

Protocol 1-1: Isolation of Plasmid DNA

General Protocol

- 1) Bacteria culture: Inoculate the single colony harboring plasmid into 20 ml LB medium containing antibiotics, 37°C, 12-16 hours.
- 2) Pellet 1–3 ml of cells by centrifugation for 1–2 minutes at 12000rpm. Decant the supernatant. (1.4ml each time and collect about 3 ml culture of E.coli in 1.5ml eppendorf tube.) Completely resuspend the cell pellet in 100 µl solution 1 by vigorous vortex.
- 3) Add 150 µl solution 2, mix by inverting the tube 4-6 times gently and incubate the tube on ice for 1-2 minutes (do not exceed this period!). The cell suspension should be clear immediately.
- 4) Add 150 µl solution 3, invert the tube gently several times, place the tube at room temperature for 5 minutes, and centrifuge at 12000rpm for 15 minutes.
- 5) Add 420 µl binding buffer to the mini-spin column. Then transfer the supernatant of procedure 3 to the same mini-spin column. Mix the supernatant and binding buffer with pipette carefully. Then place the column in another tube, centrifuge at 12000rpm for 30 and then discard waste liquid in the tube.
- 6) Add 750 µl wash buffer to the column, and centrifuge at 12000rpm for 1 minute.
- 7) Repeat procedure 5. Then centrifuge at 12000rpm for 2 minutes. Eliminate wash buffer as thoroughly as possible. The ethanol in wash buffer will impact the following enzyme-catalyzed reactions.
- 8) Carefully move the column into another clean tube. Add 50 µl Elution buffer or water into the column, place it at room temperature for 5 minutes, and centrifuge at 12000rpm for 1 minute.

Tips

- 1) Elution buffer should be added in the middle of adsorption material to guarantee all the plasmid DNA recovered.
- 2) To increase the recovery efficiency, increase the elution volume or elution times if it is necessary.
- 3) 0.5ml RNaseA (10mg/ml) can be added to the purified plasmid to eliminate RNA thoroughly.
- 4) If the molecular weight of purified plasmid exceeds 10kb, the column may be placed in 70°C water bath for 3-5 minutes to ensure plasmid DNA totally recovered.