Genlantis
Cells Delivery Expression

SoluBL21™ Competent *E. coli*

SoluBL21 Competent Cells For Toxic Clones

E. coli remains one of the most widely used hosts for the expression of recombinant proteins. Its low cost, rapid doubling time, and the development of sophisticated expression vectors can allow one to inexpensively generate milligram quantities of protein. For the study of single proteins or parallel investigations into whole proteomes, there is the need to produce sufficient quantities of protein from thousands of genes for biochemical analysis, crystallography, and protein interaction studies. As long as eukaryote-specific post-translational modifications are not required, *E. coli* represents the most versatile system.

However, there are limitations to *E. coli* as an expression host. Depending on the source of recombinant genes to be expressed, a large percentage of foreign proteins do not fold correctly; their products become insoluble, aggregating in a form called an inclusion body (for a review, see Reference 1). Recombinant proteins isolated in this form would almost certainly be devoid of their biochemical activity and be unsuitable for many downstream applications or studies. To address this problem, Genlantis has recently released the SoluBL21™ Competent *E. coli* strain, a variant of BL21(DE3), which increases the likelihood that target proteins are synthesized in soluble form. (http://www.genlantis.com/commerce/ccp1779-1029-solubl21-competent-e--coli-sb21_cp18.htm)

A second limitation often encountered is the observation that recombinant proteins can be toxic to the *E. coli* host. Many expression vectors utilize a T7 promoter to express proteins, and since BL21(DE3) contains the T7 RNA polymerase gene on the chromosome, leaky expression of the target protein prior to induction will occur. Depending on the severity of the toxic effect, this can interfere with the establishment of the target clone in the BL21(DE3) host strain or subsequent growth after transformation. In these instances, a more tightly regulated version of the strain, BL21(DE3)pLysS is frequently chosen since it limits the level of uninduced recombinant protein expression. However, some clones remain problematic even in BL21(DE3)pLysS, so other expression strategies must then be considered.

We have evaluated the SoluBL21 host to determine whether clones toxic in BL21(DE3) and BL21(DE3) pLysS would exhibit similar toxicity in this slower growing variant. We demonstrate here that in addition to improving the solubility profile of many recombinant proteins, the SoluBL21 strain also permits the establishment of many clones that are toxic to, and cannot be established in, BL21(DE3)

and BL21(DE3)pLysS. Furthermore, we show that these toxic clones produce substantial quantities of soluble protein when induced.

Results

When creating a T7-promoter based protein expression vector, the ligation mix is commonly transformed into an *E. coli* host that lacks the T7 RNA polymerase gene (such as DH5α). This is performed to limit the possibility that leaky expression of the recombinant protein will interfere with host cell growth and prevent the retrieval of the clone of interest. Once established, the expression clone is then introduced into BL21(DE3) or BL21(DE3)pLysS for production of the protein. Transformation of the ligation mix directly into BL21(DE3) is usually avoided for this reason even though an extra DNA miniprep and transformation step is necessary.

We have used the SoluBL21 electrocompetent *E. coli* cells as the primary cloning host to construct a series of expression vectors using genes from *M. tuberculosis*, Vaccinia virus, and *B. anthracis*. Twelve genes (or epitopes) from each organism were PCR amplified, directly inserted into a topoisomerse loaded vector, and electroporated into SoluBL21 cells.

