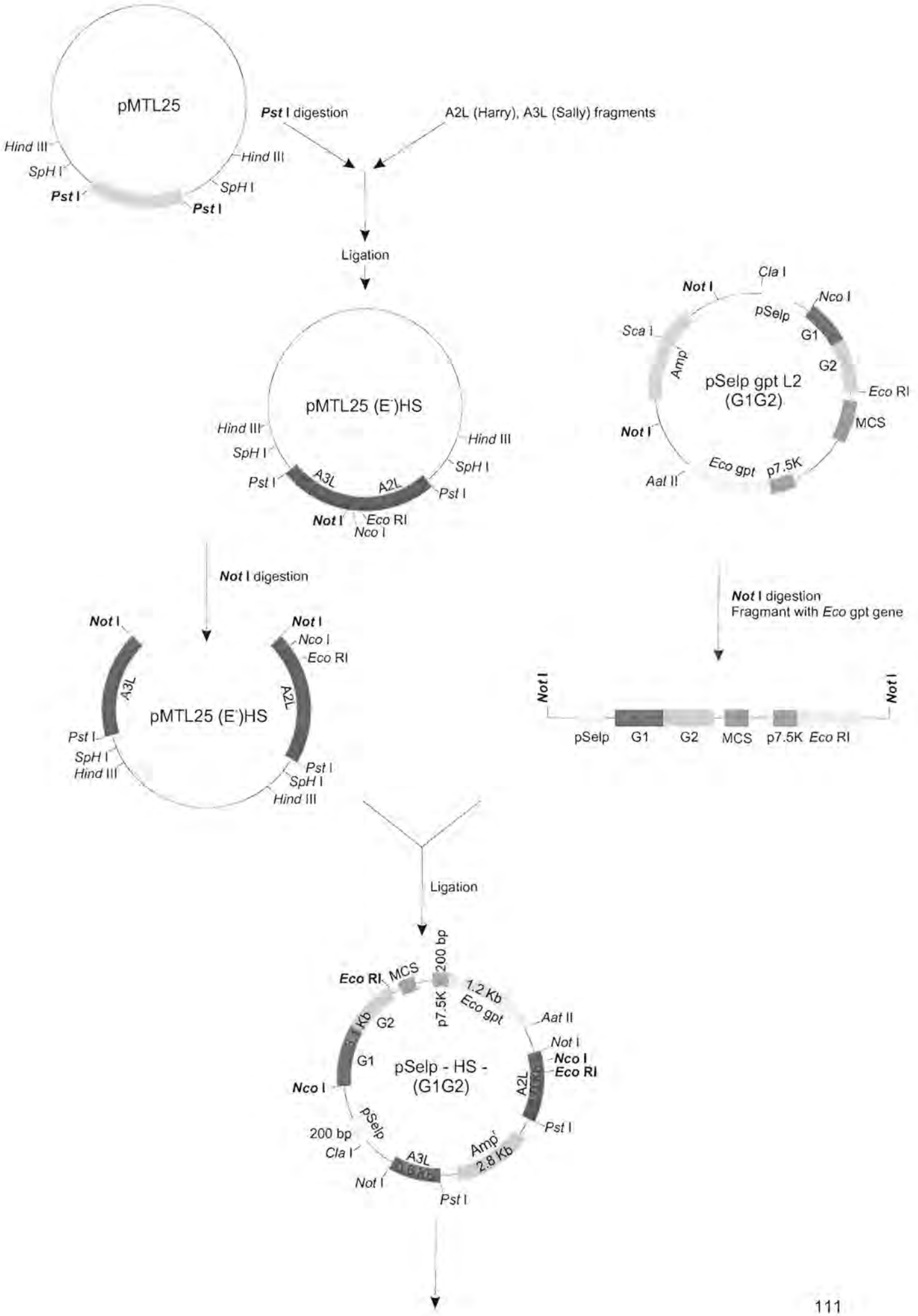
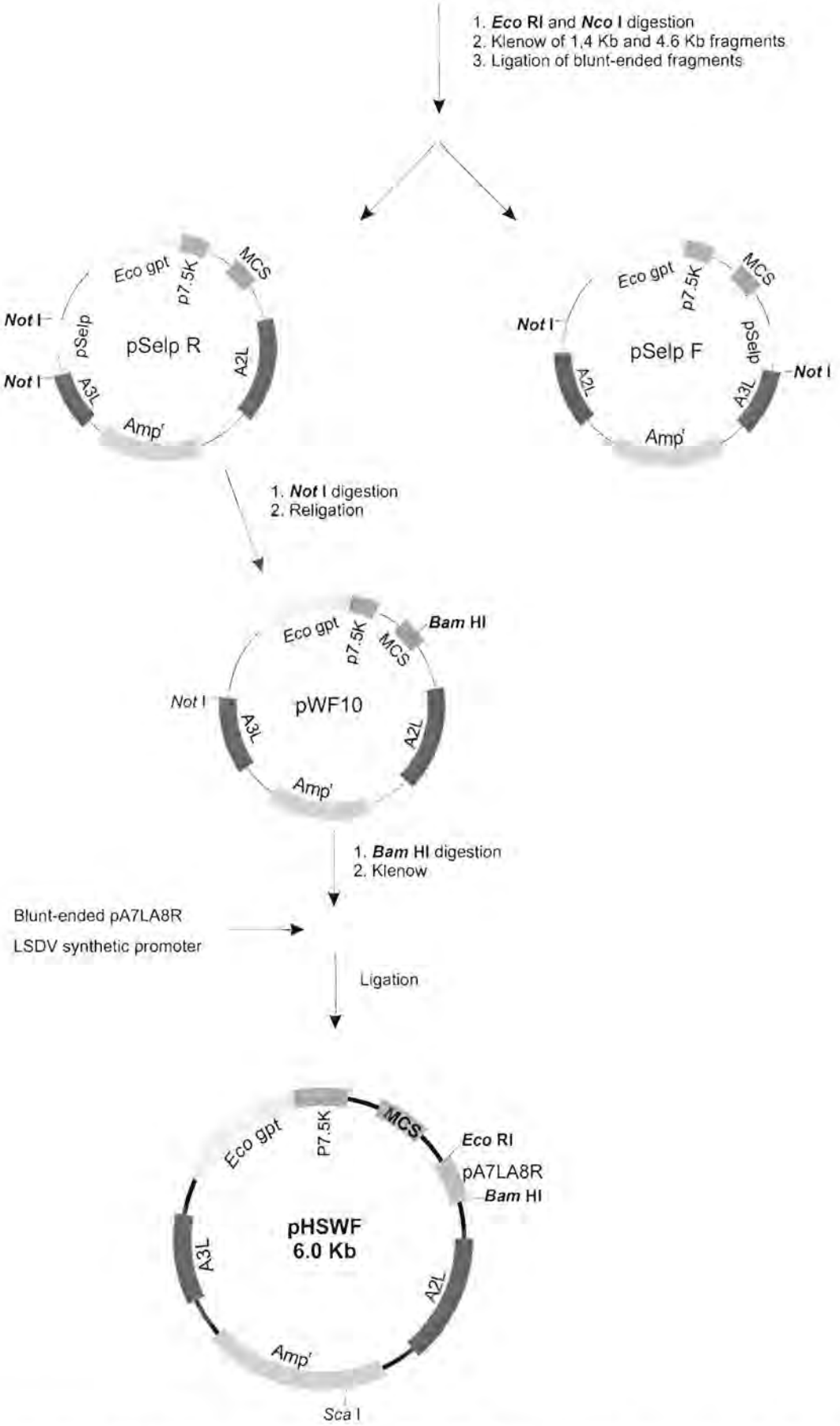
Appendix 1

