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(54) Titre : VIRUS DE RECOMBINAISON DE LA VARIOLE PORCINE
(54) Title: RECOMBINANT SWINEPOX VIRUS

(57) **Abrégé/Abstract:**

This invention provides a recombinant swinepox virus comprising a foreign DNA inserted into a swinepox virus genome, wherein the foreign DNA is inserted into a) *AccI* site within a region corresponding to a 3.2 Kb *HindIII* to *BglII* subfragment of the *HindIII* M fragment and b) an *EcoRI* site within a region corresponding to a 3.2 Kb subfragment of the *HindIII* K fragment which contains both a *HindIII* and *EcoRI* site, of the swinepox virus genome and is capable of being expressed in a host cell into which the virus is introduced. The invention further provides vaccines and methods of immunization of the recombinant swinepox virus.

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(54) Title: RECOMBINANT SWINEPOX VIRUS (57) Abstract This invention provides a recombinant swinepox virus comprising a foreign DNA inserted into a swinepox virus genome, wherein the foreign DNA is inserted into a) an AccI site within a region corresponding to a 3.2 Kb HindIII to BglII subfragment of the HindII M fragment and b) an EcoRI site within a region corresponding to a 3.2 Kb subfragment of the HindIII K fragment which contains both a HindIII and EcoRI site, of the swinepox virus genome and is capable of being expressed in a host cell into which the virus is introduced. The invention further provides vaccines and methods of immunization of the recombinant swinepox virus.		

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RECOMBINANT SWINEPOX VIRUS

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BACKGROUND OF THE INVENTION

Swinepox virus (SPV) belongs to the family *Poxviridae*.
15 Viruses belonging to this group are large, double-stranded DNA viruses that characteristically develop in the cytoplasm of the host cell. SPV is the only member of the genus *Suipoxvirus*. Several features distinguish SPV from other poxviruses. SPV exhibits species
20 specificity (18) compared to other poxviruses such as vaccinia which exhibit a broad host range. SPV infection of tissue culture cell lines also differs dramatically from other poxviruses (24). It has also been demonstrated that SPV does not exhibit antigenic cross-
25 reactivity with vaccinia virus and shows no gross detectable homology at the DNA level with the ortho, lepori, avi or entomopox virus groups (24). Accordingly, what is known and described in the prior art regarding other poxviruses does not pertain a priori to swinepox
30 virus.

SPV is only mildly pathogenic, being characterized by a self-limiting infection with lesions detected only in the skin and regional lymph nodes. Although the SPV
35 infection is quite limited, pigs which have recovered from SPV are refractory to challenge with SPV, indicating development of active immunity (18).

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The present invention concerns the use of SPV as a vector for the delivery of vaccine antigens and therapeutic agents to swine. The following properties of SPV support this rationale: SPV is only mildly pathogenic in swine, SPV is species specific, and SPV elicits a protective immune response. Accordingly, SPV is an excellent candidate for a viral vector delivery system, having little intrinsic risk which must be balanced against the benefit contributed by the vector's vaccine and therapeutic properties.

The prior art for this invention stems first from the ability to clone and analyze DNA while in bacterial plasmids. The techniques that are available are detailed for the most part in Maniatis et al., 1983 and Sambrook et al., 1989. These publications teach state of the art general recombinant DNA techniques

Among the poxviruses, five (vaccinia, fowlpox, canarypox, pigeon, and raccoon pox) have been engineered, previous to this disclosure, to contain foreign DNA sequences. Vaccinia virus has been used extensively to vector foreign genes (25) and is the subject of U.S. Patents 4,603,112 and 4,722,848. Similarly, fowlpox has been used to vector foreign genes and is the subject of several patent applications EPA 0 284 416, PCT WO 89/03429, and PCT WO 89/12684. Raccoon pox (10) and Canarypox (31) have been utilized to express antigens from the rabies virus. These examples of insertions of foreign genes into poxviruses do not include an example from the genus *Suipoxvirus*. Thus they do not teach methods to genetically engineer swinepox viruses, that is, where to make insertions and how to get expression in swinepox virus.

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The idea of using live viruses as delivery systems for antigens has a very long history going back to the first live virus vaccines. The antigens delivered were not foreign but were naturally expressed by the live virus in the vaccines. The use of viruses to deliver foreign antigens in the modern sense became obvious with the recombinant vaccinia virus studies. The vaccinia virus was the vector and various antigens from other disease causing viruses were the foreign antigens, and the vaccine was created by genetic engineering. While the concept became obvious with these disclosures, what was not obvious was the answer to a more practical question of what makes the best candidate virus vector. In answering this question, details of the pathogenicity of the virus, its site of replication, the kind of immune response it elicits, the potential it has to express foreign antigens, its suitability for genetic engineering, its probability of being licensed by regulatory agencies, etc, are all factors in the selection. The prior art does not teach these questions of utility.

The prior art relating to the use of poxviruses to deliver therapeutic agents relates to the use of a vaccinia virus to deliver interleukin-2 (12). In this case, although the interleukin-2 had an attenuating effect on the vaccinia vector, the host did not demonstrate any therapeutic benefit.

The therapeutic agent that is delivered by a viral vector of the present invention must be a biological molecule that is a by-product of swinepox virus replication. This limits the therapeutic agent in the first analysis to either DNA, RNA or protein. There are examples of therapeutic agents from each of these classes of compounds in the form of anti-sense DNA, anti-sense RNA (16), ribozymes (34), suppressor tRNAs (2), interferon-

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inducing double stranded RNA and numerous examples of protein therapeutics, from hormones, e.g., insulin, to lymphokines, e.g., interferons and interleukins, to natural opiates. The discovery of these therapeutic
5 agents and the elucidation of their structure and function does not make obvious the ability to use them in a viral vector delivery system.

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SUMMARY OF THE INVENTION

This invention provides a recombinant swinepox virus comprising a foreign DNA inserted into a swinepox virus genome, wherein the foreign DNA is inserted into an EcoRI
5 site within a region corresponding to a 3.2 Kb subfragment of the HindIII K fragment which contains both a HindIII and an EcoRI site, of the swinepox virus genome and is capable of being expressed in a host cell into which the virus is introduced.

10

This invention provides a recombinant swinepox virus comprising a foreign DNA inserted into a swinepox virus genome, wherein the foreign DNA is inserted into a) an
15 AccI site within a region corresponding to a HindIII to BglII subfragment of the HindIII M fragment and b) an EcoRI site within a region corresponding to a 3.2 Kb subfragment of the HindIII K fragment which contains both a HindIII and EcoRI site, of the swinepox virus genome and is capable of being expressed in a host cell into
20 which the virus is introduced.

The invention further provides vaccines and methods of immunization of the recombinant swinepox virus.

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BRIEF DESCRIPTION OF THE INVENTION**Figures 1A - 1B:**

5 Show a detailed diagram of SPV genomic DNA (Kasza strain) including the unique long and Terminal repeat (TR) regions. A restriction map for the enzyme *HindIII* is indicated (23). Fragments are lettered in order of decreasing size. Note that the terminal repeats are greater than 2.1 kb but less
10 than 9.7 kb in size.

Figures 2A - 2C:

15 Show the homology which exists between the 515.85.1 ORF and the Vaccinia virus 01L ORF. Figure 2A shows two maps: The first line of Figure 2A is a restriction map of the SPV *HindIII* M fragment and the second is a restriction map of the DNA insertion in plasmid 515-85.1. The location of the 515-85.1 [VV 01L-like] ORF is also indicated on the map. The
20 locations of the DNA sequences shown in Figures 2B and 2C are indicated below the map by heavy bars in Figure 2A. Figure 2B shows the homology between the VV 01L ORF and the 515-85.1 ORF at their respective N-termini. Figure 2C shows the homology between the
25 VV 01L ORF and the 515-85.1 ORF at their respective C-termini.

Figures 3A - 3C:

30 Show a description of the DNA insertion in Homology Vector 520-17.5. Figure 3A contains a diagram showing the orientation of DNA fragments assembled in plasmid 520-17.5 and table indicating the origin of each fragment. Figure 3B shows the sequences located at each of the junctions A and B between
35 fragments, and Figure 3C shows the sequences located at Junctions C and D. Figures 3B and 3C further describe the restriction sites used to generate each

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fragment as well as the synthetic linker sequences which were used to join the fragments are described for each junction. The synthetic linker sequences are underlined by a heavy bar. The location of several gene coding regions and regulatory elements are also given. The following two conventions are used: numbers in parenthesis () refer to amino acids, and restriction sites in brackets [] indicate the remnants of sites which were destroyed during construction. The following abbreviations are used, swinepox virus (SPV), early promoter 1 (EP1), late promoter 2 (LP2), lactose operon Z gene (lacZ), and *Escherichia coli* (*E. coli*).

15 **Figures 4A- 4D:**

Show a detailed description of the DNA insertion in Homology Vector 538-46.16. Figure 4A contains a diagram showing the orientation of DNA fragments assembled in plasmid 538-46.16 and a table indicating the origin of each fragment. Figure 4B shows the sequences located at Junctions A and B between fragments, Figure 4C shows sequences located at Junction C and Figure 4D shows sequences located at Junctions D and E. Figures 4B to 4D also describe the restriction sites used to generate each fragment as well as the synthetic linker sequences which were used to join the fragments are described for each junction. The synthetic linker sequences are underlined by a heavy bar. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parenthesis () refer to amino acids, and restriction sites in brackets [] indicate the remnants of sites which were destroyed during construction. The following abbreviations are used, swinepox virus (SPV), pseudorabies virus (PRV), g50 (gD), glycoprotein 63 (g63), early promoter 1 (EP1),

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late promoter 1 (LP1), late promoter 2 (LP2),
lactose operon Z gene (lacZ), and *Escherichia coli*
(*E. coli*).

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Figures 5A - 5D:

10 Show a detailed description of Swinepox Virus S-PRV-013 and the DNA insertion in Homology Vector 570-91.64. Figure 5A contains a diagram showing the orientation of DNA fragments assembled in plasmid 570-91.64 and a table indicating the origin of each fragment. Figure 5B shows the sequences located at Junctions A and B between fragments, Figure 5C shows the sequences located at Junction C, and Figure 5D shows the sequences located at Junctions D and E. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction in Figures 5B to 5D. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), *Escherichia coli* (*E. coli*), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2), gIII (gC) base pairs (BP).

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Figure 6:

35 Map showing the 5.6 kilobase pair HindIII M swinepox virus genomic DNA fragment. Open reading frames (ORF) are shown with the number of amino acids coding in each open reading frame. The swinepox virus ORFs show significant sequence identities to

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the vaccinia virus ORFs and are labeled with the vaccinia virus nomenclature (56 and 58). The I4L ORF (SEQ ID NO: 196) shows amino acid sequence homology to ribonucleotide reductase large subunit (57), and the O1L ORF (SEQ ID NO: 193) shows amino acid sequence homology to a leucine zipper motif characteristic of certain eukaryotic transcriptional regulatory proteins (13). The BglII site in the I4L ORF and the AccI site in the O1L ORF are insertion sites for foreign DNA into non-essential regions of the swinepox genome. The homology vector 738-94.4 contains a deletion of SPV DNA from nucleotides 1679 to 2452 (SEQ ID NO: 189). The black bar at the bottom indicates regions for which the DNA sequence is known and references the SEQ ID NOs: 189 and 195. Positions of restriction sites AccI, BglII, and HindIII are shown. I3L ORF (SEQ ID NO: 190), I2L ORF (SEQ ID NO: 191) and E1OR ORF (SEQ ID NO: 194) are shown. SEQ ID NO 221 contains the complete 5785 base pair sequence of the HindIII M fragment. Open reading frames within the SPV HindIII M fragment are the partial I4L ORF (445 AA; Nucl 2 to 1336); I3L ORF (275 AA; Nucl 1387 to 2211); I2L ORF (75 AA; Nucl 2215 to 2439); I1L ORF (313 AA; Nucl 2443 to 3381); O1L ORF (677 AA; Ncl 3520 to 5550); partial E1OR ORF (64 AA; Nucl 5787 to 5596).

Figures 7A - 7D:

Show a detailed description of Swinepox Virus S-SPV-015 and the DNA insertion in Homology Vector 727-54.60. Figure 7A contains a diagram showing the orientation of DNA fragments assembled in plasmid 727-54.60 and a table indicating the origin of each fragment. Figure 7B shows the sequences located at Junctions A and B between fragments, Figure 7C shows the sequences located at Junction C, and Figure 7D shows the sequences located at Junctions D and E.

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5 The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction in Figures 7B to 7D. The location of
10 several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), *Escherichia coli* (*E. coli*), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2), glycoprotein B (gB),
15 base pairs (BP).

Figures 8A-8D:

20 Detailed description of Swinepox Virus S-SPV-042 and the DNA insertion in Homology Vector 751-07.A1. Diagram showing the orientation of DNA fragments assembled in plasmid 751-07.A1. The origin of each fragment is indicated in the table. The sequence located at each of the junctions between fragments
25 is also shown. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction. Figures 8A-8D show the sequences located at Junction A (SEQ ID NOS: 197),
30 (SEQ ID NO: 198), C (SEQ ID NO: 199), D (SEQ ID NO: 200) and E (SEQ ID NO: 201) between fragments and the sequences located at the junctions. The location of several gene coding regions and regulatory elements is also given. The following
35 two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites which

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are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), chicken myelomonocytic growth factor (cMGF), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2), polymerase chain reaction (PCR), base pairs (BP).

Figures 9A-9D:

Detailed description of Swinepox Virus S-SPV-043 and the DNA insertion in Homology Vector 751-56.A1. Diagram showing the orientation of DNA fragments assembled in plasmid 751-56.A1. The origin of each fragment is indicated in the table. The sequences located at each of the junctions between fragments is also shown. Figures 9A-9D show the sequences located at Junction A (SEQ ID NOS: 202), (SEQ ID NO: 203), C (SEQ ID NO: 204), D (SEQ ID NO: 205) and E (SEQ ID NO: 206) between fragments and the sequences located at the junctions. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), chicken interferon (cIFN), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LPE2EP2), polymerase chain reaction (PCR), base pairs (BP).

Figure 10A-10D:

Detailed description of Swinepox Virus S-SPV-037 and the DNA insertion in Homology Vector 752-22.1.

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Diagram showing the orientation of DNA fragments assembled in plasmid 752-22.1. The origin of each fragment is indicated in the table. The sequences located at each of the junctions between fragments is also shown. Figures 10A-10D show the sequences located at Junction A (SEQ ID NOS: 207), (SEQ ID NO: 208), C (SEQ ID NO: 209), and D (SEQ ID NO: 210) between fragments and the sequences located at the junctions. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restrictions sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), Escherichia coli (E. coli), pox synthetic late promoter 2 early promoter 2 (LP2EP2), polymerase chain reaction (PCR), base pairs (BP).

Figures 11A-11B:

Figure 11A: Restriction Endonuclease Map and Open Reading Frames in the SPV HindIII N fragment and part of SPV HindIII M fragment. Insertions of a foreign gene into a non-essential site of the swinepox virus Hind III N and Hind III M genomic DNA include the EcoR V site (S-SPV-060), SnaB I site (S-SPV-061), Bgl II site in Hind III N (S-SPV-062), and the Bgl II site in Hind III M (S-SPV-047). Insertions of a foreign gene into the I7L ORF (SEQ ID NO. 230) and I4L ORF (SEQ ID NO. 231) indicates that the sequence of the entire open reading frame is non-essential for replication of the swinepox virus and suitable for insertion of foreign genes.

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Additional sites for insertion of foreign genes include, but are not limited to the two Hind III sites, Ava I site, and the BamHI site.

5 Figure 11B: Restriction Endonuclease Map and Open
Reading Frames in the SPV Hind III K genomic
fragment. Insertion of a foreign gene into a non-
essential site of the swinepox virus Hind III K
10 genomic DNA includes, but is not limited to the
unique EcoR I site (S-SPV-059). Three open reading
frames (ORFs) were identified within an
approximately 3.2 kB region (SEQ ID NO. 1) of the
approximately 6.7 kb SPV HindIII K fragment.
15 Insertions of a foreign DNA into a unique EcoRI site
within the SPV HindIII K genomic fragment indicates
that the sequence is non-essential for replication
of the swinepox virus and suitable for insertion of
foreign genes. The unique EcoRI site is located
20 between the 77.2 kd protein ORF and the T5 protein
ORF in an intergenic region indicating that the
intergenic region contains suitable sites for
insertion of foreign DNA. Also identified are the
77.2 kd protein ORF (SEQ ID NO:3) and the T5 protein
25 ORF (SEQ ID NO. 4) and an ORF of unknown function
(SEQ ID NO. 2) which are suitable sites for
insertion of a foreign DNA. The SPV 77.2 kd protein
ORF (SEQ ID NO. 3) has amino acid sequence homology
to rabbit fibroma virus (RFV) 77.2 kd protein. The
30 SPV T5 protein ORF has amino acid sequence homology
to rabbit fibroma virus (RFV) T5 protein. The
identified open reading frames are within an
approximately 3141 base pair segment of the SPV Hind
III K fragment (SEQ ID NO. 1). The remaining
35 approximately 3500 base pairs of the SPV Hind III K
fragment has been sequenced previously (R.F.
Massung, et al. Virology 197, 511-528 (1993)).

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Figures 12A-12C:

Detailed description of Swinepox Virus S-SPV-047 and the DNA insertion in Homology Vector 779-94.31. Diagram showing the orientation of DNA fragments assembled in plasmid 779-94.31. The origin of each fragment is indicated in the table. The sequences located at each of the junctions between fragments is also shown. Figures 12A-12C show the sequences located at Junction A (S:), C , D , and E between fragments and the sequences located at the junctions. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restrictions sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), Escherichia coli (E. coli), pox synthetic late promoter 2 early promoter 2 (LP2EP2), pox synthetic late promoter 1 (LP1), base pairs (BP).

Figures 13A-13D:

Detailed description of Swinepox Virus S-SPV-052 and the DNA insertion in Homology Vector 789-41.7. Diagram showing the orientation of DNA fragments assembled in plasmid 789-41.7. The origin of each fragment is indicated in the table. The sequences located at each of the junctions between fragments is also shown. Figures 13A-13D show the sequences located at Junction A, B, C , D , E , and F between fragments and the sequences located at the junctions. The restriction sites used to generate

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each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restrictions sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), Escherichia coli (E. coli), pox synthetic late promoter 2 early promoter 2 (LP2EP2), pox synthetic early promoter 1 late promoter 2 (EP1LP2), pox synthetic late promoter 1 (LP1), base pairs (BP).

Figures 14A-14D:

Detailed description of Swinepox Virus S-SPV-053 and the DNA insertion in Homology Vector 789-41.27. Diagram showing the orientation of DNA fragments assembled in plasmid 789-41.27. The origin of each fragment is indicated in the table. The sequences located at each of the junctions between fragments is also shown. Figures 14A-14D show the sequences located at Junction A, B, C, D, E, F, and G between fragments and the sequences located at the junctions. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restrictions sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), Escherichia coli (E. coli), pox synthetic late

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promoter 2 early promoter 2 (LP2EP2), pox synthetic
early promoter 1 late promoter 2 (EP1LP2), pox
synthetic late promoter 1 (LP1), base pairs (BP).

5 **Figures 15A-15D:**

Detailed description of Swinepox Virus S-SPV-054 and
the DNA insertion in Homology Vector 789-41.47.
Diagram showing the orientation of DNA fragments
assembled in plasmid 789-41.47. The origin of each
10 fragment is indicated in the table. The sequences
located at each of the junctions between fragments
is also shown. Figures 15A-15D show the sequences
located at Junction A, B, C (SEQ ID NO:), D , E ,
F , and G between fragments and the sequences
15 located at the junctions. The restriction sites
used to generate each fragment as well as synthetic
linker sequences which are used to join the
fragments are described for each junction. The
location of several gene coding regions and
20 regulatory elements is also given. The following
two conventions are used: numbers in parentheses,
(), refer to amino acids, and restrictions sites in
brackets, [], indicate the remnants of sites which
are destroyed during construction. The following
25 abbreviations are used: swinepox virus (SPV),
pseudorabies virus (PRV), Escherichia coli (E.
coli), pox synthetic early promoter 1 late promoter
2 (EP1LP2), pox synthetic late promoter 1 (LP1),
base pairs (BP).

30

Figures 16A-16E:

Detailed description of Swinepox Virus S-SPV-055 and
the DNA insertion in Homology Vector 789-41.73.
35 Diagram showing the orientation of DNA fragments
assembled in plasmid 789-41.73. The origin of each
fragment is indicated in the table. The sequences

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located at each of the junctions between fragments is also shown. Figures 16A-16E show the sequences located at Junction A, B, C, D, E, F, G, and H between fragments and the sequences located at the junctions. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restrictions sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), Escherichia coli (E. coli), pox synthetic late promoter 2 early promoter 2 (LP2EP2), pox synthetic early promoter 1 late promoter 2 (EP1LP2), pox synthetic late promoter 1 (LP1), base pairs (BP).

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DETAILED DESCRIPTION OF THE INVENTION

This invention provides a recombinant swinepox virus comprising a foreign DNA inserted into a swinepox virus genome, wherein the foreign DNA is inserted into an EcoRI site within a region corresponding to a 3.2 Kb subfragment of the HindIII K fragment which contains both a HindIII and an EcoRI site, of the swinepox virus genome and is capable of being expressed in a host cell into which the virus is introduced.

This invention provides a recombinant swinepox virus comprising a foreign DNA inserted into a swinepox virus genome, wherein the foreign DNA is inserted into a) an AccI site within a region corresponding to a HindIII to BglII subfragment of the larger HindIII M fragment and b) an EcoRI site within a region corresponding to a 3.2 Kb subfragment of the HindIII K fragment which contains both a HindIII and EcoRI site, of the swinepox virus genome and is capable of being expressed in a host cell into which the virus is introduced.

In another embodiment the open reading frame encodes a B18R gene. In another embodiment the open reading frame encodes a B4R gene. In another embodiment the open reading frame encodes swinepox homologue of the 77.2 kD protein gene. In another embodiment the open reading frame encodes swinepox homologue of the T5 protein gene.

In another embodiment the foreign DNA sequence is inserted within a EcoRV restriction endonuclease site within the approximately 2 kB HindIII to BamHI subfragment of the swinepox virus genome. In another embodiment the foreign DNA sequence is inserted within a SnaBI restriction endonuclease site within the

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approximately 2.0 kB HindIII to BamHI subfragment of the swinepox virus genome.

5 In another embodiment the foreign DNA sequence is inserted within an approximately 1.2 kB BamHI to HindIII subfragment of the HindIII N fragment of the swinepox virus genome. In another embodiment the foreign DNA sequence is inserted into an open reading frame within an approximately 1.2 kB BamHI to HindIII subfragment of the
10 HindIII N fragment of the swinepox virus genome. In another embodiment the foreign DNA sequence is inserted into an open reading frame which encodes a I4L gene. In another embodiment the foreign DNA sequence is inserted within a BglII restriction endonuclease site within the
15 approximately 1.2 kB BamHI to HindIII subfragment of the swinepox virus genome.

In another embodiment the recombinant swinepox virus contains the foreign DNA sequence inserted within an
20 approximately 3.6 kB larger HindIII to BglII subfragment of the HindIII M fragment of the swinepox virus genomic DNA. In another embodiment the foreign DNA sequence is inserted into an open reading frame within an approximately 3.6 kB larger HindIII to BglII subfragment
25 of the HindIII M fragment of the swinepox virus genomic DNA. In another embodiment the open reading frame encodes a I4L gene.

In one embodiment the foreign DNA sequence of the
30 recombinant swinepox virus is inserted within a non-essential Open Reading Frame (ORF) of the HindIII M fragment. Example of ORF's include, but are not limited to: I4L, I2L, 01L, and E10L.

35 In another embodiment the recombinant swinepox virus further comprises a foreign DNA sequence inserted into an open reading frame encoding swinepox virus thymidine

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kinase. In one embodiment the foreign DNA sequence is inserted into a *NdeI* site located within the open reading frame encoding the swinepox virus thymidine kinase.

5 For purposes of this invention, "a recombinant swinepox virus capable of replication" is a live swinepox virus which has been generated by the recombinant methods well known to those of skill in the art, e.g., the methods set forth in HOMOLOGOUS RECOMBINATION PROCEDURE FOR
10 GENERATING RECOMBINANT SPV in Materials and Methods and has not had genetic material essential for the replication of the recombinant swinepox virus deleted.

For purposes of this invention, "an insertion site which
15 is not essential for replication of the swinepox virus" is a region or a region which corresponds to a specific fragment in the swinepox viral genome where a sequence of DNA is not necessary for viral replication, for example, complex protein binding sequences, sequences which code
20 for reverse transcriptase or an essential glycoprotein, DNA sequences necessary for packaging, etc.

For purposes of this invention, a "promoter" is a specific DNA sequence on the DNA molecule to which the
25 foreign RNA polymerase attaches and at which transcription of the foreign RNA is initiated.

For purposes of this invention, an "open reading frame" is a segment of DNA which contains codons that can be
30 transcribed into RNA which can be translated into an amino acid sequence and which does not contain a termination codon.

The invention further provides a foreign DNA sequence or
35 foreign RNA which encodes a polypeptide. Preferably, the polypeptide is antigenic in the animal. Preferably, this antigenic polypeptide is a linear polymer of more than 10

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amino acids linked by peptide bonds which stimulates the animal to produce antibodies.

5 The S-SPV-003 swinepox virus has been deposited pursuant to the Budapest Treaty on the International Deposit of Microorganisms for the Purposes of Patent Procedure with the Patent Culture Depository of the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 U.S.A. under ATCC Accession No. VR 2335.

10

For purposes of this invention, a "polypeptide which is a detectable marker" includes the bimer, trimer and tetramer form of the polypeptide. *E. coli* β -galactosidase is a tetramer composed of four polypeptides or monomer sub-units.

15

Foreign DNA which code for a polypeptide include but are not limited to: feline leukemia virus surface protein, feline leukemia virus transmembrane protein, feline leukemia virus gag, feline leukemia virus transmembrane protease, feline immunodeficiency virus gag/protease, feline immunodeficiency virus envelope, feline leukemia virus gag/protease, feline leukemia virus envelope, canine parvovirus VP2, canine parvovirus VP1/2, bovine cytokine interleukin-12 protein 35, bovine cytokine interleukin-12 protein 40, Bovine Respiratory Syncytial Virus glycoprotein G, Newcastle Disease fusion, Infectious Rhinotracheitis Virus glycoprotein D, Canine Distemper Virus fusion, Canine Distemper Virus Hemagglutinin, DV HA, Bovine Viral Diarrhea Virus type 1 glycoprotein 45, Bovine Viral Diarrhea Virus type 1 glycoprotein 48, Bovine Viral Diarrhea Virus type 1 glycoprotein 53, Bovine Viral Diarrhea Virus type 2 glycoprotein 53.

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The present invention further provides a recombinant swinepox virus in which the foreign DNA encodes an antigenic polypeptide is: Swine Influenza Virus hemagglutinin, Swine Influenza Virus neurominidase, Swine Influenza Virus matrix, Swine Influenza Virus nucleoprotein, African Swine Fever Virus or *Mycoplasma hyopneumoniae*. Preferred embodiments of such virus are designated S-SPV-121, and S-SPV-122.

10 The present invention further provides a recombinant swinepox virus in which the foreign DNA encodes an antigenic polypeptide is: cytokine is chicken macrophage migration inhibitory factor (cMIF), chicken myelomonocytic growth factor (CMGF) or chicken interferon
15 (cIFN). Preferred embodiments of such virus are designated S-SPV-068, and S-SPV-105.

The present invention further provides a recombinant swinepox virus in which the foreign DNA encodes an
20 antigenic polypeptide is: porcine reproductive and respiratory syndrome virus (PRRS) ORF2, ORF3, ORF4, ORF5, ORF6 and ORF7, pseudorabies gB, gD, gI. Preferred embodiments of such virus are designated S-SPV-076, S-SPV-079, S-SPV-090, S-SPV-084, S-SPV-091, S-SPV-092, S-
25 SPV-093, S-SPV-094, S-SPV-095.

The present invention further provides a recombinant swinepox virus in which the foreign DNA encodes an antigenic polypeptide is: Infectious bovine
30 rhinotracheitis virus glycoprotein B, glycoprotein D and glycoprotein I, bovine respiratory syncytial virus attachment protein (BRSV G), bovine respiratory syncytial virus fusion protein (BRSV F), bovine respiratory syncytial virus nucleocapsid protein (BRSV N). Preferred
35 embodiments of such virus are designated S-SPV-109, S-

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SPV-110, S-SPV-111, S-SPV-113, S-SPV-115, S-SPV-119, S-SPV-112.

5 The present invention further provides a recombinant swinepox virus in which the foreign DNA encodes an antigenic polypeptide is: bovine viral diarrhea virus (BVDV) glycoprotein 48 or glycoprotein 53. A Preferred embodiment of such a virus is designated S-SPV-099.

10 The present invention further provides a recombinant swinepox virus in which the foreign DNA encodes an antigenic polypeptide is: feline immunodeficiency virus gag/protease and envelope, feline leukemia virus gag/protease and envelope. Preferred embodiments of such
15 viruses are designated: S-SPV-106, S-SPV-089, S-SPV-100, S-SPV-107, S-SPV-108.

The present invention further provides a recombinant swinepox virus in which the foreign DNA encodes an
20 antigenic polypeptide is: canine parvovirus VP2 and VP1/2. Preferred embodiments of such viruses are designated: S-SPV-114, S-SPV-116, S-SPV-117, S-SPV-118.

The present invention provides a recombinant swinepox
25 virus comprising a foreign DNA inserted into the swinepox virus genomic DNA, wherein the one or more foreign DNAs are inserted within each of the HindIII K fragment of the swinepox virus genomic DNA and within the HindIII M fragment of the swinepox virus genomic DNA and is capable
30 of being expressed in a swinepox virus infected host cell. Preferred embodiments of such viruses are designated: S-SPV-127, S-SPV-128, S-SPV-131, and S-SPV-132.

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The present invention provides a recombinant swinepox virus comprising a foreign DNA inserted into the swinepox virus genomic DNA, wherein the one or more foreign DNAs which encode a fusion protein are inserted within each of
5 the HindIII K fragment of the swinepox virus genomic DNA and within the HindIII M fragment of the swinepox virus genomic DNA and is capable of being expressed in a swinepox virus infected host cell. Preferred embodiments of such viruses are designated: S-SPV-130.

10

The invention further provides a recombinant swinepox virus capable of replication which contains foreign DNA encoding an antigenic polypeptide which is or is from pseudorabies virus (PRV) g50 (gD), pseudorabies virus
15 (PRV) gII (gB), Pseudorabies virus (PRV) gIII (gC), pseudorabies virus (PRV) glycoprotein H, pseudorabies virus (PRV) glycoprotein E, Transmissible gastroenteritis (TGE) glycoprotein 195, Transmissible gastroenteritis (TGE) matrix protein, swine rotavirus glycoprotein 38,
20 swine parvovirus capsid protein, *Serpulina hyodysenteriae* protective antigen, Bovine Viral Diarrhea (BVD) glycoprotein 55, Newcastle Disease Virus (NDV) hemagglutinin-neuraminidase, swine flu hemagglutinin or swine flu neuraminidase. Preferably, the antigenic
25 polypeptide is Pseudorabies Virus (PRV) g50 (gD). Preferably, the antigenic protein is Newcastle Disease Virus (NDV) hemagglutinin-neuraminidase.

The invention further provides a recombinant swinepox
30 virus capable of replication which contains foreign DNA encoding an antigenic polypeptide which is or is from *Serpulina hyodysenteriae*, Foot and Mouth Disease Virus, Hog Cholera Virus, African Swine Fever Virus or *Mycoplasma hyopneumoniae*.

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The invention further provides for a recombinant swinepox virus capable of replication which contains foreign DNA encoding RNA encoding the antigenic polypeptide Newcastle Disease Virus (NDV) hemagglutinin-neuraminidase further comprising foreign DNA encoding a polypeptide which is a detectable marker.

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from infectious bovine rhinotracheitis virus and is capable of being expressed in a host infected by the recombinant swinepox virus. Examples of such antigenic polypeptide are infectious bovine rhinotracheitis virus glycoprotein E and glycoprotein G.

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from infectious laryngotracheitis virus and is capable of being expressed in a host infected by the recombinant swinepox virus. Examples of such antigenic polypeptide are infectious laryngotracheitis virus glycoprotein G and glycoprotein I.

In one embodiment of the recombinant swinepox virus the foreign DNA sequence encodes a cytokine. In another embodiment the cytokine is chicken myelomonocytic growth factor (cMGF) or chicken interferon (cIFN). Cytokines include, but are not limited to: transforming growth factor beta, epidermal growth factor family, fibroblast growth factors, hepatocyte growth factor, insulin-like growth factor, vascular endothelial growth factor,

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interleukin 1, IL-1 receptor antagonist, interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, IL-6 soluble receptor, interleukin-7, interleukin-8, interleukin-9, interleukin-10, interleukin-11, interleukin-12, interleukin-13, angiogenin, chemokines, colony stimulating factors, granulocyte-macrophage colony stimulating factors, erythropoietin, interferon, interferon gamma, Stem cell factor (or known as mast cell growth factor, or c-kit ligand protein), leukemia inhibitory factor, oncostatin M, pleiotrophin, secretory leukocyte protease inhibitor, stem cell factor, tumor necrosis factors, and soluble TNF receptors. These cytokines are from humans, bovine, equine, feline, canine, porcine or avian.

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from a human pathogen and is capable of being expressed in a host infected by the recombinant swinepox virus.

Recombinant SPV expressing cytokines is used to enhance the immune response either alone or when combined with vaccines containing cytokines or antigen genes of disease causing microorganisms.

Antigenic polypeptide of a human pathogen which are derived from human herpesvirus include, but are not limited to: hepatitis B virus and hepatitis C virus hepatitis B virus surface and core antigens, hepatitis C virus, human immunodeficiency virus, herpes simplex virus-1, herpes simplex virus-2, human cytomegalovirus, Epstein-Barr virus, Varicella-Zoster virus, human herpesvirus-6, human herpesvirus-7, human influenza, measles virus, hantaan virus, pneumonia virus,

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rhinovirus, poliovirus, human respiratory syncytial virus, retrovirus, human T-cell leukemia virus, rabies virus, mumps virus, malaria (*Plasmodium falciparum*), *Bordetella pertussis*, Diphtheria, *Rickettsia prowazekii*,
5 *Borrelia berfordorferi*, Tetanus toxoid, malignant tumor antigens.

In one embodiment of the invention, a recombinant swinepox virus contains the foreign DNA sequence encoding
10 hepatitis B virus core protein.

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox
15 genome, wherein the foreign DNA sequence encodes a cytokine capable of stimulating an immune in a host infected by the recombinant swinepox virus and is capable of being expressed in the host infected.

20 The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an
25 antigenic polypeptide derived from an equine pathogen and is capable of being expressed in a host infected by the recombinant swinepox virus.

The antigenic polypeptide of an equine pathogen can
30 derived from equine influenza virus, or equine herpesvirus. In one embodiment the antigenic polypeptide is equine influenza neuraminidase or hemagglutinin. Examples of such antigenic polypeptide are equine
35 influenza virus type A/Alaska 91 neuraminidase, equine influenza virus type A/Prague 56 neuraminidase, equine influenza virus type A/Miami 63 neuraminidase, equine influenza virus type A/Kentucky 81 neuraminidase, equine

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influenza virus type A/Kentucky 92 neuraminidase equine
herpesvirus type 1 glycoprotein B, equine herpesvirus
type 1 glycoprotein D, *Streptococcus equi*, equine
infectious anemia virus, equine encephalitis virus,
5 equine rhinovirus and equine rotavirus.

The present invention further provides an antigenic
polypeptide which includes, but is not limited to: hog
cholera virus gE1, hog cholera virus gE2, swine influenza
10 virus hemagglutinin, neurominidase, matrix and
nucleoprotein, pseudorabies virus gB, gC and gD, and PRRS
virus ORF7.

The present invention further provides a recombinant
15 swinepox virus which comprises a foreign DNA sequence
inserted into a non-essential site of the swinepox
genome, wherein the foreign DNA sequence encodes an
antigenic polypeptide derived from bovine respiratory
syncytial virus or bovine parainfluenza virus, and is
20 capable of being expressed in a host infected by the
recombinant swinepox virus.

For example, the antigenic polypeptide of derived from
infectious bovine rhinotracheitis virus gE, bovine
25 respiratory syncytial virus equine pathogen can derived
from equine influenza virus is bovine respiratory
syncytial virus attachment protein (BRSV G), bovine
respiratory syncytial virus fusion protein (BRSV F),
bovine respiratory syncytial virus nucleocapsid protein
30 (BRSV N), bovine parainfluenza virus type 3 fusion
protein, and the bovine parainfluenza virus type 3
hemagglutinin neuraminidase.

The present invention further provides a recombinant
35 swinepox virus which comprises a foreign DNA sequence
inserted into a non-essential site of the swinepox
genome, wherein the foreign DNA sequence encodes bovine

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viral diarrhea virus (BVDV) glycoprotein 48 or glycoprotein 53, and wherein the foreign DNA sequence is capable of being expressed in a host infected by the recombinant swinepox virus.

5

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from infectious bursal disease virus and wherein the foreign DNA sequence is capable of being expressed in a host infected by the recombinant swinepox virus. Examples of such antigenic polypeptide are infectious bursal disease virus polyprotein and VP2.

The present invention further provides a recombinant swinepox virus in which the foreign DNA sequence encodes an antigenic polypeptide which includes, but is not limited to: MDV gA, MDV gB, MDV gD, NDV HN, NDV F, ILT gB, ILT gI, ILT gD, IBDV VP2, IBDV VP3, IBDV VP4, IBDV polyprotein, IBV spike, IBV matrix, avian encephalomyelitis virus, avian reovirus, avian paramyxovirus, avian influenza virus, avian adenovirus, fowl pox virus, avian coronavirus, avian rotavirus, chick anemia virus, *Salmonella* spp., *E. coli*, *Pasteurella* spp., *Bordetella* spp., *Eimeria* spp., *Histomonas* spp., *Trichomonas* spp., Poultry nematodes, cestodes, trematodes, poultry mites/lice, and poultry protozoa.

30

The invention further provides that the inserted foreign DNA sequence is under the control of a promoter. In one embodiment this is a swinepox viral promoter. In another embodiment the foreign DNA sequence is under control of an endogenous upstream poxvirus promoter. In another embodiment the foreign DNA sequence is under control of a heterologous upstream promoter.

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For purposes of this invention, promoters include but is not limited to: synthetic pox viral promoter, pox synthetic late promoter 1, pox synthetic late promoter 2 early promoter 2, pox O1L promoter, pox I4L promoter, pox I3L promoter, pox I2L promoter, pox I1L promoter, pox E10R promoter, PRV gX, HSV-1 alpha 4, HCMV immediate early, MDV gA, MDV gB, MDV gD, ILT gB, BHV-1.1 VP8 and ILT gD and internal ribosomal entry site promoter. Alternate promoters are generated by methods well known to those of skill in the art, for example, as set forth in the STRATEGY FOR THE CONSTRUCTION OF SYNTHETIC POX VIRAL PROMOTERS in Materials and Methods.

The invention provides for a homology vector for producing a recombinant swinepox virus by inserting foreign DNA into the genomic DNA of a swinepox virus. The homology vector comprises a double-stranded DNA molecule consisting essentially of a double-stranded foreign DNA sequence or (RNA) which does not naturally occur in an animal into which the recombinant swinepox virus is introduced, with at one end of the foreign DNA, double-stranded swinepox viral DNA homologous to genomic DNA located at one side of a site on the genomic DNA which is not essential for replication of the swinepox virus, and at the other end of the foreign DNA, double-stranded swinepox viral DNA homologous to genomic DNA located at the other side of the same site on the genomic DNA. Preferably, the RNA encodes a polypeptide.

In another embodiment of the present invention, the double-stranded swinepox viral DNA of the homology vectors described above is homologous to genomic DNA present within the HindIII M fragment. In another embodiment the double-stranded swinepox viral DNA of the homology vectors described above is homologous to genomic DNA present within an approximately 2 Kb HindIII to BglII sub-fragment. In a preferred embodiment the double-

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stranded swinepox viral DNA is homologous to genomic DNA present within the *Bgl*III site located in this *Hind*III to *Bgl*III subfragment.

- 5 In another embodiment the double-stranded swinepox viral DNA is homologous to genomic DNA present within the open reading frame contained in the larger *Hind*III to *Bgl*III subfragment. Preferably, the double-stranded swinepox viral DNA is homologous to genomic DNA present within the
10 *Acc*I restriction endonuclease site located in the larger *Hind*III to *Bgl*III subfragment.

- In one embodiment, the polypeptide is a detectable marker. Preferably, the polypeptide which is a
15 detectable marker is *E. coli* β -galactosidase.

- In one embodiment, the foreign DNA which codes for a polypeptide include but are not limited to: feline leukemia virus surface protein, feline leukemia virus
20 transmembrane protein, feline leukemia virus gag, feline leukemia virus transmembrane protease, feline immunodeficiency virus gag/protease, feline immunodeficiency virus envelope, feline leukemia virus gag/protease, feline leukemia virus envelope, canine
25 parvovirus VP2, canine parvovirus VP1/2, bovine cytokine interleukin-12 protein 35, bovine cytokine interleukin-12 protein 40, Bovine Respiratory Syncytial Virus glycoprotein G, Newcastle Disease fusion, Infectious Rhinotracheitis Virus glycoprotein D, Canine Distemper
30 Virus fusion, Canine Distemper Virus Hemagglutinin, DV HA, Bovine Viral Diarrhea Virus type 1 glycoprotein 45, Bovine Viral Diarrhea Virus type 1 glycoprotein 48, Bovine Viral Diarrhea Virus type 1 glycoprotein 53, Bovine Viral Diarrhea Virus type 2 glycoprotein 53.

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Other polypeptides include: pseudorabies virus (PRV) g50 (gD), pseudorabies virus (PRV) gII (gB), Pseudorabies virus (PRV) gIII (gC), Pseudorabies virus (PRV) glycoprotein H, Transmissible gastroenteritis (TGE) glycoprotein 195, Transmissible gastroenteritis (TGE) matrix protein, swine rotavirus glycoprotein 38, swine parvovirus capsid protein, *Serpulina hyodysenteriae* protective antigen, Bovine Viral Diarrhea (BVD) glycoprotein 53 and g48, Newcastle Disease Virus (NDV) hemagglutinin-neuraminidase, swine flu hemagglutinin or swine flu neuraminidase. Preferably, the antigenic polypeptide is or is from *Serpulina hyodysenteriae*, Foot and Mouth Disease Virus, Hog Cholera Virus gE1 and gE2, Swine Influenza Virus, African Swine Fever Virus or *Mycoplasma hyopneumoniae*, swine influenza virus hemagglutinin, neuraminidase and matrix and nucleoprotein, PRRS virus ORF7, and hepatitis B virus core protein.

In one embodiment, the polypeptide is antigenic in the animal.

In an embodiment of the present invention, the double stranded foreign DNA sequence in the homology vector encodes an antigenic polypeptide derived from a human pathogen.

For example, the antigenic polypeptide of a human pathogen is derived from human herpesvirus, herpes simplex virus-1, herpes simplex virus-2, human cytomegalovirus, Epstein-Barr virus, Varicell-Zoster virus, human herpesvirus-6, human herpesvirus-7, human influenza, human immunodeficiency virus, rabies virus, measles virus, hepatitis B virus and hepatitis C virus. Furthermore, the antigenic polypeptide of a human pathogen may be associated with malaria or malignant

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tumor from the group consisting of *Plasmodium falciparum*,
Bordetella pertussis, and malignant tumor.

5 In an embodiment of the present invention, the double
stranded foreign DNA sequence in the homology vector
encodes a cytokine capable of stimulating human immune
response. In one embodiment the cytokine is a chicken
myelomonocytic growth factor (cMGF) or chicken interferon
(cIFN). For example, the cytokine can be, but not
10 limited to, interleukin-2, interleukin-6, interleukin-12,
interferons, granulocyte-macrophage colony stimulating
factors, and interleukin receptors.

15 In an embodiment of the present invention, the double
stranded foreign DNA sequence in the homology vector
encodes an antigenic polypeptide derived from an equine
pathogen.

20 The antigenic polypeptide of an equine pathogen can
derived from equine influenza virus or equine
herpesvirus. Examples of such antigenic polypeptide are
equine influenza virus type A/Alaska 91 neuraminidase,
equine influenza virus type A/Prague 56 neuraminidase,
equine influenza virus type A/Miami 63 neuraminidase,
25 equine influenza virus type A/Kentucky 81 neuraminidase
equine herpesvirus type 1 glycoprotein B, and equine
herpesvirus type 1 glycoprotein D.

30 In an embodiment of the present invention, the double
stranded foreign DNA sequence of the homology vector
encodes an antigenic polypeptide derived from bovine
respiratory syncytial virus or bovine parainfluenza
virus.

35 For example, the antigenic polypeptide is derived from
infectious bovine rhinotracheitis gE, bovine respiratory
syncytial virus attachment protein (BRSV G), bovine

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respiratory syncytial virus fusion protein (BRSV F),
bovine respiratory syncytial virus nucleocapsid protein
(BRSV N), bovine parainfluenza virus type 3 fusion
protein, and the bovine parainfluenza virus type 3
5 hemagglutinin neuraminidase.

In an embodiment of the present invention, the double
stranded foreign DNA sequence of the homology vector
encodes an antigenic polypeptide derived from infectious
10 bursal disease virus. Examples of such antigenic
polypeptide are infectious bursal disease virus
polyprotein and infectious bursal disease virus VP2, VP3,
or VP4.

15 For purposes of this invention, a "homology vector" is a
plasmid constructed to insert foreign DNA in a specific
site on the genome of a swinepox virus.

In one embodiment of the invention, the double-stranded
20 swinepox viral DNA of the homology vectors described
above is homologous to genomic DNA present within the
open reading frame encoding swinepox thymidine kinase.
Preferably, the double-stranded swinepox viral DNA is
homologous to genomic DNA present within the NdeI
25 restriction endonuclease site located in the open reading
frame encoding swinepox thymidine kinase.

The invention further provides homology vectors
described above, the foreign DNA sequence of which is
30 under control of a promoter located upstream of the
foreign DNA sequence. The promoter can be an endogenous
swinepox viral promoter or an exogenous promoter.
Promoters include, but are not limited to: synthetic pox
viral promoter, pox synthetic late promoter 1, pox
35 synthetic late promoter 2 early promoter 2, pox OlL
promoter, pox I4L promoter, pox I3L promoter, pox I2L
promoter, pox IlL promoter, pox ElOR promoter, PRV gX,

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HSV-1 alpha 4, HCMV immediate early, BHV-1.1 VP8, infectious laryngotracheitis virus glycoprotein B, infectious laryngotracheitis virus gD, marek's disease virus glycoprotein A, marek's disease virus glycoprotein B, and marek's disease virus glycoprotein D.

The invention further provides a vaccine which comprises an effective immunizing amount of a recombinant swinepox virus of the present invention and a suitable carrier.

Suitable carriers for the swinepox virus are well known in the art and include proteins, sugars, etc. One example of such a suitable carrier is a physiologically balanced culture medium containing one or more stabilizing agents such as stabilized, hydrolyzed proteins, lactose, etc.

For purposes of this invention, an "effective immunizing amount" of the recombinant swinepox virus of the present invention is within the range of 10^3 to 10^9 PFU/dose.

The present invention also provides a method of immunizing an animal, wherein the animal is a human, swine, bovine, equine, caprine or ovine. For purposes of this invention, this includes immunizing the animal against the virus or viruses which cause the disease or diseases pseudorabies, transmissible gastroenteritis, swine rotavirus, swine parvovirus, *Serpulina hyodysenteriae*, bovine viral diarrhea, Newcastle disease, swine influenza, PRRS, bovine respiratory syncytial virus, bovine parainfluenza virus type 3, foot and mouth disease, hog cholera, African swine fever or *Mycoplasma hyopneumoniae*. For purposes of this invention, the method of immunizing also includes immunizing the animal against human pathogens, bovine pathogens, equine

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pathogens, avian pathogens described in the preceding part of this section.

5 The method comprises administering to the animal an effective immunizing dose of the vaccine of the present invention. The vaccine may be administered by any of the methods well known to those skilled in the art, for example, by intramuscular, subcutaneous, intraperitoneal or intravenous injection. Alternatively, the vaccine may
10 be administered intranasally or orally.

