COMPETENT CELLS

SHuffle® Sampler Pack

Instruction Manual

NEB #C3032I
Store at –80°C

NEW ENGLAND
BioLabs® Inc.
enabling technologies in the life sciences

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Kit Components

SHuffle® Competent *E. coli* (NEB #C3025H) ..... 0.2 ml

SHuffle® T7 Competent *E. coli* (NEB #C3026H) ..... 0.2 ml

SHuffle® T7 *lysY* Competent *E. coli* (NEB #C3027H) ..... 0.2 ml

SHuffle® Express Competent *E. coli* (NEB #C3028H) ..... 0.2 ml

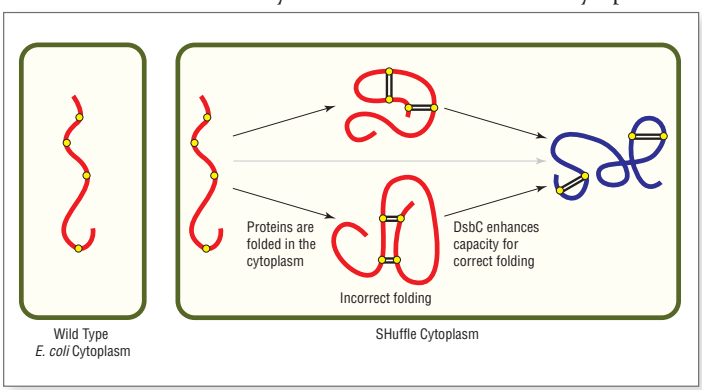
SHuffle® T7 Express Competent *E. coli* (NEB #C3029H) ..... 0.2 ml

SHuffle® T7 Express *lysY* Competent *E. coli* (NEB #C3030H) ..... 0.2 ml

Introduction

SHuffle® strains from NEB are engineered *E. coli* strains capable of expressing proteins with increasing disulfide bond complexity in the cytoplasm. SHuffle strains express the disulfide bond isomerase DsbC within the cytoplasm. DsbC isomerizes mis-oxidized substrates into their correctly folded state greatly enhancing the fidelity of disulfide bond formation. Cytoplasmic expression also results in significantly higher protein yields of disulfide bonded proteins when compared to periplasmic expression. SHuffle strains are sensitive to kan, amp, tet and in most cases, cam, which makes them able to express proteins from a wide variety of expression vectors offering greater versatility in experimental design.

SHuffle strains can correctly fold disulfide bonds in the cytoplasm



Disulfide bond formation in the cytoplasm of wild type E. coli is not favorable, while SHuffle is capable of correctly folding proteins with multiple disulfide bonds in the cytoplasm.

Advantages of NEB SHuffle Strains

- Oxidizing cytoplasmic environment enables disulfide bond formation
- DsbC (disulfide bond isomerase) directs correct disulfide bond formation
- DsbC also acts as a chaperone for protein folding
- Cytoplasmic expression increases protein yield of disulfide bonded proteins
- A wide range of antibiotics can be used for plasmid maintenance (Amp^s, Kan^s, Tet^s, Cam^s [except for *lysY* versions])
- Transformation efficiency: 1×10^6 cfu/µg pUC19 DNA (SHuffle)
  1×10^7 cfu/µg pUC19 DNA (SHuffle Express)
- Protease deficient
- TI phage resistant (*fhuA2*)
- Free of animal products

