

DNA RESTRICTION DIGEST & GEL ELECTROPHORESIS

Laboratory Kit Contents

<u>Sent Out</u>	<u>Returned</u>	
<input type="checkbox"/>	<input type="checkbox"/>	ice container (cooler)
<input type="checkbox"/>	<input type="checkbox"/>	digital camera and printer
<input type="checkbox"/>	<input type="checkbox"/>	6 small ice bath containers
<input type="checkbox"/>	<input type="checkbox"/>	6 micropipettors (2-20 ul volume)
<input type="checkbox"/>	<input type="checkbox"/>	6 boxes of micropipettor tips
<input type="checkbox"/>	<input type="checkbox"/>	1 digital heat block with 30 0.5 ml holes
<input type="checkbox"/>	<input type="checkbox"/>	3 microcentrifuges with power cord
<input type="checkbox"/>	<input type="checkbox"/>	6 fine-point permanent markers
<input type="checkbox"/>	<input type="checkbox"/>	1 FlashGel® power base unit with electrical cords
<input type="checkbox"/>	<input type="checkbox"/>	FlashGel® 1.2% agarose gels (place in orange bag)
<input type="checkbox"/>	<input type="checkbox"/>	used FlashGel agarose gels for student micropipette practice
<input type="checkbox"/>	<input type="checkbox"/>	microcentrifuge tubes w/ colored H2O for micropipette practice
<input type="checkbox"/>	<input type="checkbox"/>	1 box of disposable gloves
<input type="checkbox"/>	<input type="checkbox"/>	6 small autoclavable bags for tube and tip disposal
<input type="checkbox"/>	<input type="checkbox"/>	orange autoclavable bag for used FlashGels
<input type="checkbox"/>	<input type="checkbox"/>	CCA Science DVD
<input type="checkbox"/>	<input type="checkbox"/>	3-ring notebook with protocols and other supplementary materials
<input type="checkbox"/>	<input type="checkbox"/>	6 microcentrifuge tubes containing DNA Sample A*
<input type="checkbox"/>	<input type="checkbox"/>	6 microcentrifuge tubes containing DNA Sample B*
<input type="checkbox"/>	<input type="checkbox"/>	6 microcentrifuge tubes containing DNA Sample C*
<input type="checkbox"/>	<input type="checkbox"/>	6 microcentrifuge tubes containing DNA Sample D*
<input type="checkbox"/>	<input type="checkbox"/>	6 microcentrifuge tubes containing DNA Sample E*
<input type="checkbox"/>	<input type="checkbox"/>	6 microcentrifuge tubes containing Bgl 1 enzyme/water solution (3:1 H2O/Enzyme)
<input type="checkbox"/>	<input type="checkbox"/>	1 microcentrifuge tube containing DNA ladder/loading dye
<input type="checkbox"/>	<input type="checkbox"/>	___0.5 ml microcentrifuge tubes

*Samples A-E have a 5:1 DNA/Buffer ratio

DNA RESTRICTION DIGEST & GEL ELECTROPHORESIS

Materials and Supplies Needed:

Students work in groups totaling no more than six!

* = optional items (if students will practice pipetting prior to lab)

One per student:

- 0.5 ml microcentrifuge tubes

One per table:

- DNA Samples A-E (students collect themselves from central tubes)
- restriction enzyme Bgl I and water solution
- sterile distilled water
- one microcentrifuge tube per group of 4 students
- small ice baths
- boxes of micropipettor tips
- small autoclavable bag for used tips and tubes
- microcentrifuge tube rack
- one 20ul micropipettor
- one ultra-fine permanent marker

One per class:

- 1 box of disposable gloves
- DNA Ladder
- 3 microcentrifuges (1 per two groups = three total)
- heat block set for 37°C
- FlashGel 1.2% agarose gel with 34 loading wells
- FlashGel power base unit
- digital camera and printer

* tubes of colored water for micropipettor practice

* used Flashgels ® for well-loading practice

DNA RESTRICTION DIGEST & GEL ELECTROPHORESIS

Student Skills/Knowledge Required for Success:

- Understanding of metric units of measurement (e.g. ml, ul, etc)
- Use of micropipettor to measure small volumes using sterile tips
- Handling of microcentrifuge tubes
- Use of microcentrifuge (balance of tubes, setting parameters, etc.)
- Loading of wells in Flashgel ® agarose gels
- Safety issues (e.g. wearing gloves, handling gels, waste disposal, etc.)

