Competent Cells

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About the Kits

Description

Novagen Competent Cells enable convenient, efficient construction of plasmid recombinants. The cells are grown and made chemically competent by an optimized procedure, followed by verification of cloning efficiency and strain identity. The cells in the standard kits are provided as frozen 0.2-ml aliquots; each vial can be used for 10 transformations. The cells in the Singles™ kits are provided as single-use 50-µl aliquots for greater efficiency and convenience, and are packed in kits for either 11 or 22 transformations. Reproducible high efficiencies are available in a variety of *E. coli* strains, including NovaBlue for routine cloning, blue/white screening, and plasmid preparation, as well as T7 expression system strains (λDE3 lysogens), and isogenic control strains (non-lysogens) for superior performance in protein expression applications. The designation (DE3) indicates that the host is a lysogen of λDE3, and therefore carries a chromosomal copy of the T7 RNA polymerase gene under control of the *lacUV5* promoter (1–3). Such strains are suitable for production of protein from target genes cloned in pET vectors or other T7-driven expression vectors. pLysS is a designation given to hosts carrying a chloramphenicol-resistant plasmid with a P15A replicon that encodes T7 lysozyme, which is a natural inhibitor of T7 RNA polymerase (4–5). This strain is used to suppress basal expression of T7 RNA polymerase prior to induction and thus stabilize pET recombinants encoding target proteins that affect cell growth and viability. The pLacI designation is given to hosts carrying a compatible plasmid that encodes *lac* repressor under control of its wild type promoter. Expression hosts carrying this plasmid were specifically designed for compatibility with the pETBlue™ and pTriEx™ (1.1–4) plasmid series. The λDE3 Lysogenization Kit is also available for making new expression hosts with other genetic backgrounds.

Components

Standard 0.4 ml and 1 ml Kits

- 2 or 5 × 0.2 ml Competent Cells
- 2 or 4 × 2 ml SOC Medium
- 10 µl Test Plasmid (ampicillin resistant)

Singles Kits

- 11 or 22 × 50 µl Competent Cells
- 2 or 4 × 2 ml SOC Medium
- 10 µl Test Plasmid (ampicillin resistant)

Storage

Store all components at –70°C or below.

Strain information

Strains offered as competent cells are listed in the table below. Genotypes are found on page 14.

Strains having the designation (DE3) are lysogenic for a λ prophage that contains an IPTG-inducible T7 RNA polymerase. λDE3 lysogens are designed for protein expression from pET, pETcoco™, pETBlue™, pTriEx™, pCDF, pRSF, and Duet vectors. Strains having the pLysS designation carry a pACYC184-derived plasmid that encodes T7 lysozyme, which is a natural inhibitor of T7 RNA polymerase that serves to repress basal expression of target genes under the control of the T7 promoter. Strains having the designation pLacI carry a pACYC-derived plasmid that encodes the *lac* repressor, which serves to suppress basal expression of target genes under T7 promoter control in pETBlue and pTriEx (1.1–4) plasmids. All Rosetta™ and Rosetta 2 strains contain pRARE or pRARE2, respectively, a pACYC184-derived plasmid.

Description Strains	Resistance[1]	Derivation	Key Feature(s)
B834 is the parental strain for BL21 (6). These hosts are methionine auxotrophs and allow high specific activity labeling of target proteins with ^{35}S-methionine and selenomethionine for crystallography (7). This strain is also deficient in the *lon* (8) and *ompT* proteases.			
B834(DE3)	none	B strain	Met auxotroph; ^{35}S-met labeling
B834(DE3)pLysS	Cam		
BL21 is the most widely used host background for protein expression and has the advantage of being deficient in the *lon* (8) and *ompT* proteases.			
BL21	none	B834	*lon* and *ompT* protease deficient
BL21(DE3)	none		
BL21(DE3)pLysS	Cam		
BLR is a *recA* derivative of BL21 (9) that improves plasmid monomer yields and may help stabilize target plasmids containing repetitive sequences or whose products may cause the loss of the DE3 prophage (10). These strains are also deficient in the *lon* and *ompT* proteases.			
BLR	Tet	BL21	BL21 *recA* mutant; stabilizes tandem repeats
BLR(DE3)	Tet		
BLR(DE3)pLysS	Tet + Cam		
HMS174 strains provide the *recA* mutation in a K-12 background. Like BLR, these strains may stabilize certain target genes whose products may cause the loss of the DE3 prophage.			
HMS174	Rif	K-12	*recA* mutant, Rif resistance
HMS174(DE3)	Rif		
HMS174(DE3)pLysS	Rif + Cam		
NovaBlue is a K-12 strain ideally suited as an initial cloning host due to its high transformation efficiency, blue/white screening capability (with appropriate plasmids) and *recA endA* mutations, which result in high yields of excellent quality plasmid DNA. The **NovaBlue T1^{R}** strain has the added benefit of resistance to T1 and T5 phage. The DE3 lysogen of NovaBlue is potentially useful as a stringent host due to the presence of the *lacI^{q}* repressor encoded by the F episome. Note, however that the DE3 lysogen cannot be used for blue/white screening of recombinant plasmids.			
NovaBlue	Tet	K-12	*recA*$^{-}$, *endA*$^{-}$, *lacI^{q}*; recommended for cloning, plasmid preps (non-DE3 only)
NovaBlue(DE3)	Tet		
NovaBlue T1^{R}	Tet	K-12	*recA*$^{-}$, *endA*$^{-}$, *lacI^{q}*; *tonA* recommended for cloning, plasmid preps; resistant to T1 and T5 phage

(continued on next page; see footnotes on page 6)

Description Strains	Resistance[1]	Derivation	Key Feature(s)
NovaF– is a K-12 strain ideally suited as a cloning host due to its high transformation efficiency and *recA endA* mutations, which result in high yields of excellent quality plasmid DNA. In contrast to NovaBlue, NovaF– lacks the F episome that encodes *lacZ⊿M15* and *lacI*^{q} mutations. Therefore this strain is not appropriate for blue/white screening by α-complementation or for propagation of expression vectors that contain an *E. coli* promoter controlled by a *lac* operator sequence, i.e., *tac*, *trc*, T5, etc. in the absence of an additional source of *lac* repressor. NovaF– is recommended for the preparation of pETcoco™ recombinants (review User Protocol TB333).			
NovaF–	none	K-12	*recA*$^-$, *endA*$^-$; recommended for cloning, plasmid preps
Origami™ host strains are K-12 derivatives that have mutations in both the thioredoxin reductase (*trxB*) and glutathione reductase (*gor*) genes, which greatly enhance disulfide bond formation in the cytoplasm (11). Studies have shown that expression in Origami (DE3) yielded 10-fold more active protein than in another host even though overall expression levels were similar (12). Origami hosts are compatible with ampicillin-resistant plasmids and are ideal for use with pET-32 vectors, since the thioredoxin fusion tag further enhances the formation of disulfide bonds in the cytoplasm. The *trxB* and *gor* mutations are selectable on kanamycin and tetracycline, respectively; therefore these strains cannot be used with plasmids carrying kanamycin- or tetracycline-resistance genes. To reduce the possibility of disulfide bond formation between molecules, hosts containing the *trxB/gor* mutations are only recommended for the expression of proteins that require disulfide bond formation for proper folding.			
Origami Origami(DE3) Origami(DE3)pLysS Origami(DE3)pLacI[2]	Kan + Tet + Str[3] Kan + Tet + Str[3] Kan + Tet + Str[3] + Cam Kan + Tet + Str[3] + Cam	K-12	*trxB/gor* mutant, greatly facilitates cytoplasmic disulfide bond formation, Leu auxotroph
Origami 2 host strains are K-12 derivatives that have mutations in both the thioredoxin reductase (*trxB*) and glutathione reductase (*gor*) genes, which greatly enhances disulfide bond formation in the cytoplasm (11). Unlike the original Origami strains, the Origami 2 strains are kanamycin sensitive, making these host strains compatible with many Novagen expression vectors. The *gor* mutation is still selected for by tetracycline, as are the original strains.			
Origami 2 Origami 2(DE3) Origami 2(DE3)pLysS Origami 2(DE3)pLacI[2]	Tet + Str[3] Tet + Str[3] Tet + Str[3] + Cam Tet + Str[3] + Cam	K-12	Kan sensitive, *trxB/gor* mutant, greatly facilitates cytoplasmic disulfide bond formation, Leu auxotroph,
Origami B host strains carry the same *trxB/gor* mutations as the original Origami strain, except that they are derived from a *lacZY* mutant of BL21. Thus the Origami B strains combine the desirable characteristics of BL21, Tuner™, and Origami hosts in one strain background. The *trxB* and *gor* mutations are selectable on kanamycin and tetracycline, respectively; therefore, these strains are not compatible with kanamycin- or tetracycline-resistant plasmids.			
Origami B Origami B(DE3) Origami B(DE3)pLysS Origami B(DE3)pLacI[2]	Kan + Tet Kan + Tet Kan + Tet + Cam Kan + Tet + Cam	Tuner™ (B strain)	*trxB/gor* mutant, greatly facilitates cytoplasmic disulfide bond formation BL21 *lacZY* deletion mutant; allows precise control with IPTG

