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We, MICHIGAN STATE UNIVERSITY of East Lansing, Michigan 48824, United States of America state the following in connection with Australian Application No. 84965/91:

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   and accorded the numbers ATCC 40855 and ATCC 75039 and 75040 respectively.

Dated: 10 February 1993

By PHILLIPS ORMONDE & FITZPATRICK
Patent Attorneys for the Applicant
By:

David B Fitzpatrick
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To: The Commissioner of Patents

Our Ref: 282861

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Application ID: 84965 / 91 PCT Number : PCT/US91/05870
Applicant-Name
Michigan State University
East Lansing
MI 48824
United States Of America

Title : Marek's disease herpesvirus DNA segment encoding
glycoproteins, gD, gI and gE
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NOTICE OF ENTITLEMENT (Microorganism Deposits)

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Dated: 16 March 1992

David B Fitzpatrick

MICHIGAN STATE UNIVERSITY
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Our Ref: IRN 282861

TO: The Commissioner of Patents

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(54) Title
GENES OF MAREK'S DISEASE VIRUS GLYCOPROTEINS GD, GI AND GE

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(56) Prior Art Documents
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(57) DNA encoding glycoproteins gD, gI and gE from Marek's disease herpesvirus is described. The DNA is useful for probes to detect the DNA in the herpesvirus, for expression to produce the glycoproteins that can be used for producing the antibodies which specifically recognize the three glycoprotein antigens, and in the case of the latter two genes, for potential insertion sites for foreign genes and as possible sites for gene inactivation to attenuate MDV field isolates for vaccine purposes.

Claim

-1-

A 2.53 Kb segment of DNA with a gene coding MDV glycoprotein E (gE) precursor, between a 8488 and 9978 bp sequence of Marek's disease herpesvirus DNA and identified as part of SEQ ID No:1, and containing potential promoter sequences up to 400 nucleotides in length 5' of ~~each~~ the gene and subfragments of the DNA which selectively recognize the DNA when in the form of a probe.

-10-

A segment of DNA encoding MDV gD precursor, between a 5964 and 7175 bp nucleotide sequence of Marek's

.../2

disease herpesvirus and identified as part of SEQ ID No:1, and optionally containing a 5' regulatory region of up to 400 bp in length as shown in Figure 2 and subsegments of the segment of DNA which selectively recognize the DNA when ~~the~~ in form of a probe.

-12-

A segment of DNA with a gene encoding a glycoprotein I (gI) precursor between a 7282 and 8349 bp DNA sequence of Marek's disease herpesvirus and identified as part of SEQ ID No:1, and optionally containing a 5' regulatory region with the gene of up to 400 bp in length as shown in Figure 2 and subfragments of the segment of DNA which selectively recognize the DNA when in the form of a probe.

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(54) Title: MAREK'S DISEASE HERPESVIRUS DNA SEGMENT ENCODING GLYCOPROTEINS, gD, gI AND gE

(57) Abstract

DNA encoding glycoproteins gD, gI and gE from Marek's disease herpesvirus is described. The DNA is useful for probes to detect the DNA in the herpesvirus, for expression to produce the glycoproteins that can be used for producing the antibodies which specifically recognize the three glycoprotein antigens, and in the case of the latter two genes, for potential insertion sites for foreign genes and as possible sites for gene inactivation to attenuate MDV field isolates for vaccine purposes.

MAREK'S DISEASE HERPESVIRUS DNA SEGMENT ENCODING GLYCOPROTEINS, gD, gI and gE

~~Cross-Reference to Related Application~~

This application is a continuation-in-part of U.S. application Serial No. 07/572,711, filed August 24, ~~1990.~~

BACKGROUND OF THE INVENTION

(1) Field of the Invention

The present invention relates to segments of the Marek's Disease Herpesvirus genome, from its unique short (U_S) region encoding glycoproteins gD, gI and gE, and to novel glycoproteins produced therefrom. In particular, the present invention relates to DNA segments containing genes encoding these glycoprotein antigens and containing potential promoter sequences up to 400 nucleotides 5' of each gene, segments which are useful for probing for Marek's disease herpesvirus, as a possible source for Marek's disease virus (MDV) promoters, for gene expression to produce the glycoproteins that in turn can be used for producing antibodies which recognize the three glycoprotein antigens, and in the case of the latter two genes, for potential insertion sites for foreign genes and as possible sites for gene inactivation to attenuate MDV field isolates for vaccine purposes.

(2) Prior Art

MDV is an oncogenic herpesvirus of chickens, which is known to cause T cell lymphomas and peripheral nerve demyelination. The resulting disease, Marek's disease (MD), was the first naturally occurring lymphomatous disorder to be effectively controlled via vaccination, using either the antigenically related, yet apathogenic, herpesvirus of turkeys (HVT) or attenuated field isolates of MDV.

Because of similar biological properties, especially its lymphotropism, MDV has been classified as a

member of the gammaherpesvirus subfamily (Roizman, B., et al., Intervirology 16:201-217 (1981)). Of the three herpesvirus subfamilies, gammaherpesviruses exhibit particularly marked differences with regard to genome composition and organization. For example, the B-lymphotropic Epstein-Barr virus (EBV) of humans has a 172.3 kbp genome with 60% G+C content, is bounded by terminal 0.5 kbp direct repeats and contains a characteristic set of internal 3.07 kbp tandem repeats (Baer, R., et al., Nature (London) 310:207-211 (1984)). Herpesvirus saimiri (HVS), a T-lymphotropic herpesvirus of new-world monkeys and lower vertebrates, has an A+T rich coding sequence (112 kbp; 36% G+C; i.e. L-DNA) without any large-scale internal redundancy, but contains instead greater than 30 reiterations of a 1.44 kbp sequence of 71% G+C at the termini of the genome (H-DNA) (Banker, A. T., et al., J. Virol. 55:133-139 (1985)). Despite the structural differences between EBV and HVS, the genomes of these two viruses encode serologically related proteins and share a common organization of coding sequences which differs from that of the neurotropic alphaherpesviruses, exemplified by herpes simplex virus (HSV) and varicella-zoster virus (VZV) (Camerion, K. R., et al., J. Virol. 61:2063-2070 (1987); Davison, A. J., et al., J. Gen. Virol. 68:1067-1079 (1987); Davison, A. J., et al., J. Gen. Virol. 67:597-611 (1986); Davison, A. J., et al., J. Gen. Virol. 76:1759-1816 (1986); Davison, A. J., et al., J. Gen. Virol. 64:1927-1942 (1983); Gompels, U. A., J. of Virol. 62:757-767 (1988); and Nichols, J., et al., J. of Virol. 62:3250-3257 (1988)).

In contrast to other gammaherpesviruses, MDV has a genome structure closely resembling that of the alphaherpesviruses (Cebrian, J., et al., Proc. Natl. Acad. Sci. USA 79:555-558 (1982); and Fukuchi, K., et al., J. Virol. 51:102-109 (1984)). Members of the latter subfamily have similar genome structures consisting of covalently joined long (L) and short (S) segments. Each segment comprises a unique (U) segment (U_L, U_S) flanked by a pair

(terminal and internals) of inverted repeat regions (TR_L, IR_L; TR_S; respectively). Alphaherpesviruses include human HSV and VZV, porcine pseudorabies virus (PRV), bovine herpesvirus (BHV) and equine herpesvirus (EHV). Because MDV contains extensive repeat sequences flanking its U_L region, its genome structure most resembles that of HSV. (Cebrian, J., et al., Proc. Natl. Acad. Sci. USA 79:555-558 (1982); and Fukuchi, K., et al., J. Virol. 51:102-109 (1984)).

Recent studies (Buckmaster, A. E., et al., J. Gen. Virol. 69:2033-2042 (1988)) have shown that the two gammaherpesviruses, MDV and HVT, appear to bear greater similarity to the alphaherpesviruses, VZV and HSV, than to the gammaherpesvirus, EBV. This was based on a comparison of numerous randomly isolated MDV and HVT clones at the predicted amino acid level; not only did individual sequences exhibit greater relatedness to alphaherpesvirus genes than to gammaherpesvirus genes, but the two viral genomes were found to be generally collinear with VZV, at least with respect to the unique long (U_L) region. Such collinearity of U_L genes extends to other alphaherpesviruses such as HSV-1, HSV-2, EHV-1 and PRV as evidenced by both sequence analysis (McGeoch, D. J., et al., J. Gen. Virol. 69:1531-1574 (1988)) and DNA hybridization experiments (Davison, A. J., et al., J. Gen. Virol. 64:1927-1942 (1983)). Many of these U_L genes are shared by other herpesviruses, including the beta- and gammaherpesviruses (Davison, A. J., et al., J. Gen. Virol. 68:1067-1079 (1987)). The organization and comparison of such genes has suggested the past occurrence of large-scale rearrangements to account for the divergence of herpesviruses from a common ancestor. Unfortunately, such a hypothesis fails to account for the presence of alphaherpesvirus S component (unique short, U_S, and associated inverted/terminal repeat short, IR_S, TR_S) genes which appear unique to members of this subfamily (Davison, A. J., et al., J. Gen. Virol. 68:1067-1079 (1987); Davison,

A. J., et al., J. Gen. Virol. 67:597-611 (1986); and McGeoch, D. J., et al., J. Mol. Biol. 181:1-13 (1985)).

The DNA sequence and organization of genes in a 5.5 kbp EcoR1 fragment mapping in the U_S region of MDV strain RBIB was described by Ross, Binns and Pastorek (Ross, L. J. N., et al, Journal of General Virology 72:949-954 (1991)). The properties and evolutionary relationships of four of the predicted polypeptides was also described (Ross, L. J. N. and M. M. Binns, Journal of General Virology, 72:939-947 (1991)). In that fragment they found the homologs of HSV US2, US3, US6 (gD) and US7 (gI), as well as an MDV specific gene. For the latter, only part of the gene was present. These reports confirm the presence of four MDV U_S genes, and the evolutionary relationship proposed above. It is important to note that no evidence for US8 (gE), or the genes to the left of US2 were described.

In addition to its uniqueness compared with beta- and gammaherpesviruses, the alphaherpesvirus U_S region is particularly interesting because of marked differences in its content and genetic organization within the latter subfamily (e.g. HSV-1 U_S=13.0 kbp, 12 genes, McGeoch, D. J., et al., J. Mol. Biol. 181:1-13 (1985)); VZV U_S=5.2 kbp, 4 genes, Davison, A. J., et al., J. Gen. Virol. 76:1759-1816 (1986)). In the case of HSV-1, 11 of the 12 U_S genes have been found to be dispensable for replication in cell culture (Longnecker, R., et al., Proc. Natl. Acad. Sci. USA 84:4303-4307 (1987)). This has suggested the potential involvement of these genes in pathogenesis and/or latency (Longnecker, R., et al., Proc. Natl. Acad. Sci. USA 84:4303-4307 (1987); Meignier, B., et al., Virology 162:251-254 (1988); and Weber, P. C., et al., Science 236-576-579 (1987)). In the report by Buckmaster et al. (Buckmaster, A. E., et al., J. Gen. Virol. 69:2033-2042 (1988)), except for the identification of partial MDV sequences homologous to HSV immediate early protein alpha 22 (US1) and the serine-threonine protein kinase (US3), the

content, localization and organization of MDV S component homologs was not determined. Moreover, despite the presence of at least four HSV U_S glycoprotein genes (two in VZV), no such homologs were identified.

In application Serial No. 07/229,011 filed August 5, 1988, including Leland F. Velicer, one of the present inventors, the Marek's Disease herpesvirus DNA segment possibly containing the gene encoding the glycoprotein B antigen complex (gp100, gp60, gp49) was identified but not sequenced. Antigen B is an important glycoprotein complex because it can elicit at least partial protective immunity, and thus MDV DNA segment can be used for probes, as a possible source for promoters in the gene's 5' regulatory region, and for gene expression to produce the glycoproteins, which in turn can be used to produce antibodies that recognize the glycoprotein antigens. However, there was no discussion of the glycoproteins of the present invention. These B antigen glycoproteins are not encoded by the U_S region and thus are from a different region of the MDV genome.

In application Serial No. 07/526,790, filed May 17, 1987 by Leland F. Velicer, the MDV herpesvirus DNA segment containing the gene encoding the glycoprotein A antigen (gp57-65) is described but not sequenced. This MDV DNA segment is useful as probes, as a possible source for promoters in the gene's 5' regulatory region, and for producing antibodies by the sequence of events described above. This DNA is also important because antigen A is now known to be a homolog of HSV gC, a gene non-essential for replication in cell culture. Since that property most likely also applies to the MDV homolog, it may be useful as a site for insertion of foreign genes. However, there was no discussion of the glycoproteins of the present invention. This glycoprotein is also not encoded by the U_S region and is thus from a different region of the MDV genome.

Other glycoproteins are encoded by Marek's disease herpesvirus genome. In application Serial No.

07/572,711, filed August 24, 1990 by Leland F. Velicer, et al., the MDV DNA containing the genes encoding the MDV, gD, gI and part of gE glycoproteins is described, with MDV nucleotide sequences for the complete gD and gI genes and part of gE (MDV homologs of HSV genes US6, US7, US8, respectively). This MDV DNA segment is useful as probes, as a possible source for promoters in the gene's 5' regulatory region, and for producing antibodies by the sequence of events described above. The present invention is particularly directed to the complete gene (US8) encoding glycoprotein gE.

OBJECTS

It is an object of the present invention to provide sequenced DNA encoding glycoproteins gD, gI and gE, both together and individually. It is further an object of the present invention to provide DNA segments encoding these glycoprotein antigens and containing potential promoter sequences up to 400 nucleotides 5' of each gene; which are useful as DNA probes, as a possible source for MDV promoters, for producing antibodies which recognize the antigens and, in the case of the latter two glycoproteins, as insertion sites for foreign genes and as possible sites for gene inactivation to attenuate MDV field isolates for vaccine purposes. These and other objects will become increasingly apparent by reference to the following description and the drawings.

IN THE DRAWINGS

Figures 1A to C show map location, sequencing strategy and organization of MDV open reading frames (ORFs):

Figure 1A includes MDV genomic structure and restriction maps outlining area sequenced.

Figure 1B includes map location and sequencing strategy. Boxes define plasmid clones with BamHI, EcoRI or SalI-bound inserts that were used to generate M13mp18 and -19 templates for DNA sequencing. Rightward and leftward arrows define sequences derived from the top and bottom

strands, respectively. The restriction enzyme sites are identified as: B = BamHI, E = EcoRI, Nc = NcoI, Ns = NsiI, S = SalI, and P = PstI. Sequences derived from random libraries (Sau3A, TaqI, RsaI), specific cloned restriction fragments, Bal 31-digested libraries or using synthetically-derived oligonucleotides are denoted by a, b, c, and d, respectively.

Figure 1C includes organization of the MDV U_S ORFs. Numbers refer to homologs based on relation to HSV-1 U_S ORF nomenclature (McGeoch, D. J., et al., J. Mol. Biol. 181:1-13 (1985)). Boxes represent location of MDV ORFs. Arrows define direction of transcription/translation. Names of ORFs are displayed above boxes. Potential polyadenylation signals on the top and bottom strands are highlighted by AATAAA and AAATAA, respectively. SORF1 and SORF2 are MDV-specific S component ORFs given arbitrary names.

Figure 2 shows nucleotide and predicted amino acid sequences. The nucleotide sequence is given as the rightward 5' to 3' strand only (numbered 1 to 10350). Rightward- and leftward- directed predicted amino acid sequences are shown above and below the corresponding nucleotide sequences in single-letter code, respectively. The name of each ORF is given to the left of the first line of the amino acid sequence. Amino acid sequences are numbered from the first M (three letter code) (ATG in the DNA) at the N-terminus to the last amino acid at the C-terminus, which precedes the termination codon (identified by an *). Potential TATA consensus sites located within 400 nucleotides of the ATG are underlined and defined as sites containing at least six of seven matches to the TATA(AT)A(AT) consensus sequences defined by Corden et al. (Corden, B., et al., Science 209:1406-1414 (1980)). Underlines longer than seven nucleotides refer to areas containing overlapping TATA consensus sites.

Figure 3A shows alignment of S component homologs showing selected regions displaying maximum amino

acid conservation. Gaps have been introduced to maximize alignment of identical amino acids as described in Methods. The consensus sequence (cons) indicates residues that are shared by at least all but one of the viruses and are indicated by capital letters. In alignments between more than two sequences, asterisks (*) indicate residues conserved by all of the viruses. Amino acid numbers (with respect to 5'-ATG) of corresponding regions aligned are listed before and after each sequence.

Figure 3B shows the dot matrix analyses depicting overall homologies between selected MDV-alphaherpesvirus S segment homolog comparisons. Points were generated where at least 15 amino acids over a sliding window length of 30 were found identical or similar. The resulting diagonals illustrate regions showing greatest conservation. Amino acid numbers (with respect to 5'-ATG) of corresponding sequences are denoted above and to the right of each plot.

Figure 4 shows a comparison of overall genome organization of available S component ORFs (Audonnet, J.-C., et al., J. Gen. Virol. 71:2969-2978 (1990); McGeoch, D. J., et al., J. Gen. Virol. 68:19-38 (1987); Tikoo, S. K., et al., J. Virol. 64:5134-5142 (1990); Van Zijl, M., et al., J. Gen. Virol. 71:1747-1755 (1990); Zhang, G., et al., J. Gen. Virol. 71:2433-2441 (1990); Cullinane, A. A., et al., J. Gen. Virol. 69:1575-1590 (1988); Davison, A. J., et al., J. Gen. Virol. 76:1759-1816 (1986); McGeoch, D. J., et al., J. Mol. Biol. 181:1-13 (1985); Petrovskis, E. A., et al., Virology 159:193-195 (1987); Petrovskis, E. A., et al., J. Virol. 60:185-193 (1986); and Petrovskis, E. A., et al., J. Virol. 59:216-223 (1986)). Numbers above each ORF refer to homologs based on relation to HSV-1 U_S ORF nomenclature (McGeoch, D. J., et al., J. Mol. Biol. 181:1-13 (1985)). Alternative polypeptide designations common to each system are listed below those ORFs where applicable. Upper and lower case solid bars refer to

rightward and leftward-directed ORFs, respectively. Arrows refer to identified IR_S-U_S and/or U_S-TR_S junction sites.

