STRATAGENE
An Agilent Technologies Company

Catalog Number	**200227**
Product Name	**SURE® Electroporation-Competent Cells**
Materials Provided	SURE® electroporation-competent cells (red-orange tubes), 5 × 100 µl pUC18 control plasmid (0.1 ng/µl in TE buffer), 10 µl
Certified By	*****
Quality Controlled By	*****
Shipping Conditions	Shipped on dry ice.
Storage Conditions	Competent cells must be placed immediately at the bottom of a -80°C freezer directly from the dry ice shipping container. Do not store the cells in liquid nitrogen. Competent cells are sensitive to even small variations in temperature. Transferring tubes from one freezer to another may result in a loss of efficiency.
Guaranteed Efficiency	$\geq 1.0 \times 10^{10}$ cfu/µg pUC18 DNA
Test Conditions	Transformations are performed both with and without plasmid DNA using 40-µl aliquots of cells and 10 pg of pUC18 control DNA following the protocol outlined below. Following transformation, 2.5-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	e14$^-$(McrA$^-$) Δ*(mcrCB-hsdSMR-mrr)171 endA1 gyrA96 thi-1 supE44 relA1 lac recB recJ sbcC umuC*::Tn*5* (Kan^r) *uvrC* [F′ *proAB lacI*^{q}*ZΔM15* Tn*10* (Tet^r)]. (Genes listed signify mutant alleles. Genes on the F′ episome, however, are wild-type unless indicated otherwise). Stratagene has developed a method* to produce electroporation-competent cells that have the highest transformation efficiencies--greater than 1×10^{10} transformants/µg of DNA. These electroporation-ready cells need only be thawed, mixed with DNA, and electroporated. The SURE® (Stop Unwanted Rearrangement Events) strain** was engineered to allow the cloning of certain DNA segments that are "unclonable" in conventional *E. coli* strains. The SURE strain lacks components of the pathways that catalyze the rearrangement and deletion of nonstandard secondary and tertiary structures, including cruciforms (caused by inverted repeats) and Z-DNA, that occur frequently in eukaryotic DNA and that impede the cloning of the eukaryotic DNA in conventional strains. SURE cells are restriction minus (McrA$^-$, McrCB$^-$, McrF$^-$, Mrr$^-$, HsdR$^-$) endonuclease (*endA*) deficient, and recombination (*recB recJ*) deficient. The *lacI*^{q}*ZΔM15* gene, on the F′ episome, allows blue-white screening.
Antibiotic Resistance	**SURE electroporation-competent cells are resistant to kanamycin and tetracycline.** When transforming the SURE strain with plasmids carrying the chloramphenicol resistance gene, select for transformants on LB agar plates containing 100 µg/ml chloramphenicol. The SURE strain is resistant (Cam^r) to concentrations of <40 µg/ml chloramphenicol, but sensitive (Cam^s) to 100 µg/ml chloramphenicol.

Electroporation Protocol

1. Pre-chill two sterile electroporation cuvettes (0.1-cm gap) and two sterile 1.5-ml microcentrifuge tubes thoroughly on ice. Preheat sterile SOC medium to 37°C.
2. Set the electroporator to a voltage setting of 1700 V (17 kV/cm field strength). If using a Bio-Rad electroporator, set the resistance at 200 ohms and the capacitance at 25 µF. For best results, chill the electroporator or perform the electroporation in a cold room.
3. Thaw the cells on ice. When thawed, gently mix and aliquot 40 µl of cells into each of the two pre-chilled tubes (one tube for the experimental transformation and one tube for the pUC18 control transformation). Keep the tubes on ice.
4. Add the DNA to the cells with gentle mixing. For optimal efficiency in the experimental transformation, add 1 µl of plasmid DNA (10 pg/µl, in a low ionic strength buffer or dH_2O) to 40 µl of cells. The DNA volume may be increased up to 4 µl, but the efficiency may be reduced. Dilute the pUC18 control DNA 1:10 with sterile dH_2O, then add 1 µl of the diluted pUC18 DNA to the other 40 µl of cells.
5. Transfer the cell-DNA mixture to a **chilled** electroporation cuvette, tapping the cuvette until the mixture settles evenly to the bottom.
6. Slide the cuvette into the electroporation chamber until the cuvette sits flush against the electrical contacts.
7. Pulse the sample once, then quickly remove the cuvette. **Immediately** add 960 µl of SOC medium (held at 37°C) to resuspend the cells.
8. Transfer the cells to a sterile 14-ml BD Falcon polypropylene round-bottom tube (BD Biosciences Catalog #352059). Incubate the tube at 37°C for 1 hour with shaking at 225-250 rpm.
9. Plate 5-100 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 2.5 µl of the transformation mixture on LB-ampicillin agar plates.
10. Incubate the plates at 37°C overnight (<24 hours). If performing blue-white color screening, incubate 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).
11. For the pUC18 control, expect 250 colonies ($\geq 1 \times 10^{10}$ 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 *lacI*^{q}*ZΔM15* gene on the F′ episome) with a plasmid that provides α-complementation (e.g. Stratagene's 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.

