Aim 54

Purification of DNA Fragment from Agarose Gel using Silica

Introduction

The technique is based on the principle that under specific conditions of salt and pH the DNA binds to silica of specific size.

Requirements

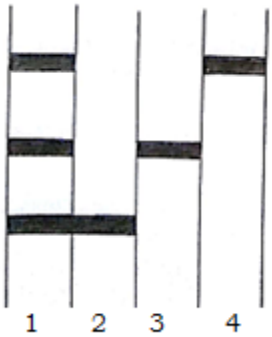
1. Wash buffer (5 mM Tris buffer, pH 8.0)
2. Silica
3. Sodium iodide solution – 6 M
4. pUC 18/Taq 1 digest
5. Tris –EDTA (TE) buffer
6. Universal staining solution
7. Agarose, TAE buffer, gel loading buffer, electrophoresis unit for agarose gel electrophoresis.
8. Microcentrifuge

Procedure

1. 1% agarose gel is prepared.
2. 5 μg of DNA is loaded with the help of micropipette in agarose gel.
3. Run the sample at 50 V till the dye has covered ¾ of the distance.
4. Excise preciously with a sharp blade the lane in which DNA has run.

5. Put the excise lane in a tray and staining dye is pour on it. Then leave the gel for 1 hour in staining solution.
6. Remove the staining dye, destain the gel by water. Water is frequently changed till the dark blue band of DNA is visible against light blue.
7. Three fragment of DNA will show three blue bands upon destaining.
8. Cut each band and place them in eppendorf tubes separately.
9. Weigh the gel pieces and add 2.5 volume of NaI solution.
10. Put the eppendorf tube in water bath at 45-50°C for 5 min to solubilise the gel.
11. Mix the contents and incubate again till the gel gets completely solubilised.
12. 15 µL of silica solution is added to solubilised gel. Keep it at room temperature for 10-15 min with mixing time to time. This adsorption of DNA molecules to the silica.
13. Spin at 8000 rpm for 2 min and discard the supernatant.
14. Silica is bounded to DNA in pellet
15. 200 µL of wash buffer is added and mixed. Spin it at 5000 rpm for one min.
16. Discard the supernatant.
17. 20 µL of TE buffer is added to the silica to elute the DNA.
18. Mix and incubate at 50°C for 10 min.
19. Centrifuge at 5000 rpm for 1 min and collect the supernatant in fresh eppendorf tube.
20. Repeat the above 2 steps again to recover the DNA.
21. Pool supernatant, add gel loading buffer.

22. Individual samples (three bands) are loaded along with PUC 18/Taq 1 digest (which serves as control) in different wells of the gel.
23. Run the electrophoresis and observe the results.



Lane 1 – PUC 18/Taq 1 digest
Lane 2 - 476 bp fragment
Lane 3 – 736 bp fragment
Lane 4 – 1444 bp fragment