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(57) Abstract

Composition comprising two pathogenic and/or infectious agents associated with multiple sclerosis, namely, a first agent comprising a human virus with reverse transcriptase activity, and related to a family of endogenous retroviral elements, or a variant thereof, and a second agent, or a variant thereof, wherein the two said pathogenic and/or infectious agents originate from the same viral strain selected from the strains respectively designated as POL-2, registered on 22.07.1992 with ECACC under accession number V92072202, and MS7PG, registered on 08.01.93 with ECACC under accession number V93010816, or from their variant strains.

(57) Abrégé

Composition comprenant deux agents pathogènes et/ou infectants associés à la sclérose en plaques, à savoir, un premier agent qui consiste en un virus humain, possédant une activité transcriptase inverse, et apparenté à une famille d'éléments rétroviraux endogènes, ou un variant dudit virus, et un second agent, ou un variant dudit second agent, ces deux agents pathogènes et/ou infectants étant issus d'une même souche virale choisie parmi les souches dénommées respectivement POL-2, déposée le 22.07.1992 auprès de l'ECACC sous le numéro d'accès V92072202 et MS7PG déposée le 08.01.93 auprès de l'ECACC sous le numéro d'accès V93010816, et parmi leurs souches variantes.

ABSTRACT

MSRV1 VIRUS AND PATHOGENIC AND/OR INFECTIVE AGENT MSRV2 ASSOCIATED WITH MULTIPLE SCLEROSIS AND THEIR NUCLEIC ACID CONSTITUENTS

Composition comprising two pathogenic and/or infective agents associated with multiple sclerosis, namely a first agent which consists of a human virus possessing reverse transcriptase activity and related to a family of endogenous retroviral elements, or a variant of said virus, and a second agent, or a variant of said second agent, these two pathogenic and/or infective agents originating from the same viral strain chosen from the strains designated, respectively, POL-2 deposited with the ECACC on July 22 1992 under Accession Number V92072202 and MS7PG deposited with the ECACC on January 8 1993 under Accession Number V93010816, and from their variant strains.

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) the cause of which remains as yet unknown.

Many studies have supported the hypothesis of a
5 viral etiology of the disease, but none of the known viruses tested has proved to be the causal agent sought: a review of the viruses sought for several years in MS has been compiled by E. Norrby (1) and R.T. Johnson (2).

Concomitantly, the possibility of an exogenous
10 and/or infectious factor is suggested by the existence of localized epidemics or "clusters" of MS, as have been observed in the Faro Islands between 1943 and 1960 (3), in Sardinia (4), in Norway (5), and also by studies on migrant populations (6). Among all the exogenous factors
15 suggested, viruses have been most often studied, and a viral etiology is traditionally called to mind.

The observation in MS of phenomena which can be likened to an autoimmunity reaction has led to an "essential" autoimmune etiological hypothesis (7 and 8). How-
20 ever, this autoimmunity directed against certain components of the CNS has been found not to be specific to MS and common in inflammation of the CNS, whether or not associated with an infection (9, 10, 11 and 12). Furthermore, none of the immunosuppressive therapies has enabled
25 decisive results to be obtained against MS (13). It now seems likely that the "autoimmune" manifestations are induced by a mechanism of viral origin: cosensitization to viral determinants associated with molecules of cellular origin, phenomena of molecular mimicry (14), or
30 by expression of retroviral superantigens (15).

Some studies have supported a hypothesis according to which a retrovirus is at the origin of the disease: the recent discovery (16) of neurological syndromes associated with the HTLV-I virus, originally
35 known as an adult T-cell leukemia agent, has led many authors (17, 18, 19, 20, 21, 22, 23) to look for an involvement of this human retrovirus in MS, however without success or with results suggesting cross-reactions.

Recently, a retrovirus different from the known human retroviruses has been isolated in patients suffering from MS (24, 25 and 26). The authors were also able to show that this retrovirus could be transmitted in vitro, that patients suffering from MS produced antibodies capable of recognizing proteins associated with the infection of leptomeningeal cells by this retrovirus, and that the expression of the latter could be strongly stimulated by the immediate-early genes of some herpesviruses (27).

All these results point to a role in MS of at least one unknown retrovirus or of a virus having reverse transcriptase activity which is detectable according to the method published by H. Perron (24) and qualified as "LM7-like RT" activity. The content of the publication identified by (24) is incorporated in the present description by reference.

Recently, the Applicant's studies have enabled two continuous cell lines infected with natural isolates originating from two different patients suffering from MS to be obtained by a culture method as described in the document WO-A-9320188, the content of which is incorporated in the present description by reference. These two lines, derived from human choroid plexus cells, designated LM7PC and PLI-2, were deposited with the ECACC on July 22 1992 and January 8 1993, respectively, under numbers 92072201 and 93010817, in accordance with the provisions of the Budapest Treaty. Moreover, the viral isolates possessing LM7-like RT activity were also deposited with the ECACC under the overall designation of "strains". The "strain" or isolate harbored by the PLI-2 line, designated POL-2, was deposited on July 22 1992 under No. V92072202. The "strain" or isolate harbored by the LM7PC line, designated MS7PG, was deposited on January 8 1993 under No. V93010816.

Starting from the cultures and isolates mentioned above, characterized by biological and morphological criteria, the next step was to endeavour to characterize the nucleic acid material associated with the viral

particles produced in these cultures.

Thus, the subjects of the invention are the following:

(i) as biological material, the composition comprising two pathogenic and/or infective agents, in the isolated or purified state, associated with multiple sclerosis, namely a first agent which consists of a human virus possessing reverse transcriptase activity and related to a family of endogenous retroviral elements, or a variant of said virus, and a second agent, or a variant of said second agent, these two pathogenic and/or infective agents originating from the same viral strain chosen from the strains designated, respectively, POL-2 deposited with the ECACC on July 22 1992 under Accession Number V92072202 and MS7PG deposited with the ECACC on January 8 1993 under Accession Number V93010816, and from their variant strains,

(ii) as biological material, the composition comprising two pathogenic and/or infective agents, in the isolated or purified state, associated with multiple sclerosis, namely a first agent consisting of a human virus possessing reverse transcriptase activity and related to a family of endogenous retroviral elements, or a variant of said virus, and a second agent, or a variant of said second agent, these two pathogenic and/or infective agents being produced by the same cell line chosen from the lines designated, respectively, PLI-2 deposited with the ECACC on July 22 1992 under Accession Number 92072201 and LM7PC deposited with the ECACC on January 8 1993 under Accession Number 93010817, and by all infected cell cultures capable of producing at least one or other of the pathogenic and/or infective agents, and/or their variants,

(iii) the composition comprising two pathogenic and/or infective agents, in the isolated or purified state, namely a first agent consisting of a virus, or a variant of said virus, whose genome comprises a nucleotide sequence chosen from SEQ ID N01, SEQ ID N02, SEQ ID N03, SEQ ID N04, SEQ ID N05, SEQ ID N06,

SEQ ID N07, SEQ ID N08, SEQ ID N09, their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 50% and preferably at least 70% homology with a nucleotide sequence chosen from SEQ ID N01, SEQ ID N02, SEQ ID N03, SEQ ID N04, SEQ ID N05, SEQ ID N06, SEQ ID N07, SEQ ID N08, SEQ ID N09 and their complementary sequences, and a second pathogenic and/or infective agent whose genome comprises a nucleotide sequence chosen from SEQ ID N010, SEQ ID N011 and SEQ ID N012, their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 70% and preferably at least 90% homology with a nucleotide sequence chosen from SEQ ID N010, SEQ ID N011, and SEQ ID N012, and their complementary sequences,

(iv) a method for detecting a first pathogenic and/or infective agent and/or a second pathogenic and/or infective agent associated with multiple sclerosis, characterized in that at least one nucleic acid fragment is employed, namely a first fragment whose nucleotide sequence comprises a nucleotide sequence chosen from SEQ ID N01, SEQ ID N02, SEQ ID N03, SEQ ID N04, SEQ ID N05, SEQ ID N06, SEQ ID N07, SEQ ID N08, SEQ ID N09, their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 50% and preferably at least 70% homology with a nucleotide sequence chosen from SEQ ID N01, SEQ ID N02, SEQ ID N03, SEQ ID N04, SEQ ID N05, SEQ ID N06, SEQ ID N07, SEQ ID N08, SEQ ID N09 and their complementary sequences, and/or a second fragment whose nucleotide sequence comprises a nucleotide sequence chosen from SEQ ID N010, SEQ ID N011, SEQ ID N012, their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 70% and preferably at least

90% homology with a nucleotide sequence chosen from SEQ ID N010, SEQ ID N011, SEQ ID N012 and their complementary sequences, each of said fragments being, in particular, a probe,

5 (v) a diagnostic, prophylactic or therapeutic composition, characterized in that it comprises at least one nucleic acid fragment, namely a first nucleic acid fragment whose nucleotide sequence comprises a nucleotide sequence chosen from SEQ ID N01, SEQ ID N02, SEQ ID N03, 10 SEQ ID N04, SEQ ID N05, SEQ ID N06, SEQ ID N07, SEQ ID N08, SEQ ID N09, their complementary sequences and the equivalent sequences, in particular a nucleotide sequence displaying, for any succession of 100 contiguous monomers, at least 50% and preferably at least 70% 15 homology with a nucleotide sequence chosen from SEQ ID N01, SEQ ID N02, SEQ ID N03, SEQ ID N04, SEQ ID N05, SEQ ID N06, SEQ ID N07, SEQ ID N08, SEQ ID N09 and their complementary sequences, and/or a second nucleic acid fragment whose nucleotide sequence 20 comprises a nucleotide sequence chosen from SEQ ID N010, SEQ ID N011, SEQ ID N012, their complementary sequences and the equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 70% and preferably at 25 least 90% homology with a nucleotide sequence chosen from SEQ ID N010, SEQ ID N011, SEQ ID N012 and their complementary sequences,

(vi) a method for detecting and/or identifying a combination of pathological and/or infective agents 30 associated with multiple sclerosis, in a biological sample, characterized in that an RNA and/or a DNA presumed to belong to at least one said pathological and/or infective agent, and/or their complementary RNA and/or DNA, is/are brought into contact with a composi- 35 tion comprising a first nucleotide fragment and a second nucleotide fragment, the nucleotide sequence of said first fragment comprising a nucleotide sequence chosen from SEQ ID N01, SEQ ID N02, SEQ ID N03, SEQ ID N04, SEQ ID N05, SEQ ID N06, SEQ ID N07, SEQ ID N08,

SEQ ID NO9, their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 50% and preferably at least 70% homology with a nucleotide sequence chosen from SEQ ID NO1, SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO9 and their complementary sequences, and the nucleotide sequence of said second fragment comprising a nucleotide sequence chosen from SEQ ID NO10, SEQ ID NO11, SEQ ID NO12, their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 70% and preferably at least 90% homology with a nucleotide sequence chosen from SEQ ID NO10, SEQ ID NO11, SEQ ID NO12 and their complementary sequences,

(vii) a method for detecting, in a biological sample, a first pathological and/or infective agent and/or a second pathological and/or infective agent associated with multiple sclerosis, characterized in that a composition comprising a first polypeptide partially or completely encoded by the first nucleotide fragment defined in (vi), and/or a second polypeptide partially or completely encoded by the second nucleotide fragment also defined in (vi), is employed,

(viii) a diagnostic, prophylactic or therapeutic composition, characterized in that it comprises the first polypeptide and/or the second polypeptide which are defined in (vii) above, or in that it comprises a first ligand, in particular antibody, specific for said first polypeptide, and/or a second ligand, in particular antibody, specific for said second polypeptide,

(ix) a cell line designated PLI-2 as deposited with the ECACC on July 22 1992 under Accession Number 92072201, or any derived cell line, or any progeny of this line, insofar as these lines and progeny are capable of producing an antibody obtained from said PLI-2 line, or any other antibody displaying an immunological

cross-reaction with said antibody,

(x) a viral strain designated POL-2 as deposited with the ECACC on July 22 1992 under Accession Number V92072202, or any derived strain, or any progeny of this strain, insofar as these strains and progeny are capable of producing an antigen obtained from said POL-2 strain, or any other antigen displaying an immunological cross-reaction with said antigen,

(xi) a cell line designated LM7PC as deposited with the ECACC on January 8 1993 under Accession Number 93010817, or any derived cell line, or any progeny of this line, insofar as these lines and progeny are capable of producing an antibody obtained from said LM7PC line, or any other antibody displaying an immunological cross-reaction with said antibody,

(xii) a viral strain designated MS7PG as deposited with the ECACC on January 8 1993 under Accession Number V93010816, or any derived strain, or any progeny of this strain, insofar as these strains and progeny are capable of producing an antigen obtained from said MS7PG strain, or any other antigen displaying an immunological cross-reaction with said antigen,

(xiii) as biological material, and in the purified or isolated state, a viral material possessing reverse transcriptase activity, associated with a family of endogenous retroviral elements and associated with multiple sclerosis, originating from a viral strain possessing reverse transcriptase activity, chosen from either of the abovementioned strains POL-2 and MS7PG, and the variant strains consisting of viruses comprising at least one antigen which is recognized by at least one antibody directed against at least one corresponding antigen of one or other of the viruses of said viral strains,

(xiv) as biological material, and in the purified or isolated state, a viral material possessing reverse transcriptase activity, associated with a family of endogenous retroviral elements, associated with multiple

sclerosis, produced by either of the cell lines PLI-2 and LM7PC, or by any infected cell culture capable of producing a virus comprising at least one antigen which is recognized by at least one antibody directed against at least one corresponding antigen of one or other of the viruses produced by said PLI-2 lines [sic],

(xv) a viral material, characterized in that its genome comprises a nucleotide sequence chosen from SEQ ID N01, SEQ ID N02, SEQ ID N03, SEQ ID N04, SEQ ID N05, SEQ ID N06, SEQ ID N07, SEQ ID N08, SEQ ID N09, their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 50% and preferably at least 70% homology with a nucleotide sequence chosen from SEQ ID N01, SEQ ID N02, SEQ ID N03, SEQ ID N04, SEQ ID N05, SEQ ID N06, SEQ ID N07, SEQ ID N08, SEQ ID N09 and their complementary sequences,

(xvi) a retroviral material associated with multiple sclerosis, characterized in that the *pol* gene of its genome comprises an equivalent nucleotide sequence, and in particular one displaying at least 50% homology, preferably at least 65%, with a nucleotide sequence belonging to the *pol* gene of the ERV-9 or HSERV-9 retrovirus genome,

(xvii) a retroviral material associated with multiple sclerosis, characterized in that the *pol* gene of its genome codes for a peptide sequence displaying at least 50% and preferably at least 70% homology with a peptide sequence encoded by the *pol* gene of the ERV-9 or HSERV-9 retrovirus genome,

(xviii) a retroviral material associated with multiple sclerosis, characterized in that the *pol* gene of its genome codes for a peptide sequence displaying, for any contiguous succession of at least 30 amino acids, at least 50% and preferably at least 70% homology with a peptide sequence encoded by a nucleotide sequence chosen from SEQ ID N01, SEQ ID N02, SEQ ID N03, SEQ ID N04, SEQ ID N05, SEQ ID N06, SEQ ID N07, SEQ ID N08,

SEQ ID NO9 and their complementary sequences,

(xix) a nucleotide fragment whose nucleotide sequence comprises a nucleotide sequence chosen from SEQ ID NO1, SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, 5 SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO9, their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 50% and preferably at least 10 70% homology with a sequence chosen from SEQ ID NO1, SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO9 and their complementary sequences,

(xx) a specific primer for the amplification by 15 polymerization of an RNA or DNA of a viral material described above, characterized in that it comprises a nucleotide sequence identical or equivalent to at least part of the nucleotide sequence of a fragment described in (xix), in particular a nucleotide sequence displaying, 20 for any succession of 10 contiguous monomers, at least 70% homology with at least part of said fragment; a preferential primer of the invention comprises a nucleotide sequence chosen from SEQ ID NO16, SEQ ID NO17, SEQ ID NO18, SEQ ID NO19, SEQ ID NO20, SEQ ID NO21, 25 SEQ ID NO22, SEQ ID NO23, SEQ ID NO24, SEQ ID NO25, SEQ ID NO26, SEQ ID NO31, SEQ ID NO32, SEQ ID NO33 and their complementary sequences,

(xxi) a probe capable of specifically hybridizing with an RNA or DNA of a viral material described above, 30 characterized in that it comprises a nucleotide sequence identical or equivalent to at least part of the nucleotide sequence of a fragment described in (xix), in particular a nucleotide sequence displaying, for any succession of 10 contiguous monomers, at least 70% 35 homology with at least part of said fragment; a preferential probe according to the invention comprises a nucleotide sequence chosen from SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO16, SEQ ID NO17, SEQ ID NO18, SEQ ID NO19, SEQ ID NO20,

SEQ ID NO21, SEQ ID NO22, SEQ ID NO23, SEQ ID NO24, SEQ ID NO25, SEQ ID NO26, SEQ ID NO31, SEQ ID NO32, SEQ ID NO33, and their complementary sequences,

(xxii) the use of a probe described in (xxi) or primer described in (xx) for detecting, separating or identifying, in a biological sample, a viral material defined above,

(xxiii) a method for detecting, separating or identifying, in a biological sample, the viral material defined above, characterized in that an RNA and/or a DNA presumed to belong to said virus, and/or their complementary DNA and/or RNA, is/are brought into contact with at least one probe described in (xxi); according to an advantageous embodiment, before the RNA and/or DNA or their complementary DNA and/or RNA is/are brought into contact with the probe, said RNA and/or said DNA is/are hybridized with at least one amplification primer described in (xx), and said RNA and/or DNA is/are amplified,

(xxiv) a method for quantifying, in a biological sample, the expression of a viral material, defined above, associated with multiple sclerosis, characterized in that an RNA and/or a DNA specific to said virus, and/or their complementary DNA and/or RNA, is/are brought into contact with at least one probe described in (xxi), amplification is carried out where appropriate and said RNA and/or DNA is/are detected,

(xxv) as biological material, and in the isolated or purified state, a pathogenic and/or infective agent different from the viral material according to (xiii), or (xiv) or (xv) or (xvi) or (xvii) or (xix), associated with multiple sclerosis, originating from either of the abovementioned viral strains POL-2 and MS7PG, and the variant strains consisting of pathogenic and/or infective agents comprising at least one antigen which is recognized by at least one antibody directed against at least one corresponding antigen of one or other of the pathogenic and/or infective agents of said viral strains, the agents being different, respectively, from either

viral material of said strains,

(xxvi) as biological material, and in the isolated or purified state, a pathogenic and/or infective agent different from the viral material according to (xiii), or (xiv) or (xv) or (xvi) or (xvii) or (xix), associated with multiple sclerosis, produced by either of the abovementioned cell lines PLI-2 and LM7PC, and all infected cell cultures capable of producing a pathogenic and/or infective agent comprising at least one antigen which is recognized by at least one antibody directed against at least one corresponding antigen of one or other of the pathogenic and/or infective agents produced by said PLI-2 and LM7PC lines, the agents being, respectively, different from either viral material of said strains,

(xxvii) a pathogenic and/or infective agent, characterized in that it comprises a nucleic acid comprising a nucleotide sequence chosen from SEQ ID N010, SEQ ID N011, SEQ ID N012, their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying at least 70% and preferably at least 90% homology with a nucleotide sequence comprising a sequence chosen from SEQ ID N010, SEQ ID N011, SEQ ID N012 and their complementary sequences,

(xxviii) a nucleotide fragment, characterized in that it comprises a nucleotide sequence chosen from SEQ ID N010, SEQ ID N011, SEQ ID N012, their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 70% and preferably at least 90% homology with a sequence chosen from SEQ ID N010, SEQ ID N011, SEQ ID N012 and their complementary sequences,

(xxix) a specific primer for the amplification by polymerization of an RNA or DNA of a pathogenic and/or infective agent defined in (xxv), or (xxvi) or (xxvii), characterized in that it comprises a nucleotide sequence identical or equivalent to at least part of the

nucleotide sequence of a fragment described in (xxviii), in particular a nucleotide sequence displaying, for any succession of 10 contiguous monomers, at least 90% homology with at least part of said fragment; a preferen-
5 tial primer according to the invention comprises a nucleotide sequence chosen from SEQ ID N013, SEQ ID N014, SEQ ID N015, SEQ ID N027, SEQ ID N028, SEQ ID N029, SEQ ID N030, SEQ ID N034, SEQ ID N035, SEQ ID N036, SEQ ID N037 and their complementary sequences,

10 (xxx) a probe capable of specifically hybridizing with an RNA or DNA of a pathogenic and/or infective agent defined in (xxv), or (xxvi) or (xxvii), characterized in that it comprises a nucleotide sequence identical or equivalent to at least part of the nucleotide sequence of
15 a fragment described in (xxviii), in particular a nucleotide sequence displaying, for any succession of 10 contiguous monomers, at least 90% homology with at least part of said fragment; a preferential probe according to the invention comprises a nucleotide sequence chosen from
20 SEQ ID N010, SEQ ID N011, SEQ ID N013, SEQ ID N014, SEQ ID N015, SEQ ID N027, SEQ ID N028, SEQ ID N029, SEQ ID N030, SEQ ID N034, SEQ ID N035, SEQ ID N036, SEQ ID N037 and their complementary sequences,

(xxxi) the use of a probe described in (xxx)
25 and/or a primer described in (xxix) for detecting and/or identifying, in a biological sample, a pathological and/or infective agent defined in (xxv), or (xxvi) or (xxvii),

(xxxii) a method for detecting, separating or
30 identifying, in a biological sample, the pathogenic and/or infective agent defined in (xxv), or (xxvi) or (xxvii), characterized in that an RNA and/or a DNA presumed to belong to said agent, and/or their complementary DNA and/or RNA, is/are brought into contact with
35 at least one probe described in (xxx); according to an advantageous embodiment, before the RNA and/or DNA or their complementary DNA and/or RNA is/are brought into contact with the probe, said RNA and/or said DNA is/are hybridized with at least one amplification primer

described in (xxxi), and said RNA and/or DNA is/are amplified,

(xxxiii) a method for quantifying in a biological sample, the expression of an infective and/or pathogenic agent, defined in (xxv), or (xxvi) or (xxvii), associated with multiple sclerosis, characterized in that an RNA and/or a DNA specific to said agent, and/or their complementary DNA and/or RNA, is/are brought into contact with at least one probe described in (xxx), and said RNA and/or DNA is/are amplified,

(xxxiv) a diagnostic, prophylactic or therapeutic composition, in particular for inhibiting the expression of at least one pathogenic and/or infective agent associated with multiple sclerosis, characterized in that it comprises at least one probe described in (xxi) or one probe described in (xxx), and/or at least one primer described in (xx) or one primer described in (xxix),

(xxxv) an RNA or DNA, and in particular replication vector, comprising a fragment described in (xix) or fragment described in (xxviii),

