Basic Transformation Procedure

1. Thaw one vial of One Shot® cells on ice per transformation.
2. Add 5–10 ng of DNA, in a volume of 1–5 µL to the cells and mix by tapping gently. **Do not mix cells by pipetting.**
3. Incubate the vial(s) on ice for 30 minutes.
4. Heat shock the cells by incubating the vial(s) for exactly 30 seconds in the 42°C water bath. **Do not mix or shake.**
5. Remove the vial(s) from the 42°C bath and quickly place on ice.
6. Add **250 µL** of pre-warmed SOC medium to the vial(s). (SOC is a rich medium; use proper sterile technique to avoid contamination.)
7. Secure the vial(s) in a microcentrifuge rack with tape. Place the rack in a shaking incubator, and shake the vial(s) at 37°C for 1 hour at 225 rpm.
8. Plate two different volumes of the transformation reaction onto LB plates containing the appropriate antibiotic for plasmid selection. Include 34 µg/mL chloramphenicol if using BL21(DE3)pLysS or BL21(DE3)pLysE cells. Select two volumes ranging from 20–200 µL to ensure well-spaced colonies on at least one plate. The remaining transformation reaction may be stored at 4°C and plated out the next day, if needed.
9. Invert the plates and incubate at 37°C overnight.
10. Select transformants from the plates and culture as described on page 9.

Note: Clones may exhibit differences in expression of heterologous genes. We recommend choosing 3–4 transformants when characterizing clones for protein expression.