Purify DNA fragment from agarose gel

Introduction

The protocol is used for the recovery of DNA fragments from agarose gels, a fundamental technique in molecular biology crucial for applications such as cloning, sequencing, and probe generation.

Reference:
1. TOOLS Gel extraction and PCR cleanup Kit (N.d.). Retrieved https://www.tools-biotech.com/upload/2023/06/19/175545691.pdf

Materials

- TOOLs Gel extraction and PCR cleanup Kit (Cat no. ST-G01)
  - DS Buffer
  - Wash Buffer (ethanol added)
  - EB Buffer
  - DE Columns
  - 2ml collection tubes

Procedure

gel fragment isolation

1. Cut the single DNA band from the agarose gel.
2. Weigh the agarose gel slice in a clean falcon tube.

   the maximum weight of the gel slice is 300 mg

Extraction

3. Add 500 ul / 1000 ul (for>2% agarose gels) of DS Buffer to the sample and mix by vortexing.
4. Incubate at 55-60°C water bath for 10-15 minutes and vortex the tube every 2-3 minutes until the gel slice dissolved completely.

   Interval vortex during incubation can accelerate the gel dissolved
5. Wait until buffer cools down for 2 minutes
6. Place a DF Column in a 2ml collection tube and move the buffer to the DF Column
7. Centrifuge 1 minute at 13000 rpm and discard the flow-through

If the sample mixture is more than 800 ul, repeat step 7 for the rest sample mixture

8. put the DF column back to the 2ml collection tube
9. Wash the DF Column by 750 ul Wash Buffer and stand for 1 minute at room temperature.
10. Centrifuge 1 minute at 13000 rpm and discard the flow-through
11. Put the DF column back to the 2ml collection tube
12. Repeat steps 9-11
13. Centrifuge for an additional 2 minutes at 13000 rpm to remove remaining Wash Buffer.

Elution

14. Place the DF Column into a clean 1.5 ml tube
15. To elute DNA, add 20-50 ul EB Buffer on the center of the DF Column
16. Stand for at least 2 minutes and centrifuge 2 minutes at 13000 rpm to collect the DNA solution
17. move the initial DNA solution back to the DF Column and repeat step 15 and 16