(xxxvi) a polypeptide having at least 5 and preferably 10 amino acids, encoded by any nucleotide sequence of the genome of a virus associated with multiple sclerosis, characterized in that it is encoded by at least part of a nucleotide fragment described in (xix) or a fragment described in (xxviii),

(xxxvii) a diagnostic and/or therapeutic and/or prophylactic composition, characterized in that it comprises at least one polypeptide defined in (xxxvi), or in that it comprises a ligand, in particular antibody, specific for at least one said polypeptide, [sic]

Before describing the invention in detail, different terms used in the description and the claims are now defined:

- strain or isolate is understood to mean any infective and/or pathogenic biological fraction containing, for example, viruses and/or bacteria and/or parasites and generating pathogenic and/or antigenic power, harbored by a culture or a living host; as an example, a

viral strain according to the above definition can contain a coinfective agent, for example a pathogenic protist,

- the term "MSRV" used in the present description denotes any pathogenic and/or infective agent associated with multiple sclerosis, in particular a viral species, the attenuated strains of said viral species or the defective-interfering particles derived from this species. Viruses, and especially viruses containing RNA, are known to have a variability resulting, in particular, from relatively high rates of spontaneous mutation (28), which will be borne in mind below for defining the notion of equivalence,

- human viruses are understood to mean a virus capable of infecting human beings,

- in view of all the natural or induced variations which may be encountered when implementing the present invention, the subjects of the latter, defined above and in the claims, have been expressed including the equivalents or derivatives of the different biological materials defined below, in particular of the homologous nucleotide or peptide sequences,

- the variant of a virus or of a pathogenic and/or infective agent according to the invention comprises at least one antigen recognized by at least one antibody directed against at least one corresponding antigen of said virus and/or said pathogenic and/or infective agent, and/or a genome any part of which is detected by at least one hybridization probe and/or at least one nucleotide amplification primer specific for said virus and/or pathogenic and/or infective agent, such as, for example, the primers having a nucleotide sequence chosen from SEQ ID NO13 through SEQ ID NO38, and their complementary sequences, under particular hybridization conditions well known to a person skilled in the art,

- according to the invention, a nucleotide fragment or an oligonucleotide or polynucleotide is an arrangement of monomers, or a biopolymer, characterized by the informational sequence of the natural nucleic

acids, which are capable of hybridizing with any other nucleotide fragment under predetermined conditions, it being possible for the arrangement to contain monomers of different chemical structures and to be obtained from a
5 molecule of natural nucleic acid and/or by genetic recombination and/or by chemical synthesis,

- thus, a monomer can be a natural nucleotide of nucleic acid whose constituent elements are a sugar, a phosphate group and a nitrogenous base; in RNA the sugar
10 is ribose, in DNA the sugar is 2-deoxyribose; depending on whether the nucleic acid is DNA or RNA, the nitrogenous base is chosen from adenine, guanine, uracil, cytosine and thymine; or the nucleotide can be modified in at least one of the three constituent elements; as an
15 example, the modification can occur in the bases, generating modified bases such as inosine, 5-methyldeoxycytidine, deoxyuridine, 5-(dimethylamino)deoxyuridine, 2,6-diaminopurine, 5-bromodeoxyuridine and any other modified base promoting hybridization; in the sugar, the
20 modification can consist of the replacement of at least one deoxyribose by a polyamide (29), and in the phosphate group, the modification can consist of its replacement by esters chosen, in particular, from diphosphate, alkyl- and arylphosphonate and phosphorothioate esters,
25 - "informational sequence" is understood to mean any ordered succession of monomers whose chemical nature and order in a reference direction constitute or otherwise an item of functional information of the same quality as that of the natural nucleic acids,
30 - hybridization is understood to mean the process during which, under suitable working conditions, two nucleotide fragments having sufficiently complementary sequences pair to form a complex structure, in particular double or triple, preferably in the form of a helix,
35 - a probe comprises a nucleotide fragment synthesized chemically or obtained by digestion or enzymatic cleavage of a longer nucleotide fragment, comprising at least six monomers, advantageously from 10 to 100 monomers and preferably 10 to 30 monomers, and possessing a

specificity of hybridization under particular conditions; preferably, a probe possessing fewer than 10 monomers is not used alone, but used in the presence of other probes of equally short size or otherwise; under certain special
5 conditions, it may be useful to use probes of size greater than 100 monomers; a probe may be used, in particular, for diagnostic purposes, such molecules being, for example, capture and/or detection probes,

- the capture probe may be immobilized on a solid
10 support by any suitable means, that is to say directly or indirectly, for example by covalent bonding or passive adsorption,

- the detection probe may be labeled by means of a label chosen, in particular, from radioactive isotopes,
15 enzymes chosen, in particular, from peroxidase and alkaline phosphatase and those capable of hydrolyzing a chromogenic, fluorogenic or luminescent substrate, chromophoric chemical compounds, chromogenic, fluorogenic or luminescent compounds, nucleotide base analogs and
20 biotin,

- the probes used for diagnostic purposes of the invention may be employed in all known hybridization techniques, and in particular the techniques termed "DOT-BLOT" (30), "SOUTHERN BLOT" (31), "NORTHERN BLOT", which
25 is a technique identical to the "SOUTHERN BLOT" technique but which uses RNA as target, and the SANDWICH technique (32); advantageously, the SANDWICH technique is used in the present invention, comprising a specific capture probe and/or a specific detection probe, on the under-
30 standing that the capture probe and the detection probe must possess an at least partially different nucleotide sequence,

- the invention also covers a probe capable of hybridizing in vivo or in vitro with RNA and/or with DNA
35 in order to block the phenomena of replication, in particular translation and/or transcription, and/or to degrade said DNA and/or RNA,

- a primer is a probe comprising at least six monomers, and advantageously from 10 to 30 monomers,

possessing a specificity of hybridization under particular conditions for the initiation of an enzymatic polymerization, for example in an amplification technique such as PCR (Polymerase Chain Reaction), in an elongation process such as sequencing, in a method of reverse transcription or the like,

- two nucleotide or peptide sequences are termed equivalent or derived with respect to one another, or with respect to a reference sequence, if functionally the corresponding biopolymers can perform substantially the same rôle, without being identical, as regards the application or use in question, or in the technique in which they participate; two sequences are, in particular, equivalent if they are obtained as a result of natural variability, in particular spontaneous mutation of the species from which they have been identified, or induced variability, as are homologous sequences, homology being defined below,

- variability is understood to mean any spontaneous or induced modification of a sequence, in particular by substitution and/or insertion and/or deletion of nucleotides and/or of nucleotide fragments, and/or extension and/or shortening of the sequence at one or both ends; an unnatural variability can result from the genetic engineering techniques used, for example the choice of synthesis primers, degenerate or otherwise, selected for amplifying a nucleic acid; this variability can manifest itself in modifications of any starting sequence, considered as reference, and capable of being expressed by a degree of homology relative to said reference sequence,

- homology characterizes the degree of identity of two nucleotide or peptide fragments compared; it is measured by the percentage identity which is determined, in particular, by direct comparison of nucleotide or peptide sequences, relative to reference nucleotide or peptide sequences,

- this percentage identity has been specifically determined for the nucleotide fragments dealt with in the

present invention which are homologous with the fragments identified by SEQ ID NO1 through NO9 (MSRV-1) on the one hand, and those which are homologous with the fragments identified by SEQ ID NO10 through NO12 (MSRV-2) on the other hand, as well as for the probes and primers homologous with the probes and primers identified by SEQ ID NO16 through NO26 and SEQ ID NO31 through NO33 on the one hand, and with the probes and primers identified by SEQ ID NO13 through NO15, SEQ ID NO27 through SEQ ID NO30 and SEQ ID NO34 through NO37 on the other hand; as an example, the smallest percentage identity observed between the different general consensus sequences of nucleic acids obtained from fragments of MSRV-1 viral RNA, originating from the LM7PC and PLI-2 lines according to a protocol detailed later, is 67% in the region described in Figure 2,

- any nucleotide fragment is termed equivalent [lacuna] or derived from a reference fragment if it possesses a nucleotide sequence equivalent to the reference sequence; according to the above definition, the following in particular are equivalent to a reference nucleotide fragment:

a) any fragment capable of hybridizing at least partially with the complement of the reference fragment

b) any fragment whose alignment with the reference fragment results in the demonstration of a larger number of identical contiguous bases than with any other fragment originating from another taxonomic group

c) any fragment resulting, or capable of resulting, from the natural variability of the species from which it is obtained

d) any fragment capable of resulting from the genetic engineering techniques applied to the reference fragment

e) any fragment containing at least eight contiguous nucleotides encoding a peptide which is homologous with or identical to the peptide encoded by the reference fragment,

f) any fragment which is different from the

reference fragment by insertion, deletion or substitution of at least one monomer, or extension or shortening at one or both of its ends; for example, any fragment corresponding to the reference fragment flanked at one or
5 both of its ends by a nucleotide sequence not coding for a polypeptide,

- polypeptide is understood to mean, in particular, any peptide of at least two amino acids, in particular an oligopeptide or protein, extracted, separated or
10 substantially isolated or synthesized through human intervention, in particular those obtained by chemical synthesis or by expression in a recombinant organism,

- polypeptide partially encoded by a nucleotide fragment is understood to mean a polypeptide possessing
15 at least 3 amino acids encoded by at least 9 contiguous monomers included in said nucleotide fragment,

- an amino acid is termed analogous to another amino acid when their respective physicochemical properties, such as polarity, hydrophobicity and/or basicity
20 and/or acidity and/or neutrality are substantially the same; thus, a leucine is analogous to an isoleucine.

- any polypeptide is termed equivalent [lacuna] or derived from a reference polypeptide if the polypeptides compared have substantially the same prop-
25 erties, and in particular the same antigenic, immunological, enzymological and/or molecular recognition properties; the following in particular are equivalent to a reference polypeptide:

a) any polypeptide possessing a sequence in which
30 at least one amino acid has been replaced by an analogous amino acid,

b) any polypeptide having an equivalent peptide sequence, obtained by natural or induced variation of said reference polypeptide and/or of the nucleotide
35 fragment coding for said polypeptide,

c) a mimotope of said reference polypeptide,

d) any polypeptide in whose sequence one or more amino acids of the L series are replaced by an amino acid of the D series, and vice versa,

e) any polypeptide into whose sequence a modification of the side chains of the amino acids has been introduced, such as, for example, an acetylation of the amine functions, a carboxylation of the thiol functions, an esterification of the carboxyl functions,

5 f) any polypeptide in whose sequence one or more peptide bonds have been modified, such as, for example, carba, retro, inverso, retro-inverso, reduced and methylenoxy bonds,

g) any polypeptide at least one antigen of which is recognized by an antigen of the reference polypeptide,

10 - the percentage identity characterizing the homology of two peptide fragments compared is, according to the present invention, at least 50% and preferably at least 70%.

In view of the fact that a virus possessing reverse transcriptase enzymatic activity may be genetically characterized equally well in RNA and in DNA form, 15 both the viral DNA and RNA will be referred to for characterizing the sequences relating to a virus possessing such reverse transcriptase activity (MSRV-1).

In view of the fact that the pathogenic and/or infective agent (MSRV)-2 [sic] has been detected both in DNA and in RNA in infected cells, it may also be characterized in DNA or RNA form.

20 The expressions of order used in the present description and the claims, such as "first nucleotide sequence", are not adopted so as to express a particular order, but so a to define the invention more clearly.

In order to further clarify the invention described, the following summarise the various aspects of the invention:

25 - method for detecting a first pathogenic and/or infective agent and/or a second pathogenic and/or infective agent associated with multiple sclerosis,

characterized in that at least one nucleic acid fragment is employed, namely a first garment whose nucleotide sequence comprises a nucleotide sequence chosen from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, their complimentary
5 sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 50% and preferably at least 70% homology with a nucleotide sequence chosen from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9 and their complimentary
10 sequences and/or a second fragment whose nucleotide sequence comprises a nucleotide sequence chosen from SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, their complimentary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 70% and preferably at least 90% homology with a nucleotide
15 sequence chosen from SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and their complementary sequences, each of said fragments being, in particular, a probe, said second fragment being different from the following sequence CGCTGAAAGCC TATCGCGTGC AGTTGCCGGA TGCCGCCTAT AGCCTC, and fragments thereof;

20 - diagnostic, prophylactic or therapeutic composition, characterized in that it comprises at least one nucleic acid fragment, namely a first nucleic acid fragment whose nucleotide sequence comprises a nucleotide sequence chose from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, their complementary sequences
25 and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 50% and preferably at least 70% homology with a nucleotide sequence chosen from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9 and their complementary sequences, said
30 first garment being different from ERV-9 or HSERV-9 sequences, and/or a second nucleic acid fragment whose nucleotide sequence comprises a nucleotide sequence chosen from SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, their

complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 70% and preferably at least 90% homology with a nucleotide sequence chosen from SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and their
5 complementary sequences, said second fragment being different from the following sequence CGCTGAAGCC TATCGCGTGC AGTTGCCGGA TGCCGCCTAT AGCCTC, and fragments thereof;

- method for detecting and/or identifying a combinations of pathological and/or infective agents associated with multiple sclerosis, in a biological sample,
10 characterized in that an RNA and/or a DNA presumed to belong to at least one said pathological and/or infective agent, and/or their complementary RNA and/or DNA, is/are brought into contact with a composition comprising a first nucleotide fragment and a second nucleotide fragment, the nucleotide sequence of said first fragment comprising a nucleotide sequence chosen from SEQ ID NO: 1, SEQ ID
15 NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 50% and preferably at least 70% homology with a nucleotide sequence chosen from SEQ ID NO: 1, SEQ ID
20 NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9 and their complementary sequences, and the nucleotide sequence of said second fragment comprising a nucleotide sequence chosen from SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, their complementary sequences and their equivalent sequences, in particular the
25 nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 70% and preferably at least 90% homology with a nucleotide sequence chosen from SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and their complementary sequences;

- viral material, characterized in that its genome comprises a nucleotide
30 sequence chosen from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9,

their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 50% and preferably at least 70% homology with a nucleotide sequence chosen from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO:
5 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9 and their complementary sequences, said viral material being different from ERV-9 or HSERV-9;

- retroviral material associated with multiple sclerosis, characterized in that the *pol* gene of its genome comprises and equivalent nucleotide sequence, and in
10 particular one displaying at least 50% homology, preferably at least 65% homology, with a nucleotide sequence belonging to the *pol* gene of the ERV-9 or HSERV-9 retrovirus genome;

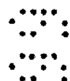
- retroviral material associated with multiple sclerosis, characterized in that the *pol* genome of its genome codes for a peptide sequence displaying at least
15 50% and preferably at least 70% homology with a peptide sequence encoded by the *pol* gene of the ERV-9 or HSERV-9 retrovirus genome;

- retroviral material associated with multiple sclerosis, characterized in that the *pol* gene of its genome codes for a peptide sequence displaying, for any contiguous succession of at least 30 amino acids, at least 50% and preferably at
20 least 70% homology with a peptide sequence encoded by a nucleotide sequence chosen from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9 and their complementary sequences;

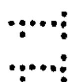
- nucleotide fragment whose nucleotide sequence comprises a nucleotide
25 sequence chosen from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 50% and preferably at least 70% homology with a sequence
30 chosen from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ

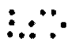
ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9 and their complementary sequences, said fragment being different from ERV-9 or HSERV-9 sequences;

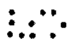
- pathogenic and/or infective agent, characterized in that it comprises a nucleic acid comprising a nucleotide sequence chosen from SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying at least 70% and preferably at least 90% homology with a nucleotide sequence comprising a sequence chosen from SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and their complementary sequences, said second fragment being different from the following sequence CGCTGAAGCC TATCGCGTGC AGTTGCCGGA TGCCGCCTAT AGCCTC and from fragments thereof;



- nucleotide fragment, characterized in that it comprises a nucleotide sequence chose from SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 70% and preferably at least 90% homology with a sequence chosen from SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and their complementary sequences, said second fragment being different from the following sequence CGCTGAAGCC TATCGCGTGC AGTTGCCGGA TGCCGCCTAT AGCCTC and from fragments thereof;







A better understanding of the invention will be gained on reading the detailed description which follows, prepared with reference to the attached figures, in which:

- Figure 1 shows the MSRV-2A type sequence obtained from LM7 cultures according to the protocol of Shih (33); this sequence is identified under the reference SEQ ID N010,

- Figure 2 shows general consensus sequences of nucleic acids of the MSRV-1B sequences amplified by the

PCR technique in the "pol" region, from viral DNA originating from the LM7PC and PLI-2 lines, identified under the references SEQ ID N03, SEQ ID N04, SEQ ID N05 and SEQ ID N06, and the common consensus with amplification
5 primers bearing the reference SEQ ID N07,

- Figure 3 shows the phylogenetic tree of the MSRV-1B type sequences obtained by PCR in the "pol" region defined by Shih (33),

- Figure 4 gives the definition of a functional
10 reading frame for each MSRV-1B/"PCR pol" type family, said families A through D being defined, respectively, by the nucleotide sequences SEQ ID N03, SEQ ID N04, SEQ ID N05 and SEQ ID N06 described in Figure 2,

- Figure 5 gives an example of consensus of the
15 MSRV-2B sequences, identified by SEQ ID N011,

- Figure 6 is a representation of the reverse transcriptase (RT) activity in dpm (disintegrations per minute) in the sucrose fractions taken from a purification gradient of the virions produced by the B
20 lymphocytes in culture from a patient suffering from MS,

- Figure 7 gives, under the same conditions as in Figure 6, the assay of the reverse transcriptase activity in the culture of a B lymphocyte line obtained from a control free from multiple sclerosis,

25 - Figure 8 shows the nucleotide sequence of the clone PSJ17 (SEQ ID N09)

- Figure 9 shows the nucleotide sequence SEQ ID N08 of the clone designated M003-P004,

- Figure 10 shows the nucleotide sequence
30 SEQ ID N02 of the clone F11-1; the portion located between two arrows in the region of the primer corresponds to a variability imposed by the choice of primer which was used for the cloning of F11-1; in this same figure, the translation into amino acids is shown,

35 - Figure 11 shows the nucleotide sequence SEQ ID N01, and a possible functional reading frame of SEQ ID N01 in terms of amino acids; on this sequence, the consensus sequences of the retroviral reverse transcriptases are underlined,

- Figure 12 shows the nucleotide sequence SEQ ID N012 of the clone designated MSRV2EL1,

- Figure 13, separated into three successive plates 13/18 to 15/18, shows the translation into amino acids of SEQ ID N012, including the primer SEQ ID N013, according to 6 possible reading frames,

- Figure 14 presents an alignment of the MSRV2-A sequence (SEQ ID N010) with the MSRV2-EL1 sequence (SEQ ID N012); in this same diagram, the hybridization region of the primer identified under the reference SEQ ID N013 (apart from the cloning tail) is boxed; that of the primer identified under the reference SEQ ID N014 is indicated between square brackets,

- Figure 15 gives the results of a PCR, in the form of a photograph under ultraviolet light of an ethidium bromide-impregnated agarose gel, of the amplification products obtained from the primers identified by SEQ ID N014 and SEQ ID N015,

- Figures 16 and 17 give the results of a PCR, in the form of a photograph under ultraviolet light of an ethidium bromide-impregnated agarose gel, of the amplification products obtained from the primers identified by SEQ ID N016, SEQ ID N017, SEQ ID N018 and SEQ ID N019.

- Figure 18 gives a representation in matrix form of the homology between SEQ ID N01 of MSRV-1 and that of an endogenous retrovirus designated HSERV9; this homology of at least 65% is demonstrated by a continuous line, the absence of a line meaning a homology of less than 65%.

EXAMPLE 1: OBTAINING MSRV-2 CLONES DESIGNATED MSRV-2A, BY AMPLIFICATION OF THE CONSERVED REGIONS OF THE GENES FOR RNA-DEPENDENT DNA POLYMERASES ON A PREPARATION OF INFECTIVE AGENT PURIFIED FROM LM7 LINE CELL CULTURE

The molecular approach consisted in using a PCR technique (33) which makes it possible to amplify a relatively conserved region of the *pol* gene of exogenous and endogenous retroviruses, but also of viruses coding for an enzyme having reverse transcriptase (RT) activity, such as, in particular, the hepatitis B virus, and,

implicitly, of any gene for RNA-dependent DNA polymerase or for an enzyme, displaying sufficient sequence homologies in the regions defined by the amplification primers used. This PCR technique was used on the nucleic acids extracted from a purified preparation of infective agent, obtained according to the protocol (34) from supernatants of the original LM7 culture (24) which were kept frozen at -80°C since that time. The fractions containing the peak of LM7-like RT activity are taken up in one volume of a buffer containing guanidine thiocyanate (35), and are stored at -80°C until the nucleic acids are extracted according to the technique described by P.Chomzynski (35).

Prior to the PCR reaction, the RNA of the sample was transcribed into complementary DNA (cDNA) with so-called "random" primers (mixed hexanucleotides) using the "cDNA synthesis system plus" kit (Amersham) according to the manufacturer's instructions, and on the basis of an approximate value, to the nearest log factor, of the amount of RNA present in the sample.

The DNA obtained after PCR amplification of the cDNA was inserted into a plasmid using the TA Cloningr [sic] kit (British Biotechnology). The 2 µl of DNA solution were mixed with 5 µl of sterile distilled water, 1 µl of a 10-fold concentrated ligation buffer "10X LIGATION BUFFER", 2 µl of "pCR™ VECTOR" (25 ng/ml) and 1 µl of "TA DNA LIGASE". This mixture was incubated overnight at 12°C. The following steps were carried out in accordance with the instructions of the TA Cloningr [sic] kit. At the end of the procedure, the white colonies of recombinant bacteria were picked out in order to be cultured and to permit extraction of the plasmids incorporated according to the so-called "miniprep" procedure (36). The plasmid preparation from each recombinant colony was cut with a suitable restriction enzyme and analyzed on agarose gel. Plasmids possessing an insert detected under UV light after staining the gel with ethidium bromide were selected for sequencing of the insert, after hybridization with a primer complementary

to the Sp6 promoter present on the cloning plasmid of the "TA cloning kit". The reaction prior to sequencing was then performed according to the method recommended for the use of the sequencing kit "Prism ready reaction kit dye deoxyterminator cycle sequencing kit" (Applied Biosystems, ref. 401384), and automatic sequencing was carried out with an Applied Biosystems model 373 A "Automatic Sequencer" apparatus according to the manufacturer's instructions.

