Non-fusion proteins expressed in *E.coli*: detection by α -complementation (blue/white selection)

Kojo Mensa-Wilmot* and Paul T.Englund

Department of Biological Chemistry, The Johns Hopkins School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205, USA

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Blue/white color selection, based on insertional inactivation of β -galactosidase (1), is a powerful tool for DNA cloning in *E. coli*. However, proteins expressed from such recombinants are fusion proteins. Although these are very valuable, non-fused proteins are much more desirable for many biochemical experiments. We describe here a general method for expression of non-fused proteins which utilizes blue/white selection.

Using PCR (2), the 5' non-coding region of a full length *Trypanosoma brucei* glycosyl phosphatidylinositol-specific phospholipase C (GPI-PLC) cDNA (3) was replaced by prokaryotic signals for translation initiation and enhancement. The upstream PCR primer (Figure 1A) contains a *Bam*HI site (GGATCC), an appropriately spaced putative translational promoter (GATCC) for highly expressed *E. coli* proteins (4), the λ *cro* Shine-Dalgarno sequence (AGGAGG), a modified *lacZ* spacer region (CAGCTAA), and 12 nucleotides (bold) of the GPI-PLC coding sequence; there are also translational termination codons (TAA; outlined) to block synthesis of any protein encoded upstream of the GPI-PLC gene. The downstream primer (Figure 1A) contains 21 nucleotides complementary to the 3' end of GPI-PLC coding sequence (bold), a *Hind*III site, *Hind*III cleavage.

Functional capacity of the regulatory elements in the upstream primer was established when GPI-PLC encoded by a similar PCR product was expressed in *E. coli*, under control of the λ *P_L* promoter, after cloning into pRLM76 (from J.D.Roberts and R.McMacken, a derivative of pHE6 (5)). Data available on request.

After PCR for 25 cycles the product was digested with *Bam*HI and *Hind*III and cloned into pBluescript SK +/- (Stratagene). The resulting construct, shown in part in Figure 1B, was transformed into *E. coli* DH5 α . Transcription from the β -galactosidase promoter (Figure 1B) should give rise to a dicistronic mRNA encoding two distinct proteins: one is a shorter form of the α fragment of β -galactosidase, truncated by an in-frame termination codon introduced by the upstream PCR primer (see Figure 1); and the other is full length GPI-PLC. White clones in a background of blue were selected, grown and induced with IPTG (1). After SDS-PAGE of all lysates (5) GPI-PLC was detected by Western blotting (6). A 39 kDa immunoreactive protein was produced, e.g. in PGPIPLC-08, which was identical in mobility to that of the *T.brucei* enzyme and to the product

in cells containing pRLM76-36. A fusion protein containing β -galactosidase sequences (predicted 43 kDa) was not detected. With a similar construct we have produced enzymatically active GPI-PLC (in preparation). This cloning method should be adaptable for expression of other authentic (non-fusion) proteins in *E. coli*.

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Figure 1. Panel A. Scheme for inserting *E. coli* translation signals upstream of *T. brucei* GPI-PLC gene by PCR. See text for explanation. **Panel B.** Plasmid construct encoding truncated (α -fragment) and full length GPI-PLC. *Hind*III and *Bam*HI double-digested PCR product was cloned into pBluescript SK +/- to generate pGPIPLC-08. *P_{LacZ}*, *LacZ* promoter; RBS, ribosome binding site and other translational control elements.

*To whom correspondence should be addressed at: Department of Zoology, 724 Biological Sciences Building, The University of Georgia, Athens, GA 30602, USA