Agarose Gel Electrophoresis

Make, load, and run a 1.5% agarose gel to visualize the products of your PCR reactions. See the end of this file for the agarose gel protocol.

Use the combs to create the space for your samples. Be sure to include a lane for the 100 bp ladder.

When you are loading the gel, use 5 uL of your reactions (each in a separate lane), and 5 uL of the 100 bp ladder (also in a separate lane).

Make a map of your gel in your lab notebook so that you will know which reaction is in which lane of the gel.

When it finishes running, be sure to take a picture of your gel.

Are there bands in each of your PCR reactions? Hopefully, there is a band for your positive control and there is no band in your negative control.

Be sure to save your PCR products - the PCR amplicons can be stored for several weeks in the refrigerator.

This material is based upon work supported by the National Science Foundation under Grant Nos. EF-2025121, EF-2025067, EF-2025009, and EF-2150107. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

Lab Skills: How to Make, Load, and Run an Agarose Gel

We will frequently use agarose gels to visualize the results of PCR reactions. Loading PCR products on the agarose gels is not difficult, but it does take some practice to get used to the muscle movements and motor control.

This protocol is for a big-size gel with two rows of combs. If you want to use any other gel sizes you need to recalculate the gel and TAE quantities to the appropriate volume of the tray.

To Make a 1.5% agarose gel:

1. Use gloves and locate your materials:

_____ 0.75g agarose

_____ 50ml **1x** TAE

_____ Hot hands (to hold hot flask)

_____ SYBR Safe DNA stain: need to keep in a dark box

_____ 250ml flask

_____ Kimwipe

2. Measure TAE and add to flask.

3. Weigh out agarose and add to flask.

4. Use a Kimwipe to insert at the top of the flask and swirl to mix.

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5. Put the flask in the microwave.

6. Push “Time” 60 (60 sec). Push “Stop” when it begins to boil if before 60 (seconds).

7. Remove the flask using Hot hands and ***gently*** swirl to mix. If it isn’t a gentle swirl, the agarose can boil over out of the flask and onto your hands. This will hurt!

8. Return to microwave and push “Time” 20. Push “Stop” if it begins to boil before 20.

9. Remove the flask using Hot hands and gently swirl.

10. Check to be sure the agarose has dissolved.

11. Microwave a third time until boiling if needed. Be sure the agarose is fully dissolved, and sometimes a fourth round is needed!

12. Let sit for 5-10 mins or until you can hold the flask comfortably in your palm.

13. While the flask is cooling, set up a gel tray with two combs.

14. Add 5 ul of SYBR Safe to the flask and very gently swirl to mix. Try not to create any bubbles in the gel.

15. Pour the agarose with SYBR Safe into the tray. Make sure you have put two combs into the tray. If you have bubbles, use a pipette tip to slowly move the bubble away from the comb or pop it.

16. Let the gel set for approximately 30 mins. You can check whether it has set with a pipette tip.

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Loading the gel

1. Locate your “loading dye” and your ladder.

2. Place the set gel into the gel chamber so the samples run towards the positive (red) electrode.

3. Make sure there is sufficient 1x TAE buffer in the chamber to cover the gel.

4. Carefully remove the combs from the gel. Pull steadily and slowly straight up; you might need to keep one finger on the edge of the gel tray to push the tray down while you pull the comb up.

5. Check to see that there is still enough buffer in the chamber to fully cover the gel by approximately 1 to 2 mm of fluid. If there is not enough, add more buffer. If there is too much, remove some buffer with a disposable pipet.

6. Load 5 ul of each PCR reaction in separate wells, then load 5 ul of 100 bp ladder into one well. Draw a map of your wells and note what will be in each well. Take your time loading the gel. Common mistakes include:
    - piercing the bottom of the gel,
    - pipetting the DNA outside of the wells,
    - pipetting an air bubble into the well which causes your DNA solution to escape the well, and
    - pipetting too quickly such that your DNA pours outside of the well.

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Running the gel

1. Place the cover on the gel chamber. Make sure it is closed properly before plugging the electrodes into the power supply.

2. Set the power supply to 80 volts (constant voltage setting) and start the gel. Be sure to note what time the gel starts. Check for bubbles rising from the positive electrode to confirm that the power supply is connected.

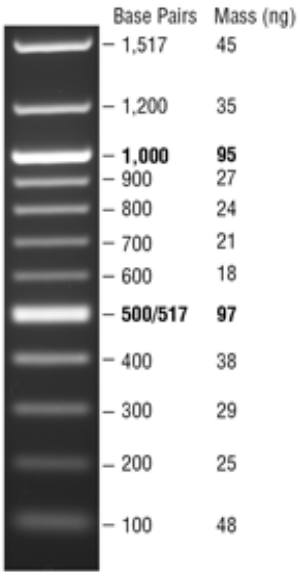
3. Run for approximately 20 to 25 minutes. Be sure to stop the gel before the first dye front from the top row reaches the bottom row of wells.

4. Once the run is complete, turn off the power supply and unplug the electrodes.

5. Carefully remove the gel tray and take to the gel imager.

6. Take a picture of your gel and record the results in your notebook. Can you see each band of the 100 bp ladder? Can you see bands in your PCR reactions?

More information on this 100 bp ladder: https://www.neb.com/products/n0551-quick-load-purple-100-bp-dna-ladder#Product%20Information



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