The sequences obtained were then analyzed using the Mac Vectorr [sic] and Geneworksr [sic] software on Genebankr [sic] computerized data bank for the nucleic acid sequences, and Swiss Protr [sic] for the amino acid sequences deduced from the reading frames revealed in the nucleic acid sequences. Analysis of the sequences obtained from the viral sample originating from the thawed LM7 supernatants, and which was purified at the peak of reverse transcriptase activity on a sucrose gradient, revealed a majority population of clones (approximately 42% of the clones), relative to the extent of individual representation of the other sequences (always less than 5%, or 10% in a small number of cases), displaying partial homologies with known retroviruses in the expected "pol" region. This clone is designated MSRV2-A and identified by SEQ ID N010 (see Fig. 1). The region amplified between the PCR primers is homologous with the corresponding sequence MSRV2-B identified by SEQ ID N011 (see Fig. 5), described in Example 2. The differences observed in the sequences located at the PCR primers is explained by the use of degenerate primers in mixture form, used under different technical conditions. Interrogation of the Genebankr [sic] data bank, fully updated, did not enable an identical sequence or one displaying significant homologies to be revealed.

This sequence is presented in Figure 1. It possesses an open reading frame in frame with the two PCR primers to be found at the ends, but it is shorter than the set of known retroviral sequences in the expected region between these primers. A deletion of 45 base pairs

(15 amino acids) is observed therein following the sequence of the upstream primer, whereas the sequences preceding the downstream primer are present. However, the reading frame is open and uninterrupted over the whole of the sequence including the primers, and the deduced amino acid sequence displays a significant homology with the corresponding region of the known retroviruses. In the sequence lying inside the PCR primers, the amino acids Glu, Arg, Gln, Pro and Asp, normally fairly well conserved in this *pol* region of retroviruses and of known viruses with reverse transcriptase activity (33), are to be found conserved at the correct positions in the reading frame of the novel sequence.

Lastly, in view of the fact that this sequence is sufficiently divergent from the retroviral sequences already described in the data banks, it may be suggested that the sequence in question belongs to a new infective and/or pathogenic agent, designated MSRV-2A. This agent is, in principle, on the basis of the analysis of the sequences obtained, related to a retrovirus but, in view of the technique used for obtaining this sequence, it may also be an RNA virus whose genome codes for an enzyme which incidentally possesses reverse transcriptase activity, as is the case, for example, with the hepatitis B virus, HBV (33). Furthermore, the random nature of the degenerate primers used for this PCR amplification technique may very well have permitted, as a result of unforeseen sequence homologies or of conserved sites in the gene for a related enzyme, the amplification of a nucleic acid originating from a prokaryotic or eukaryotic pathogenic and/or coinfective agent (protist).

EXAMPLE 2: OBTAINING CLONES DESIGNATED MSRV-1B AND MSRV-2B, DEFINING, RESPECTIVELY, A RETROVIRUS MSRV-1 AND A COINFECTIVE AGENT MSRV2, BY "NESTED" PCR AMPLIFICATION OF THE CONSERVED POL REGIONS OF RETROVIRUSES ON VIRION PREPARATIONS ORIGINATING FROM THE LM7PC AND PLI-2 LINES

A PCR technique derived from the technique published by Shih (33) was used. This technique enables

all trace of contaminant DNA to be removed by treating all the components of the reaction medium with DNase. It concomitantly makes it possible, by the use of different but overlapping primers in two successive series of PCR amplification cycles, to increase the chances of amplifying a cDNA synthesized from an amount of RNA which is small at the outset and further reduced in the sample by the spurious action of the DNAse on the RNA. In effect, the DNase is used under conditions of activity in excess which enable all trace of contaminant DNA to be removed before inactivation of this enzyme remaining in the sample by heating to 85°C for 10 minutes. This variant of the PCR technique described by Shih (33) was used on a cDNa synthesized from the nucleic acids of fractions of infective particles purified on a sucrose gradient according to the technique described by H. Perron (34) from the "POL-2" isolate (ECACC No. V92072202) produced by the PLI-2 line (ECACC No. 92072201) on the one hand, and from the MS7PG isolate (ECACC No. V93010816) produced by the LM7PC line (ECACC No. 93010817) on the other hand. These cultures were obtained according to the methods which formed the subject of the patent applications published under Nos WO 93/20188 and WO 93/20189.

After cloning the products amplified by this technique with the TA Cloning Kitr [sic] and analysis of the sequence using the automatic sequencer as has been described in Example 1, the sequences were analyzed using the Geneworksr [sic] software on the latest available version of the Genebankr [sic] data bank.

The sequences cloned and sequenced from these samples correspond, in particular, to two types of sequence: a first type of sequence, to be found in the majority of the clones (55% of the clones originating from the POL-2 isolates of the PLI-2 culture, and 67% of the clones originating from the MS7PG isolates of the LM7PC cultures), which corresponds to a family of "pol" sequences closely similar to, but different from, the endogenous human retrovirus designated ERV-9 or HSERV-9, and a second type of sequence which corresponds to

sequences very strongly homologous with the sequence attributed to an infective and/or pathogenic agent previously designated MSRV-2.

The first type of sequence, representing the majority of the clones, consists of sequences whose variability enables four subfamilies of sequences to be defined. These subfamilies are sufficiently similar to one another for it to be possible to consider them to be quasi-species originating from the same retrovirus, as is well known for the HIV-1 retrovirus (37), or to be the outcome of interference with several endogenous proviruses coregulated in the producing cells. These more or less defective endogenous elements are sensitive to the same regulatory signals possibly generated by a replicative provirus, since they belong to the same family of endogenous retroviruses (38). This new family of endogenous retroviruses, or alternatively this new retroviral species from which the generation of quasi-species has been obtained in culture, and which contains a consensus of the sequences described below, is designated MSRV-1B.

Figure 2 presents the general consensus sequences of the sequences of the different MSRV-1B clones sequenced in this experiment, these sequences being identified, respectively, by SEQ ID NO3, SEQ ID NO4, SEQ ID NO5 and SEQ ID NO6. These sequences display a homology with respect to nucleic acids ranging from 70% to 88% with the HSERV9 sequence referenced X57147 and M37638 in the Genebankr [sic] data base. The phylogenetic tree of these sequences is presented in Figure 3. In this figure, the subfamilies A, B, C and D represent the sequences which have turned up preponderantly in similar experiments repeated subsequently, in the samples of pure RNA of virions purified from the MS7PG and POL-2 isolates. From these families of sequences, four "consensus" nucleic acid sequences representative of different quasi-species of a possibly exogenous retrovirus MSRV-1B, or of different subfamilies of an endogenous retrovirus MSRV-1B, have been defined. These representative

consensus sequences are presented in Figure 4, with the translation into amino acids. A functional reading frame exists for each subfamily of these MSRV-1B sequences, and it can be seen that the functional open reading frame corresponds in each instance to the amino acid sequence appearing on the second line under the nucleic acid sequence. The general consensus of the MSRV-1B sequence, identified by SEQ ID NO7 and obtained by this PCR technique in the "pol" region, is presented in Figure 2.

The second type of sequence representing the majority of the clones sequenced is represented by the sequence MSRV-2B presented in Figure 5 and identified by SEQ ID NO11. The region amplified between the PCR primers is homologous, apart from a single base, with the MSRV2-A sequence (SEQ ID NO10 according to Fig. 1) lying inside the PCR primers, described in Example 1. The differences observed in the sequences corresponding to the PCR primers are explained by the use of degenerate primers in mixture form used under different technical conditions.

The sequences MSRV-2A (SEQ ID NO10) and MSRV-2B (SEQ ID NO11) are manifestly homologous, or even identical, derived from the same organism and sufficiently divergent from the retroviral sequences already described in the data banks for it to be suggested that the sequence region in question belongs to a new infective agent, designated MSRV-2. This infective agent would be, in principle, on the basis of the analysis of the first sequences obtained, related to a retrovirus but, in view of the technique used for obtaining this sequence, it could also be a DNA virus whose genome codes for an enzyme which incidentally possesses reverse transcriptase activity, as is the case, for example, with the hepatitis B virus, HBV (33). Furthermore, the random nature of the degenerate primers used for this PCR amplification technique may very well have permitted, as a result of unforeseen sequence homologies or of conserved sites in the gene for a related enzyme, the amplification of a nucleic acid originating from a prokaryotic or eukaryotic pathogenic and/or coinfective agent (protist).

EXAMPLE 3: OBTAINING CLONES DESIGNATED MSRV-1B AND MSRV-2B, DEFINING A FAMILY MSRV-1 and MSRV2, BY "NESTED" PCR AMPLIFICATION OF THE CONSERVED POL REGIONS OF RETROVIRUSES ON PREPARATIONS OF B LYMPHOCYTES FROM A NEW CASE OF MS

The same PCR technique, modified according to the technique of Shih (33), was used to amplify and sequence the RNA nucleic acid material present in a purified fraction of virions at the peak of "LM7-like" reverse transcriptase activity on a sucrose gradient according to the technique described by H. Perron (34), and according to the protocols mentioned in Example 2, from a spontaneous lymphoblastoid line obtained by self-immortalization in culture of B lymphocytes from an MS patient who was seropositive for the Epstein-Barr virus (EBV), after setting up the blood lymphoid cells in culture in a suitable culture medium containing a suitable concentration of cyclosporin A. A representation of the reverse transcriptase activity in the sucrose fractions taken from a purification gradient of the virions produced by this line is presented in Figure 6. Similarly, the culture supernatants of a B line obtained under the same conditions from a control free from multiple sclerosis were treated under the same conditions, and the assay of reverse transcriptase activity in the sucrose gradient fractions proved negative throughout (background), and is presented in Figure 7. Fraction 3 of the gradient corresponding to the MS B line and the same fraction without reverse transcriptase activity of the non-MS control gradient were analyzed by the same RT-PCR technique as before, derived from Shih (33), followed by the same steps of cloning and sequencing as described in Examples 1 and 2.

It is particularly noteworthy that the MSRV-1 and MSRV-2 type sequences are to be found only in the material associated with a peak of "LM7-like" reverse transcriptase activity originating from the MS B lymphoblastoid line. These sequences were not to be found with the material from the control (non-MS) B lymphoblastoid

line in 26 recombinant clones taken at random. Only Mo-MuLV type contaminant sequences, originating from the commercial reverse transcriptase used for the cDNA synthesis step, and sequences without any particular retroviral analogy were to be found in this control, as a result of the "consensus" amplification of homologous polymerase sequences which is produced by this PCR technique. Furthermore, the absence of a concentrated target which competes for the amplification reaction in the control sample permits the amplification of dilute contaminants. The difference in results is manifestly highly significant (chi-squared, $p<0.001$).

EXAMPLE 4: OBTAINING A CLONE PSJ17, DEFINING A RETROVIRUS MSRV-1, BY REACTION OF ENDOGENOUS REVERSE TRANSCRIPTASE WITH A VIRION PREPARATION ORIGINATING FROM THE PLI-2 LINE.

This approach is directed towards obtaining reverse-transcribed DNA sequences from the supposedly retroviral RNA in the isolate using the reverse transcriptase activity present in this same isolate. This reverse transcriptase activity can theoretically function only in the presence of a retroviral RNA linked to a primer tRNA or hybridized with short strands of DNA already reverse-transcribed in the retroviral particles (39). Thus, the obtaining of specific retroviral sequences in a material contaminated with cellular nucleic acids was optimized according to these authors by means of the specific enzymatic amplification of the portions of viral RNAs with a viral reverse transcriptase activity. To this end, the authors determined the particular physicochemical conditions under which this enzymatic activity of reverse transcription on RNAs contained in virions could be effective in vitro. These conditions correspond to the technical description of the protocols presented below (endogenous RT reaction, purification, cloning and sequencing).

The molecular approach consisted in using a preparation of concentrated but unpurified virion obtained from the culture supernatants of the PLI-2 line,

prepared according to the following method: the culture supernatants are collected twice weekly, precentrifuged at 10,000 rpm for 30 minutes to remove cell debris and then frozen at -80°C or used as they are for the following steps. The fresh or thawed supernatants are centrifuged on a cushion of 30% glycerol-PBS at 100,000 g (or 30,000 rpm in a type 45 T LKB-HITACHI rotor) for 2 h at 4°C. After removal of the supernatant, the sedimented pellet is taken up in a small volume of PBS and constitutes the fraction of concentrated but unpurified virion. This concentrated but unpurified viral sample was used to perform a so-called endogenous reverse transcription reaction as will now be described: a volume of 200 μl of virion purified according to the protocol described above, and containing a reverse transcriptase activity of approximately 1-5 million dpm, is thawed at 37°C until a liquid phase appears, and then placed on ice. A 5-fold concentrated buffer was prepared with the following components: 500 mM Tris-HCl pH 8.2; 75 mM NaCl; 25 mM $MgCl_2$; 75 mM DTT and 0.10% NP 40. 100 μl of 5X buffer + 25 μl of a 100 mM solution of dATP + 25 μl of a 100 mM solution of dTTP + 25 μl of a 100 mM solution of dGTP + 25 μl of a 100 mM solution of dCTP + 100 μl of sterile distilled water + 200 μl of the virion suspension (RT activity of 5 million DPM) in PBS were mixed and incubated at 42°C for 3 hours. After this incubation, the reaction mixture is added directly to a buffered phenol/chloroform/isoamyl alcohol mixture (Sigma ref. P 3803); the aqueous phase is collected and one volume of sterile distilled water is added to the organic phase to re-extract the residual nucleic acid material. The collected aqueous phases are combined, and the nucleic acids contained are precipitated by adding 3M sodium acetate pH 5.2 to 1/10 volume + 2 volumes of ethanol + 1 μl of glycogen (Boehringer-Mannheim ref. 901 393) and placing the sample at -20°C for 4 h or overnight at +4°C. The precipitate obtained after centrifugation is then washed with 70% ethanol and resuspended in 60 ml of distilled water. The products of this reaction were then purified,

cloned and sequenced according to the protocol which will now be described: blunt-ended DNAs with unpaired adenines at the ends were generated: a "filling-in" reaction was first performed: 25 μl of the previously purified DNA solution were mixed with 2 μl of a 2.5 mM solution containing, in equimolar amounts, dATP + dGTP + dTTP + dCTP/1 μl of T4 DNA polymerase (Boehringer-Mannheim ref. 1004 786) / 5 μl of 10X "incubation buffer for restriction enzyme" (Boehringer-Mannheim ref. 1417 975) / 1 μl of a 1% bovine serum albumin solution / 16 μl of sterile distilled water. This mixture was incubated for 20 minutes at 11°C. 50 μl of TE buffer and 1 μl of glycogen (Boehringer-Mannheim ref. 901 393) were added thereto before extraction of the nucleic acids with phenol/chloroform/isoamyl alcohol (Sigma ref. P 3803) and precipitation with sodium acetate as described above. The DNA precipitated after centrifugation is resuspended in 10 μl of 10 mM Tris buffer pH 7.5. 5 μl of this suspension were then mixed with 20 μl of 5X Taq buffer, 20 μl of 5 mM dATP, 1 μl (5U) of Taq DNA polymerase (Amplitaq™) and 54 μl of sterile distilled water. This mixture is incubated for 2 h at 75°C with a film of oil on the surface of the solution. The DNA suspended in the aqueous solution drawn off under the film of oil after incubation is precipitated as described above and resuspended in 2 μl of sterile distilled water. The DNA obtained was inserted into a plasmid using the TA cloning kit™. The 2 μl of DNA solution were mixed with 5 μl of sterile distilled water, 1 μl of a 10-fold concentrated ligation buffer "10X LIGATION BUFFER", 2 μl of "pCR™ VECTOR" (25 ng/ml) and 1 μl of "TA DNA LIGASE". This mixture was incubated overnight at 12°C. The following steps were carried out according to the instructions of the TA Cloningr [sic] kit (British Biotechnology). At the end of the procedure, the white colonies of recombinant (white) bacteria were picked out in order to be cultured and to permit extraction of the plasmids incorporated according to the so-called "miniprep" procedure (36). The plasmid preparation from each recombinant colony was cut with a

suitable restriction enzyme and analyzed on agarose gel. Plasmids possessing an insert detected under UV light after staining the gel with ethidium bromide were selected for sequencing of the insert, after hybrid-
5 ization with a primer complementary to the Sp6 promoter present on the cloning plasmid of the TA cloning kitr [sic]. The reaction prior to sequencing was then performed according to the method recommended for the use of the sequencing kit "Prism ready reaction kit dye
10 deoxyterminator cycle sequencing kit" (Applied Biosystems, ref. 401384), and automatic sequencing was carried out with an Applied Biosystems "model 373 A Automatic Sequencer" apparatus according to the manufacturer's instructions.

15 Discriminating analysis on the computerized data banks of the sequences cloned from the DNA fragments present in the reaction mixture enabled a retroviral type sequence to be revealed. The corresponding clone PSJ17 was completely sequenced, and the sequence obtained,
20 presented in Figure 8 and identified by SEQ ID N09, was analyzed using the "Geneworksr" [sic] software on the updated "Genebankr" [sic] data banks. An identical sequence already described could not be found by analysis of the data banks. Only a partial homology with some
25 known retroviral elements was to be found. The most useful relative homology relates to an endogenous retrovirus designated ERV-9, or HSERV-9, depending on the references (40).

EXAMPLE 5: PCR AMPLIFICATION OF THE NUCLEIC ACID
30 SEQUENCE CONTAINED BETWEEN THE 5' REGION DEFINED BY THE CLONE "POL MSRV-1B" AND THE 3' REGION DEFINED BY THE CLONE PSJ17.

Five oligonucleotides, M001, M002-A, M003-BCD, P004 and P005, were defined in order to amplify the RNA
35 originating from purified POL-2 virions. Control reactions were performed so as to check for the presence of contaminants (reaction with water). The amplification consists of an RT-PCR step according to the protocol described in Example 2, followed by a "nested" PCR

according to the PCR protocol described in the document EP-A-0569272. In the first RT-PCR cycle, the primers M001 and P004 or P005 are used. In the second PCR cycle, the primers M002-A or M003-BCD and the primer P004 [sic] are used. The primers are positioned as follows:

```
              M002-A
              M003-BCD
   M001       ___  P004P005

   ___ ___    ___   ___
_______________________________________________ARN

POL-2
<---------->                    <--------------------->
 pol MSRV-1  PSJ17
```

Their composition is:

primer M001: GGTCITICCICAIGG (SEQ ID N020)
primer M002-A: TTAGGGATAGCCCTCATCTCT (SEQ ID N021)
primer M003-BCD: TCAGGGATAGCCCCCATCTAT (SEQ ID N022)
primer P004: AACCCTTTGCCACTACATCAATTT (SEQ ID N023)
primer P005: GCGTAAGGACTCCTAGAGCTATT (SEQ ID N024)

The "nested" amplification product obtained, and designated M003-P004, is presented in Figure 9, and corresponds to the sequence SEQ ID N08.

EXAMPLE 6: AMPLIFICATION AND CLONING OF A PORTION OF THE MSRV-1 RETROVIRAL GENOME USING A SEQUENCE ALREADY IDENTIFIED, IN A SAMPLE OF VIRUS PURIFIED AT THE PEAK OF REVERSE TRANSCRIPTASE ACTIVITY

A PCR technique derived from the technique published by Frohman **(41)** was used. The technique derived makes it possible, using a specific primer at the 3' end of the genome to be amplified, to elongate the sequence towards the 5' region of the genome to be analyzed. This technical variant is described in the documentation of the firm "Clontech Laboratories Inc., (Palo-Alto California, USA) supplied with its product "5'-AmpliFINDER™ RACE Kit", which was used on a virion fraction purified as described above.

The specific 3' primers used in the kit protocol for the synthesis of the cDNA and the PCR amplification are, respectively, complementary to the following MSRV-1 sequences:

5 cDNA:TCATCCATGTACCGAAGG (SEQ ID N025)

amplification :ATGGGGTTCCCAAGTTCCCT (SEQ ID N026)

The products originating from the PCR were purified after purification on agarose gel according to 10 conventional methods (36), and then resuspended in 10 ml of distilled water. Since one of the properties of Taq polymerase consists in adding an adenine at the 3' end of each of the two DNA strands, the DNA obtained was inserted directly into a plasmid using the TA Cloning 15 kit™ (British Biotechnology). The 2 µl of DNA solution were mixed with 5 µl of sterile distilled water, 1 µl of a 10-fold concentrated ligation buffer "10X LIGATION BUFFER", 2 µl of "pCR™ VECTOR" (25 ng/ml) and 1 µl of "TA DNA LIGASE". This mixture was incubated overnight at 20 12°C. The following steps were carried out according to the instructions of the TA Cloningr [sic] kit (British Biotechnology). At the end of the procedure, the white colonies of recombinant (white) bacteria were picked out in order to be cultured and to permit extraction of the 25 plasmids incorporated according to the so-called "mini-prep" procedure (36). The plasmid preparation from each recombinant colony was cut with a suitable restriction enzyme and analyzed on agarose gel. Plasmids possessing an insert detected under UV light after staining the gel 30 with ethidium bromide were selected for sequencing of the insert, after hybridization with a primer complementary to the Sp6 promoter present on the cloning plasmid of the TA Cloning Kitr [sic]. The reaction prior to sequencing was then performed according to the method recommended 35 for the use of the sequencing kit "Prism ready reaction kit dye deoxyterminator cycle sequencing kit" (Applied Biosystems, ref. 401384), and automatic sequencing was carried out with an Applied Biosystems "model 373 A automatic sequencer" apparatus according to the

manufacturer's instructions.

This technique was applied first to two fractions of virion purified as described below on sucrose from the "POL-2" isolate produced by the PLI-2 line on the one hand, and from the MS7PG isolate produced by the LM7PC line on the other hand: the culture supernatants are collected twice weekly, precentrifuged at 10,000 rpm for 30 minutes to remove cell debris and then frozen at -80°C or used as they are for the following steps. The fresh or thawed supernatants are centrifuged on a cushion of 30% glycerol-PBS at 100,000 g (or 30,000 rpm in a type 45 T LKB-HITACHI rotor) for 2 h at 4°C. After removal of the supernatant, the sedimented pellet is taken up in a small volume of PBS and constitutes the fraction of concentrated but unpurified virions. The concentrated virus is then applied to a sucrose gradient in sterile PBS buffer (15 to 50% weight/weight) and ultracentrifuged at 35,000 rpm (100,000 g) for 12 h at +4°C in a swing-out rotor. 10 fractions are collected, and 20 µl are withdrawn from each fraction after homogenization to assay the reverse transcriptase activity therein according to the technique described by H. Perron (24). The fractions containing the peak of "LM7-like" RT activity are then diluted in sterile PBS buffer and ultracentrifuged for one hour at 35,000 rpm (100,000 g) to sediment the viral particles. The pellet of purified virion thereby obtained is then taken up in a small volume of a buffer which is appropriate for the extraction of RNA. The cDNA synthesis reaction mentioned above is carried out on this RNA extracted from purified extracellular virion. PCR amplification according to the technique mentioned above enabled the clone F1-11 to be obtained, whose sequence, identified by SEQ ID NO2, is presented in Figure 10.

This clone makes it possible to define, with the different clones previously sequenced, a region representative of the "pol" gene of the MSRV-1 retrovirus, as presented in Figure 11. This sequence, designated SEQ ID NO1, is reconstituted from different clones overlapping one another at their ends, correcting the

artefacts associated with the primers and with the amplification or cloning techniques which would artificially interrupt the reading frame of the whole.

