1. Solution I  (Resuspend the cells)
   1) EDTA chelates divalent metals (primarily magnesium and calcium). Removal of these cations destabilizes the cell membrane. It also inhibits DNases.
   2) Glucose maintains osmolarity and prevents the buffer from bursting the cells.

2. Solution II (Lyse the cells)
   1) SDS is a detergent found in many common items such as soap, shampoo and toothpaste. SDS pops holes in the cell membranes.
   2) NaOH loosens the cell walls and releases the plasmid DNA and sheared cellular DNA.

3. Solution III (Neutralization)
   1) Neutralization with potassium acetate allows only the covalently closed plasmid DNA to reanneal and to stay solubilized.
   2) Remove cell debris and SDS by centrifugation.
Plasmid Isolation (Alkaline lysis method)

1) Centrifuge 1.5m microfuge tube for 1 min at 14,000 rpm. Discard the supernatant
2) Resuspend the bacterial pellet in 200ul of Solution I (by vortexing)
3) Add 200ul of Solution II (Mix by inverting the tube gently)
4) Add 200ul of Solution III (Mix by inverting the tube gently)
5) A white precipitate forms. Centrifuge the tube for 10min at 14,000rpm.
6) Transfer the supernatant to a fresh tube. *Do not transfer any of the white pellets.
7) Add 900ul of 95% ETOH to the supernatant. (Mix well by inverting the tube several times)
8) Centrifuge at 14,000rpm for 10min.
9) Remove and discard supernatant.
10) To the DNA pellet add 100ul of ice cold 70% ETOH.
11) Centrifuge again for 30sec.
12) Remove and discard supernatant. Air dry the pellets overnight
13) Resuspend the pellet in 50ul of sterile ddH₂O or TE buffer
14) Store at -20°C. A sample of 5ul should be sufficient to see clear bands on an electrophoresis gel.