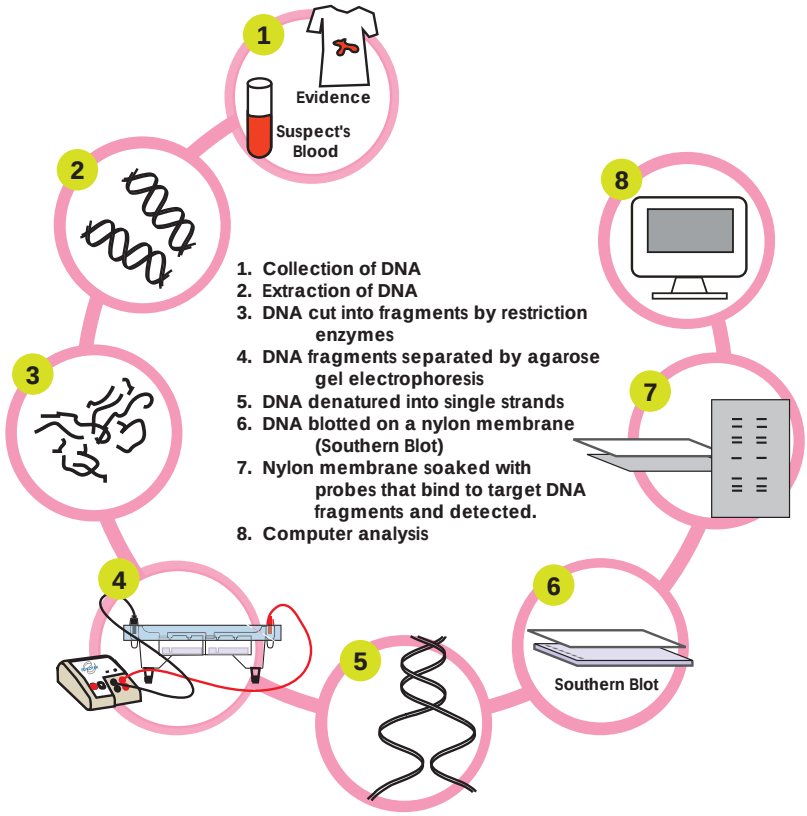


Figure 3: DNA Fingerprinting using RFLP and Southern blot analysis.

Consequently, the cleaved chromosomal DNA is visualized as a smear after staining and has no obvious banding patterns.

SOUTHERN BLOT ANALYSIS

RFLP analysis of genomic DNA is facilitated by Southern blot analysis. After electrophoresis, DNA fragments in the gel are denatured by soaking in an alkali solution. This causes double-stranded fragments to be converted into single-stranded form (no longer base-paired in a double helix). A replica of the electrophoretic pattern of DNA fragments in the gel is made by transferring (blotting) them to a sheet of nitrocellulose or nylon membrane (Figure 3). This is done by placing the membrane on the gel after electrophoresis and transferring DNA fragments to the membrane by capillary action or electrotransfer. DNA, which is not visible, becomes permanently adsorbed to the membrane, that can then be manipulated easier than gels.

Analysis of the blotted DNA is done by hybridization with a labeled oligonucleotide DNA probe. The probe is a DNA fragment that contains base sequences that are complementary to the variable arrays of tandemly repeated sequences found in the human chromosomes. Probes can be labeled with reporter molecules that are used for detection. A solution containing the single-stranded probe is incubated with the membrane containing the blotted, single-stranded (denatured) DNA fragments. Under the proper conditions, the probe will only base pair (hybridize) to those fragments containing the complementary sequences. The membrane is then washed to remove excess probe. Only DNA fragments that

DNA Fingerprinting

are hybridized to the probe will reveal their positions on the membrane. If the probes are isotopically labeled, the hybridized fragments will appear as discrete bands (fingerprint) on the film and are in the same relative positions as they were in the agarose gel after electrophoresis. Only specific DNA fragments of the hundreds of thousands of fragments present, will hybridize with the probe because of the selective nature of the hybridization process.

In forensic analysis, DNA samples can be extracted and purified from specimens of skin, blood stains, semen, or hair roots collected at the crime scene. RFLP analyses performed on these samples is then compared to those performed on samples obtained from the suspect. If RFLP patterns match, it is beyond reasonable doubt that the suspect (or biological material from the suspect, such as blood) was at the crime scene. In forensic DNA fingerprinting, different sets of probes hybridized to different types of repetitious sequences are used in DNA profile analysis in order to satisfy certain statistical criteria for positive identification.

