

High-level expression of heterologous proteins in *E. coli*

# BL21-CodonPlus™ Cells Correct Expression Problems Caused by Codon Bias

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Stratagene

To resolve the codon bias problem that can hinder the expression of heterologous proteins in *E. coli* hosts, Stratagene has developed BL21-CodonPlus™ competent cells.\* These specially engineered derivatives of BL21-Gold cells improve protein expression by supplying additional copies of specific tRNA genes that are rare in *E. coli*. Stratagene offers several varieties of BL21-CodonPlus cells, so that high-level expression can be customized for different genomes, expression vectors, and applications. BL21-CodonPlus-RIL and BL21-CodonPlus-RP competent cells rescue expression of genes derived from AT- and GC-rich genomes, respectively. BL21-CodonPlus(DE3)-RIL and BL21-CodonPlus(DE3)-RP cells feature IPTG- inducible expression of T7 promoter controlled genes.† BL21-CodonPlus(DE3)-RIL-X and BL21-CodonPlus(DE3)-RP-X cells are methionine auxotrophs for abundant production of methionine-labeled proteins for structural analysis by crystallography. Here, we compare expression levels of recombinant proteins produced in conventional BL21 versus BL21-CodonPlus competent cells.

Expression of recombinant proteins in *E. coli* offers the advantages of speed, simplicity, and high-level expression. However, expression of heterologous genes in *E. coli* is hardly infallible. The genomes of certain organisms favor sequences with codons that occur infrequently in *E. coli* hosts.<sup>23</sup> Forced high-level expression of rare codon-containing genes in *E. coli* depletes the endogenous pool of corresponding tRNAs. The deficit of tRNA molecules disrupts translation, leading to truncated protein expression or no protein expression. Other symptoms of this problem include frameshifts, codon skipping, and misincorporations. Ultimately, protein expression is slowed or aborted and mRNA is degraded.<sup>4</sup>

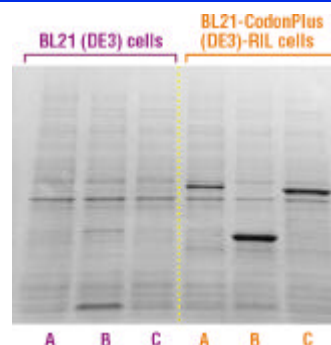
Resolving codon bias is challenging. Typical remedies include altering the codon specifications of the heterologous gene by site-directed mutagenesis or recloning the gene into eukaryotic expression systems. These solutions are not only inconvenient, they are also expensive and require a significant output of labor, time, and reagents.

## Modified Strains Contain Supplemental tRNA Genes

Stratagene has developed a better solution to the codon bias problem by engineering special BL21 strains<sup>1</sup> that supply extra copies of tRNA genes that are rare to *E. coli*. BL21-CodonPlus competent cells maintain important features of their parental BL21-Gold cells, such as Lon and OmpT protease deficiencies for preserving protein integrity, the *endA* 1 deficiency for ensuring nondegraded miniprep DNA, and the Hte phenotype for increasing the transformation efficiency. However, the BL21-CodonPlus series of competent cells contains extra copies

of tRNA genes that are rare in *E. coli* but used more frequently in other organisms, dramatically improving heterologous protein

Figure 1  
Expression of AT-Rich Genes



Cultures of BL21-(DE3) or BL21-CodonPlus (DE3)-RIL cells that contained expression vectors with one of three different proteins (A, B, and C) were induced at log phase growth with IPTG. Cell lysates were separated by SDS-PAGE and visualized by staining with Coomassie® Blue dye.

production within these *E. coli* expression systems.

