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A superior host strain for the over-expression of cloned genes using the T7 promoter based vectors

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In recent years the development of novel expression systems using the T7 RNA polymerase to direct the expression of genes under the control of a T7 promoter has been widely reported (1,2). T7 RNA polymerase is very selective for this particular promoter which is rarely found in DNA unrelated to that from the bacteriophage T7 (3). A number of modifications to this expression system have been developed to improve expression of proteins which are deleterious to the host (4). Despite being under the control of the *lac* UV5 promoter, and therefore repressed by the *lac* repressor, basal levels of T7 polymerase are present which can promote low-level transcription of toxic genes and lead to the instability of the host cell line, even with vectors which express *lacI* and include a *lac* operator sequence. Several means of overcoming this problem have been developed. T7 RNA polymerase is effectively repressed by T7 lysozyme and the presence of pLysS, which directs the constitutive expression of this enzyme, maintains tighter control of expression until cells are induced by the addition of IPTG. Despite the ingenuity of the system, absolute control over expression is still not possible but is required for the expression of very toxic enzymes (5). Another method which has allowed the expression of such proteins is the use of the bacteriophage CE6 (2). This is a bacteriphage λ derivative which allows the introduction of the polymerase into non-lysogenic host cells thus preventing uncontrolled expression occurring in the cells. Induction by this method is generally less convenient and the level of expression is often very variable, depending on the level of infection.

The T7 expression vectors require lysogenic *Escherichia coli* host strains such as HMS174(DE3) or BL21(DE3) which can provide the T7 RNA polymerase upon induction with IPTG. The most commonly used strain for this purpose is the B strain BL21(DE3), a non-suppressing host which provides an excellent background for protein expression since it lacks the *lon* and *omp T* proteases.

Here we report the finding that another *E.coli* strain, B834 (6), is a superior host for the propagation and expression of a variety of genes under the control of T7 promoters. Surprisingly BL21 was derived from this parent B834 by transduction to Met^+ (7). The B834 *E.coli* strain is a Met auxotroph and has recently become commercially available as a λDE3 derivative. This strain has been widely used for high specific [^{35}S]methionine-labelling of target proteins and more recently for selenomethionyl derivatisation of proteins for X-ray crystallography (8).

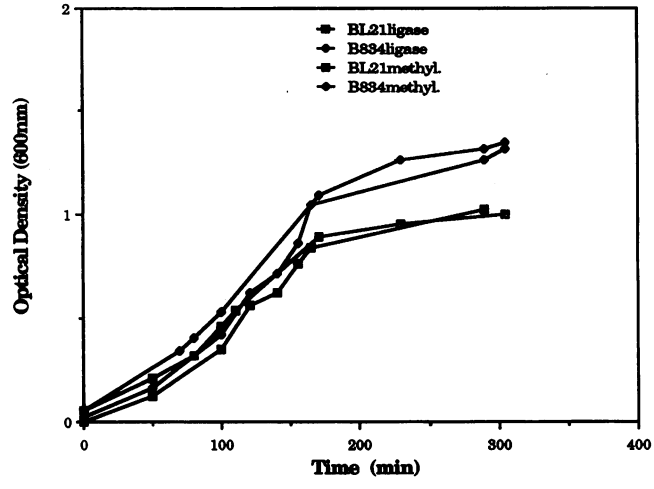

Figure 1. Growth curves of B834(DE3) and BL21(DE3) strains containing either pT7ligase or pT7methyltransferase expression vectors.

We have compared the growth rate and final yield of soluble protein from the host strains B834 and BL21 containing T7 vectors which direct expression of either the T7 DNA ligase or a 19 kDa C-terminal fragment of the *E.coli* O^6-guanosine methyltransferase. The ligase gene product is toxic to the cells and requires the use of pLysS bearing strains to control expression. One litre cultures of Luria broth were grown to an OD_{600} of either 0.5 or 0.8 for ligase and methyltransferase, respectively, and the cells induced by the addition of 1 mM IPTG. The cells were grown for a further 3 h before harvesting. The rate of culture growth was determined by taking samples at various time intervals and measuring the optical density at 600 nm. Figure 1 shows that the host cell lines have similar initial growth rates, but that they show a marked difference shortly after induction. The B834(DE3) cells grow twice as fast as BL21(DE3) which cease growing 1 h after induction. This suggests that B834(DE3) cells are more tolerant of the expressed gene product, a surprising observation considering that the two strains are thought to be almost identical. It is unclear why being Met^- would confer this resilience to the B834(DE3), and it may be that there are other differences between the strains than simply methionine auxotrophy.

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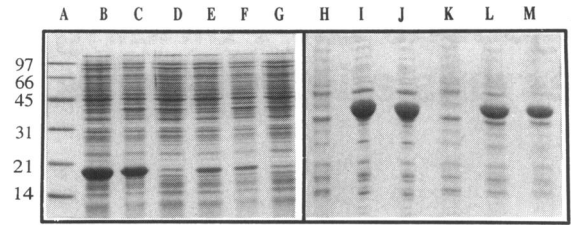

Figure 2. SDS–polyacrylamide gel of cellular proteins. (**A**) Molecular weight markers; (**B**) induced B834(DE3)[pT7meth.] (soluble); (**C**) induced B834(DE3) [pT7meth.] (total); (**D**) uninduced B834(DE3)[pT7meth.]; (**E**) induced BL21 (DE3)[pT7meth.] (soluble); (**F**) induced BL21(DE3)[pT7meth.] (total); (**G**) uninduced BL21(DE3)[pT7meth.]; (**H**) uninduced B834(DE3)[pT7lig.]; (**I**) induced B834(DE3)[pT7lig.] (total); (**J**) induced B834(DE3)[pT7lig.] (soluble); (**K**) uninduced BL21(DE3)[pT7lig.]; (**L**) induced BL21(DE3)[pT7 lig.] (total); (**M**) induced BL21(DE3)[pT7[lig.] (soluble).

Figure 2 shows the relative levels of expressed proteins before and after induction as seen on an SDS–polyacrylamide gel. The difference is very significant especially in the case of the methyltransferase.

The yield of both T7 DNA ligase and DNA methyltransferase was dramatically increased by using B834 instead of the prescribed host strain BL21(DE3). For example, the final yield of T7 DNA ligase in BL21(DE3)[pLysS] is 10–15 mg/l, which increases to ~60–70 mg/l if the host strain is changed to B834(DE3)[pLysS]. Similar results have been observed for other proteins in our laboratories but detailed comparisons have not been made. The increased expression level results from a combination of an improved growth rate after induction and a higher level of production of soluble protein. A broader screen comparing the expression of a wide variety of target genes in this host strain is needed but our results on a number of different proteins suggest that this host strain can greatly enhance the level of expression of genes cloned into the T7 promoter based expression vectors. These findings should have general significance as these vectors are among the most widely used for high level gene expression in *E.coli*.

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