Genomic DNA extracton from bacteria

- TE buffer
- 10% (w/v) sodium dodecyl sulfate (SDS)
- 20 mg/ml proteinase K
- phenol\chloroform (50:50) or chloroform:isoamil-alcohol (24:1)

1. Grow *E. coli* culture overnight in rich broth.
2. Transfer 2 ml to a 2-ml micro centrifuge tube and spin 2 min.
3. Decant the supernatant.
4. Drain well onto a Kimwipe.
5. Resuspend the pellet in 467 µl TE buffer by repeated pipetting.
6. Add 30 µl of 10% SDS and 3 µl of 20 mg/ml proteinase K, mix , and incubate 1 hr at 37 ° C.
7. Add an equal volume of phenol/chloroform and mix well but very gently to avoid shearing the DNA by inverting the tube until the phases are completely mixed.
8. Carefully transfer the DNA/phenol mixture into a Phase Lock Gel™ tube (green) and spin at 12,000 RPM for 10 min.
9. Transfer the upper aqueous phase to a new tube and add an equal volume of phenol/chloroform.
10. Again mix well and transfer to a new Phase Lock GelTM tube and spin 10 min.
11. Transfer the upper aqueous phase to a new tube.

For the next steps use the buffers and columns from the MiniPrep kit (Qiagen)

12. To the aqueous phase add 4 volumes of buffer PB (binding buffer)
13. Apply the DNA onto the MiniPrep column. Centrifuge for 60sec at full speed.
14. Wash with 500µL buffer PB. Centrifuge for 60sec at full speed. Discard flow through.
15. Wash with 750µL buffer PE. Centrifuge for 60sec at full speed. Discard flow through.
16. Dry the column by centrifugation for additional 60 sec.
17. Elute DNA with 40µL DDW or Buffer TE.
18. Measure the DNA concentration and purity by measuring the absorbance.

TE buffer:

10 mM Tris-Cl (pH, usually 7.6 or 8.0)
1 mM EDTA (pH 8.0)