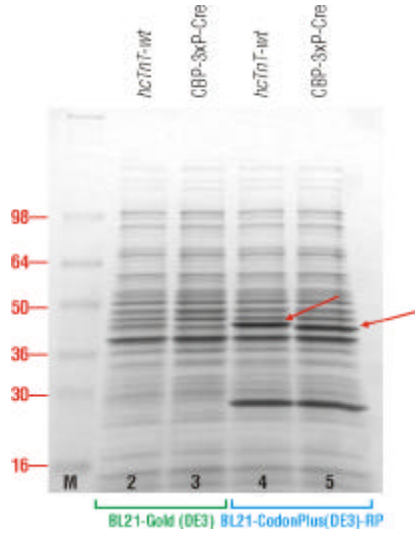
BL21-CodonPlus-RIL cells contain extra copies of the *argU*, *ileY*, and *leuW* tRNA genes, which recognize the AGA/AGG, AUA, and CUA codons, respectively. These codons are a problem predominantly in organisms with AT-rich genomes. BL21-CodonPlus-RP competent cells contain extra copies of the tRNA gene *argU*, which recognizes the AGA and AGG arginine codons, and the tRNA gene *proL*, which recognizes the proline codon, CCC. These codons appear frequently in GC-rich genomes, such as mammals.

BL21-CodonPlus(DE3)-RIL and BL21-CodonPlus(DE3)-RP cells offer IPTG-inducible protein expression, as they contain T7 RNA polymerase under the control of the lacUV5 promoter. When used in conjunction with T7 promoter-driven expression vectors, such as Stratagene's Affinity® pCAL and pET-type vectors, the expressed T7 RNA polymerase provides significant levels of transcription of heterologous genes. The newest members of the BL21-CodonPlus family are BL21-CodonPlus(DE3)-RP-X and BL21-CodonPlus(DE3)-RIL-X competent cells, which are methionine auxotrophs. These cells are designed to metabolically label proteins with methionine for analysis of protein structure by crystallography (data not shown).

## Rescuing Expression of Genes with Rare Codons

Figure 2

Expression of GC-Rich Genes



The *hcTnT-wt* (*argU* dependent) or *CBP-3xP-Cre* (*proL* dependent) test genes were expressed in BL21-Gold(DE3) and BL21-CodonPlus(DE3)-RP cells. Lanes 2 and 3: BL21-Gold(DE3) cells. Lanes 4 and 5: BL21-CodonPlus(DE3)-RP cells.

Lane M: molecular weight markers; Lane 2: *hcTnT-wt* (human cardiac troponin T) in BL21-Gold(DE3); Lane 3: *CBP-3xP-Cre* in BL21-Gold(DE3); Lane 4: *hcTnT-wt* (human cardiac troponin T) in BL21-CodonPlus-RP (DE3); Lane 5: *CBP-3xP-Cre* in BL21-CodonPlus-RP(DE3). The band around 30 kb is the chloramphenicol gene.

We transformed BL21-CodonPlus(DE3)-RIL cells and BL21(DE3) cells with plasmids that encode three different genes for which protein expression in *E. coli* would be affected by codon bias. For each plasmid, a T7 RNA polymerase responsive promoter regulated gene expression (Figure 1). Expression of each recombinant gene was greatly increased in the BL21-CodonPlus(DE3)-RIL cells when compared to expression in BL21(DE3) parental cells. Moreover, because the presence of extra copies of tRNA genes had no deleterious effects to the host cells, we conclude that BL21-CodonPlus cells can also be used for expressing genes with codons that are compatible with conventional *E. coli* strains.

In a similar experiment, we transformed BL21-CodonPlus(DE3)-RP and BL21-Gold (DE3) cells with plasmids encoding the *hcTnT-wt* (*argU* dependent) gene or a *proL* dependent derivative of the *Cre Recombinase* gene, *CBP-3xP-Cre* (Figure 2). The BL21-CodonPlus(DE3)-RP cells, but not the parental BL21-Gold(DE3) cells, demonstrated expression of both genes. These data demonstrate that BL21-CodonPlus(DE3)-RP cells are ideal for high-level expression of heterologous genes restricted by the presence of AGG/AGA and CCC codons in conventional BL21 hosts.

Conclusions

Stratagene's family of BL21-CodonPlus competent cells allow high-level expression of proteins that are difficult to express in conventional *E. coli* hosts due to the codon bias of the heterologous gene. All BL21-CodonPlus cells contain extra copies of tRNA genes that rescue protein expression that would otherwise be restricted. These cells offer a time- and money-saving alternative to previous solutions to the codon-bias

problem, such as site-directed mutagenesis or recloning into a eukaryotic expression system. We compared the expression of heterologous proteins in BL21 parental strains versus BL21-CodonPlus cells. Our results confirm that BL21-CodonPlus-RIL cells rescue expression of AT-rich genes and that BL21-CodonPlus-RP cells rescue gene expression from GC-rich organisms. All members of the BL21-CodonPlus family correct codon bias problems and preserve high-level protein expression. Therefore, these competent cells are valuable expression hosts, even in the absence of a detected codon bias problem.