Construction of the pHSWF transfer vector

The plasmids, pMTL25 and pSelp-gptL2(G_1G_2), were used as basis for the construction of the pHSWF transfer vector. The A2L and A3L gene analogs of VV (Harry and Sally) was cloned into pMTL25 as a *Pst* I-fragment and named pMTL25 (E^-)HS. The pSelp-gptL2(G_1G_2) plasmid (constructed by a co-worker at OVI) was linearised with *Not* I to give a fragment that contains the *E.coli* xanthine-guanine phosphoribosyl transferase (*Eco* gpt) gene under control of the VV early/late p7.5K promoter. The pMTL25(E^-)HS was cut with *Not* I and ligated with the above mentioned fragment to give rise to the pSelp-HS-(G_1G_2) vector. (The vector was constructed by Dr. A.L. Williamson and A.S. Cohen in the Virology Department of the Medical School at the University of Cape Town and used with permission). Three fragments with sizes 1.4 Kb, 3.1 Kb and 4.6 Kb were obtained with digestion of the pSelp-HS-(G_1G_2) vector using *Eco* RI and *Nco* I. The 1.4kb and 4.6kb fragments were blunt-ended and ligated to obtain 2 vectors named pSelpR and pSelpF. The transfer vector pHSWF was constructed by using the pSelpR vector. The pSelpR vector was digested with *Not* I, to remove the pSelp promoter, after which the vector was allowed to religate and named pWF10. pWF10 was linearised with *Bam* HI and blunt-ended to facilitate cloning of the bi-directional pA7LA8R promoter. The pA7LA8R promoter is also a blunt-ended product, obtained by annealing two complementary 68bp oligonucleotides representing the promoter.

