Suggested Transformation Protocol:

1. Thaw cells on wet ice after removing from -70°C

2. Mix cells gently by lightly flicking the tube. Aliquot ~50-100μl of cells into chilled, 17 x 100mm polypropylene tube(s). Unused cells may be refrozen, but a small drop in efficiency may result. For optimal recovery, refreeze cells in a dry ice/ ethanol bath prior to -70°C storage.

3. Add DNA solution (≤5μl per 50μl cells) to cell suspension and gently swirl tube(s) for a few seconds to mix. If a control is desired, repeat this step with 2μl of the provided pUC19 in a separate tube.

4. Incubate on ice for 30 minutes.

5. Place tube(s) in 42°C water bath for ~30 to 45 seconds without shaking.

6. Place tube(s) again on ice for ~2 minutes.

7. Dilute transformation reaction(s) to 1ml by addition of 900-950μl SOC medium**.

8. Shake tube(s) ~200 rpm for 60 minutes at 37°C.

9. Plate by spreading 5-200μl of cell transformation mixture on LB agar plates containing appropriate antibiotic and incubate overnight at 37°C.

When performing the pUC19 control transformation, plate 5μl of the transformation mixture on a LB agar plate containing 100μg/ml ampicillin. To facilitate cell spreading, place a pool of SOC (100μl) onto surface of plate prior to addition of transformation mixture.

** SOC Medium: 2% Tryptone, 0.5% Yeast Extract, 0.4% glucose, 10mM NaCl, 2.5mM KCl, 10mM MgCl₂ & 10mM MgSO₄.

Transformation Efficiency Calculation for Control DNA

Transformation Efficiency (cfu/μg pUC19 DNA) = \( \frac{\text{# colonies}}{\text{pg pUC19 transformed}} \times \frac{10^6 \text{ pg}}{\text{ug}} \times \frac{\text{Final volume (μl) of transformation mix}}{\text{Volume plated (μl)}} \)

For example:

If 40 colonies were obtained after transforming 20pg of pUC19 and plating 5μl of the final 1ml transformation mixture, the calculated transformation efficiency would be:

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\frac{40 \text{ cfu}}{20 \text{ pg pUC19}} = \frac{10^6 \text{ pg}}{\text{ug}} \times \frac{1000 \text{ul}}{5 \text{ul}} = 4 \times 10^8 \text{ cfu/ug pUC19}
\]