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(54) **NOUVEAU POLYPEPTIDE; ADN CODANT LE POLYPEPTIDE;
VECTEUR RECOMBINANT PORTANT L'ADN ET VIRUS
RECOMBINANT UTILISANT LE VECTEUR RECOMBINANT;
SON UTILISATION**
(54) **A MYCOPLASMA GALLISEPTICUM ANTIGEN, DNA
ENCODING THE ANTIGEN, RECOMBINANT VECTOR
BEARING THE DNA AND RECOMBINANT VIRUS
UTILIZING THE RECOMBINANT VECTOR AS WELL AS USE
THEREOF**

(57) A polypeptide showing an antigenicity to Mycoplasma gallisepticum, a fused polypeptide of said polypeptide ligated at the N terminus thereof with a signal membrane anchor of type II external membrane polypeptide of virus that infects to fowl, or a polypeptide showing an antigenicity which is substantially pure and reactive with Mycoplasma-immunized sera or Mycoplasma-infected sera, and having an amino acid sequence of about 32 kilodaltons, about 40 kilodaltons or about 70 kilodaltons, expression thereof or a modified polypeptide to such an extent that shows an immunogenicity equivalent to that of said polypeptide using a recombinant virus, and use of the recombinant virus as a live vaccine.



ABSTRACT

A polypeptide showing an antigenicity to Mycoplasma gallisepticum, a fused polypeptide of said polypeptide ligated at the N terminus thereof with a signal membrane anchor of type II external membrane polypeptide of virus that infects to fowl, or a polypeptide showing an antigenicity which is substantially pure and reactive with Mycoplasma-immunized sera or Mycoplasma-infected sera, and having an amino acid sequence of about 32 kilodaltons, about 40 kilodaltons or about 70 kilodaltons, expression thereof or a modified polypeptide to such an extent that shows an immunogenicity equivalent to that of said polypeptide using a recombinant virus, and use of the recombinant virus as a live vaccine.

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A MYCOPLASMA GALLISEPTICUM ANTIGEN, DNA ENCODING
THE ANTIGEN, RECOMBINANT VECTOR BEARING THE DNA AND
RECOMBINANT VIRUS UTILIZING THE RECOMBINANT VECTOR
AS WELL AS USE THEREOF

5 The present invention relates to a novel
polypeptide showing antigenicity to Mycoplasma
gallisepticum, a fused polypeptide between the said
polypeptide and a signal membrane anchor, and a
recombinant Avipox virus capable of expressing a
10 polypeptide showing antigenicity to Mycoplasma
gallisepticum, especially a polypeptide showing
antigenicity on the membrane surface of a host cell, as
well as use thereof.

15 It is expected that a polypeptide showing
antigenicity to Mycoplasma gallisepticum can be utilized
as an effective ingredient of a vaccine for Mycoplasma
gallisepticum infections, since an egg-laying rate and a
hatching rate of eggs produced by infected chickens are
20 markedly reduced when infected with Mycoplasma
gallisepticum. At present, the system using Escherichia
coli or yeast is known to prepare the antigenic protein
of Mycoplasma gallisepticum by genetic engineering
(Japanese Patent Application Laid-Open No. 2-111795).

In general, it is pointed out that the production of a polypeptide in the system using bacteria involves problems that firstly an antigen is expressed in a less amount and secondly, a pyrogen originating in a host cannot be removed. It is thus the actual situation that such a system has not been practically applied yet. For this reason, studies have been made on the preparation of a polypeptide expressing an antigenicity or a recombinant live vaccine, using a recombinant virus.

10 However, as far as Mycoplasma gallisepticum is concerned, any recombinant virus inserted with DNA encoding said protein has not been prepared.

In a virus protein where the virus infects cells, one type of a protein expressed is transported to the cell surface and the protein is expressed on the surface of a cell membrane (hereinafter such a state is sometimes merely referred to as being expressed on the cell surface) and another type of a protein that is not expressed on the cell surface. A representative example of the former protein is a glycoprotein contained in the coat of a virus. A recombinant virus that expresses such a protein efficiently exhibits the protein on the cell surface. It is thus considered that a high antibody titer can be induced in poultry infected with this recombinant virus (Japanese Patent Application Laid-Open No. 1-157381). On the other hand, an example of the latter type of protein includes a protein originating in bacteria, such as an antigenic protein of

Mycoplasma gallisepticum.

It is not expectable to induce a high antibody titer from such recombinant viruses that express these proteins, since they are expressed on the cell membrane surface merely in an extremely small quantity. However, if such a protein can be expressed on the cell membrane surface in a large quantity by genetic engineering, a high antibody titer will be induced. Thus, investigations have been made to express on the membrane surface such a protein that is not principally expressed on the membrane surface. For example, there is a report that DNA encoding a signal protein having the function of secreting a protein on the cell membrane surface and DNA encoding a membrane anchor protein having the function of retaining the secreted protein so as not to leave out of the cell membrane surface are ligated with the 5' end and the 3' end of DNA encoding an antigenic protein, respectively, and a recombinant vaccinia virus inserted with the resulting hybrid DNA expresses the antigenic protein on the cell membrane surface of a host (J. Virol., 64, 4776-4783 (1990) or Mol. Cell. Biol., 6, 3191-3199 (1986)). However, DNA encoding a signal and DNA encoding a membrane anchor are independently ligated with DNA encoding an antigenic protein in these examples so that it is hardly applicable practically due to complicated preparation of a recombinant virus.

The present inventors have made extensive studies to provide a polypeptide having antigenicity originating in Mycoplasma and showing a high
5 antigenicity, a polypeptide having antigenicity to Mycoplasma gallisepticum expressed on the cell membrane surface especially in a large quantity, DNA encoding the polypeptide, a recombinant virus inserted with the same DNA and a vaccine utilizing the virus. As a result, the
10 present invention has come to be accomplished.

Fig. 1 shows a restriction enzyme map of DNA including the open reading frame of TM-81.

Fig. 2 shows the procedure for construction of
15 TTM-1N and TTM-1C.

Fig. 3 shows the procedure for constructing pNZ7929-R1.

Fig. 4 shows the procedure for constructing pNZ87N.

20 Fig. 5 shows the procedure for constructing pNZ7929-R2.

Fig. 6 (A) and (B) show the procedure for constructing pNZ2929XM1.

Fig. 7 shows a restriction enzyme map of DNA
25 including the open reading frame of TTM-1 polypeptide.

Fig. 8 shows a restriction enzyme map of DNA including the open reading frame of TM-67 polypeptide

and the position of synthetic primers on ORF.

Fig. 9 (A) and (B) show the procedure for constructing pHZ7929-67.

Fig. 10 shows a restriction enzyme map of DNA including the open reading frame of TM-66 polypeptide and the position of synthetic primers on ORF.

Fig. 11(A), 11(B) and 11(C) show the procedure for constructing pTM66.

Fig. 12 shows the procedure for constructing pNZ7929-66.

Fig. 13 shows a restriction enzyme map of DNA encoding the full length of TM-16 polypeptide.

Fig. 14 shows a restriction enzyme map of the open reading frame of TM-16 polypeptide.

15

A novel polypeptide which is a first aspect of the present invention and shows an antigenicity which originates in Mycoplasma gallisepticum having a high antigenicity, includes a polypeptide showing an antigenicity which causes an antigen-antibody reaction with sera immunized with Mycoplasma gallisepticum or sera and which is encoded by the DNA sequence having the restriction enzyme map shown in Fig. 7 originating in Mycoplasma gallisepticum, or a modified polypeptide thereof. Specific examples of the polypeptide having such an polypeptide include those showing an antigenicity and having amino acid sequences of SEQ ID

NOS: 1, 15, 16 and 27. The modified polypeptide showing an antigenicity referred to herein is a polypeptide in which the amino acid sequence is modified by substitution, loss, deletion, insertion or addition but
5 which shows an antigenicity comparable to that of the aforesaid polypeptide. Taking SEQ ID NO: 1 as an example, a modified polypeptide is used to mean a polypeptide having the same antigenicity as in an antigenic protein having the amino acid sequence
10 equivalent thereto and having a homology of at least 70% to the amino acid sequence of said polypeptide, preferably 80% or more, most preferably 90% or more. The homology referred to in the present invention is used to mean the homology determined as an index by DNA
15 sequencing input analysis system "DNASIS" (marketed by Takara Shuzo Co.).

Hereinafter a sequence number is sometimes simply referred to as sequence in the specification. For example, Sequence No. 1 is sometimes referred to as
20 Sequence 1.

Furthermore, the DNA which encodes the polypeptide showing an antigenicity used in the present invention includes DNA encoding a polypeptide in which the amino acid is modified by deletion, addition,
25 insertion, loss, substitution, etc., so long as it causes an antigen-antibody reaction with sera immunized with Mycoplasma gallisepticum or sera and shows an antigenicity originating in Mycoplasma gallisepticum or

an antigenicity equivalent thereto.

Avipox virus which is a second aspect of the present invention is a recombinant Avipox virus inserted with a hybrid DNA in which DNA encoding the antigenic
5 polypeptide of Mycoplasma gallisepticum (hereinafter abbreviated as antigenic DNA) or DNA encoding a signal membrane anchor of Type II external membrane protein is ligated with DNA encoding a polypeptide showing an antigenicity of Mycoplasma gallisepticum. In order to
10 express large quantities of the polypeptide showing an antigenicity of Mycoplasma gallisepticum that is not basically expressed on the surface of cell membrane, it is preferred to employ the hybrid DNA.

That is, in the second aspect of the present
15 invention, there are provided a polypeptide showing an antigenicity of Mycoplasma gallisepticum (hereinafter sometimes merely referred to as antigenic protein), a fused polypeptide ligated at the N terminus of the polypeptide with a signal membrane anchor of type II
20 outer membrane protein of a virus infected to poultry (hereinafter merely referred to as signal membrane anchor), a vaccine against Mycoplasma gallisepticum infections comprising as an effective ingredient the antigenic protein or the fused polypeptide, a hybrid DNA
25 which encodes the fused polypeptide, a recombinant Avipox virus inserted into the genomic region non-essential to growth of Avipox virus (hereinafter referred to as non-essential region) with DNA encoding

the antigenic protein or the hybrid DNA, and a live vaccine against Mycoplasma gallisepticum which comprises the Avipox virus as an effective ingredient.

The signal membrane anchor which is employed
5 in the present invention as the second aspect is a polypeptide region having the function of transporting type II external membrane protein of a virus infected to poultry to the surface of cell membrane and expressing the transported protein on the surface of cell membrane,
10 and is preferably derived from a virus which is non-pathogenic to human. The DNA encoding the signal membrane anchor which is employed in the present invention (hereinafter referred to as signal membrane anchor DNA) can be readily found by amino acid sequencing analysis of the hydrophobic peptide region of type
15 II external membrane protein at the amino terminus. A specific example of the signal membrane anchor is that having the sequence shown by SEQ ID NO: 13 (Mol. Cell. Biol., 10, 449-457 (1990)). This DNA codes for 22 amino
20 acids at the amino terminus of hemagglutinin neuraminidase (hereinafter abbreviated as HN protein) of Newcastle disease virus (hereinafter abbreviated as NDV).

In order to stably exhibit the expressed
25 antigenic protein on the cell membrane, it is effective for a hydrophilic peptide to be present at the carboxy terminal of the signal membrane anchor. Accordingly, it is preferred that DNA encoding a hydrophilic peptide be

added downstream the signal membrane anchor DNA. DNA to be added comprises base pairs corresponding to 10 to 50 amino acids, preferably 20 to 30 amino acids.

Specific examples of the DNA encoding the antigenic protein in accordance with the present invention include, in addition to the four sequences as the first aspect of the present invention, DNA described in Japanese Patent Application Laid-Open No. 1-111795, a genomic DNA fragment of Mycoplasma gallisepticum containing the aforementioned DNA, DNA (hereinafter referred to as TTM-1) encoding a polypeptide of about 40 kilodaltons showing an antigenicity and having the sequence shown by SEQ. ID NO: 14 (hereinafter referred to as TTM-1' polypeptide), DNA derived from natural Mycoplasma gallisepticum substantially equivalent to TTM-1' (hereinafter referred to as TTM-1), and the like. The TTM-1 and 1' are disclosed in WO 93/24646. The DNA encoding the antigenic protein may also be DNA encoding such a polypeptide that a part of the sequence is modified by substitution, loss, deletion, insertion, addition, etc. as long as it retains an antigenicity substantially equivalent to that of the antigenic protein encoded by the nucleotide sequence.

Sources for collecting such a DNA may be any of the sources so long as they belong to Mycoplasma gallisepticum. Specific examples include S6 strain (ATCC 15302), PG31 (ATCC 19610) and the like.

The hybrid DNA which is used in the present

invention as its second aspect is the aforesaid signal membrane anchor DNA ligated with DNA encoding a polypeptide showing an antigenicity. The fused polypeptide of the present invention is a polypeptide
5 encoded by the hybrid DNA described above which contains a part of the signal membrane anchor and a part of the polypeptide showing an antigenicity in the molecule of the polypeptide. The hybrid DNA can be produced in a conventional manner, e.g., by modifying the 3' end of
10 the signal membrane anchor DNA and the 5' end of the DNA encoding the antigenic protein so as to form ligatable restriction enzyme digestion fragments, and ligating both DNAs according to the method for ligation using a ligase or the method for ligating both DNAs with a
15 ligase by inserting an appropriate linker therebetween. The signal membrane anchor and the DNA encoding the polypeptide showing an antigenicity may contain therebetween, for example, DNA encoding a hydrophilic peptide, DNA encoding other antigenic protein, linker
20 DNA, etc., so long as the signal membrane anchor DNA and the DNA encoding the polypeptide showing an antigenicity are expressed as one polypeptide. The fused polypeptide of the present invention is obtained by incubating a recombinant Avipox virus, later described, in culture
25 cells such as chick embryo fibroblast (hereinafter referred to as CEF cells) or embryonated chorioallantoic membrane cells, etc., and purifying the desired polypeptide by a method optionally chosen from

chromatography, precipitation by salting-out, density gradient centrifugation, etc. The fused polypeptide thus obtained can be used as a component vaccine which will be later described.

5 The recombinant Avipox virus of the present invention is a recombinant Avipox virus in which the aforesaid DNA or hybrid DNA is inserted in the non-essential region. The recombinant Avipox virus of the present invention may be constructed in a conventional
10 manner, e.g., by the method described in Japanese Patent Application Laid-Open No. 1-168279. That is, the non-essential region of Avipox virus is incorporated into a DNA fragment, if necessary, inserted with a promoter in the non-essential region, to construct a first
15 recombinant vector.

 As the non-essential region of Avipox virus which is used in the present invention, there are a TK gene region of quail pox virus, a TK gene region of turkey pox virus and DNA fragments described in Japanese
20 Patent Application Laid-Open 1-168279, preferably a region which causes homologous recombination with EcoRI fragment of about 7.3 Kbp, HindIII fragment of about 5.2 Kbp, EcoRI-HindIII fragment of about 5.0 Kbp, BamHI fragment of about 4.0 Kbp, described in the patent
25 specification supra.

 Examples of the vector used in the present invention include plasmids such as pBR322, pBR325, pBR327, pBR328, pUC7, pUC8, pUC9, pUC19, and the like;

phages such as λ phage, M13 phage, etc.; cosmid such as pHC79 (Gene, 11, 291, 1980) and the like.

The Avipox virus used in the present invention is not particularly limited so long as it is a virus
5 infected to poultry. Specific examples of such a virus include pigeon pox virus, fowl pox virus (hereafter abbreviated as FPV), canary pox virus, turkey pox virus, preferably turkey pox virus, pigeon pox virus and FPV, more preferably pigeon pox virus and FPV. Specific
10 examples of the most preferred Avipox virus include FPVs such as ATCC VR-251, ATCC VR-249, ATCC VR-250, ATCC VR-229, ATCC VR-288, Nishigahara strain, Shisui strain, CEVA strain and a viral strain among CEVA strain-derived viruses which forms a large plaque when infected to
15 chick embryo fibroblast, and a virus such as NP strain (chick embryo-conditioned pigeon pox virus Nakano strain), etc. which is akin to FPV and used as a fowlpox live vaccine strain. These strains are commercially available and readily accessible.

20 Then, the aforesaid antigenic DNA or hybrid DNA is inserted into the non-essential region of the first recombinant vector described above to construct a second recombinant vector. Where the hybrid DNA is employed, a promoter is generally inserted upstream the
25 hybrid DNA. The promoter used may be a promoter having any nucleotide sequence, irrespective of a synthetic or natural promoter, as far as it effectively functions as a promoter in the system of transcription possessed by

APV. Accordingly, not only a promoter inherent to APV such as a promoter of APV gene encoding thymidine kinase but also DNA derived from viruses other than APV and DNA derived from eucaryote or procaryote may also be employed in the present invention, as long as these substances meet the requirements described above. Specific examples of such a promoter include a promoter of vaccinia virus (hereinafter sometimes abbreviated as VV) described in J. Virol., 51, 662-669 (1984), more specifically a promoter of VV DNA encoding 7.5 K polypeptide, a promoter of VV DNA encoding 19 K polypeptide, a promoter of VV DNA encoding 42 K polypeptide, a promoter of VV DNA encoding thymidine kinase, a promoter of VV DNA encoding 28 K polypeptide, etc. Furthermore, there may be used a synthetic promoter obtained by modification of the Moss et al. article (J. Mol. Biol., 210, 749-776, 771-784, 1989), a promoter synthesized by Davidson, a promoter obtained by modifying a part of the Davidson promoter through deletion or change within such a range that does not lose the promoter activity (e.g., T T T T T T T T T T T T T G G C A T A T A A A T A A T A A T A A A T A C A A T A A T T A A T T A C G C G T A A A A A T T G A A A A C T A T T C T A A T T T A T T G C A C T C or T T T T T T T T T T T T T T T T T T T T T T T G G C A T A T A A A T A A T A A A T A C A A T A A T T A A T T A C G C G T A A A A A T T G A A A A A C T A T T C T A A T T T A T T G C A C T C etc.).

Further in view of easy detection of the recombinant virus, a marker DNA such as DNA encoding β -galactosidase may also be inserted.

The recombinant Avipox virus may be
5 constructed by transfecting the second recombinant vector described above to animal culture cells previously infected with Avipox virus and causing homologous recombination between the vector DNA and the viral genome DNA. The animal culture cells used herein
10 may be any cells in which Avipox can grow. Specific examples of such animal culture cells are CEF cells, embryonated egg chorioallantoic membrane cells, and the like.

The desired recombinant Avipox virus is
15 isolated from the virus infected to host cells by the method of plaque hybridization, etc. The recombinant Avipox virus may be further purified by plaque assay, etc.

The recombinant virus of the present invention
20 constructed by the method described above can be inoculated to fowl as a live vaccine against Mycoplasma gallisepticum infection.

The live vaccine of the present invention is prepared by, e.g., the following method, though the
25 process is not particularly limited. The recombinant virus of the present invention is infected to cells in which the virus can grow (hereafter referred to as host cells). After the recombinant virus grows, the cells

are recovered and homogenated. The homogenate is centrifuged with a centrifuging machine to separate into the precipitates and the high titer supernatant containing the recombinant virus in a centrifuging tube.

5 The resulting supernatant is substantially free of host cells but contains the cell culture medium and the recombinant virus and hence can be used as a live vaccine. The supernatant may be diluted by adding a pharmacologically inactive carrier, e.g., physiological

10 saline, etc. The supernatant may be freeze-dried to be provided for use as a live vaccine. A method for administration of the live vaccine of the present invention is not particularly limited and examples of the administration include a method for scratching the

15 skin and inoculating the live vaccine on the scratch, effecting the inoculation through injection, oral administration by mixing the live vaccine with feed or drinking water, inhalation by aerosol or spray, etc. In order to use as the live vaccine, the dosage may be the

20 same as ordinary live vaccine; for example, approximately 10^2 to 10^8 plaque forming unit (hereinafter abbreviated as PFU) is inoculated per chick. Where the inoculation is effected by injection, the recombinant virus of the present invention is generally suspended in

25 about 0.1 ml of an isotonic solvent such as physiological saline and the resulting suspension is provided for use. The live vaccine of the present invention may be stored under ordinary conditions and provided for use.

For example, when the recombinant virus of the present invention is freeze-dried, it is possible to store at room temperature (20 to 22°C). It is also possible to freeze the virus suspension at -20 to -70°C and store
5 the frozen suspension.

On the other hand, the component vaccine of the present invention comprises as an effective ingredient the polypeptide showing an antigenicity in accordance with the present invention, especially the
10 fused polypeptide. The component vaccine may be administered to fowl in the same manner as in the live vaccine described above. The dose is generally in the range of approximately 1 µg to 1 mg per one subject.

According to the present invention, the
15 polypeptide showing an Mycoplasma gallisepticum antigenicity and the fused polypeptide between the said polypeptide and the signal membrane anchor are obtained. In particular, this fused polypeptide is effective as a vaccine against Mycoplasma gallisepticum infections.
20 By utilizing DNA encoding the fused protein, the recombinant Avipox virus which can express the polypeptide showing an Mycoplasma gallisepticum antigenicity is obtained. The recombinant Avipox virus is effective as a potent live vaccine against Mycoplasma gallisepticum
25 infections. In addition, the novel polypeptide showing an antigenicity of the present invention and DNA encoding the same can be utilized as a component vaccine and a live vaccine, respectively.

EXAMPLES

Hereinafter the present invention will be described with reference to the examples and the reference examples but is not deemed to be limited thereto.

Reference Example 1

Obtaining of polypeptide DNA TTM-1 in which Mycoplasma gallisepticum is expressed:

- (1) Preparation of genomic DNA of Mycoplasma gallisepticum

Mycoplasma gallisepticum S6 strain was cultured at 37°C for 3 to 5 days in liquid medium prepared by supplementing 20% horse serum, 5% yeast extract, 1% glucose and a trace amount of phenol red as a pH indicator in 100 ml of PPLO broth basal medium. As Mycoplasma gallisepticum proliferated, pH of the culture broth decreased. At the point of time when the color of the pH indicator contained in the culture broth changed from red to yellow, incubation was terminated. The culture medium was centrifuged at 8000G for 20 minutes to collect the cells. The cells were then suspended in 1/10 volume of PBS based on the volume of culture medium. The suspension was again centrifuged at 10,000 rpm x G for 20 minutes to collect the cells. The collected cells were resuspended in 2.7 ml of PBS and SDS was added thereto in a final concentration of 1%. Furthermore 10 µg of RNase was added to the mixture.

The mixture was incubated at 37°C for 30 minutes to cause lysis.

The lysate was extracted 3 times with an equal volume of phenol and then 3 times with ethyl ether. The
5 extract was precipitated with ethanol to give 200 µg of genomic DNA of Mycoplasma gallisepticum.

(2) Genomic Southern hybridization of Mycoplasma gallisepticum using TM-1 DNA as a probe

After 1 µg of Mycoplasma gallisepticum DNA
10 obtained in (1) described above was digested with XbaI, the digestion product was subjected to 0.6% low melting agarose gel electrophoresis. After the electrophoresis, the gel was immersed in an alkaline denaturation solution (0.5 M NaOH, 1.5 M NaCl) for 10 minutes to
15 denature DNA and further immersed in a neutralizing solution (3 M sodium acetate, pH 5.5) for 10 minutes to neutralize. Following the neutralization, the DNA was transferred onto a nylon membrane in 6-fold SSC solution (0.7 M NaCl, 0.07 M sodium citrate, pH 7.5). After air
20 drying, the membrane was heated at 80°C for 2 hours. 4-Fold SET (0.6 M NaCl, 0.08 M Tris-HCl, 4 mM EDTA, pH 7.8)-10-fold Denhardt-0.1% SDS-0.1% Na₄P₂O₇-50 µg/ml of denatured salmon sperm DNA and pUM-1 (see Japanese Patent Application Laid-Open No. 2-111795) which had
25 been labelled in a conventional manner were added to cause hybridization at 68°C for 14 hours. The nylon membrane was overlaid on an X ray film. Autoradiography

revealed that hybridization occurred on the fragment of about 3.4 kbp.

(3) Cloning of XbaI-digested fragment of about 3.4 kbp to pUC-19 and colony hybridization

5 After 4 µg of Mycoplasma gallisepticum DNA obtained in Example 1 (1) described above was digested with restriction enzyme XbaI, the digestion product was subject to 0.6% low melting agarose gel electrophoresis. After the electrophoresis, the fragment of about 3.4 kbp
10 was recovered. The fragment was ligated with XbaI-digested pUC-19 using ligase and competent E. coli TGI strain was transformed by the ligation product. The transformants were cultured at 37°C for 15 hours in LB agar medium containing 0.003% of 5-bromo-4-chloro-3-
15 indolyl-β-D-galactopyranoside, 0.03 mM of isopropylthio-β-D-galactopyranoside and 40 µg/ml of ampicillin. White colonies grown on the agar medium were transferred onto a nylon membrane followed by hybridization in a manner similar to (2) above. Autoradiography revealed that
20 cloning was effected and, the thus obtained plasmid was named pUTTM1.

(4) Production of TTM-1' modified (TGA → TGG) not to read TTM-1-encoding protein TTMG-1 by TGA as translation termination codon (see Fig. 2)

25 After pUTTM-1 of (3) described above was digested with restriction enzymes SacI and EcoRI and the digestion product was then subjected to 0.8% low melting agarose gel electrophoresis. The 1.1 kbp fragment

containing the 5'-end of TTM-1 was recovered by treating with phenol-chloroform and precipitating with ethanol. The fragment was ligated with the fragment obtained by digestion of M13mpl1 phage with SacI and EcoRI. The
5 ligation reaction solution was mixed at m.o.i. of 0.1 with a solution obtained by culturing E. coli TGl at 37°C for 24 hours, adding IPTG thereto in a final concentration of 100 mM and further supplementing IPTG in a X-gal concentration of 2%. The resulting mixture
10 was inoculated on soft agar for solidification. Incubation was then performed at 37°C for 24 hours. Among the phage plaques formed, recombinant phage TTM-1N containing 1.1 kbp DNA of TTM-1 was collected from the phage in which the color did not change to blue.

