BL21-CodonPlus Competent Cells

Instruction Manual

Catalog #230240, #230245, #230250, #230255, #230265, #230275 and 230280

Revision C.0

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BL21-CodonPlus Competent Cells

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BL21-CodonPlus Competent Cells

MATERIALS PROVIDED

Catalog number	Materials provided	Quantity	Efficiency (cfu/μg of pUC18 DNA)[a]
#230280	BL21-CodonPlus (DE3)-RIPL competent cells (purple tube)	10 × 0.1 ml	≥1 × 10^6
	pUC18 control plasmid (0.1 ng/μl in TE buffer[b])	10 μl	—
	XL10-Gold β-mercaptoethanol mix	50 μl	—
#230240	BL21-CodonPlus-RIL competent cells (red-orange tube)	10 × 0.1 ml	≥1 × 10^7
	pUC18 control plasmid (0.1 ng/μl in TE buffer[b])	10 μl	—
	XL10-Gold β-mercaptoethanol mix	50 μl	—
#230245	BL21-CodonPlus (DE3)-RIL competent cells (yellow tube)	10 × 0.1 ml	≥1 × 10^7
	pUC18 control plasmid (0.1 ng/μl in TE buffer[b])	10 μl	—
	XL10-Gold β-mercaptoethanol mix	50 μl	—
#230250	BL21-CodonPlus-RP competent cells (clear tube)	10 × 0.1 ml	≥1 × 10^7
	pUC18 control plasmid (0.1 ng/μl in TE buffer[b])	10 μl	—
	XL10-Gold β-mercaptoethanol mix	50 μl	—
#230255	BL21-CodonPlus (DE3)-RP competent cells (green tube)	10 × 0.1 ml	≥1 × 10^7
	pUC18 control plasmid (0.1 ng/μl in TE buffer[b])	10 μl	—
	XL10-Gold β-mercaptoethanol mix	50 μl	—
#230265	BL21-CodonPlus (DE3)-RIL-X competent cells (blue tube)	10 × 0.1 ml	≥1 × 10^7
	pUC18 control plasmid (0.1 ng/μl in TE buffer[b])	10 μl	—
	XL10-Gold β-mercaptoethanol mix	50 μl	—
#230275	BL21-CodonPlus (DE3)-RP-X competent cells (red tube)	10 × 0.1 ml	≥1 × 10^7
	pUC18 control plasmid (0.1 ng/μl in TE buffer[b])	10 μl	—
	XL10-Gold β-mercaptoethanol mix	50 μl	—

[a] These competent cell efficiencies are guaranteed when cells are used according to the specifications outlined in this instruction manual.

[b] See *Preparation of Media and Reagents*.

STORAGE CONDITIONS

Store cells immediately at –80°C. Do not place the cells in liquid nitrogen.
pUC18 Control Plasmid: –80°C
XL10-Gold β-Mercaptoethanol Mix: –80°C

Revision C.0

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INTRODUCTION

BL21-CodonPlus competent cells are derived from Agilent's high-performance BL21-Gold competent cell line.[1] These cells enable efficient high-level expression of heterologous proteins in *Escherichia coli*.

Efficient production of heterologous proteins in *E. coli* is frequently limited by the rarity of certain tRNAs that are abundant in the organisms from which the heterologous proteins are derived.* Forced high-level expression of heterologous proteins can deplete the pool of rare tRNAs and stall translation. BL21-CodonPlus strains are engineered to contain extra copies of genes that encode the tRNAs that most frequently limit translation of heterologous proteins in *E. coli*. Availability of tRNAs allows high-level expression of many heterologous recombinant genes in BL21-CodonPlus cells that are poorly expressed in conventional BL21 strains.

BL21-CodonPlus-RIL and BL21-CodonPlus(DE3)-RIL cells contain extra copies of the *argU*, *ileY,* and *leuW* tRNA genes. These genes encode tRNAs that recognize the arginine codons AGA and AGG, the isoleucine codon AUA, and the leucine codon CUA, respectively (Table I). The CodonPlus-RIL strains have available the tRNAs that most frequently restrict translation of heterologous proteins from organisms that have AT-rich genomes. BL21-CodonPlus-RP and BL21-CodonPlus(DE3)-RP cells contain extra copies of the *argU* and *proL* genes. These genes encode tRNAs that recognize the arginine codons AGA and AGG and the proline codon CCC, respectively. The CodonPlus-RP strains have available the tRNAs that most frequently restrict translation of heterologous proteins of organisms that have GC-rich genomes. The BL21-CodonPlus (DE3)-RIPL cells contain extra copies of the *argU*, *ileY,* and *leuW* as well as the *proL* tRNA genes. This strain rescues expression of heterologous proteins from organisms that have either AT- or GC-rich genomes.

