Evaluation of an authentic bi-directional promoter in a new transfer vector for generating lumpy skin disease virus recombinants

BY

NADINE VOS

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dedicated to my beloved husband, Nico

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EVALUATION OF AN AUTHENTIC BI- DIRECTIONAL PROMOTER IN A NEW TRANSFER VECTOR FOR GENERATING LSDV RECOMBINANTS

ABSTRACT

Lumpy skin disease virus (LSDV), a member of the *Capripoxvirus* genus, is one of the poxviruses currently being investigated as a live recombinant system for expressing foreign genes. The strict tropism of the virus, limited to ungulates, renders it very promising as a vector for veterinary vaccine purposes. Poxvirus recombinants are generated by a process of homologous recombination, mediated by using specific transfer vectors. In this study a new transfer vector pHSWF was evaluated for its ability to generate stable LSDV recombinants that would express foreign genes from an authentic bi-directional LSDV promoter.

A reporter gene, the *E. coli LacZ*-gene encoding the β-galactosidase (β-gal) enzyme, was used in initial studies. It was placed under control of either the early or the late promoter by constructing two different transfer plasmids pHSWF/*Lac*E and pHSWF/*Lac*L. These were used to generate LSDV/*Lac*E and LSDV/*Lac*L recombinants in fetal bovine testis (FBT) cells. Southern blot analysis confirmed that the *LacZ*-gene was stably integrated into an intergenic region of the LSDV genome. Expression of the *LacZ*-gene from both the early and late promoters was confirmed by monitoring the β-gal enzyme activity in infected cells. Similar intensities of blue stained foci, indicative of β-gal activity, was observed in FBT-cells infected with the respective recombinants. This suggested that similar high levels of expression were induced by both the early and the late promoters. The results on the *LacZ* recombinants confirmed that the new transfer vector may indeed be used to generate stable recombinants and that foreign genes are expressed at high levels.

The transfer vector was then used to generate LSDV recombinants that express an immunological important gene, the VP7 gene of AHSV from the LSDV early promoter. The VP7 protein encodes the major group-specific antigen of AHSV and is a possible candidate for use in recombinant vaccines. Expression of the VP7 protein in the recombinant LSDV system was confirmed by SDS-PAGE analysis and radioactive labelling of proteins in infected cells. Characteristically, high levels of VP7 expression leads to its assembly in flat hexagonal crystals in infected cells. This property was analysed by cryoelectron microscopy. Crystal formation was indeed observed, confirming

that high levels of VP7 expression occurred in the LSDV recombinant. These results further supported the fact that the LSDV early promoter has the ability to induce high levels of foreign gene expression in the LSDV system.

The new transfer vector was also tested for its ability to generate dual LSDV recombinants that would express foreign genes from both the early and late promoter in the same recombinant. An existing plasmid vector in which the VP2 gene of AHSV was cloned under control of the late promoter was modified by placing the VP7 gene under control of the early promoter. The construct was used to generate dual recombinant viruses. PCR results confirmed that both genes were integrated into the LSDV genome, however no expression of VP2 could be observed.

In summary, this study has contributed to our knowledge of using LSDV as a live expression system. The new transfer vector allows the integration of foreign genes into a non-essential site within the LSDV genome. This procedure, whereby no LSDV genes are disrupted, should not debilitate the already attenuated LSDV virus any further. Foreign genes may be expressed from both an early and / or late promoter. The high levels of expression from the early promoter is promising for using LSDV recombinants in non-host species where the recombinants are unlikely to complete their viral replication cycle.

