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07 Extraction of Plasmid

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MATERIALS

NAME	CATALOG #	VENDOR
TIANprep Mini Plasmid Kit	/	

1 Collect the E. coli solution into the EP tube. Centrifuge at 12,000 rpm in a rotor for 1 minute. Remove the clear supernatant liquid.
00:01:00

2 Add 250μL P1 (RNase A added, kept at 4 °C)to the EP tube to suspend bacterial precipitation.
4 °C
250 µl

3 Add 250μL P2 to the EP tube, shake slightly up and down 6-8 times to lyse bacteria.
250 µl

4 Add 350μL P3 and invert the tube immediately and gently 6-8 times. Then centrifuge it at 12000rpm, 25℃ for 10 minutes.
350 µl
25 °C
00:10:00

5 Regenerate column CP3 while centrifugation. Add 500μl Buffer BL. Centrifuge for 1 min at 12,000 rpm. Discard the flow-through.
500 µl

6 Move the clear supernatant liquid to CP3, at 12000rpm, 25℃ centrifuge for 30 seconds.
25 °C
00:00:30

7 Add 600μL PW to adsorption column CP3, 12000rpm, 25℃ centrifuge for 30 seconds.
00:00:30
25 °C

8 Repeat step 7.
00:00:30
25 °C

9 Move the adsorption column CP3 to new clean centrifuge tubes and then keep them opening for 5 minutes, so that the ethanol in the PW can

be sufficiently volatilized.

00:05:00

10 Drop 50µL 65°C ddwater into the middle of the adsorption membrane, static for 2min. Then centrifuge for 2 min at 12,000 rpm to collect DNA solution in EP tube.

50 µl

65 °C