Use of the gene encoding neomycin phosphotransferase II to convert linked markers into the vaccinia virus genome

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It has previously been demonstrated that vaccinia virus (VV) recombinants containing the the bacterial neomycin phosphotransferase II (NEO) gene can be selected by virtue of their resistance to the antibiotic G418 (1). To test whether this property could be utilized to direct the co-insertion of unselected markers into the VV genome, recombinant co-expression plasmids containing both chloramphenicol acetyltransferase (CAT) and NEO genes, abutted to separate VV promoter elements [the F and 7.5 Kd promoters, respectively (2)], were constructed such that the two chimeric gene cassettes were closely-linked but in four different orientations with respect to one another, and embedded within the sequences encoding the VV thymidine kinase (tk) gene. The CAT gene was converted into the VV genome by marker transfer (2) using G418 to select for acquisition of the dominant neomycin resistant phenotype. The genomic structure of the viral recombinants were confirmed by both restriction enzyme mapping and nucleotide sequencing procedures.

VIRUS	GENOME STRUCTURE	PERCENT G418 RESISTANCE	RELATIVE CAT ACTIVITY
VV:WT	tk gene →	0.03	1
VV5.1:CAT	7.5 CAT →	-	116
VV5.1:NEO	7.5 NEO →	59	-
VV:7NCF	7.5 F → CAT ← F →	48	147
VV:7NFC	7.5 F → F ← CAT →	135	132
VV:FC7N	F → CAT → 7.5 F →	85	160
VV:CF7N	CAT → F → 7.5 F →	53	162

LEGEND	BACTERIAL CHLORAMPHENICOL ACETYL TRANSFERASE (CAT) GENE LINKED TO VV EARLY F PROMOTER (F) PLUS NON-ESSENTIAL UPSTREAM VV DNA SEQUENCES (ZZZ)	BACTERIAL NEOMYCIN PHOSPHOTRANSFERASE GENE (NEO) LINKED TO VV 7.5 Kd EARLY/LATE PROMOTER (7.5)

Independent of gene order or orientation, the VV transcriptional apparatus was apparently able to express both gene products in a functional form as judged by ability of each of the viral double recombinants to grow in the presence of G418 as well as expressing high levels of CAT enzyme (3), relative to either wild-type VV or control single recombinants. These results indicate that (i) the expression of linked foreign genes in the context of the VV genome is not sensitive to transcriptional interference from neighboring loci, and (ii) that the selectable G418/NEO co-expression system should be of use in the construction of recombinant VV strains of interest.

REFERENCES

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