Transformation Protocol

Procedure

1. Thaw chemically competent *E. coli* cells on ice. Keep cells cold at all times and avoid prolonged exposure to room temperature.
2. Prepare the following transformation mix:

Component	Volume
Competent cells	80 µL
10× KCM buffer	10 µL
Nuclease-free water	5 µL
Assembly reaction or plasmid DNA	5 µL
Total	**100 µL**

1. Mix gently by tapping or flicking the tube. Do not vortex.
2. Incubate on ice for 20 minutes.
3. Mix gently again and incubate at room temperature for 10 minutes.
4. Add 200 µL SOC or another suitable recovery medium.
5. Recover cells for 30 to 45 minutes at 37°C with shaking (approximately 150 rpm). For large constructs or assemblies prone to recombination, recovery at 30 to 32°C may improve clone stability.
6. Plate 100 µL of the transformation culture onto **LB agar plates supplemented with kanamycin**.
7. Incubate plates inverted overnight at 37°C. When lower-temperature recovery was used, incubation at 30 to 32°C may be preferred.

10× KCM Buffer

Final concentrations:

- 0.5 M KCl
- 0.15 M $CaCl_2$
- 0.25 M $MgCl_2$

Filter sterilize through a 0.22 µm filter and store aliquots at -20°C.

Note: This protocol has been validated using in-house prepared *E. coli* DH10 competent cells. Performance may differ with other strains or commercially available competent cells.