15 Likewise, pUTTM-1 was digested with EcoRI and EcoRV. After 0.8% low melting agarose gel electrophoresis, the 0.4 kbp fragment containing the 3'-end of TTM-1 was recovered from the gel. A phenol-chloroform treatment followed by ethanol precipitation
20 gave M13mpl0 phage. M13mpl0 phage was ligated with the fragment obtained by digestion with EcoRI and EcoRV using ligase. The reaction solution was treated as in the cloning of the 1.1 kbp DNA. Recombinant phage TTM-1C containing 0.4 kbp DNA of TTM-1 was thus obtained.

25 (5) Preparation of single stranded DNA from each recombinant phage

The two recombinant phage obtained in (4) described above were added at m.o.i. of 0.1,

respectively, to E. coli TGI proliferated at 37°C in 100 ml of 2 x YT medium. After shake culture at 37°C for 5 hours, centrifugation was performed at 5000G for 30 minutes to obtain the cell-free supernatant. A 0.2-fold
5 volume of polyethylene glycol/sodium chloride mixture (20% polyethylene glycol #6000, 2.5 M NaCl) was added to the supernatant. After settlement at 4°C for an hour, the mixture was centrifuged at 5000G for 20 minutes to recover the precipitates. The precipitates were
10 dissolved in 500 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). After extraction with phenol-chloroform, single stranded DNA of each recombinant phage was recovered by ethanol precipitation.

(6) Construction of site-specific mutated plasmids
15 using artificially synthesized oligonucleotide as a primer

The thus obtained DNA has TGA at the middle of the sequence. This TGA sequence is recognized as a termination codon in a normal cell so that the TGA
20 sequence does not translate the sequence added thereafter. Therefore, in order to translate the TGA portion as methionine, the basic adenine which corresponds to the third nucleotide in codon NNN must be modified to guanine. Thus, the following two oligonucleotides were
25 synthesized.

Sequence No. 17:

3'-TACGTTCTTCCTGGCAAACCTTACCACTACTT-5'

Sequence No. 18:

3'-CTACAAAGAACCTAAATATCA-5'

The oligonucleotide shown by Sequence No. 17 (SEQ ID NO: 17) is annealed to single stranded DNA of TTM-1N and the oligonucleotide shown by Sequence No. 18 to single stranded DNA of TTM-1C to cause the desired
5 mutation by the method of Frits Eckstein et al. (Nucleic Acid Research, 8749-8764, 1985). The thus obtained recombinant phages were named TTM-1N' and TTM-1C', respectively. The TTM-1N' and TTM-1C' phage DNAs thus obtained were digested with restriction enzymes SacI-
10 EcoRI and EcoRI-BglII, respectively. By 0.8% low melting agarose gel electrophoresis, the fragments of 1.1 kbp and 0.4 kbp were extracted from the agarose gel and recovered by ethanol precipitation. On the other hand, plasmid pUTTM-1 was also digested with SacI-BglII. The
15 4.8 kbp fragment bearing a vector was extracted by 0.8% low melting agarose gel electrophoresis and recovered by ethanol precipitation. The thus obtained three fragments were ligated by ligase and competent E. coli TGI strain was transformed to obtain plasmid pUTTM-1' bearing
20 TTM-1' with mutagenesis at the desired site thereof. The nucleotide sequence of TTM-1' is as shown by SEQ ID NO: 14 according to the Dideoxy method by Sanger et al. (Proc. Natl. Acad. Sci. USA, 74, 5463 (1977)). The nucleotide sequence is substantially the same as the
25 kilodalton TTM-1 polypeptide of M. gallisepticum.

Reference Example 2

Construction of vector pNZ1729R for insertion

The EcoRI fragment (about 7.3 kbp) of NP strain was inserted into pUC18 at the EcoRI digestion site (terminus at the multi-cloning site) to obtain plasmid pNZ133 (about 10.0 kbp). From the plasmid the HpaI-SpeI fragment (about 3.0 kbp fragment derived from NP strain) was excised out and rendered blunt end by Klenow fragment. Furthermore, the EcoRI-HindIII fragment (multi-cloning site of 52 bp) was removed from pUC18 and rendered blunt end by Klenow fragment. The two fragments were ligated with each other to form a plasmid. After removing the EcoRV site in the HpaI-SpeI fragment, the EcoRI-HindIII fragment (multi-cloning site of 52 bp) of pUC18 is inserted therein using HindIII linker (5'-CAAGCTTG-3') and EcoRI linker (5'-GGAATTCC-3') to construct plasmid pNZ133SR.

Sequence No. 2 (SEQ ID NO: 2) and Sequence No. 3 (SEQ ID NO: 3) (bearing FPV promoter of 17 bases and linked to a translation initiation codon for lacZ) were annealed to double strands. Sequence No. 4 (SEQ ID NO: 4) annealed to the lacZ gene (derived from pMC1871 and pMA001, Sirakawa et al., Gene, 28, 127-132, 1984) and Sequence No. 5 (SEQ ID NO: 5), Sequence No. 6 (SEQ ID NO: 6) and Sequence No. 7 (SEQ ID NO: 7), Sequence No. 8 (SEQ ID NO: 8) and Sequence No. 9 (SEQ ID NO: 9), Sequence No. 10 (SEQ ID NO: 10) and Sequence No. 11 (SEQ ID NO: 11), were ligated with each other (which contains

a modified synthetic promoter of poxvirus shown by
nucleotide sequence: T T T

nucleotide sequence: T T T T T
T T T T T T T T T T T T T G G C A T A T A A A
T A A T A A A T A C A A T A A T T A A T T A C G C
G T A A A A A T T G A A A A A C T A T T C T A A T
T T A T T G C A C T C

from the next T of AGC at the 5' end of Sequence No. 3 to C before G of Sequence No. 5 at the 3' end, and further linked to the multi-cloning site and poxvirus initial transcription termination signal on the both directions (SEQ ID NO: 12) (Yuen et al., Proc. Natl. Acad. Sci., USA, 88, 6417-6421, 1989) thereby to obtain the EcoRI-HindIII fragment (about 3.5 kbp). The EcoRI-HindIII fragment was inserted into pNZ133SR to construct plasmid pNZ1729R.

Example 1

Construction of plasmid pNZ7929-R1 for recombination
(see Fig. 3)

- 15 (1) Construction of plasmid pUTTM1P having ligated a
synthetic promoter with TTM-1' gene

In order to form the restriction enzyme DraI digestion site upstream ATG corresponding to initiation codon of TTM-1' protein in plasmid pUTTM1' (see WO 93/24646) containing the full length TTM-1' DNA obtained in Reference Example 1, the following oligonucleotide was firstly prepared.

Sequence No. 19

3'-TATAGAATTAAATTTTACTTATTC-5'

Next, after pUTM-1' was digested with restriction enzymes SacI and EcoRI, the fragment of about 2300 bp was recovered and then ligated with the fragment obtained by digestion of M13mpl0 with SacI and EcoRI to obtain recombinant phage TTM-1'. The oligonucleotide described above was annealed to single stranded TTM-1' to cause the desired variation by the method of Frits Eckstein et al. Nucleic Acid Research 13, 8749-8764 (1985). This recombinant phage DNA variant was digested with restriction enzymes SacI and EcoRI. The fragment of about 2300 bp was recovered and cloned to the vector-bearing fragment obtained by digestion of pUTM-1' again with SacI and EcoRI to obtain pUTM1D.

A synthetic promoter was prepared by synthesizing DNAs of Sequence-20 and Sequence-21 followed by annealing, whereby the digestion sites with restriction enzymes HindIII and HincII at the end.

Sequence-20 5'-AGCTTTTTTTTTTTTTTTTTTTGGCATATAAATAAATAACAATAATTAATTACGCGTAAAAATT.

Sequence-21 3'-AAAAAAAAAAAAAAAAAACCGTATATTTATTATTTATGTTATTAATTAATGCGCATTTTTAA
HindIII

GAAAACTATTCTAATTTATTGCACTCGTC -3'
CTTTTGATAAGATTAAATAACGTGAGCAG -5'
HincII

Finally, the 1200 bp fragment obtained by digestion of pUTM1D with restriction enzymes DraI and

BglIII was ligated with the synthetic promoter described above and the fragment obtained by digestion of pUC18 with HindIII and BamHI to give plasmid pUTTM1P of about 4.0 kbp.

5 (2) Construction of pNZ7929R1

After plasmid pUTTM1P obtained in (1) was digested with restriction enzymes HindIII and KpnI, the fragment of about 1300 bp was recovered. Next, vector pNZ1729R (EP-A-0520753) for FPV recombination obtained
10 in Reference Example 2 was digested with restriction enzymes HindIII and KpnI. The two fragments were ligated with each other to obtain the desired vector pNZ7929-R1 (about 10.3 kbp) for recombination.

(3) Construction of recombinant FPV fNZ7929-R11 and
15 purification thereof

NP strain, which is a fowlpox live vaccine strain, was infected to monolayered CEF at m.o.i. = 0.1. Three hours after, these cells were peeled apart from the monolayer by a treatment with trypsin to form a cell
20 suspension. After 2×10^7 cells in the suspension were mixed with 10 μ g of plasmid pNZ7929-R1 for recombination, the mixture was suspended in Saline G (0.14M NaCl, 0.5 mM KCl, 1.1 mM Na_2HPO_4 , 1.5 mM KH_2PO_4 , 0.5 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.011% glucose). The suspension was subjected to
25 electrophoresis under conditions of 3.0 kV cm^{-1} , 0.4 msec and 25°C, using Gene Pulser* (Bio-Rad) at room temperature. The plasmid-infected cells were then cultured at 37°C for 72 hours. The cells were lysed

* Trademark

by freeze and thaw 3 times to recover viruses containing the recombinant virus.

The recombinant virus recovered was selected as follows. The recovered viral solution was infected
5 to monolayered CEF and 10 ml of agar solution containing growth medium was overlaid thereon. After agar was solidified at room temperature, incubation was performed at 37°C until plaques of FPV appeared. Then agar medium containing Blue gal in a concentration of 200 µg/ml was
10 overlaid on the agar followed by incubation at 37°C for further 48 hours. Among all of the plaques, about 1% of the plaques were colored blue. These blue plaques were isolated and recovered. By the same procedures, isolation and recovery were repeated to purify the virus
15 until all the plaques were stained to blue with Blue gal. In general, the repeated procedures were terminated by 3 to 4 times. The purified virus was named fNZ7929-R1. In fNZ7929-R1, each position of the DNA inserted was confirmed by dot blotting hybridization
20 and Southern blotting hybridization.

Example 2 Obtaining of 70 K protein DNA

(1) Preparation of Mycoplasma gallisepticum genomic DNA

Using Mycoplasma gallisepticum S6 strain, 200
25 µg of Mycoplasma gallisepticum DNA was obtained in a manner similar to Reference Example 1 (1) described above.

(2) Preparation of genomic DNA library

After 4 units of restriction enzyme AluI was added to 40 µg of Mycoplasma gallisepticum genomic DNA obtained in (1), incubation was conducted at 37°C for 10 minutes for partial digestion. The partially digested genomic DNA was subjected to 0.8% low melting agarose gel electrophoresis. The DNA fragment having a strand length of approximately 1.0 kbp to 4.0 kbp was recovered from the gel. The DNA fragment was treated with phenol and then precipitated with ethanol to give 4 µg of the DNA fragment partially digested with AluI.

S-Adenosyl-L-methionine was added to 1.2 µg of the AluI-partially digested-DNA fragment in a final concentration of 80 µM and 20 units of EcoRI methylase was further added thereto to methylate the deoxy-adenosine site in the EcoRI recognition sequence, thereby to render the sequence non-sensitive to EcoRI. EcoRI linker was ligated with this DNA fragment using ligase. The ligation product was then mixed with the EcoRI digestion fragment of λgt11 DNA to ligate with each other by ligase. The reaction solution was used to effect in vitro packaging in a conventional manner (DNA Cloning, Vol. 1, A Practical Approach, DNA Cloning Vol. 1 (1985) IRL Press, edited by D.M. Glover). The resulting product was transfected to Escherichia coli Y1088 strain (Amersham) followed by incubation at 37°C for 12 hours in LB agar medium containing 0.003% of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside and 0.03 mM isopropylthio-β-D-

galactopyranoside. Among the plaques formed, a library size was estimated by the count of white plaques to prepare DNA library of 10^6 pfu (plaque forming unit).

(3) Immuno-screening of genomic DNA library

5 The phage obtained from the DNA library prepared in (2) was added to a suspension of Escherichia coli Y1090 strain (Amersham) in an aqueous solution of 10 mM $MgSO_4$ to form 500 to 1000 plaques on one plate of 8 cm ϕ to effect adsorption for 15 minutes. Furthermore
10 2.5 ml of LB soft agar medium warmed to 45°C was added and overlaid on the LB agar medium followed by incubation at 42°C for 3 to 4 hours. A nylon membrane filter was immersed in 10 mM of IPTG aqueous solution, air-dried and then overlaid on the plate described above
15 followed by incubation at 37°C for further 2 to 3 hours. After the incubation, the nylon membrane filter was peeled apart from the plate and washed with TBS (50 mM Tris-HCl, pH 8.0, 150 mM NaCl). The filter was immersed in 2% skimmed milk-containing TBS for 30 minutes and
20 then treated for an hour with anti-Mycoplasma chicken serum diluted with TBS to 500-fold. Thereafter, the filter was immersed in TBS for 15 minutes to wash the filter. The filter was further washed by immersing in TBS containing 0.05% of a surfactant (Tween*20) for 10
25 to 15 minutes. This step was repeated 4 to 5 times. Then the filter was treated with biotinylated antibody against chicken IgG for 60 minutes. After treating with a secondary antibody, the filter was washed with PBS

* Trademark

containing 0.05% of Tween 20 5 to 6 times and then treated for 60 minutes by immersing in horse radish peroxidase-avidin D solution. After the treatment, the filter washed with PBS containing 0.05% of Tween 20 5 to 6 times and then washed with 10 mM Tris-HCl, pH 8.0. Then, the filter was immersed in a buffer containing 4-chloronaphthol and hydrogen peroxide. By a series of these operations, only the plaques that expressed an antigenic protein originating in Mycoplasma
10 gallisepticum were colored purple.

By the aforesaid immuno-screening of about 5×10^4 plaques, 50 positive plaques were obtained.

(4) Production of immuno-positive recombinant λ gt11 phage DNA

15 Escherichia coli Y1090 strain was incubated at 37°C for 12 hours in LB medium supplemented with 50 μ g/ml ampicillin. The culture broth was added to a 10-fold amount of LB medium containing mM MgSO_4 . Then, recombinant λ gt11 phage which was obtained in (3) and
20 became positive by immuno-screening was added to the medium at m.o.i. = 0.05, followed by incubation at 37°C for 5 to 10 hours. After lysis of Escherichia coli, centrifugation was carried out at 8,000 rpm for 10 minutes to obtain the supernatant. To the supernatant
25 were added an equal volume of TM buffer (50 mM Tris-HCl, pH 7.4, 10 mM MgSO_4) and DNase I in a concentration of 0.016 mg/ml, followed by incubation for 15 minutes. After NaCl and polyethylene glycol (PEG 6000) were added

to the culture broth in concentrations of 0.5 M and 0.1 g/ml, respectively, the mixture was shaken at 0°C for 15 minutes. After centrifugation at 10,000 rpm for 10 minutes, the supernatant was removed. The resulting
5 pellets were dissolved in a 1/100 volume of TM buffer and an equal volume of chloroform was added thereto followed by vigorous stirring. By centrifugation at 15,000 rpm for 10 minutes, recombinant λ gt11 phage was collected in the aqueous phase to obtain the phage
10 solution.

EDTA, SDS and pronase E were added to the phage solution in final concentrations of 0.025 M, 1% and 1 mg/ml, respectively. After incubation at 37°C for 4 hours, the solution was subjected to phenol extraction
15 and ethanol precipitation to give λ gt11 phage DNA containing the cloned antigenic DNA (M-81).

(5) Construction of recombinant plasmid (pM-81)

The recombinant λ gt11 phage DNA obtained in (4) was digested with restriction enzyme EcoRI, the
20 digestion product was subjected to 0.8% low melting agarose gel electrophoresis. The genomic DNA fragment of Mycoplasma gallisepticum inserted into the genomic DNA of λ gt11 phage at the cloning site showed a strand length of about 2.8 kbp. This DNA fragment was
25 extracted from the agarose gel and then with phenol-chloroform (1 : 1) and recovered by ethanol precipitation. On the other hand, after plasmid pUC18 was digested with EcoRI, the digested pUC18 was

extracted with phenol-chloroform and recovered by ethanol precipitation, in a similar manner. Then, the phosphate at the 5' end was removed by an alkaline phosphatase treatment. After pUC18 DNA was again
5 extracted with phenol-chloroform, DNA was recovered by ethanol precipitation.

The digested pUC18 was ligated with the EcoRI digestion product (about 0.8 kbp) derived from Mycoplasma gallisepticum using ligase. Competent
10 Escherichia coli TGI strain was transformed with the ligation product. The transformants were cultured at 37°C for 15 hours in LB agar medium supplemented with 0.003% of 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside, 0.03 mM of isopropylthio- β -D-
15 galactopyranoside and 40 μ g/ml of ampicillin. Among the transformed E. coli grown on the agar medium, white colonies were cultured at 37°C for 15 hours in 40 μ g/ml ampicillin-supplemented LB liquid medium and plasmid was extracted by the method of Birnboim & Doly [Nuc. Acid
20 Res., 7, 1513 ~ (1979)]. After digestion with EcoRI, the recombinant plasmid containing the same length of DNA fragment as that of the original EcoRI fragment derived from Mycoplasma gallisepticum was detected by 0.8% low melting agarose electrophoresis; this plasmid was named
25 pM-81.

(6) Genomic Southern hybridization of Mycoplasma gallisepticum using M-81 DNA as a probe

After 1 μ g of pM81 obtained in (5) described

above was digested with EcoRI and HindIII, the digestion product was subjected to 0.6% low melting agarose gel electrophoresis. After the electrophoresis, the gel was immersed in an alkaline denaturation solution (0.5 M NaOH, 1.5 M NaCl) for 10 minutes to denature DNA. The gel was then immersed in a neutralization solution (3 M sodium acetate, pH 5.5) for 10 minutes for neutralization and then transferred onto a nylon membrane in 6-fold SSC solution (0.7 M NaCl, 0.07 M sodium citrate, pH 7.5). After air-drying, the nylon membrane was baked at 80°C for 2 hours and 4-fold SET (0.6 M NaCl, 0.08 M Tris-HCl, 4 mM EDTA, pH 7.8)-10-fold Denhardt-0.1% SDS-0.1% Na₄P₂O₇-50 µg/ml of denatured salmon sperm DNA and pM-81 (M-81 gene is contained in this plasmid) labelled in a conventional manner was added thereto to perform hybridization at 68°C for 14 hours. The nylon membrane was overlaid on an X ray film. It was confirmed by autoradiography that M-81 was hybridized to the about 5.0 kbp fragment of Mycoplasma gallisepticum.

(7) Cloning of EcoRI and HindIII-digested fragment of about 5.0 kbp to pUC19 and colony hybridization

After 4 µg of the Mycoplasma gallisepticum DNA obtained in (6) described above was digested with EcoRI and HindIII, the digestion product was subjected to 0.6% low melting agarose gel electrophoresis to recover the fragment of about 5.5 kbp. The fragment was ligated with pUC-19 cleaved by digestion with EcoRI and HindIII

using ligase. Competent Escherichia coli TGI strain was transformed with the ligation product. The transformants were cultured at 37°C for 15 hours in LB agar medium supplemented with 0.003% of 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside, 0.03 mM of isopropylthio- β -D-galactopyranoside and 40 μ g/ml of ampicillin. White colonies grown on the medium were transferred onto a nylon membrane and hybridization was carried out in a manner similar to (2) described above. It was confirmed by autoradiography that cloning was effected and this plasmid was named pUM-81.

(8) Sequence analysis of pUM-81 insert DNA

The sequence of about 5.0 kbp fragment inserted into pUM-81 prepared in (7) above was analyzed by the dideoxy method by Sanger et al.

The restriction enzyme map of the open reading frame (hereinafter abbreviated as ORF) present in this fragment is shown in Fig. 1. The nucleotide sequence of this ORF and the amino acid sequence deduced therefrom are shown by SEQ ID NO: 1. The polypeptide deduced from this ORF was named TM-81 polypeptide.

Example 3

Construction of recombinant FPV bearing hybrid DNA in which TTM-1' protein DNA was ligated downstream the signal membrane anchor DNA

(1) Cloning of the synthetic promoter to pUC18 (see Fig. 4)

The following synthetic promoters bearing the HindIII and BamHI restriction enzyme sites at both ends were synthesized.

HindIII

Sequence No. 22 5'- AGCTTTTTTTTTTTTTTTTTTTGGCATATAAATAATAAATACAATAATTAAATTACGC

Sequence No. 23 3'- AAAAAAAAAAAAAAAAAAAAAACCGTATATTTATTATTTATGTTATTAAATTAAATGCG

GTAAAAATTGAAAAACTATTCTAATTTATTGCACTCG -3'

CATTTTAACTTTTIGATAAGATTAAATAACGTGAGCCTAG -5'

BamHI

This synthetic DNA was ligated with the
5 digestion fragment of PUC18 with HindIII and BamHI to
obtain plasmid of about 2.8 kbp named PUC18P.

(2) Ligation of a gene encoding HN protein of NDV
with the synthetic promoter (see Fig. 4)

After plasmid XLIII-10H bearing HN gene of NDV
10 was fully digested with SacI, the digestion product was
then partially digested with AvaII. The fragment of
about 1800 bp was recovered by 0.8% low melting agarose
gel electrophoresis. In order to form the BamHI
cleavage site at the AvaII site of this fragment, the
15 following DNA was synthesized.

BamHI AvaII

Sequence-24 5' - GATCCAGCATG - 3'

Sequence-25 3' - GTCGTACCTG - 5'

Three of the synthetic DNA, the HN-bearing DNA

fragment of about 1800 bp and the fragment containing the synthetic promoter recovered by 2.0% low melting agarose gel electrophoresis after full digestion of pUC18P with BamHI and SacI were ligated by ligase and
5 these three fragments-ligated plasmid was extracted. The resulting plasmid of about 4.6 kbp was named pNZ87N.

(3) Change of the AluI cleavage site of pNZ7929-R1 into the EcoRI cleavage site (see Figs. 3 and 5)

In order to change the restriction enzyme AluI
10 cleavage site in the 279 nucleotide portion of SEQ ID NO: 14 into the EcoRI cleavage site, the following oligonucleotide was synthesized.

Sequence-26 5'-GGGATTTCGAATTCTATGTCT-3'

After pUTTM1P was digested with HindIII and KpnI, the fragment of about 1300 bp and ligated with the fragment
15 obtained by digestion of M13mp10 with HindIII and KpnI to obtain the single stranded recombinant phage. The oligonucleotide described above was annealed to the single stranded recombinant phage to cause the desired mutation by the method of Frits Eckstein et al. After
20 the recombinant phage DNA mutant was digested with restriction enzymes HindIII and KpnI, the fragment of about 1300 bp was recovered and ligated with the fragment obtained by digestion of pNZ1729R with restriction enzymes HindIII and KpnI using ligase to
25 obtain plasmid pNZ7929-R2 (about 10.3 kbp) with the AluI

cleavage site of pNZ7929-R1 being changed into the EcoRI cleavage site.

(4) Construction of plasmid pNZ2929XM1 for recombinant FPV (see Fig. 6(A) and 6(B))

5 Firstly pNZ87N was fully digested with restriction enzyme XbaI and the cleavage site was rendered blunt by Klenow fragment. Then EcoRI linker (5'-GGAATTC-3') was added to and ligated with the digestion product using ligase. The plasmid was
10 digested with EcoRI and HindIII. The fragment of about 300 bp was recovered by 1.2% low melting agarose gel electrophoresis. Next, pNZ7929R2 was digested with restriction enzyme EcoT22I and then partially digested with EcoRI. The fragment of about 550 bp which is a
15 part of TTM-1 DNA was recovered by 0.8% low melting agarose gel electrophoresis. Furthermore, pNZ7929RI was digested with restriction enzymes EcoT22I and HindIII and the fragment of about 9.4 kbp was recovered by 8% low melting agarose gel electrophoresis. These
20 fragments were ligated by ligase and the three fragments-ligated plasmid was extracted. The plasmid of about 10.3 kbp was named pNZ2929XM1.

(5) Construction and purification of recombinant FPV fNZ2929XM1

25 Construction and purification were carried out in a manner similar to Example 1 (3). The purified virus was named fNZ2929-XM1. By dot blotting hybridization and Southern blot hybridization, the

location of each DNA inserted was confirmed in fNZ2929-XM1.

Example 4

Expression of TTM-1 polypeptide in cells infected with
5 fNZ7929-R1 and fNZ2929XM1

In order to confirm that fNZ7929-R1 and fNZ2929XM1 express TTM-1 polypeptide in infected cells, immunofluorescent antibody technique using antisera against Mycoplasma gallisepticum S6 was employed.

