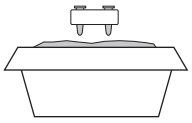
Forensic DNA Fingerprinting Kit Quick Guide

Lesson 1 Restriction Digestion

1. Place the tube containing the restriction enzyme mix, labeled ENZ, on ice.

ENZ

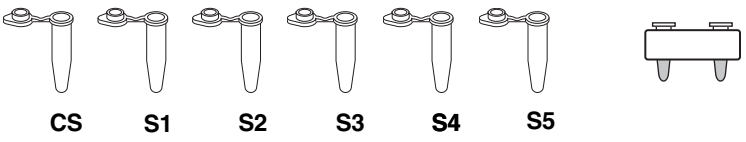


Ice

2. Label one of each colored micro test tubes as follows:

green tube	**CS** (crime scene)
blue tube	**S1** (suspect 1)
orange tube	**S2** (suspect 2)
violet tube	**S3** (suspect 3)
red tube	**S4** (suspect 4)
yellow tube	**S5** (suspect 5)

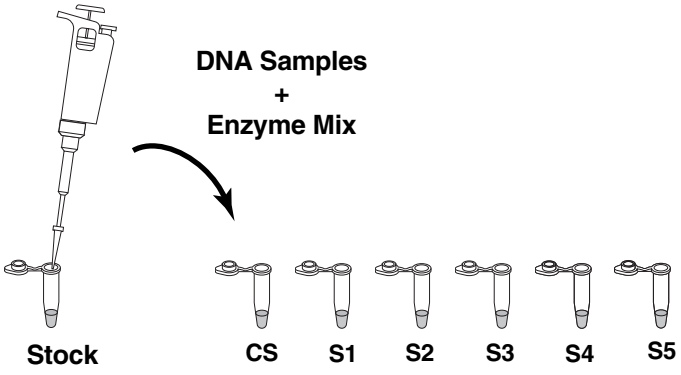
Label the tubes with your name, date, and lab period. Place the tubes in the foam micro test tube holder.



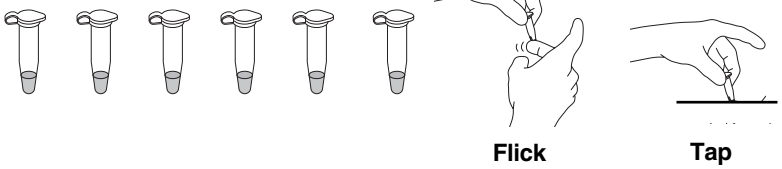
CS S1 S2 S3 S4 S5

3. Using a fresh tip for each sample, pipet 10 µl of each DNA sample from the stock tubes and transfer to the corresponding colored micro test tubes. Make sure the sample is transferred to the bottom of the tubes.

4. Pipet 10 µl of enzyme mix (ENZ) into the very bottom of each tube. Use a fresh tip to transfer the ENZ sample to each tube. Pipet up and down carefully to mix well.

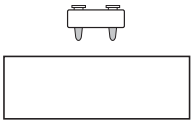


5. Tightly cap the tubes and mix the components by gently flicking the tubes with your finger. If a microcentrifuge is available, pulse-spin in the centrifuge to collect all the liquid in the bottom of the tube. Otherwise, gently tap the tube on the table top.



Flick Tap

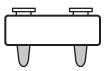
6. Place the tubes in the foam micro tube holder and incubate for 45 min at 37°C or overnight at room temperature in a large volume of water heated to 37°C.



Water bath

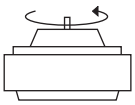
7. If required, follow the instructors directions to pour a 1% agarose gel.

8. After the incubation period, remove the tubes from the water bath and place in the refrigerator until the next laboratory period. If there is sufficient time to continue, proceed directly to step 2 of Lesson 2.



