Bacmid minipreps

(1) Grow a single colony in 3 mL of LB containing 50 μg/mL kanamycin, 7 μg/mL gentamicin, 10 μg/mL tetracycline overnight. It's best to start these cultures in the morning, since the cells grow very slowly.
(2) Make a glycerol stock by mixing 1 mL of the cells with 120 μL of 80% glycerol. Store in the -80 °C freezer.
(3) Transfer the remaining culture to a 2-mL microcentrifuge tube and spin at maximum speed for 1 min.
(4) Remove all of the supernatant using the vacuum aspirator.
(5) Resuspend the cells in 300 μL of Qiagen Buffer P1. Do not use P1 containing the blue lysis indicator dye.
(6) Add 300 μL of Qiagen Buffer P2, then incubate at room temperature for 5 min.
(7) Slowly add 300 μL of Qiagen Buffer N3, mixing gently during addition. A thick white precipitate of protein and genomic DNA will form. Place the sample on ice for 5-10 min.
(8) Centrifuge for 10 min at maximum speed. During this step, label another tube and add 800 μL isopropanol to it.
(9) Gently transfer the supernatant into the tube containing the isopropanol. Avoid transferring any of the white precipitate. Mix gently by inverting the tube a few times and place on ice for 5-10 min. Note: The sample can be stored at -20 °C overnight (or over a weekend) to get higher yield.
(10) Centrifuge for 15 min at maximum speed.
(11) Remove the supernatant and add 500 μL of 70% ethanol. Invert the tube several times to wash the pellet. Centrifuge for 10 min at maximum speed. Repeat the wash step.
(12) Carefully remove all of the supernatant using the vacuum aspirator. To generate gentle suction, use either a gel-loading pipet tip or a 10-μL tip as nozzle.
(13) Air-dry the pellet (about 5-10 min). Do not overdry.
(14) Resuspend the DNA in 40 μL of TE buffer. Do not attempt to add buffer before the ethanol has dried out, or the DNA will not go into solution.
(15) We generally store the sample at 4 °C, until it is used for transfection. After that, it can be transferred to -20 °C for long-term storage. Avoid repeated freeze-thaw cycles.