Ten-minute plasmid DNA minipreps

(Based on Zhou, C., Yujun, Y and Jong, A.Y. (1990) BioTechniques 8; 172-173)

1. Centrifuge ~1.2 ml culture[a] 20 sec in a 1.5 ml tube.
2. Remove supernatant by aspiration. Add 50 μl of Solution I[b]; vortex at hightest speed to resuspend pellet.
3. Add 300 μl TENS[c], vortex 2-5 seconds (it becomes sticky)[d].
4. Add 150 μl 3M NaOAc, pH5.2; invert 10~16 times.
5. Centrifuge at top speed for 5 minutes[d]. At the meantime, add 900 μl of 100% EtOH to new tubes.
6. Pour supernatant to the tube with EtOH, avoiding the precipitate. Mix by vortexing.
7. Centrifuge at top speed for 5 minutes[f].
8. Pour off supernatant. Add 600 μl of 70% EtOH (you can dispense 70% EtOH from a bottle). Pour off 70% EtOH, and quick spin
9. Remove most 70% EtOH; Air dry pellet briefly.
10. Resuspend in 20-40 μl TE[g]. This usually results in ~ 100 ng/μl.

Notes:

a. Using sterile technique, pick a colony into 1.5 ml LB broth in a 15 ml Falcon (2059) tube. Shake vigorously at 37ºC overnight or a few hours until cloudy.
b. Solution I (50 mM glucrose, 25 mM Tris, 10 mM EDTA, pH8.0. For 200 ml solution, 10 ml of 1M glucrose (or 1.8 g powder), 5 ml of 1M Tris.HCl, pH7.5, 4 ml of 0.5M EDTA, pH8.0, add H_2O to final 200 ml, add RNaseA to final concentration 100 μg/ml)
c. TENS=TE with 0.1N NaOH and 0.5% SDS (0.5 ml 10N NaOH, 1.25 ml 20% SDS for 50 ml solution). Store at RT.
d. Keep on ice to prevent chromosomeal degradation if you need to take more than 10 minutes before step 4.
e. This pellets cell debris and chromosomal DNA.
f. This pellets plasmid DNA and RNA.
g. Plasmid yield is 2.3 μg from a 1.5 ml culture. Usually we add 30 μl. Add more or less depending upon the size of the pellet. Use 1 μl for digestion.