DNA GEL ELECTROPHORESIS

Est. Total Time: 1.5 Hours.

Summary: DNA gel electrophoresis is a common method used by molecular biologists to separate DNA by length/size for the purposes of qualitative studies or for the purification/isolation of DNA.

Materials

- 1L Graduated Cylinder
- 10X Tris-Acetate-EDTA (TAE) Buffer
- DNA of Interest
- Deionized Water (DI H_2O)
- Agarose, Molecular Biology Grade
- DNA loading dye
- DNA Ladder
- SYBR Safe, SYBR Green, or Ethidium Bromide (EtBr) DNA Stain
- PowerPac Power Supply
- Microwave
- Micropipette, 2-20µL
- Pipette Tips 2-200 µL
- 250 mL Erlenmeyer Flask
- Autoclave Gloves
- Gel Running Box
- Gel Casting Box
- Gel Casting Balance
- Gel Casting Comb
- Gel Casting Tray

Procedure:

Reagent Prep

1. Make 500mL of 1X TAE by taking 50mL of 10X TAE and diluting it to a final volume of 500 mL. Mix well.

Gel Casting

1. Place the gel casting tray into the gel casting box. Turn the cam lever on the gel casting box and ensure that a tight seal is formed between the tray and the box.
2. Use the gel casting bubble balance to determine whether or not the tray-box combo is flat. If not, adjust the screws on the gel casting tray accordingly. Refer to the figure at below:

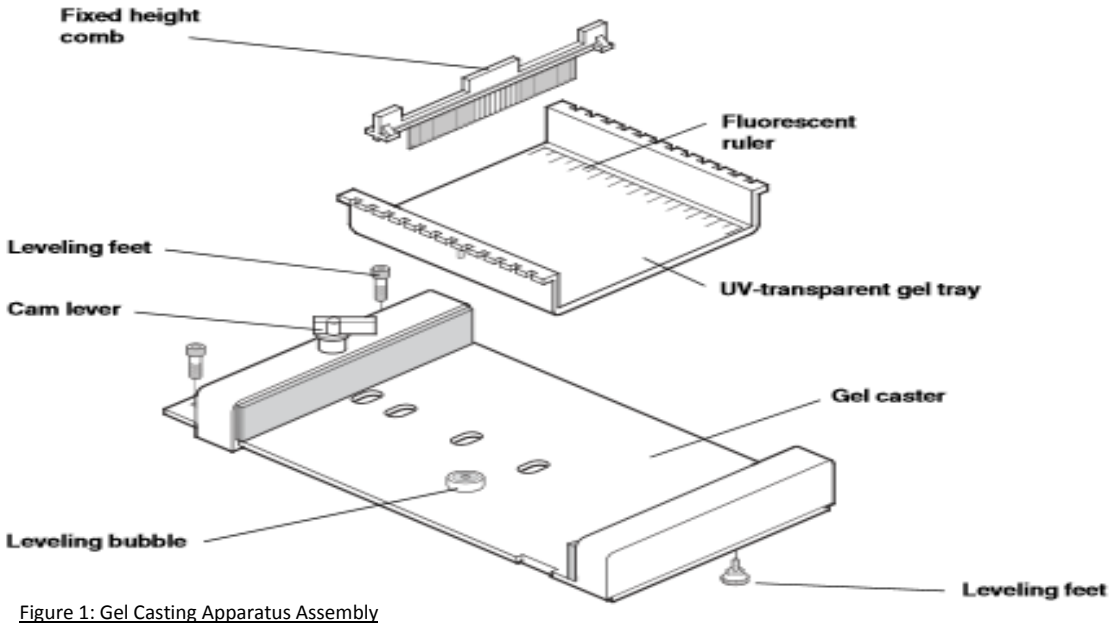

Figure 1: Gel Casting Apparatus Assembly

Gel Casting (cont'd)

3. According to the following table, weigh out agarose according to the size range of your DNA of interest; the goal is to make a 100mL solution.

Gel Concentration (%)	DNA Size (Kb)
0.50	1–30
0.75	0.8–12
1.00	0.5–10
1.25	0.4–7
1.50	0.2–3
2–5*	0.01–0.5

* Sieving agarose such as AmpliSize® agarose Figure 2: Gel Concentrations of Agarose molecular sieve for respective DNA sizes.

4. Transfer the agarose to the 250 mL Erlenmeyer Flask, and dispense 100 mL of 1X TAE buffer into the flask.
5. Heat the flask in the microwave for 10s stints, (USE AUTOCLAVE GLOVES) swirling after each stint. A total of 90s should suffice. DO NOT LET IT BOIL OVER!
6. Upon the full dissolution of the agarose, wait ~5min. for the solution to cool.
7. Add EtBr to the flask, swirl to mix.
8. Pour 50mL of the mixture into the gel casting apparatus.
9. Pop any bubbles that form with a pipette tip.
10. RINSE OUT THE REMAINDER IN THE ERLENMEYER FLASK IMMEDIATELY.
11. Wait ~30min. for the gel to solidify at room temperature. The gel will become opaque.