* A complete compilation of codon usage of the sequences in the GenBank® database is available at *http://www.kazusa.or.jp/codon/*

TABLE I

Extra Copies of tRNA Genes in BL21-CodonPlus Strains

Host strain	tRNA genes (codon recognition of gene product)	Antibiotic resistance
BL21-CodonPlus(DE3)-RIPL strain	*argU* (AGA, AGG), *ileY* (AUA) , *proL* (CCC), *leuW* (CUA)	Cam^r Strep/Spec^r
BL21-CodonPlus-RIL strain	*argU* (AGA, AGG), *ileY* (AUA), *leuW* (CUA)	Cam^r
BL21-CodonPlus(DE3)-RIL strain	*argU* (AGA, AGG), *ileY* (AUA), *leuW* (CUA)	Cam^r
BL21-CodonPlus(DE3)-RIL-X strain	*argU* (AGA, AGG), *ileY* (AUA), *leuW* (CUA)	Cam^r
BL21-CodonPlus-RP strain	*argU* (AGA, AGG), *proL* (CCC)	Cam^r
BL21-CodonPlus(DE3)-RP strain	*argU* (AGA, AGG), *proL* (CCC)	Cam^r
BL21-CodonPlus(DE3)-RP-X strain	*argU* (AGA, AGG), *proL* (CCC)	Cam^r

The BL21-CodonPlus(DE3) strains (Table II) are ideal for performing protein expression studies that utilize the T7 RNA polymerase promoter to direct high-level expression. The BL21-CodonPlus-RIL and BL21-CodonPlus-RP strains can be used for protein expression with vectors driven by non-T7 promoters. The methionine auxotrophic variants BL21-CodonPlus(DE3)-RIL-X and BL21-CodonPlus(DE3)-RP-X allow efficient labeling of recombinant proteins with selenomethionine or ^{35}S-methionine. Both strains contain a transposon in the *metA* gene that renders the cells incapable of synthesizing methionine. Derived from *E. coli* B, the BL21-CodonPlus expression strains naturally lack the Lon protease, which can degrade recombinant proteins. In addition, these strains are engineered to be deficient for a second protease, the OmpT protein.

BL21-CodonPlus competent cells also feature the Hte phenotype present in Agilent's highest efficiency competent cell strain, XL10-Gold.[2] The presence of the Hte phenotype increases the transformation efficiency of the cells. In addition, the gene that encodes endonuclease I (*endA*), an enzyme that rapidly degrades plasmid DNA isolated by most miniprep procedures, has been inactivated in these cells. These two features enable direct cloning of many protein expression constructs.

TABLE II

Host Strains and Genotypes

Host strain[a]	Genotype
BL21-CodonPlus(DE3)-RIPL strain	*E. coli* B F$^-$ *ompT hsdS*(r_B^- m_B^-) *dcm*$^+$ Tet^r *gal* λ(DE3) *endA* Hte [*argU proL* Cam^r] [*argU ileY leuW* Strep/Spec^r]
BL21-CodonPlus-RIL strain[3]	*E. coli* B F$^-$ *ompT hsdS*(r_B^- m_B^-) *dcm*$^+$ Tet^r *gal endA* Hte [*argU ileY leuW* Cam^r]
BL21-CodonPlus(DE3)-RIL strain[3]	*E. coli* B F$^-$ *ompT hsdS*(r_B^- m_B^-) *dcm*$^+$ Tet^r *gal* λ(DE3) *endA* Hte [*argU ileY leuW* Cam^r]
BL21-CodonPlus(DE3)-RIL-X strain[3]	*E. coli* B F$^-$ *ompT hsdS*(r_B^- m_B^-) *dcm*$^+$ Tet^r *gal* λ(DE3) *endA metA::Tn5*(kan^r) Hte [*argU ileY leuW* Cam^r]
BL21-CodonPlus-RP strain[3]	*E. coli* B F$^-$ *ompT hsdS*(r_B^- m_B^-) *dcm*$^+$ Tet^r *gal endA* Hte [*argU proL* Cam^r]
BL21-CodonPlus(DE3)-RP strain[3]	*E. coli* B F$^-$ *ompT hsdS*(r_B^- m_B^-) *dcm*$^+$ Tet^r *gal* λ(DE3) *endA* Hte [*argU proL* Cam^r]
BL21-CodonPlus(DE3)-RP-X strain[3]	*E. coli* B F$^-$ *ompT hsdS*(r_B^- m_B^-) *dcm*$^+$ Tet^r *gal* λ(DE3) *endA metA::Tn5*(kan^r) Hte [*argU proL* Cam^r]