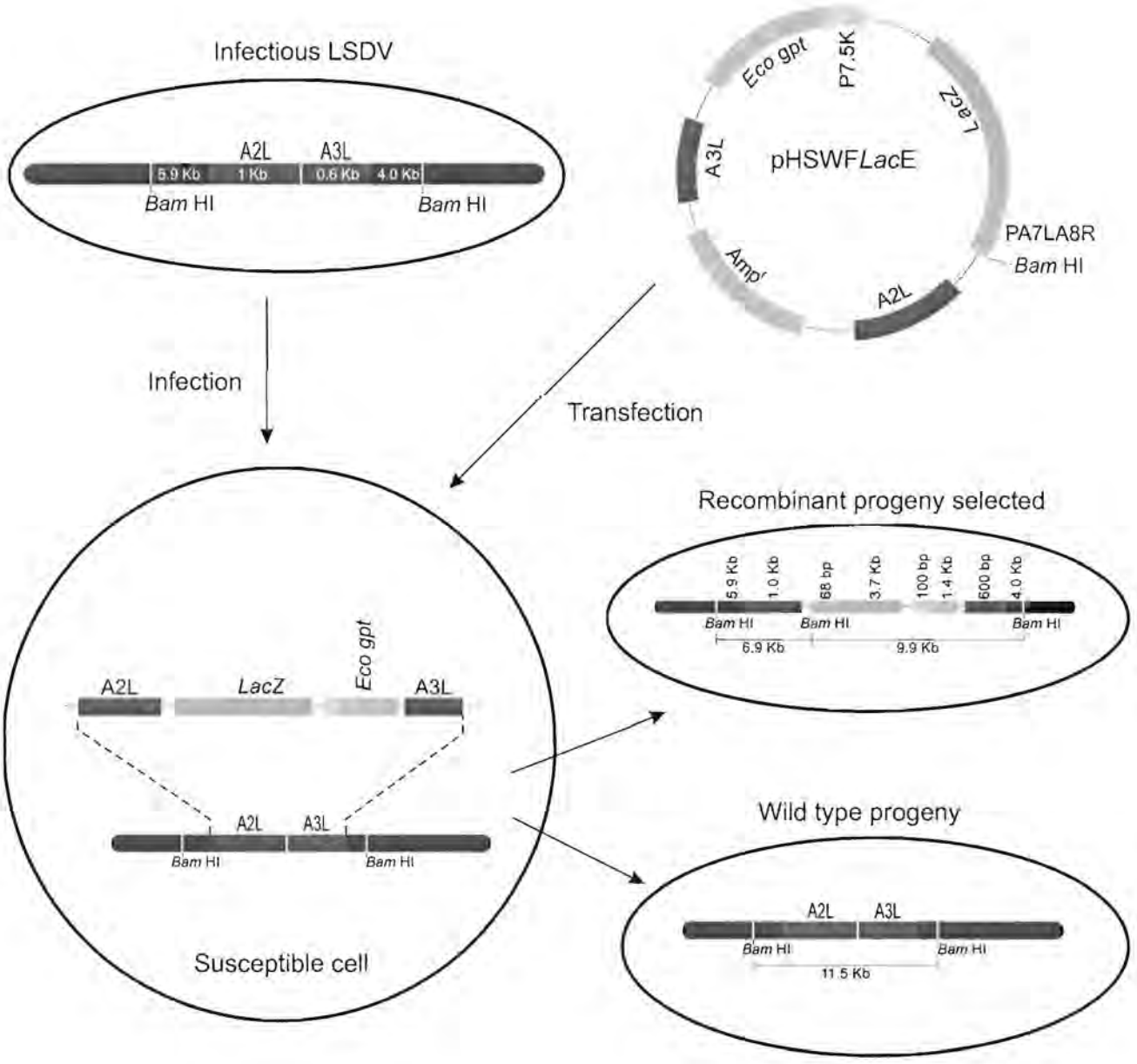

pMTL25
Hind III
SpH I
Pst I
Hind III
SpH I
Pst I
Pst I digestion
A2L (Harry), A3L (Sally) fragments
Ligation
pMTL25 (E-)HS
Hind III
SpH I
Pst I
A3L
A2L
Hind III
SpH I
Pst I
Not I
Eco RI
Nco I
Not I digestion
Not I
Cla I
Nco I
pSelp
G1
G2
Eco RI
MCS
p7.5K
Eco gpt
Aat II
Not I
Sca I
Amp
pSelp gpt L2
(G1G2)
Not I digestion
Fragmant with Eco gpt gene
Not I
Not I
pSelp
G1
G2
MCS
p7.5K
Eco RI
Not I
Not I
Nco I
Eco RI
A3L
A2L
pMTL25 (E-)HS
Pst I
SpH I
Hind III
Pst I
SpH I
Hind III
Ligation
Eco RI
MCS
200 bp
1.2 Kb
Eco gpt
p7.5K
Aat II
Not I
Nco I
Eco RI
2.8 Kb
G2
G1
pSelp - HS -
(G1G2)
Nco I
pSelp
200 bp
Cla I
A3L
Not I
Amp
2.8 Kb
A2L
Pst I
Pst I



Construction of the transfer vector pHSWF. The pWF10 plasmid was linearized with *Bam* HI and blunt-ended to facilitate cloning of the blunt-ended bi-directional pA7LA8R LSDV synthetic promoter.