10 fNZ7929-R1 and fNZ2929XM1 were infected to CEF and incubation was carried out at 37°C until plaques appeared. After fixing with cold acetone, chicken antisera (anti-S6) immunized with Mycoplasma gallisepticum S6 strain or Mycoplasma gallisepticum S6-infected chicken sera (S6
15 infected) and TTM-1 polypeptide-immunized chicken antisera (anti-TTMG-1) diluted as a primary antibody to 100 to 1000-fold were reacted. These culture cells were further reacted with anti-chicken immunoglobulin bound to a fluorescent substance (FITC) and the non-specific
20 reaction portion was washed out. Then, microscopic observation was made under the excited fluorescent wavelength light. With respect to the infected cells where no acetone fixing was performed (namely, unfixed cells), the reactivity was likewise examined. Using FPV-NP
25 strain and fNZ2337 (Japanese Patent Application Laid-Open No. 1-157381) as viruses for control, Newcastle disease virus-immunized chicken sera (anti-NDV)

and SPF chicken sera (SPF) were used in 1000-fold as primary antibody for control. The reactivity is shown in Table 1.

Table 1. Reactivity of recombinant virus-infected CEF to various antisera

Infected virus (acetone fixation)	Reactivity to primary antibody Infected				
	anti-S6	S6	anti-TTM-1	anti-NDVSPF	
fNZ2929XM1					
(acetone-fixed)	++	++	++	-	-
(non-fixed)	+	+	+	-	-
fNZ7929-R1					
(acetone-fixed)	+	+		-	-
(non-fixed)	±	±	±	-	-
fNZ2337					
(acetone-fixed)	-	-	-	+	-
(non-fixed)	-	-	-	+	-
NP					
(acetone-fixed)	-	-	-	-	-
(non-fixed)	-	-	-	-	-
None					
(acetone-fixed)	-	-	-	-	-
(non-fixed)	-	-	-	-	-

++ : strongly reacted
 + : reacted
 ± : weakly reacted
 - : not reacted

The results reveal that the cells infected
 5 with the recombinant viruses fNZ7929-R1 and fNZ2929XM1
 of the present invention are reactive with anti-S6, S6
 infection and anti-TTM-1; and that fNZ7929-R1 are

reactive with anti-S6, S6 infection and anti-TTMG-1 also in non-fixed completed cells. This indicates that fNZ2929XM1 not only expresses TTMG-1 polypeptide in the infected cells but also exhibits TTM-1 polypeptide on
5 the surface of the infected cells.

Example 5

Antibody inducing ability of recombinant FPV inoculated to chickens

After fNZ7929-R1 and fNZ2929XM1 were cultured
10 in CEF at 37°C for 48 hours, the procedure of freezing and thawing was repeated twice to recover the cell suspension. The cell suspension was adjusted to have a virus titer of 10^6 pfu/ml and then inoculated to SPF chick (Line M, Nippon Seibutsu Kagaku Kenkyusho) of 7
15 days old at the right wing web in a dose of 10 μ l. After the inoculation, generation of the pock was observed. Two weeks after the inoculation, sera were collected. The antibody titer of the sera collected was determined by ELISA. The purified TTM-1
20 polypeptide was dissolved in bicarbonate buffer in a concentration of 1 μ g/well. After adsorbing to a 96 well microtiter plate, blocking was performed with skimmed milk to prevent the following non-specific adsorption. Next, a dilution of the sample serum was
25 charged in each well and then horse radish peroxide-bound anti-chick immunoglobulin antibody (rabbit antibody) was added thereto as a secondary antibody.

After thoroughly washing, 2,2'-azinodiethylbenzothiazoline sulfonate was added to the mixture as a substrate and a relative dilution magnification of the antibody was measured with an immuno-reader in terms of absorbance at a wavelength of 405 nm. As a primary antibody for control, anti-TTM-1 polypeptide chicken serum was used. The results are shown in Table 2.

Table 2. Antibody titer of fNZ2929XM1-inoculated chick to TTM-1 polypeptide

Inoculated virus	Antibody titer to anti-TTM-1 polypeptide (dilution magnification)*
fNZ2929XM1	256
fNZ7929-R1	32
NP	1
**	1
anti-TTM-1 polypeptide	256

* dilution magnification when SPF chicken serum dilution is as 1

** not inoculated

The results reveal that both fNZ2929XM1 and fNZ2929-R1 which are the recombinant viruses of the present invention, can induce anti-TTM-1 polypeptide antibody and can be used as a vaccine for effectively preventing fowlpox and Mycoplasma gallisepticum infections.

Example 6

Collection of recombinant Avipox virus fNZ7929-67
bearing TM-67

- (1) Genomic Southern hybridization of *Mycoplasma*
5 *gallisepticum* using TM-67 gene as a probe

After 1 μ g of the *Mycoplasma gallisepticum* DNA
obtained in Reference Example (1) was digested with
XbaI, the digestion product was subjected to 0.6% low
melting agarose gel electrophoresis. After the
10 electrophoresis, the gel was immersed in an alkaline
denaturation solution (0.5 M NaOH, 1.5 M NaCl) for 10
minutes to denature DNA. The gel was then immersed in a
neutralization solution (3 M sodium acetate, pH 5.5) for
10 minutes for neutralization and then transferred onto
15 a nylon membrane in 6-fold SSC solution (0.7 M NaCl,
0.07 M sodium citrate, pH 7.5). After air-drying, the
nylon membrane was baked at 80°C for 2 hours and 4-fold
SET (0.6 M NaCl, 0.08 M Tris-HCl, 4 mM EDTA, pH 7.8)-10-
fold Denhardt-0.1% SDS-0.1% Na₄P₂O₇-50 μ g/ml of
20 denatured salmon sperm DNA and pUM-1 (cf. Japanese
Patent Application Laid-Open No. 2-111795) labelled in a
conventional manner was added thereto to perform
hybridization at 68°C for 14 hours. The nylon membrane
was overlaid on an X ray film. It was confirmed by
25 autoradiography that hybridization occurred to the about
3.4 kbp fragment different from the fragment confirmed
in Reference Example 1 (2).

(2) Cloning of the XbaI-digested fragment of about 3.4 kbp to pUC-19 and analysis of the sequence

After 4 µg of the Mycoplasma gallisepticum DNA obtained in Reference Example 1 (1) was digested with
5 restriction enzyme XbaI, the digestion product was subjected to 0.6% low melting agarose gel electrophoresis to recover the fragment of about 3.4 kbp confirmed in Example 6 (1) described above. The fragment was ligated with pUC-19 cleaved by digestion
10 with XbaI using ligase. Competent Escherichia coli TGI strain was transformed with the ligation product. The transformants were cultured at 37°C for 15 hours in LB agar medium supplemented with 0.003% of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, 0.03 mM of
15 isopropylthio-β-D-galactopyranoside and 40 µg/ml of ampicillin. Among the transformed E. coli grown on the medium, white colonies were cultured at 37°C for 15 hours in LB liquid medium supplemented with 40 µg/ml ampicillin and plasmid was extracted by the method of
20 Birnboim & Doly. After digestion with XbaI, the recombinant plasmid containing the same length as that of the XbaI fragment derived from MG was detected by 0.8% low melting agarose electrophoresis; this plasmid was named pUM67.

25 The about 3.4 kbp fragment inserted into pUM67 was analyzed by the dideoxy method by Sanger et al. Nucleic Acid Research 7, 1513-(1979).

The restriction enzyme map of the open reading frame (ORF) present in this fragment is shown in Fig. 8

and the nucleotide sequence of this ORF and the amino acid sequence are shown by SEQ ID NO: 27. The polypeptide deduced from this ORF was named TM-67 polypeptide.

- 5 (3) Construction of plasmid pTM67 bearing a modified gene (TGA → TGG) not to read TGA in ORF of TM-67 as translation termination codon (see Figs. 8 and 9(A))

TGA codons were concentrated at the downstream
10 portion in ORF of TM-67. Therefore, the EcoRI and PstI fragment of about 1300 bp containing all TGA codons were recovered from pUM67 and ligated with pUC19 digested with EcoRI and PstI to obtain PUCT1 (4.0 kbp). Next, in order to change TGA to TGG using PUCT1 as a template
15 according to polymerase chain reaction (PCR: Science, 230, 1350-1354 (1985)), primer DNAs for PCR shown by SEQ ID NOS: 28-33 were synthesized.

Primers 1 to 6 corresponding to SEQ ID NOS: 28-33 which were employed for PCR are as follows.

Primer-1 5'-GTTTCCCAGTCACGAC-3' (M13 primer)

Primer-2 3'-AACCAACCAACCCGCGATCGCTAGTCT-5'

Nhe I

Primer-3 5'-TGATTGGGCGCTAGCGATCA-3'

Nhe I

Primer-4 3'-TCCCAACCTTGTCGAAATACAA-5'

HindIII

Primer-5 5'-TGAAACAAGCTTTATGTTT-3'

HindIII

Primer-6 3'-CAGTATCGACAAAGGAC-5' (M13 RV primer)

Following the conventional procedures for PCR, the fragment of 600 bp was amplified using Primer-1 and Primer-2 and then recovered; the fragment of 360 bp using Primer-3 and Primer-4; and the fragment of 340 bp using Primer-5 and Primer-6. In addition, the fragment of 600 bp was digested with EcoRI and NheI; the fragment of 360 bp was digested with Nhe and HindIII; and the fragment of 340 bp was digested with HindIII and PstI. Thereafter each digestion product was subjected to 2.0% low melting agarose gel electrophoresis and recovered from the agarose. For cloning of each fragment, pUC19 and pUC18 were digested with DraI and then XhoI linker was inserted to obtain plasmids pUC19X and pUC18X. The fragment of 600 bp and the fragment of 360 bp treated with the respective restriction enzymes and recovered were ligated with the digestion product of pUC19X with EcoRI and HindIII by ligase. The resulting plasmid was extracted and this plasmid was named pUC19XL (about 3.6 kbp). The 340 bp fragment digested with HindIII and PstI was ligated with the fragment obtained by digesting pUC18 with HindIII and PstI, using ligase. The resulting plasmid was extracted and named pUC18R (about 3 kbp). The fragment of about 2.5 kbp obtained by digestion of pUC19XL with HindIII and XhoI, the fragment

of 180 bp obtained by digestion of pUC18R with HindIII and SpeI, and the fragment of 1.1 kbp obtained by digestion of pUC18X with XbaI and XhoI were subjected to agarose gel electrophoresis, respectively, and then
5 recovered. These fragments were ligated using ligase. The resulting plasmid was extracted and named pTM67 (about 3.7 kbp).

(4) Construction of pNZ7929-67 (Fig. 9 (B))

After pUTTM1P obtained in Example 1 (1) was
10 digested with SpeI and KpnI, the digestion product was subjected to agarose gel electrophoresis to recover the fragment of 3.9 kbp. In a similar manner, after pTM67 was digested with SpeI and KpnI, the digestion product was subjected to agarose gel electrophoresis to recover
15 the fragment of 0.9 kbp. The thus recovered fragment was ligated with the 3.9 kbp fragment described above using ligase. The resulting plasmid pUTM67 (4.8 kbp) was recovered. After this pUTM67 was digested with KpnI, the digestion product was partially digested with
20 HindIII. The product was then subjected to agarose gel electrophoresis to recover the fragment of 2.1 kbp. The thus recovered fragment was ligated with the 9.0 kbp fragment obtained by digestion of PNZ1729R (cf. Reference Example 2) with HindIII and KpnI, using
25 ligase. The resulting plasmid pNZ7929-67 (11.1 kbp) was recovered.

(5) Construction of recombinant Avipox virus fNZ7929-67 and purification

The procedures similar to Example 1 (3) were repeated using pNZ7929-67 obtained in (4) described above to obtain fNZ7929-67.

Example 7

Collection of recombinant Avipox virus fNZ7929-66 bearing TM-66

(1) Genomic Southern hybridization of Mycoplasma gallisepticum using TM-66 gene as a probe

After 1 µg of the Mycoplasma gallisepticum DNA obtained in Reference Example (1) was digested with XbaI, the digestion product was subjected to 0.6% low melting agarose gel electrophoresis. After the electrophoresis, the gel was immersed in an alkaline denaturation solution (0.5 M NaOH, 1.5 M NaCl) for 10 minutes to denature DNA. The gel was then immersed in a neutralization solution (3 M sodium acetate, pH 5.5) for 10 minutes for neutralization and then transferred onto a nylon membrane in 6-fold SSC solution (0.7 M NaCl, 0.07 M sodium citrate, pH 7.5). After air-drying, the nylon membrane was baked at 8.0°C for 2 hours and 4-fold SET (0.6 M NaCl, 0.08 M Tris-HCl, 4 mM EDTA, pH 7.8)-10-fold Denhardt-0.1% SDS-0.1% Na₄P₂O₇-50 µg/ml of denatured salmon sperm DNA and pUM-1 (cf. Japanese Patent Application Laid-Open No. 2-111795) labelled in a conventional manner was added thereto to perform

hybridization at 68°C for 14 hours. The nylon membrane was overlaid on an X ray film. It was confirmed by autoradiography that hybridization occurred to the about 6.3 kbp fragment.

5 (2) Cloning of the XbaI-digested fragment of about
 6.3 kbp to pUC-19 and analysis of the sequence
 After 4 µg of the Mycoplasma gallisepticum DNA
obtained in Reference Example 1 (1) was digested with
restriction enzyme XbaI, the digestion product was
10 subjected to 0.6% low melting agarose gel electro-
phoresis to recover the fragment of about 6.3 kbp
confirmed in Example 7 (1) described above. The
fragment was ligated with pUC-19 cleaved by digestion
with XbaI using ligase. Competent Escherichia coli TGI
15 strain was transformed with the ligation product. The
transformants were cultured at 37°C for 15 hours in LB
agar medium supplemented with 0.003% of 5-bromo-4-
chloro-3-indolyl-β-D-galactopyranoside, 0.03 mM of
isopropylthio-β-D-galactopyranoside and 40 µg/ml of
20 ampicillin. White colonies grown on the medium were
transferred to a nylon membrane and hybridization was
carried out in a manner similar to (1) described above.
Autoradiography reveals that cloning was effected and
this plasmid was named pUM66 (about 9 kbp).

25 The about 6.3 kbp fragment inserted into pUM66
was analyzed by the dideoxy method by Sanger et al.

 The restriction enzyme map of ORF present in
this fragment is shown in Fig. 10 and the nucleotide

sequence of this ORF and the amino acid sequence deduced therefrom are shown by SEQ ID NO: 16.

The polypeptide deduced from this ORF was named TM-66 polypeptide.

- 5 (3) Construction of pTM66 modified (TGA → TGG) not to read TGA in ORF encoding TM-66 as translation termination codon (see Figs. 10 and 11 (A) through (C))

10 In order to modify the TGA codon in ORF of TM-66 to TGG codon, the change was made using polymerase chain reaction (PCR: Science, 230, 1350, 1354 (1985)) as in TM-67. DNA primers for PCR synthesized for the change are shown by SEQ ID NOS: 34-43.

15 Primers 1 to 10 corresponding to SEQ ID NOS: 34-43 using PCR are as follows.

Primer-1 5'-CAGGAAACAGCTATGAC-3' (M13 RV primer)

Primer-2 3'-GTTCTTCCTGGCAAACCTTA-5'

AvaII

Primer-3 5'-AAGAAGGACCGTTTGGAATG-3'

AvaII

Primer-4 5'-GTTTCCCGAGTCACGAC-3' (M13 primer)

Primer-5 3'-CAAAGTACCTAAATATCGAATTCACCT-5'

AflII

Primer-6 5'-ATAGCTTAAGTGGAACAAACACG-3'

AflII

Primer-7 3'-GGAACCAGATCTTGTTTCCC-5'

XbaI

Primer-8 5'-GGTCTAGAACAAAGGGATTGGACA-3'

XbaI

Primer-9 3'-CTACCTACCATGGTGATGAT-5'

KpnI

Primer-10 5'-GATGGTACCACTACTATTCATGGACA-3'

KpnI

pUM66 was digested with BglII and SpeI and the fragment of about 1.2 kbp was recovered from 0.5% low melting agarose. The 1.2 kbp fragment was ligated with the digestion product of pUC19 with BamHI and XbaI to
5 obtain pUCT2 (3.9 kbp). Next, using pUCT2 as a template and using Primer-1 and Primer-2, the fragment of 620 bp was amplified following conventional procedures for PCR and then recovered; after amplification of the fragment of about 550 bp using Primer-3 and Primer-4, the
10 amplified fragment was recovered. Furthermore, the fragment of about 620 bp was digested with HindIII and AvaII; the fragment of 550 bp was digested with AvaII and BamHI. These fragments were ligated with the digestion product of pUC19 with HindIII and BamHI by
15 ligase, respectively. The resulting plasmid was extracted and named pUC19-1 (3.9 kbp).

Next, using pUCT2 as a template and using Primer-4 and Primer-6, the fragment of about 500 bp was amplified following conventional procedures for PCR and
20 then recovered; after amplification of the fragment of about 700 bp using Primer-1 and Primer-5, the amplified

fragment was recovered. Furthermore, the fragment of about 500 bp was digested with AflII and EcoRI; the fragment of about 700 bp was digested with HindIII and AflII. These fragments were ligated with the digestion product of pUC19 with HindIII and AflII by ligase. The resulting plasmid was extracted and named pUC19-2 (about 3.9 kbp). Furthermore, pUC19-1 was digested with EcoRI and the digestion product was subjected to 0.6% low melting agarose gel electrophoresis to recover the fragment of about 3.3 kbp. pUC19-2 was also digested with EcoRI and the digestion product was subjected to 2.0% low melting agarose gel electrophoresis to recover the fragment of about 550 bp. This fragment was ligated with the about 3.3 kbp fragment derived from pUC19-1 described above using ligase to obtain plasmid pUC19L bearing the fragment in which two TGA codons at the 5' end in ORF of TM-66 have been changed to TGG.

In order to change two TGA codons at the 3' end of ORF of TM66 to TGG, firstly pUM66 was digested with EcoRI and PvuII and the fragment of about 1720 bp was recovered from 0.6% low melting agarose gel. The recovered fragment was ligated with the digestion product of pUC19 with EcoRI and HincII to obtain plasmid pUCT3 (about 4.4 kbp). Using pUCT3 as a template and using Primer-4 and Primer-7, the fragment of about 820 bp was amplified following conventional procedures for PCR and also the fragment of about 900 bp using Primer-8 and Primer-1 was amplified likewise, and the both

fragments were then recovered, respectively. After this 820 bp fragment was digested with EcoRI and XbaI, the digestion product was ligated with the aforesaid about 900 bp fragment obtained by digestion with XbaI and HindIII and the digestion product of pUC19 with HindIII and EcoRI, using ligase to obtain plasmid pUCT4 (about 4.4 kbp). Next, using pUCT-4 as a template and also using Primer-4 and Primer-9, the fragment of about 880 bp was amplified following conventional procedures for PCR and also the fragment of about 900 bp using pUCT3 as a primer and using Primer-1 and Primer-10 was amplified likewise following the conventional procedures for PCR; and the fragments were then recovered, respectively. After this 880 bp fragment was digested with EcoRI and KpnI, the digestion product was ligated with the aforesaid about 850 bp fragment obtained by digestion with HindIII and KpnI and the digestion product of pUC19 with EcoRI and HindIII, using ligase to obtain plasmid pUC19R.

20 In order to obtain plasmid in which TGA codons in ORF of TM-66 are all changed to TGG, pUM66 was digested with MluI and PvuII and the fragment of about 4.8 kbp was then recovered from 0.6% low melting agarose gel. The recovered fragment was ligated with the about 25 1.0 kbp fragment obtained by the digestion of pUC19R with MluI and PstI to obtain plasmid. This plasmid was further digested with EcoT22I and NheI. The resulting fragment of about 5.2 kbp was ligated with the fragment

of about 640 bp obtained by the digestion of pUC19L with EcoT22I and NheI, using ligase to obtain plasmid bearing the full length of ORF in which TGA codons in ORF of TM-66 were all changed to TGG. This plasmid was named
5 pTM66 (about 5.8 kbp).

(4) Construction of pNZ7929-66 (Fig. 12)

After pTM66 was digested with PstI, the digestion product was partially digested with SspI to recover the fragment of about 2.4 kbp. Three of the
10 about 2.4 kbp fragment, the fragment obtained by the digestion of the synthetic promoter in Reference Example with HindIII and HincII and the fragment obtained by the digestion of pUC18 with HindIII and PstI were ligated using ligase to obtain pUTM66P (about 5.2 kbp). Next,
15 pUTM66P was digested with HindIII and BamHI and the digestion product was recovered from low melting agarose gel. This fragment (about 2.5 kbp) was ligated with the fragment obtained by the digestion of pNZ1729R with HindIII and BamHI, using ligase to obtain the desired
20 plasmid pNZ7929-66 (about 11.5 kbp).

(5) Construction of fNZ7929-66 and purification

The procedures similar to Example 1 (3) were repeated using pNZ7929-66 obtained in (4) described above to obtain fNZ7929-66.

25 Example 8

Expression of TM-67 and TM-66 polypeptides in cells infected with fNZ7929-67 and fNZ7929-66

In order to examine that fNZ7929-67 and fNZ7929XM66 express the TM-67 and TM-66 polypeptides in infected cells, the immuno-fluorescence antibody method was carried out. fNZ7929-67 and fNZ7929-66 were
 5 infected to CEF, respectively and cultured at 37°C until plaques appeared. Thereafter the medium was fixed with cold acetone. Using Mycoplasma gallisepticum S6-immunized chicken serum or Mycoplasma gallisepticum-infected chicken serum as a primary antibody, the medium
 10 was diluted to 100- to 1000-fold and the dilution was reacted. These culture cells were further reacted with fluorescence (FITC)-bound anti-chick immunoglobulin. After washing out the non-specific reaction portion, microscopic observation was made under fluorescence-
 15 excited wavelength. The reactivity is shown in Table 3.

Table 3. Reactivity of recombinant virus-infected CEF to various antisera

Infected virus	Reactivity to primary antibody Infected		
	anti-S6	S6	SPF
fNZ7929-67	+++	+++	-
fNZ7929-66	+++	+++	-
fNZ2929XM1	++	++	-
NP	-	-	-

+++ : strongly reacted over the entire surface

++ : strongly reacted

+ : reacted

± : weakly reacted

- : not reacted

The results reveal that fNZ7929-67, fNZ7929-66 and fNZ2929XM1 which are the recombinant viruses of the present invention were reactive with anti-S6 and S6 infection that are reactive with the infected cells
5 alone.

Example 9

Activity of inhibiting the growth of an induced antibody of recombinant FPV-inoculated chick

After fNZ7929-67 and fNZ7929-66 were cultured
10 in CEF at 37°C for 48 hours, the procedure of freezing and thawing was repeated twice to recover the cell suspension. The cell suspension was adjusted to have a virus titer of 10^6 pfu/ml and then inoculated through a stab needle to SPF chicken (Line M, Nippon Seibutsu
15 Kagaku Kenkyusho) of 7 days old at the right wing web in a dose of 10 μ l. After the inoculation, generation of the pock was observed. Two weeks after the inoculation, sera were collected.

On the other hand, Mycoplasma gallisepticum S6
20 was inoculated on PPLO liquid medium (modified Chanock's medium) in a 10% concentration. After incubation at 37°C for 3 days, the cell mass was removed through a membrane filter of 0.45 μ m. The filtrate was diluted with PPLO liquid medium in a cell count of 10^3 CFU/ml
25 and the resulting dilution was provided as the cell solution for determination of activity.

The cell solution was put in a polypropylene tube by 400 μ l each and 100 μ l each of standard chick serum, TMG-1 immunized serum (Japanese Patent Application Laid-Open No. 2-111795) and various sera
5 were added thereto, respectively. By incubation at 37°C for 2 to 5 days, growth inhibition test was conducted.

On Days 0, 1, 2, 3 and 4 after the incubation, 10 μ l each was collected from the culture broth for
Mycoplasma gallisepticum (hereinafter abbreviated as MG)
10 growth inhibition test and spread over PPLO agar medium followed by incubation at 37°C for 7 days. The corresponding cell count in the culture broth was deduced from the number of colonies appeared. The results of measuring the cell count on Day 3 are shown
15 in Table 4.

Table 4

Sample	Cell Count on Day 3
SPF chicken sera	1.3 x 10 ⁸
anti-TMG-1 chicken sera	1.8 x 10 ⁵
fNZ2929XMI-inoculated chicken sera	4.5 x 10 ⁵
fNZ7929-67-inoculated chicken sera	2.8 x 10 ⁴
fNZ7929-66-inoculated chicken sera	3.2 x 10 ⁴

In the culture broth of the medium in which SPF chick sera or equine sera were supplemented, there was no difference in growth rate of MG and the cell

count reached saturation on Day 3 of the incubation. In the culture broth in which fNZ7929-67- or fNZ7929-66- inoculated sera were added, the growth of MG was more effectively inhibited than the case of fNZ2929XM1 or
5 than the case of immunizing an antigen inducing an antibody for inhibiting the growth of MG as in anti-TTMG-1 chick sera. This fact indicates that TM67 polypeptide and TM66 polypeptide are antigens capable of inducing antibodies which can inhibit the growth of MG
10 more effectively than in TTMG-1.

Example 10

Obtaining of polypeptide DNA TM-16 in which Mycoplasma gallisepticum is expressed

(1) Preparation of Mycoplasma gallisepticum genomic
15 DNA

Mycoplasma gallisepticum S6 strain was incubated at 37°C for 3 to 5 days in liquid medium prepared by adding to 10 ml of PPLO broth basal medium 20% equine sera, 5% yeast extract, 1% glucose and a
20 trace amount of Phenol Red as a pH indicator. As Mycoplasma gallisepticum grew, the pH of the culture medium decreased. At the time when the color of the pH indicator contained in the medium was changed from red to yellow, the incubation was terminated. After the
25 culture medium was centrifuged at 8000G for 20 minutes, the cells were collected. The cells were suspended in PBS in a 1/10 volume of the medium. The suspension was

again centrifuged at 10,000 rpm for 20 minutes and the cells were then collected. The collected cells were again suspended in 2.7 ml of PBS. After SDS was added to the suspension in a concentration of 1% and further
5 10 µg of RNase was added thereto, incubation was performed at 37°C for 30 minutes for lysis.

