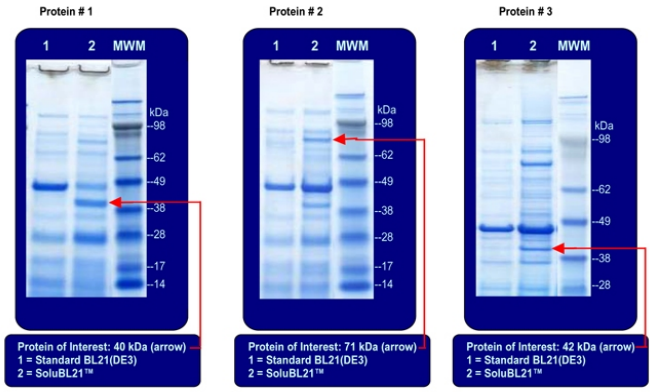
SoluBL21™ Electrocompetent *E. coli*

Using SoluBL21 Electrocompetent Cells as a Primary Cloning Host

The SoluBL21 protein expression strain was developed to increase the probability that a recombinant protein expressed in *E. coli* is produced in soluble form. For many applications such as functional analysis, protein interaction studies, X-ray crystallography, etc., producing soluble recombinant protein is essential.



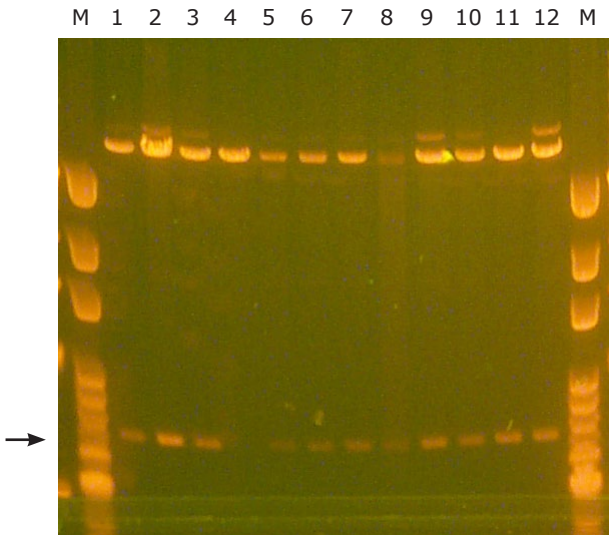
The original testing of the strain was performed using protein expression constructs that were created in DH5α, then subsequently mini-prepped and retransformed into SoluBL21 and standard BL21(DE3) for evaluation. The extra, intermediate step of creating an expression construct in DH5α, then transforming it into the expression host, is commonly done for two reasons: first, the chemically competent transformation efficiency of BL21 strains is significantly lower than DH5α, making recovery of ligated DNA plasmid unlikely; second, because leaky expression of the T7 RNA polymerase may occur in BL21(DE3), many clones are never retrieved due to their toxicity in *E. coli*.

We have addressed both of these limitations with the SoluBL21™ strain. As will be described below, electrocompetent SoluBL21 exhibit higher transformation efficiency compared with chemically competent DH5α. This improvement can result in significant savings when topoisomerase-loaded cloning vectors are used (see below). In addition, the potential for clonal toxicity commonly observed in BL21(DE3) is significantly reduced or eliminated (see SoluBL21 Toxic Clones article.

Results

In order to overcome the low chemically competent transformation efficiency of SoluBL21, we have produced these cells in electrocompetent format with an efficiency of 1 x 10^{10} cfu/μg pUC19 DNA. Using these high efficiency cells results in a significant savings in both time and money compared to chemically competent cells, as illustrated in the following experiment: we constructed a series of protein expression plasmids using PCR-generated DNA inserted into a commercially available topoisomerase-loaded cloning vector that contains a T7 promoter. The recombinant clones could therefore be expressed in SoluBL21 or parental BL21(DE3). The commercially available topoisomerase cloning kit recommends using 1 μl of the topo prepared vector per reaction (50 μl/kit; 50 reactions). When 1 μl of vector was used following manufacturer's protocol for electrocompetent transformation, we obtained > 500 colonies per 1 μl of the ligation mix, with < 50 colonies for the vector only control. Since these many potential clones were superfluous (we found multiple copies of the correct plasmid by screening 8-12 colonies by miniprep), we repeated

Figure 1. Miniprep Analysis of Clones Generated With SoluBL 21 Electrocompetent Cells



Plasmid DNA was digested with EcoR1 to liberate insert and electrophoresed though a 1% agarose gel. Arrow indicates predicted size of insert.

the cloning experiment using 0.2 μl of vector in a smaller reaction volume (see Table 1). This smaller quantity of vector still resulted in 30-200 transformants per 1 μl ligation mix using SoluBL21 electrocompetent cells (with vector only background 0-3 colonies); and multiple copies of the correct plasmid of interest were obtained by mini-prep screening of 8-12 colonies. By using 0.2 μl of the topoisomerase prepared vector, each 50-reaction kit will yield 250 reactions, a 5-fold savings in material costs of the topoisomerase vector. Additionally, since a second round of transformation was obviated by electroporating directly into SoluBL21, this feature will add to the savings both in time and materials.