(continued on next page; see footnotes on page 6)

Strains	Resistance[1]	Derivation	Description Key Feature(s)
Rosetta™ and Rosetta 2 host strains are BL21 derivatives designed to enhance the expression of eukaryotic proteins that contain codons rarely used in *E. coli*. (13–17). The original Rosetta strains supply tRNAs for the codons AUA, AGG, AGA, CUA, CCC, and GGA on a compatible chloramphenicol-resistant plasmid, pRARE (18). The Rosetta 2 strains supply a seventh rare codon (CGG) in addition to the six found in the original Rosetta strains (19). By supplying rare codons, the Rosetta strains provide for "universal" translation, where translation would otherwise be limited by the codon usage of *E. coli*. (15, 16, 20, 21). The tRNA genes are driven by their native promoters (18). In the pLysS and pLacI derivatives of these strains, the rare tRNA genes are present on the same plasmids that carry the T7 lysozyme and *lac* repressor genes, respectively.			
Rosetta Rosetta(DE3) Rosetta(DE3)pLysS Rosetta(DE3)pLacI[2]	Cam Cam Cam Cam	BL21	Expresses six rare tRNAs; facilitates expression of genes that encode rare *E. coli* codons
Rosetta 2 Rosetta 2(DE3) Rosetta 2(DE3)pLysS Rosetta 2(DE3)pLacI[2]	Cam Cam Cam Cam	BL21	Expresses seven rare tRNAs; facilitates expression of genes that encode rare *E. coli* codons
Rosetta-gami™ host strains are Origami™ derivatives that combine the enhanced disulfide bond formation resulting from *trxB/gor* mutations with enhanced expression of eukaryotic protein that contain codons rarely used in *E. coli*. These strains supply tRNAs for AGG, AGA, AUA, CUA, CCC, and GGA on a compatible chloramphenicol-resistant plasmid. In the Rosetta-gami(DE3)pLysS and Rosetta-gami(DE3)pLacI, the rare tRNA genes are present on the same plasmids that carry the T7 lysozyme and *lac* repressor genes, respectively. The Rosetta-gami strains are resistant to kanamycin, tetracycline, streptomycin, and chloramphenicol. These strains are recommended for use with expression plasmids carrying the ampicillin resistance marker *bla*. If using pCDF vectors, spectinomycin must be used for antibiotic selection because *rpsL* mutation confers streptomycin resistance.			
Rosetta-gami Rosetta-gami(DE3) Rosetta-gami(DE3)pLysS Rosetta-gami(DE3)pLacI[2]	Kan + Tet + Str[3] + Cam Kan + Tet + Str[3] + Cam Kan + Tet + Str[3] + Cam Kan + Tet + Str[3] + Cam	Origami (K-12)	Expresses rare tRNAs; facilitates expression of genes that encode rare *E. coli* codons *trxB/gor* mutant, greatly facilitates cytoplasmic disulfide bond formation, Leu auxotroph
Rosetta-gami 2 host strains combine features of Origami 2 and Rosetta 2, allowing for enhanced disulfide bond formation and enhanced expression of eukaryotic proteins that contain codons rarely used in *E. coli*. These strains are derived from Origami 2, a kanamycin-sensitive K-12 strain carrying the *trxB* and *gor* mutations for disulfide bonds formation in the cytoplasm. The cells carry the chloramphenicol-resistant plasmid, pRARE2, which supplies tRNAs for seven rare codons, AUA, AGG, AGA, CUA, CCC, GGA, and CGG under the control of their native promoter. The *gor* mutation is selectable on tetracycline.			
Rosetta-gami 2 Rosetta-gami 2(DE3) Rosetta-gami 2(DE3)pLysS Rosetta-gami 2(DE3)pLacI[2]	Tet + Str[3] + Cam Tet + Str[3] + Cam Tet + Str[3] + Cam Tet + Str[3] + Cam	Origami 2 (K-12)	Expresses seven rare tRNAs; facilitates expression of genes that encode rare *E. coli* codons Kan sensitive, *trxB/gor* mutant, greatly facilitates cytoplasmic disulfide bond formation, Leu auxotroph

(continued on next page; see footnotes on page 6)

Description Strains	Resistance[1]	Derivation	Key Feature(s)
Rosetta-gami™ B strains combine the key features of BL21 (and its Tuner™ derivative), Origami™, and Rosetta™ to enhance both the expression of eukaryotic proteins and the formation of target protein disulfide bonds in the bacterial cytoplasm. These strains are compatible with ampicillin- or spectinomycin-resistant vectors.			
Rosetta-gami B Rosetta-gami B(DE3) Rosetta-gami B(DE3)pLysS Rosetta-gami B(DE3)pLacI[2]	Kan + Tet + Cam Kan + Tet + Cam Kan + Tet + Cam Kan + Tet + Cam	Origami B (B strain)	Expresses six rare tRNAs; facilitates expression of genes that encode rare *E. coli* codons *trxB/gor* mutant, greatly facilitates cytoplasmic disulfide bond formation BL21 *lacZY* deletion mutant; allows precise control with IPTG
RosettaBlue™ host strains are NovaBlue derivatives that combine high transformation efficiency and *recA endA lacI^q* mutations with enhanced expression of eukaryotic proteins that contain codons rarely used in *E. coli*. These strains supply tRNAS for AGG, AGA, AUA, CUA, CCC, and GGA on a compatible chloramphenicol-resistant plasmid. In RosettaBlue(DE3)pLysS and RosettaBlue(DE3)pLacI, the rare tRNA genes are present on the same plasmids that carry the T7 lysozyme and *lac* repressor genes, respectively. Blue/white screening is not possible with RosettaBlue(DE3) strains due to the presence of the *lacZ* α-peptide coding sequence in the DE3 lysogenic phage.			
RosettaBlue™ RosettaBlue(DE3) RosettaBlue(DE3)pLysS RosettaBlue(DE3)pLacI[2]	Tet + Cam Tet + Cam Tet + Cam Tet + Cam	NovaBlue (K-12)	Expresses rare tRNAs; facilitates expression of genes that encode rare *E. coli* codons *recA*$^-$, *endA*$^-$, *lacI^q*; high transformation efficiency
Tuner™ strains are *lacZY* deletion mutants of BL21, which enable adjustable levels of protein expression throughout all cells in a culture. The *lac* permease (*lacY*) mutation allows uniform entry of IPTG into all cells in the population. Unlike lactose (or arabinose), IPTG is a gratuitous inducer that can enter *E. coli* cells independently from permease pathways. This allows induction with IPTG to occur in a true concentration-dependent fashion that is exceptionally uniform throughout the culture. By adjusting the concentration of IPTG, expression can be regulated from very low expression levels up to the robust, fully induced expression levels commonly associated with pET vectors. Lower level expression may enhance the solubility and activity of difficult target proteins. These strains are also deficient in the *lon* and *ompT* proteases.			
Tuner™ Tuner(DE3) Tuner(DE3)pLysS Tuner(DE3)pLacI[2]	none none Cam Cam	BL21	BL21 *lacZY* deletion mutant; allows precise control with IPTG

1. The Resistance column in the table refers to selectable resistant marker(s) possessed by the strain in the absence of target plasmids. Appropriate concentrations for selection are as follows:
   Kan: 15 µg/ml kanamycin
   Cam: 34 µg/ml chloramphenicol
   Tet : 12.5 µg/ml tetracycline
   Rif: 200 µg/ml rifampicin
   Str: 50 µg/ml streptomycin
2. Strains with the pLacI plasmid are appropriate hosts for pTriEx™ (1.1–4) and pETBlue™ vectors only.
3. These strains carry a mutation in ribosomal protein (*rpsL*) conferring resistance to streptomycin; therefore streptomycin is not necessary to maintain strain genotype. If using pCDF vectors, spectinomycin must be used for antibiotic selection because *rpsL* mutation confers streptomycin resistance.