Gel Loading

1. After the gel has solidified, loosen the cam lever on the gel casting apparatus, and transfer the gel with the gel tray and comb to the electrophoresis chamber, shown in the figure below. IGNORE THE GEL CASTING PLATES.

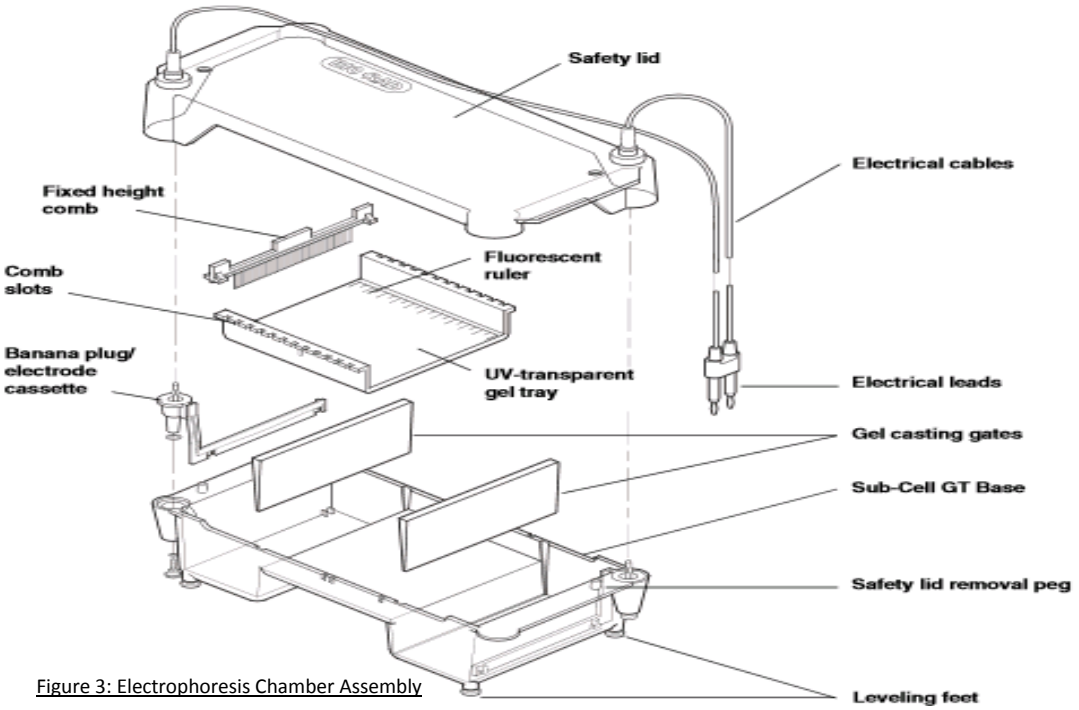

Figure 3: Electrophoresis Chamber Assembly

2. Ensure that the comb is closest to the black electrode. The electrophoresis will run to red.
3. Pour ~250 mL of the remaining 1X TAE buffer into the electrophoresis chamber. Ensure that the gel is completely submerged in the buffer.
4. Gently remove the comb from the gel.
5. Add loading dye to the samples, to dilute the loading dye to 1X with each sample.
6. To load a well:
    a. Load the sample into the pipette.
    b. Grip the pipette above the tip with the thumb and forefinger of your non-dominant hand and hold the pipette as usual in your dominant hand.
    c. Gently insert the pipette tip into the well; take care not to stab the well bottom.
    d. Slowly push down the plunger to the first stop of the pipette.
    e. If fluid remains in the pipette tip, gently proceed to continue pushing the plunger of the pipette. Avoid air bubble formation. If an air bubble forms, gently pull back the plunger of the pipette to recapture the air. If no fluid remains, proceed to the next step.
    f. Gently withdraw the pipette tip.
7. In this manner, gently pipette 10µL or 6µL of DNA ladder into the first well.
8. Load your samples into the other wells. The wells hold a maximum of 40µL and 20µL for 8 and 15 wells, respectively.

Electrophoresis

1. Place the safety lid onto the gel box such that the banana plug electrodes click into place.
2. Plug the other ends of the electrodes (such that the colors match) into the PowerPac power supply.
3. Hold the voltage constant on the power supply, and choose a voltage at which to run the gel, anywhere in the range of 90V to 120V.
4. Hit the run button (the running man).
5. Ensure that bubbles are rising from both sides of the chamber.
6. Check back once in 20 minutes, and then every 5 minutes. The gel is finished running once the dye front has run 2/3 the length of gel.
7. Hit the run button again to stop the gel from running.
8. The gel is ready to be imaged (by way of UV Transillumination, etc.).

Cleanup:

1. Clean out any solidified gel from the containers used. The gel can be wrapped in paper towels or saran wrap and thrown in the trash. Dispose of all waste.
2. Rinse off the gel electrophoresis chamber and the gel tray with deionized water.
3. TAE can be poured down the sink.
4. Replace all reagents in their proper locations.
5. Wipe down all surfaces used with 70% Ethanol or 70% Isopropanol.
6. Consult Lab Staff if unsure about any of the above.

Reference:

Bio-Rad Sub-Cell GT Agarose Gel Electrophoresis Systems Instruction Manual