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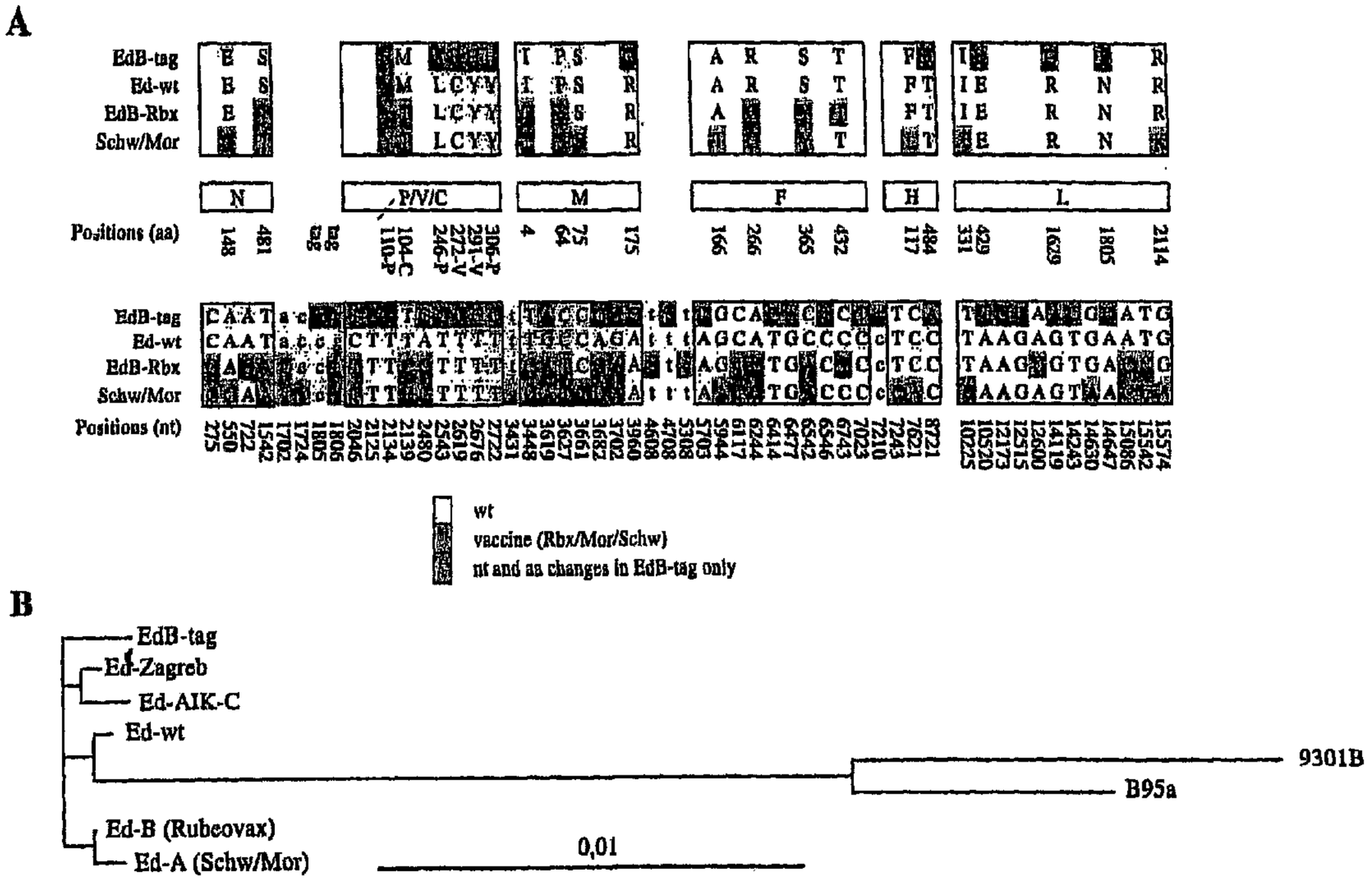





(57) Abrégé/Abstract:
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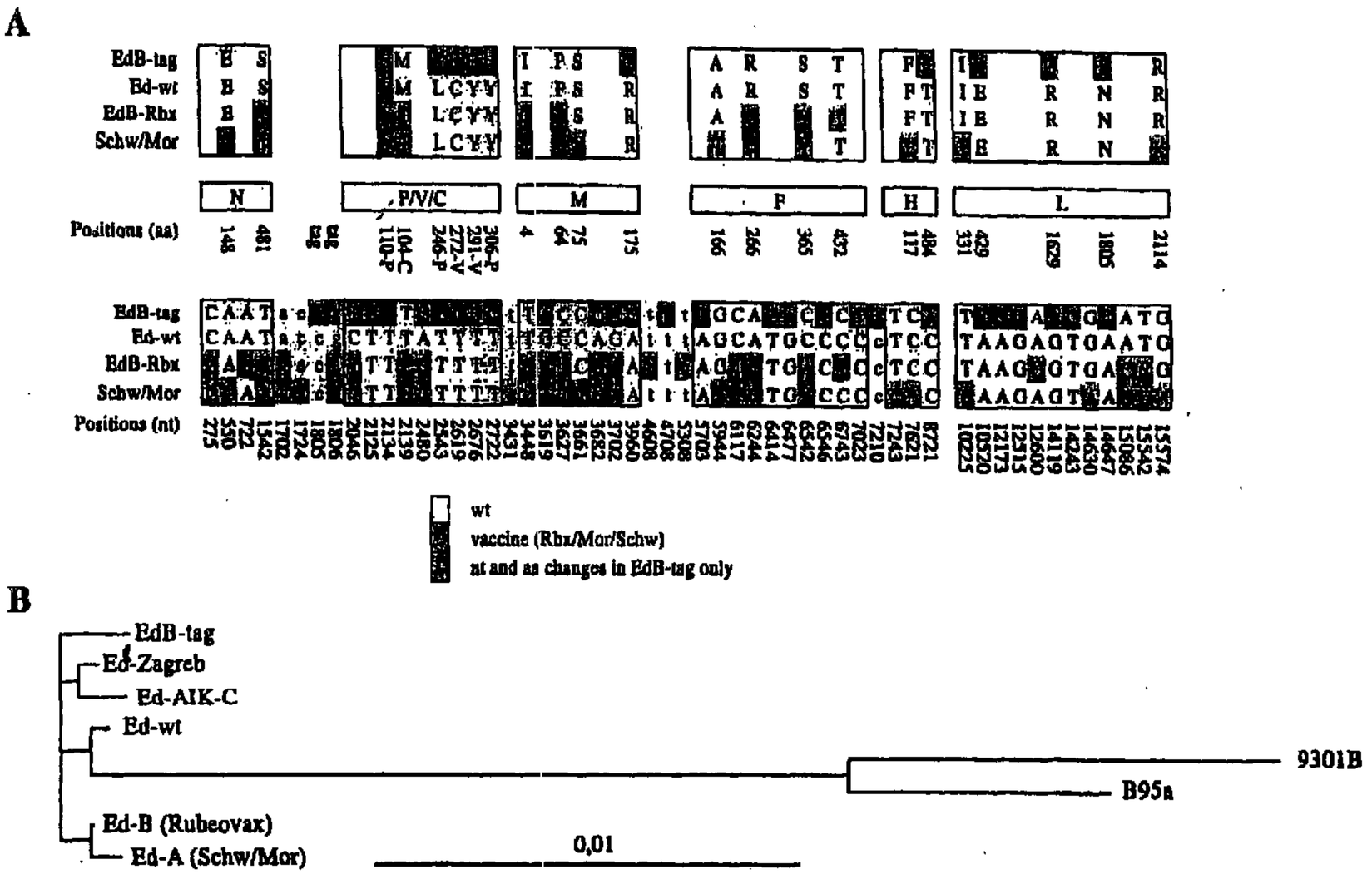
(54) Title: INFECTIOUS cDNA OF AN APPROVED VACCINE STRAIN OF MEASLES VIRUS, USE FOR IMMUNOGENIC COMPOSITIONS




(57) Abstract: The invention relates to a cDNA molecule which encodes the nucleotide sequence of the full length antigenomic (+)RNA strand of a measles virus (MV) originating from an approved vaccine strain. It also concerns the preparation of immunogenic compositions using said cDNA.

WO 2004/000876 A1

WO 2004/000876 A1

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# INFECTIOUS cDNA OF AN APPROVED VACCINE STRAIN OF MEASLES VIRUS. USE FOR IMMUNOGENIC COMPOSITIONS.

Measles virus is a member of the order *mononegavirales*, i.e., viruses with a non-segmented negative-strand RNA genome. The non segmented genome of measles virus (MV) has an antimessage polarity which results in a genomic RNA which is neither translated *in vivo* or *in vitro* nor infectious when purified.

Transcription and replication of non-segmented (-) strand RNA viruses and their assembly as virus particles have been studied and reported especially in Fields virology (*3rd edition, vol. 1, 1996, Lippincott – Raven publishers – Fields BN et al*). Transcription and replication of measles virus do not involve the nucleus of the infected cells but rather take place in the cytoplasm of said infected cells. The genome of the measles virus comprises genes encoding six major structural proteins from the six genes (designated N, P, M, F, H and L) and an additional two-non structural proteins from the P. gene. The gene order is the following : 3', N, P (including C and V), M, F, H, and L large polymerase protein at the 5' end. The genome further comprises non coding regions in the intergenic region M/F ; this non-coding region contains approximately 1000 nucleotides of untranslated RNA. The cited genes respectively encode the leader peptide (I gene), the proteins of the nucleocapsid of the virus, i.e., the nucleoprotein (N), the phosphoprotein (P), and the large protein (L) which assemble around the genome RNA to provide the nucleocapsid. The other genes encode the proteins of viral envelope including the hemagglutinin (H), the fusion (F) and the matrix (M) proteins.

The measles virus has been isolated and live attenuated vaccines have been derived from the Edmonston MV isolated in 1954 *(Enders, J. F., and T. C. Peebles. 1954. Propagation in tissue cultures od cytopathogenic agents from patients with measles. Proc. Soc. Exp. Biol. Med. 86:277-286.)*, by serially passages performed on primary human kidney or amnion cells. The used

strains were then adapted to chicken embryo fibroblasts (CEF) to produce Edmonston A and B seeds (*Griffin, D., and W. Bellini. 1996. Measles virus, p. 1267-1312. In B. Fields, D. Knipe, et al. (ed.), Virology, vol. 2. Lippincott - Raven Publishers, Philadelphia*). Edmonston B was licensed in 1963 as the first MV vaccine. Further passages of Edmonston A and B on CEF produced the more attenuated Schwarz and Moraten viruses (*Griffin, D., and W. Bellini. 1996. Measles virus, p. 1267-1312. In B. Fields, D. Knipe, et al. (ed.), Virology, vol. 2. Lippincott - Raven Publishers, Philadelphia*) whose sequences have recently been shown to be identical (*Parks, C. L., R. A. Lerch, P. Walpita, H. P. Wang, M. S. Sidhu, and S. A. Udem. 2001. Analysis of the noncoding regions of measles virus strains in the Edmonston vaccine lineage. J Virol. 75:921-933 ; Parks, C. L., R. A. Lerch, P. Walpita, H. P. Wang, M. S. Sidhu, and S. A. Udem. 2001. Comparison of predicted amino acid sequences of measles virus strains in the Edmonston vaccine lineage. J Virol. 75:910-920*). Because Edmonston B vaccine was reactogenic, it was abandoned in 1975 and replaced by the Schwarz/Moraten vaccine which is currently the most widely used measles vaccine in the world (*Hilleman, M. 2002. Current overview of the pathogenesis and prophylaxis of measles with focus on practical implications. Vaccine. 20:651-665*). Several other vaccine strains are also used: AIK-C, Schwarz F88, CAM70, TD97 in Japan, Leningrad-16 in Russia, and Edmonston Zagreb. The CAM70 and TD97 Chinese strains were not derived from Edmonston. Schwarz/Moraten and AIK-C vaccines are produced on CEF. Zagreg vaccine is produced on human diploid cells (WI-38).

The live attenuated vaccine derived from the Schwarz strain is commercialized by Aventis Pasteur (Lyon France) under the trademark Rouvax®.

In a noteworthy and pioneer work, Martin Billeter and colleagues cloned an infectious cDNA corresponding to the antigenome of Edmonston MV and established an original and efficient reverse genetics procedure to rescue the corresponding virus (*Radecke, F., P. Spielhofer, H. Schneider, K. Kaelin, M.*

*Huber, K. Dötsch, G. Christiansen, and M. Billeter., 1995. Rescue of measles viruses from cloned DNA. EMBO Journal. 14:5773-5784* and WO 97/06270*)*.

However, sequence comparison (see below) revealed that the genome cloned in this vector diverged from the Edmonston B sequence. It was closer to Edmonston-wt, an early passage on Vero cells of Edmonston isolate *(Parks, C. L., R. A. Lerch, P. Walpita, H. P. Wang, M. S. Sidhu, and S. A. Udem. 2001. Analysis of the noncoding regions of measles virus strains in the Edmonston vaccine lineage. J Virol. 75:921-933 ; Parks, C. L., R. A. Lerch, P. Walpita, H. P. Wang, M. S. Sidhu, and S. A. Udem. 2001. Comparison of predicted amino acid sequences of measles virus strains in the Edmonston vaccine lineage. J Virol. 75:910-920*), and had 10 amino acid substitutions not related to any Edmonston subgroup. Moreover, despite the fact that this vector is immunogenic in mice expressing CD46 and lacking the IFN type I receptor (19), the inventors show in the following experimental work that it is not immunogenic in non-human primates when inoculated at the standard dose of $10^4$ $TCID_{50}$.Therefore, this vector developed from a vaccine strain abandoned 25 years ago, and whose sequence diverged so much, does not appear suitable as vaccination vector, especially in human, while it certainly helps to understand some aspects of MV replication.

For these reasons, the inventors have decided that a measles vector aimed at children needs to be developed from an approved vaccine strain and have accordingly cloned an infectious cDNA starting from viral particles of the widely used Schwarz/Moraten strain of measles virus. This cDNA may allow the production of Schwarz/Moraten vaccine stocks without having to rely on the availability of seed stocks. It may also be used as a recombinant vaccination vector based on an approved and efficient vaccine strain, grown on CEF for safety reasons, and worldwide used. Such a vector may also be of interest for adult propulations in certain circumstances where a need therefore exists.

Description of the invention

The invention provides a cDNA molecule which encodes the nucleotide sequence of the full length, infectious antigenomic (+)RNA strand of a measles virus (MV) originating from the Schwarz strain or the Moraten strain, said cDNA comprising the nucleotide sequence extending from nucleotide 83 to nucleotide 15976 of the sequence SEQ ID NO.82.

The invention provides a recombinant cDNA molecule which comprises the cDNA molecule as defined therein and which optionnally comprises a heterologous DNA sequence capable of expressing a heterologous amino acid sequence which recombinant cDNA further complies with the rule of six.

The invention provides a recombinant cDNA molecule which comprises the cDNA molecule as defined therein and which further comprises a heterologous DNA sequence capable of expressing a heterologous amino acid sequence wherein the recombinant cDNA further complies with the rule of six.

The invention provides a vector comprising the cDNA molecule as defined herein.

The invention provides a vector comprising the cDNA molecule as defined therein, wherein an Additional Transcription Unit is cloned within the 5' segment of the cDNA molecule.

The invention provides a process for the preparation of infectious measles virus particles comprising:

1) expressing the cDNA according to the invention or the vector according to the invention in a helper cell line which also expresses proteins necessary for transcription, replication and encapsidation of the antigenomic (+)RNA sequence of the measles virus starting from said cDNA and under conditions enabling viral particles assembly and,
2) recovering the expressed viral particles.

The invention provides a process for the preparation of infectious measles virus particles, comprising :

1) transfecting helper cells with the cDNA as defined therein or with the vector as defined therein, wherein said helper cells are capable of expressing helper functions to express an RNA polymerase, and to express the N, P and L proteins of the MV virus;
2) co-cultivating said transfected helper cells of step 1) with passaged cells suitable for the passage of the MV vaccine strain from which the cDNA originates; and
3) recovering the infectious MV viral particles produced.

The invention provides a vaccine composition whose active principle comprises measles viral particles rescued from the cDNA molecule as defined therein, or from the vector as defined therein, in a helper-cell-based rescue system.

The invention provides a process for the preparation of the cDNA molecule as defined therein, comprising the following steps:

- purifying viral RNA from viral particles of the Schwarz strain or the Moraten strain; and
- obtaining the cDNA molecule by reverse transcription of the purified viral RNA.

The invention provides a process for the preparation of the cDNA molecule as defined herein, comprising the following steps:

- purifying viral RNA from viral particles of the Schwarz strain or the Moraten strain, wherein the genome of viral particles of the Schwarz strain or the Moraten strain comprises the nucleotide sequence extending from nucleotide 83 to nucleotide 15976 of the sequence SEQ ID NO.82; and
- obtaining the cDNA molecule by reverse transcription of the purified viral RNA.

The invention relates to a cDNA molecule which encodes the nucleotide sequence of the full length antigenomic (+)RNA strand of a measles virus (MV) originating from an approved vaccine strain.

The expression « encodes » in the above definition encompasses the capacity of the cDNA to allow transcription of a full length antigenomic (+)RNA, said cDNA serving especially as template for transcription. Accordingly, when the cDNA is a double stranded molecule, one of the strands has the same nucleotide sequence as the antigenomic (+) strand RNA of the measles virus, except that « U » nucleotides are substituted by « T » in the cDNA.

Figure 5 illustrates the sequence of a DNA molecule of the invention which comprises a cDNA sequence as defined above, be it specified that the strand of the cDNA which is represented is identical to that of the antigenomic (+)RNA strand of a MV strain except for the substitution of « U » by « T ».

The cDNA molecule according to the above definition allows the production, when placed in appropriate conditions, of an infectious antigenomic (+)RNA capable of producing infectious particles of the measles virus.

The cDNA obtained has especially the original 5'- and 3'-ends of the native antigenomic (+) strand of the viral RNA. In addition, the obtained cDNA complies with the rule of 6 which is required in order to express infectious viral particles.

The « rule of six » which is expressed in the fact that the total number of nucleotides present in the cDNA amounts to a multiple of six, rule which allows sufficient replication of genome RNA of the measles virus. It has been described in the above cited reference Fields Virology on page 1197.

The cDNA molecule of the invention which is derived from an MV approved vaccine strain can be obtained from the Schwarz or the Moraten strain.

These strains have been disclosed in several publications and used for the preparation of the currently used vaccines. The inventors propose especially the use of the Schwartz strain which is available from Aventis Pasteur (France).

According to another particular embodiment of the invention, the cDNA molecule is placed under the control of a heterologous expression control sequence.

The insertion of such a control for the expression of the cDNA, is favorable when the expression of this cDNA is sought in cell types which do not enable full transcription of the cDNA with its native control sequences.

According to a particular embodiment of the invention, the heterologous expression control sequence comprises the T7 promoter and T7 terminator sequences. These sequences are respectively located 5' and 3' of the coding sequence for the full length antigenomic (+)RNA strand of MV and from the adjacent sequences around this coding sequence.

In a particular embodiment of the invention, the cDNA molecule which is defined hereabove is modified i.e., comprises additional nucleotide sequences or motifs or comprises deletions or substitutions within said cDNA.

In a preferred embodiment, the cDNA molecule of the invention further comprises, at its 5'-end, adjacent to the first nucleotide of the nucleotide sequence encoding the full length antigenomic (+)RNA strand of the MV approved vaccine strain, a GGG motif followed by a hammerhead ribozyme sequence and which comprises, at its 3'-end, adjacent to the last nucleotide of said nucleotide sequence encoding the full length anti-genomic (+)RNA strand, the sequence of a ribozyme. The Hepatitis delta virus ribozyme ($\delta$) is appropriate to carry out the invention.

The GGG motif placed at the 5' end, adjacent to the first nucleotide of the above coding sequence improves the efficiency of the transcription of said cDNA coding sequence. As a requirement for the proper assembly of measles virus particles is the fact that the cDNA encoding the antigenomic (+)RNA complies with the rule of six, when the GGG motif is added, a ribozyme is also added at the 5' end of the coding sequence of the cDNA, 3' from the GGG

motif, in order to enable cleavage of the transcript at the first coding nucleotide of the full length antigenomic (+)RNA strand of MV.

Thus, in case where the GGG motif is added to improve efficiency of transcription, two ribozymes are added in order to ensure the cleavage of the coding sequence for the full length antigenomic (+)RNA strand of the MV.

According to the present invention, the expression "cDNA" encompasses a DNA molecule obtained by reverse transcription of an RNA molecule, including but not limited to an mRNA molecule.

Any other technique for the preparation of DNA, starting from the material disclosed in the present invention or using the disclosed features relating to the cDNA of the invention can be used, including techniques involving synthesis or PCR.

