



Duke University Model System Genomics

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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 rack or use holding ring on Erlenmeyer flask. Make sure clearance is >4 inches from bottom of tip to receptacle.
4. Equilibrate Nucleobond Midi prep column with 12 ml Equilibration buffer (EQU) by applying the buffer onto the rim of the column filter in a circular, clockwise motion. Make sure to wet the entire filter. Allow the column to empty by gravity flow.
5. Resuspend pellet in 8 ml Resuspension buffer (RES) by vortexing.
6. Add 8 ml Lysis buffer (LYS), mix by inverting. Leave at room temp for 5 minutes.
7. Add 8 ml Neutralization buffer (NEU) and mix by inverting 10 - 15 times.
8. Making sure to have a homogeneous mix in the tube, pour the lysate into the equilibrated Nucleobond column filter.
9. Wash column filter and column with 5 ml Equilibration buffer. Apply the buffer to the rim of the column filter in a circular, clockwise motion. Omitting this step or just pouring the buffer directly inside the funnel may reduce plasmid yield.
10. Discard column filter.
11. Wash the column with 8 ml Wash buffer (WASH).
12. Elute DNA into 15 ml disposable plastic Corning tube with 5 ml Elution buffer (ELU).
13. Add 3.5 ml Isopropanol and invert to mix.
14. Divide into 6 microfuge tubes and pellet DNA 15K RPM, 15 min. at 4°C.
15. Wash pellets with 0.5 ml 70% EtOH.
16. Move pellets into one tube and re-spin 15K RPM 2 min. Discard EtOH.
17. Air dry. Pellets should be translucent when dry.
18. Resuspend pellet in 30 microliters 10mM Tris pH 8.0 (EB from a miniprep kit)
19. 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. Make sure the final buffer is RNase and DNase free, and try to steer clear of EDTA. If there is flocculent when the DNA is resuspended, then there is either contamination of the isopropanol or the SDS in the Lysis buffer has crashed out of solution. Warm the Lysis buffer at 30-40°C until the precipitate dissolves and then recool it to room temperature. As for the isopropanol, get new stuff and don't share.

The Clontech kit is: NucleoBond® Xtra Midi (50 preps) Catalog number: 740410.50