In Figure 11, the potential reading frame with its translation into amino acids is presented below the nucleic acid sequence.

EXAMPLE 7: CAPTURE, AMPLIFICATION AND CLONING OF A PORTION OF THE MSRV-2 GENOME USING A SEQUENCE ALREADY IDENTIFIED, IN A CULTURE INFECTED WITH MSRV-2

The supernatants of a cell culture expressing "LM7-like" reverse transcriptase activity similar to that described by H. Perron (24) were collected regularly over several weeks and stored frozen at -80°C after adding 10% of glycerol. The set of supernatants was then thawed so as to concentrate the infective particles by ultracentrifugation and to purify them by centrifugation to equilibrium on a sucrose gradient; the reverse transcriptase activity was then measured in the different fractions collected on the gradient according to the methodology described by H. Perron (34).

The different fractions representing the peak of reverse transcriptase activity were pooled so as to extract the nucleic acids therefrom according to a protocol intended for the purification of RNA (35), but the nucleic acids extracted were not treated with DNase. A PCR amplification derived from the technique described by Shih (33) was performed directly on this nucleic acid sample not treated with DNase, according to an RNA amplification method as described in the document EP-A-0,569,272, in a total volume of 100 µl containing 200 ng of RNA, 1 µl of RNA Guard and 33 µmol of each mixture of primers (MOP) which are described by Shih (33) and identical to those used for the direct (DNA) PCR; 0.25 mM each dNTP, 10 µl of 10X buffer, 2.5 u of *Taq* enzyme and 0.4 µl of RT enzyme (RT-AMV; 10u) are also added to the samples. The amplification cycles are carried out as follows: denaturation of the RNA 65°C/10 minutes, synthesis of the cDNA 50°C/8 minutes, then the cycles are identical to those of the PCR described by Shih (33).

Control reactions were performed so as to check for the absence of contaminants (reaction with water). The products were analyzed on 10% acrylamide gel.

The samples amplified by RT-PCR were then cloned and sequenced according to the techniques described in Example 1.

The majority of the clones sequenced from the RT-PCR product corresponds to the MSRV-2A sequence and its equivalent MSRV-2B described above in Examples 1 to 3.

Moreover, after removal of the artefactual sequences, the other clones sequenced prove to correspond to MSRV-1 type sequences as are described in Examples 1 to 3.

After verification of the sequences present in this nucleic acid material originating from these purified fractions containing infective particles, at least a part of which is associated with reverse transcriptase activity, the remaining nucleic acid material was used to perform a specific capture of nucleic acids carrying the MSRV2 sequence previously identified and described in Examples 1 to 3.

In a prior step, the genetic material carrying the MSRV2 sequence was amplified by a one-directional PCR technique of 50 cycles using a single primer. This primer is coupled to a biotin molecule at its 3′ end, permits one-directional amplification from 3′ to 5′ and corresponds to the following sequence identified under SEQ ID N038:

5′ TAAAGATCTAGAATTCGGCTATAGGCGGCATCCGGCAACT 3′

Thereafter, capture was performed in solution with magnetic beads coupled to avidin (Dynabeadsr [sic]) according to the instructions of the manufacturer (Dynal) and, after a series of washes at room temperature enabling nucleic acids not coupled to a biotin to be removed, a PCR was performed directly on these washed beads with a specific primer at the 3′ end and a primer at the 5′ end provided by a solution of oligonucleotide of 10 bases (10-mer) with a random sequence.

The specific amplification primer oriented from 3′ to 5′ corresponds to the sequence identified by SEQ ID N013:

5′ GCATCCGGCAACTGCACG 3′

5 The PCR performed at 35°C over 40 cycles with these primers enabled the genetic material specifically biotinylated by the first PCR step and captured on the Dynabeadsr [sic] beads to be amplified. After cloning with the "TA cloning" kit of the DNA amplified by this 10 second PCR step and sequencing of the recombinant clones, according to the techniques described in Example 1, a sequence of 748 base pairs was obtained. This nucleic acid sequence SEQ ID N012 is presented in Figure 12. This elongated sequence will be designated hereafter 15 MSRV-2EL1.

The reverse sequence complementary to the primer SEQ ID N013 is present at the 3′ end and is boxed in Figure 12. Upstream of this primer, the sequence already identified in the MSRV-2A and MSRV-2B clones is to be 20 found.

The translation of this sequence into amino acids according to the 6 possible reading frames is presented in Figure 13.

An alignment of the MSRV2-A sequence 25 (SEQ ID N010) with the MSRV-2EL1 sequence (SEQ ID N012) is presented in Figure 14. It will be noted that the MSRV-2A sequence is strictly identical to the elongated sequence, apart from a few differences in the region corresponding to the degenerate primers used for obtain-30 ing MSRV-2A. This region is underlined in this figure; moreover, the hybridization region of the primer SEQ ID N013 (apart from the cloning tail) is boxed, that of the primer SEQ ID N014 is presented between square brackets. The true sequence of the MSRV-2 genome in this 35 region is probably that of MSRV-2EL1, where it has not been imposed by hybridized primers having low stringency as is the case for MSRV-2A (and MSRV-2B likewise).

The MSRV-2EL1 sequence hence corresponds to a new sequenced region of the MSRV-2 genome. This was verified

using new PCR primers defined in MSRV-2EL1 and MSRV-2A, which permitted a specific amplification on the nucleic acids used for the cloning described in this example.

The examples which follow present different results of specific MSRV2 amplifications which confirm the relationship with the presence of corresponding infective agent in the cell cultures described, to permit the isolation of an LM7 type virus (24), and also, in vivo, in patients suffering from MS.

The result of interrogation of the Genebankr [sic] data bank, updated in August 1994, with the MSRV-2EL1 sequence does not show any significant homology with genetic sequences known to date. However, the interrogation of the possible translations into amino acids according to the 6 potential reading frames of this MSRV-2EL1 sequence shows partial homologies with bacterial, viral or cellular sequences.

The absence of PCR amplification with specific primers on normal human DNA shows that the sequence in question is not one of cellular origin. MSRV-2 is hence an infective agent exogenous to man. However, the degenerate nature of the mixtures of primers used according to variants of the technique described by Shih (33), which enabled the first sequence elements designated MSRV-2A and MSRV-2B to be identified, may have permitted the unforeseen amplification of a genome not belonging to a retrovirus, or even to a gene coding for an RNA-dependent DNA polymerase. The almost invariable co-detection of MSRV-1 in cultures originating from MS and expressing reverse transcriptase activity may be explained by a pathological association between two different agents, at least one of which is a retrovirus (MSRV-1).

The detection in patients of these two types of sequence described in the examples which follow corroborates a pathological association. However, only one of these elements may suffice to explain the pathology induced in MS.

EXAMPLE 8: DETECTION OF SPECIFIC MSRV-2 SEQUENCES IN DIFFERENT SAMPLES OF HUMAN CELLS ORIGINATING FROM

PATIENTS SUFFERING FROM MS OR FROM CONTROLS

The MSRV-2EL1 sequence (SEQ ID NO12) enabled several pairs of oligonucleotide primers which could be used for the amplification of specific DNA or RNA by the PCR technique to be defined.

The primers defined below enabled a specific detection of the MSRV-2 genome in different human cells to be carried out by an RT-PCR step according to an RNA amplification method as described in the document EP-A-0,569,272.

The primers used are the following:

- 5′ primer, identified by SEQ ID NO14

5′GTAGTTCGATGTAGAAAGCG 3′

- 3′ primer, identified by SEQ ID NO15

5′GCATCCGGCAACTGCACG 3′

The PCR is performed according to a succession of 35 cycles linking together, after the cDNA synthesis step, 1 min at 94°C, 1 min at 54°C and 1 min at 72°C.

The total RNA extracted from different cell types (35), without DNase treatment, was used in this RT-PCR reaction.

Figure 15 presents the results of PCR using a photograph under ultraviolet light of an ethidium bromide-impregnated agarose gel, in which an electrophoresis of PCR amplification products applied separately to the different wells was performed.

Well number 1 contains a mixture of DNA molecular weight markers, and wells 2 to 9 represent, in order, the products amplified from the total RNAs of the following cells:

2- LM7PC (ECACC No. 93010817);

3- PLI2 (ECACC No. 92072201);

4- human medulloblastoma cells;

5- MRC-5 (human embryonic lung fibroblasts);

6- human blood mononuclear cells from a healthy donor;

7- cells originating from a mixture of B lymphoblastoid lines derived from the peripheral blood of different patients suffering from MS;

8- cells originating from a B lymphoblastoid line derived from the peripheral blood of a patient suffering from MS;

9- control not containing nucleic acids ("water" control).

The existence of a band of specific DNA of approximately 700 base pairs, corresponding to the expected size, which is amplified in the samples originating from patients suffering from MS (LM7PC, PLI2, B lymphocyte lines) and not in the cells tested originating from controls not suffering from MS (MRC5, blood mononuclear cells and medulloblastoma cells), can be seen.

EXAMPLE 9: DETECTION OF SPECIFIC MSRV-1 and MSRV-2 SEQUENCES IN DIFFERENT SAMPLES OF PLASMA ORIGINATING FROM PATIENTS SUFFERING FROM MS OR FROM CONTROLS.

A PCR technique similar to the one described in Example 8 was used to detect the MSRV-1 and MSRV-2 genomes in plasmas obtained after taking blood samples from patients suffering from MS and from non-MS controls onto EDTA.

Extraction of the RNAs from plasma was performed according to a technique described by P. Chomzynski (35), after adding one volume of buffer containing guanidinium thiocyanate to 1 ml of plasma stored frozen at -80°C after collection.

For MSRV-2, the PCR was performed under the same conditions and with the same primers as those described in Example 8.

However, similar results were also obtained with the following PCR primers in two successive amplifications by "nested" PCR on samples of nucleic acids not treated with DNase.

The primers used for this first step of 40 cycles with a hybridization temperature of 48°C are the following:

- 5′ primer, identified by SEQ ID N027

5′ GCCGATATCACCCGCCATGG 3′, corresponding to a 5′ MSRV-2 PCR primer, for a first PCR on patients′ sample,

- 3′ primer, identified by SEQ ID N028

5′ GCATCCGGCAACTGCACG 3′, corresponding to a 3′ MSRV-2 PCR primer, for a first PCR on patients′ sample

After this step, 10 μl of the amplification product are taken and used to carry out a second, so-called "nested" PCR amplification with primers located within the region already amplified. This second step takes place over 35 cycles, with a primer hybridization ("annealing") temperature of 50°C. The reaction volume is 100 μl.

The primers used for this second step are the following:

- 5′ primer, identified by SEQ ID N029

5′ CGCGATGCTGGTTGGAGAGC 3′, corresponding to a 5′ MSRV-2 PCR primer, for a nested PCR on patients′ sample,

- 3′ primer, identified by SEQ ID N030

5′ TCTCCACTCCGAATATTCCG 3′, corresponding to a 3′ MSRV-2 PCR primer, for a nested PCR on patients′ sample.

For MSRV-1, the amplification was performed in two steps. Furthermore, the nucleic acid sample is treated beforehand with DNase, and a control PCR without RT (AMV reverse transcriptase) is performed on the two amplification steps so as to verify that the RT-PCR amplification comes exclusively from the MSRV-1 RNA. In the event of a positive control without RT, the initial aliquot sample of RNA is again treated with DNase and amplified again.

The protocol for treatment with DNase lacking RNAse activity is as follows: the extracted RNA is aliquoted in the presence of "RNAse inhibitor" (Boehringer-Mannheim) in water treated with DEPC at a final concentration of 1 μg in 10 μl; to these 10 μl, 1 μl of "RNAse-free DNAse" (Boehringer-Mannheim) and 1.2 μl of pH 5 buffer containing 0.1 M/l [sic] sodium acetate and 5 mM/l [sic] $MgSO^4$ [sic] is [sic] added; the mixture is incubated for 15 min at 20°C and brought to 95°C for 1.5 min in a "thermocycler".

The first MSRV-1 RT-PCR step is performed according to a variant of the RNA amplification method as

described in Patent Application No. EP 0,569,272 A1. In particular, the cDNA synthesis step is performed at 42°C for one hour; the PCR amplification takes place over 40 cycles, with a primer hybridization ("annealing") temperature of 53°C. The reaction volume is 100 μl.

The primers used for this first step are the following:

- 5' primer, identified by SEQ ID N016
  5' AGGAGTAAGGAAACCCAACGGAC 3'
- 3' primer, identified by SEQ ID N017
  5' TAAGAGTTGCACAAGTGCG 3'

After this step, 10 μl of the amplification product are taken and used to carry out a second, so-called "nested" PCR amplification with primers located within the region already amplified. This second step takes place over 35 cycles, with a primer hybridization ("annealing") temperature of 53°C. The reaction volume is 100 μl.

The primers used for this second step are the following:

- 5' primer, identified by SEQ ID N018
  5' TCAGGGATAGCCCCCATCTAT 3'
- 3' primer, identified by SEQ ID N019
  5' AACCCTTTGCCACTACATCAATTT 3'.

Figure 16 presents the results of PCR in the form of photographs [sic] under ultraviolet light of ethidium bromide-impregnated agarose gels [sic], in which an electrophoresis of the PCR amplification products applied separately to the different wells was performed.

The top photograph shows the result of specific MSRV-2 amplification:

well number 8 contains a mixture of DNA molecular weight markers, and wells 1 to 7 represent, in order, the products amplified from the total RNAs of plasmas originating from 4 healthy controls free from MS (wells 1 to 4) and from 3 patients suffering from MS at different stages of the disease (wells 5 to 7).

In this series, MSRV-2 nucleic acid material is detected in the plasma of one case of MS out of the 3

tested, and in none of the 4 control plasmas. Other results obtained on more extensive series confirm these results.

The bottom photograph shows the result of specific amplification by MSRV-1 "nested" RT-PCR:

well No. 1 contains the PCR product produced with water alone, without the addition of AMV reverse transcriptase; well No. 2 contains the PCR product produced with water alone, with addition of AMV reverse transcriptase; well number 3 contains a mixture of DNA molecular weight markers; wells 4 to 13 contain, in order, the products amplified from the total RNAs extracted from sucrose gradient fractions (collected in a downward direction), on which gradient a pellet of virion originating from a supernatant of a culture infected with MSRV-1 and MSRV-2 was centrifuged to equilibrium according to the protocol described by Perron (34); to well 14 nothing was applied; to wells 15 to 17, the amplified products of RNA extracted from plasmas originating from 3 different patients suffering from MS at different stages of the disease were applied.

The MSRV-1 retroviral genome is indeed to be found in the sucrose gradient fraction containing the peak of reverse transcriptase activity measured according to the technique described by H. Perron (24), with a very strong intensity (fraction 5 of the gradient, deposited in well No. 8). A slight amplification has taken place in the first fraction (well No. 4), probably corresponding to RNA released by lysed particles which floated at the surface of the gradient; similarly, aggregated debris sedimented in the last fraction (tube bottom), carrying with it a few copies of the MSRV-1 genome which have given rise to an amplification of low intensity.

Of the 3 MS plasmas tested in this series, MSRV-1 RNA turned up in one case, producing a very intense amplification (well No. 17).

In this series, the MSRV-1 retroviral RNA genome, probably corresponding to particles of extracellular virus present in the plasma in extremely small numbers,

was detected by "nested" RT-PCR in one case of MS out of the 3 tested. Other results obtained on more extensive series confirm these results.

Furthermore, the specificity of the sequences amplified by these PCR techniques may be verified and evaluated by the "ELOSA" technique as described by F. Mallet (42) and in the document FR-2,663,040.

For MSRV-1, the products of the nested PCR described above may be tested in two ELOSA systems enabling a consensus A and a consensus B+C+D of MSRV-1 to be detected separately, corresponding to the subfamilies described in Example 2 and Figures 2, 3 and 4. In effect, the sequences closely resembling the consensus B+C+D are to be found essentially in the RNA samples originating from MSRV-1 virions purified from cultures or amplified in extracellular biological fluids of MS patients, whereas the sequences closely resembling the consensus A are essentially to be found in normal human cellular DNA.

The ELOSA/MSRV-1 system for the capture and specific hybridization of the PCR products of the subfamily A uses a capture oligonucleotide cpV1A with an amine bond at the 5′ end and a biotinylated detection oligonucleotide dpV1A having as their sequence, respectively:

- cpV1A identified by SEQ ID NO31

5′ GATCTAGGCCACTTCTCAGGTCCAGS 3′, corresponding to the ELOSA capture oligonucleotide for the products of MSRV-1 nested PCR performed with the primers identified by SEQ ID NO16 and SEQ ID NO17, optionally followed by amplification with the primers identified by SEQ ID NO18 and SEQ ID NO19 on samples from patients.

- dpV1A identified by SEQ ID NO32

5′ CATCTITTTGGICAGGCAITAGC 3′ corresponding to the ELOSA capture [sic] oligonucleotide for the subfamily A of the products of MSRV-1 nested PCR performed with the primers identified by SEQ ID NO16 and SEQ ID NO17, optionally followed by amplification with the primers identified by SEQ ID NO18 and SEQ ID NO19 on samples from patients.

The ELOSA/MSRV-1 system for the capture and specific hybridization of the PCR products of the subfamily B+C+D uses the same biotinylated detection oligonucleotide dpV1A and a capture oligonucleotide cpV1B
5 with an amine bond at the 5' end having as its sequence:

- dpV1B identified by SEQ ID NO33

5' CTTGAGCCAGTTCTCATACCTGGA 3', corresponding to the ELOSA capture oligonucleotide for the subfamily B + C + D of the products of MSRV-1 nested PCR performed with
10 the primers identified by SEQ ID NO16 and SEQ ID NO17, optionally followed by amplification with the primers identified by SEQ ID NO18 and SEQ ID NO19 on samples from patients.

This ELOSA detection system enabled it to be
15 verified that none of the PCR products thus amplified from DNase-treated plasmas of MS patients contained a sequence of the subfamily A, and that all were positive with the consensus of the subfamilies B, C and D.

For MSRV-2, a similar ELOSA technique was evalu-
20 ated on isolates originating from infected cell cultures, using the following PCR amplification primers,

- 5' primer, identified by SEQ ID NO34

5' AGTGYTRCCMCARGGCGCTGAA 3', corresponding to a 5' MSRV-2 PCR primer, for PCR on sample from cultures,

25 - 3' primer, identified by SEQ ID NO35

5' GMGGCCAGCAGSAKGTCATCCA 3', corresponding to a 3' MSRV-2 PCR primer, for PCR on sample from cultures,

and the capture oligonucleotides with an amine bond at the 5' end cpV2 and the biotinylated detection
30 oligonucleotide dpV2 having as their respective sequences:

- cpV2 identified by SEQ ID NO36

5 [sic] GGATGCCGCCTATAGCCTCTAC 3', corresponding to an ELOSA capture oligonucleotide for the products of
35 MSRV-2 PCR performed with the primers SEQ ID NO34 and SEQ ID NO35, or optionally with the degenerate primers defined by Shih (33),

- dpV2 identified by SEQ ID NO37

5' AAGCCTATCGCGTGCAGTTGCC 3', corresponding to an

ELOSA detection oligonucleotide for the products of MSRV-2 PCR performed with the primers SEQ ID NO34 and SEQ ID NO35, or optionally with the degenerate primers defined by Shih (33)

5 This PCR amplification system with a pair of primers different from those which were described previously for amplification on the samples from patients made it possible to confirm the infection with MSRV-2 of in vitro cultures and of samples of nucleic acids used for 10 the molecular biology studies.

All things considered, our first results of PCR detection of the genome of pathogenic and/or infective agents, it is possible that free "virus" may circulate in the blood stream of patients in an acute, virulent phase, 15 outside the nervous system [sic]. This is compatible with the almost invariable presence of "gaps" in the blood-brain barrier of patients in an active phase of MS.

It is thus already conceivable, as a result of the discoveries made and the methods developed by the 20 inventors, to carry out a diagnosis of MSRV-1 and/or MSRV-2 infection and/or reactivation and to evaluate a therapy in MS on the basis of its efficacy to "negative" the detection of these agents in the patients' biological fluids. Furthermore, early detection in individuals not 25 yet displaying neurological signs of MS could make it possible to institute a treatment which would be all the more effective with respect to the subsequent clinical course for the fact that it would precede the lesion stage which corresponds to the onset of neurological 30 disorders. Now, at the present time, a diagnosis of MS cannot be established before a symptomatology of neurological lesions has set in, and hence no treatment is instituted before the emergence of a clinical picture suggestive of lesions of the central nervous system which 35 are already significant. The diagnosis of an MSRV-1 and/or MSRV-2 infection and/or reactivation in man is hence of decisive importance, and the present invention provided the means of doing this.

It is thus possible, apart from carrying out a

diagnosis of MSRV-1 and/or MSRV-2 infection and/or deactivation, to evaluate a therapy in MS on the basis of its efficacy to "negative" the detection of these agents in the patients' biological fluids.

It will be understood that the term "comprises" or its grammatical variants as
5 used herein is equivalent to the term "includes" and is not to be taken as excluding the presence of other elements or features.

