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A Geno Technology, Inc. (USA) brand name

Introduction to Agarose Electrophoresis

Teacher's Guidebook

(Cat. # BE-304)

MATERIALS INCLUDED

This kit has enough materials and reagents for 24 students (six groups of 4 students).

- 1 vial DNA: Plasmid 1
- 1 vial DNA: Plasmid 1 (digested)
- 1 vial DNA: Plasmid 2
- 1 vial DNA: Plasmid 2 (digested)
- 1 pack Agarose
- 1 vial LabSafe™ Nucleic Acid Stain
- 1 vial DNA Ladder (1kb)
- 1 vial DNA Loading Buffer (6X)
- 1 bottle TAE Buffer (50X)
- 30 Centrifuge Tubes (2ml)

SPECIAL HANDLING INSTRUCTIONS

- Store all DNA: Plasmids, DNA ladder (1kb) and DNA Loading Buffer (6X) at-20°C.
- Store LabSafe™ Nucleic Acid Stain at 4°C.
- All other reagents can be stored at room temperature.

The majority of reagents and components supplied in the *BioScience Excellence*™ kits are non toxic and are safe to handle, however good laboratory procedures should be used at all times. This includes wearing lab coats, gloves and safety goggles.

For further details on reagents please review the Material Safety Data Sheets (MSDS).

ADDITIONAL EQUIPMENT REQUIRED

- Agarose electrophoresis equipment.
- Waterbath or beaker and thermometer.
- Washing trays 12cm x 12cm.
- UV Light box or transilluminator

TIME REQUIRED

- **Day 1:** 2-3 hours

OBJECTIVES

- Carry out agarose electrophoresis of DNA samples.
- Determine molecular weight of DNA with DNA ladder.
- Determine relationship between DNA samples based of their size & migration.
- Develop a restriction map of the DNA vectors using the electrophoresis results.

BACKGROUND

Agarose gel electrophoresis is routinely used in molecular biology and genetic engineering for the visualization, purification and characterization of DNA molecules. DNA molecules are negatively charged and therefore migrate through the agarose gel matrix to the positive terminal at the bottom of the gel.

Electrophoresis is used in many aspects of science for the separation of charged molecules, whether they are RNA, DNA or proteins. For the separation of nucleic acids, a compound purified from seaweed, agarose, is routinely used. Agarose, a linear polymer, is dissolved in hot running buffer and on cooling it *polymerizes* into a semi-solid matrix. The polymerization process of agarose involves the sugar groups cross-linking to form a matrix. The resulting matrix allows small molecules to move, or migrate, quickly through the gel, whereas the larger molecules are hindered by the matrix, causing them to migrate slower. This principle allows the separation of molecules by size. The addition of an electric current to the gel causes the molecules to move in one direction, towards the positive end of the gel.

Another factor affecting the rate of migration of DNA is the conformation of circular (plasmid) DNA. Three forms can exist; superhelical circular (form I), nicked circular (form II), and linear (form III). A plasmid consists of a circle of double stranded DNA, which has a helical structure. These three forms of DNA of the same molecular weight will migrate at different rates. Superhelical, or supercoiled plasmids occur when the helical coils of DNA themselves become coiled, (coiled coils), which makes the plasmid structure smaller and therefore migrate faster. The only way for the supercoils to unwind is for the plasmid to be cut to form a linear piece of DNA, which allows for all the coils to unwind. When the DNA is only partially cut, only one strand of the double stranded DNA is cut, or nicked, the DNA can partially unwind and relax its structure allowing slower migration. The linear plasmids, which are completely cut, migrate faster than nicked plasmids but not as fast as the supercoiled plasmids.

The nucleic samples are combined with a running buffer prior to loading the gel. These running buffers contain glycerol, sucrose or Ficoll that increases the density of the sample causing the samples to sink to the bottom of the well, rather than float away. A colored dye is added to aid visualization of sample loading and to monitor electrophoresis migration. The common non-reactive blue dyes used are bromophenol blue and xylene cyanol. Bromophenol blue normally migrates at 300bp, where as xylene cyanol migrates at 4kbp.

This kit supplies several DNA samples and a 1kb ladder to allow students to visualize DNA migration and calculate the size of the DNA bands. This kit does not use the carcinogen ethidium bromide for visualization, but a non-toxic fluorescent dye that allows students to visualize the DNA migrating during electrophoresis.

TEACHER'S PRE EXPERIMENT SET UP

Preparation of agarose gel

Each group of 4 students will have 4 DNA samples, so for 6 groups, there needs to be 24 wells for the samples, plus a well for the reference 1kb DNA ladder. Each well must hold 30µl sample.

Make 1-2 hours before the experiment.

Wear heat protective gloves throughout the agarose melting and pouring procedure.

