Mini-prep of BAC DNA (Alkaline lysis)

1. Inoculate a single BAC colony into 3 - 5 ml of LB medium containing the appropriate antibiotic, incubate at 200-250 rpm overnight at 37°C.
2. Collect 3 ml of the overnight culture into a single microfuge tube by repeatedly pelleting in the same tube.
3. Resuspend pellet in 100 µl of Solution I.
4. Add 200 µl of fleshly prepared Solution II mix thoroughly but gently.
5. Add 150 µl Solution III, gently mix and incubate on ice for 10 min.
6. Pellet cell debris by centrifugation at top speed for min at 4°C, carefully transfer supernatant to a new microfuge tube.
7. Add 1 ml 100% EtOH, mix by inversion, centrifuge at top speed for 15 min at 4°C.
8. Wash with 500 µl of 70% EtOH, carefully remove all liquid and air dry.
9. Dissolve DNA in 30 µl water, 10 mM Tris, or TE, etc.

Solution I: (50 mM glucose, 25 mM Tris, 10 mM EDTA, pH 8).

1M Glucose	10 ml, or 1.8 g powder
1M Tris.HCl, pH 7.5	5 ml
0.5M EDTA, pH 8.0	4 ml

Add H_20 to 200 ml, and add RNase A to 100ug/ml final concentration.

Solution II: 0.2 N NaOH, 1% SDS. Add 1 ml 2N NaOH to 8.5 ml H_2O, mix and add 0.5 ml of 20% SDS.

Solution III: 30 ml 5 M KOAc, 5.75 ml glacial acetic acid, and add H_2O to 50 ml.