The lysate was extracted 3 times with an equal volume of phenol and then 3 times with ethyl ether. By ethanol precipitation, 200 µg of Mycoplasma
10 gallisepticum genomic DNA was obtained.

(2) Genomic Southern hybridization of Mycoplasma
gallisepticum using M-16 DNA gene as a probe

After 1 µg of the Mycoplasma gallisepticum DNA obtained in (1) described above was digested with XbaI,
15 the digestion product was subjected to 0.6% low melting agarose gel electrophoresis. After the electrophoresis, the gel was immersed in an alkaline denaturation solution (0.5 M NaOH, 1.5 M NaCl) for 10 minutes to denature DNA. The gel was then immersed in a
20 neutralization solution (3 M sodium acetate, pH 5.5) for 10 minutes for neutralization and then transferred onto a nylon membrane in 6-fold SSC solution (0.7 M NaCl, 0.07 M sodium citrate, pH 7.5). After air-drying, the nylon membrane was baked at 80°C for 2 hours and 4-fold
25 SET (0.6 M NaCl, 0.08 M Tris-HCl, 4 mM EDTA, pH 7.8)-10-fold Denhardt-0.1% SDS-0.1% Na₄P₂O₇-50 µg/ml of denatured salmon sperm DNA and pUM-16 (M-16 gene is contained in this plasmid; cf. Japanese Patent

Application Laid-Open No. 2-111795) labelled in a conventional manner was added thereto to perform hybridization at 68°C for 14 hours. The nylon membrane was overlaid on an X ray film. It was confirmed by
5 autoradiography that hybridization occurred to the about 5.5 kbp fragment.

(3) Cloning of XbaI-digested fragment of about 5.5 kbp to pUC-19 and colony hybridization

After 4 µg of the Mycoplasma gallisepticum DNA
10 obtained in (1) described above was digested with XbaI, the digestion product was subjected to 0.6% low melting agarose gel electrophoresis to recover the fragment of about 5.5 kbp. The fragment was ligated with the digestion product of pUC-19 with XbaI using ligase.
15 Competent Escherichia coli TG1 strain was transformed with the ligation product. The transformants were cultured at 37°C for 15 hours in LB agar medium supplemented with 0.003% of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, 0.03 mM of isopropylthio-β-D-
20 galactopyranoside and 40 µg/ml of ampicillin. White colonies grown on the medium were transferred onto a nylon membrane and hybridization was carried out in a manner similar to (2) described above. It was confirmed by autoradiography that cloning was effected and this
25 plasmid was named pUM16.

(8) Sequence analysis of pUM-16 insert DNA

The sequence of about 5.5 kbp fragment inserted into pUM-16 prepared in (3) described above was

analyzed by the dideoxy method by Sanger et al. (Proc. Natl. Acad. Sci. USA, 74, 5463 (1977)).

The restriction enzyme map of this fragment is shown in Fig. 13. The restriction enzyme map of the
5 open reading frame present in this fragment is also shown in Fig. 14 and the nucleotide sequence of this ORF and the amino acid sequence deduced therefrom are shown by SEQ ID NO: 15. The polypeptide deduced from this ORF was named TM-16 polypeptide.

10 Hereinafter the sequences employed in the present invention are described as sequence listing. The sequences used for the primers are described basically from the 3' end. However, the primers depicted from the 5' end in the body of the
15 specification are described from the 5' end to conform to the description.

SEQUENCE LISTING

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SHIONOGI PHARMACEUTICAL CO., LTD.

(ii) Title of Invention:

A MYCOPLASMA GALLISEPTICUM ANTIGEN, DNA ENCODING
THE ANTIGEN, RECOMBINANT VECTOR BEARING THE DNA AND
RECOMBINANT VIRUS UTILIZING THE RECOMBINANT VECTOR
AS WELL AS USE THEREOF

(iii) Number of sequences: 43

(2) Information for SEQ ID NO. 1

(i) Sequence characteristics:

(A) Length of sequence: 2369

(B) Type of sequence: amino acid

(C) Number of strand: double strand

(D) Topology: linear

(E) Kind of sequence: DNA

(xi) Sequence description: SEQ ID NO: 1

GTCTGGGGTT GGTTCGATCA GCGAAAATAA ACCCGATTTA TTA	60
CTTACTG AACTTTATAT ATTCTTTAGA TAATAATAGA CGTGGTGAAC	120
GTAAGTTATT GGCTTAACTT TAAGTGAAAA GAAAAACAT	176
TTTAAAGTTT GTTAGTTTAT TAGGTATTGT TTCGTTTGTA ATG TTA	
	Met Leu
GCA GCT GCT AGT TGT ACT TCA GCA GCT ACA CCA ACT CCA AAC CCT GAA	224
Ala Ala Ala Ser Cys Thr Ser Ala Ala Thr Pro Thr Pro Asn Pro Glu	
5 10 15	
CCA AAA CCA ACT CCA AAC CCT GAA CCA AAA CCA GAT CCA ATG CCA AAC	272
Pro Lys Pro Thr Pro Asn Pro Glu Pro Lys Pro Asp Pro Met Pro Asn	
20 25 30	
CCT CCT AGT GGT GGT AAC ATG AAT GGT GGA AAC ACC AAC CCA AGT GAT	320
Pro Pro Ser Gly Gly Asn Met Asn Gly Gly Asn Thr Asn Pro Ser Asp	
35 40 45 50	
GGG CAA GGC ATG ATG AAT GCA GCT GCT AAA GAA TTA GCA GAC GCA AAA	368
Gly Gln Gly Met Met Asn Ala Ala Ala Lys Glu Leu Ala Asp Ala Lys	
55 60 65	
GCT GCT TTA ACT ACT TTG ATT AAT GGT GAA ACT GCA AAT CTT GCG TCA	416
Ala Ala Leu Thr Thr Leu Ile Asn Gly Glu Thr Ala Asn Leu Ala Ser	
70 75 80	
TAT GAA GAC TAT GCT AAG ATC AAA AGT GAA TTA ACA TCA GCG TAT GAA	464
Tyr Glu Asp Tyr Ala Lys Ile Lys Ser Glu Leu Thr Ser Ala Tyr Glu	
85 90 95	
ACA GCT AAA GCA GTT TCA GCT AAA ACT GGT GCA ACT CTA AAT GAG GTT	512
Thr Ala Lys Ala Val Ser Ala Lys Thr Gly Ala Thr Leu Asn Glu Val	
100 105 110	

AAT GAG GCA AAA ACT ACA TTA GAT GCT GCT ATT AAA AAA GCT GCT AGT	560
Asn Glu Ala Lys Thr Thr Leu Asp Ala Ala Ile Lys Lys Ala Ala Ser	
115 120 125 130	
GCT AAG AAT GAT TTT GAT GCA CAG CAC GGG TCA CTA GTG GAA GCA TAT	608
Ala Lys Asn Asp Phe Asp Ala Gln His Gly Ser Leu Val Glu Ala Tyr	
135 140 145	
AAC AAT CTA AAA GAA ACG TTA AAA GAA GAA AAA ACT AAT TTA GAT TCT	656
Asn Asn Leu Lys Glu Thr Leu Lys Glu Glu Lys Thr Asn Leu Asp Ser	
150 155 160	
CTT GCA AAC GAA AAT TAT GCA GCA ATC AGA ACT AAT CTT AAT AGT TTA	704
Leu Ala Asn Glu Asn Tyr Ala Ala Ile Arg Thr Asn Leu Asn Ser Leu	
165 170 175	
TAT GAA AAA GCC AAT ACT ATT GTT ACA GCT ACT TTA GAC CCT GCT ACT	752
Tyr Glu Lys Ala Asn Thr Ile Val Thr Ala Thr Leu Asp Pro Ala Thr	
180 185 190	
GGA AAT ATT CCT GAA GTT ATG AGT GTA ACA CAA GCT AAT CAA GAT ATT	800
Gly Asn Ile Pro Glu Val Met Ser Val Thr Gln Ala Asn Gln Asp Ile	
195 200 205 210	
ACT AAT GCA ACT TCA AGA CTA ATA GCT TGA AAA CAA AAT GCT GAT AAT	848
Thr Asn Ala Thr Ser Arg Leu Ile Ala Trp Lys Gln Asn Ala Asp Asn	
215 220 225	
TTA GCT AAC AGT TTT ATC AAA CAG TCT TTA GTT AAA AAT AAT TTG ACT	896
Leu Ala Asn Ser Phe Ile Lys Gln Ser Leu Val Lys Asn Asn Leu Thr	
230 235 240	
AGA GTT GAT GTA GCA AAT AAT CAG GAG CAA CCA GCA AAT TAC AGT TTT	944
Arg Val Asp Val Ala Asn Asn Gln Glu Gln Pro Ala Asn Tyr Ser Phe	
245 250 255	
GTT GGT TTT AGT GTT AAT GTT GAT ACT CCT AAC TGA AAT TTT GCG CAA	992
Val Gly Phe Ser Val Asn Val Asp Thr Pro Asn Trp Asn Phe Ala Gln	
260 265 270	

AGA AAA GTT TGG GCC TCT GAA AAT ACT CCT TTA GCA ACT ACA CCA GCT	1040
Arg Lys Val Trp Ala Ser Glu Asn Thr Pro Leu Ala Thr Thr Pro Ala	
275 280 285 290	
GAA GAT GCA ACA CAA CAA GCT GCA TCC TTA ACA GAT GTT TCA TGA ATC	1088
Glu Asp Ala Thr Gln Gln Ala Ala Ser Leu Thr Asp Val Ser Trp Ile	
295 300 305	
TAT AGT TTA AAT GGT GCT GAA GCT AAA TAC ACA TTA AGC TTT CGT TAC	1136
Tyr Ser Leu Asn Gly Ala Glu Ala Lys Tyr Thr Leu Ser Phe Arg Tyr	
310 315 320	
TTT GGA GCT GAA AAA ACA GCT TAC TTA TAT TTC CCT TAT AAA TTA GTT	1184
Phe Gly Ala Glu Lys Thr Ala Tyr Leu Tyr Phe Pro Tyr Lys Leu Val	
325 330 335	
AAA ACT AGT GAT AAT GTT GGT TTA CAA TAT AAG TTA AAT GGT GGT GAT	1232
Lys Thr Ser Asp Asn Val Gly Leu Gln Tyr Lys Leu Asn Gly Gly Asp	
340 345 350	
ACT AAA CAA ATT AAC TTT GTA CAA ACT CCA GCT TCT GGT TCA AGT GAT	1280
Thr Lys Gln Ile Asn Phe Val Gln Thr Pro Ala Ser Gly Ser Ser Asp	
355 360 365 370	
GTT GCT GCT AAT GAA GAA GAA ACT ATG GCT AGT CCT GCT GAA ATG CAG	1328
Val Ala Ala Asn Glu Glu Glu Thr Met Ala Ser Pro Ala Glu Met Gln	
375 380 385	
TCA GCA CCA ACT GTT Asp Asp Ile Lys Ile Ala Lys Val Ala Leu Ser	1376
Ser Ala Pro Thr Val GAC GAT ATT AAG ATT GCT AAA GTC GCT TTA TCT	
390 395 400	
AAT CTA AAA TTC AAT TCA AAC ACA ATT GAA TTT AGT GTC CCT ACA GGT	1424
Asn Leu Lys Phe Asn Ser Asn Thr Ile Glu Phe Ser Val Pro Thr Gly	
405 410 415	
AAA GCA GCT CCT ATG ATT GGA AAT ATG TAT TTA ACT TCA TCT AAT TCG	1472
Lys Ala Ala Pro Met Ile Gly Asn Met Tyr Leu Thr Ser Ser Asn Ser	
420 425 430	

GAA GTT AAT AAA AAC AAA ATT TAT GAT GAT CTA TTC GGC AAC AGC TTT	1520
Glu Val Asn Lys Asn Lys Ile Tyr Asp Asp Leu Phe Gly Asn Ser Phe	
435 440 445 450	
AAT AAT GAA AAT AAT CCA ACC GCG GTT ACT GTT GAC CTA TTA AAA GGT	1568
Asn Asn Glu Asn Asn Pro Thr Ala Val Thr Val Asp Leu Leu Lys Gly	
455 460 465	
TAT AGT CTT GCT GCT AGT TAC AGT ATA TAT GTT CGC CAA TTC AAT GAT	1616
Tyr Ser Leu Ala Ala Ser Tyr Ser Ile Tyr Val Arg Gln Phe Asn Asp	
470 475 480	
TTA AAT ATT CAA AAT GGC ACT GAT ATG GCA AGA TCT CGA ACA GTA TAC	1664
Leu Asn Ile Gln Asn Gly Thr Asp Met Ala Arg Ser Arg Thr Val Tyr	
485 490 495	
TTA GTT GGG TTA ATT GGT AGT AAT GCA AGT AGA TCA ATT AGG AAC CTA	1712
Leu Val Gly Leu Ile Gly Ser Asn Ala Ser Arg Ser Ile Arg Asn Leu	
500 505 510	
TCA AAT GTA AGA ACT TCT CCT AAC ACG GTT AGT ACC AAT AGA ACA TTT	1760
Ser Asn Val Arg Thr Ser Pro Asn Thr Val Ser Thr Asn Arg Thr Phe	
515 520 525 530	
ACA ATA TAT GTA AAT GCT CCA AAG TCA GGT GAT TAT TAT CTA AGT GGT	1808
Thr Ile Tyr Val Asn Ala Pro Lys Ser Gly Asp Tyr Tyr Leu Ser Gly	
535 540 545	
TCG TAT CTT ACA AAT CAA AAT AGA AAT ATT AAA TTC TTA AAT AGC AGC	1856
Ser Tyr Leu Thr Asn Gln Asn Arg Asn Ile Lys Phe Leu Asn Ser Ser	
550 555 560	
TCT GAT CAG ACT AGT AGT AAT TCT CTA ACA CTA AAT GTT AAG GCT CAA	1904
Ser Asp Gln Thr Ser Ser Asn Ser Leu Thr Leu Asn Val Lys Ala Gln	
565 570 575	
ACA AAT TGA GAG ACT TTA GGA AAT TTC GAT ACA TCT AAT AAT ACG AAT	1952
Thr Asn Trp Glu Thr Leu Gly Asn Phe Asp Thr Ser Asn Asn Thr Asn	
580 585 590	

ATT GTT ACT AAT AGT GGA TCA AGC ACA ACA ACA GGC CGG ACT TTA AAT	2000
Ile Val Thr Asn Ser Gly Ser Ser Thr Thr Thr Gly Arg Thr Leu Asn	
595 600 605 610	
TTA AAA CAA GGA TTA AAC AAA ATT GTT ATC AGT GGA GTA GGT AAT GGT	2048
Leu Lys Gln Gly Leu Asn Lys Ile Val Ile Ser Gly Val Gly Asn Gly	
615 620 625	
AAT ACT CCT TTC ATA GGT AAC TTA ACA TTT ACT TTG ATG GAT AAA ACA	2096
Asn Thr Pro Phe Ile Gly Asn Leu Thr Phe Thr Leu Met Asp Lys Thr	
630 635 640	
GCT AGT CCT GTA GTT GAT GAC ACT ATT TTA GAA GGA TCT ATA GAA GCT	2144
Ala Ser Pro Val Val Asp Asp Thr Ile Leu Glu Gly Ser Ile Glu Ala	
645 650 655	
GGT TCA AAA TAA AAAATTATGT TTTTAAAT CTTTTTCAA GGATCATGTT	2196
Gly Ser Lys ***	
660	
TCTGTTTAAA CGCTAAGTTA GTTAGATAAT-AAAATAAAAG TTATTGTTT TACTCCATGT	2256
AATATGGCAT GAAATCTGAA TCAAACCTCA GATTTCATGT TTTTTTATT AAGGAAGCAA	2316
ATATGAGATA CTAGCAGCCT TTTGTCTACT ATACTTATGA TCGAACTAGA TCT	2369

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(2) Information for SEQ ID NO. 2

(i) Sequence characteristics:

- 5 (A) Length of sequence: 48
(B) Type of sequence: nucleic acid
(C) Number of strand: single
(D) Topology: linear
(E) Kind of sequence: other nucleic acid,
synthetic DNA

(xi) Indication of sequence: SEQ ID NO: 2

GATCTTCCAT TTAGGATCT ATATTATTTT TTCAACGATC CGAGCTCG

48

10 (2) Information for SEQ ID NO. 3

(i) Sequence characteristics:

- 15 (A) Length of sequence: 48
(B) Type of sequence: nucleic acid
(C) Number of strand: single
(D) Topology: linear
(E) Kind of sequence: other nucleic acid,
synthetic DNA

(xi) Indication of sequence: SEQ ID NO: 3

GATCTTCCAT TTAGGATCT ATATTATTTT TTCAACGATC CGAGCTCG

48

(2) Information for SEQ ID NO. 4

20 (i) Sequence characteristics:

- (A) Length of sequence: 55
(B) Type of sequence: nucleic acid
(C) Number of strand: single
(D) Topology: linear

(E) Kind of sequence: other nucleic acid,
synthetic DNA

(xi) Indication of sequence: SEQ ID NO: 4

AGCTTTTTTT TTTTTTTTTT TTTGGCATAT AAATAATAAA TACAATAATT AATTA 55

(2) Information for SEQ ID NO. 5

5 (i) Sequence characteristics:

(A) Length of sequence: 55

(B) Type of sequence: nucleic acid

(C) Number of strand: single

(D) Topology: linear

10 (E) Kind of sequence: other nucleic acid,
synthetic DNA

(xi) Indication of sequence: SEQ ID NO: 5

CGCGTAATTA ATTATTGTAT TTATTATTTA TATGCCAAAA AAAAAAAAAA AAAAA 55

(2) Information for SEQ ID NO. 6

(i) Sequence characteristics:

15 (A) Length of sequence: 40

(B) Type of sequence: nucleic acid

(C) Number of strand: single

(D) Topology: linear

20 (E) Kind of sequence: other nucleic acid,
synthetic DNA

(xi) Indication of sequence: SEQ ID NO: 6

CGCGTAAAAA TTGAAAAACT ATTCTAATTT ATTGCACTCG 40

(2) Information for SEQ ID NO. 7

(i) Sequence characteristics:

- 5 (A) Length of sequence: 40
(B) Type of sequence: nucleic acid
(C) Number of strand: single
(D) Topology: linear
(E) Kind of sequence: other nucleic acid,
synthetic DNA

(xi) Indication of sequence: SEQ ID NO: 7

GATCCGAGTG CAATAAATTA. GAATAGTTTT TCAATTTTTA 40

10 (2) Information for SEQ ID NO. 8

(i) Sequence characteristics:

- 15 (A) Length of sequence: 42
(B) Type of sequence: nucleic acid
(C) Number of strand: single
(D) Topology: linear
(E) Kind of sequence: other nucleic acid,
synthetic DNA

(xi) Indication of sequence: SEQ ID NO: 8

GATCCCCGGG CGAGCTCGCT AGCGGGCCCG CATGCGGTAC CG 42

(2) Information for SEQ ID NO. 9

20 (i) Sequence characteristics:

- (A) Length of sequence: 42
(B) Type of sequence: nucleic acid
(C) Number of strand: single
(D) Topology: linear

(E) Kind of sequence: other nucleic acid,
synthetic DNA

(xi) Indication of sequence: SEQ ID NO: 9

TCGACGGATC CGCATGCGGG CCCGCTAGCG AGCTCGCCCG GG 42

(2) Information for SEQ ID NO. 10

5 (i) Sequence characteristics:

(A) Length of sequence: 39

(B) Type of sequence: nucleic acid

(C) Number of strand: single

(D) Topology: linear

10 (E) Kind of sequence: other nucleic acid,
synthetic DNA

(xi) Indication of sequence: SEQ ID NO: 10

TCGACCCGGT ACATTTTAT AAAAATGTAC CCGGGGATC 39

(2) Information for SEQ ID NO. 11

(i) Sequence characteristics:

15 (A) Length of sequence: 35

(B) Type of sequence: nucleic acid

(C) Number of strand: single

(D) Topology: linear

20 (E) Kind of sequence: other nucleic acid,
synthetic DNA

(xi) Indication of sequence: SEQ ID NO: 11

GATCCCCGGG TACATTTTAA TAAAAATGTA CCGGG 35

(2) Information for SEQ ID NO. 12

(i) Sequence characteristics:

- 5 (A) Length of sequence: 14
 (B) Type of sequence: nucleic acid
 (C) Number of strand: single
 (D) Topology: linear
 (E) Kind of sequence: other nucleic acid,
 synthetic DNA

(xi) Indication of sequence: SEQ ID NO: 12

ATTTTATATAA AAAT

14

10 (2) Information for SEQ ID NO. 13

(i) Sequence characteristics:

- 15 (A) Length of sequence: 66
 (B) Type of sequence: amino acid
 (C) Number of strand: single
 (D) Topology: linear
 (E) Kind of sequence: DNA

(xi) Indication of sequence: SEQ ID NO: 13

ATC GCG ATC CTA CTT TTA ACA GTA GTG ACC TTA GCC ATC TCT GCA GCC
 Ile Ala Ile Leu Leu Leu Thr Val Val Thr Leu Ala Ile Ser Ala Ala

48

5

10

15

GCC CTT GCA TAT AGT ATG

Ala Leu Ala Tyr Ser Met

66

20

(2) Information for SEQ ID NO. 14

(i) Sequence characteristics:

(A) Length of sequence: 1387

(B) Type of sequence: amino acid

5 (C) Number of strand: double

(D) Topology: linear

(E) Kind of sequence: DNA

(xi) Indication of sequence: SEQ ID NO: 14

AAAAACATCA GATTGTTAAT CTGATATCTT TGCTTAAAAA AACACAAAAT CTTCTAACAA	60
AATCCTAAAT AAATAAGCCG TTAAATTAAC TAAAAAATTA AAAAAATGGT TTTTCTTATC	120
AACCAAAATT CTCTAGTAAT AAACGCTTAT TTATTTTAT TTTTAGTCAT CTTTAAAGAT	180
ATAAATATAT CTTAATATTC T ATG AAT AAG AAA AGA ATC ATC TTA AAG ACT	231
Met Asn Lys Lys Arg Ile Ile Leu Lys Thr	
	5 10
ATT AGT TTG TTA GGT ACA ACA TCC TTT CTT AGC ATT GGG ATT TCT AGC	279
Ile Ser Leu Leu Gly Thr Thr Ser Phe Leu Ser Ile Gly Ile Ser Ser	
	15 20 25
TGT ATG TCT ATT ACT AAA AAA GAC GCA AAC CCA AAT AAT GGC CAA ACC	375
Cys Met Ser Ile Thr Lys Lys Asp Ala Asn Pro Asn Asn Gly Gln Thr	
	30 35 40
CAA TTA CAA GCA GCG CGA ATG GAG TTA ACT GAT CTA ATC AAT GCT AAA	327
Gln Leu Gln Ala Ala Arg Met Glu Leu Thr Asp Leu Ile Asn Ala Lys	
	45 50 55
GCA AGG ACA TTA GCT TCA CTA CAA GAC TAT GCT AAG ATT GAA GCT AGT	423
Ala Arg Thr Leu Ala Ser Leu Gln Asp Tyr Ala Lys Ile Glu Ala Ser	
	60 65 70
TTA TCA TCT GCT TAT AGT GAA GCT GAA ACA GTT AAC AAT AAC CTT AAT	471
Leu Ser Ser Ala Tyr Ser Glu Ala Glu Thr Val Asn Asn Asn Leu Asn	
	75 80 85 90
GCA ACA CTA GAA CAA CTA AAA ATG GCT AAA ACT AAT TTA GAA TCA GCC	519
Ala Thr Leu Glu Gln Leu Lys Met Ala Lys Thr Asn Leu Glu Ser Ala	
	95 100 105
ATC AAC CAA GCT AAT ACG GAT AAA ACG ACT TTT GAT AAT GAA CAT CCA	567
Ile Asn Gln Ala Asn Thr Asp Lys Thr Thr Phe Asp Asn Glu His Pro	
	110 115 120

AAT TTA GTT GAA GCA TAC AAA GCA CTA AAA ACC ACT TTA GAA CAA CGT 615
 Asn Leu Val Glu Ala Tyr Lys Ala Leu Lys Thr Thr Leu Glu Gln Arg
 125 130 135

GCT ACT AAC CTT GAA GGT TTA GCT TCA ACT GCT TAT AAT CAG ATT CGT 663
 Ala Thr Asn Leu Glu Gly Leu Ala Ser Thr Ala Tyr Asn Gln Ile Arg
 140 145 150

AAT AAT TTA GTG GAT CTA TAC AAT AAT GCT AGT AGT TTA ATA ACT AAA 711
 Asn Asn Leu Val Asp Leu Tyr Asn Asn Ala Ser Ser Leu Ile Thr Lys
 155 160 165 170
 ACA CTA GAT CCA CTA AAT GGG GGA ATG CTT TTA GAT TCT AAT GAG ATT 759
 Thr Leu Asp Pro Leu Asn Gly Gly Met Leu Leu Asp Ser Asn Glu Ile
 175 180 185

ACT ACA GTT AAT CGG AAT ATT AAT AAT ACG TTA TCA ACT ATT AAT GAA 807
 Thr Thr Val Asn Arg Asn Ile Asn Asn Thr Leu Ser Thr Ile Asn Glu
 190 195 200

CAA AAG ACT AAT GCT GAT GCA TTA TCT AAT AGT TTT ATT AAA AAA GTG 855
 Gln Lys Thr Asn Ala Asp Ala Leu Ser Asn Ser Phe Ile Lys Lys Val
 205 210 215

