Adapting Bio-Rad's DNA Fingerprinting kit into a Pre-cut DNA Fingerprinting Activity to Teach About DNA Electrophoresis

This protocol adapts our DNA Fingerprinting kit (166-0007EDU) to a pre-cut, ready to load DNA Fingerprinting lab where students do not perform restriction enzyme digestion themselves. Students are given ready-to-load DNA samples from a crime scene and 5 suspects. They load, run and stain agarose gels, and then analyze their results to determine who did it. This adaptation requires that the instructor pre-digest the DNA samples provided in the kit, add DNA sample loading dye to the individual digests and dispense the DNA samples for their students. Instructions for the instructor's advanced preparation and student lab are provided below, however reference is made to the DNA Fingerprinting manual provided in the kit and also available to download at explorer.bio-rad.com (bulletin number 4006096).

The DNA Fingerprinting kit provides reagents for up to 8 teams of 4 students (i.e. 32 students in total). Each team runs their own gel and analyzes their own results. Using Bio-Rad's electrophoresis chambers (Mini-Sub® Cell GT cell Catalog #166-4000EDU) two teams can run their samples on a single gel with 2 sets of wells, minimizing the number of electrophoresis chambers and power supplies needed for this activity.

Materials Required

Material	Cat #	Number Required
DNA Fingerprinting Kit	166-0007EDU	1
Distilled or deionized water	N/A	3.5 liters
1–20 µl adjustable pipettes or (20 µl fixed volume pipettes and 10 µl fixed volume piettors)	166-0506EDU or (166-0513EDU and 166-0512EDU)	8
20–200 µl pipettes	166-0507EDU	1
200–1000 µl pipettes	166-0508EDU	1
Mini-Sub® Cell GT cell (Horizontal electrophoresis chamber)	166-4000EDU	4
PowerPac Basic™ power supply or PowerPac Junior™ power supplies	164-5050EDU or 165-5048EDU	1 or 2
Pipette tips (1–200 µl)	223-9035EDU	80
Pipette tips (100–1000 µl)		2
Waterbath* or Mini-incubator*	166-0524EDU or 166-0501EDU	1
ReadyAgarose Precast Mini Gels*	161-3057EDU	4

*optional

Instructors Advanced Preparation

1. Prepare 1x TAE buffer and agarose gels as directed on page 12 of the DNA Fingerprinting manual. Note: you may want the students to pour their own gels in a previous lesson.

2. Rehydrate each DNA sample in 200 µl of sterile water (provided) (detailed instructions on page 11 of the DNA Fingerprinting manual).

3. Rehydrate lyophilized EcoR1/Pst I enzyme mix in 750 µl of sterile water and put immediately on ice (detailed instructions on page 11 of the DNA Fingerprinting manual).

4. Set up bulk digests
   a. Label 6 colored microtubes 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

   b. Using a fresh tip for each DNA:

      Add 100 µl of Crime Scene DNA to the green tube
      Add 100 µl of Suspect 1 DNA to the blue tube
      Add 100 µl of Suspect 2 DNA to the orange tube
      Add 100 µl of Suspect 3 DNA to the violet tube
      Add 100 µl of Suspect 4 DNA to the red tube
      Add 100 µl of Suspect 5 DNA to the yellow tube

   c. Then using a fresh tip for each tube add 100 µl of enzyme to each tube. Pipet up and down to mix the DNA with the enzyme and discard the tip after each addition and firmly close the tube lids.

   d. Incubate the 6 tubes for 1 hour at 37°C or overnight at room temperature to allow the enzymes to digest the DNA.

   e. After the digestion is complete, using a fresh tip for each tube, add 50 µl of DNA sample loading dye to each tube and mix by pipetting up and down.

   f. Store these digests in the refrigerator (4°C) for up to 1 week or in the freezer (-20°C) for up to 6 months.

5. Prepare DNA size markers
   a. Add 20 µl of DNA sample loading dye to the HindIII lambda digest. This is the DNA size marker.

   Note: The resolution of the marker DNA is improved if it is heated to 65°C for 5 min after the addition of DNA sample loading dye.

6. Prepare the student samples
   a. Label 8 tubes of each color as follows:

clear tube	**M**	= DNA size marker
green tube	**CS**	
blue tube	**S1**	
orange tube	**S2**	
violet tube	**S3**	
red tube	**S4**	
yellow tube	**S5**	

b. Using a fresh tip for each new bulk DNA:

Add 15 µl of DNA size marker (HindIII lambda digest + loading buffer) to each of the 8 clear tubes.

Add 25 µl of bulk digested crime scene DNA to each of the 8 green tubes.

Add 25 µl of bulk digested suspect 1 DNA to each of the 8 blue tubes.

Add 25 µl of bulk digested suspect 2 DNA to each of the 8 orange tubes.

Add 25 µl of bulk digested suspect 3 DNA to each of the 8 violet tubes.

Add 25 µl of bulk digested suspect 4 DNA to each of the 8 red tubes.

Add 25 µl of bulk digested suspect 5 DNA to each of the 8 yellow tubes.

7. Prepare Fast Blast DNA Stain.

Fast Blast can be used in a concentrated form to stain the gels quickly (15–20 min) or can be used in a more diluted form to stain the gels overnight. Directions for the preparation and use of Fast Blast are on pages 15 and 37 of the DNA Fingerprinting manual and differ depending on whether the quick or overnight protocols are used. Please note that although Fast Blast DNA Stain is non-toxic it can stain skin, clothing and furniture so care must be taken when using this stain.