Antibiotics/IPTG available separately	Size	Cat. No.
Carbenicillin	5 g	69101-3
Chloramphenicol	25 g	220551
Kanamycin Sulfate	5 g	420311
Tetracycline Hydrochloride	10 g	58346
Streptomycin Sulfate, *Streptomyces* sp.	100 g	5711
Spectinomycin, Dihydrochloride, Pentahydrate, *Streptomyces* sp.	10 g	567570
100 mM IPTG Solution	10 × 1.5 ml	70527-3
X-Gal Solution, 40 mg/ml in DMSO	3 × 1.0 ml	71077-3

Transformation Protocol for Experienced Users

Note: *See the next section for a detailed protocol.*

1. Thaw the required number of tubes of cells on ice and mix gently to ensure that the cells are evenly suspended.
2. <u>*Standard Kits:*</u>
   Place the required number of 1.5-ml polypropylene microcentrifuge tubes on ice to pre-chill. Pipet 20 µl aliquots of cells into the pre-chilled tubes.

   <u>*Singles™ Kits:*</u>
   Proceed to Step 3.
3. Add 1 µl of the DNA solution directly to the cells. Stir gently to mix.
4. Place the tubes on ice for 5 min.
5. Heat the tubes for exactly 30 s in a 42°C water bath; do not shake.
6. Place on ice for 2 min.
7. <u>*Standard Kits:*</u>
   Add **80 µl** of room temperature SOC Medium to each tube.

   <u>*Singles Kits:*</u>
   Add **250 µl** of room temperature SOC Medium to each tube.
8. Selection for transformants is accomplished by plating on media containing antibiotic for the plasmid-encoded drug resistance. Additional host-specific antibiotics may also be appropriate to insure maintenance of the host-encoded feature(s) (see chart on page 3).

   When using NovaBlue strain: if selecting for ampicillin or chloramphenicol resistance, plate 5–50 µl cells directly on selective medium (plus IPTG/X-gal for plasmids that permit blue/white screening). If selecting for kanamycin or streptomycin/spectinomycin resistance, shake at 37°C (250 rpm) for 30 min prior to plating on selective medium.
   When using strains other than NovaBlue: incubate at 37°C while shaking at 250 rpm for 60 min prior to plating on selective medium.

Transformation - Detailed Protocol

Note: When selecting for the expression of β-lactamase, the antibiotic carbenicillin is recommended instead of ampicillin. Carbenicillin is less sensitive to the drop in the pH of the growth medium that typically accompanies bacterial growth.

Competent cells in the standard kits are provided in 0.2-ml aliquots. The standard transformation reaction uses 20 µl cells, so each tube contains enough cells for 10 transformations. Singles™ competent cells are provided in 50-µl aliquots, which are used "as is" for single 50-µl transformations. Please note that there are a few steps in the protocol that vary for the Singles vs. standard kits.

DNA in ligation reactions containing high-quality reagents is suitable for direct addition to Novagen competent cells. Inactivation of the ligase is not required prior to transformation. For transformation, 1 µl of the ligation reaction usually yields sufficient numbers of colonies for screening. Up to 5 µl of the ligation reaction containing high-quality reagents can be added to Singles Competent Cells without reducing transformation efficiency.

Plasmid DNA isolated using standard miniprep procedures is also usually satisfactory; however, for maximum efficiency, the sample DNA should be free of phenol, ethanol, salts, protein, and detergents, and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) or in water. Transformation efficiencies will generally be 10- to 100-fold higher with supercoiled plasmids than with ligation reactions, so it is often necessary to dilute standard plasmid preparations in TE buffer or water prior to transformation. One microliter containing 1 to 10 ng plasmid DNA is usually sufficient to produce hundreds of colonies. For cotransformations into expression strains using two supercoiled plasmids, add 1 µl containing 10–40 ng of each plasmid into expression strain competent cells. Note that a higher concentration of DNA will yield a higher number of transformants on the plate, but the transformation efficiency of the cells will decrease.

Handling tips

1. Upon receipt from Novagen, verify that the competent cells are still frozen and that dry ice is still present in the shipping container. Immediately place the competent cells at –70°C or below. For optimal results, do not allow the cells to thaw at any time prior to use.
2. Handle only the rim of the tube and the tube cap to prevent the cells from warming. Keep the cells on ice whenever possible.
3. To mix cells, finger-flick the tube 1–3 times. *NEVER vortex the competent cells*.
4. Cells can be refrozen at –70°C and used at a later date; however, transformation efficiencies may decline several-fold with each freeze-thaw cycle. To avoid multiple freeze-thaw cycles of the standard cells, dispense the cells into aliquots after the initial thaw and store the aliquots at –70°C or below (note that Singles cells are provided as 50-µl aliquots, which are used "as is" and should not be divided). To dispense aliquots of cells from the 0.2 ml stock, remove the stock tube quickly from the ice and finger-flick 1–2 times to mix prior to opening the tube. Remove a 20-µl aliquot from the middle of the cells, and replace the tube immediately on ice. Place the aliquot immediately into the bottom of a pre-chilled 1.5-ml tube, mix by pipetting once up and down, and then immediately close the tube and replace on ice. After all of the aliquots have been removed, return any unused tubes to the freezer before proceeding with the transformation.

Procedure

1. Remove the appropriate number of competent cell tubes from the freezer (include one extra sample for the Test Plasmid positive control, if desired). Immediately place the tubes on ice, so that all but the cap is immersed in ice. Allow the cells to thaw on ice for 2–5 min.
2. Visually examine the cells to see that they have thawed and gently finger-flick the tube 1–2 times to evenly resuspend the cells. The cells are then ready for removal of an aliquot (Standard Kits), or for the addition of the DNA (Singles Kits).
3. | *Standard Kits:* | *Singles Kits:* |
   |---|---|
   | Place the required number of 1.5-ml snap-cap polypropylene tubes on ice to pre-chill. Pipet 20 µl aliquots of cells into the pre-chilled tubes. | Proceed to Step 4 or 5, depending on whether a Test Plasmid sample is included as a positive control. |
4. (Optional) To determine transformation efficiency, add 1 µl (0.2 ng) Test Plasmid to one of the tubes containing cells. Stir gently to mix and return the tube to the ice.
5. Add 1 µl of a ligation reaction or purified plasmid DNA directly to the cells. Stir gently to mix and return the tube to the ice, making sure that the tube is immersed in ice except for the cap. Repeat for additional samples.

Note: Transformation efficiencies can be increased several fold by diluting the ligation reaction 5-fold with TE or water prior to adding the DNA to the cells, or by extracting the ligation reaction twice with 1:1 TE-buffered phenol:CIAA (24:1 chloroform:isoamyl alcohol), once with CIAA, precipitating in the presence of sodium acetate, and resuspending in TE or water before adding the DNA to the cells.