Figure 5 shows the sequence of steps necessary to produce a complete segment of Marek's disease herpesvirus DNA encoding glycoprotein gI and the part of gE included in the application filed August 24, 1990.

GENERAL DESCRIPTION

The present invention relates to a segment of DNA of Marek's disease herpesvirus genome encoding multiple glycoproteins, and containing potential promoter sequences up to 400 nucleotides 5' of each gene, between a 1 and 10350 nucleotide sequence as shown in Figure 2 (and identified as SEQ ID No:1).

Further, the present invention relates to an EcoRl I segment of Marek's disease herpesvirus genome encoding the glycoprotein D precursor, and subsegments of the DNA.

Further, still, the present invention relates to a segment of DNA encoding glycoprotein gD precursor between a 5964 and 7172 nucleotide sequence of Marek's disease herpesvirus DNA, and containing potential promoter sequences up to 400 nucleotides 5' of each gene, as shown in Figure 2 (and identified as part of SEQ ID No.:1) and subsegments of the segment of DNA which recognize the DNA.

The present invention also relates to a segment of DNA encoding glycoprotein gI precursor between a 7282 and 8346 nucleotide sequence of Marek's disease herpesvirus DNA, and containing potential promoter sequences up to 400 nucleotides 5' of each gene, as shown in Figure 2 (and identified as part of SEQ ID No:1) and subsegments of the segments that recognize the DNA.

The present invention also relates to a segment of DNA encoding glycoprotein gE precursor between a 8488 and 9978 nucleotide sequence of Marek's disease herpesvirus DNA, and containing potential promoter sequences up to 400 nucleotides 5' of each gene, as shown in Figure 2 (and

(and identified as part of SEQ ID No:1) and subfragments of the DNA that recognize the DNA.

Further, the present invention relates to the novel glycoprotein precursors which are produced by expressions of the genes in the segments of DNA.

Further the present invention relates to the potential MDV gene promoters, which are located in the 400 nucleotides 5' of each coding sequence.

SPECIFIC DESCRIPTION

The present invention shows a sequence analysis of a 10.35 kbp DNA stretch encompassing a majority of the MDV U_S region. Altogether seven MDV U_S homologs, including three glycoprotein genes and two additional MDV-specific open reading frames, were identified.

Example 1

Materials and Methods

Recombinant Plasmids, M-13 subcloning and DNA sequencing

MDV EcoR1-0 and EcoR1-I of the pathogenic GA strain were previously cloned into pBR328 (Gibbs, C. P., et al., Proc. Natl. Acad. Sci. USA 81:3365-3369 (1984)), (Silva, R. F., et al., J. Virol. 54:690-696 (1985)) and made available by R. F. Silva, USDA Avian Disease and Oncology Lab, East Lansing, MI, where these clones are maintained. GA strain BamHI-A and BamHI-P1 were previously cloned into pACYC184 and pBR322, respectively (Fukuchi, K., et al., J. Virol. 51:102-109 (1984)) and kindly provided by M. Nonoyama, Showa University Research Institute, St. Petersburg, FL. GA strain clone GA-02, an EMBL-3 clone containing a partially digested MDV SalI insert, which contains BamHI-A, -P1, and additional 5' and 3' flanking sequences (kindly provided by P. Sondermeier, Intervet Intl. B. V., Boxmeer, The Netherlands) was used to extend analysis to the right of the above EcoR1 and BamH1 fragments. This phage clone was used to generate pUC18 subclones with smaller Sal I-bound inserts (psP18-A, pSP18-B, and pSP18-C) containing the 3' BamHI-P1-flanking

region. These clones (Figure 1B) were used to generate M13mp18 and -19 subclones for use as templates for nucleotide sequencing. Small- and large-scale plasmid preparations were made using the alkaline lysis procedure (Maniatis, T., et al., Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1982)).

M13mp18 and M13mp19 phage subclones to be used as templates for sequencing were generated using specific restriction subfragments determined by restriction mapping or the use of Sau3A, Taq I or RsaI-digested viral DNA pools ligated into the unique BamHI, AccI or SmaI sites of M13 RF DNA, respectively. In some cases overlapping M13 deletion clones were obtained by processive Bal31 digestions from AccI, NaeI or NsiI restriction sites in EcoR1-0 by the method of Poncz et al (Poncz, M., et al., Proc. Natl. Acad. Sci. USA 79:4298-4302 (1982)). Standard methods (Maniatis, T., et al., Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1982)) were used for restriction digestions, gel electrophoresis, purification of DNA fragments from agarose gels, ligations and fill-in of 5' overhangs with Klenow fragment.

Ligated M13 products were transformed into $CaCl_2$-competent JM107 host cells and added to melted B top agar containing 10 l of 100 mM IPTG, 50 l of 2% X-gal and 200 l of a fresh overnight JM101 culture. These contents were then plated onto B agar plates and incubated at 37°C overnight. Recombinant (clear) plaques were then used to infect 5 ml of YT media diluted 1:50 with an overnight JM101 culture and rotated at 37°C for 6 hours. The resulting cells were pelleted by centrifugation for 5 minutes at room temperature and the supernatants were removed and stored at 4°C to retain viral stocks of each recombinant clone.

Using the recovered supernatants, single-stranded M13 phage DNA to be used as templates for

DNA sequencing by the dideoxy-chain termination method was isolated according to instructions in the M13 Cloning/Dideoxy Sequencing Instruction Manual provided by Bethesda Research Laboratories. Recombinant M13mp phages were further screened by electrophoresing purified single-stranded viral DNA on 1% agarose mini-gels and selecting those templates showing reduced mobility in comparison to single-stranded M13mp 18 control DNA.

DNA sequencing with single-stranded M13 templates was performed by the dideoxy-chain termination method (Sanger, F. S., et al., Proc. Natl. Acad. Sci. USA 74:5463-5467 (1977)) employing the modified T7 DNA polymerase, Sequenase™ (United States Biochemical Corp., Cleveland, Ohio). A summary of the sequencing strategy is included in Figure 1B. For DNA sequencing reactions, the specific step by step instructions provided with the Sequenase™ sequencing kit were employed. Briefly, single-stranded M13 templates were first annealed with the universal M13 synthetic oligonucleotide primer by incubation at 65°C for 2 minutes followed by slow cooling until the incubation temperature was below 30°C. Following the addition of proper mixtures of deoxy- and dideoxynucleotide triphosphates (dNTPs and ddNTPs, respectively), radioactively labeled deoxyadenosine 5'-(alpha-thio) triphosphate (^{35}S-dATP, 1000-1500 Ci/mmol; NEN-DuPont) and the Sequenase™ enzyme, synthesis of radioactively labeled complementary strands was initiated from the annealed primer. Four separate synthesis reactions were each terminated by the incorporation of the specific ddNTP (ddATP, ddGTP, ddTTP or ddCTP) used in each tube. Reaction products were electrophoresed through 7% polyacrylamide/50% urea/Tris-Borate-EDTA gels and the labeled chains were visualized by autoradiography. Both strands were sequenced at least once. This was facilitated by the use of 16 synthetic 17-mer oligonucleotides generated based on previously determined sequences and substituted for the universal primer under similar reaction

conditions above (0.5 pmoles/reaction) according to the general approach described by Strauss (Strauss, E. C., et al., Anal. Biochem. 154:353-360 (1986)).

Analysis of sequence data

Sequences were assembled and analyzed on an IBM personal System 2/Model 50 microcomputer utilizing the IBI/Pustell (Pustell, J., et al., Nucl. Acids. Res. 14:479-488 (1986)) and Genepro (Version 4.10; Riverside Scientific Enterprises, Seattle, WA) sequence analysis software packages or programs obtained from the University of Wisconsin Genetics Computer Group (GCG; Devereaux, J., et al., Nucl. Acids. Res. 12:387-395 (1984)) and run on a VAX 8650 minicomputer. Database searches of the National Biochemical Research Foundation-Protein (NBRF-Protein, Release 21.0, 6/89) were made with the GCG program FASTA (Pearson, W. R., et al., Proc. Natl. Acad. Sci. USA 85:2444-2448 (1988)) which uses: (1) a modification of the algorithm of Wilbur and Lipman (Wilbur, W. J., et al., Proc. Natl. Acad. Sci. USA 80:726-730 (1983)) to locate regions of similarity; (2) a PAM250-based scoring system (Dayhoff, M. O., et al., p. 345-352. In M. O. Dayhoff (ed.), Atlas of protein sequence and structure, vol. 5, Suppl. 3. National Biomedical Research Foundation, Washington, D. C. (1978)) and (3) the alignment procedure of Smith and Waterman (Smith, T. F., et al., Adv. Appl. Mathematics 2:482-489 (1981)) to join together, when possible, the highest-scoring, non-overlapping regions in order to derive an alignment and its resulting, optimized score. Dot matrix homology plots were generated by using the GCG program DOTPLOT with the output file from GCG's COMPARE. The latter creates a file of the points of similarity between two predicted amino acid sequences for which a window length of 30 and a stringency of 15 (in which conservative amino acid replacements are scored positive) were chosen. Using the GCG program GAP, specific amino acid sequences were aligned using the algorithm of Needleman and Wunsch (Needleman, S. B., et al., J. Mol.

Biol. 48:443-453 (1970)); following the insertion of gaps (to maximize the number of matches) the percentage of identical and similar amino acid residues were determined. To create multiple alignments using GAP, output files of gapped MDV sequences were created following successive GAP comparisons between the MDV sequence and its homologous sequences (in descending order of homology). These output files were used as input sequences for subsequent runs of GAP until the alignment of these gapped sequences could no longer be expanded by the addition of new gaps. Following alignment, the gapped output files were displayed and a consensus sequence calculated using the GCG program PRETTY. To achieve optimal results, in some cases manual editing was employed (using GCG's LINEUP).

Results

The 10,350 nucleotide DNA sequence presented (Figure 2) appears to encompass a majority of the MDV (GA) genome's unique short (U_S) region. A summary of the sequencing strategy is included in Materials and Methods and is depicted in Figure 1B. This sequence spans the U_S fragments, EcoR1-0, EcoR1-I and extends to a SalI site 1.55 kbp downstream of the 3' end of BamHI-P_1 (Figures 1A and 1B). Fukuchi et al. (Fukuchi, K., et al., J. Virol. 51:102-109 (1984)) have previously mapped the IR_S-U_S junction to a 1.4 kb Bgl I fragment located in the second of five EcoR1 subfragments of BamHI-A (Figure 1B). Thus, the sequence presented here should lack between 2.6 and 4.0 kb of the 5'-proximal U_S region, assuming the above IR_S-U_S junction location can be independently confirmed. Because the region sequenced does not extend a sufficient distance downstream of BamHI-P_1, the MDV U_S-TR_S junction has not yet been precisely defined (Davison, A. J., et al., J. Gen. Virol. 76:1759-1816 (1986)). For VZV, EHV-4 and HSV-1, this border is located about 100 bp upstream, or 1.1 and 2.7 kb downstream, respectively, of the termination codon of their respective US8 homologs (Cullinane, A. A., et al., J. Gen. Virol. 69:1575-1590 (1988); Davison, A. J., et al.,

J. Gen. Virol. 76:1759-1816 (1986); and McGeoch, D. J., et al., J. Gen. Virol. 69:1531-1574 (1988)).

The overall G+C content of the region sequenced was found to be 41%, somewhat below the genomic MDV G+C values of 46% (Lee, L. F., et al., J. Virol. 7:289 (1971)) Observed frequencies of CpG dinucleotides in the whole-sequence, or in the coding regions only, did not differ significantly from those expected from their mononucleotide compositions (data not shown). This result agrees with those obtained from alphaherpesviruses, while contrasting with those obtained from gammaherpesviruses, such as the A+T rich HVS and the G+C rich EBV, which are both deficient in CpG dinucleotides (Honess, R. W., et al., J. Gen. Virol. 70:837-855 (1989)).

The region sequenced contains 9 complete ORFs likely to code for proteins (Fig. 1C, basis for names is given below). This prediction was based on: (1) homology and positional organization comparisons to other alphaherpesvirus genes and (2) presence of potential TATA and polyadenylation consensus sequences (Birnstiel, M. L., et al., Cell 41:349-359 (1985); and Corden, B., et al., Science 209:1406-1414 (1980)), and (3) possession of favorable contexts for translational initiation (Kozak, M., J. Cell Biool. 108:229-241 (1989)). This identification was further guided by the observation that alphaherpesviruses such as HSV and VZV tend to contain relatively tightly packed, unspliced and generally nonoverlapping coding regions (Davison, A. J., et al., J. Gen. Virol. 76:1759-1816 (1986); Davison, A. J., et al., J. Gen. Virol. 76:1759-1816 (1986); McGeoch, D. J., et al., J. Gen. Virol. 69:1531-1574 (1988); McGeoch, D. J., et al., J. Mol. Biol. 181:1-13 (1985); and McGeoch, D. J., et al., J. Gen. Virol. 68:19-38 (1987)). Such genes, especially those of the U_S regions, often share polyadenylation signals, thereby resulting in 3'-coterminal mRNA families (Rixon, F. J., et al., Nucl. Acids Res. 13:953-973 (1985)). Methods for detecting protein coding regions based on the use of

MDV-derived codon frequency tables (using these and previously published MDV sequences, Binns, M. M., et al., Virus Res. 12:371-382 (1989); Ross, L. J. N., et al., J. Gen. Virol. 70:1789-1804 (1989); and Scott, S. D., et al., J. Gen. Virol. 70:3055-3065 (1989)) or analysis of compositional bias (using the GCG programs CODONPREFERENCE and TESTCODE) were largely inconclusive, suggesting that MDV possesses relatively low codon and compositional biases compared to those prediced based on its mononucleotide composition. However, using the GCG program FRAMES, together with the MDV-derived codon frequency table above, the 9 identified ORFs clearly show a significantly low pattern of rare codon usage, which sharply contrasts with that observed in all other potentially translatable regions (data not shown).

The predicted amino acid sequences of the predicted ORFs (beginning from the first ATG codon) are shown relative to the nucleotide sequence in Figure 2. Potential TATA sites within 400 nucleotides of the initiation codon are underlined. Proposed ORF and potential polyadenylation signal locations, identification of the -3, +4 ATG context nucleotides (Kozak, M., J. Cell Biol. 108:229-241 (1989)), as well as the lengths, relative molecular masses and predicted isoelectric points of the predicted translational products are shown in Table 1.

A summary of MDV data is shown in Table 1, with location of ORFs, predicted polyadenylation signals utilized, translational context nucleotides, lengths, relative molecular sizes and isoelectric points of predicted translation products.

TABLE 1

Name	ORF Start	ORF End	Predicted Poly-adenylation Site	-3,+4 ATG[a] Context Nucleotides	Length (aa)	Predicted[b] Molecular Size (kDa)	Predicted pIc
US1	248	784	1777	A,A	179	20.4	6.5
US10	1077	1715	1777	G,G	213	23.6	8.2
SORF1	2884	1832	1790	A,A	351	40.6	8.2
US2	3923	3114	1790	A,G	270	29.7	7.6
US3	4062	5240	5394	A,G	393	43.8	6.1
SORF2	5353	5793	5904	C,G	147	16.7	9.8
US6	5964	7172	10040	G,G	403	42.6[d]	10.3[d]
US7	7282	8346	10040	G,T	355	38.3[d]	6.7[d]
US8	8488	9978	10040	A,T	497	53.7[d]	8.0[d]

[a]Nucleotides listed relative to -3, +4 positions, respectively; numbering begins with the A of the ATG (AUG) codon as position +1; nucleotides 5' to that site are assigned negative numbers.

[b]In absence of post-translational modifications.

[c]Calculated using the GCG program, ISOELECTRIC.

[d]Based on sequences that follow the predicted signal peptide cleavage site.

In the absence of previous information concerning these MDV ORFs, and to simplify identification, they have been named (Figure 1C, Table 1) based on homologous relationships to HSV-1 encoded U_S ORFs (McGeoch, D. J., et al., J. Mol. Biol. 181-1-13 (1985)). When appropriate, the letters MDV will preface the homolog's name to indicate the ORF's origin. The two MDV-specific ORFs have been arbitrarily named SORF1 and SORF2, based on their location in the S component.

According to the scanning model for translation, the 40S ribosomal subunit binds initially at the 5'-end of mRNA and then migrates, stopping at the first AUG (ATG) codon in a favorable context for initiating translation (Kozak, M., J. Cell Biol. 108:229-241 (1989)). However, in

the absence of S1 nuclease and/or primer extension analysis, definitive start sites for translation cannot be accurately predicted. Nevertheless, likely start sites are listed in Table 1; these refer to the location of the first inframe ATG codon found in the major open reading frame. According to Kozak (Kozak, M., J. Cell Biol. 108:229-241 (1989)), as long as there is a purine in position -3, deviations from the rest of the consensus only marginally impair initiation. In the absence of such a purine, however, a guanine at position +4 is essential for efficient translation. Table 1 shows that all of the ORFs, except for SORF2, contain the important purine residue in the -3 position. Nevertheless, in the case of SORF2, a compensating guanine in position +4 is indeed present.

In the case of MDV US1, two transcriptional cap sites have been tentatively identified by 5' S1 nuclease protection analysis (data not shown). These sites appear to be located 18 and 25 nucleotides downstream of a TATATAA sequence at position 200 and 207, respectively (Figure 2) Based on 3' S1 data, this transcript utilizes a polyadenylation signal located just downstream of the US10 coding region (Table 1, data not shown). Comparative Northern blot analyses of the U_S region indicate that the MDV US1 transcript appears to be the most prominent transcript expressed at late times (72h) post-infection when extensive cytopathic effects are observed (data not shown). Phosphonoacetic acid inhibition studies have indicated that MDV US1, in contrast to its immediate-early HSV1 US1 counterpart, is regulated as a late class gene (data not shown).

Using the computer program FASTA (Pearson, W. R., et al., Proc. Natl. Acad. Sci. USA 85:2444-2448 (1988)) with a K-tuple value of 1, each of the 9 predicted amino acid sequences was screened against the NBRF-Protein database (Release 21.0, 6/89), and recently published EHV-4 S segment gene sequences (11). Optimized FASTA scores of greater than 100 were generally considered to indicate a

significant degree of amino acid similarity. The results of this analysis are in Table 2.

