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 μl Buffer BL to CP4. Centrifuge for 1 min at 12,000 rpm (~13,400 × 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 (~13,400 × g) for 1 min at room temperature (15-25°C), then remove all traces of supernatant.
3. Re-suspend the bacterial pellet in 500 μl 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 μl Buffer P2 and mix thoroughly by inverting the tube 6-8 times.
5. Add 700 μl 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 (~13,400 × 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 (~13,400 × 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 l Buffer PD and centrifuging for 1 min at 12,000 rpm (~13,400 × g). Discard the flow-through and set the CP4 back into the Collection Tube.
8. Wash the Spin Column CP4 by adding 600 μl Buffer PW (ensure the ethanol (96-100%) has been added to Buffer PW) and centrifuging for 1 min at 12,000 rpm (~13,400 × g). Discard the flow-through and set the CP4 back into the Collection Tube.
9. Wash Spin Column CP4 by adding 600 μl Buffer PW and centrifuging for 1 min at 12,000 rpm (~13,400 × g).
10. Discard the flow-through, and centrifuge for an additional 2 min at 12,000 rpm (~13,400 × 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 μl 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 (~13,400 × g).