ATT CAA AAT AAT GAA CAA AGT TTT GTA GCG ACT TTT ACA AAC GCT AAT 903
 Ile Gln Asn Asn Glu Gln Ser Phe Val Gly Thr Phe Thr Asn Ala Asn
 220 225 230

GTT CAA CCT TCA AAC TAC AGT TTT GTT GCT TTT AGT GCT GAT GTA ACA 951
 Val Gln Pro Ser Asn Tyr Ser Phe Val Ala Phe Ser Ala Asp Val Thr
 235 240 245 250

CCC GTC AAT TAT AAA TAT GCA AGA AGG ACC GTT NNN AAT GGT GAT GAA 999
 Pro Val Asn Tyr Lys Tyr Ala Arg Arg Thr Val Xaa Asn Gly Asp Glu
 255 260 265

AAT TTA GTT GAA GCA TAC AAA GCA CTA AAA ACC ACT TTA GAA CAA CGT 615
 Asn Leu Val Glu Ala Tyr Lys Ala Leu Lys Thr Thr Leu Glu Gln Arg
 125 130 135

GCT ACT AAC CTT GAA GGT TTA GCT TCA ACT GCT TAT AAT CAG ATT CGT 663
 Ala Thr Asn Leu Glu Gly Leu Ala Ser Thr Ala Tyr Asn Gln Ile Arg
 140 145 150

AAT AAT TTA GTG GAT CTA TAC AAT AAT GCT AGT AGT TTA ATA ACT AAA 711
 Asn Asn Leu Val Asp Leu Tyr Asn Asn Ala Ser Ser Leu Ile Thr Lys
 155 160 165 170
 ACA CTA GAT CCA CTA AAT GGG GGA ATG CTT TTA GAT TCT AAT GAG ATT 759
 Thr Leu Asp Pro Leu Asn Gly Gly Met Leu Leu Asp Ser Asn Glu Ile
 175 180 185

ACT ACA GTT AAT CGG AAT ATT AAT AAT ACG TTA TCA ACT ATT AAT GAA 807
 Thr Thr Val Asn Arg Asn Ile Asn Asn Thr Leu Ser Thr Ile Asn Glu
 190 195 200

CAA AAG ACT AAT GCT GAT GCA TTA TCT AAT AGT TTT ATT AAA AAA GTG 855
 Gln Lys Thr Asn Ala Asp Ala Leu Ser Asn Ser Phe Ile Lys Lys Val
 205 210 215

ATT CAA AAT AAT GAA CAA AGT TTT GTA GGG ACT TTT ACA AAC GCT AAT 903
 Ile Gln Asn Asn Glu Gln Ser Phe Val Gly Thr Phe Thr Asn Ala Asn
 220 225 230

GTT CAA CCT TCA AAC TAC AGT TTT GTT GCT TTT AGT GCT GAT GTA ACA 951
 Val Gln Pro Ser Asn Tyr Ser Phe Val Ala Phe Ser Ala Asp Val Thr
 235 240 245 250

CCC GTC AAT TAT AAA TAT GCA AGA AGG ACC GTT NNN AAT GGT GAT GAA 999
 Pro Val Asn Tyr Lys Tyr Ala Arg Arg Thr Val Xaa Asn Gly Asp Glu
 255 260 265

CCT TCA AGT AGA ATT CTT GCA AAC ACG AAT AGT ATC ACA GAT GTT TCT 1047
Pro Ser Ser Arg Ile Leu Ala Asn Thr Asn Ser Ile Thr Asp Val Ser
270 275 280

Xaa ATT TAT AGT TTA GCT GGA ACA AAC ACG AAG TAC CAA TTT AGT TTT 1095
NNN Ile Tyr Ser Leu Ala Gly Thr Asn Thr Lys Tyr Gln Phe Ser Phe
285 290 295

AGC AAC TAT GGT CCA TCA ACT GGT TAT TTA TAT TTC CCT TAT AAG TTG 1143
Ser Asn Tyr Gly Pro Ser Thr Gly Tyr Leu Tyr Phe Pro Tyr Lys Leu
300 305 310

GTT AAA GCA GCT GAT GCT AAT AAC GTT GGA TTA CAA TAC AAA TTA AAT 1191
Val Lys Ala Ala Asp Ala Asn Asn Val Gly Leu Gln Tyr Lys Leu Asn
315 320 325 330

AAT GGA AAT GTT CAA CAA GTT GAG TTT GCC ACT TCA ACT AGT GCA AAT 1239
Asn Gly Asn Val Gln Gln Val Glu Phe Ala Thr Ser Thr Ser Ala Asn
335 340 345

AAT ACT ACA GCT AAT CCA ACT CAG CAG TTG ATG AGA TTA AAG TTG CTA 1287
Asn Thr Thr Ala Asn Pro Thr Gln Gln Leu Met Arg Leu Lys Leu Leu
350 355 360

AAA TCG TTT TAT CAG GTT TAA GATTTGGCCA AAACACAATC GAATTAAGTG 1338
Lys Ser Phe Tyr Gln Val ***
365

TTCCAACGGG TGAAGGAAAT ATGAATAAAG TTGCGCCAAT GATTGGCAA 1387

(2) Information for SEQ ID NO. 15

(i) Sequence characteristics:

(A) Length of sequence: 1945

(B) Type of sequence: amino acid

5 (C) Number of strand: double

(D) Topology: linear

(E) Kind of sequence: DNA

(xi) Indication of sequence: SEQ ID NO: 15

CGTACGTTTT AATGGCTATT GGGCTCTTAT TTTATTGTCA GGATTGCAC TAACAGCAGTT	60
ATAGCAAGC CCAATTAAGT CAGTAGAAGT TACAGAG ATG ATG AAT GGT CAA GAA	114
Met Met Asn Gly Gln Glu	
	5
GTC ACA ACA ACT AAA AAG ATT AGT ACG TTT GCC TTC TTA ATC AAC ATG	162
Val Thr Thr Thr Lys Lys Ile Ser Thr Phe Ala Phe Leu Ile Asn Met	
1 0 1 5 2 0	
TTA CCA AAT TAC CAA CTA AGT ACA CTT GGT TAC TTA CAG ATT ACA GCA	210
Leu Pro Asn Tyr Gln Leu Ser Thr Leu Gly Tyr Leu Gln Ile Thr Ala	
2 5 3 0 3 5	
GCT GCT GCT GGA CTT GTA GTA GGG ATT GTA TTA CTT GCA TTA GGC GCA	258
Ala Ala Ala Gly Leu Val Val Gly Ile Val Leu Leu Ala Leu Gly Ala	
4 0 4 5 5 0	
ACA TTC TTT GTT AAA ACT AGA CGT AAA ACA AAT GAA ATG CTT GCT GCA	306
Thr Phe Phe Val Lys Thr Arg Arg Lys Thr Asn Glu Met Leu Ala Ala	
5 5 6 0 6 5 7 0	
CTT CAA GAT GCT GAA GAA GAA GAA GTG GCA CAA GAA GAA CAA GCT GAA	354
Leu Gln Asp Ala Glu Glu Glu Glu Val Ala Gln Glu Glu Gln Ala Glu	
7 5 8 0 8 5	
GAA AAT GTT GAA GTC ACT CCA ACT CAA CAA GCT GAA GTT AAG ACT GAA	402
Glu Asn Val Glu Val Thr Pro Thr Gln Gln Ala Glu Val Lys Thr Glu	
9 0 9 5 1 0 0	
CAA TTA ATT GGC ACA CAA TTA GTA ACA ACT GAT GTA GCT AGC AAT CAA	450
Gln Leu Ile Gly Thr Gln Leu Val Thr Thr Asp Val Ala Ser Asn Gln	
1 0 5 1 1 0 1 1 5	
GCT GCA GGT ACT GAA CAA GTT GAA GGT GAT TTA TTA CCT CCT AGT CAA	498
Ala Ala Gly Thr Glu Gln Val Glu Gly Asp Leu Leu Pro Pro Ser Gln	
1 2 0 1 2 5 1 3 0	

CAA	CCA	ACG	GAA	ATG	CGT	CCA	GCT	CCT	TCA	CCA	ATG	GGT	AGT	CCT	AAG	546
Gln	Pro	Thr	Glu	Met	Arg	Pro	Ala	Pro	Ser	Pro	Met	Gly	Ser	Pro	Lys	
135					140					145					150	
TTA	TTA	GGT	CCA	AAC	CAA	GCT	GGT	CAT	CCA	CAA	CAC	GGA	CCA	CGT	CCG	594
Leu	Leu	Gly	Pro	Asn	Gln	Ala	Gly	His	Pro	Gln	His	Gly	Pro	Arg	Pro	
				155				160					165			
ATG	AAT	GCT	CAT	CCA	GGT	CAA	CCA	CGT	CCT	CAA	CAA	GCT	GGC	CCA	CGT	642
Met	Asn	Ala	His	Pro	Gly	Gln	Pro	Arg	Pro	Gln	Gln	Ala	Gly	Pro	Arg	
			170					175					180			
CCA	ATG	GGA	GCT	GGT	GGA	TCT	AAC	CAA	CCA	AGA	CCC	ATG	CCA	AAT	GGT	690
Pro	Met	Gly	Ala	Gly	Gly	Ser	Asn	Gln	Pro	Arg	Pro	Met	Pro	Asn	Gly	
	185						190					195				
CCA	CAA	AAC	CAA	CAA	GGT	CCA	AGA	CCA	ATG	AAC	CCT	CAA	GGC	AAT	CCT	738
Pro	Gln	Asn	Gln	Gln	Gly	Pro	Arg	Pro	Met	Asn	Pro	Gln	Gly	Asn	Pro	
	200					205					210					
CGT	CCT	GGA	CCA	GCT	GGC	CCA	CGA	CCT	AAC	GGC	CCA	CAA	AAT	TCT	CAA	786
Arg	Pro	Gly	Pro	Ala	Gly	Pro	Arg	Pro	Asn	Gly	Pro	Gln	Asn	Ser	Gln	
215					220					225					230	
CCA	CGT	CCT	CAA	CCA	GCT	GGC	CCA	CGT	CCA	ATG	GGA	GCT	GGT	AGA	TCT	834
Pro	Arg	Pro	Gln	Pro	Ala	Gly	Pro	Arg	Pro	Met	Gly	Ala	Gly	Arg	Ser	
			235					240					245			
AAC	CAA	CCA	AGA	CCA	ATG	CCA	AAT	GGT	CCA	CAA	AAC	CAA	CAA	GGT	CCA	882
Asn	Gln	Pro	Arg	Pro	Met	Pro	Asn	Gly	Pro	Gln	Asn	Gln	Gln	Gly	Pro	
			250					255				260				
AGA	CCA	ATG	AAC	CCT	CAA	GGC	AAT	CCT	CGT	CCT	CAA	CCA	GCT	GGT	GTC	930
Arg	Pro	Met	Asn	Pro	Gln	Gly	Asn	Pro	Arg	Pro	Gln	Pro	Ala	Gly	Val	
	265						270				275					
AGA	CCT	AAC	AGC	CCA	CAA	GCT	AAC	CAG	CCA	GGA	CCA	CGT	CCA	ACG	CCA	978
Arg	Pro	Asn	Ser	Pro	Gln	Ala	Asn	Gln	Pro	Gly	Pro	Arg	Pro	Thr	Pro	
	280					285				290						

AAT AAT CCT CAA GGA CCA CGG CCA ATG GGT CCA AGA CCA AAT GGA GGA 1026
 Asn Asn Pro Gln Gly Pro Arg Pro Met Gly Pro Arg Pro Asn Gly Gly
 295 300 305 310

CCA AAC CGA GCT TAATTAACCA ATAGATTAGC TCTAAATTTG AAAACAGTTC 1078
 Pro Asn Arg Ala

ATTCCTAGA AAATGAACTG TTTTTTTTAT TATTTGTAAG TAAATTTATT AATCAACCGC 1138
 TTGTTTTGTT GAATAAAGAT AGATCACAAC ATCTTCTTGA TTTACATCTT TAATTTGCAT 1198
 ATTATTGATC ATTAAAGGGA TCTTGATGAT CTGATACATC TTGTTATTCT CATAATCAAG 1258
 ATAATTAAGA TGTGAAGCAC TAAAAGCAAA TAGCTCTTGT TCAGATTGGA TTAGTTCTTT 1318
 AGCATTATTT AAGAACGACT GATCATCACT CAGTAATAAT AAGATCTGAT TCAAGTTTTT 1378
 GATATCAGTT GCTACTTCTT GATTTAACAT CAATGTTTCA TAGCGTGATA ATAAGGATTT 1438
 AAAACGGTGA ATGATTGATG TCGTTGCACT TTTCTCATCG TTGGTTTCAA CGTATTGAAA 1498
 AGTGTTCAAT AAGTTAATGT ATTCTTGCTG GTATTTCTTA TTAATCTGAT CAGGGTTATC 1558
 TGAATAGATT AAGATGTTCT TATTAGTTTG ATCAACAATA ACCATCGTTG CTTTCATTAA 1618
 AGCTCAGTAA GTAAATAGTT TTTCAATCTT ATGCTTTAAT AAAACGGGA TGATATTCTT 1678
 ATGTAGGTTA AACTTATTAA AAATAAGTTT TGCAATCTGG TTGACTAGTT TATGATCAAC 1738
 CTGGTTGATA GTTAATTTCT TAAGCATAAG AAGATTTTAA AATATTTAAA AAAACTATTG 1798
 CTGATATGTT AAAATAGTTA AGGTATAAAA ATAATAAATT AAATATGGCT CGTAGAGATG 1858
 ATCTAACCGG GCTTGGTCCT TTAGCAGGAA ATAATCGTTC TCATGCTTTA AACATTACCA 1918
 AGCGTCGTTG AACTTAAAC CTACAAA 1945

(2) Information for SEQ ID NO. 16

(i) Sequence characteristics:

(A) Length of sequence: 1935

(B) Type of sequence: amino acid

5 (D) Topology: linear

(E) Kind of sequence: DNA

(xi) Indication of sequence: SEQ ID NO: 16

TTTATTTT	TTTTCGTAA	ATCTTTTAAA	ATATAAATAT	ATTTTAATAT	TCT	ATG	56
						Met	
AAT AAA AAA AGA ATC ATC TTA AAG ACT ATT AGC TTG TTA GGT ACA ACA	104						
Asn Lys Lys Arg Ile Ile Leu Lys Thr Ile Ser Leu Leu Gly Thr Thr							
5	10	15					
TCC TTT CTT AGT ATT GGG ATT TCT AGC TGT ATG TCT ATT ACT AAA AAA	152						
Ser Phe Leu Ser Ile Gly Ile Ser Ser Cys Met Ser Ile Thr Lys Lys							
20	25	30					
GAT GCA AAC CCA AAT AAT GGC CAA ACC CAA TTA GAA GCA GCG CGA ATG	200						
Asp Ala Asn Pro Asn Asn Gly Gln Thr Gln Leu Glu Ala Ala Arg Met							
35	40	45					
GAG TTA ACA GAT CTA ATC AAT GCT AAA GCG ATG ACA TTA GCT TCA CTA	248						
Glu Leu Thr Asp Leu Ile Asn Ala Lys Ala Met Thr Leu Ala Ser Leu							
50	55	60	65				
CAA GAC TAT GCC AAG ATT GAA GCT AGT TTA TCA TCT GCT TAT AGT GAA	296						
Gln Asp Tyr Ala Lys Ile Glu Ala Ser Leu Ser Ser Ala Tyr Ser Glu							
70	75	80					
GCT GAA ACA GTT AAC AAT AAC CTT AAT GCA ACA TTA GAA CAA CTA AAA	344						
Ala Glu Thr Val Asn Asn Asn Leu Asn Ala Thr Leu Glu Gln Leu Lys							
85	90	95					
ATG GCT AAA ACT AAT TTA GAA TCA GCC ATC AAC CAA GCT AAT ACG GAT	392						
Met Ala Lys Thr Asn Leu Glu Ser Ala Ile Asn Gln Ala Asn Thr Asp							
100	105	110					
AAA ACG ACT TTT GAT AAT GAA CAC CCA AAT TTA GTT GAA GCA TAC AAA	440						
Lys Thr Thr Phe Asp Asn Glu His Pro Asn Leu Val Glu Ala Tyr Lys							
115	120	125					
GCA CTA AAA ACC ACT TTA GAA CAA CGT GCT ACT AAC CTT GAA GGT TTG	488						

Ala	Leu	Lys	Thr	Thr	Leu	Glu	Gln	Arg	Ala	Thr	Asn	Leu	Glu	Gly	Leu	
130					135					140					145	
TCA	TCA	ACT	GCT	TAT	AAT	CAA	ATT	CGC	AAT	AAT	TTA	GTG	GAT	CTA	TAC	536
Ser	Ser	Thr	Ala	Tyr	Asn	Gln	Ile	Arg	Asn	Asn	Leu	Val	Asp	Leu	Tyr	
				150						155					160	
AAT	AAA	GCT	AGT	AGT	TTA	ATA	ACT	AAA	ACA	CTA	GAT	CCA	CTA	AAT	GGG	584
Asn	Lys	Ala	Ser	Ser	Leu	Ile	Thr	Lys	Thr	Leu	Asp	Pro	Leu	Asn	Gly	
				165						170					175	
GGA	ACG	CTT	TTA	GAT	TCT	AAT	GAG	ATT	ACT	ACA	GCT	AAT	AAG	AAT	ATT	632
Gly	Thr	Leu	Leu	Asp	Ser	Asn	Glu	Ile	Thr	Thr	Ala	Asn	Lys	Asn	Ile	
				180						185					190	
AAT	AAT	ACG	TTA	TCA	ACT	ATT	AAT	GAA	CAA	AAG	ACT	AAT	GCT	GAT	GCA	680
Asn	Asn	Thr	Leu	Ser	Thr	Ile	Asn	Glu	Gln	Lys	Thr	Asn	Ala	Asp	Ala	
				195						200					205	
TTA	GCT	AAT	AGT	TTT	ATT	AAA	GAA	GTG	ATT	CAA	AAT	AAT	AAA	CAA	AGT	728
Leu	Ala	Asn	Ser	Phe	Ile	Lys	Glu	Val	Ile	Gln	Asn	Asn	Lys	Gln	Ser	
210					215					220					225	
TTT	GTA	GGA	ATG	TTT	ACA	AAC	ACT	AAT	GTT	CAA	CCT	TCA	AAC	TAT	AGT	776
Phe	Val	Gly	Met	Phe	Thr	Asn	Thr	Asn	Val	Gln	Pro	Ser	Asn	Tyr	Ser	
				230						235					240	
TTT	GTT	GCT	TTT	AGT	GCT	GAT	GTA	ACA	CCT	GTT	AAT	TAT	AAA	TAT	GCA	824
Phe	Val	Ala	Phe	Ser	Ala	Asp	Val	Thr	Pro	Val	Asn	Tyr	Lys	Tyr	Ala	
				245						250					255	
AGA	AGA	ACG	GTT	TGA	AAT	GGT	GAT	GAA	CCT	TCA	AGT	AGA	ATT	CTT	GCA	872
Arg	Arg	Thr	Val	Trp	Asn	Gly	Asp	Glu	Pro	Ser	Ser	Arg	Ile	Leu	Ala	
				260						265					270	
AAC	ACC	AAT	AGT	ATT	ACT	GAT	GTT	TCA	TGA	ATT	TAT	AGT	TTA	TCT	GGA	920
Asn	Thr	Asn	Ser	Ile	Thr	Asp	Val	Ser	Trp	Ile	Tyr	Ser	Leu	Ser	Gly	
				275						280					285	
ACA	AAC	ACG	AAA	TAC	CAA	TTT	AGT	TTT	AGC	AAC	TAC	GGT	CCA	TCA	ACT	968

Thr	Asn	Thr	Lys	Tyr	Gln	Phe	Ser	Phe	Ser	Asn	Tyr	Gly	Pro	Ser	Thr				
290					295					300					305				
GGT	TAT	TTA	TAT	TTC	CCT	TAT	AAG	TTG	GTT	AAA	GCG	GCT	GAT	GCT	AGT			1016	
Gly	Tyr	Leu	Tyr	Phe	Pro	Tyr	Lys	Leu	Val	Lys	Ala	Ala	Asp	Ala	Ser				
				310					315					320					
AAT	GTT	GGA	TTA	CAA	TAC	AAA	CTA	AAT	AAT	GGA	AAT	GTT	CAA	CCA	GTT			1064	
Asn	Val	Gly	Leu	Gln	Tyr	Lys	Leu	Asn	Asn	Gly	Asn	Val	Gln	Pro	Val				
			325					330					335						
GAG	TTT	GCC	ACT	TCA	ACT	AGC	GCA	AAT	AAT	ACT	ACA	GCT	AAT	CCA	ACT			1112	
Glu	Phe	Ala	Thr	Ser	Thr	Ser	Ala	Asn	Asn	Thr	Thr	Ala	Asn	Pro	Thr				
	340						345					350							
CCA	GCA	GTT	GAT	GAG	ATT	AAA	GTT	GCT	AAA	ATC	GTT	TTA	TCA	GGT	TTA			1160	
Pro	Ala	Val	Asp	Glu	Ile	Lys	Val	Ala	Lys	Ile	Val	Leu	Ser	Gly	Leu				
	355					360				365									
AGA	TTT	GGC	CAA	AAC	ACA	ATC	GAA	TTA	AGT	GTT	CCA	ACG	GGT	GAA	AGA			1208	
Arg	Phe	Gly	Gln	Asn	Thr	Ile	Glu	Leu	Ser	Val	Pro	Thr	Gly	Glu	Arg				
370				375					380					385					
AAT	ATG	AAT	AAA	GTT	GCC	CCA	ATG	ATT	GGT	AAT	ATG	TAT	ATT	ACT	TCA			1256	
Asn	Met	Asn	Lys	Val	Ala	Pro	Met	Ile	Gly	Asn	Met	Tyr	Ile	Thr	Ser				
			390					395					400						
TCT	AAT	GCT	GAA	GCA	AAT	AAA	AAG	CAA	ATT	TAC	GAT	AGT	ATT	TTT	GGA			1304	
Ser	Asn	Ala	Glu	Ala	Asn	Lys	Lys	Gln	Ile	Tyr	Asp	Ser	Ile	Phe	Gly				
		405					410					415							
AAC	ACT	TCA	TCA	CAA	ACT	GCT	AGC	CAA	ACA	TCT	GTT	AGT	GTT	GAT	CTA			1352	
Asn	Thr	Ser	Ser	Gln	Thr	Ala	Ser	Gln	Thr	Ser	Val	Ser	Val	Asp	Leu				
	420					425				430									
TTA	AAA	GGA	TAT	AGT	CTT	GCA	ACT	AGT	TCA	AGA	ACA	TAT	ATT	CGT	CAA			1400	
Leu	Lys	Gly	Tyr	Ser	Leu	Ala	Thr	Ser	Ser	Arg	Thr	Tyr	Ile	Arg	Gln				
	435					440				445									
TTT	ACT	GGT	TTA	ACA	GAT	AAT	GGC	GTA	CAA	ACC	TCT	GAC	CCA	GTT	TAT			1448	

610

(2) Information for SEQ ID NO. 17

(i) Sequence characteristics:

- 5 (A) Length of sequence: 32
(B) Type of sequence: nucleic acid
(C) Number of strand: single
(D) Topology: linear
(E) Kind of sequence: other nucleic acid,
synthetic DNA

(xi) Indication of sequence:

TACGTTCTTCCTGGCAAACCTTACCACTACTT

32

10 (2) Information for SEQ ID NO. 18

(i) Sequence characteristics:

- 15 (A) Length of sequence: 21
(B) Type of sequence: nucleic acid
(C) Number of strand: single
(D) Topology: linear
(E) Kind of sequence: other nucleic acid,
synthetic DNA

(xi) Indication of sequence:

CTACAAAGAACCTAAATATCA

21

(2) Information for SEQ ID NO. 19

20 (i) Sequence characteristics:

- (A) Length of sequence: 24
(B) Type of sequence: nucleic acid
(C) Number of strand: single
(D) Topology: linear

(E) Kind of sequence: other nucleic acid,
synthetic DNA

(xi) Indication of sequence:

TATAGAATTAAATTTTACTTATTC

24

(2) Information for SEQ ID NO. 20

5 (i) Sequence characteristics:

(A) Length of sequence: 97

(B) Type of sequence: nucleic acid

(C) Number of strand: single

(D) Topology: linear

10 (E) Kind of sequence: other nucleic acid,
synthetic DNA

(xi) Indication of sequence:

AGCTTTTTTT TTTTTTTTTT TTTGGCATAT AAATAATAAA TACAATAATT AATTACGCGT 60

AAAAATTGAA AAACATTTCT AATTTATTGC ACTCGTC 97

(2) Information for SEQ ID NO. 21

(i) Sequence characteristics:

15 (A) Length of sequence: 93

(B) Type of sequence: nucleic acid

(C) Number of strand: single

(D) Topology: linear

20 (E) Kind of sequence: other nucleic acid,
synthetic DNA

(xi) Indication of sequence:

AAAAAAAAAA AAAAAAAAAAC CGTATATTTA TTATTTATGT TATTAATTAA TGCGCATTTT 60
TAACTTTTTG ATAAGATTAA ATAACGTGAG CAG 93

(2) Information for SEQ ID NO. 22

(i) Sequence characteristics:

- (A) Length of sequence: 95
- 5 (B) Type of sequence: nucleic acid
- (C) Number of strand: single
- (D) Topology: linear
- (E) Kind of sequence: other nucleic acid,
synthetic DNA

10 (xi) Indication of sequence:

