



"New soluble, non-cleavable gp160 variant of hybrid form"

5 The present invention relates to a method for constructing means needed for the production of new molecules capable of inducing an immunoprotective response against a virus responsible for acquired immunodeficiency syndrome (AIDS).

10 In an individual, this disease develops following an infection of T-helper lymphocytes by an HIV (human immunodeficiency virus) retrovirus. To date, these retroviruses have been classified in two major types: type HIV-1, which is rife essentially in Europe and North America, and type HIV-2, which is characteristic of African infections. Within a given type, the HIV retroviruses exhibit some degree of variability from one
15 another, which is characterized, for example, by a difference in cellular tropism or by viral proteins that differ slightly from one another. For this reason, when it is desired to refer to a particular HIV retrovirus, the term "viral strain" is preferably employed.

20 Generally speaking, HIV retroviruses have the following structure: the genomic RNA molecule and various associated proteins are encapsulated in a capsid of protein nature (nucleocapsid). The whole is protected by a membrane of cellular origin which has incorporated the
25 envelope protein of viral origin.

This envelope protein, in various forms, is considered at the present time to be a potential therapeutic component of a vaccine against AIDS.

30 Under natural conditions, the envelope protein (env) is initially synthesized in the form of a precursor, containing at its N-terminal end a signal sequence which initiates the passage of the precursor into the endoplasmic reticulum (secretion route). This signal peptide is then removed by proteolytic cleavage. The
35 product of this cleavage is a protein referred to as gp160, which is itself subsequently cleaved into a gp40 small subunit and a gp120 large subunit. The N-terminal



end of the gp120 corresponds to the N-terminal end of the gp160, while the C-terminal end of the gp40 corresponds to the C-terminal end of the gp160.

Each of these subunits is secreted out of the infected cell. However, the gp40 remains anchored in the cell membrane via its transmembrane domain. Its C-terminal portion (intracytoplasmic domain) remains in contact with the cytoplasm, while its N-terminal portion (extracytoplasmic domain) is at the cell surface. The gp120 subunit is released outside the cell, where it interacts with the extracellular domain of the gp40 subunit. The two subunits of the envelope protein thus remain associated in the form of a complex.

The amino acid sequence of the precursors of the env proteins of various viral strains are now known. As an example, Figure 1 presents the sequence of the precursors of the gp160 proteins of the viral strains HIV-1 Bru, HIV-1 MN, HIV-1 ELI, HIV-1 RF, HIV-1 SF2C and HIV-1 SC. Similarly, Figure 2 presents the sequence of the precursor of the gp160 of the viral strain HIV-2 Rod.

Hereinafter, and to make the text easier to understand, the sequences of the gp160 proteins other than the gp160 of the strain HIV-1 Bru will be described below by reference to the sequence of the gp160 of the strain HIV-1 Bru (hereinafter referred to as gp160-Bru) as follows:

- a) gp160-Bru possesses 831 amino acids, these being numbered from 1 to 831. In addition, gp120-Bru corresponds to the sequence of the first 486 amino acids of gp160-Bru, while gp40-Bru corresponds to the sequence beginning with the amino acid at position 487 and ending with the amino acid at position 831.
- b) The sequence of a gp160 of any kind is aligned with the sequence of gp160-Bru so as to display maximum homology. For this purpose, gaps may be introduced either into the sequence of gp160-Bru or into the sequence of the gp160 of any kind. By

definition, the position of an amino acid in the gpl60 of any kind will be specified in identical fashion by the position of the homologous amino acid in gpl60-Bru.

- 5 c) Consequently, when a gap is introduced into the sequence of the gpl60 of any kind, opposite the amino acid at position x , there is no amino acid at position x in the sequence of the gpl60. However, this amino acid is considered to possess a virtual presence.
- 10
- d) When one or more gap(s) is/are introduced into the sequence of gpl60-Bru between the amino acid at position x and the amino acid at position $x+1$, it is considered that, in the gpl60 of any kind,
- 15 the position covers at least two subpositions x_a , $x_b, \dots, x_n$, each occupied by an amino acid. By definition, the downstream position x_n represents the position x .
- e) When one or more gap(s) is/are introduced into the sequence of gpl60-Bru upstream of the amino acid at position 1, it is considered that, in the gpl60 of any kind, the position 1 covers at least two subpositions 1_a , $1_b, \dots, 1_n$, each occupied by an amino acid. By definition, the NH_2 -terminal upstream position 1_n represents the position 1.
- 20
- 25 f) When one or more gap(s) is/are introduced into the sequence of the gpl60 of any kind opposite the NH_2 -terminal amino acid(s) of gpl60-Bru, it may be noted that, by definition, the amino acid at the NH_2 -terminal position in the gpl60 of any kind holds simultaneously the position 1 and its position of homologous amino acid.
- 30
- g) When one or more gap(s) is/are introduced into the sequence of the gpl60 of any kind opposite the COOH -terminal amino acid(s) of gpl60-Bru, it may be noted that, by definition, the amino acid at the COOH -terminal position in the gpl60 of any kind holds simultaneously the position 841 and
- 35



its position of homologous amino acid.

At the present time, with regard to a vaccine, envelope protein is considered to be a potentially advantageous candidate from a therapeutic standpoint. However, its multichain structure in the original state constitutes a handicap in terms of feasibility. For this reason, it has been seen to be preferable to use a single-chain protein which would retain most of the epitopes of the gp120 and those of the gp40. This type of protein has already been proposed in Patent Application WO 87/6260. It is, more especially, a non-cleavable and soluble gp160 variant.

All the gp160 proteins of type HIV-1 viral strains possess, at positions 483-486, a so-called major cleavage site, recognized by one or more proteolytic enzymes. This cleavage site is the same for all the gp160 proteins described in Figure 1, and corresponds to the sequence Arg-Glu-Lys-Arg (REKR). The proteolytic enzymes cut immediately downstream of this cleavage site to release a gp120 and a gp40.

When this cleavage site is not there, it has been demonstrated that the proteolytic enzymes recognize, albeit with lower efficiency, a so-called minor cleavage site, and to make [sic] a cut immediately downstream of the latter. Here too, this cleavage site is the same for all the gp160 proteins shown in Figure 1. It corresponds to the sequence Lys-Arg-Arg (KRR) at positions 477 - 479, according to some authors or, according to others, the sequence Lys-Ala-Lys-Arg (KAKR) at positions 475 - 478.

In addition, it may be noted that the gp160 proteins of type HIV-2 viral strains possess only a major cleavage site at positions 475 - 478, which corresponds to the sequence Lys-Glu-Arg-Lys (KEKR).

A non-cleavable gp160 variant is an artificial analog of a native gp160. Its amino acid sequence corresponds to that of a native gp160 in which the major cleavage site and/or the minor cleavage site has/have been eliminated.





Such a gp160 variant may be synthesized by means of recombinant DNA techniques. It suffices to isolate a DNA fragment coding for a native gp160, and then to modify the region coding for the major cleavage site by directed mutagenesis so as to obtain a DNA fragment coding for a gp160 variant insensitive to proteolytic action. This latter DNA fragment is subsequently expressed under suitable conditions to give said gp160 variant. Such a gp160 variant which no longer contains the major cleavage site is referred to hereinafter as a type A non-cleavable gp160 variant.