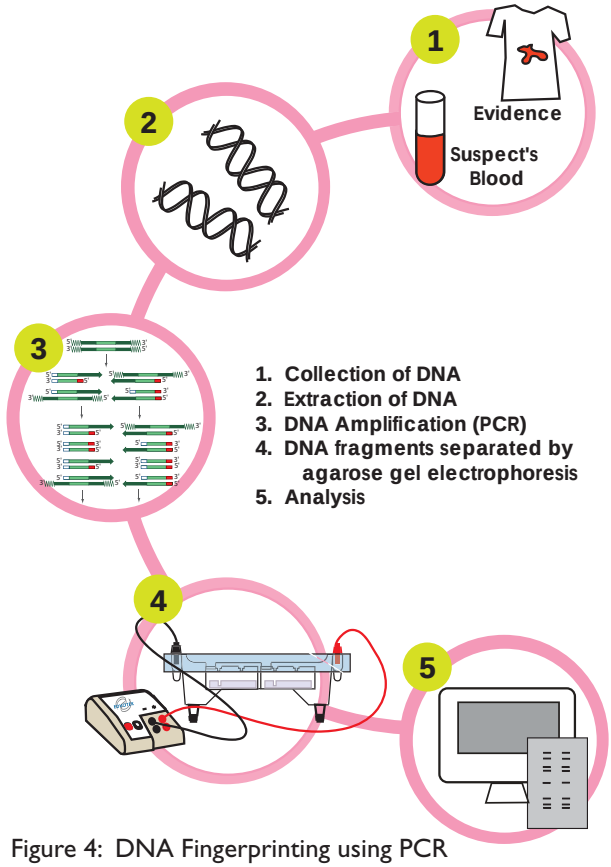


Figure 4: DNA Fingerprinting using PCR

DNA FINGERPRINTING USING POLYMERASE CHAIN REACTION (PCR)

RFLP-based DNA fingerprinting analysis has been overtaken by the Polymerase Chain Reaction (PCR) because of two important advantages. The first is the sensitivity of PCR, which allows for DNA fingerprinting identification using much smaller amounts of DNA since PCR amplifies DNA. A second advantage is the speed of PCR analysis, which allows critical questions to be answered more quickly as compared to Southern Blot analysis.

PCR amplification requires the use of a thermostable DNA polymerase, such as *Taq* polymerase. Purified from a bacterium known as *Thermus Aquaticus* that inhabits hot springs, *Taq polymerase* is commonly used in PCR because it remains stable at near-boiling temperatures. Also included in the PCR reaction are the four deoxynucleotides (dATP, dCTP, dGTP, and dTTP) and two synthetic oligonucleotides, typically 15-30 base pairs in length, known

