3. As a positive control for transformation, dilute the control pUC19 by 10 folds.

4. Thaw NEB Turbo Electrocompetent cells on ice (about 10 min) and mix cells by flicking gently. Transfer 25 μl of the cells (or the amount specified for the cuvettes) to a chilled microcentrifuge tube. Add 1 μl of the DNA solution.

5. Carefully transfer the cell/DNA mix into a chilled cuvette without introducing bubbles and make sure that the cells deposit across the bottom of the cuvette. Electroporate using the following conditions for BTX ECM 630 and Bio-Rad GenePulser electroporators: 2.1 kV, 100 μF, and 25 μF. The typical time constant is ~2.6 milliseconds.

6. Immediately add 975 μl of 37°C SOC to the cuvette, gently mix up and down twice, then transfer to the 17 mm x 100 mm round-bottom culture tube.

7. Shake vigorously (250 rpm) or rotate at 37°C for 1 hour.

8. Dilute the cells as appropriate then spread 100–200 μl cells onto a pre-warmed selective plate.

9. Incubate plates 8 hours to overnight at 37°C.

### Calculation of Electrotroporation Efficiency

Transformation efficiency is defined as the number of colony forming units (cfu) which would be produced by electrotransforming 1 μg of plasmid into a given volume of electrocompetent cells. For example, if a 10 pg transformation with pUC19 yields 100 colonies when 100 μl of a 1:10 dilution is plated, then the efficiency is 1.0 x 10^10 cfu/μg pUC19 (100 cfu/10 pg x 10^6 pg/μg x 1 ml/0.1 ml plated x 100 dilution).

### Electroporation Tips

- Electroporation cuvettes and microcentrifuge tubes should be pre-chilled on ice.
- Electrocompetent cells should be thawed on ice and suspended well by carefully flicking the tubes.
- Once DNA is added to the cells, electroporation can be carried out immediately. It is not necessary to incubate DNA with cells. The maximum recommended volume of a DNA solution to be added is 2.5 μl. Addition of a large volume of DNA decreases transformation efficiency.
- Contaminants such as salts and proteins can lower electroporation efficiency. Ideally, DNA for electroporation should be purified and suspended in water or TE. Transformation efficiency is more than 10-fold lower for ligation mixtures than the control pUC19 plasmid due to the presence of ligase and salts. If used directly, ligation reactions should be heat-inactivated at 65°C for 20 min and then diluted 10-fold. For optimal results, spin columns are recommended for clean up of ligation reactions.
- Electroporation conditions vary with different cuvettes and electroporators. If you are using electroporators not specified in the protocol, you may need to optimize the electroporation conditions. Cuvettes with 1 mm gap are recommended (e.g. BTX Model 610/613 and Bio-Rad #185–2089). Higher voltage and/or increased resistance is required for cuvettes with 2 mm gap.

### Effect of pUC19 Concentration on Electroporation Efficiency and Total Colony Output

DNA Effects of Transformation Efficiency and Colony Output. Electrotroporation efficiency remains extremely high up to about 1 ng of input DNA, then decreases at higher DNA concentrations.
Electroporation Tips (continued)
- Arcing may occur due to high concentration of salts or air bubbles.
- It is essential to add recovery medium to the cells immediately after electroporation. One minute delay can cause a 3-fold reduction of efficiency.
- Cold and dry selection plates lead to lower transformation efficiency. Pre-warm plates at 37°C for 1 hour. Using 37°C pre-warmed recovery medium increases the efficiency by about 20%.
- Refreeze unused cells in a dry ice/ethanol bath for 5 min and then store at ~80°C. Do not use liquid nitrogen. Additional freeze-thaw cycles result in lower transformation efficiency.

Solutions/Recipes
SOB:
- 2% Vegetable peptone (or Tryptone)
- 0.5% Yeast Extract
- 10 mM NaCl
- 2.5 mM KCl
- 10 mM MgCl₂
- 10 mM MgSO₄
- 2% Vegetable peptone (or Tryptone)
SOC:
- SOB + 20 mM Glucose

Blue/White Screening:
- X-gal 80 μg/ml
- IPTG* 0.3 mM

Antibiotics for Plasmid Selection

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Working Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>100 μg/ml</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>100 μg/ml</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>33 μg/ml</td>
</tr>
<tr>
<td>Kanamycin</td>
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<td>Streptomycin</td>
<td>25 μg/ml</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>15 μg/ml</td>
</tr>
</tbody>
</table>

*Omit IPTG for potentially toxic genes

Genotype: F’ proA+B- lacIq (lacZM15 / fhuA2 ∆(lac-proAB) glnV galK16 galE5 R(zgb-210::Tn10)Tetr thi-I (hsdS-mcrB5)

Strain Properties
The properties of this strain that contribute to its usefulness as a cloning strain are described below. The genotypes underlying these properties appear in parentheses.

Blue/White Screening (F’ ∆(lacZ/M15) makes ω-fragment of β-gal; ∆(lac-proAB) deletes the β-gal gene on the chromosome) pUC19 and similar plasmids code for the α-peptide of β-galactosidase (lacZ). The α-peptide can combine with the ω-fragment of β-galactosidase which is carried on the F’ (α-ω-complementation). When β-galactosidase is reconstituted in this manner it can cleave X-gal and results in blue colonies on an X-gal plate. Inserts cloned into the plasmid polylinker disrupt the α-peptide gene and the colonies are white.

Lac Promoter Control (lacP): The lac repressor blocks expression from lac, tac and trc promoters frequently carried by expression plasmids. If the level of lac repressor in E. coli cells is not sufficient to inhibit expression via these promoters during transformation or cell growth, even low levels of expression can reduce transformation efficiency and select against desired transformants. The extra molecules of lac repressor in lacP strains help to minimize promoter activity until IPTG is added.