Nov 20, 2020

# Running Gels: Electrophoresis Gel Procedure

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1 Works for me dx.doi.org/10.17504/protocols.io.bnvdme26

Bark Beetle Mycobiome Research Coordination Network

ABSTRACT

This protocol describes how to run electrophoresis gels at the UF Forest Entomology Lab.

This protocol is part of the Bark Beetle Mycobiome (BBM) Research Coordination Network. For more information on the BBM international network: Hulcr J, Barnes I, De Beer ZW, Duong TA, Gazis R, Johnson AJ, Jusino MA, Kasson MT, Li Y, Lynch S, Mayers C, Musvuugwa T, Roets F, Seltmann KC, Six D, Vanderpool D, & Villari C. 2020. Bark beetle mycobiome: collaboratively defined research priorities on a widespread insect-fungus symbiosis. Symbiosis 81: 101–113 https://doi.org/10.1007/s13199-020-00686-9.

DOI

dx.doi.org/10.17504/protocols.io.bnvdme26

DOCUMENT CITATION

Demian F Gomez, Andrew J. Johnson, You Li 2020. Running Gels: Electrophoresis Gel Procedure. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bnvdme26



CREATED

Oct 23, 2020

LAST MODIFIED

Nov 20, 2020

DOCUMENT INTEGER ID

43653

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**Materials:**

TAE buffer (In transparent plastic bottle)
Erlenmeyer flask with cap (glass petri dish)
Weigh paper



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Agarose (1% agarose in buffer)
[Hoefer PS300-B] Electrophoresis machine
[Thermo Scientific OWL Easycast B1] Gel Tray
2 gel combs

**Procedure:**

• Weigh 0.7 grams of agarose using the blue plastic spoon in the agarose jar, and put this in the flask (for small electrophoresis machine in the lab).
• Measure 70 mL of TAE buffer and pour into the flask
• Heat the flask with cap in the microwave at 2 minutes until you hear a tinkling sound – at this point take it out and swirl. Use the green PPE glove.
• Repeat microwaving in increments until you hear the tinkling sounds. Keep repeating until the agarose is completely dissolved (it will not look like there are clear granules in the liquid).
• Let the flask cool after microwaving (you may run water over the flask to cool it faster). It should cool to about 50 degrees C before you pour it. The flask shouldn't be painful to the touch.
• Ensure that the gel casting tray is clean and dry. It may be cleaned with DI water if dirty. Never use bleach or ethanol to clean the acrylic.
• Ensure that the gel container is oriented so that the current will run from black to red.
• Check that the gel container is level using the rounded level.
• Ensure that the gel casting tray is oriented so that the seals are against the walls of the gel container.
• Pour the gel from the flask into the casting tray. Use a pipette tip to remove any bubbles.
• Include 2 gel combs in the notches in the tray. Note: the combs have different volume sizes and amount of wells on each side, so be sure to pick the well volume and number of wells you want.
• The gel takes 1.5 to 2 hours to fully solidify. It can be checked intermittently during this time, and will turn opaque when solid and dry.
• Turn the gel casting tray so that the well contents will flow from black to red. If oriented from red to black, the bands will just fall off of the gel into the buffer. Pour buffer so that it is touching the electrodes.
• Once buffer is covering everything, the combs may be taken out. Be careful not to rip the gel. Pull the combs directly upwards.

**Filling Wells**

Materials:
SYBR green (In freezer)
Loading dye
Ladder 100 BP (fridge)
Approximately 6 inches of parafilm
10 uL pipette and tips
Procedure:
• Beforehand, mix 300 uL SYBR green with 600 uL loading dye and keep in freezer.
• Centrifuge the PCR products and then put in a frozen rack. Centrifuge SYBR green mixture and put in the larger frozen rack wrapped in aluminum foil. It is light sensitive.
• Add SYBR green mixture in dots on the parafilm, with 2 uL of the mixture per dot. You will need the amount of dots for your number of samples plus 1 for the ladder. (8 dots for 5 beetles, a negative and positive control, and the ladder).
• Add 6-8 uL of ladder in the first dot only, and pull the liquid up and down in the pipette tip to mix. Note: if electrophoresing two rows of wells, it can be helpful to put a ladder in the first well of the second row as well as the first.
• Add 6 uL of each PCR product in each of the other dots and mix up and down in the pipette tip (It is important at this point to make sure you know what order your samples are in).
• Set the pipette to 9.5 uL and pull the first dot up with the pipette. Be careful not to introduce air bubbles. Add the liquid to the first well as vertically as possible, and being careful not to touch the gel. Don't press the pipette button down the second half of the way so that air bubbles are not introduced. It is better to leave a very small amount of PCR product in the tip than to introduce bubbles.
• Repeat this with each of the dots into each of the wells.
• Slide the lid onto the gel container.
• Plug the red cord into the red outlet, and the black cord into the black outlet on the machine.
• Turn the machine on, and put on 100 volts for 45 minutes.
• Take a photo of the gel within 10 minutes after it finishes.

**Gel Photos Procedure:**

• Take the gel casting tray out of the gel container and place it into the tray of the Enduro machine. Make sure everything on the machine is closed, because it emits UV light to take the photo.



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• Open the Enduro GDS software on the computer, and click capture image, then click illuminate. This should make the gel with bands visible. You can adjust the illumination to make the bands more visible. Once done adjusting, you can take a photo.
• Take the gel casting tray out of the machine, making sure the UV is off first.
• Gel can be thrown away, and the gel casting tray can be cleaned with DI water. Wipe the Enduro tray with DI water as well.



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