**Materials Provided**
XL1-Blue competent cells (blue tubes), 5 × 200 μl
pUC18 control plasmid (0.1 ng/μl in TE buffer), 10 μl
β-Mercaptoethanol (1.42 M), 25 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 × 10^6 cfu/μg pUC18 DNA

**Test Conditions**
Transformations are performed both with and without plasmid DNA using 100-μl aliquots of cells and 100 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.

**Genotype and Background**
*recA1* endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [F’ proAB lacZΔM15 Tn10 (Tet)] (Genes listed signify mutant alleles. Genes on the F’ episome, however, are wild-type unless indicated otherwise.)

The XL1-Blue strain allows blue-white color screening for recombinant plasmids and is an excellent host strain for routine cloning applications using plasmid or lambda vectors. The XL1-Blue cells are endonuclease (endA) deficient, which greatly improves the quality of mini-prep DNA, and are recombination (recA) deficient, improving insert stability. The *hsdR* mutation prevents the cleavage of cloned DNA by the EcoK endonuclease system. The *lacZΔM15* gene on the F’ episome allows blue-white screening for recombinant plasmids.

**Antibiotic Resistance**
XL1-Blue competent cells are resistant to tetracycline.

**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 SOC medium 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 1.7 μl of the β-mercaptoethanol 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 μg of the experimental DNA to one aliquot of cells and add 1 μl of the pUC18 control DNA to the other aliquot. Swirl the tubes gently.
6. Incubate the tubes on ice for 30 minutes.
7. Heat-pulse the tubes in a 42°C water bath for 45 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) SOC medium 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 mixture 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 50 colonies (≥1 × 10^6 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-supercopied 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 α-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.