The present invention also provides a method for testing a swine to determine whether the swine has been vaccinated with the vaccine of the present invention,
15 particularly the embodiment which contains the recombinant swinepox virus S-SPV-008 (ATCC Accession No. VR 2339), or is infected with a naturally-occurring, wild-type pseudorabies virus. This method comprises obtaining from the swine to be tested a sample of a
20 suitable body fluid, detecting in the sample the presence of antibodies to pseudorabies virus, the absence of such antibodies indicating that the swine has been neither vaccinated nor infected, and for the swine in which antibodies to pseudorabies virus are present, detecting
25 in the sample the absence of antibodies to pseudorabies virus antigens which are normally present in the body fluid of a swine infected by the naturally-occurring pseudorabies virus but which are not present in a vaccinated swine indicating that the swine was vaccinated
30 and is not infected.

The present invention provides a recombinant SPV which when inserted with a foreign DNA sequence or gene may be employed as a diagnostic assay. In one embodiment FIV
35 *env* and *gag* genes and *D. immitis* p39 and 22kd are employed in a diagnostic assay to detect feline

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immunodeficiency caused by FIV and to detect heartworm caused by *D. immitis*, respectively.

5 The present invention also provides a host cell infected with a recombinant swinepox virus capable of replication. In one embodiment, the host cell is a mammalian cell. Preferably, the mammalian cell is a Vero cell. Preferably, the mammalian cell is an ESK-4 cell, PK-15 cell or EMSK cell.

10

For purposes of this invention a "host cell" is a cell used to propagate a vector and its insert. Infecting the cells was accomplished by methods well known to those of skill in the art, for example, as set forth in INFECTION
15 - TRANSFECTION PROCEDURE in Material and Methods.

20

Methods for constructing, selecting and purifying recombinant swinepox viruses described above are detailed below in Materials and Methods.

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EXPERIMENTAL DETAILS**Materials and Methods**

- 5 **PREPARATION OF SWINEPOX VIRUS STOCK SAMPLES.** Swinepox virus (SPV) samples were prepared by infecting embryonic swine kidney (EMSK) cells, ESK-4 cells, PK-15 cells or Vero cells at a multiplicity of infection of 0.01 PFU/cell in a 1:1 mixture of Iscove's Modified Dulbecco's
10 Medium (IMDM) and RPMI 1640 medium containing 2 mM glutamine, 100 units/ml penicillin, 100 units/ml streptomycin (these components were obtained from Sigma or equivalent supplier, and hereafter are referred to as EMSK negative medium). Prior to infection, the cell
15 monolayers were washed once with EMSK negative medium to remove traces of fetal bovine serum. The SPV contained in the initial inoculum (0.5 ml for 10 cm plate; 10 ml for T175 cm flask) was then allowed to absorb onto the cell monolayer for two hours, being redistributed every half
20 hour. After this period, the original inoculum was brought up to the recommended volume with the addition of complete EMSK medium (EMSK negative medium plus 5% fetal bovine serum). The plates were incubated at 37°C in 5% CO₂ until cytopathic effect was complete. The medium and
25 cells were harvested and frozen in a 50 ml conical screw cap tube at -70°C. Upon thawing at 37°C, the virus stock was aliquoted into 1.0 ml vials and refrozen at -70°C. The titers were usually about 10⁶ PFU/ml.
- 30 **PREPARATION OF SPV DNA.** For swinepox virus DNA isolation, a confluent monolayer of EMSK cells in a T175 cm² flask was infected at a multiplicity of 0.1 and incubated 4-6 days until the cells were showing 100% cytopathic effect. The infected cells were then harvested by scraping the
35 cells into the medium and centrifuging at 3000 rpm for 5 minutes in a clinical centrifuge. The medium was decanted, and the cell pellet was gently resuspended in

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1.0 ml Phosphate Buffer Saline (PBS: 1.5g Na_2HPO_4 , 0.2g KH_2PO_4 , 0.8g NaCl and 0.2g KCl per liter H_2O) (per T175) and subjected to two successive freeze-thaws (-70°C to 37°C). Upon the last thaw, the cells (on ice) were

5 sonicated two times for 30 seconds each with 45 seconds cooling time in between. Cellular debris was then removed by centrifuging (Sorvall RC-5B superspeed centrifuge) at 3000 rpm for 5 minutes in a HB4 rotor at 4°C . SPV virions, present in the supernatant, were then pelleted

10 by centrifugation at 15,000 rpm for 20 minutes at 4°C in a SS34 rotor (Sorvall) and resuspended in 10 mM Tris (pH 7.5). This fraction was then layered onto a 36% sucrose gradient (w/v in 10 mM tris pH 7.5) and centrifuged (Beckman L8-70M Ultracentrifuge) at 18,000 rpm for 60

15 minutes in a SW41 rotor (Beckman) at 4°C . The virion pellet was resuspended in 1.0 ml of 10 mM tris pH 7.5 and sonicated on ice for 30 seconds. This fraction was layered onto a 20% to 50% continuous sucrose gradient and centrifuged 16,000 rpm for 60 minutes in a SW41 rotor at

20 4°C . The SPV virion band located about three quarters down the gradient was harvested, diluted with 20% sucrose and pelleted by centrifugation at 18,000 rpm for 60 minutes in a SW41 rotor at 4°C . The resultant pellet was then washed once with 10 mM Tris pH 7.5 to remove traces

25 of sucrose and finally resuspended in 10 mM Tris pH 7.5. SPV DNA was then extracted from the purified virions by lysis (4 hours at 60°C) induced by the addition of EDTA, SDS, and proteinase K to final concentrations of 20 mM, 0.5% and 0.5 mg/ml, respectively. After digestion, three

30 phenol:chloroform (1:1) extractions were conducted and the sample precipitated by the addition of two volumes of absolute ethanol and incubation at -20°C for 30 minutes. The sample was then centrifuged in an Eppendorf minifuge for 5 minutes at full speed. The supernatant was

35 decanted, and the pellet air dried and rehydrated in 0.01 M Tris pH 7.5, 1 mM EDTA at 4°C .

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PREPARATION OF INFECTED CELL LYSATES. For cell lysate preparation, serum free medium was used. A confluent monolayer of cells (EMSK, ESK-4, PK-15 or Vero for SPV or VERO for PRV) in a 25 cm² flask or a 60 mm petri dish was infected with 100 μ l of virus sample. After cytopathic effect was complete, the medium and cells were harvested and the cells were pelleted at 3000 rpm for 5 minutes in a clinical centrifuge. The cell pellet was resuspended in 250 μ l of disruption buffer (2% sodium dodecyl sulfate, 2% β -mercapto-ethanol). The samples were sonicated for 30 seconds on ice and stored at -20°C.

WESTERN BLOTTING PROCEDURE. Samples of lysates and protein standards were run on a polyacrylamide gel according to the procedure of Laemmli (1970). After gel electrophoresis the proteins were transferred and processed according to Sambrook et al. (1982). The primary antibody was a swine anti-PRV serum (Shope strain; lot370, PDV8201, NVSL, Ames, IA) diluted 1:100 with 5% non-fat dry milk in Tris-sodium chloride, and sodium Azide (TSA: 6.61g Tris-HCl, 0.97g Tris-base, 9.0g NaCl and 2.0g Sodium Azide per liter H₂O). The secondary antibody was a goat anti-swine alkaline phosphatase conjugate diluted 1:1000 with TSA.

MOLECULAR BIOLOGICAL TECHNIQUES. Techniques for the manipulation of bacteria and DNA, including such procedures as digestion with restriction endonucleases, gel electrophoresis, extraction of DNA from gels, ligation, phosphorylation with kinase, treatment with phosphatase, growth of bacterial cultures, transformation of bacteria with DNA, and other molecular biological methods are described by Maniatis et al. (1982) and Sambrook et al. (1989). Except as noted, these were used with minor variation.

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DNA SEQUENCING. Sequencing was performed using the USB Sequenase Kit and ³⁵S-dATP (NEN). Reactions using both the dGTP mixes and the dITP mixes were performed to clarify areas of compression. Alternatively, compressed areas were resolved on formamide gels. Templates were double-stranded plasmid subclones or single stranded M13 subclones, and primers were either made to the vector just outside the insert to be sequenced, or to previously obtained sequence. Sequence obtained was assembled and compared using Dnastar* software. Manipulation and comparison of sequences obtained was performed with Superclone™ and Supersee™ programs from Coral Software.

CLONING WITH THE POLYMERASE CHAIN REACTION. The polymerase chain reaction (PCR) was used to introduce restriction sites convenient for the manipulation of various DNAs. The procedures used are described by Innis, et al. (1990). In general, amplified fragments were less than 500 base pairs in size and critical regions of amplified fragments were confirmed by DNA sequencing. The primers used in each case are detailed in the descriptions of the construction of homology vectors below.

HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. This method relies upon the homologous recombination between the swinepox virus DNA and the plasmid homology vector DNA which occurs in the tissue culture cells containing both swinepox virus DNA and transfected plasmid homology vector. For homologous recombination to occur, the monolayers of EMSK cells are infected with S-SPV-001 (Kasza SPV strain, 17) at a multiplicity of infection of 0.01 PFU/cell to introduce replicating SPV (i.e. DNA synthesis) into the cells. The plasmid homology vector DNA is then transfected into these cells according to the INFECTION - TRANSFECTION

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PROCEDURE. The construction of homology vectors used in this procedure is described below

INFECTION - TRANSFECTION PROCEDURE. 6 cm plates of EMSK cells (about 80% confluent) were infected with S-SPV-001 at a multiplicity of infection of 0.01 PFU/cell in EMSK negative medium and incubated at 37°C in a humidified 5% CO₂ environment for 5 hours. The transfection procedure used is essentially that recommended for Lipofectin™ Reagent (BRL). Briefly, for each 6 cm plate, 15 µg of plasmid DNA was diluted up to 100 µl with H₂O. Separately, 50 micrograms of Lipofectin Reagent was diluted to 100 µl with H₂O. The 100 µl of diluted Lipofectin Reagent was then added dropwise to the diluted plasmid DNA contained in a polystyrene 5 ml snap cap tube and mixed gently. The mixture was then incubated for 15-20 minutes at room temperature. During this time, the virus inoculum was removed from the 6 cm plates and the cell monolayers washed once with EMSK negative medium. Three ml of EMSK negative medium was then added to the plasmid DNA/lipofectin mixture and the contents pipetted onto the cell monolayer. The cells were incubated overnight (about 16 hours) at 37°C in a humidified 5% CO₂ environment. The next day the 3 ml of EMSK negative medium was removed and replaced with 5 ml EMSK complete medium. The cells were incubated at 37°C in 5% CO₂ for 3-7 days until cytopathic effect from the virus was 80-100%. Virus was harvested as described above for the preparation of virus stocks. This stock was referred to as a transfection stock and was subsequently screened for recombinant virus by the BLUOGAL* SCREEN FOR RECOMBINANT SWINEPOX VIRUS OR CPRG SCREEN FOR RECOMBINANT SWINEPOX VIRUS.

SCREEN FOR RECOMBINANT SPV EXPRESSING β-galactosidase (BLUOGAL AND CPRG ASSAYS). When the *E. coli* β-galactosidase (lacZ) marker gene was incorporated into a

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recombinant virus the plaques containing the recombinants were visualized by one of two simple methods. In the first method, the chemical Bluogal™ (Bethesda Research Labs) was incorporated (200 µg/ml) into the agarose overlay during the plaque assay, and plaques expressing active β-galactosidase turned blue. The blue plaques were then picked onto fresh cells (EMSK) and purified by further blue plaque isolation. In the second method, CPRG (Boehringer Mannheim) was incorporated (400 µg/ml) into the agarose overlay during the plaque assay, and plaques expressing active β-galactosidase turned red. The red plaques were then picked onto fresh cells (EMSK) and purified by further red plaque isolation. In both cases viruses were typically purified with three rounds of plaque purification.

SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV USING BLACK PLAQUE ASSAYS. To analyze expression of foreign antigens expressed by recombinant swinepox viruses, monolayers of EMSK cells were infected with recombinant SPV, overlayed with nutrient agarose media and incubated for 6-7 days at 37°C for plaque development to occur. The agarose overlay was then removed from the dish, the cells fixed with 100% methanol for 10 minutes at room temperature and the cells air dried. Fixation of the cells results in cytoplasmic antigen as well as surface antigen detection whereas specific surface antigen expression can be detected using non-fixed cells. The primary antibody was then diluted to the appropriate dilution with PBS and incubated on the cell monolayer for 2 hours at room temperature. To detect PRV g50 (gD) expression from S-SPV-008, swine anti-PRV serum (Shope strain; lot370, PDV8201, NVSL, Ames, IA) was used (diluted 1:100). To detect NDV HN expression from S-SPV-009, a rabbit antiserum specific for the HN protein (rabbit anti-NDV#2) was used (diluted 1:1000). Unbound antibody was then removed by washing the cells three

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times with PBS at room temperature. The secondary antibody, either a goat anti-swine (PRV g50 (gD); S-SPV-008) or goat anti-rabbit (NDV HN; S-SPV-009), horseradish peroxidase conjugate was diluted 1:250 with PBS and
5 incubated with the cells for 2 hours at room temperature. Unbound secondary antibody was then removed by washing the cells three times with PBS at room temperature. The cells were then incubated 15-30 minutes at room temperature with freshly prepared substrate solution (100
10 μ g/ml 4-chloro-1-naphthol, 0.003% H_2O_2 in PBS). Plaques expressing the correct antigen stain black.

PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS. Viral glycoproteins are purified using
15 antibody affinity columns. To produce monoclonal antibodies, 8 to 10 week old BALB/c female mice are vaccinated intraperitoneally seven times at two to four week intervals with 10^7 PFU of S-SPV-009, -014, -016, -017, -018, or -019. Three weeks after the last
20 vaccination, mice are injected intraperitoneally with 40 mg of the corresponding viral glycoprotein. Spleens are removed from the mice three days after the last antigen dose.

25 Splenocytes are fused with mouse NS1/Ag4 plasmacytoma cells by the procedure modified from Oi and Herzenberg, (41). Splenocytes and plasmacytoma cells are pelleted together by centrifugation at 300 x g for 10 minutes. One ml of a 50% solution of polyethylene glycol (m.w.
30 1300-1600) is added to the cell pellet with stirring over one minute. Dulbecco's modified Eagles's medium (5ml) is added to the cells over three minutes. Cells are pelleted by centrifugation at 300 x g for 10 minutes and resuspended in medium with 10% fetal bovine serum and
35 containing 100 mM hypoxanthine, 0.4 mM aminopterin and 16 mM thymidine (HAT). Cells (100 ml) are added to the wells of eight to ten 96-well tissue culture plates

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containing 100 ml of normal spleen feeder layer cells and incubated at 37°C. Cells are fed with fresh HAT medium every three to four days.

- 5 Hybridoma culture supernatants are tested by the ELISA ASSAY in 96-well microtiter plates coated with 100 ng of viral glycoprotein. Supernatants from reactive hybridomas are further analyzed by black-plaque assay and by Western Blot. Selected hybridomas are cloned twice by
10 limiting dilution. Ascetic fluid is produced by intraperitoneal injection of 5×10^6 hybridoma cells into pristane-treated BALB/c mice.

- Cell lysates from S-SPV-009, -014, -016, -017, -018, or
15 -019 are obtained as described in PREPARATION OF INFECTED CELL LYSATES. The glycoprotein-containing cell lysates (100 mls) are passed through a 2-ml agarose affinity resin to which 20 mg of glycoprotein monoclonal antibody has been immobilized according to manufacturer's
20 instructions (AFC Medium, New Brunswick Scientific, Edison, N.J.). The column is washed with 100 ml of 0.1% Nonidet P-40 in phosphate-buffered saline (PBS) to remove nonspecifically bound material. Bound glycoprotein is eluted with 100 mM carbonate buffer, pH 10.6 (40). Pre-
25 and posteluted fractions are monitored for purity by reactivity to the SPV monoclonal antibodies in an ELISA system.

- ELISA ASSAY.** A standard enzyme-linked immunosorbent
30 assay (ELISA) protocol is used to determine the immune status of cattle following vaccination and challenge.

- A glycoprotein antigen solution (100 ml at ng/ml in PBS) is allowed to absorb to the wells of microtiter dishes
35 for 18 hours at 4°C. The coated wells are rinsed one time with PBS. Wells are blocked by adding 250 ml of PBS containing 1% BSA (Sigma) and incubating 1 hour at 37°C.

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The blocked wells are rinsed one time with PBS containing 0.02% Tween* 20. 50 ml of test serum (previously diluted 1:2 in PBS containing 1% BSA) are added to the wells and incubated 1 hour at 37°C. The antiserum is removed and
5 the wells are washed 3 times with PBS containing 0.02% Tween 20. 50 ml of a solution containing anti-bovine IgG coupled to horseradish peroxidase (diluted 1:500 in PBS containing 1% BSA, Kirkegaard and Perry Laboratories, Inc.) is added to visualize the wells containing antibody
10 against the specific antigen. The solution is incubated 1 hour at 37°C, then removed and the wells are washed 3 times with PBS containing 0.02% Tween 20. 100 ml of substrate solution (ATBS, Kirkegaard and Perry Laboratories, Inc.) are added to each well and color is
15 allowed to develop for 15 minutes. The reaction is terminated by addition of 0.1M oxalic acid. The color is read at absorbance 410nm on an automatic plate reader.

STRATEGY FOR THE CONSTRUCTION OF SYNTHETIC POX VIRAL
20 **PROMOTERS.** For recombinant swinepox vectors synthetic pox promoters offer several advantages including the ability to control the strength and timing of foreign gene expression. Three promoter cassettes LP1, EP1 and LP2 based on promoters that have been defined in the
25 vaccinia virus (1, 7 and 8) were designed. Each cassette was designed to contain the DNA sequences defined in vaccinia flanked by restriction sites which could be used to combine the cassettes in any order or combination. Initiator methionines were also designed into each
30 cassette such that inframe fusions could be made at either EcoRI or BamHI sites. A set of translational stop codons in all three reading frames and an early transcriptional termination signal (9) were also engineered downstream of the inframe fusion site. DNA
35 encoding each cassette was synthesized according to standard techniques and cloned into the appropriate homology vectors (see Figures 3 and 4).

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VACCINATION STUDIES IN SWINE USING RECOMBINANT SWINEPOX VIRUS CONTAINING PSEUDORABIES VIRUS GLYCOPROTEIN GENES:

Young weaned pigs from pseudorabies-free herd are used to test the efficacy of the recombinant swinepox virus containing one or more of the pseudorabies virus glycoprotein genes (SPV/PRV). The piglets are inoculated intramuscularly, intradermally or orally about 10^3 to 10^7 plaque forming units (PFU) of the recombinant SPV/PRV viruses.

10

Immunity is determined by measuring PRV serum antibody levels and by challenging the vaccinated pigs with virulent strain of pseudorabies virus. Three to four weeks post-vaccination, both vaccinated and non-vaccinated groups of pigs are challenged with virulent strain of pseudorabies virus (VDL4892). Post challenge, the pigs are observed daily for 14 days for clinical signs of pseudorabies.

20 Serum samples are obtained at the time of vaccination, challenge, and at weekly intervals for two to three weeks post-vaccination and assayed for serum neutralizing antibody.

25

CLONING OF BOVINE VIRAL DIARRHEA VIRUS g48 and g53 GENES.

The bovine viral diarrhea g48 and g53 genes were cloned by a PCR CLONING procedure essentially as described by Katz et al. (42) for the HA gene of human influenza. Viral RNA prepared from BVD virus Singer strain grown in Madin-Darby bovine kidney (MDBK) cells was first converted to cDNA utilizing an oligonucleotide primer specific for the target gene. The cDNA was then used as a template for polymerase chain reaction (PCR) cloning (15) of the targeted region. The PCR primers were designed to incorporate restriction sites which permit the cloning of the amplified coding regions into vectors

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containing the appropriate signals for expression in SPV. One pair of oligonucleotides were required for each coding region. The g48 gene coding region from the BVDV Singer strain (49) was cloned using the following
5 primers: 5'-ACGTCGGATCCCTTACCAAACCACGTCTTACTCTTGTTTTCC-3' for cDNA priming and combined with 5'-ACATAGGATCCCATGGGAGAAAACATAACACAGTGGAACC-3' for PCR. The g53 gene coding region from the BVDV Singer strain (49) was cloned using the following primers: 5'-
10 CGTGGATCCTCAATTACAAGAGGTATCGTCTAC-3' for cDNA priming and combined with 5'-CATAGATCTTGTGGTGCTGTCCGACTTCGCA-3' for PCR. Note that this general strategy is used to clone the coding region of g48 and g53 genes from other strains of BVDV. The DNA fragment for BVDV g 48 was digested with
15 BamHI to yield an 678 bp fragment. The DNA fragment for BVDV g53 was digested with BglII and BamHI to yield an 1187 bp fragment. The BVDV g48 or g53 DNA fragments were cloned into the BamHI site next to the LP2EP2 promoter of the SPV homology vector to yield homology vectors, 727-
20 78.1 and 738-96, respectively.

CLONING OF BOVINE RESPIRATORY SYNCYTIAL VIRUS FUSION, NUCLEOCAPSID AND GLYCOPROTEIN GENES. The bovine respiratory syncytial virus fusion (F), nucleocapsid (N),
25 and glycoprotein (G) genes were cloned by a PCR CLONING procedure essentially as described by Katz et al. (42) for the HA gene of human influenza. Viral RNA prepared from BRSV virus grown in bovine nasal turbinate (BT) cells was first converted to cDNA utilizing an
30 oligonucleotide primer specific for the target gene. The cDNA was then used as a template for polymerase chain reaction (PCR) cloning (15) of the targeted region. The PCR primers were designed to incorporate restriction sites which permit the cloning of the amplified coding
35 regions into vectors containing the appropriate signals for expression in SPV. One pair of oligonucleotides were required for each coding region. The F gene coding region

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from the BRSV strain 375 (VR-1339) was cloned using the following primers: 5' - TGCAGGATCCTCATTCTACTAAAGGAAAGATTGTTGAT-3' for cdna priming and combined with 5' - CTCTGGATCCTACAGCCATGAGGATGATCATCAGC-3' for PCR. The N gene coding region from BRSV strain 375 (VR-1339) was cloned utilizing the following primers: 5' - CGTCGGATCCCTCACAGTTCCACATCATTGTCTTTGGGAT-3' for cdna priming and combined with 5' - CTTAGGATCCCATGGCTCTTAGCAAGGTCAAACCTAAATGAC-3' for PCR. The G gene coding region from BRSV strain 375 (VR-1339) was cloned utilizing the following primers: 5' - CGTTGGATCCCTAGATCTGTGTAGTTGATTGATTTGTGTGA-3' for cdna priming and combined with 5' - CTCTGGATCCTCATACCCATCATCTTAAATTCAAGACATTA-3' for PCR. Note that this general strategy is used to clone the coding region of F, N and G genes from other strains of BRSV. The DNA fragments for BRSV F, N, or G were digested with BamHI to yield 1722 bp, 1173 bp, or 771 bp fragments, respectively. The BRSV F, N, and G DNA fragments were cloned into the BamHI site next to the LP2EP2 promoter of the SPV homology vector to yield homology vectors, 727-20.10, 713-55.37 and 727-20.5, respectively.

25

RNA ISOLATED FROM CONCAVALIN A STIMULATED CHICKEN SPLEEN CELLS. Chicken spleens were dissected from 3 weeks old SPAFAS hatched chicks, washed, and disrupted through a syringe/needle to release cells. After allowing stroma and debris to settle out, the cells were pelleted and washed twice with PBS. The cell pellet was treated with a hypotonic lysis buffer to lyse red blood cells, and splenocytes were recovered and washed twice with PBS. Splenocytes were resuspended at 5×10^6 cells/ml in RPMI containing 5% FBS and 5 μ g/ml Concanavalin A and incubated at 39° for 48 hours. Total RNA was isolated from the cells using guanidine isothionate lysis reagents

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and protocols from the Promega RNA isolation kit (Promega Corporation, Madison WI). 4 μ g of total RNA was used in each 1st strand reaction containing the appropriate antisense primers and AMV reverse transcriptase (Promega Corporation, Madison WI). cDNA synthesis was performed in the same tube following the reverse transcriptase reaction, using the appropriate sense primers and Vent[®]* DNA polymerase (Life Technologies, Inc. Bethesda, MD).

10 **SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES.** When the E.coli β -glucuronidase (uidA) marker gene was incorporated into a recombinant virus the plaques containing recombinants were visualized by a simple assay. The enzymatic substrate was incorporated
15 (300 μ g/ml) into the agarose overlay during the plaque assay. For the uidA marker gene the substrate X-Glucuro Chx (5-bromo-4-chloro-3-indolyl- β -D-glucuronic acid Cyclohexylammonium salt, Biosynth AG) was used. Plaques that expressed active marker enzyme turned blue. The
20 blue plaques were then picked onto fresh ESK-4 cells and purified by further blue plaque isolation. In recombinant virus strategies in which the enzymatic marker gene is removed the assay involves plaque purifying white plaques from a background of parental blue plaques. In both
25 cases viruses were typically purified with three rounds of plaque purification.

HOMOLOGY VECTOR 515-85.1. The plasmid 515-85.1 was constructed for the purpose of inserting foreign DNA into
30 SPV. It contains a unique AccI restriction enzyme site into which foreign DNA may be inserted. When a plasmid, containing a foreign DNA insert at the AccI site, is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing the foreign
35 DNA will result. A restriction map of the DNA insert in homology vector 515-85.1 is given in Figures 3A-3C. It may be constructed utilizing standard recombinant DNA

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techniques (22 and 29), by joining two restriction fragments from the following sources. The first fragment is an approximately 2972 base pair *Hind*III to *Bam*HI restriction fragment of pSP64 (Promega). The second
5 fragment is an approximately 3628 base pair *Hind*III to *Bgl*II restriction sub-fragment of the SPV *Hind*III restriction fragment M (23).

HOMOLOGY VECTOR 520-17.5. The plasmid 520-17.5 was
10 constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli* β -galactosidase (*lacZ*) marker gene flanked by SPV DNA. Upstream of the marker gene is an approximately 2149 base pair fragment of SPV DNA. Downstream of the marker gene is an approximately
15 1484 base pair fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the marker gene will result. Note that the β -galactosidase (*lacZ*) marker gene is under the control of
20 a synthetic early/late pox promoter. A detailed description of the plasmid is given in Figures 3A-3C. It may be constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the synthetic DNA
25 sequences indicated in Figures 3A-3C. The plasmid vector was derived from an approximately 2972 base pair *Hind*III to *Bam*HI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 2149 base pair *Hind*III to *Acc*I restriction sub-fragment of the SPV *Hind*III
30 restriction fragment M (23). Fragment 2 is an approximately 3006 base pair *Bam*HI to *Pvu*II restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 1484 base pair *Acc*I to *Bgl*II restriction sub-fragment of the SPV *Hind*III fragment M (23).

35

HOMOLOGY VECTOR 538-46.16. The plasmid 538-46.16 was constructed for the purpose of inserting foreign DNA into

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SPV. It incorporates an *E. coli* β -galactosidase (*lacZ*) marker gene and the PRV g50 (gD) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (*lacZ*) marker gene is under the control of a synthetic late pox promoter (LP1) and the g50 (gD) gene is under the control of a synthetic early/late pox promoter (EP1LP2). A detailed description of the plasmid is given in 3A-3C. It may be constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in Figures 3A-3C. The plasmid vector was derived from an approximately 2972 base pair *Hind*III to *Bam*HI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 2149 base pair *Hind*III to *Acc*I restriction sub-fragment of the SPV *Hind*III restriction fragment M (23). Fragment 2 is an approximately 3006 base pair *Bam*HI to *Pvu*II restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 1571 base pair *Eco*RI to *Stu*I restriction sub-fragment of the PRV *Bam*HI fragment 7 (21). Note that the *Eco*RI site was introduced in to this fragment by PCR cloning. In this procedure the primers described below were used along with a template consisting of a PRV *Bam*HI #7 fragment subcloned into pSP64. The first primer 87.03 (5'-CGCGAATTCGCTCG CAGCGCTATTGGC-3') sits down on the PRV g50 (gD) sequence (26) at approximately amino acid 3 priming toward the 3' end of the gene. The second primer 87.06 (5'-GTAGGAGTGGCTGCTGAAG-3') sits down on the opposite strand at approximately amino acid 174 priming toward the 5' end of the gene. The PCR product may be digested with *Eco*RI and *Sal*I to produce an approximately

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509 base pair fragment. The approximately 1049 base pair *SalI* to *StuI* sub-fragment of PRV *BamHI* #7 may then be ligated to the approximately 509 base pair *EcoRI* to *SalI* fragment to generate the approximately 1558 base pair *EcoRI* to *StuI* fragment 3. Fragment 4 is an approximately 1484 base pair *AccI* to *BglIII* restriction sub-fragment of the SPV *HindIII* fragment M (23).

10 **HOMOLOGY VECTOR 570-91.41.** The plasmid 570-91.41 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli* β -galactosidase (*lacZ*) marker gene and the PRV *gIII* (*gC*) gene flanked by SPV DNA. Upstream of the foreign DNA genes is an
15 approximately 2149 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding
20 for the foreign genes will result. Note that the β -galactosidase (*lacZ*) marker gene is under the control of a synthetic late pox promoter (LP1), and the *gIII* (*gC*) gene is under the control of a synthetic early late pox promoter (EP1LP2). A detailed description of the plasmid
25 is given in Figures 5A-5D. It may be constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in 5A-5D. The plasmid vector was derived from an approximately
30 2972 base pair *HindIII* to *BamHI* restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair *BglIII* to *AccI* restriction sub-fragment of the SPV *HindIII* restriction fragment M (23). Fragment 2 is an approximately 3002 base pair *BamHI* to *PvuII* restriction
35 fragment of plasmid pJF751 (11). Fragment 3 is an approximately 2378 base pair *NcoI* to *NcoI* fragment of plasmid 251-41.A, a subfragment of PRV *BamHI* #2 and #9.

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EcoRI linkers have replaced the *NcoI* and *NcoI* sites at the ends of this fragment. Fragment 4 is an approximately 2149 base pair *AccI* to *HindIII* restriction sub-fragment of the SPV *HindIII* fragment M (23). The

5 *AccI* sites in fragments 1 and 4 have been converted to *PstI* sites using synthetic DNA linkers.

HOMOLOGY VECTOR 570-91.64. The plasmid 570-91.64 was constructed for the purpose of inserting foreign DNA into

10 SPV. It incorporates an *E. coli* β -galactosidase (*lacZ*) marker gene and the PRV *gIII* (*gC*) gene flanked by SPV DNA. Upstream of the foreign DNA genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149

15 base pair fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (*lacZ*) marker gene is under the control of

20 a synthetic late pox promoter (LP1), and the *gIII* (*gC*) gene is under the control of a synthetic late early pox promoter (LP2EP2). A detailed description of the plasmid is given in Figures 7A-7D. It may be constructed utilizing standard recombinant DNA techniques (22 and

25 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in 5 A-5D. The plasmid vector was derived from an approximately 2972 base pair *HindIII* to *BamHI* restriction fragment of pSP64 (Promega). Fragment 1 is an

30 approximately 1484 base pair *BglII* to *AccI* restriction sub-fragment of the SPV *HindIII* restriction fragment M (23). Fragment 2 is an approximately 3002 base pair *BamHI* to *PvuII* restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 2378 base pair *NcoI*

35 to *NcoI* fragment of plasmid 251-41.A, a subfragment of PRV *BamHI* #2 and #9. *EcoRI* linkers have replaced the *NcoI* and *NcoI* sites at the ends of this fragment.

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Fragment 4 is an approximately 2149 base pair *AccI* to *HindIII* restriction sub-fragment of the SPV *HindIII* fragment M (23). The *AccI* sites in fragments 1 and 4 have been converted to *PstI* sites using synthetic DNA linkers.

HOMOLOGY VECTOR 727-54.60. The plasmid 727-54.60 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli* β -galactosidase (*lacZ*) marker gene and the pseudorabies virus (PRV) *gII* (*gB*) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (*lacZ*) marker gene is under the control of a synthetic late pox promoter (LP1), and the PRV *gB* gene is under the control of a synthetic late/early pox promoter (LP2EP2). A detailed description of the plasmid is given in Figures 7A-7D. It may be constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in Figures 7A-7D. The plasmid vector was derived from an approximately 2972 base pair *HindIII* to *BamHI* restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair *BglIII* to *AccI* restriction sub-fragment of the SPV *HindIII* restriction fragment M (23). Fragment 2 is an approximately 3500 base pair fragment which contains the coding sequence for the PRV *gB* gene within the *KpnI* C fragment of genomic PRV DNA(21). Fragment 2 contains an approximately 53 base pair synthetic fragment containing the amino terminus of the PRV *gB* gene, an approximately 78 base pair *SmaI* to

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Nhe I fragment from the PRV KpnI C genomic fragment, and an approximately 3370 base pair *NheI* to *EcoRI* fragment from the PRV KpnI C genomic fragment (21). Fragment 3 is an approximately 3010 base pair *BamHI* to *PvuII* restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair *AccI* to *HindIII* subfragment of the SPV *HindIII* fragment M. The *AccI* sites in fragments 1 and 4 were converted to unique *NotI* sites using *NotI* linkers.

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HOMOLOGY VECTOR 751-07.A1. The plasmid 751-07.A1 was used to insert foreign DNA into SPV. It incorporates an *E. coli* β -galactosidase (*lacZ*) marker gene and the chicken interferon (*cIFN*) gene flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the β -galactosidase (*lacZ*) marker gene is under the control of a synthetic late pox promoter (LP1) and the *cIFN* gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair *HindIII* to *BamHI* restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1146 base pair *BglII* to *AccI* restriction sub-fragment of the SPV *HindIII* fragment M (23). Fragment 2 is an approximately 577 base pair *EcoRI* to *BglII* fragment coding for the *cIFN* gene (54) derived by reverse transcription and polymerase chain reaction (PCR) (Sambrook, et al., 1989) of RNA ISOLATED FROM CONCANAVALLIN A STIMULATED CHICKEN SPLEEN CELLS. The antisense primer (6/94.13) used for reverse transcription

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and PCR was 5'-CGACGGATCCGAGGTGCGTTTGGGGCTAAGTGC-3' (SEQ ID NO: 211). The sense primer (6/94.12) used for PCR was 5'-CCACGGATCCAGCACAACGCGAGTCCCACCATGGCT-3' (SEQ ID NO: 212). The BamHI fragment resulting from reverse transcription and PCR was gel purified and used as a template for a second PCR reaction to introduce a unique EcoRI site at the 5' end and a unique BglII site at the 3' end. The second PCR reaction used primer 6/94.22 (5'-CCACGAATTCGATGGCTGTGCCTGCAAGCCCACAG-3'; SEQ ID NO: 213) at the 5' end and primer 6/94.34 (5'-CGAAGATCTGAGGTGCGTTTGGGGCTAAGTGC-3'; SEQ ID NO: 214) at the 3' end to yield an approximately 577 base pair fragment. The DNA fragment contains the coding sequence from amino acid 1 to amino acid 193 of the chicken interferon protein (54) which includes a 31 amino acid signal sequence at the amino terminus and 162 amino acids of the mature protein encoding chicken interferon. Fragment 3 is an approximately 3002 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2156 base-pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The AccI site in the SPV homology vector was converted to a unique NotI site.

HOMOLOGY VECTOR 751-56.A1. The plasmid 751-56.A1 was used to insert foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the chicken myelomonocytic growth factor (cMGF) gene flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the β -galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the cMGF gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining

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restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega).
5 Fragment 1 is an approximately 1146 base pair BglII to AccI restriction sub-fragment of the SPV HindIII fragment M (23). Fragment 2 is an approximately 640 base pair EcoRI to BamHI fragment coding for the cMGF gene(55) derived by reverse transcription and polymerase chain
10 reaction (PCR) (Sambrook, et al., 1989) of RNA ISOLATED FROM CONCANAVALLIN A STIMULATED CHICKEN SPLEEN CELLS. The antisense primer (6/94.20) used for reverse transcription and PCR was 5'-CGCAGGATCCGGGGCGTCAGAGGCGGGCGAGGTG-3' (SEQ ID NO: 215). The sense primer (5/94.5) used for PCR
15 was 5'-GAGCGGATCCTGCAGGAGGAGACACAGAGCTG-3' (SEQ ID NO: 216). The BamHI fragment derived from PCR was subcloned into a plasmid and used as a template for a second PCR reaction using primer 6/94.16 (5'-GCGCGAATTCCATGTGCTGCCTCACCCCTGTG-3'; SEQ ID NO: 217) at
20 the 5' end and primer 6/94.20 (5'-CGCAGGATCCGGGGCGTCAGAGGCGGGCGAGGTG-3'; SEQ ID NO: 218) at the 3' end to yield an approximately 640 base pair fragment. The DNA fragment contains the coding sequence from amino acid 1 to amino acid 201 of the cMGF protein
25 (55) which includes a 23 amino acid signal sequence at the amino terminus and 178 amino acids of the mature protein encoding cMGF. Fragment 3 is an approximately 3002 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2156
30 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The AccI site in the SPV homology vector was converted to a unique NotI site.

35 **HOMOLOGY VECTOR 752-22.1.** The plasmid 752-22.1 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ)

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marker gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a swinepox virus O1L gene promoter. The homology vector also contains the synthetic late/early promoter (LP2EP2) into which a second foreign gene is inserted into a unique BamHI or EcoRI site. A detailed description of the plasmid is given in Figures 10A-10D. It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in Figures 10A-10D. The plasmid vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5'-GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with SphI and BglII ends. Fragment 2 is a 3002 base pair BamHI to PvuII fragment derived from plasmid pJF751 (49) containing the *E. coli* lacZ gene. Fragment 3 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5'-GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

HOMOLOGY VECTOR 752-29.33. The plasmid 759.33 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli* β -galactosidase (lac Z)

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marker gene and an equine herpesvirus type 1 gB gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 113
5 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of
10 a swinepox virus 01L gene promoter and the EHV-1 gB gene is under the control of the late/early promoter (LP2EP2). The LP2EP2 promoter-EHV-1 gB gene cassette was inserted into a NotI site of homology vector 738-94.4. Homology vector 752-29.33 was constructed utilizing standard
15 recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is
20 an approximately 855 base pair sub-fragment of the SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5'-GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce
25 an 855 base pair fragment with SphI and BglII ends. Fragment 2 is a 3002 base pair BamHI to PvuII fragment derived from plasmid pJF751 (49) containing the *E. coli* lacZ gene. Fragment 3 is the product of a PCR reaction (EcoRI to BamHI) and a restriction fragment (BamHI to
30 PmeI) ligated together to yield an EHV-1 gB gene which is an EcoRI to PmeI fragment approximately 2941 base pairs (979 amino acids) in length. The PCR fragment is an approximately 429 base pair fragment having a synthetic EcoRI site at the 5' end of the gene and a natural BamHI
35 site at the 3' end within the BamHI "a" fragment of EHV-1 genomic DNA. The restriction fragment is an approximately 2512 base pair fragment from BamHI to PmeI

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within the BamHI "I" fragment of EHV-1 genomic DNA. In the procedure to produce the 5' end PCR fragment, the primers described below were used with a template consisting of the EHV-1 BamHI "a" and "i" fragments.

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The first primer 5/94.3 (5'-CGGAATTCCTCTGGTTCGCCGT-3') sits down on the EHV-1 gB sequence at amino acid number 2 and introduces an EcoRI site at the 5' end of the EHV-1 gB gene and an ATG start codon. The second primer 5/94.4 (5'-GACGGTGGATCCGGTAGGCGGT-3') sits down on the EHV-1 gB sequence at approximately amino acid 144 on the opposite strand to primer 5/94.3 and primes toward the 5' end of the gene. The PCR product was digested with EcoRI and BamHI to yield a fragment 429 base pairs in length corresponding to the 5' end of the EHV-1 gB gene. Fragment 3 consists of the products of the PCR reaction (EcoRI to BamHI) and the restriction fragment (BamHI to PmeI) ligated together to yield an EHV-1 gB gene which is an EcoRI to PmeI fragment approximately 2941 base pairs (979 amino acids) in length. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5'-GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

HOMOLOGY VECTOR 746-94.1. The plasmid 746-94.1 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and an infectious bovine rhinotracheitis virus glycoprotein E (gE) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV,

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a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a swinepox virus O1L gene promoter and the IBRV gE gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. A 1250 base pair EcoRI to BamHI fragment coding for amino acids 1 to 417 of the IBRV gE gene (missing 158 amino acids of the carboxy terminal transmembrane region) was inserted into unique EcoRI and BamHI sites of homology vector 752-22.1 (Figures 10A-10D). The 1250 base pair EcoRI to BamHI fragment was synthesized by polymerase chain reaction (15) using IBRV (Cooper) genomic DNA as a template and primer 10/94.23 (5'-GGGGAATTCAATGCAACCCACCGCGCCGCCCC-3'; SEQ ID NO: 219) at the 5' end of the IBRV gE gene (amino acid 1) and primer 10/94.22 (5'-GGGGGATCCTAGGGCGCGCCCGCCGGCTCGCT-3'; SEQ ID NO: 220) at amino acid 417 of the IBRV gE gene.

HOMOLOGY VECTOR 767-67.3. The plasmid 767-67.3 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and an bovine viral diarrhea virus glycoprotein 53 (BVDV gp53) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a swinepox virus O1L gene promoter and the BVDV gp53 gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30),

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- by joining restriction fragments from the following sources with the synthetic DNA sequences. A 1187 base pair BamHI fragment coding for the BVDV gp53 was inserted into the unique BamHI sites of homology vector 752-22.1 (Figures 10A-10D). The 1187 base pair BamHI fragment was synthesized by polymerase chain reaction (15) as described in CLONING OF BOVINE VIRAL DIARRHEA VIRUS gp48 AND gp53 GENES.
- 10 **HOMOLOGY VECTOR 771-55.11.** The plasmid 771-55.11 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and an bovine viral diarrhea virus glycoprotein 48 (BVDV gp48) gene flanked by SPV DNA.
- 15 Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV,
- 20 a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a swinepox virus O1L gene promoter and the BVDV gp48 gene is under the control of the late/early promoter (LP2EP2). It was constructed
- 25 utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. A 678 base pair BamHI fragment coding for the BVDV gp48 was inserted into the unique BamHI sites of homology vector 752-22.1
- 30 (Figures 10A-10D). The 678 base pair BamHI fragment was synthesized by polymerase chain reaction (15) as described in CLONING OF BOVINE VIRAL DIARRHEA VIRUS gp48 AND gp53 GENES.
- 35 **PLASMID 551-47.23.** The plasmid 551-47.23 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates the E. coli β -glucuronidase (β -glu) marker

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gene under the control of a late/early pox promoter (LP2EP2). It is useful to insert the marker gene into sites in the SPV genome to produce a recombinant swinepox virus. It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources. The plasmid vector was derived from an approximately 3005 base pair HindIII restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 1823 base pair EcoRI to SmaI fragment of the plasmid pRAJ260 (Clonetech). Note that the EcoRI and SmaI sites were introduced by PCR cloning. Plasmid 551-47.23 was used to make recombinant swinepox viruses S-SPV-059, S-SPV-060, S-SPV-061, and S-SPV-062.

HOMOLOGY VECTOR 779-94.31. The plasmid 779-94.31 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the pseudorabies virus (PRV) gB (gII) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 538 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1180 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the PRV gB gene is under the control of a synthetic late/early pox promoter (LP2EP2). A detailed description of the plasmid is given in Figures 12A-12E. It was constructed utilizing standard recombinant DNA techniques (22, and 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2986 base pair HindIII to PstI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 542 base pair HindIII to BglII restriction sub-fragment of the SPV HindIII restriction

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fragment M (23). Fragment 2 is an approximately 3500 base pair fragment which contains the coding sequence for the PRV gB gene within the KpnI C fragment of genomic PRV DNA (21). Fragment 2 contains an approximately 53 base pair synthetic fragment containing the amino terminus of the PRV gB gene, an approximately 78 base pair SmaI to Nhe I fragment from the PRV KpnI C genomic fragment, and an approximately 3370 base pair NheI to EcoRI fragment from the PRV KpnI C genomic fragment (21). Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 1180 base pair BglII to PstI subfragment of the SPV HindIII fragment M. The BglII sites in fragments 1 and 4 were converted to unique HindIII sites using HindIII linkers.

HOMOLOGY VECTOR 789-41.7. The plasmid 789-41.7 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene, the pseudorabies virus (PRV) gB (gII) gene and the PRV gD (g50) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the PRV gB gene is under the control of a synthetic late/early pox promoter (LP2EP2), and the PRV gD gene is under the control of a synthetic early/late pox promoter (EP1LP2). A detailed description of the plasmid is given in Figures 13A-13D. It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid

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vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII
5 restriction fragment M (23). Fragment 2 is an approximately 1552 base pair subfragment of the PRV BamHI #7 fragment which contains the coding sequence of the PRV gD gene from amino acid 3 to amino acid 279. The EcoRI site and the ATG translation start codon are derived from
10 a polymerase chain reaction using a 5' primer with an EcoRI site. The StuI site at the 3' end is normally within the PRV gI gene 3' to the PRV gD gene. The entire open reading frame beginning at the EcoRI site codes for 405 amino acids. Fragment 3 is an approximately 48 base
15 pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 3500 base pair fragment which contains the coding sequence for the PRV gB gene within the KpnI C fragment of genomic PRV DNA(21). Fragment 4 contains an approximately 53 base
20 pair synthetic fragment containing the amino terminus of the PRV gB gene, an approximately 78 base pair SmaI to Nhe I fragment from the PRV KpnI C genomic fragment, and an approximately 3370 base pair NheI to EcoRI fragment from the PRV KpnI C genomic fragment (21). Fragment 5 is
25 an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 6 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites
30 using PstI linkers. The NdeI sites in fragments 3 and 6 were converted to unique HindIII sites using HindIII linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104;) of the SPV HindIII M fragment has been deleted which would span SPV
35 fragments 3 and 6.

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HOMOLOGY VECTOR 789-41.27. The plasmid 789-41.27 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene, the pseudorabies virus (PRV) gB (gII) gene and the PRV gC (gIII) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the PRV gB gene is under the control of a synthetic late/early pox promoter (LP2EP2), and the PRV gC gene is under the control of a synthetic early/late pox promoter (EP1LP2). A detailed description of the plasmid is given in Figures 14A-14D. It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1560 base pair HindIII to NdeI subfragment of the SPV HindIII fragment M. Fragment 2 is an approximately 3500 base pair fragment which contains the coding sequence for the PRV gB gene within the KpnI C fragment of genomic PRV DNA(21). Fragment 2 contains an approximately 53 base pair synthetic fragment containing the amino terminus of the PRV gB gene, an approximately 78 base pair SmaI to Nhe I fragment from the PRV KpnI C genomic fragment, and an approximately 3370 base pair NheI to EcoRI fragment from the PRV KpnI C genomic fragment (21). Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 48 base pair AccI to NdeI subfragment of

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the SPV HindIII M fragment. Fragment 5 is an approximately 2378 base pair NcoI to NcoI fragment of plasmid 251-41.A, a subfragment of PRV BamHI #2 and #9. EcoRI linkers have replaced the NcoI sites at the ends of the fragment. Fragment 6 is an approximately 1484 base pair AccI to BglII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The NdeI sites in fragments 1 and 4 were converted to unique HindIII sites using HindIII linkers. The AccI site in fragments 4 and 6 were converted to unique PstI sites using PstI linkers. An approximately 545 base pair NdeI to NdeI (Nucleotides 1560 to 2104;) subfragment of the SPV HindIII M fragment has been deleted which would span SPV fragments 4 and 6.

HOMOLOGY VECTOR 789-41.47. The plasmid 789-41.47 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene, the pseudorabies virus (PRV) gC (gIII) gene and the PRV gD (g50) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the PRV gC gene is under the control of a synthetic early/late pox promoter (EP1LP2), and the PRV gD gene is under the control of a synthetic early/late pox promoter (EP1LP2). A detailed description of the plasmid is given in Figures 15A-15D. It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega).