DNA Fingerprinting

The Experiment

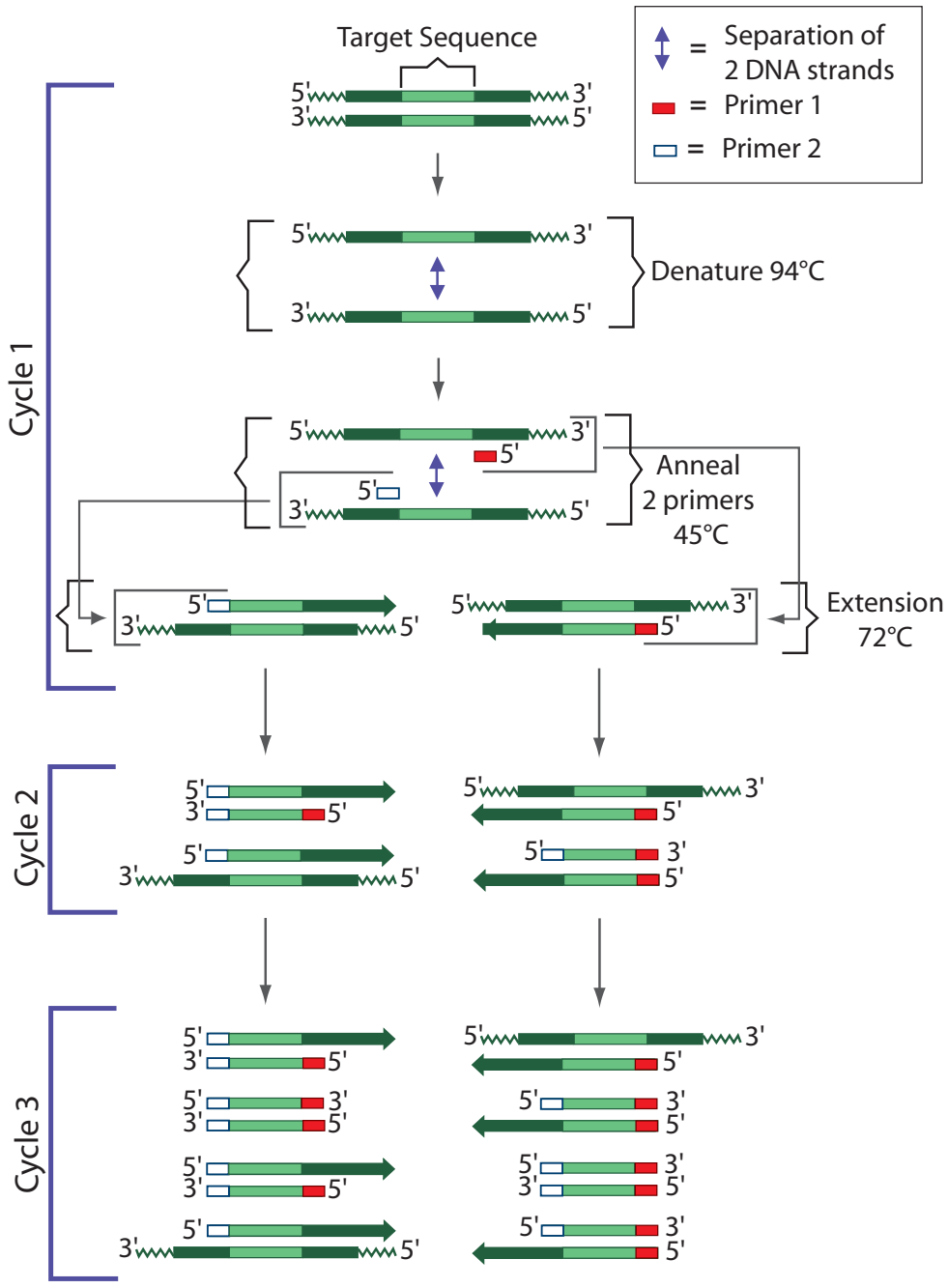


Figure 5: The Polymerase Chain Reaction

DNA Fingerprinting

as "primers". These components, together with the DNA to be amplified, are incubated in an appropriate buffer that contains Mg2+. The primers are designed to correspond to the start and end of the DNA to be amplified, known as the "target".

The PCR reaction mixture (which contains the DNA polymerase, buffer, deoxynucleotides, primers, and template) is subjected to sequential heating/cooling cycles at three different temperatures (Figure 5).

- In the first step, the template is heated to near boiling (92° - 96°C.) to denature or "melt" the DNA. This step, known as "denaturation" disrupts the hydrogen bonds between the two complimentary DNA strands and causes their separation.
- In the second PCR step, the mixture is cooled to a temperature that is typically in the range of 45° - 65°. In this step, known as "annealing", the primers, present in great excess to the template, bind to the separated DNA strands.
- In the third PCR step, known as "extension", the temperature is raised to an intermediate value, usually 72°C. At this temperature the *Taq* polymerase is maximally active and adds nucleotides to the primers to complete the synthesis of the new complimentary strands.

DNA fingerprinting analysis has become increasingly significant in court cases involving murder, rape, physical battery, and other types of crimes. Jurors are often asked to determine the validity of DNA evidence, resulting in both acquittals and convictions of suspected criminals. To ensure greater accuracy, scientists have incorporated standardization procedures in DNA analysis. Standard DNA Fragments are used to determine the exact size of individual DNA fragments in a DNA fingerprint. It is generally accepted that DNA fingerprints are identical only in the case of identical twins.

In this experiment, emphasis is placed on concepts related to RFLP analysis. The experiment activities will focus on the identification of DNA by analyzing restriction fragmentation patterns separated by agarose gel electrophoresis.

THIS EXPERIMENT DOES NOT CONTAIN HUMAN DNA.