6. Incubate the tubes on ice for 5 min.
7. Heat the tubes for exactly 30 s in a 42°C water bath; do not shake.

Note: This "heat shock" step is most easily accomplished if the tubes are in a rack that leaves the lower halves of the tubes exposed. Hold the rack in the water bath so that the lower halves of the tubes are submerged for 30 s, and then replace the rack on ice.

8. Place the tubes on ice for 2 min.
9. | *Standard Kits:* | *Singles Kits:* |
   |---|---|
   | Add **80 µl** of room temperature SOC medium to each tube. Keep the tubes on ice until all have received SOC. | Add **250 µl** of room temperature SOC medium to each tube. Keep the tubes on ice until all have received SOC. |

Selection for transformants is accomplished by plating on medium containing antibiotic(s) for the plasmid-encoded drug resistance(s). Additional host-specific antibiotics also may be appropriate to insure maintenance of the host encoded feature(s) (see chart on page 3).

When using NovaBlue: if selecting for ampicillin or chloramphenicol resistance, plate 5–50 µl cells directly on selective media (plus IPTG/X-gal for plasmids which permit blue/white screening). If selecting for kanamycin or streptomycin/spectinomycin resistance, shake at 37°C (250 rpm) for 30 min prior to plating on selective media.
When using strains other than NovaBlue: incubate at 37°C while shaking at 250 rpm for 60 min prior to plating on selective media.

Notes: The outgrowth incubation is conveniently performed in a shaking incubator using a test tube rack anchored to the shaking platform. Place each transformation tube in an empty 13 mm × 100 mm glass test tube in the rack. The snap-caps on the transformation tubes prevent them from falling to the bottom of the test tubes, and all transformation tubes remain vertical.

During the outgrowth (or earlier if omitting outgrowth), place the plates at 37°C. If the plates contain a lot of moisture, place them cover-side up and open the cover ~1/3 of the way to allow

the plates to dry for 30–45 min. If the plates do not need drying, keep them closed and place them cover-side down in the 37°C incubator for ~20 min prior to plating.

10. Refer to "Plating Technique" in the subsequent section for specific instructions. Spread 5–50 µl of each transformation on LB agar plates containing the appropriate antibiotic(s) for the plasmid and host strain (see page 3). When plating less than 25 µl, first pipet a "pool" of SOC onto the plate and then pipet the cells into the SOC. Please see the next section for additional details on plating technique.

Important: *The appropriate amount of transformation mixture to plate varies with the efficiency of both the ligation and the competent cells. For recombinants in NovaBlue, expect 10^5–10^7 transformants/µg plasmid, depending on the particular insert and the ligation efficiency. Transformations with the pETcoco™ plasmid require a plating volume of 50 µl to obtain sufficient colonies because the pETcoco plasmid is large (12,272 bp).*

***When using the Test Plasmid**, plate no more than 5 µl of the final NovaBlue transformation mix or plate 10 µl of any strain with a 2×10^6 efficiency in a pool of SOC on an LB agar plate containing 50 µg/ml carbenicillin or ampicillin (because the Test Plasmid carries the ampicillin resistance gene,* bla*).*

***For blue/white screening of recombinants**, also include IPTG and X-gal in the LB agar. These can be pre-spread on the plates and allowed to soak in for about 30 min prior to plating. Use 35 µl of 50 mg/ml X-gal in dimethyl formamide and 20 µl 100 mM IPTG (in water) per 82 mm plate. Alternatively, X-gal and IPTG can be added to the LB agar at a final concentration of 70 µg/ml and 80 µM, respectively, just prior to pouring the plates.*

11. Set the plates on the bench for several min to allow excess liquid to be absorbed, and then invert and incubate overnight at 37°C.

Plating techniques

1. Remove the plates from the incubator. If plating less than 25 µl of the transformation, we recommend plating onto a pool of SOC, which facilitates even colony distribution on the plate surface. Using a sterile pipet tip, place 40–60 µl of SOC in the center of a plate for a plating cushion.
2. To remove the transformation sample, finger-flick the transformation tube 5–8 times, open the cap and immediately remove the sample volume from the middle of the transformation reaction.
3. Transfer the sample to the plate by dispensing the sample volume into the SOC cushion. After the sample is expelled, use the same tip to pipet up the same volume of SOC from the cushion edge and dispense the fluid back into the cushion. (This effectively rinses out your pipet tip.)

Plating with ColiRollers™ Plating Beads

ColiRollers Plating Beads (Cat. No. 71013-3) are sterile glass beads that eliminate the use of the spreader and alcohol flame while evenly distributing cells without damaging the cells.

To use ColiRollers, simply dispense 10–20 beads per plate. Cover the plate with its lid and rock the plate back and forth several times. The rolling action of the beads distributes the cells. Several plates can be stacked and shaken at the same time. After all plates have been spread, discard the ColiRollers and incubate (step 4 below).

Plating with a standard spreader

1. Completely immerse the plating spreader (bent glass rod or equivalent) into ethanol and flame to sterilize. After the flame is extinguished, allow the spreader to cool ~10 s prior to placing the spreader on the plate. To further cool the spreader before spreading the cells, place the spreader on the LB agar at the outside of the plate (not touching the pool of cells).
2. *Slowly* rotate the plate while supporting the weight of the spreader.

Important: *Do not press down on the spreader – use just enough contact to spread the cells.*

3. Spread until the sample is evenly distributed on the plate. If the plates are fairly dry, the sample and cushion will quickly absorb into the plate. If the plates are wet, spread until the

sample is evenly distributed. Do not continue to spread until the sample and cushion have absorbed completely into the plate, as overspreading is lethal to the cells. Instead, after spreading briefly, set the plates upright at room temperature for ~15 min prior to placing them inverted in the 37ºC incubator. This will allow excess moisture to absorb into the plates.

4. Incubate all plates, cover-side down, in a 37ºC incubator for 15–18 h. To obtain larger colonies, extend the incubation time slightly (1–2 h), but beware of the potential for development of satellite colonies with extended incubations (usually > 36 h at 37°C). Satellites are not commonly observed when using carbenicillin or kanamycin. Strains having thioredoxin reductase (*trxB*) and glutathione reductase (*gor*) mutations (i.e., Origami and Rosetta-gami strains) may take 24 h or longer for efficient colony formation. If performing blue/white screening, place the plates at 4ºC for a few hours after the colonies have reached the desired size to enhance color development.

Troubleshooting

Problems rarely occur if the above protocols are carefully followed. The Test Plasmid is included with all Novagen competent cells to use as a positive control.

