

- ELIMINATES EXPRESSION PROBLEMS DUE TO CODON BIAS
- PROTEASE DEFICIENT FOR HIGH-YIELD PROTEIN EXPRESSION
- EXPRESSION CONTROL DERIVATIVES AVAILABLE

Easier Protein Expression in *E. coli*

When you need to produce recombinant protein an *E. coli* expression system is always your first choice because it is fast, easy, and provides extremely high yields. However, sometimes *E. coli* expression fails due to the problem of codon bias. Our **BL21-CodonPlus[®] competent cells*** overcome expression problems caused by codon bias, providing high-level expression of many proteins that are difficult or impossible to express in conventional *E. coli* hosts.

Low or non-existent protein synthesis, early termination, and misincorporation of amino acids in the expressed protein are common symptoms of codon bias, a common problem associated with conventional *E. coli* expression.

Extra Codons Make the Difference

Expression of recombinant proteins in *E. coli* is difficult when the codon use in the recombinant gene differs from the codon use in the host cells. Forced high-level expression of a gene with codons that are rarely used by *E. coli* causes depletion of the of the internal tRNA pools. This leads to delayed translation of the recombinant RNA, resulting in degraded RNA or codon substitutions and misincorporations that destroy the functional characteristics of the protein. To solve this problem, we increased the supply of the rarest codons in *E. coli* to make expression of these proteins possible.

Choose the Right Strain for Your Genome

The problem of codon bias has been most thoroughly documented for the arginine codons AGA and AGG, which are the rarest codons in *E. coli*. However, codons for isoleucine (AUA), leucine (CUA), and proline (CCC) are also known to affect the amount and quality of protein produced in *E. coli* hosts (Table 1). Our BL21-CodonPlus[®] RIL strains contain extra copies of the *E. coli* *argU*, *ileY*, and *leuW* tRNA genes while the BL21-CodonPlus[®] RP strains contain the *argU* and *proL* tRNA genes. Use the –RIL strains for AT-rich genomes and the –RP strains for GC-rich genomes (Figures 1 and 2).

	AGG arginine	AGA arginine	CUA leucine	AUA isoleucine	CCC proline
<i>Escherichia coli</i>	1.2	2.1	3.9	4.4	5.5
<i>Homo sapiens</i>	11.4	11.5	6.5	6.9	20.0
<i>Drosophila melanogaster</i>	6.4	5.1	8.2	9.2	18.0
<i>Caenorhabditis elegans</i>	4.0	15.4	8.0	9.7	4.5
<i>Saccharomyces cerevisiae</i>	9.3	21.3	13.4	17.8	6.8
<i>Plasmodium falciparum</i>	4.1	20.2	15.2	33.2	8.5
<i>Clostridium pasteurianum</i>	2.4	29.4	6.2	50.0	0.9
<i>Pyrococcus horikoshii</i>	30.1	20.1	18.2	44.5	10.2
<i>Thermus aquaticus</i>	14.3	1.3	3.6	1.4	38.8
<i>Arabidopsis thaliana</i>	10.9	18.8	10.0	12.7	5.3

Table 1
Codon Usage in Various Organisms

Codon frequencies are expressed as codons used per 1000 codons encountered. The arginine codons AGG and AGA are recognized by the same tRNA and should therefore be combined. Codon frequencies of more than 15 codons/1000 codons are shown in bold to help identify a codon bias that may cause problems for high-level expression in *E. coli*. These frequencies are updated regularly. A complete compilation of codon usage of the sequences in the gene bank database can be found at www.kazusa.or.jp/codon/.

