Agarose Gel Electrophoresis

Overview

Agarose gel electrophoresis is a simple and highly effective method for separating, identifying, and purifying 0.5 to 25 kb DNA fragments. Voltage applied at the ends of an agarose gel generates an electric field with a strength defined by the length of the gel and the potential difference at the ends (V/cm). DNA molecules exposed to this electric field migrate toward the anode (positive end) due to the negatively charged phosphates along the DNA backbone. The frictional force imposed by the gel matrix limits the migration speed. While charge and/or size can affect the rate at which macromolecules will pass through the gel, the charge to mass ratio is the same for DNA molecules of different lengths. The size of the DNA determines the rate at which it passes through the gel, thereby allowing an effective separation of a mixture of different DNA fragment-lengths by electrophoresis. To visualize the DNA, Ethidium bromide is added to the gel. This dye intercalates between the stacked bases of nucleic acids and fluoresces red-orange (560 nm) when illuminated with UV light (260 to 360 nm).

Reagents and materials

- 6X DNA loading buffer
- 1 X TAE buffer (agarose gel running buffer)
- Ethidium bromide 10 mg/ml (20,000x stock)

Note: Ethidium bromide is a DNA-intercalating molecule and, therefore, a mutagen! **You must wear proper PPE and specifically nitrile gloves when handling EtBr**. Dispose of all tips in the labeled receptacle in fume hood. Gels should be disposed of in the waste container (bucket) for gels only (**do not** dispose of paper towels, gloves, etc. with the gels). TAE buffer containing Ethidium Bromide is filtered through special bottle filters. Each filter can be used to remove the Ethidium Bromide from up to 10 liters of buffer. Between uses, the filter should be stored in the Satellite Accumulation Area (SAA). When it has been used to filter the maximum volume of 10 liters, it is treated as chemical waste - placed in a plastic bag, tagged appropriately, and placed in the SAA for collection.

Procedure

1. To an Erlenmeyer flask, add agarose in an appropriate weight to volume (see below). The final gel should be about 8mm thick. If you're unsure of how much agarose solution is needed to fill the casting tray, pour water from a graduated cylinder containing a known volume. In this way, you can determine how much volume is needed to fill the tray. **Note:** Avoid entirely covering the comb with liquid.

Agarose (%)	Size of fragment to be separated
0.5	15 kb to 30 kb
0.7	1 kb-12 kb
1.0	500 bp to 8 kb
1.2	400 bp to 5 kb
1.5	200 bp to 3 kb
2.0	Less than 200 bp

2. Add appropriate amount of 1X TAE to the flask. ****Do not make your agarose in water!**** Bring slurry to a boil in a microwave (heat about 2 minutes on high power), swirl carefully, and repeat as necessary until no "streaks" or chunks remain.
**** Be careful with molten agar and agarose. It can boil over suddenly and cause burns. Use an orange autoclave mitten or the rubber bottle mittens.**

3. Once agarose is completely dissolved, add Ethidium bromide **in the hood,** to a final concentration of 0.5 µg/ml (WEAR GLOVES!) – for example 5 µl in 100 ml of agarose solution. Swirl to mix.

4. Set up the gel tray in the gel box (in the casting orientation, which is perpendicular to the long axis of the gel box) and insert a comb. Pour molten agar into the gel box in the hood. Any air bubbles in the solution can be removed with a pipet tip.

5. The agar is completely set when it becomes opaque (< 20 min). If you prepare your gel ahead of time, wrap it in plastic wrap and store it at 4°C.

6. Add the loading buffer/dye to your DNA samples to a final concentration of 1X. DNA loading buffer is 6X.

7. Set up your gel box for the run. The casting tray has to be placed so that the open ends of the tray are continuous with the buffer reservoirs at either end of the gel box. Add 1X TAE buffer (same as the buffer used to make the agarose gel) to the box. The gel should be covered by buffer (about 5-8 mm deep above the gel); this allows current to pass from one electrode to the other, through the gel.

8. Before loading samples on the gel, make sure that the wells contain buffer. You can gently flush out any air bubbles that may be in the well with the 1X TAE buffer. Load samples on the gel. Remember to also load a molecular weight marker of the appropriate sizes. See the end of this protocol for the patterns of commonly used 1 kb and 100 bp molecular weight markers available in lab.

9. Run the gel at 80-120V. If time permits, run the gel at a voltage that is at the lower end of the range. Runs at high voltage disrupt resolution and the DNA bands will appear fuzzy. **Note:** The bromophenol blue (dark blue) in the loading dye will migrate with an apparent molecular weight of 300-500 bp and the xylene cyanol (light blue) at 2-4 kb.

10. When the gel has run far enough (the bromophenol blue should still be in the gel, especially if you are interested in bands that are less than 300 bp), view the gel using the imager and the UV box in the dark room. If the bands have clearly separated and you can determine the sizes, the run can be stopped and you can take a picture of the gel.

Note on electrophoresis of RNA samples:
You may wish to check the integrity of RNA samples by running a very small volume on an agarose gel. In this case, use the smallest gel tray and comb that forms the smallest wells available. Since the reagents (agarose, TAE, etc.) are unlikely to be free of RNases (that degrade RNA), the gel has to be loaded quickly and run only for the amount of time needed to resolve the two prevalent rRNA types found in total RNA preparations. Alternatively, TAE buffer can be made using DEPC (RNAse-free) water.

Solutions

6X DNA loading buffer
2.5% Ficoll
11 mM EDTA
3.3 mM Tris-HCl
0.017% SDS
0.015% bromophenol blue
0.015% xylene cyanol
pH 8.0@25°C

Make a volume in a 50 ml Falcon tube, dispense into aliquots, and store at RT.

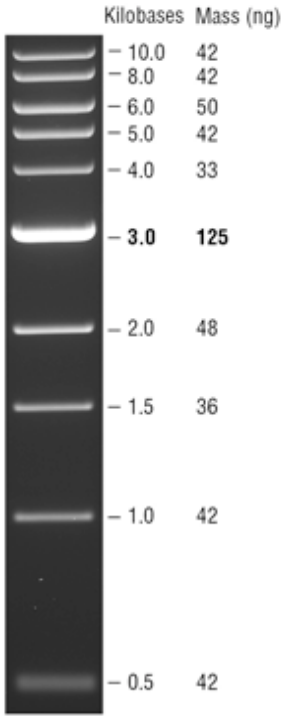
1X TAE (working solution)
40 mM Tris-acetate
2 mM $Na_2EDTA{\cdot}2H_2O$

The stock buffer purchased for lab is 25X; to make 10 liters of 1X, mix 0.4 liter of 25X stock with 9.6 liters dH_2O.

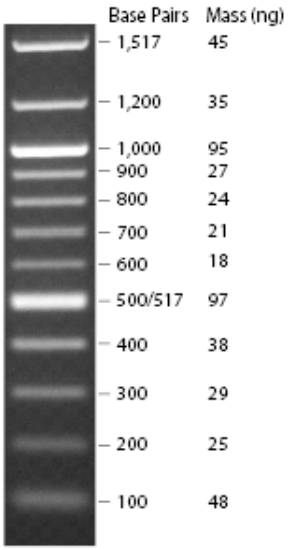
50X TAE stock
242 g Tris base
52.1 ml glacial acetic acid
37.2 g $Na_2EDTA{\cdot}2H_2O$ (Ethylene Dinitrile Tetraacetic disodium salt)
add dH_2O for a final volume of 1 liter

DNA ladders (New England Biolabs)

1 kb ladder



100 bp ladder



7.15 Experimental Molecular Genetics

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