ArcticExpress Competent Cells and ArcticExpress (DE3) Competent Cells

Instruction Manual

Catalog #230191 ArcticExpress Competent Cells
#230192 ArcticExpress (DE3) Competent Cells

Revision B

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MATERIALS PROVIDED

Catalog number	Materials provided	Quantity	Efficiency (cfu/μg of pUC18 DNA)[a]
#230191	ArcticExpress competent cells (purple tubes)	10 × 0.1 ml	≥5 × 10^6
	pUC18 control plasmid (0.1 ng/μl in TE buffer[b])	10 μl	—
	XL10-Gold β-mercaptoethanol mix	50 μl	—
#230192	ArcticExpress (DE3) competent cells (red tubes)	10 × 0.1 ml	≥5 × 10^6
	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

ADDITIONAL MATERIAL REQUIRED

Low-temperature (10–13°C) incubator with shaker
14-ml BD Falcon polypropylene round-bottom tubes (BD Biosciences Catalog #352059)
Gentamycin sulfate
Isopropyl-1-thio-β-D-galactopyranoside (IPTG)

Revision B
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INTRODUCTION

ArcticExpress Competent Cells are engineered to address the common bacterial gene expression hurdle of protein insolubility. These cells are derived from the high-performance Agilent BL21-Gold competent cells, enabling efficient high-level expression of heterologous proteins in *Escherichia coli*.[1]

Overcoming Protein Misfolding and Insolubility

Forced high-level expression of a heterologous protein in *E. coli* can result in the production of large amounts of incorrectly folded protein, generating aggregates of inactive protein known as inclusion bodies. While the aggregated protein may be easy to purify, obtaining active protein from inclusion bodies typically requires protein-specific and labor-intensive *in vitro* re-folding steps, with no guarantee of obtaining biologically active product. The ArcticExpress series of competent cells provides an *in vivo* approach to increasing the yield of soluble protein produced in *E. coli*.

The mesophilic host *E. coli* is suitable for expression of a wide range of heterologous proteins. At standard cultivation temperatures, however, high-level expression of a heterologous protein can impair the cell's ability to properly process the recombinant protein. Low-temperature cultivation represents one strategy for increasing the recovery of soluble protein.[2] An obstacle to using this approach, however, is that *E. coli* chaperonins, which facilitate proper protein folding by binding to and stabilizing unfolded or partially folded proteins, lose activity at reduced temperatures. Specifically, it has been shown that the activity of the *E. coli* chaperonin complex GroEL/ES retains only about 30% refolding activity at 12°C, compared to its activity at the temperature optimum of 30°C.[3] To overcome this obstacle, ArcticExpress competent cells have been engineered for improved protein processing at low temperatures. These cells co-express the cold-adapted chaperonins Cpn10 and Cpn60 from the psychrophilic bacterium, *Oleispira antarctica*. The Cpn10 and Cpn60 chaperonins from *O. antarctica* have 74% and 54% amino acid identity to the *E. coli* GroEL and GroES chaperonins, respectively, and show high protein refolding activities at temperatures of 4–12°C.[3] When expressed in ArcticExpress cells, these chaperonins confer improved protein processing at lower temperatures, potentially increasing the yield of active, soluble recombinant protein.

Protein Expression Systems and Induction Methods

The ArcticExpress strains are designed for expression of recombinant proteins either from the T7 promoter using the DE3 lysogen strain, or from IPTG-inducible, non-T7 promoters using the non-DE3 lysogen strain (See Table I).

Expression from the T7 Promoter

For recombinant proteins expressed from vectors driven by the T7 promoter, such as the pCAL vectors and the pET vectors, use the DE3 lysogen ArcticExpress (DE3) host strain. This host strain contains a chromosomally integrated cassette in which the T7 RNA polymerase is expressed from the *lacUV5* promoter. Induction of T7 RNA polymerase expression with IPTG results in expression of the T7 promoter-driven recombinant protein.

Expression from Other IPTG-Inducible Promoters

For recombinant proteins expressed from IPTG-inducible promoters other than the T7 promoter, such as the *tac* or *trc* promoter, use the non-DE3 lysogen ArcticExpress host strain.

