Materials Provided
XL10-Gold ultracompetent cells (yellow tubes), 10 x 100 μl
pUC18 control plasmid (0.1 ng/μl in TE buffer), 10 μl
XL10-Gold β-Mercaptoethanol (β-ME) mix, 50 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
≥ 5 x 10^9 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, 5-μl samples of the culture 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.

Antibiotic Resistance
XL10-Gold cells are tetracycline and chloramphenicol resistant.

Genotype and Background
Tet(ΔmcrA183 ΔmcrCB-hsdSMR-merR173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac HsdS [F' proAB lacZΔM15 Tn10 (Tet') Amy Cam']
(Genes listed signify mutant alleles. Genes on the F’ episome, however, are wild-type unless indicated otherwise.)

XL10-Gold® ultracompetent cells were created for transformation of large DNA molecules with high efficiency. These cells exhibit the Hsd phenotype, which increases the transformation efficiency of ligated and large DNA molecules. XL10-Gold ultracompetent cells are ideal for constructing plasmid DNA libraries because they decrease size bias and produce larger, more complex plasmid libraries. XL10-Gold cells are deficient in all known restriction systems [ΔmcrA183 ΔmcrCB-hsdSMR-merR173]. The strain is endonuclease deficient (endA), greatly improving the quality of miniprep DNA, and recombination deficient (recA), helping to ensure insert stability. The lacZΔM15 gene on the F’ episome allows blue-white screening for recombinant plasmids.

Transformation Protocol
1. Pre-chill two 14-ml BD Falcon polypropylene round-bottom tubes on ice. (One tube is for the experimental transformation and one tube is for the pUC18 control.) Preheat NZY broth to 42°C.
2. Thaw the cells on ice. When thawed, gently mix and aliquot 100 μl of cells into each of the two pre-chilled tubes.
3. Add 4 μl of the β-ME mix provided with this kit to each aliquot of cells.
4. Swirl the tubes gently. Incubate the cells on ice for 10 minutes, swirling gently every 2 minutes.
5. Add 0.1-0.5 ng of the experimental DNA (or 2 μl of a ligation mixture) to one aliquot of cells. Dilute the pUC18 control DNA 1:10 with sterile dH₂O, then add 1 μl of the diluted pUC18 DNA to the other aliquot of cells.
6. Swirl the tubes gently, then incubate the tubes on ice for 30 minutes.
7. Heat-pulse the tubes in a 42°C water bath for 30 seconds. The duration of the heat pulse is critical.
8. Incubate the tubes on ice for 2 minutes.
9. Add 0.9 ml of preheated (42°C) NZY broth and incubate the tubes at 37°C for 1 hour with shaking at 225-250 rpm.
10. Plate 200 μl of the transformation mixture on LB agar plates containing the appropriate antibiotic (and containing IPTG and X-gal if color screening is desired). For the pUC18 control transformation, plate 5 μl of the transformation on LB-ampicillin agar plates.
11. Incubate the plates at 37°C overnight. If performing blue-white color screening, incubate the plates at 37°C for at least 17 hours to allow color development (color can be enhanced by subsequent incubation of the plates for 2 hours at 4°C).
12. For the pUC18 control, expect 250 colonies (≥5 x 10^9 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.

Blue-White Color Screening
Blue-white color screening for recombinant plasmids is available when transforming this host strain (containing the lacZΔM15 gene on the F’ episome) with a plasmid that provides c-complementation (e.g. the pBluescript II vector). When lacZ expression is induced by IPTG in the presence of the chromogenic substrate X-gal, colonies containing plasmids with inserts will be white, while colonies containing plasmids without inserts will be blue. If an insert is suspected to be toxic, plate the cells on media without X-gal and IPTG. Color screening will be eliminated, but lower levels of the potentially toxic protein will be expressed in the absence of IPTG.