Lot: 2

C3026H

SHuffle® T7 Competent *E. coli*

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6 x 0.05 ml/tube **Lot: 2**

Store at –80°C

Description: Chemically competent *E. coli* K12 cells engineered to form disulfide bonded proteins in the cytoplasm. Suitable for T7 promoter driven protein expression.

Features:

- Transformation efficiency: 1 x 10^6 cfu/µg pUC19 DNA
- Engineered *E. coli* K12 to promote disulfide bond formation in the cytoplasm
- Expresses constitutively a chromosomal copy of the disufide bond isomerase DsbC
- DsbC promotes the correction of mis-oxidized proteins into their correct form (1,3)
- The cytoplasmic DsbC is also a chaperone that can assist in the folding of proteins that do not require disulfide bonds (4)
- Expresses a chromosomal copy of T7 RNAP
- Tight control of expression by *lacI*^{q} allows potentially toxic genes to be cloned
- Resistance to phage T1 (*fhuA2*)

Reagents Supplied:

6 x 0.05 ml/tube of chemically competent SHuffle Competent *E. coli* cells **(Store at –80°C)**

Quality Control Assays

Transformation Efficiency: 100 pg of pUC19 plasmid DNA was used to transform one tube of SHuffle® Competent *E. coli* following the high efficiency protocol provided. 1 x 10^6 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 ϕ80, a standard test for resistance to phage T1, and sensitivity to ampicillin, chloramphenicol, kanamycin and tetracycline. Cells are resistant to streptomycin and spectinomycin.

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.
4. Heat shock at exactly 42°C for exactly 30 seconds. Do not mix.
5. Place on ice for 5 minutes. Do not mix.

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.

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 OD_{600} 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, innoculate 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.

DNA Contaminants to Avoid

Contaminant	Removal Method
Detergents	Ethanol precipitate
Phenol	Extract with chloroform and ethanol precipitate
Ethanol or Isopropanol	Dry pellet before resuspending
PEG*	Column purify or phenol/chloroform extract and ethanol precipitate
DNA binding proteins* (e.g. Ligase)	Column purify or phenol/chloroform extract and ethanol precipitate

*Ideally, DNA for transformation should be purified and resuspended in water or TE. However, up to 10 µl of DNA directly from a ligation mix can be used with only a two-fold loss of transformation efficiency. Where it is necessary to maximize the number of transformants (e.g. a library), a purification step, either a spin column or phenol/chloroform extraction and ethanol precipitation should be added.

Solutions/Recipes

SOB:		SOC:	
2%	Vegetable peptone (or Tryptone)	SOB + 20 mM Glucose	
0.5%	Yeast Extract	LB agar:	
10 mM	NaCl	1%	Tryptone
2.5 mM	KCl	0.5%	Yeast Extract
10 mM	$MgCl_2$	0.17 M	NaCl
10 mM	$MgSO_4$	1.5%	Agar

Antibiotics for plasmid selection

Antibiotic	Working Concentration
Ampicillin	100 µg/ml
Carbenicillin	100 µg/ml
Chloramphenicol	33 µg/ml
Kanamycin	30 µg/ml
Streptomycin	25 µg/ml
Tetracycline	15 µg/ml

Genotype: F´ *lac, pro, lacI^q / Δ(ara-leu)7697 araD139 fhuA2 lacZ::T7 gene1 Δ(phoA) PvuII phoR ahpC* galE (or U) galK λatt::pNEB3-r1-cDsbC* (Spec^R, *lacI^q*) *ΔtrxB rpsL150*(Str^R) *Δgor Δ(malF)3*

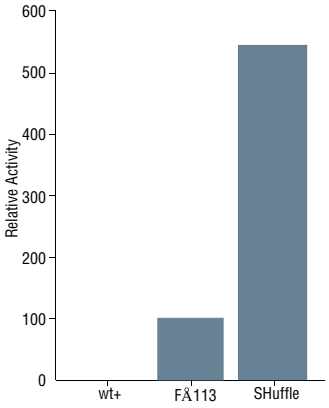

Figure 1, vtPA activity assayed from crude lysates: *Truncated tissue plasminogen activator (vtPA), which contains nine disulfide bonds when folded and oxidized correctly, was expressed from a pTrc99a plasmid in the cytoplasm of E. coli cells. After induction, cells were harvested and crude cell lysates were prepared. vtPA was assayed using a chromogenic substrate Chromozym t-PA (Roche #11093037001) and standardized to protein concentration using Bradford reagent. E. coli wt+ cells are DHB4, which is the parent of FÅ113 (Origami™).*

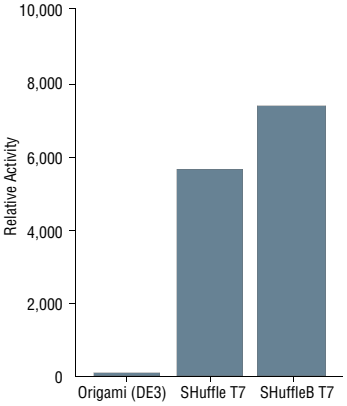

Figure 2, PfCHT1 chitinase activity assayed from crude lysates: *Plasmodium falciparum chitinase (PfCHT1) with three cysteines were expressed from a plasmid under the regulation of T7 promoter. After induction, cells were harvested and crude cell lysates were prepared. PfCHT1 was assayed using a chromogenic substrate (CalBioChem #474550) and standardized to protein concentration using Bradford reagent.*

Strain Properties

The properties of this strain that contribute to its usefulness as a protein expression strain are described below. The genotypes underlying these properties appear in parentheses.

Lac Promoter Control (*lacI^q*): The *lac* repressor blocks expression from *lac, tac* and *trc* promoters frequently carried by expression plasmids. If the level of *lac* repressor in *E. coli* cells is not sufficient to inhibit expression via these promoters during transformation or cell growth, even low levels of expression can reduce transformation efficiency and select against desired transformants. The extra molecules of *lac* repressor in *lacI^q* strains help to minimize promoter activity until IPTG is added.

M13 phage sensitive (F´): Infection by M13 and other similar phage requires *E. coli* surface features conferred by the F plasmid carried by some *E. coli* strains. Infection by these phage allows production of single-stranded DNA and the generation of phage display libraries. The F plasmid is frequently modified to carry other useful DNA in the cell (e.g. *Δ(lacZ)* M15 in this cell line) and when modified is called F´

Disulfide bond formation in the cytoplasm: Normally reductases in the *E. coli* cytoplasm keep cysteines in their reduced form, thereby reducing any disulfide bond that may form in this compartment. SHuffle has deletions of the genes for glutaredoxin reductase and thioredoxin reductase (*Δgor ΔtrxB*), which allows disulfide bonds to form in the cytoplasm. This combination of mutations is normally lethal, but the lethality is suppressed by a mutation in the peroxiredoxin enzyme (*ahpC**). In addition, SHuffle expresses a version of the periplasmic disulfide bond isomerase DsbC which lacks its signal sequence, retaining it in the cytoplasm. This enzyme has been shown to act on proteins with multiple disulfide bonds, to correct mis-oxidized bonds and promote proper folding. The gene for the cytoplasmic DsbC is present on the chromosome.

Usage Note: "NEB recommends using SHuffle Express strains for best performance"

References

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

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