1. Prepare running buffer: In a clean two-liter container, add the entire contents of the TAE buffer (50X) and add two liters of ultra pure water to make a 1X TAE buffer solution. Stir until thoroughly mixed.

2. Prepare agarose: In a clean, glass 1000ml container add the entire contents of the agarose pack and add 500ml of the 1X TAE buffer from step 1.

3. Heat the solution in a microwave on full power, using 10 second bursts, or use a boiling waterbath. Check to see if all the agarose has dissolved. Continue until agarose has dissolved.

   
   DO NOT BOIL. The agarose gets very hot, very quickly and can cause severe burns. Wear heat protective gloves throughout the melting and pouring procedure.

4. Once the agarose has cooled to the point it can be held comfortably in your hand, add the entire contents of the LabSafe™ Nucleic Acid Stain to the agarose and swirl to mix.

5. Pour the agarose into the gel casting mould as per the manufacturer's instructions. You will need 25 wells that each holds 30µl for each group, use an appropriate size comb.

6. Once the gels have set, remove the comb, transfer to the running apparatus and cover with the running buffer until ready to use.

Prepare the reference markers

1. Add 25µl ultra pure water to the lyophilized DNA ladder (1kb), dissolve by gently pipetting up and down 5-6 times.

2. Add 5µl DNA Loading Buffer (6X), mix by gently pipetting up and down 5-6 times.

3. Load 10µl into each reference well.

Prepare the DNA samples

1. Add 150µl ultra pure water to each tube of DNA sample; dissolve by gently pipetting up and down 5-6 times.

2. OPTIONAL: Aliquot 25µl DNA vector into clean tubes and give one tube of each DNA vector to each group of students

MATERIALS FOR EACH GROUP

Supply each group with the following components. Several components are shared by the whole class and should be kept on a communal table.

- 1 vial DNA: Plasmid 1
- 1 vial DNA: Plasmid 1 (digested)
- 1 vial DNA: Plasmid 2
- 1 vial DNA: Plasmid 2 (digested)
- 1 vial DNA Loading buffer
- 4 Centrifuge Tubes (2ml)

PROCEDURE

Each group of students is supplied with 4 DNA samples. The DNA samples consist of 2 different DNA plasmids that are undigested or digested with a restriction enzyme. The aim of this experiment is to determine the size of the two vectors and determine which vectors you have been supplied with using the restriction map.

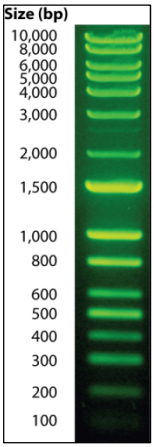
1. Label the 4 tubes with your groups name and number them 1 to 4.

2. Add 5µl of DNA loading buffer (6X) to each tube.

3. Each student in the group sets up one of the four reactions. Transfer 25µl of DNA sample to the appropriate tube, as shown below:

Tube Number	DNA Sample
1	Plasmid 1
2	Plasmid 1 (Digested)
3	Plasmid 2
4	Plasmid 2 (Digested)

4. The agarose gels should have been prepared by your teacher/supervisor. Each student takes turn in loading a sample into a well. Your teacher/ supervisor will load a reference lane to determine the size of the DNA (see image for reference markers).



5. Once the samples are all loaded, apply a current and migrate at 12-15V/cm. For an 8cm long gel run at 96-120 volts.

6. Once the blue dye front has migrated ¾ the length of the gel, turn off the power and carefully transfer the gel to a UV Light box.

CAUTION: Wear UV Safety Specs when working around the UV Light Box.

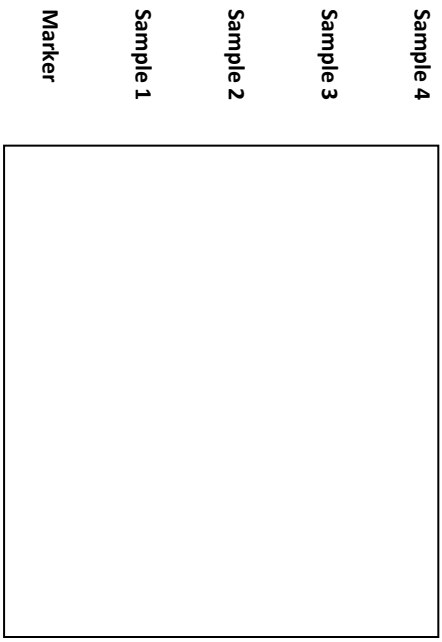
7. Determine the size of the vectors and digested fragments. Determine which vector was used on the worksheets.