[a] These strains, derivatives of *E. coli* B, are general protein expression strains that lack both the Lon protease and the OmpT protease, which can degrade proteins during purification.[4] Dcm methylase, naturally lacking in *E. coli* B, is inserted into the genomes.

Host Strain Features

Protein Expression Systems and Induction Methods

- The BL21-CodonPlus(DE3)-RIPL, BL21-CodonPlus(DE3)-RIL and BL21-CodonPlus(DE3)-RP competent cells are all-purpose strains for high-level protein expression and easy induction in T7 expression systems.

- The strains of the BL21-CodonPlus series of competent cells provide varying levels of expression control with T7 promoter-driven vectors such as the pCAL vectors and the pET vectors (Table III). BL21-CodonPlus-RIL and BL21-CodonPlus-RP cells require infection with the CE6 bacteriophage for T7 promoter-driven expression. With expression induced by the CE6 bacteriophage, the BL21-CodonPlus-RIL and BL21-CodonPlus-RP cells provide the tightest control of protein expression.

- BL21-CodonPlus-RIL and BL21-CodonPlus-RP cells can be used for protein expression with vectors driven by non-T7 promoters.

tRNA Expression Plasmids

- BL21-CodonPlus-RIL, BL21-CodonPlus(DE3)-RIL, and BL21-CodonPlus(DE3)-RIL-X cells contain a ColE1-compatible, pACYC-based plasmid containing extra copies of the *argU*, *ileY*, and *leuW* tRNA genes.

- BL21-CodonPlus-RP, BL21-CodonPlus(DE3)-RP, and BL21-CodonPlus(DE3)-RP-X cells contain a ColE1-compatible, pACYC-based plasmid containing extra copies of the *argU* and *proL* tRNA genes.

- BL21-CodonPlus(DE3)-RIPL cells contain a ColE1-compatible, pACYC-based plasmid containing extra copies of the *argU* and *proL* tRNA genes and a ColE1- and pACYC-compatible pSC101-based plasmid containing extra copies of the *argU*, *ileY*, and *leuW* tRNA genes.

Protein Labeling Strains

- BL21-CodonPlus(DE3)-RIL-X and BL21-CodonPlus(DE3)-RP-X cells are methionine auxotrophic variants of the BL21-CodonPlus(DE3)-RIL and BL21-CodonPlus(DE3)-RP strains, respectively, and are designed to allow efficient labeling of recombinant proteins with selenomethionine or ^{35}S-methionine.

Table III

Expression strains	Induction	Advantages	Disadvantages
BL21-CodonPlus-RIL competent cells	Infection with lambda bacteriophage CE6	Tightest control of uninduced expression	Induction is not as efficient as in DE3 derivatives and induction (infection) process is more cumbersome
BL21-CodonPlus-RP competent cells			
BL21-CodonPlus(DE3)-RIPL competent cells	Isopropyl-1-thio-β-D-galactopyranoside (IPTG) induction of T7 polymerase from *lacUV5* promoter	High-level expression	Leaky expression of T7 polymerase can lead to uninduced expression of potentially toxic proteins
BL21-CodonPlus(DE3)-RIL competent cells			
BL21-CodonPlus(DE3)-RP competent cells			
BL21-CodonPlus(DE3)-RIL-X competent cells	Isopropyl-1-thio-β-D-galactopyranoside (IPTG) induction of T7 polymerase from *lacUV5* promoter	High-level expression Efficient labeling of recombinant proteins with methionine derivatives	Leaky expression of T7 polymerase can lead to uninduced expression of potentially toxic proteins
BL21-CodonPlus(DE3)-RP-X competent cells			

Considerations

BL21-CodonPlus(DE3) Strains and Protein Toxicity

Proteins expressed from the T7 promoter can often be toxic to *E. coli* host cells. If using a BL21-CodonPlus(DE3) strain as the primary host strain for expression, some caution should be exercised because even low-level expression can result in accumulation of a toxic gene product. If the gene to be expressed is suspected of being toxic to *E. coli*, we recommend transforming BL21-CodonPlus-RIL or BL21-CodonPlus-RP cells with the gene of interest and then inducing expression with CE6 bacteriophage (see *Induction of Target Protein by Infection with Lambda CE6*); this procedure provides the tightest control of expression for genes under the control of the T7 promoter. For less toxic proteins, transform BL21-CodonPlus(DE3) cells with plasmid DNA for IPTG-induced expression (see *Induction of Target Protein Using IPTG*).

