

Plasmid extraction

Material

TIAN prep Mini Plasmid Kit II(TIANGEN Code No. DP106-02)

Procedure

1. Column equilibration: Place a Spin Column CP4 in a clean collection tube, and add 500 μ l Buffer BL to CP4. Centrifuge for 1 min at 12,000 rpm (\sim 13,400 \times g) in a table-top microcentrifuge. Discard the flow-through, and set the Spin Column CP4 back into the collection tube.

2. Harvest 5-15 ml bacterial cells in a microcentrifuge tube by centrifugation at 12,000 rpm (\sim 13,400 \times g) for 1 min at room temperature (15-25°C), then remove all traces of supernatant.

3. Re-suspend the bacterial pellet in 500 μ l Buffer P1 (Ensure that RNase A has been added to Buffer P1). The bacteria should be resuspended completely by vortex or pipetting up and down until no cell clumps remain.

4. Add 500 μ l Buffer P2 and mix thoroughly by inverting the tube 6-8 times.

5. Add 700 μ l Buffer P3 and mix immediately and thoroughly by inverting the tube 6-8 times. The lysate should become cloudy. Centrifuge for 10 min at 12,000 rpm (\sim 13,400 \times g) in a table-top centrifuge. A compact white pellet will form.

6. Carefully transfer the supernatant from step 5 to the Spin Column CP4 and please note not to touch precipitate. Centrifuge for 1 min at 12,000 rpm (\sim 13,400 \times g). Discard the flow-through and set the Spin Column CP4 back into the Collection Tube.

7. (optional) Wash the Spin Column CP4 by adding 500 μ l Buffer PD and centrifuging for 1 min at 12,000 rpm (\sim 13,400 \times g). Discard the flow-through and set the CP4 back into the Collection Tube.

8. Wash the Spin Column CP4 by adding 600 μ l Buffer PW (ensure the ethanol (96-100%) has been added to Buffer PW) and centrifuging for 1 min at 12,000 rpm (\sim 13,400 \times g). Discard the flow-through and set the CP4 back into the Collection Tube.

9. Wash Spin Column CP4 by adding 600 μ l Buffer PW and centrifuging for 1 min at 12,000 rpm (\sim 13,400 \times g).

10. Discard the flow-through, and centrifuge for an additional 2 min at 12,000 rpm (\sim 13,400 \times g) to remove residual wash buffer PW.

11. Place the Spin Column CP4 in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 100-300 μ l Buffer EB or water (pH 7.0-8.5) to the center of the Spin Column CP4, let stand for 2 min, and centrifuge for 1 min at 12,000 rpm (\sim 13,400 \times g).