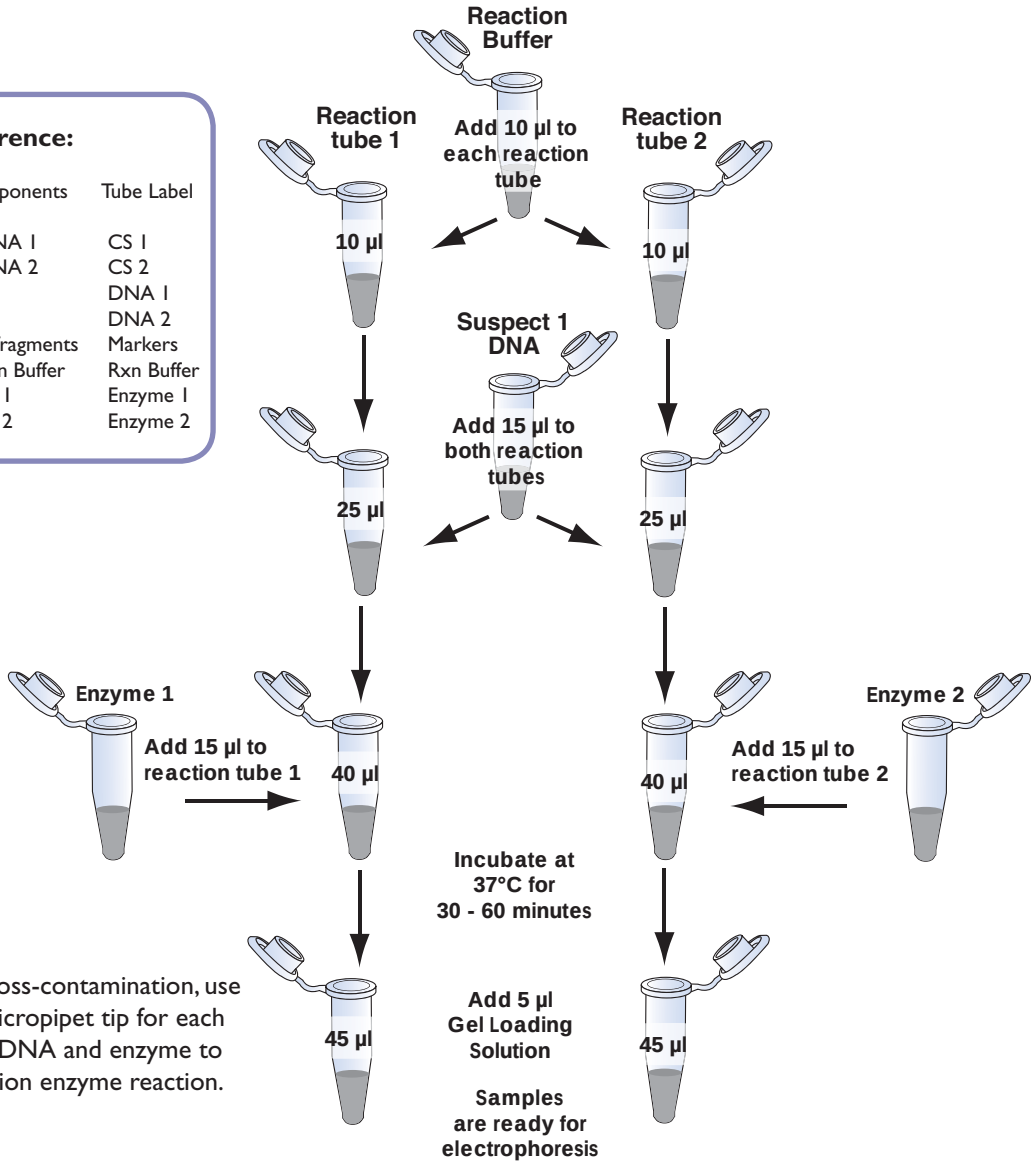
The Experiment

Crime Scene Investigation - Restriction Enzyme Digestion

In this experiment, the DNA from two suspects are each digested with two restriction enzymes in separate reactions and compared to crime scene samples after agarose gel electrophoresis. This flow chart outlines the procedure used for the restriction enzyme digestion of DNA obtained from Suspect 1. The DNA from Suspect 2 is digested in the same manner, using reaction tubes 3 and 4 (not shown).

Quick Reference:

Dispensed Components	Tube Label
Crime scene DNA 1	CS 1
Crime scene DNA 2	CS 2
Suspect 1 DNA	DNA 1
Suspect 2 DNA	DNA 2
Standard DNA fragments	Markers
Enzyme Reaction Buffer	Rxn Buffer
Diluted Enzyme 1	Enzyme 1
Diluted Enzyme 2	Enzyme 2



To avoid cross-contamination, use a FRESH micropipet tip for each transfer of DNA and enzyme to the restriction enzyme reaction.

Restriction Enzyme Digestion

The enzymes used in this experiment are stored and shipped in Dryzyme™ form (lyophilized). A buffer has been added to reconstitute the enzyme to liquid form.