Therefore, the expression "cDNA" used for the description of the nucleotide sequence of the molecule of the invention merely relates to the fact that originally said molecule is obtained by reverse transcription of the full length genomic (-)RNA strand of the genome of viral particles of the measles virus. This should not be viewed as a limitation for the methods used for its preparation. Purified nucleic acids, including DNA are thus encompassed within the meaning cDNA according to the invention, provided said nucleic acid, especially DNA fulfils the above-given definitions.

The invention also concerns a cDNA molecule according to one of the above definitions which is comprised in a plasmid capable of replication.

Many plasmids can be prepared in order to fulfil the requirement of the invention and the invention especially relates to plasmid pTM-MVSchw which is represented on figure 2.

Plasmid pTM-MVSchw has been deposited at the CNCM (Paris, France) on June 12, 2002 under number I-2889. This plasmid is described in the examples and figures which follow. It is a plasmid vector derived from Bluescript, comprising the full length sequence coding for the measles virus, strain Schwarz,

placed under the control of the promoter of the T7 RNA polymerase; its size is 18967 nucleotide.

The invention especially also relates to a cDNA molecule which is capable of producing infectious viral particles of the MV approved vaccine strain, preferably using the previously reported rescue system involving 293-3-46 helper cells (Radecke et al. and WO 97/06270), 293-3-46 helper cells expressing proteins necessary for transcription, replication of the RNA genome-sequence of MV from said cDNA and under conditions enabling viral particles assembly.

293-3-46 cells are cited as example for the preparation of the viral particles. They can however be replaced by any other appropriate cell line suitable for constituting helper cells.

Methods for the production of such infectious particles are given in the examples of the present application.

Particular preferred cDNA molecules according to the invention are the molecules having a nucleotide sequence selected among the following sequences:

- the cDNA molecule which comprises the nucleotide sequence extending from nucleotide 83 to nucleotide 15977 of the sequence represented on figure 5,
- the cDNA molecule which comprises the nucleotide sequence extending from nucleotide 29 to nucleotide 16202 of the sequence represented on figure 5,
- the cDNA molecule which comprises the nucleotide sequence extending from nucleotide 26 to nucleotide 16202 of the sequence represented on figure 5,
- the cDNA molecule which comprises the nucleotide sequence extending from nucleotide 9 to nucleotide 16202 of the sequence represented on figure 5,

- the cDNA molecule which comprises the nucleotide sequence extending from nucleotide 29 to nucleotide 15977 of the sequence represented on figure 5,
- the cDNA molecule which comprises the nucleotide sequence extending from nucleotide 26 to nucleotide 15977 of the sequence represented on figure 5,
- the cDNA molecule which comprises the nucleotide sequence extending from nucleotide 9 to nucleotide 15977 of the sequence represented on figure 5.

The invention of course relates to each of the particular sequences described hereabove.

A particular cDNA molecule which is preferred to carry out the invention is the molecule which comprises the insert contained in plasmid pTMMVschw deposited at the CNCM under number I-2889, wherein said insert encodes a nucleotide sequence of the full length antigenomic (+)RNA strand of the measles virus. One particular insert is the one which is comprised within the sequence defined by the following restriction sites: NotI (located at position 1 on figure 5) and NotI (located at position 16203 on figure 5).

In a particular embodiment of the invention, the cDNA molecule is the product of the reverse transcription of the viral RNA purified from viral particles of the measles virus.

The preparation of the cDNA from viral purified RNA advantageously limits the presence of cellular components and especially cellular DNA or RNA which could be present in cells used for the cultivation of the virus. It limits especially the presence of viral genomic RNA which would be incomplete or mutated and which are present in cells, and limits the presence of viral mRNA present in large quantities in the cells.

The invention further relates to a cDNA molecule having the above defined features, which is capable of inducing an immune response against at least one antigen of a measles virus, when administered *in vivo*.

The invention also relates to a recombinant *mononegavirales* virus comprising the cDNA molecule of a measles virus according to anyone of the above definitions and a DNA sequence of a RNA virus, which recombinant virus is capable of eliciting *in vivo* a humoral and/or a cellular response against measles virus or against said RNA virus, or against both measles virus and RNA virus.

The invention also concerns a recombinant cDNA molecule as defined above, which further comprises a heterologous DNA sequence cloned therein in conditions enabling its expression as a heterologous amino acid sequence, said cloning being performed in such a way that the obtained recombinant cDNA complies with the rule of six.

Heterologous coding sequences especially DNA sequences are advantageously selected among sequences capable of expressing antigens or epitopes of antigens, having immunogenic properties and especially having the capacity of eliciting or favoring an immunogenic response in a host to which they are administered. Such heterologous DNA sequences can be derived for instance from antigens of pathogens.

The invention advantageously enables the insertion of such heterologous DNA sequences in a sequence which is designated an Additional Transcription Unit (ATU) especially an ATU as disclosed by Billeter et al. in WO 97/06270.

This ATU is especially represented on Figure 4.

When used for the performance of the invention, the ATU is advantageously located in the N-terminal sequence of the cDNA molecule encoding the full-length (+)RNA strand of the antigenome of the MV and is especially located between the P and M genes of this virus or between the H and L genes. It has been observed that the transcription of the viral RNA of MV follows a gradient from the 5' to the 3' end. This explains that when inserted in the 5' end of the coding sequence of the cDNA, the ATU will enable a more efficient expression of the heterologous DNA sequence that it contains.

The invention also relates to a vector comprising a cDNA molecule as defined above including a recombinant cDNA. A particular vector is vector for cloning and/or expressing of this cDNA.

According to a preferred embodiment of the invention, the vector is a plasmid and is especially pTM-MVSchw deposited at the CNCM on June 12, 2002 under No. I-2889.

Other vectors, designated pTM-MVSchw2-gfp deposited at the CNCM under n° I-2890 on June 12, 2002 or designated pTM-MVSchw2-GFPbis deposited at the CNCM under n° I-3034 on May 26, 2003 are encompassed within the invention.

These vectors are derived from pTM-MVSchw, and are accordingly plasmid vectors derived from Bluescript, comprising the full length sequence coding for the measles virus, strain Schwarz, placed under the control of the promoter of the T7 RNA polymerase, and further containing the gfp gene coding for the GFP protein, said gene being inserted in an ATU at position 2 (i.e., between the N and P genes of MV).

The size of pTM-MvSchw is 18967 nucleotides. The size of pTM-MVSchw2-gfp is 19800 nucleotides.

The difference between pTM-MVSchw2-gfp and pTM-MVSchw2-GFPbis corresponds to a mutation in the ATU sequence where a C nucleotide is substituted as illustrated on figure 4B at the end of the ATU, to provide pTM-MVSchw2-GFPbis.

The invention also relates to a process for the preparation of infectious measles virus particles comprising :

1) expressing the cDNA of the invention according to one of the above definitions or the vector containing such cDNA in a helper cell line which also expresses proteins necessary for transcription, replication and encapsidation of the antigenomic (+)RNA sequence of MV from said cDNA and under conditions enabling viral particles assembly and

2) recovering the expressed viral particles.

According to a particular embodiment of this process, it comprises :

1) transfecting helper cells with a cDNA according to the above definition with a vector above defined, wherein said helper cells are capable of expressing helper functions to express an RNA polymerase, and to express the N, P and L proteins of the MV virus ;
2) co-cultivating said transfected helper cells of setp 1) with passaged cells suitable for the passage of the MV vaccine strain from which the cDNA originates ;
3) recovering the infectious MV viral particles produced.

According to a preferred embodiment, helper cells are derived from human embryonic kidney cell line 293, which cell line 293 is deposited with the ATCC under No. CRL-1573.

According to another aspect of this process, the cells suitable for passage are CEF cells.

CEF cells can be prepared from fertilized chicken eggs as obtained from EARL Morizeau, 8 rue Moulin, 28190 Dangers, France, or from any other producer of fertilized chicken eggs.

The process which is disclosed according to the present invention is used advantageously for the production of infectious measles virus appropriate for use as vaccine compositions.

The invention thus relates to an immunogenic composition whose active principle comprises infection measles viral particles obtained by the process disclosed above.

The invention also concerns a vaccine composition. Such a vaccine composition has advantageously an active principle which comprises measles virus particles rescued from the cDNA of the vector which has been defined hereabove, which is expressed in a helper cell based rescue system.

Advantageously, such a vaccine composition is suitable for protection against measles virus. According to the embodiment where the cDNA is recombined with a heterologous DNA sequence encoding an immunogenic

amino acid sequence, the vaccine composition can further be suitable for protection against the pathogen from which the immunogenic DNA sequence derives.

The invention also concerns a cell which is recombined with a cDNA molecule according to the invention or with a vector as defined above. A preferred cell is a prokaryotic cell such as E. coli or Salmonella.

Another preferred cell is a eukaryotic cell, especially a cell selected among yeasts, such as Saccharomyces Cerevisiae.

A cell within the definition of the invention, can be characterized according to a particular embodiment by the fact that this comprises nucleotide sequences expressing helper functions necessary to express an RNA polymerase and to express the N, P and L proteins of the MV virus. Such a cell can thus be used for the rescue of the viral particles.

The examples and figures which follow provide additional features for the characterization of the invention.

**Figure 1**. Comparison of MV genomes. (A) Nucleotide changes for each coding region (capital letters in boxes) and in non-coding regions (lower case letters) are shown in the lower part. Amino acid changes are shown in the upper part (one-letter amino acid symbol). Yellow color in the grid shows the wt substitutions. Blue color indicates the substitutions corresponding to the Rubeovax/Scwharz/moraten vaccine type. The red color shows the nucleotide and amino acid changes that are present only in the EdB-tag sequence. Nucleotide changes in positions 1805 and 1806 of EdB-tag correspond to the tag introduced. (B) Phylogenetic tree showing the EdB-tag among the Edmonston group and two wt isolates (Takeda, M., A. Kato, F. Kobune, H. Sakata, Y. Li, T. Shioda, Y. Sakai, M. Asakawa, and Y. Nagai. 1998. Measles virus attenuation associated with transcriptional impediment and a few amino acid changes in the polymerase and accessory proteins. J Virol. 72:8690-8696 ; Takeuchi, K., N. Miyajima, F. Kobune, and M. Tashiro. 2000. Comparative nucleotide sequence analyses of the entire genomes of B95a cell-isolated and

vero cell-isolated measles viruses from the same patient. Virus Genes. 20:253-257). The sequences were aligned using Clustal W (Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673-4680). Nucleotide sequence distance were determined with Dnadist of the Phylip package version 3.5 'Felsenstein, J. 1989. Cladistics. 5:164-166. The tree was derived by neighbor-joining analysis applied to pairwise sequence distances calculated using a variety of methods including the Kimura two-parameter method to generate unrooted trees. The final output was generated with Treeview (Page, R. D. 1996. TreeView: an application to display phylogenetic trees on personal computers. Comput Appl Biosci. 12:357-358).

**Figure 2**. Schematic map of the pTM-MV Schw plasmid. To construct the complete sequence, the six fragments represented in the upper part were generated and recombined step by step using the unique restriction sites indicated. T7 = T7 promoter; hh = hammerhead ribozyme; hδv = hepatitis delta ribozyme; T7t = T7 RNA polymerase terminator. The following oligonucleotides were used for the sequencing of the MV Schwarz cDNA and the Schwarz MV rescued from the cDNA.

| N° Sens | Position | N° Antisens |
|---|---|---|
| 1 ATCCGAGATGGCCACACTTT | 101 | 1a AAAGTGTGGCCATCTCGGAT |
| 2 TGATTCTGGGTACCATCCTA | 601 | 2a TAGGATGGTACCCAGAATCA |
| 3 TATGCCATGGGAGTAGGAGT | 1110 | 3a ACTCCTACTCCCATGGCATA |
| 4 TGGCAGGAATCTCGGAAGAA | 1609 | 4a TTCTTCCGAGATTCCTGCCA |
| 5 GCATCAAGCACTGGGTTACA | 2110 | 5a TGTAACCCAGTGCTTGATGC |
| 6 TACAGGAGTGGACACCCGAA | 2651 | 6a TTCGGGTGTCCACTCCTGTA |
| 7 AGGACAGCTGCTGAAGGAAT | 3096 | 7a ATTCCTTCAGCAGCTGTCCT |
| 8 TTGTTGAGGACAGCGATTCC | 3610 | 8a GGAATCGCTGTCCTCAACAA |
| 9 AGAGTGAAGTCTACTCTGCC | 4120 | 9a GGCAGAGTAGACTTCACTCT |
| 10 TGACACAAGGCCACCACCAG | 4608 | 10a CTGGTGGTGGCCTTGTGTCA |
| 11 AGCTCCCAGACTCGGCCATC | 5169 | 11a GATGGCCGAGTCTGGGAGCT |
| 12 CCAGCCATCAATCATTAGTC | 5603 | 12a GACTAATGATTGATGGCTGG |

| | | |
|---|---|---|
| 13 AGTTTACGGGACCCCATATC | 6115 | 13a GATATGGGGTCCCGTAAACT |
| 14 GGAACCTAATAGCCAATTGT | 6608 | 14a ACAATTGGCTATTAGGTTCC |
| 15 CTCTTCGTCATCAAGCAACC | 7151 | 15a GGTTGCTTGATGACGAAGAG |
| 16 TCACTTGGTGTATCAACCCG | 7677 | 16a CGGGTTGATACACCAAGTGA |
| 17 AACTGTATGGTGGCTTTGGG | 8126 | 17a CCCAAAGCCACCATACAGTT |
| 18 TGTGTATTGGCTGACTATCC | 8620 | 18a GGATAGTCAGCCAATACACA |
| 19 ATCAGGCATACCCACTAGTG | 9162 | 19a CACTAGTGGGTATGCCTGAT |
| 20 GCACAGCTCCCAGTGGTTTG | 9701 | 20a CAAACCACTGGGAGCTGTGC |
| 21 TCATGAGTTAACTGAAGCTC | 10214 | 21a GAGCTTCAGTTAACTCATGA |
| 22 GTCACGGAGGCTTGTAGATG | 10715 | 22a CATCTACAAGCCTCCGTGAC |
| 23 GTACTGCCTTAATTGGAGAT | 11231 | 23a ATCTCCAATTAAGGCAGTAC |
| 24 TGATGGGCTACTTGTGTCCC | 11747 | 24a GGGACACAAGTAGCCCATCA |
| 25 ACCCTTACTCAGCAAATCTT | 12223 | 25a AAGATTTGCTGAGTAAGGGT |
| 26 TCTATGCGAGGCCACCTTAT | 12726 | 26a ATAAGGTGGCCTCGCATAGA |
| 27 TTGTCCGAGTGGCGAGGTAT | 13144 | 27a ATACCTCGCCACTCGGACAA |
| 28 CAATTGGGCATTTGATGTAC | 13712 | 28a GTACATCAAATGCCCAATTG |
| 29 GAGGCTATGTTATCTCCAGC | 14172 | 29a GCTGGAGATAACATAGCCTC |
| 30 AGTTGGCCTTGTCGAACACA | 14723 | 30a TGTGTTCGACAAGGCCAACT |
| 31 CTGGACTTATAGGTCACATC | 15190 | 31a GATGTGACCTATAAGTCCAG |
| 32 GGTTTGAAACGTGAGTGGGT | 15693 | 32a ACCCACTCACGTTTCAAACC |

**Figure 3**. Growth kinetics of rescued Schwarz and EdB-tag viruses on Vero and CEF cells. Cells on 35 mm dishes were infected with Schwarz MV rescued from pTM-MVSchw plasmid (-■-), EdB-tag MV (-O-), and industrial Schwarz virus (-Δ-) at different MOI (as indicated). At each time point, cells were collected and cell-associated virus titers were determined using the $TCID_{50}$ method on Vero cells. (A) Vero cells incubated at 37°C, (B) CEF incubated at 37°C, (C) CEF incubated at 32°C.

**Figure 4**. A: Schematic representation the additional transcription unit (ATU) and Schwarz MV vector plasmid. (AA) Cis-acting elements of the ATU inserted in position 2 between phosphoprotein (P) and matrix (M) MV open reading frames. (AB). Representation of the three positions of ATU insertion in the Schwarz MV vector plasmid.

B: ATU sequence: small letters represent additional sequences (copy of the N-P intergenic region of measles virus) plus cloning

sites. Capital letters correspond to the inserted enhanced GFP sequence. This sequence is inserted at the SpeI site (position 3373) of the cDNA sequence of the Schwarz strain of the measles virus for ATU2 and at the SpeI site (position 9174) for the ATU3. The mutation which distinguishes normal ATU from bis (in pTM-MVSchw2-gfp and pTM-MVSchw2-GFPbis) is a substituted C (Capital letter) at the end of ATU.

**Figure 5**. Complete nucleotide sequence of the pTM-MVSchw plasmid. The sequence can be described as follows with reference to the position of the nucleotides :

- 1-8 *NotI* restriction site
- 9-28 T7 promoter
- 29-82 Hammer head ribozyme
- 83-15976 MV Schwarz antigenome
- 15977-16202 HDV ribozyme and T7 terminator
- 16203-16210 *NotI* restriction site
- 16211-16216 *ApaI* restriction site
- 16220-16226 *KpnI* restriction site
- 16226-18967 pBluescript KS(+) plasmid (Stratagene)

**Figure 6**. Detection of anti-MV antibodies in macaques immunized with different MV vaccine strains. Anti-MV antibodies were detected by ELISA one month after immunization of rhesus macaques (2 monkeys per group) with Schwarz virus (gray bars), EdB-tag virus (white bars) and Rouvax vaccine (black bars) at the doses indicated. Immune status ratio (ISR) were calculated as described in materials and methods. Only ISR values higher than 0.9 were considered as positive (determinations were done in triplicate on 1/20 dilution of serum samples and results are expressed as the mean values ± SD).

**Figure 7**. Antibody titers to MV in mice immunized with different MV vaccine strains. Anti-MV antibodies were detected by ELISA one month after immunization of CD46 (A) and CD46/IFNAR (B) mice with $10^4$ $TCID_{50}$ of EdB-tag virus (white bars), Schwarz virus (gray bars) and Rouvax vaccine (black bars). Results are expressed as mean OD values ± SD (4 mice per group) determined in serial dilutions of sera.

**Figure 8**. Detection of anti-MV antibodies in macaques immunized with different Schwarz measles virus preparations. Anti-MV antibodies were detected by ELISA at different time points after immunization of cynomolgus macaques (2 monkeys per group) with $10^4$ $TCID_{50}$ of bulk industrial Schwarz virus (white marks), Schwarz virus rescued from pTM-MVSchw plasmid and grown on CEF (gray bars) or Vero cells (black bars). Immune status ratio (ISR) were calculated as described in materials and methods.

**Figure 9**. Changes in the number of circulating leukocytes and MV-specific T-cell response in macaques immunized with different Schwarz MV preparations. Enumeration of white blood cells (A), lymphocytes (B), monocytes (C), and MV hemaglutinin-specific IFN-γ-ELISpots (D) in PBMC of cynomolgus macaques collected at different time points after immunization with $10^4$ $TCID_{50}$of bulk industrial Schwarz virus (white marks), Schwarz virus rescued from pTM-MVSchw plasmid and grown on CEF (gray bars) or Vero cells (black bars). IFN-γ-ELISpots were deteced after stimulation of PBMC for 24 hours with a recombinant MVA expressing the MV hemaglutinin. The background obtained with MVA-wt stimulation was subtracted and the results are expressed as MVA-HMV specific γ-IFN producing cells per million PBMC.

**Figure 10**. Schematic representation of the pTM-MVSchw-ATU plasmids (A) and GFP expression in Vero cells infected by rescued recombinant viruses (B). Vero cells were infected with recombinant Schwarz MV-GFP either in position ATU2 (left side) or position ATU3 (right side) and the GFP fluorescence was observed in syncytia.