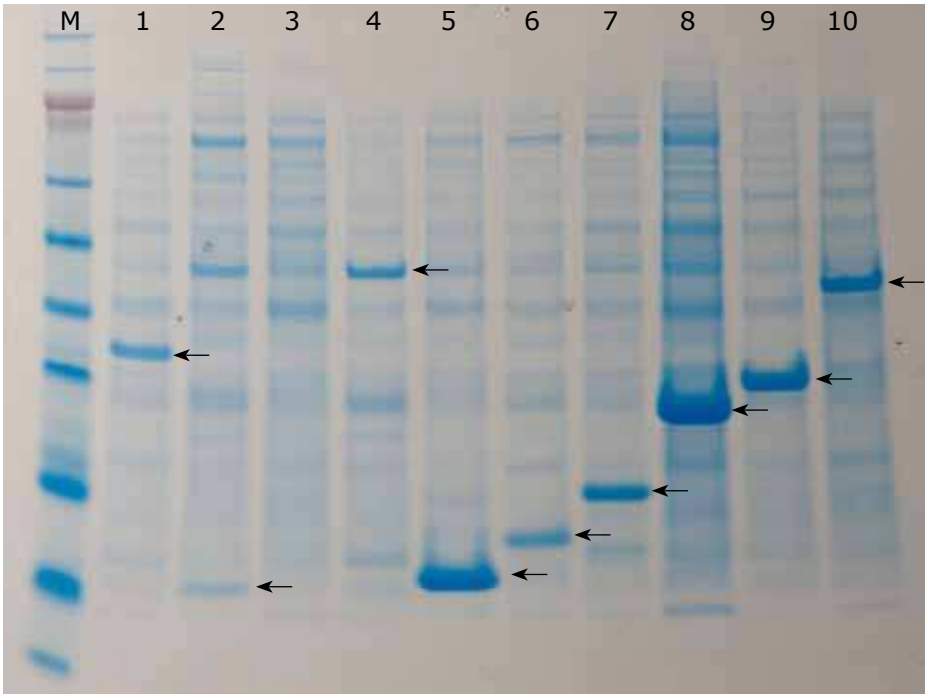
The 36 clones (12 from each organism) were then transformed into standard BL21(DE3) in order to compare expression levels and solubility profiles of these proteins. Of the 36, 10 were unable to form colonies in BL21(DE3) or BL21(DE3)pLysS at either 37ºC or room temperature (see Figure 1). Each of these 10 was readily re-established in SoluBL21 at both temperatures and were able to transform DH5α at 37ºC. The lack of colony formation in BL21(DE3) and the pLysS hosts suggested that small quantities of the products were toxic to these strains. The ability to readily form colonies in SoluBL21 at both room temperature and 37ºC indicated that (1) the proteins were not toxic, (2) expression of the T7 RNA polymerase was more tightly regulated, or (3) that the target proteins were not expressed, and hence, exhibited no toxicity. Since 10 independent proteins from three organisms (two bacterial and a mammalian virus) could be established in SoluBL21, this suggested that the ability to allow propagation of toxic clones is a global effect of the host strain rather than a protein-specific phenomenon. To address the possibility that the proteins were not expressible in SoluBL21 cells, these "toxic" clones were grown and induced following the recommended conditions (M9 minimal media at room temperature). We observed recombinant protein for all 10 clones, with many exhibiting abundant quantities of soluble product (see Figure 2). This indicates that the ability of SoluBL21 cells to withstand the toxic effects of these clones is not achieved by blocking recombinant protein expression. Further studies into the mechanism by which SoluBL21 *E. coli* avoids the clonal toxicity are ongoing. The identities of 6 of these 10 clones are indicated in Table 1.

TABLE 1: Identities of Toxic Clones*

Clone #	Organism	Gene Product
1	*B. anthracis*	FeS assembly ATPase
2	*B. anthracis*	Small acid-soluble sporulation protein
5	*M. tuberculosis*	lppD lipoprotein
6	*M. tuberculosis*	Transcription regulatory protein
9	*M. tuberculosis*	Short chain dehydrogenase
10	*M. tuberculosis*	Sugar transferase
*Identities of clones 3,4,7,and 8 cannot be shown due to confidentiality agreements.		

Figure 1. Transformation of Plasmid DNAs (Toxic Clones) into standard BL21(DE3), BL21(DE3)pLysS and SoluBL21 Competent *E. coli*

Figure 2. Soluble Protein Fractions of "Toxic" Clones in SoluBL 21 (DE3) *E. coli*



Soluble protein fractions were obtained using SoluLyse Bacterial Protein Extraction Reagent (cat # L100125) and purified with Ni-coated magnetic beads. The proteins were electrophoresed through a 4-20% polyacrylamide gel and stained with colloidal blue. Arrows indicate predicted protein (or epitope) migration. Lanes 1-10 correspond to toxic clones 1-10.

Conclusions

Many recombinant proteins can be toxic to *E. coli*, limiting its utility as a protein expression host. Genlantis has recently introduced a BL21(DE3) variant called SoluBL21 that produces a greater quantity of recombinant protein in the soluble fraction, even in clones that were essentially 100% insoluble in wild type BL21(DE3). In addition to this substantial benefit, we have now shown that a number of clones that cannot be established in BL21(DE3) or BL21(DE3)pLysS can readily be established in SoluBL21 cells and produce substantial quantities of soluble recombinant product. This makes SoluBL21 *E. coli* a very versatile host strain that can solve two intransigent protein expression problems simultaneously without any additional effort to re-clone genes or add purification tags.

Reference

1. Dyson, M.R., Shadbolt, S. P., Vincent, K.J., Perera, R.L., and McCafferty, J. (2004). Production of soluble mammalian proteins in Escherichia coli: identification of protein features that correlate with successful expression. BMC Biotechnology 2004, 4:32-49.

Catalog Number	Description	Quantity
C700200	SoluBL21™ Chemically Competent *E. coli*	10 x 50 µl
C700210	SoluBL21™ Electrocompetent *E. coli*	10 x 20 µl

Genlantis
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