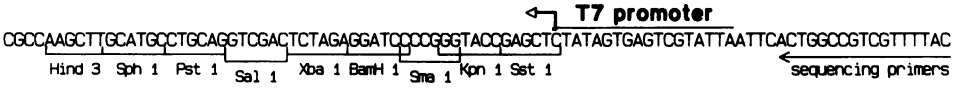
Volume 16 Number 17 1988 Nucleic Acids Research

A plasmid vector for cloning directly at the transcription initiation site of a bacteriophage T7 promoter

Ian T.D.Petty

Department of Plant Pathology, University of California, Berkeley, CA 94720, USA
Submitted June 14, 1988

The polylinker sequences in available plasmid vectors containing the bacteriophage T7 promoter are located several base-pairs from the transcription initiation site (TIS). For some applications eg. *in vitro* synthesis of biologically active viral RNAs (1,2), it is necessary to produce transcripts which have a minimal number of extra 5' nucleotides. Plasmid vectors which contain the SP6 promoter with either *Stu*I (2) or *Bam*HI (3) sites at the TIS have been described. I have now constructed a multifunctional plasmid, pT7E19(+), which has an *Sst*I site at the TIS of the T7 promoter. The 2.8 kb plasmid is a pUC19 derivative which has the bacteriophage f1 replication origin from pTZ18U (United States Biochemical), and has a T7 promoter inserted into the polylinker. The oligo insertion destroyed the *Eco*RI site, but maintains the *lac*Z' reading-frame, allowing colour screening of transformants with XGal. The figure shows the sequence of the ssDNA encapsidated after superinfection with M13K07 (4), the TIS and direction of transcription are indicated by the angled arrow. DNA fragments can be cloned at the TIS by removing the *Sst*I 3'-ends with T4 DNA polymerase (1 extra 5' G transcribed), or by over-digestion with mung bean nuclease (3) (no extra 5' nucleotides). Dideoxy sequencing from universal primers proceeds in the direction of *in vitro* transcription, facilitating analysis of the junction between the promoter and a cloned fragment.



ACKNOWLEDGEMENTS

This work was supported by USDA grant #85-CRCR-1-1777.

REFERENCES

(1) Janda, M. *et al.* (1987) Virology 158, 259-262.
(2) Bujarski, J.J. & Kaesberg, P. (1987) Nucl. Acids. Res. 15, 1337.
(3) Kang, C. & Wu, C-W. (1987) Nucl. Acids. Res. 15, 2279-2294.
(4) Vieira, J. & Messing, J. (1987) Methods Enzymol. 153, 3-11.