Composition of Miniprep DNA Prepared from Transformed Cells

Miniprep DNA obtained following transformation of the competent cells with the expression plasmid of choice will be a mixture containing both the expression plasmid and the tRNA-encoding plasmid(s). Each of the BL21-CodonPlus strains contains a ColE1-compatible pACYC-based plasmid that confers chloramphenicol resistance. This plasmid is ~3.5 kb and will be observed when analyzing miniprep results by gel electrophoresis. The BL21-CodonPlus(DE3)-RIPL strain also contains a ColE1- and pACYC-compatible pSC101-based plasmid that confers streptomycin resistance. Although the 4.7 kb pSC101-based plasmid is maintained at relatively low copy number, the plasmid may be observed when analyzing BL21-CodonPlus(DE3)-RIPL strain miniprep DNA by gel electrophoresis.

Confirming the Presence of the tRNA-Encoding Plasmids Before Induction

The presence of the pACYC-based plasmid can be confirmed by verifying the chloramphenicol-resistance phenotype using LB-agar medium supplemented with 34 µg/ml chloramphenicol. Before inducing expression of the protein of interest, we recommend growing the overnight culture in the presence of the expression plasmid-specific antibiotic and 50 µg/ml chloramphenicol to assure the presence of the pACYC-based plasmids at the time of induction. When analyzing a cell extract by gel electrophoresis, chloramphenicol acetyl transferase, the protein that provides chloramphenicol resistance, will be observed at ~25,660 Da.

For the BL21-CodonPlus(DE3)-RIPL strain, the presence of the pSC101-based plasmid can be confirmed by the streptomycin- or spectinomycin-resistance phenotype. If phenotypic verification is desired, we recommend using LB-agar medium supplemented with 75 µg/ml streptomycin. It is **not**, however, necessary to add streptomycin to the growth medium prior to induction in order to maintain the pSC101-based plasmid. The plasmid contains a functional partitioning locus that ensures proper plasmid segregation, preventing plasmid loss even in the absence of a selective agent. When analyzing cell extracts by gel electrophoresis followed by Coomassie® staining, the 37.5-kD protein that confers streptomycin resistance is **not** typically detected.

Growth of Methionine Auxotrophic Strains

The growth rate of the kanamycin-resistant methionine auxotrophic strains BL21-CodonPlus(DE3)-RIL-X and BL21-CodonPlus(DE3)-RP-X in methionine-supplemented minimal medium is approximately half the growth rate as in rich medium.

TRANSFORMATION GUIDELINES

Important *For optimal transformation efficiency, please read the guidelines outlined in this section before proceeding with the* Transformation Protocol.

Storage Conditions

The competent cells are very sensitive to slight variations in temperature. In order to prevent a loss of transformation efficiency, store the competent cells at the bottom of a –80°C freezer directly from the dry ice shipping container. Transferring tubes from one freezer to another may result in a loss of efficiency. The transformation efficiency of the competent cells is guaranteed when the competent cells are used according to the specifications outlined in this instruction manual.

Use of 14-ml BD Falcon Polypropylene Round-Bottom Tubes

It is important to use 14-ml BD Falcon polypropylene round-bottom tubes (BD Biosciences Catalog #352059) for the *Transformation Protocol* because the critical incubation period during the heat pulse is optimized specifically for the thickness and shape of these tubes.

Aliquoting Cells

Store the competent cells on ice at all times while aliquoting. It is essential to place the 14-ml polypropylene tubes on ice before the competent cells are thawed and to aliquot the competent cells directly into the prechilled 14-ml polypropylene tubes. It is also important to use 100 µl of competent cells/transformation. Using an inadequate volume of competent cells results in lower transformation efficiencies.

