

Product Data Sheet

Revised 24/11/99

Product name(s):	pJY2 plasmid
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Catalogue number:	DW 8720	Batch number:	Z02423	Expiry date:	12/00
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Product information:	
<p>5' - AAGCTTGGCTGCAGGTCACCTTGTGATACATGAAAATACGGGTTTTCTTG ATTCAGACGCGCAGCGGTGTGCGTTTGTTCGCGCTATAGCGAAATAA ATCAGAAAATCAGACGCGGTTCACCTTGTTCAGCAACC [AGA---- ATC] AGCCAGCTTGCCAGGTGTGTCTGAGGTCATGGAACGGAAATC TTCAATTCTGCACGACGACAAGCTGATAGCCATGATGCTTGTCTGTC GATGCGCATCTTCTTGACCGCAGGCGTTGATGTTCCATCTGCTCGCT AGCCGCCCTTGGTATGTGACGGATCC - 3'</p> <p>pJY2 map. Top: diagram of vector. Except for the LysS⁵ and argU⁶ genes, the sequence of pJY2 is identical to the sequence of pACYC184. Selected unique restriction sites are shown with their map positions. Bottom: sequences flanking argU gene. Underlined sites are BamHI (5') and HindIII. The start and stop codons of the argU gene are bracketed and shown in bold.</p>	<p>The AGA arginine codon is rarely used in <i>E. coli</i> but is common in eukaryotic genes. The low level of tRNA_{UCU}^{Arg} can lead to low expression, and mis-incorporation of lysine for arginine, during expression of genes containing AGA codons in <i>E. coli</i>^{1, 2}. The chloramphenicol-selectable plasmid pJY2¹ is designed to facilitate the expression of such genes cloned into pET vectors: pJY2 encodes tRNA_{UCU}^{Arg} (to suppress lysine mis-incorporation at AGA codons) and T7 lysozyme (to depress constitutive expression of the cloned gene).</p> <p><i>E. coli</i> cells harbouring pJY2 can be employed to suppress lysine mis-incorporation and achieve high-level expression of pET-encoded target genes without modification of AGA codons in the target gene sequence. For example, pJY2 allows for translationally faithful expression of pET3a-encoded mutant ubiquitins harbouring a high density of AGA codons (up to 14% of total codons)¹.</p> <p>pJY2 can also be used as a source of tRNA_{UCU}^{Arg} with expression systems, such as the pGEX series of vectors, that do not rely on T7 polymerase. The AGG arginine codon is also infrequently utilised in <i>E. coli</i>³. As tRNA_{UCU}^{Arg} decodes AGG when over-expressed³, pJY2 is also likely to ameliorate problems associated with the expression of genes containing AGG codons¹.</p> <p>Recommended use¹: To make a generalised host strain: transform pJY2 into competent <i>E. coli</i> host strain (such as BL21(DE3)) and select transformants on plates containing chloramphenicol (30 µg/ml). Make mineral-competent BL21(DE3)pJY2 cells by standard procedures⁴, and use as host strain for chemical transformation with target gene plasmid.</p>

Analytical and physico-chemical data:	
Purity:	Determined to be ≥95% by agarose gel electrophoresis.
Form:	Supplied at a concentration of 0.4mg/mL in 10mM Tris, 1mM EDTA, pH8.0, i.e. 5µg in 12.5µL.
Solubility:	Soluble in aqueous buffers.

Stability, storage and specific hazard data:	
Store solutions at -20C for up to twelve months.	

References:	
<ol style="list-style-type: none"> You, J., Cohen, R.E. and Pickart, C.M. Construct for high level expression and low misincorporation of lysine for arginine during expression of pET-encoded eukaryotic proteins in <i>E. coli</i>. <i>BioTechniques</i>, 27, 950-954 (1999). Calderone, T. L., Stevens, R.D. and Oas, T.G. High-level mis-incorporation of lysine for arginine at AGA codons in a fusion protein expressed in <i>Escherichia coli</i>. <i>J. Mol. Biol.</i>, 262, 407-412 (1996). Spanjaard, R. A., Chen, K., Walker, J.R. and van Duijn, J. Frameshift suppression at tandem AGA and AGG codons by cloned tRNA genes: assigning a codon to argU tRNA and T4 tRNA(Arg). <i>Nucleic Acids Res.</i>, 18, 5031-5036 (1990). Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Smith, J.A. and Stuhl, K. <i>Current protocols in molecular biology</i>. John Wiley and Sons, New York (1995) Studier, F.W. Use of bacteriophage T7 lysozyme to improve an inducible T7 expression system. <i>J. Mol. Biol.</i>, 219, 37 (1991). Saxena, P. and Walker, J.R. Expression of argU, the <i>Escherichia coli</i> gene coding for a rare arginine tRNA. <i>J. Bact.</i>, 174, 1956-1964 (1992). 	