Plasmid DNA Miniprep: Standard Method

1. Inoculate 3-5 ml of LB + antibiotic in 15 ml round bottom tube with a single bacterial colony. Or, inoculate 1.5 ml of TYGPN + antibiotic in 2.0 ml tube. Grow to saturation at 30 – 37°C.
   - This method may be scaled up for 10 mls of culture. Double volumes in steps 3 - 5. Use 0.6 volumes (0.5 ml) of isopropanol instead of ethanol in step 7. Resuspend in 100 µl TE at step 10.

2. Place tubes on ice. Pellet cells (spin 10 min in IEC or 1 min in microfuge). Discard supernatant.

3. Resuspend pellet in 100 µl GTE solution. If using 15 ml tube, transfer to 1.5 ml microfuge tube.

4. Add 200 µl 0.2 N NaOH/1% SDS solution. Mix completely by inverting tube several times. Do not vortex. Place on ice for 5 minutes.
   - NaOH/SDS solution: freshly dilute 2 M NaOH and 10% SDS each at 1 in 10.

5. Add 150 µl of 3 M potassium acetate, pH 5. Immediately vortex for exactly 3 seconds to mix. Place on ice for 5 minutes.

6. Spin 3 minutes at maximum speed in microfuge to pellet cell debris and chromosomal DNA.

7. Transfer supernatant to fresh 1.5 ml tube. Add 2 volumes (0.8 ml) of 100 % ethanol. Vortex to mix well. Hold at room temperature 1 minute to precipitate nucleic acids.

8. Spin 1 minute at room temperature in microfuge to pellet nucleic acids. Discard supernatant.

9. Add 0.5 ml of 70% EtOH. Dislodge pellet (scrape across microfuge tube rack). Hold at room temperature for 5 minutes. Spin 1 minute. Discard supernatant. Spin again for a few seconds. Discard remaining 70% EtOH. Dry pellet in Speed-Vac for 5 minutes (no heat).

10. Resuspend pellet in 25 - 50 µl TE, pH 8.0 + 10 µg/ml RNase A (dilute 1 µl 10 mg/ml RNase A into 1 ml TE). Use 1-2 µl of resuspended DNA for restriction digest.

PEG Precipitation of Miniprep DNA

1. Resuspend pellet in 50 µl TE and add RNase A as described above. Digest for 30 minutes at 37°C.

2. Chill on ice. Microfuge for 10 minutes at top speed at 4°C. Transfer supernatant to fresh tube.

3. Add an equal volume of sterile 1.6 M NaCl + 13% (w/v) PEG 8000. Vortex well to mix completely.

4. Hold on ice 1 hour to overnight. Overnight will give ~100% yield; 1 hour will give ~50% yield.

5. Spin for 30 minutes at top speed at 4°C. Remove supernatant carefully. Pellet may be small.

6. Add 0.5 ml 70% EtOH and vortex briefly.

7. Spin in microfuge for 5 minutes at top speed at 25°C. Remove 70% EtOH.

8. Speed-Vac for ~5 minutes to dry pellet. Resuspend pellet in original volume of TE buffer.