1. Label microtest tubes 1 through 4 for four restriction enzyme digestion reactions. Put your initials or group number on the tubes.
2. Tap all the tubes (see Quick Reference at left) on the lab bench to collect all the contents at the bottom of the tube.
3. Use an automatic micropipet to dispense 10 µl of Enzyme Reaction Buffer (Rxn Buffer) to each of four reaction tubes labeled 1 through 4.
4. Add DNA and enzyme to the reaction tubes as summarized in Chart 1. Use a FRESH micropipet tip for each transfer of DNA and enzyme.

Chart 1: Summary of Restriction Enzyme Digestion Reactions

	Reaction Tube	Reaction Buffer	DNA 1 (µl)	DNA 2 (µl)	Enzyme 1 (µl)	Enzyme 2 (µl)	Final Volume (µl)
Crime Scene Samples	Crime Scene DNA, cut with enzyme 1 ready for electrophoresis				X	--	45 *
	Crime Scene DNA, cut with enzyme 2 ready for electrophoresis				--	X	45 *
Suspect 1	1	10	15	--	15	--	40
	2	10	15	--	--	15	40
Suspect 2	3	10	--	15	15	--	40
	4	10	--	15	--	15	40

* 10x Gel loading solution has already been added to the crime scene samples.

5. Cap the reaction tubes and tap gently to mix. Then tap each tube on the lab bench to collect contents at the bottom.
6. Incubate reaction tubes in a 37°C waterbath for 30 minutes (or 60 minutes if time allows).

After the 30 or 60 minute incubation is completed:

7. Add 5 µl of 10x gel loading solution to reaction tubes 1 - 4 to stop the reactions. Cap and mix by tapping.

OPTIONAL STOPPING POINT

After addition of 10x gel loading solution to stop the reaction, samples are ready for electrophoresis. Samples may be stored in the refrigerator for electrophoresis.

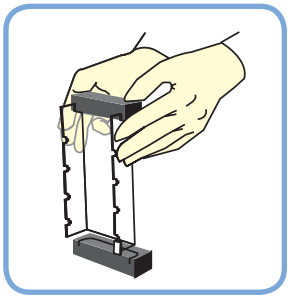
Electrophoresis - Agarose Gel Preparation

AGAROSE GEL REQUIREMENTS FOR THIS EXPERIMENT

- Recommended gel size: 7 x 7 cm or 7 x 14 cm
- Number of sample wells required: 7 - 8
- Well-former template (comb):

  For EDVOTEK units:
  7 x 14 cm gel - two standard 6-well combs in the first and middle set of notches
  7 x 7 cm gel - one 8-well comb in the first set of notches
- Agarose gel concentration: 0.8%

PREPARING THE GEL BED

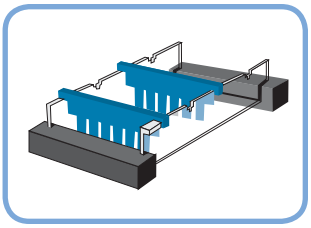


1. Close off the open ends of a clean and dry gel bed (casting tray) by using rubber dams or tape.

   A. Using Rubber dams:
   - Place a rubber dam on each end of the bed. Make sure the rubber dam fits firmly in contact with the sides and bottom of the bed.

   B. Taping with labeling or masking tape:
   - With 3/4 inch wide tape, extend the tape over the sides and bottom edge of the bed.
   - Fold the extended edges of the tape back onto the sides and bottom. Press contact points firmly to form a good seal.

2. Placement of well-former template (comb):
   For EDVOTEK units:
   7 x 14 cm gel - two standard 6-well combs in the first and middle set of notches
   7 x 7 cm gel - one 8-well comb in the first set of notches



Electrophoresis - Agarose Gel Preparation

CASTING THE AGAROSE GEL(S)

3. Use a 250 ml flask or beaker to prepare the gel solution.

IMPORTANT

Check with your instructor regarding the concentration of the buffer you are using to prepare your gel. Use the appropriate table (A.1 or A. 2) below.

If preparing the gel with concentrated (50x) buffer, use Table A.1.

Table A.1

Individual 0.8%* UltraSpec-Agarose™ Gel
DNA Staining with InstaStain® MetBlue

Size of Gel (cm)	Amt of Agarose (g)	+	Concentrated Buffer (50x) (ml)	+	Distilled Water (ml)	=	Total Volume (ml)
7 x 7	0.23		0.6		29.4		30
7 x 14	0.46		1.2		58.8		60

*0.77% UltraSpec-Agarose™ gel percentage rounded up to 0.8%

If preparing the gel with diluted (1x) buffer, use Table A.2.

Diluted buffer is one volume of concentrated buffer to every 49 volumes of distilled or deionized water. See Table B.

