Materials Provided
LC1062 competent cells, 50 x 100 μl

Storage Conditions
Place cells at the bottom of a -80°C freezer directly from the shipping container. Do not store in liquid nitrogen. Competent cells are sensitive to small temperature changes. Transferring tubes between freezers may result in a loss of efficiency.

Guaranteed Efficiency
≥1.0 x 10^7 cfu/μg pUC18 DNA

Test Conditions
Transformations are performed both with and without plasmid DNA using 100-μl aliquots of cells and 10 pg of pUC18 control DNA following the protocol outlined below. Following transformation, the cultures are plated in duplicate on LB agar plates with 100 μg/ml ampicillin. The plates are incubated at 37°C overnight and the efficiency is calculated based on the average number of colonies per plate.

Transformation Protocol
1. Thaw the appropriate number of tubes containing competent cells in an ice slurry for approximately 5 minutes. When thawed, gently mix the cells by flicking the tubes.
2. Add the experimental DNA to one tube of cells and add 1 μl of 10 pg/μl pUC18 DNA to another tube. Swirl the tubes gently.
3. Incubate the tubes on ice for 15 minutes.
4. Heat-pulse the tubes in a 37°C water bath for 15 minutes.
5. Incubate the tubes on ice for 2 minutes.
6. Plate the entire volume of the transformation mixture on an LB agar plate containing the appropriate antibiotic. For the pUC18 control transformation, plate the transformation mixture on LB-ampicillin agar.
7. Incubate the plates at 37°C overnight.
8. For the pUC18 control, expect ≥100 colonies (≥1 x 10^7 cfu/μg pUC18 DNA). For the experimental DNA, the number of colonies will vary according to the size and form of the transforming DNA, with larger and non-supercoiled DNA producing fewer colonies.

Preparation of Media and Reagents

LB Agar (per Liter)
10 g of NaCl
10 g of tryptone
5 g of yeast extract
20 g of agar
Add deionized H2O to a final volume of 1 liter
Adjust pH to 7.0 with 5 N NaOH and then autoclave
Pour into petri dishes (~25 ml/100-mm plate)

LB-Ampicillin Agar (per Liter)
1 liter of LB agar, autoclaved and cooled to 55°C
Add 10 ml of 10 mg/ml filter-sterilized ampicillin
Pour into petri dishes (~25 ml/100-mm plate)