Purification of plasmids

Introduction

The Gene JET™ Plasmid Midiprep Kit was used for plasmid isolation and purification procedure. The kit utilizes an exclusive silica-based membrane technology in the form of a convenient spin column. Each Gene JET spin column can recover up to 20 µg of plasmid DNA. The pelleted bacterial cells were re-suspended and subjected to SDS/alkaline lysis to liberate the plasmid DNA. The resulting lysate was neutralized to create appropriate conditions for binding of plasmid DNA on the silica membrane in the spin column. Cell debris and SDS precipitate were pelleted by centrifugation, and the supernatant containing the plasmid DNA was loaded onto the spin column membrane. The adsorbed DNA was washed to remove contaminants, and then eluted with a small volume of the Elution Buffer (10 mM Tris-HCl, pH 8.5). Resuspension Solution has RNase A to degrade the RNA in the solution. Ethanol (96-100%) in Wash Solution helps to remove the organic contaminates.

Materials

- Gene JET™ Plasmid Midiprep Kit
- LB media
- Antibiotic
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Procedure

Cell preparation

1. 20mL of LB medium was prepared with antibiotic (Kanamycine 25µg/mL) in 50mL of falcon tube.
2. A single colony of DH5α was transferred to 20mL of LB media.
3. Incubated at 37^{O}C overnight in shaker incubator at the speed of 120rpm.
4. The culture was then centrifuged at 6500rpm and 4^{O}C

Plasmid isolation and purification

5. The pellet was re-suspended in 250µL of the re-suspension solution and ensured the total suspension of cells in solution
6. Then the re-suspended cells were transferred to a micro-centrifuge tube.
7. Then 250µL of lysis solution was added and mixed properly.
8. The 350µL of neutralizing solution was added and immediately mixed.
9. The above mixture was pelleted-down by centrifuging it at 12,000rpm for 5minutes.

10. The resulted supernatant was transferred to Gene-JET-spin column.
11. Centrifuged for 1minute, the flow-through was discarded and the column was placed back to the same collection tube.
12. 500 µL of wash solution was added to the Gene-JET-spin column. Then centrifuged at 12,000rpm for 30-60seconds.
13. The collected flow-through was discarded and the column was placed back to the collection tube.
14. The above mentioned step was repeated by using 500µL of wash buffer.
15. The collected flow-through was discarded.
16. Centrifuged at 12,000rpm for additional 1minute to remove the residual wash solution.
17. The Gene-JET-column was placed on a fresh autoclaved 1.5mL microcentrifuge tube and 25µL of elution buffer was added to the centre of Gene-JET-column.
18. It was incubated for 2minutes then centrifuged at 12,000rpm for 2minutes.
19. Then again 25µL of elution buffer was added to the centre of Gene-JET-column.
20. It was incubated for 2minutes then centrifuged for 2minutes at 12,000rpm.
21. Finally, the sample was collected and both the sample and column were stored at -20^{O}C.