# Plasmid DNA purification using QIAprep® Spin Miniprep Kit

This protocol is designed for purification of up to 20 µg of high-copy plasmid DNA from 1-5mL overnight cultures in LB mediul.

## Procedure

**1.** Pellet 1-5mL bacterial overnight culture by centrifugation at >8000 rpm ( we used 13000 ) for 3mn at room temperature

**2.** Resuspend pelleted bacterial cells in 250µL buffer P1 and transfer to a microcentrifuge tube

**3.** Add 250µL buffer P2 and mix thoroughly by inverting the tybe 4-6 times until the solution becomes clear. Do not allow the lysis reaction to proceed for more than 5mn.

**4.** Add 350µL buffer N3 and mix immediately and thoroughly by inverting the tube 4-6 times.

**5.** Centrifuge for 10mn at 13000 rpm in a table-top microcentrifuge

**6.** Apply the supernatant from step 5 to the QIAprep spin column by decanting or pipetting. Centrifuge for 30-60s and discard the flow-through.

**7.Recommended if the strain is endA+** Wash the QIAprep spin column by adding 0.5mL buffer PB. Centrifuge for 30-60s and discard the flow-through.

**8.** Wash the QIAprep spin column by adding 0.75 mL buffer PE. Centrifuge for 30-60s and discard the flow-through.

**9.** Centrifuge for 1mn to remove residual wash buffer

**10.** Place the QIAprep column in a clean 1.5mL microcentrifuge tube. To elute DNA, add 50 µL buffer EB ( 10mM Tris.Cl, pH 8.5 ), let stand for 1mn and centrifuge for 1mn.

This protocol is extracted from "QIAprep Miniprep Handbook" by **QIAGEN**