Table I

Expression Strain	Compatible Expression Vectors	Induction Method
ArcticExpress (DE3) competent cells	T7 promoter-driven expression vectors (e.g. pCAL and pET vectors)	Isopropyl-1-thio-β-D-galactopyranoside (IPTG) induction of T7 RNA polymerase from *lacUV5* promoter
ArcticExpress competent cells	*lac, tac,* and *trc* promoter-driven expression vectors	Isopropyl-1-thio-β-D-galactopyranoside (IPTG) induction of the promoter driving recombinant gene expression (e.g. *lac*, *tac,* or *trc* promoter)

Host Strain Genotype and Features

Derived from *E. coli* B, the ArcticExpress 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. These strains also feature the Hte phenotype* present in Agilent's highest efficiency competent cell strain, XL10-Gold.[4] 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. The *Oleispira antarctica* chaperonins Cpn10 and Cpn60 are constitutively expressed in these strains from a ColE1-compatible, pACYC-based plasmid that contains a gentamycin-resistance gene. See Table II for complete genotype information.

Table II: Host Strain Genotypes

Host strain[a]	Genotype
ArcticExpress (DE3) strain	*E. coli* B F$^-$ *ompT hsdS*(r_B^- m_B^-) *dcm*$^+$ Tet^r *gal* λ(DE3) *endA* Hte [*cpn10 cpn60* Gent^r]
ArcticExpress strain	*E. coli* B F$^-$ *ompT hsdS*(r_B^- m_B^-) *dcm*$^+$ Tet^r *gal endA* Hte [*cpn10 cpn60* Gent^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.[5] Dcm methylase, naturally lacking in *E. coli* B, is inserted into the genomes.

Selection for the Chaperonin-Encoding Plasmid Before Induction

Selection for the *cpn10/cpn60* chaperonin expression plasmid prior to induction is recommended. Gentamycin should be added to the overnight LB broth culture at a final concentration 20 µg/ml. (See the *Protein Expression Protocol* in the *Protocols* section.) When analyzing cell extracts by gel electrophoresis followed by Coomassie® staining, the protein that confers gentamycin resistance is **not** typically detected.

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 the expression plasmid and the *cpn10/cpn60*-encoding pACYC-based plasmid (~5.5 kb). The pACYC-based plasmid is typically observed during gel analysis of miniprep DNA.

*U. S. Patent No. 6,706,525.

PROTOCOLS

Protocol Overview

- Transform the appropriate host strain with the protein expression plasmid, using a 37°C cultivation temperature.
- Pick several transformants and grow overnight cultures in medium containing gentamycin and antibiotic for selection of the expression plasmid at 37°C.
- Grow the cells without antibiotic selection for 3 hours at 30°C.
- Induce expression of the recombinant protein with IPTG at 10–13°C, and continue growth after induction at 10–13°C for 24 hours.
- Analyze protein expression in induced cultures and non-induced controls by SDS-PAGE.

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 heat pulse duration, which is critical for obtaining the highest efficiencies, 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 transfer 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 transferred directly into each **prechilled** polypropylene tube.*

2. Gently mix the competent cells. For each expression plasmid transformation, transfer 100 µl of competent cells into a prechilled 14-ml BD Falcon polypropylene round-bottom tube. 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.**

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 selection of the expression plasmid.

    For the pUC18 control plasmid, plate 100 µl on an LB–ampicillin agar plate.[§]

    Notes *It is **not** necessary to add streptomycin or gentamycin to the transformation plates.*

    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.

13. Transformants will appear as colonies following overnight incubation at 37°C. For the pUC18 control transformation, ≥50 cfu are expected, indicating a transformation efficiency $\geq 5 \times 10^6$ cfu/µg pUC18 DNA.

