E. coli plasmid boiling prep

(The new and improved Judelson method)

1. Grow up ovemíght culture (ca. 2 ml LB or TB). Note: this protocol can't be scaled up.
2. Pour ca. 1.5 ml of culture into microfuge tubes. Centrifuge 4 min, 5k rpm (or 1 min, 10K rpm)
3. Pour off supernatant and add 0.35 ml of the followíng "boiling prep buffer" and resuspend by vortexíng:

   8% sucrose
   0.5% Triton X-100 or NP4O
   50 mM EDTA
   10 mM Tris-CI pH 8.0

   (this is stored at 4C)
4. Add 25 µl of fresh lysozyme solution (10 mg/ml in 10 mM Tris-CI pH 8.0). Gently invert a few times to mix. Incubate 5 min at room temp.
5. Place for 1 min (~45-75 sec) in boiling water. Make sure the caps don't "pop."
6. Spin 10 min in microfuge, room temp, full speed.
7. Remove "clot" with a toothpick and discard.
8. To the remaining liquid, add more boiling prep buffer as needed to equalize the volumes in each tube (to roughly 0.3 to 0.4 ml).
9. Add 40 µl 3M Sodium acetate pH 5.5. Using a P1000 measure the volume ot the líquid ín a typical tube.
10. Add 0.75 volumes room temp isopropanol (too much will precipitate proteins; too little will poorly precipitate the DNA).
11. Invert the tubes and sit at room temperature 1 min.
12. Spin for 10 min at room temp,full speed in microfuge. It you orient the tubes with the hinge side up, it will be easier to handle the tubes in later steps.
13. Carefully pour off the líquid. Do this either one tube or a time, or, keeping the tubes in a rack and using a pipette the keep the tubes from falling off, invert the rack.
14. GENTLY add 250 µl of 70% ethanol (room temp). Wait 1 min. CAREFULLY pour off the liquid as before. BIot lip of tube on a paper towel.
15. Dry pellets by (a) laying the tube on its side and waiting 10-20 min or (b) spinning the tube for 20 sec, pipetting off the liquid with a P200, then waiting ca. 1 min).
16. Resuspend the pellet in 40 µl TE (10 mM Tris 8.0, 1 mM EDTA) containing about 20 µg/ml DNAse-free RNAse (this will help degrade the RNA, which otherwise would inhibit restriction enzymes.
17. For high copy plasmids, 1-2 µl should be about 0.5 µg. The pellet will likely not dissolve completely, leaving a milky white clump. When removing DNA for digests, etc., just take the liquid and ignore the garbage.