**Affordable method for the large-scale preparation of transfection quality plasmid DNA from *E.coli***

1. Spin 750 ml of transformed *E. coli* cells containing the plasmid of interest at 3000 x *g* for 5 minutes at 4ºC.
2. Re-suspend the cell pellet in 15 ml of 25 mM Tris/HCl pH 8.0, 50 mM glucose, 10 mM EDTA.
3. Split into two 50 ml centrifuge tubes. Add into each tube 2.5 ml of the same buffer (2) plus 25 mg lysozyme per tube. Incubate at room temperature for 5 minutes.
4. Add 15 ml 0.2 M NaOH 1% SDS. Incubate at room temperature for 5 minutes.
5. Add 11.25 ml of 3 M potassium acetate pH 5.5 into each tube. Incubate at room temperature for 5 minutes.
6. Spin at 3000 x *g* for 10 minutes at 4ºC.
7. Filter supernatant through miracloth into a clean 50 ml centrifuge tube. Add 0.6 volumes ispropanol. Incubate at room temperature for 5 minutes.
8. Spin at 3000 x *g* for 10 minutes at 4ºC.
9. Re-suspend each pellet in 15 ml of 10 mM Tris/HCl pH 8.0, 10 mM EDTA.
10. Add 1 volume of 5M LiCl (pre-chilled at -20ºC). This precipitates RNA and proteins. Incubate on ice for 5 minutes. Spin 3000 x *g* at 4ºC.
11. Add 0.6 volumes of isopropanol to precipitate the supernatant. Incubate at room temperature for 5 minutes, spin at 3000 x *g* for 10 minutes at 4ºC.
12. Re-suspend and combine the 2 pellets in 10 ml Tris/HCl, 1 mM EDTA. Vortex vigorously.
13. Add 50 µl of 10 mg/ml heat-treated RNase A. Incubate at room temperature for 15 minutes.
14. Add 0.25 Volumes of 30% PEG 6000, 2.5 M NaCl. Incubate on ice for 30 minutes.
15. Spin at 3000 x *g* for 10 minutes at 4ºC.
16. Re-suspend the pellet in 10 ml of 10 mM Tris/HCl pH 8.0, 1.0 mM EDTA.

 **Chloroform-extract PEG:**

1. Add 2 ml of chloroform, vortex briefly and spin at 3000 x *g* for 1 minute at 4ºC. Retain the top aqueous layer with a pipette.
2. Ethanol DNA precipitation:
3. Add 0.1 volumes of 5M NaCl (or 3 M sodium acetate) and 3 volumes of absolute ethanol. Mix by inverting the tube a few times.
4. Spin at 3000 x *g* for 10 minutes at 4ºC.
5. Wash with 70% EtOH and spin at 3000 x *g* for 10 minutes at 4ºC.
6. Leave the tube upside down to dry overnight.
7. Re-suspend into an appropriate volume of filter sterilized 18 MΩcm H2O, commercial DNA elution buffer or 10 mM Tris/HCl pH 8.0, 0.1 mM EDTA.