

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

**BY**

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**Evaluation of an authentic bi-directional promoter in a new transfer vector  
for generating LSDV recombinants**

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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  $\beta$ -galactosidase ( $\beta$ -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/*LacE* and pHSWF/*LacL*. These were used to generate LSDV/*LacE* and LSDV/*LacL* 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  $\beta$ -gal enzyme activity in infected cells. Similar intensities of blue stained foci, indicative of  $\beta$ -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	beta-mercapto-ethanol
CaCl <sub>2</sub>	calcium chloride
ccc	covalently closed circular
cDNA	complementary deoxyribonucleic acid
Ci	Curie
cm	centimeter
cm <sup>2</sup>	centimeter square
CO <sub>2</sub>	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 <sub>2</sub> O	double distilled water
DEPC	diethylpyrocarbonate
dH <sub>2</sub> O	distilled water
DIG	digoxigenin
DMEM	Dubelco's Modified Eagle's Medium
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
DNAse	deoxyribonuclease

dNTP	2'-deoxy-nucleotide-5'-triphosphate
ds	double stranded
dsDNA	double stranded ribonucleic acid
DTT	dithiothreitol
<i>E.coli</i>	<i>Escherichia coli</i>
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	<i>Escherichia coli</i> xanthine-guanine phosphoribosyl transferase
HA	haemagglutinin gene
HAT	hypoxanthine, aminopterin and thymidine
HCl	hydroxyl chloride
HN	haemagglutinin-neuraminidase
H <sub>2</sub> O	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 <sub>2</sub>	magnesium chloride
min	minute
ml	milliliter
MM/mMol	millimolar
M.O.I.	multiplicity of infection



MPA	mycophenolic acid
mrRNA	messenger ribonucleic acid
MgSO <sub>4</sub>	magnesium sulphate
MVA	modified vaccinia Ankara
NaAc	sodium acetate
NaCl	sodium chloride
Nal	sodium iodine
NaOH	sodium hydroxide
NDV	newcastle disease virus
ng	nanogram
NLS	N-lauryl sarcosinate
nm	nanometer
OD <sub>550</sub>	optical density at 550 nanometer
OH	hydroxyl
O/N	overnight
OVI	Onderstepoort veterinary institute
φX	PhiX174/ <i>Hae</i> 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	<i>Spodoptera frugiperda</i>

SMII	DNA molecular weight marker II
SPV	swinepox virus
ST	sodium chloride Tris HCl
TAE	Tris acetate EDTA
<i>Taq</i>	<i>Thermus aquaticus</i>
TBE	Tris-boric acid EDTA buffer
TBST	Tris-buffered saline Tween
TE	Tris EDTA
TEMED	N,N,N',N'-Tetra-methylethylenediamin
TGS	Tris glycine sodium dodecyl sulphate buffer
TK	thymidine kinase
tRNA	transfer ribonucleic acid
Tris	Tris-(hydroxymethyl)-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
$\mu$ Ci	microCurie
$\mu$ g	microgram
$\mu$ l	microliter

## CONTENTS

	PAGE
<b>AKNOWLEDGEMENTS</b>	
<b>ABSTRACT</b>	
<b>ABREVIATIONS</b>	
<b>LIST OF FIGURES</b>	
<b>LIST OF TABLES</b>	
<b>CHAPTER 1</b>	
<b>LITERATURE REVIEW</b>	1
1.1 INTRODUCTION	1
1.2 A GENERAL INTRODUCTION TO POXVIRUSES	3
1.2.1 Introduction	3
1.2.2 Virion structure	3
1.2.3 The poxvirus genome	5
1.2.4 Replication of poxviruses	5
1.2.5 Capripoxviruses	6
1.2.6 Genomic relationship between capripoxviruses	7
1.3 LUMPY SKIN DISEASE VIRUS (LSDV)	8
1.3.1 History	8
1.3.2 Antigenic relationship between different isolates of LSDV	9
1.3.3 Epidemiology and transmission of LSDV	9
1.3.4 Clinical signs	9
1.4 POXVIRUSES AS EXPRESSION VECTORS	10
1.4.1 Introduction	10
1.4.2 Recombinant vaccines based on mammalian poxvirus vectors	11
1.5 HOST-RANGE RESTRICTED POXVIRUSES USED AS VECTORS	13
1.6 CONSTRUCTION OF RECOMBINANT POXVIRUSES	16
1.7 AIMS OF THIS INVESTIGATION	21