## EXAMPLES

**Sequence comparison between EdB-tag and measles vaccine strains.**

In a nice analysis previously reported (Parks, C. L., R. A. Lerch, P. Walpita, H. P. Wang, M. S. Sidhu, and S. A. Udem. 2001. Analysis of the noncoding regions of measles virus strains in the Edmonston vaccine lineage. J Virol. 75:921-933 ; Parks, C. L., R. A. Lerch, P. Walpita, H. P. Wang, M. S. Sidhu, and S. A. Udem. 2001. Comparison of predicted amino acid sequences of measles virus strains in the Edmonston vaccine lineage. J Virol. 75:910-920), the coding and non-coding sequences of Edmonston-derived vaccine virus strains were compared to that of a low-passage isolate of the Edmonston wild-type measles virus. The authors identified 10 amino acids substitutions shared by almost all the vaccine strains. We compared the genomic sequences of these Edmonston-derived vaccine strains and two primary isolates (Takeda, M., A. Kato, F. Kobune, H. Sakata, Y. Li, T. Shioda, Y. Sakai, M. Asakawa, and Y. Nagai. 1998. Measles virus attenuation associated with transcriptional impediment and a few amino acid changes in the polymerase and accessory proteins. J Virol. 72:8690-8696 ; Takeuchi, K., N. Miyajima, F. Kobune, and M. Tashiro. 2000. Comparative nucleotide sequence analyses of the entire genomes of B95a cell-isolated and vero cell-isolated measles viruses from the same patient. Virus Genes. 20:253-257) to that of the previously reported Edmonston B infectious cDNA (EdB-tag) (Radecke, F., P. Spielhofer, H. Schneider, K. Kaelin, M. Huber, K. Dötsch, G. Christiansen, and M. Billeter. 1995. Rescue of measles viruses from cloned DNA. EMBO Journal. 14:5773-5784). Figure 1 shows that the nucleotide sequence of EdB-tag differed from that of Rubeovax (Edmonston B vaccine) by 0.24% (38 mutations) and from that of Schwarz/Moraten by 0.27% (44 mutations). Schwarz/Moraten and Emonston B (Rubeovax®) sequences differed only by 0.1% (16 mutations). Among the 38 differences between EdB-tag and Rubeovax, 17 were amino acid substitutions in coding regions and 7

were located in non-coding regions. Among the 44 differences between EdB-tag and Schwarz/Moraten, 22 were amino acid substitutions and 9 were in non-coding regions. The 10 amino acids substitutions shared by almost all the Edmonston vaccine strains Parks, C. L., R. A. Lerch, P. Walpita, H. P. Wang, M. S. Sidhu, and S. A. Udem. 2001. Analysis of the noncoding regions of measles virus strains in the Edmonston vaccine lineage. J Virol. 75:921-933 ; Parks, C. L., R. A. Lerch, P. Walpita, H. P. Wang, M. S. Sidhu, and S. A. Udem. 2001. Comparison of predicted amino acid sequences of measles virus strains in the Edmonston vaccine lineage. J Virol. 75:910-920) were conserved in EdB-tag cDNA. However, 5 of them were specific of the AIK-C and Zabreg subgroup, indicating that the virus from which EdB-tag was cloned diverged from Edmonston B and did not correspond to any approved vaccine strain. Moreover, 10 amino acid substitutions in EdB-tag were not related to any Edmonston subgroup, probably reflecting the adaptation to growth on HeLa cells and/or errors introduced during the cloning procedure. Among these specific changes, 5 were located in the P/V/C coding sequences and 3 were located in the L polymerase gene, thus possibly affecting the replicative capacity of the virus *in vivo*. These changes and others in cis-acting sequences may influence the immunogenicity or pathogenicity of the virus recovered from the EdB-tag cDNA. Indeed, MV adaptation to growth on Vero cells has been shown to be associated with loss of pathogenic potential (Kobune, F., H. Sakata, and A. Sugiura. 1990. Marmoset lymphoblastoid cells as a sensitive host for isolation of measles virus. J Virol. 64:700-705) and with a few amino acid changes located in the polymerase (L) and accessory (P /V/C) proteins resulting in transcriptional attenuation in lymphoid cells (Takeda, M., A. Kato, F. Kobune, H. Sakata, Y. Li, T. Shioda, Y. Sakai, M. Asakawa, and Y. Nagai. 1998. Measles virus attenuation associated with transcriptional impediment and a few amino acid changes in the polymerase and accessory proteins. J Virol. 72:8690-8696 ; Takeuchi, K., N. Miyajima, F. Kobune, and M. Tashiro. 2000. Comparative nucleotide sequence analyses of the entire genomes of B95a cell-isolated and

vero cell-isolated measles viruses from the same patient. Virus Genes. 20:253-257.

**Construction of a cDNA corresponding to the antigenome of the Schwarz vaccine strain of measles virus**.

Viral particles were purified from a measles Schwarz vaccine batch kindly provided by Aventis Pasteur (Lyon, France). This bulk vaccine preparation (50 ml, 3 $10^4$ $TCID_{50}$/ml) was obtained by scraping of infected CEF cells, freeze-thawing cells and medium, and filtration of cellular debris. Particles were concentrated by centrifugation through a 30% sucrose cushion. Viral RNA was purified from lysed particles using a silica-gel-based membrane (QIAmp, Qiagen). Viral RNA was reverse-transcribed into cDNA using a mixture of random hexameres (pdN6, 1 µM) and a specific oligonucleotide representing the 32 first nucleotides of MV genome (MVSchwRT1 5'-ACCAAACAAAGTTGGGTAAGGATAGTTCAATC-3', 10 µM), as primers. In order to ensure the fidelity of reverse transcription and the yield of full-length products, the SuperScript II* DNA polymerase was used (GibcoBRL). A set of six overlapping fragments covering the full viral cDNA (numbered 1 to 6 in Fig. 2) were generated by PCR using *PfuTurbo* DNA polymerase (Stratagene) and a set of specific primers closed to unique restriction sites. Fragment 1 at the 5' end of the viral antigenome was engineered by PCR with specific primers in order to contain a T7 RNA polymerase promoter with the GGG motif necessary for a full efficiency and a hammerhead ribozyme sequence inserted between the T7 promoter and the first viral nucleotide. To generate this fragment, two overlapping oligonucleotides were annealed together : Leader 1 (5'-TATGCGGCCGCTAATACGACT CACTATAGGGCCAACTTTGTTTGGTCTGA-3') containing a *NotI* site, the T7 promoter (underlined) and the 19 first nucleotides of hammerhead ribozyme sequence, and Leader 2 (5'-GGTGACCCGGGACTCCGGGTTTCGTCCTCACGGACTCATCAGACCAAACA-3') containing the hammer head sequence with a *SmaI/XmaI* site. After PCR

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amplification, the resulting fragment was linked by PCR extension to a second fragment also generated by PCR from Schwarz cDNA using oligonucleotides MVSchw1 (5'-GAGTCCCGGGTCACCAA ACAAAGTTGGGTAAG-3') overlapping with hammerhead sequence (underlined) and covering MV Schwarz genome 1-15, and MVSchw160 (5'-GGTTTGTCCTTGTTTCTTTT-3', MV Schwarz genome 141-160). Fragment 2 (2173 nucleotides long, see Fig. 2) was amplified using oligonucleotides MVSchwRT1 (5'-ACCAAACAAAGTTGGGTAAGGATAGTTCAAT C-3', MV Schwarz genome 1-32), and MVSchw2173 (5'-ATTCCCTTAACCGCTTCACC-3', MV Schwarz genome 2155-2174). Fragment 3 (3142 nucleotides long) was amplified using oligonucleotides MV Schw1901 (5'-CTATGGCAGCATGGTCAGAAATA-3', MV Schwarz genome 1901-1924) and MVSch5043 (5'-ATTGTCGATGGTTGGGTGCT-3', MV Schwarz genome 5024-5043). Fragment 4 (4349 nucleotides long) was amplified using oligonucleotides MVSchw4869 (5'-AAACTTAGGGCCAAGGAACATAC-3', MV Schwarz genome 4869-4891) and MVSchw9218 (5'-GGACCCTACGTTTTTCTTAATTCTG-3', MV Schwarz genome 9194-9218). Fragment 5 (6200 nucleotides long) was amplified using oligonucleotides MVSchw9119 (5'-AGATAGGGCTGCTAGTGAACCAAT-3', MV Schwarz genome 9119-9142) and MVSchw15319 (5'-ATCAGCACCTGCTCTATAGGTGTAA-3', MV Schwarz genome 15295-15319). To obtain fragment 6, two overlapping fragments generated by PCR were annealed together. The following oligonucleotides were used : MVSchw15155 (5'-GCAGCAGATAATTGAATCATCTGTGAGGACTTCAC, MV Schwarz genome 15155-15190) and MVSchw15570 (5'-CCCGGAGTAAAGAAGAATGTGCCCCCAGAATTTGC-3',
MV Schwarz genome 15535-15570). This fragment was annealed to a fragment containing the hepatitis delta virus (HDV) ribozyme linked to the T7 terminator that was previously obtained by PCR amplification of p(MV+) plasmid (a kind gift from M. Billeter) using oligonucleotides MVSchw15547 (5'-GGCACATTCTTCTTTACTCCGGGAACAAAAAGTTG-3', MV Schwarz genome

15547-15581) and MVSchwEnd (5'-ATAGGGCCCGCGGCCGCATCCGG ATATAGTTCCTCCTTTCA-3' containing an *ApaI* restriction site linked to the last nucleotides of T7 terminator. The 6 fragments thus generated were cloned in pCR®2.1-TOPO® vector (Invitrogen, Groningen, Netherlands) and sequenced. In order to assemble the MV Schwarz full length DNA, a modified BlueScript KS (+) plasmid was constructed : two internally complementary oligonucleotides yielding a *NotI*, *KasI/NarI*, *SpeI*, *ApaI* polylinker were annealed and inserted in pTM plasmid digested with *NotI/ApaI* (pTM was derived from pBluescript KS (+) (Stratagene) by deletion of the T7 promoter (Tangy, F., A. McAllister, and M. Brahic. 1989. Molecular cloning of the complete genome of Theiler's virus, strain GDVII, and production of infectious transcripts. J. Virol. 63:1101-11066). The 6 MV Schwarz cDNA fragments were assembled together step by step using unique restriction sites. Fragments 1 and 2 were assembled together using *BlpI* site in MV sequence (Fig. 2) and *BglII* site in pCR®2.1-TOPO® vector backbone. The resulting plasmid was assembled with fragment 3 using *SbfI* site in MV sequence and *BglII* site in pCR®2.1-TOPO® vector backbone, yielding plasmid pCR®2.1-TOPO®-MVSchw-1-2-3 containing MV Schwarz fragments 1-3. After *NotI/NarI* digestion of this plasmid, the fragment containing the T7 promoter, hammer head ribozyme and the 4922 first nucleotides of MV Schwarz antigenome was inserted in *NotI/NarI* digested pTM vector, yielding pTM-MVL. At the same time, fragments 5 and 6 were assembled together using *BsmBI* site in MV sequence (Fig. 2) and *BssHII* site in pCR®2.1-TOPO® vector backbone, yielding plasmid pCR®2.1-TOPO®-MVSchw-5-6 containing MV Schwarz fragments 5-6. After *SpeI/ApaI* digestion of this plasmid, the fragment containing the 6720 last nucleotides of MV Schwarz antigenome, HDV ribozyme and T7 terminator sequences was inserted in *SpeI/ApaI* digested pTM vector, yielding pTM-MVT. For the final assembling, four fragments were prepared and ligated together : 1) a *SapI/SapI* fragment of pTM-MVL (4367 nucleotides long) containing a part of pTM backbone, the T7 promoter, hammer head ribozyme, and the 1813 first

nucleotides of MV antigenome, 2) a *SapI/NarI* fragment of pTM-MVL (3110 nucleotides long) containing nucleotides 1813-4923 from MV Schwarz antigenome, 3) a *NarI/SpeI* fragment of pCR®2.1-TOPO®-MVSchw-3 (4253 nucleotides long) containing nucleotides 4923-12157 of MV Schwarz antigenome, and 4) a *SpeI/SapI* fragment of pTM-MVT (7235 nucleotides long) containing nucleotides 12157-15894 of MV Schwarz antigenome, HDV ribozyme, T7 terminator and a part of pTM vector backbone. After ligation and cloning, several full constructs were obtained. The resulting plasmid, named pTM-MVSchw, was fully sequenced (Acc. Num. CNCM I-2889). No mutation was found between this cDNA and the previously reported sequence of Schwarz genome (Parks, C. L., R. A. Lerch, P. Walpita, H. P. Wang, M. S. Sidhu, and S. A. Udem. 2001. Analysis of the noncoding regions of measles virus strains in the Edmonston vaccine lineage. J Virol. 75:921-933 ; Parks, C. L., R. A. Lerch, P. Walpita, H. P. Wang, M. S. Sidhu, and S. A. Udem. 2001. Comparison of predicted amino acid sequences of measles virus strains in the Edmonston vaccine lineage. J Virol. 75:910-920).

**Recovery of infectious Schwarz virus from pTM-MVSchw plasmid.**

To recover the Schwarz virus from the pTM-MVSchw cDNA, we used the helper-cell-based rescue system described by Radecke et al. (Radecke, F., P. Spielhofer, H. Schneider, K. Kaelin, M. Huber, K. Dötsch, G. Christiansen, and M. Billeter. 1995. Rescue of measles viruses from cloned DNA. EMBO Journal. 14:5773-5784) and modified by Parks et al. (Parks, C. L., R. A. Lerch, P. Walpita, M. S. Sidhu, and S. A. Udem. 1999. Enhanced measles virus cDNA rescue and gene expression after heat shock. J Virol. 73:3560-3566). Human helper cells stably expressing T7 RNA polymerase and measles N and P proteins (293-3-46 cells, disclosed by Radecke et al (17) were transfected using the calcium phosphate procedure with pTM-MVSchw plasmid (5 µg) and a plasmid expressing the MV polymerase L gene (pEMC-La, 20 ng, disclosed by Radecke et al (17). After overnight incubation at 37° C, the transfection medium

was replaced by fresh medium and a heat shock was applied (43° C for two hours) (12). After two days of incubation at 37° C, transfected cells were transferred on a CEF cells layer and incubated at 32° C in order to avoid any adaptation of the Schwarz vaccine that was originally selected on CEF cells and is currently grown on these cells for safety considerations. The above chicken embryo fibroblastic cells (CEF) were prepared as follows. Fertilized chicken eggs (EARL Morizeau, 8 rue Moulin, 28190 Dangers, France) was incubated at 38°C for 9 days. Embryos were collected sterilely. Head, limbs and viscera were removed and embryos were sliced up and trypsinized for 5-10 minutes at 37°C (Trypsine/EDTA 2.5 g/L). After filtration (70 µm) and several washes in DMEM high glucose/10% FCS, cells were seeded (5-7 $10^6$ cells/pertri dish) and incubated overnight at 37°C. Infectious virus was easily recovered between 3 and 7 days following cocultivation. Syncytia appeared occasionally in CEF, but not systematically. The Schwarz virus was also rescued by the same technique after cocultivation of transfected 293-3-46 helper cells at 37° C with primate Vero cells (african green monkey kidney). In this case, syncytia appeared systematically in all transfections after 2 days of coculture. In order to test for viral adaptation to Vero cells, a preparation of cloned Schwarz virus rescued on Vero cells was passaged two times on Vero cells. Viral particles were purified and viral RNA was reverse-transcribed as described above with the primers used for the cloning (see above). The viral genome was fully sequenced. Two nucleotide changes out of 15894 were found between the rescued/passaged virus and the cDNA used for transfection. These mutations were found in 7 and 8 respectively out of 10 different clones of the same region, indicating a high percentage of mutation among the viral population. Moreover, both mutations resulted in amino acid changes in the fusion protein (F) : G->R in position 266 and Y->S in position 365.

In contrast, the genomic sequence of the virus recovered and passaged on CEF cells at 32 °C was identical to that of the original Schwarz virus. This

observation indicates that changing the host cell of Schwarz virus leads to a rapid adaptation that may affect the properties of the vaccine.

**Growth capacity of the rescued virus**.

The capacity of the Schwarz virus rescued from cDNA to grow on CEF and Vero cells was analyzed and compared to the industrial bulk Schwarz vaccine from which it was derived (obtained from Aventis Pasteur) and to the EdB-tag virus rescued from its cDNA. Monolayers of Verocells in 6-well plates were infected with viruses at different multiplicity of infection. A various time post infection (pi), the cells were seraped into culture medium. After freezing and thawing, infectivity titers were determined by measuring the $TCID_{50}$ in Vero cells. *Growth curves* : Monolayers of Vero cells in 6-well plates were infected with viruses at different multiplicities of infection (MOI). At various times postinfection (pi), the cells were scraped into culture medium. After freezing and thawing, infectivity titers were determined by measuring the $TCID_{50}$ in Vero cells.

*$TCID_{50}$ titration* : Vero cells were seeded into 96-well plate (7500 cells/well) and infected by serial 1:10 dilutions of virus sample in DMEM/5% FCS. After incubation at 37°C for 4-5 days for Ed-B virus and 7 days for Schwarz virus, cells were stained with crystal violet and the virus dilution that resulted in infection in 50% of test unit was determined. The 50% end point described as tissue culture infectious dose ($TCID_{50}$) was calculated by the Kärber method (Karber, G. 1931. Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Arch Exp Path Pharmak. 162:480-483). Tested on Vero cells, the growth kinetics of Schwarz and EdB-tag viruses rescued from their respective cDNA were similar (Fig. 3). The Schwarz viral production on Vero cells reached high yields ($10^7$-$10^8$ $TCID_{50}$/ml after two days of infection using a multiplicity of infection of 0.01). Tested on CEF cells, the Schwarz virus was

able to grow as well at 32° C as at 37° C, while the EdB-tag was not (Fig. 3 ). This observation confirms that the virus from which EdB-tag was cloned was not adapted to CEF cells. The yield of Schwarz virus on CEF was lower than on Vero cells ($10^6$ $TCID_{50}$/ml after 4 days of infection using a multiplicity of infection of 0.05). Similar growth curves and similar titers were observed when CEF cells were infected with the original Schwarz virus from which it was cloned (Fig. 3). These observations demonstrate that the Schwarz virus rescued from its cDNA had the same growth characteristics than the original vaccine batch from which it was cloned.

**Introduction of an additional transcription unit in the Schwarz cDNA**. In the previous work reporting the cloning of EdB-tag virus (17), the authors developed an original method to adapt the viral cDNA as a vector suitable for the expression of foreign transgenes. They inserted an additional transcription unit (ATU) in different positions of the viral genome. This ATU is a copy of the MV N-P intergenic region containing the cis-acting sequences necessary for MV-dependant expression of a transgene inserted into a multiple cloning sites cassette. Largely tested by the authors and ourselves, the expression of foreign transgenes inserted in this ATU was very efficient, depending on the position of insertion in the genome. The different MV genes are expressed according to a transcriptional gradient down the genome, leading to a high expression of the N gene to a low expression of the L gene (Lamb, R., and D. Kolakofsky. 1996. Paramyxoviridae: the viruses ans their replication, p. 1177-1199. *In* B. Fileds, D. Knipe, *et al.* (ed.), Fields Virology. Lippincott-Raven Publishers, Philadelphia).

The insertion of the ATU takes advantage of this gradient, allowing high or low expression of the transgene, depending on the position of insertion. Moreover, in this context the foreign transgenes are expressed using the same controls and pathways as authentic MV genes.

In order to transform the Schwarz cDNA as a vector, we constructed a similar ATU that was inserted in two different positions of the cDNA (Figure 4). The cDNA was sequenced and no mutation was found.

**Immunogenicity of Schwarz MV recovered from cDNA in Macaques.**

First experiment: comparison with Schwarz vaccine.

The immunogenicity of the virus rescued from pTM-MVSchw plasmid and passaged two times on CEF cells was compared to the immunogenicity of Schwarz vaccine in *cynomolgus* macaques. The conditions for passage were the following:

- After rescue, isolated syncytia were picked from the CEF cells cocultivated with 293-3-46 helper cells and a single syncytium was diluted in 600 µl of OptiMEM 1 (Gibco) and vortexed. This inoculum was used to infect fresh CEF cells (80-90% confluent) in a 35 mm well or a T-25 flask. After 2 hours of adsorption at 37°C, the inoculum was replaced by DMEM/5% FCS and cells were incubated at 32°C for 1-2 days. When small syncytia appeared, infected cells were expanded to T-75 flasks : cells were washed with PBS and detached with PBS/1 mM EDTA/0.25% trypsin for 1 minute, then transferred to T-75 flasks together with fresh CEF cells (1/4 of a confluent T-75 flask culture). After 4-7 days of incubation at 32°C in DMEM/5% FCS, the virus (passage 1) was harvested : culture medium was removed and infected cells were scraped in 3 ml of OptiMEM 1. After one cycle of freezing and thawing, cell debris were discarded by centrifugation (1500 rpm, 5 minutes, room temperature). This stock seed was kept frozen at –80 °C and used to infect fresh CEF in the same way to prepare the passage 2 stock.

Different formulations of the vaccine were tested using both the unpassaged bulk preparation from Aventis Pasteur, and the same preparation passaged two times on CEF cells. Viruses were prepared as follows : CEF cells (obtained from chick embryos incubated during 9 days) were infected at a MOI

of 0,05 and incubated at 32° C during 7 days. Viruses were purified by scraping infected cells, freeze/thawing and low speed clarification of cells debris. Stabilizing agents used in the preparation of MV vaccine were obtained from Aventis Pasteur. Different bulk vaccine preparations with and without stabilizing agents were compared at the same dose to the lyophilized final product (Rouvax, Aventis Pasteur). All vaccine preparations were titrated using the $TCID_{50}$ method on Vero cells. Monkeys were injected sub-cutaneaously and blood samples were taken at different time points. In order to compare both humoral and cellular responses, the presence of anti-MV antibodies was looked for in serums by ELISA (Trinity Biotech, USA) and the presence of anti-MV T-cells was looked for by ELISPOT in PBMCs.

