

FavorPrep™ Plasmid DNA Extraction Mini Kit protocol

1. Transfer 1-4 mL of bacterial culture to a microcentrifuge tube.
2. Bacterial cells were harvested by centrifugation at 14 K for 1 min and the supernatant was discarded.
3. Add 200 µL of FAPD1 Buffer (RNase A added) to the pellet and the cells were resuspended.
4. Add 200 µL of FAPD2 Buffer and gently invert the tube 10 times to lyse the cells and incubated at room temperature for 2 min.
5. Add 300 µL of FAPD3 Buffer and immediately invert the tube 10 times. .
6. Centrifuge for 5 minutes at full speed. During centrifuging, place a FAPD Column in a Collection Tube.
7. Transfer the supernatant carefully to the FAPD Column. Centrifuge for 30 seconds then discard the flow-through and place the FAPD Column back in the Collection Tube.
8. Add 400 µL of W1 Buffer to FAPD Column. Centrifuge for 30 seconds then discard the flow-through and place the FAPD Column back in the Collection Tube.
9. Add 600 µL of Wash Buffer (96%-100% ethanol added) to FAPD Column. Centrifuge for 30 seconds then discard the flow-through and place the FAPD Column back in the Collection Tube.
10. Centrifuge again for an additional 3 min to dry the column.
11. Place FAPD Column to a new 1.5 mL microcentrifuge tube.
12. Add 50 µL - 100 µL of Elution Buffer or ddH₂O to the membrane center of FAPD Column. Stand the column at room temperature for 2 minutes.
13. Centrifuge for 1 min to elute plasmid DNA.
14. Purified plasmid DNA was stored at 4°C or -20°C.

Invitrogen PureLink® Quick Plasmid Miniprep Kits

protocol

1. Centrifuge 1–5 mL of the overnight LB-culture. Remove all medium.
2. Add 250 µL Resuspension Buffer (R3) with RNase A to the cell pellet and resuspend the pellet until it is homogeneous.
3. Add 250 µL Lysis Buffer (L7). Mix gently by inverting the capped tube until the mixture is homogeneous. (Do not vortex.)
4. Incubate the tube at room temperature for 5 minutes.
5. Add 350 µL Precipitation Buffer (N4). Mix immediately by inverting the tube, or for large pellets, vigorously shake the tube until the mixture is homogeneous. (Do not vortex.)
6. Centrifuge the lysate at $>12,000 \times g$ for 10 minutes.
7. Load the supernatant from step 6 onto a spin column in a 2-mL wash tube. Centrifuge the column at $12,000 \times g$ for 1 minute. Discard the flow-through and place the column back into the wash tube.
8. Optional Wash. (Recommended for endA⁺ strains). Add 500 µL Wash Buffer (W10) with ethanol to the column. Incubate the column for 1 minute at room temperature. Centrifuge the column at $12,000 \times g$ for 1 minute. Discard the flow-through and place column back into the wash tube.
9. Add 700 µL Wash Buffer (W9) with ethanol to the column. Centrifuge the column at $12,000 \times g$ for 1 minute. Discard the flow-through and place the column into the wash tube. Centrifuge the column at $12,000 \times g$ for 1 minute. Discard the wash tube with the flow-through.
10. Place the Spin Column in a clean 1.5-mL recovery tube. Add 75 µL of preheated TE Buffer (TE) to the center of the column. Incubate the column for 1 minute at room temperature.
11. Centrifuge the column at $12,000 \times g$ for 2 minutes. The recovery tube contains the purified plasmid DNA. Discard the column. Store plasmid DNA at 4°C (short-term) or store the DNA in aliquots at –20°C (long-term).