Appendix 2A

Homologous recombination and the results of double cross-over events between wt LSDV-DNA and pHSWF/*Lac*E



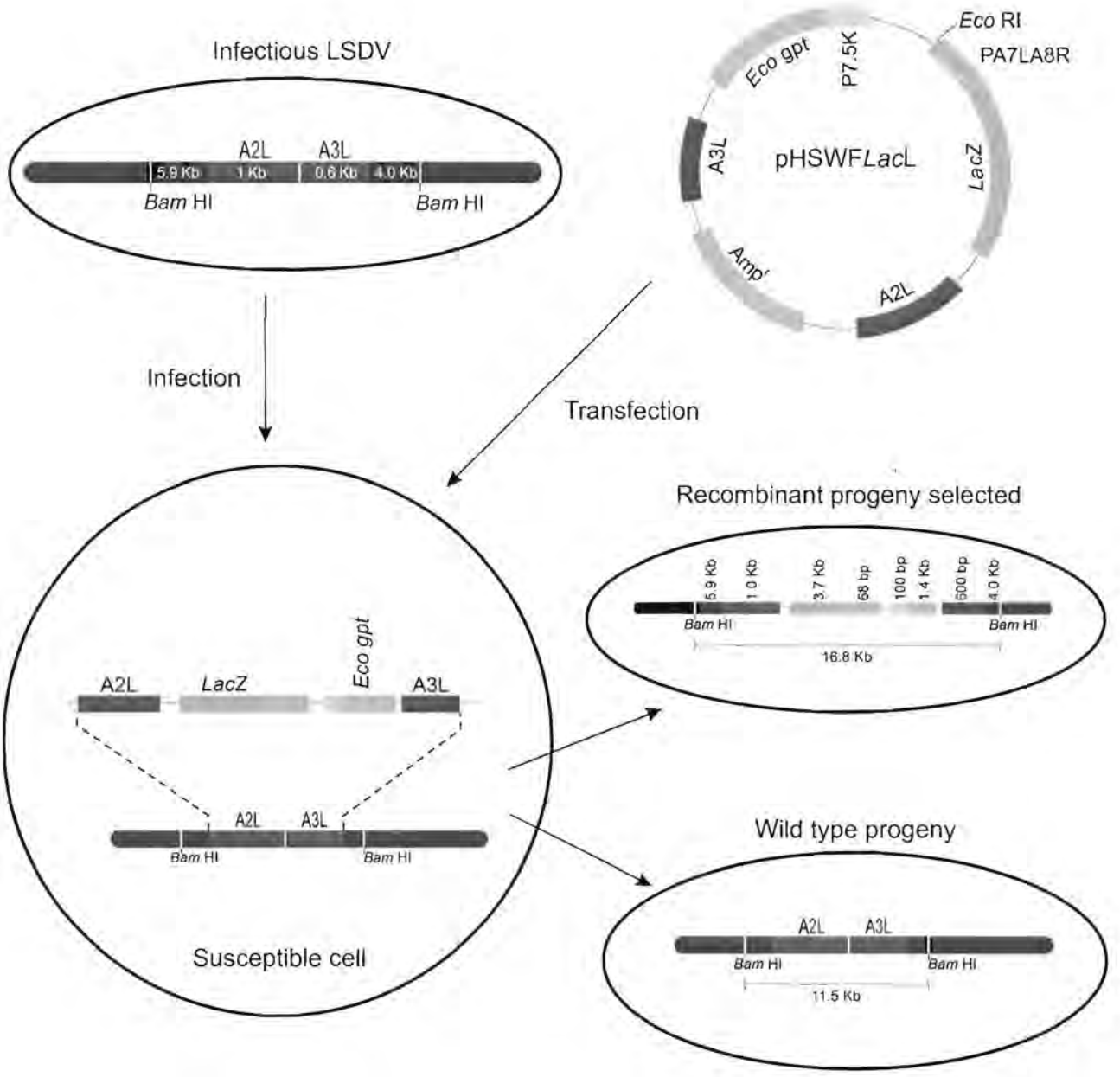
Fragments expected with *Bam* HI digested recombinant LSDV/*Lac*E DNA, hybridized with *Lac*Z- and pHSWF-DIG probes:

- Fragments with sizes 6.9 Kb and 9.9 Kb each, if no residing wt LSDV is still present in the recombinant.

- If parental LSDV resides in recombinant, an extra fragment of 11.5 Kb will be obtained.

Appendix 2B

Homologous recombination and the results of double cross-over events between wt LSDV-DNA and pHSWF/*Lac*L