Second experiment: comparison with EdB-tag strain

Colony-bred rhesus (*Macaca mulatta*) or cynomolgus (*Macaca fascicularis*) macaques that were seronegative for simian type D retrovirus, simian T-cell lymphotropic virus, simian immunodeficiency virus, and measles virus were housed in accordance with the American Association for Accreditation of Laboratory Animal Care. Monkeys were inoculated subcutaneously with different doses ($10^3$-$10^5$ $TCID_{50}$) of EdB-tag or Schwarz MV diluted in OptiMEM (GibcoBRL) or with $10^4$ $TCID_{50}$ of the lyophilized Rouvax MV vaccine (Aventis Pasteur, Marcy l'Etoile, France) diluted in the solution provided by the supplier. Blood samples were collected at different time after inoculation.

The presence of anti-MV antibodies in serum was looked for by ELISA (Trinity Biotech, USA) one month after vaccination. Each determination was done in triplicate on 1/20 dilution of serum samples. A mixture of 5 samples from negative monkeys was used as the negative control. To determine the immune status ratio (ISR) of each sample, the absorbance of the negative control was subtracted from the absorbance of the positive sample and the

result was divided by the absorbance of a calibrator supplied in the ELISA kit, as recommended by the supplier. Only ISR values higher than 0.9 were considered as positive in this test.

Cellular immune responses were determined by γ-IFN ELISpot assays. Frozen PBMC were thawed and incubated overnight in RPMI,10% FCS and 4 U/ml rh-IL2 (Boehringer Mannheim). Multiscreen-HA 96-wells plates were coated overnight at 4°C with 4 µg/ml of capture anti-γ-IFN (GZ-4, MAbTech) in PBS, washed, then incubated with 100 µl RPMI, 10%FCS for 1 h at 37°C. The medium was replaced by $5.10^5$ PBMC in suspension in 100 µl of RPMI-10% FCS and 100 µl of stimulating agent. The stimulating agent consisted of$10^7$ pfu of recombinant Modified Vaccine Ankara (32) MVA-$H_{MV}$ or MVA-wt as a control Cells were stimulated for 24 h at 37°C. Phytohemaglutinin A (2.5 µg/ml, Sigma) was used as positive control and RPMI as a negative control. The plates were washed twice with PBS, 4 times with PBS, 0.05% Tween 20* (Sigma), and twice again with PBS. A biotinylated anti-γ-IFN antibody (7-B6-1, MabTech, 100µl, 1 µg/ml in PBS) was added and the plates were incubated for 2-4 h at 37°C. Streptravidin-Alkaline Phosphatase (AP) conjugate (Roche, 100µl, 1/2000 dilution in PBS) was added And spots were developed with BCIP/NBT (Promega) in 1 M Tris pH 9.5, 1.5 M NaCl, 0.05 M MgCl2. After drying overnight at room temperature, spots were counted using an automated image analysis system (ELISpot Reader, Bio-Sys). The low background obtained after MVA-wt stimulation was subtracted and the results were expressed as MVA-$H_{MV}$ specific γ-IFN producing cells per million PBMC.

Mice immunization and characterization of humoral immune responses. FVB mice heterozygous for the CD46 transgene (33), were crossed with 129sv IFN-$\alpha/\beta R^{-/-}$ mice which lack the type I interferon receptor (30). The F1 progeny was screened by PCR and the CD46 $^{+/-}$ animals were crossed again with 129sv IFN-$\alpha/\beta R^{-/-}$ mice. IFN-$\alpha/\beta R^{-/-}$ CD46 $^{+/-}$ animals were selected and used for

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immunization experiments. These mice are susceptible to MV infection (27, 29). Six-week-old female $CD46^{+/-}$ or $CD46^{+/-}$ IFN-$\alpha/\beta R^{-/-}$ (IFNAR) mice were inoculated intraperitoneally with $10^4$ $TCID_{50}$ of the different vaccine preparations (4 mice per group). The presence of anti-MV antibodies was looked for by ELISA (Trinity Biotech, USA) in sera collected one month after vaccination. In this case, an anti mouse IgG Mab (Amersham) was used as secondary antibody. Each determination was done in triplicate. The absorbence determined with a mixture of negative mice sera was subtracted from the absorbence measured in positive mice. Because it was not possible in this case to use the ISR to compare samples, serial dilutions of mice sera were tested to determine the endpoint limit positive dilution.

## RESULTS

**Comparison of humoral immune responses after vaccination of macaques and mice with EdB-tag and Schwarz MV vaccines.** EdB-tag MV is a molecularly cloned MV derived from the Edmonston B strain (16). We compared its immunogenicity in macaques with that of the Schwarz commercial MV vaccine. The EdB-tag virus was prepared in Vero cells infected at a multiplicity of infection (MOI) of 0.05. When syncytia occupied 80-90% of the culture, the cells were scraped, cells and medium were freeze/thawed and cell debris were eliminated by low speed centrifugation. The Schwarz MV, obtained from Aventis Pasteur (Marcy l'Etoile, France), was prepared in the same way from infected chick embryo fibroblasts (CEF) grown at 32°C, the temperature at which this strain has been adapted to CEF. The titers of both vaccine preparations were determined by endpoint dilution assays in Vero cells and expressed as $TCID_{50}$. Different doses ($10^3$ to $10^5$ $TCID_{50}$) of EdB-tag and Schwarz MV were injected subcutaneously to macaques (2 monkeys per dose). As a control, animals were also injected with $10^4$ $TCID_{50}$ of the lyophilized

commercial Schwarz vaccine (Rouvax, Aventis Pasteur). Anti-MV antibodies levels were determined by ELISA in macaques' sera collected one month after vaccination. Macaques inoculated with $10^3$ and $10^4$ $TCID_{50}$ of the Schwarz MV had antibody levels similar to those induced by a standard dose of Rouvax vaccine (Fig. 6). Macaques inoculated with $10^4$ $TCID_{50}$ of EdB-tag virus remained negative (not shown). The injection of a tenfold higher dose ($10^5$ $TCID_{50}$) induced only a weak response that was lower than that observed with $10^3$ $TCID_{50}$ of Schwarz MV (Fig. 6). Vaccination with the commercial vaccine induced the best response probably due to the adjuvant effect of lyophilization.

The different vaccine preparations were also tested in genetically modified mice obtained as described in Materials and Methods. Two types of mice were used : mice expressing CD46 (33), the human receptor for MV vaccine strains (34), and mice expressing CD46 and lacking the IFN type I receptor (29). Six-week-old mice were inoculated intraperitoneally with $10^4$ $TCID_{50}$ of the different vaccine preparations (4 mice per group). Figure 7 shows the detection of anti-MV antibodies in sera of both types of mice collected one month after vaccination. In CD46 mice, the EdB-tag virus was less immunogenic than the Schwarz vaccine. The average titer obtained with the former was 1/80, whereas it was 1/1280 with the latter. The EdB-tag virus was also less immunogenic in CD46 mice lacking the IFN type I receptor but the difference was less pronounced than in CD46 immuno-competent mice, possibly indicating a difference in sensitivity to IFN $\alpha/\beta$ between the two viral strains.

**Immunogenicity of Schwarz MV recovered from cDNA**. The immunogenicity for cynomolgus macaques of the virus rescued from pTM-MVSchw plasmid and passaged two times on CEF or Vero cells was compared to that of the industrial Schwarz vaccine. Cynomolgus macaques were used in this experiment because of the difficulty of obtaining rhesus macaques from China that were MV negative. These macaques are as sensitive to MV as rhesus macaques, as shown by several studies (28, 26). Monkeys (2 animals

per preparation) were injected sub-cutaneaously with $10^4$ $TCID_{50}$ of Schwarz MV vaccine from Aventis or Schwarz MV rescued from pTM-MVSchw plasmid and grown either on CEF or Vero cells. The presence of anti-MV antibodies was determined in sera collected at different time points (Fig. 8). All the vaccinated macaques became positive. No statistically significant difference was observed, one or two months after immunization, between the different vaccine preparations tested. This result demonstrates that the virus rescued from the pTM-MVSchw plasmid has the same immunogenicity in non human primates as the parental Schwarz vaccine. No difference was detected between the rescued viruses grown on CEF or Vero cells, indicating that the two mutations generated in the F protein by the passages on Vero cells did not affect the immunogenicity of the virus.

Changes in the number of total white blood cells (WBC), lymphocytes and monocytes were observed during the first month following inoculation (Fig. 9). There was a mild leukopenia during the first week, as previously observed after MV vaccination (1). During the second week a clear increase in the number of circulating lymphocytes and monocytes was observed. It coincided with a peak of the number of MV-specific T-lymphocytes as detected by a $\gamma$-IFN ELISpot assay (Fig. 9 D). No statistically significant difference was detected between the specific cellular immune responses induced by the Schwarz MV rescued from plasmid and the Schwarz vaccine prepared by Aventis.

**DISCUSSION**

In the present work we describe cloning and rescuing the Schwarz/Moraten attenuated strain of measles virus, the constituent of two widely used measles vaccines, Attenuavax (Merck and Co. Inc., West Point, USA) and Rouvax (Aventis Pasteur, Marcy l'Etoile, France), and of the combined measles, mumps, and rubella vaccine (MMR) (35). To be used in a pediatric clinical trial, a live attenuated MV produced from a cDNA must be as safe and efficient as the parental vaccine. Assuming that safety and efficiency

depend ultimately on the genomic sequence of the attenuated strain, we cloned the MV Schwarz cDNA from viral particles prepared from an industrial batch of vaccine using procedures optimized for fidelity of cloning. As a result, the sequence of the clone that we obtained was identical to that of the parental Schwarz MV genome. To maximize yield during rescue, the viral antigenomic cDNA was placed under the control of a T7 RNA polymerase promoter with the GGG motif necessary for full efficiency. A hammerhead ribozyme was inserted between this GGG motif and the first viral nucleotide to allow the exact cleavage of the viral RNA. In order to avoid adapting the Schwarz vaccine to non-certified cells during rescue, helper cells transfected with the engineered cDNA were cocultivated with CEF, the cells on which this vaccine was selected originally and is currently prepared. The rescued virus was passaged two times on CEF and its genome was entirely sequenced. No mutation was found when the sequence was compared to that of the original virus. Moreover, the growth kinetics and the yield of the rescued virus and the original Schwarz virus on CEF were identical.

The Schwarz virus was also rescued after co-cultivation of transfected helper cells with Vero cells, which are very permissive to MV. In this case, however, two mutations appeared in the viral fusion protein (F) after two passages on Vero cells. This rapid adaptation correlated with a much more fusogenic phenotype on Vero cells. In contrast, the rescued Schwarz MV was not fusogenic on CEF (only rare syncytia could be observed in infected CEF). The two mutations occurred in the F protein (G->R in position 266 and Y->S in position 365). These mutations are present in the EdB-tag virus (see Figure 6) which is grown on Vero cells. They are also present in the Hallé strain, which is highly related to Edmonston strain and does not infect CEF (31). These two mutations appear thus to correlate with enhanced fusion in Vero cells. The rapid adaptation of the F protein after only two passages of the Schwarz virus on Vero cells shows that in order to keep its genetic integrity the vaccine must be grown on CEF.

The virus rescued from the pTM-Schw plasmid had the same immunogenicity in macaques as the parental Schwarz vaccine. It is important to emphasize that in these experiments macaques were inoculated with the low dose of virus used for human immunization. Therefore, it will be possible to conduct human clinical trials with this virus using standard vaccine doses ($10^4$ $TCID_{50}$). In contrast, the previously cloned EdB-tag MV was not immunogenic in macaques and poorly immunogenic in mice transgenic for CD46, when used at the same dose as the cloned Schwarz MV.

What could be the reason for the higher immunogenicity of the Schwarz MV strain? Inducing good immunogenicity with a live attenuated viral vaccine requires replication in tissues at a level high enough to prime the immune system adequately. Several of the mutations between the Schwarz and the EdB-tag MV genomes are located in the P/V/C and L genes, suggesting difference in replication efficiency. It is possible that the Schwarz MV replicates in lymphoid cells *in vivo* more efficiently than the EdB-tag MV even though they replicated at the same rate in Vero cells. Efficient replication *in vivo* requires some evasion mechanism from the IFN-α/β response. Vero cells, on which the EdB-tag virus was adapted, do not respond to IFN-α/β stimulation. Therefore the EdB-tag MV was selected in the absence of an IFN-α/β response and might be particularly sensitive to this host defense mechanism. Indeed, it has been shown that passaging wild type MV on Vero cells changes the phenotype of the virus from non-IFN-inducer to IFN-inducer (36). Also, the fact that the Ed-tag MV was immunogenic in mice transgenic for the CD46 receptor providing they were also knock-out for the IFN-α/β receptor suggest that this virus is particularly IFN-sensitive. Interestingly, the IFN-α/β response helps priming the specific immune response against the vaccine. Therefore a good live vaccine must at the same time induce an IFN-α/β response and evade it to some extent. For this reason selecting attenuated viral vaccines on primary cells with a strong IFN-α/β response, such as CEF, might be a good strategy.

The MV products which contribute to IFN resistance have not been identified. However, the nonstructural C protein of the closely related Sendai virus has been shown to counteract the IFN-induced antiviral state (37). The 5 mutations not related to any Edmonston subgroup that we found in the EdB-tag P/V/C gene might be responsible for its low immunogenicity in macaques. On the other hand, the two mutations generated in the F protein by passaging the Schwarz virus on Vero cells did not affect its immune potential, indicating that the fusogenic property of the viral envelope proteins may not play a significant role in immunogenicity.

The pTM-MVSchw plasmid was engineered for the expression of foreign genes by the introduction of two ATU at different positions of the genome. Rescued Schwarz recombinant MV expressed the green fluorescent protein, thus showing that this new measles vaccine functions as a vector. In conclusion, this molecular clone will allow producing MV vaccine without having to rely on seed stocks. With its ATUs, it will be possible to use it as a vector to produce recombinant vaccines based on an approved, efficient and worldwide used vaccine strain.

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# SEQUENCE LISTING

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<210> 33
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oligonucleotide

<400> 33
aactgtatgg tggctttggg 20

<210> 34
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<220>
<223> Description of Artificial Sequence:
oligonucleotide

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<210> 36
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<210> 39
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<210> 48
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<210> 52
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<210> 59
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<223> Description of Artificial Sequence:
oligonucleotide

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<400> 62
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oligonucleotide

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oligonucleotide

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<400> 74
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oligonucleotide

<400> 79
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<210> 80
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oligonucleotide

<400> 80
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nucleotide sequence of the pTM-MVSChw plasmid
(CNCM I-2889)

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**WHAT IS CLAIMED IS:**

1. A cDNA molecule which encodes the nucleotide sequence of the full length, infectious antigenomic (+)RNA strand of a measles virus (MV) originating from the Schwarz strain or the Moraten strain, said cDNA comprising the nucleotide sequence extending from nucleotide 83 to nucleotide 15976 of the sequence SEQ ID NO.82.

2. The cDNA molecule according to claim 1, which is placed under the control of a heterologous expression control sequence appropriate for the transcription of the antigenomic (+)RNA strand starting from the cDNA molecule.

3. The cDNA molecule according to claim 2, wherein the heterologous expression control sequence of said cDNA comprises the T7 promoter and T7 terminator sequences.

4. The cDNA molecule according to any one of claims 1 to 3, which further comprises, at its 5'-end, adjacent to the first nucleotide of the nucleotide sequence encoding the full length, infectious antigenomic (+)RNA strand of the Schwarz strain or the Moraten strain, a GGG motif followed by a hammerhead ribozyme sequence and which comprises, at its 3'-end, adjacent to the last nucleotide of said nucleotide sequence encoding the full length, infectious anti-genomic (+)RNA strand, the sequence of a hepatitis delta virus ribozyme.

5. The cDNA molecule according to any one of claims 1 to 4, which is capable of producing infectious viral particles of the Schwarz strain or the Moraten strain in a helper cell capable of expressing proteins necessary for transcription, and replication of the RNA genome-sequence of the measles virus from said cDNA and under conditions enabling viral particles assembly.

6. The cDNA molecule according to any one of claims 1 to 5, which is comprised in a plasmid capable of replication.

7. The cDNA molecule according to any one of claims 1 to 6, which is comprised in a plasmid pTM-MVSchw deposited at the CNCM on June 12, 2002 under number I-2889.

8. The cDNA molecule according to any one of claims 1 to 7, which comprises the nucleotide sequence of SEQ ID NO.82.

9. The cDNA molecule according to any one of claims 1 to 7, which comprises the nucleotide sequence extending from nucleotide 83 to nucleotide 15977 of the sequence SEQ ID NO.82.

10. The cDNA molecule according to any one of claims 1 to 7, which comprises the nucleotide sequence extending from nucleotide 29 to nucleotide 16202 of the sequence SEQ ID NO.82 or from nucleotide 29 to nucleotide 15977 of the sequence SEQ ID NO.82.

11. The cDNA molecule according to any one of claims 1 to 7, which comprises the nucleotide sequence extending from nucleotide 26 to nucleotide 16202 of the sequence SEQ ID NO.82.

12. The cDNA molecule according to any one of claims 1 to 7, which comprises the nucleotide sequence extending from nucleotide 9 to nucleotide 16202 of the sequence SEQ ID NO.82.

13. The cDNA molecule according to claim 1 which is the insert contained in plasmid pTM-MVSchw deposited at the CNCM on June 12, 2002 under number I-2889, wherein said insert encodes the nucleotide sequence of the full length antigenomic (+)RNA strand of the measles virus.

14. The cDNA molecule according to any one of claims 1 to 13, which is the product of the reverse transcription of the viral RNA purified from viral particles of the measles virus.

15. The cDNA molecule according to any one of claims 1 to 14, which is capable of inducing an immune response against at least one antigen of a measles virus, when administered *in vivo*.

16. A recombinant cDNA molecule which comprises the cDNA molecule according to any one of claims 1 to 13 and which further comprises a heterologous DNA sequence capable of expressing a heterologous amino acid sequence, wherein the recombinant cDNA further complies with the rule of six.

17. The recombinant cDNA according to claim 16, which further comprises an Additional Transcription Unit used for cloning the heterologous DNA sequence.

18. The recombinant cDNA according to claim 17, wherein the Additional Transcription Unit is cloned upstream of the N gene of the measles virus or between the P and M gene of the measles virus, or between the H and L genes.

19. The recombinant cDNA according to any one of claims 16 to 18, wherein the heterologous DNA sequence codes for an immunogenic sequence of a pathogen.

20. A vector comprising the cDNA molecule according to any one of claims 1 to 15.

21. The vector of claim 20, which is plasmid pTM-MVSchw deposited at the CNCM on June 12, 2002 under number I-2889.

22. The vector according to claim 20 or 21, which comprises a heterologous DNA sequence inserted therein provided the obtained recombinant cDNA molecule complies with the rule of six.

23. The vector according to claim 22, wherein the cDNA molecule and the heterologous DNA sequence are contained within a replicon and said replicon complies with the rule of six.

24. The vector according to claim 22 or 23, wherein the heterologous DNA sequence is inserted within an Additional Transcription Unit (ATU) cloned into said cDNA molecule.

25. The vector according to claim 24, wherein the ATU is cloned upstream from the M gene of the measles virus contained in the cDNA molecule.

26. The vector according to claim 24, wherein the ATU is cloned between the N and P genes of the measles virus contained in the cDNA molecule.

27. The vector of claim 26, which is the plasmid pTM-MV Schw2-gfp deposited at the CNCM under number I-2890, or the plasmid pTM-MVSchw2-GFPbis deposited at the CNCM under number I-3034.

28. A process for the preparation of infectious measles virus particles comprising :

1) expressing the cDNA according to any one of claims 1 to 19 or the vector according to any one of claims 20 to 27 in a helper cell line which also expresses proteins necessary for transcription, replication and encapsidation of the antigenomic (+)RNA sequence of the measles virus starting from said cDNA and under conditions enabling viral particles assembly and,
2) recovering the expressed viral particles.

29. A process for the preparation of infectious measles virus particles, comprising :

1) transfecting helper cells with the cDNA according to any one of claims 1 to 19 or with the vector according to any one of claims 20 to 27, wherein said helper cells are capable of expressing helper functions to express an RNA polymerase, and to express the N, P and L proteins of the MV virus;

2) co-cultivating said transfected helper cells of step 1) with passaged cells suitable for the passage of the MV vaccine strain from which the cDNA originates; and

3) recovering the infectious MV viral particles produced.

