Agarose Gel Electrophoresis Lab

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GOAL:

To determine the presence or absence of PCR products and quantify the size (length of the DNA molecule) of the product. Gel electrophoresis is a widely used technique for the preparation and analysis of DNA and proteins. We will be using agarose gel electrophoresis to determine the presence and size of *Wolbachia 16S rDNA* amplified by PCR.

INTRODUCTION:

Electrophoresis is a method of separating substances based on the rate of movement while under the influence of an electric field. Agarose is a polysaccharide purified from seaweed. An agarose gel is created by suspending dry agarose in a buffer solution, boiling until the solution becomes clear, and then pouring it into a casting tray and allowing it to cool. The result is a flexible gelatin-like slab. During electrophoresis, the gel is submersed in a chamber containing a buffer solution and a positive and negative electrode. The DNA to be analyzed is pulled through the pores of the gel by the electrical current. Under an electrical field, DNA will move to the positive electrode (red) and away from the negative electrode (black). Several factors influence how fast the DNA moves, including; the strength of the electrical field, the concentration of agarose, and most importantly, the size of the DNA molecules. Smaller DNA molecules move through the agarose faster than larger molecules. DNA itself is not visible within an agarose gel. The DNA will be visualized by the use of a dye that binds to DNA.

TEACHING TIME: 90 minutes or two class periods of 45min each

MATERIALS (per group of two students)

- Your 6 PCR products
- 4 Gloves
- 1 Micron pen or sharpie
- 1 box of P200 pipet tips
- 1 box of P20 pipet tips
- P200 and P20 pipets
- 1 rack for holding PCR tubes
- 6X Loading Buffer (Fisher TAK-9156)
- DNA ladder (Fisher PR-G3161)
- 10X TAE Buffer (Fisher PR-V4271)
- Agarose (Ward's 944 V 3700)
- Gel casting tray and combs
- QUIKView DNA stain (Ward's 38 V 9014)
- Staining trays for the class
- Electronic balance for class

- Weighing dishes or paper
- Spatula
- 500ml flask
- 100ml graduated cylinder
- Microwave for class
- Plate or water bath for class
- Oven mitt or tongs for class
- Masking tape for class
- Saftey goggles

Agarose Gel Electrophoresis Protocol

Preparing the agarose gel

1. Measure 1.25 g Agarose powder and add it to a 500 ml flask
2. Add 125 ml TAE Buffer to the flask. (1% solution; note the total gel volume well vary depending on the size of the casting tray)
3. Melt the agarose in a microwave or hot water bath until the solution becomes clear.
   (i) if using a microwave, heat the solution for several short intervals - do not let the solution boil for long periods as it may boil out of the flask.
4. Let the solution cool to about 50-55°C, swirling the flask occasionally so it cools evenly.
5. Seal the ends of the casting tray with two layers of masking tape.
6. Place the combs in the gel casting tray.
7. Pour the melted agarose solution into the casting tray and let cool until it is solid (it turns a light blue as it solidifies).
8. Carefully pull out the combs and remove the tape.
9. Place the gel in the electrophoresis chamber.
10. Add enough TAE Buffer so that there is about 2-3 mm of buffer over the gel.

Loading the gel

1. Add 6 μl of 6X Loading Buffer to each 25 μl PCR reaction
2. Record the order each sample will be loaded on the gel, including who prepared the sample, the DNA template - what organism the DNA came from, controls and ladder.

Lane #	*Prepared by*	*DNA Template*	*notes*

3. Carefully pipette 20 μl of each sample/Sample Loading Buffer mixture into separate wells in the gel.
4. Pipette 10 μl of the DNA ladder standard into at least one well of each row on the gel.

Running the gel

1. Place the lid on the gel box, connecting the electrodes appropriately (positive (red) and negative (black))
2. Turn on the power supply to about 100 volts. Maximum allowed voltage will vary depending on the size of the electrophoresis chamber – it should not exceed 5 volts/ cm between electrodes!
3. Check to make sure the current is running through the buffer by looking for bubbles forming on each electrode.
4. Check to make sure that the current is running in the correct direction by observing the movement of the blue loading dye – this will take a couple of minutes.
5. Let the power run until the blue dye approaches the end of the gel, then turn off the power, disconnect the electrodes, remove the lid and the gel using gloves.

Gel Staining

1. Place into the staining dish.
2. Add warmed (50-55°) staining mix.
3. Allow gel to stain for at least 25-30 minutes (the entire gel will become dark blue).
4. Pour off the stain (the stain can be saved for future use).
5. Rinse the gel and staining tray with water to remove residual stain.
6. Fill the tray with warm tap water (50-55°). Change the water several times as it turns blue. Gradually the gel will become lighter, leaving only dark blue DNA bands. Destain completely overnight for best results.
7. View the gel against a white light box or bright surface.
8. Record the data while the gel is fresh, very light bands may be difficult to see with time.

CLASS SUMMARY

Date _____

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Making Your Own Recipes:

1X TAE Buffer
4.84 g Tris Base
1.14 ml Glacial Acetic Acid
2 ml 0.5M EDTA (pH 8.0)
- bring the total volume up to 1L with water

Add Tris base to ~900 ml H_2O. Add acetic acid and EDTA to solution and mix. Pour mixture into 1 L graduated cylinder and add H_2O to a total volume of 1 L. Note – for convenience a concentrated stock of TAE buffer (either 10X or 50X) is often made ahead of time and diluted with water to 1X concentration prior to use.

6X Sample Loading Buffer
1 ml sterile H_2O
1 ml Glycerol
enough bromophenol blue to make the buffer deep blue
(~ 0.05 mg)
-for long term storage, keep sample loading buffer frozen.

QUIKView DNA Stain
25 ml WARDS QUIKView DNA Stain
475 ml warm water (50-55° C)