Quantity of DNA

Greatest transformation efficiencies (i.e., transformants/microgram of DNA) are observed when each 100-µl aliquot of competent cells is transformed with 1 µl of DNA at a concentration of 0.1 ng/µl. Although the overall transformation efficiency may be lower, a greater number of colonies will be obtained when the cells are transformed with up to 50 ng of DNA.

Use of β-Mercaptoethanol

β-Mercaptoethanol has been shown to increase transformation efficiency. For optimal efficiency, use 2.0 µl of a 1:10 dilution of the XL10-Gold β-mercaptoethanol mix provided in this kit. (Using an alternative source of β-ME may reduce transformation efficiency.)

Length of the Heat Pulse

Optimal transformation efficiencies are observed when transformation reactions are heat-pulsed for 20–25 seconds. Transformation efficiencies decrease sharply when the duration of the heat pulse is <20 seconds or >25 seconds.

TRANSFORMATION PROTOCOL

1. Thaw the competent cells on ice.

   Note *Store the competent cells **on ice at all times** while aliquoting. It is essential that 14-ml BD Falcon polypropylene round-bottom tubes are placed on ice before the competent cells are thawed and that 100 µl of competent cells are aliquoted directly into each **prechilled** polypropylene tube. Do not pass the frozen competent cells through more than one freeze–thaw cycle.*

2. Gently mix the competent cells. Aliquot 100 µl of the competent cells into the appropriate number of prechilled 14-ml BD Falcon polypropylene round-bottom tubes. Prepare an additional 100-µl aliquot of cells for use as a transformation control.

3. Dilute XL10-Gold β-mercaptoethanol mix provided with this kit 1:10 with dH_2O. Each 100-µl aliquot of cells requires 2 µl of diluted β-mercaptoethanol.

4. Add 2.0 µl of the 1:10 dilution of β-mercaptoethanol to each of the 100-µl aliquots of competent cells. (Using an alternative source of β-ME may reduce transformation efficiency.)

5. Swirl the contents of the tubes gently. Incubate the cells on ice for 10 minutes, swirling gently every 2 minutes.

6. Add 1–50 ng of expression plasmid DNA containing the gene of interest to each tube of cells and swirl gently. For the control transformation reaction, add 1 µl of the pUC18 control plasmid to a separate 100-µl aliquot of competent cells and swirl gently.

7. Incubate the reactions on ice for 30 minutes.

8. Preheat SOC medium (see *Preparation of Media and Reagents*) in a 42°C water bath for use in step 11.

9. Heat-pulse each transformation reaction in a 42°C water bath for 20 seconds. **The duration of the heat pulse is critical for optimal transformation efficiencies (see *Length of the Heat Pulse*).**

10. Incubate the reactions on ice for 2 minutes.

11. Add 0.9 ml of preheated (42°C) SOC medium to each transformation reaction and incubate the reactions at 37°C for 1 hour with shaking at 225–250 rpm.

12. Using a sterile spreader, spread ≤ 200 µl of the cells transformed with the experimental DNA onto LB agar[§] plates that contain the appropriate antibiotic.

    For the pUC18 control plasmid, plate the volume of the control transformation reaction listed in Table IV, according to the BL21-CodonPlus strain used, on an LB–ampicillin agar plate.[§]

    Notes *The cells may be concentrated by centrifuging at 200 ×g for 3–5 minutes at 4°C if desired. Resuspend the pellet in 200 µl of SOC broth.*

    If plating ≤100 µl of the transformation reaction, first place a 100-µl pool of SOC medium onto the plate. Pipet the cells from the transformation reaction into the pool of SOC and then spread the mixture. If plating >100 µl, spread the cell suspension onto the plates directly.

    When spreading the cells, tilt and tap the spreader to remove the last drop of cells.

13. Transformants will appear as colonies following overnight incubation at 37°C. See Table IV for the results expected for the pUC18 control transformation.

TABLE IV

Transformation Summary for the pUC18 Control Plasmid

Host strain	Plating quantity	Expected colony number	Efficiency (cfu/µg of pUC18 DNA)
BL21-CodonPlus(DE3)-RIPL competent cells	200 µl	≥ 20 cfu	$\geq 1 \times 10^6$
BL21-CodonPlus-RIL competent cells	100 µl	≥100 cfu	$\geq 1 \times 10^7$
BL21-CodonPlus(DE3)-RIL competent cells	100 µl	≥100 cfu	$\geq 1 \times 10^7$
BL21-CodonPlus(DE3)-RIL-X competent cells	100 µl	≥100 cfu	$\geq 1 \times 10^7$
BL21-CodonPlus-RP competent cells	100 µl	≥100 cfu	$\geq 1 \times 10^7$
BL21-CodonPlus(DE3)-RP competent cells	100 µl	≥100 cfu	$\geq 1 \times 10^7$
BL21-CodonPlus(DE3)-RP-X competent cells	100 µl	≥100 cfu	$\geq 1 \times 10^7$

