Plasmid DNA Extraction Procedure

Plasmid DNA was extracted using the AxyPrep Plasmid Miniprep Kit according to the manufacturer's instructions, with the following steps:

A single bacterial colony was inoculated into 20 mL of LB medium supplemented with kanamycin (50 μg/mL) and cultured at 37 °C with shaking at 220 rpm for approximately 16 h. A 2–4 mL aliquot of the culture was transferred to a sterile 2 mL microcentrifuge tube and centrifuged at 12,000 rpm for 1 min to collect the bacterial pellet.

The pellet was resuspended in 250 μL prechilled Buffer S1 containing RNase A. Complete resuspension was ensured to achieve efficient cell lysis.

A total of 250 μL Buffer S2 was added, and the tube was gently inverted 4–6 times until the solution became viscous. Vigorous mixing was avoided to prevent genomic DNA contamination, and the lysis reaction was not prolonged to minimize plasmid degradation.

A total of 350 μL Buffer S3 was immediately added and mixed by gentle inversion. The sample was centrifuged at 12,000 rpm for 10 min to pellet the precipitate. The clear supernatant was carefully collected, with extended centrifugation applied if residual flocculent material was observed.

The supernatant was transferred to a CP spin column and incubated for 2 min to allow plasmid DNA binding. The column was centrifuged at

12,000 rpm for 1 min, and the flow-through was discarded.

The column was sequentially washed with 500 μL Buffer W1 (pre-mixed with absolute ethanol) and 700 μL Buffer W2, followed by centrifugation at 12,000 rpm for 1–2 min. A final centrifugation for 2 min was performed to remove residual ethanol completely.

The column was placed into a new sterile 1.5 mL microcentrifuge tube. A total of 25 μL elution buffer, preheated to 65 °C, was applied to the membrane and incubated for 2 min, followed by centrifugation at 12,000 rpm for 2 min. The eluted plasmid DNA was collected and stored at −20 °C.