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Fragment 1 is an approximately 1484 base pair BglII to
AccI restriction sub-fragment of the SPV HindIII
restriction fragment M (23). Fragment 2 is an
approximately 1552 base pair subfragment of the PRV BamHI
5 #7 fragment which contains the coding sequence of the PRV
gD gene from amino acid 3 to amino acid 279. The EcoRI
site and the ATG translation start codon are derived from
a polymerase chain reaction using a 5' primer with an
EcoRI site. The StuI site at the 3' end is normally
10 within the PRV gI gene 3' to the PRV gD gene. The entire
open reading frame beginning at the EcoRI site codes for
405 amino acids. Fragment 3 is an approximately 48 base
pair AccI to NdeI subfragment of the SPV HindIII M
fragment. Fragment 4 is an approximately 3010 base pair
15 BamHI to PvuII restriction fragment of plasmid pJF751
(11). Fragment 5 is an approximately 2378 base pair NcoI
to NcoI fragment of plasmid 251-41.A, a subfragment of
PRV BamHI #2 and #9. EcoRI linkers have replaced the NcoI
sites at the ends of the fragment. Fragment 6 is an
20 approximately 1560 base pair NdeI to HindIII subfragment
of the SPV HindIII fragment M. The AccI sites in
fragments 1 and 3 were converted to unique PstI sites
using PstI linkers. The NdeI sites in fragments 3 and 6
were converted to unique HindIII sites using HindIII
25 linkers. An approximately 545 base pair NdeI to NdeI
subfragment (Nucleotides 1560 to 2104) of the SPV
HindIII M fragment has been deleted which would span SPV
fragments 3 and 6.

30 **HOMOLOGY VECTOR 789-41.73.** The plasmid 789-41.73 was
constructed for the purpose of inserting foreign DNA into
SPV. It incorporates an E. coli β -galactosidase (lacZ)
marker gene, the pseudorabies virus (PRV) gB (gII) gene,
the PRV gC (gIII) gene and the PRV gD (g50) gene flanked
35 by SPV DNA. Upstream of the foreign genes is an
approximately 1484 base pair fragment of SPV DNA.
Downstream of the foreign genes is an approximately 1560

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base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the PRV gB gene is under the control of a synthetic late/early pox promoter (LP2EP2), the PRV gC gene is under the control of a synthetic early/late promoter (EP1LP2), and the PRV gD gene is under the control of a synthetic late/early pox promoter (LP2EP2). A detailed description of the plasmid is given in Figures 16A-16E. It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 1552 base pair subfragment of the PRV BamHI #7 fragment which contains the coding sequence of the PRV gD gene from amino acid 3 to amino acid 279. The EcoRI site and the ATG translation start codon are derived from a polymerase chain reaction using a 5' primer with an EcoRI site. The StuI site at the 3' end is normally within the PRV gI gene 3' to the PRV gD gene. The entire open reading frame beginning at the EcoRI site codes for 405 amino acids. Fragment 3 is an approximately 2378 base pair NcoI to NcoI fragment of plasmid 251-41.A, a subfragment of PRV BamHI #2 and #9. EcoRI linkers have replaced the NcoI sites at the ends of the fragment. Fragment 4 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 5 is an approximately 3500 base pair fragment which contains the coding sequence for the PRV gB gene within the KpnI C fragment of genomic PRV DNA(21). Fragment 5 contains an approximately 53 base pair

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synthetic fragment containing the amino terminus of the PRV gB gene, an approximately 78 base pair SmaI to Nhe I fragment from the PRV KpnI C genomic fragment, and an approximately 3370 base pair NheI to EcoRI fragment from the PRV KpnI C genomic fragment (21). Fragment 6 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 7 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 6 were converted to unique HindIII sites using HindIII linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 6.

HOMOLOGY VECTOR 791-63.19. The plasmid 791-63.19 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequence. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an

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approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers.

HOMOLOGY VECTOR 791-63.41. The plasmid 791-63.41 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers.

HOMOLOGY VECTOR 796-18.9. The plasmid 796-18.9 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ)

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marker gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic early pox promoter (EP1). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers.

HOMOLOGY VECTOR 783-39.2. The plasmid 783-39.2 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and an bovine viral diarrhea virus glycoprotein 53 (BVDV gp53) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the

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BVDV gp53 gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 1187 base pair BamHI fragment coding for the BVDV gp53. The 1187 base pair BamHI fragment was synthesized by polymerase chain reaction (15) as described in CLONING OF BOVINE VIRAL DIARRHEA VIRUS gp48 AND gp53 GENES. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

HOMOLOGY VECTOR 749-75.78. The plasmid 749-75.78 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the infectious bursal disease virus (IBDV) polymerase gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the IBDV polymerase gene is under the control of a synthetic late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques

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(22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 2700 EcoRI to AscI restriction fragment synthesized by cDNA cloning and polymerase chain reaction (PCR) from an IBDV RNA template. cDNA and PCR primers (5'-CACGAATTCTGACATTTTCAACAGTCCACAGGCGC-3'; 12/93.4) and 5'-GCTGTTGGACATCACGGGCCAGG-3'; 9/93.28) were used to synthesize an approximately 1400 base pair EcoRI to BclI fragment at the 5' end of the IBDV polymerase gene. cDNA and PCR primers (5'-ACCCGGAACATATGGTCAGCTCCAT-3'; 12/93.2) and 5'-GGCGCGCCAGGCGAAGGCCGGGGATACGG-3'; 12/93.3) were used to synthesize an approximately 1800 base pair BclI to AscI fragment at the 3' end of the IBDV polymerase gene. The two fragments were ligated at the BclI site to form the approximately 2700 base pair EcoRI to BclI fragment. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

HOMOLOGY VECTOR 761-75.B18. The plasmid 761-75.B18 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lac Z) marker gene and a feline immunodeficiency virus (FIV) protease (gag) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV,

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a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a swinepox virus 01L gene promoter and the FIV gag gene is under the control of the late/early promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5' GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with SphI and BglII ends. Fragment 2 is a 3002 base pair BamHI to PvuII fragment derived from plasmid pJF751 (49) containing the E. coli lacZ gene. Fragment 3 is an approximately 1878 base pair EcoRI to BglII restriction fragment synthesized by polymerase chain reaction (PCR) using cDNA from the FIV (PPR strain) (61). The primer (5' GCGTGAATTCGGGGAATGGACAGGGGCGAGAT-3'; 11/94.9) synthesizes from the 5' end of the FIV gag gene, introduces an EcoRI site at the 5' end of the gene and an ATG start codon. The primer (5'-GAGCCAGATCTGCTCTTTTACTTTCCC-3'; 11/94.10) synthesizes from the 3' end of the FIV gag gene. The PCR product was digested with EcoRI and BglII to yield a fragment 1878 base pairs in length corresponding to the FIV gag gene. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5' GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

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HOMOLOGY VECTOR 781-84.C11. The plasmid 781-84.C11 was used to insert foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the feline immunodeficiency virus (FIV) envelope (env) gene flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the FIV env gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII fragment M (23). Fragment 3 is an approximately 2564 base pair BamHI to BamHI fragment of the FIV env gene (61) synthesized by CLONING WITH THE POLYMERASE CHAIN REACTION. The template for the PCR reaction was FIV strain PPR genomic cDNA (61). The upstream primer 10/93.21 (5'-GCCCGGATCCTATGGCAGAAGGGTTTGCAGC-3') was synthesized corresponding to the 5' end of the FIV env gene starting at nucleotide 6263 of FIV strain PPR genomic cDNA, and the procedure introduced a BamHI site at the 5' end. The BamHI site was destroyed during the cloning of the PCR fragment. The downstream primer 10/93.20 (5'-CCGTGGATCCGGCACTCCATCATTCCTCCTC-3') was synthesized corresponding to the 3' end of the FIV env gene starting at nucleotide 8827 of FIV PPR genomic cDNA, and the procedure introduced a BamHI site at the 3' end. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to

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HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The AccI site in the SPV homology vector was converted to a unique NotI site.

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EXAMPLESExample 1

5 Homology Vector 515-85.1. The homology vector 515-85.1
is a plasmid useful for the insertion of foreign DNA into
SPV. Plasmid 515-85.1 contains a unique *AccI* restriction
site into which foreign DNA may be cloned. A plasmid
containing such a foreign DNA insert may be used
10 according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
GENERATING RECOMBINANT SPV to generate a SPV containing
the foreign DNA. For this procedure to be successful it
is important that the insertion site (*AccI*) be in a
region non-essential to the replication of the SPV and
15 that the site be flanked with swinepox virus DNA
appropriate for mediating homologous recombination
between virus and plasmid DNAs. *AccI* site in homology
vector 515-85.1 is used to insert foreign DNA into at
least three recombinant SPV (see examples 2-4).

20 In order to define an appropriate insertion site, a
library of SPV *HindIII* restriction fragments was
generated. Several of these restriction fragments
(*HindIII* fragments G, J, and M see Figures 1A-1B) were
25 subjected to restriction mapping analysis. Two
restriction sites were identified in each fragment as
potential insertion sites. These sites included *HpaI* and
NruI in fragment G, *BalI* and *XbaI* in fragment J, and *AccI*
and *PstI* in fragment M. A β -galactosidase (*lacZ*) marker
30 gene was inserted in each of the potential sites. The
resulting plasmids were utilized in the HOMOLOGOUS
RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV.
The generation of recombinant virus was determined by the
SCREEN FOR RECOMBINANT SPV EXPRESSING β -GALACTOSIDASE
35 ASSAYS. Four of the six sites were found to generate
recombinant virus, however the ability of each of these
viruses to be purified away from the parental SPV varied

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greatly. In one case, the virus could not be purified above the level of 1%, in another case the virus could not be purified above the level of 50%, and in a third case the virus could not be purified above the level of 90%. The inability to purify these viruses indicates instability at the insertion site. This makes the corresponding sites inappropriate for insertion of foreign DNA. However the insertion at one site, the AccI site of Homology vector 515-85.1, resulted in a virus which was easily purified to 100% (see example 2), clearly defining an appropriate site for the insertion of foreign DNA.

The homology vector 515-85.1 was further characterized by DNA sequence analysis. Two regions of the homology vector were sequenced. The first region covers a 599 base pair sequence which flanks the unique AccI site. The second region covers the 899 base pairs upstream of the unique HindIII site. The sequence of the first region codes for an open reading frame (ORF) which shows homology to amino acids 1 to 115 of the vaccinia virus (VV) O1L open reading frame identified by Goebel et al., 1990 (see Figures 2A-2C). The sequence of the second region codes for an open reading frame which shows homology to amino acids 568 to 666 of the same vaccinia virus O1L open reading frame (see Figures 2A-2C). These data suggest that the AccI site interrupts the presumptive VV O1L-like ORF at approximately amino acid 41, suggesting that this ORF codes for a gene non-essential for SPV replication. Goebel et al. suggest that the VV O1L ORF contains a leucine zipper motif characteristic of certain eukaryotic transcriptional regulatory proteins, however they indicate that it is not known whether this gene is essential for virus replication.

The DNA sequence located upstream of the VV O1L-like ORF would be expected to contain a swinepox viral promoter.

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This swinepox viral promoter will be useful as the control element of foreign DNA introduced into the swinepox genome.

5 Example 2

S-SPV-003

10 S-SPV-003 is a swinepox virus that expresses a foreign gene. The gene for *E.coli* β -galactosidase (lacZ gene) was inserted into the SPV 515-85.1 ORF. The foreign gene (lacZ) is under the control of a synthetic early/late promoter (EP1LP2).

15 S-SPV-003 was derived from S-SPV-001 (Kasza strain). This was accomplished utilizing the homology vector 520-17.5 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by
20 the SCREEN FOR RECOMBINANT SPV EXPRESSING β -GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-003. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple
25 passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable and expressing the foreign gene. The assays described here were carried
30 out in VERO cells as well as EMSK cells, indicating that VERO cells would be a suitable substrate for the production of SPV recombinant vaccines. S-SPV-003 has been deposited with the ATCC under Accession No. VR 2335.

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Example 3S-SPV-008

5 S-SPV-008 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ gene) and the gene for pseudorabies virus (PRV) g50 (gD) (26) were inserted into the SPV 515-85.1 ORF. The lacZ gene is under the control of a synthetic late
10 promoter (LP1) and the g50 (gD) gene is under the control of a synthetic early/late promoter (EP1LP2).

S-SPV-008 was derived from S-SPV-001 (Kasza strain). This was accomplished utilizing the homology vector 538-46.16
15 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). The final result of red
20 plaque purification was the recombinant virus designated S-SPV-008. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds
25 of purification, all plaques observed were blue indicating that the virus was pure, stable and expressing the marker gene.

S-SPV-008 was assayed for expression of PRV specific
30 antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Swine anti-PRV serum was shown to react specifically with S-SPV-008 plaques and not with S-SPV-009 negative control plaques. All S-SPV-008 observed plaques reacted with the swine antiserum
35 indicating that the virus was stably expressing the PRV foreign gene. The black plaque assay was also performed on unfixed monolayers. The SPV plaques on the unfixed

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monolayers also exhibited specific reactivity with swine anti-PRV serum indicating that the PRV antigen is expressed on the infected cell surface.

- 5 To confirm the expression of the PRV g50 (gD) gene product, cells were infected with SPV and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. The
10 swine anti-PRV serum was used to detect expression of PRV specific proteins. The lysate from S-SPV-008 infected cells exhibits a specific band of approximately 48 kd, the reported size of PRV g50 (gD) (35).
- 15 PRV g50 (gD) is the g50 (gD) homologue of HSV-1 (26). Several investigators have shown that VV expressing HSV-1 g50 (gD) will protect mice against challenge with HSV-1 (6 and 34). Therefore the S-SPV-008 should be valuable as a vaccine to protect swine against PRV disease.
- 20 It is anticipated that several other PRV glycoproteins will be useful in the creation of recombinant swinepox vaccines to protect against PRV disease. These PRV glycoproteins include gII (28), gIII (27), and gH (19).
- 25 The PRV gIII coding region has been engineered behind several synthetic pox promoters. The techniques utilized for the creation of S-SPV-008 will be used to create recombinant swinepox viruses expressing all four of these PRV glycoprotein genes. Such recombinant swinepox
30 viruses will be useful as vaccines against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek[®]*, gI HerdChek[®]* and ClinEase[®]*) which are utilized to distinguish vaccinated
35 animals from infected animals. S-SPV-008 has been deposited with the ATCC under Accession No. VR 2339.

* Trade-mark

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Example 6S-SPV-013

5 S-SPV-013 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and the gene for pseudorabies virus gIII (gC) were inserted into the unique *Pst*I restriction site (*Pst*I linkers inserted into a unique *Acc*I site) of the homology
10 vector 570-33.32. The lacZ gene is under the control of the synthetic late promoter (LP1) and the PRV gIII (gC) gene is under the control of the synthetic late early promoter (LP2EP2).

15 S-SPV-013 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 570-91.64 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by
20 the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-013. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple
25 passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

30 S-SPV-013 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal goat anti-PRV gIII (gC) antibody was shown to react specifically with
35 S-SPV-013 plaques and not with S-SPV-001 negative control plaques. All S-SPV-013 observed plaques reacted with the swine anti-PRV serum indicating that the virus was stably

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expressing the PRV foreign gene. The assays described here were carried out in EMSK and VERO cells, indicating that EMSK cells would be a suitable substrate for the production of SPV recombinant vaccines.

5

To confirm the expression of the PRV gIII (gC) gene product, cells were infected with SPV and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Polyclonal goat anti-PRV gIII (gC) antibody was used to detect expression of PRV specific proteins. The lysate from S-SPV-013 infected cells exhibits two specific bands which are the reported size of PRV gIII (gC) (37)—a 92 kd mature form and a 74 kd pre-Golgi form.

Recombinant-expressed PRV gIII (gC) has been shown to elicit a significant immune response in mice and swine (37, 38). Furthermore, when gIII (gC) is coexpressed with gII (gB) or g50 (gD), significant protection from challenge with virulent PRV is obtained. (39) Therefore S-SPV-013 is valuable as a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals. S-SPV-013 has been deposited with the ATCC under Accession No. 2418.

30

Protection against Aujeszky's disease using recombinant swinepox virus vaccines S-SPV-008 and S-SPV-013.

A vaccine containing S-SPV-008 and S-SPV-013 (1 x 10⁶PFU/ml) (2ml of a 1:1 mixture of the two viruses) was given to two groups of pigs (5 pigs per group) by intradermal inoculation or by oral/pharyngeal spray. A

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control group of 5 pigs received S-SPV-001 by both intradermal and oral/pharyngeal inoculation. Pigs were challenged three weeks post-vaccination with virulent PRV, strain 4892, by intranasal inoculation. The table presents a summary of clinical responses. The data support an increase in protection against Aujeszky's disease in the S-SPV-008/S-SPV-013 vaccinates compared to the S-SPV-001 vaccinate controls.

10	Vaccine	Route of inoculation	Post-challenge Respiratory Signs: (# with signs/total number)	Post-challenge CNS signs: (# with signs/total number)	Post-challenge Group average: (Days of clinical signs)
	S-SPV-008 + S-SPV-013	Intradermal	3/5	0/5	2.6
	S-SPV-008 + S-SPV-013	Oral/pharyngeal	3/5	0/5	2.2
15	S-SPV-001 (Control)	Intradermal + Oral/Pharyngeal	5/5	2/5	7.8

Example 720 S-SPV-015

S-SPV-015 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and the gene for pseudorabies virus (PRV) gII (gB) were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the PRV gB gene is under the control of the synthetic late/early promoter (LP2EP2).

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S-SPV-015 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 727-54.60 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-015. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-015 was assayed for expression of PRV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-PRV serum was shown to react specifically with S-SPV-015 plaques and not with S-SPV-001 negative control plaques. All S-SPV-015 observed plaques reacted with the antiserum indicating that the virus was stably expressing the PRV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the PRV gII gene product, cells were infected with SPV-015 and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Polyclonal swine anti-PRV serum was used to detect expression of PRV specific proteins. The lysate from S-SPV-015 infected cells exhibited bands corresponding to 120 kd, 67 kd and 58 kd, which are the expected size of the PRV gII glycoprotein.

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S-SPV-015 is useful as a vaccine in swine against pseudorabies virus. A superior vaccine is formulated by combining S-SPV-008 (PRV g50), S-SPV-013 (PRV gIII), and S-SPV-015 for protection against pseudorabies in swine.

5

Therefore S-SPV-015 should be valuable as a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals. S-SPV-015 has been deposited with the ATCC under Accession No. 2466.

15 Example 8

Recombinant swinepox virus expressing more than one pseudorabies virus (PRV) glycoproteins, which can elicit production of neutralizing antibodies against pseudorabies virus, is constructed in order to obtain a recombinant swinepox virus with enhanced ability to protect against PRV infection than that which can be obtained by using a recombinant swinepox virus expressing only one of those PRV glycoproteins.

25

There are several examples of such recombinant swinepox virus expressing more than one PRV glycoproteins: a recombinant swinepox virus expressing PRV g50 (gD) and gIII (gC), a recombinant swinepox virus expressing PRV g50 (gD) and gII (gB); a recombinant swinepox virus expressing PRV gII (gB) and gIII (gC); and a recombinant swinepox virus expressing PRV g50 (gD), gIII (gC) and gII (gB). Each of the viruses cited above is also engineered to contain and express *E. coli* β -galactosidase (lac Z) gene, which will facilitate the cloning of the recombinant swinepox virus.

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Listed below are three examples of a recombinant swinepox virus expressing PRV g50 (gD), PRV gIII (gC), PRV gII (gB) and *E. coli* β -galactosidase (lacZ):

5 a) Recombinant swinepox virus containing and
expressing PRV g50 (gD) gene, PRV gIII (gC) gene,
PRV gII (gB) gene and lacZ gene. All four genes are
10 inserted into the unique AccI restriction
endonuclease site within the *HindIII* M fragment of
the swinepox virus genome. PRV g50 (gD) gene is
under the control of a synthetic early/late promoter
(EP1LP2), PRV gIII (gC) gene is under the control of
a synthetic early promoter (EP2), PRV gII (gB) gene
15 is under the control of a synthetic late/early
promoter (LP2EP2) and lacZ gene is under the control
of a synthetic late promoter (LP1).

20 b) Recombinant swinepox virus containing and
expressing PRV g50 (gD) gene, PRV gIII (gC) gene,
PRV gII (gB) gene and lacZ gene. All four genes are
inserted into the unique AccI restriction
endonuclease site within the *HindIII* M fragment of
the swinepox virus genome. PRV g50 (gD) gene is
under the control of a synthetic early/late promoter
25 (EP1LP2), PRV gIII (gC) gene is under the control of
a synthetic early/late promoter (EP1LP2), PRV gII
(gB) gene is under the control of a synthetic
late/early promoter (LP2EP2) and lacZ gene is under
the control of a synthetic late promoter (LP1).

30 c) Recombinant swinepox virus containing and
expressing PRV g50 (gD) gene, PRV gIII (gC) gene,
PRV gII (gB) gene and lacZ gene. All four genes are
inserted into the unique AccI restriction
endonuclease site within the *HindIII* M fragment of
35 the swinepox virus genome. PRV g50 (gD) gene is
under the control of a synthetic early/late promoter

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(EP1LP2), PRV gIII (gC) gene is under the control of a synthetic late/early promoter (LP2EP2), PRV gII (gB) gene is under the control of a synthetic late/early promoter (LP2EP2) and lacZ gene is under the control of a synthetic late promoter (LP1).

Protection against Aujeszky's disease using recombinant swinepox virus vaccines S-SPV-008, S-SPV-013 and S-SPV-015.

10

A vaccine containing S-SPV-008, S-SPV-013, or S-SPV-015 (2 ml of 1×10^7 PFU/ml of the virus) or a mixture of S-SPV-008, S-SPV-013, and S-SPV-015 (2ml of a 1:1:1 mixture of the three viruses; 1×10^7 PFU/ml) was given to four groups of pigs (5 pigs per group) by intramuscular inoculation. A control group of 5 pigs received S-SPV-001 by intramuscular inoculation. Pigs were challenged four weeks post-vaccination with virulent PRV, strain 4892, by intranasal inoculation. The pigs were observed daily for 14 days for clinical signs of pseudorabies, and the table presents a summary of clinical responses. The data show that pigs vaccinated with S-SPV-008, S-SPV-013, or S-SPV-015 had partial protection and pigs vaccinated with the combination vaccine S-SPV-008/S-SPV-013/S-SPV-015 had complete protection against Aujeszky's disease caused by pseudorabies virus compared to the S-SPV-001 vaccinate controls.

Vaccine	Route of inoculation	Post-challenge	Post-challenge	Post-challenge
		Respiratory Signs: (# with signs/total number)	CNS signs: (# with signs/total number)	Group average: (Days of clinical signs)
30 S-SPV-008	Intramuscular	2/5	2/5	2.0
S-SPV-013	Intramuscular	1/5	0/5	0.4

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	S-SPV-015	Intramuscular	3/5	0/5	1.0
	S-SPV-008 +	Intramuscular	0/5	0/5	0.0
	S-SPV-013 +				
	S-SPV-015				
5	S-SPV-001	Intramuscular	5/5	2/5	3.6
	(Control)				

Example 17

10

The development of vaccines utilizing the swinepox virus to express antigens from various disease causing microorganisms can be engineered.

15

TRANSMISSIBLE GASTROENTERITIS VIRUS

The major neutralizing antigen of the transmissible gastroenteritis virus (TGE), glycoprotein 195, for use in the swinepox virus vector has been cloned. The clone of the neutralizing antigen is disclosed in U.S.

20

Patent No. 5,223,424. It is contemplated that the procedures that have been used to express PRV g50 (gD) in SPV and are disclosed herein are applicable to TGE.

25

PORCINE PARVOVIRUS

The major capsid protein of the porcine (swine) parvovirus (PPV) was cloned for use in the swinepox virus vector. The clone of the capsid protein is disclosed in U.S. Patent No. 5,068,192 issued November 26, 1991. It is contemplated that the procedures that have been used to express PRV g50 (gD) in SPV and are disclosed herein are applicable to PPV.

30

SWINE ROTAVIRUS

35

The major neutralizing antigen of the swine rotavirus, glycoprotein 38, was cloned for use in the swinepox virus vector. The clone of glycoprotein 38 is disclosed

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in U.S. Patent No. 5,068,192 issued November 26, 1991. It is contemplated that the procedures that have been used to express PRV g50 (gD) in SPV and are disclosed herein are applicable to SRV.

5

HOG CHOLERA VIRUS

The major neutralizing antigen of the bovine viral diarrhea (BVD) virus was cloned as disclosed in U.S. Patent No. 5,223,424.

10 Since the BVD and hog cholera viruses are cross protective (31), the BVD virus antigen has been targeted for use in the swinepox virus vector. It is contemplated that the procedures that have been used to express PRV g50 (gD) in SPV and are disclosed herein are applicable to BVD
15 virus.

SERPULINA HYODYSENTERIAE

A protective antigen of *Serpulina hyodysenteriae* (3), for use in the swinepox virus vector has been cloned.

20 It is contemplated that the procedures that have been used to express PRV g50 in SPV and are disclosed herein are also applicable to *Serpulina hyodysenteriae*.

Antigens from the following microorganisms may also be
25 utilized to develop animal vaccines: swine influenza virus, foot and mouth disease virus, African swine fever virus, hog cholera virus, *Mycoplasma hyopneumoniae*, porcine reproductive and respiratory syndrome/swine infertility and respiratory syndrome
30 (PRRS/SIRS).

Antigens from the following microorganisms may also be utilized for animal vaccines: 1) canine - herpesvirus, canine distemper, canine adenovirus type 1 (hepatitis),
35 adenovirus type 2 (respiratory disease), parainfluenza, *Leptospira canicola*, icterohemorrhagia, parvovirus, coronavirus, *Borrelia burgdorferi*, canine herpesvirus,

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Bordetella bronchiseptica, *Dirofilaria immitis* (heartworm) and rabies virus. 2) Feline - Fiv gag and env, feline leukemia virus, feline immunodeficiency virus, feline herpesvirus, feline infectious peritonitis virus, canine herpesvirus, canine coronavirus, canine parvovirus, parasitic diseases in animals (including *Dirofilaria immitis* in dogs and cats), equine infectious anemia, *Streptococcus equi*, coccidia, emeria, chicken anemia virus, *Borrelia bergdorferi*, bovine coronavirus, *Pasteurella haemolytica*.

Example 24

15

Homology Vector 738-94.4

Homology Vector 738-94.4 is a swinepox virus vector that expresses one foreign gene. The gene for *E. coli* β -galactosidase (lacZ) was inserted into the the O1L open reading frame. The lacZ gene is under the control of the O1L promoter. The homology vector 738-94.4 contains a deletion of SPV DNA from nucleotides 1679 to 2452 (SEQ ID NO: 189) which deletes part of the O1L ORF.

The upstream SPV sequences were synthesized by polymerase chain reaction using DNA primers 5'-GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with BglII and SphI ends. The O1L promoter is present on this fragment. The downstream SPV sequences were synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5'-GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

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A recombinant swinepox virus was derived utilizing homology vector 738-94.4 and S-SPV-001 (Kasza strain) in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was
5 screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification is the recombinant virus. This virus is assayed for β -galactosidase expression, purity, and insert stability by multiple
10 passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign gene. Recombinant swinepox
15 viruses derived from homology vector 738-94.4 are utilized as an expression vector to express foreign antigens and as a vaccine to raise a protective immune response in animals to foreign genes expressed by the recombinant swinepox virus. Other promoters in addition
20 to the O1L promoter are inserted into the deleted region including LP1, EP1LP2, LP2EP2, HCMV immediate early, and one or more foreign genes are expressed from these promoters.

25 Example 24B

Homology Vector 752-22.1 is a swinepox virus vector that is utilized to express two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) was inserted into
30 the the O1L open reading frame. The lacZ gene is under the control of the O1L promoter. A second foreign gene is expressed from the LP2EP2 promoter inserted into an EcoRI or BamHI site following the LP2EP2 promoter sequence. The homology vector 752-22.1 contains a
35 deletion of SPV DNA from nucleotides 1679 to 2452 (SEQ ID NO: 189) which deletes part of the O1L ORF. The homology vector 752-22.1 was derived from homology

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vector 738-94.4 by insertion of the LP2EP2 promoter fragment (see Materials and Methods). The homology vector 752-22.1 is further improved by placing the lacZ gene under the control of the synthetic LP1 promoter.

5 The LP1 promoter results in higher levels of lacZ expression compared to the SPV O1L promoter

Example 25

10 S-SPV-041:

S-SPV-041 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and the gene for equine herpesvirus type 1 glycoprotein B (gB) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1679 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox O1L promoter, and the EHV-1 gB gene is under the control of the

15

20 synthetic late/early promoter (LP2EP2).

S-SPV-041 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 752-29.33 (see Materials and Methods) and virus S-SPV-

25 001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant

30 virus designated S-SPV-041. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all

35 plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

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S-SPV-041 is useful as a vaccine in horses against EHV-1 infection and is useful for expression of EHV-1 glycoprotein B.

5 S-SPV-045:

10 S-SPV-045 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and the gene for infectious bovine
15 rhinotracheitis virus glycoprotein E (gE) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1679 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox O1L promoter, and the IBRV gE gene is under
20 the control of the synthetic late/early promoter (LP2EP2).

25 S-SPV-045 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector
30 746-94.1 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final
35 result of red plaque purification was the recombinant virus designated S-SPV-045. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods.
40 After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

45 S-SPV-045 is useful for expression of IBRV glycoprotein E.

S-SPV-049

S-SPV-049 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and the gene for bovine viral diarrhea virus glycoprotein 48 (gp48) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1679 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox O1L promoter, and the BVDV gp48 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-049 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 771-55.11 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-049. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-049 is useful as a vaccine in cattle against BVDV infection and is useful for expression of BVDV glycoprotein 48.

S-SPV-050:

S-SPV-050 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and the gene for the bovine viral diarrhea virus glycoprotein 53 (gp53) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF;

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Deletion of nucleotides 1679 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox O1L promoter, and the IBRV gE gene is under the control of the synthetic late/early promoter (LP2EP2).

5

S-SPV-050 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 767-67.3 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
10 GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-050. This virus was assayed for
15 β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus
20 was pure, stable, and expressing the foreign gene.

S-SPV-050 is useful as a vaccine in cattle against BVDV infection and is useful for expression of BVDV glycoprotein 53.

25

Example 26

Recombinant swinepox virus, S-SPV-042 or S-SPV-043, expressing chicken interferon (cIFN) or chicken
30 myelomonocytic growth factor (cMGF), respectively, are useful to enhance the immune response when added to vaccines against diseases of poultry. Chicken myelomonocytic growth factor (cMGF) is homologous to mammalian interleukin-6 protein, and chicken interferon
35 (cIFN) is homologous to mammalian interferon. When used in combination with vaccines against specific avian diseases, S-SPV-042 and S-SPV-043 provide enhanced

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mucosal, humoral, or cell mediated immunity against avian disease-causing viruses including, but not limited to, Marek's disease virus, Newcastle disease virus, infectious laryngotracheitis virus, infectious
5 bronchitis virus, infectious bursal disease virus.

Example 26A

S-SPV-042:

10

S-SPV-042 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and the gene for chicken interferon (cIFN) were inserted into the SPV 617-48.1 ORF (a unique NotI
15 restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the cIFN gene is under the control of the synthetic late/early promoter (LP2EP2).

20

S-SPV-042 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 751-07.A1 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
25 GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-042. This virus was assayed for
30 β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus
35 was pure, stable, and expressing the foreign gene.

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S-SPV-042 has interferon activity in cell culture. Addition of S-SPV-042 conditioned media to chicken embryo fibroblast (CEF) cell culture inhibits infection of the CEF cells by vesicular stomatitis virus or by herpesvirus of turkeys. S-SPV-042 is useful to enhance the immune response when added to vaccines against diseases of poultry.

Example 26B

10

S-SPV-043:

S-SPV-043 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and the gene for chicken myelomonocytic growth factor (cMGF) were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the cMGF gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-043 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 751-56.A1 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-043. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

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S-SPV-043 is useful to enhance the immune response when added to vaccines against diseases of poultry.

Example 27

5

Insertion into a non-essential site in the 2.0 kb HindIII to BglII region of the swinepox virus HindIII M fragment.

- 10 A 2.0 kb HindIII to BglII region of the swinepox virus HindIII M fragment is useful for the insertion of foreign DNA into SPV. The foreign DNA is inserted into a unique BglII restriction site in the region Nucleotide 540 of SEQ ID NOs: 195). A plasmid
15 containing a foreign DNA insert is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV to generate an SPV containing the foreign DNA. For this procedure to be successful, it is important that the insertion site be in a region non-
20 essential to the replication of the SPV and that the site be flanked with swinepox virus DNA appropriate for mediating homologous recombination between virus and plasmid DNAs. The unique BglII restriction site in the 2.0 kb HindIII to BglII region of the swinepox virus
25 HindIII M fragment is located within the coding region of the SPV I4L open reading frame. The I4L ORF has sequence similarity to the vaccinia virus and smallpox virus ribonucleotide reductase (large subunit) gene (56-58). The ribonucleotide reductase (large subunit)
30 gene is non-essential for DNA replication of vaccinia virus and is an appropriate insertion site in swinepox virus.

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Example 28S-SPV-047

5 S-SPV-047 is a swinepox virus that expresses two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for pseudorabies virus gB (gII) were inserted into a unique HindIII site (HindIII linker inserted into the BglII restriction endonuclease
10 site within the 2.0 kb BglII to HindIII subfragment of the HindIII M fragment.) The BglII insertion site is within the SPV I4L open reading frame which has significant homology to the vaccinia virus ribonucleoside-diphosphate reductase gene. The lacZ
15 gene is under the control of the synthetic late promoter (LP1), and the PRV gB (gII) gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-047 was derived from S-SPV-001 (Kasza Strain).
20 This was accomplished utilizing the homology vector 779-94.31 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING
25 β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-047. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue
30 plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

35 S-SPV-047 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-

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PRV serum was shown to react specifically with S-SPV-047 plaques and not with S-SPV-001 negative control plaques. All S-SPV-047 observed plaques reacted with the swine anti-PRV serum indicating that the virus was stably expressing the PRV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

10

To confirm the expression of the PRV gB gene product, cells were infected with S-SPV-047 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRV serum was used to detect expression of PRV specific proteins. The cell lysate and supernatants from S-SPV-047 infected cells exhibited bands corresponding to 120 kD, 67 kD and 58 kD, which are the expected size of the PRV glycoprotein B.

15
20

SPV recombinant-expressed PRV gB has been shown to elicit a significant immune response in swine (37, 38; See example 8). Furthermore, PRV gB is expressed in recombinant SPV, significant protection from challenge with virulent PRV is obtained. (See Examples 6 and 8) Therefore S-SPV-047 is valuable as a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals.

30
35

S-SPV-052

S-SPV-052 is a swinepox virus that expresses three foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for pseudorabies virus gB (gII) were inserted into the unique HindIII restriction site (HindIII linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104) of the SPV HindIII M fragment has been deleted). The gene for PRV gD (g50) was inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The lacZ gene is under the control of the synthetic late promoter (LP1), the PRV gB (gII) gene is under the control of the synthetic late/early promoter (LP2EP2), and the PRV gD (g50) gene is under the control of the synthetic early/late promoter (EP1LP2).

S-SPV-052 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 789-41.7 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 052. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-052 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-PRV serum was shown to react specifically with S-SPV-

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052 plaques and not with S-SPV-001 negative control plaques. All S-SPV-052 observed plaques reacted with the swine anti-PRV serum indicating that the virus was stably expressing the PRV foreign gene. The assays
5 described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

10 To confirm the expression of the PRV gB and gD gene products, cells were infected with S-SPV-052 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed
15 using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRV serum was used to detect expression of PRV specific proteins. The cell lysate and supernatants from S-SPV-052 infected cells exhibited bands corresponding to 120 kD, 67 kD and 58 kD, which
20 are the expected size of the PRV glycoprotein B; and a 48 kD which is the expected size of the PRV glycoprotein D.

SPV recombinant-expressed PRV gB and gD has been shown
25 to elicit a significant immune response in swine (37, 38; See example 8). Furthermore, PRV gB and gD are expressed in recombinant SPV, significant protection from challenge with virulent PRV is obtained. (See Examples 6 and 8) Therefore S-SPV-052 is valuable as
30 a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated
35 animals from infected animals.

S-SPV-053

S-SPV-053 is a swinepox virus that expresses three foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for pseudorabies virus gB (gII) were inserted into the unique HindIII restriction site (HindIII linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104) of the SPV HindIII M fragment has been deleted). The gene for PRV gC (gIII) was inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The lacZ gene is under the control of the synthetic late promoter (LP1), the PRV gB (gII) gene is under the control of the synthetic late/early promoter (LP2EP2), and the PRV gC (gIII) gene is under the control of the synthetic early/late promoter (EP1LP2).

S-SPV-053 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 789-41.27 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 053. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-053 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-PRV serum was shown to react specifically with S-SPV-

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- 053 plaques and not with S-SPV-001 negative control plaques. All S-SPV-053 observed plaques reacted with the swine anti-PRV serum indicating that the virus was stably expressing the PRV foreign gene. The assays
- 5 described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.
- 10 To confirm the expression of the PRV gB and gC gene products, cells were infected with S-SPV-053 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed
- 15 using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRV serum was used to detect expression of PRV specific proteins. The cell lysate and supernatants from S-SPV-053 infected cells exhibited bands corresponding to 120 kD, 67 kD and 58 kD, which
- 20 are the expected size of the PRV glycoprotein B; and a 92 kD which is the expected size of the PRV glycoprotein C.
- SPV recombinant-expressed PRV gB and gC has been shown
- 25 to elicit a significant immune response in swine (37, 38; See example 8). Furthermore, PRV gB and gC are expressed in recombinant SPV, significant protection from challenge with virulent PRV is obtained. (See Examples 6 and 8) Therefore S-SPV-053 is valuable as
- 30 a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated
- 35 animals from infected animals.

S-SPV-054

S-SPV-054 is a swinepox virus that expresses three foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for pseudorabies virus gC (gIII) were inserted into the unique HindIII restriction site (HindIII linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104) of the SPV HindIII M fragment has been deleted). The gene for PRV gD (g50) was inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The lacZ gene is under the control of the synthetic late promoter (LP1), the PRV gC (gIII) gene is under the control of the synthetic early/late promoter (EP1LP2), and the PRV gD (g50) gene is under the control of the synthetic early/late promoter (EP1LP2).

S-SPV-054 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 789-41.47 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 054. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-054 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-

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PRV serum was shown to react specifically with S-SPV-054 plaques and not with S-SPV-001 negative control plaques. All S-SPV-054 observed plaques reacted with the swine anti-PRV serum indicating that the virus was stably expressing the PRV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

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To confirm the expression of the PRV gC and gD gene products, cells were infected with S-SPV-054 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRV serum was used to detect expression of PRV specific proteins. The cell lysate and supernatants from S-SPV-054 infected cells exhibited a band corresponding to 92 kD which is the expected size of the PRV glycoprotein C and a 48 kD which is the expected size of the PRV glycoprotein D.

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SPV recombinant-expressed PRV gC and gD has been shown to elicit a significant immune response in swine (37, 38; See example 8). Furthermore, PRV gC and gD are expressed in recombinant SPV, significant protection from challenge with virulent PRV is obtained. (See Examples 6 and 8) Therefore S-SPV-054 is valuable as a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals.

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S-SPV-055

S-SPV-055 is a swinepox virus that expresses four foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for pseudorabies virus gB (gII) were inserted into the unique HindIII restriction site (HindIII linkers inserted into a unique NdeI site in the SPV O1L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104) of the SPV HindIII M fragment has been deleted). The gene for PRV gD (g50) and PRV gC (gIII) were inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV O1L open reading frame). The lacZ gene is under the control of the synthetic late promoter (LP1), the PRV gB (gII) gene is under the control of the synthetic late/early promoter (LP2EP2), the PRV gD (g50) gene is under the control of the synthetic late/early promoter (LP2EP2), and the PRV gC (gIII) gene is under the control of the synthetic early/late promoter (EP1LP2).

S-SPV-055 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 789-41.73 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 055. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-055 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE

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EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-PRV serum was shown to react specifically with S-SPV-055 plaques and not with S-SPV-001 negative control plaques. All S-SPV-055 observed plaques reacted with the swine anti-PRV serum indicating that the virus was stably expressing the PRV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the PRV gB, gC and gD gene products, cells were infected with S-SPV-055 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRV serum was used to detect expression of PRV specific proteins. The cell lysate and supernatants from S-SPV-055 infected cells exhibited a bands corresponding to 120 kD, 67 kD, and 58 kD which is the expected size of the PRV glycoprotein B; a 92 kD which is the expected size of the PRV glycoprotein C; and a 48 kD which is the expected size of the PRV glycoprotein D.

SPV recombinant-expressed PRV gB, gC and gD has been shown to elicit a significant immune response in swine (37, 38; See example 8). Furthermore, PRV gB, gC and gD are expressed in recombinant SPV, significant protection from challenge with virulent PRV is obtained. (See Examples 6 and 8) Therefore S-SPV-055 is valuable as a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI

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HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals.

Example 29

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S-SPV-059

S-SPV-059 is a swinepox virus that expresses one foreign gene. The gene for E. coli B-glucuronidase (uidA) was inserted into the unique EcoRI restriction site in the SPV B18R open reading frame within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic late/early promoter (LP2EP2). Partial sequence of the SPV 3.2 kb region of the SPV 6.5 kb HindIII K fragment indicates three potential open reading frames. The SPV B18R ORF shows sequences homology to the vaccinia virus B18R gene, 77.2K protein from rabbit fibroma virus, vaccinia virus C19L/B25R ORF and an ankyrin repeat region from a human brain variant. The B18R gene codes for a soluble interferon receptor with high affinity and broad specificity. The SPV B4R open reading frame shows sequence homology to the T5 protein of rabbit fibroma virus.

25 S-SPV-059 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 796-50.31 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. Homology vector 796-50.31 was generated by insertion of a blunt ended NotI fragment containing the LP2EP2 promoter uidA cassette from plasmid 551-47.23 (see Materials and Methods) into a unique EcoRI site (blunt ended) in the SPV 6.5 kb HindIII K fragment, (Figure 29B). The transfection stock was screened by the SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES. The final result of blue plaque purification was the recombinant virus designated S-SPV-059. This virus

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was assayed for B-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-059 has been purified and expresses the foreign gene, *E. coli uidA*, indicating that the *EcoRI* site within the 6.5 kb *HindIII* K fragment is a stable insertion site for foreign genes. Recombinant swinepox virus utilizing this insertion site is useful for expression of foreign antigen genes, as a vaccine against disease or as an expression vector to raise antibodies to the expressed foreign gene.

S-SPV-060

S-SPV-060 is a swinepox virus that expresses one foreign gene. The gene for *E. coli* B-glucuronidase (*uidA*) was inserted into the unique *EcoRV* restriction site within the SPV *HindIII* N genomic fragment. The *uidA* gene is under the control of the synthetic late/early promoter (LP2EP2). Partial sequence of the SPV 3.2 kb *HindIII* N fragment (SEQ ID NO.) indicates two potential open reading frames. The SPV I7L ORF shows sequences homology to protein I7 of vaccinia virus. The SPV I4L open reading frame shows sequence homology to the ribonucleoside diphosphate reductase gene of vaccinia virus. Two potential open reading frames I5L and I6L, between I4L and I7L ORF are of unknown function.

S-SPV-060 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 796-71.31 and virus S-SPV-001 in the HOMOLOGOUS

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RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. Homology vector 796-71.31 was generated by insertion of a blunt ended NotI fragment containing the LP2EP2 promoter uidA cassette from plasmid 551-47.23 (see Materials and Methods) into a unique EcoRV site in the SPV 3.2 kb HindIII N fragment (Figure 11A). The transfection stock was screened by the SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES. The final result of blue plaque purification is the recombinant virus designated S-SPV-060. This virus is assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-SPV-060 is purified and expresses the foreign gene, E. coli uidA, indicating that the EcoRI site within the 3.2 kb HindIII N fragment is a stable insertion site for foreign genes. Recombinant swinepox virus utilizing this insertion site is useful for expression of foreign antigen genes, as a vaccine against disease or as an expression vector to raise antibodies to the expressed foreign gene.

S-SPV-061

S-SPV-061 is a swinepox virus that expresses one foreign gene. The gene for E. coli β -glucuronidase (uidA) was inserted into the unique SnaBI restriction site within the SPV HindIII N genomic fragment. The uidA gene is under the control of the synthetic late/early promoter (LP2EP2). Partial sequence of the SPV 3.2 kb HindIII N fragment indicates two potential open reading frames. The SPV I7L ORF shows sequence

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homology to protein 17 of vaccinia virus. The SPV I4L open reading frame shows sequence homology to the ribonucleoside diphosphate reductase gene of vaccinia virus. Two potential open reading frames I5L and I6L, between I4L ORF and I7L ORF are of unknown function.

S-SPV-061 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 796-71.41 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. Homology vector 796-71.41 was generated by insertion of a blunt ended NotI fragment containing the LP2EP2 promoter uidA cassette from plasmid 551-47.23 (see Materials and Methods) into a unique SnaBI site in the SPV 3.2 kb HindIII N fragment. The transfection stock was screened by the SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES. The final result of blue plaque purification is the recombinant virus designated S-SPV-061. This virus is assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-SPV-061 is purified and expresses the foreign gene, E. coli uidA, indicating that the SnaBI site within the 3.2 kb HindIII N fragment is a stable insertion site for foreign genes. Recombinant swinepox virus utilizing this insertion site is useful for expression of foreign antigen genes, as a vaccine against disease or as an expression vector to raise antibodies to the expressed foreign gene.

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S-SPV-062

S-SPV-062 is a swinepox virus that expresses one foreign gene. The gene for E. coli β -glucuronidase (uidA) was inserted into the unique BglII restriction site within the SPV HindIII N genomic fragment (Figure 11A). The uidA gene is under the control of the synthetic late/early promoter (LP2EP2). Partial sequence of the SPV 3.2 kb HindIII N fragment indicates two potential open reading frames. The SPV I7L ORF shows sequence homology to protein 17 of vaccinia virus. The SPV I4L open reading frame shows sequence homology to the ribonucleoside diphosphate reductase gene of vaccinia virus. Two potential open reading frames I5L and I6L, between I4L ORF and I7L ORF are of unknown function.

S-SPV-062 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 796-71.51 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. Homology vector 796-71.51 was generated by insertion of a blunt ended NotI fragment containing the LP2EP2 promoter uidA cassette from plasmid 551-47.23 (see Materials and Methods) into a unique BglII site in the SPV 3.2 kb HindIII N fragment. The transfection stock was screened by the SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES. The final result of blue plaque purification is the recombinant virus designated S-SPV-062. This virus is assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-SPV-062 is purified and expresses the foreign gene, E. coli uidA, indicating that the BglII site within the

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3.2 kb HindIII N fragment is a stable insertion site for foreign genes. Recombinant swinepox virus utilizing this insertion site is useful for expression of foreign antigen genes, as a vaccine against disease or as an expression vector to raise antibodies to the expressed foreign gene.

Example 30:

Recombinant swinepox virus expressing E coli β -galactosidase (lacZ) under the control of a synthetic early or synthetic late pox promoter.

Three recombinant swinepox viruses, S-SPV-056, S-SPV-057, and S-SPV-058 expressing E coli β -galactosidase (lacZ) under the control of a synthetic pox promoter, LP1, LP2, and EP1, respectively, have been constructed.

S-SPV-056 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 791-63.19 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). S-SPV-057 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 791-63.41 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). S-SPV-058 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 796-18.9 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by

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the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification were the recombinant viruses designated S-SPV-056, S-SPV-057 and S-SPV-058.

5 The viruses were assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were
10 blue indicating that the virus was pure, stable, and expressing the foreign gene.

Recombinant swinepox virus expresses a foreign gene such as E. coli β -galactosidase in a human cell line
15 but does not replicate in the human cell line. To optimize expression of the foreign gene, S-SPV-056, S-SPV-057 and S-SPV-058 are used to compare optimal expression levels of E. coli β -galactosidase under the control of early or late synthetic pox viral promoters.
20 The human cell lines in which expression of recombinant swinepox virus has been detected include, but are not limited to 143B (osteosarcoma), A431 (epidermoid carcinoma), A549 (lung carcinoma), Capan-1 (liver carcinoma), CF500 (foreskin fibroblasts), Chang Liver
25 (liver), Detroit (down's foreskin fibroblasts), HEL-199 (embryonic lung), HeLa (cervical carcinoma), HEp-2 (epidermal larynx carcinoma), HISM (intestinal smooth muscle), HNK (neonatal kidney), MRC-5 (embryonic lung), NCI-H292 (pulmonary mucoepidermoid carcinoma), OVCA-3
30 (ovarian carcinoma), RD (rhabdosarcoma), THP (monocyte leukemia), WIL2-NS (B lymphocyte line, non-secreting), WISH (amnion).