For the nucleotide sequences described and listed herein in accordance with the invention, the bases in each sequence are represented using a one-letter code for nucleotide sequence characters which conforms with WIPO STANDARD
10 ST.23:

Symbol	Meaning	Origin of Destination
A	A	*A*denine
G	G	*G*uanine
C	C	*C*ytosine
T	T	*T*hymine
U	U	*U*ridine*
R	G or A	pu*R*ine
Y	T/U or C	p*Y*rimidine
M	A or C	a*M*ino
K	G or T/U	*K*eto
S	G or C	*S*trong interactions 3H-bonds
W	A or T/U	*W*eak interactions 2H-bonds
B	G or C or T/U	not A
D	A or G or T/U	not C
H	A or C or T/U	not G
V	A or G or C	not T, not U
N	(A or G or C or T/U) or (unknown or other)	a*N*y

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SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT:
(A) NAME: BIOMERIEUX
5 (B) STREET: NONE
(C) CITY: MARCY L'ETOILE
(E) COUNTRY: FRANCE
(F) POSTAL CODE: 69280

(ii) TITLE OF INVENTION: MS EXTENSION

10 (iii) NUMBER OF SEQUENCES: 38

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC com-
15 patible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version
20 #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1158 base pairs
(B) TYPE: nucleotide
25 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
CCCTTTGCCA CTACATCAAT TTTAGGAGTA AGGAAACCCA ACGGACAGTG GAGGTTAGTG   60
CAAGAACTCA GGATTATCAA TGAGGCTGTT GTTCCTCTAT ACCCAGCTGT ACCTAACCCT  120
TATACAGTGC TTTCCCAAAT ACCAGAGGAA GCAGAGTGGT TTACAGTCCT GGACCTTAAG  180
GATGCCTTTT TCTGCATCCC TGTACGTCCT GACTCTCAAT TCTTGTTTGC CTTTGAAGAT  240
CCTTTGAACC CAACGTCTCA ACTCACCTGG ACTGTTTTAC CCCAAGGGTT CAGGGATAGC  300
CCCCATCTAT TTGGCCAGGC ATTAGCCCAA GACTTGAGTC AATTCTCATA CCTGGACACT  360
CTTGTCCTTC AGTACATGGA TGATTTACTT TTAGTCGCCC GTTCAGAAAC CTTGTGCCAT  420
CAAGCCACCC AAGAACTCTT AACTTTCCTC ACTACCTGTG GCTACAAGGT TTCCAAACCA  480
AAGGCTCGGC TCTGCTCACA GGAGATTAGA TACTNAGGGC TAAAATTATC CAAAGGCACC  540
AGGGCCCTCA GTGAGGAACG TATCCAGCCT ATACTGGCTT ATCCTCATCC CAAAACCCTA  600
AAGCAACTAA GAGGGTTCCT TGGCATAACA GGTTTCTGCC GAAAACAGAT TCCCAGGTAC  660
ASCCCAATAG CCAGACCATT ATATACACTA ATTANGCAAA CTCAGAAAGC CAATACCTAT  720
TTAGTAAGAT GGACACCTAC AGAAGTGGCT TTCCAGGCCC TAAAGAAGGC CCTAACCCAA  780
GCCCCAGTGT TCAGCTTGCC AACAGGGCAA GATTTTTCTT TATATGCCAC AGAAAAAACA  840
CGAATAGCTC TAGGAGTCCT TACGCAGGTC TCAGGGATGA GCTTGCAACC CGTGGTATAC  900
CTCAGTAAGG AAATTGATGT AGTGGCAAAG GGTTGGCCTC ATNGTTTATG GGTAATGGNG  960
GCAGTAGCAG TCTNAGTATC TGAAGCACTT AAAATAATAC AGGGAAGAGA TCTTNCTGTG1020
TGGACATCTC ATGATGTGAA CGGCATACTC ACTGCTAAAG GAGACTTGTG GTTGTCAGAC1080
AACCATTTAC TTAANTATCA GGCTCTATTA CTTGAAGAGC CAGTGCTCNG ACTGCGCACT1140
TGTGCAACTC TTAAACCC                                              1158
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 297 base pairs

(B) TYPE: nucleotide

5 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
CCCTTTGCCA CTACATCAAT TTTAGGAGTA AGGAAACCCA ACGGACAGTG GAGGTTAGTG   60
CAAGAACTCA GGATTATCAA TGAGGCTGTT GTTCCTCTAT ACCCAGCTGT ACCTAACCCT  120
TATACAGTGC TTTCCCAAAT ACCAGAGGAA GCAGAGTCGT TTACAGTCCT GGACCTTAAG  180
GATGCCTTTT TCTGCATCCC TGTACGTCCT GACTCTCAAT TCTTGTTTGC CTTTGAAGAT  240
CCTTTGAACC CAACGTCTCA ACTCACCTGG ACTGTTTTAC CCCAAGGGTT CAAGGGA     297
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleotide
5 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GTTTAGGGAT ANCCCTCATC TCTTTGGTCA GGTACTGGCC CAAGATCTAG GCCACTTCTC 60
AGGTCCAGSN ACTCTGTYCC TTCAG 85

(2) INFORMATION FOR SEQ ID NO: 4:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GTTCAGGGAT AGCCCCCATC TATTTGGCCA GGCACTAGCT CAATACTTGA GCCAGTTCTC 60
ATACCTGGAC AYTCTYGTCC TTCGGT 86

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
20 (B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
GTTCARRGA TAGCCCCCATC TATTTGGCCW RGYATTAGCC CAAGACTTGA GYCAATTCTC   60
ATACCTGGA CACTCTTGTCC TTYRG                                         85
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 85 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
GTTCAGGGAT AGCTCCCATC TATTTGGCCT GGCATTAACC CGAGACTTAA GCCAGTTCTY   60
ATACGTGGAC ACTCTTGTCC TTTGG                                         85
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 111 base pairs

(B) TYPE: nucleotide

15 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
GTGTTGCCAC AGGGGTTTAR RGATANCYCY CATCTMTTTG GYCHRGYAYT RRCYCRAKAY   60
YTRRGYCAVT TCTYAKRYSY RGSNAYTCTB KYCCTTYRGT ACATGGATGA C           111
```

(2) INFORMATION FOR SEQ ID NO: 8:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 645 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
TCAGGGATAG CCCCCATCTA TTTGGCCAGG CATTAGCCCA AGACTTGAGT CAATTCTCAT  60
ACCTGGACAC TCTTGTCCTT CAGTACATGG ATGATTTACT TTTAGTCGCC CGTTCAGAAA 120
CCTTGTGCCA TCAAGCCACC CAAGAACTCT TAACTTTCCT CACTACCTGT GGCTACAAGG 180
TTTCCAAACC AAAGGCTCGG CTCTGCTCAC AGGAGATTAG ATACTNAGGG CTAAAATTAT 240
CCAAAGGCAC CAGGGCCCTC AGTGAGGAAC GTATCCAGCC TATACTGGCT TATCCTCATC 300
CCAAAACCCT AAAGCAACTA AGAGGGTTCC TTGGCATAAC AGGTTTCTGC CGAAAACAGA 360
TTCCCAGGTA CASCCCAATA GCCAGACCAT TATATACACT AATTANGGAA ACTCAGAAAG 420
CCAATACCTA TTTAGTAAGA TGGACACCTA CAGAAGTGGC TTTCCAGGCC CTAAAGAAGC 480
CCCTAACCCA AGCCCCAGTG TTCAGCTTGC CAACAGGGCA AGATTTTTCT TTATATGCCA 540
CAGAAAAAAC AGGAATAGCT CTAGGAGTCC TTACGCAGGT CTCAGGGATG AGCTTGCAAC 600
CCGTGGTATA CCTGAGTAAG GAAATTGATG TAGTGGCAAA GCCTT                 645
```

5 (2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 741 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

10 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
CAAGCCACCC AAGAACTCTT AAATTTCCTC ACTACCTGTG GCTACAAGGT TTCCAAACCA  60
AAGGCTCAGC TCTGCTCACA GGACATTAGA TACTTAGGGT TAAAATTATC CAAAGGCACC 120
AGGGGCCTCA GTGAGGAACG TATCCAGCCT ATACTGGGTT ATCCTCATCC CAAAACCCTA 180
AAGCAACTAA GAGGGTTCCT TAGCATGATC AGGTTTCTGC CGAAAACAAG ATTCCCAGGT 240
ACAACCAAAA TAGCCAGACC ATTATATACA CTAATTAACG AAACTCAGAA AGCCAATACC 300
TATTTAGTAA GATGGACACC TAAACAGAAG GCTTTCCAGC CCCTAAAGAA GGCCCTAACC 360
CAAGCCCCAG TGTTCAGCTT GCCAACAGGG CAAGATTTTT CTTTATATGG CACAGAAAAA 420
ACAGGAATCG CTCTAGGAGT CCTTACACAG GTCCGAGGGA TGAGCTTGCA ACCCGTGGCA 480
TACCTGAATA AGGAAATTCA TGTAGTGGCA AAGGGTTGGC CTCATNGTTT ATGGGTAATG 540
CNGGCAGTAG CAGTCTNAGT ATCTGAAGCA GTTAAAATAA TACAGGGAAG AGATCTTNCT 600
GTGTGGACAT CTCATCATGT GAACGGCATA CTCACTGCTA AAGGAGACTT GTGGTTGTCA 660
GACAACCATT TACTTAANTA TCACGCTCTA TTACTTGAAG AGCCAGTGCT GNGACTGCGC 720
ACTTGTGCAA CTCTTAAACC C                                           741
```

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 93 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
TGGAAAGTGT TGCCACAGGG CGCTGAAGCC TATCGCGTGC AGTTGCCGGA TCCCGCCTAT 60
AGCCTCTACA TGCATGACAT CCTGCTGGCC TCC                              93
```

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 96 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
TTGGATCCAG TGYTGCCACA GGGCGCTGAA GCCTATCGCG TGCAGTTGCC GGATGCCGCC  60
TATAGCCTCT ACGTCGATGA CCTSCTGAAC CTTGAG                            96
```

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 748 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```
TGCAAGCTTC ACCGCTTGCT GGATGTAGGC CTCAGTACCG GNGTGCCCCG CGCGCTGTAG   60
TTCGATGTAG AAAGCGCCCG GAAACACGCG GGACCAATGC GTCGCCAGCT TGCGCGCCAG  120
CGCCTCGTTG CCATTGGCCA GCGCCACGCC CATATCACCC GCCATGGCGC CGGAGAGCGC  180
CAGCAGACCG GCGGCCAGCG GCGCATTCTC AACGCCGGGC TCGTCGAACC ATTCGGGGGC  240
GATTTCCGCA CGACCGCGAT GCTGGTTGGA GAGCCAGGCC CTGGCCAGCA ACTGGCACAG  300
GTTCAGGTAA CCCTGCTTGT CCCGCACCAA CAGCAGCAGG CGGGTCGGCT TGTCGCGCTC  360
GTCGTGATTG GTGATCCACA CGTCAGCCCC GACGATGGCC TTCACGCCCT TGCCACGCGC  420
TTCCTTGTAG ANGCGCACCA GCCCGAAGGC ATTGGCGAGA TCGGTCAGCG CCAAGGCGCC  480
CATGCCATCT TTGGCGGCAG CCTTGACGCC ATCGTCGAGA CGGACATTGC CATCGACGAC  540
GGAATATTCG GAGTGGAGAC GGAGGTGGAC GAAGCGCGGC GAATTCATCC GCGTATTGTA  600
ACGGTGACA CCTTCCCGCAA ACCATTCCGG ACGTGCCCGA TTGACCCGGA GCAACCCCGC  660
ACGGCTGCGC GGGCAGTTAT AATTTCGGCT TACGAATCAA CGGGTTACCC CAGGGCGCTG  720
AAGCCTATCG CGTGCAGTTG CCGGATGC                                      748
```

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs

(B) TYPE: nucleotide

5 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GCATCCGGCA ACTGCACG 18

(2) INFORMATION FOR SEQ ID NO: 14:

10 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GTAGTTCGAT GTAGAAAGCG 20

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 18 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

GCATCCGGCA ACTGCACG 18

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

AGGAGTAAG GAAACCCAACG CAC 23

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

TAAGAGTTGC ACAAGTGCG 19

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

TCAGGGATAG CCCCCATCTA T 21

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: base pairs
(B) TYPE: 24 nucleotide
5 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

AACCCTTTGC CACTACATCA ATTT 24

(2) INFORMATION FOR SEQ ID NO: 20:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA
(ix) FEATURE:
(B) LOCATION: 5,7,10,13
(C) OTHER INFORMATION: G represents inosine (i)

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

GGTCGTGCCG CAGGG 15

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
25 (B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

TTAGGGATA CCCCTCATCTC T 21

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

TCAGGGATAG CCCCCATCTA T 21

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23

AACCCTTTGC CACTACATCA ATTT 24

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24

GCGTAAGGAC TCCTAGAGCTA TT 22

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 18 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25

TCATCCATGT ACCGAAGC 18

10 (2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

15 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26

ATGGGGTTCC CAAGTTCCCT 20

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 20 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27

GCCGATATCA CCCGCCATGG 20

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

5

(A) LENGTH: 18 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28

GCATCCGGCA ACTGCACG 18

10 (2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single

15

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29

CGCGATGCTG GTTGGAGAGC 20

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

20

(A) LENGTH: 20 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30

TCTCCACTCC GAATATTCCG 20

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

5

(A) LENGTH: 26 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31

GATCTAGGCC ACTTCTCAGG TCCAGS 26

10

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single

15

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(ix) FEATURE

(B) LOCATION: 6, 12, 19

20

(D) OTHER INFORMATION: G represents inosine (i)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32

CATCTGTTTC C[illegible]CAGGCAGT AGC 23

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

25

(A) LENGTH: 24 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33

CTTGAGCCAG TTCTCATACC TGGA 24

(2) INFORMATION FOR SEQ ID NO: 34:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34

AGTGYTRCCH CARGGCGCTG AA 22

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:
15 (A) LENGTH: 22 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35

GMGCCCAGCA GSAKGTCATC CA 22

20 (2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36

GGATGCCGCCT ATAGCCTCTAC 20

(2) INFORMATION FOR SEQ ID NO: 37:

5 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37

AAGCCTATCG CGTGCAGTTG CC 22

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs

15 (B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

TAAAGATCTA GAATTCGGCT ATAGGCGGCA TCCGGCAAGT 40

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. Method for detecting a first pathogenic and/or infective agent and/or a second pathogenic and/or infective agent associated with multiple sclerosis, characterized in that at least one nucleic acid fragment is employed, namely a first fragment whose nucleotide sequence comprises a nucleotide sequence chosen from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 50% and preferably at least 70% homology with a nucleotide sequence chosen from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9 and their complementary sequences and/or a second fragment whose nucleotide sequence comprises a nucleotide sequence chosen from SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 70% and preferably at least 90% homology with a nucleotide sequence chosen from SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and their complementary sequences, each of said fragments being, in particular, a probe, said second fragment being different from the following sequence CGCTGAAGCC TATCGCGTGC AGTTGCCGGA TGCCGCCTAT AGCCTC, and fragments thereof.

2. Diagnostic, prophylactic or therapeutic composition, characterized in that it comprises at least one nucleic acid fragment, namely a first nucleic acid fragment whose nucleotide sequence comprises a nucleotide sequence chosen from SEQ ID NO: 1, SEQ ID NO: 2,

SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 50% and preferably at least 70% homology with a nucleotide sequence chosen from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9 and their complementary sequences, said first fragment being different from ERV-9 or HSERV-9 sequences, and/or a second nucleic acid fragment whose nucleotide sequence comprises a nucleotide sequence chosen from SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 70% and preferably at least 90% homology with a nucleotide sequence chosen from SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and their complementary sequences, said second fragment being different from the following sequence CGCTGAAGCC TATCGCGTGC AGTTGCCGGA TGCCGCCTAT AGCCTC, and fragments thereof.

3. Method for detecting and/or identifying a combination of pathological and/or infective agents associated with multiple sclerosis, in a biological sample, characterized in that an RNA and/or a DNA presumed to belong to at least one said pathological and/or infective agent, and/or their complementary RNA and/or DNA, is/are brought into contact with a composition comprising a first nucleotide fragment and a second nucleotide fragment, the nucleotide sequence of said first fragment comprising a nucleotide sequence chosen from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, their complementary sequences and their

equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 50% and preferably at least 70% homology with a nucleotide sequence chosen from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7,
5 SEQ ID NO:8, SEQ ID NO:9 and their complementary sequences, and the nucleotide sequence of said second fragment comprising a nucleotide sequence chose from SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 70% and
10 preferably at least 90% homology with a nucleotide sequence chosen from SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 and their complementary sequences.

4. Method for detecting, in a biological sample, a first pathological and/or infective agent and/or a second pathological and/or infective agent associated with multiple sclerosis, characterized in that a composition comprising a first
15 polypeptide partially or completely encoded by the first nucleotide fragment defined in claim 1, and/or a second polypeptide partially or completely encoded by the second nucleotide fragment defined in claim 1, is employed.

5. Diagnostic, prophylactic or therapeutic composition, characterized in that it comprises the first polypeptide and/or the second polypeptide which are defined in
20 claim 4.

6. Diagnostic, prophylactic or therapeutic composition, characterized in that it comprises a first antibody specific for the first polypeptide, and/or a second antibody specific for the second polypeptide, said first and second polypeptides being defined in claim 4.

25 7. Cell line designated LM7PC as deposited with the ECACC on January 8, 1993 under Accession Number 93010817, or any derived cell line, or any progeny of this line, insofar as these lines and progeny are capable of producing an antigen obtained from said LM7PC line, or any other antigen displaying an immunological cross-reaction with said antigen.

8. Viral strain designated MS7PG as deposited with the ECACC on January 8, 1993 under Accession Number V93010816, or any derived strain, or any progeny of this strain, insofar as these strains and progeny are capable of producing an antigen obtained from said MS7PG strain, or any other antigen displaying an
5 immunological cross-reaction with said antigen.

9. Viral material, in the purified or isolated state, possessing reverse transcriptase activity, associated with a family of endogenous retroviral elements and associated with multiple sclerosis, originating from a viral strain possessing reverse transcriptase activity, chosen from strain of claim 8, and variant strains
10 consisting of viruses at least one antigen which is recognized by at least one antibody directed against at least one corresponding antigen of the virus of the above-mentioned viral strain MS7PG.

10. Viral material, in the purified or isolated state, possessing reverse transcriptase activity, associated with a family of endogenous retroviral elements,
15 associated with multiple sclerosis, produced by cell line of claim 7, or by any infected cell culture capable of producing a virus comprising at least one antigen which is recognized by at least one antibody directed against at least one-corresponding antigen of the virus produced by the above-mentioned line LM7PC.

11. Viral material according to claim 9 or 10, characterized in that is genome
20 comprises a nucleotide sequence chosen from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 50% and preferably at least 70% homology
25 whit a nucleotide sequence chosen from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and their complementary sequences, sad viral material being different from ERV-9 or HSERV-9.

12. Retroviral material according to claim 9 or 10, which is associated with

multiple sclerosis, and characterized in that the *pol* gene of its genome comprises an equivalent nucleotide sequence, and in particular one displaying at least 50% homology, preferably at least 65% homology, with a nucleotide sequence belonging to the *pol* gene of the ERV-9 or HSERV-9 retrovirus genome.

5 13. Retroviral material according to claim 9 or 10, which is associated with multiple sclerosis, and characterized in that the *pol* gene of its genome codes for a peptide sequence displaying at least 50% and preferably at least 70% homology with a peptide sequence encoded by the *pol* gene of the ERV-9 or HSERV-9 retrovirus genome.

10 14. Retroviral material according to claim 9 or 10, which is associated with multiple sclerosis, and characterized in that the *pol* gene of its genome codes for a peptide sequence displaying, for any contiguous succession of at least 30 amino acids, at least 50% and preferably at least 70% homology with a

peptide sequence encoded by a nucleotide sequence chosen from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9 and their complementary 5 sequences.

15. Nucleotide fragment whose nucleotide sequence comprises a nucleotide sequence chosen from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, 10 SEQ ID NO: 9, their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 50% and preferably at least 70% homology with a sequence chosen from SEQ ID NO: 1, SEQ ID 15 NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9 and their complementary sequences, said fragment being different from ERV-9 or HSERV-9 sequences.

16. Specific primer for the amplification by 20 polymerization of an RNA or DNA of a viral material according to any one of claims 9 to 14, characterized in that it comprises a nucleotide sequence identical or equivalent to at least part of the nucleotide sequence of a fragment according to claim 15, in particular a 25 nucleotide sequence displaying, for any succession of 10 contiguous monomers, at least 70% homology with at least part of said fragment.

17. Primer according to claim 16, characterized in that it comprises a nucleotide sequence chosen from 30 SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO:21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33 and their complementary sequences.

18. Probe capable of specifically hybridizing with an RNA or DNA of a viral material according to any one of claims 9 to 14, characterized in that it comprises a nucleotide sequence identical or equivalent to at least
5 part of the nucleotide sequence of a fragment according to claim 15, in particular a nucleotide sequence displaying, for any succession of 10 contiguous monomers, at least 70% homology with at least part of said fragment.

19. Probe according to claim 18, characterized
10 in that it comprises a nucleotide sequence chosen from SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24,
15 SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33 and their complementary sequences.

20. Use of a probe according to claim 18 or 19 or primer according to claim 16 or 17 for detecting,
20 separating or identifying, in a biological sample, a viral material according to any one of claims 9 to 14.

21. Method for detecting, separating or identifying, in a biological sample, the viral material according to one of claims 9 to 14, characterized in that
25 an RNA and/or a DNA presumed to belong to said virus, and/or their complementary DNA and/or RNA, is/are brought into contact with at least one probe according to claim 19.

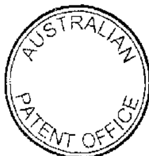
22. Method according to claim 21, characterized
30 in that, before the RNA and/or DNA or their complementary DNA and/or RNA is/are brought into contact with the probe, said RNA and/or said DNA is/are hybridized with at least

one amplification primer according to claim 17, and said RNA and/or DNA is/are amplified.

23. Method for quantifying, in a biological sample, the expression of a viral material associated with 5 multiple sclerosis according to one of claims 9 to 14, characterized in that an RNA and/or a DNA specific to said virus, and/or their complementary DNA and/or RNA, is/are brought into contact with at least one probe according to claim 19, amplification is carried out where appropriate 10 and said RNA and/or DNA is/are detected.

24. Pathogenic and/or infective agent, in the purified or isolated state, different from the viral material according to one of claims 9 to 14, associated with multiple sclerosis, originating from a viral strain 15 chosen from strain of claim 8, and the variant strains consisting of pathogenic and/or infective agents comprising at least one antigen which is recognized by at least one antibody directed against at least one corresponding antigen of one or other of the pathogenic 20 and/or infective agents of the above-mentioned viral strain MS7PG, the agents being different, respectively, from viral material of said strain.

25. Pathogenic and/or infective agent, in the purified or isolated state, different from the viral 25 material according to one of claims 9 to 14, associated with multiple sclerosis, produced by the cell line of claim 7, and by any infected cell culture capable of producing a pathogenic and/or infective agent comprising at least one antigen which is recognized by at least one 30 antibody directed against at least one corresponding antigen of one or other of the pathogenic and/or infective agents produced by the above-mentioned line LM7PC, the agents being, respectively, different from viral material of said strain.



26. Pathogenic and/or infective agent, characterized in that it comprises a nucleic acid comprising a nucleotide sequence chosen from SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, their
5 complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying at least 70% and preferably at least 90% homology with a nucleotide sequence comprising a sequence chosen from SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and their complementary
10 sequences, said second fragment being different from the following sequence
CGCTGAAGCC TATCGCGTGC AGTTGCCGGA TGCCGCCTAT AGCCTC and from fragments thereof.

27. Nucleotide fragment, characterized in that
15 it comprises a nucleotide sequence chosen from SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, their complementary sequences and their equivalent sequences, in particular the nucleotide sequences displaying, for any succession of 100 contiguous monomers, at least 70% and preferably at
20 least 90% homology with a sequence chosen from SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and their complementary sequences, said second fragment being different from the following sequence
CGCTGAAGCC TATCGCGTGC AGTTGCCGGA TGCCGCCTAT AGCCTC, and
25 from fragments thereof.

28. Specific primer for the amplification by polymerization of an RNA or DNA of a pathogenic and/or infective agent according to any one of claims 24 to 26, characterized in that it comprises a nucleotide sequence
30 identical or equivalent to at least part of the nucleotide sequence of a fragment according to claim 27, in particular a nucleotide sequence displaying, for any succession of 10 contiguous monomers, at least 90% homology with at least part of said fragment.

29. Primer according to claim 28, characterized in that it comprises a nucleotide sequence chosen from SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37 and their complementary sequences.