TABLE 2. PAIRWISE COMPARISONS OF MDV AND ALPHAHERPESVIRUS S COMPONENT HOMOLOGS

		US1					US10			
	Virus	MDV	HSV-1	VZV	PRV	EHV-4	MDV	HSV-1	VZV	EHV-4
% similar / %identical	MDV	-	47/26	43/27	51/33	48/30	-	45/24	40/24	45/29
	HSV-1	47/26	-	49/29	43/25	50/29	45/24	-	49/27	49/27
	VZV	43/27	49/29	-	51/35	54/36	40/24	49/27	-	55/32
	PRV	51/33	43/25	51/35	-	56/41	a	a	a	a
	EHV-4	48/30	50/29	54/36	56/41	-	45/29	49/27	55/32	-
FASTA scores	MDV	891	101	160	218	208	1,071	134	147	251
	HSV-1	101	2,047	119	201	150	134	1,617	123	180
	VZV	160	119	1,378	340	359	147	123	978	191
	PRV	218	201	340	1,724	525	a	a	a	a
	EHV-4	208	150	359	525	1,308	251	180	191	1,312
	length (aa)	179	420	278	364	*273	213	312	180	259

		US2			US3			
		MDV	HSV-1	PRV	MDV	HSV-1	VZV	PRV
% similar / %identical	MDV	-	51/33	48/26	-	56/38	54/33	55/33
	HSV-1	51/33	-	50/31	56/38	-	57/41	59/36
	VZV	a	a	a	54/33	57/41	-	58/35
	PRV	48/26	50/31	-	55/33	59/36	58/35	-
	EHV-4	a	a	a	a	a	a	a
FASTA scores	MDV	1,421	335	**118	1,931	611	616	563
	HSV-1	335	1,554	112	611	2,409	717	620
	VZV	a	a	a	616	717	1,960	595
	PRV	**168	112	1,240	563	620	595	1,948
	EHV-4	a	a	a	a	a	a	a
	length (aa)	270	291	256	393	481	393	390

		US6					US7				
	Virus	MDV	HSV-1	PRV	EHV-1	BHV-1	MDV	HSV-1	VZV	PRV	EHV-1
% similar / %identical	MDV	-	42/21	44/23	43/21	42/33	-	39/22	46/23	43/25	41/23
	HSV-1	42/21	-	47/27	44/22	50/28	39/22	-	43/24	41/26	42/23
	VZV	b	b	b	b	b	46/23	43/24	-	47/25	46/29
	PRV	44/23	47/27	-	51/30	57/38	43/25	41/26	47/25	-	51/30
	EHV-1	43/21	44/22	51/30	-	52/30	41/23	42/23	46/29	51/30	-
	BHV-1	42/33	50/28	57/38	52/30	-	a	a	a	a	a
FASTA scores	MDV	2,068	211	279	246	291	1,816	145	228	184	242
	HSV-1	211	1,999	294	253	304	145	1,880	234	198	249
	VZV	b	b	b	b	b	228	234	1,705	198	298
	PRV	279	294	2,116	428	730	188	188	198	1,652	274
	EHV-1	246	253	428	1,995	494	242	249	298	274	1,979
	BHV-1	291	304	730	494	2,148	a	a	a	a	a
	length (aa)	403	394	402	395	417	355	390	354	350	424

		US8				
		MDV	HSV-1	VZV	PRV	EHV-1
% similar / %identical	MDV	-	44/22	43/22	46/28	47/22
	HSV-1	44/22	-	46/27	49/28	41/23
	VZV	43/22	46/27	-	47/25	46/29
	PRV	46/28	49/28	49/29	-	54/34
	EHV-1	47/22	41/23	50/28	54/34	-
	BHV-1	a	a	a	a	a
FASTA scores	MDV	2,489	192	376	**243	399
	HSV-1	192	2,751	357	257	274
	VZV	376	357	3,171	329	468
	PRV	**217	257	329	2,923	417
	EHV-1	399	274	468	417	2,821
	BHV-1	a	a	a	a	a
	length (aa)	497	550	623	577	552

a existence of homolog undetermined
b no homolog present in genome
* actual length will differ somewhat, since probable initiation codon not defined
** different score when order of comparison reversed

While SORF1 and SORF2 do not appear to share any significant homology to any of the sequences in the database (data not shown), apart from MDV US3, the other six ORFs (MDV US1, 10, 2, 6, 7, and 8; Tables 1, 2) were found to be homologous to alphaherpesvirus S segment genes exclusively (Table 2). Because the US3 ORF represents a member of the serine-threonine protein kinase superfamily (Hanks, S. K., et al., Science 241:42- (1988)), a relatively large number of scores above 150 were obtained. Nevertheless, these scores were 3-4 fold lower than those obtained in comparisons with US3 homologs of HSV, PRV and VZV. To compare with previously established alphaherpesvirus S segment homologies, all possible FASTA comparisons between the seven groups of alphaherpesvirus-related sequences are included. The program GAP was used in similar pairwise comparisons to generate optimal alignments in order to determine the total percentage of identical and similar amino acids shared by the two sequences. As shown in Table 2, homology comparisons between MDV S segment ORFs and their alphaherpesvirus counterparts were comparable to those previously observed between the other alphaherpesvirus S segment homologs themselves. In some cases MDV ORFs were found to be more related to alphaherpesvirus homologs than those same homologs were to their other alphaherpesvirus counterparts (compare MDV/EHV-4 vs. HSV-1/EHV-4 US1 and MDV/EHV-4 vs. HSV-1/EHV-4 US10 homologies). Moreover, despite the fact that VZV lacks US2 and US6 homologs, MDV, although formally considered a gammaherpesvirus, clearly does possess US2 and US6 homologs. The results of limited multiple alignments for each of the seven homologs in which areas showing best conservation are depicted in Figure 3A.

Dot matrix homology plots depicting overall homologies between selected MDV-alphaherpesvirus S segment homolog comparisons are included in Figure 3B. (Using a sliding window length of 30 amino acids, in which points are generated where at least 15 amino acids are found

identical or similar). The resulting diagonals illustrate the regions showing greatest conservation. Such regions include and in some cases extend upon those regions depicted in Figure 3A.

More sensitive attempts to identify other related proteins not detected with FASTA were made using the GCG programs PROFILE and PROFILESEARCH. Use of these programs permit database comparisons which rely on information available from structural studies and, in this case, from information implicit in the alignments of related S component ORFs (including MDV sequences using GAP) (Gribskov, M., et al., Proc. Natl. Acad. Sci. USA 84:4355-4358 (1987)); nevertheless, such analyses failed to extend upon the groups of related proteins described here.

Herpesvirus glycoprotein homologs have generally been found to contain similar patterns of conserved cysteine residues. In comparing the gB homologs of seven different herpesviruses included in the alpha-, beta- and gammaherpesvirus subclasses, there is complete conservation of 10 cysteine residues (Ross, L. J. N., et al., J. Gen. Virol. 70:1789-1804 (1989)). HSV-1 US6 (gD) contains 7 cysteine residues: six appear critical for correct folding, antigenic structure and extent of oligosaccharide processing (Wilcox, W. C., et al., J. Virol. 62:1941-1947 (1988)). Not only is this same general pattern of cysteines conserved in the gD homologs of HSV-2 (McGeoch, D. J., et al., J. Gen. Virol. 68:19-38 (1987)) and PRV (Petrovskis, E. A., et al., J. Virol. 59:216-223 (1986)), but they are conserved in the MDV gD homolog as well (full alignment not shown). Figure 3A depicts portions of cysteine conservation patterns observed among the US6 (gD), US7 (gI), and US8 (gE) homologs (in which case 4, 3, and 6 conserved cysteine residues are shown, respectively). While the MDV, VZV, PRV, and EHV-1 US8 homologs (Audonnet, J.-C., et al., J. Gen. Virol. 71:2969-2978 (1990); Davison, A. J., et al., J. Gen. Virol. 76:1759-1816 (1986); and Petrovskis, E. A., et al., J. Virol. 60:185-193 (1986)) all

share a similar pattern of four conserved cysteine residues near their amino termini, the HSV-1 and -2 counterparts carry only two of these (McGeoch, D. J., J. Gen. Virol. 71:2361-2367 (1990); data not shown). It is quite possible that the unique pattern of four conserved cysteines could facilitate the formation of different secondary and tertiary structures which might impart important functional consequences. These might be reflected by findings which show that HSV-1 gE has Fc receptor activity (Johnson, D. C., et al., J. Virol. 62:1347-1354 (1988)), while its PRV and VZV counterparts do not (Edson, C. M., et al., Virology, 161:599-602 (1987); and Zuckerman, F. A., et al., J. Virol. 62:4622-4626 (1988)).

Careful inspection of the N-terminal regions of the MDV gD, gI and gE homologs has revealed that they contain the three basic building blocks of signal peptide sequences: a basic, positively charged N-terminal region (n-region), a central hydrophobic region (h-region), and a more polar terminal region (c-region) that seems to define the cleavage site (von Heijne, G. J. Mol. Biol. 184:99-105 (1985)). Using a recently improved method for predicting signal sequence cleavage sites (von Heijne, G. Nucl. Acids Res. 14: 4683-4690 (1986)), Table 3 shows the likely position of these sites, the location of the hydrophobic transmembrane and charged cytoplasmic domains near the C-terminal end and the location of potential N-glycosylation sites.

Table 3 shows MDV U_S glycoprotein data on predicted signal peptide cleavage sites and locations of transmembrane and cytoplasmic domains and potential N-glycosylation sites (with respect to the ATG initiation codon).

TABLE 3

Name	Predicted Signal Peptide Cleavage Site	Trans-membrane Domain	Cyto-plasmic Domain	N-glycosylation Sites
US6	G_{30}-D_{31}	358-374	375-403	87,138,230,306
US7	S_{18}-I_{19}	269-288	289-355	147,167,210,245, 253
US8	T_{18}-A_{19}	394-419	420-497	60,133,148,203, 229,277,366,388

Like the other gI homologs, MDV's counterpart contains a relatively long cytoplasmic domain. However, in contrast to the other gD homologs, MDV gD's signal peptide contains a relatively long n-region (18 residues), that is unusually highly charged (+4; Figure 2) considering an overall mean value of +1.7 among eukaryotes, which generally does not vary with length (von Heijne, G, J. Mol. Biol. 184:99-105 (1985)). Although a more distal methionine codon exists directly before the initiation codon (as in the PRV gD homolog, Petrovskis, E. A., et al., J. Virol. 59:216-223 (1986)) the scanning model for translation (Gribskov, M., et al., Proc. Natl. Acad. Sci. USA 84:4355-4358 (1987)) favors usage of the more 5'-proximal initiation codon (at position 5964, Figure 2). Further support is based on an overall translation context that appears at least as good as, if not better than, the one corresponding to the downstream ATG. Despite such a prediction, a possible mRNA cap site location between the two ATG sites, which would preclude such a prediction, cannot be ruled out at this point.

One final point concerning MDV gD requires mention. Using the 10,350 nucleotide DNA sequence as a probe for screening the GenBank (62.0, 12/89) and EMBL (19.0, 5/89) nucleic acid databases with the computer program FASTA (K-tuple=6), an optimized score of 1027, corresponding to 91.5% nucleotide identity in a 342 bp overlap between MDV gD coding sequences (6479-6814; aa#173-aa#284; Figure 2) and a previously reported 467 bp

MDV DNA segment (Wen, L.-T., et al., J. Virol. 62:3764-3771 (1988)). The latter sequence has been reported to contain a 60 bp segment protected against DNAse digestion by binding of a 28kD MDV nuclear antigen (MDNA) expressed only in "latently" infected MDV-transformed lymphoblastoid cells. In view of similarities between MDV and VZV, these authors suggested that MDNA may function in a manner analogous to that of EBNA-1 in immortalizing primate cells. In their report, Wen et al. (Wen, L.-T., et al., J. Virol. 62:3764-3771 (1988)) mapped the MDNA binding site to the same EcoRI subfragment of BamHI-A in which MDV gD is located (EcoRI-I, Figure 1). Although our sequence covering this region is consistent with a complete, uninterrupted ORF containing all the characteristic features of a glycoprotein, and showing significant homology to HSV gD, their sequence contains about 140 bases of 5'-proximal sequence unrelated to any determined from our 5.3 kbp EcoRl-I fragment or its adjoining 3.5 kb sequences. The remaining 327 bp sequence (which contains the putative nuclear antigen binding site) while clearly resembling our gD coding sequence, upon computer translation fails to yield any ORF longer than 30 aa.

Discussion

Recent data have shown that despite MDV's classification as a gammaherpesvirus, based on lymphotropic properties shared with other members of this subfamily, its genome structure (Cebrian, J., et al., Proc. Natl. Acad. Sci. USA 79:555-558 (1982); and Fukuchi, K., et al., J. Virol. 51:102-109 (1984)) and genetic organization of primarily its U_L region (Buckmaster, A. E., et al., J. Gen. Virol. 69:2033-2042 (1988)) more closely resembles that of the neurotropic alphaherpesviruses. Moreover, in cases where polypeptide sequences were found conserved among the three herpesvirus subfamilies (e.g. U_L genes), significantly higher homology scores were consistently observed against the respective alpha- rather than beta- or gammaherpesvirus counterparts (Davison, A. J., et al., J.

Gen. Virol. 67:597-611 (1986); Buckmaster, A. E., et al., J. Gen. Virol. 69:2033-2042 (1988); Ross, L. J. N., et al., J. Gen. Virol. 70:1789-1804 (1989); and Scott, S. D., et al., J. Gen. Virol. 70:3055-3065 (1989)). Alphaherpesvirus S segment genes have previously been found to be unique to members of this taxonomic subfamily (Davison, A. J., et al., J. Gen. Virol. 68:1067-1079 (1987); and Davison, A. J., et al., J. Gen. Virol. 67:597-611 (1986)). The identification of seven MDV homologs of alphaherpesvirus S segment genes in this study is consistent with the idea that MDV shares a closer evolutionary relationship with alphaherpesviruses than gammaherpesviruses. This is further supported by dinucleotide frequency analysis which fails to show a lack of CpG suppression as observed among all gammaherpesviruses thus far studied (Efstathiou, S., et al., J. Gen. Virol. 71:1365-1372 (1990); and Honess, R. W., et al., J. Gen. Virol. 70:837-855 (1989)). The above situation resembles a similar one observed with human herpesvirus-6 (HHV-6), in which case its T-lymphotropism suggested provisional classification as a gammaherpesvirus (Lopez, C., et al., J. Infect. Dis. 157:1271-1273 (1988)). However, subsequent genetic analysis has shown a greater relatedness between HHV-6 and the betaherpesvirus, human cytomegalovirus (HCMV; Lawrence, G. L., et al., J. Virol. 64:287-299 (1990)).

A comparison of the genetic organization of alphaherpesvirus S segment genes is presented in Figure 4. The organization of these genes in some cases vary greatly in overall length, organization and degree of homology. Nevertheless, the overall gene layouts displayed are consistent with a model to account for the divergence of alphaherpesviruses from a common ancestor by a number of homologous recombination events which result in expansion or contraction of the inverted repeat regions and a concomitant loss or gain of U_S gene(s). In the case of VZV, six S segment homologs are lacking compared to HSV-1 (US2, US4, US5, US6, US11, US12). Some genes, such as the

US1 homologs, show particular sequence and length divergences. Compared to HSV-1, the MDV, VZV and EHV-4 US1 homologs lack approximately 120 aa of sequence comparable to the 5'-proximal portion of HSV-1 US1 (alpha 22) Based on Northern blot analysis, S1 nuclease protection analysis and phosphonoacetic acid inhibition studies, in contrast to its relatively uncharacterized immediate-early HSV-1 counterpart, the MDV US1 gene appears to be regulated as an abundantly expressed late class gene (data not shown). In contrast to the other alphaherpesviruses, MDV contains two apparently MDV-specific ORFs. Moreover, the MDV U_S region appears to contain approximately 2.6 to 4.0 kb of additional 5'-proximal sequences. Based on a comparison of Figure 4 and consideration of the expansion-contraction recombination scheme, it appears likely that there are additional MDV-specific U_S genes.

Since MDV has long been regarded as a gammaherpesvirus, much of the previous work interpreting their properties has proceeded by analogy with the association between EBV and B cells (Nonoyama, M. p. 333-341. In B. Roizman (ed.), The herpesviruses, vol. 1. Plenum Press (1982); and Wilbur, W. J., et al., Proc. Natl. Acad. Sci. USA 80:726-730 (1983)). Because of a closer genetic relationship to the alphaherpesviruses, and keeping in mind the analysis of HHV-6 above, we agree with Lawrence et al. (Lawrence, G. L., et al., J. Virol. 64:287-299 (1990)) that the lymphotropic properties of MDV and HVT are unlikely to be determined by molecules homologous to EBV and that a delineation of molecular differences between MDV and the neurotropic alphaherpesviruses would be more fruitful in explaining the observed biological differences than employing analogies based on properties of gammaherpesviruses such as EBV and HVS.

To account for such differences, the MDV U_S region may be particularly important. With few exceptions, each HSV-1 L component gene possesses an equivalent in VZV (McGeoch, D. J., et al., J. Gen. Virol. 69:1531-1574

(1988)); a considerable number of these are related to beta- and gammaherpesvirus genes as well (29 of 67 EBV counterparts to VZV U_L genes; Davison, A. J., et al., J. Gen. Virol. 68:1067-1079 (1987)). In contrast, the S segments of HSV-1 and VZV differ significantly in size and appear to be among the least related parts of the two genomes (Davison, A. J., et al., J. Gen. Virol. 67:597-611 (1986; and Davison, A. J., et al., J. Gen. Virol. 64:1927-1942 (1983)). Recent studies have shown that 11 of 12 open reading frames contained in the HSV-1 S component are dispensable for growth in cell culture (Longnecker, R., et al., Proc. Natl. Acad. Sci. USA 84:4303-4307 (1987); and Weber, P. C., et al., Science 236:576-579 (1987)). The maintenance and evolution of such a dispensable gene cluster suggests the presence of functions relevant to the viruses survival in its specific ecological niche in the natural or laboratory animal host, rather than the presence of functions necessary for replication (Longnecker, R., et al., Proc. Natl. Acad. Sci. USA 84:4303-4307 (1987); and Weber, P. C., et al., Science 236:576-579 (1987)). Consistent with such a hypothesis are findings that HSV mutants carrying different S component gene-specific deletions were significantly less pathogenic and exhibited a reduced capacity for latency establishment in mice (Meignier, B., et al., Virology 162:251-254 (1988)). In regard to the latter, there is evidence suggesting that RNA transcribed from the HSV U_S region may be involved in the establishment and maintenance of an <u>in vitro</u> latency system employing human fetus lung fibroblast cells (Scheck, A. C., et al., Intervirology 30:121-136 (1989)). Taken together, the above evidence suggest(s) potentially important role(s) for MDV's U_S genes in tissue tropism, latency, and/or induction of cell transformation.