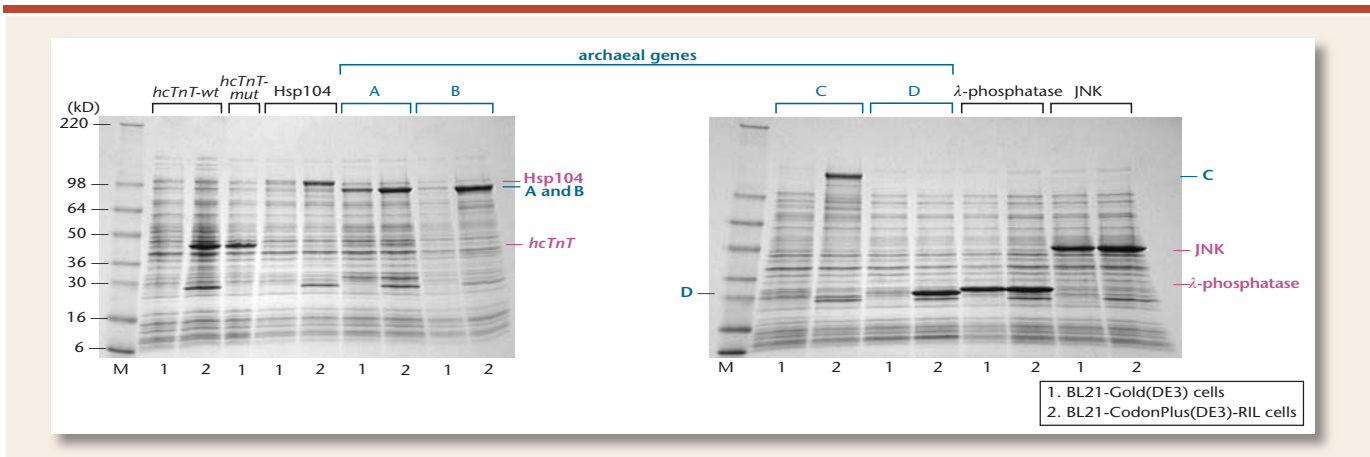


Figure 1
Expression of AT-Rich Genome
 A 1-ml aliquot of each of the indicated cultures was induced at mid-logarithmic growth with 1 mM IPTG for 2 hours. Then, 15 µl of each induced culture was denatured by boiling in reducing loading buffer and loaded on a 4-20% gradient polyacrylamide gel. The separated proteins were then visualized by staining with Coomassie® blue. Selected recombinant genes of human (cardiac troponin-T, *hcTnT*), yeast (Hsp104), or archaeal origin (genes A-D) were expressed in either BL21-Gold(DE3) cells (designated 1) or BL21-CodonPlus®(DE3)-RIL cells (designated 2). Rare arginine codons were removed from the recombinant *hcTnT* gene by

synonymous replacement with arginine codons more commonly used in *E. coli* and expressed in conventional BL21(DE3) cells and designated *hcTnT* 1-mut. λ -phosphatase and JNK (jun-N-terminal kinase) are recombinant genes that are well expressed in conventional BL21-Gold(DE3) cells as well as BL21-CodonPlus(DE3) cells. All recombinant genes were expressed from vectors using a T7 RNA polymerase responsive promoter. The relative positions of the recombinant gene products are indicated. The band visible in all BL21-CodonPlus(DE3)-RIL samples is the ~26 kD product of the chloramphenicol resistance marker gene.

Expression Control Choices

BL21-CodonPlus derivatives offer different levels of expression control with T7 promoter-driven vectors, such as the Affinity® pCAL vectors^{**},^{***} and the pET vectors. The BL21-CodonPlus(DE3)-RIL and (DE3)-RP competent cells are all-purpose strains for high-level protein expression and easy induction with IPTG. When used with the CE6 bacteriophage, the BL21-CodonPlus-RIL and -RP cells provide the tightest control of protein expression, which is important for extremely toxic proteins. In addition, the BL21-CodonPlus-RIL and -RP cells are excellent hosts for non-T7 RNA polymerase expression systems.

*Patent pending.
 See license reference 3 on page 68.
 ** See license reference 4 on page 68.
 *** See license reference 5 on page 68.

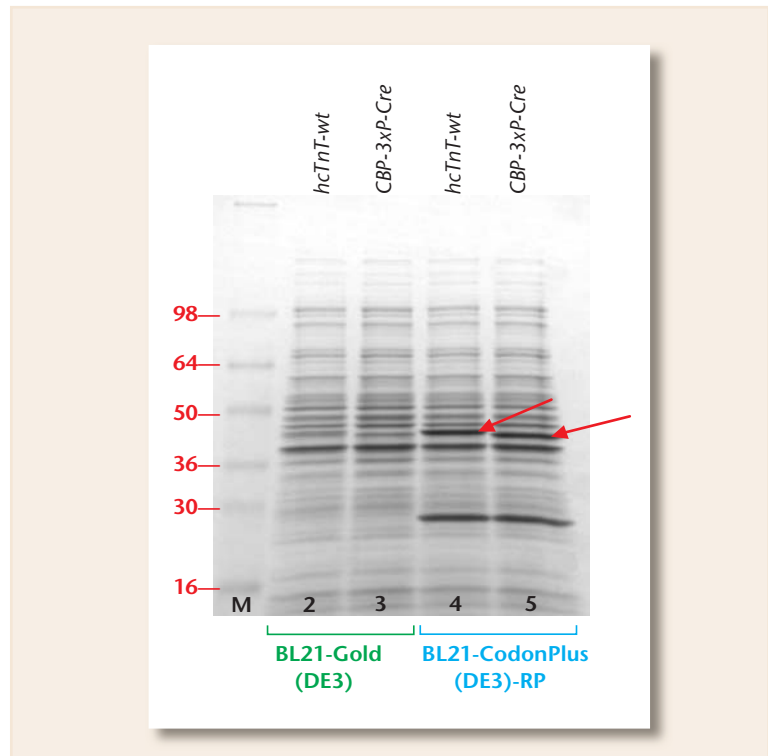


Figure 2
Expression of GC-Rich Genome
 The *hcTnT*-wt (*argU* dependent) or CBP-3xP-Cre (*proL* dependent) test genes were expressed in BL21-Gold(DE3) and BL21-CodonPlus®(DE3)-RP cells. The band around 30 kb is the chloramphenicol-resistance gene.

BL21-CodonPlus® Competent Cells	Quantity	Catalog
BL21-CodonPlus®-RIL Competent Cells	10 x 0.1 ml	#230240
BL21-CodonPlus® (DE3)-RIL Competent Cells	10 x 0.1 ml	#230245
BL21-CodonPlus®-RP Competent Cells	10 x 0.1 ml	#230250
BL21-CodonPlus® (DE3)-RP Competent Cells	10 x 0.1 ml	#230255