C. Mini Plasmid

1	Preservation of Bacteria	① remove and ignite the alcohol lights; ② folder 1.5mL centrifuge tube with a pair of tweezers and cover the tube cover; ③ take 700μL bacteria from the liquid medium into the 1.5mL centrifuge tube beside alcohol lights; ④ take 700μL glycerol into the 1.5mL centrifuge tube; ⑤ place the centrifuge tube in -20 ℃ refrigerator.
2	Pretreatment	Column equilibration: Place a Spin Column CP3 in a clean collection tube, and add 500 μl Buffer BL to CP3. Centrifuge for 1 min at 12,000 rpm (~13,400 × g) in a table-top microcentrifuge. Discard the flow-through, and put the Spin Column CP3 back into the collection tube. (Please use freshly treated spin column).
3	Collect Bacteria	Harvest 1-5 ml bacterial cells in a microcentrifuge tube by centrifugation at 12,000 rpm (~13,400 × 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.)
4	Remove RNA	Re-suspend the bacterial pellet in 250 μl Buffer P1 (Ensure that RNase A has been added). The bacteria should be resuspended completely by vortex or pipetting up and down until no cell clumps remain.
5	Lysis of Cells	Add 250 μl Buffer P2 and mix gently and thoroughly by inverting the tube 6-8 times.

6	Remove Protein	Add 350 μl 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 (~13,400 × g) in a table-top microcentrifuge. Transfer the supernatant from step 5 to the Spin Column CP3 (place CP3 in a collection tube) by decanting or pipetting. Centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through and set the Spin Column CP3 back into the Collection Tube.
7	Wash	Wash the Spin Column CP3 by adding 600 μl Buffer PW (ensure that ethanol (96%-100%) has been added) and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through, and put the Spin Colum CP3 back into the Collection Tube. Repeat the previous step . Centrifuge for an additional 2 min at 12,000 rpm (~13,400 × g) to remove residual wash Buffer PW.
8	Collection of Plasmids	Place the Spin Column CP3 in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50-100 μl Buffer EB to the center of the Spin Column CP3, incubate for 2 min, and centrifuge for 2 min at 12,000 rpm (~13,400 × g).