A consideration of the three gD, gI and gE homologs identified in this invention raises two other questions of relevance to future vaccine development. The 11 HSV-1 U_S region genes dispensible for growth in tissue

culture described above include HSV-1 US7 (gI) and US8 (gE) (Longnecker, R., et al., Proc. Natl. Acad. Sci. USA 84:4303-4307 (1987); and Weber, P. C., et al., Science 236:576-579 (1987)). Assuming the MDV homologs have the same properties, these genes may be useful as sites for insertion of foreign genes. Further the same two MDV homologs, and especially US8 (gE), may very likely be involved in the pathogenicity-related issues introduced above. Specifically HSV's gE seem to play a role in HSV-1's ability to establish lethal infections and latency in mice (Meignier, B., et al., Virology 162:251-254 (1988)). Further, the gI and gE homologs of PRV of swine play a clear role in PRV virulence for 1-day-old chickens and young pigs (Mettenleiter, Thomas C., et al., Journal of Virology, p. 4030-4032 (Dec. 1987)). Assuming the same holds true for the MDV US7 (gI) and US8 (gE) homologs, it may be possible to inactivate one or both of these genes from very virulent MDV isolates which cause outbreaks not prevented by current vaccines, and thereby creating an attenuated vaccine viruses more closely related to field virus causing disease outbreaks.

A further consideration of the three (gD, gI and gE) homologs identified in this invention raises another interesting question. Fully enveloped infectious MDV virions are only known to be produced in feather follicle epithelial cells (Payne, L. N. p. 347-431. In B. Roizman (ed.), The herpesviruses, vol. 1. Plenum Press (1982)). Because of this, MDV studies have had to rely on limited fibroblast cell cultures which only promote the spread of cell-associated infections in vitro. Over the last 20 years, studies aimed at identifying immunogenic surface antigens have relied on this in vitro culture system and altogether only two glycoprotein antigens (A antigen/gC homolog; B antigen) have been routinely identified and characterized (Binns, M. M., et al., Virus Res. 12:371-382 (1989); Coussens, P. M., et al., J. Virol. 62:2373-2379 (1988); Isfort, R. J., et al., J. Virol. 59:411-419 (1986);

Isfort, R. J., et al., J. Virol. 57:464-474 (1986); and Sithole, I., et al., J. Virol. 62:4270-4279 (1988)). This is despite findings of three MDV gD, gI and gE homologs of the present invention and two additional glycoprotein homologs (gB and gH, Buckmaster, A. E., et al., J. Gen. Virol. 69:2033-2042 (1988); and Ross, L. J. N., et al., J. Gen. Virol. 70:1789-1804 (1989)). While immune chicken sera (ICS) from naturally infected birds is likely to react with many, if not all, MDV-encoded surface antigens, this complex polyclonal sera would only be useful to the extent that antigen expression/processing in semi-productive cell culture resembles that in feather follicle epithelial cells. Northern blot analysis using MDV gD-specific probes suggests that MDV gD mRNA is either not expressed or poorly expressed in DEF cells at a time when extensive cytopathic effects are observed (data not shown). In light of the fact that VZV lacks a gD homolog and is strongly cell-associated, it will be interesting to see whether the block in MDV virion formation in primary avian fibroblast cells is found to correlate with lack of expression (in these cells) of a glycoprotein, such as gD, and/or some other S component gene(s).

Because the protection against MD conferred by attenuated MDV strains (serotype 2) or HVT (serotype 3) appears to have an immunological basis, there is considerable interest in identifying common antigens. In view of this invention identifying seven MDV U_S homologs to U_S genes of HSV (the latter of which is clearly less related to MDV than HVT is), it would be surprising if the previous report showing lack of homology between MDV-HVT U_S regions (Igarashi, T., et al., Virology 157:351-358 (1987)) were proven correct. Such negative results may reflect the limitations regarding homology estimates based on hybridization, rather than sequence analysis studies.

Example 2 shows the molecular cloning of a construct containing the DNA encoding the complete MDV US7 (gI) gene and part of the MDV US8 (gE) gene. As can be

seen, this is accomplished using segments of DNA spanning the gI and part of the gE coding region.

Example 2

MOLECULAR CLONING OF A CONSTRUCT CONTAINING THE DNA ENCODING THE COMPLETE MDV US7 (gI) and PART OF MDV US8 (gE)

Construction of a recombinant clone (pKS-MDgI1.59) containing the complete MDV US7 (gI) coding sequence and a portion of the MDV US8 (gE) coding sequence requires two preexisting MDV clones, pKS-MDgD1.75 and p19P1 (Fig. 5). pKS-MDgD1.75 is a recombinant plasmid containing the 1.75 kbp NcoI-SstII subfragment of MDV EcoR1-I ligated into the SmaI-Sst II site of the cloning vector, pBluescript KS-. This clone contains the complete MDV US6 (gD) coding sequence and additional sequences at the 3' end which code for the first 39 amino acids (aa) of MDV gI. p19P1 is a recombinant plasmid containing the 1.5 kbp BamHI-P_1 subfragment of MDV cloned into the unique BamHI site of pUC19. This clone contains the entire MDV gI coding sequence, except for the first 9 aa of its signal sequence. In addition, at the 3' end, p19P1 contains the first 104 aa of the MDV US8 (gE) coding region.

To generate pKS-MDgI1.59, pKS-MgD1.75 is first cut with HincII, which cuts once in the multiple cloning site of the pBluescript vector and once about 180 bp upstream of the insert's SstII terminus. This results in two fragments: one fragment (1.6 kbp) consists primarily of insert sequences encoding MDV US6 (gD); the larger fragment (3.1 kbp) consists of pBluescript vector sequences, in addition to about 180 bp which encode the N-terminus of MDV gI. The 3.1 kb fragment is gel purified and self-ligated by way of the two HincII ends. The resulting recombinant plasmid, pKS-MDgI0.18, is then cut with SstI (in the multiple cloning site, just downstream of the SstII site). Prior to subsequent digestion with SstII, the cohesive SstI ends is made blunt-ended with T4 DNA polymerase. The resulting 3.1 kbp SstII-SstI (blunt) fragment of pMDgI0.18 is gel purified and used in the final ligation step to

create pKS-MDgI1.59. While the enzymatic manipulations of pKS-MDgD1.75 and pKS-MDgI0.18 are taking place, p19P1 is cut with HindIII, which cuts just downstream of the partial MDV US8 (gE) coding sequence in the multiple cloning site of pUC19. Prior to digestion with SstIId, the cohesive HindIII ends is made blunt-ended using Klenow fragment. The smaller SstII-HindIII (blunt) fragment (1.4 kbp) contains a majority of the MDV US7 (gI) coding sequence, in addition to 312 nucleotides at the 3' end which code for the 5' end of MDV gE. This 1.4 kbp SstII-HindIII(blunt) fragment is gel purified and ligated to the 3.1 kbp SstII-SstI(blunt) fragment of pKS-MDgD0.18. The resulting recombinant, pKS-MDgI1.59, contains the complete coding sequence for MDV gI and a portion of the N-terminal gE coding sequence. Digestion of pKS-MDgI1.59 with KpnI yields two fragments; the smaller 1.15 kbp fragment contains the complete coding sequence for MDV gI.

Example 3 shows molecular subcloning of a construct containing the complete MDV US8 (gE) gene.

Example 3

MOLECULAR CLONING OF A CONSTRUCT ENCODING THE COMPLETE MDV US8 (gE)

Construction of a recombinant clone (p18-MDgE2.53) containing the complete MDV US8 (gE) coding sequence requires a clone other than the BamHI or EcoR1 clones used previously. GA strain clone GA-02, an EMBL-3 clone containing a partially digested MDV SalI insert, which contains BamHI-A, -P1, and additional 5' and 3' flanking sequences (kindly provided by P. Sondermeier, Intervet Intl. B. V., Boxmeer, The Netherlands) was used to extend analysis 3' of the EcoR1-I and BamH1-P1 fragments. Smaller SalI subfragments located at the 3' end of this phage clones MDV insert were gel purified and ligated to pUC18 linearized to SalI (pSP18-A, pSP18-B, and pSP18-C, Fig. 1B). The pUC18 subclone, pSP18-A contains the entire MDV US8 (gE) coding sequence and is designated p18-MDgE2.53 for ATCC deposit purposes.

Index of definition of letters in Figure 2.

Table 4 showing the amino acids with both their single letter and three letter symbols.

TABLE 4

	A	Ala	Alanine	M	Met	Methionine
	C	Cys	Cysteine	N	Asn	Asparagine
	D	Asp	Aspartic Acid	P	Pro	Proline
	E	Glu	Glutamic Acid	Q	Gln	Glutamine
	F	Phe	Phenylalanine	R	Arg	Arginine
	G	Gly	Glycine	S	Ser	Serine
	H	His	Histidine	T	Thr	Threonine
	I	Ile	Isoleucine	V	Val	Valine
	K	Lys	Lysine	W	Trp	Tryptophan
	L	Leu	Leucine	Y	Tyr	Tyrosine

When the DNA segments encoding glycoproteins gI and gE are altered by insertional, site-directed or deletion mutagenesis, the pathogenicity of the MDV may be reduced. Also, the segments of DNA encoding the non-essential gI and gE can be used as insertion sites for segments of foreign DNA which encode proteins that are antigenically active for the purpose of producing a recombinant vaccine.

~~ATCC Deposit~~

~~The gene for MDV US6 (MDV gD) has been deposited~~ in a plasmid (phagemid) pKS-MDgD1.75, as ATCC 40855, with The American Type Culture Collection, Rockville, MD, 20852, USA.

The gene for MDV US7 (MDV gI) has been deposited in a plasmid (phagemid) pKS-MDgI1.59, as ATCC 75040, with The American Type Culture Collection, Rockville, MD, 20852, USA.

The gene for MDV US8 (MDV gE) has been deposited in a plasmid p18-MDgE 2.53, as ATCC 75039, with The American Type Culture Collection, Rockville, MD, 20852, USA.

Attached are Sequence Listings for Sequence ID ~~NOS. 1, 2 and 3 as previously described in the application.~~

ATCC Deposit

The gene for MDV US6 (MDV gD) was deposited in a plasmid (phagemid) pKS-MDgD1.75, on 2 August 1990 as ATCC 40855, with The American Type Culture Collection, Rockville, MD, 20852, USA.

The gene for MDV US7 (MDV gI) was deposited in a plasmid (phagemid) pKS-MDgI1.59, on 20 June 1991 as ATCC 75040, with The American Type Culture Collection, Rockville, MD, 20852, USA.

The gene for MDV US8 (MDV gE) was deposited in a plasmid p18-MDgE 2.53, on 20 June 1991 as ATCC 75039, with The American Type Culture Collection, Rockville, MD, 20852, USA.

Attached are Sequence Listings for Sequence ID NOS. 1, 2 and 3 as previously described in the application.

VS

APPENDIX I

(1) GENERAL INFORMATION:

(i) Applicants: Leland F. Velicer, Peter Brunovskis, and Paul Coussens

(ii) Title of Invention: Marek's Disease Herpesvirus DNA Segment Encoding Glycoproteins gD, gI and gE

(iii) Number of Sequences: 3

(iv) Correspondence Address:

(A) Addressee: Ian C. McLeod

(B) Street: 2190 Commons Parkway

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(D) State: Michigan

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(A) Medium Type: 1.44 Mb 3 1/2" floppy diskette

(B) Computer: IBM PS2, Model 50

(C) Operating System: MS-DOS 5.0

(D) Software: PC-Write 3.02

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(ix) Telecommunication Information:

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(2) Information for SEQ ID NO: 1

(i) Sequence Charateristics:

(A) Length: 10,350 base pairs

(B) Type: nucleic acid

(C) Strandedness: double

(D) Topology: linear

(ii) Molecule Type: genomic DNA

(iii) HYPOTHETICAL: Yes

(v) ANTI-SENSE: No

(vi) ORIGINAL SOURCE:

(A) Organism: MDV, GA strain

(vii) IMMEDIATE SOURCE:

(A) Library: genomic

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

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GAATTCCTTG AAATTGGAGT GAAATCTTTA GGGAGGGAGG TTTACCATTG TGGAGAATAT   60

ATAGAGCAAG TAGTACATTA GGGGCTGGGT TAAAGACCAA GTAATTTTTG ACCGGATATC  120

ACGTGATGTA AATTCTAGCA ATTATTGTTC CTAGCAGAAG ATAAAAGCTG GTAGCTATAT  180

AATACAGGCC AAAGTCTCCA AATTACACTT GAGCAGAAAA CCTGCTTTCG GCTCCATCGG  240

AGGCAAC ATG AGT CGT CAT CGA GAT CGA GCC AGA CCC GAT ACA CGA TTA      289
       Met Ser Arg Asp Arg Asp Arg Ala Arg Pro Asp Thr Arg Leu
        1               5                  10

TCA TCG TCA GAT AAT GAG AGC GAC GAC GAA GAT TAT CAA CTG CCA CAT     337
Ser Ser Ser Asp Asn Glu Ser Asp Asp Glu Asp Tyr Gln Leu Pro His
 15                  20                  25                  30

TCA CAT CCG GAA TAT GGC AGT GAC TCG TCC GAT CAA GAC TTT GAA CTT     385
Ser His Pro Glu Tyr Gly Ser Asp Ser Ser Asp Gln Asp Phe Glu Leu
                35                  40                  45

AAT AAT GTG GGC AAA TTT TGT CCT CTA CCA TGG AAA CCC GAT GTC GCT     433
Asn Asn Val Gly Lys Phe Cys Pro Leu Pro Trp Lys Pro Asp Val Ala
            50                  55                  60

CGG TTA TGT GCG GAT ACA AAC AAA CTA TTT CGA TGT TTT ATT CGA TGT     481
Arg Leu Cys Ala Asp Thr Asn Lys Leu Phe Arg Cys Phe Ile Arg Cys
        65                  70                  75

CGA CTA AAT AGC GGT CCG TTC CAC GAT GCT CTT CGG AGA GCA CTA TTC     529
Arg Leu Asn Ser Gly Pro Phe His Asp Ala Leu Arg Arg Ala Leu Phe
    80                  85                  90

GAT ATT CAT ATG ATT GGT CGA ATG GGA TAT CGA CTA AAA CAA GCC GAA     577
Asp Ile His Met Ile Gly Arg Met Gly Tyr Arg Leu Lys Gln Ala Glu
 95                  100                 105                 110

TGG GAA ACT ATC ATG AAT TTG ACC CCA CGC CAA AGT CTA CAT CTG CGC     625
Trp Glu Thr Ile Met Asn Leu Thr Pro Arg Gln Ser Leu His Leu Arg
                115                 120                 125

AGG ACT CTG AGG GAT GCT GAT AGT CGA AGC GCC CAT CCT ATA TCC GAT     673
Arg Thr Leu Arg Asp Ala Asp Ser Arg Ser Ala His Pro Ile Ser Asp
            130                 135                 140

ATA TAT GCC TCC GAT AGC ATT TTT CAC CCA ATC GCT GCG TCC TCG GGA     721
Ile Tyr Ala Ser Asp Ser Ile Phe His Pro Ile Ala Ala Ser Ser Gly
        145                 150                 155

ACT ATT TCT TCA GAC TGC GAT GTA AAA GGA ATG AAC GAT TTG TCG GTA     769
Thr Ile Ser Ser Asp Cys Asp Val Lys Gly Met Asn Asp Leu Ser Val
    160                 165                 170

GAC AGT AAA TTG CAT TAA CTATCCAGAC TTGAAGAGAA AGCTCTTATT            817
Asp Ser Lys Leu His End
175
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ATATAATTTT AATTGTTAGA CATAGAGCCG ACATTCTTTG ATCTATCTAA TGAGATAAAA   877

TAATAGATTT TGGATTTATT TGTCATGATC TGTTGCAACA AACGCTGACC CCCCCCATCC   937

ATGAAGGGGC GTGTCAAATA ACGTGTTGCC TTTTTGTTGT ATATGAAGAT ATTTAATGTG   997

GCGTTGAGCC TAATGAGAGG AGAACGTGTT TGAATACTGG AGACCAGCGC CGTGTAAGAT   1057

TAAAACATAT TGGAGAGGT  ATG GCC ATG TGG TCT CTA CGG CGC AAA TCT        1106
                      Met Ala Met Trp Ser Leu Arg Arg Lys Ser
                       1               5                  10

AGC AGG AGT GTG CAA CTC CGG GTA GAT TCT CCA AAA GAA CAG AGT TAT     1154
Ser Arg Ser Val Gln Leu Arg Val Asp Ser Pro Lys Glu Gln Ser Tyr
                  15                  20                  25

GAT ATA CTT TCT GCC GGC GGG GAA CAT GTT GCG CTA TTG CCT AAA TCT     1202
Asp Ile Leu Ser Ala Gly Gly Glu His Val Ala Leu Leu Pro Lys Ser
             30                  35                  40

GTA CGC AGT CTA GCC AGG ACC ATA TTA ACC GCC GCT ACG ATC TCC CAG     1250
Val Arg Ser Leu Ala Arg Thr Ile Leu Thr Ala Ala Thr Ile Ser Gln
         45                  50                  55

GCT GCT ATG AAA GCT GGA AAA CCA CCA TCG TCT CGT TTG TGG GGT GAG     1298
Ala Ala Met Lys Ala Gly Lys Pro Pro Ser Ser Arg Leu Trp Gly Glu
     60                  65                  70

ATA TTC GAC AGA ATG ACT GTC ACG CTT AAC GAA TAT GAT ATT TCT GCT     1346
Ile Phe Asp Arg Met Thr Val Thr Leu Asn Glu Tyr Asp Ile Ser Ala
 75                  80                  85                  90