30. Probe capable of specifically hybridizing with an RNA or DNA of a pathogenic and/or infective agent according to any one of claims 24 to 26, characterized in that it comprises a nucleotide sequence identical or equivalent to at least part of the nucleotide sequence of a fragment according to claim 27, in particular a nucleotide sequence displaying, for any succession of 10 contiguous monomers, at least 90% homology with at least part of said fragment.

31. Probe according to claim 30, characterized in that it comprises a nucleotide sequence chosen from SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37 and their complementary sequences.

32. Use of a probe according to claim 30 or 31 and/or a primer according to claim 28 or 29 for detecting and/or identifying, in a biological sample, a pathological and/or infective agent according to any one of claims 24 to 26.

33. Method for detecting, separating or identifying, in a biological sample, the pathogenic and/or infective agent according to any one of claims 24 to 26, characterized in that an RNA and/or a DNA presumed to belong to said agent, and/or their complementary DNA

and/or RNA, is/are brought into contact with at least one probe according to claim 31.

34. Method according to claim 33, characterized in that, before the RNA and/or DNA or their complementary DNA and/or RNA is/are brought into contact with the probe, said RNA and/or said DNA is/are hybridized with at least one amplification primer according to claim 29, and said RNA and/or DNA is/are amplified.

35. Method for quantifying in a biological sample, the expression of an infective and/or pathogenic agent associated with multiple sclerosis according to any one of claims 24 to 26, characterized in that an RNA and/or a DNA specific to said agent, and/or their complementary DNA and/or RNA, is/are brought into contact with at least one probe according to claim 31, and said RNA and/or DNA is/are amplified.

36. Diagnostic, prophylactic or therapeutic composition, in particular for inhibiting the expression of at least one pathogenic and/or infective agent associated with multiple sclerosis, characterized in that it comprises at least one probe according to claim 18 or 19 or one probe according to claim 30 or 31, and/or at least one primer according to claim 13 or 14 or one primer according to claim 28 or 29.

37. RNA or DNA, and in particular replication vector, comprising a fragment according to claim 15 and/or a fragment according to claim 27.

38. Polypeptide having at least 5 and preferably 10 amino acids, encoded by any nucleotide sequence of the genome of a pathogenic and/or infective agent associated with multiple sclerosis, characterized in that it is encoded by at least one part of a nucleotide

fragment according to claim 20 or of a nucleotide fragment according to claim 27.

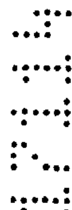
39. Diagnostic and/or therapeutic and/or prophylactic composition, characterized in that it comprises at least one polypeptide according to claim 38.

40. Diagnostic and/or therapeutic and/or prophylactic composition, 5 characterized in that it comprises an antibody specific for at least one polypeptide according to claim 38.

41. A method according to claim 1, substantially as hereinbefore described, with reference to the Examples.

42. A diagnostic, prophylactic or therapeutic composition substantially as 10 hereinbefore described, with reference to the Examples.

43. A method for detecting and/or identifying a combination of pathological and/or infective agents associated with multiple sclerosis in a biological sample, substantially as hereinbefore described with reference to the Examples.



44. A viral material according to claim 9 or 10, substantially as hereinbefore 15 described with reference to the Examples.

45. A retroviral material according to claim 12, 13 or 14, substantially as hereinbefore described with reference to the Examples.

46. A nucleotide fragment according to claim 15 or 27, substantially as hereinbefore described with reference to the Examples.

20 47. A pathogenic and/or infective agent according to claim 26, substantially as hereinbefore described with reference to the Examples.

Bio Merieux
By its Registered Patent Attorneys
25 **Freehills Patent Attorneys**

10 January 1999

FIG 1

pol SHH	TGGAAAGTGT TGCCACAGGG CGCTGAAGCC TATCGCGTGC AGTTGCCGGA	50
pol SHH	TGCCGCCTAT AGCCTCTACA TGGATGACAT CCTGCTGGCC TCC	93

SEQ ID NO 10

FIG 2

Consensus	GTTTAGGGAT ANCCCTCATC TCTTTGGTCA GGTACTGGCC CAAGATCTAG	50
Consensus	GCCACTTCTC AGGTCCAGSN ACTCTGTYCC TTCAG	85

SEQ ID NO3

Consensus	GTTCAGGGAT AGCCCCCATC TATTTGGCCA GGCACTAGCT CAATACTTGA	50
Consensus	GCCAGTTCTC ATACCTGGAC AYTCTYGTCC TTCGGT	86

SEQ ID NO4

Consensus	GTTCARRGAT AGCCCCCATC TATTTGGCCW RGYATTAGCC CAAGACTTGA	50
Consensus	GYCAATTCTC ATACCTGGAC ACTCTTGTCC TTYRG	85

SEQ ID NO5

Consensus	GTTCAGGGAT AGCTCCCATC TATTTGGCCT GGCATTAACC CGAGACTTAA	50
Consensus	GCCAGTTCTY ATACGTGGAC ACTCTTGTCC TTTGG	85

SEQ ID NO6

Consensus	GTGTTGCCAC AGGGGTTTAR RGATANCYCY CATCTMTTTG GYCWRGYAYT
Consensus	RRCYCRAKAY YTRRGYCAVT TCTYAKRYSY RGSNAYTCTB KYCCTTYRGT
Consensus	ACATGGATGA C

SEQ ID NO7

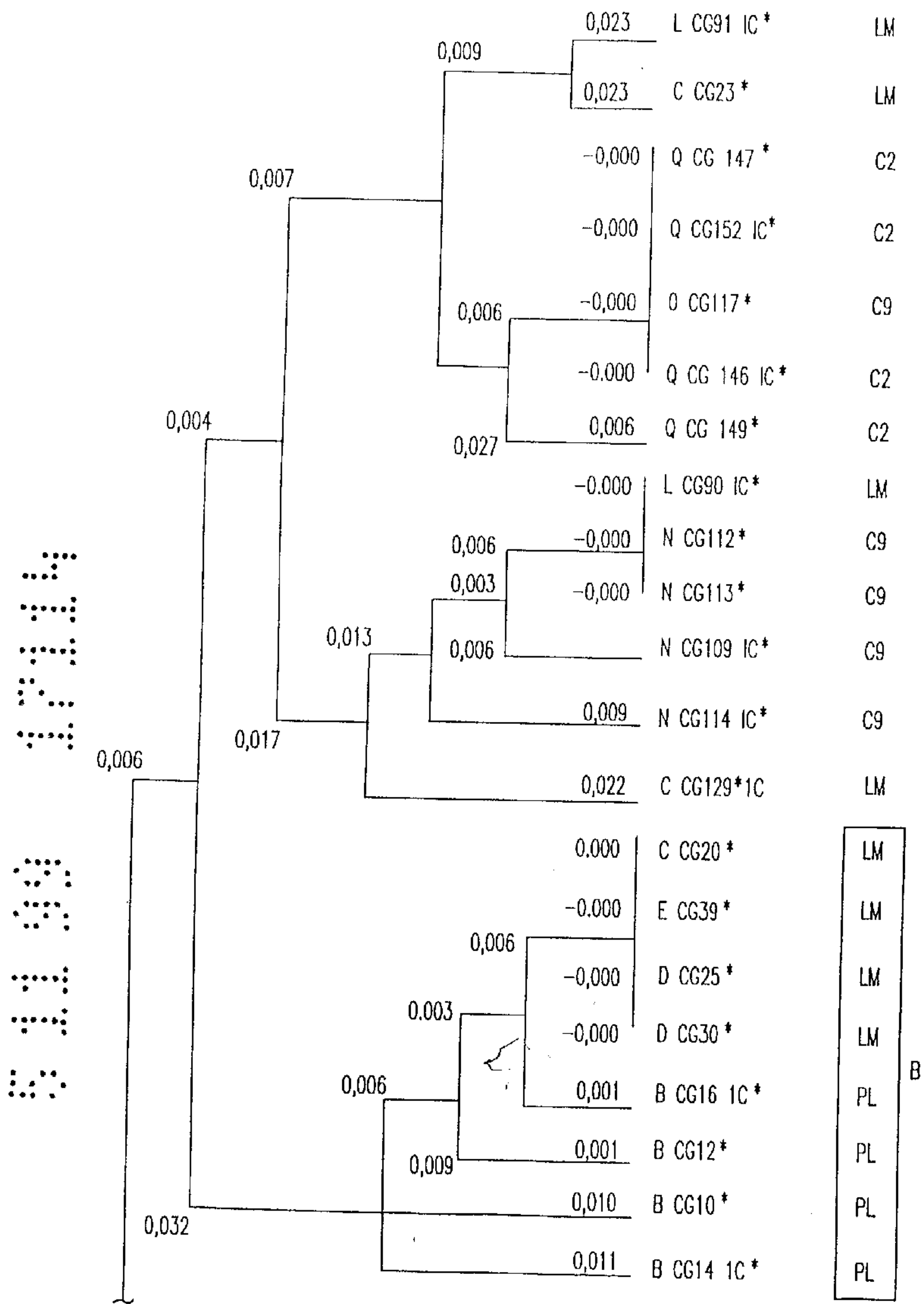


FIG.3A

FIG.3B

0.015
0,000 0,011 H CG62* LM
0,005 0,011 J CG149* PL
0,011 E CG133* LM
0.006
-0,000 K CG83* PL
0.006
0.013
-0,000 K CG84 1C* PL
0,005
0,015
K CG85* PL
0,012
0.006
0,026 D CG28* LM
C
0,031 M CG106* C9
0,007
0,006
0,043 E CG35* LM
0,064 D CG32* LM
-0,000 C DG130* LM
0,006
0,000 J CG141* PL
D
0,071
0,006 I CG681C* LM
-0,000 I CG136* LM
-0,000 R CG158* C4
0,006
0,000 R CG156 1C* C4
0,000 R CG 159* C4
-0,000 R CG 154* C4
0,089
0,006 -0,000 R CG163* C4
0,014 R CG 155* C4
0,006 -0,000 L CG93 1C* LM
0,005
0,000 L CG95 1C* LM
0,004
0,006 J CG74 1C* PL
A
0,014 E CG134 1C* LM
0,166
0,019 I CG139* LM

FIG 4

CONSENSUS A

```
GTTTAGGGATAGCCC TCATCTCTTTGGTCA GGTACTGGCCCAAGA TCTAGGCCACTTCTC   60
V  .  G  .  P   S  S  L  W  S   G  T  G  P  R   S  R  P  L  L
 F  R  D  S  P   H  L  F  G  Q   V  L  A  Q  D   L  G  H  F  S
  L  G  I  A  L   I  S  L  V  R   Y  W  P  K  I   .  A  T  S  Q

AGGTCCAGGCACTCT GTTCCTTCAG                                        85
R  S  R  H  S   V  P  S
 G  P  G  T  L   F  L  Q
  V  Q  A  L  C   S  F
```

CONSENSUS B

```
GTTCAGGGATAGCCC CCATCTATTTGGCCA GGCACTAGCTCAATA CTTGAGCCAGTTCTC   60
V  Q  G  .  P   P  S  I  W  P   G  T  S  S  I   L  E  P  V  L
 F  R  D  S  P   H  L  F  G  Q   A  L  A  Q  Y   L  S  Q  F  S
  S  G  I  A  P   I  Y  L  A  R   H  .  L  N  T   .  A  S  S  H

ATACCTGGACACTCT TGTCCTTCGGT                                       86
I  P  G  H  S   C  P  S
 Y  L  D  T  L   V  L  R
  T  W  T  L  L   S  F  G
```

CONSENSUS C

```
GTTCAGGGATAGCCC CCATCTATTTGGCCA GGCATTAGCCCAAGA CTTGAGTCAATTCTC   60
V  Q  G  .  P   P  S  I  W  P   G  I  S  P  R   L  E  S  I  L
 F  R  D  S  P   H  L  F  G  Q   A  L  A  Q  D   L  S  Q  F  S
  S  G  I  A  P   I  Y  L  A  R   H  .  P  K  T   .  V  N  S  H

ATACCTGGACACTCT TGTCCTTCAG                                        85
I  P  G  H  S   C  P  S
 Y  L  D  T  L   V  L  Q
  T  W  T  L  L   S  F
```

CONSENSUS D

```
GTTCAGGGATAGCTC CCATCTATTTGGCCT GGCATTAACCCGAGA CTTAAGCCAGTTCTC   60
V  Q  G  .  L   P  S  I  W  P   G  I  N  P  R   L  K  P  V  L
 F  R  D  S  S   H  L  F  G  L   A  L  T  R  D   L  S  Q  F  S
  S  G  I  A  P   I  Y  L  A  W   H  .  P  E  T   .  A  S  S  H

ATACGTGGACACTCT TGTCCTTTGG                                        85
I  R  G  H  S   C  P  L
 Y  V  D  T  L   V  L  W
  T  W  T  L  L   S  F
```

FIG 5

Consensus	TTGGATCCAG TGYTGCCACA GGGCGCTGAA GCCTATCGCG TGCAGTTGCC	50
Consensus	GGATGCCGCC TATAGCCTCT ACGTGGATGA CCTSCTGAAG CTTGAG	96

SEQ ID NO 11

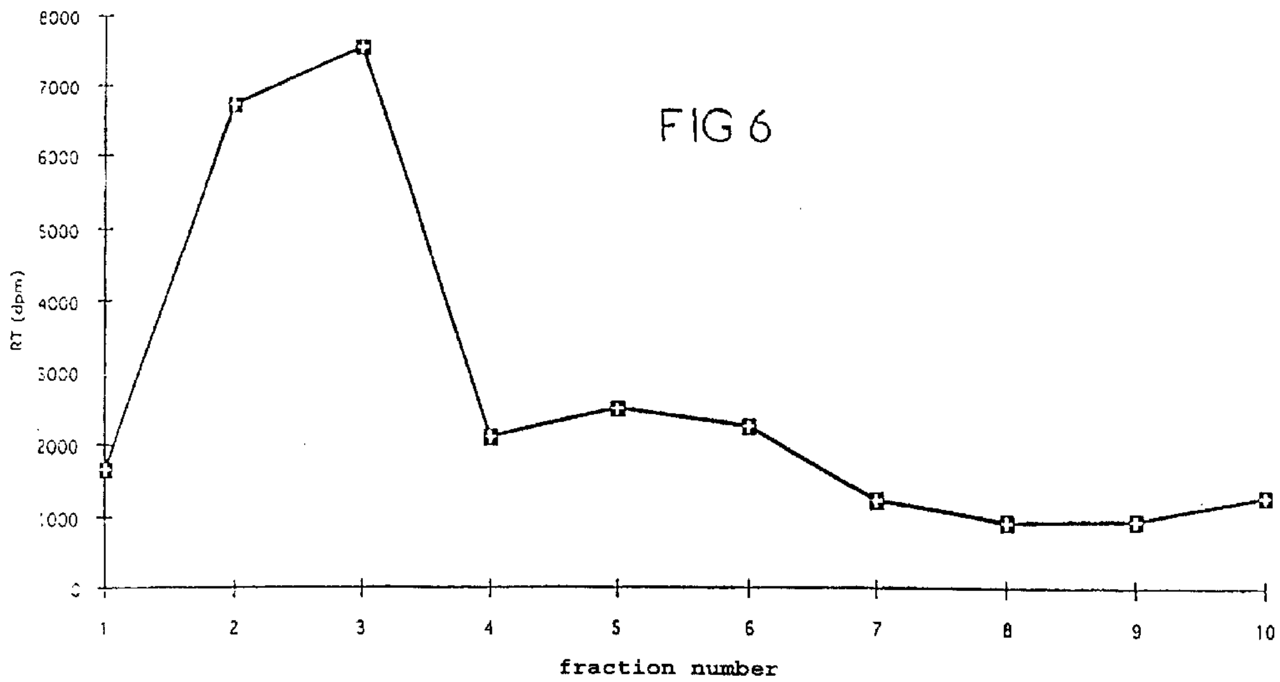


FIG 6
RT (dpm)
8000
7000
6000
5000
4000
3000
2000
1000
0
1
2
3
4
5
6
7
8
9
10
fraction number

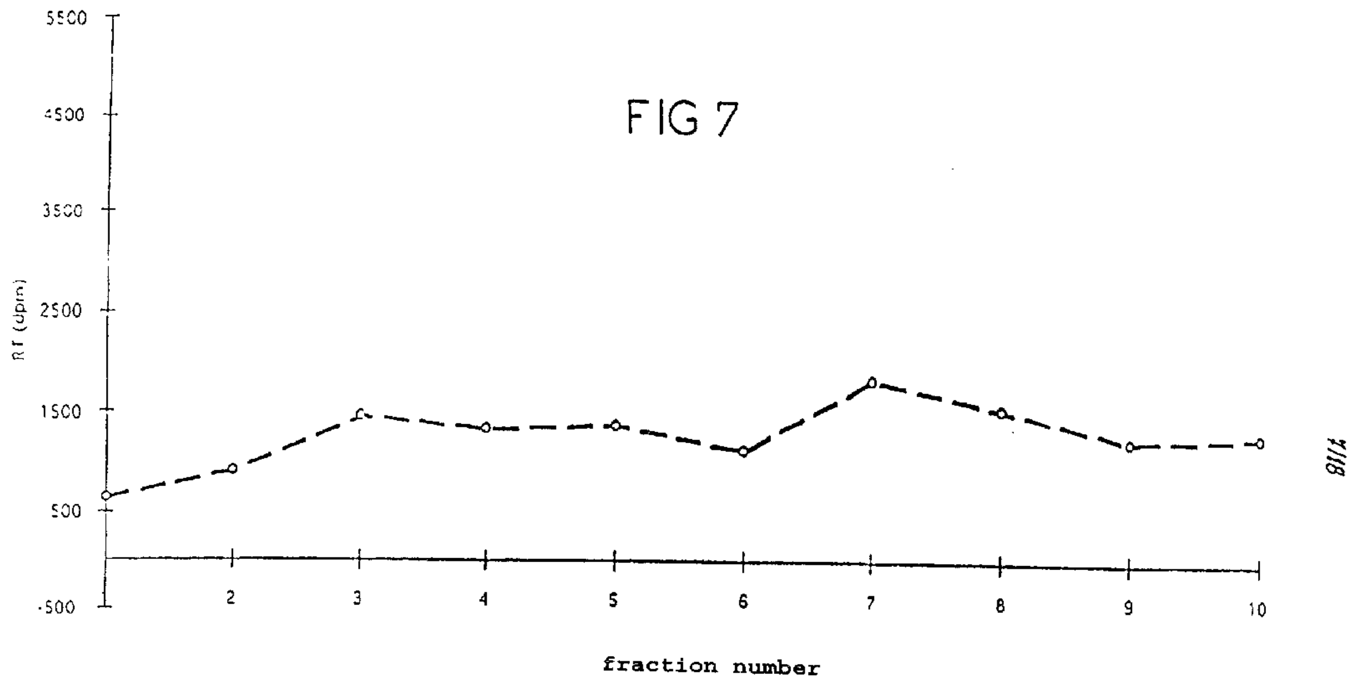


FIG 7
RT (dpm)
5500
4500
3500
2500
1500
500
-500
2
3
4
5
6
7
8
9
10
fraction number

FIG 8

```
CAAGCCACCC AAGAACTCTT AAATTTCCTC ACTACCTGTG GCTACAAGGT    50
TTCCAAACCA AAGGCTCAGC TCTGCTCACA GGAGATTAGA TACTTAGGGT   100
TAAAATTATC CAAAGGCACC AGGGGCCTCA GTGAGGAACG TATCCAGCCT   150
ATACTGGGTT ATCCTCATCC CAAAACCCTA AAGCAACTAA GAGGGTTCCT   200
TAGCATGATC AGGTTTCTGC CGAAAACAAG ATTCCCAGGT ACAACCAAAA   250
TAGCCAGACC ATTATATACA CTAATTAAGG AAACTCAGAA AGCCAATACC   300
TATTTAGTAA GATGGACACC TAAACAGAAG GCTTTCCAGG CCCTAAAGAA   350
GGCCCTAACC CAAGCCCCAG TGTTCAGCTT GCCAACAGGG CAAGATTTTT   400
CTTTATATGG CACAGAAAAA ACAGGAATCG CTCTAGGAGT CCTTACACAG   450
GTCCGAGGGA TGAGCTTGCA ACCCGTGGCA TACCTGAATA AGGAAATTGA   500
TGTAGTGGCA AAGGGTTGGC CTCATNGTTT ATGGGTAATG GNGGCAGTAG   550
CAGTCTNAGT ATCTGAAGCA GTTAAAATAA TACAGGGAAG AGATCTTNCT   600
GTGTGGACAT CTCATGATGT GAACGGCATA CTCACTGCTA AAGGAGACTT   650
GTGGTTGTCA GACAACCATT TACTTAANTA TCAGGCTCTA TTACTTGAAG   700
AGCCAGTGCT GNGACTGCGC ACTTGTGCAA CTCTTAAACC C            741
```

SEQ ID NO9

FIG 9

TCAGGGATAGCCCCCATCTATTTGGCCAGGCATTAGCCCAAGACTTGAGTC
AATTCTCATACCTGGACACTCTTGTCCTTCAGTACATGGATGATTTACTTT
TAGTCGCCCGTTCAGAAACCTTGTGCCATCAAGCCACCCAAGAACTCTTAA
CTTTCCTCACTACCTGTGGCTACAAGGTTTCCAAACCAAAGGCTCGGCTCT
GCTCACAGGAGATTAGATACTNAGGGCTAAAATTATCCAAAGGCACCAGG
GCCCTCAGTGAGGAACGTATCCAGCCTATACTGGCTTATCCTCATCCCAAA
ACCCTAAAGCAACTAAGAGGGTTCCTTGGCATAACAGGTTTCTGCCGAAA
ACAGATTCCCAGGTACASCCCAATAGCCAGACCATTATATACACTAATTA
NGGAAACTCAGAAAGCCAATACCTATTTAGTAAGATGGACACCTACAGAA
GTGGCTTTCCAGGCCCTAAAGAAGGCCCTAACCCAAGCCCCAGTGTTCAGC
TTGCCAACAGGGCAAGATTTTTCTTTATATGCCACAGAAAAAACAGGAAT
AGCTCTAGGAGTCCTTACGCAGGTCTCAGGGATGAGCTTGCAACCCGTGGT
ATACCTGAGTAAGGAAATTGATGTAGTGGCAAAGGGTT