Table A.2

Individual 0.8%* UltraSpec-Agarose™ Gel
DNA Staining with InstaStain® MetBlue

Size of Gel (cm)	Amt of Agarose (g)	+	Diluted Buffer (1x) (ml)
7 x 7	0.23		30
7 x 14	0.46		60

*0.77% UltraSpec-Agarose™ gel percentage rounded up to 0.8%

4. Swirl the mixture to disperse clumps of agarose powder.

5. With a marking pen, indicate the level of the solution volume on the outside of the flask.

Electrophoresis - Agarose Gel Preparation

The Experiment

At high altitudes, it is recommended to use a microwave oven to reach boiling temperatures.

6. Heat the mixture to dissolve the agarose powder. The final solution should appear clear (like water) without any undissolved particles.

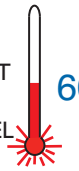
   A. Microwave method:
   - Cover the flask with plastic wrap to minimize evaporation.
   - Heat the mixture on High for 1 minute.
   - Swirl the mixture and heat on High in bursts of 25 seconds until all the agarose is completely dissolved.

   B. Hot plate method:
   - Cover the flask with aluminum foil to prevent excess evaporation.
   - Heat the mixture to boiling over a burner with occasional swirling. Boil until all the agarose is completely dissolved.

   Check the solution carefully. If you see "crystal" particles, the agarose is not completely dissolved.

7. Cool the agarose solution to 60°C with careful swirling to promote even dissipation of heat. If detectable evaporation has occurred, add distilled water to bring the solution up to the original volume marked on the flask in step 6.

DO NOT POUR BOILING HOT AGAROSE INTO THE GEL BED.



60˚C

Hot agarose solution may irreversibly warp the bed.

After the gel is cooled to 60°C:

If you are using rubber dams, go to step 9.

If you are using tape, continue with step 8.

8. Seal the interface of the gel bed and tape to prevent the agarose solution from leaking.
   - Use a transfer pipet to deposit a small amount of cooled agarose to both inside ends of the bed.
   - Wait approximately 1 minute for the agarose to solidify.

9. Pour the cooled agarose solution into the bed. Make sure the bed is on a level surface.

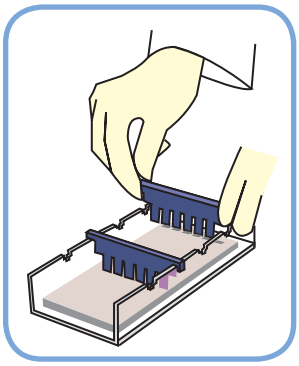
10. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes.

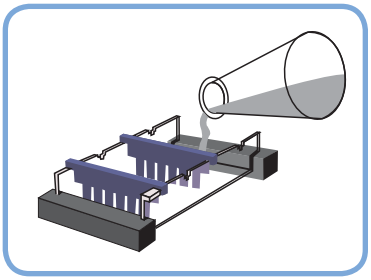
Electrophoresis - Agarose Gel Preparation

PREPARING THE GEL FOR ELECTROPHORESIS

11. After the gel is completely solidified, carefully and slowly remove the rubber dams or tape from the gel bed.

    Be especially careful not to damage or tear the gel wells when removing the rubber dams. A thin plastic knife, spatula or pipet tip can be inserted between the gel and the dams to break possible surface tension.





12. Remove the comb by slowly pulling straight up. Do this carefully and evenly to prevent tearing the sample wells.

13. Place the gel (on its bed) into the electrophoresis chamber, properly oriented, centered and level on the platform.

14. Fill the electrophoresis apparatus chamber with the appropriate amount of diluted (1x) electrophoresis buffer.

15. Make sure that the gel is completely submerged under buffer before proceeding to loading the samples and conducting electrophoresis.

For DNA analysis, the recommended electrophoresis buffer is Tris-acetate-EDTA, pH 7.8. The formula for diluting EDVOTEK (50x) concentrated buffer is one volume of buffer concentrate to every 49 volumes of distilled or deionized water. Prepare buffer as required for your electrophoresis unit.

IMPORTANT: Check with your instructor to determine if the buffer has previously been diluted. Pour the appropriate amount of 1x buffer into the electrophoresis chamber according to Table B below.