Preferably, the region coding for the minor cleavage site can be, in addition, modified for identical purposes. Under these conditions, a type A' non-cleavable gp160 variant, which contains neither the major cleavage site nor the minor cleavage site, is obtained.

For this purpose, it may be noted, as an example, that the cleavage site REKR, KEKR or KAKR and the cleavage site KRR may be replaced, respectively, by the sequences Asn-Glu-His-Gln (NEHQ) and Gln-Asn-His (QNH).

Moreover, it is also necessary for a gp160 to be obtained in soluble form. Such a gp160 corresponds to a native gp160 in which the transmembrane region of hydrophobic nature has been eliminated. This transmembrane region is located in the zone corresponding to the gp40, from the amino acid residue at position 659 to the amino acid residue at position 680. Additionally, but unnecessarily, another hydrophobic region, extending from the amino acid residue at position 487 to the amino acid residue at position 514, could be deleted.

Similarly, the means needed for the synthesis of a soluble gp160 variant obviously include the corresponding DNA fragment, which must be obtained by modification of the original DNA fragment.

A comparison of the sequences of the different gp160 proteins already known has demonstrated at least three domains whose sequence is hypervariable from one gp160 to another. These three domains are commonly



referred to as the V_1 , V_2 and V_3 domains (or loops).

The first two domains, V_1 and V_2 , are located between the cysteine residue at position 96 and the cysteine residue at position 171, while the third domain, V_3 , is located from the cysteine residue at position 271 to the cysteine residue at position 306.

There is also a final domain exhibiting some degree of variability, albeit considered to be lesser. This is the site of binding to the CD4 receptor of T-helper lymphocytes; it being located approximately from the amino acid residue at position 340 to the amino acid residue at position 440.

Irrespective of the gp160 under consideration, vaccination experiments have shown that the third hypervariable domain is essential for obtaining appropriate immunity. However, as a result of the hypervariable nature of this domain, the protection developed will be effective only against the viral strain from which the gp160 used is derived.

Lastly, it would appear that the first and the second hypervariable domains, as well as the CD4 receptor binding site, have an influence on the degree of immunity that could be obtained.

A vaccine of general applicability should enable individuals to be protected against the majority of HIV viral strains which are rife in the world. Consequently, a gp160-based vaccine should contain various gp160 proteins, each derived from a different viral strain. In order to employ such a vaccine, it is appropriate, in the first place, to construct the means intended for the production of the non-cleavable and soluble gp160 variant corresponding to each viral strain. As a first approach, this would hence involve cloning in each instance the DNA fragment coding for the gp160 of a particular viral strain, determining its sequence, modifying this DNA fragment in order to eliminate cleavage sites and hydrophobic regions by substitution or deletion and then, lastly, placing this DNA fragment thus modified in

suitable conditions of expression. Since this type of operation would have to be repeated for each gp160, the preparation of such a vaccine is anticipated to be lengthy and expensive.

5 Furthermore, the viral strains can vary with the passage of time, e.g. by mutation. A particular strain which is a major cause of infection in a particular region of the world at a particular time can regress as an infectious agent, and another particular strain appear
10 as a replacement. It is hence important to be able to adapt a vaccine rapidly to the epidemiological conditions and, in the present case, to have a gp160 variant available in the shortest possible time.

For this purpose, a new method has now been found
15 for obtaining a gp160 variant which is suitable irrespective of the viral strain in question. Inasmuch as an expression cassette intended for the production of a non-cleavable and soluble gp160 variant derived from a first viral strain is made available, this method makes it
20 possible to obtain a similar variant derived from a second viral strain while avoiding the complete cloning of the DNA fragment coding the gp160 of the second viral strain, and the modifications which would have to ensue therefrom.

25 Consequently, the invention proposes a method for constructing an expression cassette containing a DNA unit coding either for a precursor of a non-cleavable and soluble hybrid gp160 variant, or for a non-cleavable and soluble hybrid gp160 variant; said variant having an
30 amino acid sequence comprising:

- i) a first region derived from the gp160 of a first strain of HIV virus and located in this latter gp160 from the amino acid at position X to the amino acid at position Y, X being a
35 number from 1 to 271 and Y being a number from 306 to 482;
- ii) a second region derived from a type A non-cleavable, soluble gp160 variant originating



from a second strain of HIV virus and located in this latter gp160 variant from the amino acid at position Y+1 to the amino acid at the C-terminal position, Y being as defined above; and

iii) when X is other than 1, a third region derived from the gp160 of said second strain of HIV virus and located in this latter gp160 from the amino acid at position 1 to the amino acid at position X-1, X being as defined above;

said method comprising:

a) the act of cloning a DNA fragment coding for said first region; and

b) the act of inserting the DNA fragment cloned in a) into a site of a cassette which comprises:

m) upstream of the site and in sequence:

i) a promotor,

ii) a translation initiation codon,

iii) optionally, a first DNA region coding for a signal peptide, and

iv) when X is as defined above but other than 1, a second DNA region coding for said third region of the amino acid sequence of said hybrid gp160 variant; and

n) downstream of the site and in sequence:

i) when Y is as defined above, a third DNA region coding for said second region of the amino acid sequence of said hybrid gp160 variant, and

ii) a translation termination codon;

to obtain an expression cassette containing said DNA unit.

Such an expression cassette is the indispensable tool which enables a soluble and non-cleavable hybrid gp160 variant to be produced in a heterologous expression system.



In the method according to the invention, the DNA fragment coding for said first region may be cloned according to any method in common use. It may be noted, however, that this cloning may be advantageously performed by the PCR technique starting with the genomic DNA of cells infected with said second strain of HIV virus. Insertion of the cloned DNA fragment takes place in restriction sites initially present in the cassette or created for this purpose, for example by the suitable use of a polylinker. An illustration of this method is presented below in the examples.

"DNA unit coding for a polypeptide of any kind" is understood to mean a DNA segment whose first codon at the 5' position and last codon at the 3' position code, respectively, for the first amino acid at the N-terminal position and the last amino acid at the C-terminal position of the polypeptide.

According to a preferred embodiment, the method according to the invention is carried out in order to construct an expression cassette containing a DNA unit coding for a precursor of a non-cleavable and soluble hybrid gp160 variant.

"Precursor of a gp160 variant according to the invention" means a polypeptide containing a signal peptide and a mature gp160 variant; the N-terminal end of said variant being combined with the C-terminal end of the signal peptide via a peptide bond. This precursor is the initial product of expression of the DNA unit. It is, in particular, present in the cytoplasm of the host cell, combined at the N-terminal position with a translation initiation methionine residue. The signal peptide enables the transfer of the gp160 variant to the endoplasmic reticulum to be initiated. During this transfer, the signal peptide is relinquished by proteolytic cleavage to give the mature form of the gp160 variant. In the remainder of the text, the term "gp160 variant" refers exclusively to its mature form.



The signal peptide can be any signal peptide in common use. It must, however, be chosen taking into account the host organism intended for the production of the gp160 variant according to the invention. For example, if the host organism is a mammalian cell, the signal peptide may be advantageously selected from the signal peptides of the precursors of the gp160 proteins of the different strains of HIV virus, independently of the origin of the first, second and third regions of the variant according to the invention. Alternatively, synthetic signal peptides may be used. Such signal peptides are, for example, hybrid signal peptides whose N-terminal end is derived from the precursor of the gp160 of said second strain of HIV virus and whose C-terminal end is derived from the precursor of the gp160 of said first strain of HIV virus. As a guide, it may be mentioned furthermore that the signal peptide of the precursor of the glycoprotein of a strain of the rabies virus may also be used. Lastly, a person skilled in the art should understand that this list is non-limiting.