Lesson 2 Agarose Gel Electrophoresis

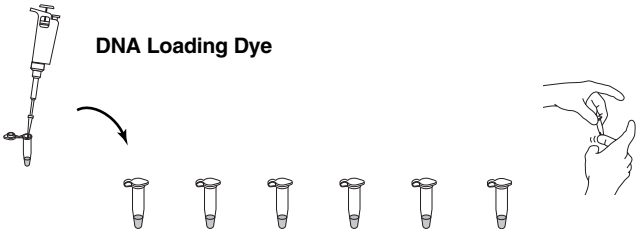
1. Remove the digested DNA samples from the refrigerator (if applicable).



Centrifuge

2. If a centrifuge is available, pulse spin the tubes in the centrifuge to bring all of the liquid into the bottom of the tube or gently tap on the table top.

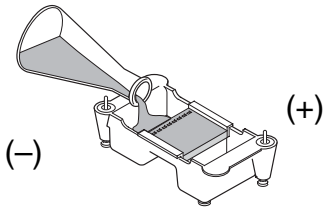
3. Using a separate tip for each sample, add 5 µl of loading dye "LD" into each tube. Cap the tubes and mix by gently flicking the tube with your finger. Collect the sample at the bottom of the tube by tapping it gently on the table or by pulse-spinning in a centrifuge.



DNA Loading Dye

4. Remove the agarose gel from the refrigerator (if applicable) and remove the plastic wrap.

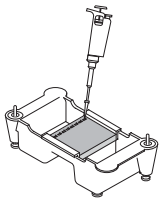
5. Place the agarose gel in the electrophoresis apparatus. Fill the electrophoresis chamber with 1x TAE buffer* to cover the gel, using approximately 275 ml of buffer for a Bio-Rad Mini-Sub Cell, horizontal electrophoresis chamber.



(–) (+)

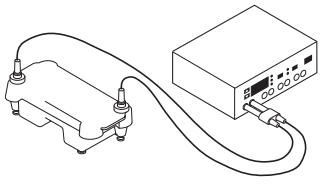
6. Check that the wells of the agarose gels are near the black (–) electrode and the bottom edge of the gel is near the red (+) electrode.

7. Using a separate tip for each sample, load the indicated volume of each sample into 7 wells of the gel in the following order:



Lane 1: **M**, DNA size marker, 10 µl
Lane 2: **CS**, green tube, 20 µl
Lane 3: **S1**, blue tube, 20 µl
Lane 4: **S2**, orange tube, 20 µl
Lane 5: **S3**, violet tube, 20 µl
Lane 6: **S4**, red tube, 20 µl
Lane 7: **S5**, yellow tube, 20 µl

8. Carefully place the lid on the electrophoresis chamber. The lid will attach to the base in only one orientation. The red and black jacks on the lid of the horizontal electrophoresis chambers will match with the red and black jacks on the base. Plug the electrodes into the power supply, red to red and black to black.

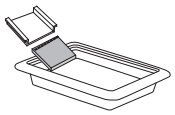


9. Turn on the power and electrophorese your samples at 100 V for 30 minutes.

* or 0.25x TAE if using the Fast Gel Protocol

Visualization of DNA Fragments

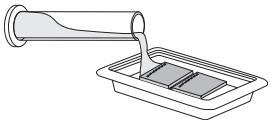
1. When the electrophoresis run is complete, turn off the power and remove the top of the chamber. Carefully remove the gel and tray from the gel box. Be careful — the gel is very slippery. Slide the gel into the staining tray.



2. You have two options for staining your gel:

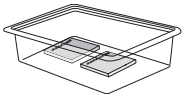
   Quick staining (requires 12–15 minutes)

   a. Add 120 ml of **100x** Fast Blast DNA stain into a staining tray (2 gels per tray).



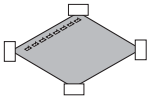
   b. Stain the gels for 2 minutes with gentle agitation. Save the used stain for future use.

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



   d. Destain by washing twice in warm tap water for 5 minutes each with gentle shaking for best results.

   e. Record results.

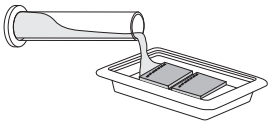


   f. Trim away any unloaded lanes.