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Example 31:S-SPV-051

S-SPV-051 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for the bovine viral diarrhea virus glycoprotein 53 (g53) were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the BVDV g53 gene is under the control of the synthetic late/early promoter (LP2EP2).

10

S-SPV-051 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 783-39.2 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 051. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

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S-SPV-051 was assayed for expression of BVDV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. A mouse monoclonal antibody to BVDV g53 was shown to react specifically with S-SPV-051 plaques and not with S-SPV-001 negative control plaques. All S-SPV-051 observed plaques reacted with the monoclonal antibody to BVDV g53 indicating that the virus was stably expressing the BVDV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4

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cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the BVDV g53 gene product,
5 cells were infected with S-SPV-051 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A mouse monoclonal
10 antibody to BVDV g53 was used to detect expression of BVDV specific proteins. The cell lysate and supernatant from S-SPV-051 infected cells exhibited bands at 53 kd and higher indicating glycosylated and unglycosylated forms of the BVDV g53 protein.

15 S-SPV-051 is useful as a vaccine in cattle against BVDV infection and is useful for expression of BVDV glycoprotein 53.

20 **Example 32:**

S-SPV-044:

S-SPV-044 is a swinepox virus that expresses at least
25 two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for the infectious bursal disease virus (IBDV) polymerase protein were inserted into the 617-48.1 ORF (a unique NotI site has replaced a unique AccI restriction site). The lacZ gene is under the
30 control of the synthetic late promoter (LP1), and the IBDV polymerase gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-044 was derived from S-SPV-001 (Kasza Strain).
35 This was accomplished utilizing the homology vector 749-75.78 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR

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GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-044. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-044 is useful for expression of IBDV polymerase protein. S-SPV-044 is useful in an *in vitro* approach to a recombinant IBDV attenuated vaccine. RNA strands from the attenuated IBDV strain are synthesized in a bacterial expression system using T3 or T7 promoters (pBlueScript plasmid; Stratagene, Inc.) to synthesize double stranded short and long segments of the IBDV genome. The IBDV double stranded RNA segments and S-SPV-044 are transfected into CEF cells. The swinepox virus expresses the IBDV polymerase but does not replicate in CEF cells. The IBDV polymerase produced from S-SPV-044 synthesizes infectious attenuated IBDV virus from the double stranded RNA genomic templates. The resulting attenuated IBDV virus is useful as a vaccine against infectious bursal disease in chickens.

Example 33:**S-SPV-046:**

S-SPV-046 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and the gene for the feline immunodeficiency virus (FIV) gag protease (gag) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L

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ORF; Deletion of nucleotides 1669 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox O1L promoter, and the FIV gag gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-046 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 761-75.B18 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 046. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

To confirm the expression of the FIV gag gene product, cells were infected with S-SPV-046 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Feline anti-FIV (PPR strain) sera was used to detect expression of FIV specific proteins. The cell lysate and supernatant from S-SPV-046 infected cells exhibited bands at 26 kd and 17 kd which are the expected sizes of the processed form of the FIV gag protein. The recombinant swinepox virus expressed FIV gag protein is processed properly and secreted into the culture media.

S-SPV-048

S-SPV-048 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for feline immunodeficiency virus (FIV) envelope (env) were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the FIV env gene is under the control of the synthetic late/early promoter (LP2EP2).

10

S-SPV-048 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 781-84.C11 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 048. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

25

S-SPV-046 and S-SPV-048 are useful alone or in combination as a vaccine in cats against FIV infection and are useful for expression of the FIV env and gag proteins. A recombinant swinepox virus expressing both the FIV env and gag proteins is useful as a vaccine in cats against FIV infection.

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Recombinant swinepox virus expressing human respiratory syncytial virus F and G proteins is useful as a vaccine against the human disease.

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Example 34

In Vitro Properties of Chicken IFN Expressed in Recombinant Pox viruses.

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Growth properties of recombinant viruses in cell culture. Growth properties of recombinant S-SPV-042 were not effected in embryonic swine kidney cells (ESK-4) compared to wild-type swinpox virus.

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Western blot analysis was performed on supernatants from cells infected with SPV/cIFN recombinant virus. Rabbit and mouse antisera were raised against cIFN from concentrated SPV/cIFN infected supernatants and pre-cleared against ESK-4 cells infected with wild-type SPV in preparation for western analysis. Rabbit and mouse anti-cIFN antisera were reacted with denatured proteins on nitrocellulose from recombinant SPV/cIFN and SPV wild type virus infected supernatants. A reactive band with an estimated molecular weight size range of 17-20 kilodaltons was present in the SPV/cIFN lanes, and absent in the SPV wild type control lanes.

25

Effect of cIFN expressed in supernatants from SPV/cIFN (S-SPV-042), FPV/cIFN, and FPV/cIFN/NDV infected cells on the growth of Vesicular Stomatitis Virus.

Virion cleared supernatants from SPV/cIFN, FPV/cIFN and FPV/cIFN/NDV infected cells were tested for the presence of viral inhibitory activity, results shown in Table 1. Briefly, CEF cells were incubated with serially diluted viral supernatants. Subsequently, 40,000 plaque forming units (pfu)/well of vesicular stomatitis virus (VSV) were added and 48 hours later, wells were scored for the presence of VSV cytopathic effect (CPE). Recombinant viral supernatants

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containing cIFN were shown to inhibit VSV induced CPE, whereas, control viral supernatants did not. VSV induced cytopathic effect could be reversed in the presence of rabbit anti-cIFN sera.

5

Table 1.

	Recombinant Viral Supernatants.	cIFN Activity (units/ml). ^a
	SPV/IFN	2,500 000
10	SPV	<100
	FPV/IFN	250,000
	FPV/cIFN/NDV	250,000
	FPV	<100

15 ^a. One unit of cIFN activity is defined as the dilution of pox virus supernatant at which 100% VSV CPE was inhibited.

20 Effect of cIFN expressed from supernatants of SPV/cIFN infected cells on herpes virus of turkeys.

Supernatant containing recombinant cIFN from ESK-4 cells infected with SPV/cIFN virus, was tested for its ability to inhibit the growth of herpes virus of turkeys (HVT) in CEF cells, results shown in Table 2. Briefly, serially diluted supernatants were incubated with CEF cells, and then subsequently infected with 100 pfu/well of wild-type HVT. Plaques were counted in all wells after 48 hours. It was shown that 10-100 units of cIFN activity inhibited plaque formation of HVT(100 pfu/well). Supernatants from wild type SPV did not inhibit HVT plaque formation.

35

Table 2.

SPV/cIFN Supernatant		Number of HVT plaques	
(units/ml ^a)			
5	0	99	
	1000	0	
	100	0	
	10	45	

10 ^{a.} One unit of cIFN activity is defined as the dilution of pox virus supernatant at which 100% VSV CPE was inhibited.
 Induction of NO by chicken macrophages after treatment with cIFN expressed in supernatants from SPV/cIFN infected cells.

15 HD 11 cells or bone marrow adherent cells were incubated with 1000unit/ml of cIFN from SPV/cIFN supernatants, lipopolysaccharide (LPS) (6ng/ml) or with both cIFN and LPS, results shown in Table 3. After 24 hours, supernatant fluids were collected and nitrite
20 levels were measured. These data demonstrate that cIFN expressed from SPV/cIFN supernatants has the ability to activate chicken macrophages in the presence of LPS.

25 Table 3.

Nitrite (micro/mol) levels following stimulation with :			
Cell source	LPS	SPV/cIFN	LPS + SPV/cIFN
HD11	10.76	6.4	35.29
BMAC	13.1	5.8	35.10

30

Conclusions:

1. Recombinant swinepox viruses express biologically active chicken interferon into the supernatants of infected cells, as measured by protection of CEF cells from VSV infection.
2. Chicken interferon expressed in supernatants from recombinant SPV/cIFN infected cells has been shown to protect CEF cells against infection with HVT in a dose dependent manner.
3. Chicken interferon expressed from SPV/cIFN acted synergistically with LPS to activate chicken macrophages as detected by nitric oxide induction.
4. The foregoing data indicate that recombinant swinepox viruses expressing chicken IFN may have beneficial applications as immune modulating agents in vitro, in vivo and in ovo.

Example 35

As an alternative to the construction of a IBD vaccine using a viral vectored delivery system and/or subunit approaches, IBD virus RNA is directly manipulated re-constructing the virus using full length RNA derived from cDNA clones representing both the large (segment A) and small (segment B) double-stranded RNA subunits. Generation of IBD virus in this manner offers several advantages over the first two approaches. First, if IBD virus is re-generated using RNA templates, one is able to manipulate the cloned cDNA copies of the viral genome prior to transcription (generation of RNA). Using this approach, it is possible to either attenuate a virulent IBD strain or replace the VP2 variable region of the attenuated vaccine backbone with that of virulent strains. In doing so, the present invention provides protection against the virulent IBDV strain

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while providing the safety and efficacy of the vaccine strain. Furthermore, using this approach, the present invention constructs and tests temperature sensitive IBD viruses generated using the RNA polymerase derived from the related birnavirus infectious pancreatic necrosis virus (IPNV) and the polyprotein derived from IBDV. The IPNV polymerase has optimum activity at a temperature lower than that of IBDV. If the IPNV polymerase recognizes the regulatory signals present on IBDV, the hybrid virus is expected to be attenuated at the elevated temperature present in chickens. Alternatively, it is possible to construct and test IBD viruses generated using the RNA polymerase derived from IBDV serotype 2 virus and the polyprotein derived from IBDVserotype 1 virus..

cDNA clones representing the complete genome of IBDV (double stranded RNA segments A and B) is constructed, initially using the BursaVac vaccine strain (Sterwin Labs). Once cDNA clones representing full length copies of segment A and B are constructed, template RNA is prepared. Since IBDV exists as a bisegmented double-stranded RNA virus, both the sense and anti-sense RNA strands of each segment are produced using the pBlueScript plasmid; Stratagene, Inc.). These vectors utilize the highly specific phage promoters SP6 or T7 to produce substrate amounts of RNA *in vitro*. A unique restriction endonuclease site is engineered into the 3' PCR primer to linearize the DNA for the generation of run-off transcripts during transcription.

The purified RNA transcripts (4 strands) are transfected into chick embryo fibroblasts (CEF) cells to determine whether the RNA is infectious. If IBD virus is generated, as determined by black plaque assays using IBDV specific Mabs, no further manipulations are required and engineering of the

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vaccine strain can commence. The advantage of this method is that engineered IBD viruses generated in this manner will be pure and require little/no purification, greatly decreasing the time required to generate new vaccines. If negative results are obtained using the purified RNA's, functional viral RNA polymerase is required by use of a helper virus. Birnaviruses replicate their nucleic acid by a strand displacement (semi-conservative) mechanism, with the RNA polymerase binding to the ends of the double-stranded RNA molecules forming circularized ring structures (Muller & Nitschke, Virology 159, 174-177, 1987). RNA polymerase open reading frame of about 878 amino acids in swinepox virus is expressed and this recombinant virus (S-SPV-044) is used to provide functional IBDV RNA polymerase *in trans*. Swinepox virus expressed immunologically recognizable foreign antigens in avian cells (CEF cells), where there are no signs of productive replication of the viral vector. In the present invention the IBDV polymerase protein is expressed in the same cells as the transfected RNA using the swinepox vector without contaminating the cells with SPV replication.

With the demonstration that IBD virus is generated *in vitro* using genomic RNA, an improved live attenuated virus vaccines against infectious bursal disease is developed. Using recombinant DNA technology along with the newly defined system of generating IBD virus, specific deletions within the viral genome, facilitating the construction of attenuated viruses are made. Using this technology, the region of IBDV responsible for virulence and generate attenuated, immunogenic IBDV vaccines are identified. The present invention provides a virulent IBD strain or replacement of the VP2 variable region of the attenuated vaccine backbone with that of a virulent strain, thus

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protecting against the virulent strain while providing the safety and efficacy of the vaccine strain.

5 Example 36

Effects of Rabbit anti-chicken interferon (cIFN) antibody on the growth of Herpes Virus of Turkeys.

10 Supernatants from SPV/cIFN (SPV 042) infected ESK-4 cells were harvested 48 hours after infection and then concentrated 5-10 times, by Centricon* 10 columns (Amicon). One ml of concentrated supernatant was
15 injected into a rabbit 3 times, at 3 week intervals, and then bled. This rabbit antisera was then used in culture to study the effect of interferon on the growth of HVT. It was shown that anti-cIFN reverses the block to HVT (1:200) and VSV(1:80) growth induced by the addition of cIFN in plaque assays. Furthermore,
20 it was shown that the addition of anti-cIFN (1:100) in the media of CEFs transiently transfected with sub-plaqueing levels of HVT viral DNA, enhances the formation of HVT plaques (200 plaques/well). CEFs transfected with HVT DNA in the absence of anti-cIFN
25 did not yield plaques.

HVT is highly susceptible to interferon produced from CEFs and that when cIFN is blocked, HVT growth is enhanced.

30 Applications include: (1) Use antibody to cIFN as an additive to increase HVT titers in vaccine stocks; (2) Use antibody to cIFN as an additive to facilitate the formation of new recombinant HVT viruses via cosmid
35 reconstructions.

* Trade-mark

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S-SPV-063

S-SPV-063 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for swine influenza virus (SIV) NP (H1N1) were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the SIV NP gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-063 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 807-41.3 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 063. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-063 was assayed for expression of SIV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-SIV serum or a polyclonal goat anti-NP serum was shown to react specifically with S-SPV-063 plaques and not with S-SPV-001 negative control plaques. All S-SPV-063 observed plaques reacted with the swine anti-SIV serum or goat anti-NP serum indicating that the virus was stably expressing the SIV foreign gene. The assays

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described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

5

To confirm the expression of the SIV NP gene products, cells were infected with S-SPV-063 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis, 10 The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-SIV serum or a polyclonal goat anti-NP serum was used to detect expression of SIV specific proteins. The cell lysate and supernatant from S-SPV-063 infected cells exhibited 15 bands corresponding to 56 kd, which is the expected size of the SIV NP protein.

S-SPV-063 is useful as a vaccine in swine against SIV infection and is useful for expression of SIV NP. S- 20 SPV-063 is useful as a vaccine in combination with S-SPV-066 which expresses NA and S-SPV-065 which expresses SIV HA.

S-SPV-064

25

S-SPV-064 is a swinepox virus that expresses one foreign gene. The gene for E. coli β -glucuronidase (uidA) was inserted into the unique XhoI restriction site within the 6.9 kb SPV HindIII J genomic fragment. 30 The uidA gene is under the control of the synthetic late/early promoter (LP2EP2). The HindIII J genomic fragment contains part of the A50R ORF (aa 227 to 552). The unique XhoI site is not within the A50R ORF. The XhoI site is 25 kb from the 3'end of the swinepox virus 35 genome (62).

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S-SPV-064 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 807-42.28 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV.

5 Homology vector 807-42.28 was generated by insertion of a NotI fragment containing the LP2EP2 promoter uidA gene cassette from plasmid 551-47.23 (see Materials and Methods) into a NotI site (unique XhoI site converted to NotI by a DNA linker) in the SPV 6.9 kb HindIII J

10 fragment. The transfection stock was screened by the SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATICAL MARKER GENES. The final result of blue plaque purification is the recombinant virus designated S-SPV-064. This virus is assayed for β -glucuronidase

15 expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, plaques observed are blue indicating that the virus is pure, stable, and

20 expressing the foreign gene.

S-SPV-064 is purified and expresses the foreign gene, E. coli uidA, indicating that the XhoI site within the 6.9 kb HindIII J fragment is a site non-essential for

25 virus growth and a stable insertion site for foreign genes. Recombinant swinepox virus utilizing this insertion site is useful for expression of foreign antigen genes, as a vaccine against disease or as an expression vector to raise antibodies to the expressed

30 foreign gene.

S-SPV-065

S-SPV-065 is a swinepox virus that expresses at least

35 two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for swine influenza virus (SIV) HA (H1N1) were inserted into the SPV 617 48.1 ORF (a

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unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the SIV HA gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-065 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 807-84.8 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 065. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene. S-SPV-065 was assayed for expression of SIV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-SIV serum or a Polyclonal goat anti-HA serum was shown to react specifically with S-SPV-065 plaques and not with S-SPV-001 negative control plaques. All S-SPV-065 observed plaques reacted with the swine anti-SIV serum or the SIV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the SIV NP gene products, cells were infected with S-SPV-065 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis.

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The gel was blotted and analyzed using the WESTERN
BLOTTING PROCEDURE. A Polyclonal swine anti-SIV serum
or a Polyclonal goat anti-HA serum was used to detect
expression SIV specific proteins. The cell lysate and
5 supernatant from S-SPV-065 infected cells exhibited
bands corresponding to 64 kd, which is the expected
size of the SIV-HA protein.

S-SPV-065 is useful as a vaccine in swine against SIV
10 infection and is useful for expression of SIV HA. S-
SPV-065 is useful as a vaccine in combination with S-
SPV-066 which expresses NA and S-SPV-063 which
expresses SIV NP.

15 S-SPV-066

S-SPV-066 is a swinepox virus that expresses at least
two foreign genes. The gene for E. coli β -galactosidase
(lacZ) and the gene for swine influenza virus (SIV) NA
20 (H1N1) were inserted into the SPV 617--48.1 ORF (a
unique NotI restriction site has replaced a unique AccI
restriction site). The lacZ gene is under the control
of the synthetic late promoter (LP1), and the SIV NA
gene is under the control of the synthetic late/early
25 promoter (LP2EP2).

S-SPV-066 was derived from S-SPV-001 (Kasza Strain).
This was accomplished utilizing the homology vector
807-84.35 (see Materials and Methods) and virus S-SPV-
30 001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
GENERATING RECOMBINANT SPV. The transfection stock was
screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING
 β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final
result of red plaque purification was the recombinant
35 virus designated S-SPV 066. This virus was assayed for
 β -galactosidase expression, purity, and insert
stability by multiple passages monitored by the blue

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plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

5

To confirm the expression of the SIV NA gene products, cells were infected with S-SPV-066 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A Polyclonal swine anti-SIV serum or a Polyclonal goat anti-NA serum was used to detect expression of SIV specific proteins. The cell lysate and supernatant from S-SPV-066 infected cells exhibited bands corresponding to 64 kd, which is the expected size of the SIV HA protein.

S-SPV-066 is useful as a vaccine in swine against SIV infection and is useful for expression of SIV-NA. S-SPV-066 is useful as a vaccine in combination with S-SPV-065 which expresses HA and S-SPV-063 which expresses SIV NP.

S-SPV-071

25

S-SPV-071 is a swinepox virus that expresses at least four foreign genes. The gene for E. coli β -galactosidase (lacZ) and the genes for swine influenza virus (SIV) HA (H1N1) and NA (H1N1) were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the SIV HA, and NA genes are under the control of the synthetic late/early promoter (LP2EP2).

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S-SPV-071 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 817-86.35 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
5 GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 071. This virus was assayed for
10 β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus
15 was pure, stable, and expressing the foreign gene.

S-SPV-071 was assayed for expression of SIV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal goat anti-HA
20 serum was shown to react specifically with S-SPV-071 plaques and not with S-SPV-001 negative control plaques. All S-SPV-071 observed plaques reacted with the goat anti-HA serum indicating that the virus was stably expressing the SIV foreign gene. The assays
25 described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

30 To confirm the expression of the SIV HA and NA gene products, cells were infected with S-SPV-071 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed
35 using the WESTERN BLOTTING PROCEDURE. A Polyclonal swine anti-SIV serum or a Polyclonal goat anti-HA serum

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was used to detect expression of SIV specific proteins. The cell lysate and supernatant from S-SPV-071 infected cells exhibited bands corresponding to 64 kd and 52 kd, which is the expected size of the SIV HA and NA protein.

S-SPV-071 is useful as a vaccine in swine against SIV infection and is useful for expression of SIV-HA and NA. S-SPV-071 is useful as a vaccine in combination with S-SPV-063 which expresses SIV NP.

S-SPV-074

S-SPV-074 is a swinepox virus that expresses at least four foreign genes. The gene for E. coli β -glucuronidase (uidA) and the genes for swine influenza virus (SIV) HA (H1N1) and NA (H1N1) were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The uidA gene is under the control of the synthetic late/early promoter (LP2EP2), and the SIV HA and NA genes are under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-074 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 817.14.2 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 074. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue

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plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

5

S-SPV-074 was assayed for expression of SIV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-SIV serum was shown to react specifically with S-SPV-074 plaques and not with S-SPV-001 negative control plaques. All S-SPV-074 observed plaques reacted with the goat anti-HA serum indicating that the virus was stably expressing the SIV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-074 is useful as a vaccine in a swine against SIV infection and is useful for expression of SIV HA and NA. S-SPV-074 is useful as a vaccine in combination with S-SPV-063 which expresses SIV NP. S-SPV-063, -065, -066, -071, and -074, are useful alone or in combination as a vaccine in swine against swine influenza infection and are useful for expression of the SIV NP, HA, and NA proteins.

S-SPV-068:

S-SPV-068 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and the gene for chicken macrophage migration inhibitory factor (cMIF) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV 01L ORF; Deletion of nucleotides 1679 to 2452, SEQ ID NO: 189).

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The lacZ gene is under the control of the swinepox OIL promoter, and the cMIF gene is under the control of the synthetic late/early promoter (LP2EP2).

5 S-SPV-068 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 802-95.A1 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
10 GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-068. This virus was assayed for β -galactosidase expression, purity, and insert
15 stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

20 To confirm the expression of the cMIF gene product, cells were infected with S-SPV-068 and samples of infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted
25 and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal goat anti-human cMIF antibody was used to detect expression of cMIF specific proteins. The cell lysate from S-SPV-068 infected cells exhibited a band corresponding to approximately 15 kd, which is the
30 expected size of the cMIF protein.

S-SPV-068 is useful as a vaccine in chickens to inhibit migration of macrophages and to stimulate an immune response against infection by avian pathogens. S-SPV-
35 068 is useful for expression of cMIF.

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HOMOLOGY VECTOR 802-95.A1. The plasmid 802-95.A1 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli* β -galactosidase (lac Z) marker gene and an chicken macrophage migration inhibitory factor (cMIF) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a swinepox virus 01L gene promoter and the cMIF gene is under the control of the late/early promoter (LP2EP2). The LP2EP2 cMIF gene cassette was inserted into a BamHI site of homology vector 752-22.1. Homology vector 802-95.A1 was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5' GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with SphI and BglII ends. Fragment 2 is a 3002 base pair BamHI to PvuII fragment derived from plasmid pJF751 (49) containing the *E. coli* lacZ gene. Fragment 3 is an approximately 363 base pair BglII fragment coding for the cMIF gene (63) derived by reverse transcription and polymerase chain reaction

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(PCR) (Sambrook, et al., 1989) of RNA ISOLATED FROM CONCANAVALLIN A STIMULATED CHICKEN SPLEEN CELLS. The antisense primer used for reverse transcription and PCR was 5' TCGAAGATCTTCTCATGCAAAGGTGGAACCGTTC -3' (6/95.28; SEQ ID NO: 58). The sense primer used for PCR was 5' TCGAAGATCTCATGCCTATGTTTACCATCCACAC -3' (6/95.27; SEQ ID NO: 59). The DNA fragment contains the open reading frame of 121 amino acids of the chicken macrophage migration inhibitory factor protein. The native methionine codon of cMIF is preceded by amino acid codons for met-asn-ser-asp-lys. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' (and 5' GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

S-SPV-069

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S-SPV-069 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for human respiratory syncytial virus (HRSV) fusion (F) protein were inserted into the SPV 738-94.4 ORF (a 773 base pair deletion of the SPV OIL ORF; Deletion of nucleotides 1669 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox P_{OIL} promoter and the HRSV F gene is under the control of the synthetic late/early promoter (LP2EP2).

30 S-SPV-069 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 810-29.A2 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was

35 screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING

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5 β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 069. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

10

S-SPV-069 was assayed for expression of HRSV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Monoclonal antibody 621 (Biodesign, Inc.) against HRSV F was shown to react specifically with S-SPV-069 plaques and not with S-SPV-001 negative control plaques. All S-SPV-069 observed plaques reacted with the monoclonal antibody 621 indicating that the virus was stably expressing the PRV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

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S-SPV-078

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S-SPV-078 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for human respiratory syncytial virus (HRSV) attachment (G) protein were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late/early promoter (LP2EP2), and the HRSV G gene is under the control of the synthetic late/early promoter (LP2EP2).

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S-SPV-078 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 822-52G.7 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
5 GENERATING RECOMBINANT SPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification is the recombinant virus designated S-SPV-078. This virus is assayed for
10 β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is
15 pure, stable, and expressing the foreign gene.

S-SPV-069 and S-SPV-078 are useful individually or in combination as a vaccine in swine against human respiratory syncytial virus infection and are useful
20 for expression of HRSV F and G genes.

HOMOLOGY VECTOR 810-29.A2. The plasmid 810-29.A2 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase
25 (lac Z) marker gene and a human respiratory syncytial virus (HRSV) fusion (F) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair
30 fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the
35 control of a swinepox virus 01L gene promoter and the

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HRSV F gene is under the control of the late/early promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5' GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with SphI and BglII ends. Fragment 2 is a 3002 base pair BamHI to PvuII fragment derived from plasmid pJF751 (49) containing the E. coli lacZ gene. Fragment 3 is an approximately 1728 base pair EcoRI restriction fragment synthesized by reverse transcriptase and polymerase chain reaction (PCR) (15, 42) using RNA from the HRSV Strain A2 (ATCC VR-1302). The primer (5' GCCGAATTCGCTAATCCTCAAAGCAAATGCAAT-3'; 4/95.23) synthesizes from the 5' end of the HRSV F gene, introduces an EcoRI site at the 5' end of the gene and an ATG start codon. The primer (5'-GGTGAATTCTTTATTAGTTACTAAATGCAATATTATTT-3'; 4/95.24) synthesizes from the 3' end of the HRSV F gene and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 1728 base pairs in length corresponding to the HRSV F gene. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5'

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GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

HOMOLOGY VECTOR 822-52G.7. The plasmid 822-52G.7 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli* β -galactosidase (lacZ) marker gene and the human respiratory syncytial virus (HRSV) attachment (G) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a synthetic late/early pox promoter (LP2EP2) and the HRSV G gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 1484 base pair AccI to BglII restriction sub-fragment of the SPV HindIII fragment M (23). Fragment 2 is an approximately 3006 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 899 base pair EcoRI restriction fragment synthesized by reverse transcriptase and polymerase chain reaction (PCR) (15, 42) using RNA from the HRSV Strain A2 (ATCC VR-1302). The primer (5' GCCGAATTCCAAAAACAAGGACCAACGCAC-3'; 4/95.25) synthesizes from the 5' end of the HRSV F gene, introduces an EcoRI site at the 5' end of the

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gene and an ATG start codon. The primer (5'-GCCGAATTCACTACTGGCGTGGTGTGTTG-3'; 4/95.26) synthesizes from the 3' end of the HRSV G gene and was used for reverse transcription and polymerase chain reaction.

5 The PCR product was digested with EcoRI to yield a fragment 899 base pairs in length corresponding to the HRSV G gene. Fragment 4 is an approximately 2149 base pair HindIII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23).

10

HOMOLOGY VECTOR 807-41.3. The plasmid 807-41.3 was used to insert foreign DNA into SPV. It incorporates an E. coli B-galactosidase (lacZ) marker gene and the swine influenza virus (SIV) nucleoprotein (NP) gene flanked

15 by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the B galactosidase (lacZ) marker gene is under the control of a synthetic

20 late pox promoter (LP1) and the SIV NP gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following

25 sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair Hindiii to Bam HI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base BglII to AccI restriction sub-fragment of the SPV

30 HindIII fragment M(23). Fragment 2 is an approximately 1501 base pair EcoRI to EcoRI fragment of the SIV NP gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the SIV H1N1 strain (NVSL). The primer

35 (5'CATGAATTCTCAAGGCACCAAACGATCATATGAAC-3'; 6/95.13) synthesizes from the 5' end of the SIV NP gene and introduces an EcoRI site at the 5'-

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ATTTGAATTCAATTGTCATACTCCTCTCGCATTGTCT-3';6/95.14)
synthesizes from the 3' end of the SIV NP gene,
introduces an EcoRI site at the 3' end of the gene, and
was used for reverse transcription and polymerase chain
5 reaction. The PCR product was digested with EcoRI to
yield a fragment 1501 base pairs in length
corresponding to the SIV NP gene. Fragment 3 is
approximately 3010 base pair BamHI to PuvII restriction
fragment of plasmid pJF751 (11). Fragment 4 is
10 approximately 2149 base pair AccI to HindIII
restriction sub-fragment of the SPV Hind III
restriction fragment M (23)

HOMOLOGY VECTOR 807-84.8. The plasmid 807-84.8 was used
15 to insert foreign DNA into SPV. It incorporates an E.
coli B-galactosidase (lacZ) marker gene and the swine
influenza virus (SIV) hemmagglutinin (HA) gene flanked
by SPV DNA. When this plasmid was used according to the
HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING
20 RECOMBINANT SPV a virus containing DNA coding for the
foreign genes results. Note that the B-galactosidase
(lacZ) marker gene is under the control of a synthetic
late pox promoter (LP1) and the SIV HA gene is under
the control of a synthetic late/early promoter
25 (LP2EP2). The homology vector was constructed utilizing
standard recombinant DNA techniques (22 and 30), by
joining restricting fragments from the following
sources with the appropriate synthetic DNA sequences.
The plasmid vector was derived from an approximately
30 2972 base pair HindIII to BamHI restriction fragment of
pSP64 (Promega). Fragment 1 is an approximately 1484
base pair BglII to AccI restriction sub-fragment of the
SPV HindIII fragment M(23). Fragment 2 is an
approximately 1721 base pair BamHI to BamHI fragment of
35 the SIV HA gene synthesized by reverse transcription
(RT) and polymerase chain reaction (PCR) (15,42) using
RNA from the SIV H1N1 strain (NVSL). The primer

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(5' CCGAGGATCCGGCAATACTATTAGTCTTGCTATGTACAT-3'; 6/95.5) synthesizes from the 5' end of the SIV HA gene and introduces an BamHI site at the 5' end of the gene. The primer (5'- CTCTGGATCCTAATTAAATACATATTCTGCACTGTS-3'; 6/95.6) synthesizes from the 3' end of the SIV HA gene, introduces a Bam HI site at the 3' end of the gene, and was used for the reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 1721 base pairs in length corresponding to the SIV HA gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to fragment M (23).

HOMOLOGY VECTOR 807-84.35. The plasmid 807-84.35 was used to insert foreign DNA into SPV. It incorporates an E. coli B-galactosidase (lacZ) marker gene and the swine influenza virus (SIV) neuraminidase (NA) gene flanked by SPV DNA. When this PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the B-galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the SIV NA gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30) by joining restricting fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII fragment M (23). Fragment 2 is an approximately 1414 base pair EcoRI to BglII fragment of the SIV NA gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the SIV H1N1 strain (NVSL). The primer (5'

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AATGAATTCAAATCAAAAAATAATAACCATTTGGGTCAAT-3'; 6.95.12) synthesizes from the 3' end of the SIV NA gene, introduces an EcoRI site at the 5' end of the gene. The primer (5'-GGAAGATCTACTTGTCAATGGTGAATGGCAGATCAG-3'; 6/95.13) synthesizes from the 3' end of the SIV NA gene, introduces an BglIII site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 1414 base pairs in length corresponding to the SIV NA gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23).

HOMOLOGY VECTOR 807-86.35. The plasmid 807-86.35 was used to insert foreign DNA into SPV. It incorporates an E. coli B-galactosidase (lacZ) marker gene and the swine influenza virus (SIV) HEMAGGLUTININ (HA) and neuraminidase (NA) gene flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the B-galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the SIV NA and HA genes are each under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is approximately 1484 base pair BglIII to AccI restriction sub-fragment of the SPV HindIII fragment M (23).

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Fragment 2 is an approximately 1721 base pair BamHI to BamHI fragment for the SIV HA gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the SIV H1N1 strain (NVSL). The primer (5' - CCGAGGATCCGGCAATACTATTAGTCTTGCTATGTACAT-3'; 6/95.5 synthesizes from the 5' end of the SIV HA gene and introduces an Bam HI site at the 5' end of the gene. The primer (5' - CTCTGGGATCCTAATTTTAAATACATATTCTGCACTGTA-3'; 6/95.6) synthesizes from the 3' end of the SIV HA gene, introduces an BamHI site at the 3' end of the gene, introduces an BamHI site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 1721 base pairs in length corresponding to the SIV HA gene. Fragment 3 is an approximately 1414 base pair EcoRI to BglIII fragment of the SIV NA gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the SIV H1N1 strain (NVSL). The primer (5' - AATGAATTCAAATCAAAAAATAATAACCATTGGGTCAAT-3'; 6/95.12) synthesizes from the 5' end of the SIV NA gene and introduces an EcoRI site at the 5' end of the gene. The primer (5' - GGAAGATCTACTTGTCAATGGTGAATGGCAGATCAG-3'; 6/95.13) synthesizes from the 3' end of the SIV NA gene, introduces an BglIII site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 1414 base pairs in length corresponding to the SIV NA gene. Fragment 4 is an approximately 2149 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23).

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HOMOLOGY VECTOR 817-14.2. The plasmid 817-14.2 was used to insert foreign DNA into SPV. It incorporates an E.

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coli B-galactosidase (lacZ) marker gene and the swine influenza virus (SIV) HEMAGGLUTININ (HA) and neuraminidase (NA) gene flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the B-galactosidase (uidA) marker gene is under the control of a synthetic late/early pox promoter (LP2EP2) and the SIV NA and HA genes are each under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction subfragment to the SPV HindIII fragment M (23). Fragment 2 is an approximately 1721 base pair BamHI to BamHI fragment of the SIV HA gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the SIV H1N1 strain (NVSL). The primer (5' CCGAGGATCCGGCAATACTATTAGTCTTGCTATGTACAT-3'; 6/95.5) synthesizes from the 5' end of the SIV HA gene and introduces an BamHI site at the 5' end of the gene. The primer (5'-CTCTGGGATCCTAATTTTAAATACATATTCTGCACTGTA-3'; 6/95.6) synthesizes from the 3' end of the SIV HA gene, introduces an BamHI site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 1721 base pairs in length corresponding to the SIV HA gene. Fragment 3 is an approximately 1414 base pair EcoRI to BglII fragment of the SIV HA gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using

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RNA for the SIV H1N1 strain (NVSL). The primer (5' AATGAATTCAAATCAAAAAATAATAACATTGGGTCAAT-3'; 6/95.12) synthesizes from the 5' end of the SIV NA gene, introduces an EcoRI site at the 5' end of the gene. The
5 primer (5'-GGAAGATCTACTTGTCAATGGTGAATGGCAGATCAG-3'; 6/95.13) synthesizes from the 3' end of the SIV NA gene, introduces an BglII site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested
10 with EcoRI to yield a fragment 1414 base pairs in length corresponding to the SIV NA gene. Fragment 4 is an approximately 1823 base pair NotI restriction fragment of plasmid pRAJ260 (Clonetech). Fragment 5 is an approximately 2149 base pair AccI to HindIII
15 restriction sub-fragment of the SPV HindIII restriction fragment M (23).

PRRS HOMOLGY VECTORS CONTAINING SINGLE OR MULTIPLE
PRRS GENES (ORF2, ORF3, ORF4, ORF5, ORF6 or ORF7: The
20 PRRS homology vector is constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli B-galactosidase (lacZ) marker gene and a porcine reproductive and respiratory syndrome virus (PRRS) ORF2, ORF3, ORF4, ORF5, ORF6 or ORF7 gene flanked by
25 SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION
30 PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the B-galactosidase (lacZ) marker gene is under the control of a swinepox virus 01L gene promoter and the PRRS gene is under the control of the
35 late/early promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA sequences. The plasmid vector was derived from an

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approximately 2519 base pair HINDIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV polymerase chain reaction using DNA primers 5' GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with SphI and BglII ends. Fragment 2 is a 3002 base pair BamHI to PvuII fragment derived from plasmid pJF751 (49) containing the E. coli lacZ gene. Fragment 3 is an EcoRI to BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the NVSL (Reference strain). Each homology vector contains one or multiple of the PRRS virus ORF2 through 7. To synthesize PRRS ORF2, the primer (5' AATGAATTCGAAATGGGTCCATGCAAAGCCTTTTGTG-3'; 1/96.15) synthesizes from the 5' end of the PRRS ORF2 gene, introduces an EcoRI site at the 5' end of the gene. The primer (5'- CAAGGATCCCACACCGTGTAATTCAGTGTGAGTTCG-3'; 1/96.16) is used for reverse transcription and PCR and synthesizes from the 3' end of the PRRS ORF2 gene. The PCR product was digested with EcoRI and BamHI to yield a fragment 771 base pairs in length corresponding to the PRRS ORF2 gene. To synthesize PRRS ORF3, the primer (5' TTCGAATTCGGCTAATAGCTGTACATTCCTCCATATTT-3'; 1/96.7) synthesizes from the 5' end of the PRRS ORF3 gene, introduces an EcoRI site at the 5' end of the gene. The primer (5'- GGGGATCCTATCGCCGTACGGCACTGAGGG-3'; 1/96.8) is used for reverse transcription and PCR and synthesizes from the 3' end of the PRRS ORF3 gene. To synthesize PRRS ORF4, the primer (5' CCGAATTCGGCTGCGTCCCTTCTTTTCCTCATGG-3'; 1/96.11) synthesizes from the 5' end of the PRRS ORF4 gene, introduces an EcoRI site at the 5'-CTGGATCCTTCAAATTGCCAACAGAATGGCAAAAAGAC-3'; 1/96.12) is used for reverse transcription and PCR and synthesizes

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from the 3' end of the PRRS ORF4 gene. The PCR product was digested with EcoRI and BamHI to yield a fragment 537 base pairs in length corresponding to the PRRS ORF4 gene. To synthesize PRRS ORF5, the primer (5' TTGAATTCGTTGGAGAAATGCTTGACCGCGGGC-3'; 1/96.13) synthesizes from the 5' end of the PRRS ORF5 gene, introduces an EcoRI site at the 5' end of the gene. The primer (5'- GAAGGATCCTAAGGACGACCCCATTTGTTCCGCTG-3'; 1/96.14) is used for reverse transcription and PCR and synthesizes from the 3' end of the PRRS ORF5 gene. The PCR product was digested with EcoRI and BamHI to yield a fragment 603 base pairs in length corresponding to the PRRS ORF5 gene. To synthesize PRRS ORF6, the primer (5' CGGGAATTCGGGGTCGTCCTTAGATGACTTCTGCC-3'; 1/96.17) synthesizes from the 5' end of the PRRS ORF6 gene, introduces an EcoRI site at the 5' end of the gene. The primer (5' - GCGGATCCTTGTTATGTGGCATATTTGACAAGGTTTAC-3'; 1/96.18) is used for reverse transcription and PCR and synthesizes from the 3' end of the PRRS ORF6 gene. The PCR product was digested with EcoRI and BamHI to yield a fragment 525 base pairs in length corresponding to the PRRS ORF6 gene. To synthesize PRRS ORF7, the primer (5' GTCGAATTCGCCAAATAACAACGGCAAGCAGCAGAAG-3'; 1/96.19) synthesizes from the 3' end of the PRRS ORF7 gene. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'- CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5' GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAT-3' to produce and 1113 base pair fragment with SalI and HindIII ends.

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Recombinant swinepox virus expressing pseudorabies genes

5 S-SPV-076 is a swinepox virus that expresses at least three foreign genes. The gene for E. coli B-galactosidase (lacZ) and the genes for pseudorabies virus (PRV) gD and gI were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under
10 the control of the synthetic late promoter (LP1), and the PRV gD and gI genes are under the control of the synthetic late/early promoter (LP2EP2).

15 S-SPV-077 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for pseudorabies virus (PRV) gI were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the
20 control of the synthetic late promoter (LP1), and the PRV gI gene is under the control of the synthetic late/early promoter (LP2EP2).

25 S-SPV-079 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for pseudorabies virus (PRV) gI were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the
30 control of the synthetic late promoter (LP1), and the PRV gB gene is under the control of the synthetic late/early promoter (LP2EP2).

35 S-SPV-076, S-SPV-077, S-SPV-079 have been tested by BLACK PLAQUE ASSAY and WESTERN BLOT for expression of the PRV glycoproteins.

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S-SPV-076, S-SPV-077, and S-SPV-079 were derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing a homology vector and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock were screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-076, S-SPV-077, and S-SPV-079. The viruses were assayed for B-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-076, S-SPV-077, and S-SPV-079 are useful as a vaccine in swine against PRV infection and is useful for expression of PRV gD, gI or gB. S-SPV-071 is useful as a vaccine in combination with a recombinant swinepox virus which expresses PRV gC, such as S-SPV-011, S-SPV-012 or S-SPV-013.

25	143B	carcinoma* osteosarcoma*
	A431	epidermoid carcinoma*
	A549	lung carcinoma*
	Capan-1	liver carcinoma*
	CF500	foreskin fibroblasts
30	Chang Liver	liver
	Detroit	Downs'foreskin fibroblasts
	HEL-199	embryonic lung
	HeLa	cervical carcinoma*
	Hep-2	epidermal larynx carcinoma
35	HISM	intestinal smooth muscle

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	HNK	neonatal kidney
	MRC-5	embryonic lung
	NCI-H292	pulmonary mucoepidermoid
	OVCAR-3	ovarian carcinoma*
5	RD	rhabdosarcoma*
	THP	monocyte (leukemia)*
	WIL2-NS	B lymphocyte line, non-secreting
	WISH	amnion
10	PBL	peripheral blood lymphocytes

15

20

Example 38

Recombinant swinepox virus expressing PRRS genes ORF2,
 25 ORF3, ORF4, ORF5 and ORF6

S-SPV-080 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for porcine reproductive and
 30 respiratory syndrome virus (PRRS) ORF2 were inserted into the SPV 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, (SEQ ID NO: 189). The lacZ gene is under the control of the swinepox P_{01L} promoter and the PRRS ORF2 gene is
 35 under the control of the synthetic late/early promoter (LP2EP2).

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S-SPV-081 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF3 were inserted
5 into the SPV 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, (SEQ ID NO: 189). The lacZ gene is under the control of the swinepox P_{o1L} promoter and the PRRS ORF3 gene is under the control of the synthetic late/early promoter
10 (LP2EP2).

S-SPV-082 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for porcine reproductive and
15 respiratory syndrome virus (PRRS) ORF4 were inserted into the SPV 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, (SEQ ID NO: 189). The lacZ gene is under the control of the swinepox P_{o1L} promoter and the PRRS ORF4 gene is
20 under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-083 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase
25 (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF5 were inserted into the SPV 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, (SEQ ID NO: 189). The lacZ gene is under the control of
30 the swinepox P_{o1L} promoter and the PRRS ORF5 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-084 is a swinepox virus that expresses at least
35 two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF6 were inserted

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into the SPV 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, (SEQ ID NO: 189). The lacZ gene is under the control of the swinepox P_{oil} promoter and the PRRS ORF6 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-085 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF7 were inserted into the SPV 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, (SEQ ID NO: 189). The lacZ gene is under the control of the swinepox P_{oil} promoter and the PRRS ORF7 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-080, S-SPV-081, S-SPV-082, S-SPV-083, S-SPV-084, S-SPV-085 were derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector Material and Methods (PRRS HOMOLOGY VECTORS) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-080, S-SPV-081, S-SPV-082, S-SPV-083, S-SPV-084, S-SPV-085. This virus was assayed for B-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

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S-SPV-080, S-SPV-081, S-SPV-082, S-SPV-083, S-SPV-084, S-SPV-085 are useful individually or in combination as vaccines in swine against PRRS infection and are useful for expression of PRRS ORF2, ORF3, ORF4, ORF5, ORF6 and ORF7.

Example 39

The following experiment was performed to determine the ability of swinepox virus to infect human cells in culture and express a foreign DNA as lacZ.

S-SPV-003 was absorbed to the human cell lines listed in the Table below at an MOI=0.1 for 2 to 3 hours. Cells were rinsed three times with PBS, growth media was added, and cells were incubated at 37°C for four days. Cells were harvested and a lysate prepared in 200 microliters of PBS by freeze/thaw three times. Cell debris was pelleted, and 10 microliters of supernatant was assayed for -galactosidase activity by ONPG assay at 37°C for 1 1/2 hours. The table shows the results of infection of various human cell lines with S-SPV-003 and the relative levels of cytopathetic effect and expression of lacZ.

The results show that various human cell lines vary in the ability to take up S-SPV-003 and express lacZ. CPE was minimal in all cases and did not result in viral replication. One exception A549 cells which did show some rounding of cells and lifting off the plate in one instance, and another instance of ten-fold increase in titer during passage suggesting limited viral replication. Several cell lines show significant lacZ activity with no cytopathetic effect.

Different pox promoters express lacZ from recombinant swinepox virus in a number of human cell lines. Six

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different swinepox viruses were constructed which expressed lacZ from EP1, LP1, LP2, EP1LP2, LP2EP2, or the SPV PO1L promoter. Viruses were each used to infect A549, Chang liver, or 143B cells at 0.1 moi, and
5 cells were rinsed between 2 and 3 hours later and then incubated for 4 days at 37°C. Each cell line maintained a different hierarchy of promoter activity, which was reproducible in following experiments.

10 For example, the EP1, LP2EP2, and PO1L promoters gave the most expression in 143B cells, while the LP2 was strongest in Chang liver cells, and the EP1LP2 in A549. In the Chang liver and A549 cells, expression from the PO1L promoter was poorest, whereas in 143B, expression
15 from LP2 was poorest. Therefore, different human cell lines utilize pox promoters in dissimilar ways. This may reflect how far the swinepox virus can proceed along the replication pathway in different cell lines.

20 These early and late promoters exhibited lower or higher lacZ activity depending on the human cell type infected by the recombinant swinepox virus. By choosing different promoters for different target tissues, one is able to regulate the amounts of foreign
25 gene product delivered by the swinepox virus to target tissues.

Recombinant swinepox virus is useful as a vaccine for human infectious disease and to deliver therapeutic
30 agents to humans. Recombinant swinepox virus is useful as a vaccine against viral or bacterial infection in humans, and as a therapeutic for cancer or genetic disease to deliver antibodies, tumor antigens, cell surface ligands and receptors, immune modulating
35 molecules such as cytokines

Example 40:

S-SPV-003 Expression of lacZ in human cell lines
Measurement of cytopathic effect and lacZ expression

	Cell Type	Cytopathetic Effect*	LacZ Expression**
5	A431 epidermoid carcinoma*	_____	_____
	A549 lung carcinoma*	++	+++
10	Capan-1 liver carcinoma*	_____	_____
	CF500 foreskin fibroblasts	+	+
	Chang Liver	+	+++
15			
20			
25	Detroit Down's foreskin fibroblasts	+/-	_____
	HEL-199 embryonic lung	+/-	+++
30	HEp-2 epidermal larynx carcinoma*	_____	_____
	HISM intestinal smooth muscle	+	+
	HNK neonatal kidney	_____	++
35	MRC-5 embryonic lung	+/-	+
	NCI-H292 pulmonary mucoepidermoid carcinoma*	_____	+++

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	OVCAR-3	_____	+++
	ovarian carcinoma*		
	RD	_____	+
	rhabdosarcoma*		
5	THP	_____	+
	monocyte (leukemia)*		
	WIL2-NS	_____	_____
	B lymphocyte line, non-secreting		
10	WISH	+/-	++
	amnion		
	HeLa	_____	+++
	PBL	_____	_____
15	peripheral blood lymphocytes		

* When human cells are infected with SPV, a cytopathic effect is sometimes seen. In most cell lines, this cytopathic effect is evidenced by a change in the appearance of the cells, with cells becoming thinner and more ragged along the edges; cells look stressed. This phenomenon was assessed as follows:

25 - indicates no difference between infected & uninfected cells;

+/- indicates that the monolayer is visibly different from uninfected, though most cells appear normal;

30 + indicates that the monolayer is obviously affected, with most cells looking stressed. It should be noted that in certain cell lines (HeLa, CF500, 143B), in which titers were obtained after serial passage, there was no evidence for replication of SPV, with one exception.

