Alkaline Lysis Method for Plasmid Purification:

modified from: Zhou A., Jiang X., & Xu X. (1997). Improved alkaline lysis method for rapid isolation of plasmid DNA. *Biotechniques*. **23**, 592-594.

1. Grow *E. coli* transformed with the plasmid of inerest in 3 mL LB medium containing appropriate antibiotics in a tube (1.5 x 15cm). After overnight (15-16 h) growth at 37°C, harvest the bacterial cells by centrifugation at top speed for 20 s in a microcentrifuge.

2. Resuspend the bacterial pellets completely in 120 μL ice-cold **GTE** by vortex mixing for several seconds.

3. Add 240 μl freshly prepared **lysis buffer**; mix quickly by inverting and swirling the tube several times until the contents become clear. Do not vortex mix.

4. Precipitate cellular debris by adding 360 μL ice-cold **4M potassium acetate solution**. Mix the contents thoroughly by immediately swirling the tube. Do not vortex mix.

5. Centrifuge at top speed for 10 min at room temperature and transfer about 660 μL of the supernatant into a clean tube with a disposable pipet. Avoid collecting floating film of denatured material by submerging the pipet tip well into the solution before suction.

6. Add 330 μL isopropanol and invert tubes to mix well. Centrifuge at top speed for 3 min. Pour out the supernatant and place each tube back in the rotor in the original orientation. Quickly centrifuge the tubes at maximum speed for several seconds. Aspirate all residual supernatant from the tubes carefully.

7. Resuspend the pellet in 200 μL of **TE** containing 20 μg/mL DNase-free RNase A. Keep warm at 37°C for 30 min.

8. Add 100 μL of 40% PEG-8000 in 30 mM $MgCl_2$, mix well and then centrifuge at top speed for 5 min at room temperature.

9. Rinse the pellet with 1 mL ice-cold 75% ethanol, resuspending and centrifuging 1 min to remove PEG. Discard all the residual supernatant as described in step 6.

10. Air-dry and dissolve the plasmid DNA in 100 μL TE.

Note: For srains like BL-21 a phenol:choloroform extraction needs to be done after step 5.

Solutions:

GTE:

50mM glucose
25mM Tris-HCl pH 8.0
10mM EDTA, pH 8.0

Lysis Buffer:

0.2M NaOH,
1% sodium dodecyl sulfate (SDS)

4 M Potassium Acetate Solution:

80 mL 5M potassium acetate
11.5 µL glacial acetic acid
8.5 mL sterile Millipore H_2O

TE

10mM Tris-HCl, pH 8.0
1mM EDTA, pH 8.0