A single-stranded insertion vector (SSIV) for introducing foreign genes into the vaccinia virus genome

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We have previously reported that single-stranded DNA from recombinant M13 phage can be used to direct the targeted insertion of heterologous sequences into the genome of infectious vaccinia virus in a transcriptionally active configuration (VV,1). Taking advantage of this observation, we have constructed a generic M13-based insertion vector whose structure is shown below. Foreign DNA can be inserted into M13 SSIV RF, transformed into E. coli, and the appropriate recombinant phage grown up as the source of ssDNA for recombinational insertion of the foreign DNA into VV. The main advantages of this vector system are the ability to clone in phage instead of plasmids, higher marker transfer efficiencies, and the suitability of ssDNA to serve as template for sequencing and site-directed mutagenesis procedures.

The polylinker region of M13mp18 was removed using EcoRI and HindIII restriction endonucleases. The sticky-ends were filled in and a 1311 base-pair DNA fragment (HindIII - HpaII) containing the vaccinia virus thymidine kinase (TK) gene was inserted (2). The VV 7.5Kd promoter abutted to a multiple cloning site (MCS) was inserted into the EcoRI site at position 771 in the middle of the VV TK gene (3). The restriction sites indicated in bold type are unique and can be used for the insertion of foreign genetic material.

REFERENCES
1. Wilson et al. (1986) Gene 49, 207-213.
2. Rice et al. (1985) J. Virol. 56, 227-239.
3. Hruby et al. (1983) Proc. Natl. Acad. Sci. USA 80, 3411-3415.