The invention likewise proposes a non-cleavable and soluble hybrid gp160 variant which comprises:

- i) a first region derived from the gp160 of a first strain of HIV virus and located in this latter gp160 from the amino acid at position X to the amino acid at position Y, X being a number from 1 to 271 and Y being a number from 306 to 482;
- ii) a second region derived from a type A non-cleavable, soluble gp160 variant originating from a second strain of HIV virus and located in this latter gp160 variant from the amino acid at position Y+1 to the amino acid at the C-terminal position, Y being as defined above; and
- iii) when X is other than 1, a third region derived from the gp160 of said second strain of HIV virus and located in this latter gp160 from the





amino acid at position 1 to the amino acid at position X-1, X being as defined above.

The first and also the second and third regions of the gp160 variant according to the invention may be derived from the gp160 of any strain of HIV virus, on condition that the first strain is different from the second. Preferably, the first region is derived from the gp160 of a viral strain selected from the strains HIV-2 Rod, HIV-1 Eli, HIV-1 RF, HIV-1 SF2C, HIV-1 SC and HIV-1 MN, the latter being more especially preferred. Similarly, the second and third regions preferably originate from the gp160 of the strain HIV-1 Bru.

"Type A non-cleavable gp160 variant" is understood to mean a gp160 variant:

- either derived from a gp160 of a type HIV-1 viral strain no longer containing the major cleavage site,

- or derived from a gp160 of a type HIV-2 viral strain no longer containing the cleavage site.

When the gp160 variant is derived from a gp160 of a type HIV-1 viral strain, this variant is preferably of type A'; that is to say, containing neither the major cleavage site nor the minor cleavage site.

"Soluble gp160 variant" is understood to mean a gp160 variant derived from a native gp160 which no longer contains a transmembrane region or whose native transmembrane region has been mutated so that it can no longer perform its function of anchorage in the membrane. In addition, it is advantageously possible for such a soluble variant no longer to contain a hydrophobic region.

"Expression cassette" is understood to mean a DNA segment comprising a DNA unit to be expressed, as well as the elements needed for expression of the latter. The expression of a DNA unit is obtained by transcription of this DNA unit into messenger RNA and by translation of this messenger RNA into protein. Consequently, the essential elements are a constitutive or inducible



promoter (transcription initiation), a translation initiation codon (ATG codon) and a translation termination codon (TAG, TAA or TGA codon). In addition, an expression cassette can also contain other elements; for example, a transcription terminator.

A person skilled in the art is obviously capable of choosing the appropriate promoter and terminator in accordance with the host organism in which it is desired to express the DNA unit, and in accordance with the vector into which the expression cassette has to be inserted in order to provide for its replication.

In keeping with the foregoing, the invention likewise proposes an expression cassette containing a DNA unit coding either for a precursor of a gp160 variant according to the invention, or for a gp160 variant according to the invention, as well as the elements needed for expression of said DNA unit.

In order to provide for its autonomous application in a host organism, an expression cassette according to the invention may be inserted into different types of vector having an origin of replication suited to the host organism; for example a plasmid or a virus. Viral type vectors have, in particular, the capacity to integrate in their genome a substantial amount of foreign DNA without impairing their capacity for replication. These vectors include, for example, pox viruses such as vaccinia virus, canarypox virus and fowlpox virus; and baculoviruses such as adenoviruses such as Adenovirus-2 or Adenovirus-5.

Apart from their use in a heterologous production system, some of these viral type vectors can be functional as a vaccinating agent. This applies, in particular, to the pox viruses and adenoviruses.

Thus, the invention likewise relates to a viral vector, into the genome of which an expression cassette according to the invention is inserted.

In another aspect of the invention, a cell transformed with an expression cassette according to the invention is also obtained. The expression cassette which

transforms the cell may be transported by a plasmid or, alternatively, be integrated in the genome of the host cell.

5 The host organism intended for the production of the gp160 variant according to the invention can be any type of cell, preferably eukaryotic. This includes, for example: fungi such as yeasts, insect cells and mammalian cells.

10 The invention proposes, in addition, two alternative processes directed towards the production of a gp160 variant according to the invention:

- the first process comprises the act of culturing a cell according to the invention and the act of harvesting said variant from the culture,

15 - the second process comprises the act of infecting a cell culture with a viral vector according to the invention and the act of harvesting said variant from the culture.

20 A gp160 variant and also a viral vector according to the invention possess a vaccinal activity against an HIV virus, and are consequently useful as pharmaceutical products intended especially for the treatment or prevention of AIDS.

Consequently, the invention proposes:

25 i) a pharmaceutical composition intended for the curative or preventive treatment of AIDS, which comprises as therapeutic agent at least one gp160 variant or one viral vector according to the invention,

30 ii) a method of curative or preventive treatment of AIDS, which comprises the act of administering a therapeutically effective amount of a gp160 variant or of a viral vector according to the invention to a patient needing such a treatment,

35 iii) the use of a gp160 variant or of a viral vector according to the invention as therapeutic agent intended for the curative or preventative treatment of AIDS.



Preferably, a pharmaceutical composition according to the invention can contain several gp160 variants according to the invention; each gp160 variant possessing at least one third hypervariable domain (V₃ loop) different from the other variants present in the composition. Such a pharmaceutical composition should hence enable an individual to be correctly protected with respect to various HIV strains.

A pharmaceutical composition according to the invention can consist, in addition, of other therapeutic agents such as, for example, a peptide essentially corresponding to the third hypervariable domain of a gp160 (hereinafter referred to as V₃ peptide). Preferably, such a peptide has a sequence substantially identical to the sequence of the third domain of a gp160 variant contained in the pharmaceutical composition according to the invention. Similarly, several V₃ peptides may be present in the composition. In particular, if the pharmaceutical composition according to the invention contains different gp160 variants, the corresponding V₃ peptides can obviously be added thereto.

A pharmaceutical composition according to the invention may be manufactured in a conventional manner. In particular, a gp160 variant according to the invention is combined with a diluent or a vehicle which is acceptable from a pharmaceutical standpoint. Lastly, a composition according to the invention can contain a vaccination adjuvant such as alum. Alternatively, this adjuvant may be added to a composition according to the invention immediately before use.

A composition according to the invention may be administered by any conventional route in common use in the vaccine field, especially subcutaneously, for example in the form of an injectable solution or suspension. The administration can take place in a single dose or a dose repeated one or more times after a certain time interval. The appropriate dosage varies in accordance with various parameters, for example with the individual being treated



or the mode of administration.

Alternatively, a pharmaceutical composition according to the invention may be presented as part of a treatment kit. As an example, it may be noted that such a kit can contain:

- on the one hand a pharmaceutical composition containing at least one gp160 variant according to the invention, and
- on the other hand a pharmaceutical composition containing at least one V₃ peptide,
- as well as a leaflet specifying the instructions relating to the sequential or concomitant administration of the pharmaceutical compositions contained in the kit.

