Preparation of BAC DNA by double acetate precipitation and CsCl gradient

1. Pellet cells from 1 L growth in a 500 ml bottle with two consecutive spin 5,000 rpm for 15 min using a GS-3 rotor.
2. Resuspend cells in 40 ml of 10 m*M* EDTA, pH8.0 and transfer to a 300 ml bottle.
3. Add 80 ml of alkaline lysis solution (0.2*N* NaOH and 1% SDS----2 ml of 10*N* NaOH, 5 ml of 20% SDS for 100 ml solution). Mix by swirling and incubate for 5 min at RT.
4. Add 60 ml of cold 2 *M* KOAc (50 ml of 7.5*M* KOAc, 23 ml of glacial acetic acid, and 127 ml of dH_2O, stored at 4°C). Mix by swirling and incubate on ice for 5 min.
5. Spin at 10,000 rpm with a GSA rotor for 15 min at 4°C.
6. Transferred supernatant into a 500 ml bottle. Add 180 ml of isopropanol. Mix by swirling.
7. Spin at 5,000 rpm for 15 min in a GS-3 rotor. Decant the supernatant.
8. Dissolve the DNA pellet in 18 ml of 10:50 TE (1 ml of 1*M* Tris, 10 ml of 0.5*M* EDTA into 100 ml solution). Add 9 ml of 7.5 *M* KOAc and mix and incubate at –70°C for 30 min.
9. Thaw solution and centrifuged at 6,000 rpm for 10 min at 4°C with a GSA rotor.
10. Transfer supernatant to a new tube and add 2.5 volumes of ethanol.
11. Spin 8,000 rpm for 15 min at 4°C to precipitate the DNA.
12. Gently resuspend pellet (while still moist) in 4 ml TE. Place tubes in an ice bucket and put onto a rotary shaker for 5-10minutes until fully resuspended (may have to pipette gently to fully resuspend). Bring up volume to 4.4 ml. Add 5.1g of CsCl and mix gently to dissolve. Add 0.5 ml Ethidium Bromide (10mg/ml) and mix immediately. Spin (room temp) at 3000 rpm in a table-top centrifuge for 5 min to pellet protein precipitate. Remove the solution and load into a Beckman Quickseal tube (1/2 x 2in., #342412) using a syringe and 18-gauge needle. Seal tubes and place in a vTi80 rotor (tighten to 80 inch-pounds). Spin at either 80,000 rpm for 3h or 65,000 rpm O/N 18°C.

13. Use 23-gauge needle to poke a hole in the top of the tube, 18-gauge needle to pull band. Carefully remove band (bottom band if there are two) with the needle bevel up. Take the band and no more (usually about 200 μl). Bring it up to 1 ml with TE. Extract 4-5 times with NaCl-saturated-butanol (Add 20 ml of 3*M* NaCl to 100 ml of butanol) until there is no more orange (add ButOH, mix gently, let sit 30 sec to allow for separation, remove and discard top layer).
14. Add 2 volumes of H_2O and then 2.5~3.0 volumes of EtOH and mix. If there is no sign of precipitation, place at 20°C for one hour. Spin down DNA and resuspend in 0.5 ml of 0.3*M* NaOAc. Move to eppendorf tube and add 1ml EtOH. Spin, and wash with 70% EtOH, resuspend gently in TE.