SEQ ID NO 8

FIG 10

SEQ ID NO 2

```
          10        20        30        40        50        60        70
           *         *         *         *         *         *         *
CCC TTT GCC ACT ACA TCA ATT TTA GGA GTA AGG AAA CCC AAC GGA CAG TGG AGG TTA GTG CAA GAA CTC AGG
 P   F   A   T   T   S   I   L   G   V   R   K   P   N   G   Q   W   R   L   V   Q   E   L   R>
___a___a___a___a___a___a___a___a___TRANSLATION OF F11-1 [A]____a___a___a___a___a___a___a___a___>

        80        90       100       110       120       130       140
         *         *         *         *         *         *         *
ATT ATC AAT GAG GCT GTT GTT CCT CTA TAC CCA GCT GTA CCT AAC CCT TAT ACA GTG CTT TCC CAA ATA CCA
 I   I   N   E   A   V   V   P   L   Y   P   A   V   P   N   P   Y   T   V   L   S   Q   I   P>
___a___a___a___a___a___a___a___a___TRANSLATION OF F11-1 [A]____a___a___a___a___a___a___a___a___>

  150       160       170       180       190       200       210
    *         *         *         *         *         *         *
GAG GAA GCA GAG TGG TTT ACA GTC CTG GAC CTT AAG GAT GCC TTT TTC TGC ATC CCT GTA CGT CCT GAC TCT
 E   E   A   E   W   F   T   V   L   D   L   K   D   A   F   F   C   I   P   V   R   P   D   S>
___a___a___a___a___a___a___a___a___TRANSLATION OF F11-1 [A]____a___a___a___a___a___a___a___a___>

  220       230       240       250       260       270       280
    *         *         *         *         *         *         *
CAA TTC TTG TTT GCC TTT GAA GAT CCT TTG AAC CCA ACG TCT CAA CTC ACC TGG ACT GTT|TTA CCC CAA GGG
 Q   F   L   F   A   F   E   D   P   L   N   P   T   S   Q   L   T   W   T   V   L   P   Q   G>
___a___a___a___a___a___a___a___a___TRANSLATION OF F11-1 [A]____a___a___a___a___a___a___a___a___>

290
 *
TTC AAG GGA
 F   K   G>
___a___a___>
```

FIG. 11A

```
        10          20          30          40          50          60          70
         *           *           *           *           *           *           *
CCC TTT GCC ACT ACA TCA ATT TTA GGA GTA AGG AAA CCC AAC GGA CAG TGG AGG TTA GTG CAA GAA CTC AGG
 P   F   A   T   T   S   I   L   G   V   R   K   P   N   G   Q   W   R   L   V   Q   E   L   R>
___a___a___a___a___a___a___a___TRANSLATION OF MSRV-1 POL   (A)___a___a___a___a___a___a___a___>

        80          90         100         110         120         130         140
         *           *           *           *           *           *           *
ATT ATC AAT GAG GCT GTT GTT CCT CTA TAC CCA GCT GTA CCT AAC CCT TAT ACA GTG CTT TCC CAA ATA CCA
 I   I   N   E   A   V   V   P   L   Y   P   A   V   P   N   P   Y   T   V   L   S   Q   I   P>
___a___a___a___a___a___a___a___TRANSLATION OF MSRV-1 POL   (A)___a___a___a___a___a___a___a___>

  150         160         170         180         190         200         210
    *           *           *           *           *           *           *
GAG GAA GCA GAG TGG TTT ACA GTC CTG GAC CTT AAG GAT GCC TTT TTC TGC ATC CCT GTA CGT CCT GAC TCT
 E   E   A   E   W   F   T   V   L   D   L   K   D   A   F   F   C   I   P   V   R   P   D   S>
___a___a___a___a___a___a___a___TRANSLATION OF MSRV-1 POL   (A)___a___a___a___a___a___a___a___>

  220         230         240         250         260         270         280
    *           *           *           *           *           *           *
CAA TTC TTG TTT GCC TTT GAA GAT CCT TTG AAC CCA ACG TCT CAA CTC ACC TGG ACT|GTT TTA CCC CAA GGG|
 Q   F   L   F   A   F   E   D   P   L   N   P   T   S   Q   L   T   W   T | V   L   P   Q   G>|
___a___a___a___a___a___a___a___TRANSLATION OF MSRV-1 POL   (A)___a___a___a___a___a___a___a___>

  290         300         310         320         330         340         350         360
    *           *           *           *           *           *           *           *
TTC AGG GAT AGC CCC CAT CTA TTT GGC CAG GCA TTA GCC CAA GAC TTG AGT CAA TTC TCA TAC CTG GAC ACT
 F   R   D   S   P   F   E   D   P   L   N   P   T   S   Q   L   T   W   T   V   L   P   Q   G>
___a___a___a___a___a___a___a___TRANSLATION OF MSRV-1 POL   (A)___a___a___a___a___a___a___a___>

              370         380         390         400         410         420
    *           *           *           *           *           *           *
CTT GTC CTT CAG|TAC ATG GAT GAT|TTA CTT TTA GTC GCC CGT TCA GAA ACC TTG TGC CAT CAA GCC ACC CAA
 L   V   L   Q | Y   M   D   D | L   L   L   V   A   R   S   E   T   L   C   M   Q   A   T   Q>
___a___a___a___a___a___a___a___TRANSLATION OF MSRV-1 POL   (A)___a___a___a___a___a___a___a___>

       440         450         460         470         480         490         500
         *           *           *           *           *           *           *
GAA CTC TTA ACT TTC CTC ACT ACC TGT GGC TAC AAG GTT TCC AAA CCA AAG GCT CGC CTC TGC TCA CAG GAG
 E   L   L   T   F   L   T   T   C   G   Y   K   V   S   K   F   K   A   K   L   C   I   Q   F>
___a___a___a___a___a___a___a___TRANSLATION OF MSRV-1 POL   (A)___a___a___a___a___a___a___a___>

       510         520         530         540         550         560         570
         *           *           *           *           *           *           *
ATT AGA TAC TNA GGG CTA AAA TTA TCC AAA GGC ACC AGG GCC CTC AGT GAG GAA CGT ATC CAG CCT ATA CTG
 I   R   Y   X   G   L   K   L   S   K   G   T   R   A   L   S   E   E   R   I   Q   F   I   L>
___a___a___a___a___a___a___a___TRANSLATION OF MSRV-1 POL   (A)___a___a___a___a___a___a___a___>

  580         590         600         610         620         630         640
    *           *           *           *           *           *           *
GCT TAT CCT CAT CCC AAA ACC CTA AAG CAA CTA AGA GGG TTC CTT GGC ATA ACA GGT TTC TGC CGA AAA CAG
 A   Y   P   M   P   K   T   L   K   Q   L   K   G   F   L   G   I   T   G   F   C   R   K   Q>
___a___a___a___a___a___a___a___TRANSLATION OF MSRV-1 POL   (A)___a___a___a___a___a___a___a___>

650         660         670         680         690         700         710         720
  *           *           *           *           *           *           *           *
ATT CCC AGG TAC ASC CCA ATA GCC AGA CCA TTA TAT ACA CTA ATT ANG GAA ACT CAG AAA GCC AAT ACC TAT
 I   F   R   Y   X   P   I   A   R   P   L   Y   T   L   I   X   K   T   Q   K   A   M   T   Y>
___a___a___a___a___a___a___a___TRANSLATION OF MSRV-1 POL   (A)___a___a___a___a___a___a___a___>

       730         740         750         760         770         780         790
         *           *           *           *           *           *           *
TTA GTA AGA TGG ACA CCT ACA GAA GTG GCT TTC CAG GCC CTA AAG AAG GCC CTA ACC CAA GCC CCA GTG TTC
 L   V   R   W   T   P   T   E   V   A   F   Q   A   L   K   K   A   L   T   Q   A   P   V   F>
___a___a___a___a___a___a___a___TRANSLATION OF MSRV-1 POL   (A)___a___a___a___a___a___a___a___>
```

FIG. 11B

```
  800          810          820          830          840           850           860
   *            *            *            *            *             *             *
AGC TTG CCA ACA GGG CAA GAT TTT TCT TTA TAT GCC ACA GAA AAA ACA GGA ATA GCT CTA GGA GTC CTT ACG
 S   L   P   T   G   Q   D   F   S   L   Y   A   T   E   K   T   G   I   A   L   G   V   L   T>
___a___a___a___a___a___a___a___TRANSLATION OF MSRV-1 POL   (A)____a___a___a___a___a___a___a___>
  870          860          890          900          910          920          930
   *            *            *            *            *            *            *
CAG GTC TCA GGG ATG AGC TTG CAA CCC GTG GTA TAC CTG AGT AAG GAA ATT GAT GTA GTG GCA AAG GGT TGG
 Q   V   S   G   M   S   L   Q   P   V   V   Y   L   S   K   E   I   D   V   V   A   K   G   W>
___a___a___a___a___a___a___a___TRANSLATION OF MSRV-1 POL   (A)____a___a___a___a___a___a___a___>
  940          950          960          970          980          990          1000
   *            *            *            *            *            *            *
CCT CAT NGT TTA TGG GTA ATG GNG GCA GTA GCA GTC TNA GTA TCT GAA GCA GTT AAA ATA ATA CAG GGA AGA
 P   H   X   L   W   V   M   X   A   V   A   V   X   V   S   E   A   V   K   I   I   Q   G   R>
___a___a___a___a___a___a___a___TRANSLATION OF MSRV-1 POL   (A)____a___a___a___a___a___a___a___>
1010         1020         1030         1040         1050         1060         1070         1080
   *            *            *            *            *            *            *            *
GAT CTT NCT GTG TGG ACA TCT CAT GAT GTG AAC GGC ATA CTC ACT GCT AAA GGA GAC TTG TGG TTG TCA GAC
 D   L   X   V   W   T   S   H   D   V   N   C   I   L   T   A   K   G   D   L   W   L   S   D>
___a___a___a___a___a___a___a___TRANSLATION OF MSRV-1 POL   (A)____a___a___a___a___a___a___a___>
             1090         1100         1110         1120         1130         1140         1150
               *            *            *            *            *            *            *
AAC CAT TTA CTT AAN TAT CAG GCT CTA TTA CTT GAA GAG CCA GTG CTG NGA CTG CGC ACT TGT GCA ACT CTT
 N   H   L   L   X   Y   Q   A   L   L   ??  E   P   V   L   X   L   H   T   C   A   T   L
___a___a___a___a___a___a___a___TRANSLATION OF MSRV-1 POL   (A)____a___a___a___a___a___a___a___>
AAA CCC
 K   P>
___a___>
```

SEQ ID NO 1

FIG 12

SEQ ID NO 12

```
        10             20             30             40
TGCAAGCTTCACCGC TTGCTGGATGTAGGC CTCAGTACCGGNGTG
     50             60             70             80             90
CCCCGCGCGCTGTAG TTCGATGTAGAAAGC GCCCGGAAACACGCG
        100            110            120            130
GGACCAATGCGTCGC CAGCTTGCGCGCCAG CGCCTCGTTGCCATT
     140            150            160            170            180
GGCCAGCGCCACGCC GATATCACCCGCCAT GGCGCCGGAGAGCGC
        190            200            210            220
CAGCAGACCGGCGGC CAGCGGCGCATTCTC AACGCCGGGCTCGTC
     230            240            250            260            270
GAACCATTCGGGGGC GATTTCCGCACGACC GCGATGCTGGTTGGA
        280            290            300            310
GAGCCAGGCCCTGGC CAGCAACTGGCACAG GTTCAGGTAACCCTG
     320            330            340            350            360
CTTGTCCCGCACCAA CAGCAGCAGGCGGGT CGGCTTGTCGCGCTC
        370            380            390            400
GTCGTGATTGGTGAT CCACACGTCAGCCCC GACGATGGGCTTCAC
     410            420            430            440            450
GCCCTTGCCACGCGC TTCCTTGTAGANGCG CACCAGCCCGAAGGC
        460            470            480            490
ATTGGCGAGATCGGT CAGCGCCAAGGCGCC CATGCCATCTTTGGC
     500            510            520            530            540
GGCAGCCTTGACGGC ATCGTCGAGACGGAC ATTGCCATCGACGAC
        550            560            570            580
GGAATATTCGGAGTG GAGACGGAGGTGGAC GAAGCGCGGCGAATT
     590            600            610            620            630
CATCCGCGTATTGTA ACGGGTGACACCTTC CGCAAAGCATTCCGG
        640            650            660            670
ACGTGCCCGATTGAC CCGGAGCAACCCCGC ACGGCTGCGCGGGCA
     680            690            700            710            720
GTTATAATTTCGGCT TACGAATCAACGGGT TACCCCAGGGCGCTG
        730            740
AAGCCTATCGGGTGC AGTTCCCGGATGC
```