ABBREVIATIONS

AHS	african horsesickness
AHSV-9	african horsesickness virus serotype 9
ATG	adenosine thymidine guanosine
ATP	adenosine triphosphate
ATV	activated trypsin versene
bp	base pairs
BTV	bluetongue virus
BudR	bromodioxyuridine
β-gal	β-galactosidase
βME	βeta-mercapto-ethanol
$CaCl_2$	calcium chloride
ccc	covalently closed circular
cDNA	complementary deoxiribonucleic acid
Ci	Curie
cm	centimeter
cm^2	centimeter square
CO_2	carbon dioxide
CPE	cytopathic effect
cpm	counts per minute
CTL	cytotoxic T lymphocytes
°C	degrees Celcius
dATP	2'-deoxyadenosine-5'-triphosphate
dCTP	2'-deoxycytidine-5'-triphosphate
dGTP	2'-deoxyguanosine-5'-triphosphate
dTTP	2'-deoxythymidine-5'-triphosphate
dUTP	2'-deoxy-uracil-5'-triphosphate
ddH_2O	double distilled water
DEPC	diethylpyrocarbonate
dH_2O	distilled water
DIG	digoxigenin
DMEM	Dubelco's Modified Eagle's Medium
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
DNAse	deoxyribonuclease

dNTP	2'-deoxy-nucleotide-5'-trophosphate
ds	double stranded
dsDNA	double stranded ribonucleic acid
DTT	dithiothreitol
E.coli	*Escherichia coli*
EDTA	ethylenediaminetetra-acetic acid
EtBr	ethidium bromide
EtOH	ethanol
F	fusion gene of rinderpest
FBT	foetal bovine testis
ffu	focus forming units
Fig	figure
FPV	fowlpox virus
GMP	guanine monophosphate
gpt	*Escherichia coli* xanthine-guanine phosphoribosyl transferase
HA	haemagglutinin gene
HAT	hypoxanthine, aminopterin and thymidine
HCl	hydroxyl chloride
HN	haemagglutinin-neuraminidase
H_2O	water
hr	hour
ITR's	inverted terminal repetitions
K	kiloDalton
Kb	kilobase pairs
kPa	kiloPascal
kV	kiloVolt
LB broth	Luria-Bertani medium
LiCl	litium chloride
LSDV	lumpy skin disease virus
M	molar
mA	miliAmpere
$MgCl_2$	magnesium chloride
min	minute
ml	milliliter
MM/mMol	millimolar
M.O.I.	multiplicity of infection

MPA	mycophenolic acid
mRNA	messenger ribonucleic acid
$MgSO_4$	magnesium sulphate
MVA	modified vaccinia Ankara
NaAc	sodium acetate
NaCl	sodium chloride
NaI	sodium Iodine
NaOH	soidium hydroxide
NDV	newcastle disease virus
ng	nanogram
NLS	N-lauryl sarcosinate
nm	nanometer
OD_{550}	optical density at 550 nanometer
OH	hydroxyl
O/N	overnight
OVI	Onderstepoort veterinary institute
φX	PhiX174/*Hae* Marker
PBS	phosphate buffered saline
PBS(A)	phosphate buffered saline without calcium and magnesium containing 0.2 % gentamycin
PCR	polymerase chain reaction
p.i.	post infection
pmol	picomole
PPR	peste des petits ruminants
PSB	protein solvent buffer
r.e.	restriction endonuclease
RNA	ribonucleic acid
rpm	revolutions per minute
RP	rinderpest
RPV	rinderpest virus
RT	room temperature
SA	South-Africa
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
sec	second
Sf9	*Spodoptera frugiperda*

SMII	DNA molecular weight marker II
SPV	swinepox virus
ST	sodium chloride Tris HCl
TAE	Tris acetate EDTA
Taq	*Thermus aquaticus*
TBE	Tris-boric acid EDTA buffer
TBST	Tris-buffered saline Tween
TE	Tris EDTA
TEMED	N,N,N',N'-Tetra-methylethylendiamin
TGS	Tris glysine sodium dodecyl sulphate buffer
TK	thymidine kinase
tRNA	transfer ribonucleic acid
Tris	Tris-(hydroximethyl)-aminomethane
U	unit
UP	University of Pretoria
USA	United States of America
UV	ultraviolet
V	volt
VV	vaccinia virus
WHO	World Health Organization
wt	wild-type
w/v	weight per volume ratio
X-Gal	5-bromo-4-chlor-3-indolyl-3-D-galactopyranoside
µCi	microCurie
µg	microgram
µl	microliter

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