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Chapter 27

Agarose gel electrophoresis of DNA: Principles, protocols, and applications in molecular analysis

Yashdeep Srivastava[1], Keshawanand Tripathi[1*], Narendra Kumar[2]
[1] *Department of Biotechnology, Invertis University, Bareilly, Uttar Pradesh, India.*
[2]*School of Biotechnology and Bioengineering, Institute of Advanced Research, Gandhinagar, Gujarat, India.*
[*]Email: tripathikn009@gmail.com

1. Introduction:

Agarose gel electrophoresis, a cornerstone technique in molecular biology, segregates DNA fragments based on size, leveraging electrophoresis principles (Sambrook and Russell, 2001). The gel matrix, composed of agarose, forms a porous network facilitating DNA migration. Gel concentration determines the size range of separated fragments, with higher concentrations suited for smaller fragments. Agarose powder is mixed with a buffer—usually. Tris-borate-EDTA (TBE) or Tris-acetate-EDTA (TAE)—and then heated upto 65 degree and poured onto a gel casting tray fitted with a comb to produce wells (Srivastava et al., 2022). DNA samples, mixed with a loading dye for visibility and sinking, are loaded into wells (Brown, 2010).

Electrophoresis in a buffer-filled chamber applies an electric current, propelling DNA towards the positive electrode. Migration rate inversely correlates with fragment size, yielding distinct bands post-electrophoresis. Staining with ethidium bromide or SYBR Safe enables visualization under UV light, aiding fragment size determination, PCR validation, or specific sequence confirmation. Agarose gel electrophoresis is a versatile tool in molecular biology, pivotal in research, diagnostics, and DNA profiling (Wilson, and Walker, J. 2010; Tripathi et al., 2013a, b).

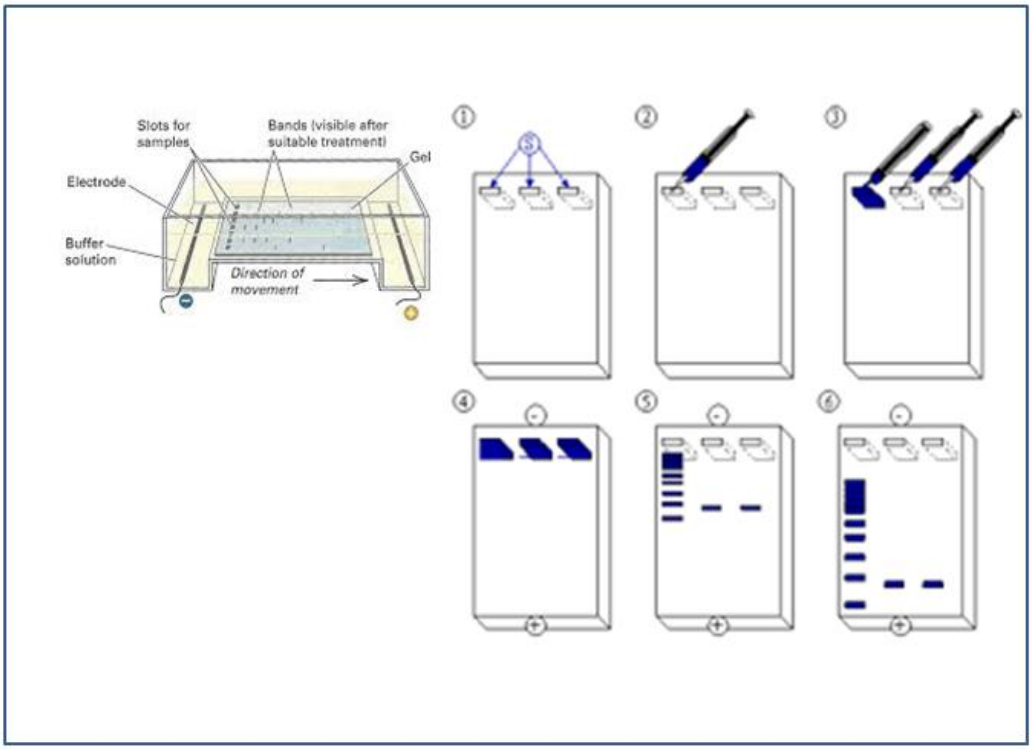

Figure: 1 Agarose gel electrophoresis

2. Procedure:

1. Ensure the correct amount of agarose is used based on the gel rig/tray being used. Make gels with a concentration of 1 to 1.5%.
2. The small size contains a total of 30 ml - The large size contains a total of 50 ml
3. Take 0.45 g of agarose in powdered form and add into a 100 ml Erlenmeyer flask.
4. Further, add 30 ml of 1X Tris-borate-EDTA (TBE) buffer.
5. Then heat the contents in the microwave for 60 seconds, stirring at 15 and 30 second intervals.
6. Apply tape to the edges of the tray to create a seal, then place the combs inside.
7. Include 3 µl of Ethidium Bromide into the agarose
8. Once the gel has reached a warm temperature (not hot), carefully pour it into the tray and allow it to set for 10-15 minutes.
9. Fill a flask with distilled water and heat it in the microwave to remove any remaining gel residue.
10. Dispose of waste in the designated bin for EtBr.
11. Take out the combs and remove any plastic adhesive tape
12. Place TBE 1X buffer into the gel tank
13. Insert the tray into the tank and ensure that the gel is completely submerged in the buffer solution.
14. Include 6 µl of DNA ladder mixed with dye

15. Add 6 µl of DNA samples containing dye into wells of the gel.
16. Connect the +red and –blue wires and set the voltage to 80 volts for 1 hour or 125 volts for 20 minutes.
17. Please power off the machine and carefully take off the lid.
18. Clean the UV transilluminator
19. Carefully remove the tray and slide the gel out.
20. Ensure your safety by using a protective shield to view the gel.
21. Capture the picture of gel-by-gel documentation system containing camera.
22. Then, Dispose of the gel in the appropriate hazardous waste container.

3. Precautions:

1. Prepare agarose gel with appropriate concentration and stain according to the size range of DNA fragments.
2. Handle agarose powder and gel staining dyes carefully, wearing gloves to avoid skin contact.
3. Use clean and sterile equipment throughout the gel preparation process to prevent contamination.
4. Ensure the gel casting tray and comb are properly aligned to create straight and uniform wells.
5. Mix DNA samples with loading dye thoroughly but gently to avoid shearing DNA molecules.
6. Load DNA samples into wells carefully to prevent spillage or damaging the gel structure.
7. Use appropriate voltage and run time settings based on the size of DNA fragments for optimal separation.
8. Monitor the electrophoresis process closely to prevent overheating and potential gel damage.
9. Handle stained gels with care to avoid damaging or tearing the gel during staining and destaining steps.
10. Dispose of used agarose gel, staining solutions, and contaminated materials according to laboratory waste disposal guidelines.

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