30. The process according to claim 29, wherein the transfection step is followed by replacement of the transfection medium by fresh medium and applying a heat shock prior to incubation.

31. The process according to claim 29 or 30, wherein the helper cells are derived from human embryonic kidney cell line 293 (ATCC CRL-1573).

32. The process according to claim 29, wherein the cells suitable for passage are CEF cells.

33. The process according to any one of claims 28 to 32, which is used for the production of infectious measles virus appropriate for use as active principle in a vaccine composition.

34. A vaccine composition whose active principle comprises measles viral particles rescued from the cDNA molecule according to any one of claims 16 to 19, or from the vector according to any one of claims 22 to 27, in a helper-cell-based rescue system.

35. The vaccine composition according to claim 34, which is suitable for protection against measles virus.

36. The vaccine composition according to claim 34, which is suitable for protection against measles virus and against a pathogen from which the immunogenic sequence derives.

37. A process for the preparation of the cDNA molecule as defined in any one of claims 1 to 19, comprising the following steps:

- purifying viral RNA from viral particles of the Schwarz strain or the Moraten strain, wherein the genome of viral particles of the Schwarz strain or the Moraten strain comprises the nucleotide sequence extending from nucleotide 83 to nucleotide 15976 of the sequence SEQ ID NO.82; and
- obtaining the cDNA molecule by reverse transcription of the purified viral RNA.

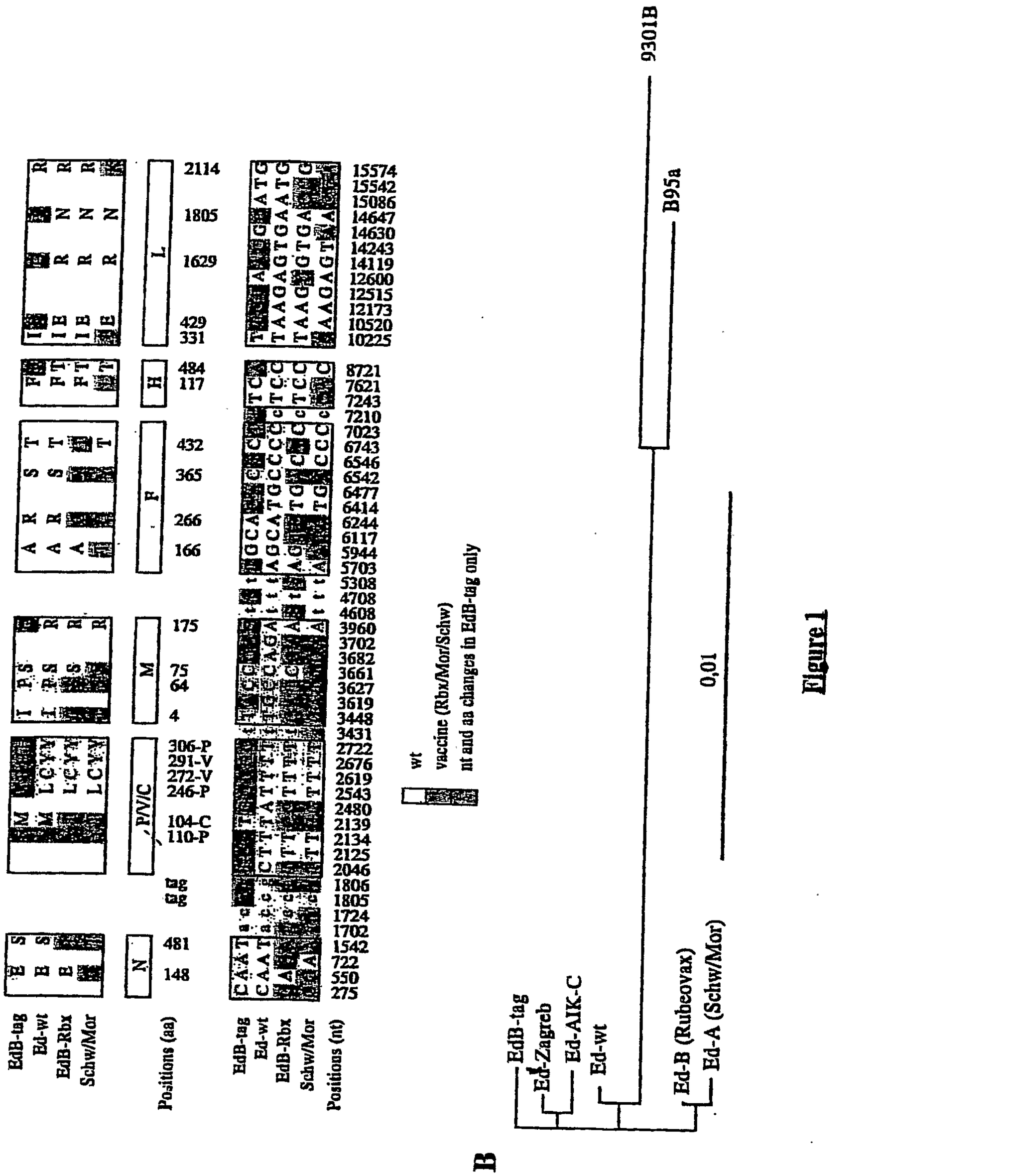
A
EdB-tag
Ed-wt
EdB-Rbx
Schw/Mor
Positions (aa)
Positions (nt)
2114
1805
1629
429
331
484
117
432
365
266
166
175
75
64
4
306-P
291-V
272-V
246-P
104-C
110-P
481
148
15574
15542
15086
14647
14630
14243
14119
12600
12515
12173
10520
10225
8721
7621
7243
7210
7023
6743
6546
6542
6477
6414
6244
6117
5944
5703
5308
4708
4608
3960
3702
3682
3661
3627
3619
3448
3431
2722
2676
2619
2543
2480
2139
2134
2125
2046
1806
1805
1724
1702
1542
722
550
275
L
H
F
M
P/V/C
wt
vaccine (Rbx/Mor/Schw)
nt and aa changes in EdB-tag only
B
9301B
B95a
EdB-tag
Ed-Zagreb
Ed-AIK-C
Ed-wt
Ed-B (Rubeovax)
Ed-A (Schw/Mor)
0,01

Figure 1

pTM-MVSchw

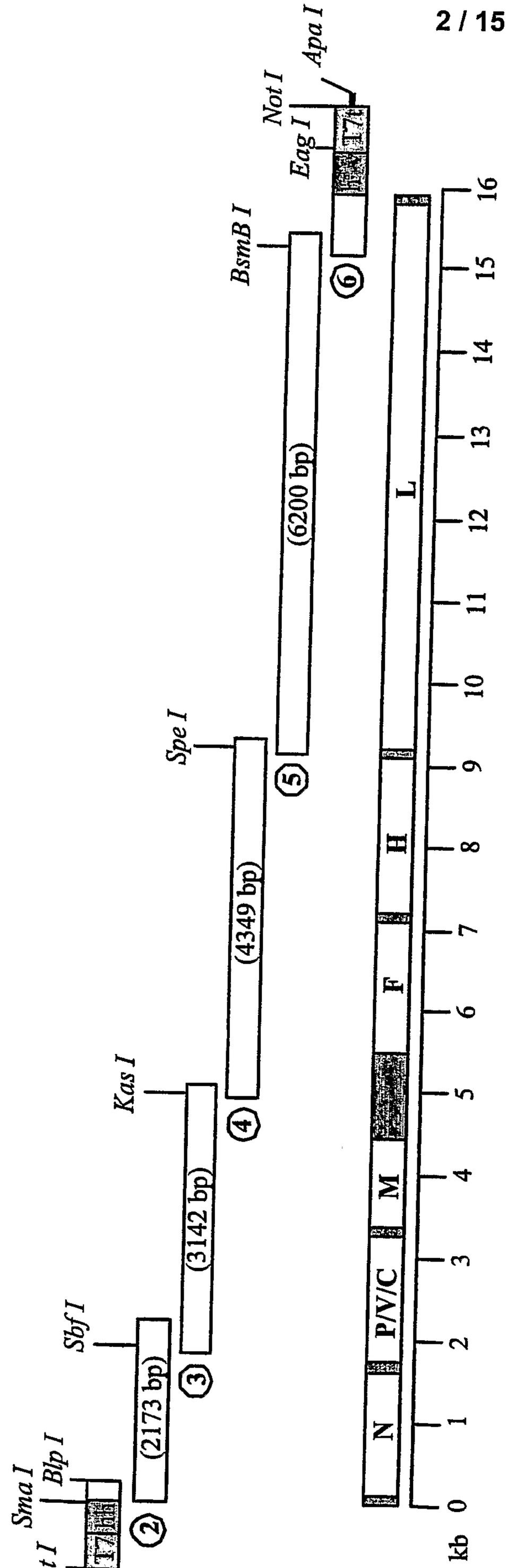


**Figure 2**

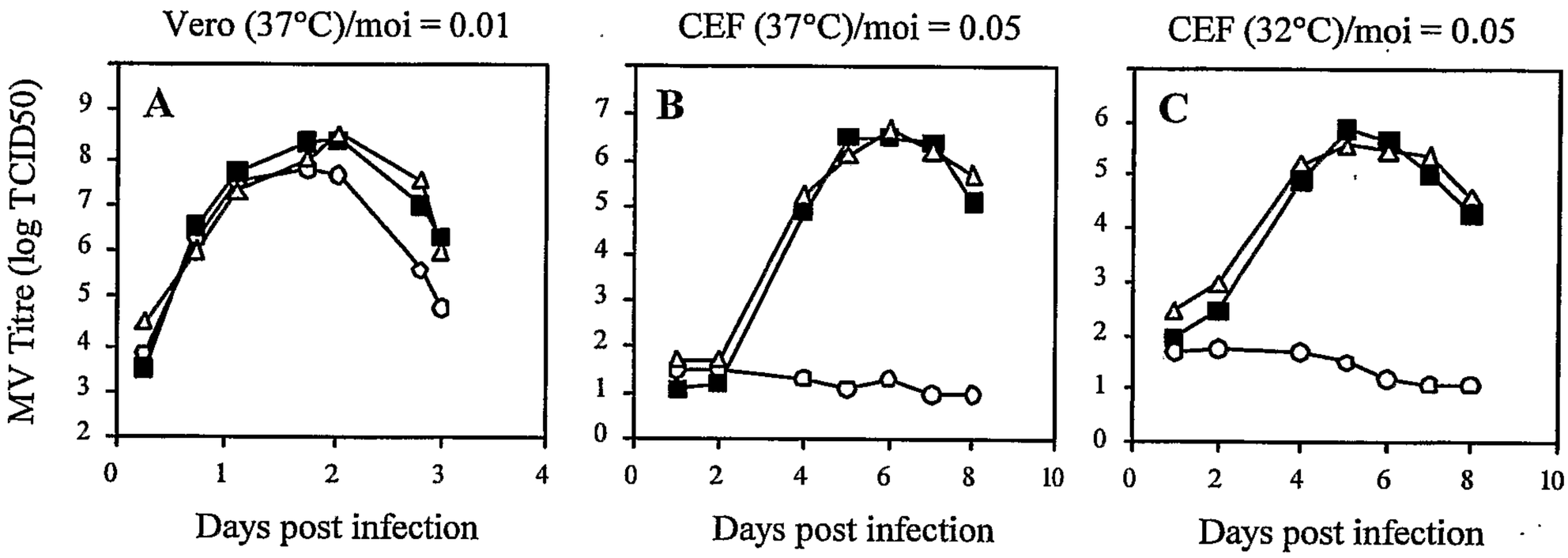
Vero (37°C)/moi = 0.01
CEF (37°C)/moi = 0.05
CEF (32°C)/moi = 0.05
A
B
C
MV Titre (log TCID50)
Days post infection
Days post infection
Days post infection

Figure 3

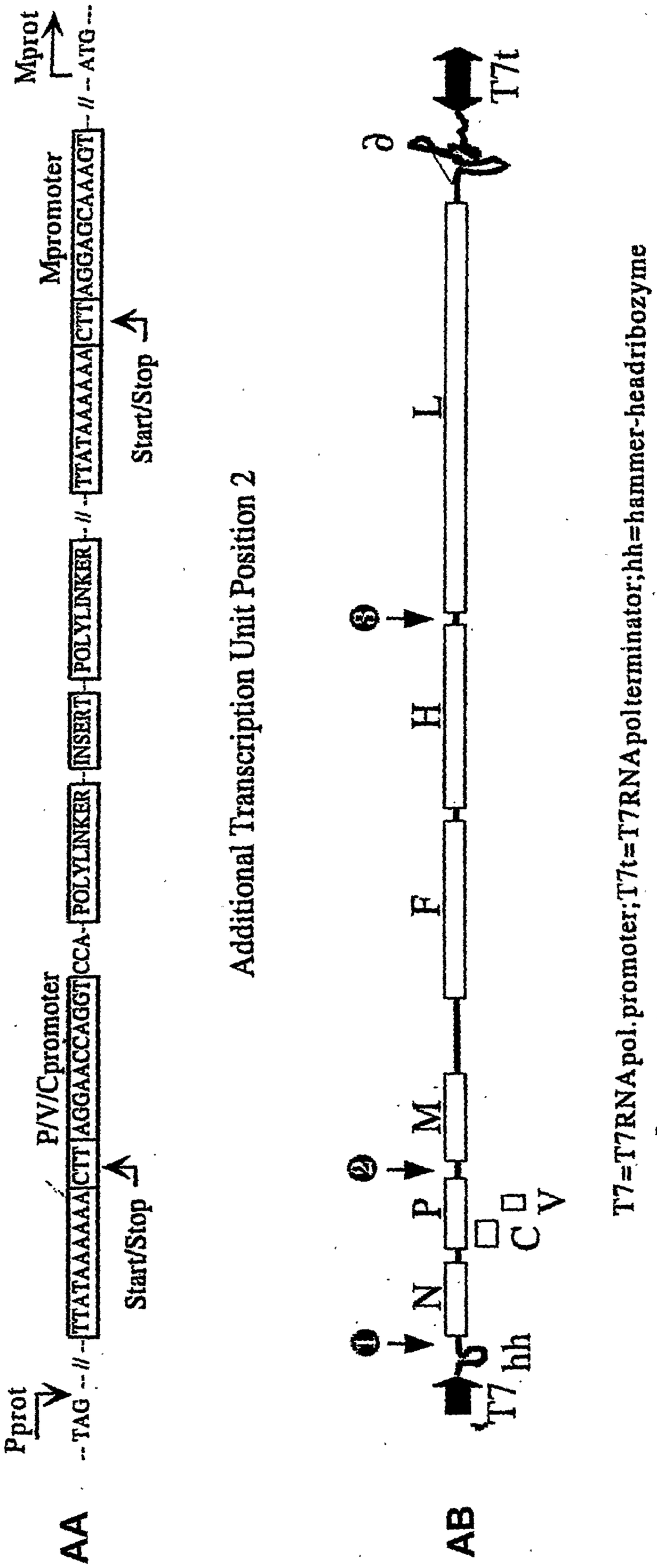
AA
Pprot
-- TAG -- // --
TTATAAAAAA
Start/Stop
P/V/Cpromoter
CTT
AGGAACCAGGT
CCA-
POLYLINKER
INSERT
Additional Transcription Unit Position 2
T7=T7RNApol.promoter;T7t=T7RNApolterminator;hh=hammer-headribozyme
∂=hepatitisdeltavirus(HDV)genomicsenseribozyme;plasmide=pTM
❶ ❷ ❻ =additionaltranscriptionunits(ATU)
AB
T7 hh
N
P
M
F
H
L
C
V
∂
T7t

Figure 4A

Sequence of ATU :

actagcctaccctccatcattgttataaaaaacttaggaaccaggtccacacagccgccagcccatcaacgcgtacgtagcgcgcATGGTGAGCAAGGGCGAGGAGCTGTT
CACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAG
GGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGT
GACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCA
TGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTT
CGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAA
GCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAG
ATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCG
TGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGT
CCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAGtgagcgcgcagcgctgacgtctcgcgatC
gatgctagc

**Figure 4B**

GCGGCCGCTA ATACGACTCA CTATAGGGcc aactttgttt ggtctgatga gtccgtgagg 60
acgaaacccg gagtcccggg tcACCAAACA AAGTTGGGTA AGGATAGTTC AATCAATGAT 120
CATCTTCTAG TGCACTTAGG ATTCAAGATC CTATTATCAG GGACAAGAGC AGGATTAGGG 180
ATATCCGAGA TGGCCACACT TTTAAGGAGC TTAGCATTGT TCAAAAGAAA CAAGGACAAA 240
CCACCCATTA CATCAGGATC CGGTGGAGCC ATCAGAGGAA TCAAACACAT TATTATAGTA 300
CCAATCCCTG GAGATTCCTC AATTACCACT CGATCCAGAC TTCTGGACCG GTTGGTGAGG 360
TTAATTGGAA ACCCGGATGT GAGCGGGCCC AAACTAACAG GGGCACTAAT AGGTATATTA 420
TCCTTATTTG TGGAGTCTCC AGGTCAATTG ATTCAGAGGA TCACCGATGA CCCTGACGTT 480
AGCATAAGGC TGTTAGAGGT TGTCCAGAGT GACCAGTCAC AATCTGGCCT TACCTTCGCA 540
TCAAGAGGTA CCAACATGGA GGATGAGGCG GACCAATACT TTTCACATGA TGATCCAATT 600
AGTAGTGATC AATCCAGGTT CGGATGGTTC GGGAACAAGG AAATCTCAGA TATTGAAGTG 660
CAAGACCCTG AGGGATTCAA CATGATTCTG GGTACCATCC TAGCCCAAAT TTGGGTCTTG 720
CTCGCAAAGG CGGTTACGGC CCCAGACACG GCAGCTGATT CGGAGCTAAG AAGGTGGATA 780
AAGTACACCC AACAAAGAAG GGTAGTTGGT GAATTTAGAT TGGAGAGAAA ATGGTTGGAT 840
GTGGTGAGGA ACAGGATTGC CGAGGACCTC TCCTTACGCC GATTCATGGT CGCTCTAATC 900
CTGGATATCA AGAGAACACC CGGAAACAAA CCCAGGATTG CTGAAATGAT ATGTGACATT 960
GATACATATA TCGTAGAGGC AGGATTAGCC AGTTTTATCC TGACTATTAA GTTTGGGATA 1020
GAAACTATGT ATCCTGCTCT TGGACTGCAT GAATTTGCTG GTGAGTTATC CACACTTGAG 1080
TCCTTGATGA ACCTTTACCA GCAAATGGGG GAAACTGCAC CCTACATGGT AATCCTGGAG 1140
AACTCAATTC AGAACAAGTT CAGTGCAGGA TCATACCCTC TGCTCTGGAG CTATGCCATG 1200
GGAGTAGGAG TGGAACTTGA AAACTCCATG GGAGGTTTGA ACTTTGGCCG ATCTTACTTT 1260
GATCCAGCAT ATTTTAGATT AGGGCAAGAG ATGGTAAGGA GGTCAGCTGG AAAGGTCAGT 1320
TCCACATTGG CATCTGAACT CGGTATCACT GCCGAGGATG CAAGGCTTGT TTCAGAGATT 1380
GCAATGCATA CTACTGAGGA CAAGATCAGT AGAGCGGTTG GACCCAGACA AGCCCAAGTA 1440
TCATTTCTAC ACGGTGATCA AAGTGAGAAT GAGCTACCGA GATTGGGGGG CAAGGAAGAT 1500
AGGAGGGTCA AACAGAGTCG AGGAGAAGCC AGGGAGAGCT ACAGAGAAAC CGGGCCCAGC 1560
AGAGCAAGTG ATGCGAGAGC TGCCCATCTT CCAACCGGCA CACCCCTAGA CATTGACACT 1620
GCAACGGAGT CCAGCCAAGA TCCGCAGGAC AGTCGAAGGT CAGCTGACGC CCTGCTTAGG 1680
CTGCAAGCCA TGGCAGGAAT CTCGGAAGAA CAAGGCTCAG ACACGGACAC CCCTATAGTG 1740
TACAATGACA GAAATCTTCT AGACTAGGTG CGAGAGGCCG AGGGCCAGAA CAACATCCGC 1800
CTACCATCCA TCATTGTTAT AAAAAACTTA GGAACCAGGT CCACACAGCC GCCAGCCCAT 1860
CAACCATCCA CTCCCACGAT TGGAGCCAAT GGCAGAAGAG CAGGCACGCC ATGTCAAAAA 1920
CGGACTGGAA TGCATCCGGG CTCTCAAGGC CGAGCCCATC GGCTCACTGG CCATCGAGGA 1980
AGCTATGGCA GCATGGTCAG AAATATCAGA CAACCCAGGA CAGGAGCGAG CCACCTGCAG 2040
GGAAGAGAAG GCAGGCAGTT CGGGTCTCAG CAAACCATGC CTCTCAGCAA TTGGATCAAC 2100
TGAAGGCGGT GCACCTCGCA TCCGCGGTCA GGGACCTGGA GAGAGCGATG ACGACGCTGA 2160
AACTTTGGGA ATCCCCCCAA GAAATCTCCA GGCATCAAGC ACTGGGTTAC AGTGTTATTA 2220
CGTTTATGAT CACAGCGGTG AAGCGGTTAA GGGAATCCAA GATGCTGACT CTATCATGGT 2280
TCAATCAGGC CTTGATGGTG ATAGCACCCT CTCAGGAGGA GACAATGAAT CTGAAAACAG 2340
CGATGTGGAT ATTGGCGAAC CTGATACCGA GGGATATGCT ATCACTGACC GGGGATCTGC 2400
TCCCATCTCT ATGGGGTTCA GGGCTTCTGA TGTTGAAACT GCAGAAGGAG GGGAGATCCA 2460
CGAGCTCCTG AGACTCCAAT CCAGAGGCAA CAACTTTCCG AAGCTTGGGA AAACTCTCAA 2520
TGTTCCTCCG CCCCCGGACC CCGGTAGGGC CAGCACTTCC GGGACACCCA TTAAAAAGGG 2580
CACAGACGCG AGATTAGCCT CATTTGGAAC GGAGATCGCG TCTTTATTGA CAGGTGGTGC 2640
AACCCAATGT GCTCGAAAGT CACCCTCGGA ACCATCAGGG CCAGGTGCAC CTGCGGGGAA 2700
TGTCCCCGAG TGTGTGAGCA ATGCCGCACT GATACAGGAG TGGACACCCG AATCTGGTAC 2760
CACAATCTCC CCGAGATCCC AGAATAATGA AGAAGGGGGA GACTATTATG ATGATGAGCT 2820
GTTCTCTGAT GTCCAAGATA TTAAAACAGC CTTGGCCAAA ATACACGAGG ATAATCAGAA 2880
GATAATCTCC AAGCTAGAAT CACTGCTGTT ATTGAAGGGA GAAGTTGAGT CAATTAAGAA 2940
GCAGATCAAC AGGCAAAATA TCAGCATATC CACCCTGGAA GGACACCTCT CAAGCATCAT 3000
GATCGCCATT CCTGGACTTG GGAAGGATCC CAACGACCCC ACTGCAGATG TCGAAATCAA 3060

