



Midi Prep Protocol

This is EXACTLY how we make DNA for injections. We take measures to keep the DNA out of re-usable glassware (potential soap residue contaminants) and to keep it RNase free.

1. Grow 50 ml LB+0.1 mg/ml AMP overnight
2. Spin down in 45 ml Oak Ridge tube in SS34 rotor 5000 RPM, 15 minutes.
3. Put column into Qiagen rack or use holding ring on Erlenmeyer flask. Make sure clearance is >4 inches from bottom of tip to receptacle.
4. Equilibrate Midi prep column with 4 ml QBT.
5. Resuspend Pellet in 4 ml P1 by vortexing
6. Add 4 ml P2, mix by inverting. Leave at room temp. for 5 min.
7. Add 4 ml chilled P3, mix by inverting
8. Place mixture into Qiafilter syringe. Leave at room temp. for 10 min.
9. Quirt onto column, discard syringe.
10. Wash 2X 10ml QC.
11. Elute DNA into 15 ml disposable plastic Corning tube with 5 ml QF
12. Add 3.5 ml Isopropanol, invert to mix.
13. Divide into 6 microfuge tubes (~1.4 ml each), and pellet DNA 15K RPM , 15 min. at 4°.
14. Wash pellets with 0.5 ml 70% EtOH
15. Move pellets into one tube using P1000, re-spin 15K RPM 2 min. Discard 70% EtOH.
16. Air dry
17. Resuspend pellet in 30 microliters of H₂O or EB
18. Spec to determine concentration.

The water we use for the 70% washes and to resuspend the pellets is RNase/DNase free DEPC water purchased from Sigma. If you really don't want to resuspend your pellet in water, then resuspend in 10mM Tris, pH 8.0. Make sure the final buffer is RNase and DNase free, and try to steer clear of EDTA.

The Qiagen kit is: QIAFilter Plasmid Mini Kit (25 columns) Catalog number: 12243