Pre-Lab Preparations:

1. The heat block will be pre-set for 37° C. On the left hand side of the panel, there is a button and a green light. Press the start button until the green light comes on. Double check to make sure the setting is at 37° C.
2. Set out supplies at each lab table of 4 students. Each lab table should have:
 - set of DNA samples (A, B, C, D, E) in labeled microcentrifuge tubes
 - one microcentrifuge tube containing Bgl I restriction enzyme solution
 - one small ice bath for above microcentrifuge tubes
 - four 0.5 ml microcentrifuge tubes
 - one microcentrifuge tube rack
 - one ultrafine permanent marker
 - one 2-20 ul micropipettor
 - one box of sterile tips for micropipettor
 - one small autoclavable bag for used tubes and tips

DNA RESTRICTION DIGEST & GEL ELECTROPHORESIS

Procedures for Part A - DNA Restriction Digest

Student Guide

SET UP

1. Immediately place the microcentrifuge tubes of the five DNA samples A, B, C, D and E, DNA ladder/loading dye, and Bgl I restriction enzyme in a small ice bath at each table and keep on ice for the duration of the lab period. It is very important that the contents of these tubes be kept cold, as you will be sharing these tubes with students in other lab classes throughout the day.
2. The restriction digest reactions will be performed in tubes A, B, C, D, and E. Use the permanent marker to label the lids of 5 microcentrifuge tubes... "A", "B", "C," "D," "E." "A" = DNA sample A, "B" = DNA sample B, "C" = DNA sample C, and so forth to be used to compare electrophoresis migration distances and estimate the sizes (in base pairs) of the restriction fragments.

Write your initials or some other distinguishing mark on the sides of your five tubes so that you can tell them apart from those of other students when they are all placed in the microcentrifuge in step 11.

CHART

3. Set-up a chart in your lab notebook (similar to the one below) showing which reagents and volumes of reagents are added to each microcentrifuge tube. Based on the following steps outlined in this lab protocol, complete the blank boxes of this chart. Shaded boxes indicate when that reagent is not added to a particular tube. This chart can be used as a checklist to make sure the tubes are prepared correctly for the restriction digest.

	Tube A	Tube B	Tube C	Tube D	Tube E
DNA Sample A / Buffer Solution					
DNA Sample B / Buffer Solution					
DNA Sample C / Buffer Solution					
DNA Sample D / Buffer Solution					
DNA Sample E / Buffer Solution					
BgI I enzyme/ H ₂ O Solution					

4. Using the micropipettor and a sterile tip, add 6 ul of the DNA Sample A / Buffer Solution to Tube A. Dispose of the used tip in the autoclavable waste bag at your table.
5. Using the micropipettor with a new sterile tip, add 6 ul of the DNA Sample B / Buffer Solution to Tube B. Dispose of the used tip in the autoclavable waste bag at your table.
6. Using the micropipettor with a new sterile tip, add 6 ul of the DNA Sample C / Buffer Solution to Tube C. Dispose of the used tip in the autoclavable waste bag at your table.
7. Using the micropipettor with a new sterile tip, add 6 ul of the DNA Sample D / Buffer Solution to Tube D. Dispose of the used tip in the autoclavable waste bag at your table.
8. Using the micropipettor with a new sterile tip, add 6 ul of the DNA Sample E/ Buffer Solution to Tube E. Dispose of the used tip in the autoclavable waste bag at your table.
9. Using the micropipettor with a new sterile tip, add 4 ul of the BgI I enzyme / H₂O Solution to Tube A. Dispose of the used tip in the autoclavable waste bag at your table.

10. Using the micropipettor with a new sterile tip, add 4 ul of the BgI I enzyme / H₂O Solution to Tube B. Dispose of the used tip in the autoclavable waste bag at your table.

11. Using the micropipettor with a new sterile tip, add 4 ul of the BgI I enzyme / H₂O Solution to Tube C. Dispose of the used tip in the autoclavable waste bag at your table.

12. Using the micropipettor with a new sterile tip, add 4 ul of the BgI I enzyme / H₂O Solution to Tube D. Dispose of the used tip in the autoclavable waste bag at your table.