8. Set up student workstations:

DNA size marker (M) - clear tube

Crime Scene sample (CS) -green tube

Sample from suspect 1 (S1) - blue tube

Sample from suspect 2 (S2) - orange tube

Sample from suspect 3 (S3) - violet tube

Sample from suspect 4 (S4) - red tube

Sample from suspect 5 (S5) - yellow tube

Agarose gel in casting tray

Electrophoresis Buffer

1–20 µl adjustable volume pipettor or 20 µl and 10 µl fixed volume pipettors

Horizontal electrophoresis chamber

Pipet tips

Student Laboratory Procedure

1. If a centrifuge is available, pulse spin your colored microtubes to bring the contents to the bottom of the tube. Otherwise, gently tap the tubes on the table top.

2. Place the casting tray with the solidified gel in it, into the platform in the electrophoresis chamber. The wells should be at the (-) cathode end of the chamber, where the black lead is connected. If necessary, very carefully, remove the comb from the gel by gently pulling it straight up.

3. Pour ~ 275 ml of electrophoresis buffer into the electrophoresis chamber. Pour buffer in the chamber until it just covers the wells of the gel by 1–2 mm.

4. Using a fresh pipet tip load 10 µl of the DNA size marker (M) from the clear tube into lane 1 of your agarose gel. Gels are read from left to right. The first sample is loaded in the well at the top left hand corner of the gel.

5. Then using a fresh pipet tip for each sample load 20 µl of each of the samples from the crime scene and suspects into the other lanes in the following order:

    Lane 2: CS, green, 20 µl
    Lane 3: S1, blue, 20 µl
    Lane 4: S2, orange, 20 µl
    Lane 5: S3, violet, 20 µl
    Lane 6: S4, red, 20 µl
    Lane 7: S5, yellow, 20 µl

6. Secure the lid on the electrophoresis chamber. 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.Turn on the power supply. Set it for 100 V and electrophorese the samples for 30–40 minutes.

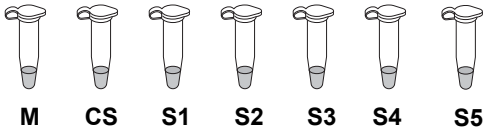
7. When the electrophoresis is complete, turn off the power supply and remove the lid from the chamber. Carefully remove the gel tray and the gel from the electrophoresis chamber. Be careful, the gel is very slippery!

8. Instructions for staining your gel will be given by your instructor.

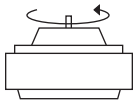
Quick Guide for Electrophoresis of Prepared DNA Samples

1. Obtain the DNA samples from the crime scene and suspects.

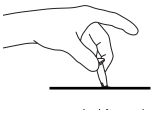
Clear tube	**M**	(DNA Size Marker)
Green tube	**CS**	(Crime scene DNA)
Blue tube	**S1**	(Suspect 1 DNA)
Orange tube	**S2**	(Suspect 2 DNA)
Violet tube	**S3**	(Suspect 3 DNA)
Red tube	**S4**	(Suspect 4 DNA)
Yellow tube	**S5**	(Suspect 5 DNA)



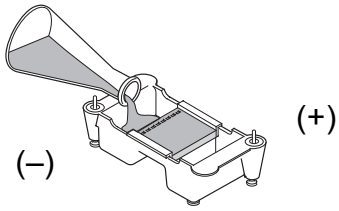
2. If a centrifuge is available, pulse spin the tubes in the centrifuge to bring all of the liquid into the bottom of the tube or gently tap on the table top.


Centrifuge

or


Tap

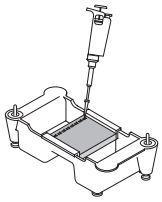
3. Place an agarose gel in the electrophoresis chamber. Fill the electrophoresis chamber with 1x TAE buffer to cover the gel, using approximately 275 ml of buffer.



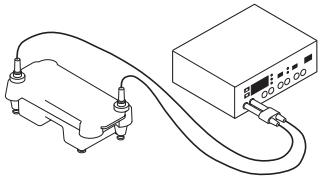
4. Check that the wells of the agarose gels are near the black (–) electrode and the bottom edge of the gel is near the red (+) electrode.

5. 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, 20 µl
Lane 3:	**S1**, blue, 20 µl
Lane 4:	**S2**, orange, 20 µl
Lane 5:	**S3**, violet, 20 µl
Lane 6:	**S4**, red, 20 µl
Lane 7:	**S5**, yellow, 20 µl



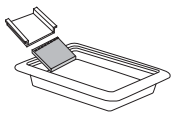
6. Carefully place the lid on the electrophoresis chamber. The lid will attach to the base in only one orientation. The red and black jacks on the lid will match with the red and black jacks on the base. Plug the electrodes into the power supply, red to red and black to black.



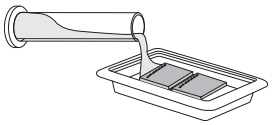
7. Turn on the power and electrophorese your samples at 100 V for 30 minutes.

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.



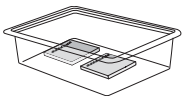
2. You have two options for staining your gel:
   Quick staining (requires 12–15 minutes)



   a. Add 120 ml of **100x** Fast Blast 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.

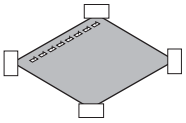


   d. Destain by washing twice in warm tap water for 5 minutes each with gentle shaking for best results.

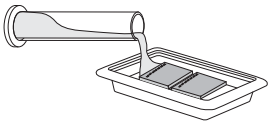
   e. Record results.

   f. Trim away any unloaded lanes.

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

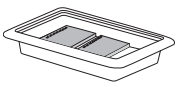


   Overnight staining



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

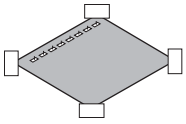
   b. Let the gels stain overnight, with gentle shaking for best results. No destaining is required.



   c. Pour off the water 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.