DNA Gel Extraction Procedure

Genomic DNA was recovered using the AxyPrep DNA Gel Extraction Kit according to the manufacturer's protocol, with the following steps:

1. The agarose gel was visualized under a UV transilluminator, and the target DNA band was excised with a sterile blade and transferred into a 2.0 mL microcentrifuge tube.
2. Buffer DE-A was added at a volume of 1–3 times the gel slice volume. The tube was incubated in a 75 °C water bath for 8–10 min until the gel slice was completely dissolved.
3. An equal volume of Buffer DE-B was added and mixed thoroughly. For DNA fragments smaller than 400 bp, an additional equal volume of isopropanol was added to improve recovery efficiency.
4. The DNA binding column was placed into a 2 mL collection tube. The dissolved gel mixture was carefully applied to the column, followed by centrifugation at 12,000 rpm for 1 min. The flow-through was discarded.
5. A total of 500 μL Buffer W1 was added to the column, incubated for 1 min, and centrifuged at 12,000 rpm for 1 min. The flow-through was discarded.
6. A total of 700 μL Buffer W2 was added, incubated for 1 min, and centrifuged at 12,000 rpm for 1 min. This step was repeated once to ensure complete washing.

7. The column was transferred to a new 2 mL collection tube and centrifuged at 12,000 rpm for 1 min to remove residual liquid. The column was then air-dried at room temperature to completely evaporate any remaining ethanol.
8. The column was placed into a new sterile 2 mL microcentrifuge tube, and 25 μL of preheated sterile water (55 °C) was added. After incubation for 2 min, DNA was eluted by centrifugation at 12,000 rpm for 2 min and collected in the tube.