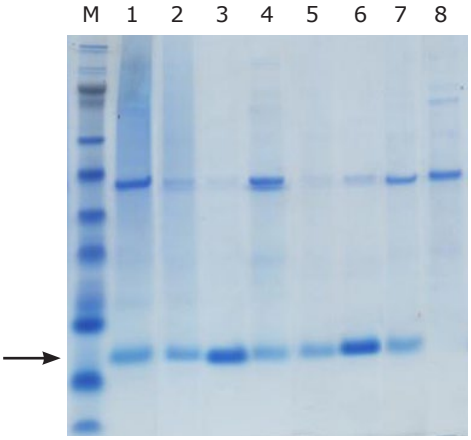
Figure 1 shows miniprep analysis of 12 independent clones obtained from 1 ml of SoluBL21 cells grown overnight in LB at 37°C . The miniprep DNA was digested with *Eco*RI to liberate the insert (if present); 11 of 12 random clones contained the insert of expected size. These plasmids were further screened to determine orientation relative to the T7 promoter.

The 7 properly oriented constructs were then tested for protein expression following the standard SoluBL21 protocol of growth in M9 minimal media, induction with 1 mM IPTG at OD_{600} of 0.4, and overnight expression at room temperature. Cells were lysed using Solulyse reagent (Cat# L100125), recombinant proteins were purified from soluble fractions using magnetic-coated nickel beads, and samples were run on polyacrylamide gels. As shown in Figure 2, all 7 clones expressed soluble protein of the expected size (lanes 1-7). Lane 8 was the SoluBL21 strain without a plasmid.

BL21(DE3) cells are not usually recommended as a primary cloning host due to the leaky expression of the T7 RNA polymerase that resides on the chromosome. This constitutive synthesis of T7 RNA polymerase may result in the production of small quantities of the target protein prior to induction. If this protein product is toxic (or sub-lethal) to the host, this may prevent retrieval of the clone upon transformation or electroporation. When clonal toxicity is observed, the BL21(DE3)pLysS host is frequently chosen since the constitutive level of T7 RNA polymerase is lower and the background levels of the target protein are concomitantly reduced. However, expression in the BL21(DE3)pLysS strain typically results in overall lower protein expression levels - a drawback not seen with the SoluBL21 strain.

In the accompanying article, *SoluBL21 for Toxic Clones*, we present evidence of the ability of SoluBL21 to propagate and robustly express many proteins that could not be established in either BL21(DE3) or BL21(DE3)pLysS at any temperature. This feature eliminates much of the concern when using the expression host as the primary cloning strain.

Figure 2. PAGE Analysis of Soluble Fractions From 8 Clones in SoluBL21 Electrocomp Cells



1.5 ml SoulBL21 cultures were grown in M9 minimal media (+0.3% glycerol) and induced with 1 mM IPTG overnight at room temperature. Soluble fractions were obtained using Solulyse reagent (Cat# L100125) and target proteins purified using Ni-coated magnetic beads. Lanes 1-7: 7 independent clones. Lane 8: Cells only control.

Conclusions

Construction of protein expression plasmids usually goes through a non-expression strain intermediate such as DH5α. We have produced electrocompetent cells of the SoluBL21 expression host that not only results in a highly efficient host strain, but also in a strain able to tolerate toxic clones. The higher efficiency results in great cost savings when using topoisomerase-loaded vector systems and faster throughput in screening expression constructs.

Table 1. Optimized Topoisomerase Cloning Conditions *

Manufacturer Recommendation	Conditions with SoluBL21™
1.0 µl Topoisomerase loaded vector	0.2 µl topoisomerase loaded vector
1.0 µl salt solution (diluted)	0.5 µl salt solution (diluted)
4.0 µl PCR product + H_2O	2.3 µl PCR product + H_2O
*1 µl of ligation mix is recommended for electroporation	

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

1. Dyson, *et. al.* (2004) *BMC Biotechnology* **4**: 32-50.

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