Strain Properties Selection Chart

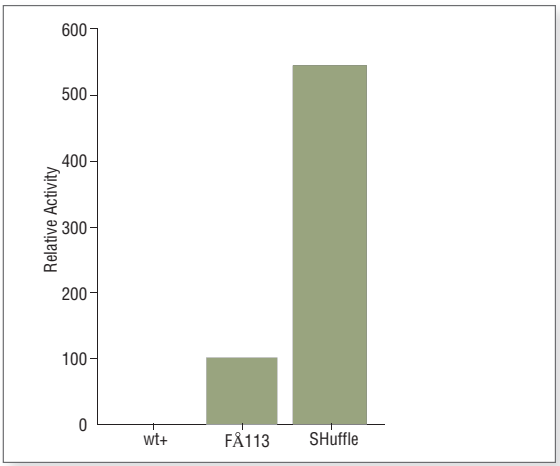
STRAIN PROPERTIES	SHuffle	SHuffle T7	SHuffle T7 *lysY*	SHuffle Express	SHuffle T7 Express	SHuffle T7 Express *lysY*
NEB #	C3025	C3026	C3027	C3028	C3029	C3030
Transformation efficiency (cfu/µg)	1 x 10^6	1 x 10^6	1 x 10^6	1 x 10^7	1 x 10^7	1 x 10^7
Strain	K12	K12	K12	B	B	B
T1 Phage Resistant	●	●	●	●	●	●
lacI^q	●	●	●	●	●	●
lysY	–	–	●	–	–	●
EndoI Deficient[1]	–	–	–	●	●	●
Protease Deficient[2]	–	–	–	●	●	●
F′	●	●	–	–	–	–
RecA$^-$	–	–	–	–	–	–
T7 RNA Polymerase	–	●	●	–	●	●
Cytoplasmic disulfide bond formation[3]	●	●	●	●	●	●
Drug Resistance[4]	str, spec	str, spec	cam, str, spec	spec	spec[5]	cam, spec[5]

(1) Important for plasmid preparation.
(2) Lacks Lon and OmpT protease activity.
(3) Constitutively expresses a chromosomal copy of the disulfide bond isomerase DsbC.
(4) nit = nitrofurantoin, tet = tetracycline, cam = chloramphenicol, str = streptomycin, spec = spectinomycin
(5) Resistance to low levels of streptomycin may be observed.

Getting Started

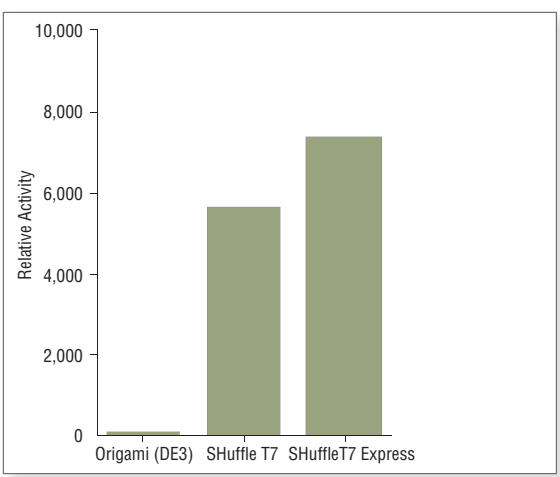
For those unfamiliar with SHuffle strains, NEB recommends starting with SHuffle Express Competent *E. coli* (NEB #C3028) at 30°C or below and induction in late log phase (OD_{600} ~ 0.8).

Figure 1: Express higher levels of biologically active protein with SHuffle.



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: SHuffle offers robustness and less toxicity than origami strains.



Plasmodium falciparum chitinase (PfCHT1) with three cysteines was 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.

Transformation Protocol

1. Thaw a tube of SHuffle Competent *E. coli* cells on ice until the last crystals disappear. Mix gently and carefully pipette 50 µl of cells into a transformation tube on ice.
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.
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 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.

Expression Protocol

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 OD_{600} 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 precipitation
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:

2%	Vegetable peptone (or Tryptone)
0.5%	Yeast Extract
10 mM	NaCl
2.5 mM	KCl
10 mM	$MgCl_2$
10 mM	$MgSO_4$

SOC:

SOB + 20 mM Glucose

LB agar:

1%	Tryptone
0.5%	Yeast Extract
0.17 M	NaCl
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

SHuffle® Competent *E. coli*

#C3025 (sample size) **0.2 ml** **Store at –80°C**

Description: Chemically competent *E. coli* K12 cells engineered to form disulfide bonded proteins in the cytoplasm.

Features:

- Transformation efficiency: 1 x 10^6 cfu/µg pUC19 DNA
- Engineered *E. coli* K12 strain 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)
- Tight control of expression by *lacI*^{q} allows potentially toxic genes to be cloned
- Resistance to phage T1 (*fhuA2*)

Genotype:
F´ *lac pro lacI*^{q} */ Δ(ara-leu)7697 araD13 fhuA2 Δ(lac)X74 Δ(phoA)PvuII phoR ahpC* galE (or U) galK Δλatt::pNEB3-r1-cDsbC* (Spec^R, *lacI*^{q}) *ΔtrxB rpsL150*(Str^R) *Δgor Δ(malF)3*

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 80, a standard test for resistance to phage T1, and sensitivity to ampicillin, kanamycin, tetracycline and chloramphenicol. Cells are resistant to streptomycin and spectinomycin.