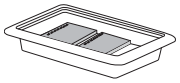
   g. Air-dry the gel on gel support film and tape the dried gel into your laboratory notebook.

   Overnight staining

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



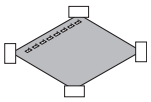
   b. Let the gels stain overnight, with gentle shaking for best results. No destaining is required.



   c. Pour off the stain into a waste beaker.

   d. Record results.

   e. Trim away any unloaded lanes.



   f. Air-dry the gel on gel support film and tape the dried gel into your laboratory notebook.

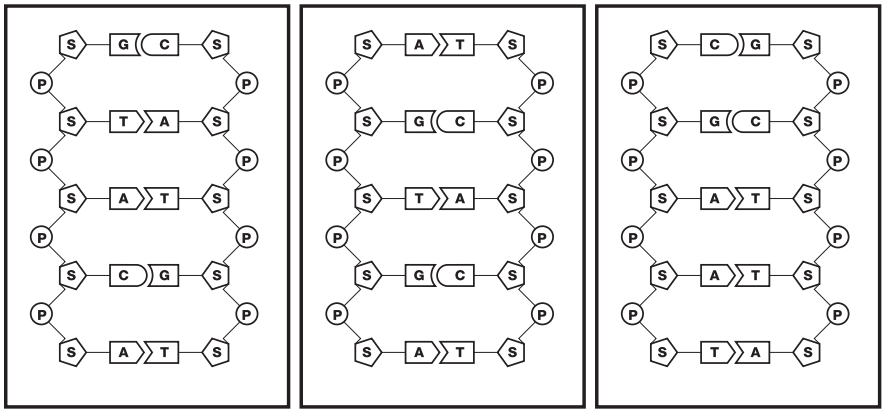
Student Manual

Pre-Lab Introduction to DNA Fingerprinting

You are about to perform a procedure known as DNA fingerprinting. The data obtained may allow you to determine if the samples of DNA that you will be provided with are from the same individual or from different individuals. For this experiment it is necessary to review the structure of DNA molecules.

DNA consists of a series of nitrogenous base molecules held together by weak hydrogen bonds. These base pairs are in turn bonded to a sugar-phosphate backbone. The four nitrogenous bases are **adenine**, **thymine**, **guanine**, and **cytosine** (**A**, **T**, **G**, and **C**). Remember the base-pairing rule is **A** - **T** and **G** - **C**. Refer to the figure below of a DNA molecule.

The Structure of DNA



The schematics above represent a very small section of DNA from three different individuals. In this representation of DNA the symbol system is as follows:

Backbone:

S = Five carbon sugar molecule known as deoxyribose

P = Phosphate group

DNA Nucleotide Bases:

A = adenine **C** = cytosine **G** = guanine **T** = thymine

Analysis of the three DNA samples above (see next page) might help us detect similarities and differences in samples of DNA from different people.

Pre-Lab Focus Questions: Introduction to DNA Fingerprinting

Consideration What is the structure of DNA?

1. Compare the "backbone" of the sugar-phosphate arrangement in the side chains of all three figures. Are there any differences?

2. In the above figure, do all three samples contain the same bases? Describe your observations.

3. Are the bases paired in an identical manner in all three samples? Describe the pattern of the base pair bonding.

4. In your attempt to analyze DNA samples from three different individuals, what conclusions can you make about the similarities and differences of the DNA samples?

5. What will you need to compare between these DNA samples to determine if they are identical or non-identical?

Lesson 2 Agarose Gel Electrophoresis (Laboratory Procedure)

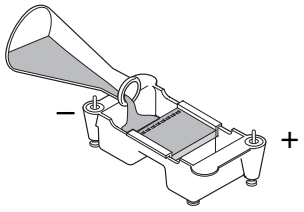
1. Obtain a prepoured agarose gel from your teacher, or if your teacher instructs you to do so, prepare your own gel.
2. After preparing the gel, remove your digested samples from the refrigerator.