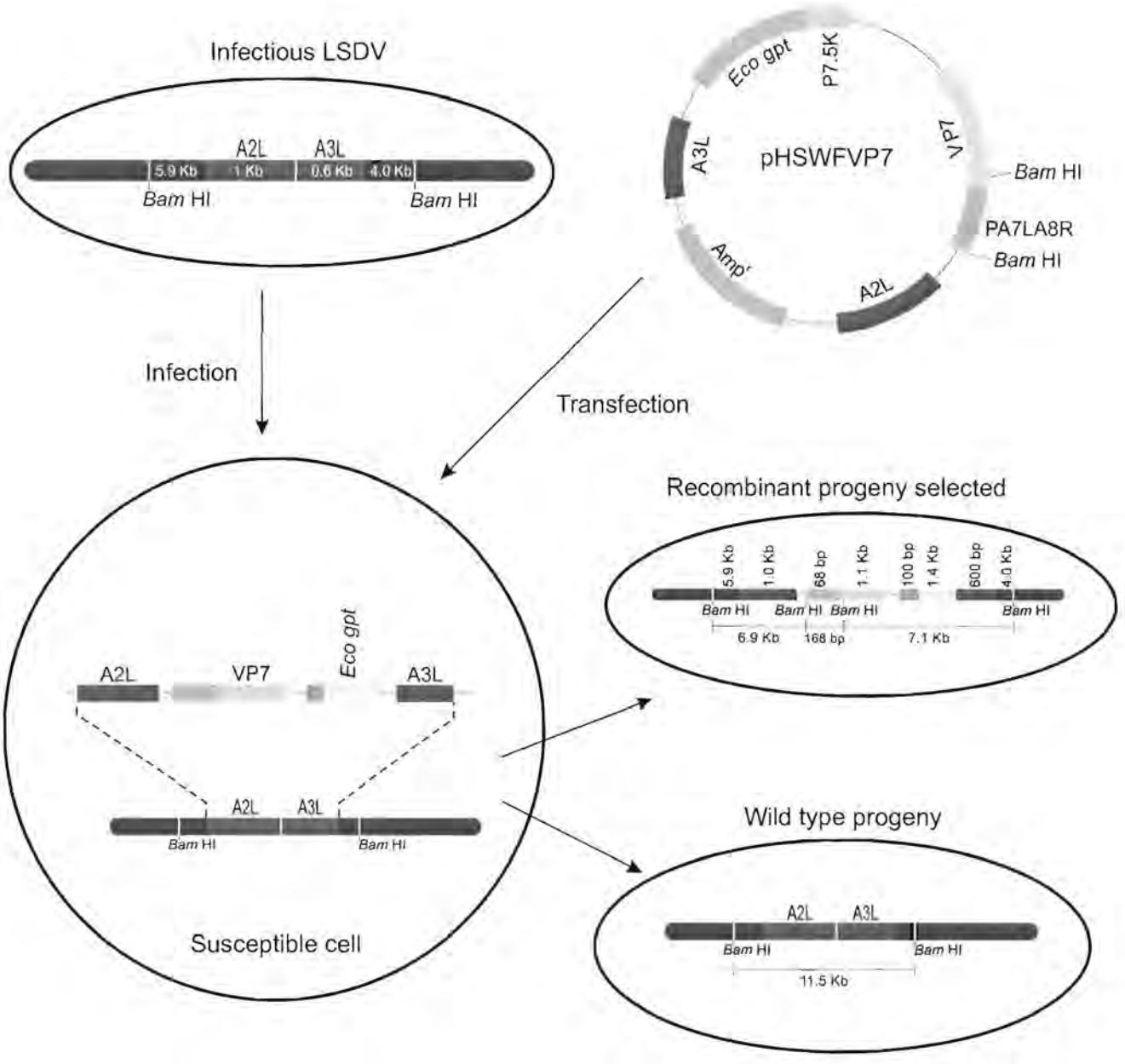
Fragments expected with *Bam* HI digested recombinant LSDV/*Lac*L DNA, hybridized with *LacZ*- and pHSWF-DIG probes:

- A fragment with size 16.8 Kb,if no residing wt LSDV is still present in the recombinant.

- If parental LSDV resides in recombinant, an extra fragment of 11.5 Kb will be obtained.

Appendix 3

Homologous recombination and the results of double cross-over events between wt LSDV-DNA and pHSWF/VP7



Fragments expected with *Bam* HI digested recombinant LSDV/VP7 DNA, hybridized with VP7- and pHSWF-DIG probes:

- Fragments with sizes 168 bp, 6.9 Kb and 7.1 Kb each, if no residing wt LSDV is still present in the recombinant.

- If parental LSDV resides in recombinant, an extra fragment of 11.5 Kb will be obtained.

REFERENCES

An, G., Hidaka, K. & Siminovitch, L. (1982). Expression of bacterial β-galactosidase in animal cells. *Molecular and Cellular Biology* **2**, 1628-1632.

Angove, H. (1995). PhD thesis, University of Hertfordshire, England.

Barcena, J. & Blasco, R. (1998). Recombinant swinepox virus expressing β-galactosidase: Investigation of viral host range and gene expression levels in cell culture. *Virology* **243**, 396-405.

Baroudy, B. M., Venkatensan, S. & Moss, B. (1982). Incompletely base-paired flip-flop terminal loops link the two DNA strands of the vaccinia virus genome into one uninterrupted polynucleotide chain. *Cell* **28**, 315-324.

Basak, A.K., Gouet, P., Grimes, J., Roy, P. & Stuart, D. (1996). Crystal structure of the top domain of African horse sickness virus VP7: Comparisons with Bluetongue virus VP7. *Journal of Virology* **70** (6), 3797-3806.

Baxby, D. (1993). Recombinant poxvirus vaccines. *Reviews in Medical Microbiology* **4**, 80-88.

Bennink, J. R., Yewdell, J. W., Smith, G. L., Moller, C. & Moss, B. (1984). Recombinant vaccinia virus primes and stimulates influenza haemagglutinin-specific cytotoxic T cells. *Nature, London* **311**, 578-579.

Black, D. N., Hammond, J. M. & Kitching, R. P. (1986). Genomic relationship between capripoxviruses. *Virus Research* **5**, 277-292.

Boursnell. M. E. G., Green, P. F., Samson, A. C. R., Cambell, J. I. A., Deuter, A., Peters, R. W., Millar, N. S., Emmerson, P. T. & Binns, M. M. (1990). A recombinant fowlpox virus expressing the hemagglutinin-neuraminidase gene of newcastle disease virus (NDV) protects chickens against challenge by NDV. *Virology* **178**, 297-300.