Problem	Possible solution
Experimental DNA produces no colonies or very low number of colonies, but Test Plasmid (included with the kit) yields expected efficiency	a. Experimental DNA contains an inhibitor of ligation. Make sure input DNA is free of contaminants (e.g., excess salts, EDTA, proteins, etc.) that inhibit ligation. Gel purify and/or extract the vector and insert prior to ligation. b. Experimental DNA contains an inhibitor of transformation. Mix Test Plasmid with the ligation and transform on carbenicillin or ampicillin plates. If the expected number of colonies is produced, this is not the problem. If colony number is low, dilute the ligation 5-fold in TE buffer or extract, precipitate, and resuspend the ligation in TE buffer prior to transformation. c. Vector and/or insert have damaged or otherwise incompatible ends. Recheck cloning strategy, including vector:insert ratio, and use fresh, reliable reagents for DNA preparation. If cloning PCR products, it is likely to be faster to clone them first using a Perfectly Blunt® or **A**ccep**T**or™ Vector Kit. Then, if needed, transfer into another vector using restriction enzymes to excise the fragment. d. Insert is not tolerated in *E. coli*. If possible, check the target sequence for strong *E. coli* promoters or other potentially toxic elements, as well as inverted repeats. Occasionally, certain repeated elements (usually found only in genomic DNA) are not well-maintained in NovaBlue or other multi-purpose *E. coli* strains. These sequences can sometimes be cloned in *recJ* $^{-}$ strains. Inserts may have a methylation pattern incompatible with the host strain. e. Verify that IPTG was NOT added to the plate when attempting to transform a DE3 lysogen-based host strain. IPTG will induce the expression of T7 RNA polymerase in DE3 hosts and any target gene on a T7 promoter-based plasmid. This typically results in decreased fitness of the cell and will likely be selected against over time. IPTG induction of DE3 hosts should be performed ONLY after a stable transformant has been isolated. f. Avoid exceeding the recommended volume of input DNA. DNA volumes greater than 1 µl of a ligation reaction per 20 µl of competent cells may lead to reduced transformation efficiencies. g. Transformations of pETcoco™ constructs typically require plating 50 µl of the transformation mixture to obtain sufficient colonies, because the vectors are large (>12 kbp) and they transform with a lower efficiency.
No colonies or low colony numbers with the Test Plasmid	a. If no colonies are observed, the incorrect selective drug or the wrong concentration of the correct selective drug may have been used in the plates. Use ampicillin or carbenicillin at 50 µg/ml with the Test Plasmid. b. Incorrect or toxic media components, or plates too old/dry. Recheck media formulations. c. Incorrect incubator temperature. Make sure incubator is set to 37°C. d. Cells were handled incorrectly. Handle the cells very gently at all times. Never vortex or mix vigorously. To resuspend cells, finger-flick or gently pipet up and down without generating bubbles. Make sure the cells are stored at –70°C or below. Thaw on ice and keep on ice except where indicated in the procedure. Gently resuspend the cells before plating if they settle out during outgrowth. Use a very light touch with the spreader when plating or use ColiRollers™ Plating Beads.
Small satellite colonies present	a. Plates were incubated at 37°C too long. β-lactamase is secreted by amp-resistant bacteria and thus can eventually clear a zone of surrounding media from the drug, allowing non-recombinants to grow. In general, colonies are sufficiently large for analysis after 18 h for NovaBlue, 24 h for Origami™ and Origami-derivatives, or 15 h for all other strains. b. Antibiotic stock is degraded, plates are old, or drug was added when the media was too hot. Use freshly prepared antibiotics and correct plate preparation. For the *bla* gene, use the ampicillin analog carbenicillin, which appears to be less susceptible to degradation.

Ordering Information

T7 Expression Host Strains: λDE3 Lysogens	Size	Cat. No.
B834(DE3) Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	69041-3 69041-4
B834(DE3)pLysS Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	69042-3 69042-4
BL21(DE3) Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	69450-3 69450-4
BL21(DE3)pLysS Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	69451-3 69451-4
BLR(DE3) Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	69053-3 69053-4
BLR(DE3)pLysS Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	69956-3 69956-4
HMS174(DE3) Competent Cells guaranteed transformation efficiency 5×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	69453-3 69453-4
HMS174(DE3)pLysS Competent Cells guaranteed transformation efficiency 5×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	69454-3 69454-4
NovaBlue(DE3) Competent Cells guaranteed transformation efficiency 1×10^8 cfu/µg Test Plasmid	0.4 ml 1 ml	69284-3 69284-4
Origami™(DE3) Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	70627-3 70627-4
Origami(DE3)pLysS Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	70628-3 70628-4
Origami 2(DE3) Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	71345-3 71345-4
Origami 2(DE3)pLysS Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	71346-3 71346-4
Origami B(DE3) Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	70837-3 70837-4
Origami B(DE3)pLysS Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	70839-3 70839-4
Rosetta™(DE3) Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	70954-3 70954-4
Rosetta(DE3)pLysS Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	70956-3 70956-4
Rosetta 2(DE3) Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	71397-3 71397-4
Rosetta 2(DE3)pLysS Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	71403-3 71403-4
Rosetta-gami™(DE3) Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	71055-3 71055-4
Rosetta-gami(DE3)pLysS Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	71057-3 71057-4
Rosetta-gami 2(DE3) Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	71351-3 71351-4
Rosetta-gami 2(DE3)pLysS Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	71352-3 71352-4

T7 Expression Host Strains: λDE3 Lysogens (continued)	Size	Cat. No.
Rosetta-gami™ B(DE3) Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	71136-3 71136-4
Rosetta-gami B(DE3)pLysS Competent Cells guaranteed transformation efficiency 1.0×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	71137-3 71137-4
RosettaBlue™(DE3) Competent Cells guaranteed transformation efficiency 1×10^8 cfu/µg Test Plasmid	0.4 ml 1 ml	71059-3 71059-4
RosettaBlue(DE3)pLysS Competent Cells guaranteed transformation efficiency 1×10^8 cfu/µg Test Plasmid	0.4 ml 1 ml	71034-3 71034-4
Tuner™(DE3) Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	70623-3 70623-4
Tuner(DE3)pLysS Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	70624-3 70624-4

T7 Expression Strain Competent Cell Sets	Cat. No.
(DE3) Competent Cell Set 1 BL21(DE3), BLR(DE3), HMS174(DE3), NovaBlue(DE3), Tuner(DE3): 0.2 ml each, SOC & Test Plasmid	71207-3
(DE3) Competent Cell Set 2 Origami™(DE3), Origami B(DE3), Rosetta™ 2(DE3), Rosetta-gami(DE3), Rosetta-gami B(DE3), RosettaBlue(DE3): 0.2 ml each, SOC, & Test Plasmid	71208-3
(DE3)pLysS Competent Cell Set 1 BL21(DE3)pLysS, BLR(DE3)pLysS, HMS174(DE3)pLysS, Tuner(DE3)pLysS: 0.2 ml each, SOC, & Test Plasmid	71209-3
(DE3)pLysS Competent Cell Set 2 Origami(DE3)pLysS, Origami B(DE3)pLysS, Rosetta 2(DE3)pLysS, Rosetta-gami(DE3)pLysS, Rosetta-gami B(DE3)pLysS, RosettaBlue(DE3)pLysS: 0.2 ml each, SOC, & Test Plasmid	71210-3
BL21 Competent Cell Set BL21, BL21(DE3), BL21(DE3)pLysS: 2 × 0.2 ml each, SOC, & Test Plasmid	70232-3
BLR Competent Cell Set BLR, BLR(DE3), BLR(DE3)pLysS: 2 × 0.2 ml each, SOC & Test Plasmid	70233-3
HMS174 Competent Cell Set HMS174, HMS174(DE3), HMS174(DE3)pLysS: 2 × 0.2 ml each, SOC, & Test Plasmid	70234-3
Origami Competent Cell Set Origami, Origami(DE3), Origami(DE3)pLysS: 2 × 0.2 ml each, SOC, & Test Plasmid	70670-3
Origami 2 Competent Cell Set Origami 2, Origami 2(DE3), Origami 2(DE3)pLysS: 2 × 0.2 ml each, SOC, & Test Plasmid	71434-3
Origami B Competent Cell Set Origami B, Origami B(DE3), Origami B(DE3)pLysS: 2 × 0.2 ml each, SOC, & Test Plasmid	70911-3