AGCTTTTTTT TTTTTTTTTT TTTGGCATAT AAATAATAAA TACAATAATT AATTACGCGT 60
AAAAATTGAA AACTATTCT AATTATTGC ACTCG 95

(2) Information for SEQ ID NO. 23

(i) Sequence characteristics:

- (A) Length of sequence: 96
- (B) Type of sequence: nucleic acid
- 15 (C) Number of strand: single
- (D) Topology: linear
- (E) Kind of sequence: other nucleic acid,
synthetic DNA

(xi) Indication of sequence:

AAAAAAAAAA AAAAAAAAAA CCGTATATTT ATTATTTATG TTATTAATTA ATGCGCATTT 60
TTAACTTTTT GATAAGATTA AATAACGTGA GCCTAG 96

(2) Information for SEQ ID NO. 24

(i) Sequence characteristics:

- (A) Length of sequence: 11
- 5 (B) Type of sequence: nucleic acid
- (C) Number of strand: single
- (D) Topology: linear
- (E) Kind of sequence: other nucleic acid,
synthetic DNA

10 (xi) Indication of sequence:

GATCCAGCATG 11

(2) Information for SEQ ID NO. 25

(i) Sequence characteristics:

- (A) Length of sequence: 10
- (B) Type of sequence: nucleic acid
- 15 (C) Number of strand: single
- (D) Topology: linear
- (E) Kind of sequence: other nucleic acid,
synthetic DNA

(xi) Indication of sequence:

GTCGTACCTG 10

(2) Information for SEQ ID NO. 26

(i) Sequence characteristics:

- (A) Length of sequence: 21
- (B) Type of sequence: nucleic acid
- 5 (C) Number of strand: single
- (D) Topology: linear
- (E) Kind of sequence: other nucleic acid,
synthetic DNA

(xi) Indication of sequence:

GGGATTTCGAATTCTATGTCT

21

10 (2) Information for SEQ ID NO. 27

(i) Sequence characteristics:

- (A) Length of sequence: 2346
- (B) Type of sequence: amino acid
- (C) Number of strand: single
- 15 (D) Topology: linear
- (E) Kind of sequence: DNA

(xi) Indication of sequence: SEQ ID NO: 27

AAAAACATCA GATTGTTAAT CTGATATCTT TGCTTAAAAA AACACAAAAT CTTCTAACAA	60
AATCCTAAAT AAATAAGCCG TTAAATTAAC TAAAAAATTA AAAAAATGGT TTTTCTTATC	120
AACCAAATT CTCTAGTAAT AAACGCTTAT TTATTTTAT TTTTAGTCAT CTTTAAAGAT	180
ATAAATATAT CTTAATATTC T ATG AAT AAG AAA AGA ATC ATC TTA AAG ACT	231
Met Asn Lys Lys Arg Ile Ile Leu Lys Thr	
5 10	
ATT AGT TTG TTA GGT ACA ACA TCC TTT CTT AGC ATT GGG ATT TCT AGC	279
Ile Ser Leu Leu Gly Thr Thr Ser Phe Leu Ser Ile Gly Ile Ser Ser	
15 20 25	
TGT ATG TCT ATT ACT AAA AAA GAC GCA AAC CCA AAT AAT GGC CAA ACC	327
Cys Met Ser Ile Thr Lys Lys Asp Ala Asn Pro Asn Asn Gly Gln Thr	
30 35 40	
CAA TTA CAA GCA GCG CGA ATG GAG TTA ACT GAT CTA ATC AAT GCT AAA	375
Gln Leu Gln Ala Ala Arg Met Glu Leu Thr Asp Leu Ile Asn Ala Lys	
45 50 55	
GCA AGG ACA TTA GCT TCA CTA CAA GAC TAT GCT AAG ATT GAA GCT AGT	423
Ala Arg Thr Leu Ala Ser Leu Gln Asp Tyr Ala Lys Ile Glu Ala Ser	
60 65 70	
TTA TCA TCT GCT TAT AGT GAA GCT GAA ACA GTT AAC AAT AAC CTT AAT	471
Leu Ser Ser Ala Tyr Ser Glu Ala Glu Thr Val Asn Asn Asn Leu Asn	
75 80 85 90	
GCA ACA CTA GAA CAA CTA AAA ATG GCT AAA ACT AAT TTA GAA TCA GCC	519
Ala Thr Leu Glu Gln Leu Lys Met Ala Lys Thr Asn Leu Glu Ser Ala	
95 100 105	
ATC AAC CAA GCT AAT ACG GAT AAA ACG ACT TTT GAT AAT GAA CAT CCA	567
Ile Asn Gln Ala Asn Thr Asp Lys Thr Thr Phe Asp Asn Glu His Pro	
110 115 120	
AAT TTA GTT GAA GCA TAC AAA GCA CTA AAA ACC ACT TTA GAA CAA CGT	615

Asn	Leu	Val	Glu	Ala	Tyr	Lys	Ala	Leu	Lys	Thr	Thr	Leu	Glu	Gln	Arg	
		125					130					135				
GCT	ACT	AAC	CTT	GAA	GGT	TTA	GCT	TCA	ACT	GCT	TAT	AAT	CAG	ATT	CGT	663
Ala	Thr	Asn	Leu	Glu	Gly	Leu	Ala	Ser	Thr	Ala	Tyr	Asn	Gln	Ile	Arg	
		140				145						150				
AAT	AAT	TTA	GTG	GAT	CTA	TAC	AAT	AAT	GCT	AGT	AGT	TTA	ATA	ACT	AAA	711
Asn	Asn	Leu	Val	Asp	Leu	Tyr	Asn	Asn	Ala	Ser	Ser	Leu	Ile	Thr	Lys	
155					160					165					170	
ACA	CTA	GAT	CCA	CTA	AAT	GGG	GGA	ATG	CTT	TTA	GAT	TCT	AAT	GAG	ATT	759
Thr	Leu	Asp	Pro	Leu	Asn	Gly	Gly	Met	Leu	Leu	Asp	Ser	Asn	Glu	Ile	
				175					180					185		
ACT	ACA	GTT	AAT	CGG	AAT	ATT	AAT	AAT	ACG	TTA	TCA	ACT	ATT	AAT	GAA	807
Thr	Thr	Val	Asn	Arg	Asn	Ile	Asn	Asn	Thr	Leu	Ser	Thr	Ile	Asn	Glu	
			190						195					200		
CAA	AAG	ACT	AAT	GCT	GAT	GCA	TTA	TCT	AAT	AGT	TTT	ATT	AAA	AAA	GTG	855
Gln	Lys	Thr	Asn	Ala	Asp	Ala	Leu	Ser	Asn	Ser	Phe	Ile	Lys	Lys	Val	
		205				210						215				
ATT	CAA	AAT	AAT	GAA	CAA	AGT	TTT	GTA	GGG	ACT	TTT	ACA	AAC	GCT	AAT	903
Ile	Gln	Asn	Asn	Glu	Gln	Ser	Phe	Val	Gly	Thr	Phe	Thr	Asn	Ala	Asn	
		220				225						230				
GTT	CAA	CCT	TCA	AAC	TAC	AGT	TTT	GTT	GCT	TTT	AGT	GCT	GAT	GTA	ACA	951
Val	Gln	Pro	Ser	Asn	Tyr	Ser	Phe	Val	Ala	Phe	Ser	Ala	Asp	Val	Thr	
235					240					245				250		
CCC	GTC	AAT	TAT	AAA	TAT	GCA	AGA	AGG	ACC	GTT	TGG	AAT	GGT	GAT	GAA	999
Pro	Val	Asn	Tyr	Lys	Tyr	Ala	Arg	Arg	Thr	Val	Trp	Asn	Gly	Asp	Glu	
				255					260					265		
CCT	TCA	AGT	AGA	ATT	CTT	GCA	AAC	ACG	AAT	AGT	ATC	ACA	GAT	GTT	TCT	1047
Pro	Ser	Ser	Arg	Ile	Leu	Ala	Asn	Thr	Asn	Ser	Ile	Thr	Asp	Val	Ser	
			270						275					280		
TGG	ATT	TAT	AGT	TTA	GCT	GGA	ACA	AAC	ACG	AAG	TAC	CAA	TTT	AGT	TTT	1095

Trp Ile Tyr Ser Leu Ala Gly Thr Asn Thr Lys Tyr Gln Phe Ser Phe	
285 290 295	
AGC AAC TAT GGT CCA TCA ACT GGT TAT TTA TAT TTC CCT TAT AAG TTG	1143
Ser Asn Tyr Gly Pro Ser Thr Gly Tyr Leu Tyr Phe Pro Tyr Lys Leu	
300 305 310	
GTT AAA GCA GCT GAT GCT AAT AAC GTT GGA TTA CAA TAC AAA TTA AAT	1191
Val Lys Ala Ala Asp Ala Asn Asn Val Gly Leu Gln Tyr Lys Leu Asn	
315 320 325 330	
AAT GGA AAT GTT CAA CAA GTT GAG TTT GCC ACT TCA ACT AGT GCA AAT	1239
Asn Gly Asn Val Gln Gln Val Glu Phe Ala Thr Ser Thr Ser Ala Asn	
335 340 345	
AAT ACT ACA GCT AAT CCA ACT CCA GCA GTT GAT GAG ATT AAA GTT GCT	1287
Asn Thr Thr Ala Asn Pro Thr Pro Ala Val Asp Glu Ile Lys Val Ala	
350 355 360	
AAA ATC GTT TTA TCA GGT TTA AGA TTT GGC CAA AAC ACA ATC GAA TTA	1335
Lys Ile Val Leu Ser Gly Leu Arg Phe Gly Gln Asn Thr Ile Glu Leu	
365 370 375	
AGT GTT CCA ACG GGT GAA GGA AAT ATG AAT AAA GTT GCG CCA ATG ATT	1383
Ser Val Pro Thr Gly Glu Gly Asn Met Asn Lys Val Ala Pro Met Ile	
380 385 390	
GGC AAC ATT TAT CTT AGC TCA AAT GAA AAT AAT GCT GAT AAG ATC TAC	1431
Gly Asn Ile Tyr Leu Ser Ser Asn Glu Asn Asn Ala Asp Lys Ile Tyr	
395 400 405 410	
AAT GAT ATC TTT GGT AAC ACA ATC AAC CAA CAG AAT AAT GCT ATT TCT	1479
Asn Asp Ile Phe Gly Asn Thr Ile Asn Gln Gln Asn Asn Ala Ile Ser	
415 420 425	
GTA ATG GTT AAT ATG GTT GAG GCA TAT AAT TTA GCT AGT AGT TAT TCT	1527
Val Met Val Asn Met Val Glu Gly Tyr Asn Leu Ala Ser Ser Tyr Ser	
430 435 440	
CCA GCA TAT AAA CCA ATT AAT GTT TCC ACT GGT GGT GGT CAA ACT CAA	1575

Pro	Ala	Tyr	Lys	Pro	Ile	Asn	Val	Ser	Thr	Gly	Gly	Gly	Gln	Thr	Gln		
			445				450					455					
CCA	TAT	TAT	GTA	ATT	GGT	TGA	TTG	GGC	GCT	AGT	GAT	CAG	AAC	CCT	AGA	1623	
Pro	Tyr	Tyr	Val	Ile	Gly	Trp	Leu	Gly	Ala	Ser	Asp	Gln	Asn	Pro	Arg		
			460				465					470					
AAC	GCT	GTG	GGA	ACC	AAC	ATG	AAC	GTA	CAA	AGA	GTT	CCA	GCA	ACA	AAT	1671	
Asn	Ala	Val	Gly	Thr	Asn	Met	Asn	Val	Gln	Arg	Val	Pro	Ala	Thr	Asn		
			475				480					485			490		
AGC	AAC	CAA	GGC	GGA	TAT	GCT	AGA	TAT	GTC	TCT	TTT	TAT	GTT	AAT	GCT	1719	
Ser	Asn	Gln	Gly	Gly	Tyr	Ala	Arg	Tyr	Val	Ser	Phe	Tyr	Val	Asn	Ala		
				495					500					505			
CCA	CAA	GCT	GGT	TCA	TAT	TAT	ATT	AGT	GGT	AAC	TAT	AAT	AGT	TTA	ACA	1767	
Pro	Gln	Ala	Gly	Ser	Tyr	Tyr	Ile	Ser	Gly	Asn	Tyr	Asn	Ser	Leu	Thr		
				510					515					520			
GCT	AGA	GGT	CTA	GCT	GTG	TCT	ACT	GAG	AAA	ACA	TTT	ACA	ACC	AAT	GTG	1815	
Ala	Arg	Gly	Leu	Ala	Val	Ser	Thr	Glu	Lys	Thr	Phe	Thr	Thr	Asn	Val		
			525						530					535			
ATC	AAG	ATC	ACT	CAC	TTA	CAA	GTA	ATT	AAT	GCC	ACT	AAT	AGA	ATC	TTA	1863	
Ile	Lys	Ile	Thr	His	Leu	Gln	Val	Ile	Asn	Ala	Thr	Asn	Arg	Ile	Leu		
			540				545							550			
ACC	TTT	GAT	ACT	AAA	ACA	AAA	AGA	GGA	ACT	GAT	AGT	AAT	AAC	GGT	AAT	1911	
Thr	Phe	Asp	Thr	Lys	Thr	Lys	Arg	Gly	Thr	Asp	Ser	Asn	Asn	Gly	Asn		
			555				560					565			570		
ATT	ACA	TTA	GAA	GCA	AAC	AAA	GAC	ACA	ATA	ACA	TTA	ACT	AAG	GGT	TGA	1959	
Ile	Thr	Leu	Glu	Ala	Asn	Lys	Asp	Thr	Ile	Thr	Leu	Thr	Lys	Gly	Trp		
				575					580					585			
AAC	AAA	CTT	TAT	GTT	TCA	GGT	AAT	AAT	AAT	GAC	ACT	GTA	GGT	ATT	GGT	2007	
Asn	Lys	Leu	Tyr	Val	Ser	Gly	Asn	Asn	Asn	Asp	Ser	Val	Gly	Ile	Gly		
				590					595					600			
AAT	CTT	ACT	TTT	ACA	TTA	ATG	CCA	CCA	CAA	ACT	AAT	TCA	TAATTAAGAT			2056	

Asn Leu Thr Phe Thr Leu Met Pro Pro Gln Thr Asn Ser

605

610

615

ATATTAAACA TACCCATTTA GATAATCTAA ATGGGTATCT TTTTATTGA AAATGCGGCA 2116
 TGATGAAATC AAAGTTAAGT TCACTAGTGC TTGATAAAT TAGATCAGCT TTAGAANNAT 2176
 CTTCACTACT GCCATGGGTA ATGACAACAG CTTTCATTTT GNCTGCTTCG ATCGCTTTCA 2236
 ATCCTGAGAT CGCATCTTCA AACCCAATAN CTINATCATT GCTGATATCT AAGTCTTCTN 2296
 CAGCTTTAAG ATAGATATCA GCTNCTGGTT TACCTTGTTT AATCTCACTT 2346

(2) Information for SEQ ID NO. 28

(i) Sequence characteristics:

(A) Length of sequence: 17

(B) Type of sequence: nucleic acid

5 (C) Number of strand: single

(D) Topology: linear

(E) Kind of sequence: other nucleic acid,
 synthetic DNA

(xi) Indication of sequence: SEQ ID NO: 28

GTTTTCCCAGTCACGAC

17

10 (2) Information for SEQ ID NO. 29

(i) Sequence characteristics:

(A) Length of sequence: 27

(B) Type of sequence: nucleic acid

(C) Number of strand: single

15 (D) Topology: linear

(E) Kind of sequence: other nucleic acid,
 synthetic DNA

(xi) Indication of sequence: SEQ ID NO: 29

AACCAACCAACCCGATCGCTAGTCT

27

(2) Information for SEQ ID NO. 30

(i) Sequence characteristics:

(A) Length of sequence: 20

5 (B) Type of sequence: nucleic acid

(C) Number of strand: single

(D) Topology: linear

(E) Kind of sequence: other nucleic acid,
synthetic DNA

10 (xi) Indication of sequence: SEQ ID NO: 30

TGATTGGGCGCTAGCGATCA

20

(2) Information for SEQ ID NO. 31

(i) Sequence characteristics:

(A) Length of sequence: 23

(B) Type of sequence: nucleic acid

15 (C) Number of strand: single

(D) Topology: linear

(E) Kind of sequence: other nucleic acid,
synthetic DNA

(xi) Indication of sequence: SEQ ID NO: 31

TCCCAACCTTGTTGAAATACAA

23

20 (2) Information for SEQ ID NO. 32

(i) Sequence characteristics:

(A) Length of sequence: 19

- (B) Type of sequence: nucleic acid
- (C) Number of strand: single
- (D) Topology: linear
- (E) Kind of sequence: other nucleic acid,

5

DNA

(xi) Indication of sequence: SEQ ID NO: 32

TGAAACAAGCTTTATGTTT

19

(2) Information for SEQ ID NO. 33

(i) Sequence characteristics:

(A) Length of sequence: 17

10

(B) Type of sequence: nucleic acid

(C) Number of strand: single

(D) Topology: linear

(E) Kind of sequence: other nucleic acid,

DNA

15 (xi) Indication of sequence: SEQ ID NO: 33

CAGTATCGACAAAGGAC

17

(2) Information for SEQ ID NO. 34

(i) Sequence characteristics:

(A) Length of sequence: 17

(B) Type of sequence: nucleic acid

20

(C) Number of strand: single

(D) Topology: linear

(E) Kind of sequence: other nucleic acid,

synthetic DNA

(xi) Indication of sequence: SEQ ID NO: 34

CAGGAAACAGCTATGAC

17

(2) Information for SEQ ID NO. 35

(i) Sequence characteristics:

(A) Length of sequence: 20

5 (B) Type of sequence: nucleic acid

(C) Number of strand: single

(D) Topology: linear

(E) Kind of sequence: other nucleic acid,
synthetic DNA

10 (xi) Indication of sequence: SEQ ID NO: 35

GTTCTTCCTGGCAAACCTTA

20

(2) Information for SEQ ID NO. 36

(i) Sequence characteristics:

(A) Length of sequence: 20

(B) Type of sequence: nucleic acid

15 (C) Number of strand: single

(D) Topology: linear

(E) Kind of sequence: other nucleic acid,
synthetic DNA

(xi) Indication of sequence: SEQ ID NO: 36

AAGAAGGACCGTTTGGGAATG

20

20 (2) Information for SEQ ID NO. 37

(i) Sequence characteristics:

(A) Length of sequence: 17

(B) Type of sequence: nucleic acid

(C) Number of strand: single

(D) Topology: linear

(E) Kind of sequence: other nucleic acid,

5 synthetic DNA

(xi) Indication of sequence: SEQ ID NO: 37

GTTTTCCCAGTCACGAC

17

(2) Information for SEQ ID NO. 38

(i) Sequence characteristics:

(A) Length of sequence: 27

10 (B) Type of sequence: nucleic acid

(C) Number of strand: single

(D) Topology: linear

(E) Kind of sequence: other nucleic acid,

synthetic DNA

15 (xi) Indication of sequence: SEQ ID NO: 38

CAAAGTACCTAAATATCGAATTCACCT

27

(2) Information for SEQ ID NO. 39

(i) Sequence characteristics:

(A) Length of sequence: 23

(B) Type of sequence: nucleic acid

20 (C) Number of strand: single

(D) Topology: linear

(E) Kind of sequence: other nucleic acid,

synthetic DNA

(xi) Indication of sequence: SEQ ID NO: 39

ATAGCTTAAGTGGAACAAACACG

23

(2) Information for SEQ ID NO. 40

(i) Sequence characteristics:

(A) Length of sequence: 20

5 (B) Type of sequence: nucleic acid

(C) Number of strand: single

(D) Topology: linear

(E) Kind of sequence: other nucleic acid,
synthetic DNA

10 (xi) Indication of sequence: SEQ ID NO: 40

GGAACCAGATCTTGTTTCCC

20

(2) Information for SEQ ID NO. 41

(i) Sequence characteristics:

(A) Length of sequence: 21

(B) Type of sequence: nucleic acid

15 (C) Number of strand: single

(D) Topology: linear

(E) Kind of sequence: other nucleic acid,
synthetic DNA

(xi) Indication of sequence: SEQ ID NO: 41

GGTCTAGAACAAAGGGATTGGACA

21

20 (2) Information for SEQ ID NO. 42

(i) Sequence characteristics:

(A) Length of sequence: 20

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(B) Type of sequence: nucleic acid

(C) Number of strand: single

(D) Topology: linear

(E) Kind of sequence: other nucleic acid,

5

synthetic DNA

(xi) Indication of sequence: SEQ ID NO: 42

CTACCTACCATGGTGATGAT

20

(2) Information for SEQ ID NO. 43

(i) Sequence characteristics:

(A) Length of sequence: 27

10

(B) Type of sequence: nucleic acid

(C) Number of strand: single

(D) Topology: linear

(E) Kind of sequence: other nucleic acid,

synthetic DNA

15 (xi) Indication of sequence: SEQ ID NO: 43

GATGGTACCACTACTATTTCATGGACA

27

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A recombinant Avipox virus inserted with DNA encoding a polypeptide showing antigenicity to Mycoplasma gallisepticum, wherein said inserted DNA is DNA encoding a polypeptide showing an antigenicity which is reactive with Mycoplasma gallisepticum-immunized sera or Mycoplasma gallisepticum-infected sera and is substantially pure, its nucleotide sequence being shown by SEQ ID NO: 1, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16 or SEQ ID NO: 27.

2. The recombinant Avipox virus according to claim 1, wherein said DNA encoding a signal membrane anchor is DNA encoding a signal membrane anchor of Newcastle disease virus.

3. A substantially pure antigenic protein which is reactive with Mycoplasma gallisepticum-immunized sera or Mycoplasma gallisepticum-infected sera and encoded by a gene derived from Mycoplasma gallisepticum having a nucleotide sequence shown by SEQ ID NO: 1, or a degenerate sequence thereof.

4. A gene encoding an antigenic protein according to claim 3.

5. A substantially pure antigenic protein which is reactive with Mycoplasma gallisepticum-immunized sera or Mycoplasma gallisepticum-infected sera and encoded by a gene derived from Mycoplasma gallisepticum having a nucleotide sequence shown by SEQ ID NO: 27, or a degenerate sequence thereof.

6. A gene encoding an antigenic protein according to claim 5.

7. A substantially pure antigenic protein which is reactive with Mycoplasma gallisepticum-immunized sera and Mycoplasma gallisepticum-infected sera and encoded by a gene derived from Mycoplasma gallisepticum having a nucleotide sequence shown by SEQ ID NO: 16, or a degenerate sequence thereof.

8. A gene encoding an antigenic protein according to claim 7.

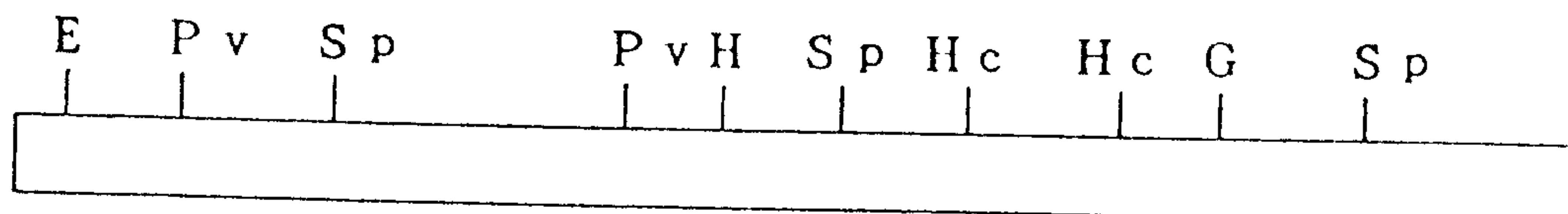
9. A component vaccine comprising as an effective ingredient a protein according to claim 3, 5 or 7.