TCG CCA TTC CAC CCG ACA GAC CCG ACG AGA AAA ATT GTA GGC CGG GCT     1394
Ser Pro Phe His Pro Thr Asp Pro Thr Arg Lys Ile Val Gly Arg Ala
                 95                 100                 105

TTA CGG TGT ATT GAA CGT GCT CCT CTT ACA CAC GAA GAA ATG GAC ACT     1442
Leu Arg Cys Ile Glu Arg Ala Pro Leu Thr His Glu Glu Met Asp Thr
            110                 115                 120

CGG TTT ACT ATC ATG ATG TAT TGG TGT TGT CTT GGA CAT GCT GGA TAC     1490
Arg Phe Thr Ile Met Met Tyr Trp Cys Cys Leu Gly His Ala Gly Tyr
        125                 130                 135

TGT ACT GTT TCG CGC TTA TAT GAG AAG AAT GTC CGT CTT ATG GAC ATA     1538
Cys Thr Val Ser Arg Leu Tyr Glu Lys Asn Val Arg Leu Met Asp Ile
    140                 145                 150

GTA GGT TCG GCA ACG GGC TGT GGA ATA AGT CCA CTC CCC GAA ATA GAG     1586
Val Gly Ser Ala Thr Gly Cys Gly Ile Ser Pro Leu Pro Glu Ile Glu
155                 160                 165                 170

TCT TAT TGG AAA CCT TTA TGT CGT GCC GTC GCT ACT AAG GGG AAT GCA     1634
Ser Tyr Trp Lys Pro Leu Cys Arg Ala Val Ala Thr Lys Gly Asn Ala
                175                 180                 185

GCA ATC GGT GAT GAT GCT GAA TTG GCA CAT TAT CTG ACA AAT CTT CGG     1682
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Ala Ile Gly Asp Asp Ala Glu Leu Ala His Tyr Leu Thr Asn Leu Arg
          190                 195                 200

GAA TCG CCA ACA GGA GAC GGG GAA TCC TAC TTA TAA CTAATCGCAC          1728
Glu Ser Pro Thr Gly Asp Gly Glu Ser Tyr Leu End
       205                 210

AATTATTAAT AGGATTTTAG GAAAAACTGC TACTAACGTT GTTTAAATAA TAAAATTTTA   1788

TTTTCAATAA GGCATTACAG TGTTGTCATG ATTGTATGTA TTATATGGGG TATGCATGAG   1848

GATTACTTCG ATTGAAACTT TGTCTAAATG TCTGTAGGAT TTTACTATTC ATTAGTCTGG   1908

ATCGAGGCGG ACGTAAATGG AGATTGCGGC AAATGTAGGG GTGCTGGTAC ATAAGACCTC   1968

CAACATCCAT TCGACTCATC GGCCTGCGTC CAAATGGATA TGTTGATGTA CCTTGTAAAG   2028

TTATGACATT AGAAGATCGA TGGTGAATAG TGGGATCTAT ATCCATGCTA TTCTCAATAT   2088

TGCATGATAT GCAATGTTCC CGGTTAGGTT TGATAAGATC ATGTATGGTT CTATAATACA   2148

ACTCCTCTTC AGAAGAATCA TTTATTTTAT GTCCACTGTC CTTGGATATT CCAGTTTCTG   2208

TCAATCGATT CGCTTGCATT TGCGTGCAGC ATGTCTTGAT GGCATTTCCT ATGCTATCAT   2268

CCGGCAGGCC TAAGGGTGTT CTATACTCGC ACACAGGTAG AGCAAGAACC ACGGCATATC   2328

GAGCTACCTC TATTGCCCCG CTAAGGACAT TTCTTGCAGA CTGTATTGTC ATGAACATAT   2388

TTCGTGTATT GTGTCGATCA TAACCCTTGT TGATTCCTAT GGAAAGCATT GTGGTCCAGT   2448

TTTCCAGATG AAATGAAAAC AATGCGGGCA AAAATGGTCC CACCTGTTTC ATCTTCAATG   2508

CATCTCTCAC ATCCCAAGTT CTATAGAATA TTCTCCACTG ACCAGTTTCG GTAAGATCAG   2568

TTTCTGTAAA ATTTGTGATA GTTTCAATCG AAAACATTTT GTCCATCATG GCAAAAAATC   2628

TATAGGCAGA CCAGATAACC ATTTGACACC ACATATCCTT GTGTATATCA AACGATGTAA   2688

TAGATCCCTC GTTAGTAGAT ATGGTACATA AAAGGCCTAA TCTCTCTCGG GCTTCCATAC   2748

ATTGAACGAT TCCTTCTGTG AATTCATCAA CAACCACATG CCAAAAATTT ACATTAGTAA   2808

TCTTTCTCGG TGGCTTACCA AATCGTCCTC TTGGTATATC CATATCATCG AACATTGTAG   2868

CATTGACTCT GCTCATCGTT GTCTTTCAAA TGCGCTCGAT TGTTGAATCT CTCCTGATGT   2928

TAGAAGTATA TGGAAGATAG CCTGGATACA TAAGTGATCT AGAAGGGTTT GTTATTGCAC   2988

TAATATACAA ATTATACGTG ACACTATAGC GACGGTTGTA GCGATCCACC TAATCGTAAT   3048

GTGTATACGC CCCATCATGT AATTATATCT AATTGGTAGC AAGTAGGTCT GTCGAATAAC   3108

AGCTAATGAC TACCGGCTCT ACATTTTTTC TGTATTCGTG ACTTTCCTGT CGCAGTGTAA   3168

CGAACCGGAA TTGCAATCGC ATCTCTATCT TCTTTCTTGC AACATTTTCC ACAACAGAAT   3228
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AATCTGCCGG GTGTACTACT CATTTGAGGT GGTTCGATTT CCGGAGGTTT TAGAGGATTG   3288

GGTGGGGACC CGAGGATTTT GTATACACAT ACCATATCAC TGTCGCAAAA ATCCGCTCTA   3348

TCTTCTGGGG TGTCGAACTT CGGTTCCCAT GTAGATGTCA AGAGAGTTTG AATATTGTCG   3408

GGAATGGCCC ACGGCATACC GGACCAGGTC CCAGACACTT TGATTGCAAG TAACCTTTTT   3468

GGCAAAGGAA TACATTCGAG CGCAATGGCA CATATATCTG CCGCCCCAAC TATCCACAAG   3528

CTATGTGGAG CATTACCAGA AACTTCAGAT TCCAACATCA AATATCCAGA TAGAACATCC   3588

TGCCATTCTG TGGAACATCC TGCAACATCT TCAAATAGCC GCACTATAAA CGAATCCCTA   3648

GTTCCGGCCA ATCCGGTACC ACGAACTCCA GTTCCATCTG GTGGCTTTGT CCTTACTATC   3708

GGTCGATGTT GCCGAGGAAG AATTAACATG GGTTTGGCAA AACGGAATAG GTCTGCAGCT   3768

CTGGCGATTA TGGGCACACC CACATCATCC TGTATTTGTT CCATACATTG CTTTATAAGG   3828

AATATCCATA AAGTAGATGC AGCATCTCTA GATCTTCCTG GCAATCGATC GCATTCATCT   3888

AGAAGTGTGA CTATAGTTAT CATGGACACA CCCATCTTCA CCTCCACCAA TAATCTTTTT   3948

TATTGTTAAT AACTGGGCCG GTCTGATCTC CAAATCTTAT ACTCTGGTAG AATATGAAAC   4008

AGGGTTAAAA CTAGGTAATA GACTGGATGT CTTCGACTCC GGAGGCAGAA ACG ATG      4064
                                                           Met
                                                            1

GAA TGT GGC ATT TCT TCG TCG AAA GTA CAC GAC TCT AAA ACT AAT ACT      4112
Glu Cys Gly Ile Ser Ser Ser Lys Val His Asp Ser Lys Thr Asn Thr
             5                  10                  15

ACC TAC GGA ATT ATA CAT AAC AGC ATC AAT GGT ACG GAT ACG ACG TTG      4160
Thr Tyr Gly Ile Ile His Asn Ser Ile Asn Gly Thr Asp Thr Thr Leu
         20                  25                  30

TTT GAT ACT TTT CCC GAC AGT ACC GAT AAC GCG GAA GTG ACG GGG GAT      4208
Phe Asp Thr Phe Pro Asp Ser Thr Asp Asn Ala Glu Val Thr Gly Asp
     35                  40                  45

GTG GAC GAT GTG AAG ACT GAG AGC TCT CCC GAG TCC CAA TCT GAA GAT      4256
Val Asp Asp Val Lys Thr Glu Ser Ser Pro Glu Ser Gln Ser Glu Asp
 50                  55                  60                  65

TTG TCA CCT TTT GGG AAC GAT GGA AAT GAA TCC CCC GAA ACG GTG ACG      4304
Leu Ser Pro Phe Gly Asn Asp Gly Asn Glu Ser Pro Glu Thr Val Thr
                 70                  75                  80

GAC ATT GAT GCA GTT TCA GCT GTG CGA ATG CAG TAT AAC ATT GTT TCA      4352
Asp Ile Asp Ala Val Ser Ala Val Arg Met Gln Tyr Asn Ile Val Ser
             85                  90                  95

TCG TTA CCG CCC GGA TCT GAA GGG TAT ATC TAT GTT TGT ACA AAG CGT      4400
Ser Leu Pro Pro Gly Ser Glu Gly Tyr Ile Tyr Val Cys Thr Lys Arg
         100                 105                 110
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GGG GAT AAT ACC AAG AGA AAA GTC ATT GTG AAA GCT GTG ACT GGT GGC      4448
Gly Asp Asn Thr Lys Arg Lys Val Ile Val Lys Ala Val Thr Gly Gly
    115                 120                 125

AAA ACC CTT GGG AGT GAA ATT GAT ATA TTA AAA AAA ATG TCT CAC CGC      4496
Lys Thr Leu Gly Ser Glu Ile Asp Ile Leu Lys Lys Met Ser His Arg
130                 135                 140                 145

TCC ATA ATT AGA TTA GTT CAT GCT TAT AGA TGG AAA TCG ACA GTT TGT      4544
Ser Ile Ile Arg Leu Val His Ala Tyr Arg Trp Lys Ser Thr Val Cys
                150                 155                 160

ATG GTA ATG CCT AAA TAC AAA TGC GAC TTG TTT ACG TAC ATA GAT ATC      4592
Met Val Met Pro Lys Tyr Lys Cys Asp Leu Phe Thr Tyr Ile Asp Ile
            165                 170                 175

ATG GGA CCA TTG CCA CTA AAT CAA ATA ATT ACG ATA GAA CGG GGT TTG      4640
Met Gly Pro Leu Pro Leu Asn Gln Ile Ile Thr Ile Glu Arg Gly Leu
        180                 185                 190

CTT GGA GCA TTG GCA TAT ATC CAC GAA AAG GGT ATA ATA CAT CGT GAT      4688
Leu Gly Ala Leu Ala Tyr Ile His Glu Lys Gly Ile Ile His Arg Asp
    195                 200                 205

GTA AAA ACT GAA AAT ATA TTT TTG GAT AAA CCT GAA AAT GTA GTA TTG      4736
Val Lys Thr Glu Asn Ile Phe Leu Asp Lys Pro Glu Asn Val Val Leu
210                 215                 220                 225

GGG GAC TTT GGG GCA GCA TGT AAA TTA GAT GAA CAT ACA GAT AAA CCC      4784
Gly Asp Phe Gly Ala Ala Cys Lys Leu Asp Glu His Thr Asp Lys Pro
                230                 235                 240

AAA TGT TAT GGA TGG AGT GGA ACT CTG GAA ACC AAT TCG CCT GAA CTG      4832
Lys Cys Tyr Gly Trp Ser Gly Thr Leu Glu Thr Asn Ser Pro Glu Leu
            245                 250                 255

CTT GCA CTT GAT CCA TAC TGT ACA AAA ACT GAT ATA TGG AGT GCA GGA      4880
Leu Ala Leu Asp Pro Tyr Cys Thr Lys Thr Asp Ile Trp Ser Ala Gly
        260                 265                 270

TTA GTT CTG TTT GAG ATG TCA GTA AAA AAT ATA ACC TTT TTT GGC AAA      4928
Leu Val Leu Phe Glu Met Ser Val Lys Asn Ile Thr Phe Phe Gly Lys
    275                 280                 285

CAA GTA AAC GGC TCA GGT TCT CAG CTG AGA TCC ATA ATT AGA TGC CTG      4976
Gln Val Asn Gly Ser Gly Ser Gln Leu Arg Ser Ile Ile Arg Cys Leu
290                 295                 300                 305

CAA GTC CAT CCG TTG GAA TTT CCA CAG AAC AAT TCT ACA AAC TTA TGC      5024
Gln Val His Pro Leu Glu Phe Pro Gln Asn Asn Ser Thr Asn Leu Cys
                310                 315                 320

AAA CAC TTC AAG CAG TAC GCG ATT CAG TTA CGA CAT CCA TAT GCA ATC      5072
Lys His Phe Lys Gln Tyr Ala Ile Gln Leu Arg His Pro Tyr Ala Ile
            325                 330                 335

CCT CAG ATT ATA CGA AAG AGT GGT ATG ACG ATG GAT CTT GAA TAT GCT      5120
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Pro Gln Ile Ile Arg Lys Ser Gly Met Thr Met Asp Leu Glu Tyr Ala
        340                 345                 350

ATT GCA AAA ATG CTC ACA TTC GAT CAG GAG TTT AGA CCA TCT GCC CAA      5168
Ile Ala Lys Met Leu Thr Phe Asp Gln Glu Phe Arg Pro Ser Ala Gln
    355                 360                 365

GAT ATT TTA ATG TTG CCT CTT TTT ACT AAA GAA CCC GCT GAC GCA TTA      5216
Asp Ile Leu Met Leu Pro Leu Phe Thr Lys Glu Pro Ala Asp Ala Leu
370                 375                 380                 385

TAC ACG ATA ACT GCC GCT CAT ATG TAA ACACCCGTCA AAAATAACTT            5263
Tyr Thr Ile Thr Ala Ala His Met End
                390

CAATGATTCA TTTTATAATA TATACTACGC GTTACCTGCA ATAATGACAA CATTCGAAGT    5323

CTTTGAAGAT TCGCAGACCT TTTTTGCGGA ATG GCA CCT TCG GGA CCT ACG CCA      5376
                                Met Ala Pro Ser Gly Pro Thr Pro
                                 1               5

TAT TCC CAC AGA CCG CAA ATA AAG CAT TAT GGA ACA TTT TCG GAT TGC      5424
Tyr Ser His Arg Pro Gln Ile Lys His Tyr Gly Thr Phe Ser Asp Cys
     10                  15                  20

ATG AGA TAT ACT CTA AAC GAT GAG AGT AAG GTA GAT GAT AGA TGT TCA      5472
Met Arg Tyr Thr Leu Asn Asp Glu Ser Lys Val Asp Asp Arg Cys Ser
 25                  30                  35                  40

GAC ATA CAT AAC TCC TTA GCA CAA TCC AAT GTT ACT TCA AGC ATG TCT      5520
Asp Ile His Asn Ser Leu Ala Gln Ser Asn Val Thr Ser Ser Met Ser
                45                  50                  55

GTA ATG AAC GAT TCG GAA GAA TGT CCA TTA ATA AAT GGA CCT TCG ATG      5568
Val Met Asn Asp Ser Glu Glu Cys Pro Leu Ile Asn Gly Pro Ser Met
            60                  65                  70

CAG GCA GAG GAC CCT AAA AGT GTT TTT TAT AAA GTT CGT AAG CCT GAC      5616
Gln Ala Glu Asp Pro Lys Ser Val Phe Tyr Lys Val Arg Lys Pro Asp
        75                  80                  85

CGA AGT CGT GAT TTT TCA TGG CAA AAT CTG AAC TCC CAT GGC AAT AGT      5664
Arg Ser Arg Asp Phe Ser Trp Gln Asn Leu Asn Ser His Gly Asn Ser
     90                  95                  100

GGT CTA CGT CGT GAA AAA TAT ATA CGT TCC TCT AAG AGG CGA TGG AAG      5712
Gly Leu Arg Arg Glu Lys Tyr Ile Arg Ser Ser Lys Arg Arg Trp Lys
105                 110                 115                 120

AAT CCC GAG ATA TTT AAG GTA TCT TTG AAA TGT GAA TCA ATT GGC GCT      5760
Asn Pro Glu Ile Phe Lys Val Ser Leu Lys Cys Glu Ser Ile Gly Ala
                125                 130                 135

GGT AAC GGA ATA AAA ATT TCA TTC TCA TTT TTC TAA CATTATAATA          5806
Gly Asn Gly Ile Lys Ile Ser Phe Ser Phe Phe End
            140                 145
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TATCAGATCG TTTCTTATAT ACTTATTTTC ATCGTCGGGA TATGACTAAC GTATACTAAG   5866

TTACAAGAAA CAACTGCTTA ACGTCGAACA TAACGGAAAT AAAAATATAT ATAGCGTCTC   5926

CTATAACTGT TATATTGGCA CCTTTTAGAG CTTCGGT ATG AAT AGA TAC AGA TAT    5981
                                          Met Asn Arg Tyr Arg Tyr
                                          -30                -25

GAA AGT ATT TTT TTT AGA TAT ATC TCA TCC ACG AGA ATG ATT CTT ATA      6029
Glu Ser Ile Phe Phe Arg Tyr Ile Ser Ser Thr Arg Met Ile Leu Ile
               -20                 -15                 -10

ATC TGT TTA CTT TTG GGA ACT GGG GAC ATG TCC GCA ATG GGA CTT AAG      6077
Ile Cys Leu Leu Leu Gly Thr Gly Asp Met Ser Ala Met Gly Leu Lys
            -5                  1               5

AAA GAC AAT TCT CCG ATC ATT CCC ACA TTA CAT CCG AAA GGT AAT GAA      6125
Lys Asp Asn Ser Pro Ile Ile Pro Thr Leu His Pro Lys Gly Asn Glu
     10                  15                  20

