Transforming DH10Bac *E. coli*, continued

**Transformation procedure**

Follow the procedure below to transform MAX Efficiency® DH10Bac chemically competent cells with your pFastBac construct. We recommend including positive controls for transposition (i.e., pFastBac expression plasmid) and transformation (i.e., pUC19) in your experiment to help you evaluate your results.

1. Thaw on ice one tube (50uL) of homemade DH10Bac competent cells for each transformation

2. Add the appropriate amount of plasmid DNA to the cells and mix gently. **Do not pipet up and down to mix**
   - Your pFastBac construct: 20 ng
   - Optional: pFastBac control plasmid: 1 ng
   - Optional: pUC19 control: 50 pg (5 uL)

3. Incubate cells on ice for 30 minutes.

4. Heat-shock the cells for 45 seconds at 42°C without shaking.

5. Immediately transfer the tubes to ice and chill for 2 minutes.

6. Add 450 uL of room temperature S.O.C. Medium.

7. For pFastBac™ transformations: Shake tubes at 37°C at 225 rpm for 4 hours.
   For pUC19 transformation: Shake tube at 37°C at 225 rpm for 1 hour.

8. For each pFastBac™ transformation: Plate 100 uL of each construct on an LB-agar plate containing 50 ug/mL kanamycin, 7 ug/mL gentamicin, 10 ug/mL tetracycline, 100 ug/mL Bluo-gal, and 40 ug/mL IPTG.
   For the pUC19 transformation: Dilute the cells 1:100 with S.O.C. Medium. Plate 100 uL of the dilution on an LB agar plate containing 100 ug/mL ampicillin.

9. Incubate plates for 48 hours at 37°C. Pick white colonies for analysis (see the next page for recommendations).
   **Note:** We do not recommend picking colonies earlier than 48 hours as it may be difficult to distinguish between white and blue colonies.

**IMPORTANT!**

Insertions of the mini-Tn7 into the mini-attTn7 attachment site on the bacmid disrupt the expression of the LacZα peptide, so colonies containing the recombinant bacmid are white in a background of blue colonies that harbor the unaltered bacmid. **Select white colonies for analysis.** True white colonies tend to be large; therefore, to avoid selecting false positives, choose the largest, most isolated white colonies. Avoid picking colonies that appear gray or are darker in the center as they can contain a mixture of cells with empty bacmid and recombinant bacmid.