E. coli Boiling Lysis Plasmid Preparation

This protocol yields plasmid DNA that is suitable for restriction digests and cloning purposes. This preparation method works well on *E. coli* strains containing the *endA* mutation, such as XL1-Blue, DH5-α, but not HB101.

1. Grow a 2-3 ml culture in rich media with appropriate antibiotic selection.

2. Pellet 1.5 ml in a microfuge tube for 30 sec-2 min.

3. Pour off supernatant and vortex pellet until a homogenous cell paste is obtained.

4. Add 110 μl STETL* and vortex 1 sec.

5. Place tubes in boiling water bath or 95-100°C heat block for 30 sec.

6. Spin for 10 min maximum speed in a microfuge.

7. Remove pellet with a sterile toothpick and discard pellet.

8. Add 110 μl isopropanol and spin in a microfuge for 10 min at maximum speed. pour off supernatant and wash briefly with 0.4 ml cold 70% ethanol. Pour off ethanol wash and spin 30 sec in microfuge. Remove remaining liquid with a micropipet and dry pellet in a vacuum.

Pellet will be large and contaminated with much protein. These proteins will not interfere with subsequent restriction digests, but may require removal for future cloning steps.

9. Dissolve pellet in 20 μl TE buffer. Use one to three microliters for restriction digests. Store plasmid prep at 4° for days-weeks or -20° for months-years.

Notes:

More DNA can be prepared if a rich broth or more cells are used. In this case, increase the volume of STETL and isopropanol proportionally.

STET: 8% sucrose, 5% Triton X-100, 50 mM Tris pH 8.0, 50 mM EDTA pH 8.0
Store at room temperature.

*STETL: add lysozyme (0.5 mg/ml final concentration) to STET.