Miniprep – 5-prime kit

A method to isolate plasmids from transformed cells during cloning

Procedure

1. Pellet 1.5 ml of fresh bacterial culture at maximum speed for 1 minute in the provided 2 ml Culture Tube.

2. Remove medium by decanting, taking care not to disturb bacterial pellet.
3. Add 400 μL of ***ICE-COLD*** Complete Lysis Solution.

4. Mix thoroughly by constant vortexing at the highest setting for a full 30 seconds. This step is critical for obtaining maximum yield.

5. Incubate the lysate at room temperature for 3 minutes.

6. Transfer the lysate to a Spin Column Assembly by decanting or pipetting.

7. Centrifuge the Spin Column Assembly for 60 seconds at maximum speed.

8. Add 400 μL of DILUTED Wash Buffer to the Spin Column Assembly.

9. Centrifuge the Spin Column Assembly for 60 seconds at maximum speed.

10. Remove the Spin Column from the centrifuge and decant the filtrate from the Spin Column Assembly Waste Tube. Place the Spin Column back into the Waste Tube and return it to the centrifuge.

11. Centrifuge at maximum speed for 1 minute to dry the Spin Column.

12. Transfer the Spin Column into a Collection Tube.

13. Add 50 μL of Elution Buffer directly to the center of the Spin Column membrane and cap the Collection Tube over the Spin Column.

14. Centrifuge at maximum speed for 60 seconds.

15. Remove and discard the Spin Column.

16. The eluted DNA can be used immediately for downstream applications or stored at -20°C.

Materials required

Overnight bacterial cultures of successful clones
5-prime (Eppendorf) Miniprep kit

200 and 1000 μL pipette tips
1.5 mL microcentrifuge tubes

Equipment required

200 μL micropipetter
1000 μL micropipetter
Benchtop centrifuge capable of 20,800 x g
Benchtop vortexer