T7 Expression Strain Competent Cell Sets (continued)	Cat. No.
Rosetta™ Competent Cell Set Rosetta, Rosetta(DE3), Rosetta(DE3)pLysS: 2 × 0.2 ml each, SOC, & Test Plasmid	70987-3
Rosetta 2 Competent Cell Set Rosetta 2, Rosetta 2(DE3), Rosetta 2(DE3)pLysS: 2 × 0.2 ml each, SOC, & Test Plasmid	71405-3
RosettaBlue™ Competent Cell Set RosettaBlue, RosettaBlue(DE3), RosettaBlue(DE3)pLysS: 2 × 0.2 ml each, SOC & Test Plasmid	71079-3
Rosetta-gami™ Competent Cell Set Rosetta-gami, Rosetta-gami(DE3), Rosetta-gami(DE3)pLysS: 2 × 0.2 ml each, SOC, & Test Plasmid	71080-3
Rosetta-gami 2 Competent Cell Set Rosetta-gami 2, Rosetta-gami 2(DE3), Rosetta-gami 2(DE3)pLysS: 2 × 0.2 ml each, SOC, & Test Plasmid	71432-3
Rosetta-gami B Competent Cell Set Rosetta-gami B, Rosetta-gami B(DE3), Rosetta-gami B(DE3)pLysS: 2 × 0.2 ml each, SOC & Test Plasmid	71177-3
Tuner™ Competent Cell Set Tuner, Tuner(DE3), Tuner(DE3)pLysS: 2 × 0.2 ml each, SOC & Test Plasmid	70726-3

pETBlue™ and pTriEx™ Expression Strains	Size	Cat. No.
Origami™(DE3)pLacI Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	70629-3 70629-4
Origami 2(DE3)pLacI Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	71347-3 71347-4
Origami B(DE3)pLacI Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	70838-3 70838-4
Rosetta(DE3)pLacI Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	70920-3 70920-4
Rosetta 2(DE3)pLacI Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	71404-3 71404-4
Rosetta-gami(DE3)pLacI Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	71056-3 71056-4
Rosetta-gami 2(DE3)pLacI Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	71353-3 71353-4
Rosetta-gami B(DE3)pLacI Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	71138-3 71138-4
RosettaBlue(DE3)pLacI Competent Cells guaranteed transformation efficiency 1×10^8 cfu/µg Test Plasmid	0.4 ml 1 ml	71060-3 71060-4
Tuner(DE3)pLacI Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	70625-3 70625-4

Non-λDE3 Lysogen Host Strains	Size	Cat. No.
Non-λDE3 Lysogen Competent Cell Set BL21, NovaBlue, Origami™ B, Rosetta™ 2, Rosetta-gami™ B: 0.2 ml each, SOC, & Test Plasmid		71211-3
BL21 Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	69449-3 69449-4
BLR Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	69052-3 69052-4
HMS174 Competent Cells guaranteed transformation efficiency 5×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	69452-3 69452-4
NovaBlue Competent Cells guaranteed transformation efficiency 1×10^8 cfu/µg Test Plasmid	0.4 ml 1 ml	69825-3 69825-4
NovaF⁻ Competent Cells guaranteed transformation efficiency 1×10^8 cfu/µg Test Plasmid	0.4 ml 1.0 ml	71133-3 71133-4
Origami Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	70626-3 70626-4
Origami 2 Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	71344-3 71344-4
Origami B Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	70836-3 70836-4
Rosetta 2 Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	71402-3 71402-4
Rosetta-gami Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	71054-3 71054-4
Rosetta-gami 2 Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	71350-3 71350-4
Rosetta-gami B Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	71135-3 71135-4
RosettaBlue™ Competent Cells guaranteed transformation efficiency 1×10^8 cfu/µg Test Plasmid	0.4 ml 1 ml	71058-3 71058-4
Tuner™ Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	0.4 ml 1 ml	70622-3 70622-4

Singles™ Competent Cells	Size	Cat. No.
BL21(DE3) Singles Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	11 rxn 22 rxn	70235-3 70235-4
BL21(DE3)pLysS Singles Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	11 rxn 22 rxn	70236-3 70236-4
NovaBlue Singles Competent Cells guaranteed transformation efficiency 1.5×10^8 cfu/µg Test Plasmid	11 rxn 22 rxn	70181-3 70181-4
NovaBlue T1^R Singles Competent Cells guaranteed transformation efficiency 1.5×10^8 cfu/µg Test Plasmid	11 rxn 22 rxn	71318-3 71318-4
Origami(DE3) Singles Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	11 rxn 22 rxn	70630-3 70630-4
Origami(DE3)pLysS Singles Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	11 rxn 22 rxn	70631-3 70631-4

Singles™ Competent Cells (continued)	Size	Cat. No.
Origami™ 2(DE3) Singles Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	11 rxn 22 rxn	71408-3 71408-4
Origami 2(DE3)pLysS Singles Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	11 rxn 22 rxn	71409-3 71409-4
Rosetta™(DE3) Singles Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	11 rxn 22 rxn	71099-3 71099-4
Rosetta(DE3)pLysS Singles Competent Cells guaranteed transformation efficiency 2×10^6 cfu/ug Test Plasmid	11 rxn 22 rxn	71100-3 71100-4
Rosetta 2(DE3) Singles Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	11 rxn 22 rxn	71400-3 71400-4
Rosetta 2(DE3)pLysS Singles Competent Cells guaranteed transformation efficiency 2×10^6 cfu/µg Test Plasmid	11 rxn 22 rxn	71401-3 71401-4

Strains Genotypes

Strain	Genotype
B834	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm met*
B834(DE3)	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm met* (DE3)
B834(DE3)pLysS	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm met* (DE3) pLysS (Cam^R)
BL21	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm*
BL21(DE3)	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* (DE3)
BL21(DE3)pLysS	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* (DE3) pLysS (Cam^R)
BL21(DE3)pLacI	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* (DE3) pLacI (Cam^R)
BLR	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* Δ(*srl-recA*)*306*::Tn*10* (Tet^R)
BLR(DE3)	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* (DE3) Δ(*srl-recA*)*306*::Tn*10* (Tet^R)
BLR(DE3)pLysS	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* (DE3) Δ(*srl-recA*)*306*::Tn*10* pLysS (Cam^R, Tet^R)
HMS174	F⁻ *recA1 hsdR*(r_{K12}^- m_{K12}^+) (Rif^R)
HMS174(DE3)	F⁻ *recA1 hsdR*(r_{K12}^- m_{K12}^+) (DE3) (Rif^R)
HMS174(DE3)pLysS	F⁻ *recA1 hsdR*(r_{K12}^- m_{K12}^+) (DE3) pLysS (Cam^R, Rif^R)
NovaBlue	*endA1 hsdR17*(r_{K12}^- m_{K12}^+) *supE44 thi-1 recA1 gyrA96 relA1 lac* F'[*proA*$^+$*B*$^+$ *lacI*^{q} *Z*Δ*M15*::Tn*10*] (Tet^R)
NovaBlue(DE3)	*endA1 hsdR17*(r_{K12}^- m_{K12}^+) *supE44 thi-1 recA1 gyrA96 relA1 lac* (DE3) F'[*proA*$^+$*B*$^+$ *lacI*^{q} *Z*Δ*M15*::Tn*10*] (Tet^R)
NovaBlue T1^R	*endA1 hsdR17*(r_{K12}^- m_{K12}^+) *supE44 thi-1 recA1 gyrA96 relA1 lac tonA* F'[*proA*$^+$*B*$^+$ *lacI*^{q} *Z*Δ*M15*::Tn*10*] (Tet^R)
NovaF⁻	F⁻ *endA1 hsdR17*(r_{K12}^- m_{K12}^+) *supE44 thi-1 recA1 gyrA96 relA1 lac*
Origami™[1]	Δ(*ara–leu*)*7697* Δ*lacX74* Δ*phoA PvuII phoR araD139 ahpC galE galK rpsL* F'[*lac*$^+$ *lacI* ^{q}*pro*] *gor522*::Tn*10 trxB* (Kan^R, Str^R, Tet^R)[4]
Origami(DE3)[1]	Δ(*ara–leu*)*7697* Δ*lacX74* Δ*phoA PvuII phoR araD139 ahpC galE galK rpsL* F'[*lac*$^+$ *lacI*^{q}*pro*] (DE3) *gor522*::Tn*10 trxB* (Kan^R, Str^R, Tet^R)[4]
Origami(DE3)pLysS[1]	Δ(*ara–leu*)*7697* Δ*lacX74* Δ*phoA PvuII phoR araD139 ahpC galE galK rpsL* F'[*lac*$^+$ *lacI*^{q} *pro*] (DE3) *gor522*::Tn*10 trxB* pLysS (Cam^R, Kan^R, Str^R, Tet^R)[4]
Origami(DE3)pLacI[1]	Δ(*ara–leu*)*7697* Δ*lacX74* Δ*phoA PvuII phoR araD139 ahpC galE galK rpsL* F'[*lac*$^+$ *lacI*^{q} *pro*] (DE3) *gor522*::Tn*10 trxB* pLacI (Cam^R, Kan^R, Str^R, Tet^R)[4]
Origami 2[1]	Δ(*ara–leu*)*7697* Δ*lacX74* Δ*phoA PvuII phoR araD139 ahpC galE galK rpsL* F'[*lac*$^+$ *lacI*^{q} *pro*] *gor522*::Tn*10 trxB* (Str^R, Tet^R)[4]
Origami 2(DE3)[1]	Δ(*ara–leu*)*7697* Δ*lacX74* Δ*phoA PvuII phoR araD139 ahpC galE galK rpsL* F'[*lac*$^+$ *lacI*^{q} *pro*] (DE3) *gor522*::Tn*10 trxB* (Str^R, Tet^R)[4]
Origami 2(DE3)pLysS[1]	Δ(*ara–leu*)*7697* Δ*lacX74* Δ*phoA PvuII phoR araD139 ahpC galE galK rpsL* F'[*lac*$^+$ *lacI*^{q} *pro*] (DE3) *gor522*::Tn*10 trxB* pLysS (Cam^R, Str^R, Tet^R)[4]
Origami 2(DE3)pLacI[1]	Δ(*ara–leu*)*7697* Δ*lacX74* Δ*phoA PvuII phoR araD139 ahpC galE galK rpsL* F'[*lac*$^+$ *lacI*^{q} *pro*] (DE3) *gor522*::Tn*10 trxB* pLacI (Cam^R, Str^R, Tet^R)[4]
Origami B[1]	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm lacY1 aphC gor522*::Tn*10 trxB* (Kan^R, Tet^R)
Origami B(DE3)[1]	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm lacY1 aphC* (DE3) *gor522*::Tn*10 trxB* (Kan^R, Tet^R)
Origami B(DE3)pLysS[1]	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm lacY1 aphC* (DE3) *gor522*::Tn*10 trxB* pLysS (Cam^R, Kan^R, Tet^R)
Origami B(DE3)pLacI[1]	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm lacY1 aphC* (DE3) *gor522*::Tn*10 trxB* pLacI (Cam^R, Kan^R, Tet^R)

