Agarose Gel Electrophoresis Protocol Generally Utilised

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Abstract

Agarose gel electrophoresis is a method of gel electrophoresis used in biochemistry, molecular biology, genetics, and clinical chemistry to separate a mixed population of macromolecules such as DNA or proteins in a matrix of agarose, one of the two main components of agar. The proteins may be separated by charge and/or size (isoelectric focusingagarose electrophoresis is essentially size independent), and the DNA and RNA fragments by length.[1] Biomolecules are separated by applying an electric field to move the charged molecules through an agarose matrix, and the biomolecules are separated by size in the agarose gel matrix.[2]

Introduction

Agarose gel is easy to cast, has relatively fewer charged groups, and is particularly suitable for separating DNA of size range most often encountered in laboratories, which accounts for the popularity of its use. The separated DNA may be viewed with stain, most commonly under UV light, and the DNA fragments can be extracted from the gel with relative ease. Most agarose gels used are between 0.7 - 2% dissolved in a suitable electrophoresis buffer.

Materials Required:

Buffers and Solutions:

Agarose solutions.

Ethidium bromide.

Electrophoresis buffer.

Nucleic Acids and Oligonucleotides:

Dna Samples.

Dna Ladders.

(Samples of DNAs of known size are typically generated by restriction enzyme digestion of a plasmid or bacteriophage DNA of known sequence).

The equipment and supplies necessary for conducting agarose gel electrophoresis are relatively simple and include:

- An electrophoresis chamber and power supply.
- Gel casting trays, which are available in a variety of sizes and composed of UV-transparent plastic.
- Sample combs, around which molten agarose is poured to form sample wells in the gel.
- Electrophoresis buffer, usually Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE).
- Loading buffer, which contains something dense (e.g. glycerol) to allow the sample to "fall" into the

sample wells, and one or two tracking dyes, which migrate in the gel and allow visual monitoring or how far the electrophoresis has proceeded.

- Ethidium bromide, a fluorescent dye used for staining nucleic acids.(1,2)
- Transilluminator (an ultraviolet light box), which is used to visualize ethidium bromide-stained DNA in gels.

NOTE:

Always wear protective eyewear when observing DNA on a Transilluminator to prevent damage to the eyes from UV light.

1. Prepare a 50x stock solution of TAE buffer in 1000m of distilled H_2O:

   For this weigh 242 g of Tris base in a chemical balance. Transfer this to a 1000ml beaker. Prepare EDTA solution (pH 8.0, 0.5M) by weighing 9.31g of EDTA and dissolve it in 40ml distilled water. EDTA is insoluble and it can be made soluble by adding sodium hydroxide pellets. Check the pH using pH meter. Make the solution 100ml by adding distilled water. Pipette out 57.1 ml of glacial acetic acid.

   Mix the Tris base, EDTA solution and glacial acetic acid and add distilled water to make the volume to 1000ml

2. Prepare sufficient electrophoresis buffer (usually 1x TAE) to fill the electrophoresis tank and to cast the gel:

   For this we take 2ml of TAE stock solution in an Erlenmeyer flask and make the volume to 100ml by adding 98ml of distilled water. The 1x working solution is 40 mMTris-acetate/1 mM EDTA

   It is important to use the same batch of electrophoresis buffer in both the electrophoresis tank and the gel preparation.(3,4)

3. Prepare a solution of agarose in electrophoresis buffer at an appropriate concentration:

   For this usually 2 grams of agarose is added to 100ml of electrophoresis buffer.

Agarose Concentration in Gel (% [w/v])	Range of Separation of Linear DNA Molecules (kb)
0.3	5-60
0.6	1-20
0.7	0.8-10
0.9	0.5-7
1.2	0.4-6
1.5	0-2-3
2.0	0.1-2

1. Loosely plug the neck of the Erlenmeyer flask. Heat the slurry in a boiling-water bath or a microwave oven until the agarose dissolves. The agarose solution can boil over very easily so keep checking it. It is good to stop it after 45 seconds and give it a swirl. It can become superheated and NOT boil until you take it out whereupon it boils out all over you hands. So wear gloves and hold it at arm's length. You can use a Bunsen burner instead of a microwave - just remember to keep watching it.
2. Use insulated gloves or tongs to transfer the flask/bottle into a water bath at 55°C. When the molten gel has cooled, add 0.5µg/ml of ethidium bromide. Mix the gel solution thoroughly by gentle swirling.

   (For the preparation of ethidium bromide adds 1 g of ethidium bromide to 100 ml of H_2O. Stir on a magnetic stirrer for several hours to ensure that the dye has dissolved. Wrap the container in aluminum foil or transfer the 10 mg/ml solution to a dark bottle and store at room temperature.)
3. While the agarose solution is cooling, choose an appropriate comb for forming the sample slots in the gel.
4. Pour the warm agarose solution into the mold.

   (The gel should be between 3 - 5 mm thick. Check that no air bubbles are under or between the teeth of the comb.)
5. Allow the gel to set completely (30-45 minutes at room temperature), then pour a small amount of electrophoresis buffer on the top of the gel, and carefully remove the comb. Pour off the electrophoresis buffer. Mount the gel in the electrophoresis tank.
6. Add just enough electrophoresis buffers to cover the gel to a depth of approx. 1mm.
7. Mix the samples of DNA with 0.20 volumes of the desired 6x gel-loading buffer.
8. Slowly load the sample mixture into the slots of the submerged gel using a disposable micropipette or an automatic micropipettor or a drawn-out Pasteur pipette or a glass capillary tube. Load size standards into slots on both the right and left sides of the gel.
9. Close the lid of the gel tank and attach the electrical leads so that the DNA will migrate toward the positive anode (red lead). Apply a voltage of 1-5 V/cm (measured as the distance between the positive and negative electrodes). If the electrodes are 10cm apart then run the gel at 50V. It is fine to run the gel slower than this but do not run it any faster. Above 5V/cm the agarose may heat up and begin to melt with disastrous effects on your gel's resolution. If the leads have been attached correctly, bubbles should be generated at the anode and cathode.(5)
10. Run the gel until the bromophenol blue and xylenecyanol FF have migrated an appropriate distance through the gel.

    (The presence of ethidium bromide allows the gel to be examined by UV illumination at any stage during electrophoresis).
11. The gel tray may be removed and placed directly on a transilluminator. When the UV is switched on we can see orange bands of DNA.

Procedure for operating the virtual lab:

Check whether you have done all the steps listed below:

- Prepare TAE buffer.
- Transfer 100ml of the buffer to a conical flask.
- Weigh 2grams of agarose and add to the 100ml buffer solution.
- Keep in oven.
- Take the solution from oven.

- Add ethidium bromide.
- Pour the solution to a gel caster.
- Place the comb.
- Pour the 100ml buffer solution to the electrophoretic chamber.
- Place the gel in the caster in the electrophoretic chamber.
- Connect the electrodes and switch on the current.
- Switch off the power supply.
- Remove the gel from the electrophoretic chamber.
- Place the gel in the UV Transilluminator.
- Switch on the Transilluminator.

Caution:

- Ethidium bromide is a mutagen and should be handled as a hazardous chemical (so wear gloves while handling)

Differences Encountered In Real Laboratory:

1. Make sure that the Agarose is fully dissolved in the buffer. If it is not dissolved well, again melt it some more time to dissolve completely.
2. Before casting the gel, the tray and comb should wipe with ethanol.
3. Make sure that the gel in the Chamber is immersed in the TAE Buffer.
4. Labelings should be proper.
5. Ensure that the connections should be proper.
6. Before the incubation step, ensure that the water bath is set at the correct temperature that we required or not.

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