Bracken-Grissom Lab: xDNA Gel Electrophoresis

MATERIALS & EQUIPMENT

Agarose powder
Refrigerated tris-acetate-EDTA (TAE) buffer
Gel Red fluorescent dye
DNA loading dye
1kb gel ladder

Electrophoresis power supply
Gel electrophoresis buffer chamber, electrode lid, and casting tray (small, medium or large)
Gel combs (various sizes)
Small bull's eye level
Microwave
Thermal protection gloves

Weigh scale
Spatula and weigh boat
250mL Erlenmeyer flask
100mL graduated cylinder
1000mL plastic beaker filled with room temperature water

Parafilm
Pipettes & Pipette tips

Enduro GDS UV Gel imager with computer and software

PROTOCOL

1. Determine the size of gel to be run based on the number of xDNA samples to be tested.
    a. Small gels can have one or two rows of combs, with two 10-well combs, and one 6-well comb to choose from. Therefore, it can hold a maximum of 18 samples with 2 ladders across two rows.

    b. Medium gels can have 1 – 4 rows of combs, with four 20-well combs, and four 25-well combs to choose from. Therefore, it can hold a maximum of 96 samples with 4 ladders across four rows.

    c. Large gels can have 1 – 3 rows of combs, with three 50-well combs to choose from. Therefore, it can hold a maximum of 147 samples with 4 ladders across three rows.

2. Assemble the gel electrophoresis gel system.
    a. The casting tray, with rubber gaskets, should fit snuggly inside the center of buffer chamber. Press it halfway down, then use the bull's eye level to level the tray.

    b. The electrode lid, and gel combs should remain detached.

3. Cast the gel
    a. Measure out enough agarose and TAE buffer for a 1% gel based on the gel size.

        i. Small = 0.5g agarose
            50mL TAE

        ii. Medium = 0.8g agarose
            80mL TAE

        iii. Large = 2g agarose
            ……..200mL TAE

    b. Combine the agarose and TAE buffer in Erlenmeyer flask. Mix thoroughly

    c. Microwave mixture until gel is perfectly clear fluid with no visible powder or un-melted crystals.
        i. 1-minute intervals for 2 minutes, then 15 - 30 -second intervals until gel is adequately melted. Mix gel between each interval

        ii. Use thermal gloves to prevent burning your hands off!!!!

    d. Transfer melted gel to room-temperate water bath. Cool gel until it can be held on palm of hand for ~10 seconds.

    e. Pipette 0.5uL of Gel Red dye into mixture. Swirl to mix into gel.

    f. Slowly pour gel into casting tray. Use pipette tip to pop air bubbles, or move them to the edge of tray.
        i. Pour gel so that it flows across casting tray from top to bottom, based on where combs will be inserted. The "top" is where the first comb will be.

            This helps ensure any bubbles or impurities flow to bottom of gel, mitigating any impact they may have on the movement of DNA across the gel.

    g. Insert combs into gel.

    h. Allow gel to set for 20-30 minutes.

    i. Remove combs carefully, so as not to tear the gel.

    j. Rotate casting tray 90° so top of gel (where wells are closer to edge) is on the negative (black) electrode side of the buffer chamber. It should sit all the way down into middle part of buffer chamber.
        i. The DNA will flow in direction of positive electrode. Arrange casting tray so the DNA must flow across the gel.

    k. Fill buffer chamber with refrigerated TAE buffer until gel is completely submerged.
        i. TAE buffer is reused multiple times. Make sure the buffer you use isn't too dirty before use. If it is, pour down drain with running water. Refill container with fresh TAE from BG Lab TAE Buffer Carboy.

4. Pipette 0.5uL of 1kb gel ladder to the first well of each row in the gel

5. Loading xDNA samples into gel
    a. Cut off a strip of parafilm.
        i. It's expensive, so be frugal, but make sure it's large enough for the following steps.

    b. Pipette 3uL drops of DNA loading dye directly onto waxy side of parafilm; 1 drop for every xDNA sample.

    c. Pipette 2uL of xDNA sample into one drop of loading dye. Mix together by back-pipetting until the mixture turns a deep blue color.

    d. Pipette ~4.2uL of blue xDNA droplet into next available well of the gel
        i. Leave some sample on the parafilm to ensure no air is taken into the pipette tip. Air can cause the xDNA to come out of the pipette tip in spurts, which can cause it to plume out of the well, contaminating adjacent wells.
        ii. The loading dye makes the droplet dense, so it will sink straight down into well. Therefore, you don't have to insert the pipette tip into the well. As long as the tip is in the TAE buffer, and over the well, the DNA will sink into the well.

    e. Repeat steps "c" and "d" until all xDNA samples are loaded into gel.

6. Starting electrophoresis power supply
    a. Attach electrode lid to buffer chamber, and plug into gel electrophoresis power supply. The plugs are color-coded to help ensure they get plugged in correctly.

    b. The BG lab has two different power supplies
        i. For black FB103 Power Supply:
            1. Set "Voltage Range" to "Lo." Ensure "Voltage" is selected on right side of front panel, not Amps.
            2. Turn on power by flipping the power switch on front of power supply. The gel will immediately begin running. Observe rising bubbles on ends of buffer chamber to verify.
            3. Rotate voltage dial until the readout shows ~90 to ~110 volts.
            4. For gels with one row, run for ~80 minutes maximum.
               For gels with two rows, run for ~40 minutes maximum.
               For gels with 3+ rows, run for ~30 minutes maximum.
            5. Return to gel after allotted time, and turn off power supply.
        ii. For white FB1000 Power Supply:
            1. Turn on power by pressing the power button on front of power supply.
            2. Select "Manual"
            3. Select "Constant VOLTS"
                a. Set "Constant" to anything between 90 and 110 Volts.
                   Amperage should read 500mA
                   Wattage should read 250 Watts
            4. Select "Time, V-hours"
                a. For gels with one row, run for ~80 minutes maximum.
                   For gels with two rows, run for ~40 minutes maximum.
                   For gels with 3+ rows, run for ~30 minutes maximum.
            5. Select "Run." The gel will begin running. It will stop automatically after the specified V-hours has been reached

7. Visualize your gel
    a. Remove casting tray with gel from buffer chamber

    b. Transfer gel (without the casting tray) to Enduro GDS Gel Imager.
        i. Orientate gel so wells are on top side of image

    c. Login to computer next to Gel Imager
        i. User: brackengrissomlab
        Password: labuser

    d. Create a folder with your name on desktop if one doesn't already exist. Inside that folder, create another "xDNA" folder.

    e. Open Enduro GDS software
        i. Visualize gel by clicking "Acquire" in upper left corner of window.
        ii. Turn on UV light with "UV" button in upper left corner of window.
            1. Adjust UV exposure with UV slider
        iii. Capture image with "Capture Image" button in upper center of window.
        iv. Save image to your xDNA folder. Name the image as follows:

            xDNA_[YourInitials##]_[DayMonYear]

            Example: xDNA_xDNA01_01Jan2018

        v. Email image to yourself. Access FIU_SECURE_WIFI using our PantherSoft login information.

8. Clean up Enduro GDS Gel Imager
    a. Dispose of gel in the trash.
    b. Use Chemwipes to wipe off / dry Enduro GDS Gel Imager
    c. Use DI water, or 80% EtOH if the imager looks dirty

9. Clean up Gel Station
    a. Pour TAE buffer from buffer chamber back into its original container. Return it to the refrigerator.

    b. Rinse gel casting tray, buffer chamber, and combs with DI water from the sink. Set in draining rack next to sink to dry.

    c. Return 1kb Gel Ladder to -20°C freezer.

END