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 LacZa 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.