The invention is illustrated below by reference to the following figures:

Figure 1 presents the amino acid sequence of the precursors of the native gp160 proteins of the viral strains HIV-1 Bru (a), HIV-1 MN (b), HIV-1 Eli (c), HIV-1 RF (d), HIV-1 SC (e), and HIV-1 SF2C (f). The amino acid residues of the signal sequences are numbered from position -1 to -29. The methionine residue at position -30 corresponds to the translation initiation codon. The amino acid residues of the mature gp160 proteins are numbered from position 1 to 841. The asterisk (*) symbolizes the identity of the amino acid residues at a given position, while the dot (.) indicates a conservative change (different amino acids but belonging to the same class).

Figure 2 presents the amino acid sequence of the precursors of the native gp160 proteins of the viral strains HIV-1 Bru (a) and HIV-2 Rod (b). The amino acid residues of the signal sequences are numbered from position -1 to -29. The methionine residue at position -30 corresponds to the translation initiation codon. The amino acid residues of the mature gp160 proteins are numbered from position 1 to 841. The asterisk (*) symbolizes the identity of the amino acid residues at a





given position, while the dot (.) indicates a conservative change (different amino acids but belonging to the same class).

5 Figure 3 presents the nucleotide sequence of the *PstI-PstI* fragment of the vector pTG1163 which contains the sequence coding for a precursor of a soluble, non-cleavable gp160-Bru variant (transmembrane domain absent), as well as the amino acid sequence of this precursor.

10 Figure 4 shows diagrammatically the steps in the construction of the bacteriophages M13TG4168 and M13TG4174.

15 Figure 5 presents the nucleotide sequence coding for the precursor of gp120-MN, as well as the amino acid sequence of this precursor. The oligonucleotides OTG2624 and OTG2625, intended for the amplification of a DNA fragment coding at least for the third hypervariable domain of gp120-MN, are shown above their hybridization region.

20 Figure 6 presents the nucleotide sequence coding for the precursor of gp120-Eli, as well as the amino acid sequence of this precursor. The oligonucleotides OTG2624 and OTG2625, intended for the amplification of a DNA fragment coding at least for the third hypervariable domain of gp120-MN, are shown above their hybridization region.

25 Figure 7 presents the nucleotide sequence coding for the precursor of gp120-RF, as well as the amino acid sequence of this precursor. The oligonucleotides OTG2624 and OTG2625, intended for the amplification of a DNA fragment coding at least for the third hypervariable domain of gp120-RF, are shown above their hybridization region.

30 Figure 8 presents the nucleotide sequence coding for the precursor of gp120-SF2C, as well as the amino acid sequence of this precursor. The oligonucleotides OTG2624 and OTG2625, intended for the amplification of a DNA fragment coding at least for the third hypervariable domain of gp120-SF2C, are shown above their hybridization

region.

In the examples which follow, to make the text easier to write and to understand, "signal sequence" is understood to mean a signal sequence including the translation initiation methionine residue.

Example 1: Construction of an insertion cassette carried by the bacteriophage M13TG4168.

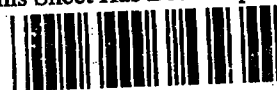
As shown in Figure 3, the *PstI*-*PstI* DNA fragment of plasmid pTG1163 described in Patent Application EPA 245, 136 contains a DNA sequence coding for a precursor of a non-cleavable and soluble gp160-Bru variant. This *PstI*-*PstI* DNA fragment is inserted into the bacteriophage M13mp701 (described by M.P. Kieny et al. Gene (1983) 26:91) previously digested with *PstI*, to give the bacteriophage M13TG4137 (Figure 4). The numbering of the nucleotides of the *PstI*-*PstI* fragment as shown in Figure 3 serves as a reference in the remainder of Example 1.

Plasmid pTG1163 is cut with *PstI* and *KpnI*, and the DNA fragment corresponding to nucleotides 1 to 138 (Figure 3) is inserted into the bacteriophage M13TG130 (described by M.P. Kieny et al. (1983), *supra*) previously digested with *PstI* and *KpnI*. The bacteriophage M13TG4147 is thereby obtained (Figure 4).

The bacteriophage M13TG4137 is cut with *BglII*, treated with the Klenow enzyme (Boehringer Mannheim) to obtain blunt ends and then cut with *EcoRI*. The *EcoRI*-*BglII** fragment derived from this digestion and containing the sequence corresponding to nucleotides 1424 to 2644 (Figure 3) is inserted into the bacteriophage M13TG4147 previously digested with *EcoRV* and *EcoRI*. The bacteriophage M13TG4158, which contains:

- i) a DNA fragment corresponding to nucleotides 1 to 138,
- ii) the remaining sequence of the polylinker of the bacteriophage M13TG4147, that is to say ATCGCATGCG,





iii) a DNA fragment corresponding to nucleotides 1424 to 2644, is thereby obtained.

The single-stranded antisense bacteriophage M13TG4158 serves as a template for a mutation-deletion, performed using the Amersham kit and the oligonucleotide OTG2623 whose sequence is as follows:

$\overbrace{\hspace{1.5cm}}^{SphI} \quad \overbrace{\hspace{1.5cm}}^{SmaI}$
 GGGGGTGGAAATGGGGCA GCATGC AT CCCGGG CACAGAATCACGTGGTGC.

This mutagenesis makes it possible simultaneously to delete a 184-base pair fragment which contains nucleotides 67 to 138, the sequence ATCGCATGCG and nucleotides 1424 to 1525, and to create the cleavage sites for the enzymes *SphI* at the 5' end and *SmaI* at the 3' end, which are inserted between nucleotides 66 and 1526. The bacteriophage M13TG4168, which comprises:

- i) a DNA fragment corresponding to nucleotides 1 to 66,
 - ii) the DNA sequence GCATGCATCCCG containing the cleavages sites of the enzymes *SphI* and *SmaI*, and
 - iii) a DNA fragment corresponding to nucleotides 1526 to 2644,
- is thereby obtained.

Example 2: Construction of an expression cassette intended for the synthesis of a Bru-MN hybrid gp160 variant.

As shown in Figure 5, the DNA fragment coding for the larger part of a precursor of gp120-MN is cloned by the PCR (Polymerase Chain Reaction) gene amplification technique, carried out starting with genomic DNA of CEM human cells infected with the viral strain HIV1-MN. This cloning is performed using the oligonucleotides OTG2624 and OTG2625, which introduce, respectively, a cleavage site for the enzyme *SphI* at the 5' end of the amplified DNA fragment and a cleavage site for the enzyme *SmaI* at



the 3' end of the amplified DNA fragment. The sequences of these oligonucleotides are as follows:

┌─── *Sph*I ───┐

OTG2624 : GGCA GCATGC TCCTTGGGATATTGATGATCTG

5

┌─── *Sma*I ───┐

OTG2625 : CTTTG CCCGGG TGGGTGCTACTCCTAATGGTTC

10 The amplified *Sph*I-*Sma*I DNA fragment corresponds to nucleotides 53 to 1505 shown in Figure 5, taking into account the sequence modifications brought about by the oligonucleotides OTG2624 and OTG2625. This fragment is inserted into the bacteriophage M13TG4168 cut with *Sph*I and *Sma*I. The bacteriophage M13TG4174, which contains a DNA fragment coding for a precursor of a Bru-MN hybrid gp160 protein, is thereby obtained. The *Pst*I fragment of 15 the bacteriophage M13TG4174 which codes for the Bru-MN hybrid gp160 variant is inserted into the transfer plasmid pTG186poly (described by M.P. Kieny et al., Biotechnology, (1986) 4: 790), downstream of the E7.5k promoter and within the vaccinia virus gene coding for 20 thymidine kinase. Plasmid pTG5156 is thereby obtained.