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A549 was given a ++ for cytopathic effect in one instance, when cells appeared to round up and come off the plate during infection, though this observation was not repeated. A549 also showed evidence in another case
5 of a ten-fold increase in titer during passage, suggesting that it might support limited viral replication.

** B-galactosidase activity in A_{260} units per cell
10 lysate from 1/20 of a 35 mm dish:
- No activity
+ 0.2-0.9 A_{260} unit
++ 0.9-1.6 A_{260} unit
+++ greater than 1.6 A_{260} units.

15

20

Example 41: BOVINE CONSTRUCTS AND VACCINES

S-SPV-112

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S-SPV-112 is a swinepox virus that expresses three foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for bovine respiratory syncytial virus (BRSV) attachment (G) were inserted into the
30 unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV O1L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The
35 gene for BRSV fusion (F) was inserted into the unique PstI restriction site (PstI linkers inserted into a

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unique AccI site in the SPV 01L open reading frame). The lacZ gene is under the control of the synthetic late promoter (LP1), the BRSV G and F genes are each under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-112 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 848-02 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 112. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-112 is useful as a vaccine in bovine against disease caused by bovine respiratory syncytial virus. The BRSV antigens are key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. The swinepox virus is useful for cloning other subtypes of BRSV to protect against rapidly evolving variants in this disease. S-SPV-112 is also useful as an expression vector for expressing BRSV antigens. Such BRSV antigens are useful to identify antibodies directed against the wild-type BRSV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such

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antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR
5 PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 848-02. The plasmid 848-02 was constructed for the purpose of inserting foreign DNA
10 into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene, the bovine respiratory syncytial virus (BRSV) attachment (G) and fusion (F) genes flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA.
15 Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will
20 result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the BRSV F and G genes are under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard
25 recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment
30 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 1722 base pair Bam HI fragment generated by PCR which contains the coding sequence of the BRSV F gene.
35 Fragment 3 is an approximately 48 base pair AccI to

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NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 771 base pair Bam HI fragment generated by PCR which contains the coding sequence for the BRSV G gene. The BRSV F and G genes were synthesized by PCR as described in the CLONING OF BOVINE RESPIRATORY SYNCYTIAL VIRUS FUSION, NUCLEOCAPSID AND GLYCOPROTEIN GENES. Fragment 5 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 6 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 6 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 6.

20

Recombinant swinepox virus expressing BRSV F and G fusion protein

S-SPV-130:

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S-SPV-130 is a swinepox virus that expresses three foreign genes. The gene E. coli β -galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The genes for bovine respiratory syncytial virus (BRSV) attachment (G) and BRSV fusion (F) are inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to

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2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the BRSV F/G fusion gene is under the control of the synthetic late/early promoter (LP2EP2). The BRSV F/G fusion gene comprises approximately 1560 nucleotides of the F gene (520 amino acids including the amino terminus) fused in frame to approximately 580 nucleotides of the G gene (193 amino acids including the carboxy terminus).

10

S-SPV-130 is derived from S-SPV-001 (Kasza Strain). This is accomplished utilizing the HOMOLOGY VECTOR 807-75.41 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The recombinant virus is isolated by red plaque purification. This virus is assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus was pure, stable, and expressing the foreign gene.

25

S-SPV-130 is useful as a vaccine in bovine against disease caused by bovine respiratory syncytial virus. The BRSV F/G fusion protein is particularly effective and key to raising a protective immune response in the animal. The BRSV F/G fusion protein contains the intact amino terminus of the F protein and the intact carboxy terminus of the G protein which includes the known immunogenic region of each protein. The BRSV F/G fusion protein provides an improved immune response compared

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to expressing the BRSV F and G proteins separately or
expressing isolated epitopes of the BRSV F and G
proteins. The swinepox virus is useful for cloning
other subtypes of BRSV to protect against rapidly
5 evolving variants in this disease. Recombinant swinepox
virus is also useful as an expression vector for
expressing BRSV antigens. Such BRSV antigens are useful
to identify antibodies directed against the wild-type
BRSV. The virus is also useful as a source of antigens
10 for the production of monospecific polyclonal or
monoclonal antibodies. Such antibodies are useful in
the development of diagnostic tests specific for the
viral proteins. Monoclonal or polyclonal antibodies are
generated in mice utilizing these viruses according to
15 the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS
FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 807-75.41. The homology vector 807-
75.41 was constructed for the purpose of inserting
20 foreign DNA into SPV. It incorporates an E. coli β -
galactosidase (lacZ) marker gene, a fusion protein of
the bovine respiratory syncytial virus (BRSV)
attachment (G) and fusion (F) genes flanked by SPV DNA.
Upstream of the foreign genes is an approximately 1484
25 base pair fragment of SPV DNA. Downstream of the
foreign genes is an approximately 1560 base pair
fragment of SPV DNA. When the plasmid is used according
to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
GENERATING RECOMBINANT SPV, a virus containing DNA
30 coding for the foreign genes will result. Note that the
 β galactosidase (lacZ) marker gene is under the control
of a synthetic late pox promoter (LP1), the BRSV F/G
fusion gene is under the control of a synthetic
late/early pox promoter (LP2EP2). It was constructed
35 utilizing standard recombinant DNA techniques (22, 30),

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by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector is derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 1560 base pair Bam HI fragment generated by PCR which contains the coding sequence of the BRSV F gene. The F gene coding region from the BRSV strain 375 (VR-1339) was cloned using the following primers: 5'-GCGGATCCGGCGCGCCGGATTTTCCTACATCTACACT-3' (5/96.26; SEQ ID NO 12) for cDNA priming and combined with 5'-CTAAAATTGAATTGTAAT-3' (1/95.19; SEQ ID NO 13) for PCR. The DNA encodes 520 amino acids at the amino terminus of the BRSV F protein. Fragment 5 is an approximately 580 base pair AscI fragment generated by PCR which contains the coding sequence for the BRSV G gene. The G gene coding region from the BRSV strain 375 (VR-1339) was cloned using the following primers: 5' TTGGCGCGCCCTAGATCTGTGTAGTTGATTGATTG-3' (5/96.28; SEQ ID NO 14) for cDNA priming and combined with 5' TACGGCGCGCCGGGAAATGCTAAAGCCAAGCCCACA-3' (5/96.27; SEQ ID NO 15) for PCR. The DNA product encodes 193 amino acids (including a translation stop codon) of the carboxy terminus of the BRSV G protein. The BRSV F and G coding sequences are fused in the correct translational reading frame. Fragment 6 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI

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sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 6 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 6.

10 S-SPV-099

S-SPV-099 is a swinepox virus that expresses two foreign genes. The gene E. coli β -galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for bovine viral diarrhea virus type 2 (BVDV-2) (strain 890) glycoprotein 53 (gp53) was inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the BVDV-2 gp53 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-099 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 815-73.16A (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant

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virus designated S-SPV 099. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-099 is useful as a vaccine in bovine against disease caused by bovine viral diarrhea virus. The BVDV-2 gp53 antigen is key to raising a protective immune response in the animal. The recombinant virus is useful alone or in combination as an effective vaccine. S-SPV-099 is also useful as an expression vector for expressing BVDV antigens. Such BVDV antigens are useful to identify antibodies directed against the wild-type BVDV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

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HOMOLOGY VECTOR 815-73.16A. The homology vector 815-73.16A was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and bovine viral diarrhea virus type 2 (BVDV-2) glycoprotein 53 (gp53) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION

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PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the BVDV gp53 gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 1113 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the BVDV gp53 gene. BVDV gp53 gene coding region was cloned by reverse transcription and PCR using RNA from BVDV type 2 (Strain 890) as an RNA template for reverse transcription and the following PCR using primers: 5'-TTCGGATCCTGCTCAGACAGTATTGTGTATGTTATCAAGAGC-3' (2/96.32; SEQ ID NO 16) at the 3' end of the BVDV gp53 gene for reverse transcription and PCR combined with 5'-CCATGAATTCCTTCCCTGAATGCAAGGAGGGCTTC-3' (2/96.15; SEQ ID NO 17) at the 5' end of the BVDV gp53 gene for PCR. The DNA encodes approximately 373 amino acids of the BVDV gp53 protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and

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3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment
5 (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

S-SPV-109

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S-SPV-109 is a swinepox virus that expresses two foreign genes. The gene E. coli β -galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L
15 open reading frame). The gene for infectious bovine rhinotracheitis virus (IBRV) glycoprotein D (gD) was inserted into the unique HindIII restriction site (HindIII linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545
20 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the IBRV gD gene is under the control of the synthetic late/early promoter
25 (LP2EP2).

S-SPV-109 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 835-57.5 (see Materials and Methods) and virus S-SPV-
30 001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant
35 virus designated S-SPV 109. This virus was assayed for

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5 β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

10 S-SPV-109 is useful as a vaccine in bovine against disease caused for infectious bovine rhinotracheitis virus. The IBRV gD antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-109 is also useful as an expression vector for expressing IBRV antigens. Such
15 IBRV antigens are useful to identify antibodies directed against the wild-type IBRV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic
20 tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

25

HOMOLOGY VECTOR 835-57.5. The homology vector 835-57.5 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the infectious
30 bovine rhinotracheitis virus (IBRV) glycoprotein D (gD) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is
35 used according to the HOMOLOGOUS RECOMBINATION

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PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the IBRV gD gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 1320 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the IBRV gD gene. The IBRV gD gene coding region was cloned by PCR using the HindIII K fragment of the IBRV Cooper strain (pSY 524) as DNA template and the following PCR primers: 5'-CGGGATCCTCACCCGGGCAGCGCGCTGTA-3' (4/96.12; SEQ ID NO 18) at the 3' end of the IBRV gD gene and combined with 5'-CGGAATTCACAAGGGCCGACATTGGCC-3' (4/96.11; SEQ ID NO 19) at the 5' end of the IBRV gD gene. The DNA encodes approximately 440 amino acids of the IBRV gD protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I

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sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

5

S-SPV-110

S-SPV-110 is a swinepox virus that expresses two foreign genes. The gene E. coli β -galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for infectious bovine rhinotracheitis virus (IBRV) glycoprotein I (gI) was inserted into the unique HindIII restriction site (HindIII linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the IBRV gI gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-110 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 835-58.5 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 110. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods.

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After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

5 S-SPV-110 is useful as a vaccine in bovine against
disease caused for infectious bovine rhinotracheitis
virus. The IBRV gI antigen is key to raising a
protective immune response in the animal. The
recombinant viruses are useful alone or in combination
10 as an effective vaccine. S-SPV-110 is also useful as an
expression vector for expressing IBRV antigens. Such
IBRV antigens are useful to identify antibodies
directed against the wild-type IBRV. The virus is also
useful as a source of antigens for the production of
15 monospecific polyclonal or monoclonal antibodies. Such
antibodies are useful in the development of diagnostic
tests specific for the viral proteins. Monoclonal or
polyclonal antibodies are generated in mice utilizing
these viruses according to the PROCEDURE FOR
20 PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS
DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 835-58.5. The homology vector 835-58.5
was constructed for the purpose of inserting foreign
25 DNA into SPV. It incorporates an E. coli β -
galactosidase (lacZ) marker gene and the infectious
bovine rhinotracheitis virus (IBRV) glycoprotein I (gI)
gene flanked by SPV DNA. Upstream of the foreign genes
is an approximately 1484 base pair fragment of SPV DNA.
30 Downstream of the foreign genes is an approximately
1560 base pair fragment of SPV DNA. When the plasmid is
used according to the HOMOLOGOUS RECOMBINATION
PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus
containing DNA coding for the foreign genes will
35 result. Note that the β galactosidase (lacZ) marker

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gene is under the control of a synthetic late pox promoter (LP1), the IBRV gI gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 1140 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the IBRV_gI gene. The IBRV gI gene coding region was cloned by PCR using the HindIII K fragment of the IBRV Cooper strain (pSY 524) as DNA template and the following PCR primers: 5'-ATCGGGATCCCGTTATTCTTCGCTGATGGTGG-3' (4/96.18; **SEQ ID NO 20**) at the 3' end of the IBRV gI gene and combined with 5'-ATCGGAATTCGCGGTGCCTGTTGCTCTGGATG-3' (4/96.17; **SEQ ID NO 21**) at the 5' end of the IBRV gI gene. The DNA encodes approximately 380 amino acids of the IBRV gI protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to

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2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

S-SPV-111

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S-SPV-111 is a swinepox virus that expresses two foreign genes. The gene E. coli β -galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for infectious bovine rhinotracheitis virus (IBRV) glycoprotein B (gB) was inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the IBRV gB gene is under the control of the synthetic late/early promoter (LP2EP2). The direction of transcription of the IBRV gB gene is opposite the direction of transcription of the lacZ gene and the SPV 01L gene.

S-SPV-111 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 847-15.1C (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 111. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods.

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After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

5 S-SPV-111 is useful as a vaccine in bovine against disease caused for infectious bovine rhinotracheitis virus. The IBRV gB antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination
10 as an effective vaccine. S-SPV-111 is also useful as an expression vector for expressing IBRV antigens. Such IBRV antigens are useful to identify antibodies directed against the wild-type IBRV. The virus is also useful as a source of antigens for the production of
15 monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR
20 PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 847-15.1C. The homology vector 847-15.1C was constructed for the purpose of inserting
25 foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the infectious bovine rhinotracheitis virus (IBRV) glycoprotein B (gB) gene flanked by SPV DNA. The direction of transcription of the IBRV gB gene is opposite the direction of
30 transcription of the lacZ gene and the SPV O1L gene. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according
35 to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR

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GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the IBRV gB gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglIII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 2800 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the IBRV gB gene. The IBRV gB gene coding region was cloned by PCR using the HindIII A fragment of the IBRV Cooper strain (pSY 830-71) as DNA template and the following PCR primers: 5' - CTTCGGATCCTCATGCCCCCGACGTCGGCCATC-3' (4/96.15; SEQ ID NO 22) at the 3' end of the IBRV gB gene and combined with 5'- TCATGAATTCTGGCCGCTCGCGGCGGTGCTGAACGC-3' (4/96.10; SEQ ID NO 23) at the 5' end of the IBRV gB gene. The DNA encodes approximately 932 amino acids of the IBRV gB protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I sites using NotI linkers. An

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approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

5

S-SPV-113

10 S-SPV-113 is a swinepox virus that expresses two foreign genes. The gene E. coli β -galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for infectious bovine rhinotracheitis virus (IBRV) glycoprotein C (gC) was
15 inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been
20 deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the IBRV gC gene is under the control of the synthetic late/early promoter (LP2EP2).

25 S-SPV-113 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 848-08 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was
30 screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 113. This virus was assayed for β -galactosidase expression, purity, and insert
35 stability by multiple passages monitored by the blue

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plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

5

S-SPV-113 is useful as a vaccine in bovine against disease caused by infectious bovine rhinotracheitis virus. The IBRV gC antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-113 is also useful as an expression vector for expressing IBRV antigens. Such IBRV antigens are useful to identify antibodies directed against the wild-type IBRV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 848-08. The homology vector 848-08 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the infectious bovine rhinotracheitis virus (IBRV) glycoprotein C (gC) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will

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result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the IBRV gC gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 1563 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the IBRV gC gene. The IBRV gC gene coding region was cloned by PCR using the HindIII I fragment of the IBRV Cooper strain (pSY 830-71) as DNA template and the following PCR primers: 5'-CGGGATCCCTAGGGCGCGGAGCCGAGGGC-3' (4/96.14; **SEQ ID NO 24**) at the 3' end of the IBRV gC gene and combined with 5'-CGGAATTCAGGCCCGCTGGGGCGAGCGTGG-3' (4/96.13; **SEQ ID NO 25**) at the 5' end of the IBRV gC gene. The DNA encodes approximately 521 amino acids of the IBRV gC protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to

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2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

5 S-SPV-115

S-SPV-115 is a swinepox virus that expresses two foreign genes. The gene E. coli β -galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV O1L open reading frame). The gene for infectious bovine rhinotracheitis virus (IBRV) glycoprotein B (gB) was inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV O1L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the IBRV gB gene is under the control of the synthetic late/early promoter (LP2EP2). The direction of transcription of the IBRV gB gene is the same as the direction of transcription of the lacZ gene and the SPV O1L gene.

25 S-SPV-115 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 847-19.59 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 115. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue

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plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

5

S-SPV-115 is useful as a vaccine in bovine against disease caused for infectious bovine rhinotracheitis virus. The IBRV gB antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-115 is also useful as an expression vector for expressing IBRV antigens. Such IBRV antigens are useful to identify antibodies directed against the wild-type IBRV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 847-19.59. The homology vector 847-19.59 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the infectious bovine rhinotracheitis virus (IBRV) glycoprotein B (gB) gene flanked by SPV DNA. The direction of transcription of the IBRV gB gene is the same as the direction of transcription of the lacZ gene and the SPV O1L gene. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according

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to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the IBRV gB gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 2800 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the IBRV gB gene. The IBRV gB gene coding region was cloned by PCR using the HindIII A fragment of the IBRV Cooper strain (pSY 830-71) as DNA template and the following PCR primers: 5' - CTTCGGATCCTCATGCCCCCGACGTCGGCCATC-3' (4/96.15; **SEQ ID NO 26**) at the 3' end of the IBRV gB gene and combined with 5' - TCATGAATTCGGCCGCTCGCGGCGGTGCTGAACGC-3' (4/96.10; **SEQ ID NO 27**) at the 5' end of the IBRV gB gene. The DNA encodes approximately 932 amino acids of the IBRV gB protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were

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converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span
5 SPV fragments 3 and 5.

S-SPV-119

10 S-SPV-119 is a swinepox virus that expresses three foreign genes. The gene E. coli β -galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The genes for infectious bovine
15 rhinotracheitis virus (IBRV) glycoprotein D (gD) and glycoprotein I (gI) were inserted into the unique HindIII restriction site (HindIII linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment
20 (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the IBRV gD and gI genes are each under the control of the synthetic late/early promoter (LP2EP2).

25

S-SPV-119 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 835-83 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
30 GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 119. This virus was assayed for
35 β -galactosidase expression, purity, and insert

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stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus was
5 pure, stable, and expressing the foreign gene.

S-SPV-119 is useful as a vaccine in bovine against disease caused for infectious bovine rhinotracheitis virus. The IBRV gD and gI antigens are key to raising
10 a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-119 is also useful as an expression vector for expressing IBRV antigens. Such IBRV antigens are useful to identify antibodies
15 directed against the wild-type IBRV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or
20 polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

25 **HOMOLOGY VECTOR 835-83.** The homology vector 835-83 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the infectious bovine rhinotracheitis virus (IBRV) glycoprotein D (gD) and
30 glycoprotein I (gI) genes flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS
35 RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV,

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a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the IBRV gI gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 1320 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the IBRV gD gene. The IBRV gD gene coding region was cloned by PCR using the HindIII K fragment of the IBRV Cooper strain (pSY 524) as DNA template and the following PCR primers: 5'-CGGGATCCTCACCCGGGCAGCGCGCTGTA-3' (4/96.12; **SEQ ID NO 18**) at the 3' end of the IBRV gD gene and combined with 5'-CGGAATTCACAAGGGCCGACATTGGCC-3' (4/96.11; **SEQ ID NO 19**) at the 5' end of the IBRV gD gene. The DNA encodes approximately 440 amino acids of the IBRV gD protein. Fragment 5 is an approximately 1140 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the IBRV gI gene. The IBRV gI gene coding region was cloned by PCR using the HindIII K fragment of the IBRV Cooper strain (pSY 524) as DNA template and the following PCR primers: 5'-

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ATCGGGATCCCGTTATTCTTCGCTGATGGTGG-3' (4/96.18; SEQ ID NO
20) at the 3' end of the IBRV gI gene and combined with
5'-ATCGGAATTCGCGGTGCCTGTTGCTCTGGATG-3' (4/96.17; SEQ ID
NO 21) at the 5' end of the IBRV gI gene. The DNA
5 encodes approximately 380 amino acids of the IBRV gI
protein. Fragment 6 is an approximately 1560 base pair
NdeI to HindIII subfragment of the SPV HindIII fragment
M. The AccI sites in fragments 1 and 3 were converted
to unique PstI sites using PstI linkers. The NdeI sites
10 in fragments 3 and 6 were converted to unique Not I
sites using NotI linkers. An approximately 545 base
pair NdeI to NdeI subfragment (Nucleotides 1560 to
2104; SEQ ID NO. 189) of the SPV HindIII M fragment has
been deleted which would span SPV fragments 3 and 6.

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Example 42: CANINE CONSTRUCTS AND VACCINESS-SPV-114

5 S-SPV-114 is a swinepox virus that expresses two foreign genes. The gene E. coli β -galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for canine parvovirus
10 (CPV) VP2 protein was inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV
15 HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the CPV VP2 gene is under the control of the synthetic late/early promoter (LP2EP2). The direction of transcription of the CPV VP2 gene is the same as the
20 direction of transcription of the lacZ gene and the SPV 01L gene.

S-SPV-114 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector
25 848-15.14 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final
30 result of red plaque purification was the recombinant virus designated S-SPV 114. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods.
35 After the initial three rounds of purification, all

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plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

5 S-SPV-114 was assayed for expression of CPV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Canine antiserum to CPV (from NVSL) was shown to react specifically with S-SPV-114 plaques and not with S-SPV-003 negative control plaques. All S-SPV-114 observed plaques reacted with
10 the antiserum indicating that the virus was stably expressing the CPV foreign gene.

To confirm the expression of the CPV VP2 gene product, cells were infected with S-SPV-114 and samples of
15 infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A canine antiserum to CPV (from NVSL) was used to detect expression of CPV specific proteins. The cell lysate
20 from S-SPV-114 infected cells exhibited bands corresponding to 60 kd, which are the expected size of the CPV VP2 protein.

S-SPV-114 is useful as a vaccine in canine against
25 disease caused by canine parvovirus. The CPV VP2 antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-114 is also useful as an expression vector for expressing CPV
30 antigens. Such CPV antigens are useful to identify antibodies directed against the wild-type CPV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the
35 development of diagnostic tests specific for the viral

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proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

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HOMOLOGY VECTOR 848-15.14. The homology vector 848-15.14 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and for canine parvovirus (CPV) VP2 protein gene flanked by SPV DNA. The direction of transcription of the CPV VP2 gene is the same as the direction of transcription of the lacZ gene and the SPV O1L gene. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the CPV VP2 gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII

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M fragment. Fragment 4 is an approximately 1758 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the CPV VP2 gene. The CPV VP2 gene coding region was cloned by PCR using DNA
5 from CPV 2B field isolate (NVSL) as DNA template and the following PCR primers: 5'-
CGGGATCCTTAATATAATTTTCTAGGTGCTAGTTG -3' (4/96.26; **SEQ ID NO 28**) at the 3' end of the CPV VP2 gene and combined with 5'- CGGAATTCGATGAGTGATGGAGCAGTTCAA -3'
10 (4/96.25; **SEQ ID NO 29**) at the 5' end of the CPV VP2 gene. The DNA encodes approximately 586 amino acids of the CPV VP2 protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and
15 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV
20 HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

S-SPV-116

25 S-SPV-116 is a swinepox virus that expresses two foreign genes. The gene E. coli β -galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for canine parvovirus
30 (CPV) VP2 protein was inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV
35 HindIII M fragment has been deleted). The lacZ gene is

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under the control of the synthetic late promoter (LP1), the CPV VP2 gene is under the control of the synthetic late/early promoter (LP2EP2). The direction of transcription of the CPV VP2 gene is opposite the direction of transcription of the lacZ gene and the SPV 01L gene.

S-SPV-116 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 848-15.13 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 116. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-116 was assayed for expression of CPV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Canine antiserum to CPV (from NVSL) was shown to react specifically with S-SPV-116 plaques and not with S-SPV-003 negative control plaques. All S-SPV-116 observed plaques reacted with the antiserum indicating that the virus was stably expressing the CPV foreign gene.

To confirm the expression of the CPV VP2 gene product, cells were infected with S-SPV-116 and samples of infected cell lysates were subjected to SDS

-199-

polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A canine antiserum to CPV (from NVSL) was used to detect expression of CPV specific proteins. The cell lysate
5 from S-SPV-116 infected cells exhibited bands corresponding to 60 kd, which are the expected size of the CPV VP2 protein.

S-SPV-116 is useful as a vaccine in canine against
10 disease caused by canine parvovirus. The CPV VP2 antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-116 is also useful as an expression vector for expressing CPV
15 antigens. Such CPV antigens are useful to identify antibodies directed against the wild-type CPV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the
20 development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

25

HOMOLOGY VECTOR 848-15.13. The homology vector 848-15.13 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and for canine
30 parvovirus (CPV) VP2 protein gene flanked by SPV DNA. The direction of transcription of the CPV VP2 gene is opposite the direction of transcription of the lacZ gene and the SPV O1L gene. Upstream of the foreign genes is an approximately 1484 base pair fragment of
35 SPV DNA. Downstream of the foreign genes is an

-200-

approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the CPV VP2 gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 1758 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the CPV VP2 gene. The CPV VP2 gene coding region was cloned by PCR using DNA from CPV 2B field isolate (NVSL) as DNA template and the following PCR primers: 5'-CGGGATCCTTAATATAATTTTCTAGGTGCTAGTTG -3' (4/96.26; **SEQ ID NO 30**) at the 3' end of the CPV VP2 gene and combined with 5'-CGGAATTCGATGAGTGATGGAGCAGTTCAA -3' (4/96.25; **SEQ ID NO 31**) at the 5' end of the CPV VP2 gene. The DNA encodes approximately 586 amino acids of the CPV VP2 protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI

DEMANDES OU BREVETS VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET
COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2

NOTE: Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

2760759

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE
THAN ONE VOLUME

THIS IS VOLUME 1 OF 2

NOTE: For additional volumes please contact the Canadian Patent Office

Claims:

1. A recombinant swinepox virus comprising both a) a first foreign DNA inserted into a swinepox virus genome, wherein the first foreign DNA is inserted into an EcoRI site within a region corresponding to a 3.2 kb subfragment of a HindIII K fragment which contains both a HindIII and an EcoRI site, wherein said 3.2 kb subfragment comprises the nucleotide sequence shown in SEQ ID NO:1, and b) a second foreign DNA inserted into a swinepox virus genome, wherein the second foreign DNA is inserted into an AclI site within a region corresponding to a 3.6 kb HindIII to BglII subfragment of a HindIII M fragment, and wherein both the first foreign DNA and the second foreign DNA are expressed in a host cell into which the virus is introduced.
2. The recombinant swinepox virus of claim 1, wherein the first foreign DNA encodes a polypeptide.
3. The recombinant swinepox virus of claim 1, wherein the second foreign DNA encodes a polypeptide.
4. The recombinant swinepox virus of claim 1, wherein the first foreign DNA encodes *E. coli* beta-galactosidase or *E. coli* beta-glucuronidase.
5. The recombinant swinepox virus of claim 1, wherein the second foreign DNA encodes *E. coli* beta-galactosidase or *E. coli* beta-glucuronidase.
6. The recombinant swinepox virus of claim 1, wherein the first foreign DNA is under control of a heterologous upstream promoter.
7. The recombinant swinepox virus of claim 1, wherein the second foreign DNA is under control of a heterologous upstream promoter.
8. The recombinant swinepox virus of claim 6 or 7, wherein the promoter is: synthetic pox viral promoter, pox synthetic late promoter 1, pox early promoter 2, pox synthetic late promoter 2, pox synthetic early promoter

- 2, pox O1L promoter, pox I4L promoter, pox I3L promoter, pox 12L promoter, pox I1L promoter, pox E1OR promoter, PRV gX, HSV-I alpha 4, internal ribosomal entry site, or HCMV immediate early.
9. The recombinant swinepox virus of claim 2 or 3, wherein the polypeptide is: swine influenza virus hemagglutinin, swine influenza virus neuraminidase, swine influenza virus matrix, or swine influenza virus nucleoprotein.
10. The recombinant swinepox virus of claim 1,

wherein said virus (S-SPV-120) comprises the gene for *E. coli* β -galactosidase (*lacZ*) and the gene for *E. coli* β -glucuronidase (*uidA*);
and

wherein said *lacZ* gene is inserted into said *AccI* site, and said *uidA* gene is inserted into a unique *NotI* site inserted into said *EcoRI* site of said 3.2 kb region.
11. The recombinant swinepox virus of claim 1, wherein the first foreign DNA encodes a cytokine.
12. The recombinant swinepox virus of claim 1, wherein the second foreign DNA encodes a cytokine.
13. The recombinant swinepox virus of claim 2 or 3. wherein the polypeptide is: swine influenza virus hemagglutinin, swine influenza virus neuraminidase, swine influenza virus matrix, swine influenza virus nucleoprotein, pseudorabies virus glycoprotein B, pseudorabies virus glycoprotein C, pseudorabies virus glycoprotein D, porcine respiratory and reproductive virus ORF2, porcine respiratory and reproductive virus ORF3, porcine respiratory and reproductive virus ORF4, porcine respiratory and reproductive virus ORF5, porcine respiratory and reproductive virus ORF6, or porcine respiratory and reproductive virus ORF7.

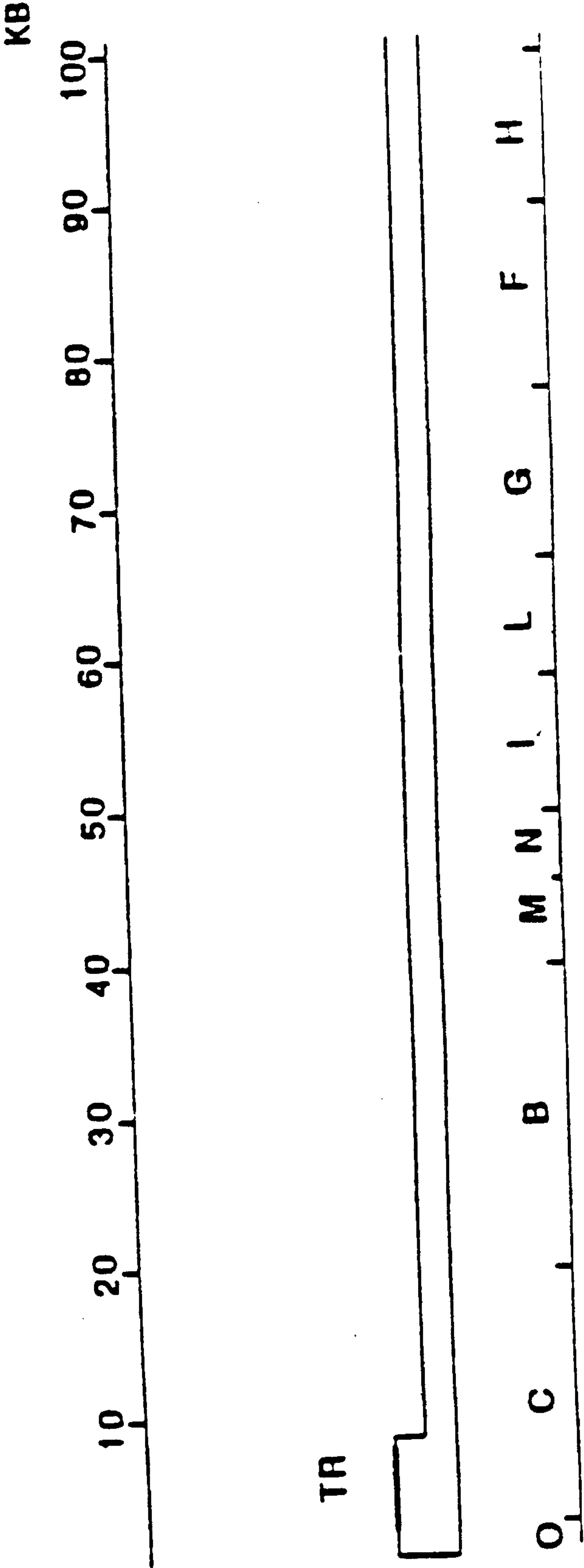
14. The recombinant swinepox virus of claim 2 or 3, wherein the polypeptide is: feline leukemia virus surface protein, feline leukemia virus transmembrane protein, feline leukemia virus gag, feline leukemia virus transmembrane protease, feline immunodeficiency virus gag/protease, feline immunodeficiency virus envelope, feline leukemia virus gag/protease, feline leukemia virus envelope, canine parvovirus VP2, or canine parvovirus VP1/2.
15. The recombinant swinepox virus of claim 2 or 3, wherein the polypeptide is: bovine cytokine interleukin-12 protein 35, bovine cytokine interleukin-12 protein 40, Bovine Respiratory Syncytial Virus glycoprotein G, Bovine Respiratory Syncytial Virus fusion, Newcastle Disease fusion, Newcastle Disease hemagglutinin, Infectious Rhinotracheitis Virus glycoprotein D, Canine Distemper Virus fusion, Canine Distemper Virus hemagglutinin, DV HA, Bovine Viral Diarrhea Virus type 1 glycoprotein 45, Bovine Viral Diarrhea Virus type 1 glycoprotein 48, Bovine Viral Diarrhea Virus type 1 glycoprotein 53, or Bovine Viral Diarrhea Virus type 2 glycoprotein 53.
16. The recombinant swinepox virus of claim 1, wherein said virus (S-SPV-217) further comprises a third foreign DNA and a fourth foreign DNA, wherein said first foreign DNA is the gene for *E. coli* β -galactosidase (*lacZ*), said second foreign DNA is the gene for *E. coli* β -glucuronidase (*uidA*), said third foreign DNA is the porcine reproductive and respiratory virus (PRRS) ORF5, and said fourth foreign DNA is the PRRS ORF6; and wherein said PRRS ORF5 and said *lacZ* gene are inserted into a unique NotI site inserted into said AccI site; wherein said PRRS ORF6 and said *uidA* gene are inserted into a unique NotI site inserted in said EcoRI site of said 3.2 kb region; and

wherein said first foreign DNA, said second foreign DNA, said third foreign DNA, and said fourth foreign DNA are expressed in a host cell into which the virus is introduced.

17. A vaccine useful for immunizing an animal against swinepox virus which comprises an effective immunizing amount of the recombinant swinepox virus of any one of claims 1 to 16 and a suitable carrier.
18. The recombinant swinepox virus of any one of claims 1 to 17 for use as a vaccine.
19. Use of the recombinant swinepox virus of any one of claims 1 to 18 in the manufacture of a medicament for immunizing an animal against a swinepox virus.

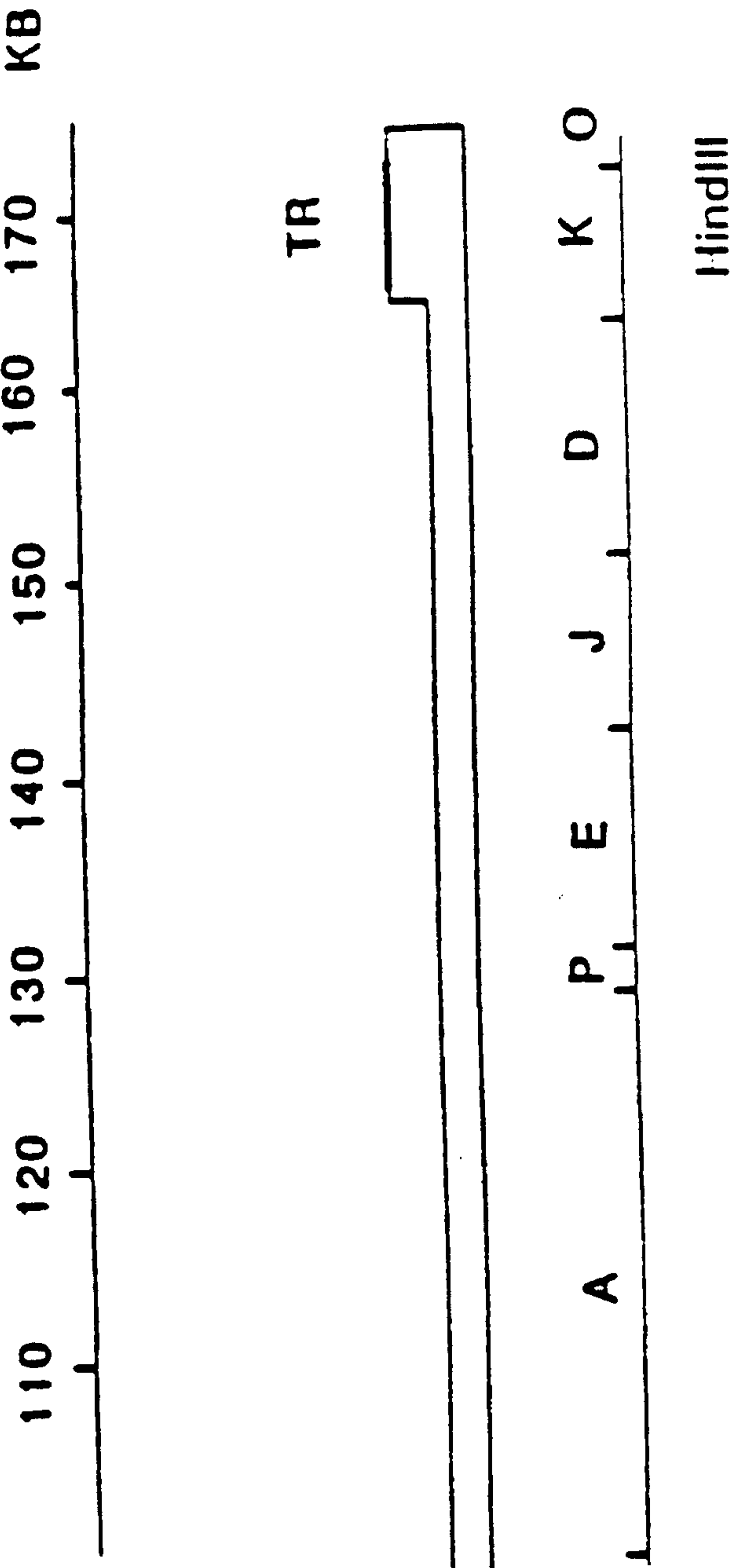
FIGURE 1A

FIGURE 1A	FIGURE 1B
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FIGURE 1B



3/55

FIGURE 2A

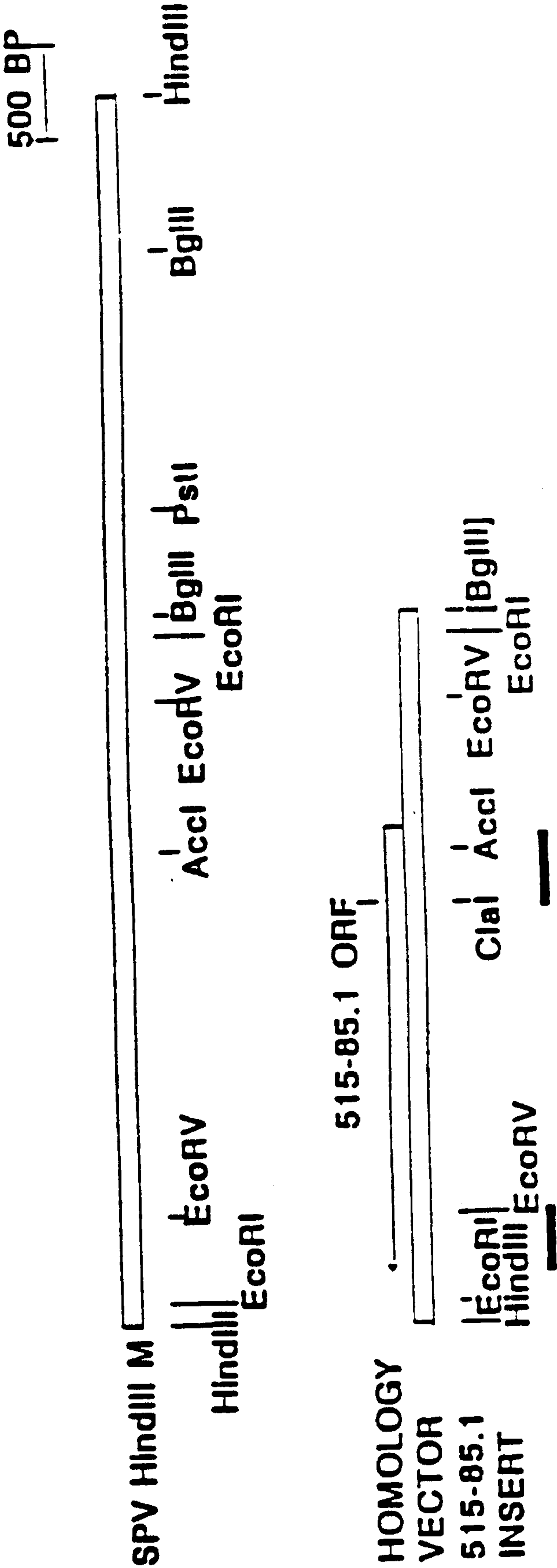


FIGURE 2B

[illegible]

	80	90	100	110	120
(A) VV	★	★	★	★	★
orf 01L	:	:	:	:	:
(B) SPV					
AccI-Clai					

FIGURE 2C

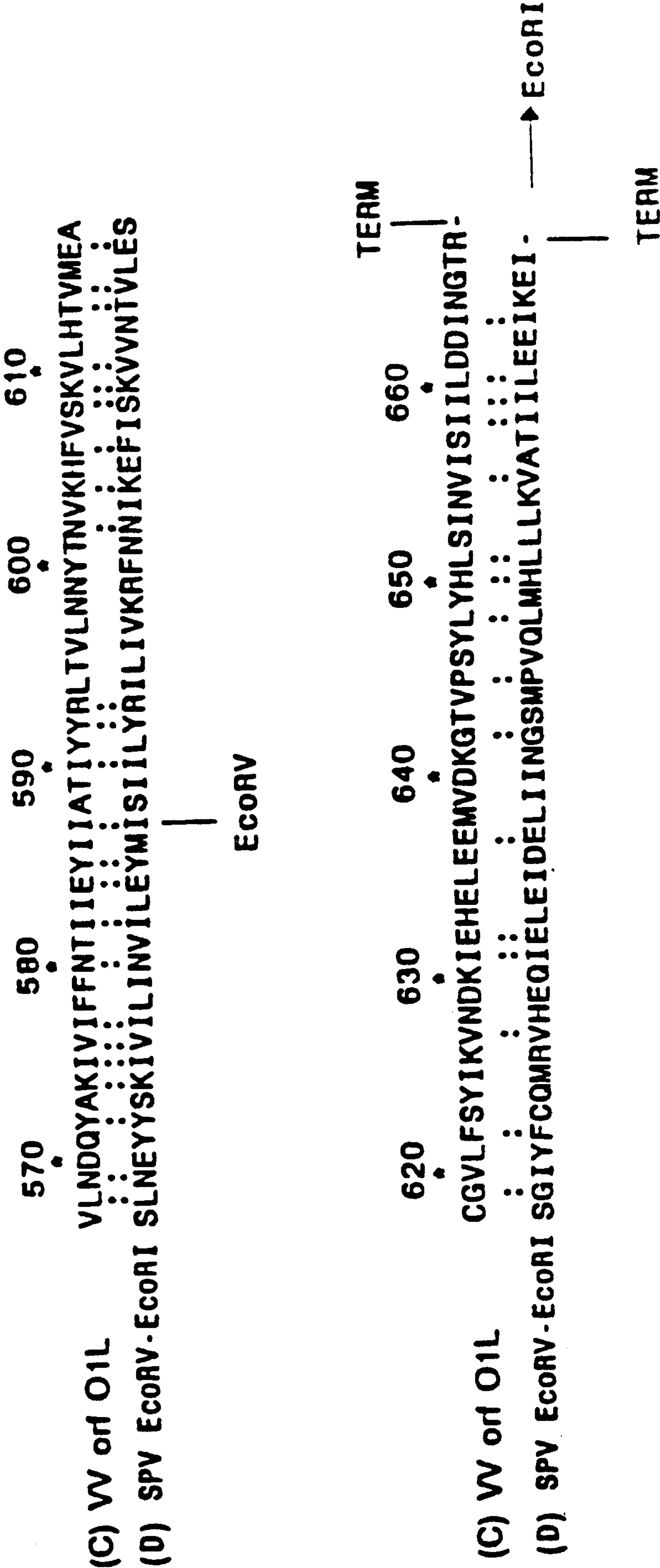
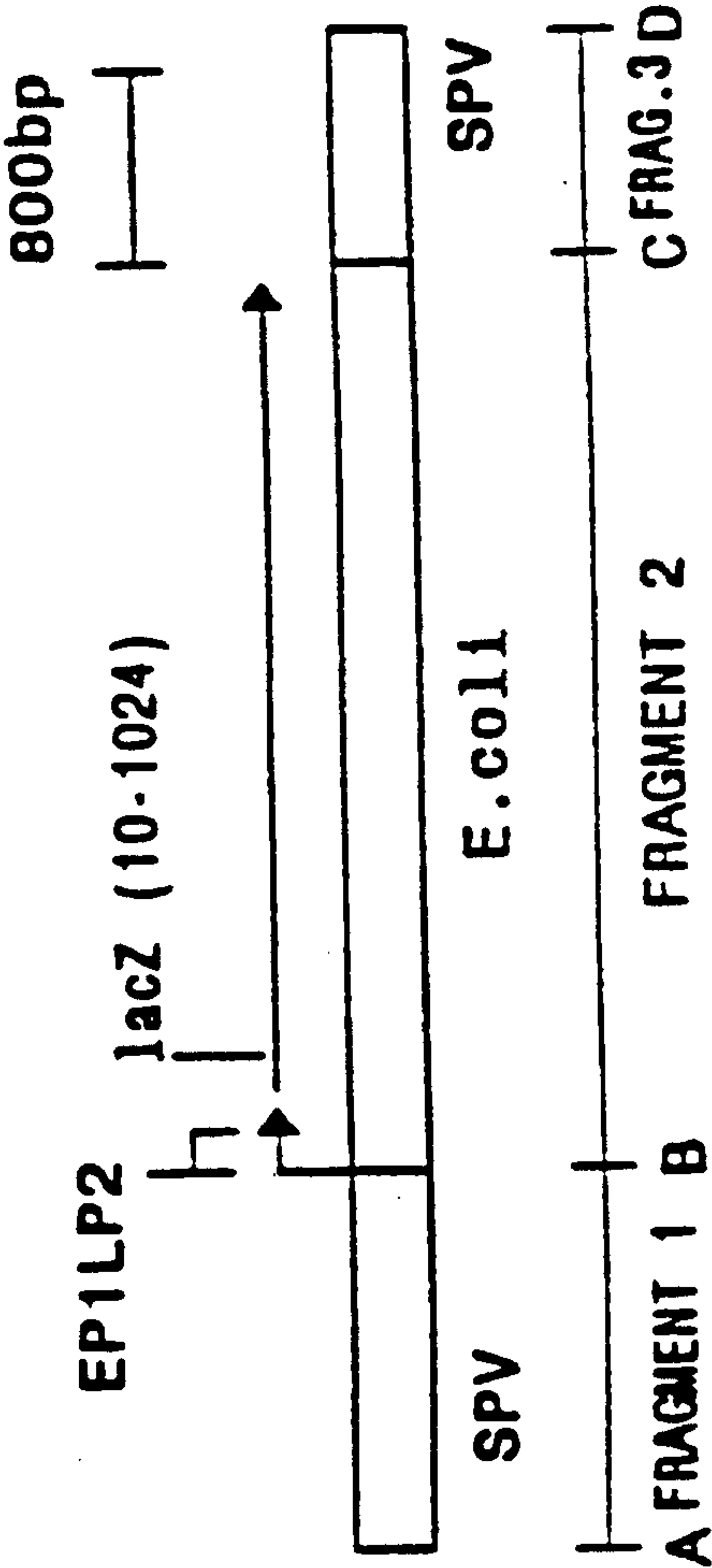