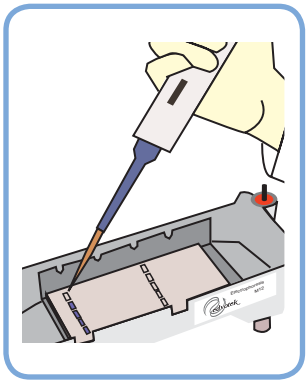
Table B

Electrophoresis (Chamber) Buffer

EDVOTEK Model #	Total Volume Required (ml)	Dilution: 50x Conc. Buffer (ml)	+ Distilled Water (ml)
M6+	300	6	294
M12	400	8	392
M36 (blue)	500	10	490
M36 (clear)	1000	20	980

The Experiment

Electrophoresis - Conducting Electrophoresis



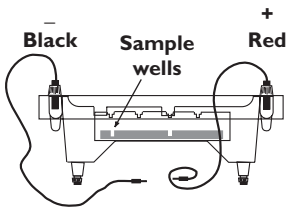
LOAD THE SAMPLES

1. Optional Step:
   Heat the samples, including the Standard DNA fragments for two minutes a 65°C. Allow the samples to cool for a few minutes.

2. Load 40 µl of each of the DNA samples in the following manner (7 x 14 cm gel):

Reminder:

During electrophoresis, the DNA samples migrate through the agarose gel towards the positive electrode. Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.



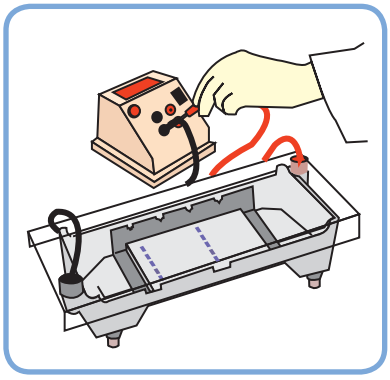
First Row

Lane	Tube	
1	Markers	Standard DNA Fragments
2	CS 1	DNA from crime scene cut with Enzyme 1
3	CS 2	DNA from crime scene cut with Enzyme 2
4	1	DNA from Suspect 1 cut with Enzyme 1
5	2	DNA from Suspect 1 cut with Enzyme 2

Second Row

Lane	Tube	
1	Markers	Standard DNA Fragments
2	3	DNA from Suspect 2 cut with Enzyme 1
3	4	DNA from Suspect 2 cut with Enzyme 2

RUNNING THE GEL



1. After the DNA samples are loaded, carefully snap the cover down onto the electrode terminals.

   Make sure that the negative and positive color-coded indicators on the cover and apparatus chamber are properly oriented.

2. Insert the plug of the black wire into the black input of the power source (negative input). Insert the plug of the red wire into the red input of the power source (positive input).

Electrophoresis - Conducting Electrophoresis

3. Set the power source at the required voltage and conduct electrophoresis for the length of time determined by your instructor. General guidelines are presented in Table C.

Table C
Time and Voltage Recommendations

Volts	EDVOTEK Electrophoresis Model M6+ Minimum / Maximum	EDVOTEK Electrophoresis Model M12 & M36 Minimum / Maximum
150	15 / 20 min	25 / 35 min
125	20 / 30 min	35 / 45 min
70	35 / 45 min	60 / 90 min
50	50 / 80 min	95 / 130 min

4. Check to see that current is flowing properly - you should see bubbles forming on the two platinum electrodes.

5. After the electrophoresis is completed, turn off the power, unplug the power source, disconnect the leads and remove the cover.

6. Remove the gel from the bed for staining.

ABOUT DNA GEL STAINING

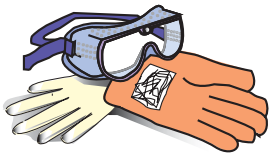
After electrophoresis, the agarose gels require staining in order to visualize the separated DNA samples. This experiment features a proprietary stain called InstaStain® Methylene Blue. Two options are provided for using the InstaStain® Methylene Blue cards. Check with your instructor regarding which staining method you should use.

Method 1: One-step Staining and Destaining with InstaStain® MetBlue

Method 2: Staining with InstaStain® Methylene Blue

Electrophoresis - Staining and Visualization of DNA

The Experiment



Wear gloves and safety goggles

Do not stain gel(s) in the electrophoresis apparatus.

METHOD 1: ONE-STEP STAINING AND DESTAINING WITH INSTASTAIN® METHYLENE BLUE

Agarose gels can be stained and destained in one easy step with InstaStain™ Methylene Blue cards. This one-step method can be completed in approximately 3 hours, or can be left overnight.

1. Remove the 7 x 14 cm agarose gel from its bed and completely submerse the gel in a small, clean tray containing 150 ml of distilled or deionized water, or used electrophoresis buffer. The agarose gel should be completely covered with liquid.

