Volume 15 Number 9 1987 Nucleic Acids Research

pBD7, a novel cell-free expression vector with efficient translation initiation signal

Bimalendu Dasmahapatra, Edward J.Rozhon and Jerome Schwartz

Department of Antiviral Chemotherapy, Research Division, Schering Corporation, Bloomfield, NJ 07003, USA

Submitted February 27, 1987

A plasmid vector for the cell-free expression of genes or gene segments which lack the translation initiation signal is presented. The expression plasmid pBD7 (see Figure), uses the strong T7 promoter (1) to direct transcription, and the highly efficient translational start signal of black bettle virus (BBV) RNA1 (2) to initiate protein synthesis. In addition, plasmid pBD7 contains several unique restriction sites immediately after the initiator ATG to provide multiple cloning sites for genes or gene segments to be expressed. The chimeric RNAs produced will, therefore, have a built-in efficient translation start signal fused to the coding sequence of the gene to be expressed. Plasmid pBD7 has been used successfully in the cell-free expression of biologically active coxsackievirus 3C protease and also the functionally active β-galactosidase (3). The translational efficiences of eucaryotic messenger RNAs are regulated by 5' untranslated leader sequences of m-RNAs, the site where eucaryotic ribosomes bind and initiate protein synthesis (4). The molecular basis of this phenomenon is not well understood. Some eucaryotic m-RNAs and most procaryotic m-RNAs are not efficiently translated in cell extracts containing eucaryotic ribosomes (5). The plasmid pBD7 offers, for the first time, a unique opportunity for the cell-free expression of (i) a gene or a gene segment, or a part of polyprotein, lacking its own translation initiation signal, or (ii) a gene containing an inefficient translation initiation signal.

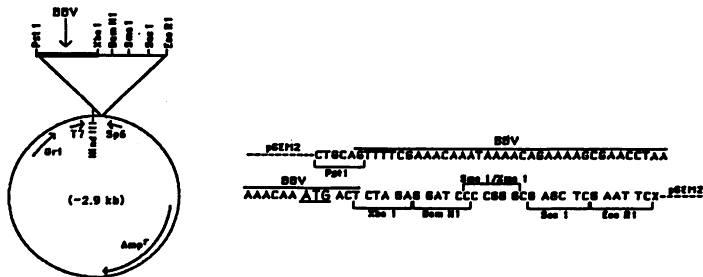

Diagram of pBD7 and the sequence of the 5' untranslated region of BBV RNA1 and those of multiple cloning sites. The BBV sequence (highlighted under the line), derived from the plasmid p1B9SP (5), is engineered and cloned into the pGEM-2 plasmid (Promega Biotech. Inc., Madison, WI). T7 and SP6 promoters and their polarities are shown by the arrows.

Refs
1. Chamberlin, M. et. al. (1970) Nature 228, 227-231.
2. Dasmahapatra, B. et al. (1985). J. Mol. Biol. 182, 183-190.
3. Dasmahapatra, B. et. al. (Manuscript in preparation).
4. Kozak, M., Microbial Rev. (1983) 47, 1-45.
5. Dasmahapatra, B. et. al. (1986) Proc. Natl. Acad. Sci. USA 83, 63-66.