Gel Electrophoresis Protocol

Standard

Class Preparation: Running Buffer Working Solution

1. Together with your class, prepare a working solution of 1X electrophoresis running buffer.

Prepare Lab Space

2. Remove all unnecessary items from your lab station.
3. Put on nitrile gloves and clean all surfaces by wiping down with 70% Ethanol.

Prepare the Gel with DNA Stain

4. Measure 1g agarose powder and add it to a 500mL flask.
5. Add 100mL running buffer to the flask. (1% solution; note the total gel volume will vary depending on the size of the casting tray; refer to manufacturer instructions)
6. Melt the agarose in a microwave or hot water bath until the solution becomes clear. Look for "lenses" in the liquid by holding the flask up to the light. If using a microwave, place a ball of Kimwipe tissue in the flask opening, or cover with parafilm and poke a hole for venting. Heat the solution for several short (~20 second) intervals, do not let the solution boil over.
7. Let the solution cool to about 50-55°C, swirling the flask occasionally so it cools evenly. The flask should be warm, but not too hot to touch.
8. Seal the ends of the casting tray by placing it into the gel electrophoresis tank, or by sealing the ends with wide lab tape. Refer to the manual of your electrophoresis system for more information.
9. Add 10uL GelRed (Biotium), or comparable DNA stain, to the agarose. Swirl to mix.
10. Select a comb that will accommodate all samples. Place the comb(s) in the gel casting tray.
11. Slowly pour the melted agarose solution into the casting tray. Gently pop any bubbles with a pipette tip. *Note: Fill the gel in the tray so the teeth of the comb are immersed under the gel, but the base of the comb is above the gel. You do not have to use all the melted agarose.*
12. Let gel cool undisturbed on a solid, flat surface until it is opaque and solid. This may take at least 15-30 minutes. Moving the tray or not waiting until the gel is fully set may affect your results.
13. Carefully pull out the combs and remove the tape or lift casting tray out of the electrophoresis chamber.
14. Place the gel in the electrophoresis chamber with the wells oriented near the (-) electrode.
15. Add enough running buffer so there is 2-3mm of buffer over the gel.

Prepare to Load the Gel

16. Fill out the Loading Key on the next page.
17. If your PCR Master Mix has no loading dye (your PCR products are clear), follow 17a. If your PCR products are colored, move to *Load the Gel*.
    (a) Pipette 2uL drops of 5X loading buffer onto a piece of Parafilm or wax paper. Add 10uL from each of your PCR reactions to a drop of loading buffer. Then mix well by gently pipetting up and down several times until the color of the liquid is homogenous.

Load the Gel

18. Pipette 10uL of the DNA ladder in the first well. Hover your pipette above the well, and slowly empty your pipette. Do not press to the second stop.

19. Continue in this manner, carefully pipetting 10uL of each sample/sample loading buffer mixture into separate wells in the gel. Change tips between each sample and store remaining PCR products in the freezer.

Run the Gel

20. Place the lid on the gel box, connecting the electrodes appropriately (positive is red, negative is black). The negative electrode should be near the wells of the gel, your DNA should "run to red". Also connect your electrodes to your power supply.
21. Turn on the power supply to about 100 volts. Maximum allowed voltage will vary depending on size of the electrophoresis chamber, and will be printed on the label.

Gel #1 Loading Key: Arthropod

Lane	Sample
1	DNA Ladder
2	
3	
4	(+) Arthropod Control
5	(-) Arthropod Control
6	(+) DNA Control
7	Water

22. Check to make sure current is running through the buffer by looking for bubbles that form on each electrode.
23. Check to make sure the current is moving in the correct direction by observing the movement of the loading dye. This may take a few minutes.
24. Let the power run until the yellow (or bottom) band in the loading dye is ¾ down the gel. Then, turn off the power, disconnect the electrodes, and remove the lid and the gel using gloves.

Obtain an Image of the Gel

CAUTION; UV LIGHT CAN DAMAGE EYES!!! EYE PROTECTION REQUIRED!

25. Place the gel on the transilluminator or other imaging equipment. Put on eye protection before turning on the transilluminator. Use the equipment as directed, following the manual.
26. Note the presence or absence of bands in each lane. Use the DNA ladder, with fragments of a known size, to determine the size of each of the PCR products.
27. Document your results on the next page.

Repeat Steps 4-27 for the Wolbachia Gel Electrophoresis

Clean your Work Station

28. Discard used tips and wipe down the bench with 70% ethanol.
29. Refer to your equipment's manual to clean, dry, and store the electrophoresis system.

Label the Gel(s)

30. Transfer the gel images to a computer and use a program such as Powerpoint, Google Slides or Preview to label each gel (Arthropod/*Wolbachia*) and corresponding lanes.

Gel #2 Loading Key: *Wolbachia*

Lane	Sample
1	DNA Ladder
2	
3	
4	(+) Arthropod Control
5	(-) Arthropod Control
6	(+) DNA Control
7	Water