Agarose Electrophoresis

E. Crandall/P.Barber

Agarose electrophoresis is performed to visualize your PCR products. This step allows you to determine whether your PCR was successful, whether the resulting product is the correct size, whether other products were amplified as well, and whether the concentration of the resulting product is suitable for cycle sequencing. All gel work should be performed in the **HIGH DNA** area.

Precautions

1. Always wear gloves! This is essential to limit the spread of PCR products around the lab as well as protecting against exposure to Ethidium Bromide.
2. Treat Ethidium Bromide with respect (it is a powerful mutagen) and ensure that it does not get spread around the lab.
3. Wash hands when finished.

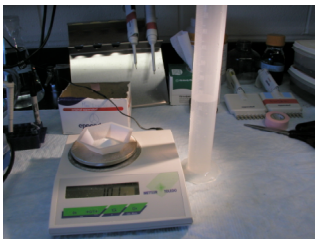
Materials

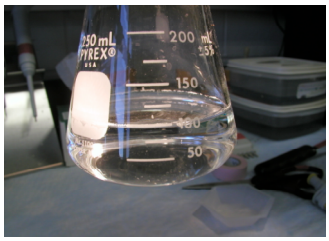
1. Agarose
2. 1X SB
3. weighing paper

Equipment

1. Balance
2. Erlenmeyer flask
3. Microwave
4. Gel box with comb

IF GEL IS PREMADE, PROCEED TO STEP 3.





Step 1: Mixing Gel

1. On the scale, weigh 1 gram of agarose onto a piece of weighing paper.
2. Add agarose to Erlenmeyer flask.
3. Add 100mL of 1X SB to Erlenmeyer flask.
4. Swirl vigorously to thoroughly mix agarose. Slurry will by opaque.

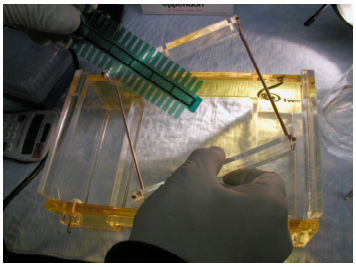
Step 2: Melting Gel

1. Put agarose and 1x SB slurry into microwave.
2. Heat on HIGH for 30 seconds at a time.
3. After 30 seconds, remove from microwave and swirl. Be careful, as the mixture will be HOT.
4. After 1 minute of heating, repeat heating and swirling procedure every 10 seconds until mixture is clear.
5. If the mixture begins to boil, stop and IMMEDIATELY remove the agarose. Swirl until mixture is clear.

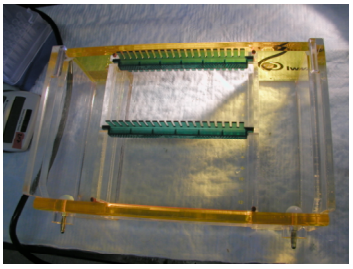
NOTE: Watch carefully to ensure that agarose mixture DOES NOT boil over in the microwave.

Step 3: Pouring the Gel

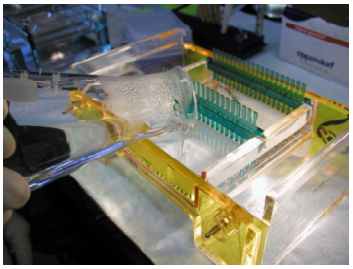
1. Before pouring, gel must be cool enough for you to hold the Erlenmeyer flask in your hand. Pouring boiling hot gel will warp the gel boxes.



2. Place gel tray into casting chamber.
3. Add casting comb(s) into the appropriate slot(s).
4. Pour agarose into gel tray to about 5-7mm.
5. Let sit for at least 30 min, until gel is cool to touch and is opaque in appearance.



6. Once set, place gel and tray into gel rig, with wells on the left (cathode) side. Then, fill gel rig with 1x SB sufficient to cover the entire gel.
7. Carefully remove combs by pulling them upwards firmly and smoothly in a continuous motion. The remaining depressions are the wells into which your samples will be loaded.

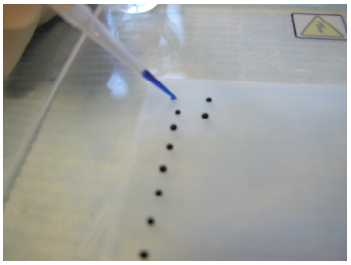


NOTE: When pouring the gel, avoid creating bubbles as this will prevent current from flowing through the gel.

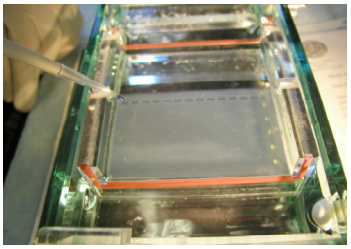
NOTE: Do not pull comb out too quickly as wells will form holes, resulting in the loss of your sample.

