

ArcticExpress™ Competent Cells

Cold-Adapted Protein Folding for Increased Solubility

- + Low growth temperatures enhance protein folding and solubility
- + Feature chaperonins Cpn60 and Cpn10 from psychrophilic bacterium, *Oleispira antarctica*
- + Correct for codon bias and enhance solubility at the same time

CROSS THE PROTEIN SOLUBILITY BARRIER WITH STRATAGENE'S ARCTICEXPRESS™ COMPETENT CELLS, FEATURING COLD-ADAPTED TECHNOLOGY. LOWER GROWTH TEMPERATURES (11-13°C) AND PROTEIN FOLDING CHAPERONINS PROVIDED BY ANTARCTIC ISOLATE, *Oleispira antarctica*, ALLOW THESE CELLS TO PROPERLY PROCESS YOUR PREVIOUSLY INSOLUBLE PROTEINS WITHOUT FUSION TAGS OR COMPLICATED *IN VITRO* REFOLDING METHODS.

Increase Protein Solubility

E. coli is often the first choice for recombinant protein expression because it is fast, simple, and typically produces a lot of protein. High protein yield, however, can be accompanied by incorrect folding, resulting in inactive protein aggregates known as inclusion bodies. Aggregated protein can be easier to purify, but *in vitro* refolding requires many processing steps and there is no guarantee that it will yield a comparable amount of biologically active product. Our ArcticExpress™ Competent Cells* are optimized to facilitate proper protein folding, resulting in increased production of soluble, potentially active protein (Figure 1).

Cold-Adapted Chaperonins

It is generally known that the mesophilic host *E. coli* is suitable for expression of a wide range of heterologous proteins. On a large scale production level, however, the cell's ability to properly process recombinant proteins is impaired and insoluble; enzymatically inactive protein can result. Lower cultivation temperatures have been shown to increase levels of active protein, but slower growth and reduced synthesis rates can result in lower protein yields.

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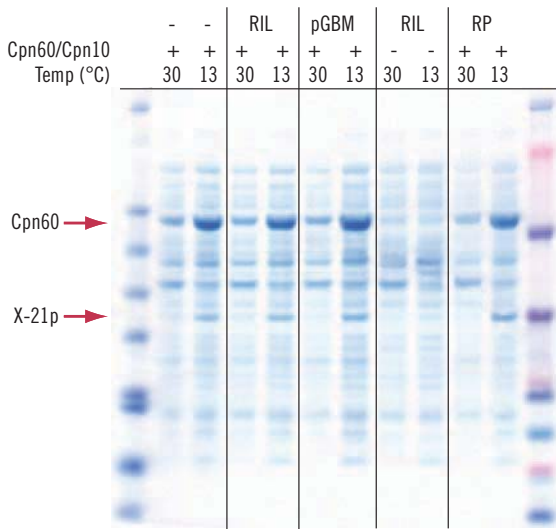


Figure 2
Enhanced Protein Solubility at Lower Growth Temperatures

We used our cold-adapted ArcticExpress™ Competent Cells with and without our CodonPlus® technology to express protein X-21p at both 13°C and 30°C. Solubility of X-21p was enhanced at the lower growth temperature in all cells expressing chaperonin Cpn60 and co-chaperonin Cpn10. Expression of extra tRNA molecules in BL21-CodonPlus® cells or the presence of the CodonPlus® vector, pGBM, without tRNA genes did not hinder this effect.

Our ArcticExpress competent cells have been engineered to express cold-adapted chaperonin Cpn60 and co-chaperonin Cpn10 from psychrophilic bacterium, *Oleispira antarctica*. Chaperonins are known to facilitate proper protein folding by binding to and stabilizing unfolded or partially folded proteins. The chaperonins Cpn60 and Cpn10 show high protein refolding activities at temperatures of 4-12°C. When expressed in our ArcticExpress cells, these chaperonins confer an enhanced ability to grow at lower temperatures and properly process recombinant proteins, thus increasing the amount of available soluble protein (Figure 2).

Solubility Enhancement and Codon Bias Correction

We provide a full portfolio of solutions for all of your protein expression problems. In addition to enhancing protein solubility, we have combined our cold-adapted technology with our premier BL21-CodonPlus® Competent Cell lines to further increase your chances of protein expression success. Codon bias occurs when the ratio of codons in the recombinant protein coding sequence is inconsistent with the *E. coli* tRNA pool, resulting in a paucity of tRNA molecules for efficient synthesis of the heterologous protein. The end result is usually poor or no protein synthesis, early termination of the polypeptide chain, or misincorporation of amino acids in the expressed protein. In the past, solutions to this problem have been mutagenesis of 'rare codons' to more common *E. coli* codons or recloning of the gene for a different host system.

Our BL21-CodonPlus competent cell lines eliminate the need for mutagenesis or recloning. They contain extra copies of *argU*, *ileY*, *leuW*, and *proL* tRNA genes, which are able to rescue expression of genes restricted by rare arginine, isoleucine, leucine, and proline codons. With our cold-adapted ArcticExpress RIL and ArcticExpress RP competent cell lines you can enhance protein solubility and correct for codon bias at the same time (Figure 3).



Figure 3
Solubility Enhancement and Codon Bias Correction

Expression of human cardiac troponinT (hcTnT) protein is significantly enhanced by extra *argU* tRNA genes in ArcticExpress™ (DE3)-RIL and ArcticExpress™ (DE3)-RP Competent Cells. Codon bias correction does not occur in the presence of CodonPlus® vector, pGBM, without tRNA genes. Chaperonin Cpn60 and co-chaperonin Cpn10 do not interfere with codon bias correction.

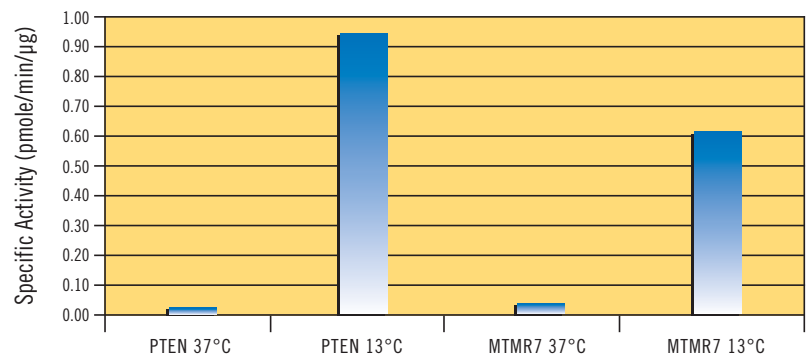


Figure 1
Improved Protein Activity with Cold-Adapted Expression Technology

ArcticExpress™ Competent Cells (13°C) and BL21-Gold Competent Cells (37°C) were used to express phosphatase proteins PTEN and MTMR7 with GST epitope tags. Phosphatase activity was enhanced in proteins expressed in ArcticExpress™ Competent Cells at 13°C.

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ArcticExpress™ Competent Cells		
ArcticExpress™ Competent Cells	10 x 0.1 ml	230191
ArcticExpress™ (DE3) Competent Cells	10 x 0.1 ml	230192
ArcticExpress™ (DE3)RIL Competent Cells	10 x 0.1 ml	230193
ArcticExpress™ (DE3)RP Competent Cells	10 x 0.1 ml	230194
ArcticExpress™ RIL Competent Cells	10 x 0.1 ml	230195
ArcticExpress™ RP Competent Cells	10 x 0.1 ml	230196

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a. Patents pending.
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