Plasmid pTG5156 is subsequently used to transfer the block for expression of the Bru-MN hybrid gp160 protein into the genome of vaccinia virus, Copenhagen strain, according to the method described by M.P. Kieny 25 et al. Nature (1984), 312, 163-166). The vaccinia vector VVTG5156 is thereby obtained.

Example 3: Construction of an expression cassette intended for the synthesis of a Bru-Eli hybrid gp160 variant.

30 Plasmid pTG186poly is cut with *Bam*HI, treated with the Klenow enzyme, cut with *Sma*I to delete the larger part of the polylinker and then religated with itself to give plasmid pTG186PE. The polylinker now retains only the cleavage sites for the enzymes *Pst*I, *Sal*I and *Eco*RI.

35 The *Pst*I-*Pst*I DNA fragment of the bacteriophage

M13TG4168 described above, which contains:

- i) a DNA fragment corresponding to nucleotides 1 to 66 of the DNA fragment shown in Figure 3,
- 5 ii) the DNA sequence GCATGCATCCCG containing the cleavage sites of the enzymes *SphI* and *SmaI*, and
- 10 iii) a DNA fragment corresponding to nucleotides 1526 to 2644 of the DNA fragment shown in Figure 3,

is inserted into plasmid pTG186PE previously cut with *PstI*, downstream of the E7.5k promoter and within the vaccinia virus gene coding for thymidine kinase. Plasmid pTG5160 is thereby obtained.

15 As shown in Figure 6, the DNA fragment coding for the larger part of a precursor of gp120-Eli is cloned by the PCR gene amplification technique, carried out starting with the genomic DNA of CEM human cells infected with HIV1-Eli virus. This cloning is performed using the
20 oligonucleotides OTG2624 and OTG2625, which introduce, respectively, a cleavage site for the enzyme *SphI* at the 5' end of the amplified DNA fragment and a cleavage site for the enzyme *SmaI* at the 3' end of the amplified DNA fragment. The amplified *SphI*-*SmaI* DNA fragment corresponds
25 to nucleotides 53 to 1490 shown in Figure 6, taking into account the sequence modifications brought about by the oligonucleotides OTG2624 and OTG2625. This fragment is inserted into the bacteriophage M13TG131 (described by M.P.Kieny et al. (1983), *supra*) previously cut with *SphI*
30 and *SmaI*, to give the bacteriophage M13TG4197. The *SphI*-*SmaI* fragment of the bacteriophage M13TG4197 is then inserted into the transfer plasmid pTG5160 (described above) previously cut with *SphI* and *SmaI*. pTG5193 is thereby obtained.

35 Plasmid pTG5193 is subsequently used to transfer the block for expression of the Bru-Eli hybrid gp160 protein into the genome of vaccinia virus, Copenhagen





strain, according to the method described by M.P.Kieny et al. (1984) *supra*. The vaccinia vector VVTG5193 is thereby obtained.

Example 4: Construction of an expression cassette intended for the synthesis of a Bru-RF hybrid gp160 variant.

As shown in Figure 7, the DNA fragment coding for the larger part of a precursor of a gp120-RF protein is cloned by the PCR gene amplification technique, carried out starting with the genomic DNA of CEM human cells infected with HIV1-RF virus. This cloning is performed using the oligonucleotides OTG2624 and OTG2625, which introduce, respectively, a cleavage site for the enzyme *SphI* at the 5' end of the amplified DNA fragment and a cleavage site for the enzyme *SmaI* at the 3' end of the amplified DNA fragment. The amplified *SphI*-*SmaI* DNA fragment corresponds to nucleotides 53 to 1523 shown in Figure 7, taking into account the sequence modifications brought about by the oligonucleotides OTG2624 and OTG2625. This fragment is inserted into the bacteriophage M13TG131 previously cut with *SphI* and *SmaI*, to give the bacteriophage M13TG4198. The *SphI*-*SmaI* fragment of the bacteriophage M13TG4198 is then inserted into the transfer plasmid pTG5160 (described in Example 3) previously cut with *SphI* and *SmaI*. pTG5194 is thereby obtained.

Plasmid pTG5194 is subsequently used to transfer the block for expression of the Bru-RF hybrid gp160 protein into the genome of vaccinia virus, Copenhagen strain, according to the method described by M.P.Kieny et al. (1984) *supra*. The vaccinia vector VVTG5194 is thereby obtained.

Example 5: Construction of an expression cassette intended for the synthesis of a Bru-SF2C hybrid gp160 variant.

As shown in Figure 8, the DNA fragment coding for the larger part of a precursor of gp120-SF2C is cloned by the PCR gene amplification technique, carried out starting with the genomic DNA of CEM human cells infected with



HIV1-SF2C virus. This cloning is performed using the oligonucleotides OTG2624 and OTG2625, which introduce, respectively, a cleavage site for the enzyme *SphI* at the 5' end of the amplified DNA fragment and a cleavage site for the enzyme *SmaI* at the 3' end of the amplified DNA fragment. The amplified *SphI-SmaI* DNA fragment corresponds to nucleotides 53 to 1493 shown in Figure 8, taking into account the sequence modifications brought about by the oligonucleotides OTG2624 and OTG2625. This fragment is then inserted into the bacteriophage M13TG131 previously cut with *SphI* and *SmaI*, to give the bacteriophage M13TG4199. The *SphI-SmaI* fragment of the bacteriophage M13TG4199 is inserted into the transfer plasmid pTG5160 (described in Example 3) previously cut with *SphI* and *SmaI*. pTG5195 is thereby obtained.

Plasmid pTG5195 is subsequently used to transfer the block for expression of the Bru-SF2C hybrid gp160 protein into the genome of vaccinia virus, Copenhagen strain, according to the method described by M.P.Kieny et al. (1984) *supra*. The vaccinia vector VVTG5195 is thereby obtained.

Examples 6 to 9: Production and purification of Bru-MN, Bru-Eli, Bru-RF and Bru SF2C hybrid gp160 proteins:

BHK-21 cells are cultured in a GMEM medium (Gibco) supplemented with 10 % of fetal calf serum (FCS). When the cells are at confluence (5.8×10^6 cells/ml), the culture medium is removed and the cell lawn is washed twice with 50 ml of PBS (Dubecco's phosphate buffer salt; Seromed). Fresh GMEM medium without FCS is then added. One of the vaccinia viruses VVTG5156, VVTG5193, VVTG5194 and VVTG5195, described above in Examples 2 to 5, is then added at an infectivity of 1 pfu/cell (plaque forming unit), and infection is continued for 72 h. The lysate is then centrifuged for 20 min at 10,000 g in order to remove cell debris, and the supernatant containing, *inter alia*, a hybrid gp160 and virions is recovered.

To 20 ml of culture supernatant obtained as

described above, 1.3 ml of 1 M zinc chloride (ZnCl_2) solution is added so as to have a final ZnCl_2 concentration of 60 mM. The mixture is left for 1 h in ice; the precipitation supernatant is then recovered after centrifugation at 3000 rpm for 20 min in a Heraeus RF Minifuge centrifuge. This method removes by precipitation the virions as well as the majority of contaminant proteins. In each case, a purified solution of the hybrid gp160 variant is thereby obtained.