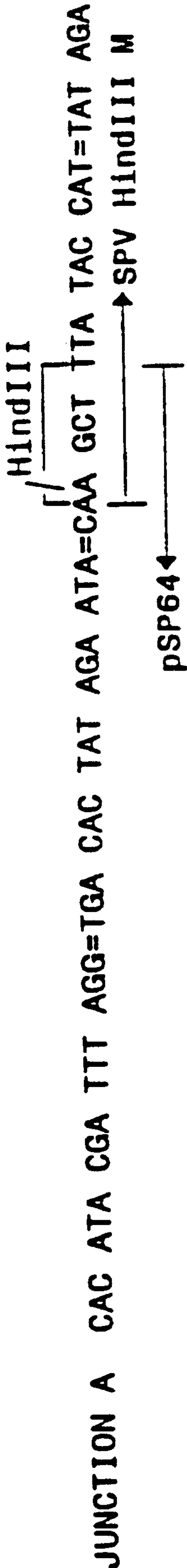
FIGURE 3A

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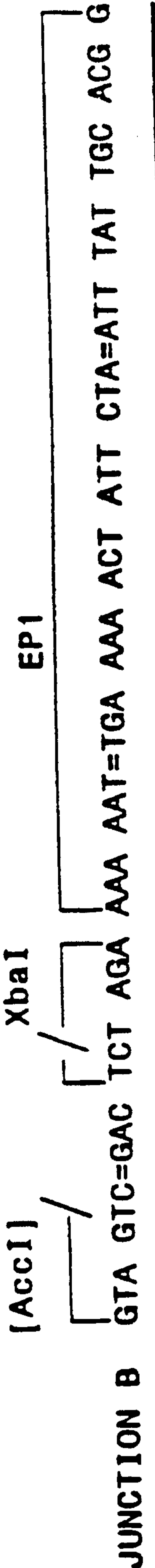
DNA	ORIGIN	SLICES	SIZE
VECTOR	pSP64	HindIII-BamHI	~2972 BP
FRAGMENT 1	SPV HindIII	M HindIII-AccI	~2149 BP
FRAGMENT 2	pJF751	BamHI-PvuII	~3002 BP
FRAGMENT 3	SPV HindIII	M AccI-BglII	~1484 BP

FIGURE 3B



JUNCTION A (CONTINUED)

TAC ATT ACC=TTG TCC GAC GTG TAG=AAT TCA TGC CAA AGA=AGA ATT AAC TAA



SPV HindIII M ←

EcoRI BamHI

JUNCTION B (CONTINUED)

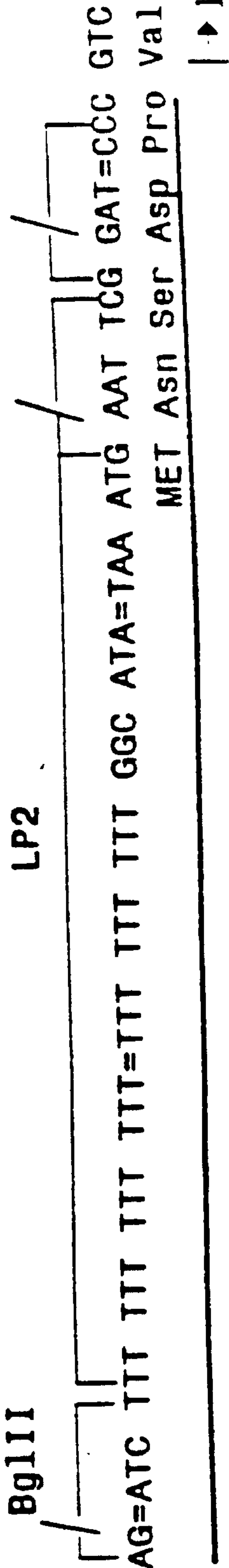


FIGURE 3C

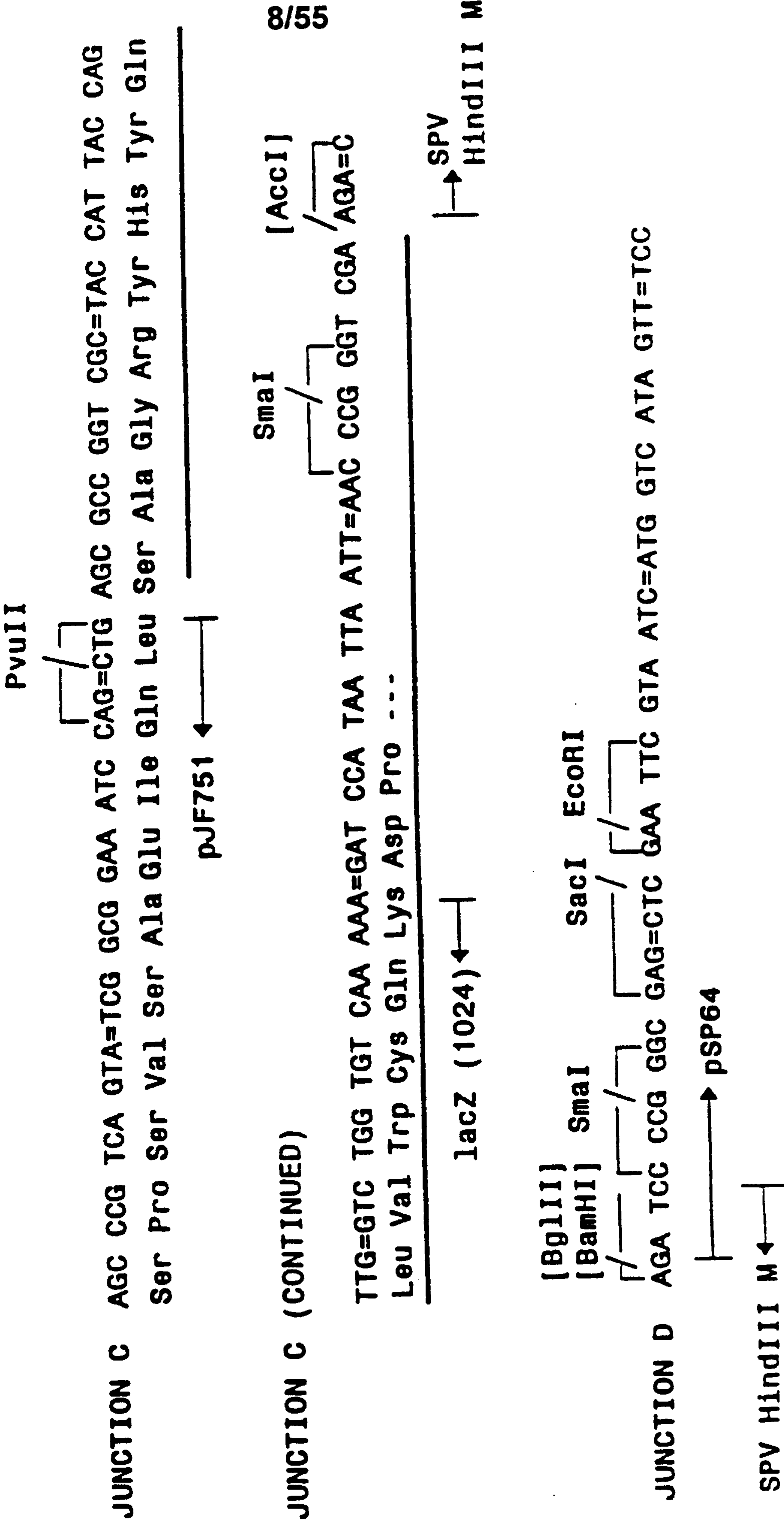
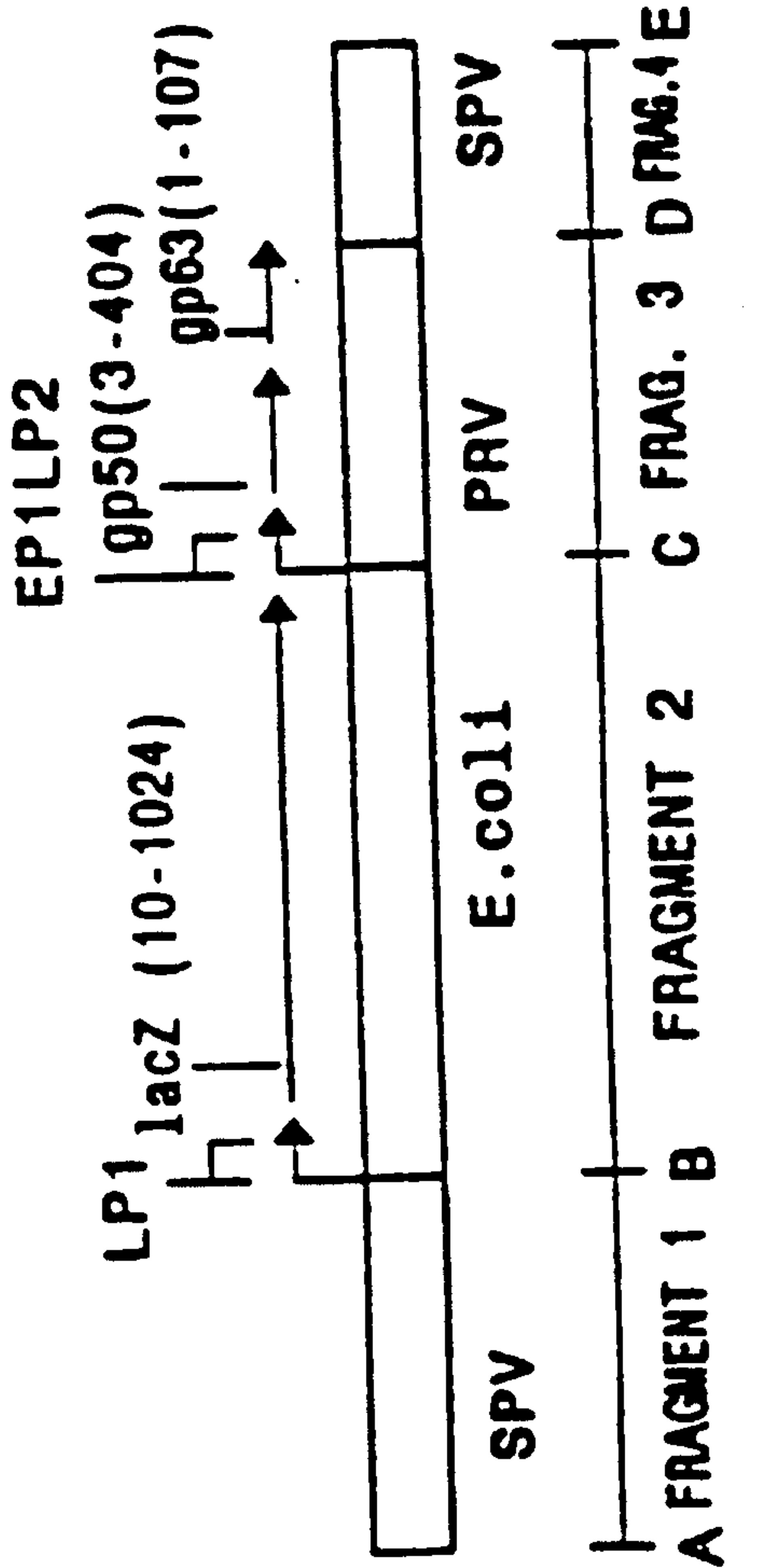


FIGURE 4A

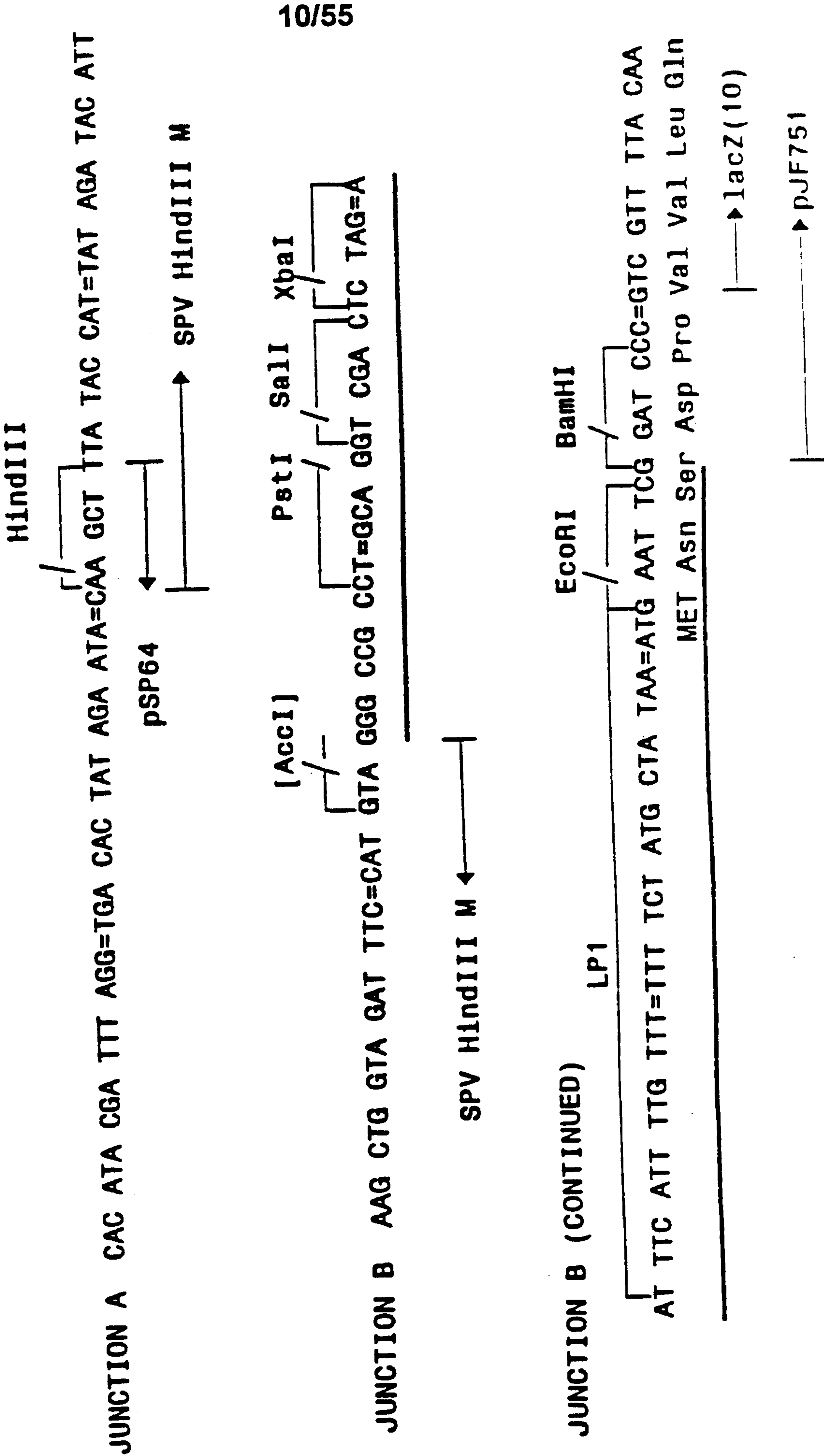
9/55



DNA	ORIGIN	SLICES	SIZE
VECTOR	pSP64	HindIII-BamHI	~2972 BP
FRAGMENT 1	SPV HindIII M	HindIII-AccI	~2149 BP
FRAGMENT 2	pJF751	BamHI-PvuII	~3002 BP
FRAGMENT 3	PRV BamHI #7	EcoRI-StuI	~1558 BP
FRAGMENT 4	SPV HindIII M	AccI-BglII	~1484 BP

• INTRODUCED VIA CLONING

FIGURE 4B



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FIGURE 4C

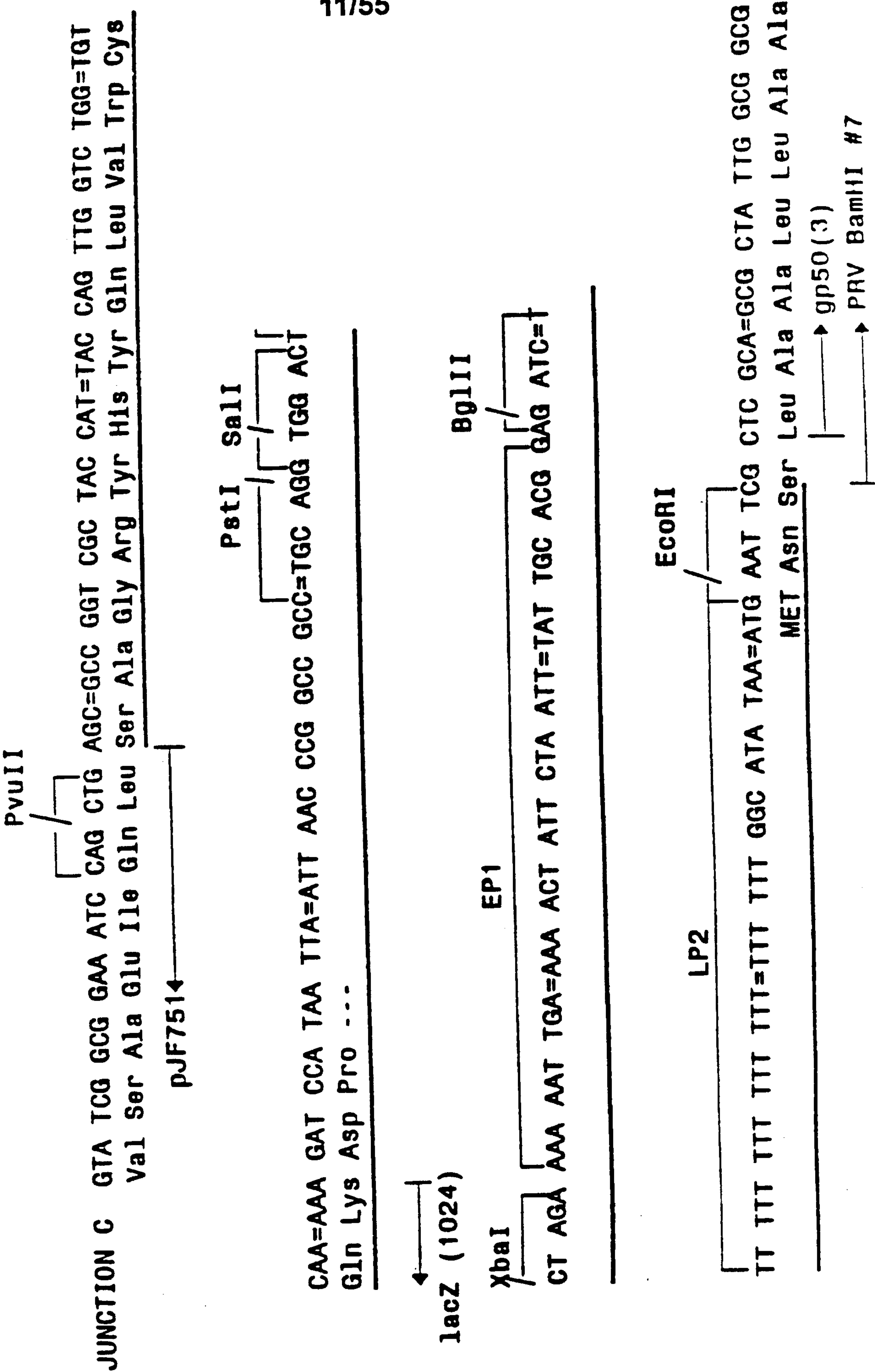
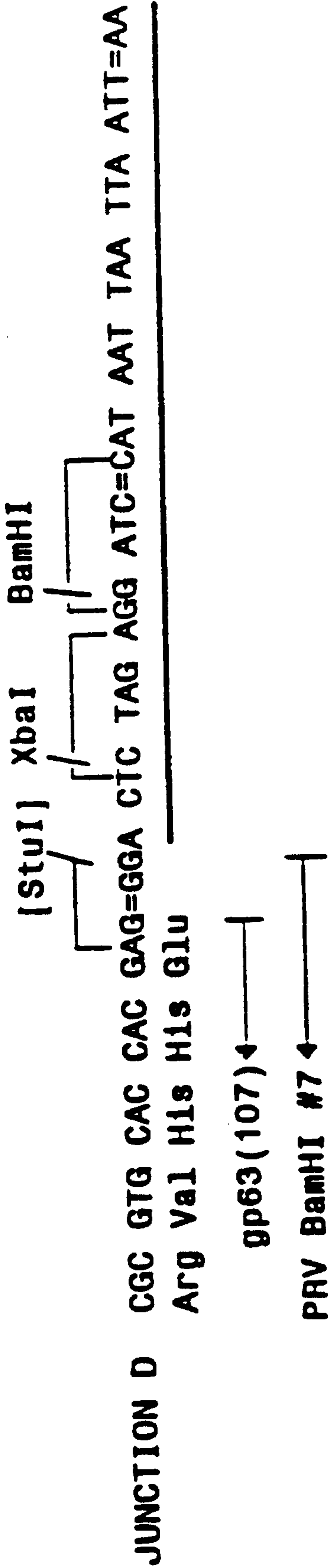


FIGURE 4D



JUNCTION D (CONTINUED)

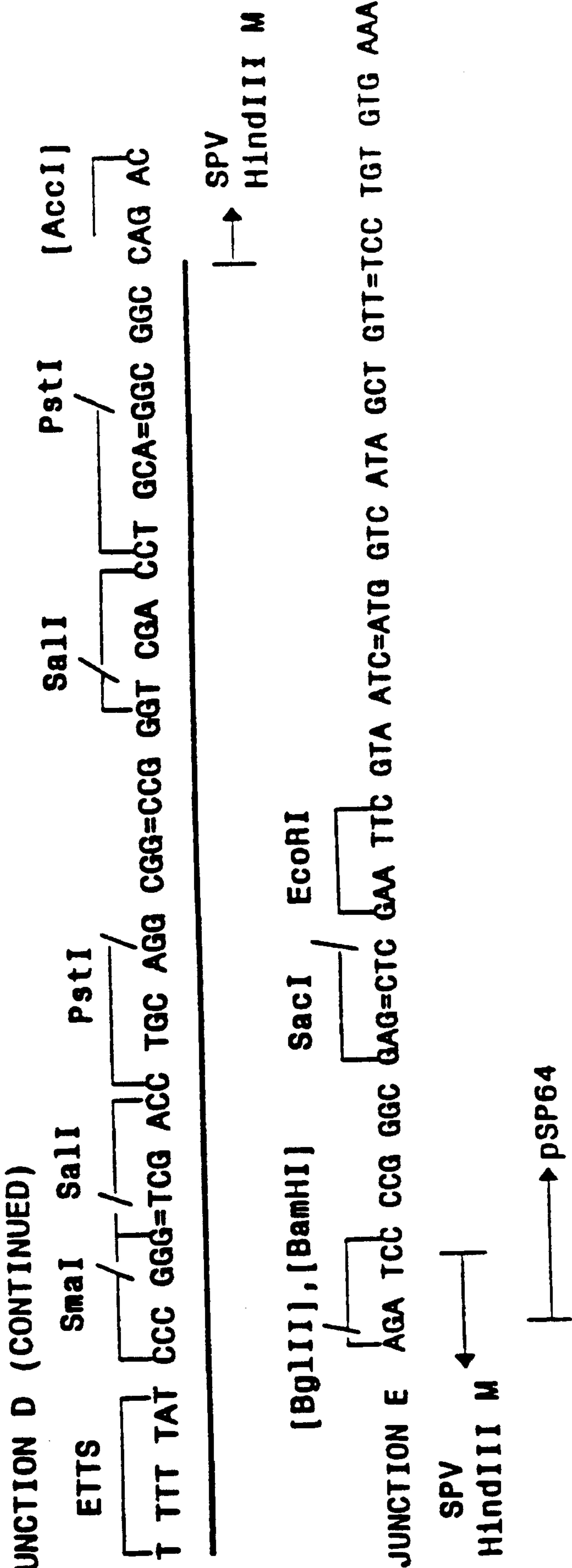


FIGURE 5A

DNA	Origin	Sites	Size
Vector	pSP64	Hind III—Bam HI	~2972 BP
Fragment 1	SPV HindIII M	Bgl II—Acc I	~1484 BP
Fragment 2	pJF751	Bam HI—Pvu II	~3002 BP
Fragment 3	PRV BamHI 2 & 9	Nco I—Nco I	~2378 BP
Fragment 4	SPV HindIII M	Acc I—Hind III	~2149 BP

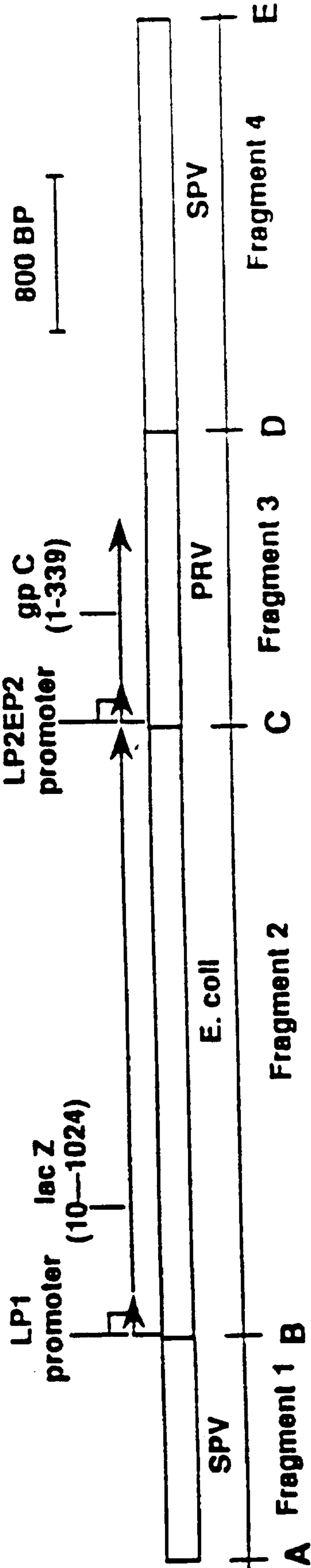
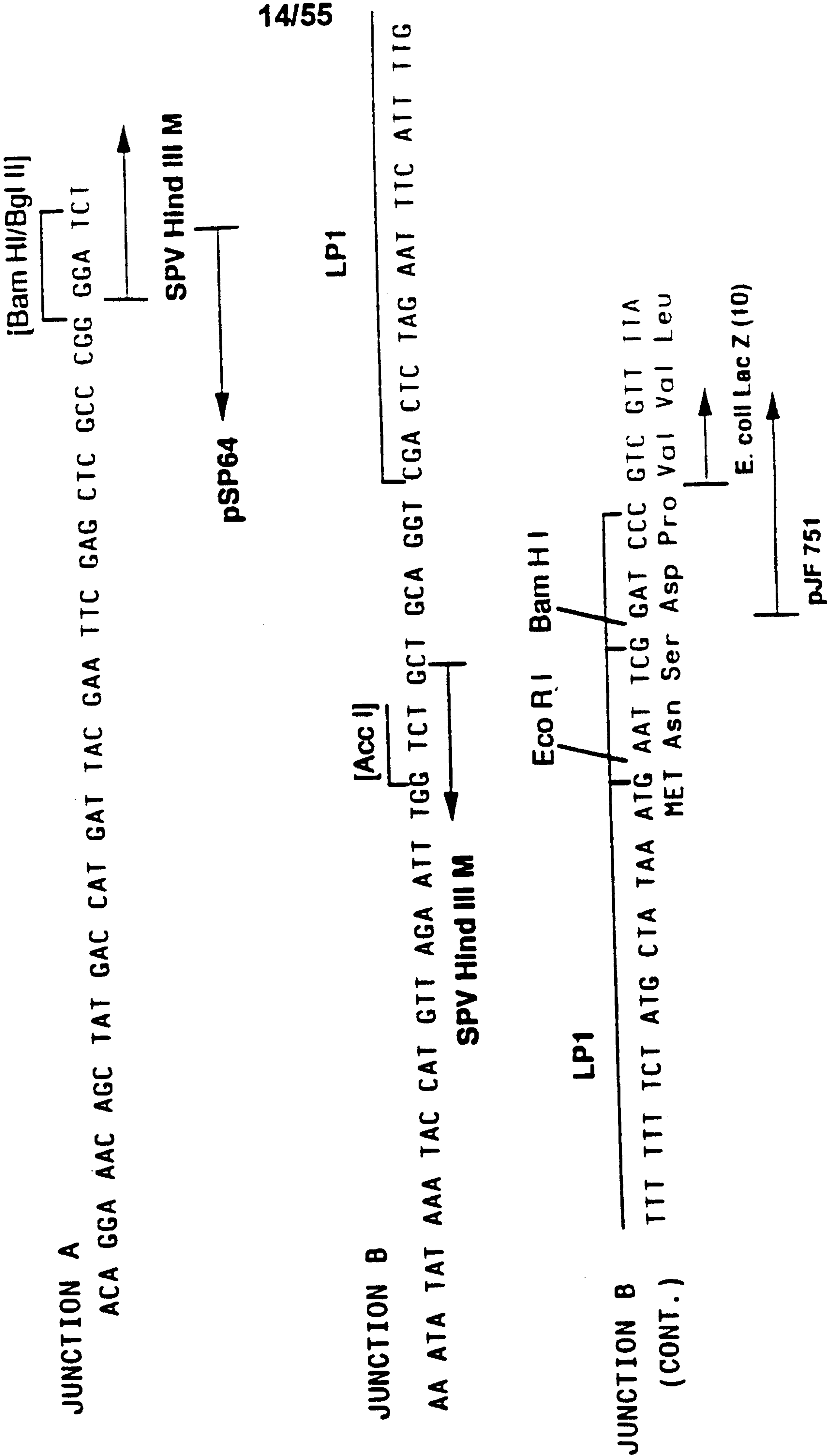


FIGURE 5B



JUNCTION C

GAA	ATC	CAG	CTG	AGC	GCC	GGT	CGC	TAC	CAT	TAC	CAG	TTG	GTC	TGG	TGT	CAA	AAA	GAT
GIU	Ile	Gln	Leu	Ser	Ala	Gly	Arg	Tyr	His	Tyr	Gln	Leu	Val	Trp	Cys	Gln	Lys	Asp

PLF 751

JUNCTION C (CONT.)

CCA TAA TTA ATT AAC CCG GTC GAC TCT AGA TTT TTT TTT TTT TTT TTT TTT TTT TTT

PRO .

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JUNCTION C (CONT.)

EcoRI

[Nco I]

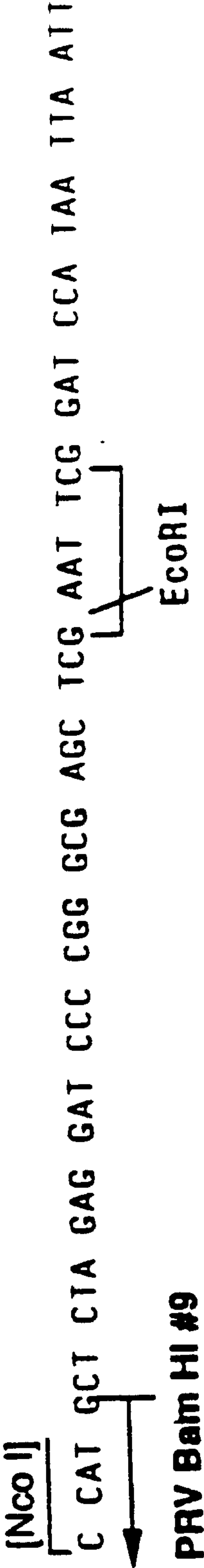
23

TAG	ATC	TGT	ATC	CTA	AAA	TTG	AAT	TGT	AAT	TAT	CGA	TAA	TAA	ATG	AAT	TCC	GGC	AIG	GCC	ICG	
															MET	Asn	Ser	Gly	Met	Ala	Ser

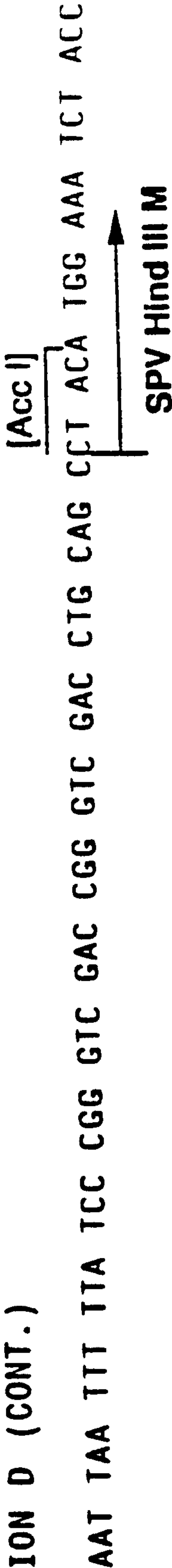
PRV gpC (1)

FIGURE 5D

JUNCTION D



JUNCTION D (CONT.)



JUNCTION E

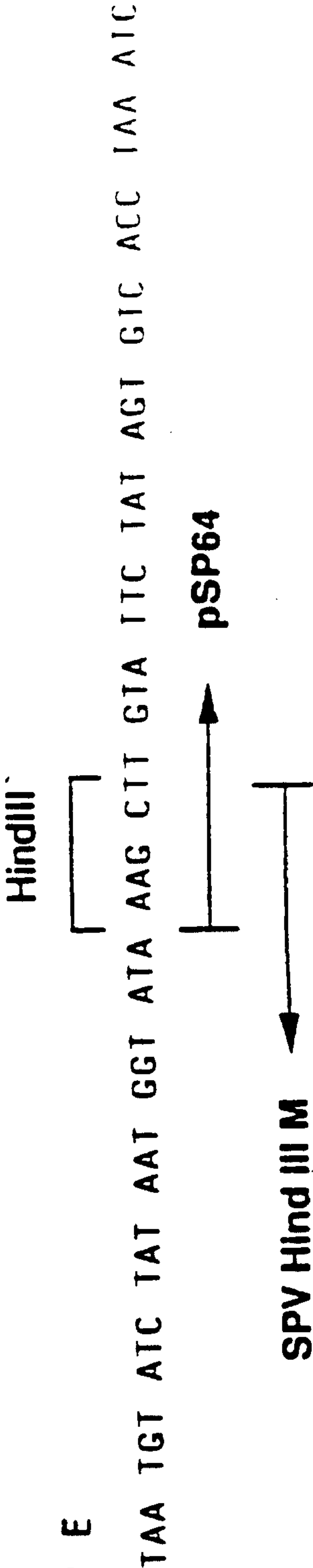
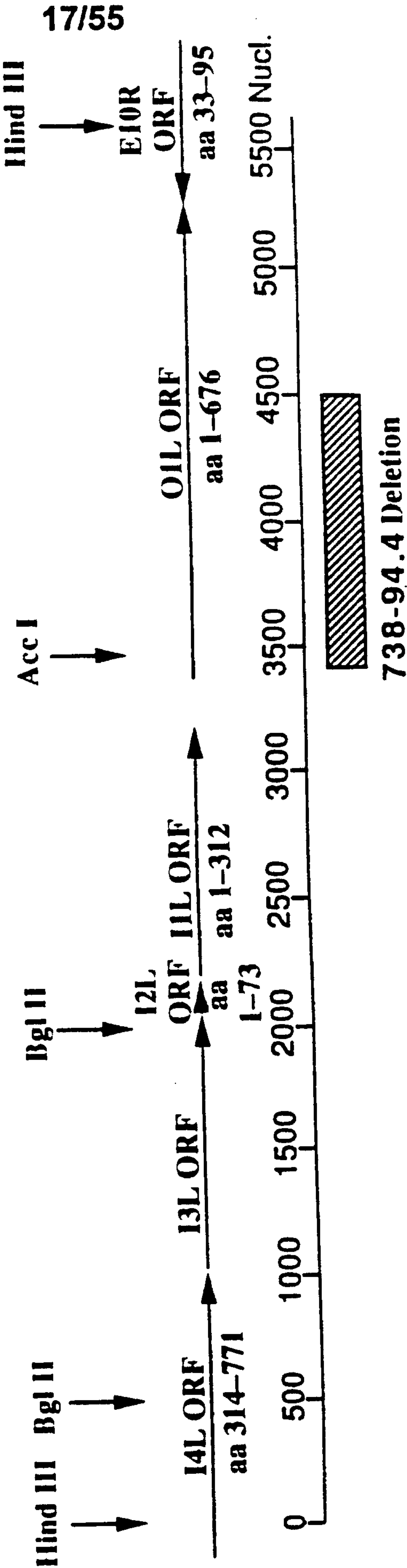


FIGURE 6



SEQ ID NO. 195

SEQ ID NO. 189

FIGURE 7A

DNA	Origin	Sites	Size
Vector	pSP64	Hind III-Bam HI	~2972 BP
Fragment 1	SPV Hind III M	Bgl II-Acc I	~1484 BP
Fragment 2	PRV Kpn I C	Sma I-Sac I	~3500 BP
Fragment 3	pJF751	Bam HI-Pvu II	~3010 BP
Fragment 4	SPV Hind III M	Acc I-Hind III	~2149 BP

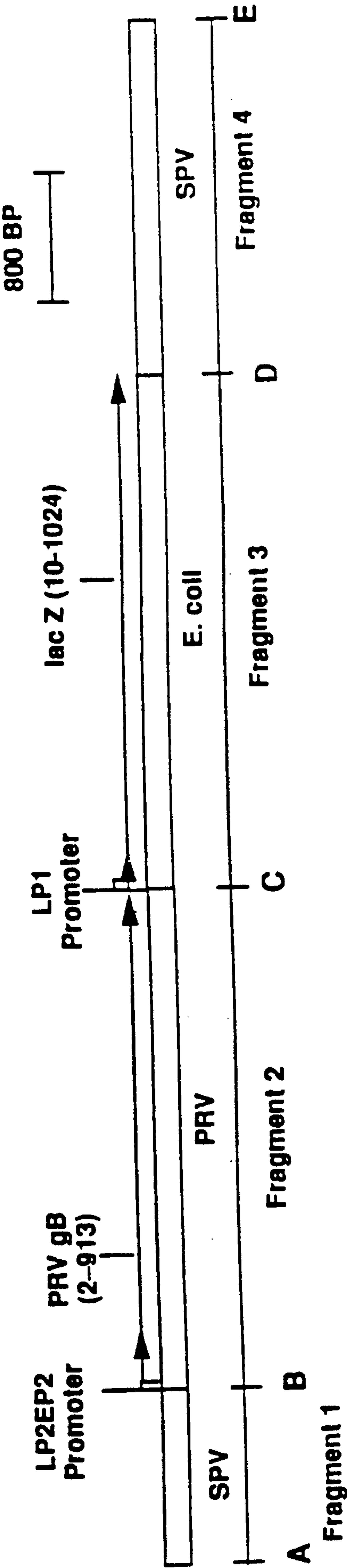


FIGURE 7B

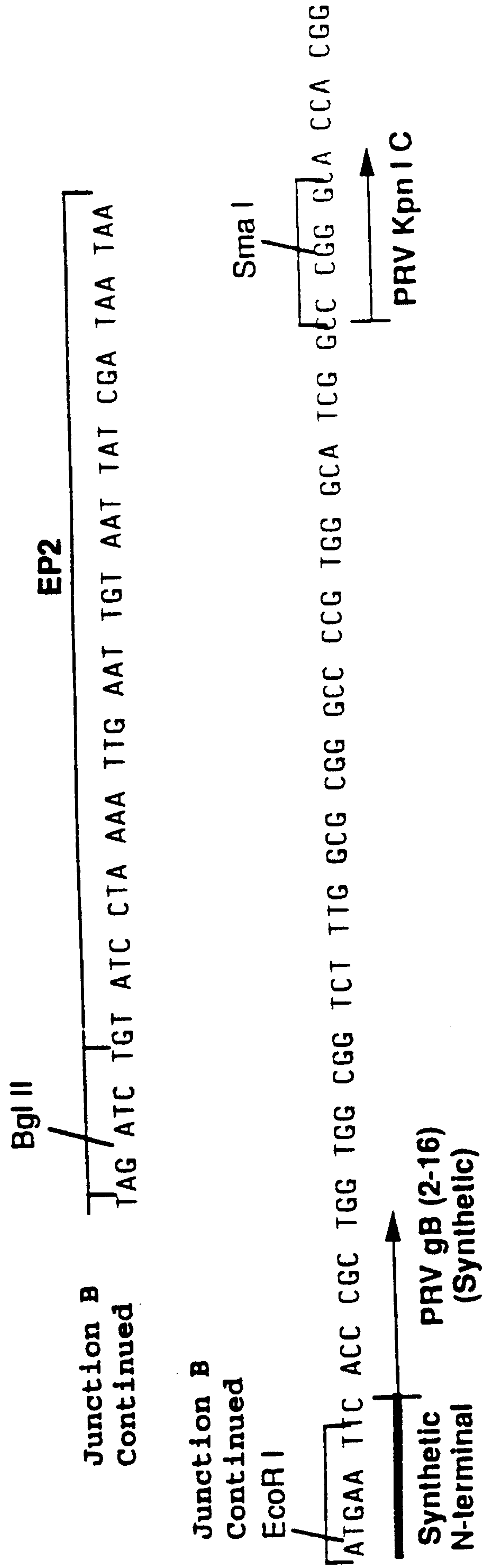
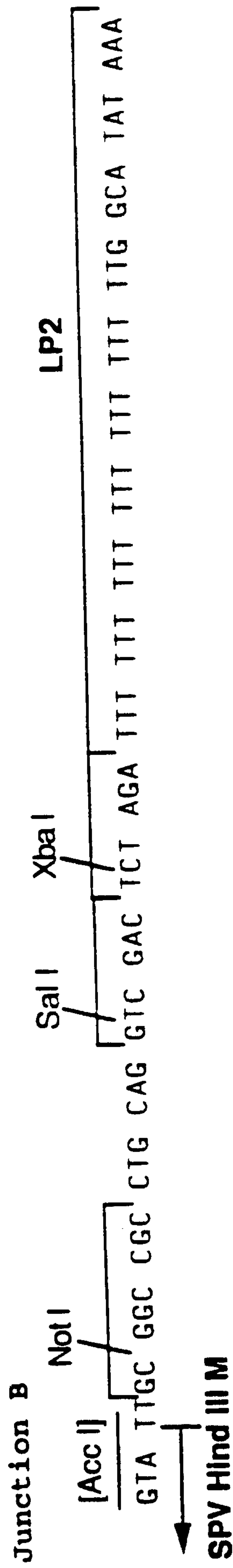
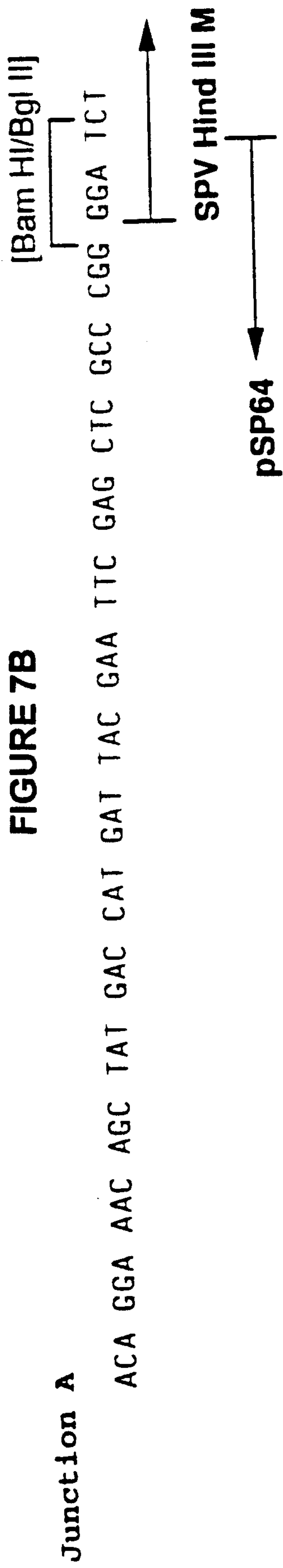
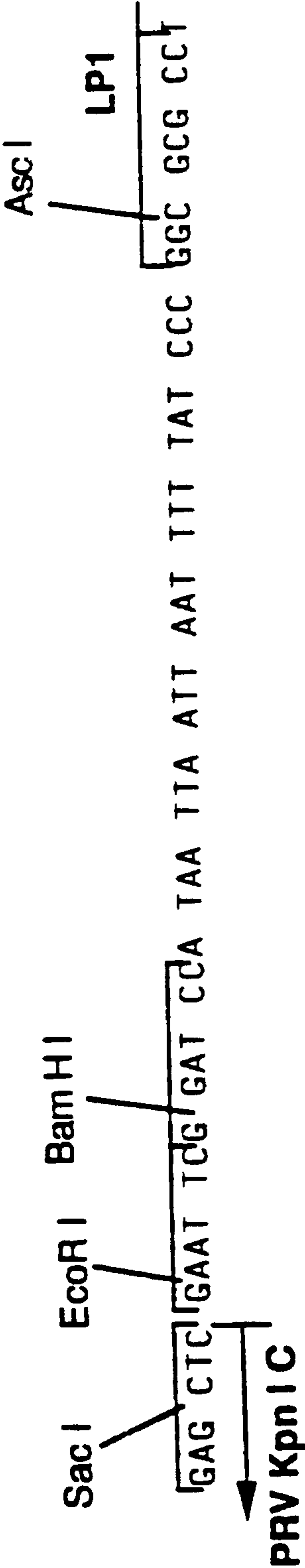


FIGURE 7C

Junction C



Junction C Continued

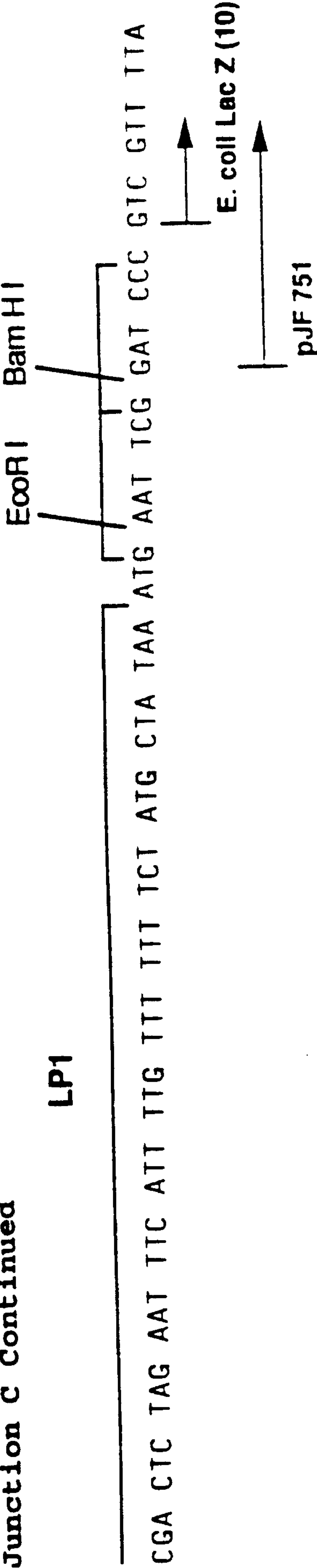


FIGURE 7D

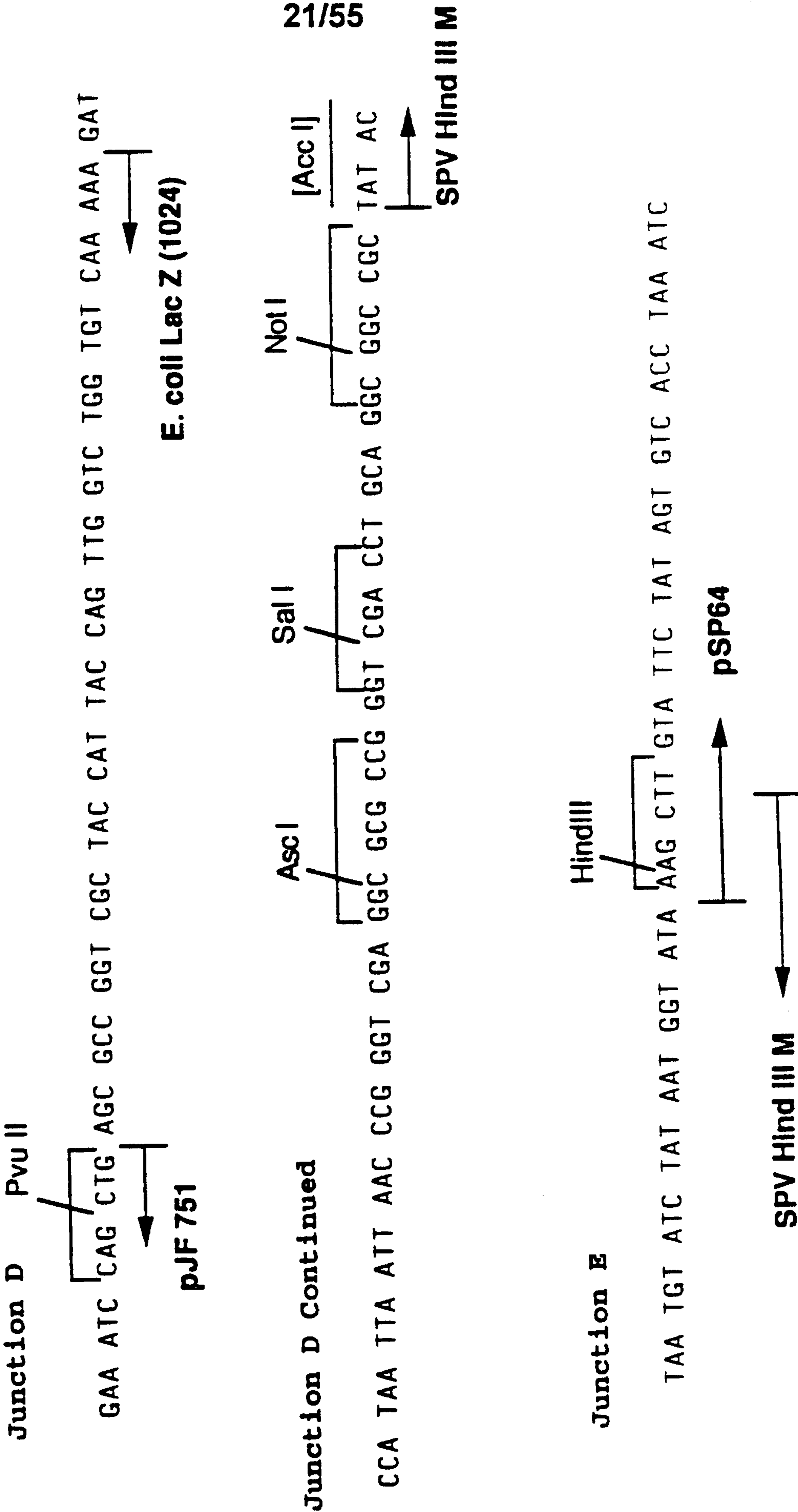


FIGURE 8A

DNA	Origin	Sites	Size
Vector	pSP64	Hind III-Bam HI	~2972 BP
Fragment 1	SPV Hind III M	Bgl II-Acc I	~1484 BP
Fragment 2	chicken MGF	EcoR I†-BamH I†	~640 BP
Fragment 3	pJF751	Bam HI-Pvu II	~3002 BP
Fragment 4	SPV Hind III M	Acc I-Hind III	~2149 BP

