An efficient expression vector (pPLEX) for bacterial synthesis of non-fusion proteins and protein domains

Georg Sczakiel*, Alfred Wittinghofer and Jane Tucker¹

Abteilung Biophysik, Max-Planck-Institut für medizinische Forschung, Jahnstr. 29 and ¹Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-6900 Heidelberg, FRG

Submitted December 30, 1986

An *E. coli* plasmid vector (pPLEX) for the bacterial expression of non-fusion proteins and protein domains was constructed. Depending on the restriction sites used for the insertion of coding DNA, the resulting polypeptides do not have any, or only a minimal amount of additional vector-derived amino acids. Thus biochemical and structural properties of expressed proteins or protein domains should not be affected.

Construction of pPLEX was as follows: the completely HindIII and partially EcoRI digested plasmid ptac-rasv (1) was ligated with synthetic deoxyoligonucleotides, carrying the translational start and stop signals and unique restriction sites. Subsequently the ptac promoter was replaced by the phage λ P₁ promoter, which also changed the sequence of the ribosomal binding site to that of plasmid pPlc245 (2). **Description:** Downstream of the strong and temperature inducible λ P₁ promoter (3) are located the ribosomal

binding site and the translational start codon (ATG) which is part of an NcoI site. Unique restriction sites (NcoI, SalI, AccI, HindIII, HpaI, BclI) follow. Stop codons in all three reading frames and the λ P₁ terminator are upstream of the galactokinase gene from pKM-1 (4). The latter is not detectably expressed in the uninduced state, whereas in the induced state high amounts of galactokinase are produced as detected by SDS-PAGE. This allows one to monitor the effectiveness of the promoter during induction. A blunt-ended coding DNA sequence may be connected in frame with the ATG by cutting pPLEX with NcoI, AccI or SalI and

filling up the recessed 3'-ends with Klenow-fragment. pPLEX was used to express large quantities of an internal portion of the rabbit skeletal muscle LMM in *E. coli* (>10% of soluble proteins). pPLEX was used also to express mouse β_2 microglobulin in *E. coli*. Both expression products were isolated from the soluble protein fraction (manuscripts in preparation).

References

- (1) Tucker, J., Sczakiel, G., Feuerstein, J., John, J., Goody, R.S. and Wittinghofer, A. (1986) *EMBO J.* **5**, 1351-1358.
- (2) Remaut, E., Stanssens, P. and Fiers, W. (1983) *Nucl. Acid. Res.* **11**, 4677-4688.
- (3) Remaut, E., Stanssens, P. and Fiers, W. (1981) *Gene* **15**, 81-93.
- (4) deBoer, H.A., Comstock, L.J. and Vassen, M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 21-25.

*to whom correspondence or plasmid requests should be addressed