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CLAIMS

1. A method for constructing an expression cassette containing a DNA unit coding either for a precursor of a non-cleavable and soluble hybrid gp160 variant, or for a non-cleavable and soluble hybrid gp160 variant; said gp160 variant having an amino acid sequence comprising:

i) a first region derived from the gp160 of a first strain of HIV virus and located in this latter gp160 from the amino acid at position X to the amino acid at position Y, X being a number from 1 to 271 and Y being a number from 306 to 482;

ii) a second region derived from a type A non-cleavable, soluble gp160 variant originating from a second strain of HIV virus and located in this latter gp160 variant from the amino acid at position Y+1 to the amino acid at the C-terminal position, Y being as defined above; and

iii) when X is other than 1, a third region derived from the gp160 of said second strain of HIV virus and located in this latter gp160 from the amino acid at position 1 to the amino acid at position X-1, X being as defined above;

said method comprising:

a) the act of cloning a DNA fragment coding for said first region; and

b) the act of inserting the DNA fragment cloned in a) into a site of a cassette which comprises:

m) upstream of the site and in sequence:

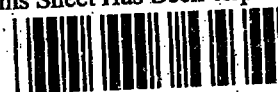
i) a promotor,

ii) a translation initiation codon,

iii) optionally, a first DNA region coding for a signal peptide, and

iv) when X is as defined above but other than 1, a second DNA region coding for said third region of the amino acid sequence of said hybrid gp160





variant; and

n) downstream of the site and in sequence:

i) when Y is as defined above, a third DNA region coding for said second region of the amino acid sequence of said hybrid gp160 variant, and

ii) a translation termination codon;

to obtain an expression cassette containing said DNA unit.

2. A method according to claim 1, for constructing an expression cassette containing a DNA unit coding for a precursor of a non-cleavable and soluble hybrid gp160 variant having an amino acid sequence which comprises:

i) a first region derived from the gp160 of a first strain of HIV virus and located in this latter gp160 from the amino acid at position X to the amino acid at position Y, X being a number from 1 to 271 and Y being a number from 306 to 482;

ii) a second region derived from a type A non-cleavable, soluble gp160 variant originating from a second strain of HIV virus and located in this latter gp160 variant from the amino acid at position Y+1 to the amino acid at the C-terminal position, Y being as defined above; and

iii) when X is other than 1, a third region derived from the gp160 of said second strain of HIV virus and located in this latter gp160 from the amino acid at position 1 to the amino acid at position X-1, X being as defined above;

said method comprising:

a) the act of cloning a DNA fragment coding for said first region; and

b) the act of inserting the DNA fragment cloned in a) into a site of a cassette which comprises:

m) upstream of the site and in sequence:

i) a promotor,





- 5 ii) a translation initiation codon,
 iii) a first DNA region coding for a
 signal peptide, and
 iv) when X is as defined above but other
 than 1, a second DNA region coding
 for said third region of the amino
 acid sequence of said hybrid gp160
 variant; and
n) downstream of the site and in sequence:
10 i) when Y is as defined above, a third
 DNA region coding for said second
 region of the amino acid sequence of
 said hybrid gp160 variant, and
 ii) a translation termination codon;
15 to obtain an expression cassette containing said DNA
 unit.
3. A method according to claim 1, for constructing an
 expression cassette containing a DNA unit coding either
 for a precursor of a non-cleavable and soluble hybrid
20 gp160 variant, or for a non-cleavable and soluble hybrid
 gp160 variant; said gp160 variant having an amino acid
 sequence comprising:
 i) a first region derived from the gp160 of a
 first strain of HIV virus and located in this
25 latter gp160 from the amino acid at position X
 to the amino acid at position Y, X being a
 number from 1 to 271 and Y being a number from
 306 to 476;
 ii) a second region derived from a type A' non-
30 cleavable, soluble gp160 variant originating
 from a second strain of HIV virus and located
 in this latter gp160 variant from the amino
 acid at position Y+1 to the amino acid at the
 C-terminal position, Y being as defined above;
35 and
 iii) when X is other than 1, a third region derived
 from the gp160 of said second strain of HIV
 virus and located in this latter gp160 from the





amino acid at position 1 to the amino acid at position X-1, X being as defined above;
said method comprising:

- 5 a) the act of cloning a DNA fragment coding for said first region; and
- b) the act of inserting the DNA fragment cloned in a) into a site of a cassette which comprises:
 - m) upstream of the site and in sequence:
 - 10 i) a promotor,
 - ii) a translation initiation codon,
 - iii) optionally, a first DNA region coding for a signal peptide, and
 - iv) when X is as defined above but other than 1, a second DNA region coding for said third region of the amino acid sequence of said hybrid gp160 variant; and
 - 15 n) downstream of the site and in sequence:
 - 20 i) when Y is as defined above, a third DNA region coding for said second region of the amino acid sequence of said hybrid gp160 variant, and
 - ii) a translation termination codon;

25 to obtain an expression cassette containing said DNA unit.

4. A method according to claim 1 or 3, for constructing an expression cassette containing a DNA unit coding either for a precursor of a non-cleavable and soluble hybrid gp160 variant, or for a non-cleavable and soluble hybrid gp160 variant; said variant having an amino acid sequence comprising:

- 35 i) a first region derived from the gp160 of a first strain of HIV virus and located in this latter gp160 from the amino acid at position X to the amino acid at position Y, X being a number from 1 to 271 and Y being a number from 306 to 482;





- 5 ii) a second region derived from a type A non-cleavable, soluble gp160 variant originating from a second strain of HIV virus and located in this latter gp160 variant from the amino acid at position Y+1 to the amino acid at the C-terminal position, Y being as defined above and said second strain being the strain HIV1-Bru; and
- 10 iii) when X is other than 1, a third region derived from the gp160 of said second strain of HIV virus and located in this latter gp160 from the amino acid at position 1 to the amino acid at position X-1, X being as defined above;
- said method comprising:
- 15 a) the act of cloning a DNA fragment coding for said first region; and
- b) the act of inserting the DNA fragment cloned in a) into a site of a cassette which comprises:
- m) upstream of the site and in sequence:
- 20 i) a promotor,
- ii) a translation initiation codon,
- iii) optionally, a first DNA region coding for a signal peptide, and
- iv) when X is as defined above but other
- 25 than 1, a second DNA region coding for said third region of the amino acid sequence of said hybrid gp160 variant; and
- n) downstream of the site and in sequence:
- 30 i) when Y is as defined above, a third DNA region coding for said second region of the amino acid sequence of said hybrid gp160 variant, and
- ii) a translation termination codon;
- 35 to obtain an expression cassette containing said DNA unit.
5. A method according to any one of claims 1, 3 and 4, for constructing an expression cassette containing a





DNA unit coding either for a precursor of a non-cleavable and soluble hybrid gp160 variant, or for a non-cleavable and soluble hybrid gp160 variant; said variant having an amino acid sequence comprising:

