Transformation Efficiency: 1 x 10^10ับชิพชี่ยง is a chromosomal copy of T7 RNAP. The cytoplasmic DsbC is also a chaperone that can assist in the folding of proteins that do not require disulfide bonds (4). Deficient in proteases Lon and OmpT. Activity of nonspecific endonuclease I (endA1) eliminated for highest quality plasmid preparations. Deficient in proteases Lon and OmpT. Resistance to phage T1 (fhuA2).

Reagents Supplied:
6 x 0.05 ml/tube of chemically competent SHuffle T7 Express Competent E. coli (Store at −80°C)

Quality Control Assays
Transformation Efficiency: 100 pg of pUC19 plasmid DNA was used to transform one tube of SHuffle® T7 Express Competent E. coli following the high efficiency protocol provided. 1 x 10^5 colonies formed/µg after an overnight incubation on LB-ampicillin plates at 37°C.

Disulfide bond formation: The Serratia marcescens extracellular nuclease NucA requires disulfide bonds for its stability. When expressed cytoplasmically at 37°C in E. coli, NucA is toxic to cells only in its oxidized disulfide-bonded state. Transformation of a plasmid that expresses a MBP-NucA fusion in the cytoplasm was used to test the ability of SHuffle strains to form cytoplasmic disulfide bonds. 100 pg pMBP-NucA was used to transform SHuffle, resulting in no transformants. Empty pMAL vector was used to calculate transformation efficiency and the wild type parent of SHuffle was used as a control.

Untransformed cells were tested for resistance to phage q80, a standard test for resistance to phage T1, and sensitivity to ampicillin, chloramphenicol, kanamycin and tetracycline. Cells are resistant to streptomycin * and spectinomycin.

*Resistance to low levels of streptomycin may be observed.

High Efficiency Transformation Protocol
Perform steps 1–7 in the tube provided.
1. Thaw a tube of SHuffle Competent E. coli cells on ice for 10 minutes.
2. Add 1–5 µl containing 1 pg–100 ng of plasmid DNA to the cell mixture. Carefully flick the tube 4–5 times to mix cells and DNA. Do not vortex.
3. Place the mixture on ice for 30 minutes. Do not mix.
5. Place on ice for 5 minutes. Do not mix.
6. Pipette 950 µl of room temperature SOC into the mixture.
7. Place at 30°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Warm selection plates to 30°C.
9. Mix the cells thoroughly by flicking the tube and inverting, then perform several 10-fold serial dilutions in SOC.
10. Spread 50–100 µl of each dilution onto a selection plate and incubate overnight at 30°C. Alternatively, incubate at 25°C for 48 hours.

5 Minute Transformation Protocol
A shortened transformation protocol resulting in approximately 10% efficiency compared to the standard protocol may be suitable for applications where a reduced total number of transformants is acceptable. Follow the High Efficiency Transformation Protocol above with the following changes:
1. Steps 3 and 5 are reduced to 2 minutes.
2. Omit outgrowth (step 7) completely for ampicillin-resistant plasmids or reduce the outgrowth time for other selective media as appropriate.

Protocol for Expression Using SHuffle
1. Transform expression plasmid into SHuffle. Plate on antibiotic selection plates and incubate 24 hours at 30°C.
2. Resuspend a single colony in 10 ml liquid medium with antibiotic.
3. Incubate at 30°C until OD₆₀₀ reaches 0.4–0.8.
4. Add the appropriate inducer, e.g. 40 µl of a 100 mM stock of IPTG. Incubate for 4 hours at 30°C or 16°C overnight.
5. Check for expression either by Coomassie stained protein gel, Western Blot or activity assay. Check expression in both the total cell extract (soluble + insoluble) and the soluble fraction alone.
6. For large scale, inoculate 1 L of liquid medium (with antibiotic) with a freshly grown colony or 10 ml of freshly grown culture. Incubate at 30°C until reaches 0.4–0.8. Add the appropriate inducer, e.g. IPTG to 0.4 mM. Induce 4 hours or 16°C overnight.

Transformation Protocol Variables
Thawing: Cells are best thawed on ice and DNA added as soon as the last bit of ice in the tube disappears. Cells can also be thawed by hand, but warming above 0°C will decrease the transformation efficiency.

Incubation of DNA with Cells on Ice: For maximum transformation efficiency, cells and DNA should be incubated together on ice for 30 minutes. Expect a 2-fold loss in transformation efficiency for every 10 minutes you shorten this step.

Heat Shock: Both the temperature and the timing of the heat shock step are important and specific to the transformation volume and vessel. Using the transformation tube provided, 30 seconds at 42°C is optimal.