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FIG. 1

(RESTRICTION ENZYME MAP OF TM-81)

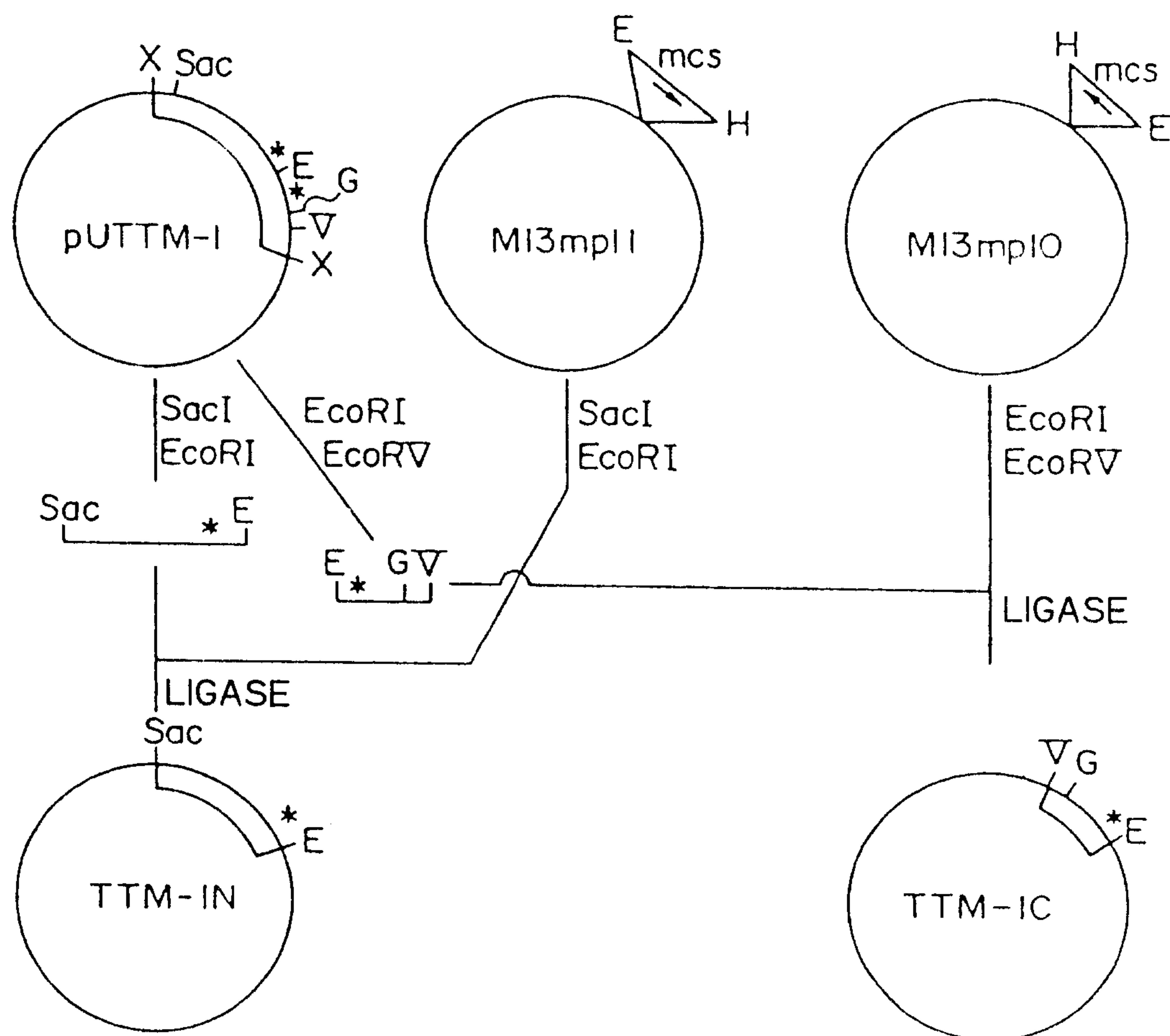


E: EcoRI, Pv: PvuII, Sp: SmaI, H: HindIII
Hc: HincII, G: BglII

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FIG. 2



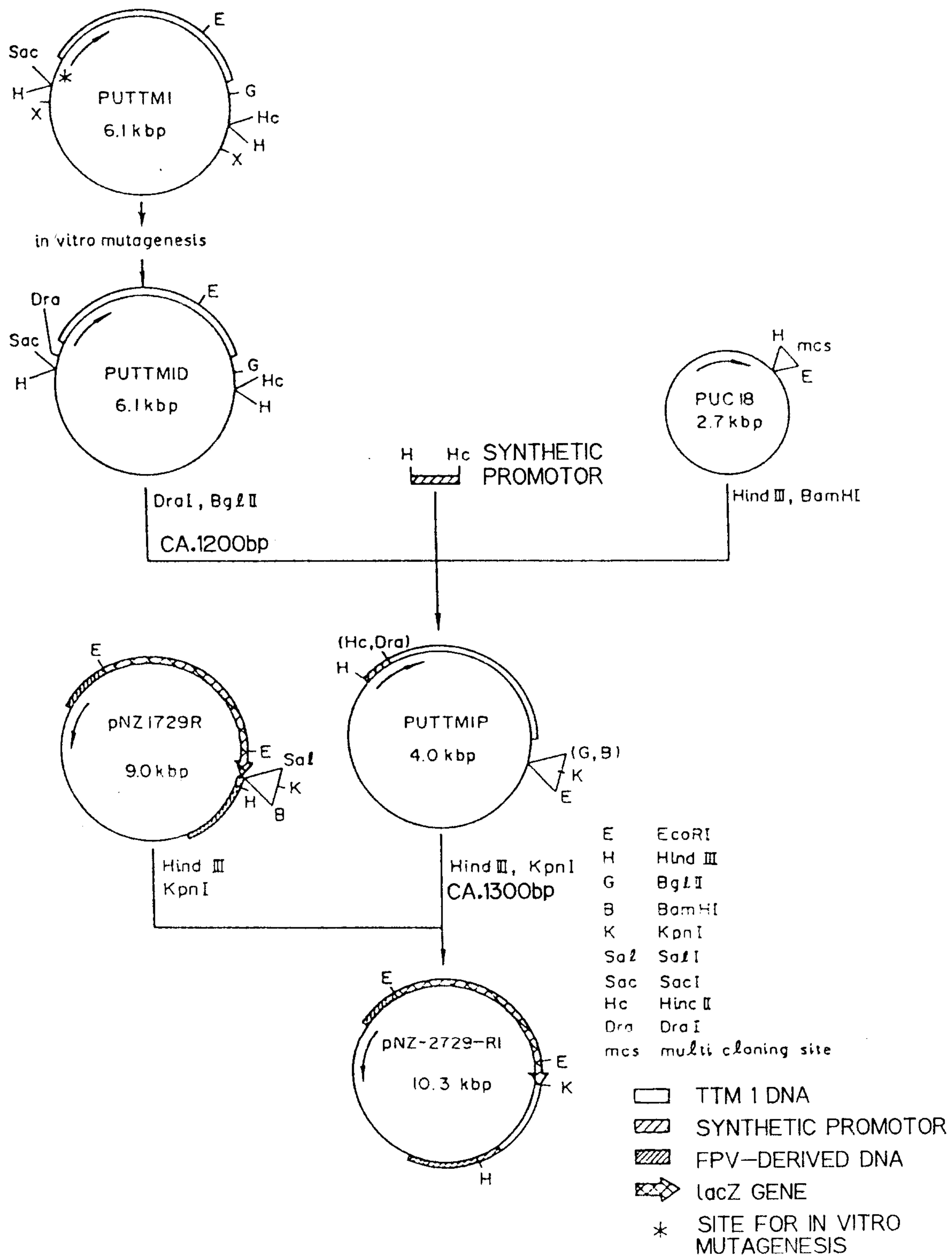
E : EcoRI
 V : EcoRV
 G : Bgl II
 Sac : SacI
 X : XbaI
 Ss : SspI
 Sp : SpeI

* MUTAGENETIC SITE OF NUCLEOTIDE

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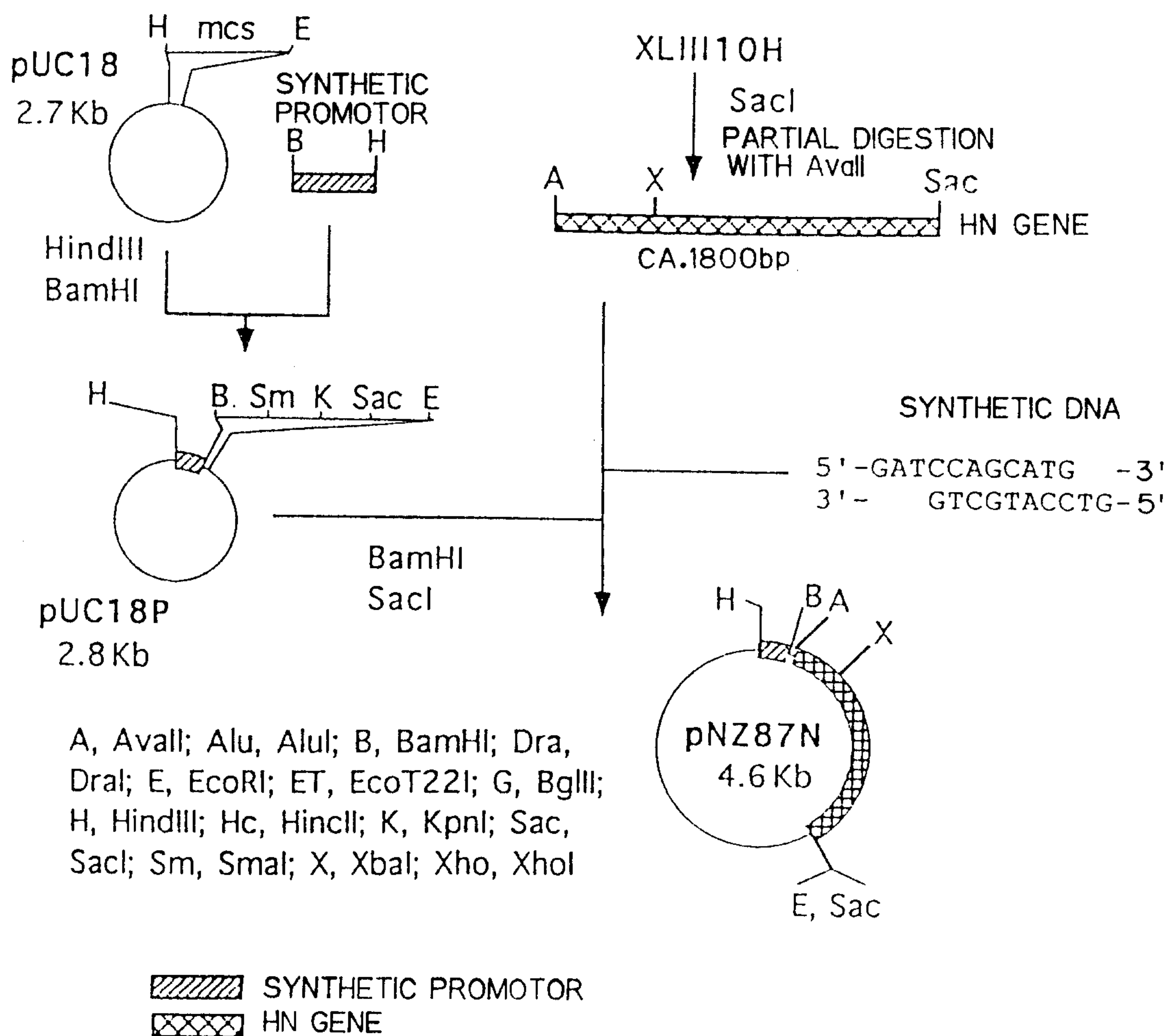
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FIG. 3



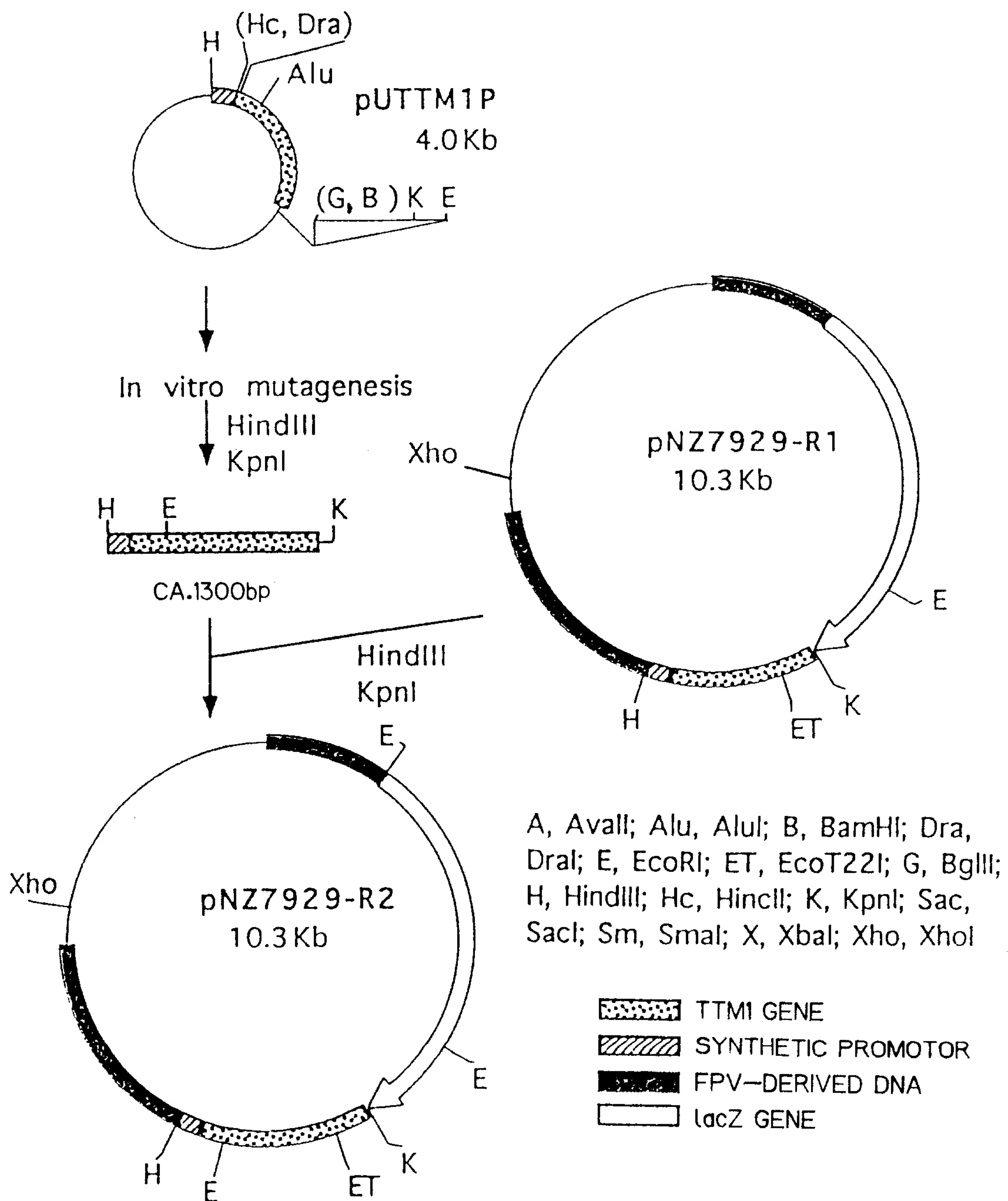
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FIG. 4



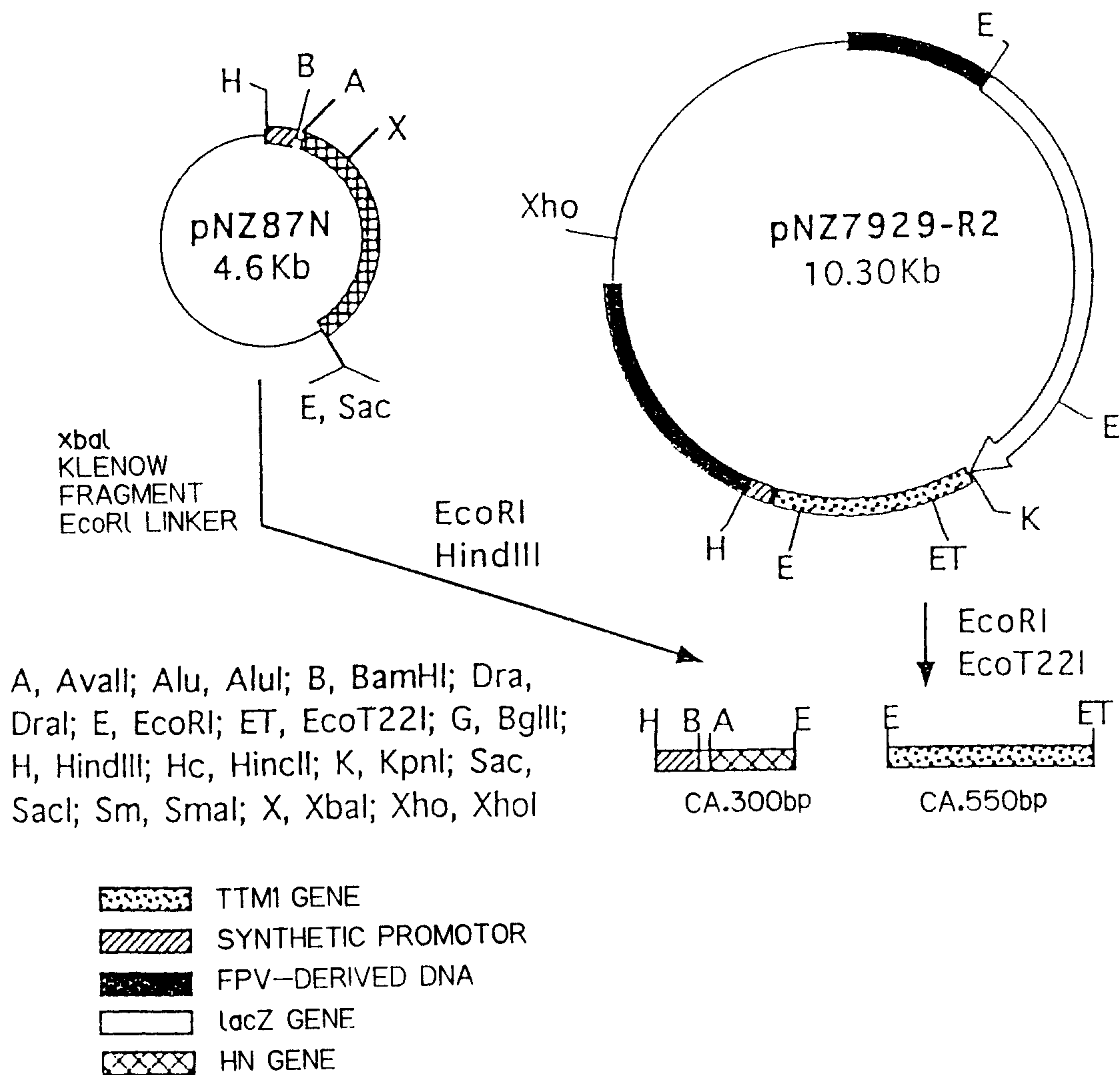
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FIG. 5



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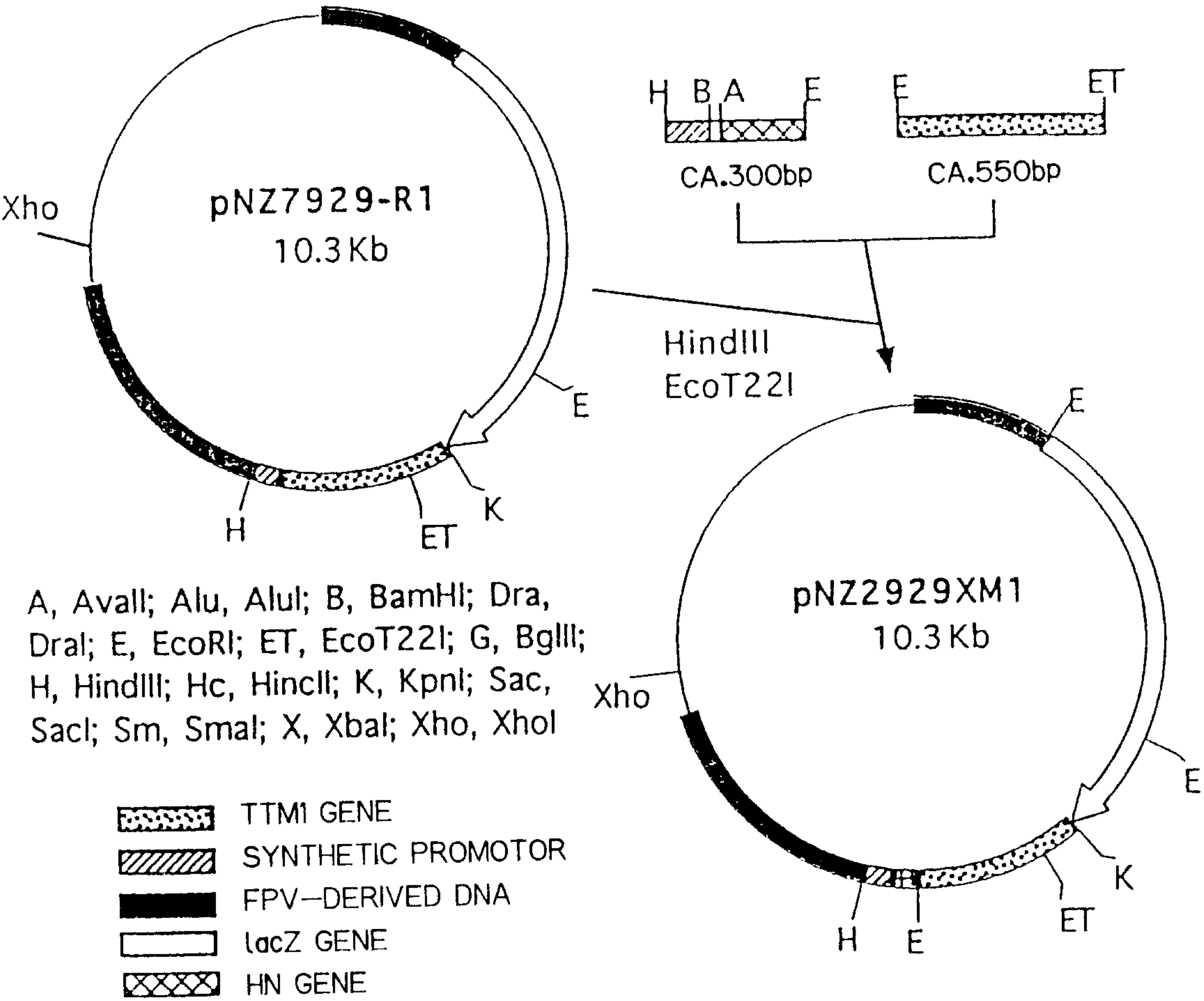
FIG. 6(A)



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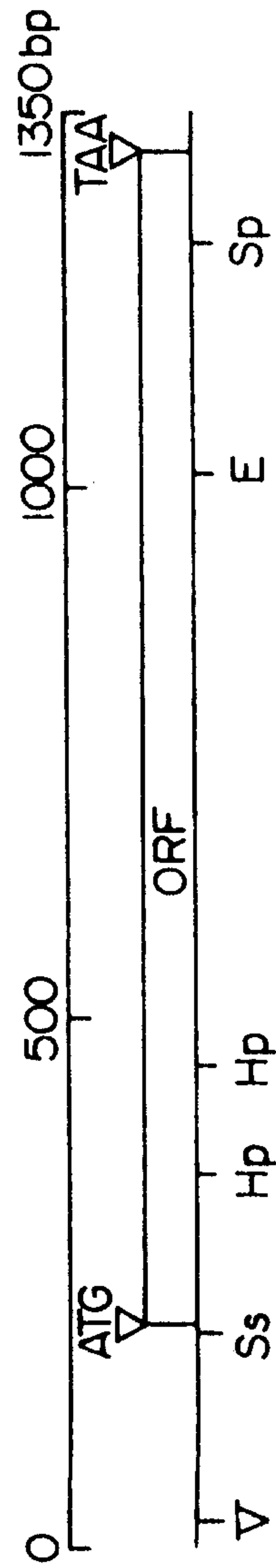
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FIG. 6(B)



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FIG. 7



100bp

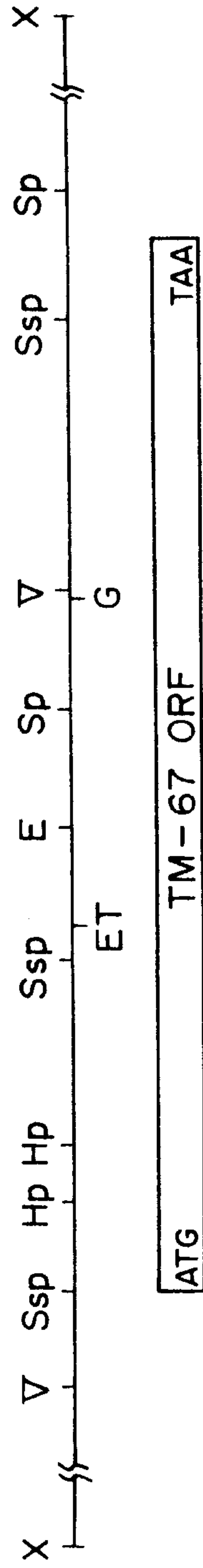
Hp : HpaI
 Ss : SspI
 E : EcoRI
 Sp : SpeI
 V : EcoRV
 ATG : TRANSLATION INITIATION CODON
 TAA : TRANSLATION TERMINATION CODON