(continued on next page; see pages 19–20 for footnotes)

Strain	Genotype
Rosetta™	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* pRARE[2] (Cam^R)
Rosetta™(DE3)	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* (DE3) pRARE[2] (Cam^R)
Rosetta(DE3)pLysS	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* (DE3) pLysSRARE[2] (Cam^R)
Rosetta™(DE3)pLacI	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* (DE3) pLacIRARE[2] (Cam^R)
Rosetta 2	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* pRARE2[3] (Cam^R)
Rosetta 2(DE3)	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* (DE3) pRARE2[3] (Cam^R)
Rosetta 2(DE3)pLysS	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* (DE3) pLysSpRARE2[3] (Cam^R)
Rosetta 2(DE3)pLacI	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* (DE3) pLacIpRARE2[3] (Cam^R)
Rosetta-gami™[1]	Δ*(ara–leu)7697 ΔlacX74 ΔphoA PvuII phoR araD139 ahpC galE galK rpsL* F'[*lac*$^+$ *lacI*^{q} *pro*] *gor522*::Tn*10 trxB* pRARE[2] (Cam^R, Kan^R, Str^R, Tet^R)[4]
Rosetta-gami(DE3)[1]	Δ*(ara–leu)7697 ΔlacX74 ΔphoA PvuII phoR araD139 ahpC galE galK rpsL* (DE3) F'[*lac*$^+$ *lacI*^{q} *pro*] *gor522*::Tn*10 trxB* pRARE[2] (Cam^R, Kan^R, Str^R, Tet^R)[4]
Rosetta-gami(DE3)pLysS[1]	Δ*(ara–leu)7697 ΔlacX74 ΔphoA PvuII phoR araD139 ahpC galE galK rpsL* (DE3) F'[*lac*$^+$ *lacI*^{q} *pro*] *gor522*::Tn*10 trxB* pLysSRARE[2] (Cam^R, Kan^R, Str^R, Tet^R)[4]
Rosetta-gami(DE3)pLacI[1]	Δ*(ara–leu)7697 ΔlacX74 ΔphoA PvuII phoR araD139 ahpC galE galK rpsL* (DE3) F'[*lac*$^+$ *lacI*^{q} *pro*] *gor522*::Tn*10 trxB* pLacIRARE[2] (Cam^R, Kan^R, Str^R, Tet^R)[4]
Rosetta-gami 2[1]	Δ*(ara–leu)7697 ΔlacX74 ΔphoA PvuII phoR araD139 ahpC galE galK rpsL* F'[*lac*$^+$ *lacI*^{q} *pro*] *gor522*::Tn*10 trxB* pRARE2[3] (Cam^R, Str^R, Tet^R)[4]
Rosetta-gami 2(DE3)[1]	Δ*(ara–leu)7697 ΔlacX74 ΔphoA PvuII phoR araD139 ahpC galE galK rpsL* (DE3) F'[*lac*$^+$ *lacI*^{q} *pro*] *gor522*::Tn*10 trxB* pRARE2[3] (Cam^R, Str^R, Tet^R)[4]
Rosetta-gami 2(DE3)pLysS[1]	Δ*(ara–leu)7697 ΔlacX74 ΔphoA PvuII phoR araD139 ahpC galE galK rpsL* (DE3) F'[*lac*$^+$ *lacI*^{q} *pro*] *gor522*::Tn*10 trxB* pLysSRARE2[3] (Cam^R, Str^R, Tet^R)[4]
Rosetta-gami 2(DE3)pLacI[1]	Δ*(ara–leu)7697 ΔlacX74 ΔphoA PvuII phoR araD139 ahpC galE galK rpsL* (DE3) F'[*lac*$^+$ *lacI*^{q} *pro*] *gor522*::Tn*10 trxB* pLacIRARE2[3] (Cam^R, Str^R, Tet^R)[4]
Rosetta-gami B[1]	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm lacY1 aphC gor522*::Tn*10 trxB* pRARE[2] (Cam^R, Kan^R, Tet^R)
Rosetta-gami B(DE3)[1]	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm lacY1 aphC* (DE3) *gor522*::Tn*10 trxB* pRARE[2] (Cam^R, Kan^R, Tet^R)
Rosetta-gami B(DE3)pLysS[1]	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm lacY1 aphC* (DE3) *gor522*::Tn*10 trxB* pLysSRARE[2] (Cam^R, Kan^R, Tet^R)
Rosetta-gami B(DE3)pLacI[1]	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm lacY1 aphC* (DE3) *gor522*::Tn*10 trxB* pLacIRARE[2] (Cam^R, Kan^R, Tet^R)
RosettaBlue™	*endA1 hsdR17*(r_{K12}^- m_{K12}^+) *supE44 thi-1 recA1 gyrA96 relA1 lac* [F' *proA*$^+$*B*$^+$ *lacI*^{q} *ZΔM15* ::Tn*10*] pRARE[2] (Cam^R, Tet^R)
RosettaBlue(DE3)	*endA1 hsdR17*(r_{K12}^- m_{K12}^+) *supE44 thi-1 recA1 gyrA96 relA1 lac* (DE3) [F' *proA*$^+$*B*$^+$ *lacI*^{q} *ZΔM15* ::Tn*10*] pRARE[2] (Cam^R, Tet^R)
RosettaBlue(DE3)pLysS	*endA1 hsdR17*(r_{K12}^- m_{K12}^+) *supE44 thi-1 recA1 gyrA96 relA1 lac* (DE3) [F' *proA*$^+$*B*$^+$ *lacI*^{q} *ZΔM15* ::Tn*10*] pLysSRARE[2] (Cam^R, Tet^R)
RosettaBlue(DE3)pLacI	*endA1 hsdR17*(r_{K12}^- m_{K12}^+) *supE44 thi-1 recA1 gyrA96 relA1 lac* (DE3) [F' *proA*$^+$*B*$^+$ *lacI*^{q} *ZΔM15* ::Tn*10*] pLacIRARE[2] (Cam^R, Tet^R)
Tuner™	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm lacY1*
Tuner(DE3)	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm lacY1* (DE3)
Tuner(DE3)pLysS	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm lacY1* (DE3) pLysS (Cam^R)
Tuner(DE3)pLacI	F⁻ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm lacY1* (DE3) pLacI (Cam^R)

1. The original *trxB/gor* double mutant (10) required reducing agent in the growth medium to support normal growth rates. The Origami and Rosetta-gami strains are a derivative (FA113) of the original strain that carry a mutation (*ahpC*) which allows normal growth rates in the absence of supplemental reducing agent (11–22). The Origami B and Rosetta-gami B strains are a derivative of the Tuner strain which also carries the *aphC* mutation.