13. Using the micropipettor with a new sterile tip, add 4 ul of the BgI I enzyme / H₂O Solution to Tube E. Dispose of the used tip in the autoclavable waste bag at your table.

15. Tightly close the caps on all five tubes (A, B, C, D, and E) and place them in the microcentrifuges along with those of other students using 0.5 ml tube adaptors. It is essential that the tubes be placed in the microcentrifuges so that they are balanced i.e. placed opposite each other and spaced equally (ask your teacher for help if you don't understand). You can also use a blank tube for extra balancing. An unbalanced centrifuge will spin unevenly and the rotor mechanism will break.

16. Secure the lid on top of the tubes and close the lid of the microcentrifuges. **WITH THE MICROCENTRIFUGES BALANCED**, spin the tubes down in the microcentrifuges for approximately 10 seconds. When the rotor stops you can then open the microcentrifuge tubes and remove your set of tubes. The liquid will all be at the bottom of the tubes, so do not shake or invert the tubes as you carry them back to your lab table.

17. Set the heat block timer for 40 minutes using the buttons on the right-hand side if the timer is not already set for you..

Place tubes A, B, C, D, and E in a 37°C heat block and start the timer, using the timer start button on right-hand side.

The restriction digest of the DNA samples will occur during this incubation.

*Note: Following the incubation and removal of the tubes from the water bath, you can proceed directly to Part B. Or if time does not allow, you may place all of your tubes (A, B, C, D, and E) in the freezer until the next lab session.

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Procedures for Part B — Gel Electrophoresis

Student Guide

You will work with your classmates to share the agarose FlashGels used to display your PCR results. These FlashGels are quite expensive so it is necessary to share them. Each gel has 34 small wells (holes) – one for each student's DNA sample. One class can use a single gel effectively. A DNA ladder/size marker will be placed in a center lane (#6 on top and bottom) for comparison with the student results on either side.

Your teacher will open the gel and prepare it for a small amount of your sample to be loaded into the FlashGel. This procedure requires concentration and a minimal amount of disturbance to the gel.

1. Carefully load **5ul** of your sample into one of the wells. It sometimes works best if you hold the micropipettor in one hand and steady it by bracing with the other hand. Gently lower the micropipettor tip with your sample into the well and release.
2. Make a note of the number your teacher has written above the well you used so that you can check on the progress of your sample as it is developed!