	PAGE
<b>CHAPTER TWO</b>	
<b>EVALUATION OF A LSDV BI-DIRECTIONAL PROMOTER ELEMENT FOR EXPRESSING A REPORTER GENE IN LSDV RECOMBINANTS</b>	23
<b>2.1 INTRODUCTION</b>	23
<b>2.2 MATERIALS AND METHODS</b>	25
2.2.1 Construction of transfer plasmids	25
2.2.2 Restriction enzyme digestion	25
2.2.3 Agarose gel electrophoresis	26
2.2.4 Purification of DNA excised from gels	26
2.2.5 Generation of blunt-ended fragments	27
2.2.6 Dephosphorylation of vector	27
2.2.7 Ligation procedure	27
2.2.8 Preparation of competent <i>Escherichia coli</i> cells	28
2.2.9 Transformation procedure	28
2.2.10 Purification of plasmid DNA	29
2.2.11 Characterisation of transfer plasmids	30
2.2.12 Sterile conditions for cell culture work	30
2.2.13 Primary cell culture preparation	30
2.2.14 Passaging culture cells	31
2.2.15 Transfections	32
2.2.16 X-gal staining	33
2.2.17 Generation of LSDV recombinants	33
2.2.18 Purification of LSDV DNA	34
2.2.19 Restriction enzyme digestions of virus DNA	35
2.2.20 Non-radioactive labelling of DNA	35
2.2.21 Southern blot analysis	36
<b>2.3 RESULTS</b>	39
2.3.1 Construction of plasmids containing the <i>LacZ</i> -gene	39
2.3.2 Generation of LSDV recombinants	48
2.3.2.1 Transient expression of the <i>LacZ</i> -gene	48
2.3.2.2 Generation of LSDV recombinants	49
2.3.3 Confirmation of LSDV recombinants	52
<b>2.4 DISCUSSION</b>	55

	PAGE
<b>CHAPTER THREE</b>	
<b>GENERATION OF LSDV RECOMBINANTS EXPRESSING IMMUNOLOGICAL IMPORTANT ANTIGENS</b>	57
<b>3.1 INTRODUCTION</b>	57
<b>3.2 MATERIALS AND METHODS</b>	60
3.2.1 Construction of transfer plasmids	60
3.2.2 Transfection procedure	60
3.2.3 Generation of LSDV recombinants	60
3.2.4 Purification of LSDV DNA for analysis by PCR	61
3.2.5 Polymerase chain reaction	62
3.2.6 Nick-translation	64
3.2.7 Hybridisation of <sup>32</sup> P-labeled probes	64
3.2.8 Virus purification	65
3.2.9 Virus titration	66
3.2.10 Virus titer	66
3.2.11 Total RNA isolation	67
3.2.12 Dot blot analysis of RNA	67
3.2.13 Radio-labelling of proteins	68
3.2.14 Protein gel electrophoresis	69
3.2.15 Western blot analysis	69
3.2.16 Separation of cytoplasmic- and nucleus fractions	71
3.2.17 Electron microscopy	71
3.2.18 Generation of LSDV recombinants without residing wt LSDV	72
<b>3.3 RESULTS OF THE SINGLE RECOMBINANT</b>	73
3.3.1 Construction of a plasmid containing the AHSV-9 VP7 gene	73
3.3.2 Generation of LSDV/VP7 recombinants	78
3.3.3 Confirmation of LSDV recombinants	79
3.3.4 The titer of LSDV/VP7	81
3.3.5 Analysis of RNA transcription in LSDV/VP7	81
3.3.6 Expression of VP7 in the LSDV/VP7 recombinant	82
3.3.7 Generation of LSDV recombinants without residing wt LSDV	88

	PAGE
<b>3.4 RESULTS OF THE DUAL RECOMBINANT</b>	92
3.4.1 Construction of a dual plasmid containing both the VP2- and VP7-genes of AHSV-9	92
3.4.2 Generation of dual LSDV recombinants	97
<b>3.5 DISCUSSION</b>	102
<b>CHAPTER FOUR</b>	
<b>CONCLUDING DISCUSSION</b>	105
<b>APPENDICES</b>	
APPENDIX 1	110
Construction of the transfer vector, pHSWF	
APPENDIX 2A	113
Homologous recombination and the results of double cross- over events between wt LSDV-DNA and pHSWF/ <i>LacE</i>	
APPENDIX 2B	114
Homologous recombination and the results of double cross- over events between wt LSDV-DNA and pHSWF/ <i>LacL</i>	
APPENDIX 3	115
Homologous recombination and the results of double cross- over events between wt LSDV-DNA and pHSWF/ <i>VP7</i>	
<b>REFERENCE LIST</b>	116