   Using a new tip for each sample add 5 µl of sample loading dye "LD" to each tube:

DNA Samples	Loading dye
Crime Scene [CS]	5 µl
Suspect 1 [S1]	5 µl
Suspect 2 [S2]	5 µl
Suspect 3 [S3]	5 µl
Suspect 4 [S4]	5 µl
Suspect 5 [S5]	5 µl

   Tightly cap each tube. Mix the components by gently flicking the tubes with your finger. If a centrifuge is available, pulse spin the tubes to bring the contents to the bottom of the tube. Otherwise, gently tap the tubes on the table top.

3. Place the casting tray with the solidified gel in it, into the platform in the gel box. The wells should be at the (–) cathode end of the box, where the black lead is connected. Very carefully, remove the comb from the gel by pulling it straight up.
4. Pour ~ 275 ml of electrophoresis buffer into the electrophoresis chamber. Pour buffer in the gel box until it **just covers** the wells of the gel by 1–2 mm.



5. Obtain the tube of *Hin*dIII lambda digest (DNA marker). The loading dye should already have been added by your instructor.

6. Using a separate pipet tip for each sample, load your digested DNA samples into the gel. Gels are read from left to right. The first sample is loaded in the well at the left hand corner of the gel.

   Lane 1: *Hin*dIII DNA size marker, clear tube, 10 µl

   Lane 2: CS, green tube, 20 µl

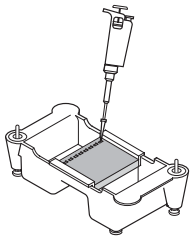
   Lane 3: S1, blue tube, 20 µl

   Lane 4: S2, orange tube, 20 µl

   Lane 5: S3, violet tube, 20 µl

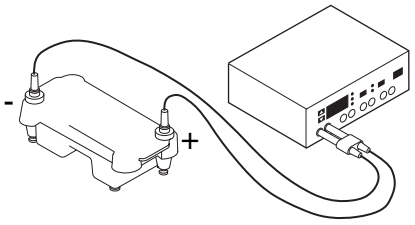
   Lane 6: S4, red tube, 20 µl

   Lane 7: S5, yellow tube, 20 µl



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 electrical leads to the power supply.

8. Turn on the power supply. Set it for 100 V and electrophorese the samples for at least 30 min. The gel can be run for up to 40 min to improve resolution if the time is available. The Fast Gel Protocol in Appendix D allows the gel to be run in 20 min at 200 V.



While you are waiting for the gel to run, you may begin the review questions on the following page.

9. When the electrophoresis is complete, turn off the power supply and remove the lid from the gel box. Carefully remove the gel tray and the gel from the electrophoresis chamber. Be careful, the gel is very slippery! Proceed to pg 35 for detailed instructions on staining your gel.

Lesson 2 Agarose Gel Electrophoresis

Review Questions

1. The electrophoresis apparatus creates an electrical field with positive and negative poles at the ends of the gel. DNA molecules are negatively charged. To which electrode pole of the electrophoresis field would you expect DNA to migrate? (+ or -)? Explain.

2. What color represents the negative pole?

3. After DNA samples are loaded into the sample wells, they are "forced" to move through the gel matrix. What size fragments (large vs. small) would you expect to move toward the opposite end of the gel most quickly? Explain.

4. Which fragments (large vs. small) are expected to travel the shortest distance from the well? Explain.

Staining DNA with Fast Blast DNA Stain (Laboratory Procedure)

Consideration Are any of the DNA samples from the suspects the same as that of the individual at the crime scene?