SHuffle® T7 Competent *E. coli*

#C3026 (sample size) **0.2 ml** **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*)

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*

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 80, a standard test for resistance to phage T1, and sensitivity to ampicillin, kanamycin, tetracycline and chloramphenicol. Cells are resistant to streptomycin and spectinomycin.

SHuffle® T7 *lysY* Competent *E. coli*

#C3027 (sample size) **0.2 ml** **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 of toxic proteins.

Features:

- Transformation efficiency: 1 x 10^6 cfu/µg pUC19 DNA
- Engineered *E. coli* K12 strain 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
- Inactive mutant lysozyme expressed from miniF
- Allows for the expression of toxic proteins
- No Cam required
- Resistance to phage T1 (*fhuA2*)

Genotype: MiniF *lysY* (Cam^R) */ 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*

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 80, a standard test for resistance to phage T1, and sensitivity to ampicillin, kanamycin and tetracycline. Cells are resistant to chloramphenicol, streptomycin and spectinomycin.

SHuffle® Express Competent *E. coli*

#C3028 (sample size) **0.2 ml** **Store at –80°C**

Description: Chemically competent *E. coli* B cells engineered to form disulfide bonded proteins in the cytoplasm.

Features:

- Transformation efficiency: 1 x 10^7 cfu/µg pUC19 DNA
- Engineered *E. coli* B strain 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)
- Enhanced BL21 derivative
- Activity of nonspecific endonuclease I (*endA1*) eliminated for highest quality plasmid preparations
- Deficient in proteases Lon and OmpT
- Resistance to phage T1 (*fhuA2*)

Genotype: *fhuA2 [lon] ompT ahpC gal λatt::pNEB3-r1-cDsbC* (Spec^R, *lacI*^{q}) *ΔtrxB sulA11 R(mcr-73::miniTn10*--Tet^S*)2 [dcm] R(zgb-210::Tn10* --Tet^S*) endA1 Δgor Δ(mcrC-mrr)114::IS10*

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 10^7 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 80, a standard test for resistance to phage T1, and sensitivity to ampicillin, kanamycin, tetracycline, chloramphenicol and streptomycin. Cells are resistant to spectinomycin.

SHuffle® T7 Express Competent *E. coli*

#C3029 (sample size) **0.2 ml** **Store at –80°C**

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

Features:

- Transformation efficiency: 1 x 10^7 cfu/µg pUC19 DNA
- Engineered *E. coli* B strain 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)
- Enhanced BL21 derivative
- Expresses a chromosomal copy of T7 RNAP
- Activity of nonspecific endonuclease I (*endA1*) eliminated for highest quality plasmid preparations
- Deficient in proteases Lon and OmpT
- Resistance to phage T1 (*fhuA2*)

Genotype: *fhuA2 lacZ::T7 gene1 [lon] ompT ahpC gal λatt::pNEB3-r1-cDsbC* (Spec^R, *lacI^q*) *ΔtrxB sulA11 R(mcr-73::miniTn10*--Tet^S*)2 [dcm] R(zgb-210::Tn10* --Tet^S*) endA1 Δgor Δ(mcrC-mrr)114::IS10*

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 10^7 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 80, a standard test for resistance to phage T1, and sensitivity to ampicillin, kanamycin, chloramphenicol and tetracycline. Cells are resistant to streptomycin* and spectinomycin.

*Resistance to low levels of streptomycin may be observed.

SHuffle® T7 Express *lysY* Competent *E. coli*

#C3030 (sample size) **0.2 ml** **Store at –80°C**

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

Features:

- Transformation efficiency: 1 x 10^7 cfu/µg pUC19 DNA
- Engineered *E. coli* B strain 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
- Inactive mutant lysozyme expressed from miniF
- Allows for the expression of toxic proteins
- No Cam required
- Enhanced BL21 derivative
- Resistance to phage T1 (*fhuA2*)

Genotype: MiniF *lysY* (Cam^R) */ fhuA2 lacZ::T7 gene1 [lon] ompT ahpC gal* λ*att::pNEB3-r1-cDsbC* (Spec^R, *lacI^q*) *ΔtrxB sulA11 R(mcr-73::miniTn10--*Tet^S*)2 [dcm] R(zgb-210::Tn10 --*Tet^S*) endA1 Δgor Δ(mcrC-mrr)114::IS10*

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 10^7 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 80, a standard test for resistance to phage T1, and sensitivity to ampicillin, kanamycin and tetracycline. Cells are resistant to chloramphenicol, streptomycin* and spectinomycin.