Step 4: Loading the Gel

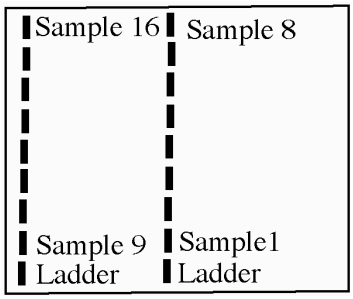
1. Cut a piece of parafilm and place it flat on the bench top. You may have to rub or scratch it to stick it down.



2. Using a pipette, place small dots of 6X loading dye (about 1-2uL) onto parafilm, in rows of 8, 1 dot for each PCR sample that will loaded on the gel. It is not necessary to be exact, and it is not necessary to change the tip.
3. Using a pipette take 3 µl of product and pipette it onto its corresponding dye dot, then mix the sample and dye by pipetting up and down. Then, pipette up the dye/sample solution and pipette it into the proper well.

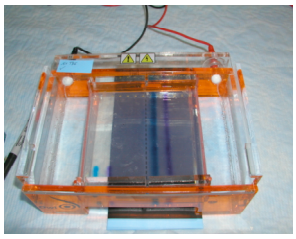


NOTE: By convention, samples are loaded from left to right starting with well number 2, leaving the first well in each row of wells for the DNA ladder.



NOTE: Insert pipette tip into well at an angle to avoid putting a hole in the bottom of the well.

4. After loading the first sample into the well, wash out your pipette tip by pipetting up and down several times in the buffer, then blot tip dry with a Kimwipe. In this way, all of your samples may be loaded using **ONE tip**.
5. Once all samples have been loaded into the gel, using a **fresh tip**, pipette 3uL of the DNA ladder into well number 1 of each row of wells.
6. Put gel box cover into place (this step is essential for your gel to run and to minimize the risk of electric shock). Turn on power supply. Run the gel for 20 minutes at 200 volts.
7. Check for bubbles at the cathode side to ensure that gel rig is running. Clean up, deglove, and wash hands.



Tips to improve your gels

A. Do not shake the gel chamber while loading as this will facilitate diffusion of samples out of the wells
B. Insert pipette tips into wells at an angle to avoid puncturing the well, which will result in loss of sample.
C. Always make sure that your gel is running before leaving.
D. Work as fast as you can without sacrificing accuracy and safety. The longer your samples sit in the buffer, the more diffuse your bands will be when you visualize the gel.
E. Until you are comfortable, only load 1 row of samples at a time, run the gel for 1 minute, then load the next row of samples. This will minimize sample diffusion.

Step 5: Visualizing the Gel

1. Turn off power supply. Using gloved hands, remove the cover from the gel box.
2. Remove the gel and casting tray from rig, avoiding dripping buffer all over the bench top.
3. Carefully slide the gel off of the casting tray and into ethidium bromide solution. Caution: ethidium bromide is a mutagen and should be treated with respect. If you get ethidium bromide on you gloves, immediately change your gloves, placing the contaminated gloves into the ethidium bromide trash. Do not spread ethidium bromide around the lab.
4. Soak the gel in the ethidium bromide solution for 10-15 minutes to stain. Using a spatula, remove the gel from ethidium bromide and rinse for 30 seconds in a tray 1x buffer. The gel may be left in the 1X buffer for longer to "destain" the gel and improve contrast of stain, but this step is often unnecessary.
5. Using the spatula, carefully remove the gel from the destaining buffer and place the gel on UV light box, avoiding creating bubbles underneath the gel.
6. Put on UV protecting face shield and turn on UV light box to see stained sample bands and DNA ladder. Ensure that all around you are protected for UV exposure.

7. If bands are strong, take a picture using the Polaroid camera (below). If bands are weak, return gel to ethidium bromide stain bath as the gel has been insufficiently stained. If the ladder is strong, but the PCR bands are weak, this indicates that the PCR reaction did not work well. Consult professor or TF on how to improve your PCR.

Step 6: Taking a picture. Remember that the gel has been soaking in Ethidium Bromide. All objects and surfaces that the gel comes into contact with will be contaminated with Ethidium Bromide. Always wear gloves and limit the spread of Ethidium Bromide to protect yourself and your lab mates.

1. Ensure that you are still wearing your gloves and UV face shield.
2. With the gel flat on the UV light box, position the hood of Polaroid camera over the gel so that the hood is centered over the gel.
3. Turn on the UV light box.
4. Keeping the camera steady, pull the trigger and **HOLD the trigger** until there is silence (i.e. the shutter has opened and closed. This may take 0.5-2.0 seconds depending on the cameras exposure settings).
5. Turn off the UV light box.
6. Pull WHITE film tab on camera to remove film. Wait 1 minute
7. After one minute has passed, pull backing off of film to check whether the gel imaged properly.
8. Once picture is developed and you have a good picture of your gel, remove your gel and put in gel disposal box.
9. Wipe down the UV light box with Kimwipes and dispose of in gel disposal box. Remember that all of these surfaces and wipes are contaminated with Ethidium Bromide.
10. Remove your gloves and dispose of in the Ethidium Bromide trash.
11. Wash hands thoroughly.
12. Label your picture.

NOTE: Do not change the shutter speed or F-stop on the camera unless instructed to do so by the professor or T.F.