   Examples of small trays include large weigh boats, or small plastic food containers

2. Gently float two 7 x 7 cm cards of InstaStain® MetBlue with the stain side (blue) facing the liquid.
3. Let the gel soak undisturbed in the liquid for approximately 3 hours. The gel can be left in the liquid overnight (cover with plastic wrap to prevent evaporation).
4. After staining and destaining, the gel is ready for visualization and photography.

STORAGE AND DISPOSAL OF INSTASTAIN® METHYLENE BLUE CARDS AND GELS

- Stained gels may be stored in the refrigerator for several weeks. Place the gel in a sealable plastic bag with destaining liquid.

  DO NOT FREEZE AGAROSE GELS!

- Used InstaStain® cards and destained gels can be discarded in solid waste disposal.
- Destaining solutions can be disposed down the drain.

Electrophoresis - Staining and Visualization of DNA

Wear gloves and safety goggles

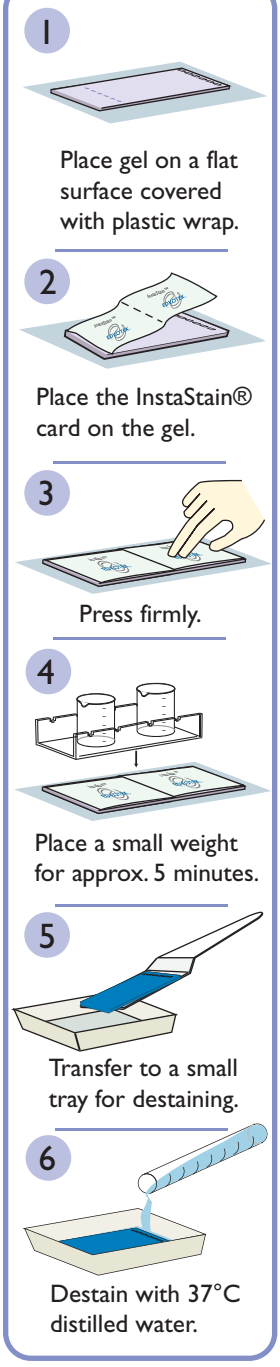
METHOD 2: STAINING WITH INSTASTAIN® METHYLENE BLUE CARDS

1. After electrophoresis, place the agarose gel on a flat surface covered with plastic wrap.
2. Wearing gloves, place the blue dye side of two InstaStain® Methylene Blue cards on the gel.
3. Firmly run your fingers several times over the entire surface of the InstaStain® cards to establish good contact between the InstaStain® cards and the gel.
4. To ensure continuous contact between the gel and the InstaStain® cards, place a gel casting tray and weight, such as a small empty beaker, on top of the InstaStain® cards.
5. Allow the InstaStain® Methylene Blue to sit on the gel for 5 to 10 minutes.
6. After staining, remove the InstaStain® cards.

   If the color of the gel appears very light, wet the gel surface with buffer or distilled water and place the InstaStain® cards back on the gel for an additional 5 minutes.

Destaining and Visualization of DNA

7. Transfer the gel to a large weigh boat or small plastic container.
8. Destain with distilled water.*
   - Add approximately 150 ml of distilled water to cover the gel.
9. Repeat destaining by changing the distilled water as needed.



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The Experiment

Electrophoresis - Staining and Visualization of DNA

The larger DNA bands will initially be visible as dark blue bands against a lighter blue background. When the gel is completely destained, the larger DNA bands will become sharper and the smaller bands will be visible. With additional destaining, the entire background will become uniformly light blue.

10. Carefully remove the gel from the destain solution and examine the gel on a Visible Light Gel Visualization System. To optimize visibility, use the amber filter provided with EDVOTEK equipment.

11. If the gel is too light and bands are difficult to see, repeat the staining and destaining procedures.

* Destaining Notes

- Warmed distilled water at 37°C will accelerate destaining. Destaining will take longer with room temperature water.
- DO NOT EXCEED 37°C ! Warmer temperatures will soften the gel and may cause it to break.
- The volume of distilled water for destaining depends upon the size of the tray. Use the smallest tray available that will accommodate the gel. The gel should be completely submerged during destaining.
- Do not exceed 3 changes of water for destaining. Excessive destaining will cause the bands to be very light.

STORAGE AND DISPOSAL OF INSTASTAIN® METHYLENE BLUE CARDS AND GELS

- Stained gels may be stored in the refrigerator for several weeks. Place the gel in a sealable plastic bag with destaining liquid.

  DO NOT FREEZE AGAROSE GELS!

- Used InstaStain® cards and destained gels can be discarded in solid waste disposal.
- Destaining solutions can be disposed down the drain.