Bostock, C. J. (1990). Viruses as vectors. *Veterinary Microbiology* **23**, 55-71.

Boyle, D. B. (1992). Quantitative assessment of poxvirus promoters in fowlpox and vaccinia virus recombinants. *Virus Genes* **6**, 281-290.

Bremer, C. W., Huismans, H. & Van Dijk, A. A. (1990). Characterisation and cloning of the African horsesickness virus genome. *Journal of General Virology* **71**, 793-799.

Burrage, T. G., Trevejo, R., Stone-Marschat, M. & Laegreid, W. W. (1993). Neutralizing epitopes of African horsesickness virus serotype 4 are located on VP2. *Virology* **196**, 799-803.

Burroughs, J.N., O'Hara, R.S., Smale, C.J., Hamblin, C., Walton, A., Armstrong, R. & Mertens, P.P. (1994). Purification and properties of virus particles, infectious subviral particles, cores and VP7 crystals of African horsesickness virus serotype 9. *Journal of General Virology* **75**, 1849-1857.

Capstick, P. B. & Coakley, W. (1961). Protection of cattle against lumpy skin disease. *Research in Veterinary Science* **2**, 362-375.

Chakrabarti, S., Brechling, K. & Moss, B. (1985). Vaccinia virus expression vector: Co-expression of β-galactosidase provides visual screening of recombinant virus plaques. *Molecular and Cellular Biology* **5**, 3403-3409.

Cochran, M. A., Puckett, C. & Moss, B. (1985). In vitro mutagenesis of the promoter region for a vaccinia virus gene: evidence for tandem early and late regulatory signals. *Journal of Virology* **54**, 30-37.

Coupar, B. E. H., Andrew, M. E., Both, G. W. & Boyle, D. B. (1986). Temporal regulation of influenza hemagglutinin expression in vaccinnia virus recombinants and effects on the immune response. *European Journal of Immunology* **16**, 1479-1487.

Davies, F. G. & Oedema, C. (1981). Relationships of capripox viruses found in Kenya with two Middle Eastern strains and some orthopox viruses. *Research in Veterinary Science* **31**, 253-255.

Dubochet, J. A. M., Richter, K., Garces J. & Wittek R. (1994). Structure of intracellular mature vaccinia virus observed by cryoelctron microscopy. *Journal of Virology* **68**, 1935-1941.

Esposito, J., Condit, R. and Obijeski, J. (1981). The preparation of orthopoxvirus DNA. *Journal of Virology Methods* **2**, 175-179.

Falkner, F. G. & Moss, B. (1990). Transient dominant selection of recombinant vaccinia viruses. *Journal of Virolofy* **64** (6), 3108-3111.

Feinberg, A. P. & Vogelstein, B. (1983). A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Analytical Biochemistry* **132,** 6-13.

Fenner, F., Henderson, D. A., Arita, I., Jezek, Z. & Ladnyi, I. D. (1988). Smallpox and its Eradication, pp 69-168. World Health Organization, Geneva.

Fick, W. C. & Viljoen, G. J. (1994). Early and late transcriptional phases in the replication of lumpy-skin-disease virus. *Onderstepoort Journal of Veterinary Research* **61**, 255-261.

Fick, W. C. & Viljoen, G. J. (1999). Identification and characterisation of an early/late bi-directional promoter of the capripoxvirus, lumpy skin disease virus. *Archives of Virology* **144**, 1229-1239.

Gershon, P. D. & Black, D. N. (1987). Physical characterization of the genome of a cattle isolate of capripoxvirus. *Virology* **160**, 473-476.

Gershon, P. D. & Black, D. N. (1988). A comparison of the genomes of capripoxvirus isolates of sheep, goats and cattle. *Virology* **164**, 341-349.

Gershon, P. D., Ansell, D. M. & Black, D. N. (1989). A comparison of the genome organization of a capripoxvirus with that of the orthopocviruses. *Journal of Virology* **63**, 4703-4708.

Giavedoni, L., Jones, L., Mebus, C. & Yilma, T. (1991). A vaccinia virus double recombinant expressing the F and H genes of rinderpest virus protects cattle against riderpest and causes no pock lesions. *Proceedings of the National Academy of Sciences U.S.A* **88**, 8011-8015.

Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982). Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Molecular and Cellular Biology* **2**, 1044-1051.

Green, H. F. (1959). Lumpy skin disease: its effect on hides and leather and a comparison in this respect with some other skin diseases. *Bulletin of Epizootic Diseases of Africa* **7**, 63-66.

Grubman, M. J. & Lewis, S. A. (1992). Identification and characterisation of the structural and non-structural proteins of African horsesickness virus and determination of the genome coding assignments. *Virology* **186**, 444-451.

Holmes, I. H. (1991). Family *Reoviridae*. In: Classification and Nomenclature of Viruses. *Archives of Virology, Supplementum* **2**, 188-189.

House, C., House, J. A. & Mebus, C. A. (1992). A review of African horsesickness with emphasis on selected vaccines. *Annuals of the New York Academy Science* **653**, 228-232.

Howell, P. G. (1962). The isolation and identification of further antigenic types of African horsesickness virus. *Onderstepoort Journal of Veterinary Research* **29**, 139-149.