†Restriction sites introduced by PCR cloning

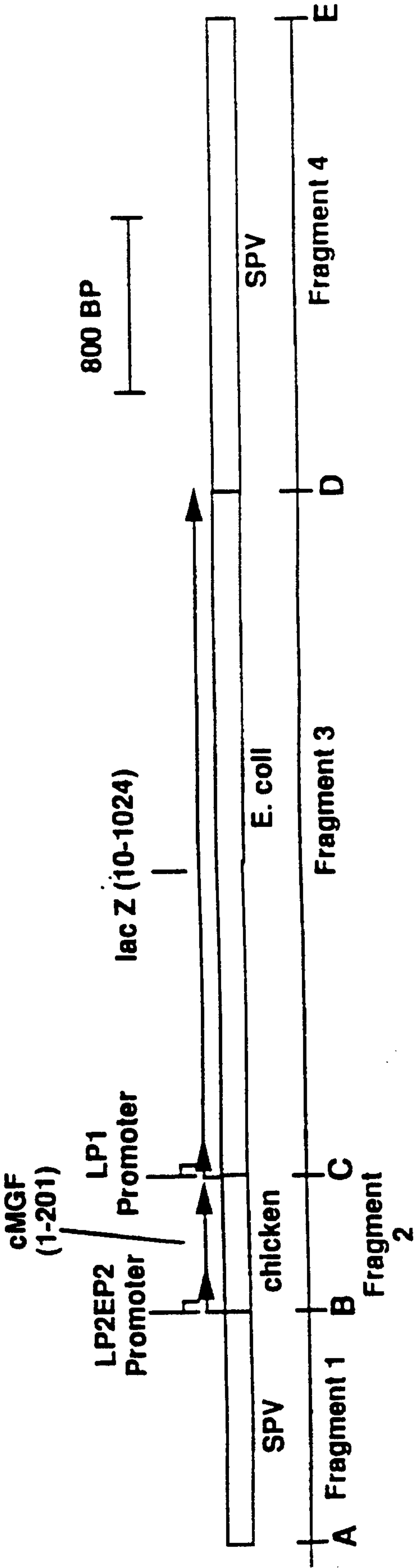


FIGURE 8B

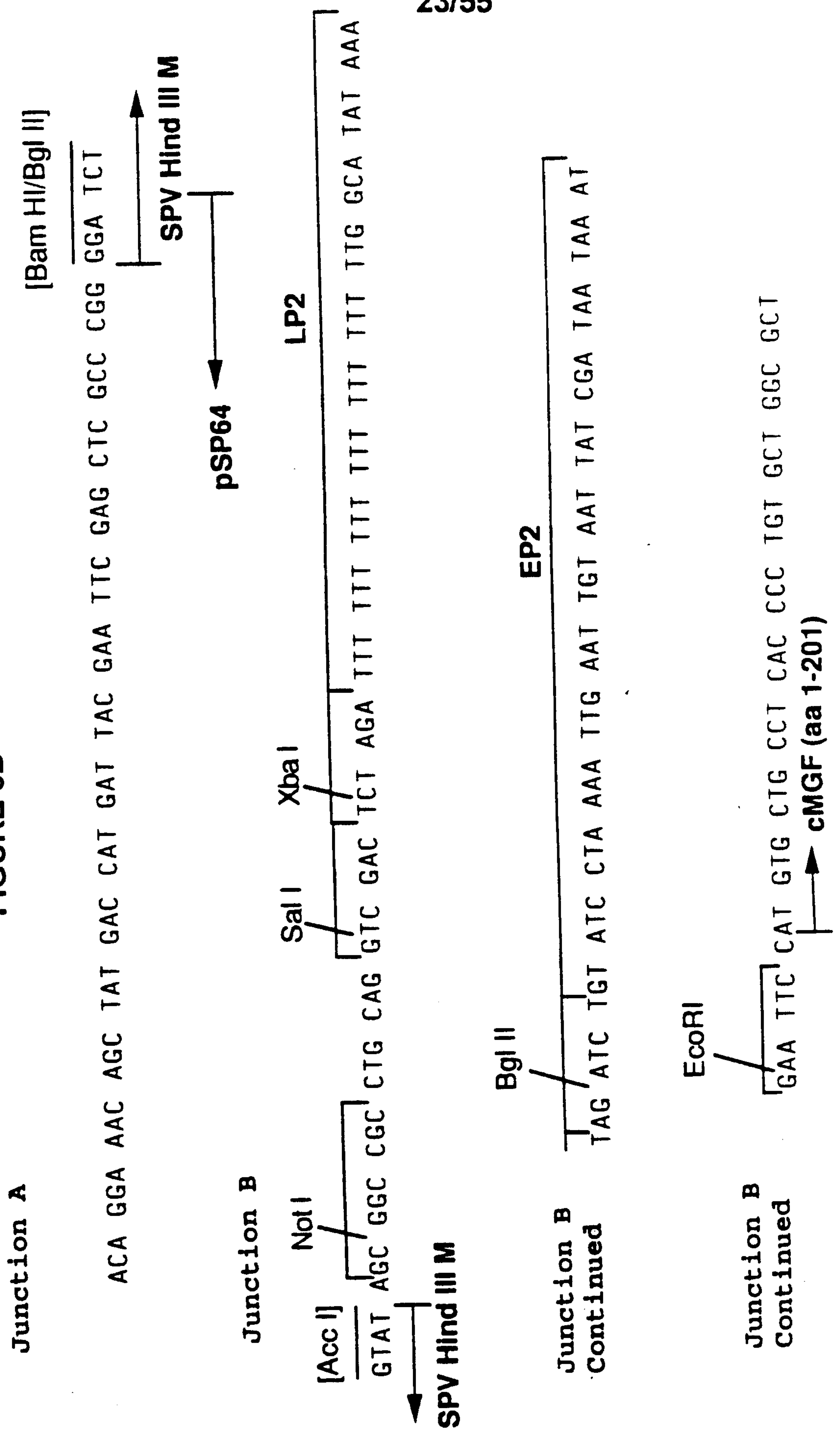


FIGURE 8C

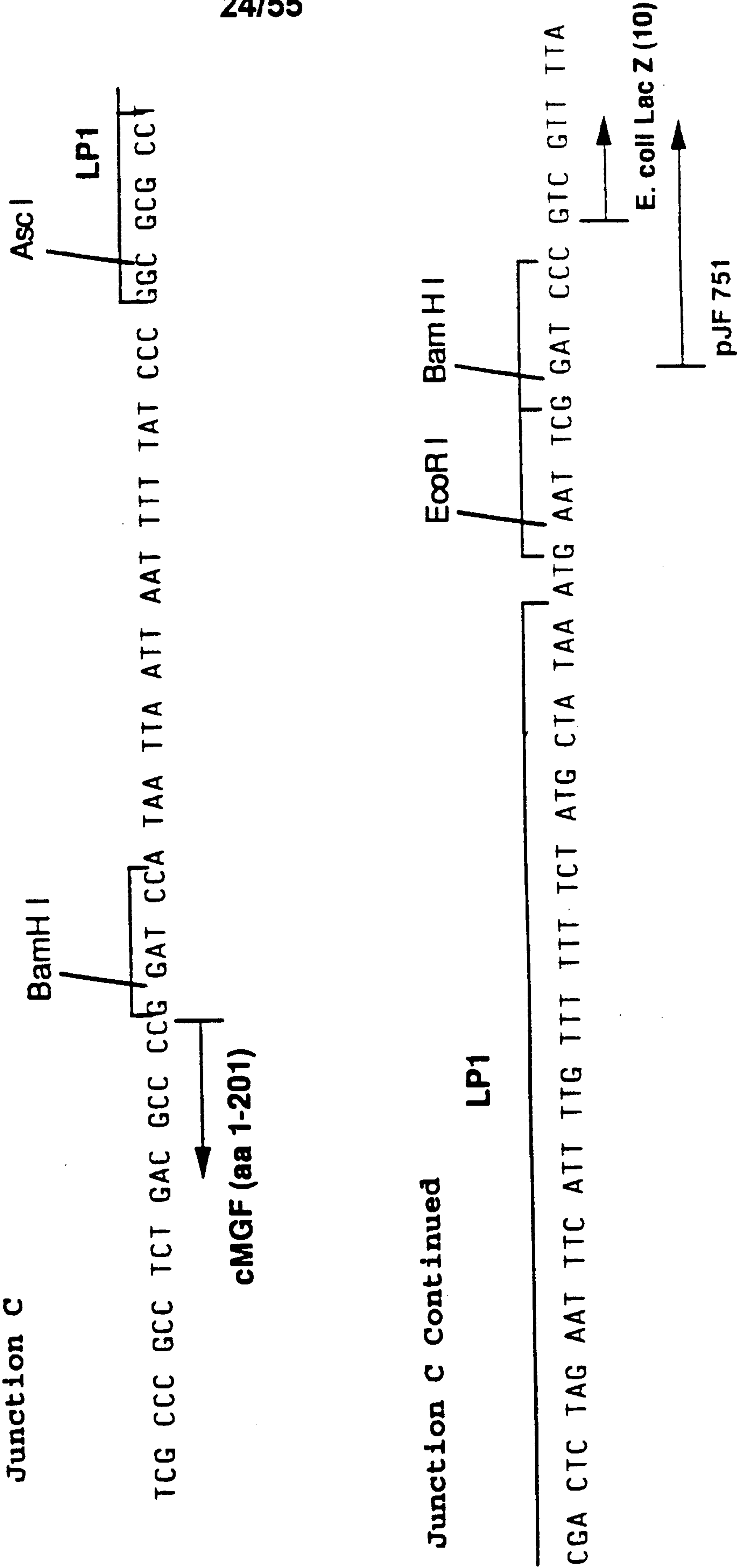
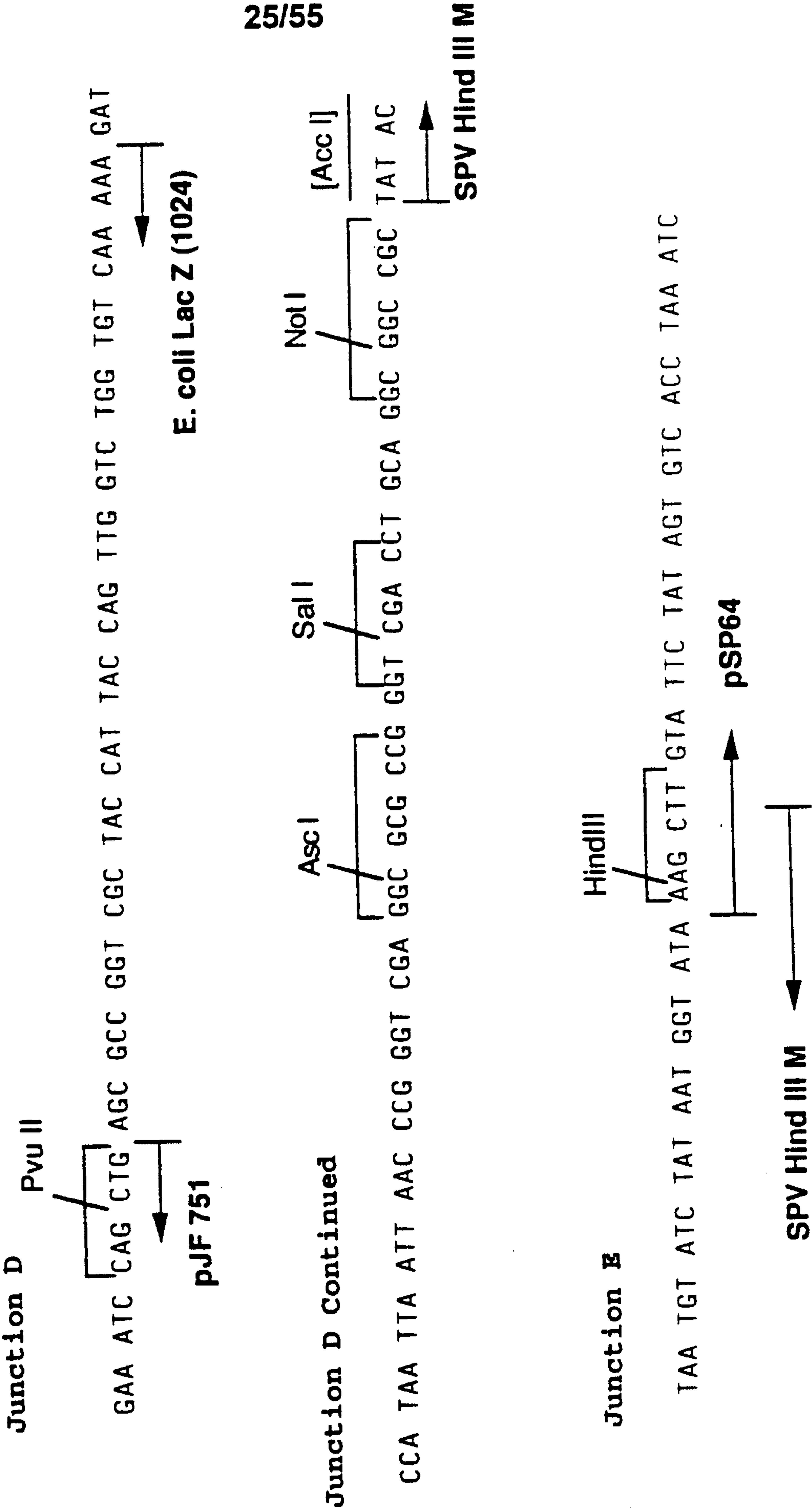


FIGURE 8D



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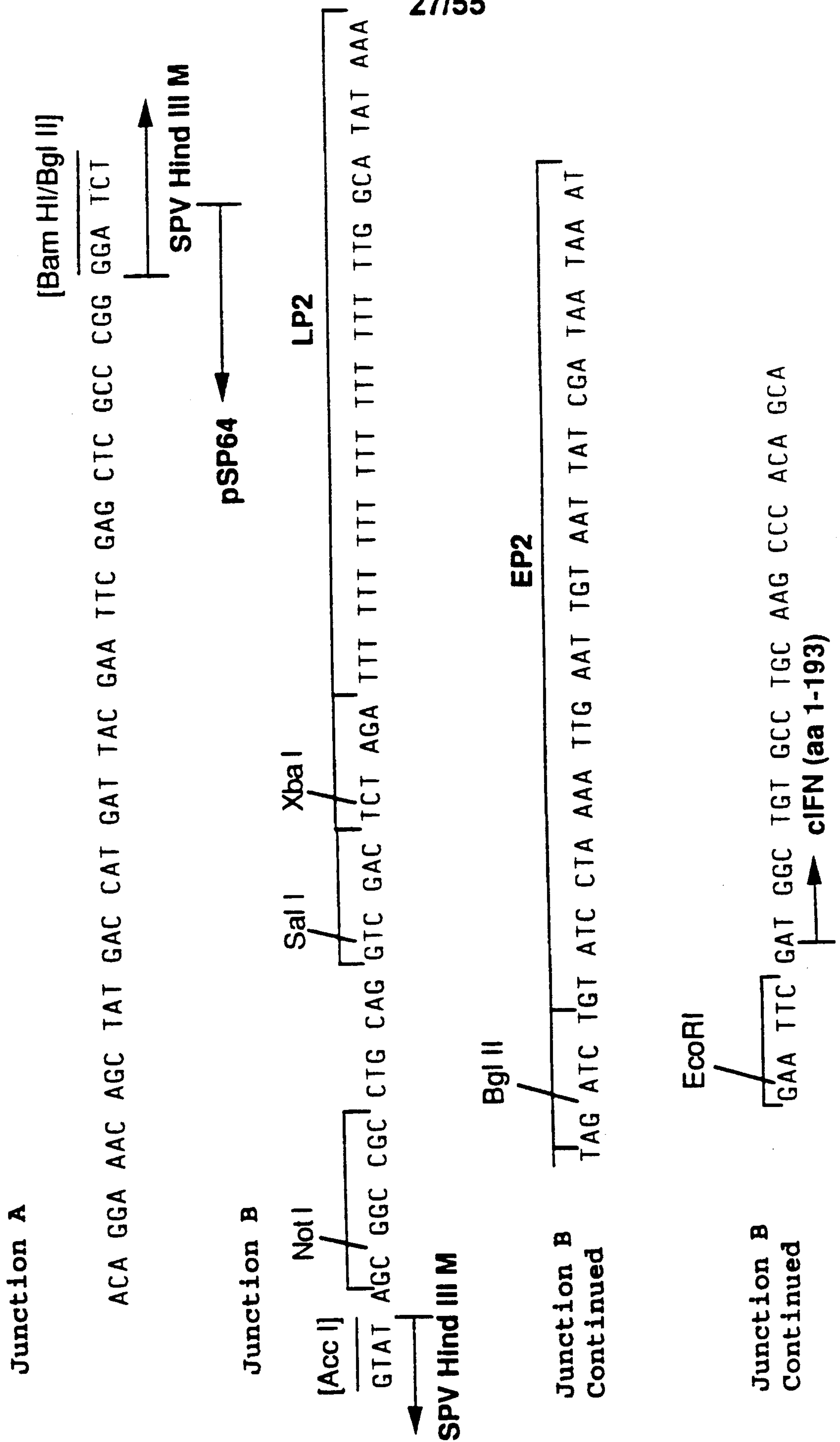


FIGURE 9C

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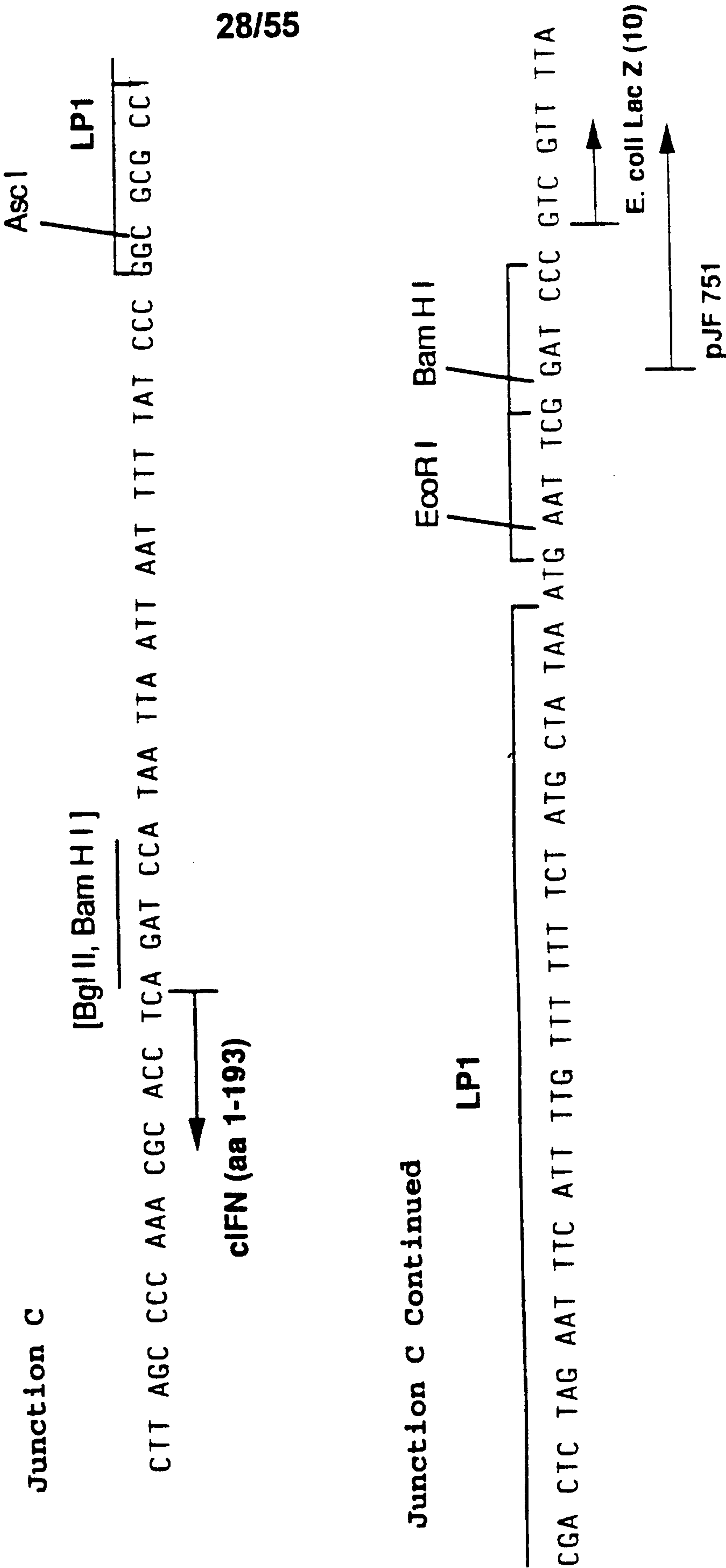


FIGURE 9D

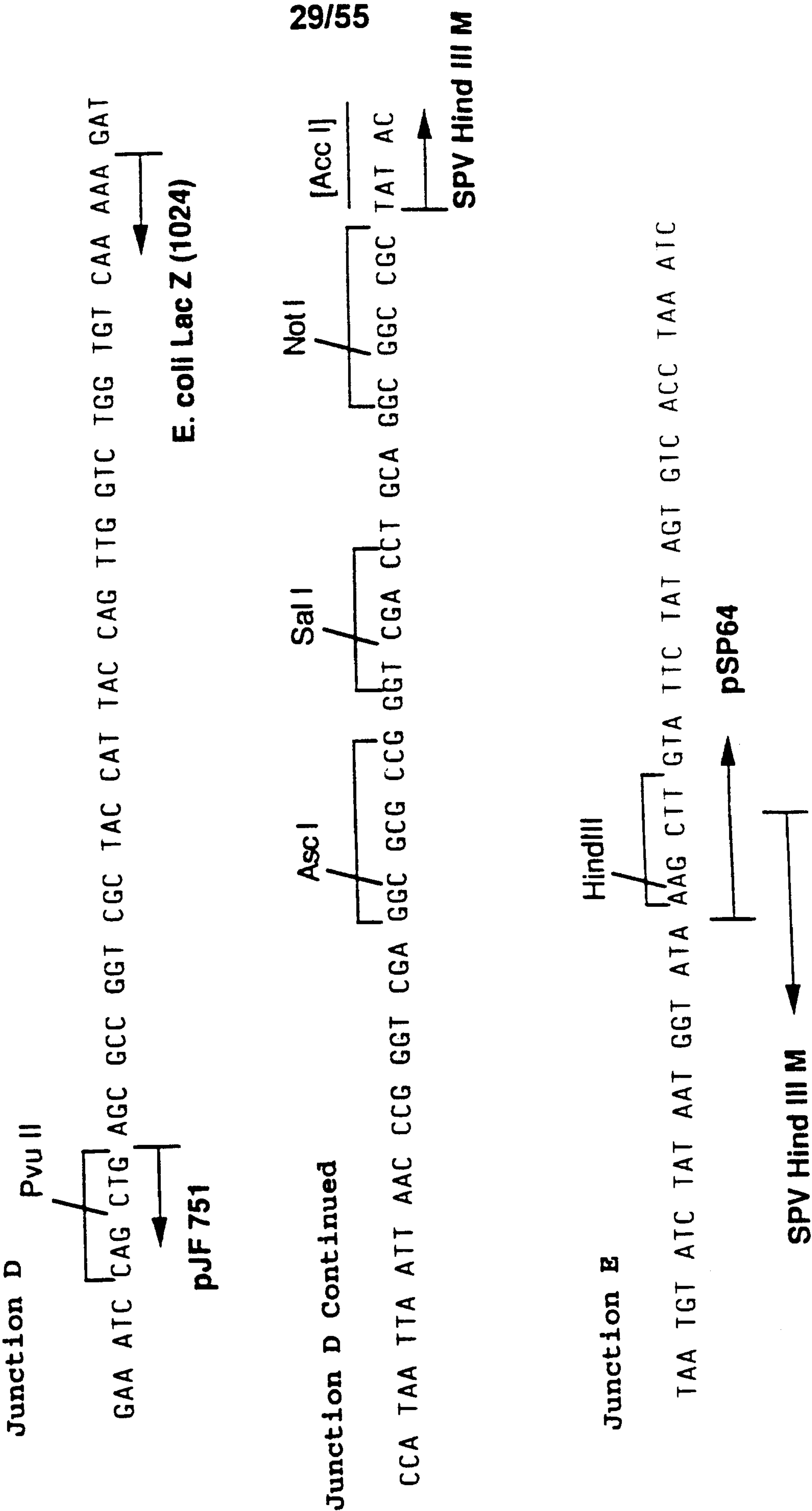


FIGURE 10A

DNA	Origin	Sites	Size
Vector	pSP65	Hind III-Sph I	~2519 BP
Fragment 1	SPV Hind III M	Sph I-Bgl II†	~855 BP
Fragment 2	pJF751	Bam HI-Pvu II	~3002 BP
Fragment 3	SPV Hind III M	Sal I†-Hind III	~1113 BP

†Restriction sites introduced by PCR cloning

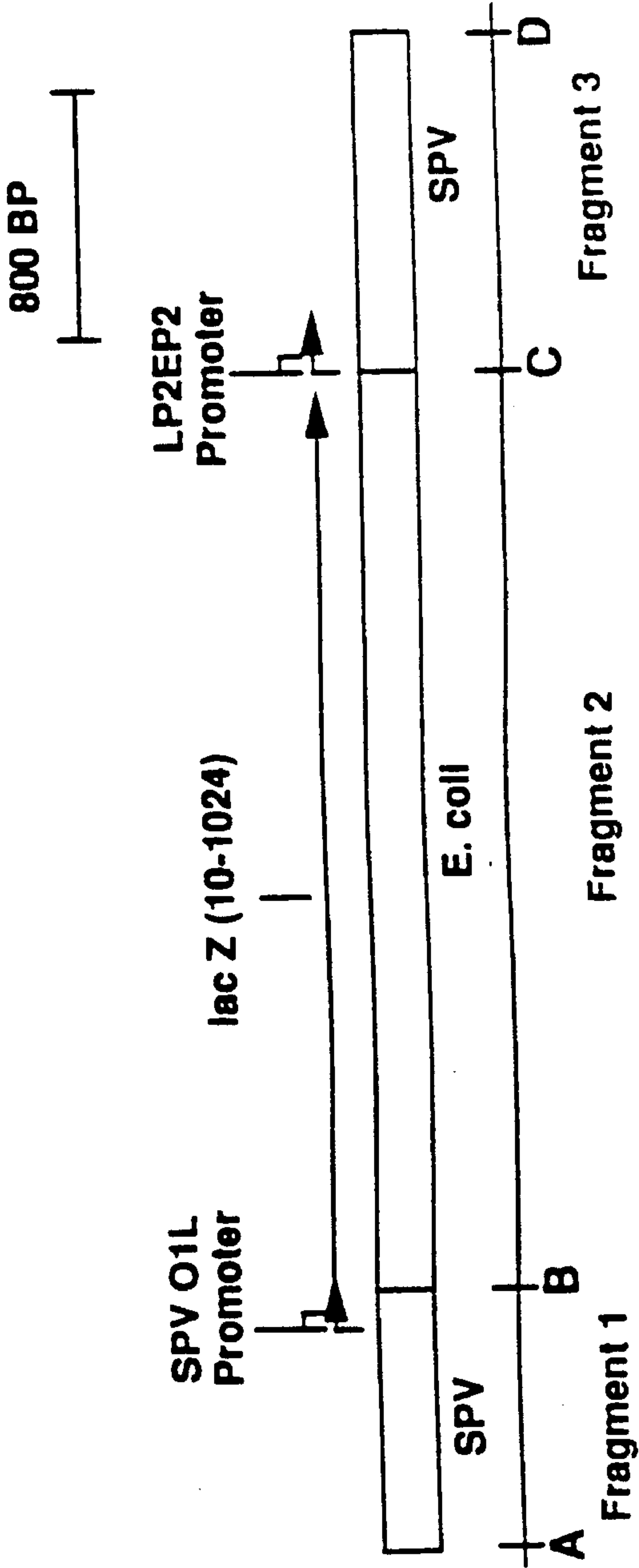
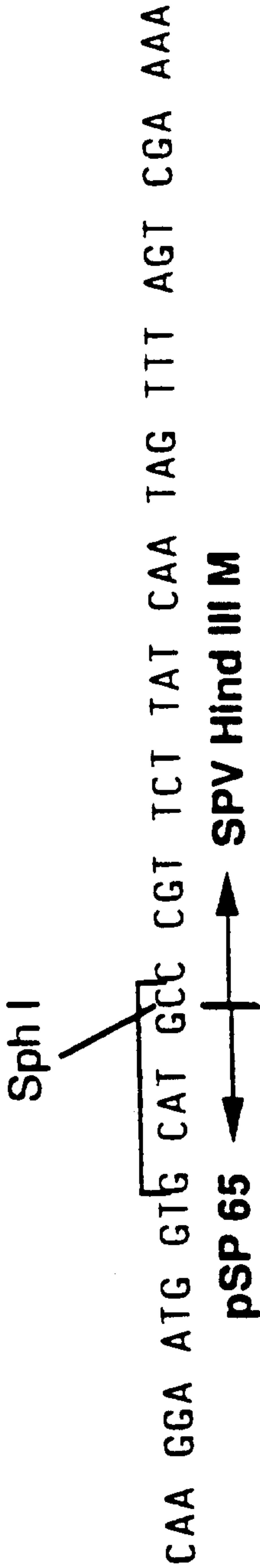


FIGURE 10B

Junction A



Junction B

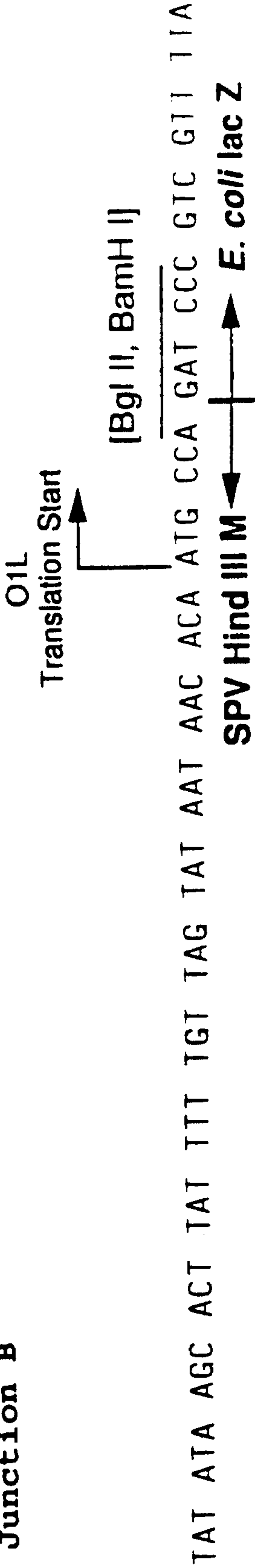


FIGURE 10C

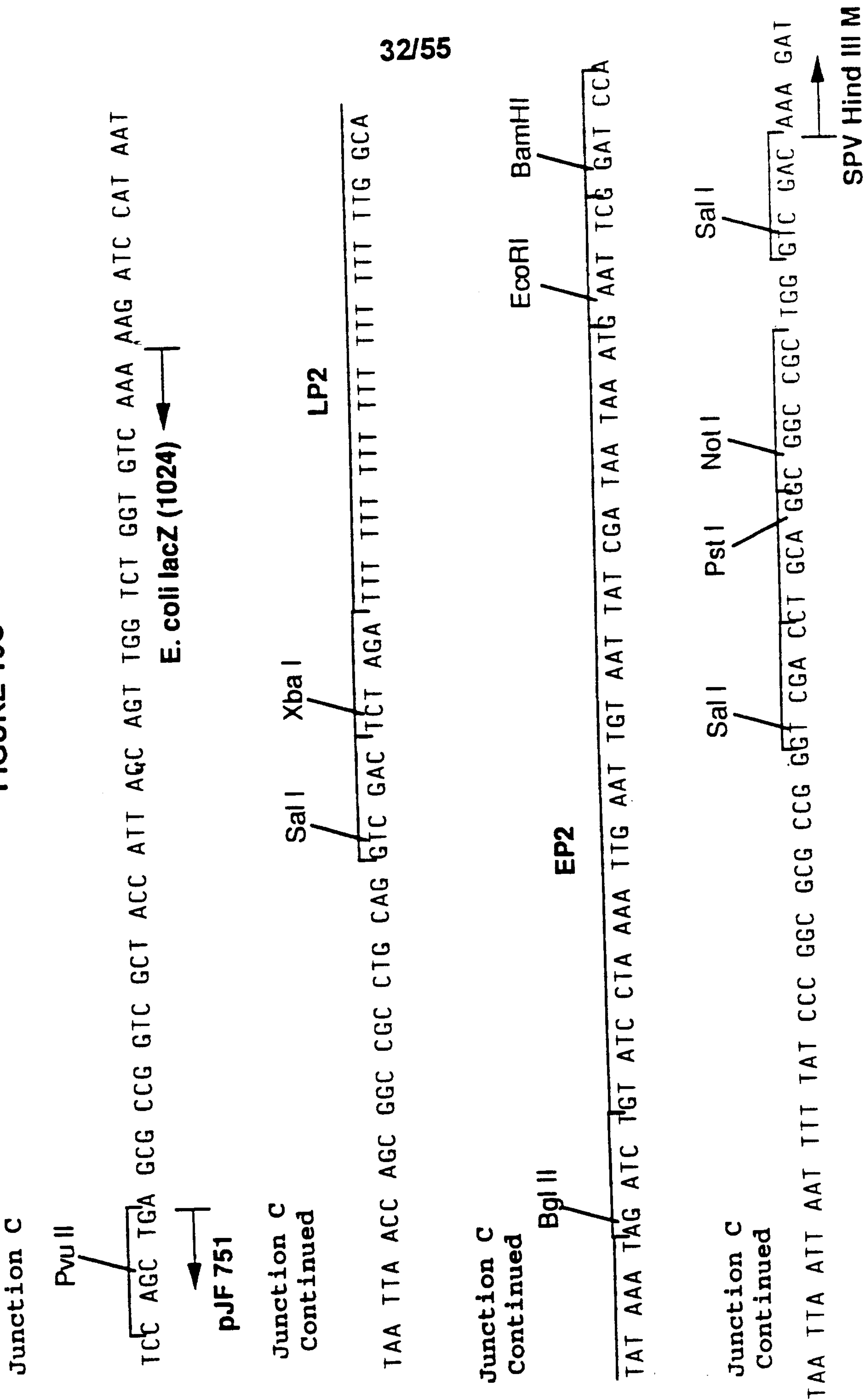


FIGURE 10D

Junction D

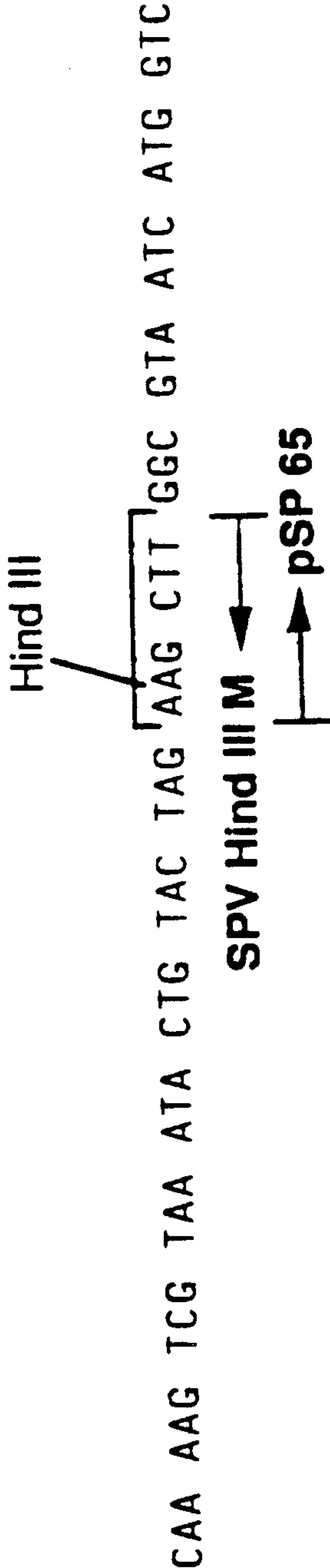


FIGURE 11A

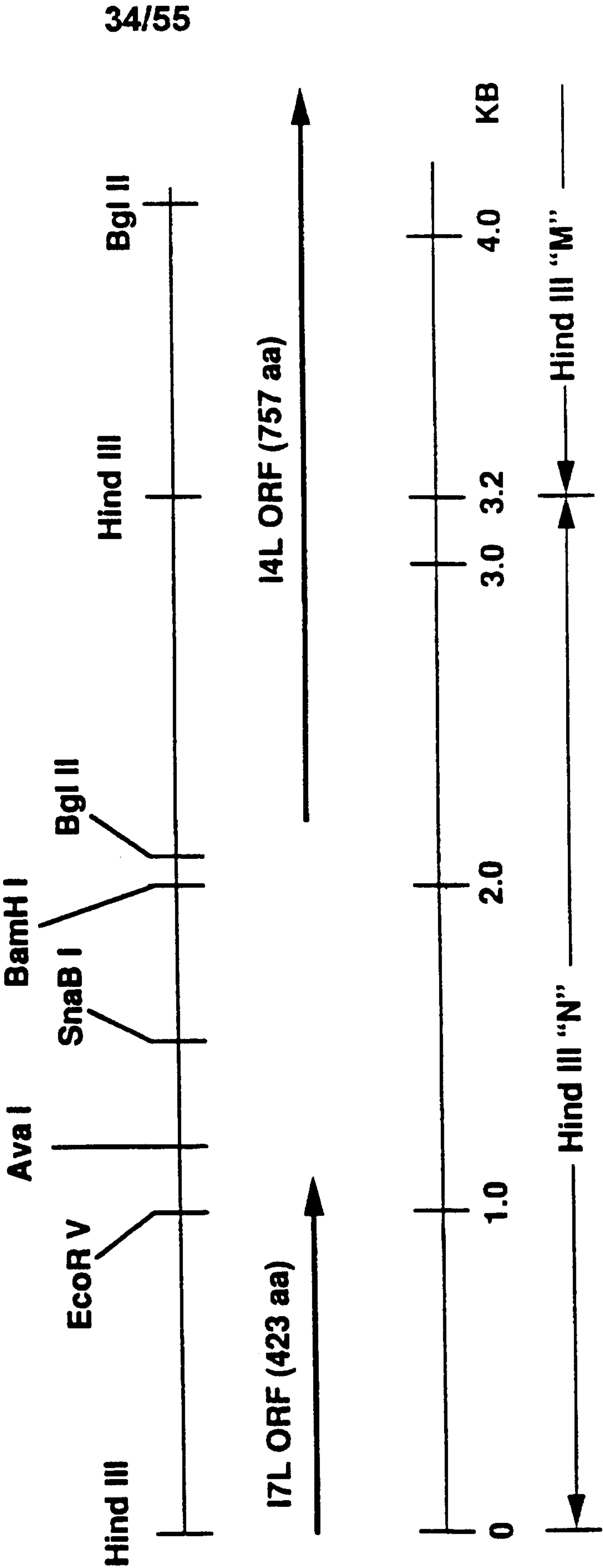


FIGURE 11B
SPV HindIII K Genomic Fragment
6.7 kb

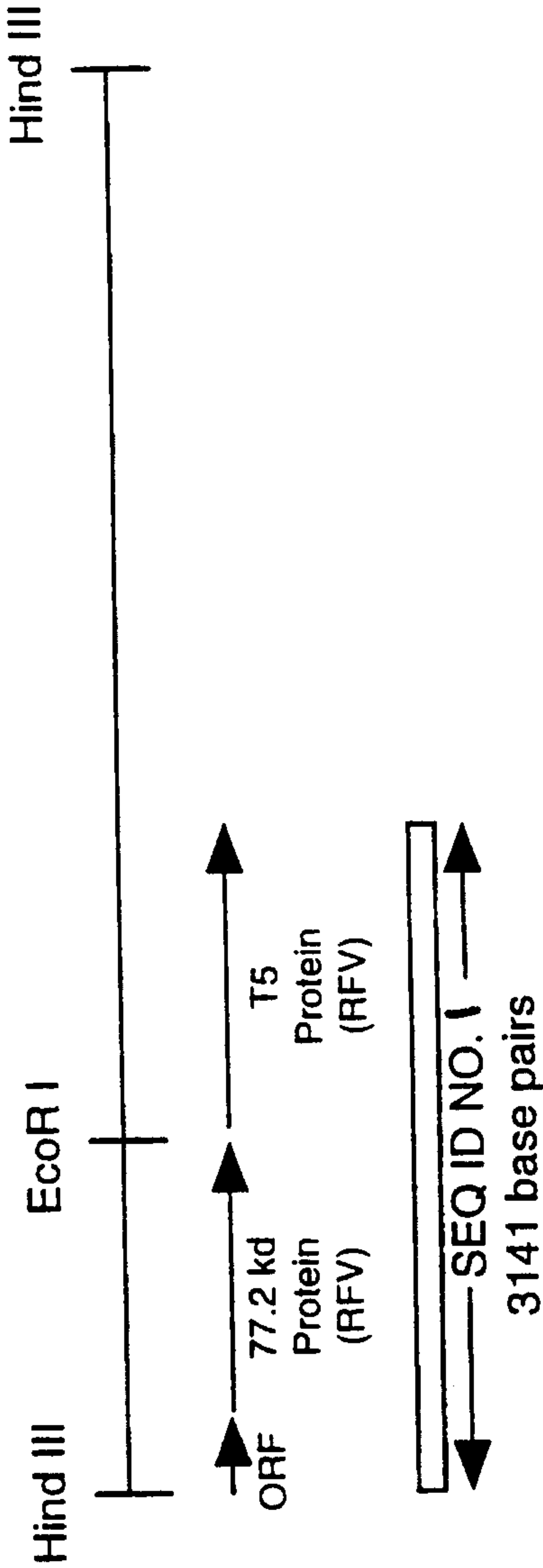


FIGURE 12A

DNA	Origin	Sites	Size
Vector	pSP64	Pst I-Hind III	~2986 BP
Fragment 1	SPV Hind III M	Hind III-Bgl II	~542 BP
Fragment 2	PRV Kpn I C	Sma I-Sac I	~3500 BP
Fragment 3	pJF751	Bam HI-Pvu II	~3010 BP
Fragment 4	SPV Hind III M	Bgl II-Pst I	~1180 BP

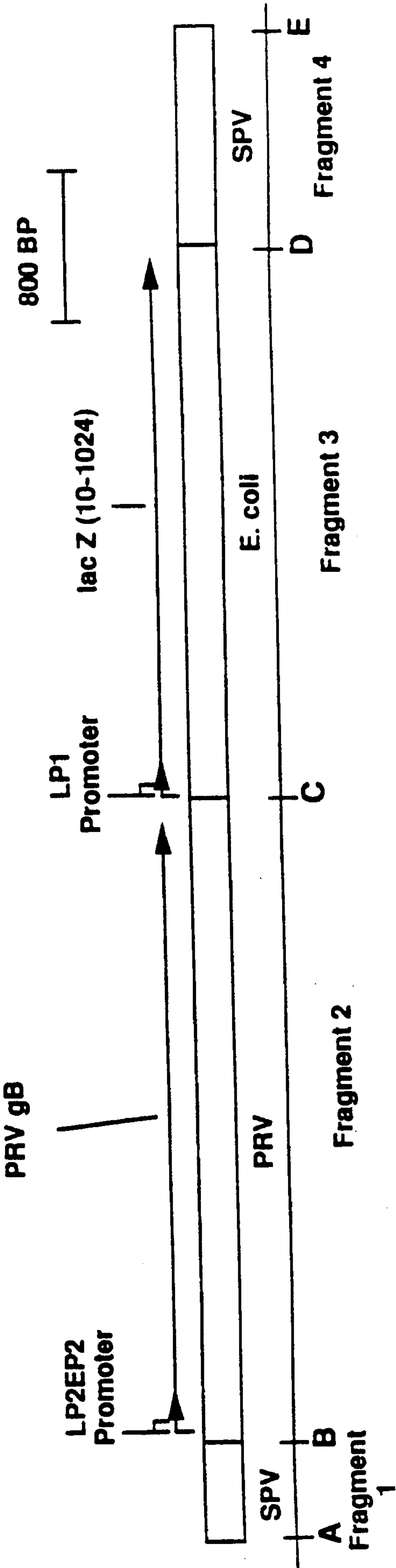
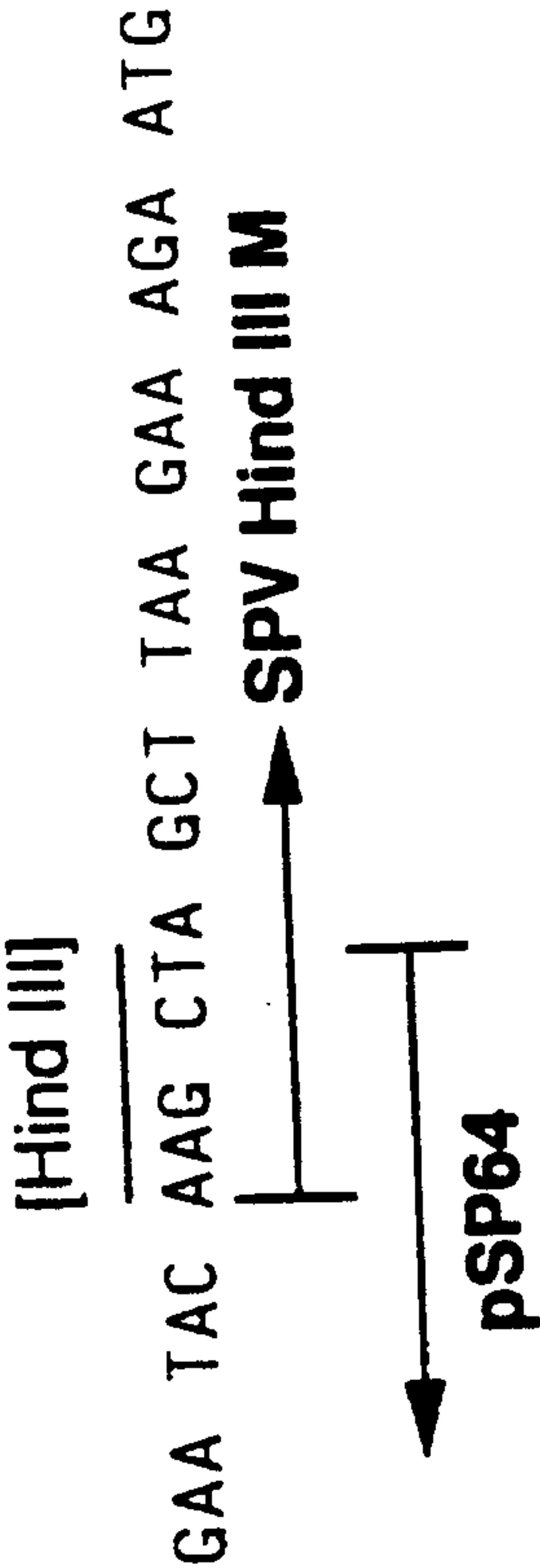