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

Protein Expression Protocol

Note *The induction phase of this protocol requires incubation of cell cultures during induction at 10–13°C. This may be achieved using a low-temperature incubator equipped with a shaker or by placing a water bath, equipped with a shaker, in a cold room and adjusting the water bath to 10–13°C.*

The following induction protocol is a general guide for expression of genes under the control of IPTG-inducible promoters on an analytical scale (3 ml of induced culture). Optimization of induction conditions (IPTG concentration and duration of growth periods preceding and following induction) may improve active protein yield for some recombinant proteins.

Preparation of Liquid Cultures Prior to Induction

1. Inoculate 1 ml aliquots of LB broth (containing 20 µg/ml of gentamycin and the appropriate antibiotic for selection of the expression plasmid) with single colonies from the transformation plates. Incubate these cultures at 37°C with shaking at 220–250 rpm overnight.

   Note *It is prudent to test more than one colony as colony-to-colony variations in protein expression are possible.*

2. The next morning, subculture the cells in 50-ml culture tubes by pipetting 60 µl of each culture into 3-ml of LB broth containing no selection antibiotics. Incubate these cultures at 30°C with shaking at 220–250 rpm for 3 hours.

Induction of Target Protein Using IPTG

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

4. Transfer the culture tubes to 10–13°C and incubate with shaking at 220–250 rpm for ~10 minutes. After the culture has equilibrated to 10–13°C, add IPTG to each tube to a final concentration of 1 mM. Incubate at 10–13°C, with shaking at 220–250 rpm, for 24 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.

Analysis of Protein Expression by SDS-PAGE

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 SDS-PAGE, the Cpn60 protein will be observed at ~57 kDa, and the Cpn10 protein may be observed at ~10 kDa.*

TROUBLESHOOTING

Observations	Suggestions
Plasmid instability	An insert that contains a repeated sequence or secondary structures may undergo rearrangement (the 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 ArcticExpress competent cells.
Gel analysis of miniprep restriction digestion results in multiple bands	The pACYC-based chaperonin expression plasmid (5.5 kb) harbored in the ArcticExpress strains may be detected in miniprep DNA. Insert cloning and verification should be performed in a general cloning strain (e. g., XL1-Blue competent cells) prior to expression in the ArcticExpress strains.
Gel analysis of cell extract results in an extra protein band at ~57 kDa	The Cpn60 protein (~57 kDa), which is constitutively expressed from the pACYC-based plasmid, is typically detected in protein gel analysis of ArcticExpress cell lysates. An additional band, corresponding to Cpn10 (~10 kDa) may also be observed.
No increase in expression of the recombinant protein after induction	Optimize the induction conditions, including IPTG concentration, length of the 30°C-incubation period prior to induction, and length of the 10–13°C-incubation period after induction.
	If induction of recombinant protein expression cannot be verified by Coomassie staining, analyze protein levels by Western blot, when possible, for greater sensitivity and reduced background.
	Verify that the expression strain chosen is compatible with the promoter driving expression of the recombinant protein [e.g. pET vectors are compatible only with the ArcticExpress (DE3) competent cells].
	Verify the integrity of the expression plasmid to ensure that the plasmid contained in the expression strain does not include a mutation that impairs expression of functional protein, such as a premature stop codon. If non-functional clones are obtained from multiple independent cloning attempts, functional clones may be under negative selection due to toxicity.
	Some DNA sequences may adopt strong secondary structures (e.g. hairpin structures) at low temperatures that impede transcription of the gene of interest.
	Protein expression may be limited by tRNA availability. Try expressing the protein in an ArcticExpress strain with CodonPlus technology: ArcticExpress (DE3)RIL competent cells (catalog #230193), ArcticExpress (DE3)RP competent cells (catalog #230194), ArcticExpress RIL competent cells (catalog #230195), or ArcticExpress RP competent cells (catalog #230196).
Protein is produced in insoluble form	Some recombinant proteins are not substrates for the Cpn60/Cpn10 chaperonins. Consider alternative approaches to increasing the solubility of the recombinant protein, such as the Agilent VariFlex bacterial protein expression system, which allows the convenient addition of solubility-enhancement and purification tags to the protein of interest.

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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ENDNOTES

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MSDS INFORMATION

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