Take a moment to think about how you will perform the analysis of your gel. In the final two steps, you will:

A. Visualize DNA fragments in your gel.

B. Analyze the number and positions of visible DNA bands on your gel.

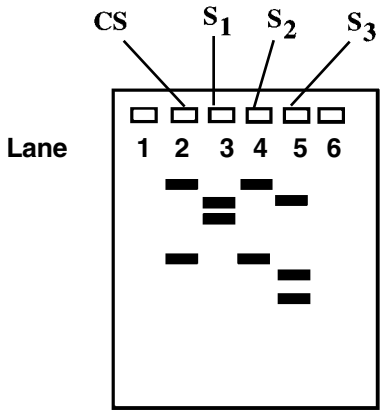
Making DNA Fragments Visible

Since DNA is naturally colorless, it is not immediately visible in the gel. Unaided visual examination of the gel after electrophoresis indicates only the positions of the loading dyes and not the positions of the DNA fragments. DNA fragments are visualized by staining the gel with a blue stain called Fast Blast DNA stain. The blue stain molecules are positively charged and have a high affinity for the DNA. These blue stain molecules strongly bind to the DNA fragments and allow DNA to become visible. These visible bands of DNA may then be traced, photographed, sketched, or retained as a permanently dried gel for analysis. Detained instruction on staining your gel are found on the following pages.

The drawing below represents an example of a stained DNA gel after electrophoresis. For fingerprinting analysis, the following information is important to remember:

- Each lane has a different sample of DNA
- Each DNA sample was treated with the same restriction endonucleases.

With reference to the numbered lanes, analyze the bands in the gel drawing below, then answer the questions on page 40. Note that this picture is an example and it may not correspond to the pattern of bands that you will see in the lab.



Staining DNA with Fast Blast DNA Stain (Laboratory Procedure)

There are two protocols for using Fast Blast DNA stain in the classroom. Use option 1 for quick staining of gels to visualize DNA bands in 12–15 minutes, and option 2 for overnight staining. Depending on the amount of time available, your teacher will decide which protocol to use. Two student teams will stain the gels per staining tray (you may want to notch gel corners for identification). Mark staining trays with initials and class period before beginning this activity.

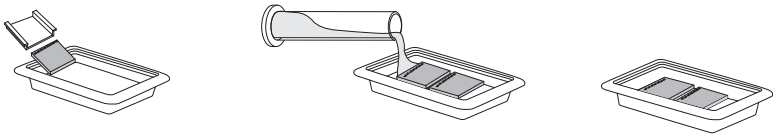
WARNING
Although Fast Blast DNA stain is nontoxic and noncarcinogenic, latex or vinyl gloves should be worn while handling the stain or stained gels to keep hands from becoming stained blue. Lab coats or other protective clothing should be worn to avoid staining clothes.

Protocol 1: Quick Staining of Agarose Gels in 100x Fast Blast DNA Stain

This protocol allows quick visualization of DNA bands in agarose gels within 15 minutes. For quick staining, Fast Blast DNA stain (500x) should be diluted to a **100x** concentration. We recommend using 120 ml of 100x Fast Blast to stain two 7 x 7 cm or 7 x 10 cm agarose gels in individual staining trays provided in Bio-Rad's education kits. If alternative staining trays are used, add a sufficient volume of staining solution to completely submerge the gels.

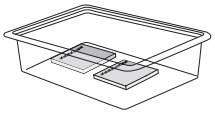
Following electrophoresis, agarose gels must be removed from their gel trays before being placed in the staining solution. This is easily accomplished by holding the base of the gel tray in one hand and gently pushing out the gel with the thumb of the other hand. Because the gel is fragile, special attention must be given when handling it. We highly recommend using a large spatula or other supportive surface to transfer the gel from one container to another. Destaining requires the use of at least one large-volume container, capable of holding at least 500 ml, at each student workstation. Each student team may utilize separate washing containers for each wash step, or simply use a single container that is emptied after each wash and refilled for the next wash.

1. Label the staining trays with your initials and class period. You will stain 2 gels per tray.
2. **Stain gels**
   Remove each gel from the gel tray and carefully slide it into the staining tray. Pour approximately 120 ml of 100x stain into the staining tray. If necessary, add more 100x stain to completely submerge the gels. Stain the gels for 2–3 minutes, but not for more than 3 minutes. Using a funnel, pour the 100x stain into a storage bottle and save it for future use. **The stain can be reused at least 7 times**.