AAC CTC CGG GCT ACT CTC AAT GAA TAC AAA ATC CCG TCT CCA CTG TTT      6173
Asn Leu Arg Ala Thr Leu Asn Glu Tyr Lys Ile Pro Ser Pro Leu Phe
 25                  30                  35                  40

GAT ACA CTT GAC AAT TCA TAT GAG ACA AAA CAC GTA ATA TAT ACG GAT      6221
Asp Thr Leu Asp Asn Ser Tyr Glu Thr Lys His Val Ile Tyr Thr Asp
                 45                  50                  55

AAT TGT AGT TTT GCT GTT TTG AAT CCA TTT GGC GAT CCG AAA TAT ACG      6269
Asn Cys Ser Phe Ala Val Leu Asn Pro Phe Gly Asp Pro Lys Tyr Thr
             60                  65                  70

CTT CTC AGT TTA CTG TTG ATG GGA CGA CGC AAA TAT GAT GCT CTA GTA      6317
Leu Leu Ser Leu Leu Leu Met Gly Arg Arg Lys Tyr Asp Ala Leu Val
         75                  80                  85

GCA TGG TTT GTC TTG GGC AGA GCA TGT GGG AGA CCA ATT TAT TTA CGT      6365
Ala Trp Phe Val Leu Gly Arg Ala Cys Gly Arg Pro Ile Tyr Leu Arg
     90                  95                  100

GAA TAT GCC AAC TGC TCT ACT AAT GAA CCA TTT GGA ACT TGT AAA TTA      6413
Glu Tyr Ala Asn Cys Ser Thr Asn Glu Pro Phe Gly Thr Cys Lys Leu
105                 110                 115                 120

AAG TCC CTA GGA TGG TGG GAT AGA AGA TAT GCA ATG ACG AGT TAT ATC      6461
Lys Ser Leu Gly Trp Trp Asp Arg Arg Tyr Ala Met Thr Ser Tyr Ile
                125                 130                 135

GAT CGA GAT GAA TTG AAA TTG ATT ATT GCA GCA CCC AGT CGT GAG CTA      6509
Asp Arg Asp Glu Leu Lys Leu Ile Ile Ala Ala Pro Ser Arg Glu Leu
            140                 145                 150

AGT GGA TTA TAT ACG CGT TTA ATA ATT ATT AAT GGA GAA CCC ATT TCG      6557
Ser Gly Leu Tyr Thr Arg Leu Ile Ile Ile Asn Gly Glu Pro Ile Ser
        155                 160                 165

AGT GAC ATA TTA CTG ACT GTT AAA GGA ACA TGT AGT TTT TCG AGA CGG      6605
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Ser Asp Ile Leu Leu Thr Val Lys Gly Thr Cys Ser Phe Ser Arg Arg
    170                 175                 180

GGG ATA AAG GAT AAC AAA CTA TGC AAA CCG TTC AGT TTT TTT GTC AAT     6653
Gly Ile Lys Asp Asn Lys Leu Cys Lys Pro Phe Ser Phe Phe Val Asn
185                 190                 195                 200

GGT ACA ACA CGG CTG TTA GAC ATG GTG CGA ACA GGA ACC CCG AGA GCC     6701
Gly Thr Thr Arg Leu Leu Asp Met Val Arg Thr Gly Thr Pro Arg Ala
                205                 210                 215

CAT GAA GAA AAT GTG AAG CAG TGG CTT GAA CGA AAT GGT GGT AAA CAT     6749
His Glu Glu Asn Val Lys Gln Trp Leu Glu Arg Asn Gly Gly Lys His
            220                 225                 230

CTA CCA ATC GTC GTC GAA ACA TCT ATG CAA CAA GTC TCA AAT TTG CCG     6797
Leu Pro Ile Val Val Glu Thr Ser Met Gln Gln Val Ser Asn Leu Pro
        235                 240                 245

AGA AGT TTT AGA GAT TCA TAT TTA AAA TCA CCT GAC GAC GAT AAA TAT     6845
Arg Ser Phe Arg Asp Ser Tyr Leu Lys Ser Pro Asp Asp Asp Lys Tyr
    250                 255                 260

AAT GAC GTC AAA ATG ACA TCG GCC ACT ACT AAT AAC ATT ACC ACC TCC     6893
Asn Asp Val Lys Met Thr Ser Ala Thr Thr Asn Asn Ile Thr Thr Ser
265                 270                 275                 280

GTG GAT GGT TAC ACT GGA CTC ACT AAT CGG CCC GAG GAC TTT GAG AAA     6941
Val Asp Gly Tyr Thr Gly Leu Thr Asn Arg Pro Glu Asp Phe Glu Lys
                285                 290                 295

GCA CCA TAC ATA ACT AAA CGA CCG ATA ATC TCT GTC GAG GAG GCA TCC     6989
Ala Pro Tyr Ile Thr Lys Arg Pro Ile Ile Ser Val Glu Glu Ala Ser
            300                 305                 310

AGT CAA TCA CCT AAA ATA TCA ACA GAA AAA AAA TCC CGA ACG CAA ATA     7037
Ser Gln Ser Pro Lys Ile Ser Thr Glu Lys Lys Ser Arg Thr Gln Ile
        315                 320                 325

ATA ATT TCA CTA GTT GTT CTA TGC GTC ATG TTT TGT TTC ATT GTA ATC     7085
Ile Ile Ser Leu Val Val Leu Cys Val Met Phe Cys Phe Ile Val Ile
    330                 335                 340

GGG TCT GGT ATA TGG ATC CTT CGC AAA CAC CGC AAA ACG GTG ATG TAT     7133
Gly Ser Gly Ile Trp Ile Leu Arg Lys His Arg Lys Thr Val Met Tyr
345                 350                 355                 360

GAT AGA CGT CGT CCA TCA AGA CGG GCA TAT TCC CGC CTA TAA             7175
Asp Arg Arg Arg Pro Ser Arg Arg Ala Tyr Ser Arg Leu End
                365                 370

CACGTGTTTG GTATGGGCGT GTCGCTATAG TGCATAAGAA GTTGACTACA TTGATCAATG   7235

ACATTATATA GCTTCTTTGG TCAGATAGAC GGCGTGTGTG ATTGCG ATG TAT GTA      7290
                                                   Met Tyr Val

CTA CAA TTA TTA TTT TGG ATC CGC CTC TTT CGA GGC ATC TGG TCT ATA     7338
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Leu Gln Leu Leu Phe Trp Ile Arg Leu Phe Arg Gly Ile Trp Ser Ile
-15                 -10                 -5                  1

GTT TAT ACT GGA ACA TCT GTT ACG TTA TCA ACG GAC CAA TCT GCT CTT      7386
Val Tyr Thr Gly Thr Ser Val Thr Leu Ser Thr Asp Gln Ser Ala Leu
            5                  10                  15

GTT GCG TTC CGC GGA TTA GAT AAA ATG GTG AAT GTA CGC GGC CAA CTT      7434
Val Ala Phe Arg Gly Leu Asp Lys Met Val Asn Val Arg Gly Gln Leu
        20                  25                  30

TTA TTC CTG GGC GAC CAG ACT CGG ACC AGT TCT TAT ACA GGA ACG ACG      7482
Leu Phe Leu Gly Asp Gln Thr Arg Thr Ser Ser Tyr Thr Gly Thr Thr
    35                  40                  45

GAA ATC TTG AAA TGG GAT GAA GAA TAT AAA TGC TAT TCC GTT CTA CAT      7530
Glu Ile Leu Lys Trp Asp Glu Glu Tyr Lys Cys Tyr Ser Val Leu His
50                  55                  60                  65

GCG ACA TCA TAT ATG GAT TGT CCT GCT ATA GAC GCC ACG GTA TTC AGA      7578
Ala Thr Ser Tyr Met Asp Cys Pro Ala Ile Asp Ala Thr Val Phe Arg
                70                  75                  80

GGC TGT AGA GAC GCT GTG GTA TAT GCT CAA CCT CAT GGT AGA GTA CAA      7626
Gly Cys Arg Asp Ala Val Val Tyr Ala Gln Pro His Gly Arg Val Gln
            85                  90                  95

CCT TTT CCC GAA AAG GGA ACA TTG TTG AGA ATT GTC GAA CCC AGA GTA      7674
Pro Phe Pro Glu Lys Gly Thr Leu Leu Arg Ile Val Glu Pro Arg Val
        100                 105                 110

TCA GAT ACA GGC AGC TAT TAC ATA CGT GTA TCT CTC GCT GGA AGA AAT      7722
Ser Asp Thr Gly Ser Tyr Tyr Ile Arg Val Ser Leu Ala Gly Arg Asn
    115                 120                 125

ATG AGC GAT ATA TTT AGA ATG GTT GTT ATT ATA AGG AGT AGC AAA TCT      7770
Met Ser Asp Ile Phe Arg Met Val Val Ile Ile Arg Ser Ser Lys Ser
130                 135                 140                 145

TGG GCC TGT AAT CAC TCT GCT AGT TCA TTT CAG GCC CAT AAA TGT ATT      7818
Trp Ala Cys Asn His Ser Ala Ser Ser Phe Gln Ala His Lys Cys Ile
                150                 155                 160

CGC TAT GTC GAC CGT ATG GCC TTT GAA AAT TAT CTG ATT GGA CAT GTA      7866
Arg Tyr Val Asp Arg Met Ala Phe Glu Asn Tyr Leu Ile Gly His Val
            165                 170                 175

GGC AAT TTG CTG GAC AGT GAC TCG GAA TTG CAT GCA ATT TAT AAT ATT      7914
Gly Asn Leu Leu Asp Ser Asp Ser Glu Leu His Ala Ile Tyr Asn Ile
        180                 185                 190

ACT CCC CAA TCC ATT TCC ACA GAT ATT AAT ATT GTA ACG ACT CCA TTT      7962
Thr Pro Gln Ser Ile Ser Thr Asp Ile Asn Ile Val Thr Thr Pro Phe
    195                 200                 205

TAC GAT AAT TCG GGA ACA ATT TAT TCA CCT ACG GTT TTT AAT TTG TTT      8010
Tyr Asp Asn Ser Gly Thr Ile Tyr Ser Pro Thr Val Phe Asn Leu Phe
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210                 215                 220                 225

AAT AAC AAT TCC CAT GTC GAT GCA ATG AAT TCG ACT GGT ATG TGG AAT     8058
Asn Asn Asn Ser His Val Asp Ala Met Asn Ser Thr Gly Met Trp Asn
                230                 235                 240

ACC GTT TTA AAA TAT ACC CTT CCA AGG CTT ATT TAC TTT TCT ACG ATG     8106
Thr Val Leu Lys Tyr Thr Leu Pro Arg Leu Ile Tyr Phe Ser Thr Met
            245                 250                 255

ATT GTA CTA TGT ATA ATA GCA TTG GCA ATT TAT TTG GTC TGT GAA AGG     8154
Ile Val Leu Cys Ile Ile Ala Leu Ala Ile Tyr Leu Val Cys Glu Arg
        260                 265                 270

TGC CGC TCT CCC CAT CGT AGG ATA TAC ATC GGT GAA CCA AGA TCT GAT     8202
Cys Arg Ser Pro His Arg Arg Ile Tyr Ile Gly Glu Pro Arg Ser Asp
    275                 280                 285

GAG GCC CCA CTC ATC ACT TCT GCA GTT AAC GAA TCA TTT CAA TAT GAT     8250
Glu Ala Pro Leu Ile Thr Ser Ala Val Asn Glu Ser Phe Gln Tyr Asp
290                 295                 300                 305

TAT AAT GTA AAG GAA ACT CCT TCA GAT GTT ATT GAA AAG GAG TTG ATG     8298
Tyr Asn Val Lys Glu Thr Pro Ser Asp Val Ile Glu Lys Glu Leu Met
                310                 315                 320

GAA AAA CTG AAG AAG AAA GTC GAA TTG TTG GAA AGA GAA GAA TGT GTA     8346
Glu Lys Leu Lys Lys Lys Val Glu Leu Leu Glu Arg Glu Glu Cys Val
            325                 330                 335

TAG GTTTGAGAAA CTATTATAGG TAGGTGGTAC CTGTTAGCTT AGTATAAGGG         8399
End

GAGCAGCCGT TTCTTGTTTT AAAGACACGA ACACAAGGCC GTAAGTTTTA TATGTGAATT   8459

TTGTGCATGT CTGCGAGTCA GCGTCATA ATG TGT GTT TTC CAA ATC CTG ATA      8511
                               Met Cys Val Phe Gln Ile Leu Ile
                                           -15

ATA GTG ACG ACG ATC AAA GTA GCT GGA ACG GCC AAC ATA AAT CAT ATA     8559
Ile Val Thr Thr Ile Lys Val Ala Gly Thr Ala Asn Ile Asn His Ile
-10                 -5                  1                   5

GAC GTT CCT GCA GGA CAT TCT GCT ACA ACG ACG ATC CCG CGA TAT CCA     8607
Asp Val Pro Ala Gly His Ser Ala Thr Thr Thr Ile Pro Arg Tyr Pro
            10                  15                  20

CCA GTT GTC GAT GGG ACC CTT TAC ACC GAG ACG TGG ACA TGG ATT CCC     8655
Pro Val Val Asp Gly Thr Leu Tyr Thr Glu Thr Trp Thr Trp Ile Pro
        25                  30                  35

AAT CAC TGC AAC GAA ACG GCA ACA GGC TAT GTA TGT CTG GAA AGT GCT     8703
Asn His Cys Asn Glu Thr Ala Thr Gly Tyr Val Cys Leu Glu Ser Ala
    40                  45                  50

CAC TGT TTT ACC GAT TTG ATA TTA GGA GTA TCC TGC ATG AGG TAT GCG     8751
His Cys Phe Thr Asp Leu Ile Leu Gly Val Ser Cys Met Arg Tyr Ala
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55                  60                  65                  70

GAT GAA ATC GTC TTA CGA ACT GAT AAA TTT ATT GTC GAT GCG GGA TCC     8799
Asp Glu Ile Val Leu Arg Thr Asp Lys Phe Ile Val Asp Ala Gly Ser
                75                  80                  85

ATT AAA CAA ATA GAA TCG CTA AGT CTG AAT GGA GTT CCG AAT ATA TTC     8847
Ile Lys Gln Ile Glu Ser Leu Ser Leu Asn Gly Val Pro Asn Ile Phe
            90                  95                  100

CTA TCT ACG AAA GCA AGT AAC AAG TTG GAG ATA CTA AAT GCT AGC CTA     8895
Leu Ser Thr Lys Ala Ser Asn Lys Leu Glu Ile Leu Asn Ala Ser Leu
        105                 110                 115

CAA AAT GCG GGT ATC TAC ATT CGG TAT TCT AGA AAT GGG ACG AGG ACT     8943
Gln Asn Ala Gly Ile Tyr Ile Arg Tyr Ser Arg Asn Gly Thr Arg Thr
    120                 125                 130

GCA AAG CTG GAT GTT GTT GTG GTT GGC GTT TTG GGT CAA GCA AGG GAT     8991
Ala Lys Leu Asp Val Val Val Val Gly Val Leu Gly Gln Ala Arg Asp
135                 140                 145                 150

CGC CTA CCC CAA ATG TCC AGT CCT ATG ATC TCA TCC CAC GCC GAT ATC     9039
Arg Leu Pro Gln Met Ser Ser Pro Met Ile Ser Ser His Ala Asp Ile
                155                 160                 165

AAG TTG TCA TTA AAA AAC TTT AAA GCA TTA GTA TAT CAC GTG GGA GAT     9087
Lys Leu Ser Leu Lys Asn Phe Lys Ala Leu Val Tyr His Val Gly Asp
            170                 175                 180

ACT ATC AAT GTC TCG ACG GCG GTT ATA CTA GGA CCT TCT CCG GAG ATA     9135
Thr Ile Asn Val Ser Thr Ala Val Ile Leu Gly Pro Ser Pro Glu Ile
        185                 190                 195

TTC ACA TTG GAA TTT AGG GTG TTG TTC CTC CGT TAT AAT CCA ACG TGC     9183
Phe Thr Leu Glu Phe Arg Val Leu Phe Leu Arg Tyr Asn Pro Thr Cys
    200                 205                 210

AAG TTC GTC ACG ATT TAT GAA CCT TGT ATA TTT CAC CCC AAA GAA CCA     9231
Lys Phe Val Thr Ile Tyr Glu Pro Cys Ile Phe His Pro Lys Glu Pro
215                 220                 225                 230

GAG TGT ATT ACT ACT GCA GAA CAA TGG GTA TGT CAT TTC GCA TCC AAC     9279
Glu Cys Ile Thr Thr Ala Glu Gln Ser Val Cys His Phe Ala Ser Asn
                235                 240                 245

ATT GAC ATT CTG CAG ATA GCC GCC GCA CGT TCT GAA AAT TGT AGC ACA     9327
Ile Asp Ile Leu Gln Ile Ala Ala Ala Arg Ser Glu Asn Cys Ser Thr
            250                 255                 260

GGG TAT CGT AGA TGT ATT TAT GAC ACG GCT ATC GAT GAA TCT GTG CAG     9375
Gly Tyr Arg Arg Cys Ile Tyr Asp Thr Ala Ile Asp Glu Ser Val Gln
        265                 270                 275

GCC AGA TTA ACA TTC ATA GAA CCA GGA ATT CCT TCC TTT AAA ATG AAA     9423
Ala Arg Leu Thr Phe Ile Glu Pro Gly Ile Pro Ser Phe Lys Met Lys
    280                 285                 290
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GAT GTC CAG GTA GAC GAT GCT GGA TTG TAT GTG GTT GTG GCT TTA TAC      9471
Asp Val Gln Val Asp Asp Ala Gly Leu Tyr Val Val Val Ala Leu Tyr
295                 300                 305                 310

AAT GGA CGT CCA AGT GCA TGG ACT TAC ATT TAT TTG TCA ACG GTG GAA      9519
Asn Gly Arg Pro Ser Ala Trp Thr Tyr Ile Tyr Leu Ser Thr Val Glu
                315                 320                 325

ACA TAT CTT AAT GTA TAT GAA AAC TAC CAC AAG CCG GGA TTT GGG TAT      9567
Thr Tyr Leu Asn Val Tyr Glu Asn Tyr His Lys Pro Gly Phe Gly Tyr
            330                 335                 340