5 11 99 17114

FIG. 13A

```
        10             20             30             40             50             60             70             80             90
         *              *              *              *              *              *              *              *              *
TGC AAG CTT CAC CGC TTG CTG GAT GTA GGC CTC AGT ACC GGN GTG CCC CGC GCO CTO TAG TTC GAT GTA GAA AGC GCC CGG AAA CAC GCG GGA CCA
 C   K   L   H   R   L   L   D   V   G   L   S   T   G   V   P   R   A   L   *   F   D   V   E   S   A   R   K   H   A   G   P>
___a___a___a___a___a___a___a___a___a___a___a___a_TRANSLATION OF MSRV-2EL1 (A)__a___a___a___a___a___a___a___a___a___a___a___a___>
 A   S   F   T   A   C   W   M   *   A   S   V   P   X   C   P   A   R   C   S   S   M   *   K   A   P   G   N   T   R   D   Q>
___b___b___b___b___b___b___b___b___b___b___b___b_TRANSLATION OF MSRV-2EL1 (B)__b___b___b___b___b___b___b___b___b___b___b___b___>
   Q   A   S   P   L   A   G   C   R   P   Q   Y   R   X   A   P   R   A   V   V   R   C   R   K   R   P   E   T   R   G   T>
___c___c___c___c___c___c___c___c___c___c___c___c_TRANSLATION OF MSRV-2EL1 (C)__c___c___c___c___c___c___c___c___c___c___c___c___>
<C   A   E   G   S   A   P   H   L   G   *   Y   R   X   A   G   R   A   T   T   R   H   L   F   R   G   S   V   R   P   V   L
<__d___d___d___d___d___d___d___d___d___d___d___d_TRANSLATION OF MSRV-2EL1 (D)__d___d___d___d___d___d___d___d___d___d___d___d___
 <A   L   K   V   A   Q   Q   I   Y   A   E   T   G   X   H   G   A   R   Q   L   E   I   Y   F   A   G   P   F   V   R   S   W
<__e___e___e___e___e___e___e___e___e___e___e___e_TRANSLATION OF MSRV-2EL1 (E)__e___e___e___e___e___e___e___e___e___e___e___e___
  <L   S   *   R   K   S   S   T   P   R   L   V   P   T   G   R   A   S   Y   N   S   T   S   L   A   R   F   C   A   P   G
<__f___f___f___f___f___f___f___f___f___f___f___f_TRANSLATION OF MSRV-2EL1 (F)__f___f___f___f___f___f___f___f___f___f___f___f___

100            110            120            130            140            150            160            170            180            190
 *              *              *              *              *              *              *              *              *              *
ATG CGT CGC CAG CTT GCG CGC CAG CGC CTC GTT GCC ATT GGC CAG CGC CAC GCC GAT ATC ACC CGC CAT GGC GCC GGA GAG CGC CAG CAG ACC GGC
 M   R   R   Q   L   A   R   Q   R   L   V   A   I   G   Q   R   H   A   D   I   T   R   H   G   A   G   E   R   Q   Q   T   G>
___a___a___a___a___a___a___a___a___a___a___a___a_TRANSLATION OF MSRV-2EL1 (A)__a___a___a___a___a___a___a___a___a___a___a___a___>
 C   V   A   S   L   R   A   S   A   S   L   P   L   A   S   A   T   P   I   S   P   A   M   A   P   E   S   A   S   R   P   A>
___b___b___b___b___b___b___b___b___b___b___b___b_TRANSLATION OF MSRV-2EL1 (B)__b___b___b___b___b___b___b___b___b___b___b___b___>
N   A   S   P   A   C   A   P   A   P   R   C   H   W   P   A   P   R   R   Y   H   P   P   W   R   R   R   A   P   A   D   R>
___c___c___c___c___c___c___c___c___c___c___c___c_TRANSLATION OF MSRV-2EL1 (C)__c___c___c___c___c___c___c___c___c___c___c___c___>
 <A   D   G   A   Q   A   G   A   G   R   Q   W   Q   G   A   G   R   R   Y   *   G   G   H   R   R   L   A   G   A   S   R   R
<__d___d___d___d___d___d___d___d___d___d___d___d_TRANSLATION OF MSRV-2EL1 (D)__d___d___d___d___d___d___d___d___d___d___d___d___
 <H   T   A   L   K   R   A   L   A   E   N   G   N   A   L   A   V   G   I   D   G   A   M   A   G   S   L   A   L   L   G   A
<__e___e___e___e___e___e___e___e___e___e___e___e_TRANSLATION OF MSRV-2EL1 (E)__e___e___e___e___e___e___e___e___e___e___e___e___
<I   R   R   W   S   A   R   W   R   R   T   A   M   P   W   R   W   A   S   I   V   R   W   P   A   P   S   R   W   C   V   P
<__f___f___f___f___f___f___f___f___f___f___f___f_TRANSLATION OF MSRV-2EL1 (F)__f___f___f___f___f___f___f___f___f___f___f___f___
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5 11 99 17114

FIG. 13B

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         200        210           220           230           240           250           260           270           280
          *          *             *             *             *             *             *             *             *
GGC CAG CGG CGC ATT CTC AAC GCC GGG CTC GTC GAA CCA TTC GGG GGC GAT TTC CGC ACG ACC GCG ATG CTG GTT GGA GAG CCA GGC CCT GGC CAG
 G   Q   R   R   I   L   N   A   G   L   V   E   P   F   G   G   D   F   R   T   T   A   M   L   V   G   E   P   G   F   G   Q>
___a___a___a___a___a___a___a___a___a___a___a___a_TRANSLATION OF MSRV-2EL1 (A)__a___a___a___a___a___a___a___a___a___a___a___a___>
  A   S   G   A   F   S   T   P   G   S   S   N   H   S   G   A   I   S   A   R   P   R   C   W   L   E   S   Q   A   L   A   S>
___b___b___b___b___b___b___b___b___b___b___b___b_TRANSLATION OF MSRV-2EL1 (B)__b___b___b___b___b___b___b___b___b___b___b___b___>
R   P   A   A   H   S   Q   R   R   A   R   R   T   I   R   G   R   F   P   H   D   R   D   A   G   W   R   A   R   P   W   P>
___c___c___c___c___c___c___c___c___c___c___c___c_TRANSLATION OF MSRV-2EL1 (C)__c___c___c___c___c___c___c___c___c___c___c___c___>
 <G   A   A   C   E   *   R   R   A   R   R   V   M   R   P   R   N   G   C   S   R   S   A   P   Q   L   A   L   G   Q   G   A
___d___d-__d___d___d___d___d___d___d___d___d___d_TRANSLATION OF MSRV-2EL1 (D)__d___d___d___d___d___d___d___d___d___d___d___d___
 <A   L   P   A   N   E   V   G   P   E   D   F   W   E   P   A   I   E   A   R   G   R   H   Q   N   S   L   W   A   R   A   L
<__e___e___e___e___e___e___e___e___e___e___e___e_TRANSLATION OF MSRV-2EL1 (E)__e___e___e___e___e___e___e___e___e___e___e___e___
<P  W   R   R   M   R   L   A   P   S   T   S   G   N   P   P   S   K   R   V   V   A   I   S   T   P   S   G   P   G   P   W
___f___f___f___f___f___f___f___f___f___f___f___f_TRANSLATION OF MSRV-2EL1 (F)__f___f___f___f___f___f___f___f___f___f___f___f___

290         300           310           320           330           340           350           360           370           380
 *           *             *             *             *             *             *             *             *             *
CAA CTG GCA CAG GTT CAG GTA ACC CTG CTT GTC CCG CAC CAA CAG CAG CAG GCG GGT CGG CTT GTC GCG CTC GTC GTG ATT GGT GAT CCA CAC GTC
 Q   L   A   Q   V   Q   V   T   L   L   V   P   H   Q   Q   Q   Q   A   G   R   L   V   A   L   V   V   I   G   D   P   H   V>
___a___a___a___a___a___a___a___a___a___a___a___a_TRANSLATION OF MSRV-2EL1 (A)__a___a___a___a___a___a___a___a___a___a___a___a___>
  N   W   H   R   F   R   *   P   C   L   S   R   T   N   S   S   R   R   V   G   L   S   R   S   S   *   L   V   I   H   T   S>
___b___b___b___b___b___b___b___b___b___b___b___b_TRANSLATION OF MSRV-2EL1 (B)__b___b___b___b___b___b___b___b___b___b___b___b___>
A   T   G   T   G   S   G   N   P   A   C   P   A   P   T   A   A   G   G   S   A   C   R   A   R   R   D   W   *   S   T   R>
___c___c___c___c___c___c___c___c___c___c___c___c_TRANSLATION OF MSRV-2EL1 (C)__c___c___c___c___c___c___c___c___c___c___c___c___>
 <V   ?   V   P   E   P   L   G   A   Q   G   A   G   V   A   A   P   P   D   A   Q   R   A   R   R   S   Q   H   D   V   R   *
<__d___d___d___d___d___d___d___d___d___d___d___d_TRANSLATION OF MSRV-2EL1 (D)__d___d___d___d___d___d___d___d___d___d___d___d___
 <L   Q   C   L   N   L   Y   G   Q   K   D   R   V   L   L   L   L   R   T   P   K   D   R   E   D   H   N   T   I   W   V   D
<__e___e___e___e___e___e___e___e___e___e___e___e_TRANSLATION OF MSRV-2EL1 (E)__e___e___e___e___e___e___e___e___e___e___e___e___
<C  S   A   C   T   *   T   V   R   S   T   G   C   W   C   C   C   A   P   R   S   T   A   S   T   T   I   P   S   G   C   T
<__f___f___f___f___f___f___f___f___f___f___f___f_TRANSLATION OF MSRV-2EL1 (F)__f___f___f___f___f___f___f___f___f___f___f___f___
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5 11 99 17114

FIG. 13C

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    390        400        410        420        430    440        450        460        470        480
     *          *          *          *          *      *          *          *          *          *
AGC CCC GAC GAT GGG CTT CAC GCC CTT GCC ACG CGC TTC CTT GTA GAN GCG CAC CAG CCC GAA GGC ATT GGC GAG ATC GGT CAG CGC CAA GGC GCC
 S   P   D   D   G   L   H   A   L   A   T   R   F   L   V   X   A   H   Q   P   E   G   I   G   E   I   G   Q   R   Q   G   A>
___a___a___a___a___a___a___a___a___a___a___a___a_TRANSLATION OF MSRV-2EL1 (A)__a___a___a___a___a___a___a___a___a___a___a___a___>
 A   P   T   M   G   F   T   P   L   P   R   A   S   L   *   X   R   T   S   P   K   A   L   A   R   S   V   S   A   K   A   P>
___b___b___b___b___b___b___b___b___b___b___b___b_TRANSLATION OF MSRV-2EL1 (B)__b___b___b___b___b___b___b___b___b___b___b___b___>
Q   P   R   R   W   A   S   R   P   C   H   A   L   P   C   R   X   A   P   A   R   R   H   W   R   D   R   S   A   P   R   R>
___c___c___c___c___c___c___c___c___c___c___c___c_TRANSLATION OF MSRV-2EL1 (C)__c___c___c___c___c___c___c___c___c___c___c___c___>
 <G  R   R   H   A   E   R   G   Q   W   A   S   G   Q   L   X   A   G   A   R   L   C   Q   R   S   R   D   A   G   L   R   G
<__d___d___d___d___d___d___d___d___d___d___d___d_TRANSLATION OF MSRV-2EL1 (D)__d___d___d___d___d___d___d___d___d___d___d___d___
 <A  G   V   I   P   K   V   G   K   G   R   A   E   K   Y   X   R   V   L   G   F   A   N   A   L   D   T   L   A   L   A   G
<__e___e___e___e___e___e___e___e___e___e___e___e_TRANSLATION OF MSRV-2EL1 (E)__e___e___e___e___e___e___e___e___e___e___e___e___
<L   G   S   S   P   S   *   A   R   A   V   R   K   R   T   S   A   C   W   G   S   P   M   P   S   I   P   *   R   W   P   A
<__f___f___f___f___f___f___f___f___f___f___f___f_TRANSLATION OF MSRV-2EL1 (F)__f___f___f___f___f___f___f___f___f___f___f___f___

         490        500        510        520        530        540        550        560        570
          *          *          *          *          *          *          *          *          *
CAT GCC ATC TTT GGC GGC AGC CTT GAC GGC ATC GTC GAG ACG GAC ATT GCC ATC GAC GAC GGA ATA TTC GGA GTG GAG ACG GAG GTG GAC GAA GCG
 H   A   I   F   G   G   S   L   D   G   I   V   E   T   D   I   A   I   D   D   G   I   F   G   V   E   T   E   V   D   E   A>
___a___a___a___a___a___a___a___a___a___a___a___a_TRANSLATION OF MSRV-2EL1 (A)__a___a___a___a___a___a___a___a___a___a___a___a___>
 M   P   S   L   A   A   A   L   T   A   S   S   R   R   T   L   P   S   T   T   E   Y   S   E   W   R   R   R   W   T   K   R>
___b___b___b___b___b___b___b___b___b___b___b___b_TRANSLATION OF MSRV-2EL1 (B)__b___b___b___b___b___b___b___b___b___b___b___b___>
P   C   H   L   W   R   Q   P   *   R   H   R   R   D   G   H   C   H   R   R   R   N   I   R   S   G   D   G   G   R   S>
___c___c___c___c___c___c___c___c___c___c___c___c_TRANSLATION OF MSRV-2EL1 (C)__c___c___c___c___c___c___c___c___c___c___c___c___>
 <H  W   R   Q   R   C   G   Q   R   C   R   R   S   P   C   Q   W   R   R   R   F   I   R   L   P   S   P   P   P   R   L   A
___d___d___d___d___d___d___d___d___d___d___d___d_TRANSLATION OF MSRV-2EL1 (D)__d___d___d___d___d___d___d___d___d___d___d___d___
 <M  G   D   K   A   A   A   K   V   A   D   D   L   R   V   N   G   D   V   V   S   Y   E   S   H   L   R   L   H   V   F   R
<__e___e___e___e___e___e___e___e___e___e___e___e_TRANSLATION OF MSRV-2EL1 (E)__e___e___e___e___e___e___e___e___e___e___e___e___
<W   A   M   K   P   P   L   R   S   P   M   T   S   V   S   M   A   M   S   S   P   I   N   P   T   S   V   S   T   S   S   A
___f___f___f___f___f___f___f___f___f___f___f___f_TRANSLATION OF MSRV-2EL1 (F)__f___f___f___f___f___f___f___f___f___f___f___f___
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FIG. 13D

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580          590           600          610          620           630          640          650          660          670
   *            *            *            *            *            *            *            *            *            *
CGC CGA ATT CAT CCG CGT ATT GTA ACG GGT GAC ACC TTC CGC AAA GCA TTC CGG ACG TGC CCG ATT GAC CCG GAG CAA CCC CGC ACG GCT GCG CGG
 R   R   I   H   P   R   I   V   T   G   D   T   F   R   K   A   F   R   T   C   P   I   D   P   E   Q   P   R   T   A   A   P>
___a___a___a___a___a___a___a___a___a___a___a___a_TRANSLATION OF MSRV-2EL1 (A)__a___a___a___a___a___a___a___a___a___a___a___a___>
  G   E   F   I   R   V   L   *   R   V   T   P   S   A   K   H   S   G   R   A   R   L   T   R   S   N   P   A   R   L   R   G>
___b___b___b___b___b___b___b___b___b___b___b___b_TRANSLATION OF MSRV-2EL1 (B)__b___b___b___b___b___b___b___b___b___b___b___b___>
A   A   N   S   S   A   Y   C   N   G   *   H   L   P   Q   S   I   P   D   V   P   D   *   P   G   A   T   P   H   G   C   A>
___c___c___c___c___c___c___c___c___c___c___c___c_TRANSLATION OF MSRV-2EL1 (C)__c___c___c___c___c___c___c___c___c___c___c___c___>
 <A   F   E   D   A   Y   Q   L   P   H   C   R   G   C   L   M   G   S   T   G   S   Q   G   P   A   V   G   C   P   Q   A   P
<__d___d___d___d___d___d___d___d___d___d___d___d_TRANSLATION OF MSRV-2EL1 (D)__d___d___d___d___d___d___d___d___d___d___d___d___
 <P   S   N   M   R   T   N   Y   R   T   V   G   E   A   F   C   E   P   R   A   R   N   V   R   L   L   G   A   R   S   R   P
<__e___e___e___e___e___e___e___e___e___e___e___e_TRANSLATION OF MSRV-2EL1 (E)__e___e___e___e___e___e___e___e___e___e___e___e___
<R   R   I   *   G   R   I   T   V   P   S   V   K   R   L   A   N   R   V   H   G   I   S   G   S   C   G   R   V   A   A   R
<__f___f___f___f___f___f___f___f___f___f___f___f_TRANSLATION OF MSRV-2EL1 (F)__f___f___f___f___f___f___f___f___f___f___f___f___

    680          690           700          710          720     730          740
       *            *            *            *            *            *            *
GCA GTT ATA ATT TCG GCT TAC GAA TCA ACG GGT TAC CCC ACG GCG CTG AAG CCT ATC GCG TGC AGT TGC CGC ATG C
 A   V   I   I   S   A   Y   E   S   T   G   Y   P   R   A   L   K   P   I   A   C   S   C   R   M   X>
___a___a___a___a___a___a___a___a____TRANSLATION OF MSRV-2EL1 (A)___a___a___a___a___a___a___a___a___a_>
  Q   L   *   F   R   L   T   N   Q   R   V   T   P   G   R   *   S   L   S   R   A   V   A   G   C>
___b___b___b___b___b___b___b___b____TRANSLATION OF MSRV-2EL1 (B)___b___b___b___b___b___b___b___b___b_>
G   S   Y   N   F   G   L   R   I   N   G   L   P   Q   G   A   E   A   Y   R   V   Q   L   P   D   A
___c___c___c___c___c___c___c___c____TRANSLATION OF MSRV-2EL1 (C)___c___c___c___c___c___c___c___c___c_>
 <L   *   L   K   P   K   R   I   L   P   N   G   W   P   A   S   A   *   R   T   C   N   G   S   A
<__d___d___d___d___d___d___d___d____TRANSLATION OF MSRV-2EL1 (D)___d___d___d___d___d___d___d___d___d_
 <C   N   Y   N   R   S   V   F   *   R   T   V   G   P   R   Q   L   R   D   R   A   T   A   P   H
<__e___e___e___e___e___e___e___e____TRANSLATION OF MSRV-2EL1 (E)___e___e___e___e___e___e___e___e___
<A   T   I   I   E   A   *   S   D   V   P   *   G   L   A   S   F   G   I   A   H   L   Q   R   I
<__f___f___f___f___f___f___f___f____TRANSLATION OF MSRV-2EL1 (F)___f___f___f___f___f___f___f___f___
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SEQ ID NO 12

FIG 13

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290       300       310       320       330       340       350       360       370       380
CAA CTG GCA CAG GTT CAG GTA ACC CTG CTT GTC CCG CAC CAA CAG CAG CAG GCG GGT CGG CTT GTC GCG CTC GTC GTG ATT GGT GAT CCA CAC GTC
 Q   L   A   Q   V   Q   V   T   L   L   V   P   H   Q   Q   Q   Q   A   G   R   L   V   A   L   V   V   I   G   D   P   H   V>
___a___a___a___a___a___a___a___a___a___a___a___a_TRANSLATION OF MSRV-2EL1 [A]__a___a___a___a___a___a___a___a___a___a___a___a___>
 N   W   H   R   F   R   *   P   C   L   S   R   T   N   S   S   R   R   V   G   L   S   R   S   S   *   L   V   I   H   T   S>
___b___b___b___b___b___b___b___b___b___b___b___b_TRANSLATION OF MSRV-2EL1 [B]__b___b___b___b___b___b___b___b___b___b___b___b___>
 A   T   G   T   G   S   G   N   P   A   C   P   A   P   T   A   A   G   G   S   A   C   R   A   R   R   D   W   *   S   T   R>
___c___c___c___c___c___c___c___c___c___c___c___c_TRANSLATION OF MSRV-2EL1 [C]__c___c___c___c___c___c___c___c___c___c___c___c___>
<V   P   V   P   E   P   L   G   A   Q   G   A   G   V   A   A   P   P   D   A   Q   R   A   R   R   S   Q   H   D   V   R   *
<___d___d___d___d___d___d___d___d___d___d___d___d_TRANSLATION OF MSRV-2EL1 [D]__d___d___d___d___d___d___d___d___d___d___d___d___
<L   Q   C   L   N   L   Y   G   Q   K   D   R   V   L   L   L   L   R   T   P   K   D   R   E   D   H   N   T   I   W   V   D
<___e___e___e___e___e___e___e___e___e___e___e___e_TRANSLATION OF MSRV-2EL1 [E]__e___e___e___e___e___e___e___e___e___e___e___e___
<?   S   A   C   T   *   T   V   R   S   T   G   C   W   C   C   C   A   P   R   S   T   A   S   T   T   I   P   S   G   C   T
<___f___f___f___f___f___f___f___f___f___f___f___f_TRANSLATION OF MSRV-2EL1 [F]__f___f___f___f___f___f___f___f___f___f___f___f___

  390       400       410       420       430       440       450       460       470       480
AGC CCC GAC GAT GGG CTT CAC GCC CTT GCC ACG CGC TTC CTT GTA GAN GCG CAC CAG CCC GAA GGC ATT GGC GAG ATC GGT CAG CGC CAA GGC GCC
 S   P   D   D   G   L   H   A   L   A   T   R   F   L   V   X   A   H   Q   P   E   G   I   G   E   I   G   Q   R   Q   G   A>
___a___a___a___a___a___a___a___a___a___a___a___a_TRANSLATION OF MSRV-2EL1 [A]__a___a___a___a___a___a___a___a___a___a___a___a___>
 A   P   T   M   G   F   T   P   L   P   R   A   S   L   *   X   R   T   S   P   K   A   L   A   R   S   V   S   A   X   A   P>
___b___b___b___b___b___b___b___b___b___b___b___b_TRANSLATION OF MSRV-2EL1 [B]__b___b___b___b___b___b___b___b___b___b___b___b___>
 Q   P   R   R   W   A   S   R   P   C   H   A   L   P   C   R   X   A   P   A   R   R   H   W   R   D   R   S   A   P   R   R>
___c___c___c___c___c___c___c___c___c___c___c___c_TRANSLATION OF MSRV-2EL1 [C]__c___c___c___c___c___c___c___c___c___c___c___c___>
<G   R   R   H   A   E   R   G   Q   W   A   S   G   Q   L   X   A   G   A   R   L   C   Q   R   S   R   D   A   G   L   R   G
<___d___d___d___d___d___d___d___d___d___d___d___d_TRANSLATION OF MSRV-2EL1 [D]__d___d___d___d___d___d___d___d___d___d___d___d___
<A   G   V   I   P   K   V   G   K   G   R   A   E   K   Y   X   R   V   L   G   F   A   N   A   L   D   T   L   A   L   A   G
<___e___e___e___e___e___e___e___e___e___e___e___e_TRANSLATION OF MSRV-2EL1 [E]__e___e___e___e___e___e___e___e___e___e___e___e___
<L   G   S   S   P   S   *   A   R   A   V   R   X   R   T   S   A   C   W   G   S   P   M   P   S   I   P   *   R   W   P   A
<___f___f___f___f___f___f___f___f___f___f___f___f_TRANSLATION OF MSRV-2EL1 [F]__f___f___f___f___f___f___f___f___f___f___f___f___

        490       500       510       520       530       540       550       560       570
CAT GCC ATC TTT GGC GGC AGC CTT GAC GGC ATC GTC GAG ACG GAC ATT GCC ATC GAC GAC GGA ATA TTC GGA GTG GAG ACG GAG GTG GAC GAA GCG
 H   A   I   F   G   G   S   L   D   G   I   V   E   T   D   I   A   I   D   D   G   I   F   G   V   E   T   E   V   D   E   A>
___a___a___a___a___a___a___a___a___a___a___a___a_TRANSLATION OF MSRV-2EL1 [A]__a___a___a___a___a___a___a___a___a___a___a___a___>
 H   P   S   L   A   A   A   L   T   A   S   S   R   R   T   L   P   S   T   T   E   Y   S   E   W   R   R   R   W   T   K   P>
___b___b___b___b___b___b___b___b___b___b___b___b_TRANSLATION OF MSRV-2EL1 [B]__b___b___b___b___b___b___b___b___b___b___b___b___>
 P   C   H   L   W   R   Q   P   *   R   H   R   R   D   G   H   C   H   R   R   R   N   I   R   S   G   D   G   G   G   R   S>
___c___c___c___c___c___c___c___c___c___c___c___c_TRANSLATION OF MSRV-2EL1 [C]__c___c___c___c___c___c___c___c___c___c___c___c___>
<H   W   R   Q   R   C   G   Q   R   C   R   R   S   P   C   Q   W   R   R   R   F   I   R   L   P   S   P   P   P   R   L   A
<___d___d___d___d___d___d___d___d___d___d___d___d_TRANSLATION OF MSRV-2EL1 [D]__d___d___d___d___d___d___d___d___d___d___d___d___
<M   G   D   K   A   A   A   X   V   A   D   D   L   R   V   N   G   D   V   V   S   Y   E   S   H   L   R   L   H   V   F   R
<___e___e___e___e___e___e___e___e___e___e___e___e_TRANSLATION OF MSRV-2EL1 [E]__e___e___e___e___e___e___e___e___e___e___e___e___
<W   A   M   K   P   P   L   R   S   P   H   T   S   V   S   M   A   M   S   S   P   I   N   P   T   S   V   S   T   S   S   A
<___f___f___f___f___f___f___f___f___f___f___f___f_TRANSLATION OF MSRV-2EL1 [F]__f___f___f___f___f___f___f___f___f___f___f___f___
 580       590       600       610       620       630       640       650       660       670
```

FIG 13

```
CGG CGA ATT CAT CCG CGT ATT GTA ACG GGT GAC ACC TTC CGC AAA GCA TTC CGG ACG TGC CCG ATT GAC CCG GAG CAA CCC CGC ACG GCT GCG CGG
 R   R   I   H   P   R   I   V   T   G   D   T   F   R   K   A   F   R   T   C   P   I   D   P   E   Q   P   R   T   A   A   P>
___a___a___a___a___a___a___a___a___a___a___a___a_TRANSLATION OF MSRV-2EL1 (A)__a___a___a___a___a___a___a___a___a___a___a___a___>
  G   E   F   I   R   V   L   *   R   V   T   P   S   A   K   H   S   G   R   A   R   L   T   R   S   N   P   A   R   L   R   G>
___b___b___b___b___b___b___b___b___b___b___b___b_TRANSLATION OF MSRV-2EL1 (B)__b___b___b___b___b___b___b___b___b___b___b___b___>
A   A   N   S   S   A   Y   C   N   G   *   H   L   P   Q   S   I · P   D   V   P   D   *   P   G   A   T   P   H   G   C   A>
___c___c___c___c___c___c___c___c___c___c___c___c_TRANSLATION OF MSRV-2EL1 (C)__c___c___c___c___c___c___c___c___c___c___c___c___>
 <A   F   E   D   A   Y   Q   L   P   H   C   R   Q   C   L   M   Q   S   T   Q   S   Q   G   P   A   V   G   C   P   Q   A   P
<___d___d___d___d___d___d___d___d___d___d___d___d_TRANSLATION OF MSRV-2EL1 (D)__d___d___d___d___d___d___d___d___d___d___d___d___
<P   S   N   M   R   T   N   Y   R   T   V   G   E   A   F   C   E   P   R   A   R   N   V   R   L   L   G   A   R   S   R   P
<___e___e___e___e___e___e___e___e___e___e___e___e_TRANSLATION OF MSRV-2EL1 (E)__e___e___e___e___e___e___e___e___e___e___e___e___
<R   R   I   *   G   R   I   T   V   P   S   V   K   R   L   A   N   R   V   H   Q   I   S   Q   S   C   G   R   V   A   A   R
<___f___f___f___f___f___f___f___f___f___f___f___f_TRANSLATION OF MSRV-2EL1 (F)__f___f___f___f___f___f___f___f___f___f___f___f___

         680         690         700         710         720         730         740
          *           *           *           *           *           *           *
GCA GTT ATA ATT TCG GCT TAC GAA TCA ACG GGT TAC CCC AGG GCG CTG AAG CCT ATC GCG TGC AGT TGC CGG ATG C
 A   V   I   I   S   A   Y   E   S   T   G   Y   P   R   A   L   K   P   I   A   C   S   C   R   M   X>
___a___a___a___a___a___a___a___a____TRANSLATION OF MSRV-2EL1 (A)___a___a___a___a___a___a___a___a___a_>
  Q   L   *   F   R   L   T   N   Q   R   V   T   P   Q   R   *   S   L   S   R   A   V   A   Q   C>
___b___b___b___b___b___b___b___b____TRANSLATION OF MSRV-2EL1 (B)___b___b___b___b___b___b___b___b___b_>
G   S   Y   N   F   G   L   R   I   N   Q   L   P   Q   G   A   E   A   Y   R   V   Q   L   P   D   A>
___c___c___c___c___c___c___c___c____TRANSLATION OF MSRV-2EL1 (C)___c___c___c___c___c___c___c___c___c_>
 <L   *   L   K   P   K   R   I   L   P   N   G   W   P   A   S   A   *   R   T   C   N   Q   S   A
<___d___d___d___d___d___d___d___d____TRANSLATION OF MSRV-2EL1 (D)___d___d___d___d___d___d___d___d___d_
<C   N   Y   N   R   S   V   F   *   R   T   V   G   P   R   Q   L   R   D   R   A   T   A   P   H
<___e___e___e___e___e___e___e___e___TRANSLATION OF MSRV-2EL1 (E)___e___e___e___e___e___e___e___e___
<n   I   I   I   E   A   *   S   D   V   P   *   G   L   A   S   F   G   I   A   H   L   Q   R   I
<___f___f___f___f___f___f___f___f__TRANSLATION OF MSRV-2EL1 (F)_f___f___f___f___f___f___f___f___f_
```

FIG 14

```
                   10         20          30         40         50          60         70         80         90
                    .          .           .          .          .           .          .          .          .
MSRV-2A     TGGAAAGTGTTGCCA CAGGGCGCTGAAGCC TATCGCGTGCAGTTG CCGGATGCCGCCTAT AGCCTCTACATGGAT GACATCCTGCTGGCC TCC

              700       710        720          730        740
            __cAAcGgGTTaCCc CAGGGCGCTGAAGCC TATCGCGTGCAGTTG CCGGATGC
              ** * *** **   *************** *************** ********
MSRV-2A       GAAAGTGTTGCCA CAGGGCGCTGAAGCC TATCGCGTGCAGTTG CCGGATGC
```

FIG 15

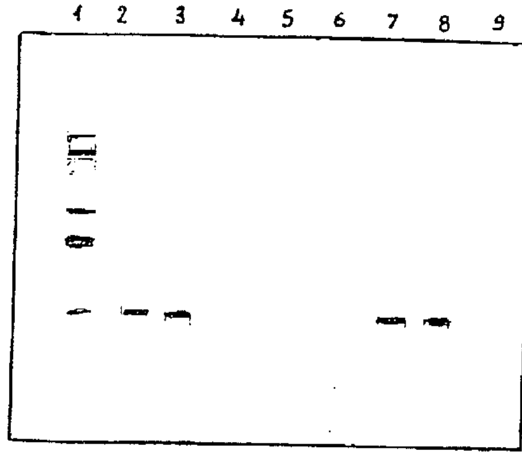

FIG 18

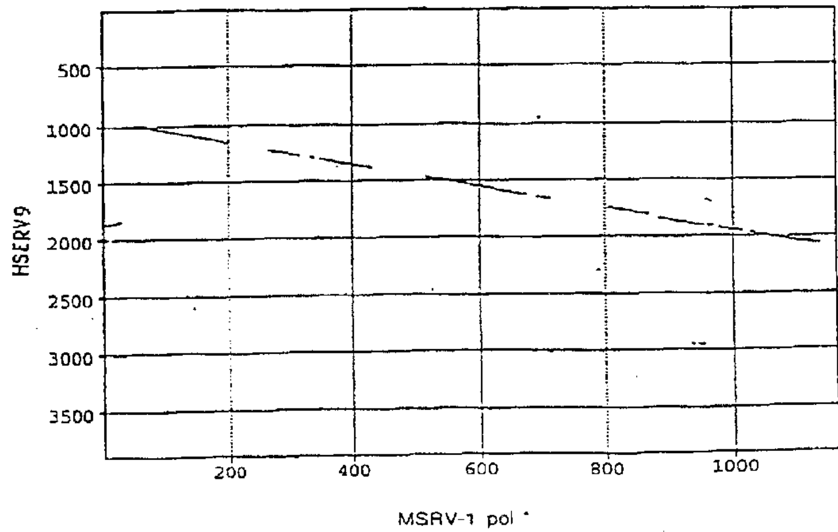

FIG 16

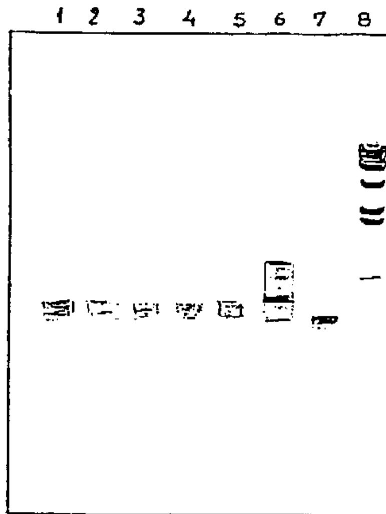

FIG 17