FIGURE 5A

TCCCGACTTG AAACCCATCA TAGGCAGAGA TTCAGGCCGA GCACTGGCCG AAGTTCTCAA 3120
GAAACCCGTT GCCAGCCGAC AACTCCAAGG AATGACAAAT GGACGGACCA GTTCCAGAGG 3180
ACAGCTGCTG AAGGAATTTC AGCTAAAGCC GATCGGGAAA AAGATGAGCT CAGCCGTCGG 3240
GTTTGTTCCT GACACCGGCC CTGCATCACG CAGTGTAATC CGCTCCATTA TAAAATCCAG 3300
CCGGCTAGAG GAGGATCGGA AGCGTTACCT GATGACTCTC CTTGATGATA TCAAAGGAGC 3360
CAATGATCTT GCCAAGTTCC ACCAGATGCT GATGAAGATA ATAATGAAGT AGCTACAGCT 3420
CAACTTACCT GCCAACCCCA TGCCAGTCGA CCCAACTAGT ACAACCTAAA TCCATTATAA 3480
AAAACTTAGG AGCAAAGTGA TTGCCTCCCA AGGTCCACAA TGACAGAGAC CTACGACTTC 3540
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GATGGCAGGC TGGTGCCCCA GGTCAGAGTC ATAGATCCTG GTCTAGGCGA CAGGAAGGAT 3660
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CCAATCGGGC GAGCATTTGG GTTCCTGCCC TTAGGTGTTG GCAGATCCAC AGCAAAGCCC 3780
GAAAAACTCC TCAAAGAGGC CACTGAGCTT GACATAGTTG TTAGACGTAC AGCAGGGCTC 3840
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GTCCTAACAA CAGGGAGTGT CTTCAACGCA AACCAAGTGT GCAATGCGGT TAATCTGATA 3960
CCGCTCGATA CCCCGCAGAG GTTCCGTGTT GTTTATATGA GCATCACCCG TCTTTCGGAT 4020
AACGGGTATT ACACCGTTCC TAGAAGAATG CTGGAATTCA GATCGGTCAA TGCAGTGGCC 4080
TTCAACCTGC TGGTGACCCT TAGGATTGAC AAGGCGATAG GCCCTGGGAA GATCATCGAC 4140
AATACAGAGC AACTTCCTGA GGCAACATTT ATGGTCCACA TCGGGAACTT CAGGAGAAAG 4200
AAGAGTGAAG TCTACTCTGC CGATTATTGC AAAATGAAAA TCGAAAAGAT GGGCCTGGTT 4260
TTTGCACTTG GTGGGATAGG GGGCACCAGT CTTCACATTA GAAGCACAGG CAAAATGAGC 4320
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GACCAAGGAC TATTCAAAGT TCTGTAGACC GTAGTGCCCA GCAATGCCCG AAAACGACCC 4560
CCCTCACAAT GACAGCCAGA AGGCCCGGAC AAAAAAGCCC CCTCCGAAAG ACTCCACGGA 4620
CCAAGCGAGA GGCCAGCCAG CAGCCGACGG CAAGCGCGAA CACCAGGCGG CCCCAGCACA 4680
GAACAGCCCT GACACAAGGC CACCACCAGC CACCCCAATC TGCATCCTCC TCGTGGGACC 4740
CCCGAGGACC AACCCCCAAG GCTGCCCCCG ATCCAAACCA CCAACCGCAT CCCCACCACC 4800
CCCGGGAAAG AAACCCCCAG CAATTGGAAG GCCCCTCCCC CTCTTCCTCA ACACAAGAAC 4860
TCCACAACCG AACCGCACAA GCGACCGAGG TGACCCAACC GCAGGCATCC GACTCCCTAG 4920
ACAGATCCTC TCTCCCCGGC AAACTAAACA AAACTTAGGG CCAAGGAACA TACACACCCA 4980
ACAGAACCCA GACCCCGGCC CACGGCGCCG CGCCCCCAAC CCCCGACAAC CAGAGGGAGC 5040
CCCCAACCAA TCCCGCCGGC TCCCCCGGTG CCCACAGGCA GGGACACCAA CCCCCGAACA 5100
GACCCAGCAC CCAACCATCG ACAATCCAAG ACGGGGGGGC CCCCCCAAAA AAAGGCCCCC 5160
AGGGGCCGAC AGCCAGCACC GCGAGGAAGC CCACCCACCC CACACACGAC CACGGCAACC 5220
AAACCAGAAC CCAGACCACC CTGGGCCACC AGCTCCCAGA CTCGGCCATC ACCCCGCAGA 5280
AAGGAAAGGC CACAACCCGC GCACCCCAGC CCCGATCCGG CGGGGAGCCA CCCAACCCGA 5340
ACCAGCACCC AAGAGCGATC CCCGAAGGAC CCCCGAACCG CAAAGGACAT CAGTATCCCA 5400
CAGCCTCTCC AAGTCCCCCG GTCTCCTCCT CTTCTCGAAG GGACCAAAAG ATCAATCCAC 5460
CACACCCGAC GACACTCAAC TCCCCACCCC TAAAGGAGAC ACCGGGAATC CCAGAATCAA 5520
GACTCATCCA ATGTCCATCA TGGGTCTCAA GGTGAACGTC TCTGCCATAT TCATGGCAGT 5580
ACTGTTAACT CTCCAAACAC CCACCGGTCA AATCCATTGG GGCAATCTCT CTAAGATAGG 5640
GGTGGTAGGA ATAGGAAGTG CAAGCTACAA AGTTATGACT CGTTCCAGCC ATCAATCATT 5700
AGTCATAAAA TTAATGCCCA ATATAACTCT CCTCAATAAC TGCACGAGGG TAGAGATTGC 5760
AGAATACAGG AGACTACTGA GAACAGTTTT GGAACCAATT AGAGATGCAC TTAATGCAAT 5820
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GGAAACTACT AATCAGGCAA TTGAGACAAT CAGACAAGCA GGGCAGGAGA TGATATTGGC 6060
TGTTCAGGGT GTCCAAGACT ACATCAATAA TGAGCTGATA CCGTCTATGA ACCAACTATC 6120
TTGTGATTTA ATCGGCCAGA AGCTCGGGCT CAAATTGCTC AGATACTATA CAGAAATCCT 6180

FIGURE 5B

GTCATTATTT GGCCCCAGTT TACGGGACCC CATATCTGCG GAGATATCTA TCCAGGCTTT 6240
GAGCTATGCG CTTGGAGGAG ACATCAATAA GGTGTTAGAA AAGCTCGGAT ACAGTGGAGG 6300
TGATTTACTG GGCATCTTAG AGAGCGGAGG AATAAAGGCC CGGATAACTC ACGTCGACAC 6360
AGAGTCCTAC TTCATTGTCC TCAGTATAGC CTATCCGACG CTGTCCGAGA TTAAGGGGGT 6420
GATTGTCCAC CGGCTAGAGG GGGTCTCGTA CAACATAGGC TCTCAAGAGT GGTATACCAC 6480
TGTGCCCAAG TATGTTGCAA CCCAAGGGTA CCTTATCTCG AATTTTGATG AGTCATCGTG 6540
TACTTTCATG CCAGAGGGGA CTGTGTGCAG CCAAAATGCC TTGTACCCGA TGAGTCCTCT 6600
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TTTTGGGAAC CGGTTCATTT TATCACAAGG GAACCTAATA GCCAATTGTG CATCAATCCT 6720
TTGCAAGTGT TACACAACAG GAACGATCAT TAATCAAGAC CCTGACAAGA TCCTAACATA 6780
CATTGCTGCC GATCACTGCC CGGTAGTCGA GGTGAACGGC GTGACCATCC AAGTCGGGAG 6840
CAGGAGGTAT CCAGACGCTG TGTACTTGCA CAGAATTGAC CTCGGTCCTC CCATATCATT 6900
GGAGAGGTTG GACGTAGGGA CAAATCTGGG GAATGCAATT GCTAAGTTGG AGGATGCCAA 6960
GGAATTGTTG GAGTCATCGG ACCAGATATT GAGGAGTATG AAAGGTTTAT CGAGCACTAG 7020
CATAGTCTAC ATCCTGATTG CAGTGTGTCT TGGAGGGTTG ATAGGGATCC CCGCTTTAAT 7080
ATGTTGCTGC AGGGGGCGTT GTAACAAAAA GGGAGAACAA GTTGGTATGT CAAGACCAGG 7140
CCTAAAGCCT GATCTTACGG GAACATCAAA ATCCTATGTA AGGTCGCTCT GATCCTCTAC 7200
AACTCTTGAA ACACAAATGT CCCACAAGTC TCCTCTTCGT CATCAAGCAA CCACCGCACC 7260
CAGCATCAAG CCCACCTGAA ATTATCTCCG GCTTCCCTCT GGCCGAACAA TATCGGTAGT 7320
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CCTTCTACAA AGATAACCCC CATCCCAAGG GAAGTAGGAT AGTCATTAAC AGAGAACATC 7440
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TCGGGTTGCT AGCCATTGCA GGCATTAGAC TTCATCGGGC AGCCATCTAC ACCGCAGAGA 7560
TCCATAAAAG CCTCAGCACC AATCTAGATG TAACTAACTC AATCGAGCAT CAGGTCAAGG 7620
ACGTGCTGAC ACCACTCTTC AAAATCATCG GTGATGAAGT GGGCCTGAGG ACACCTCAGA 7680
GATTCACTGA CCTAGTGAAA TTAATCTCTG ACAAGATTAA ATTCCTTAAT CCGGATAGGG 7740
AGTACGACTT CAGAGATCTC ACTTGGTGTA TCAACCCGCC AGAGAGAATC AAATTGGATT 7800
ATGATCAATA CTGTGCAGAT GTGGCTGCTG AAGAGCTCAT GAATGCATTG GTGAACTCAA 7860
CTCTACTGGA GACCAGAACA ACCAATCAGT TCCTAGCTGT CTCAAAGGGA AACTGCTCAG 7920
GGCCCACTAC AATCAGAGGT CAATTCTCAA ACATGTCGCT GTCCCTGTTA GACTTGTATT 7980
TAGGTCGAGG TTACAATGTG TCATCTATAG TCACTATGAC ATCCCAGGGA ATGTATGGGG 8040
GAACTTACCT AGTGGAAAAG CCTAATCTGA GCAGCAAAAG GTCAGAGTTG TCACAACTGA 8100
GCATGTACCG AGTGTTTGAA GTAGGTGTTA TCAGAAATCC GGGTTTGGGG GCTCCGGTGT 8160
TCCATATGAC AAACTATCTT GAGCAACCAG TCAGTAATGA TCTCAGCAAC TGTATGGTGG 8220
CTTTGGGGGA GCTCAAACTC GCAGCCCTTT GTCACGGGGA AGATTCTATC ACAATTCCCT 8280
ATCAGGGATC AGGGAAAGGT GTCAGCTTCC AGCTCGTCAA GCTAGGTGTC TGGAAATCCC 8340
CAACCGACAT GCAATCCTGG GTCCCCTTAT CAACGGATGA TCCAGTGATA GACAGGCTTT 8400
ACCTCTCATC TCACAGAGGT GTTATCGCTG ACAATCAAGC AAAATGGGCT GTCCCGACAA 8460
CACGAACAGA TGACAAGTTG CGAATGGAGA CATGCTTCCA ACAGGCGTGT AAGGGTAAAA 8520
TCCAAGCACT CTGCGAGAAT CCCGAGTGGG CACCATTGAA GGATAACAGG ATTCCTTCAT 8580
ACGGGGTCTT GTCTGTTGAT CTGAGTCTGA CAGTTGAGCT TAAAATCAAA ATTGCTTCGG 8640
GATTCGGGCC ATTGATCACA CACGGTTCAG GGATGGACCT ATACAAATCC AACCACAACA 8700
ATGTGTATTG GCTGACTATC CCGCCAATGA AGAACCTAGC CTTAGGTGTA ATCAACACAT 8760
TGGAGTGGAT ACCGAGATTC AAGGTTAGTC CCTACCTCTT CACTGTCCCA ATTAAGGAAG 8820
CAGGCGAAGA CTGCCATGCC CCAACATACC TACCTGCGGA GGTGGATGGT GATGTCAAAC 8880
TCAGTTCCAA TCTGGTGATT CTACCTGGTC AAGATCTCCA ATATGTTTTG GCAACCTACG 8940
ATACTTCCAG GGTTGAACAT GCTGTGGTTT ATTACGTTTA CAGCCCAAGC CGCTCATTTT 9000
CTTACTTTTA TCCTTTTAGG TTGCCTATAA AGGGGGTCCC CATCGAATTA CAAGTGGAAT 9060
GCTTCACATG GGACCAAAAA CTCTGGTGCC GTCACTTCTG TGTGCTTGCG GACTCAGAAT 9120
CTGGTGGACA TATCACTCAC TCTGGGATGG TGGGCATGGG AGTCAGCTGC ACAGTCACCC 9180
GGGAAGATGG AACCAATCGC AGATAGGGCT GCTAGTGAAC CAATCACATG ATGTCACCCA 9240
GACATCAGGC ATACCCACTA GTGTGAAATA GACATCAGAA TTAAGAAAAA CGTAGGGTCC 9300

FIGURE 5C

AAGTGGTTCC CCGTTATGGA CTCGCTATCT GTCAACCAGA TCTTATACCC TGAAGTTCAC 9360
CTAGATAGCC CGATAGTTAC CAATAAGATA GTAGCCATCC TGGAGTATGC TCGAGTCCCT 9420
CACGCTTACA GCCTGGAGGA CCCTACACTG TGTCAGAACA TCAAGCACCG CCTAAAAAAC 9480
GGATTTTCCA ACCAAATGAT TATAAACAAT GTGGAAGTTG GGAATGTCAT CAAGTCCAAG 9540
CTTAGGAGTT ATCCGGCCCA CTCTCATATT CCATATCCAA ATTGTAATCA GGATTTATTT 9600
AACATAGAAG ACAAAGAGTC AACGAGGAAG ATCCGTGAAC TCCTCAAAAA GGGGAATTCG 9660
CTGTACTCCA AAGTCAGTGA TAAGGTTTTC CAATGCTTAA GGGACACTAA CTCACGGCTT 9720
GGCCTAGGCT CCGAATTGAG GGAGGACATC AAGGAGAAAG TTATTAACTT GGGAGTTTAC 9780
ATGCACAGCT CCCAGTGGTT TGAGCCCTTT CTGTTTTGGT TTACAGTCAA GACTGAGATG 9840
AGGTCAGTGA TTAAATCACA AACCCATACT TGCCATAGGA GGAGACACAC ACCTGTATTC 9900
TTCACTGGTA GTTCAGTTGA GTTGCTAATC TCTCGTGACC TTGTTGCTAT AATCAGTAAA 9960
GAGTCTCAAC ATGTATATTA CCTGACATTT GAACTGGTTT TGATGTATTG TGATGTCATA 10020
GAGGGGAGGT TAATGACAGA GACCGCTATG ACTATTGATG CTAGGTATAC AGAGCTTCTA 10080
GGAAGAGTCA GATACATGTG GAAACTGATA GATGGTTTCT TCCCTGCACT CGGGAATCCA 10140
ACTTATCAAA TTGTAGCCAT GCTGGAGCCT CTTTCACTTG CTTACCTGCA GCTGAGGGAT 10200
ATAACAGTAG AACTCAGAGG TGCTTTCCTT AACCACTGCT TTACTGAAAT ACATGATGTT 10260
CTTGACCAAA ACGGGTTTTC TGATGAAGGT ACTTATCATG AGTTAACTGA AGCTCTAGAT 10320
TACATTTTCA TAACTGATGA CATACATCTG ACAGGGGAGA TTTTCTCATT TTTCAGAAGT 10380
TTCGGCCACC CCAGACTTGA AGCAGTAACG GCTGCTGAAA ATGTTAGGAA ATACATGAAT 10440
CAGCCTAAAG TCATTGTGTA TGAGACTCTG ATGAAAGGTC ATGCCATATT TTGTGGAATC 10500
ATAATCAACG GCTATCGTGA CAGGCACGGA GGCAGTTGGC CACCGCTGAC CCTCCCCCTG 10560
CATGCTGCAG ACACAATCCG GAATGCTCAA GCTTCAGGTG AAGGGTTAAC ACATGAGCAG 10620
TGCGTTGATA ACTGGAAATC TTTTGCTGGA GTGAAATTTG GCTGCTTTAT GCCTCTTAGC 10680
CTGGATAGTG ATCTGACAAT GTACCTAAAG GACAAGGCAC TTGCTGCTCT CCAAAGGGAA 10740
TGGGATTCAG TTTACCCGAA AGAGTTCCTG CGTTACGACC CTCCCAAGGG AACCGGGTCA 10800
CGGAGGCTTG TAGATGTTTT CCTTAATGAT TCGAGCTTTG ACCCATATGA TGTGATAATG 10860
TATGTTGTAA GTGGAGCTTA CCTCCATGAC CCTGAGTTCA ACCTGTCTTA CAGCCTGAAA 10920
GAAAAGGAGA TCAAGGAAAC AGGTAGACTT TTTGCTAAAA TGACTTACAA AATGAGGGCA 10980
TGCCAAGTGA TTGCTGAAAA TCTAATCTCA AACGGGATTG GCAAATATTT TAAGGACAAT 11040
GGGATGGCCA AGGATGAGCA CGATTTGACT AAGGCACTCC ACACTCTAGC TGTCTCAGGA 11100
GTCCCCAAAG ATCTCAAAGA AAGTCACAGG GGGGGGCCAG TCTTAAAAAC CTACTCCCGA 11160
AGCCCAGTCC ACACAAGTAC CAGGAACGTG AGAGCAGCAA AAGGGTTTAT AGGGTTCCCT 11220
CAAGTAATTC GGCAGGACCA AGACACTGAT CATCCGGAGA ATATGGAAGC TTACGAGACA 11280
GTCAGTGCAT TTATCACGAC TGATCTCAAG AAGTACTGCC TTAATTGGAG ATATGAGACC 11340
ATCAGCTTGT TTGCACAGAG GCTAAATGAG ATTTACGGAT TGCCCTCATT TTTCCAGTGG 11400
CTGCATAAGA GGCTTGAGAC CTCTGTCCTG TATGTAAGTG ACCCTCATTG CCCCCCCGAC 11460
CTTGACGCCC ATATCCCGTT ATATAAAGTC CCCAATGATC AAATCTTCAT TAAGTACCCT 11520
ATGGGAGGTA TAGAAGGGTA TTGTCAGAAG CTGTGGACCA TCAGCACCAT TCCCTATCTA 11580
TACCTGGCTG CTTATGAGAG CGGAGTAAGG ATTGCTTCGT TAGTGCAAGG GGACAATCAG 11640
ACCATAGCCG TAACAAAAAG GGTACCCAGC ACATGGCCCT ACAACCTTAA GAAACGGGAA 11700
GCTGCTAGAG TAACTAGAGA TTACTTTGTA ATTCTTAGGC AAAGGCTACA TGATATTGGC 11760
CATCACCTCA AGGCAAATGA GACAATTGTT TCATCACATT TTTTTGTCTA TTCAAAAGGA 11820
ATATATTATG ATGGGCTACT TGTGTCCCAA TCACTCAAGA GCATCGCAAG ATGTGTATTC 11880
TGGTCAGAGA CTATAGTTGA TGAAACAAGG GCAGCATGCA GTAATATTGC TACAACAATG 11940
GCTAAAAGCA TCGAGAGAGG TTATGACCGT TACCTTGCAT ATTCCCTGAA CGTCCTAAAA 12000
GTGATACAGC AAATTCTGAT CTCTCTTGGC TTCACAATCA ATTCAACCAT GACCCGGGAT 12060
GTAGTCATAC CCCTCCTCAC AAACAACGAC CTCTTAATAA GGATGGCACT GTTGCCCGCT 12120
CCTATTGGGG GGATGAATTA TCTGAATATG AGCAGGCTGT TTGTCAGAAA CATCGGTGAT 12180
CCAGTAACAT CATCAATTGC TGATCTCAAG AGAATGATTC TCGCCTCACT AATGCCTGAA 12240
GAGACCCTCC ATCAAGTAAT GACACAACAA CCGGGGGACT CTTCATTCCT AGACTGGGCT 12300
AGCGACCCTT ACTCAGCAAA TCTTGTATGT GTCCAGAGCA TCACTAGACT CCTCAAGAAC 12360
ATAACTGCAA GGTTTGTCCT GATCCATAGT CCAAACCCAA TGTTAAAAGG ATTATTCCAT 12420