Outgrowth: Outgrowth at 30°C for 1 hour is best for cell recovery and for expression of antibiotic resistance. Expect a 2-fold loss in transformation efficiency for every 15 minutes you shorten this step. SOC gives 2-fold higher transformation efficiency than LB medium; and incubation with shaking or rotating the tube gives 2-fold higher transformation efficiency than incubation without shaking.

Plating: Selection plates can be used warm or cold, wet or dry without significantly affecting the transformation efficiency. However, warm, dry plates are easier to spread and allow for the most rapid colony formation.

STORAGE AND HANDLING: Competent cells should be stored at −80°C. Storage at −20°C will result in a significant decrease in transformation efficiency. Cells lose efficiency whenever they are warmed above −80°C, even if they do not thaw.
DNA Contaminants to Avoid

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Removal Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detergents</td>
<td>Ethanol precipitate</td>
</tr>
<tr>
<td>Phenol</td>
<td>Extract with chloroform and ethanol precipitate</td>
</tr>
<tr>
<td>Ethanol or Isopropanol</td>
<td>Dry pellet before resuspending</td>
</tr>
<tr>
<td>PEG*</td>
<td>Column purity or phenol/chloroform extract and ethanol precipitate</td>
</tr>
<tr>
<td>DNA binding proteins* (e.g. Ligase)</td>
<td>Column purity or phenol/chloroform extract and ethanol precipitate</td>
</tr>
</tbody>
</table>

*Solutions/Recipes

<table>
<thead>
<tr>
<th>SOB:</th>
<th>SDC:</th>
</tr>
</thead>
<tbody>
<tr>
<td>2% Vegetable peptone (or Tryptone)</td>
<td>SOB + 20 mM Glucose</td>
</tr>
<tr>
<td>0.5% Yeast Extract</td>
<td>LB agar:</td>
</tr>
<tr>
<td>10 mM NaCl</td>
<td>1% Tryptone</td>
</tr>
<tr>
<td>2.5 mM KCl</td>
<td>0.5% Yeast Extract</td>
</tr>
<tr>
<td>10 mM MgCl2</td>
<td>0.17 M NaCl</td>
</tr>
<tr>
<td>10 mM MgSO4</td>
<td>1.5% Agar</td>
</tr>
</tbody>
</table>

Endonuclease I Deficient (endA1): The plasmidic space of wide type E. coli cells contains a nonspecific endonuclease. Extreme care must be taken to avoid degradation of plasmids prepared from these cells. The endA1 mutation deletes this endonuclease and can significantly improve the quality of plasmid preparations.

Protease Deficient (Ion ompT): E. coli B strains are “naturally” deficient in the lon protease which in K-12 strains serves to degrade misfolded proteins and to prevent some cell cycle-specific proteins from accumulating. The OmpT protease resides at the surface of wild type E. coli in both K-12 and B strains, presumably helping the cells to derive amino acids from their external environment. Cells deficient in both these proteases are much more amenable to the production of proteins from cloned genes. Mutations of other genes can help to ameliorate the sometimes-detrimental effects of these protease defects (e.g. suI below).

T1 Phage Resistant (tha2): T1, an extremely virulent phage requires the E. coli ferric hydroxamate uptake receptor for infectivity. Deletion of this gene confers resistance to this type of phage, but does not significantly affect the transformation or growth characteristics of the cell.

Usage Note: “NEB recommends using Shuffle Express strains for best performance”

References


New England Biolabs, Inc.: U.S. Patent No. 6,569,669


Academic and Non-Commercial Use Assurances

The T7 expression system is based on technology developed at Brookhaven National Laboratory. As such, the T7 promoter is under the control of the U.S. Department of Energy and is the subject of a patent application assigned to Brookhaven Science Associates, LLC (BSA). BSA will grant a non-exclusive license for use of this technology, including the encoded materials, based upon the following assurances:

1. Three materials must be used for noncommercial research purposes only. A separate license is required for any commercial use, including the use of these materials to research products or products containing the same or any component of the same. Information about the technology has been obtained from the United States Office of Technology Assessment, Brookhaven National Laboratory, Upton, NY 11973-5000; telephone: 631-344-7134; fax: 631-344-3729.

2. No materials that contain the cloned copy of T7 gene 1, the gene for T7 RNA polymerase, may be distributed further to third parties outside of your laboratory, unless the recipient is a copy of the license and agrees to be bound by its terms. This limitation applies to strains ER2566, C2833, C3010, C3013, C3016, C3022, SHuffle T7, SHuffle T7 Express, SHuffle T7 Express (LysY) and their competent derivatives, and any derivatives you may make of them, including such strains containing recombinant vectors.

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