Miniprep (Promega Kit)

All centrifugation steps are carried out at 17.900 g in a conventional table-top microcentrifuge if not stated differently. Prewarm elution buffer to 70 – 80 °C.

1. Pellet **5 ml** bacterial overnight culture in a 2 ml microcentrifuge tube by centrifugation at 6800 g for 3 min (**or at 17.900 g for 1 min each**)
2. Resuspend pelleted bacterial cells in 600 µl Buffer TE (not supplied)
3. Add 100 µl Cell Lysis Buffer (blue) and mix by inverting 6 times.
4. Add 350 µl of cold Neutralization Solution and mix by inverting 6 times.
5. Centrifuge for 3 min, longer if the cell debris pellet is not properly seperated from the supernatant
6. Apply supernatant spin column by pipetting.
7. Centrifuge for 30 s and discard the flow-through.
8. Add 200µl of Endotoxin Removal Wash (ERB) to the minicolumn. Centrifuge at maximum speed in a microcentrifuge for 30 seconds
9. Add 400µl of Column Wash Solution (CWC) to the minicolumn. Centrifuge at maximum speed in a microcentrifuge for 30 seconds.
10. Centrifuge for 60 s to remove residual wash buffer. Place spin column in microcentrifuge tube into thermo mixer at 70-80 °C until ethanol smell has vanished.
11. Place the spin column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 30-50 µl Buffer EB or water to the center of the spin column. Let stand for 1 min, then centrifuge for 1 min.
12. **Pipette the elution into the center of the spin column and centrifuge again for 1 min.**