FIGURE 5D

GATGACAGTA AAGAAGAGGA CGAGGGACTG GCGGCATTCC TCATGGACAG GCATATTATA 12480
GTACCTAGGG CAGCTCATGA AATCCTGGAT CATAGTGTCA CAGGGGCAAG AGAGTCTATT 12540
GCAGGCATGC TGGATACCAC AAAAGGCTTG ATTCGAGCCA GCATGAGGAA GGGGGGGTTA 12600
ACCTCTCGAG TGATAACCAG ATTGTCCAAT TATGACTATG AACAATTCAG AGCAGGGATG 12660
GTGCTATTGA CAGGAAGAAA GAGAAATGTC CTCATTGACA AAGAGTCATG TTCAGTGCAG 12720
CTGGCGAGAG CTCTAAGAAG CCATATGTGG GCGAGGCTAG CTCGAGGACG GCCTATTTAC 12780
GGCCTTGAGG TCCCTGATGT ACTAGAATCT ATGCGAGGCC ACCTTATTCG GCGTCATGAG 12840
ACATGTGTCA TCTGCGAGTG TGGATCAGTC AACTACGGAT GGTTTTTTGT CCCCTCGGGT 12900
TGCCAACTGG ATGATATTGA CAAGGAAACA TCATCCTTGA GAGTCCCATA TATTGGTTCT 12960
ACCACTGATG AGAGAACAGA CATGAAGCTT GCCTTCGTAA GAGCCCCAAG TCGATCCTTG 13020
CGATCTGCTG TTAGAATAGC AACAGTGTAC TCATGGGCTT ACGGTGATGA TGATAGCTCT 13080
TGGAACGAAG CCTGGTTGTT GGCTAGGCAA AGGGCCAATG TGAGCCTGGA GGAGCTAAGG 13140
GTGATCACTC CCATCTCAAC TTCGACTAAT TTAGCGCATA GGTTGAGGGA TCGTAGCACT 13200
CAAGTGAAAT ACTCAGGTAC ATCCCTTGTC CGAGTGGCGA GGTATACCAC AATCTCCAAC 13260
GACAATCTCT CATTTGTCAT ATCAGATAAG AAGGTTGATA CTAACTTTAT ATACCAACAA 13320
GGAATGCTTC TAGGGTTGGG TGTTTTAGAA ACATTGTTTC GACTCGAGAA AGATACCGGA 13380
TCATCTAACA CGGTATTACA TCTTCACGTC GAAACAGATT GTTGCGTGAT CCCGATGATA 13440
GATCATCCCA GGATACCCAG CTCCCGCAAG CTAGAGCTGA GGGCAGAGCT ATGTACCAAC 13500
CCATTGATAT ATGATAATGC ACCTTTAATT GACAGAGATG CAACAAGGCT ATACACCCAG 13560
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CTGCTCATAG AGCCAAGATT ATTCACTATC TACTTGGGCC AGTGTGCGGC CATCAATTGG 13800
GCATTTGATG TACATTATCA TAGACCATCA GGGAAATATC AGATGGGTGA GCTGTTGTCA 13860
TCGTTCCTTT CTAGAATGAG CAAAGGAGTG TTTAAGGTGC TTGTCAATGC TCTAAGCCAC 13920
CCAAAGATCT ACAAGAAATT CTGGCATTGT GGTATTATAG AGCCTATCCA TGGTCCTTCA 13980
CTTGATGCTC AAAACTTGCA CACAACTGTG TGCAACATGG TTTACACATG CTATATGACC 14040
TACCTCGACC TGTTGTTGAA TGAAGAGTTA GAAGAGTTCA CATTTCTCTT GTGTGAAAGC 14100
GACGAGGATG TAGTACCGGA CAGATTCGAC AACATCCAGG CAAAACACTT ATGTGTTCTG 14160
GCAGATTTGT ACTGTCAACC AGGGACCTGC CCACCAATTC GAGGTCTAAG ACCGGTAGAG 14220
AAATGTGCAG TTCTAACCGA CCATATCAAG GCAGAGGCTA TGTTATCTCC AGCAGGATCT 14280
TCGTGGAACA TAAATCCAAT TATTGTAGAC CATTACTCAT GCTCTCTGAC TTATCTCCGG 14340
CGAGGATCGA TCAAACAGAT AAGATTGAGA GTTGATCCAG GATTCATTTT CGACGCCCTC 14400
GCTGAGGTAA ATGTCAGTCA GCCAAAGATC GGCAGCAACA ACATCTCAAA TATGAGCATC 14460
AAGGCTTTCA GACCCCCACA CGATGATGTT GCAAAATTGC TCAAAGATAT CAACACAAGC 14520
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AGAATCGGGT TGAACTCATC TGCTTGCTAC AAAGCTGTTG AGATATCAAC ATTAATTAGG 14640
AGATGCCTTG AGCCAGGGGA GGACGGCTTG TTCTTGGGTG AGGGATCGGG TTCTATGTTG 14700
ATCACTTATA AAGAGATACT TAAACTAAAC AAGTGCTTCT ATAATAGTGG GGTTTCCGCC 14760
AATTCTAGAT CTGGTCAAAG GGAATTAGCA CCCTATCCCT CCGAAGTTGG CCTTGTCGAA 14820
CACAGAATGG GAGTAGGTAA TATTGTCAAA GTGCTCTTTA ACGGGAGGCC CGAAGTCACG 14880
TGGGTAGGCA GTGTAGATTG CTTCAATTTC ATAGTTAGTA ATATCCCTAC CTCTAGTGTG 14940
GGGTTTATCC ATTCAGATAT AGAGACCTTG CCTGACAAAG ATACTATAGA GAAGCTAGAG 15000
GAATTGGCAG CCATCTTATC GATGGCTCTG CTCCTGGGCA AAATAGGATC AATACTGGTG 15060
ATTAAGCTTA TGCCTTTCAG CGGGGATTTT GTTCAGGGAT TTATAAGTTA TGTAGGGTCT 15120
CATTATAGAG AAGTGAACCT TGTATACCCT AGATACAGCA ACTTCATCTC TACTGAATCT 15180
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AAGCAACTAA GCTGCATACA AGCAATTGTG GGAGACGCAG TTAGTAGAGG TGATATCAAT 15360
CCTACTCTGA AAAAACTTAC ACCTATAGAG CAGGTGCTGA TCAATTGCGG GTTGGCAATT 15420
AACGGACCTA AGCTGTGCAA AGAATTGATC CACCATGATG TTGCCTCAGG GCAAGATGGA 15480
TTGCTTAATT CTATACTCAT CCTCTACAGG GAGTTGGCAA GATTCAAAGA CAACCAAAGA 15540

FIGURE 5E

AGTCAACAAG GGATGTTCCA CGCTTACCCC GTATTGGTAA GTAGCAGGCA ACGAGAACTT 15600
ATATCTAGGA TCACCCGCAA ATTCTGGGGG CACATTCTTC TTTACTCCGG GAACAAAAAG 15660
TTGATAAATA AGTTTATCCA GAATCTCAAG TCCGGCTATC TGATACTAGA CTTACACCAG 15720
AATATCTTCG TTAAGAATCT ATCCAAGTCA GAGAAACAGA TTATTATGAC GGGGGGTTTG 15780
AAACGTGAGT GGGTTTTTAA GGTAACAGTC AAGGAGACCA AAGAATGGTA TAAGTTAGTC 15840
GGATACAGTG CCCTGATTAA GGACTAATTG GTTGAACTCC GGAACCCTAA TCCTGCCCTA 15900
GGTGGTTAGG CATTATTTGC AATATATTAA AGAAAACTTT GAAAATACGA AGTTTCTATT 15960
CCCAGCTTTG TCTGGTGGCC GGCATGGTCC CAGCCTCCTC GCTGGCGCCG GCTGGGCAAC 16020
ATTCCGAGGG GACCGTCCCC TCGGTAATGG CGAATGGGAC GCGGCCGATC CGGCTGCTAA 16080
CAAAGCCCGA AAGGAAGCTG AGTTGGCTGC TGCCACCGCT GAGCAATAAC TAGCATAACC 16140
CCTTGGGGCC TCTAAACGGG TCTTGAGGGG TTTTTTGCTG AAAGGAGGAA CTATATCCGG 16200
ATGCGGCCGC GGGCCCTATG GTACCCAGCT TTTGTTCCCT TTAGTGAGGG TTAATTCCGA 16260
GCTTGGCGTA ATCATGGTCA TAGCTGTTTC CTGTGTGAAA TTGTTATCCG CTCACAATTC 16320
CACACAACAT AGGAGCCGGA AGCATAAAGT GTAAAGCCTG GGGTGCCTAA TGAGTGAGGT 16380
AACTCACATT AATTGCGTTG CGCTCACTGC CCGCTTTCCA GTCGGGAAAC CTGTCGTGCC 16440
AGCTGCATTA ATGAATCGGC CAACGCGCGG GGAGAGGCGG TTTGCGTATT GGGCGCTCTT 16500
CCGCTTCCTC GCTCACTGAC TCGCTGCGCT CGGTCGTTCG GCTGCGGCGA GCGGTATCAG 16560
CTCACTCAAA GGCGGTAATA CGGTTATCCA CAGAATCAGG GGATAACGCA GGAAAGAACA 16620
TGTGAGCAAA AGGCCAGCAA AAGGCCAGGA ACCGTAAAAA GGCCGCGTTG CTGGCGTTTT 16680
TCCATAGGCT CGGCCCCCCT GACGAGCATC ACAAAAATCG ACGCTCAAGT CAGAGGTGGC 16740
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ATCGTCTTGA GTCCAACCCG GTAAGACACG ACTTATCGCC ACTGGCAGCA GCCACTGGTA 17040
ACAGGATTAG CAGAGCGAGG TATGTAGGCG GTGCTACAGA GTTCTTGAAG TGGTGGCCTA 17100
ACTACGGCTA CACTAGAAGG ACAGTATTTG GTATCTGCGC TCTGCTGAAG CCAGTTACCT 17160
TCGGAAAAAG AGTTGGTAGC TCTTGATCCG GCAAACAAAC CACCGCTGGT AGCGGTGGTT 17220
TTTTTGTTTG CAAGCAGCAG ATTACGCGCA GAAAAAAAGG ATCTCAAGAA GATCCTTTGA 17280
TCTTTTCTAC GGGGTCTGAC GCTCAGTGGA ACGAAAACTC ACGTTAAGGG ATTTTGGTCA 17340
TGAGATTATC AAAAAGGATC TTCACCTAGA TCCTTTTAAA TTAAAAATGA AGTTTTAAAT 17400
CAATCTAAAG TATATATGAG TAAACTTGGT CTGACAGTTA CCAATGCTTA ATCAGTGAGG 17460
CACCTATCTC AGCGATCTGT CTATTTCGTT CATCCATAGT TGCCTGACTG CCCGTCGTGT 17520
AGATAACTAC GATACGGGAG GGCTTACCAT CTGGCCCCAG TGCTGCAATG ATACCGCGAG 17580
ACCCACGCTC ACCGGCTCCA GATTTATCAG CAATAAACCA GCCAGCCGGA AGGGCCGAGC 17640
GCAGAAGTGG TCCTGCAACT TTATCCGCCT CCATCCAGTC TATTAATTGT TGCCGGGAAG 17700
CTAGAGTAAG TAGTTCGCCA GTTAATAGTT TGCGCAACGT TGTTGCCATT GCTACAGGCA 17760
TCGTGGTGTC ACGCTCGTCG TTTGGTATGG CTTCATTCAG CTCCGGTTCC CAACGATCAA 17820
GGCGAGTTAC ATGATCCCCC ATGTTGTGAA AAAAAGCGGT TAGCTCCTTC GGTCCTCCGA 17880
TCGTTGTCAG AAGTAAGTTG GCCGCAGTGT TATCACTCAT GCTTATGGCA GCACTGCATA 17940
ATTCTCTTAC TGTCATGCCA TCCGTAAGAT GCTTTTCTGT GACTGGTGAG TACTCAACCA 18000
AGTCATTCTG AGAATAGTGT ATGCGGCGAC CGAGTTGCTC TTGCCCGGCG TCAATACGGG 18060
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GGCGAAAACT CTCAAGGATC TTACCGCTGT TGAGATCCAG TTCGATGTAA CCCACTCGTG 18180
CACCCAACTG ATCTTCAGCA TCTTTTACTT TCACCAGCGT TTCTGGGTGA GCAAAAACAG 18240
GAAGGCAAAA TGCCGCAAAA AAGGGAATAA GGGCGACACG GAAATGTTGA ATACTCATAC 18300
TCTTCCTTTT TCAATATTAT TGAAGCATTT ATCAGGGTTA TTGTCTCATG AGCGGATACA 18360
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TGCCACCTGA AATTGTAAAC GTTAATATTT TGTTAAAATT CGCGTTAAAT TTTTGTTAAA 18480
TCAGCTCATT TTTTAACCAA TAGGCCGAAA TCGGCAAAAT CCCTTATAAA TCAAAAGAAT 18540
AGACCGAGAT AGGGTTGAGT GTTGTTCCAG TTTGGAACAA GAGTCCACTA TTAAAGAACG 18600
TGGACTCCAA CGTCAAAGGG CGAAAAACCG TCTATCAGGG CGATGGCCCA CTACGTGAAC 18660

FIGURE 5F

```
CATCACCCTA ATCAAGTTTT TTGGGGTCGA GGTGCCGTAA AGCACTAAAT CGGAACCCTA 18720
AAGGGAGCCC CCGATTTAGA GCTTGACGGG GAAAGCCGGC GAACGTGGCG AGAAAGGAAG 18780
GGAAGAAAGC GAAAGGAGCG GGCGCTAGGG CGCTGGCAAG TGTAGCGGTC ACGCTGCGCG 18840
TAACCACCAC ACCCGCCGCG CTTAATGCGC CGCTACAGGG CGCGTCCCAT TCGCCATTCA 18900
GGCTGCGCAA CTGTTGGGAA GGGCGATCGG TGCGGGCCTC TTCGCTATTA CGCCAGCCAC 18960
CGCGGTG                                                           18967
```

FIGURE 5G

Figure 6

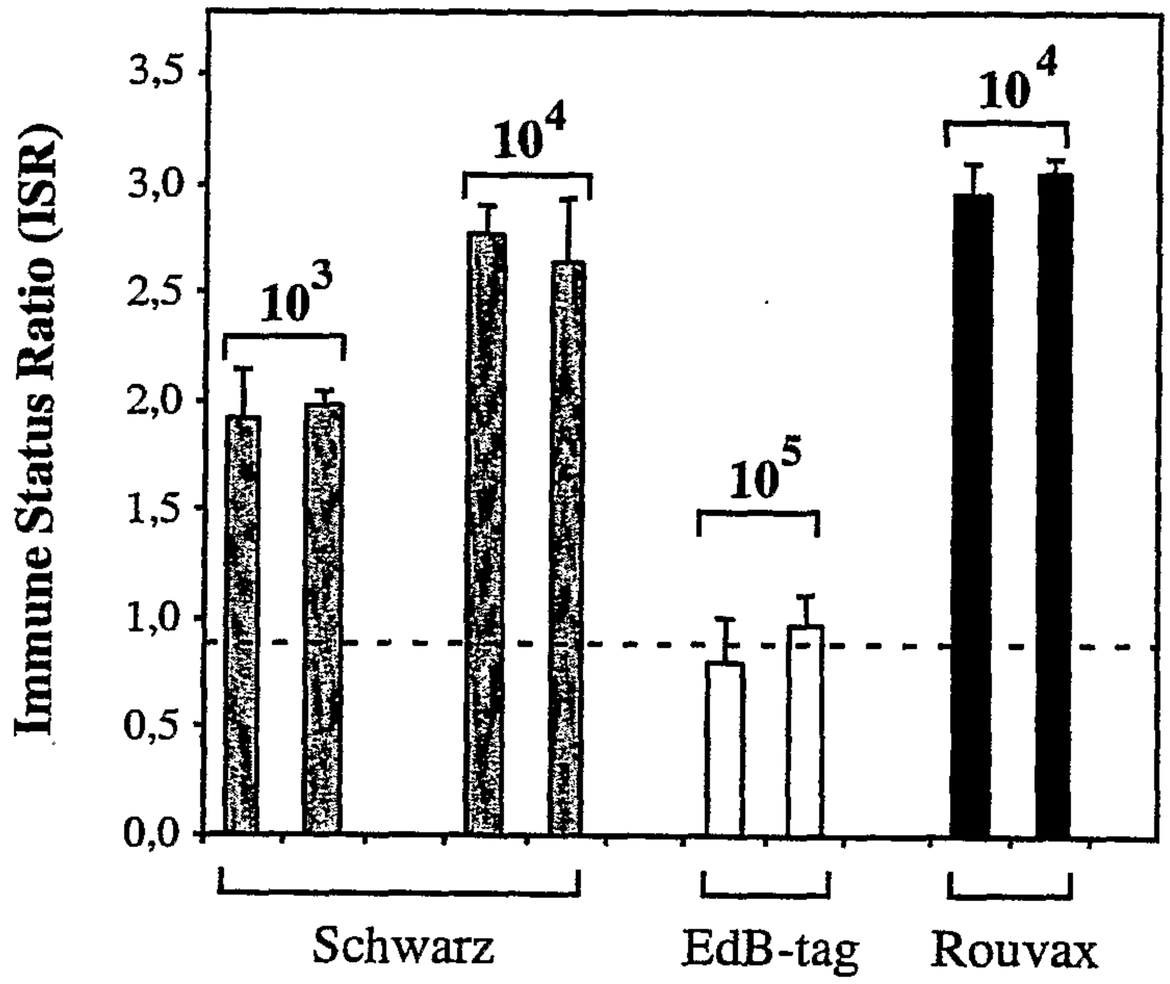

Immune Status Ratio (ISR)
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
10^3
10^4
10^5
10^4
Schwarz
EdB-tag
Rouvax