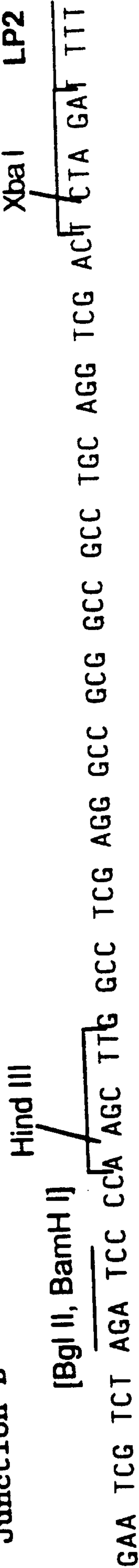
FIGURE 12B

779-94.31
SPV-047

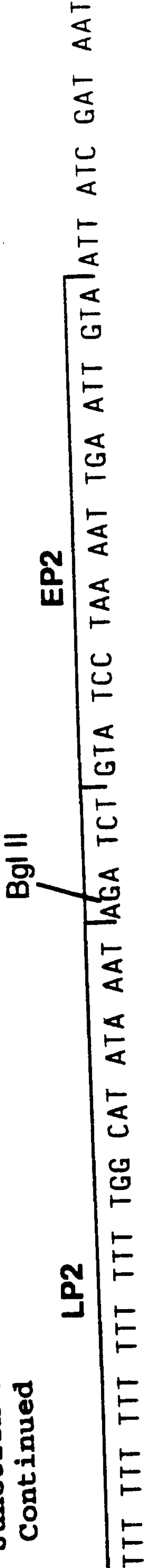
Junction A



Junction B



Junction B
Continued



Junction B
Continued

EcoR I



FIGURE 12C

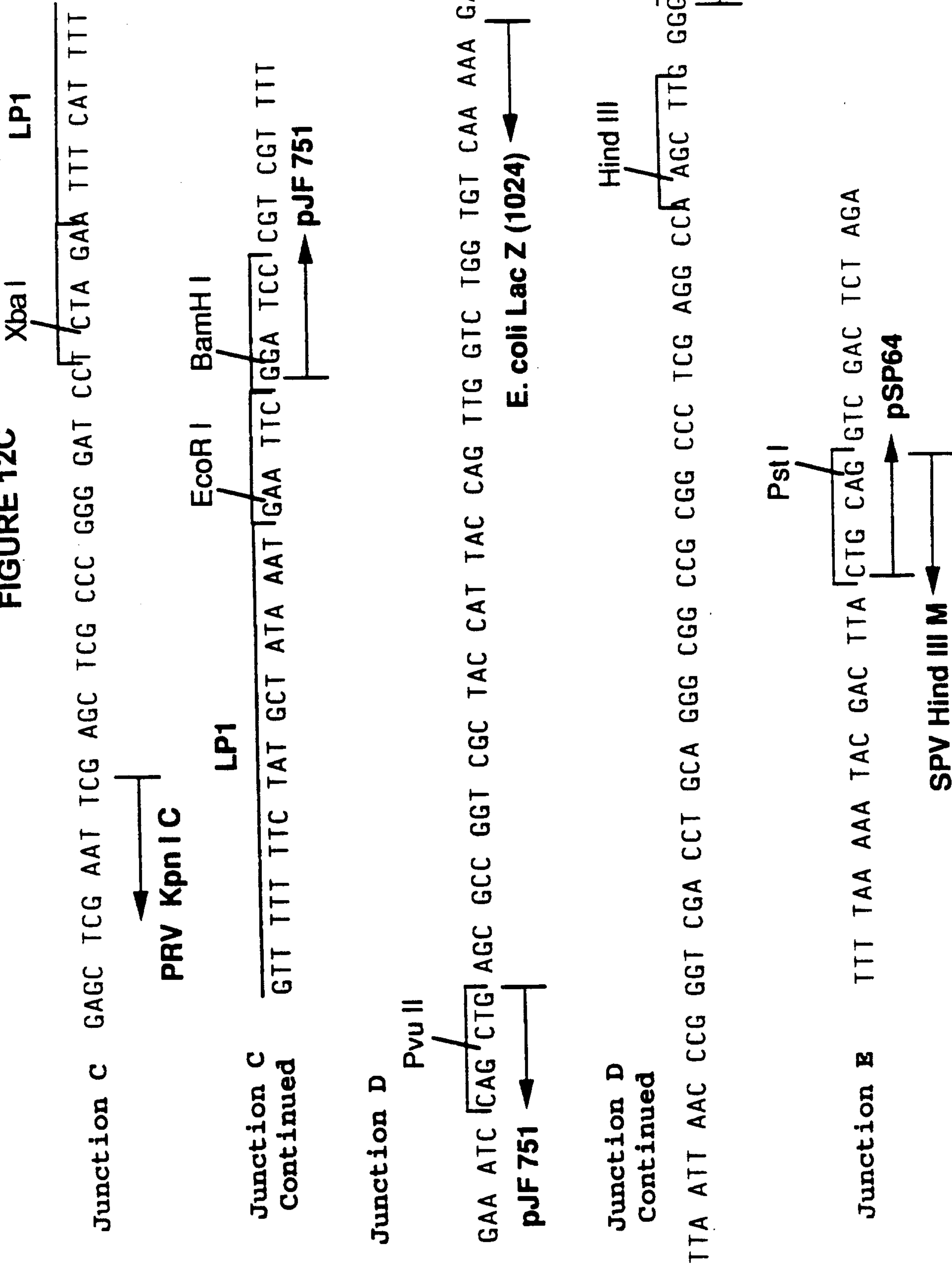


FIGURE 13A

DNA	Origin	Sites	Size
Vector	pSP64	Hind III-BamH I	~2972 BP
Fragment 1	SPV Hind III M	Bgl II-Acc I	~1484 BP
Fragment 2	PRV BamHI #7	EcoR I-Stu I	~1552 BP
Fragment 3	SPV Hind III M	Acc I-Nde I	~48 BP
Fragment 4	PRV Kpn I C	Sma I-EcoR I	~3500 BP
Fragment 5	pJF751	Bam HI-Pvu II	~3010 BP
Fragment 6	SPV Hind III M	Nde I-Hind III	~1560 BP

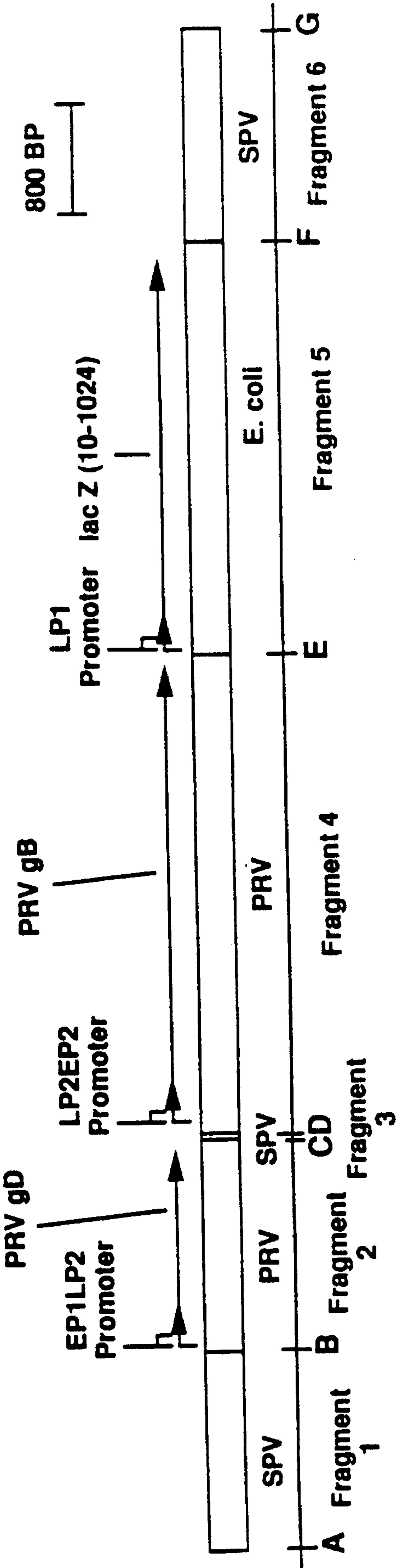
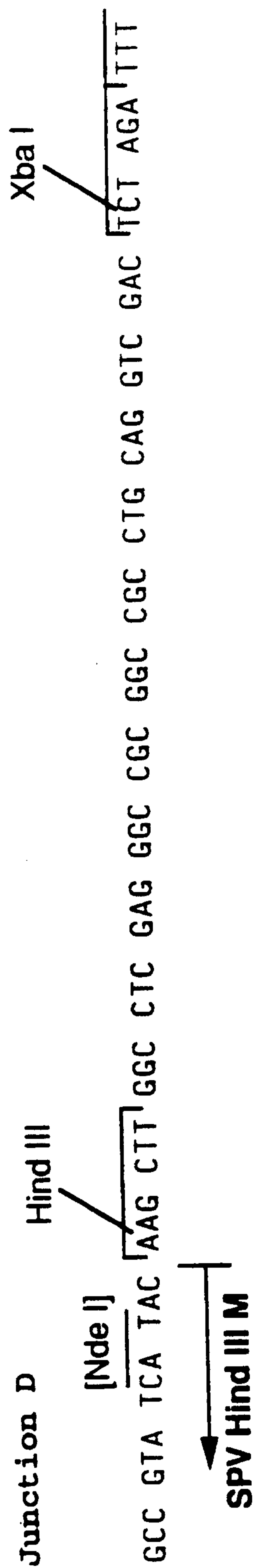


FIGURE 13C



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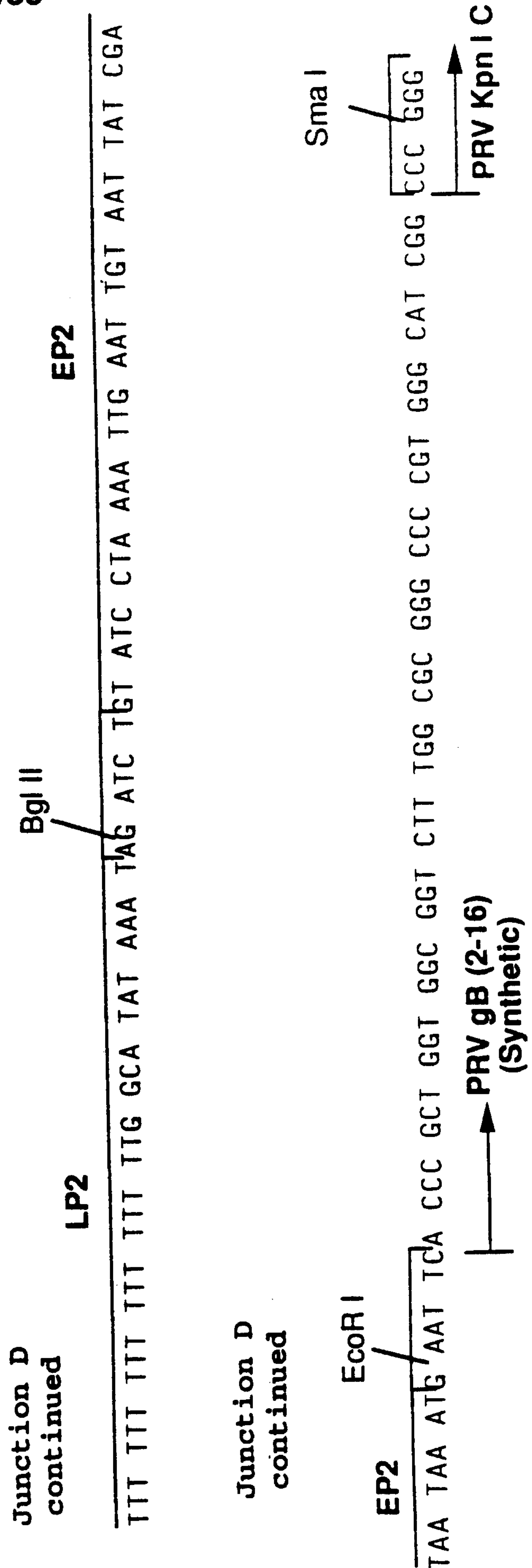


FIGURE 13D

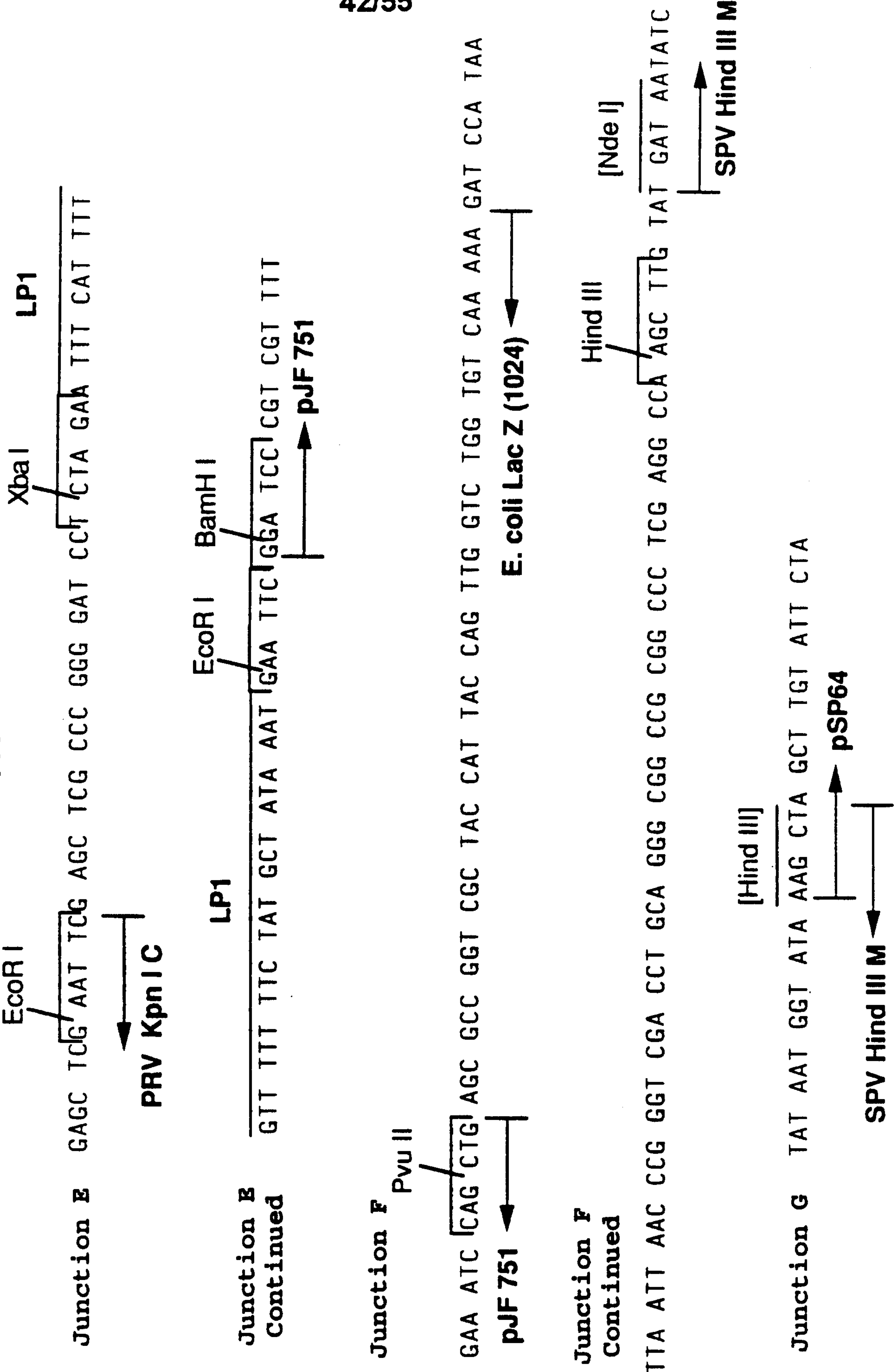
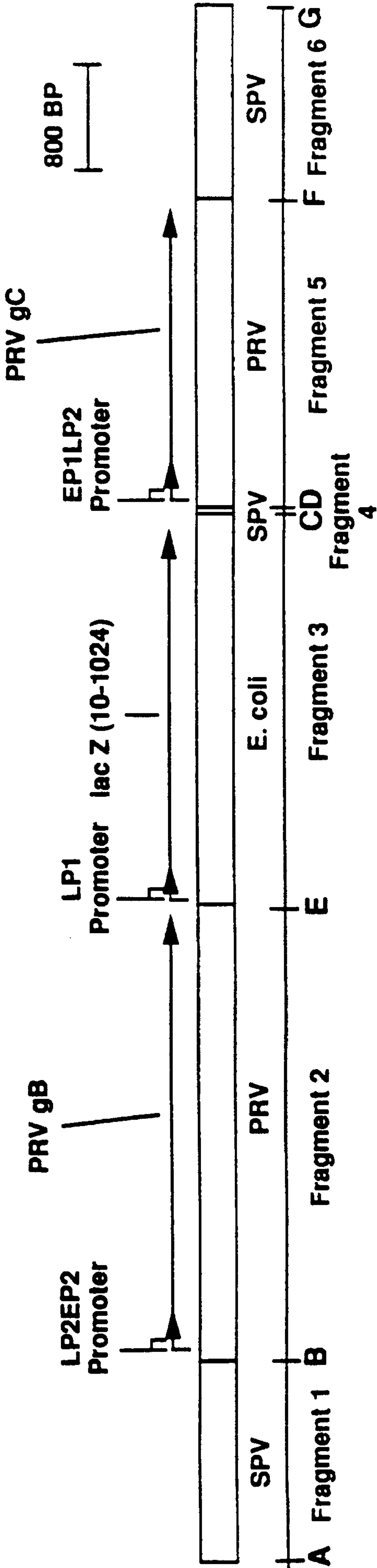


FIGURE 14A

DNA	Origin	Sites	Size
Vector	pSP64	Hind III-BamH I	~2972 BP
Fragment 1	SPV Hind III M	Hind III-Nde I	~1560 BP
Fragment 2	PRV Kpn I C	Sma I-EcoR I	~3500 BP
Fragment 3	pJF751	Bam HI-Pvu II	~3010 BP
Fragment 4	SPV Hind III M	Nde I-Acc I	~48 BP
Fragment 5	PRV BamH I #2 & #9	Nco I-Nco I	~2378 BP
Fragment 6	SPV Hind III M	Acc I-Bgl II	~1484 BP

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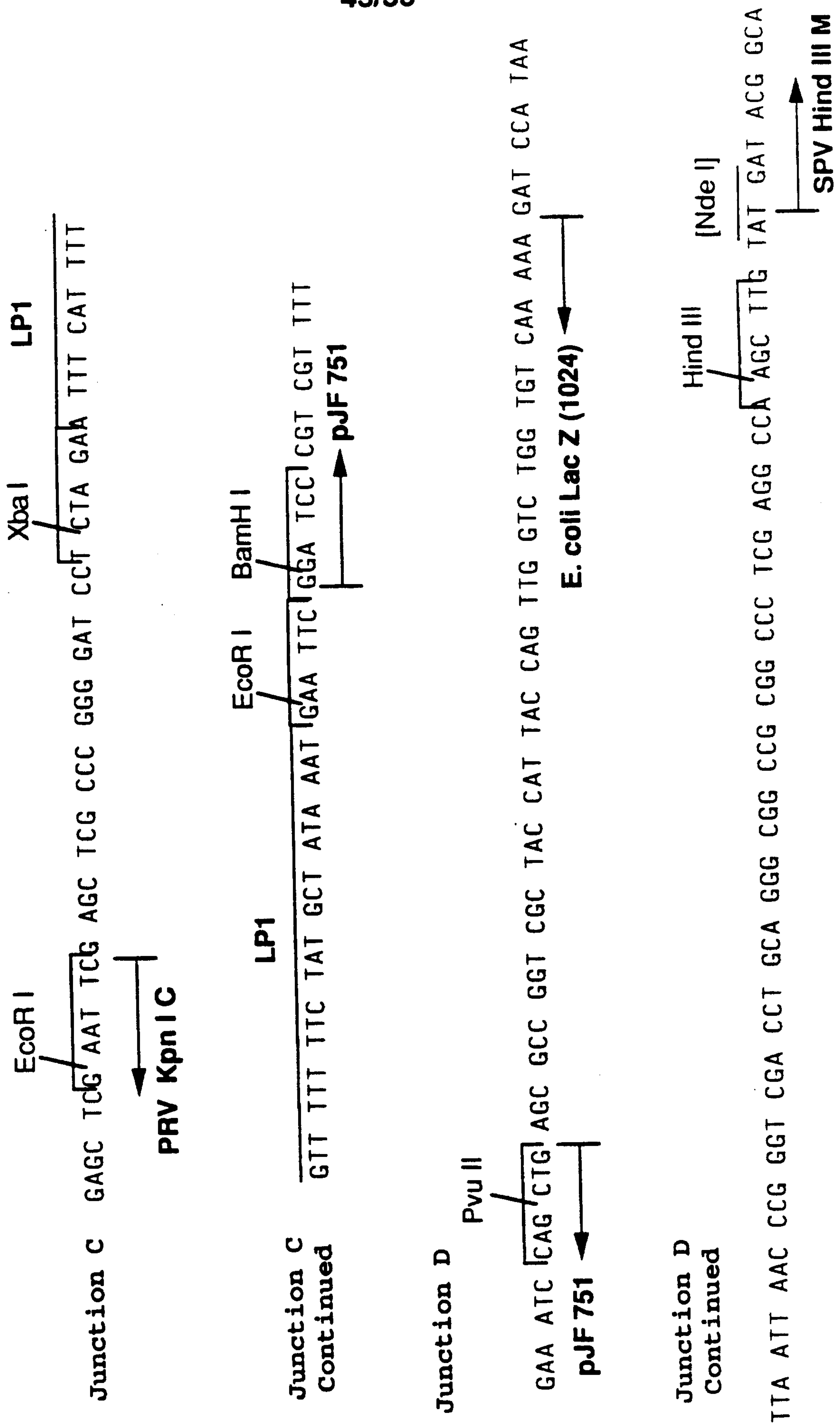


FIGURE 14D

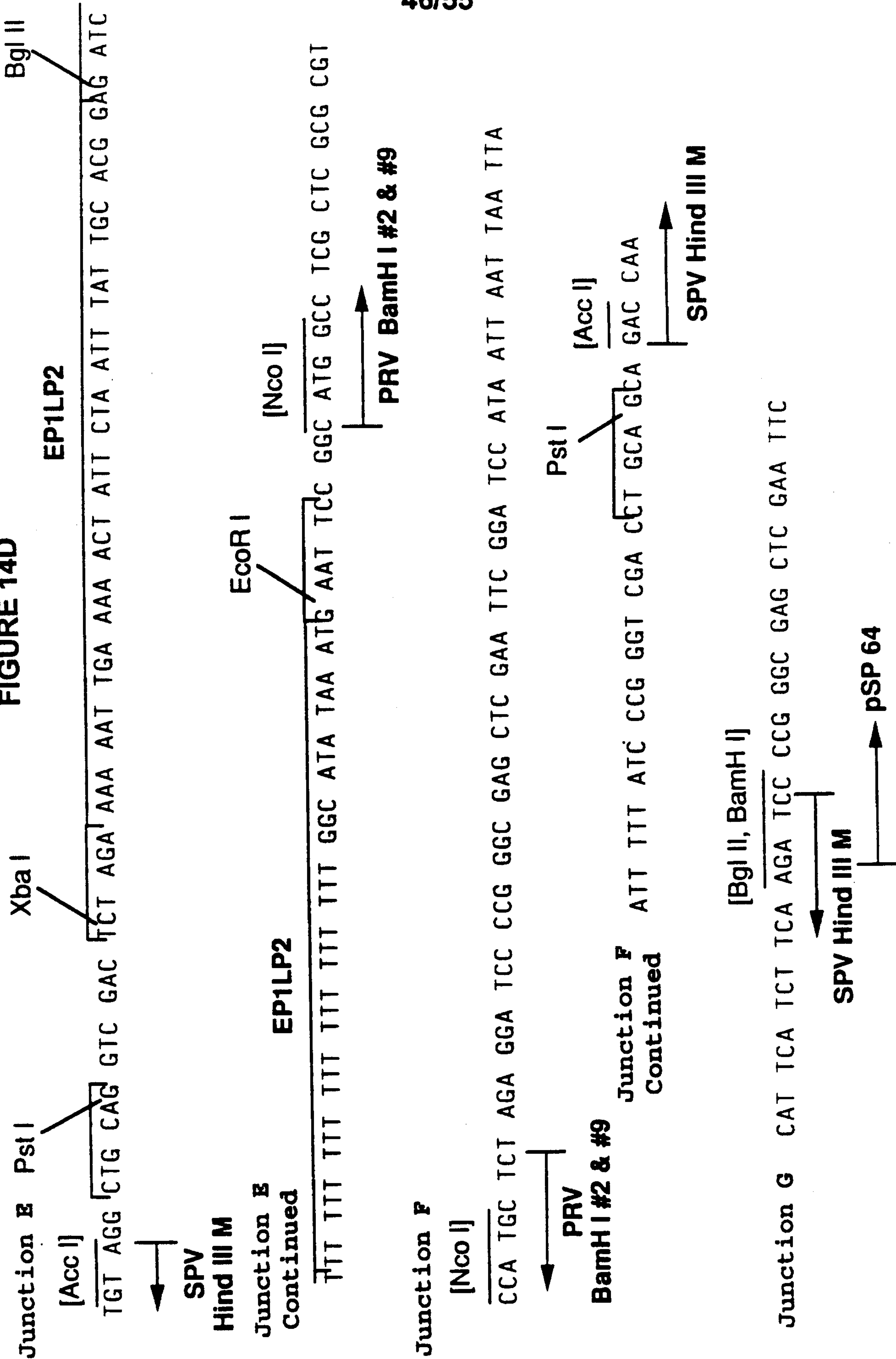


FIGURE 15A

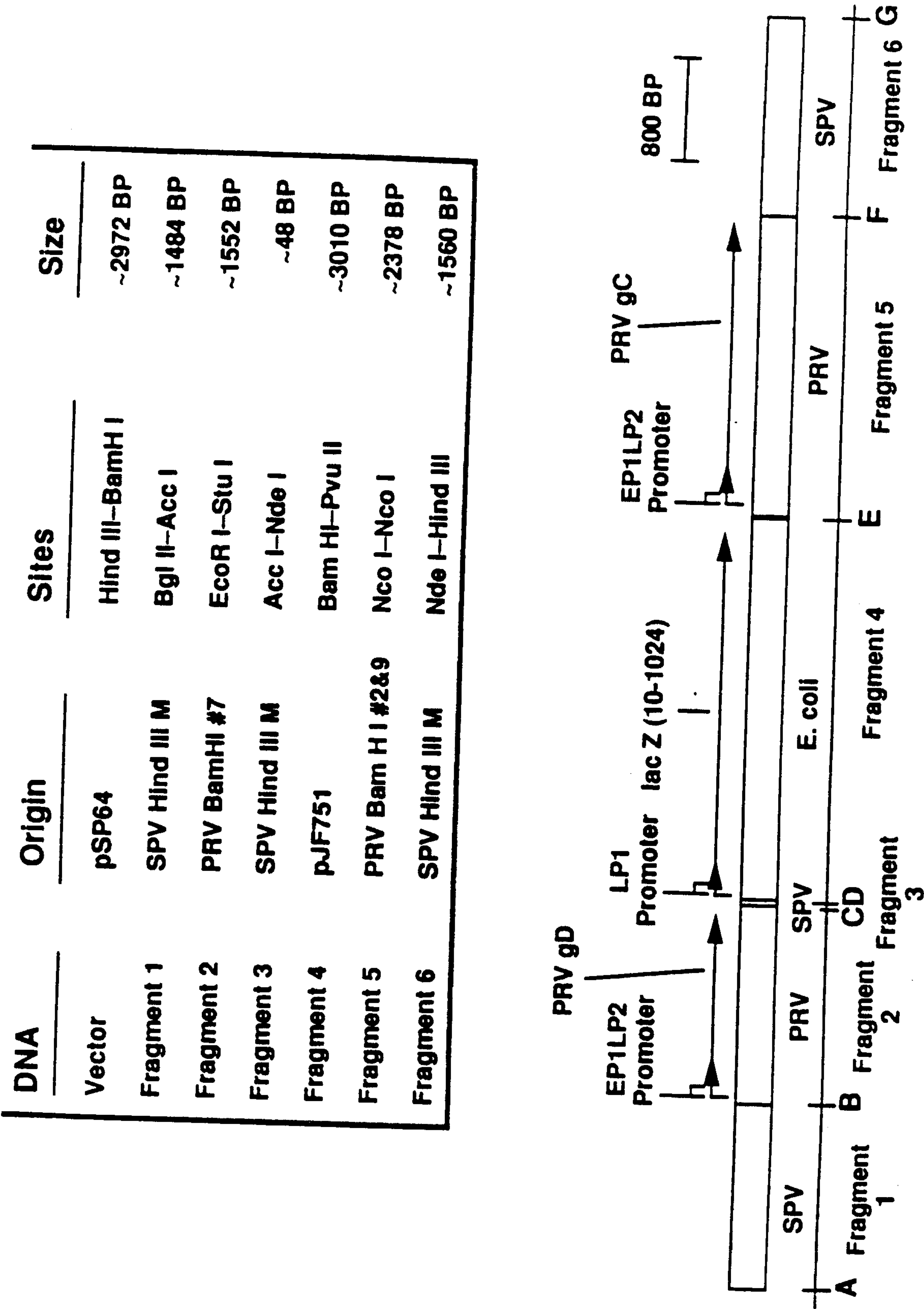


FIGURE 15B

789-41.47
SPV-054

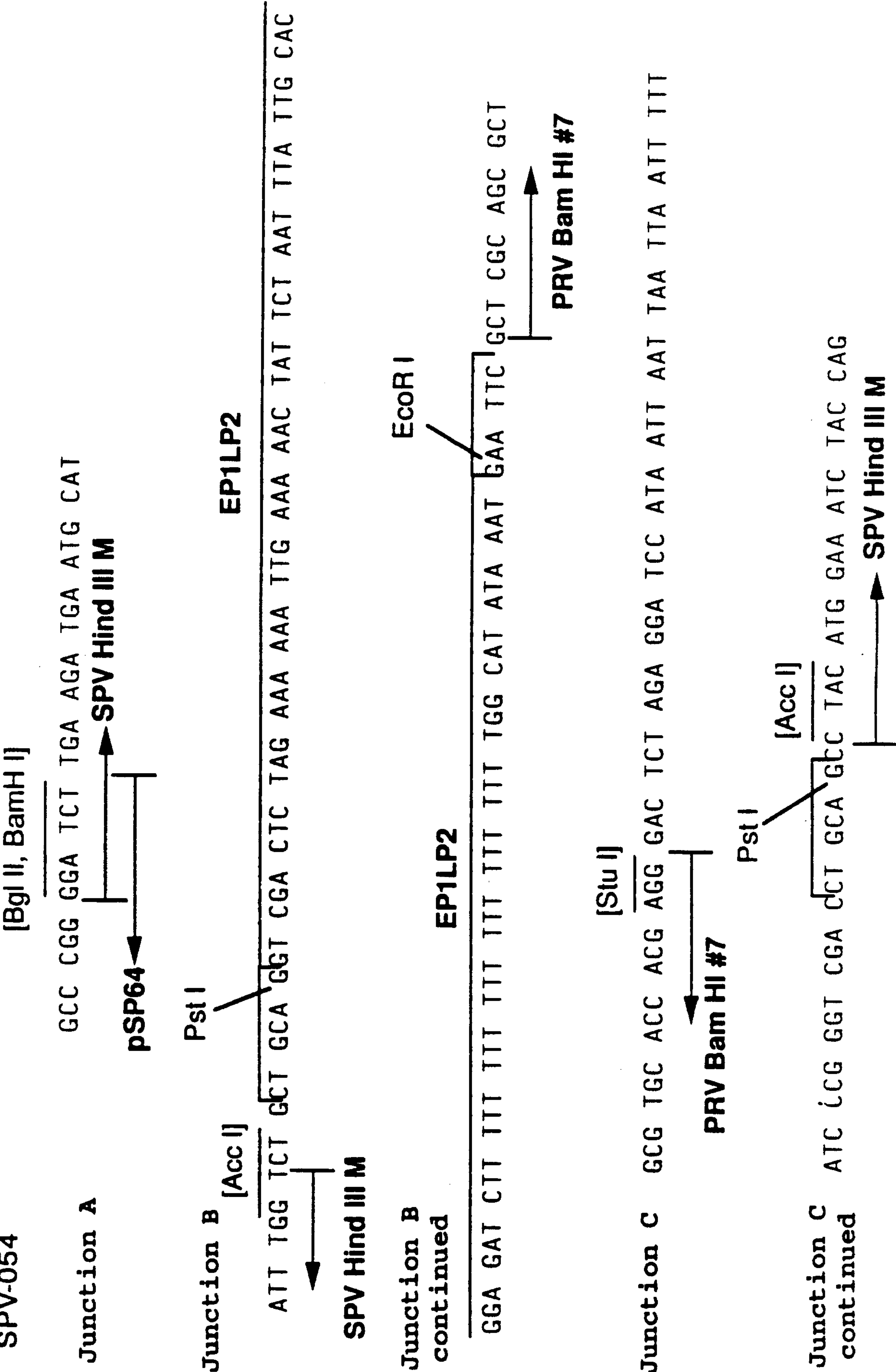


FIGURE 15C

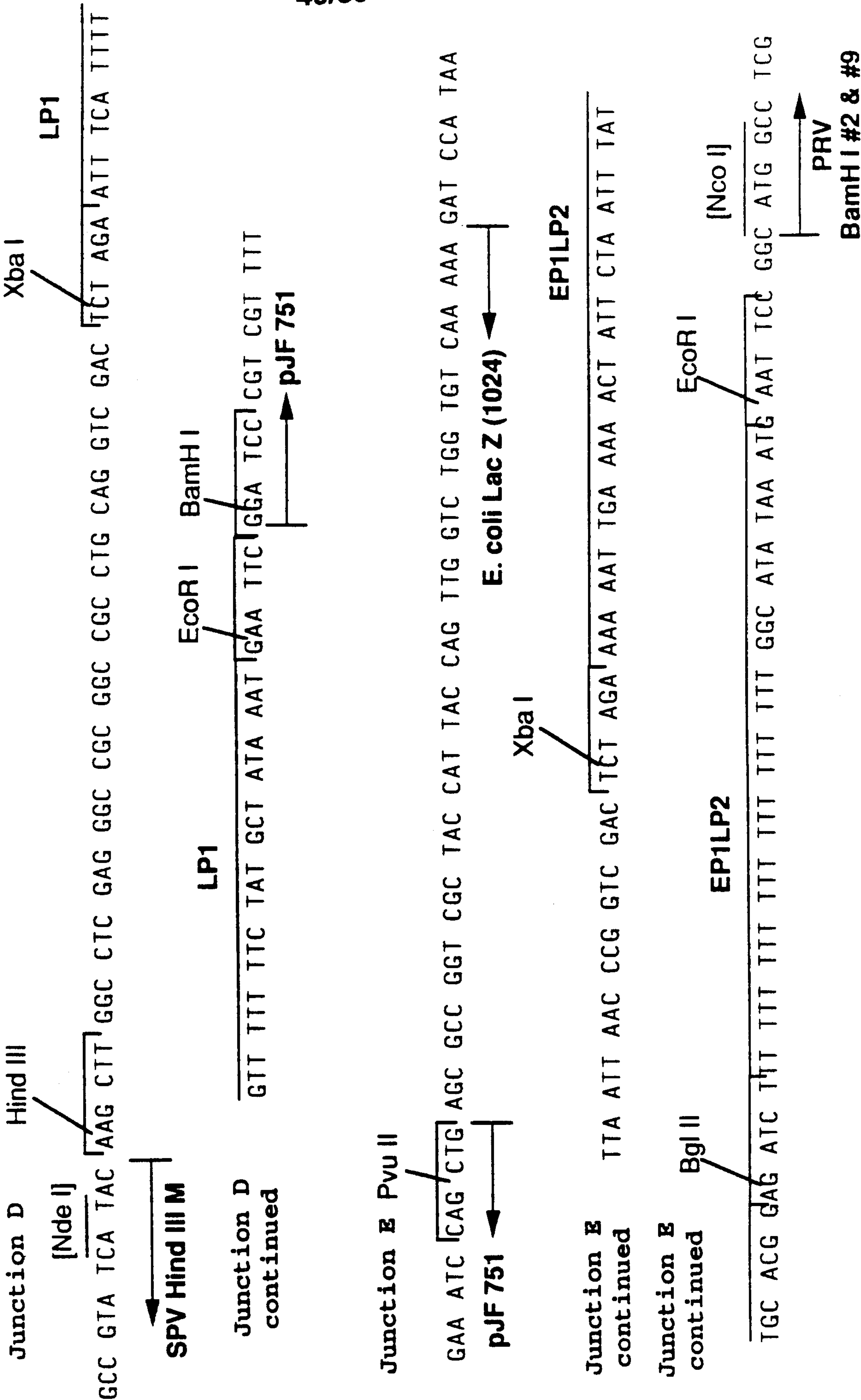


FIGURE 15D

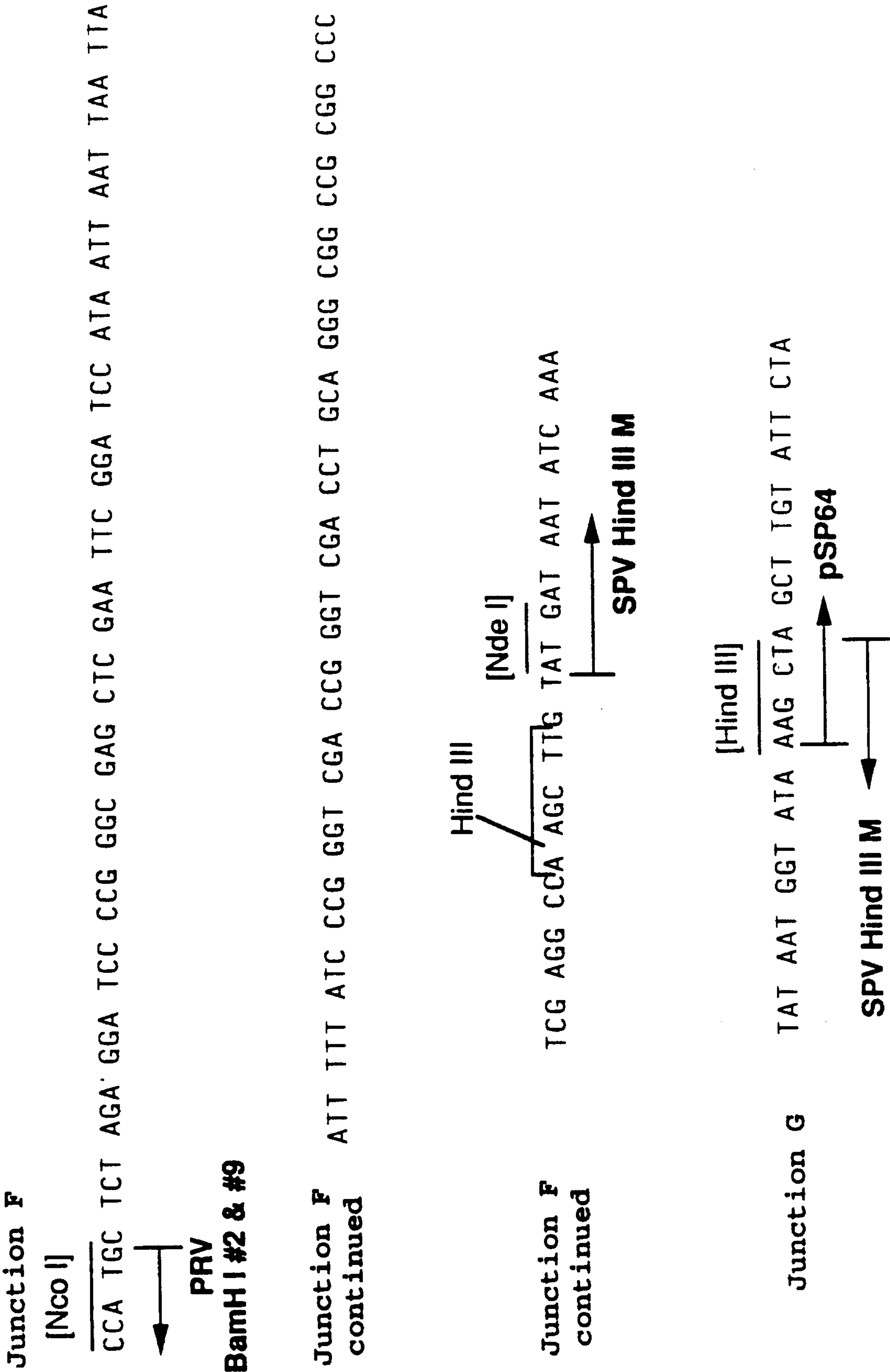
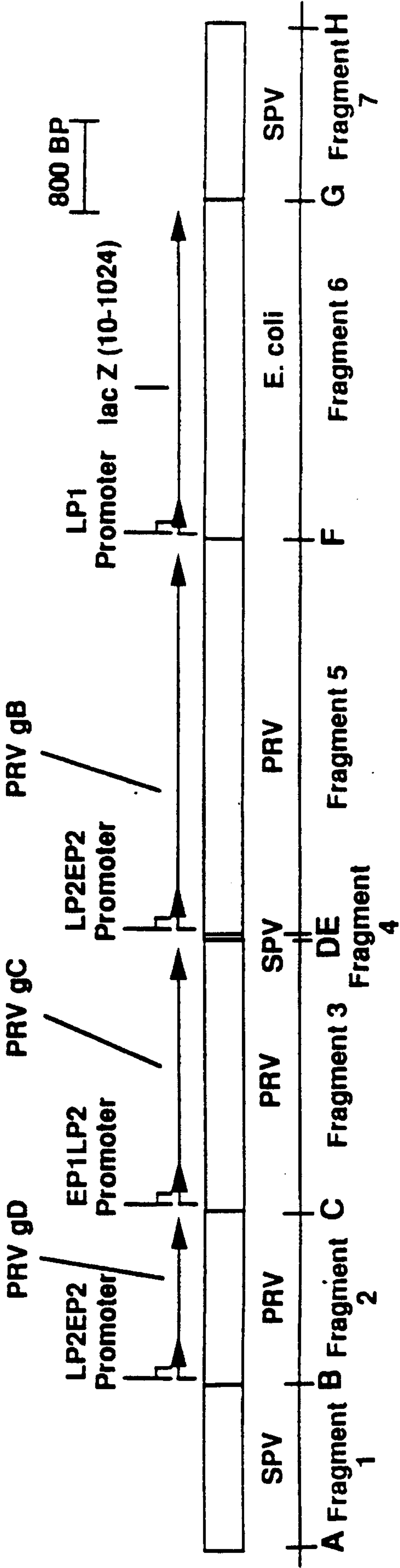


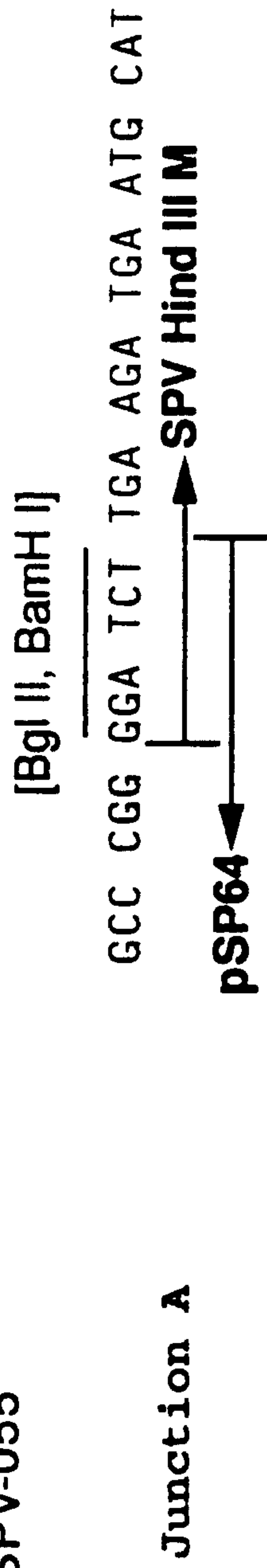
FIGURE16A

DNA	Origin	Sites	Size
Vector	pSP64	Hind III-BamH I	~2972 BP
Fragment 1	SPV Hind III M	Bgl II-Acc I	~1484 BP
Fragment 2	PRV BamHI #7	EcoR I-Stu I	~1552 BP
Fragment 3	PRV BamHI #2		Nco I-Nco I	~2378 BP
Fragment 4	SPV Hind III M	Acc I-Nde I	~48 BP
Fragment 5	PRV Kpn I C	Sma I-EcoR I	~3500 BP
Fragment 6	pJF751	Bam HI-Pvu II	~3010 BP
Fragment 7	SPV Hind III M	Nde I-Hind III	~1560 BP



789-41.73
SPV-055

FIGURE 16B



Junction B

[Acc I]

ATT TGG TCT GCT GCA GGT CGA CTC TAG ATT TTT TTT TTT TTT GGC ATA TAA ATA

←

SPV Hind III M

Pst I

LP2EP2

Junction B
continued

LP2EP2

EcoR I

GAT CTG TAT CCT AAA ATT GAA TTG TAA TTA TCG ATA ATA AAT GAA TTC GCT CGC AGC GCT

→

PRV Bam HI #7

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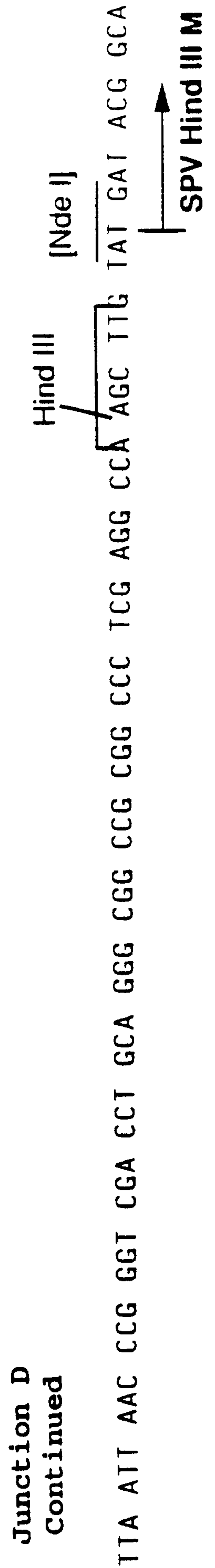


FIGURE 16D

Junction E

GCC GTA TCA TAC [Nde I] AAG CTT GGC CTC GAG GGC CGC GGC CTG CAG GTC GAC TCT AGA TTT

Hind III Xba I

SPV Hind III M

**Junction E
continued**

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Bgl II

LP2 EP2

TTT TTT TTT TTT TTG GCA TAT AAA TAG ATC TGT ATC CTA AAA TTG AAT TGT AAT TAT CGA

Junction E
continued

EP2 **EcoRI**

TAA TAA ATG AAT TCA CCC GCT GGT GGC GGT CTT TGG CGC GGG CCC CGT GGG CAT CGG

Sma I

CCC GGG

PRV gB (2-16)
(Synthetic)

PRV Kpn I C

FIGURE 16E