- 5 i) a first region derived from the gp160 of a first strain of HIV virus and located in this latter gp160 from the amino acid at position X to the amino acid at position Y, X being a number from 1 to 271 and Y being a number from 306 to 482 and said first strain being selected from the strains HIV-1 MN, HIV-1 Eli, HIV-1 RF, HIV-1 SF2C and HIV-1 SC;
- 10
- ii) a second region derived from a type A non-cleavable, soluble gp160 variant originating from a second strain of HIV virus and located in this latter gp160 variant from the amino acid at position Y+1 to the amino acid at the C-terminal position, Y being as defined above; and
- 15
- iii) when X is other than 1, a third region derived from the gp160 of said second strain of HIV virus and located in this latter gp160 from the amino acid at position 1 to the amino acid at position X-1, X being as defined above;
- 20
- 25 said method comprising:
- a) the act of cloning a DNA fragment coding for said first region; and
- b) the act of inserting the DNA fragment cloned in a) into a site of a cassette which comprises:
- 30 m) upstream of the site and in sequence:
- i) a promotor,
- ii) a translation initiation codon,
- iii) optionally, a first DNA region coding for a signal peptide, and
- 35 iv) when X is as defined above but other than 1, a second DNA region coding for said third region of the amino acid sequence of said hybrid gp160





variant; and

- n) downstream of the site and in sequence:
- i) when Y is as defined above, a third DNA region coding for said second region of the amino acid sequence of said hybrid gp160 variant, and
 - ii) a translation termination codon;

to obtain an expression cassette containing said DNA unit.

6. A method according to claim 5, for constructing an expression cassette containing a DNA unit coding either for a precursor of a non-cleavable and soluble hybrid gp160 variant, or for a non-cleavable and soluble hybrid gp160 variant; said variant having an amino acid sequence comprising:

i) a first region derived from the gp160 of a first strain of HIV virus and located in this latter gp160 from the amino acid at position X to the amino acid at position Y, X being a number from 1 to 271 and Y being a number from 306 to 482 and said first strain being the strain HIV-1 MN;

ii) a second region derived from a type A non-cleavable, soluble gp160 variant originating from a second strain of HIV virus and located in this latter gp160 variant from the amino acid at position Y+1 to the amino acid at the C-terminal position, Y being as defined above; and

iii) when X is other than 1, a third region derived from the gp160 of said second strain of HIV virus and located in this latter gp160 from the amino acid at position 1 to the amino acid at position X-1, X being as defined above;

said method comprising:

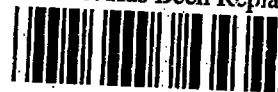
- a) the act of cloning a DNA fragment coding for said first region; and





- b) the act of inserting the DNA fragment cloned in
a) into a site of a cassette which comprises:
m) upstream of the site and in sequence:
i) a promotor,
5 ii) a translation initiation codon,
iii) optionally, a first DNA region
coding for a signal peptide, and
iv) when X is as defined above but other
10 than 1, a second DNA region coding
for said third region of the amino
acid sequence of said hybrid gp160
variant; and
n) downstream of the site and in sequence:
i) when Y is as defined above, a third
15 DNA region coding for said second
region of the amino acid sequence of
said hybrid gp160 variant, and
ii) a translation termination codon;
to obtain an expression cassette containing said DNA
20 unit.
7. A method according to any one of claims 1 and 3
to 6, for constructing an expression cassette containing
a DNA unit coding either for a precursor of a non-
cleavable and soluble hybrid gp160 variant, or for a non-
25 cleavable and soluble hybrid gp160 variant; said variant
having an amino acid sequence comprising:
i) a first region derived from the gp160 of a
first strain of HIV virus and located in this
latter gp160 from the amino acid at position X
30 to the amino acid at position Y, X being a
number from 1 to 271 and Y being a number from
450 to 482;
ii) a second region derived from a type A' non-
cleavable, soluble gp160 variant originating
35 from a second strain of HIV virus and located
in this latter gp160 variant from the amino
acid at position Y+1 to the amino acid at the
C-terminal position, Y being as defined above;





and

- iii) when X is other than 1, a third region derived from the gp160 of said second strain of HIV virus and located in this latter gp160 from the amino acid at position 1 to the amino acid at position X-1, X being as defined above;

said method comprising:

- a) the act of cloning a DNA fragment coding for said first region; and

- b) the act of inserting the DNA fragment cloned in a) into a site of a cassette which comprises:

- m) upstream of the site and in sequence:

- i) a promotor,
ii) a translation initiation codon,
iii) optionally, a first DNA region coding for a signal peptide, and
iv) when X is as defined above but other than 1, a second DNA region coding for said third region of the amino acid sequence of said hybrid gp160 variant; and

- n) downstream of the site and in sequence:

- i) when Y is as defined above, a third DNA region coding for said second region of the amino acid sequence of said hybrid gp160 variant, and
ii) a translation termination codon;

to obtain an expression cassette containing said DNA unit.

8. A method according to any one of claims 1 and 3 to 7, for constructing an expression cassette containing a DNA unit coding either for a precursor of a non-cleavable and soluble hybrid gp160 variant, or for a non-cleavable and soluble hybrid gp160 variant; said variant having an amino acid sequence comprising:

- i) a first region derived from the gp160 of a first strain of HIV virus and located in this latter gp160 from the amino acid at position X



to the amino acid at position Y, X being a number from 1 to 97 and Y being a number from 306 to 482;

5 ii) a second region derived from a type A' non-cleavable, soluble gp160 variant originating from a second strain of HIV virus and located in this latter gp160 variant from the amino acid at position Y+1 to the amino acid at the C-terminal position, Y being as defined above; and

10 iii) when X is other than 1, a third region derived from the gp160 of said second strain of HIV virus and located in this latter gp160 from the amino acid at position 1 to the amino acid at position X-1, X being as defined above;

15 said method comprising:

 a) the act of cloning a DNA fragment coding for said first region; and
 b) the act of inserting the DNA fragment cloned in
20 a) into a site of a cassette which comprises:

 m) upstream of the site and in sequence:
 i) a promotor,
 ii) a translation initiation codon,
 iii) optionally, a first DNA region
25 coding for a signal peptide, and
 iv) when X is as defined above but other than 1, a second DNA region coding for said third region of the amino acid sequence of said hybrid gp160 variant; and

30 n) downstream of the site and in sequence:
 i) when Y is as defined above, a third DNA region coding for said second region of the amino acid sequence of said hybrid gp160 variant, and
35 ii) a translation termination codon;

to obtain an expression cassette containing said DNA unit.





9. A method according to any one of claims 1 and 3 to 8, for constructing an expression cassette containing a DNA unit coding either for a precursor of a non-cleavable and soluble hybrid gp160 variant, or for a non-cleavable and soluble hybrid gp160 variant; said variant having an amino acid sequence comprising:

5 i) a first region derived from the gp160 of a first strain of HIV virus and located in this latter gp160 from the amino acid at position X to the amino acid at position Y, X being a number from 1 to 97 and Y being a number from 10 306 to 482;

15 ii) a second region derived from a type A' non-cleavable, soluble gp160 variant originating from a second strain of HIV virus and located in this latter gp160 variant from the amino acid at position Y+1 to the amino acid at the C-terminal position, Y being as defined above; and

20 iii) when X is other than 1, a third region derived from the gp160 of said second strain of HIV virus and located in this latter gp160 from the amino acid at position 1 to the amino acid at position X-1, X being as defined above;

25 said method comprising:

a) the act of cloning a DNA fragment coding for said first region by PCR technique starting with the genomic DNA of cells infected with said second strain of HIV virus; and

30 b) the act of inserting the DNA fragment cloned in a) into a site of a cassette which comprises:

m) upstream of the site and in sequence:
i) a promotor,
ii) a translation initiation codon,
35 iii) optionally, a first DNA region coding for a signal peptide, and
iv) when X is as defined above but other than 1, a second DNA region coding





for said third region of the amino acid sequence of said hybrid gp160 variant; and

- 5 n) downstream of the site and in sequence:
- i) when Y is as defined above, a third DNA region coding for said second region of the amino acid sequence of said hybrid gp160 variant, and
- ii) a translation termination codon;
- 10 to obtain an expression cassette containing said DNA unit.

