Agarose Gel Equipment

There are a lot of ways to run the gel. Here is one of the ways. Make sure the operator has more than 3 h on the task of running the gel

Gel Preparation

The base of the gel is 0.5× TBE with 11 mM magnesium and agarose powder. To visualise the DNA, EtBr should be added into the gel. It takes more than 0.5 h to prepare the gel.

1. Wash and dry the 125 mL Erlenmeyer flask. Weight 0.5 g agarose powder (for one piece of 1 % gel). Add the powder into the flask and add TBE buffer to the level of 50 mL. Get the 500 mL Erlenmeyer flask ready for washing the smaller flask once the boiling of the gel is finished
2. Heat the 125 mL flask with the heating pad in the fume hood (adjust the temperature to 400 as the value is not accurate). Add tap water into the 500 mL flask and put it in the fume hood with paper towel under it and by the side of the heating pad
3. Once the mixture in the 125 mL flask is boiling, use thermal resistant glove to move it off the heating pad, on to the paper towel. At the same time, place the 500 mL flask on the heating pad. Adjust the temperature setting to 250
4. Quickly get ready the horizontal dock, the holder for the gel and the 8-bit comb ready for solidification of the gel (in the fume hood). It has to be ready by step 6. This is time sensitive as the gel is cooling
5. Wait until the 125 mL flask is about 80 °C (can hold at the neck by thin gloves for 1 – 2 s), add 5 µL of EtBr original solution (10mg/mL). If the visualisation is not good, add 10 µL or even more. Try not to touch the agarose solution with the pipette tip. Stir the flask with hand (thermal resistant glove) for evenly distributed reddish pigment
6. Wait until the 125 mL flask is about 60 °C (can hold at the neck by thin gloves for 10 s), pour the agarose solution into the holder for the gel (make sure the comb is well-placed). Double check the liquid level is horizontal and even
7. Pour ~75 mL warm water from 500 mL flask into the empty 125 mL flask. Place the 500 mL flask on the paper towel and 125 mL on the heating pad. Adjust the temperature setting to 400. Wait for boiling
8. Once the water boils, use thermal resistant glove to pour the water into the small sink inside the fume hood. After this, use the rest of warm water in the 500 mL flask to rinse the 125 mL flask twice. Use the tube brush if needed. The wastewater goes into the regular sink. After warm water, use tap water to rinse it a few times until it is clean
9. Somewhere during step 8, the gel should be solidified. Check it by blow soft wind or tilt on corner of the horizontal dock. If there is no flow, it is a solid piece. Carefully remove the horizontal dock assembly from the fume hood and place it outside once the electric power parts are ready

Electric Power

During the last few steps of gel preparation, the operator should get the electric part ready

1. Take the running dock out and install the electrodes. There are two sets. Pick the shiny set. Check with the cabled covering to avoid the wrong order
2. Carefully remove the holder for the gel together with the gel and comb from the horizontal dock. This might need some practice since it is very easy to rip the edge of the gel. Do not pull the comb out from the gel yet

3. Place the holder in the running dock out. Make sure the comb is at the side of the negative electrode (since DNA is negatively charged, it will move from negative to positive).
4. Add TBE buffer to the dock. Make sure the buffer goes on both sides and just a little over the level of the gel. Once all the gel is submerged in the buffer, carefully remove the comb. Sometimes the gel can move as the comb is removed. This is not a big problem. Just use clean gloves to gently push the gel back to its place
5. Mix the sample to be run in the gel with loading buffer. Depending on the concentration of loading buffer, the mixing ratio is different. For example, if use 6× loading buffer, mix 15 µL sample with 3 µL loading buffer. The maximum loading volume should be 20 µL. Prepare a ladder with loading buffer. If the running ladder is available, it can be used directly. Otherwise, the stocking ladder is a concentrated solution. Calculate the amount needed for stocking ladder, loading buffer and DI water so that the final concentration of ladder and loading buffer are all 1×
6. Add ladder and all the samples with loading buffer in the 8 wells. Normally ladder is placed at one of the two sides (either #1 from the left or right). Sometimes it can be really hard to see the place of the well. Try different directions and feel that with the pipette tip. Once the samples are loaded, make sure the blue-purple liquid is not escaping
7. Put the cabled covering on and plug the cables into the power supply. Check the electric current again. The DNA samples should be places near the negative side. If the order is wrong, simple reverse the cables in the power supply
8. Adjust the voltage to 20 V. Test run to see if there are any error codes. Sometimes there can be an error stating no current. This could be a bad electrode. Try to clear the rusty part may solve the issue. If there is nothing wrong, both electrodes in the buffer should be surrounded by tiny bubbles. If not, wait a bit or do the same procedure as if there is no current

Running and Checking the Gel

When have time, rinse all the used equipment and dry them on the paper towel

1. Wait for 10 min at 20 V or until the directing of sample movements is visible. If the direction of moving is wrong, flip the cables in the power supply
2. Add the voltage to 60 V. Let it run for 2 h. If condition permits, check it frequently and add ice packs to keep the temperature low
3. Once the colour bands from the loading dye have pass the half of the gel, it can be taken out for visualisation under UV light
4. When taking the holder for the gel and the gel together from the buffer, make sure it is tilted to one side so that the buffer above the gel can flow back down into the running dock. Use the gloves to hold the lower side so that the gel won't slip down with the buffer. The 'exhausted' buffer can be reused for the next gel
5. Use the Spectroline TE-312S UV Transilluminator to shine UVB from the back side of the gel and holder. The bands should be separated. If not well-separated, the gel can be placed back to the buffer for further running
6. For better visualisation and photos, the operator may remove the gel from the holder. Be really careful in case the gel breaks or fails on the floor. The operator may also open

the plastic covering of the transilluminator. Before opening it, UV face shield should be placed

7. Recovering origami from the gel can be referred to the instruction manual of 'Freeze N Squeeze' on BIO-RAD website
8. Clean everything related to the gel. The gel itself and anything in contact with concentration EtBr (gloves, paper towels and sometimes blades) must be disposed into the special trash can for EtBr