Qualitative analysis of isolated plasmid by gel electrophoresis

Introduction

Agarose gel electrophoresis is the effective method for the size- and shape-based separation of DNA molecules. Before loading onto the gel, the plasmid samples were treated with a loading buffer solution. The loading buffer contains glycerol (or high molecular weight polysaccharides) in order to increase the density of the sample and thus facilitate the efficient sample loading. This buffer also contains a loading dye (most often bromophenol blue), which has slightly higher mobility than the DNA molecules to be separated. Thus, by inspecting the progress of the blue spot, the proper termination time of the run can be determined. Ethidium bromide, the DNA dye traditionally used for visualising DNA molecules within the gel, has a mutagenic effect. Ethidium bromide is a ring-containing compound that is able to intercalate between the bases within the double helix of DNA. The DNA complexes of dye produce orange-coloured fluorescent light upon illumination by UV radiation. Superhelically packed circular plasmid DNA has a compact structure, and its hydrodynamic size is much smaller—and its electrophoretic mobility is therefore greater—than that of linear DNA molecules of the same size, as the latter form a freely moving entropic chain. When one of the strands of the superhelical plasmid DNA is cut, it adopts a relaxed circular form. The mobility of this form is smaller than even that of linear DNA.

Materials

- TAE buffer of pH8
- agarose
- Ethidium bromide
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Procedure

1. TAE buffer of pH8 was prepared and autoclaved.
2. Electrophoretic apparatus were wiped with 70% alcohol and dried.
3. 0.35grams of agarose was weighed and added to the 50ml buffer solution to get 0.7% agarose.
4. Boiled in oven.
5. 8µL of Ethidium bromide was added when the gel temperature declined to $60^{O}C$.
6. Poured the solution to a gel caster.
7. The comb was properly inserted to gel.
8. The electrophoretic chamber was filled with 500ml of tank buffer solution.
9. Placed the gel with the caster in the electrophoretic chamber.
10. Sample was prepared by adding 5µL of plasmid and 2µL of 6X dye.

11. Samples were loaded onto the wells.
12. The electrodes were connected and current flow was initiated 50Volts.
13. Switched off the power supply.
14. The gel was retrieved from the electrophoretic chamber.
15. Placed the gel on the UV Transilluminator.
16. Visualized the band.