Table 1: Transformation efficiencies of pIVEX vectors into BL21(DE3) strain

Resident plasmid (p15A derivative)	Incoming plasmid (pIVEX vector)	Transformation efficiency (transformants per μg of DNA)[a]	Colony fluorescence[b]
none	pIVEX2.4d	$3.1 \times 10^4 \pm 0.8$	-
none	pIVEX-GFP	$3.5 \times 10^4 \pm 0.6$	85 % F 15 % NF
pLysS	pIVEX2.4d	$4.5 \times 10^4 \pm 0.8$	-
pLysS	pIVEX-GFP	$4.6 \times 10^4 \pm 0.7$	90 % F 10 % NF
pDIA17	pIVEX2.4d	$7.5 \times 10^8 \pm 1.5$	-
pDIA17	pIVEX-GFP	$8.2 \times 10^8 \pm 2.5$	100 % F

a Results are mean of three experiments ± SD on LB plates containing antibiotics.
b Transformation plates containing 100 to 200 colonies were observed under UV illumination, NF; non-fluorescent, F; fluorescent

Some investigations revealed that less than optimal expression in this cell-free system could be explained by the presence of stable secondary structures in mRNAs [4]. Indeed, transcription levels are kept very high due to the highly active T7 RNA polymerase. Consequently, no real coupling of transcription and translation can take place *in vitro*, and unprotected mRNAs may form stable secondary structures, notably in the translation initiation region that inhibit ribosome binding and limit expression levels. There are mRNA folding algorithms that can predict such unfavourable intramolecular secondary structures, but they do not give information about expression levels. Although similar regulatory mechanisms exist in *E. coli* [5], it is simple and useful to assess *in vivo* expression levels from a pIVEX plasmid which gave poor protein yields *in vitro*. The influence of mRNA secondary structures on translation is not identical in both contexts. However, transformation of the most widely used T7 RNA polymerase-producing BL21(λDE3) strain [6] by pIVEX is impaired because the absence of the *lacI* gene, coding the lactose repressor, in these high copy number plasmids. Here, we report how to solve this problem by designing a simple protocol with a compatible plasmid carrying the lactose repressor gene. This method allows the direct comparison of *in vitro* and bacterial expression from pIVEX vectors.

Results and Discussion

Unlike other T7 promoter-based vectors, the pIVEX do not contain a *lac* operator sequence downstream of the T7 promoter. Since expression from pIVEX is not repressed by LacI, they do not contain the corresponding gene. In fact, first attempts to transform, by a standard chemical procedure, the BL21(λDE3) strain with different pIVEX failed since no transformant was obtained on LB ampicillin-agar plates. On the assumption that basal expression from pIVEX may have adverse effects on bacterial growth, we decided to test various plasmid/host combinations in order to control more tightly transcription both at the *lacUV5* promoter of the T7 RNA polymerase gene in the host chromosome, and consequently at the T7 promoter in the pIVEX plasmid. Furthermore, other investigations indicated that the transformation efficiency of this *E. coli* B strain could be critical [7]. Therefore, we electroporated freshly BL21(λDE3) competent cells containing either the pLysS plasmid encoding T7 lysozyme [8], a natural inhibitor of T7 RNA polymerase, or the pDIA17 plasmid [9] harboring the *lacI* gene. Both resident plasmids are chloramphenicol resistant and compatible with pIVEX since they carry the origin of replication from plasmid p15A. To facilitate the analysis of expression among the recombinants, a pIVEX-GFP plasmid encoding the green fluorescence protein, GFP, was used for fluorescence screening of single colonies on agar plates. As a control for toxic expression, the same strains were also transformed with an empty pIVEX plasmid (pIVEX2.4d).

The results presented in Table 1 show that 1) the presence of the cloned gene coding GFP into pIVEX has no influence on transformation efficiency, 2) pLysS did not increase the tolerance of BL21(λDE3) for either of the pIVEX plasmids employed, and 3) in contrast, pDIA17 increases the transformation efficiency by 4 orders of magnitude. These results suggest that the basal production of T7 RNA polymerase in BL21(λDE3) was incompatible with a high-copy number plasmid which does not encode the *lacI* gene, even without a cloned gene downstream the T7 promoter. Yet, a substantial increase in lactose repressor from pDIA17 in BL21(λDE3) cells is sufficient to permit the presence of pIVEX.

Furthermore, the production of GFP could be directly assessed from the transformation plates. At 37°C in the absence of IPTG, GFP production from pIVEX-GFP, after overnight cell growth on solid LB medium, was sufficient to give fluorescent colonies under these conditions. These