Making bacmids by recombination

You will need Lennox LB-agar plates (10 g peptone, 5 g yeast extract, 5 g NaCl, 12 g agar per liter) supplemented with:

50 μg/mL kanamycin
7 μg/mL gentamicin
10 μg/mL tetracycline
100 μg/mL Bluo-Gal
40 μg/mL IPTG

Step 1: Transposition of pFastBac into DH10Bac plates

(1) Thaw the DH10Bac competent cells on ice.
(2) Dispense 30 μL of the cells into a 14-mL round-bottom polypropylene tube.
(3) Add approximately 5 ng of the donor pFastBac plasmid (maximum 1.5 μL volume) to the cells and gently mix by tapping the side of the tube.
(4) Incubate in ice for 30 min.
(5) Heat shock at 42 °C for 45 sec.
(6) Incubate in ice for 2 min.
(7) Add 500 μL of SOC.
(8) Grow the cells in a shaking incubator for **4 h** at 37 °C with medium agitation (225 rpm).
(9) Plate on 50 μL of the cells on Lennox LB-agar (preferably pre-warmed).
(10) Incubate the plate upside-down at 37 °C for 2 days. Blue coloring does not fully develop until day 2. White colonies are generally larger than the blue colonies.

Step 2: Pick 2-3 clones (white colonies) and re-streak. After 2 days, pick 1 isolated colony for each clone for a bacmid miniprep.