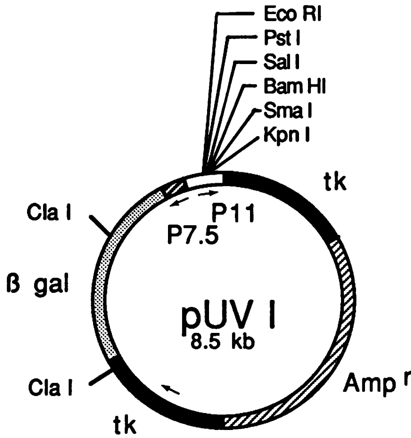
Volume 15 Number 17 1987 Nucleic Acids Research

pUV I: a new vaccinia virus insertion and expression vector

Falko G.Falkner, Sekhar Chakrabarti* and Bernard Moss

Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892, USA
Submitted July 30, 1987

A plasmid vector, that can be used for the convenient formation of recombinant vaccinia viruses which express foreign genes behind the strong late promotor of the vaccinia virus 11K gene (1), was constructed. The 500 base pair Cla I-Eco RI fragment upstream of the 11K gene was used as the P11 promotor. The initiation codon, derived from the 11K gene, is followed immediately by a series of restriction endonuclease sites each of which, except for Xba I, can be used as a unique cloning site. The Escherichia coli ß-galactosidase gene, regulated by the early/late promotor of the vaccinia virus 7.5K gene (2), is oriented opposite to the P11 promotor. Thymidine kinase (tk) gene sequences flank the two expression units. The structure of the plasmid and the sequence of the initiation codon and the restriction sites are shown below.



DNA containing a continous open reading frame followed by a termination codon may be ligated into one of six indicated restriction endonuclease sites in the 8.5 kilobase pair plasmid.

Insertion into vaccinia virus by homologous recombination, selection for tk⁻ recombinant virus, and blue colour plaque screening with ß-galactosidase indicator can be carried out as described (3). The pUV I vector has been used in our laboratory over the past year to express a variety of foreign genes and the derived sequence of the plasmid has been submitted to the GenBank.

```
                                                   Kpn
P11 Promotor-ATG AAT TCC TGC AGG TCG ACT CTA GAG GAT CCC CGG GTA CCG TCG ACC
                 Eco RI    PstI    SalI  XbaI    BamHI  SmaI            SalI
```

*Present address: Institute of Immunology, New Delhi, India

References:
1. Bertholet, C., Drillien, R. and Wittek, R. (1985) Proc. Natl. Acad. Sci. USA 82, 2096-2100. 2. Cochran, M. A., Puckett, C. and Moss, B. (1985) J. Virol. 54, 30-37. 3. Chakrabarti, S., Brechling, K., and Moss, B. (1985) Mol . Cell. Biol. 5, 3403-3409.