Davison, A.J., and Moss, B. (1990) New vaccinia virus recombination plasmids incorporating a synthetic late promoter for high level expression of foreign proteins. Nucleic Acids Research, 18 (14). p. 4285. ISSN 0305-1048

Copyright © 1990 Oxford University Press

A copy can be downloaded for personal non-commercial research or study, without prior permission or charge

The content must not be changed in any way or reproduced in any format or medium without the formal permission of the copyright holder(s)

When referring to this work, full bibliographic details must be given

http://eprints.gla.ac.uk/72374

Deposited on: 19 December 2012

Enlighten – Research publications by members of the University of Glasgow
http://eprints.gla.ac.uk

New vaccinia virus recombination plasmids incorporating a synthetic late promoter for high level expression of foreign proteins

Andrew J.Davison[1] and Bernard Moss

Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA and [1]MRC Virology Unit, Institute of Virology, Church Street, Glasgow G11 5JR, UK

Submitted June 11, 1990

Vaccinia virus has found considerable utility as a vector for expressing foreign genes in eukaryotic cells. Foreign genes may be regulated by early or late vaccinia promoters, but much higher levels of expression may be achieved with the latter (1, 2). Based on a detailed analysis of vaccinia late promoters, we designed a synthetic promoter that is at least half as strong again as the most active poxvirus natural late promoters presently used in expression systems (3). We describe here a pair of recombination plasmids incorporating this powerful late promoter.

The plasmids, pMJ601 and pMJ602, are 7.2 kbp in size, and comprise a cassette inserted into the EcoRI site of the vaccinia virus thymidine kinase (TK) gene, which is carried on a portion of pBR328 that includes the betalactamase gene. They were made by insertion of synthetic oligonucleotide duplexes into a parental plasmid, pMJ7 (4). The cassette consists of the powerful late promoter immediately upstream from a multiple cloning site (MCS) containing 11 unique restriction endonuclease sites and lacking ATG codons, followed by *lac*Z under the control of the vaccinia 7.5-kD early promoter. The two plasmids differ only in the orientation of the MCS. Features of pMJ601 and the sequence upstream of *lac*Z are shown in Fig. 1. The mRNA starts within the indicated AAA sequence, and contains a poly(A) leader.

A DNA fragment containing a continuous ATG-initiated open reading frame may be inserted into the MCS, and the resulting cassette recombined into the vaccinia virus genome at the TK locus (4). Recombinant virus may be isolated by selecting for lack of TK expression and by screening for expression of betagalactosidase from *lac*Z (4). We have used pMJ601 and pMJ602 to construct several recombinant viruses that express herpesvirus proteins. As an example, Fig. 2 shows the proteins expressed by two recombinant viruses. The virus made using the parental plasmid pMJ601 expressed betagalactosidase at a high level (lane 1) because, in the absence of an insert, *lac*Z is regulated by both the powerful late and 7.5-kD early promoters. The virus made using the 3′ portion of varicella-zoster virus gene 61 inserted into pMJ601 expressed a much lower level of betagalactosidase (lane 2), as *lac*Z is regulated only by the 7.5-kD early promoter. This virus also synthesized an abundant novel protein comprising a carboxy terminal fragment of the gene 61 protein. The level of expression of a foreign protein by a recombinant virus is unpredictable, since it depends on the inserted sequences. Indeed, in some cases we have been unable to isolate recombinant viruses, presumably because expression of certain proteins at high levels is lethal to the growth of vaccinia virus.

The plasmids and their DNA sequences may be obtained from Dr. Bernard Moss.