RESULTS, ANALYSIS & ASSESSMENT

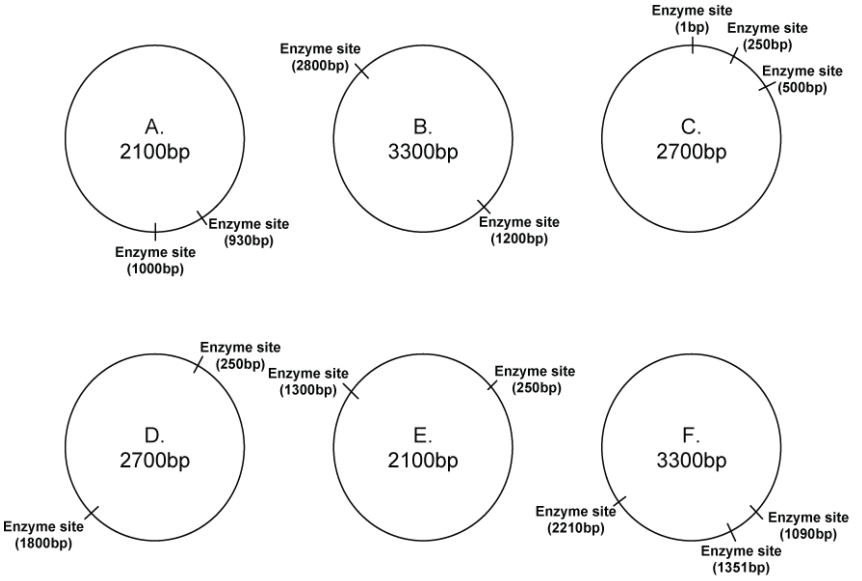
1. Draw a representation of the bands visualized on the gel below:

Write the sizes of the bands seen in the table below:

Sample	Approximate DNA band sizes
Sample 1	3300bp
Sample 2	2200bp, 850bp, 250bp
Sample 3	2700bp
Sample 4	1500bp, 1200bp



From the five vector maps below, select which map best represents your two plasmids



Plasmid 1 is F; plasmid 2 is D.

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2. Add 5µl of DNA loading buffer (6X) to each tube.

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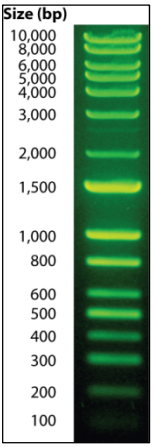

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Write the sizes of the bands seen in the table below:

Sample	Approximate DNA band sizes
Sample 1	
Sample 2	
Sample 3	
Sample 4	

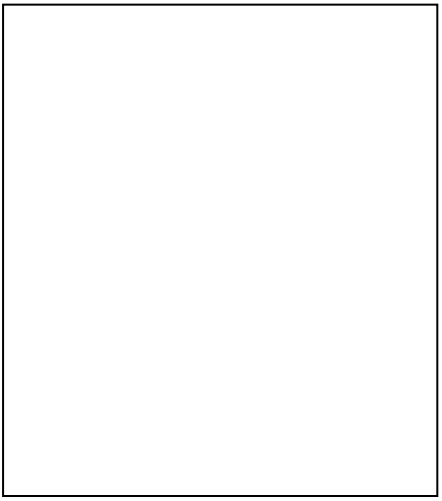
Marker

Sample 1

Sample 2

Sample 3

Sample 4



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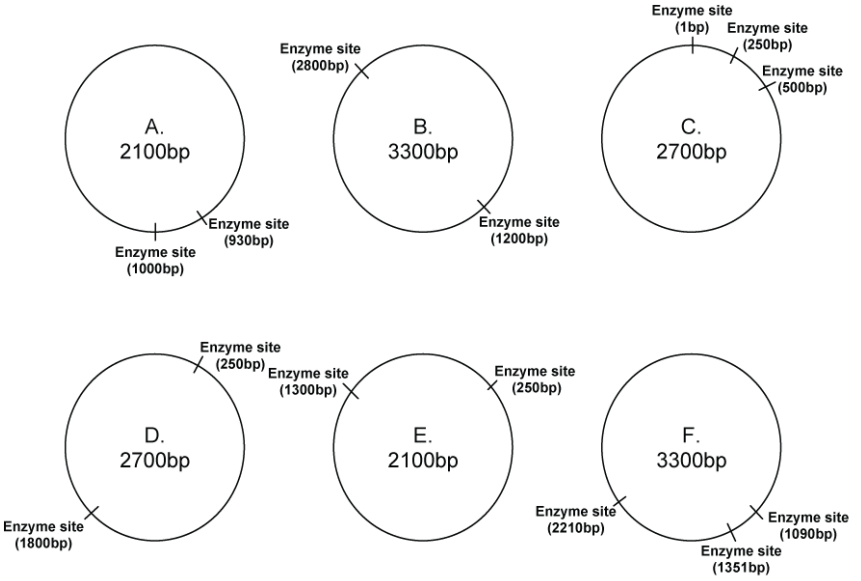

Plasmid 1 is _____; plasmid 2 is _____.

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