2. pRARE, pLysSRARE, and pLacIRARE contain the tRNA genes *argU*, *argW*, *ileX*, *glyT*, *leuW*, *proL*, *metT*, *thrT*, *tyrU*, *and thrU*. The rare codons AGG, AGA, AUA, CUA, CCC, and GGA are supplemented.
3. pRARE2, pLysSRARE2, and pLacIRARE2 contains the tRNA gene *argX* which recognizes the CGG codon for arginine in addition to tRNA genes supplied in the pRARE plasmid (see note 2).
4. These strains carry a mutation in ribosomal protein (*rpsL*) conferring resistance to streptomycin; therefore streptomycin is not necessary to maintain strain genotype.

Genetic Marker Descriptions

Marker	Description
ahpC	Mutation in alkyl hydroperoxide reductase conferring disulfide reductase activity.
Δ*ara-leu*	Unable to utilize arabinose and requires leucine for growth on minimal media.
ara	Unable to utilize arabinose.
dcm	No methylation of cytosines in the sequence CCWGG.
DE3	Contains a lambda prophage in which the gene for T7 RNA polymerase is under control of the *lacUV5* promoter.
endA	Endonuclease I activity absent; thought to improve quality of plasmid minipreps.
F^-	Strain does not contain the F episome.
F^+	Strain contains the single copy F plasmid.
F'	Strain contains an F plasmid which harbors some bacterial chromosomal DNA.
gal	Unable to utilize galactose.
gor	Abolishes glutathione reductase. Allows formation of disulfide bonds in *E. coli* cytoplasm.
gyr	Mutation in DNA gyrase. Confers resistance to naladixic acid.
hfl	High frequency of lysogenization by phage λ.
hsdR	Abolishes restriction but not methylation of certain sequences ($r^- m^+$).
hsdS	Abolishes both restriction and methylation of DNA at certain sites ($r^- m^-$).
lac	Unable to utilize lactose.
lacI^q	Produces a high level of *lac* repressor.
Δ(*lac*)X74	Deletion of entire *lac* operon from the chromosome.
lacY	Abolishes *lac* permease.
*lacZ*Δ*M15*	Lacks coding region for amino terminal portion of β-galactosidase (aa 11-41).
lon	Deficient for an ATP-dependent protease; thought to stabilize some foreign proteins.
met	Requires methionine for growth on minimal medium.

Marker	Description
mtl	Unable to utilize mannitol.
ompT	Lacks an outer membrane protease; improves recovery of intact recombinant proteins.
pLacI	Contains a Cam^R plasmid (pACYC184) that carries the gene for *lac* repressor.
pLacIRARE and pLacIRARE2	Contain a Cam^R plasmid (pACYC184) that carries the gene for *lac* repressor, plus tRNA genes for several codons rarely used in *E. coli*.
pLysE, pLysS	Contains a Cam^R plasmid (pACYC184) that carries the gene for T7 lysozyme.
pLysSRARE and pLysSRARE2	Contain a Cam^R plasmid (pACYC184) that carries the gene for T7 lysozyme plus tRNA genes for several codons rarely used in *E. coli*.
pRARE and pRARE2	Contains a Cam^R plasmid (pACYC184) that carries the tRNA genes for several codons rarely used in *E. coli*.
proAB	Requires proline for growth on minimal medium.
recA	Abolishes homologous recombination.
rpsL	Carries a mutation in a ribosomal protein conferring resistance to streptomycin.
srl	Unable to utilize sorbitol.
strA	(Same as *rpsL.*)
supE	Amber suppressor strain; inserts gln suppressor tRNA for UAG codon.
supF	Amber suppressor strain; inserts tyr suppressor tRNA for UAG codon; required for lytic growth of *Sam* 7 or S100λ.
thi	Requires thiamine for growth in minimal medium.
Tn *10*	Contains the Tet^R transposable element, Tn*10*.
tonA	Confers resistance to T1 and T5 phage.
traD	Defective for ability to transfer F episome DNA.
trp	Requires tryptophan for growth in minimal medium.
trxB	Abolishes thioredoxin reductase. Allows formation of disulfide bonds in *E. coli* cytoplasm.
xyl	Unable to utilize xylose.

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Academic and Non-profit Laboratory Assurance Letter

The T7 expression system is based on technology developed at Brookhaven National Laboratory under contract with the U.S. Department of Energy and is the subject of patent applications assigned to Brookhaven Science Associates (BSA). This technology, including bacteria, phages, and plasmids that carry the gene for T7 RNA polymerase, is made available on the following conditions:

1. The T7 expression system is to be used for noncommercial research purposes only. A license is required for any commercial use, including use of the T7 system for research purposes or for production purposes by any commercial entity. Information about commercial licenses may be obtained from the Patent Office, Brookhaven National Laboratory, Upton, New York, 11973, Telephone: (631) 344-7134. Contact: Christine Brakel.
2. No materials that contain the cloned gene for T7 RNA polymerase may be distributed further to third parties outside of your laboratory, unless the recipient receives a copy of this assurance letter and agrees to be bound by its terms. This limitation applies to any of the following materials that are included in this kit and to any derivatives you may make of them:

E. coli B834(DE3)	*E. coli* Rosetta(DE3)pLysS
E. coli B834(DE3)pLysS	*E. coli* Rosetta(DE3)pLacI
E. coli BL21(DE3)	*E. coli* Rosetta 2(DE3)
E. coli BL21(DE3)pLysS	*E. coli* Rosetta 2(DE3)pLysS
E. coli BL21(DE3)pLysE	*E. coli* Rosetta 2(DE3)pLacI
E. coli BL26(DE3)pLysE	*E. coli* RosettaBlue™(DE3)
E. coli BLR(DE3)	*E. coli* RosettaBlue(DE3)pLysS
E. coli BLR(DE3)pLysS	*E. coli* RosettaBlue(DE3)pLacI
E. coli HMS174(DE3)	*E. coli* Rosetta-gami™(DE3)
E. coli HMS174(DE3)pLysS	*E. coli* Rosetta-gami(DE3)pLysS
E. coli HMS174(DE3)pLysE	*E. coli* Rosetta-gami(DE3)pLacI
E. coli NovaBlue(DE3)	*E. coli* Rosetta-gami 2(DE3)
E. coli Origami™(DE3)	*E. coli* Rosetta-gami 2(DE3)pLysS
E. coli Origami(DE3)pLysS	*E. coli* Rosetta-gami 2(DE3)pLacI
E. coli Origami(DE3)pLacI	*E. coli* Rosetta-gami B(DE3)
E. coli Origami 2(DE3)	*E. coli* Rosetta-gami B(DE3)pLysS
E. coli Origami 2(DE3)pLysS	*E. coli* Rosetta-gami B(DE3)pLacI
E. coli Origami 2(DE3)pLacI	*E. coli* Tuner™(DE3)
E. coli Origami B(DE3)	*E. coli* Tuner(DE3)pLysS
E. coli Origami B(DE3)pLysS	*E. coli* Tuner(DE3)pLacI
E. coli Origami B(DE3)pLacI	Bacteriophage λCE6
E. coli Rosetta™(DE3)	Bacteriophage λDE3

3. The initial purchaser may refuse to accept the above conditions by returning the kit unopened and the enclosed materials unused. By accepting or using the kit or the enclosed materials, you agree to be bound by the foregoing conditions.

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