Isolation of BAC clones: Occasionally, more than one BAC clone may be represented in a single well of the BAC library. It is recommended that the following procedure for the initial analysis and isolation of BAC clones.

1. Streak some of the BAC culture from the stab tube to a LB plate containing 12.5 μg/ml chloramphenicol. Grow at 37ºC for 1 day.
2. Pick 12 (or so) single colonies and restreak each to another LB with chloramphenicol plate. Grow 1 day at 37ºC. The streak plates may be saved, wrapped in parafilm, for up to month at 4ºC.

Verification of clones: All cones should be verified after isolation using Southern blot hybridization or PCR prior to further studies. A rapid method for verifying clones by PCR involves "whole cell PCR". In this method, cells from an isolated colony are suspended in 500 μl dH_2O. Use 10 μl of this suspension directly in a standard PCR reaction.

After clones are verified, a glycerol stock should be made for each clone desired.

Glycerol stock preparation: Use a sterile loop to inoculate a tube containing 2ml of LB with chloramphenicol (12.5 μg/ml) with the clone stab. Incubate the inoculum overnight, or until turbid, at 37ºC with shaking. Add sterile glycerol to achieve a final glycerol concentration of 20%. Mix thoroughly. Dispense into ultra-low temperature vials and freeze at –70ºC or less. Revive the BAC clone by transferring a small portion of the frozen sample onto an LB agar plate with chloramphenicol (12.5 μg/ml).

BAC mini-prep protocol (for 1.5 ml of overnight culture)

1. Spin 1.5 ml cells 30 sec at full speed in the microfuge.
2. Aspirate medium.
3. Resuspend pellet in 100 μl chilled solution I by vortex.
4. Place tubes on ice; add 200 μl freshly prepared Solution II.
5. Cap and invert tubes 7-9 times; replace on ice.
6. Add 150 μl Solution III.
7. Cap and invert 7-9 times.
8. Spin 6 minutes at full speed in the microfuge at room temperature.
9. Transfer supernatant by pouring to a new tube (remove any debris with a toothpick).
10. Add 1 ml room temperature ethanol, and mix by inverting.
11. Spin 6 minutes at full speed in the microfuge at room temperature.
12. Pour off supernatant; add 500 μl 70% ethanol at room temperature to rinse pellet.
13. Pour off and drain tube upside down on a clean paper towel. Air dry.
14. Resuspend pellet in 20 μl TE.
15. For restriction digestion, cut 5 μl in a reaction of 20 μl.

Solution I: 50 mM glucose, 20 mM Tris-Cl, pH8.0, 10 mM EDTA, pH8.0.
Solution II: 0.2N NaOH, 1% SDS.
Solution III: Potassium Acetate (pH4.8). This solution is made by adding glacial acetic acid to a fully saturated solution of 3M potassium acetate to achieve a pH of 4.8. This is accomplished by adding a minimal amount of water to potassium acetate and then adding acetic acid until the potassium acetate is dissolve and the pH has reached 4.8.