## LIST OF FIGURES

Figure number and Title	PAGE
Fig. 1.1 Structural features of vaccinia viral DNA. A representation of the entire double-stranded DNA genome and an expansion of the 10 000 bp inverted terminal repetition	5
Fig. 2.1 pSV- $\beta$ -Galactosidase Vector circle map	39
Fig. 2.2 Agarose gel electrophoretic analysis of vector pSV- $\beta$ -Galactosidase	41
Fig. 2.3 Agarose gel electrophoretic analysis of the gene-cleaned recovered DNA	41
Fig. 2.4 Agarose gel electrophoretic analysis of vector pHSWF	41
Fig. 2.5 Agarose gel electrophoretic analysis of gene-cleaned recovered linearised vector, pHSWF	41
Fig. 2.6 Agarose gel electrophoretic analysis of gene-cleaned recovered DNA following dephosphorylation	42
Fig. 2.7 Agarose gel electrophoretic analysis of putative <i>LacZ</i> recombinants with the <i>LacZ</i> -gene cloned downstream of the early promoter, pA8R	42
Fig. 2.8 Agarose gel electrophoretic analysis of putative <i>LacZ</i> recombinants with the <i>LacZ</i> -gene cloned downstream of the late promoter, pA7L	42
Fig. 2.9a Schematic representation of the cloning of <i>LacZ</i> into the transfer vector pHSWF under control of the early LSDV promoter (pA8R)	44
Fig. 2.9b Schematic representation of the cloning of <i>LacZ</i> into the transfer vector pHSWF under control of the late LSDV promoter (pA7L)	45
Fig. 2.10 Agarose gel electrophoretic analysis of putative plasmids containing the <i>LacZ</i> -gene, pHSWF/ <i>LacE</i> to establish the transcriptional orientation	46
Fig. 2.11 Agarose gel electrophoretic analysis of putative plasmids containing the <i>LacZ</i> -gene, pHSWF/ <i>LacL</i> to establish the transcriptional orientation	46
Fig. 2.12 Agarose gel electrophoretic analysis of plasmids pHSWF/ <i>LacE</i> and pHSWF/ <i>LacL</i> digested with <i>Sac</i> I to confirm the correct orientation	47

	PAGE
Fig. 2.13	47
Agarose gel electrophoretic analysis of plasmids pHSWF/ <i>LacE</i> and pHSWF/ <i>LacL</i> digested with <i>Sma</i> I and <i>Sal</i> I to confirm the full length <i>LacZ</i>	
Fig. 2.14	50
Transient expression of enzyme $\beta$ -Galactosidase in LSDV-infected cells	
Fig. 2.15	51
Expression of the enzyme $\beta$ -Galactosidase in FBT-cells infected with recombinant LSDV	
Fig.2.16 A	54
Genomic analysis of parental and recombinant LSDV DNA. <i>Bam</i> HI digested DNA fragments were separated by means of 0.6% agarose gel electrophoresis	
B	54
An autoradiograph after hybridisation between the <i>LacZ</i> -gene, pHSWF plasmid and SMI DIG-labelled probes and the <i>Bam</i> HI-digested LSDV DNA	
Fig. 3.1.	73
Plasmid pBS/VP7 in T3	
Fig. 3.2	74
Agarose gel electrophoretic analysis of pBS/VP7	
Fig. 3.3	74
Agarose gel electrophoretic analysis of gene-cleaned recovered VP7-DNA	
Fig. 3.4	74
Agarose gel electrophoretic analysis of linearised vector, pHSWF	
Fig. 3.5	74
Agarose gel electrophoretic analysis of gene-cleaned recovered linearised vector, pHSWF	
Fig. 3.6	76
Schematic representation of the cloning of AHSV-9 VP7 into the transfer vector pHSWF, under control of the early LSDV promoter (pA8R)	
Fig. 3.7	77
Agarose gel electrophoretic analysis of putative plasmids containing VP7	
Fig. 3.8	77
Agarose gel electrophoretic analysis of plasmids digested with <i>Bam</i> HI to confirm orientation	
Fig. 3.9	77
Agarose gel electrophoretic analysis of a selected plasmid to confirm cloning of the full-length VP7-gene in the correct orientation	
Fig. 3.10	78
Agarose gel electrophoretic analysis of putative LSDV/VP7 recombinants. PCR amplification using the VP7-specific primer-set, SON2a and SON2b	