[§] See *Preparation of Media and Reagents.*

INDUCTION OF TARGET PROTEIN USING IPTG

The following induction protocol is a general guide for expression of genes under the control of IPTG-inducible promoters on an analytical scale (1 ml of induced culture). Most commonly, this protocol is used to analyze protein expression of individual transformants when using BL21(DE3) host strains in combination with plasmids containing T7 promoter constructs (e.g. pET vectors). Expression cassettes under the control of the *trp/lac* hybrid promoter, *tac*, can be also induced using this protocol. In the case of *tac* promoter constructs, non-DE3 lysogen strains can be employed as hosts.

Note *The transformation procedure described above will produce varying numbers of colonies depending on the efficiency of transformation obtained using the expression plasmid. It is prudent to test more than one colony as colony-to-colony variations in protein expression are possible.*

1. Inoculate 1 ml aliquots of LB broth (containing 50 µg/ml of chloramphenicol and the appropriate antibiotic) with single colonies from the transformation. Shake at 220–250 rpm at 37°C overnight.

   Note *Chloramphenicol is required in the medium to maintain the pACYC plasmid in the BL21-Codon Plus strains. The antibiotic required for maintenance of the expression plasmid must also be added to the medium. (If using the BL21-CodonPlus(DE3)-RIPL strain, the additional pSC101-based plasmid is maintained without antibiotic selection. It is* ***not*** *necessary to add streptomycin to growth medium.)*

2. The next morning, pipet 50 µl of each culture into fresh 1-ml aliquots of LB broth containing no selection antibiotics. Incubate these cultures with shaking at 220–250 rpm at 37°C for 2 hours.

3. Pipet 100 µl of each of the cultures into clean microcentrifuge tubes and place the tubes on ice until needed for gel analysis. These will serve as the non-induced control samples.

4. To the rest of the culture in each tube add IPTG to a final concentration of 1 mM. Incubate with shaking at 220–250 rpm at 37°C for 2 hours.

   Note *These values for IPTG concentration and induction time are starting values only and may require optimization depending on the gene expressed.*

5. After the end of the induction period, place the cultures on ice.

6. Pipet 20 µl of each of the induced cultures into clean microcentrifuge tubes. Add 20 µl of 2× SDS gel sample buffer (see *Preparation of Media and Reagents*) to each microcentrifuge tube.

7. Mix the non-induced samples held on ice to resuspend the cells. Pipet 20 µl from each tube into a clean microcentrifuge tube. Add 20 µl of 2× SDS gel sample buffer to each of the 20-µl aliquots of cells.

8. Heat all tubes to 95°C for 5 minutes. Load the associated non-induced and induced samples in adjacent lanes for analysis by SDS-PAGE. Stain the protein gel with Coomassie Brilliant Blue stain.

Note *When analyzing a cell extract by gel electrophoresis, chloramphenicol acetyl transferase, the protein that provides chloramphenicol resistance, will be observed at ~25,660 Da.*

INDUCTION OF TARGET PROTEIN BY INFECTION WITH LAMBDA CE6

Expression of genes under the control of the T7 promoter (e.g. genes in pET vectors) can be achieved in non-DE3 lysogen host strains (e.g. BL21) if the strain harboring the expression plasmid is subsequently infected with lambda CE6. Lambda CE6 expresses T7 polymerase, which in turn drives the transcription of the gene downstream of the T7 promoter. The following protocols describe the growth and maintenance of lambda CE6 and the use of lambda CE6 for infecting host strains. We offer the Lambda CE6 Induction Kit (Catalog #235200) for use in protein expression protocols that incorporate CE6 infection.

Growth and Maintenance of High-Titer Bacteriophage Lambda CE6 Stocks

1. Inoculate 5 ml of modified* NZY broth§ with a single colony of LE392 host cells. Shake overnight at 37°C at 220–250 rpm.

2. Centrifuge the overnight culture for 15 minutes at 1700–2000 × *g* at 4°C. Resuspend the cells in 10 mM $MgSO_4$ to a final OD_{600} of 0.5.