DNA RESTRICTION DIGEST & GEL ELECTROPHORESIS

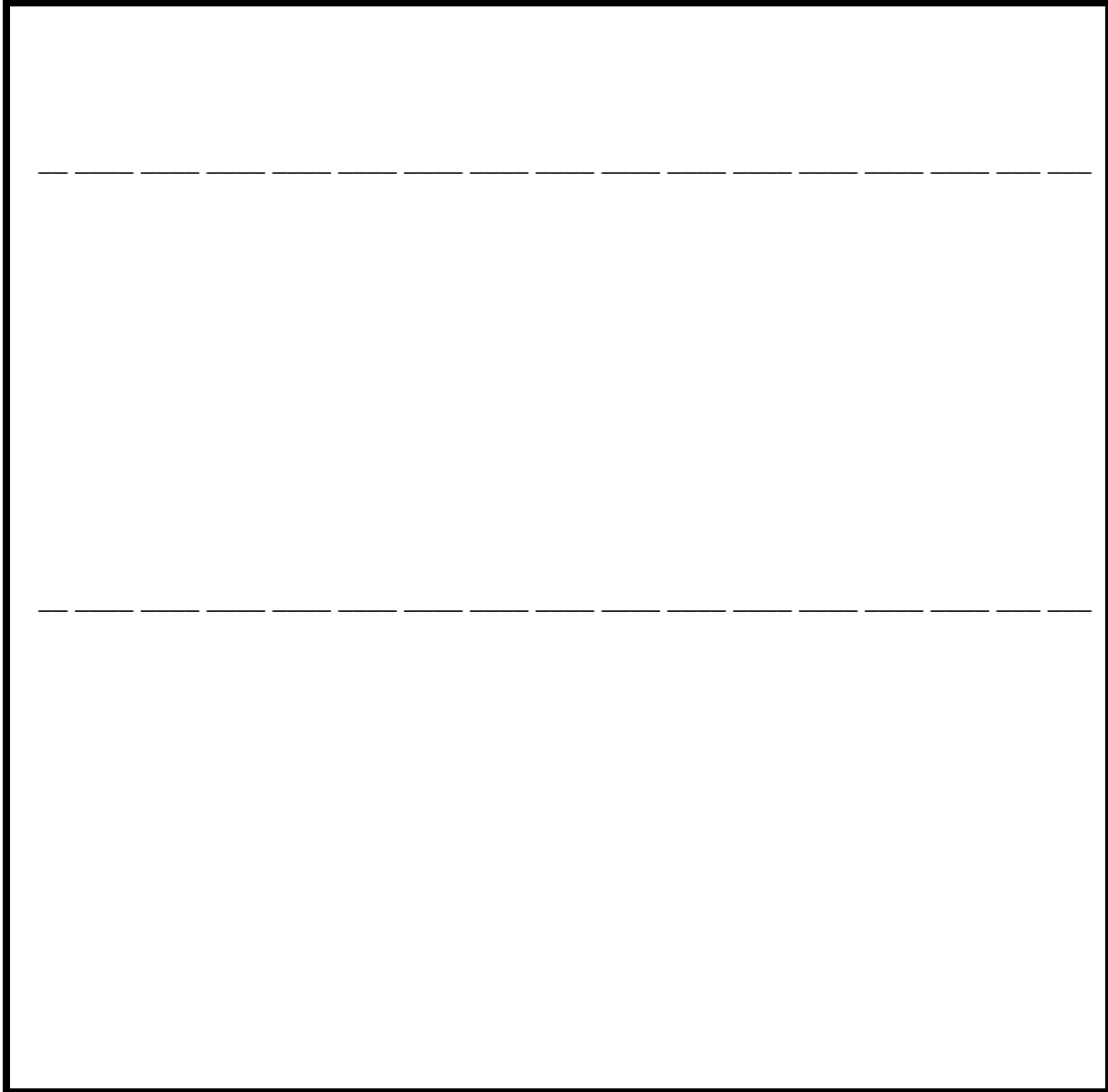
Procedures for Part B — Gel Electrophoresis

Teacher Guide

1. Wearing a pair of disposable gloves, open a package containing a 1.2% agarose FlashGel. It is very important that anyone handling these gels wear gloves and handle the gels only at their edges. These prepared gels contain a chemical which will allow the DNA fragments to be seen with an ultra violet light source, but should not come into contact with your skin or be ingested. Avoid rubbing your eyes or face after handling these gels and wash your hands thoroughly after completing this part of the lab activity. Once the cassette has been removed from the bag, remove the white well seals. Remove the elongated thin seal first, followed by the larger seal. Use caution when removing the larger seal by pulling forward (towards yourself) and not straight up. This reduces the amount of air bubbles in the gel. **DO NOT remove the clear, circular side vent seals.**
2. Insert the cassette into the dock by sliding it in gently. Use the FlashGel mask strip in place, underneath the wells of the bottom (or second) row. It is a flexible strip, so it can be moved to block the glowing light from the wells if it is not in place correctly.
3. Mark each well with a number, using the fine-point permanent marker provided
4. Flood the sample wells with distilled water. Tilt the cassette to move excess fluid to the edge and blot off the excess with a kimwipe. **DO NOT** blot the wells.
5. If there are still large amounts of air bubbles in the gel, gently press on the cassette with your fingers and manipulate the bubbles towards the wells for escape. Do not touch the wells or push on the cassette too hard.
6. Carefully **load 5ul of the DNA Ladder into the first well(s).**
7. Once there are only a few air bubbles left, supervise students as they load **5ul** of the sample in the wells and make note of the number on the well they use.

8. Plug in the voltage cords to their corresponding outlet in the power supply, i.e. black to black and red to red. Plug in the lower voltage light supply to an available outlet. Set the voltage to **275V**
9. Turn on the light and the voltage and **watch the DNA travel down the gel** with the aid of the built in light source.
10. Run the gel for **2-5 minutes** or until separation of desired fragments is complete.
11. Take digital photographs of each FlashGel using the digital camera.
12. Print pictures using the printer included with the camera.
13. While wearing gloves, remove the FlashGel ® from the docking unit and dispose of in the provided waste bag or box to be returned to CCA.

Flash Gel Wells



* Please initial and label your samples for each well on this paper. The first well(s) will be saved for the DNA ladder.