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FIG. 8

RESTRICTION ENZYME MAP OF TM-67 ORF AND
POSITION OF SYNTHETIC PRIMERS ON ORF



200bp

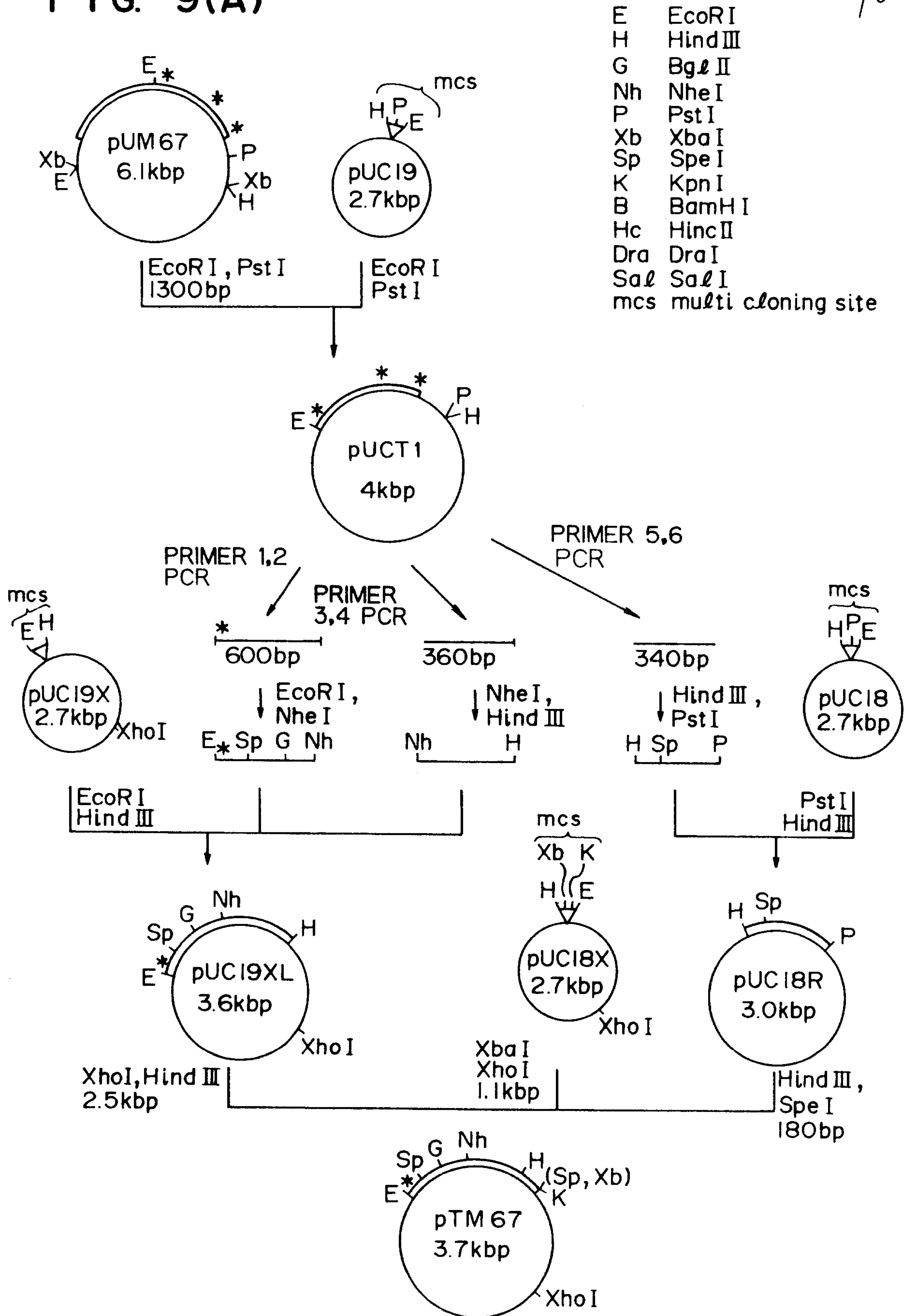
PRIMER 2 ←
PRIMER 3 →
PRIMER 4 ←
PRIMER 5 →

E: EcoRI, G: BglII, Ssp: SspI, Hp: HpaI,
V: EcoRV, Sp: SpeI, ET: EcoT22I

Handwritten signature

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FIG. 9(A)



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FIG. 9(B)

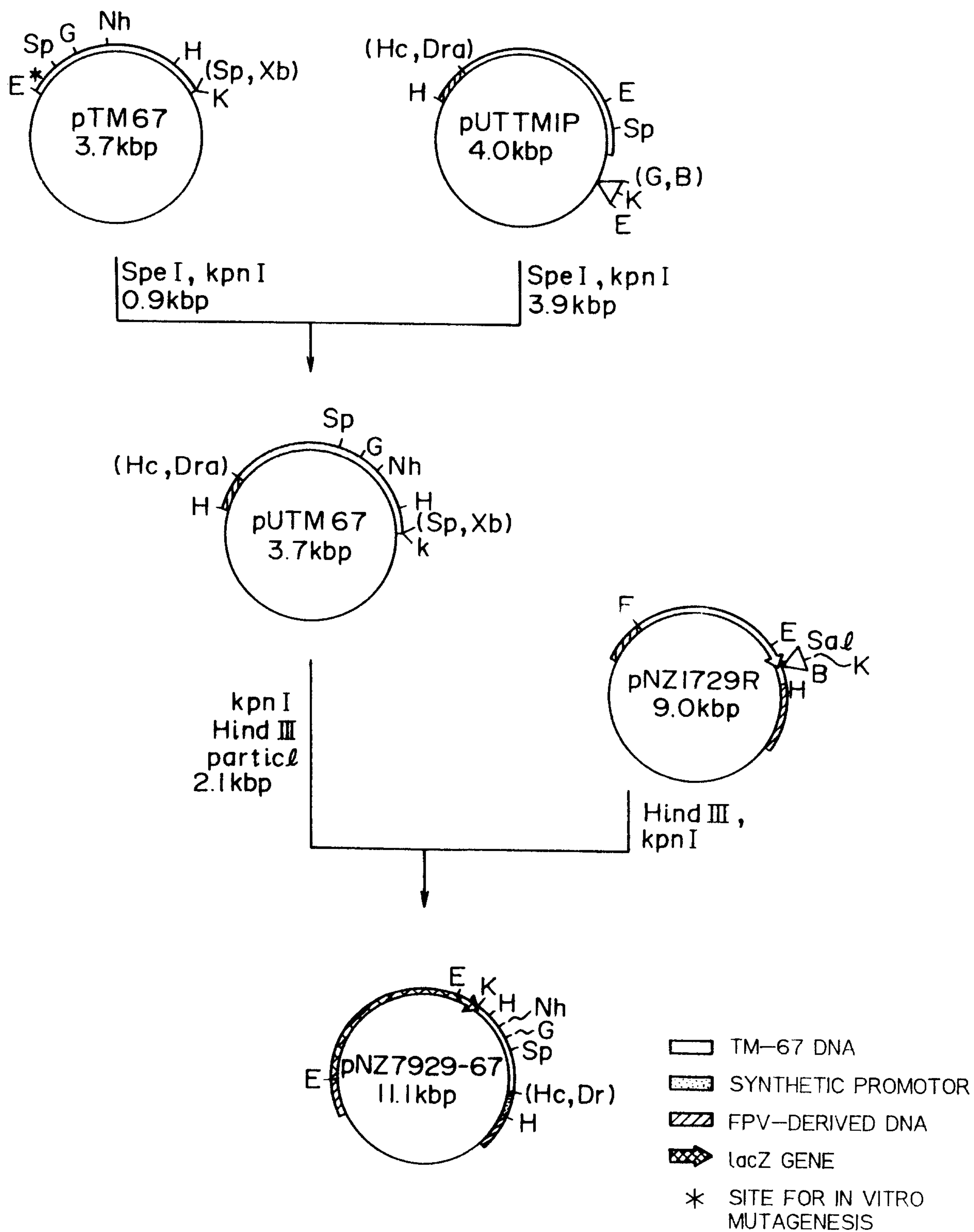
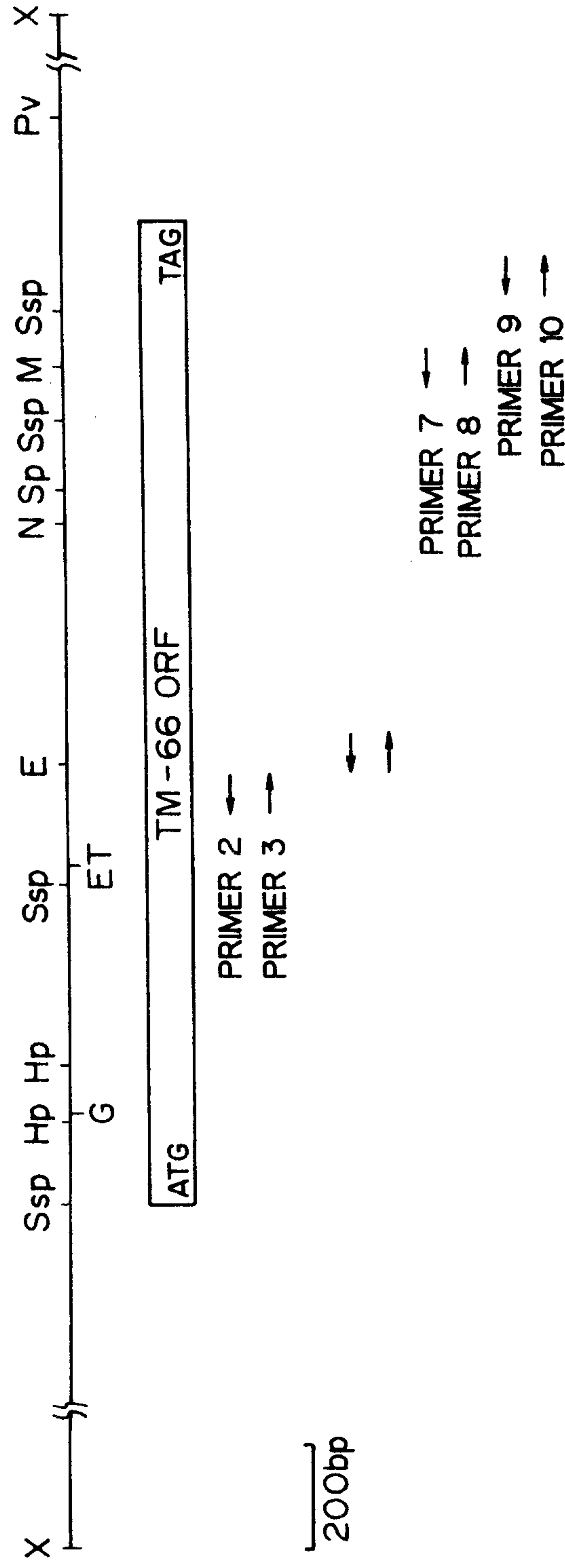


FIG. 10

RESTRICTION ENZYME MAP OF TM-66 ORF AND
POSITION SYNTHETIC PRIMERS ON ORF



E: EcoRI, G: BglII, Ssp: SspI, Hp: HpaI, X: XbaI
N: NheI, Sp: SpeI, ET: EcoT22I, M: MluI, Pv: PvuII

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mcs
B Xb
E H

pUC19
2.7kbp

pUM66
9kbp

Sp ** Pv
G Xb E H

E EcoRI
B BamHI
Xb XbaI
H HindIII
Pv PvuII
Sp SpeI
G BglII
E22 EcoT22I (=AvaIII)
Av AvaII
Nh NheI
Af AflII
M MluI
k kpnI
P PstI
Ss SspI
Hc HincII
mcs MULTI CLONING SITE

BamHI XbaI | BglII, SpeI | 1200bp

E22 * E
(G, B) Nhe (Sp, Xb)
E H

pUCT 2
3.9kbp

PRIMER 1,2 PCR
PRIMER 3,4 PCR
PRIMER 1,5 PCR
PRIMER 4,6 PCR

620bp 550bp 700bp 500bp

mcs
B H E

pUC19
2.7kbp

HindIII
AvaII

H E22 Av Av E Nh B

AvaII
EcoRI

H E Af Af Nh E

HindIII
AflII

AflII
EcoRI

mcs
H E

pUC19
2.7kbp

HindIII
BamHI

E22 Av E Nh B
H

pUC19-1
3.9kbp

EcoRI 3300bp

HindIII
EcoRI

E Af Nh E
H

pUC19-2
3.9kbp

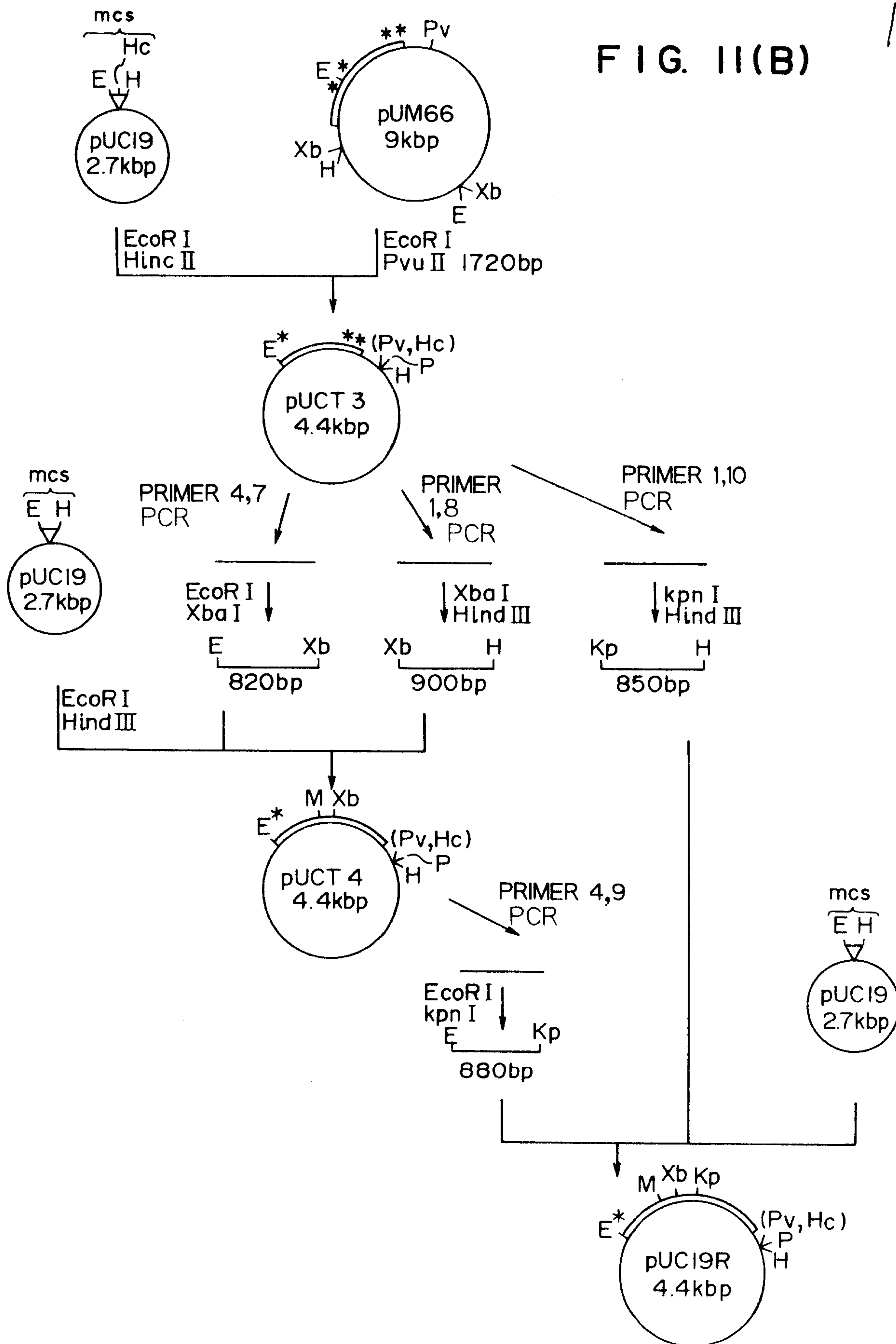
EcoRI 550bp

Av
E22 E Af
H Nh E

pUC19-L
3.9kbp

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FIG. II(B)



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FIG. II(C)

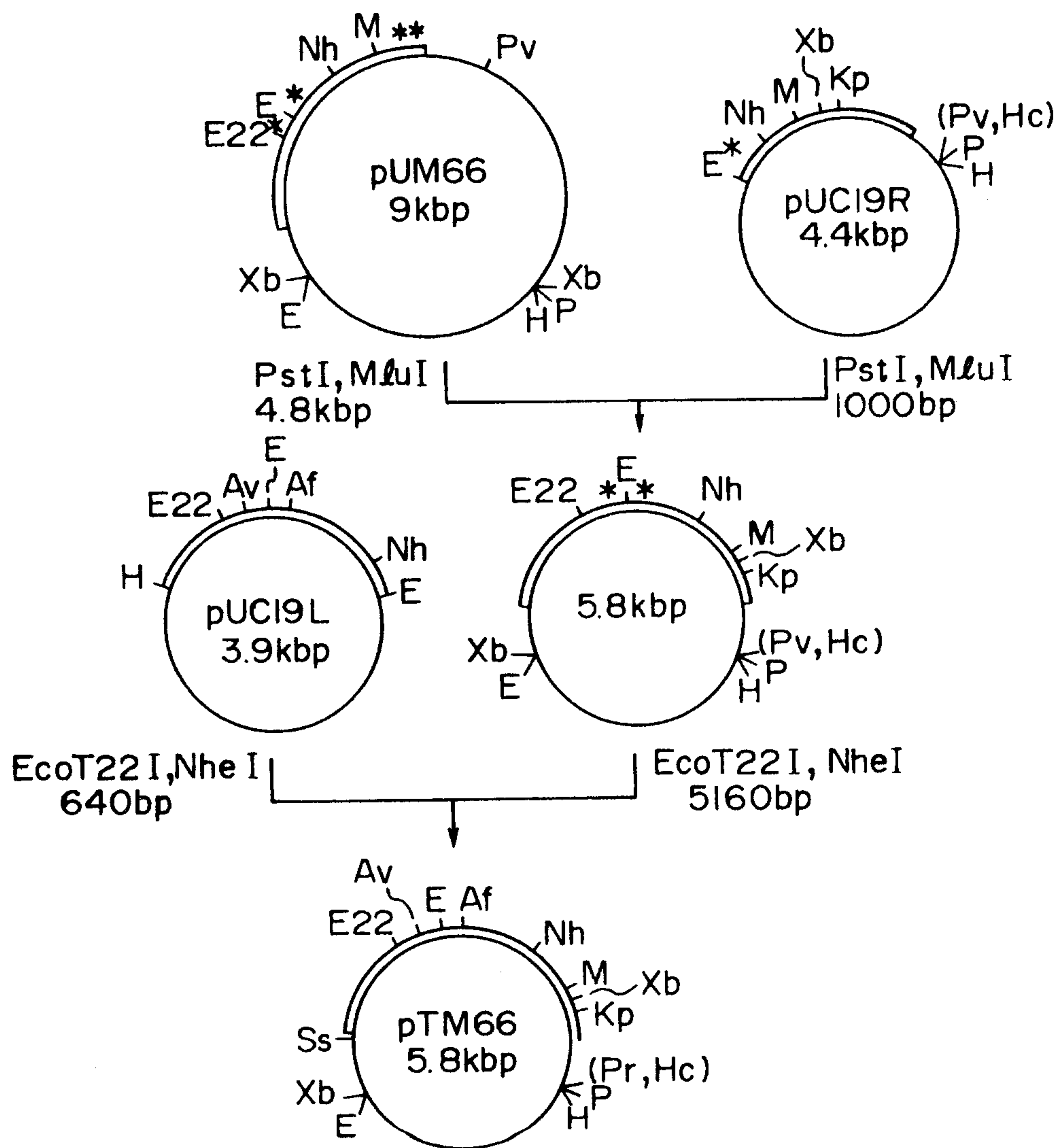
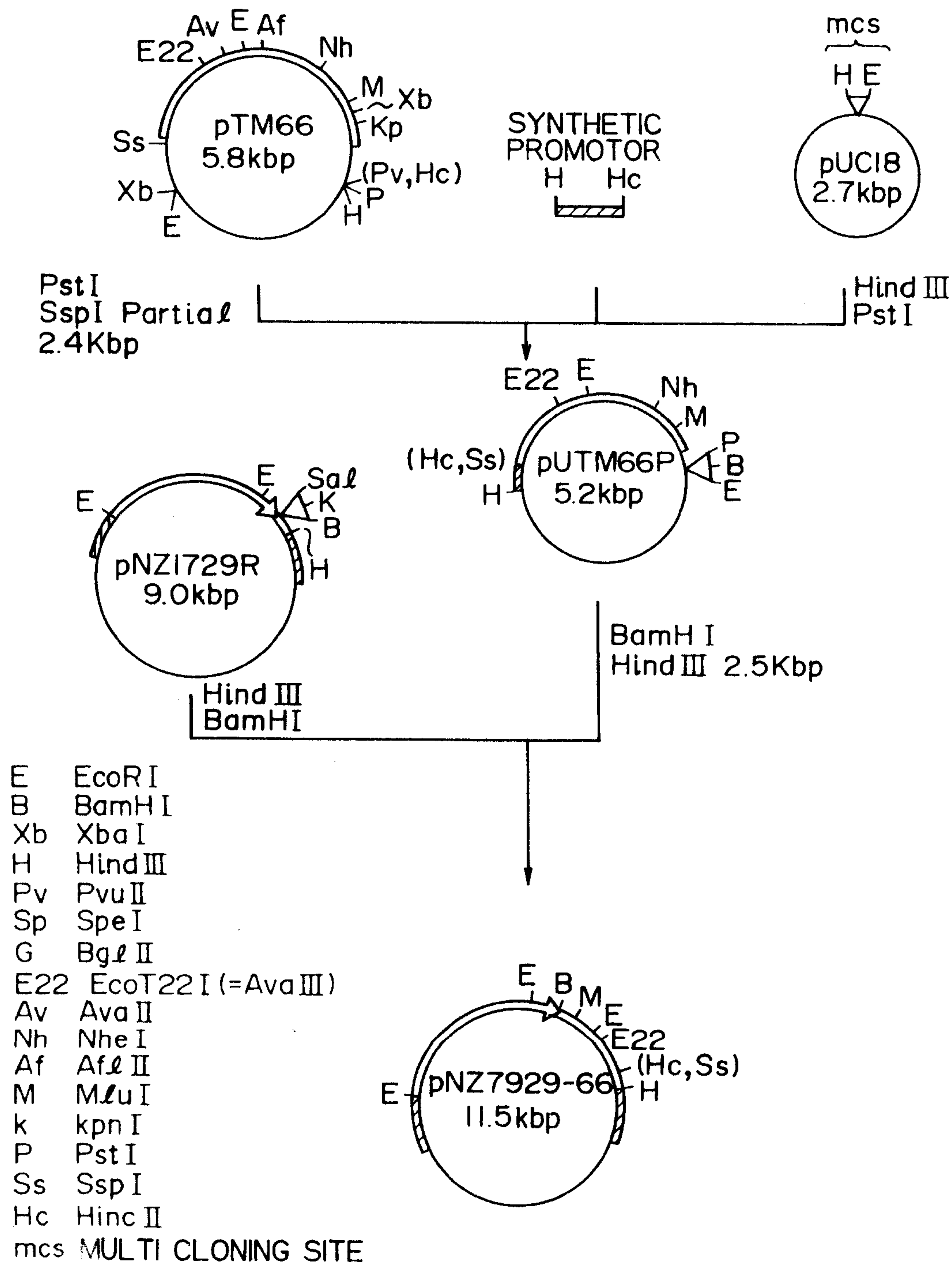
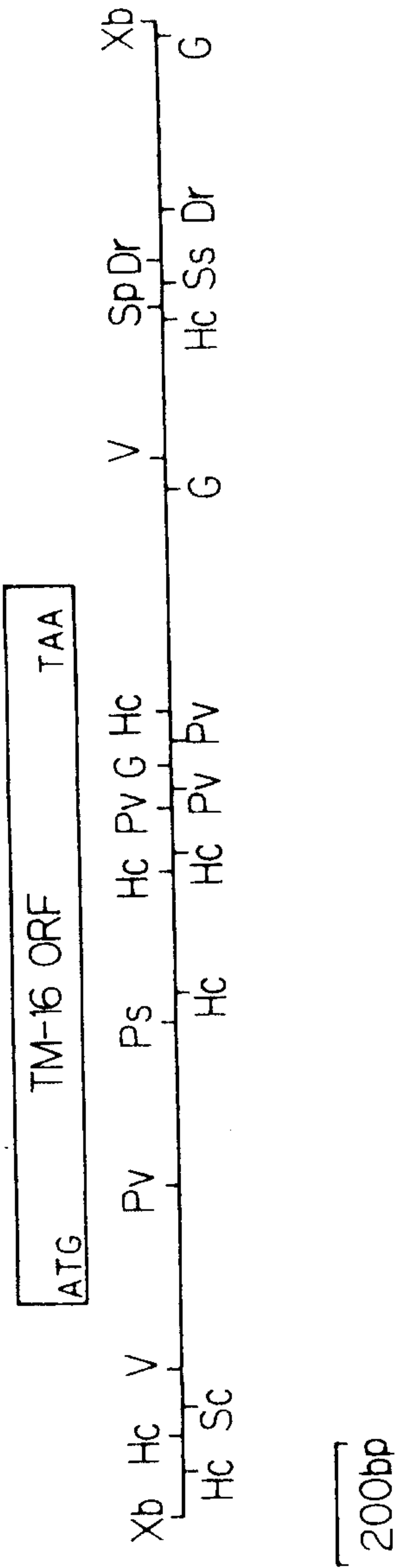


FIG. 12

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FIG. 13

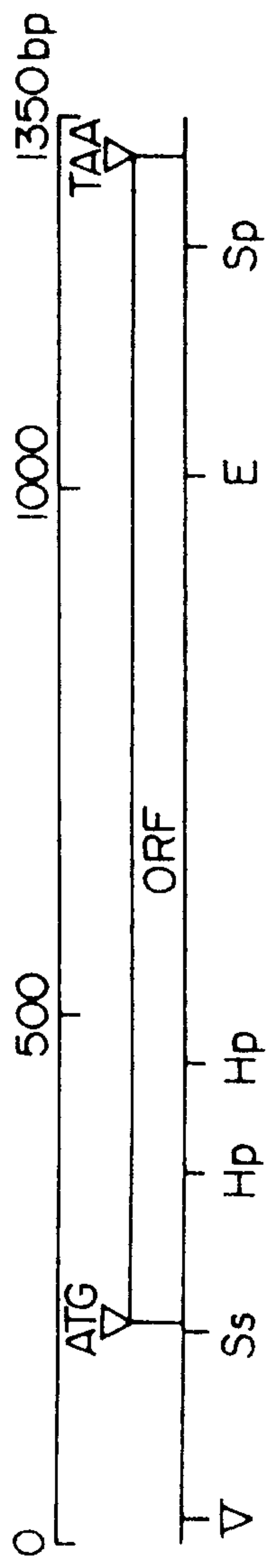


Hc:Hinc II,Pv:Pvu II,Ps:Pst I,G:Bgl II

Marks a Clark

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FIG. 14



Hp : HpaI
Ss : SspI
E : EcoRI
Sp : SpeI
V : EcoRV

ATG : TRANSLATION INITIATION CODON
TAA : TRANSLATION TERMINATION CODON

Meekes & Clark