ACKNOWLEDGEMENTS

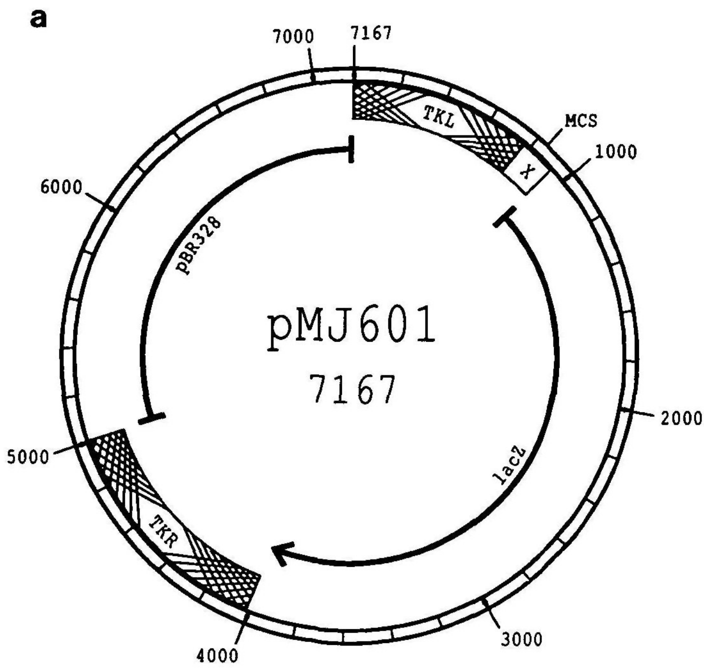
We are grateful to David Stevenson for constructing the gene 61 insertion plasmid and to Charles Cunningham for supplying the autoradiograph.

REFERENCES

1. Falkner,F.G. and Moss,B. (1988) *J. Virol.* **62**, 1849–1854.
2. Patel,D.D., Ray,C.A., Drucker,R.P. and Pickup,D.J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 9431–9435.
3. Davison,A J. and Moss,B. (1989) *J. Mol. Biol.* **210**, 771–784.
4. Davison,A.J. and Moss,B. (1989) *J. Mol. Biol.* **210**, 749–769.
5. Davison,A.J. and Scott,J.E. (1986) *J. Gen. Virol.* **67**, 1759–1816.

Downloaded from http://nar.oxfordjournals.org/ at University of Glasgow on December 19, 2012

a



b

```
       synthetic late promoter    mRNA>>>
AATTGGATCAGCTTTTTTTTTTTTTTTTTTGGCATATAAATAAG

SalI  SmaI  AflII NarI  BspMIIBamHI ApaI  NheI
GTCGACCCCGGGCTTAAGGGCGCCTCCGGAGGATCCGGGCCCGC

    SacII KpnI  HindIII          7.5-kD early
TAGCCCGCGGGGTACCAAGCTTCGACAAGCTCGTAAAAGTAGAA

promoter      mRNA> >XhoI
AATATATTCTAATTTATTGCACTCGAGCATG - lacZ
```

Figure 1 (a) Features of pMJ601. Vaccinia virus DNA sequences are shaded, and comprise sequences upstream of and downstream from the EcoRI site in the TK gene (TKL and TKR, respectively). Region X contains the synthetic late promoter, the MCS and the early promoter for *lacZ*, as shown in Fig. 1(b). **(b)** DNA sequence of region X in Fig. 1(a), which is located between the EcoRI site in the vaccinia virus TK gene and the initation codon for betagalactosidase. The MCS is underlined. It is inverted in pMJ602, and thus all the cloning sites are in reverse order.

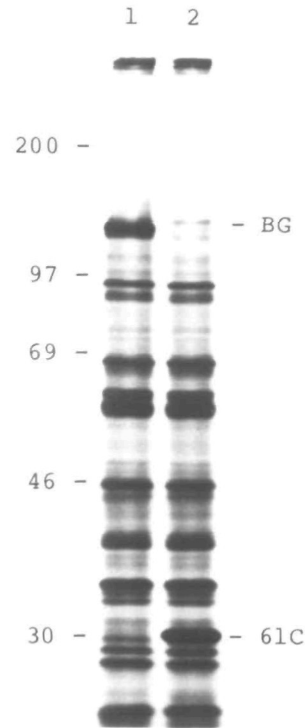


Figure 2. Autoradiograph showing proteins expressed by two recombinant vaccinia viruses. Monolayers of CV-1 cells were infected at a multiplicity of 10 p.f.u per cell and labelled with [^{35}S]-methionine for 48 h. Aliquots of lysed cells were then subjected to SDS-PAGE. Lane 1 shows the result for a virus made using the parental plasmid pMJ601, and lane 2 shows that for a virus made using the 3′ portion of varicella-zoster virus gene 61 (5) inserted into pMJ601. The inserted sequence was arranged so that protein synthesis initiates at an AUG codon that is normally located internally, and in frame, in the gene 61 mRNA. The positions of molecular weight markers ($\times$ 10^{-3}) are shown on the left, and bands representing betagalactosidase (BG) and the carboxy terminal fragment of the gene 61 protein (61C) are indicated on the right.

Downloaded from http://nar.oxfordjournals.org/ at University of Glasgow on December 19, 2012