AAA TCA TTT CTA CAG AAC AGT AGT ATC GTC GAC GAA AAT GAG GCT AGC      9615
Lys Ser Phe Leu Gln Asn Ser Ser Ile Val Asp Glu Asn Glu Ala Ser
        345                 350                 355

GAT TGG TCC AGC TCG TCC ATT AAA CGG AGA AAT AAT GGT ACT ATC ATT      9663
Asp Trp Ser Ser Ser Ser Ile Lys Arg Arg Asn Asn Gly Thr Ile Ile
    360                 365                 370

TAT GAT ATT TTA CTC ACA TCG CTA TCA ATT GGG GCG ATT ATT ATC GTC      9711
Tyr Asp Ile Leu Leu Thr Ser Leu Ser Ile Gly Ala Ile Ile Ile Val
375                 380                 385                 390

ATA GTA GGG GGT GTT TGT ATT GCC ATA TTA ATT AGG CGT AGG AGA CGA      9759
Ile Val Gly Gly Val Cys Ile Ala Ile Leu Ile Arg Arg Arg Arg Arg
                395                 410                 415

CGT CGC ACG AGG GGG TTA TTC GAT GAA TAT CCC AAA TAT ATG ACG CTA      9807
Arg Arg Thr Arg Gly Leu Phe Asp Glu Tyr Pro Lys Tyr Met Thr Leu
            420                 425                 430

CCA GGA AAC GAT CTG GGG GGC ATG AAT GTA CCG TAT GAT AAT ACA TGC      9855
Pro Gly Asn Asp Leu Gly Gly Met Asn Val Pro Tyr Asp Asn Thr Cys
        435                 440                 445

TCT GGT AAC CAA GTT GAA TAT TAT CAA GAA AAG TCG GCT AAA ATG AAA      9903
Ser Gly Asn Gln Val Glu Tyr Tyr Gln Glu Lys Ser Ala Lys Met Lys
    450                 455                 460

AGA ATG GGT TCG GGT TAT ACC GCT TGG CTA AAA AAT GAT ATG CCG AAA      9951
Arg Met Gly Ser Gly Tyr Thr Ala Trp Leu Lys Asn Asp Met Pro Lys
465                 470                 475                 480

ATT AGG AAA CGC TTA GAT TTA TAC CAC TGA TATGTACATA TTTAAACTTA       10001
Ile Arg Lys Arg Leu Asp Leu Tyr His End
                485

ATGGGATATA GTATATGGAC GTCTATATGA CGAGAGTAAA TAAACTGACA ATGCAAATGA   10061

AGCTGATCTA TATTGTGCTT TATATTGGGA CAAACCACTC GCACAAGCTC ATTCAACACA   10121

TCCACTCTTG CTATTAAATT CCCCATTATA TAACAATACT GACATAACAC TCATATTAAG   10181

GGGAGAAAAT AAATATGCAT GGCCGATCAT ATTTTATTGA GATCCGAAAA TATATCATGC   10241
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AAATAAGCAT GTTCTAGCAC CACTGCAACA TGTGGTTTAT CGATTTCCGG AAAGAATAGT 10301

TGAACCATTG CCTCCGAGCA GTTGGCGATC CGTTGACCTG CAGGTCGAC 10350

(3) Information for SEQ ID NO: 2

(i) Sequence Characteristics:

(A) Length: 10,350 base pairs

(B) Type: nucleic acid

(C) Strandedness: double

(D) Topology: linear

(ii) Molecule Type:

(A) Description: genomic DNA

(iii) HYPOTHETICAL: Yes

(iv) ANTI-SENSE: Yes

(vi) ORIGINAL SOURCE:

(A) Organism: MDV, GA strain

(vii) IMMEDIATE SOURCE:

(A) Library: genomic

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

GTCGACCTGC AGGTCAACGG ATCGCCAACT GCTCGGAGGC AATGGTTCAA CTATTCTTTC 60

CGGAAATCGA TAAACCACAT GTTGCAGTGG TGCTAGAACA TGCTTATTTG CATGATATAT 120

TTTCGGATCT CAATAAAATA TGATCGGCCA TGCATATTTA TTTTCTCCCC TTAATATGAG 180

TGTTATGTCA GTATTGTTAT ATAATGGGGA ATTTAATAGC AAGAGTGGAT GTGTTGAATG 240

AGCTTGTGCG ACTGGTTTGT CCCAATATAA AGCACAATAT AGATCAGCTT CATTTGCATT 300

GTCAGTTTAT TTACTCTCGT CATATAGACG TCCATATACT ATATCCCATT AAGTTTAAAT 360

ATGTACATAT CAGTGGTATA AATCTAAGCG TTTCCTAATT TTCGGCATAT CATTTTTTAG 420

CCAAGCGGTA TAACCCGAAC CCATTCTTTT CATTTTAGCC GACTTTTCTT GATAATATTC 480

AACTTGGTTA CCAGAGCATG TATTATCATA CGGTACATTC ATGCCCCCCA GATCGTTTCC 540

TGGTAGCGTC ATATATTTGG GATATTCATC GAATAACCCC CTCGTGCGAC GTCGTCTCCT 600

ACGCCTAATT AATATGGCAA TACAAACACC CCCTACTATG ACGATAATAA TCGCCCCAAT 660

TGATAGCGAT GTGAGTAAAA TATCATAAAT GATAGTACCA TTATTTCTCC GTTTAATGGA 720

CGAGCTGGAC CAATCGCTAG CCTCATTTTC GTCGACGATA CTACTGTTCT GTAGAAATGA 780

TTTATACCCA AATCCCGGCT TGTGGTAGTT TTCATATACA TTAAGATATG TTTCCACCGT 840

TGACAAATAA ATGTAAGTCC ATGCACTTCG ACGTCCATTG TATAAAGCCA CAACCACATA 900

CAATCCAGCA TCGTCTACCT GGACATCTTT CATTTTAAAG GAAGGAATTC CTGGTTCTAT 960

GAATGTTAAT CTGGCCTGCA CAGATTCATC GATAGCCGTG TCATAAATAC ATCTACGATA 1020

CCCTGTGCTA CAATTTTCAG AACGTGCGGC GGCTATCTGC AGAATGTCAA TGTTGGATGC 1080

GAAATGACAT ACCGATTGTC CTGCAGTAGT AATACACTCT GGTTCTTTGG GGTGAAATAT 1140

ACAAGGTTCA TAAATCGTGA CGAACTTGCA CGTTGGATTA TAACGGAGGA ACAACACCCT 1200

AAATTCCAAT GTGAATATCT CCGGAGAAGG TCCTAGTATA ACCGCCGTCG AGACATTGAT 1260

AGTATCTCCC ACGTGATATA CTAATGCTTT AAAGTTTTTT AATGACAACT TGATATCGGC 1320

GTGGGATGAG ATCATAGGAC TGGACATTTG GGGTAGGCGA TCCCTTGCTT GACCCAAAAC 1380

GCCAACCACA ACAACATCCA GCTTTGCAGT CCTCGTCCCA TTTCTAGAAT ACCGAATGTA 1440

GATACCCGCA TTTTGTAGGC TAGCATTTAG TATCTCCAAC TTGTTACTTG CTTTCGTAGA 1500

TAGGAATATA TTCGGAACTC CATTCAGACT TAGCGATTCT ATTTGTTTAA TGGATCCCGC 1560

ATCGACAATA AATTTATCAG TTCGTAAGAC GATTTCATCC GCATACCTCA TGCAGGATAC 1620

TCCTAATATC AAATCGGTAA AACAGTGAGC ACTTTCCAGA CATACATAGC CTGTTGCCGT 1680

TTCGTTGCAG TGATTGGGAA TCCATGTCCA CGTCTCGGTG TAAAGGGTCC CATCGACAAC 1740

TGGTGGATAT CGCGGGATCG TCGTTGTAGC AGAATGTCCT GCAGGAACGT CTATATGATT 1800

TATGTTGGCC GTTCCAGCTA CTTTGATCGT CGTCACTATT ATCAGGATTT GGAAAACACA 1860

CATTATGACG CTGACTCGCA GACATGCACA AAATTCACAT ATAAAACTTA CGGCCTTGTG 1920

TTCGTGTCTT TAAAACAAGA AACGGCTCCT CCCCTTATAC TAAGCTAACA GGTACCACCT 1980

ACCTATAATA GTTTCTCAAA CCTATACACA TTCTTCTCTT TCCAACAATT CGACTTTCTT 2040

CTTCAGTTTT TCCATCAACT CCTTTTCAAT AACATCTGAA GGAGTTTCCT TTACATTATA 2100

ATCATATTGA AATGATTCGT TAACTGCAGA AGTGATGAGT GGGGCCTCAT CAGATCTTGG 2160

TTCACCGATG TATATCCTAC GATGGGGAGA GCGGCACCTT TCACAGACCA AATAAATTGC 2220

CAATGCTATT ATACATAGTA CAATCATCGT AGAAAAGTAA ATAAGCCTTG GAAGGGTATA 2280

TTTTAAAACG GTATTCCACA TACCAGTCGA ATTCATTGCA TCGACATGGG AATTGTTATT 2340

AAACAAATTA AAAACCGTAG GTGAATAAAT TGTTCCCGAA TTATCGTAAA ATGGAGTCGT 2400

TACAATATTA ATATCTGTGG AAATGGATTC GGGAGTAATA TTATAAATTG CATGCAATTC 2460

CGAGTCACTG TCCAGCAAAT TGCCTACATG TCCAATCAGA TAATTTTCAA AGGCCATACG 2520

GTCGACATAG CGAATACATT TATGGGCCTG AAATGAACTA GCAGAGTGAT TACAGGCCCA 2580

AGATTTGCTA CTCCTTATAA TAACAACCAT TCTAAATATA TCGCTCATAT TTCTTCCAGC 2640

GAGAGATACA CGTATGTAAT AGCTGCCTGT ATCTGATACT CTGGGTTCGA CAATTCTCAA 2700

CAATGTTCCC TTTTCGGGAA AAGGTTGTAC TCTACCATGA GGTTGAGCAT ATACCACAGC 2760

GTCTCTACAG CCTCTGAATA CCGTGGCGTC TATAGCAGGA CAATCCATAT ATGATGTCGC 2820

ATGTAGAACG GAATAGCATT TATATTCTTC ATCCCATTTC AAGATTTCCG TCGTTCCTGT 2880

ATAAGAACTG GTCCGAGTCT GGTCGCCCAG GAATAAAAGT TGGCCGCGTA CATTCACCAT 2940

TTTATCTAAT CCGCGGAACG CAACAAGAGC AGATTGGTCC GTTCATAACG TAACAGATGT 3000

TCCAGTATAA ACTATAGACC AGATGCCTCG AAAGAGGCGG ATCCAAAATA ATAATTGTAG 3060

TACATACATC GCAATCACAC ACGCCGTCTA TCTGACCAAA GAAGCTATAT AATGTCATTG 3120

ATCAATGTAG TCAAGTTCTT ATGCACTATA GCGACACGCC CATACCAAAC ACGTGTTATA 3180

GGCGGGAATA TGCCCGTCTT GATGGACGAC GTCTATCATA CATCACCGTT TTGCGGTGTT 3240

TGCGAAGGAT CCATATACCA GACCCGATTA CAATGAAACA AAACATGACG CATAGAACAA 3300

CTAGTGAAAT TATTATTTGC GTTCGGGATT TTTTTTCTGT TGATATTTTA GGTGATTGAC 3360

TGGATGCGTC CTCGACAGAG ATTATCGGTC GTTTAGTTAT GTATGGTGCT TTCTCAAAGT 3420

CCTCGGGCCG ATTAGTGAGT CCAGTGTAAC CATCCACGGA GGTGGTAATG TTATTAGTAG 3480

TGGCCGATGT CATTTTGACG TCATTATATT TATCGTCGTC AGGTGATTTT AAATATGAAT 3540

CTCTAAAACT TCTCGGCAAA TTTGAGACTT GTTGCATAGA TGTTTCGACG ACGATTGGTA 3600

GATGTTTACC ACCATTTCGT TCAAGCCACT CTTCACATT TTCTTCATGG GCTCTCGCGG 3660

TTCCTGTTCG CACCATGTCT AACAGCCGTG TTGTACCATT GACAAAAAAA CTGAACGGTT 3720

TGCATAGTTT GTTATCCTTT ATCCCCCGTC TCGAAAAACT ACATGTTCCT TTAACAGTCA 3780

GTAATATGTC ACTCGAAATG GGTTCTCCAT TAATAATTAT TAAACGCGTA TATAATCCAC 3840

TTAGCTCACG ACTGGGTGCT GCAATAATCA ATTTCAATTC ATCTCGATCG ATATAACTCG 3900

TCATTGCATA TCTTCTATCC CACCATCCTA GGGACTTTAA TTTACAAGTT CCAAATGGTT 3960

CATTAGTAGA GCAGTTGGCA TATTCACGTA AATAAATTGG TCTCCCACAT GCTCTGCCCA 4020

AGACAAACCA TGCTACTAGA GCATCATATT TGCGTCGTCC CATCAACAGT AAACTGAGAA 4080

GCGTATATTT CGGATCGCCA AATGGATTCA AAACAGCAAA ACTACAATTA TCCGTATATA 4140

TTACGTGTTT TGTCTCATAT GAATTGTCAA GTGTATCAAA CAGTGGAGAC GGGATTTTGT 4200

ATTCATTGAG AGTAGCCCGG AGGTTTTCAT TACCTTTCGG ATGTAATGTG GGAATGATCG 4260

GAGAATTGTC TTTCTTAAGT CCCATTGCGG ACATGTCCCC AGTTCCCAAA AGTAAACAGA 4320

TTATAAGAAT CATTCTCGTG GATGAGATAT ATCTAAAAAA AATACTTTCA TATCTGTATC 4380

TATTCATACC GAAGCTCTAA AAGGTGCCAA TATAACAGTT ATAGGAGACG CTATATATAT 4440

TTTTATTTCC GTTATGTTCG ACGTTAAGCA GTTGTTTCTT GTAACTTAGT ATACGTTAGT 4500

CATATCCCGA CGATGAAAAT AAGTATATAA GAAACGATCT GATATATTAT AATGTTAGAA 4560

AAATGAGAAT GAAATTTTTA TTCCGTTACC AGCGCCAATT GATTCACATT TCAAAGATAC 4620

CTTAAATATC TCGGGATTCT TCCATCGCCT CTTAGAGGAA CGTATATATT TTTCACGACG 4680

TAGACCACTA TTGCCATCGG AGTTCAGATT TTGCCATGAA AAATCACGAC TTCGGTCAGG 4740

CTTACGAACT TTATAAAAAA CACTTTTAGG GTCCTCTGCC TGCATCGAAG GTCCATTTAT 4800

TAATGGACAT TCTTCCGAAT CGTTCATTAC AGACATGCTT GAAGTAACAT TGGATTGTGC 4860

TAAGGAGTTA TGTATGTCTG AACATCTATC ATCTACCTTA CTCTCATCGT TTAGAGTATA 4920

TCTCATGCAA TCCGAAAATG TTCCATAATG CTTTATTTGC GGTCTGTGGG AATATGGCGT 4980

AGGTCCCGAA GGTGCCATTC GCAAAAAAGG TCTGCGAATC TTCAAAGACT TCGAATGTTG 5040

TCATTATTGC AGGTAACGCG TAGTATATAT TATAAAATGA ATCATTGAAG TTATTTTTGA 5100

CGGGTGTTTA CATATGAGCG GCAGTTATCG TGTATAATGC GTCAGCGGGT TCTTTAGTAA 5160

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AAAGAGGCAA CATTAAAATA TCTTGGGCAG ATGGTCTAAA CTCCTGATCG AATGTGAGCA   5220

TTTTTGCAAT AGCATATTCA AGATCCATCG TCATACCACT CTTTCGTATA ATCTGAGGGA   5280

TTGCATATGG ATGTCGTAAC TGAATCGCGT ACTGCTTGAA GTGTTTGCAT AAGTTTGTAG   5340

AATTGTTCTG TGGAAATTCC AACGGATGGA CTTGCAGGCA TCTAATTATG GATCTCAGCT   5400

GAGAACCTGA GCCGTTTACT TGTTTGCCAA AAAAGGTTAT ATTTTTTACT GACATCTCAA   5460

ACAGAACTAA TCCTGCACTC CATATATCAG TTTTTGTACA GTATGGATCA AGTGCAAGCA   5520

GTTCAGGCGA ATTGGTTTCC AGAGTTCCAC TCCATCCATA ACATTTGGGT TTATCTGTAT   5580

GTTCATCTAA TTTACATGCT GCCCCAAAGT CCCCCAATAC TACATTTTCA GGTTTATCCA   5640

AAAATATATT TTCAGTTTTT ACATCACGAT GTATTATACC CTTTTCGTGG ATATATGCCA   5700

ATGCTCCAAG CAAACCCCGT TCTATCGTAA TTATTTGATT TAGTGGCAAT GGTCCCATGA   5760

TATCTATGTA CGTAAACAAG TCGCATTTGT ATTTAGGCAT TACCATACAA ACTGTCGATT   5820

TCCATCTATA AGCATGAACT AATCTAATTA TGGAGCGGTG AGACATTTTT TTTAATATAT   5880

CAATTTCACT CCCAAGGGTT TTGCCACCAG TCACAGCTTT CACAATGACT TTTCTCTTGG   5940

TATTATCCCC ACGCTTTGTA CAAACATAGA TATACCCTTC AGATCCGGGC GGTAACGATG   6000

AAACAATGTT ATACTGCATT CGCACAGCTG AAACTGCATC AATGTCCGTC ACCGTTTCGG   6060

GGGATTCATT TCCATCGTTC CCAAAAGGTG ACAAATCTTC AGATTGGGAC TCGGGAGAGC   6120

TCTCAGTCTT CACATCGTCC ACATCCCCCG TCACTTCCGC GTTATCGGTA CTGTCGGGAA   6180

AAGTATCAAA CAACGTCGTA TCCGTACCAT TGATGCTGTT ATGTATAATT CCGTAGGTAG   6240

TATTAGTTTT AGAGTCGTGT ACTTTCGACG AAGAAATGCC ACATTCCATC GTTTCTGCCT   6300

CCGGAGTCGA AGACATCCAG TCTATTACCT AGTTTTAACC CTGTTTCATA TTCTACCAGA   6360

GTATAAGATT TGGAGATCAG ACCGGCCCAG TTATTAACAA TAAAAAAGAT TATTGGTGGA   6420

GGTGAAG  ATG GGT GTG TCC ATG ATA ACT ATA GTC ACA CTT CTA GAT GAA     6469
        Met Gly Val Ser Met Ile Thr Ile Val Thr Leu Leu Asp Glu
         1               5                  10

