

Purification of bacteria plasmids :

1. Harvest 1-5 ml bacterial cells in a microcentrifuge tube by centrifugation at 12,000 rpm ( $\sim 13,400 \times g$ ) in a conventional, table-top microcentrifuge for 1 min at room temperature (15-25°C), then remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained (For large volume of bacterial cells, please harvest to one tube by several centrifugation step.)
2. Re-suspend the bacterial pellet in 250  $\mu$ l Buffer P1 (Ensure that Buffer P1 is marked with "✓"). The bacteria should be resuspended completely by vortex or pipetting up and down (Better choice) until no cell clumps remain.
3. Add 250  $\mu$ l Buffer P2 and **mix gently** and thoroughly by inverting the tube 6-8 times. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min. If the lysate is still not clear, please reduce bacterial pellet.
4. Add 350  $\mu$ l Buffer P3 and mix immediately and gently by inverting the tube 6-8 times. The solution should become cloudy. Centrifuge for 10 min at 12,000 rpm ( $\sim 13,400 \times g$ ) in a table-top microcentrifuge.
5. Column equilibration: Place a Spin Column CP3 in a clean collection tube and add 500  $\mu$ l Buffer BL to CP3. Centrifuge for 1 min at 12,000 rpm ( $\sim 13,400 \times g$ ) in a table-top microcentrifuge. Discard the flow-through and put the Spin Column CP3 back into the collection tube.
6. Transfer the supernatant from step 4 to the Spin Column CP3 (place CP3 in a collection tube) by decanting or pipetting. Centrifuge for 30-60 s at 12,000 rpm ( $\sim 13,400 \times g$ ). Discard the flow-through and set the Spin Column CP3 back into the Collection Tube.
7. Wash the Spin Column CP3 by adding 500  $\mu$ l Buffer PD (Ensure ethanol has been added.) and centrifuge for 30-60 s at 12,000 rpm ( $\sim 13,400 \times g$ ). Discard the flow-through and put Spin Column CP3 back to the collection tube.
8. Centrifuge for an additional 2 min at 12,000 rpm ( $\sim 13,400 \times g$ ) to remove residual wash Buffer PW.
9. Place the Spin Column CP3 in a clean microcentrifuge tube. Open the lid for about 5 min to get rid of residual ethanol.
10. To elute DNA, add 40  $\mu$ l ddH<sub>2</sub>O to the centre of the Spin Column CP3, incubate for 5 min, and centrifuge for 2 min at 12,000 rpm ( $\sim 13,400 \times g$ ).