Huismans, H. (1979). Protein synthesis in Bluetongue virus-infected cells. *Virology* **92**, 385-396.

Kates, J. R. & McAuslan, B. R. (1967). Poxvirus DNA-dependent RNA polymerase. *Proceedings of the National Academy of Sciences USA* **58**, 134-141.

Kitching, R. P. & Taylor, W. P. (1985). Clinical and antigenic relationship between isolates of sheep and goat pox viruses. *Tropical Animal Health and Production* **17**, 64-74.

Kitching, R. P. (1986). Passive protection of sheep against capripoxvirus. *Research in Veterinary Science* **41**, 247-250.

Kitching, R. P. & Mellor, P. S. (1986). Insect transmission of capripoxviruses. *Research in Veterinary Science* **40**, 255-258.

Kitching, R. P., Hammond, J. M. & Black, D. N. (1986). Studies on the major common precipitating antigen of capripoxvirus. *Journal of General Virology* **67**, 139-148.

Kitching, R. P., Bhat, P. P. & Black, D. N. (1989). The characterisation of African strains of capripoxvirus. *Epidemiology and Infection* **102**, 335-343.

Levy, J. A., Fraenkel-Conrat H. & Owens, R. A. (1994). *Virology*. Third ed. Prentice Hall, Englewood Cliffs, New Jersey.

MacDonald, R. A. S. (1931). Pseudo-urticaria of cattle. Annual Report 1930, Department of Animal Health, Northern Rhodesia, 20-21.

Mackett, M., Smith, G. L. & Moss, B. (1982). Vaccinia virus: a selectable eukaryotic cloning and expression vector. *Proceedings of the National Academy of Sciences USA* **79**, 7415-7419.

Mackett, M., Smith, G. L. & Moss, B. (1985). In *DNA Cloning : A practical Approach,* Vol 2, pp. 191-211. Edited by D.M. Glover. IRL Press, Washington, D.C.

Mandel, M. & Higa, A. (1970). Calsium dependant bacteriophage DNA infection. *Journal of Molecular Biology* **53**, 159-162.

McIntosh, B, M. (1958). Immunological types of horsesickness virus and their significance in immunization. *Onderstepoort Journal of Veterinary Research* **27**, 465-538.

Medson, E. L. & Bauer, H. (1970). Structural features of vaccinia virus revealed by negative staining. *Virology* **40**, 860-867.

Moss, B. (1996). *Poxviridae*: The viruses and their replication. In *Virology*, pp 2637-2671. Edited by B. N. Fields, D. M. Knipe & P. M. Howley, with associate editors R. M. Chanock, J. L. Melnick, T. P. Monath, B. Roizman & S. E. Straus. Philadelphia: Lippincott-Raven Publishers.

Mulligan, R. C. & Berg, P. (1981). Selection for animal cells that express the *E. coli* gene coding for xanthine-guanine phosphoribosyltransferase. *Proceedings of the National Academy of Sciences USA* **78**, 2072-2076.

Oelermann, R. A., Els, H. J. & Erasmus, B. J. (1970). Characterisation of African horsesickness virus. *Archives Ges Virusforsch* **29**, 163-174.

Panicali, D. L. & Paoletti, E. (1982). Construction of poxviruses as cloning vectors: insertion of the thymidine kinase gene of herpes simplex virus into the DNA of infectious vaccinia virus. *Proceedings of the National Academy of Sciences USA* **79,** 4927-4931.

Pastoret, P. P. & Brochier, B. (1996). The development and use of a vaccinia-rabies recombinant oral vaccine for the control of wildlife rabies: a link between Jenner and Pasteur. *Epidemiology and Infection* **116**, 235-240.

Perkus, M. E.,. Piccini, A., Lipinskas, B. R. & Paoletti, E. (1985). Recombinant vaccinia virus: immunization against multiple pathogens. *Science* **229**, 981-984.

Prydie, J. & Coackley, W. (1959). Lumpy skin disease - tissue culture studies. *Bulletin of Epizootic Diseases of Africa* **7**, 37-50.

Romero, C. H., Barrett, T., Evans, S. A., Kitching, R. P., Gershon, P. D., Bostock, C. & Black, D. N. (1993). Single capripoxvirus recombinant vaccine for the protection of cattle against rinderpest and lumpy skin disease. *Vaccine* **11**, 737-742.

Romero, C. H., Barrett, T., Chamberlain, R. W., Kitching, R. P., Fleming, M. & Black, D. N. (1994). Recombinant capripoxvirus expressing the hemagglutinin protein gene of rinderpest virus: protection of cattle against rinderpest and lumpy skin disease virus. *Virology* **204**, 425-429.

Roy,P. (1992). Bluetongue virus proteins. *Journal of General Virology* **73**, 3051-3064.

Roy, P., Mertens, P. P. C. & Casal, I. (1994). African horsesickness virus structure. *Comparative Immunology and Microbiology of Infectious diseases* **17**, 1237-1241.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). Molecular cloning, a laboratory manual. Second edition. Cold Spring Harbor Laboratory Press.

