Materials Provided
StrataClone SoloPack Competent Cells, 21 single-tube transformations (50 µl each)
pUC18 control plasmid (0.1 ng/µl in TE buffer), 10 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.

Description
StrataClone SoloPack competent cells are used in the StrataClone PCR cloning system. The cells express Cre recombinase, in order to circularize the linear DNA molecules produced by topoisomerase I-mediated ligation in the StrataClone system. The cells are provided in a convenient single-tube transformation format.

StrataClone SoloPack competent cells contain the lacZAM15 mutation for blue-white screening with the product of StrataClone PCR cloning, pSC-A, which contains the lacZα-complementation cassette. It is not necessary to induce lacZα expression with IPTG when performing blue-white screening with this strain.

StrataClone SoloPack competent cells are optimized for high efficiency transformation and recovery of high-quality recombinant DNA. The cells are endonuclease (endA), and recombination (RecA) deficient, and are restriction-minus. The cells lack the tonA receptor, conferring resistance to T1, T5, and phi80 bacteriophage infection, and lack the F’ episome.

Antibiotic Resistance
StrataClone SoloPack competent cells are streptomycin resistant.

Guaranteed Efficiency
≥5 × 10⁷ cfu/µg pUC18 DNA

StrataClone PCR Cloning Protocol
1. Prepare insert DNA by PCR using Tag DNA polymerase or a polymerase blend qualified for PCR cloning applications.
2. Prepare the PCR cloning reaction mixture by combining the following components. Add the components in the order given below and mix gently by repeated pipetting.
   - 3 µl StrataClone Cloning Buffer
   - 2 µl of PCR product (5–50 ng, typically a 1:10 dilution of a robust PCR reaction)
   - 1 µl StrataClone Vector Mix
3. Incubate the reaction at room temperature for 5 minutes, then place the reaction on ice. Proceed to the Transformation Protocol.

Test Conditions
The transformation efficiency of the competent cells is tested by transformation with the pUC18 control DNA. Transformations are performed both with and without plasmid DNA in duplicate using 50-µl aliquots of cells and 10 pg of pUC18, following the Transformation Protocol provided below. Following transformation, 25-µl samples from each culture are plated 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. For each transformation reaction, thaw an aliquot of StrataClone SoloPack competent cells on ice.
2. When the cells have thawed, swirl the tubes to gently mix the cells.
3. For PCR cloning, add 1 μl of the completed StrataClone PCR cloning reaction mixture to a tube of cells. (Up to 2 μl of the cloning reaction may be added to the competent cells for long or challenging inserts.) For the pUC18 control transformation, dilute the provided pUC18 DNA 1:10 with sterile dH2O, then add 1 μl of the diluted pUC18 DNA to a tube of cells.
4. Swirl the tubes gently, then incubate the tubes on ice for 20 minutes.
5. Preheat SOC to 42°C for use as the outgrowth medium in step 7.
6. Heat-pulse the tubes in a 42°C water bath for 45 seconds.
7. Add 250 μl of preheated (42°C) SOC medium and incubate the tubes at 37°C for 1 hour with agitation. (Position the tubes on the shaker horizontally for better aeration.)
8. During the outgrowth period, prepare LB-ampicillin agar plates for PCR cloning color screening by spreading 40 μl of 2% X-gal on each plate.
9. For PCR cloning, plate 5 μl and 100 μl of the transformation mixture on the LB-ampicillin-X-gal plates. For the pUC18 control transformation, plate 30 μl of the transformation reaction on LB-ampicillin agar plates. (When plating <50 μl, pipette the cells into a 50-μl pool of SOC medium before spreading.)
10. Incubate the plates at 37°C overnight.
11. For PCR cloning reactions, pick white or light blue colonies for DNA analysis.

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 dH2O to a final volume of 1 liter and then autoclave
Add 10 ml of filter-sterilized 1 M MgCl2 and 10 ml of filter-sterilized 1 M MgSO4 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 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)

2% X-Gal (per 10 ml)
0.2 g of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal)
10 ml of dimethylformamide (DMF)
Store at −20°C

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