

DNA mini prep

1. Grow 10 ml of cells till late log phase
2. Harvest cells in 15 ml falcon tube
3. wash once with 5 ml of sterile water
4. Resuspend in 0.5 ml of lysis buffer 1.
5. Add 0.4 ml of glass beads
6. Add 25 μ l of 5M NaCl
7. Vortex at high setting for 2-3 min
8. Spin at 2000 RPM for 2 min.
9. Transfer the liquid to an eppendorf tube using P-1000.
10. Add 500 μ l of phenol, vortex and spin for one min.
11. Take aqueous layer and extract once with 500 μ l of chloroform and isoamyl alcohol.
12. Add 1 ml of 95% alcohol and precipitate for 1 hr in -20°C .
13. Pellet DNA by spinning down for 5 min at high speed.
14. Wash pellet with 70% alcohol.
15. Resuspend in 250 μ l of 1XTE
16. Add 5 μ l of proteinase K (10 mg/ml stock) and incubate at 37°C for 30 min.
17. Add 250 μ l of 5M ammonium acetate and repeat step 12-14
18. Resuspend the DNA in 100 μ l of 1XTE