Sekar, C., Breshling, K. & Moss, B. (1985). Vaccinia virus expression vector: coexpression of β-galactosidase provides visual screening of recombinant virus plaques. *Molecular and Cellular Biology* **5** (12), 3403-3409.

Selden, R. F., Burke-Howie, K., Rowe, M. E., Goodman, H. M. & Moore, D. D. (1986). Human growth hormone as a reporter gene in regulation studies employing transient gene expression. *Molecular and Cellular Biology* **6**, 3173-3179.

Smith, G. L. & Mackett, M. (1992). The design, construction and use of vaccinia virus recombinants. In *Recombinant Poxviruses*, pp 81-122. Edited by M.M. Binns & G. L. Smith. Florida: CRC Press.

Southern, E. M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology* **98**, 503-517.

Stone-Marschat, M. A., Moss, S. R., Burrage, T. G., Barber, M. L., Roy, P. & Laegreid, W. W. (1996). Immunization with VP2 is sufficient for protection against lethal challenge with African horsesickness virus type 4. *Virology* **220**, 219-222.

Sutter, G. & Moss, B. (1992). Nonreplicating vaccinia vector efficiently expresses recombinant genes. *Proceedings of the National Academy of Sciences USA* **89**, 10847-10851.

Taylor, W.P. (1979). Protection of goats against peste des petits ruminants with attenuated rinderpest virus. *Research in Veterinary Science* **27**, 321-324.

Taylor, J., Weinberg, R., Languet, B., Desmettre, P. & Paoletti, E. (1988a). Recombinant fowlpox virus inducing protective immunity in non-avian species. *Vaccine* **6**, 497-502.

Taylor, J., Weinberg, R., Kawaoka, Y., Webster, R. G. & Paoletti, E. (1988b). Protective immunity against avian influenza induced by a fowlpox virus recombinant. *Vaccine* **6**, 504-508.

Taylor, J., edbauer, C., Rey-Senelonge, A., Bouquet, J-F., Norton, E., Goebel, S., Desmettre, P. & Paoletti, E. (1990). Newcastle disease virus fusion protein expressed in a fowlpox virus recombinant confers protection in chickens. *Journal of Virology* **64**(4), 1441-1450.

Taylor, J., Trimarchi, C., Weinberg, R., Languet, B., Guillemin, F., Desmettre, P. & Paoletti, E. (1991). Efficacy studies on a canarypox-rabies recombinant virus. *Vaccine* **9**, 190-193.

Tuboly, J., Nagy, E. & Derbyshire, J. B. (1993). Potential viral vectors for the stimulation of mucosal antibody responses against enteric viral antigens in pigs. *Research in Veterinary Science* **54**(3), 345-350.

Van der Leek, M. L., Feller, J. A., Sorensen, G., Isaacson, W., Adams, C. L., Borde, D. J., Pfeiffer, N., Tran, T., Moyer, R. W. & Gibbs, E.P. (1994). Evaluation of swinepox virus as a vaccine vector in pigs using an Aujeszky's disease (pseudorabies) virus gene insert coding for glycoproteins gp50 and gp63. *Veterinary Record* **134**(1), 13-18.

Vreede, F. T. & Huismans, H. (1994). Cloning, characterization and expression of the gene that encodes the major neutralization-specific antigen of African horsesickness virus serotype 3. *Journal of General Virology* **75**, 3629-3633.

Wade-Evans, A. M., Romero, C. H., Mellor, P., Takamatsu, H., Anderson, J., Thevasagayam, J., Fleming, M. J., Mertens, P. P. C. & Balck, D. N. (1996). Expression of the major core structural protein (VP7) of bluetongue virus by a recombinant capripox virus, provides partial protection of sheep against a virulent heterotypic bluetongue virus challenge. *Virology* **220**, 227-231.

Wade-Evans, A.M., Pullen, L., Hamblin, C., O'Hara, R., Burroughs, J.N. & Mertens, P.P.C. (1997). African horsesickness virus VP7 sub-unit vaccine protects mice against a lethal, heterologous serotype challenge. *Journal of General Virology* **78**, 1611-1616.

Weiss, K. E. (1968). Lumpy skin disease virus. *Virology Monographs* **3**, 111-131.

Wiktor, T. J., MacFarlan, R. I., Reagan, K.. J., Dietzschold, B., Curtis, P.J. Wunner, W. H. Kieny, M. -P., Lathe, R., Lecocq, J. P., Mackett, M., Moss, B. & Koprowski, H. (1984). Protection from rabies by a vaccinia virus recombinant containing the rabies virus glycoprotein gene. *Proceedings of the National Academy of Sciences U.S.A* **81**, 7194-7198.

World Health Organization Meeting(1989). Potential use of live viral and bacterial vectors for vaccines. *Vaccine* (1990) **8**, 425-437.

Yamanouchi, K., Barrett, T. & Kai, C. (1998). New approaches to the development of virus vaccines for veterinary use. *Rev. sci. tech. Off. Int. Epiz.* **17** (3), 641-653.

Yilma, T., Hsu, D., Jones, L., Owens, S., Grubman, M., Mebus, C., Yamanka, M. & Dale, B. (1988). Protection of cattle against rinderpest with infectious vaccinia virus recombinants expressing the HA or F gene. *Science* **242**, 1058-1061.