3. Combine 250 µl of cells (at OD_{600} = 0.5) with 1 × 10^6 pfu of lambda CE6 in 14-ml BD Falcon polypropylene round-bottom tubes in triplicate. Incubate at 37°C for 15 minutes.

4. Add 3 ml of melted NZY top agar§ (equilibrated to 48°C prior to addition) to each cell suspension and plate on warm agarose plates.§ Incubate the plates overnight at 37°C.

5. Flood each plate with 5 ml of SM solution§ and rock the plates for 2 hours at room temperature.

* NZY broth to be used for lambda infection protocols should be supplemented with maltose at a final concentration of 0.2%. Add 1 ml of 20% maltose solution (filter-sterilized) per 100 ml of NZY broth to achieve the correct final concentration of maltose.

§ See *Preparation of Media and Reagents.*

6. Remove the SM solution (which contains the lambda CE6) from each plate and pool the volumes in a 50-ml conical tube.

7. Centrifuge the SM solution at 1700–2000 × *g* for 15 minutes at 4°C.

8. Remove the supernatant and determine the titer of the solution.

9. Store the lambda CE6 stock at 4°C.

Phage Amplification

If the titer decreases over time, or if more phage are needed, grow up LE392 cells in 10 ml of medium and add bacteriophage lambda CE6 at a multiplicity of infection of 1:1000 (lambda CE6-to-cell ratio). Continue growing the culture at 37°C for 5–6 hours and then spin down the cellular debris. Titer of the supernatant should be $\geq 5.0 \times 10^9$ pfu/ml. For general information regarding phage amplification, see reference 5.

Infection with Lambda CE6

Note *This protocol is designed for induction in a 50-ml culture volume. If induction of larger volumes of culture is desired, it will be necessary to increase the volume of the overnight culture in step 1. The increased volume of overnight culture is necessary to achieve the required cell density ($A_{600} \leq 1$) in the larger volume of broth the following day.*

1. Inoculate 5 ml of modified* NZY broth containing 50 µg/ml chloramphenicol and the antibiotic required to maintain the expression plasmid with a single colony of BL21 cells (not the DE3 lysogen) harboring the expression plasmid. Shake overnight at 37°C at 200–250 rpm.

2. In the morning, centrifuge 1.0 ml of the overnight culture, resuspend the cells in 1.0 ml of fresh modified* NZY broth, and pipet the resuspended cells into a flask containing 50 ml of fresh modified* NZY broth (no selection antibiotics).

3. Record the A_{600} of the diluted culture. It should be ≤ 0.1. If the A_{600} is > 0.1, use more fresh modified* NZY broth to dilute the culture to $A_{600} \leq 0.1$. If the A_{600} is < 0.1, the time required to reach an A_{600} of 0.3 (in step 4) will be extended.

4. Grow the culture to an A_{600} of 0.3 and then add glucose to a final concentration of 4 mg/ml (e.g. 1.0 ml of a 20% glucose solution to the 50-ml culture).

* NZY broth to be used for lambda infection protocols should be supplemented with maltose at a final concentration of 0.2%. Add 1 ml of 20% maltose solution (filter-sterilized) per 100 ml of NZY broth to achieve the correct final concentration of maltose.

5. Grow the culture to an A_{600} of 0.6–1.0 and then add $MgSO_4$ to a final concentration of 10 mM (e.g. 500 µl of a 1.0 M solution of $MgSO_4$ to the 50-ml culture).

6. Remove a portion of the culture to serve as the uninduced control and infect the rest with bacteriophage lambda CE6 at a multiplicity of infection (MOI) of 5–10 particles per cell. (To optimize induction, cultures may be split into 3 or 4 aliquots and infected with varying dilutions of bacteriophage lambda CE6. The subsequent induction can be monitored by SDS-PAGE or by a functional assay, if available.)

7. Grow the culture for 2–3 hours.

8. Remove 5–20 µl of the culture for determination by SDS-PAGE, and harvest the remaining culture by centrifugation. Store the pellets at –70°C.