*Resistance to low levels of streptomycin may be observed.

Frequently Asked Questions

What applications are SHuffle strains useful for?

SHuffle strains are ideal for the expression of proteins that require disulfide bonds for their folding. The DsbC isomerase present in the chromosome of SHuffle strains has also been shown to be an effective chaperone (4) and can assist in the folding of target proteins, independent of disulfide bond formation (6).

Does my protein have disulfide bonds?

All known eukaryotic and prokaryotic cell cytoplasms contain reductases which participate in reducing disulfide bonds. Thus, proteins are oxidized to form disulfide bonds only in certain extra-cytoplasmic compartments such as the periplasmic space in gram negative prokaryotes or endoplasmic reticulum in eukaryotes. Exceptions to this may occur in certain thermophilic archaea such as *Crenarchaea* and a few thermophilic bacteria (e.g. *Aquifex* and *Thermotoga*). There are web sites such as Phobius (http://phobius.binf.ku.dk) or SignalP (http://www.cbs.dtu.d/services/SignalP/) that can be used to predict whether a protein is in the oxidizing periplasm or endoplasmic reticulum. One can get an idea of the importance of cysteines in a protein of interest by analyzing how conserved the cysteine residues are in close homologs.

Which SHuffle strain should I use?

For non-T7 promoters (e.g. lac and ara promoter expression vectors) we recommend the *E. coli* B strain, SHuffle Express (NEB #C3028H). For T7 promoter expression vectors, we recommend SHuffle T7 Express (NEB #C3029H). For toxic T7 promoter expression, we recommend SHuffle T7 Express *lysY* (NEB #C3030H). If SHuffle Express strains are not optimal, the *E. coli* K12 Shuffle strains may prove more effective.

Is there anything I can do to increase protein yield when using SHuffle strains?

Try varying the induction strength, duration and temperature to find optimum expression conditions. Lowering the induction strength may improve yield.

What are the growth characteristics of the SHuffle strains?

SHuffle strains reach similar final ODs as their wild type parental strains. However, we have observed a prolonged lag phase in SHuffle strains compared to wild type cells.

How do SHuffle strains aid in cytoplasmic disulfide bond formation?

SHuffle is a mutant *E. coli* strain lacking the two reductases (trxB and gor) with an additional suppressor mutation (ahpC) which restores viability, allowing the formation of stable disulfide bonds in the cytoplasm. Under these conditions thioredoxins are in their oxidized state, converting them from reductases to oxidases. Proteins that require disulfide bonds for their folding thus can be oxidized and form stable disulfide bonds within the cytoplasm. Additionally, SHuffle strains express the disulfide bond isomerase DsbC within the cytoplasm. This feature greatly enhances the fidelity of disulfide bond formation in the cytoplasm, and proteins with multiple disulfide bonds are correctly oxidized to significantly higher yields.

How should I express my protein of interest in SHuffle?

For initial conditions we recommend using rich media at 30°C. Otherwise, overnight at 16°C is possible. At 30°C or 16°C, innoculate to 1% from an overnight culture and grow cells at 30°C for 3 hours until OD_{600} ~ 0.8. Then induce expression of protein for at least 5 hours at 30°C or overnight at 16°C. If using 37°C, innoculate to 1% from an overnight culture and grow cells for 2 hours at 37°C until OD_{600}~0.8. Then induce expression of protein for at least 6 hours at 37°C.

Do streptomycin and/or spectinomycin need to be added to SHuffle strains?

No, these antibiotic resistance genes are on the chromosome and were used in the construction of the strain.

Strain Properties

The properties of these strains that contribute to their usefulness as protein expression strains 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.

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

Protease Deficient ([lon] 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-deleterious effects of these protease defects (e.g. sulA, below).

T1 Phage Resistant (fhuA2): 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.

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