Critical Success Factors and Troubleshooting

Aliquoting Cells: Keep the cells on ice at all times during aliquoting. It is essential that the microcentrifuge tubes that the cells will be aliquoted into are placed on ice before the cells are thawed and that the cells are aliquoted directly into the pre-chilled tubes.
Cuvette Gap Width: Use a cuvette with a 0.1-cm gap to maximize the transformation efficiency and to minimize the possibility of arcing. A cuvette with a 0.2-cm gap is not recommended because the transformation efficiency is lower and the possibility of arcing is higher.
Quantity and Volume of DNA: The greatest efficiency is obtained from the transformation of 1 µl of 0.01 ng/µl DNA per 40 µl of cells. The volume of DNA may be increased to up to 4 µl but the transformation efficiency may be reduced and the possibility of arcing may be increased if the DNA solution contains salts. A greater number of colonies may be obtained by increasing the amount of DNA added to the cells, although the overall efficiency may be lower.
Ionic Strength of DNA Solution: The sample DNA to be electroporated must be in a low-ionic-strength buffer, such as TE buffer or water. DNA samples containing too much salt will cause arcing at high voltage, possibly damaging both the sample and the machine.
Reduction of Satellite Colonies: Due to the high concentration of bacteria in electroporation, the transformation plates should be incubated at 37°C for less than 24 hours. If using an incubation period >24 hours, Stratagene recommends selecting for transformants on LB plates containing tetracycline (30 µg/ml) in addition to the antibiotic used to select for the transforming plasmid. Including tetracycline helps suppress satellite colony growth and does not affect the transformation efficiency. In addition, all colonies that grow in the presence of tetracycline contain an F′ episome, improving blue-white color selection.
Plating the Transformation Mixture: If plating <100 µl of cells, pipet the cells into a 200-µl pool of SOC medium and then spread the mixture with a sterile spreader. If plating ≥100 µl, the cells can be spread on the plates directly. Tilt and tap the spreader to remove the last drop of cells.

Preparation of Media and Reagents

SOB Medium (per Liter)
20.0 g of tryptone
5.0 g of yeast extract
0.5 g of NaCl
Add dH_2O to a final volume of 1 liter and then autoclave
Add 10 ml of filter-sterilized 1 M $MgCl_2$ and 10 ml of filter-sterilized 1 M $MgSO_4$ prior to use

SOC Medium (per 100 ml)
Prepare immediately before use
2 ml of filter-sterilized 20% (w/v) glucose or 1 ml of filter-sterilized 2 M glucose
SOB medium (autoclaved) to a final volume of 100 ml

LB Agar (per Liter)
10 g of NaCl
10 g of tryptone
5 g of yeast extract
20 g of agar
Add deionized H_2O 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)

TE Buffer
10 mM Tris-HCl (pH 7.5)
1 mM EDTA

Plates for Blue-White Color Screening
Prepare the LB agar and when adding the antibiotic, also add 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) to a final concentration of 80 µg/ml [prepared in dimethylformamide (DMF)] and isopropyl-1-thio-β-D-galactopyranoside (IPTG) to a final concentration of 20 mM (prepared in sterile dH_2O). Alternatively, 100 µl of 10 mM IPTG and 100 µl of 2% X-gal may be spread on solidified LB agar plates 30 minutes prior to plating the transformations. (For consistent color development across the plate, pipet the X-gal and the IPTG into a 100-µl pool of SOC medium and then spread the mixture across the plate. Do not mix the IPTG and the X-gal before pipetting them into the pool of SOC medium because these chemicals may precipitate.)

Limited Product Warranty

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Endnotes

*U.S. Patent Nos. 6,338,965 and 6,040,184.
**U.S. Patent Nos. 6,017,748, 5,552,314 and equivalent foreign patents.
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