   Note *If induction will be monitored using Coomassie stain, silver stain, or another nonspecific protein stain, we recommend running a control of CE6-infected BL21 cells harboring the plasmid without a cloned insert.*

TROUBLESHOOTING

Observations	Suggestions
Plasmid instability	Rearrangement of an insert that contains a repeated sequence or secondary structures (the BL21-CodonPlus series of competent cells are $recA^+$). Establish the insert in a recombination-deficient host strain, such as SURE competent cells ($recB^-$ and $recJ^-$) or XL1-Blue competent cells ($recA^-$), prior to expression in BL21-CodonPlus competent cells.
Clone toxicity	More tightly controlled induction may be achieved by infecting BL21-CodonPlus cells with bacteriophage CE6 than by IPTG-induction of BL21-CodonPlus(DE3) cells.
Gel analysis of miniprep restriction digestion results in multiple bands	The tRNA expression plasmid(s) harbored in the BL21-CodonPlus strains may be detected in miniprep DNA. A pACYC-based plasmid (3.5 kB) is present in all BL21-CodonPlus strains, and an additional 4.7 kb pSC101-based plasmid is present in the BL21-CodonPlus(DE3)-RIPL strain. Insert cloning and verification should be performed in a general cloning strain (e. g., XL1-Blue competent cells) prior to expression in the BL21-CodonPlus strains.
Gel analysis of cell extract results in an extra protein band at ~25,660 Da	The chloramphenicol acetyl transferase expressed from the pACYC-based plasmid is typically detected in protein gel analysis of cell extracts from the BL21-CodonPlus strains.

PREPARATION OF MEDIA AND REAGENTS

LB Broth (per Liter)

10 g of NaCl
10 g of tryptone
5 g of yeast extract
Add dH_2O to a final volume of 1 liter
Adjust pH to 7.0 with 5 N NaOH
Autoclave
Pour into petri dishes (~25 ml/100-mm plate)

LB–Ampicillin Agar (per Liter)

1 liter of LB agar, autoclaved
Cool to 55°C
Add 10 ml of 10-mg/ml filter-sterilized ampicillin
Pour into petri dishes (~25 ml/100-mm plate)

LB Agar (per Liter)

10 g of NaCl
10 g of tryptone
5 g of yeast extract
20 g of agar
Add deionized H_2O to a final volume of 1 liter
Adjust pH to 7.0 with 5 N NaOH
Autoclave
Cool to 55°C, and then add antibiotic, if required
Pour into petri dishes (~25 ml/100-mm plate)

SOB Medium (per Liter)

20.0 g of tryptone
5.0 g of yeast extract
0.5 g of NaCl
Add deionized H_2O to a final volume of 1 liter
Autoclave
Add 10 ml of filter-sterilized 1 M $MgCl_2$ and 10 ml of filter-sterilized 1 M $MgSO_4$ prior to use

SOC Medium (per 100 ml)

Note *This medium should be prepared immediately before use*

1 ml of a 2 M filter-sterilized glucose solution **or** 2 ml of 20% (w/v) glucose
SOB medium to a final volume of 100 ml
Filter sterilize

TE Buffer

10 mM Tris-HCl (pH 7.5)
1 mM EDTA

2× SDS gel sample buffer

100 mM Tris-HCl (pH 6.5)
4% SDS (electrophoresis grade)
0.2% bromophenol blue
20% glycerol

Note *Add dithiothreitol to a final concentration in the 2× buffer of 200 mM prior to use.* ***This sample buffer is useful for denaturing, discontinuous acrylamide gel systems only.***

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SM Solution 5 g of NaCl 2 g of $MgSO_4 \cdot 7H_2O$ 50 ml of 1 M Tris-HCl (pH 7.5) 5 ml 2% gelatin Add deionized H_2O to a final volume of 1 liter Adjust the pH to 7.5 Autoclave	**NZY Broth, (per Liter)** 5 g of NaCl 2 g of $MgSO_4 \cdot 7H_2O$ 5 g of yeast extract 10 g of NZ amine (casein hydrolysate) Add deionized H_2O to a final volume of 1 liter Adjust the pH to 7.5 with NaOH Autoclave
Agarose Plates (per Liter) Melt 20 g of agarose in 500 ml of deionized H_2O Add the following: 5 g of NaCl 5 g of yeast extract 10 g of tryptone Add deionized H_2O to a final volume of 1 liter Autoclave Pour into petri dishes (~25 ml/100-mm plate)	**NZY Top Agar (per Liter)** Prepare 1 liter of NZY broth Add 0.7% (w/v) agarose Autoclave Prior to use, melt agar in microwave, then hold at 48°C in water bath

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5. Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

ENDNOTES

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MSDS INFORMATION

Material Safety Data Sheets (MSDSs) are provided online at *http://www.genomics.agilent.com*. MSDS documents are not included with product shipments.