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\* Patents Pending

‡‡ See inside front cover.

See next page for a list of BL21-CodonPlus™ competent cells!



BL21-CodonPlus™ Competent Cells		
<b>BL21-CodonPlus™ -RIL Competent Cells</b> 10 x 0.1-ml aliquots pUC18 DNA control plasmid	<ul style="list-style-type: none"> <li>Use for non-T7 polymerase systems</li> <li>Use with lambda CE6 for for extremely toxic proteins</li> <li>1 x 10<sup>7</sup> cfu/μg of supercoiled DNA</li> </ul>	#230240
<b>BL21-CodonPlus™ (DE3)-RIL Competent Cells</b> 10 x 0.1-ml aliquots pUC18 DNA control plasmid	<ul style="list-style-type: none"> <li>Use with pET or pCAL vectors</li> <li>1 x 10<sup>7</sup> cfu/μg of supercoiled DNA</li> </ul>	#230245
<b>BL21-CodonPlus™ -RP Competent Cells</b> 10 x 0.1-ml aliquots pUC18 DNA control plasmid	<ul style="list-style-type: none"> <li>Use for non-T7 polymerase systems</li> <li>Use with lambda CE6 for extremely toxic proteins</li> <li>1 x 10<sup>7</sup> cfu/μg of supercoiled DNA</li> </ul>	#230250
<b>BL21-CodonPlus™ (DE3)-RP Competent Cells</b> 10 x 0.1-ml aliquots pUC18 DNA control plasmid	<ul style="list-style-type: none"> <li>Use with pET or pCAL vectors</li> <li>1 x 10<sup>7</sup> cfu/μg of supercoiled DNA</li> </ul>	#230255
<b>BL21-CodonPlus™ (DE3)-RIL-X Competent Cells</b> 10 x 0.1-ml aliquots pUC18 DNA control plasmid	<ul style="list-style-type: none"> <li>Use for labeling cells</li> </ul>	#230265
<b>BL21-CodonPlus™ (DE3)-RIL-X Competent Cells</b> 10 x 0.1-ml aliquots pUC18 DNA control plasmid	<ul style="list-style-type: none"> <li>Use for labeling cells</li> </ul>	#230275
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<b>BL21-Gold (DE3) Competent Cells</b> 10 x 0.1-ml aliquots pUC18 DNA control plasmid	<ul style="list-style-type: none"> <li>Use with pET or pCAL vectors</li> <li><i>endA</i>- for direct cloning many expression constructs</li> <li>1x10<sup>8</sup> cfu/μg of supercoiled DNA</li> </ul>	#230132
<b>BL21-Gold (DE3)pLysS Competent Cells</b> 10 x 0.1-ml aliquots pUC18 DNA control plasmid	<ul style="list-style-type: none"> <li>For use with both toxic and non-toxic proteins.</li> <li>Use with pET or pCAL vectors</li> <li><i>endA</i>- for cloning many expression constructs</li> <li>1x10<sup>8</sup> cfu/μg of supercoiled DNA</li> </ul>	#230134
BL21 Competent Cells		
<b>BL21 Competent Cells</b> 5 x 0.2-ml aliquots pUC18 DNA control plasmid	<ul style="list-style-type: none"> <li>Use with non-T7 polymerase-based systems</li> <li>Use with lambda CE6 for for extremely toxic proteins</li> </ul>	#200133
<b>BL21(DE3) Competent Cells</b> 5 x 0.2-ml aliquots pUC18 DNA control plasmid	<ul style="list-style-type: none"> <li>Use with pET or pCAL vectors</li> </ul>	#200131
<b>BL21(DE3)pLysS Competent Cells</b> 5 x 0.2-ml aliquots pUC18 DNA control plasmid	<ul style="list-style-type: none"> <li>Use with both toxic and non-toxic proteins.</li> <li>Use with pET or pCAL vectors</li> </ul>	#200132

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\* Patent pending