10. A non-cleavable and soluble hybrid gp160 variant having an amino acid sequence which comprises:

- 15 i) a first region derived from the gp160 of a first strain of HIV virus and located in this latter gp160 from the amino acid at position X to the amino acid at position Y, X being a number from 1 to 271 and Y being a number from 306 to 482;
- 20 ii) a second region derived from a type A non-cleavable, soluble gp160 variant originating from a second strain of HIV virus and located in this latter gp160 variant from the amino acid at position Y+1 to the amino acid at the
- 25 C-terminal position, Y being as defined above; and
- iii) when X is other than 1, a third region derived from the gp160 of said second strain of HIV virus and located in this latter gp160 from the
- 30 amino acid at position 1 to the amino acid at position X-1, X being as defined above.

11. A non-cleavable and soluble hybrid gp160 variant according to claim 10, having an amino acid sequence which comprises:

- 35 i) a first region derived from the gp160 of a first strain of HIV virus and located in this latter gp160 from the amino acid at position X to the amino acid at position Y, X being a





number from 1 to 271 and Y being a number from 306 to 476;

ii) a second region derived from a type A' non-cleavable, soluble gp160 variant originating from a second strain of HIV virus and located in this latter gp160 variant from the amino acid at position Y+1 to the amino acid at the C-terminal position, Y being as defined above; and

iii) when X is other than 1, a third region derived from the gp160 of said second strain of HIV virus and located in this latter gp160 from the amino acid at position 1 to the amino acid at position X-1, X being as defined above.

12. A non-cleavable and soluble hybrid gp160 variant according to claim 10 or 11, having an amino acid sequence which comprises:

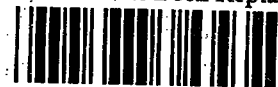
i) a first region derived from the gp160 of a first strain of HIV virus and located in this latter gp160 from the amino acid at position X to the amino acid at position Y, X being a number from 1 to 271 and Y being a number from 306 to 482;

ii) a second region derived from a type A non-cleavable, soluble gp160 variant originating from a second strain of HIV virus and located in this latter gp160 variant from the amino acid at position Y+1 to the amino acid at the C-terminal position, Y being as defined above and said second strain being the strain HIV-1 Bru; and

iii) when X is other than 1, a third region derived from the gp160 of said second strain of HIV virus and located in this latter gp160 from the amino acid at position 1 to the amino acid at position X-1, X being as defined above.

13. A non-cleavable and soluble hybrid gp160 variant according to any one of claims 10 to 12, having an amino





acid sequence which comprises:

- 5 i) a first region derived from the gp160 of a first strain of HIV virus and located in this latter gp160 from the amino acid at position X to the amino acid at position Y, X being a number from 1 to 271 and Y being a number from 306 to 482 and said first strain being selected from the strains HIV-1 MN, HIV-1 Eli, HIV-1 RF, HIV-1 SF2C and HIV-1 SC;
- 10 ii) a second region derived from a type A non-cleavable, soluble gp160 variant originating from a second strain of HIV virus and located in this latter gp160 variant from the amino acid at position Y+1 to the amino acid at the C-terminal position, Y being as defined above;
- 15 and
- 20 iii) when X is other than 1, a third region derived from the gp160 of said second strain of HIV virus and located in this latter gp160 from the amino acid at position 1 to the amino acid at position X-1, X being as defined above.
14. A non-cleavable and soluble hybrid gp160 variant according to claim 13, which comprises:
- 25 i) a first region derived from the gp160 of a first strain of HIV virus and located in this latter gp160 from the amino acid at position X to the amino acid at position Y, X being a number from 1 to 271 and Y being a number from 306 to 482 and said second strain being the strain HIV-1 MN;
- 30 ii) a second region derived from a type A non-cleavable, soluble gp160 variant originating from a second strain of HIV virus and located in this latter gp160 variant from the amino acid at position Y+1 to the amino acid at the C-terminal position, Y being as defined above;
- 35 and





5 iii) when X is other than 1, a third region derived from the gp160 of said second strain of HIV virus and located in this latter gp160 from the amino acid at position 1 to the amino acid at position X-1, X being as defined above.

15. A non-cleavable and soluble hybrid gp160 variant according to any one of claims 10 to 14, having an amino acid sequence which comprises:

10 i) a first region derived from the gp160 of a first strain of HIV virus and located in this latter gp160 from the amino acid at position X to the amino acid at position Y, X being a number from 1 to 271 and Y being a number from 450 to 482;

15 ii) a second region derived from a type A' non-cleavable, soluble gp160 variant originating from a second strain of HIV virus and located in this latter gp160 variant from the amino acid at position Y+1 to the amino acid at the C-terminal position, Y being as defined above; and

20 iii) when X is other than 1, a third region derived from the gp160 of said second strain of HIV virus and located in this latter gp160 from the amino acid at position 1 to the amino acid at position X-1, X being as defined above.

25 16. A non-cleavable and soluble hybrid gp160 variant according to any one of claims 10 to 15, having an amino acid sequence which comprises:

30 i) a first region derived from the gp160 of a first strain of HIV virus and located in this latter gp160 from the amino acid at position X to the amino acid at position Y, X being a number from 1 to 97 and Y being a number from 306 to 482;

35 ii) a second region derived from a type A' non-cleavable, soluble gp160 variant originating from a second strain of HIV virus and located





in this latter gp160 variant from the amino acid at position Y+1 to the amino acid at the C-terminal position, Y being as defined above; and

- 5 iii) when X is other than 1, a third region derived from the gp160 of said second strain of HIV virus and located in this latter gp160 from the amino acid at position 1 to the amino acid at position X-1, X being as defined above.
- 10 17. An expression cassette obtained by a method according to any one of claims 1 to 9.
18. An expression cassette containing a DNA unit coding either for a precursor of a gp160 variant according to any one of claims 10 to 16, or for a gp160 variant
- 15 according to any one of claims 10 to 16, and the elements needed for expression of said DNA unit.
19. A cell transformed with an expression cassette according to claim 17 or 18.
- 20 20. A viral vector, into the genome of which an expression cassette according to claim 17 or 18 is inserted.
21. A process for producing a gp160 variant according to any one of claims 10 to 16, which comprises the act of culturing a cell according to claim 19 and the act of
- 25 harvesting said variant from the culture.
22. A process for producing a gp160 variant according to any one of claims 10 to 16, which comprises the act of infecting a cell culture with a viral vector according to claim 20 and the act of harvesting said variant from the
- 30 culture.
23. A pharmaceutical composition intended for the prevention or treatment of AIDS, which comprises as therapeutic agent a gp160 variant according to any one of claims 10 to 16.
- 35 24. A pharmaceutical composition intended for the prevention or treatment of AIDS, which comprises as therapeutic agent a viral vector according to claim 20.