	PAGE	
Fig. 3.11	An autoradiograph of a Southern blot hybridisation between <i>Bam</i> HI digested LSDV/VP7 DNA and $\alpha$ - <sup>32</sup> P probes of the VP7-insert and pHSWF	80
Fig. 3.12	Autoradiograph of total RNA isolated at different times from cells infected with LSDV/VP7 and hybridised to the purified VP7-insert	82
Fig. 3.13 A	15% Polyacrylamide gel of LSDV infected FBT-cells. At the different times post infection, the uninfected and infected cells were labelled for 10hr using <sup>35</sup> S-methionine	84
	B Autoradiograph of the 15 % polyacrylamide gel in (A)	84
Fig. 3.14	Expression of VP7 analysed by a Western blot	85
Fig.3.15	VP7 crystal formation as analysed by Jeol scanning electron microscopy	87
Fig. 3.16	Agarose gel electrophoretic analysis of LSDV/VP7 recombinants by PCR. The LSDV-specific primer-set OP48 and OP49 was used	89
Fig. 3.17	PCR amplification of LSDV/VP7 recombinants using the primers SON2a and SON2b	89
Fig. 3.18	Identification of LSDV recombinants with residing wt LSDV. PCR amplification was performed using the primer-set Harry-WF and Sally-WF	89
Fig. 3.19	Analysis of non-specific amplification using the primer-set Harry-WF and Sally-WF	89
Fig. 3.20 A	LSDV/VP7 recombinants digested with <i>Bam</i> HI analysed by 0.6% agarose gel	91
	B An autoradiograph after hybridisation between VP7 and SMII DIG-labelled probes and the <i>Bam</i> HI-digested LSDV DNA	91
Fig. 3.21	Schematic representation of the construction of pHSWF(VP2/VP7) – the AHSV-9 VP7-gene was cloned into the transfer vector pHSWF/VP2 and placed under control of the early LSDV promoter (pA8R)	93
Fig. 3.22	Agarose gel electrophoretic analysis of pBS/VP7	94
Fig. 3.23	Agarose gel electrophoretic analysis of gene-cleaned recovered VP7-DNA	94

	PAGE
Fig. 3.24 Agarose gel electrophoretic analysis of linearised vector, pHSWF/VP2	94
Fig. 3.25 Agarose gel electrophoretic analysis of gene-cleaned recovered linearised vector, pHSWF-VP2	94
Fig. 3.26 A Agarose gel electrophoretic analysis of putative dual plasmids	95
B Agarose gel electrophoretic analysis of putative dual plasmids	95
Fig. 3.27 Agarose gel electrophoretic analysis of the dual plasmids digested with <i>Sac</i> I to confirm the correct orientation of VP7	96
Fig. 3.28 Agarose gel electrophoretic analysis of a putative dual transfer plasmid to confirm cloning of the full-length VP7 gene in the correct orientation	96
Fig. 3.29 Agarose gel electrophoretic analysis of putative dual LSDV(VP2/VP7) recombinants. PCR amplification using the VP7-specific primer-set, SON2a and SON2b	98
Fig. 3.30 A Agarose gel electrophoretic analysis of putative dual LSDV(VP2/VP7) recombinants. PCR amplification using the VP2-specific primer-set.	98
B Agarose gel electrophoretic analysis of putative dual LSDV(VP2/VP7) recombinants. PCR amplification using the VP2-specific primer-set.	98
Fig. 3.31 Amplification of the specific VP2-fragment to determine the external contamination in the DNA of the uninfected cells and wt LSDV	99
Fig. 3.32 A Confirmation of the presence of the AHSV-9 VP7 gene in selected putative dual LSDV recombinants. The primer-set (SON2a and SON2b) was used for amplification of the VP7-gene.	100
B Southern blot hybridisation between the VP7-amplified product and VP7, SMII and $\phi$ X DIG-labelled probes	100
Fig. 3.33 A Confirmation of the presence of the AHSV-9 VP2 gene in selected putative LSDV recombinants. The primer-set AHSV-9 VP2 (for and rev) was used to amplify the specific VP2-fragment	101
B Southern blot hybridisation between the VP2-amplified product and VP2 and $\phi$ X DIG-labelled probes	101

## LIST OF TABLES

	PAGE
1.1 Family <i>Poxviridae</i> : Genera and members	4
3.1 List of primers	63
3.2 Sets of primers used and the sizes of expected specific amplification products from a given source of DNA	63
3.3 List of primers specific for AHSV-9 VP2	97
3.4 AHSV-9 VP2 primer-set used and the size of the expected specific amplified product	97