2–3 minutes

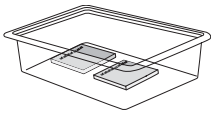
3. **Rinse gels**
   Transfer the gels into a large container containing 500–700 ml of clean, warm (40–55°C) tap water. Gently shake the gel in the water for ~10 seconds to rinse.



10 seconds

4. Wash gels

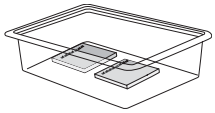
Transfer the gel into a large container with 500–700 ml of clean, warm tap water. Gently rock or shake the gel on a rocking platform for 5 minutes. If no rocking platform is available, move the gels gently in the water once every minute.



5 minutes

5. Wash gels

Perform a second wash as in step 4.



5 minutes

6. Record results

Pour off the water and examine the stained gels for expected DNA bands. The bands may appear fuzzy immediately after the second wash, but will begin to develop into sharper bands within 5–15 minutes after the second wash. This is due to Fast Blast stain molecules migrating into the gel and binding more tightly to the DNA.

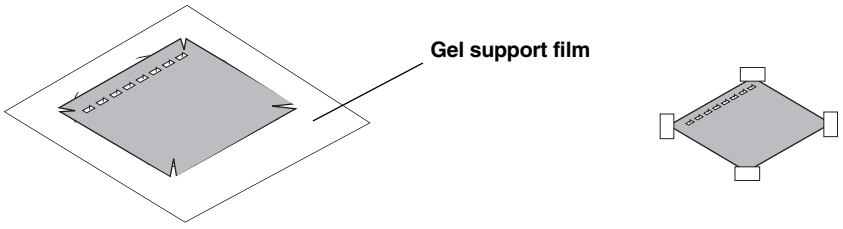
To obtain maximum contrast, additional washes in warm water may be necessary. Destain to the desired level, but do not wash the gel in water overnight. If you cannot complete the destaining in the allocated time, you may transfer the gel to 1x Fast Blast stain for overnight staining. **See Protocol 2**.

a. Place your gel on a light background and record your results by making a diagram as follows. Place a clear sheet of plastic sheet or acetate over the gel. With a permanent marker, trace the wells and band patterns onto the plastic sheet to make a replica picture of your gel. Remove the plastic sheet for later analysis. Alternatively, gels can be photocopied on a yellow piece of transparent film for optimal contrast.

b. Dry the agarose gel as a permanent record of the experiment.

   i. Trim away any unloaded lanes with a knife or razor blade. Cut your gel from top to bottom to remove the lanes that you did not load samples into, leaving only lanes 1–4.

   ii. Place the gel directly upon the hydrophilic size of a piece of gel support film. (Water will form beads on the hydrophobic side of a piece of gel support film.) Center the gel on the film and remove bubbles that may form between the gel and film. Place the film on a paper towel and let the gel dry in a well-ventilated area, making sure to avoid direct exposure to light. As the gel dries it will bond to the film but will not shrink. If left undisturbed on the support film, the gel will dry completely at room temperature after 2–3 days. The result will be a flat, transparent, and durable record for the experiment.



Post-Lab: Thought Questions

1. What can you assume is contained within each band?

2. If this were a fingerprinting gel, how many samples of DNA can you assume were placed in each separate well?

3. What would be a logical explanation as to why there is more than one band of DNA for each of the samples?

4. What caused the DNA to become fragmented?

5. Which of the DNA samples have the same number of restriction sites for the restriction endonucleases used? Write the lane numbers.

6. Which sample has the smallest DNA fragment?

7. Assuming a circular piece of DNA (plasmid) was used as starting material, how many restriction sites were there in lane three?

8. From the gel drawing on page 35, which DNA samples appear to have been “cut” into the same number and size of fragments?

9. Based on your analysis of the example gel drawing on page 35, what is your conclusion about the DNA samples in the drawing? Do any of the samples seem to be from the same source? If so, which ones? Describe the evidence that supports your conclusion.