Figure 7

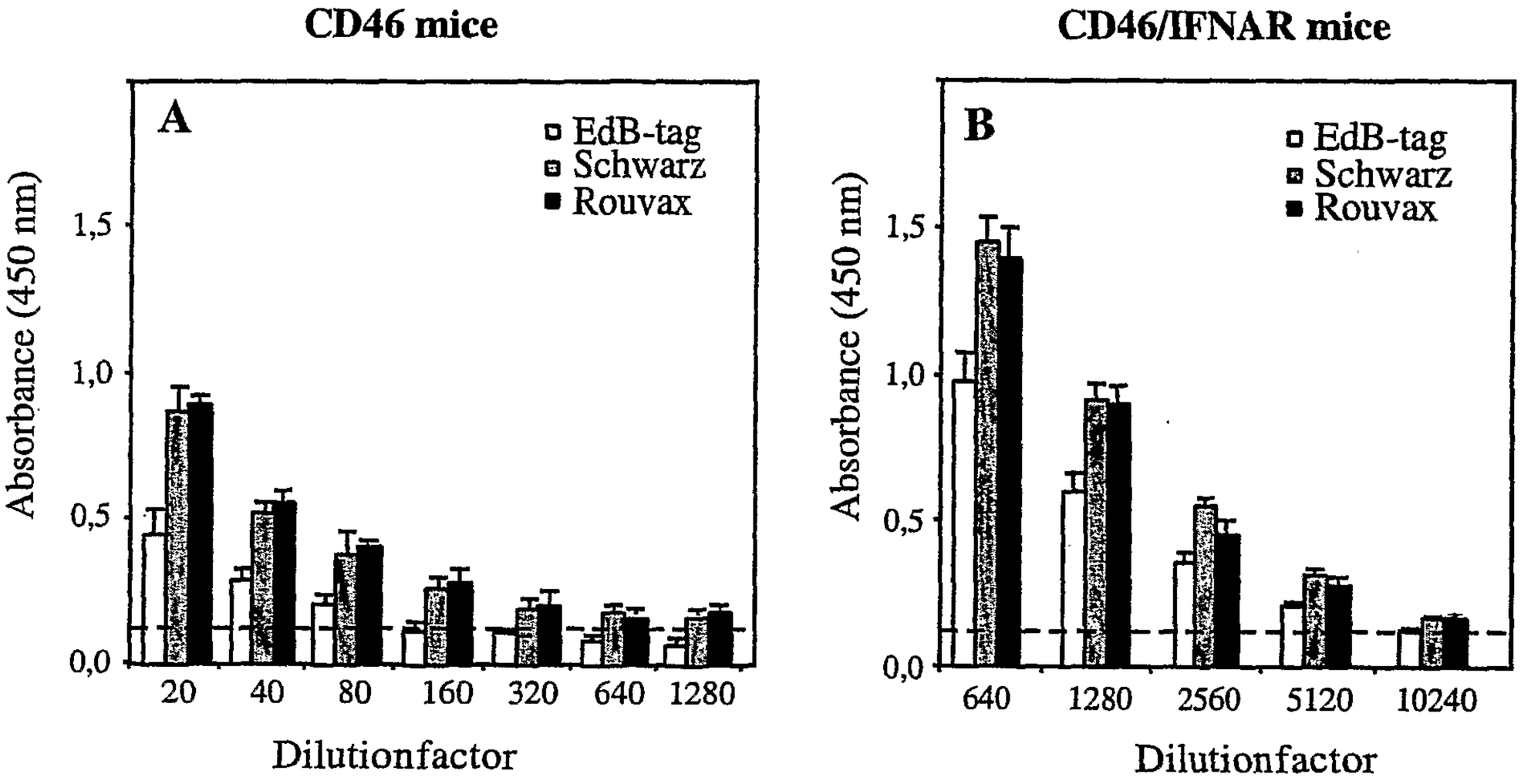

CD46 mice
A
EdB-tag
Schwarz
Rouvax
Absorbance (450 nm)
1,5
1,0
0,5
0,0
20
40
80
160
320
640
1280
Dilutionfactor
CD46/IFNAR mice
B
EdB-tag
Schwarz
Rouvax
Absorbance (450 nm)
1,5
1,0
0,5
0,0
640
1280
2560
5120
10240
Dilutionfactor

Figure 8

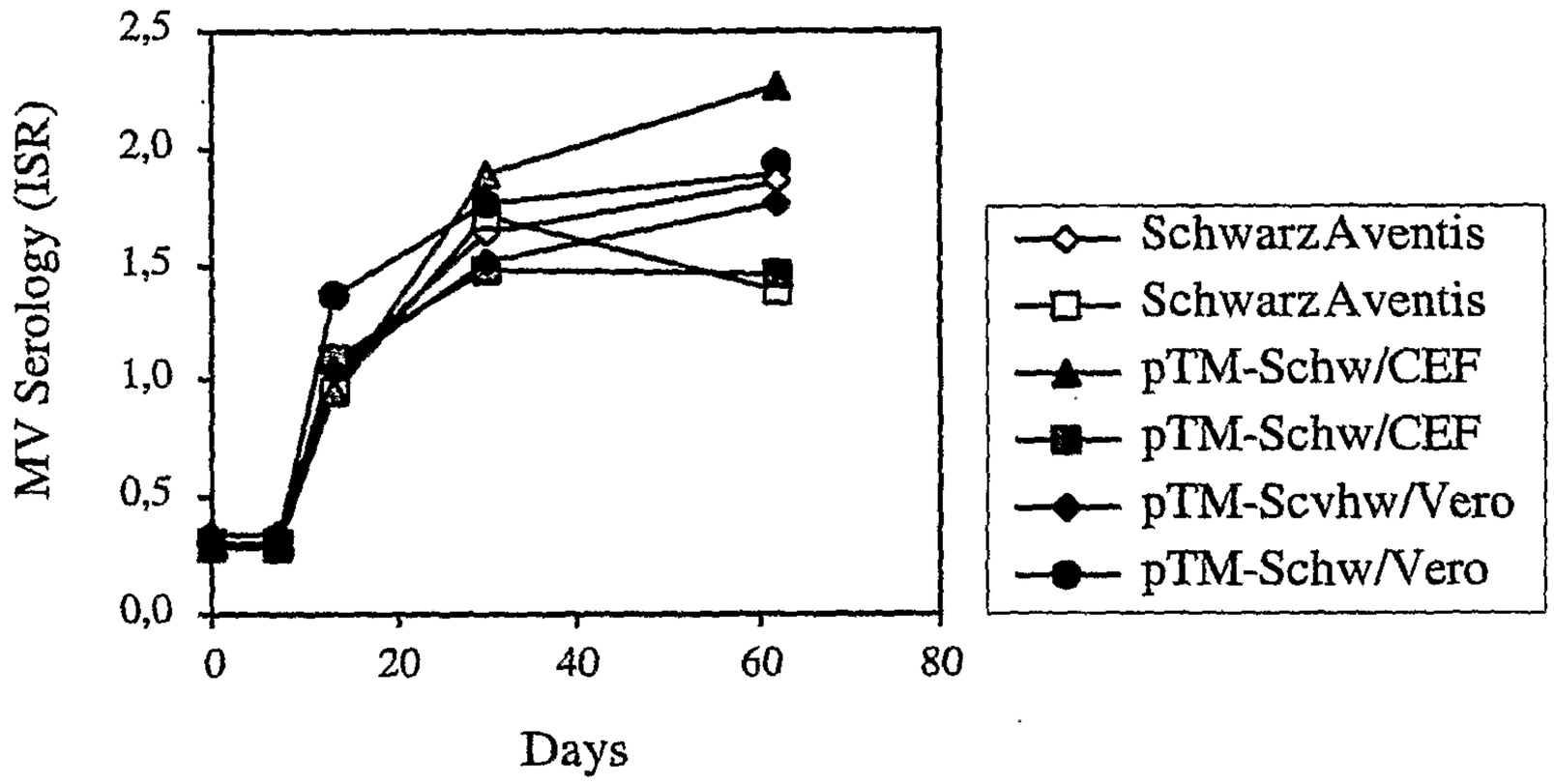

MV Serology (ISR)
2,5
2,0
1,5
1,0
0,5
0,0
0
20
40
60
80
Days
SchwarzAventis
SchwarzAventis
pTM-Schw/CEF
pTM-Schw/CEF
pTM-Scvhw/Vero
pTM-Schw/Vero

**Figure 9**

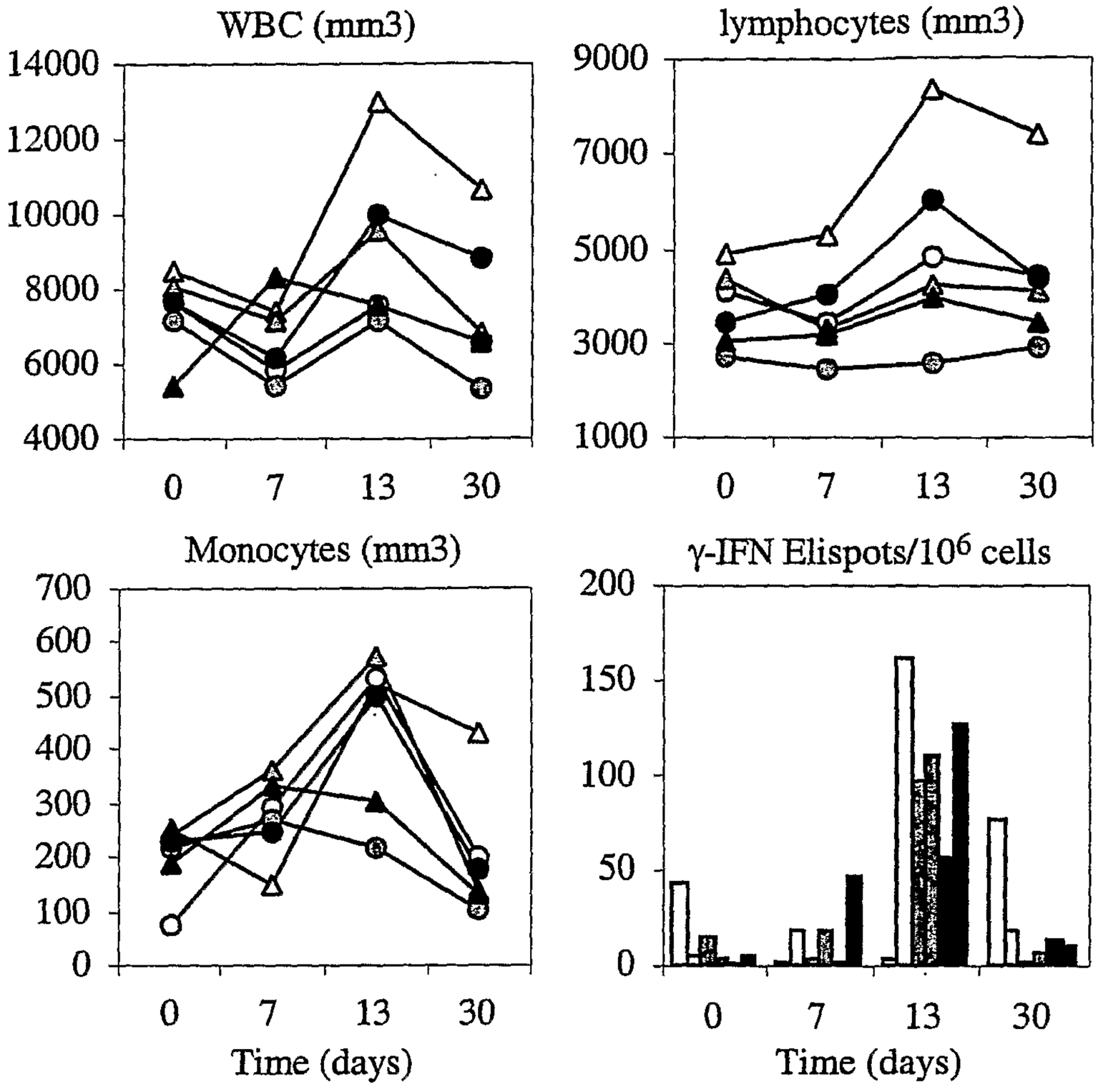
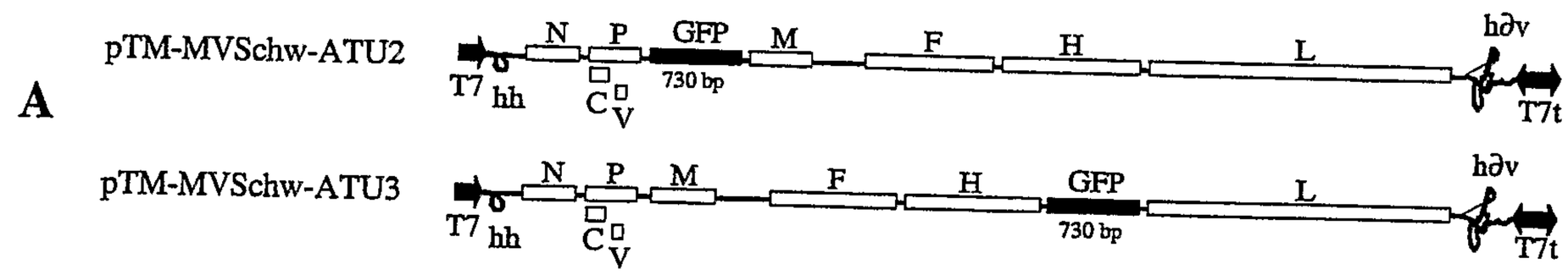

WBC (mm3)
14000
12000
10000
8000
6000
4000
0
7
13
30
lymphocytes (mm3)
9000
7000
5000
3000
1000
0
7
13
30
Monocytes (mm3)
700
600
500
400
300
200
100
0
0
7
13
30
Time (days)
γ-IFN Elispots/10^6 cells
200
150
100
50
0
0
7
13
30
Time (days)

Figure 10


A
pTM-MVSchw-ATU2
N
P
GFP
M
F
H
L
hδv
T7
hh
C
V
730 bp
T7t
pTM-MVSchw-ATU3
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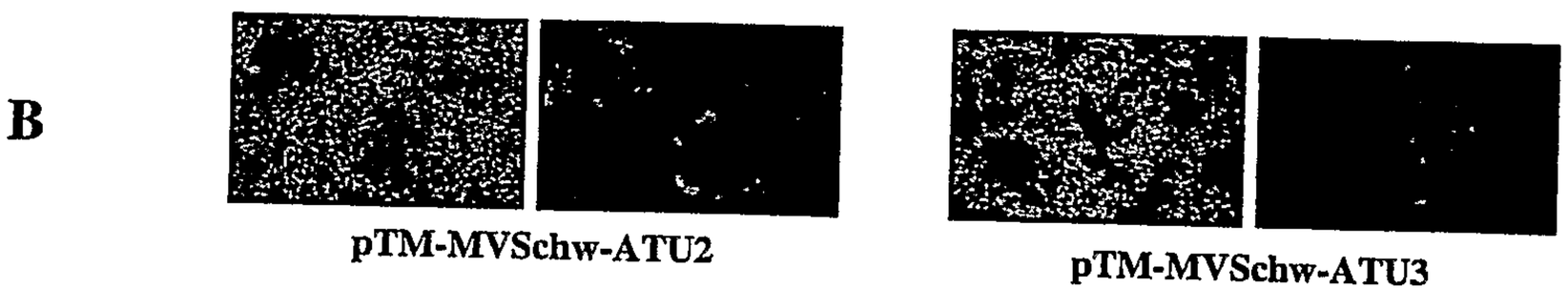
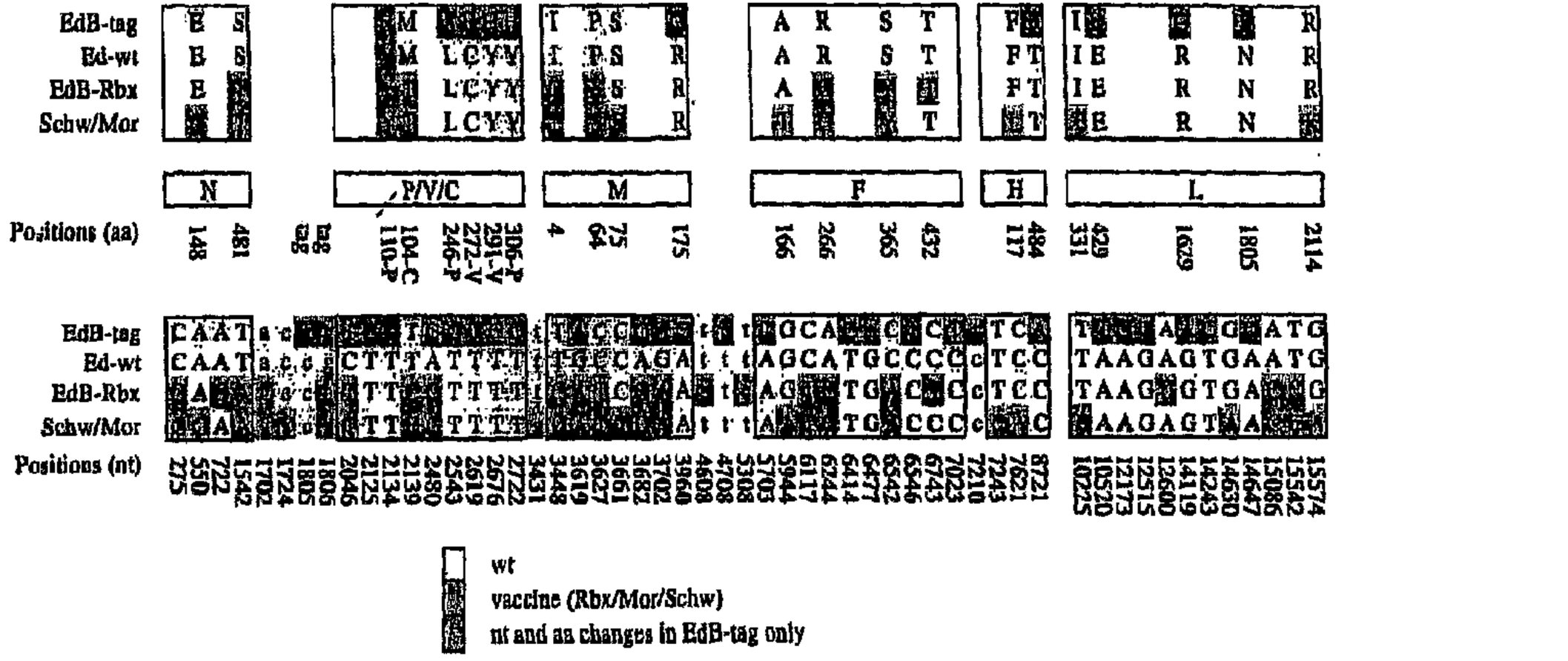
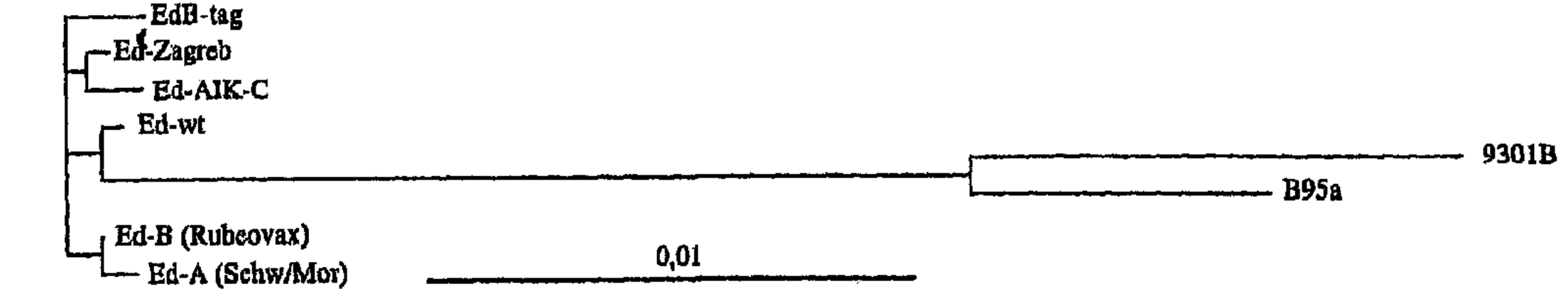
P
M
F
H
GFP
L
hδv
T7
hh
C
V
730 bp
T7t


B
pTM-MVSchw-ATU2
pTM-MVSchw-ATU3

A


EdB-tag
Ed-wt
EdB-Rbx
Schw/Mor
N
P/V/C
M
F
H
L
Positions (aa)
148
481
Bag
Eag
110-P
104-C
272-V
246-P
306-P
291-V
4
94
75
175
166
266
366
432
484
117
429
331
1629
1805
2114
Positions (nt)
275
550
722
1542
1702
1724
1805
1809
2046
2125
2134
2136
2480
2543
2619
2676
2722
3431
3448
3619
3627
3691
3682
3702
3960
4608
4708
5308
5703
5944
6117
6244
6414
6477
6542
6546
6943
7023
7210
7243
7321
8721
10225
10520
12173
12515
12600
14119
14243
14630
14647
15086
15542
15574
wt
vaccine (Rbx/Mor/Schw)
nt and aa changes in EdB-tag only

B


EdB-tag
Ed-Zagreb
Ed-AIK-C
Ed-wt
9301B
B95a
Ed-B (Rubeovax)
Ed-A (Schw/Mor)
0,01