Gel Electrophoresis

Purpose

Agarose gel electrophoresis will be used in order to determine the success of PCR reactions, restriction digestions, ligations and mini-prepped plasmids after cloning and transformation.

Materials

- DNA (Label with name)
- Nuclease-free H2O
- 4 µLLoading Dye (6X)

- PCR tubes
- 250 mL Erlenmeyer flask
- DNA samples for loading onto gel (PCR, Restriction digestion product, Ligation product, MIni-prepped plasmid)
- Nuclease-free H2O
- Double-distilled H2O
- RedSafe Nucleic Acid Staining Solution (20,000X)
- 1X TAE Buffer - 40 mM Tris, 20 mM acetic acid, 1 mM Na2EDTA
- Agarose
- Gel Loading Dye, Purple (6X)
- 1 kb Plus DNA Ladder
- 50 bp DNA Ladder

Equipment

- Top-loading balance
- Bio-Rad Gel imaging station
- Electrophoresis power source
- Agarose gel casting apparatus
- Microwave Oven

Procedure

Preparing the Agarose Gel

- Weigh the appropriate amount of agarose necessary based on the agarose concentration determined above. For a 1.5% gel, weigh 1.5 g of agarose.
- Add 100 mL of 1X TAE to a 250 mL Erlenmeyer. Add the agarose powder. Swirl to mix.

- Place the Erlenmeyer inside the microwave oven. Microwave on high for 2 minutes. Watch the contents of the microwave closely to ensure that the contents do not boil over.
    a. If the solution is not complete clear and free of agarose particles, then microwave on high for another 30 seconds.
- Place the hot Erlenmeyer flask on a heat-resistant surface and allow it to cool.
- When the surface of the Erlenmeyer is warm, but not hot to the touch, add 5 µL of RedSafe Nucleic Acid Staining Solution (20,000X) to the agarose solution. Swirl the flask gently to mix and avoid forming bubbles.
- Place an agarose gel tray inside the gel caster and tightly seal the caster against the open sides of the gel tray. Place a comb inside the casting apparatus at the appropriate grooves on the side of the tray.
    a. If you poured water into the casting unit, no liquid should leak from any side.
- Pour the agarose solution into the gel tray. You should be immersing between half and a quarter of the comb length in agarose.
- Leave on a heat-resistant surface and allow to cool and solidify. This will take approximately 20 minutes.
- When the gel is cool, carefully pull out the comb.
- Place the solidified gel kept in the casting tray inside the electrophoresis chamber. The wells should be located at the BLACK negative end of the apparatus.
- Pour 1X TAE buffer on top of the gel into the electrophoresis chamber so that it covers the gel with about 3 mm of liquid.

Sample Preparation, Loading and Gel Electrophoresis

- Label each PCR tube with the name of sample being tested.
- For each tube, add the volumes of nuclease-free water, DNA sample and 6X loading dye calculated above. Pipette up and down gently to mix.
- Load the first well of the gel with 5 µL of the ladder chosen above.
- Load the wells of the gel with the samples prepared in PCR tubes.
    a. Do not move too slowly when loading the wells, otherwise the first samples will begin to diffuse as the last samples are being loaded.
- Place the lid on the gel electrophoresis chamber, taking care that the red electrode matches the red end on the chamber.
- Turn on the electrophoresis power source. Plug the electrodes connected to the gel electrophoresis chamber lid to the electrophoresis power source. Please consult SOP #17 Electrophoresis power supply for additional details prior to usage.
- Run the gel electrophoresis at a constant voltage of 120 V for 60 minutes. Monitor the progression of the gel to ensure that the band representing the loading dye does not run off the gel.
    a. Stop the electrophoresis if this band is about to run off.
    b. Extend the duration of electrophoresis if this band does not pass ~75% of the gel.
- Stop the run and turn off the gel electrophoresis power supply.

Gel Imaging

- Remove the gel tray containing the gel from the gel electrophoresis unit.
- Open the door of the transilluminator (BioRad Gel Doc XR+) and place the gel on the transilluminator surface. Close door.
    - Consult SOP #19 Bio-Rad Gel Imager for more information
- Select the “GelRed” protocol and follow computer instructions for changing the filter settings. Run the protocol and either utilize automatic exposure or manual exposure.
- Acquire the image and save as jpeg in team folder. Send image through email on the laptop.

Safety Precautions

- Heat, electrical current, UV light
- Always wear safety goggles, lab coat and disposable gloves.
- Wash hands in the designated hand-washing sink once finished.
- Wear appropriate skin and eye protection when working with UV light.
- Ensure all switches and indicators are in proper working condition.
- Ensure power cords and leads are undamaged and properly insulated.
- Wear insulated work gloves when heating and transferring agarose gel. Do not cap the agarose gel container when heating.

Reference

- https://www.thermofisher.com/content/dam/LifeTech/global/brands/Documents/1114/general-recommendations-dna-electrophoresis.pdf
- IBEHS 2P03 Laboratory Manual
- SOPs supplied by ETB 311