TGC GAT CGA TTG CCA GGA AGA TCT AGA GAT GCT GCA TCT ACT TTA TGG      6517
Cys Asp Arg Leu Pro Gly Arg Ser Arg Asp Ala Ala Ser Thr Leu Trp
 15                  20                  25                  30

ATA TTC CTT ATA AAG CAA TGT ATG GAA CAA ATA CAG GAT GAT GTG GGT      6565
Ile Phe Leu Ile Lys Gln Cys Met Glu Gln Ile Gln Asp Asp Val Gly
                35                  40                  45

GTG CCC ATA ATC GCC AGA GCT GCA GAC CTA TTC CGT TTT GCC AAA CCC      6613
Val Pro Ile Ile Ala Arg Ala Ala Asp Leu Phe Arg Phe Ala Lys Pro
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            50                  55                  60

ATG TTA ATT CTT CCT CGG CAA CAT CGA CCG ATA GTA AGG ACA AAG CCA     6661
Met Leu Ile Leu Pro Arg Gln His Arg Pro Ile Val Arg Thr Lys Pro
        65                  70                  75

CCA GAT GGA ACT GGA GTT CGT GGT ACC GGA TTG GCC GGA ACT AGG GAT     6709
Pro Asp Gly Thr Gly Val Arg Gly Thr Gly Leu Ala Gly Thr Arg Asp
    80                  85                  90

TCG TTT ATA GTG CGG CTA TTT GAA GAT GTT GCA GGA TGT TCC ACA GAA     6757
Ser Phe Ile Val Arg Leu Phe Glu Asp Val Ala Gly Cys Ser Thr Glu
 95                 100                 105                 110

TGG CAG GAT GTT CTA TCT GGA TAT TTG ATG TTG GAA TCT GAA GTT TCT     6805
Trp Gln Asp Val Leu Ser Gly Tyr Leu Met Leu Glu Ser Glu Val Ser
                115                 120                 125

GGT AAT GCT CCA CAT AGC TTG TGG ATA GTT GGG GCG GCA GAT ATA TGT     6853
Gly Asn Ala Pro His Ser Leu Trp Ile Val Gly Ala Ala Asp Ile Cys
            130                 135                 140

GCC ATT GCG CTC GAA TGT ATT CCT TTG CCA AAA AGG TTA CTT GCA ATC     6901
Ala Ile Ala Leu Glu Cys Ile Pro Leu Pro Lys Arg Leu Leu Ala Ile
        145                 150                 155

AAA GTG TCT GGG ACC TGG TCC GGT ATG CCG TGG GCC ATT CCC GAC AAT     6949
Lys Val Ser Gly Thr Trp Ser Gly Met Pro Trp Ala Ile Pro Asp Asn
    160                 165                 170

ATT CAA ACT CTC TTG ACA TCT ACA TGG GAA CCG AAG TTC GAC ACC CCA     6997
Ile Gln Thr Leu Leu Thr Ser Thr Trp Glu Pro Lys Phe Asp Thr Pro
175                 180                 185                 190

GAA GAT AGA GCG CAT TTT TGC GAC AGT GAT ATG GTA TGT GTA TAC AAA     7045
Glu Asp Arg Ala His Phe Cys Asp Ser Asp Met Val Cys Val Tyr Lys
                195                 200                 205

ATC CTC GGG TCC CCA CCC AAT CCT CTA AAA CCT CCG GAA ATC GAA CCA     7093
Ile Leu Gly Ser Pro Pro Asn Pro Leu Lys Pro Pro Glu Ile Glu Pro
            210                 215                 220

CCT CAA ATG AGT AGT ACA CCC GGC AGA TTA TTC TGT TGT GGA AAA TGT     7141
Pro Gln Met Ser Ser Thr Pro Gly Arg Leu Phe Cys Cys Gly Lys Cys
        225                 230                 235

TGC AAG AAA GAA GAT AGA GAT GCG ATT GCA ATT CCG GTT CGT TAC ACT     7189
Cys Lys Lys Glu Asp Arg Asp Ala Ile Ala Ile Pro Val Arg Tyr Thr
    240                 245                 250

GCG ACA GGA AAG TCA CGA ATA CAG AAA AAA TGT AGA GCC GGT AGT CAT     7237
Ala Thr Gly Lys Ser Arg Ile Gln Lys Lys Cys Arg Ala Gly Ser His
255                 260                 265                 270

TAG  CTGTTATTCG ACAGACCTAC TTGCTACCAA TTAGATATAA TTACATGATG
End
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GGGCGTATAC ACATTACGAT TAGGTGCATC GCTACAACCG TCGCTATAGT GTCACGTATA   7350

ATTTGTATAT TAGTGCAATA ACAAACCCTT CTAGATCACT TATGTATCCA GGCTATCTTC   7410

CATATACTTC TAACATCAGG AGAGATTCAA CAATCGAGCG CATTTGAAAG ACAACG ATG   7469
                                                              Met
                                                               1

AGC AGA GTC AAT GCT ACA ATG TTC GAT GAT ATG GAT ATA CCA AGA GGA      7517
Ser Arg Val Asn Ala Thr Met Phe Asp Asp Met Asp Ile Pro Arg Gly
             5                  10                  15

CGA TTT GGT AAG CCA CCG AGA AAG ATT ACT AAT GTA AAT TTT TGG CAT      7565
Arg Phe Gly Lys Pro Pro Arg Lys Ile Thr Asn Val Asn Phe Trp His
         20                  25                  30

GTG GTT GTT GAT GAA TTC ACA GAA GGA ATC GTT CAA TGT ATG GAA GCC      7613
Val Val Val Asp Glu Phe Thr Glu Gly Ile Val Gln Cys Met Glu Ala
     35                  40                  45

CGA GAG AGA TTA GGC CTT TTA TGT ACC ATA TCT ACT AAC GAG GGA TCT      7661
Arg Glu Arg Leu Gly Leu Leu Cys Thr Ile Ser Thr Asn Glu Gly Ser
 50                  55                  60                  65

ATT ACA TCG TTT GAT ATA CAC AAG GAT ATG TGG TGT CAA ATG GTT ATC      7709
Ile Thr Ser Phe Asp Ile His Lys Asp Met Trp Cys Gln Met Val Ile
                 70                  75                  80

TGG TCT GCC TAT AGA TTT TTT GCC ATG ATG GAC AAA ATG TTT TCG ATT      7757
Trp Ser Ala Tyr Arg Phe Phe Ala Met Met Asp Lys Met Phe Ser Ile
             85                  90                  95

GAA ACT ATC ACA AAT TTT ACA GAA ACT GAT CTT ACC GAA ACT GGT CAG      7805
Glu Thr Ile Thr Asn Phe Thr Glu Thr Asp Leu Thr Glu Thr Gly Gln
         100                 105                 110

TGG AGA ATA TTC TAT AGA ACT TGG GAT GTG AGA GAT GCA TTG AAG ATG      7853
Trp Arg Ile Phe Tyr Arg Thr Trp Asp Val Arg Asp Ala Leu Lys Met
     115                 120                 125

AAA CAG GTG GGA CCA TTT TTG CCC GCA TTG TTT TCA TTT CAT CTG GAA      7901
Lys Gln Val Gly Pro Phe Leu Pro Ala Leu Phe Ser Phe His Leu Glu
 130                 135                 140                 145

AAC TGG ACC ACA ATG CTT TCC ATA GGA ATC AAC AAG GGT TAT GAT CGA      7949
Asn Trp Thr Thr Met Leu Ser Ile Gly Ile Asn Lys Gly Tyr Asp Arg
                 150                 155                 160

CAC AAT ACA CGA AAT ATG TTC ATG ACA ATA CAG TCT GCA AGA AAT GTC      7997
His Asn Thr Arg Asn Met Phe Met Thr Ile Gln Ser Ala Arg Asn Val
             165                 170                 175

CTT AGC GGG GCA ATA GAG GTA GCT CGA TAT GCC GTG GTT CTT GCT CTA      8045
Leu Ser Gly Ala Ile Glu Val Ala Arg Tyr Ala Val Val Leu Ala Leu
         180                 185                 190
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CCT GTG TGC GAG TAT AGA ACA CCC TTA GGC CTG CCG GAT GAT AGC ATA      8093
Pro Val Cys Glu Tyr Arg Thr Pro Leu Gly Leu Pro Asp Asp Ser Ile
    195                 200                 205

GGA AAT GCC ATC AAG ACA TGC TGC ACG CAA ATG CAA GCG AAT CGA TTG      8141
Gly Asn Ala Ile Lys Thr Cys Cys Thr Gln Met Gln Ala Asn Arg Leu
210                 215                 220                 225

ACA GAA ACT GGA ATA TCC AAG GAC AGT GGA CAT AAA ATA AAT GAT TCT      8189
Thr Glu Thr Gly Ile Ser Lys Asp Ser Gly His Lys Ile Asn Asp Ser
                230                 235                 240

TCT GAA GAG GAG TTG TAT TAT AGA ACC ATA CAT GAT CTT ATC AAA CCT      8237
Ser Glu Glu Glu Leu Tyr Tyr Arg Thr Ile His Asp Leu Ile Lys Pro
            245                 250                 255

AAC CGG GAA CAT TGC ATA TCA TGC AAT ATT GAG AAT AGC ATG GAT ATA      8285
Asn Arg Glu His Cys Ile Ser Cys Asn Ile Glu Asn Ser Met Asp Ile
        260                 265                 270

GAT CCC ACT ATT CAC CAT CGA TCT TCT AAT GTC ATA ACT TTA CAA GGT      8333
Asp Pro Thr Ile His His Arg Ser Ser Asn Val Ile Thr Leu Gln Gly
    275                 280                 285

ACA TCA ACA TAT CCA TTT GGA CGC AGG CCG ATG AGT CGA ATG GAT GTT      8381
Thr Ser Thr Tyr Pro Phe Gly Arg Arg Pro Met Ser Arg Met Asp Val
290                 295                 300                 305

GGA GGT CTT ATG TAC CAG CAC CCC TAC ATT TGC CGC AAT CTC CAT TTA      8429
Gly Gly Leu Met Tyr Gln His Pro Tyr Ile Cys Arg Asn Leu His Leu
                310                 315                 320

CGT CCG CCT CGA TCC AGA CTA ATG AAT AGT AAA ATC CTA CAG ACA TTT      8477
Arg Pro Pro Arg Ser Arg Leu Met Asn Ser Lys Ile Leu Gln Thr Phe
            325                 330                 335

AGA CAA AGT TTC AAT CGA AGT AAT CCT CAT GCA TAC CCC ATA TAA          8522
Arg Gln Ser Phe Asn Arg Ser Asn Pro His Ala Tyr Pro Ile End
        340                 345                 350

TACATACAAT CATGACAACA CTGTAATGCC TTATTGAAAA TAAAATTTTA TTATTTAAAC   8582

AACGTTAGTA GCAGTTTTTC CTAAAATCCT ATTAATAATT GTGCGATTAG TTATAAGTAG   8642

GATTCCCCGT CTCCTGTTGG CGATTCCCGA AGATTTGTCA GATAATGTGC CAATTCAGCA   8702

TCATCACCGA TTGCTGCATT CCCCTTAGTA GCGACGGCAC GACATAAAGG TTTCCAATAA   8762

GACTCTATTT CGGGGAGTGG ACTTATTCCA CAGCCCGTTG CCGAACCTAC TATGTCCATA   8822

AGACGGACAT TCTTCTCATA TAAGCGCGAA ACAGTACAGT ATCCAGCATG TCCAAGACAA   8882

CACCAATACA TCATGATAGT AAACCGAGTG TCCATTTCTT CGTGTGTAAG AGGAGCACGT   8942

TCAATACACC GTAAAGCCCG GCCTACAATT TTTCTCGTCG GGTCTGTCGG GTGGAATGGC   9002

GAAGCAGAAA TATCATATTC GTTAAGCGTG ACAGTCATTC TGTCGAATAT CTCACCCCAC   9062
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AAACGAGACG ATGGTGGTTT TCCAGCTTTC ATAGCAGCCT GGGAGATCGT AGCGGCGGTT 9122

AATATGGTCC TGGCTAGACT GCGTACAGAT TTAGGCAATA GCGCAACATG TTCCCCGCCG 9182

GCAGAAAGTA TATCATAACT CTGTTCTTTT GGAGAATCTA CCCGGAGTTG CACACTCCTG 9242

CTAGATTTGC GCCGTAGAGA CCACATGGCC ATACCTCTCC AATATGTTTT AATCTTACAC 9302

GGCGCTCGTC TCCAGTATTC AAACACGTTC TCCTCTCATT AGGCTCAACG CCACATTAAA 9362

TATCTTCATA TACAACAAAA AGGCAACACG TTATTTGACA CGCCCCTTCA TGGATGGGGG 9422

GGGTCAGCGT TTGTTGCAAC AGATCATGAC AAATAAATCC AAAATCTATT ATTTTATCTC 9482

ATTAGATAGA TCAAAGAATG TCGGCTCTAT GTCTAACAAT TAAAATTATA TAATAAGAGC 9542

TTTCTCTTCA AGTCTGGATA GTTAATGCAA TTTACTGTCT ACCGACAAAT CGTTCATTCC 9602

TTTTACATCG CAGTCTGAAG AAATAGTTCC CGAGGACGCA GCGATTGGGT GAAAAATGCT 9662

ATCGGAGGCA TATATATCGG ATATAGGATG GGCGCTTCGA CTATCAGCAT CCCTCAGAGT 9722

CCTGCGCAGA TGTAGACTTT GGCGTGGGGT CAAATTCATG ATAGTTTCCC ATTCGGCTTG 9782

TTTTAGTCGA TATCCCATTC GACCAATCAT ATGAATATCG AATAGTGCTC TCCGAAGAGC 9842

ATCGTGGAAC GGACCGCTAT TTAGTCGACA TCGAATAAAA CATCGAAATA GTTTGTTTGT 9902

ATCCGCACAT AACCGAGCGA CATCGGGTTT CCATGGTAGA GGACAAAATT TGCCCACATT 9962

ATTAAGTTCA AAGTCTTGAT CGGACGAGTC ACTGCCATAT TCCGGATGTG AATGTGGCAG 10022

TTGATAATCT TCGTCGTCGC TCTCATTATC TGACGATGAT AATCGTGTAT CGGGTCTGGC 10082

TCGATCTCGA TCACGACTCA TGTTGCCTCC GATGGAGCCG AAAGCAGGTT TTCTGCTCAA 10142

GTGTAATTTG GAGACTTTGG CCTGTATTAT ATAGCTACCA GCTTTTATCT TCTGCTAGGA 10202

ACAATAATTG CTAGAATTTA CATCACGTGA TATCCGGTCA AAAATTACTT GGTCTTTAAC 10262

CCAGCCCCTA ATGTACTACT TGCTCTATAT ATTCTCCACA ATGGTAAACC TCCCTCCCTA 10322

AAGATTTCAC TCCAATTTCA AGGAATTC 10350

(4) Information for SEQ ID NO: 3

(i) Sequence Charteristics:

(A) Length: 497 amino acids

(B) Type: peptide

(C) Strandedness: single

(D) Topology: linear

(ii) Molecule Type:

(A) Description: polypeptide

(vi) ORIGINAL SOURCE:

(A) Organism: MDV, GA strain

(vii) IMMEDIATE SOURCE:

(A): Library: genomic

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

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Met Cys Val Phe Gln Ile Leu Ile Ile Val Thr Thr Ile Lys Val Ala
            -15                 -10                  -5

Gly Thr Ala Asn Ile Asn His Ile Asp Val Pro Ala Gly His Ser Ala
         1              5                  10

Thr Thr Thr Ile Pro Arg Tyr Pro Pro Val Val Asp Gly Thr Leu Tyr
 15                  20                  25                  30

Thr Glu Thr Trp Thr Trp Ile Pro Asn His Cys Asn Glu Thr Ala Thr
                 35                  40                  45

Gly Tyr Val Cys Leu Glu Ser Ala His Cys Phe Thr Asp Leu Ile Leu
             50                  55                  60

Gly Val Ser Cys Met Arg Tyr Ala Asp Glu Ile Val Leu Arg Thr Asp
         65                  70                  75

Lys Phe Ile Val Asp Ala Gly Ser Ile Lys Gln Ile Glu Ser Leu Ser
     80                  85                  90

Leu Asn Gly Val Pro Asn Ile Phe Leu Ser Thr Lys Ala Ser Asn Lys
 95                  100                 105                 110

Leu Glu Ile Leu Asn Ala Ser Leu Gln Asn Ala Gly Ile Tyr Ile Arg
                115                 120                 125

Tyr Ser Arg Asn Gly Thr Arg Thr Ala Lys Leu Asp Val Val Val Val
            130                 135                 140

Gly Val Leu Gly Gln Ala Arg Asp Arg Leu Pro Gln Met Ser Ser Pro
        145                 150                 155

Met Ile Ser Ser His Ala Asp Ile Lys Leu Ser Leu Lys Asn Phe Lys
    160                 165                 170

Ala Leu Val Tyr His Val Gly Asp Thr Ile Asn Val Ser Thr Ala Val
175                 180                 185                 190

Ile Leu Gly Pro Ser Pro Glu Ile Phe Thr Leu Glu Phe Arg Val Leu
                195                 200                 205

Phe Leu Arg Tyr Asn Pro Thr Cys Lys Phe Val Thr Ile Tyr Glu Pro
            210                 215                 220

Cys Ile Phe His Pro Lys Glu Pro Glu Cys Ile Thr Thr Ala Glu Gln
        225                 230                 235

Ser Val Cys His Phe Ala Ser Asn Ile Asp Ile Leu Gln Ile Ala Ala
    240                 245                 250

Ala Arg Ser Glu Asn Cys Ser Thr Gly Tyr Arg Arg Cys Ile Tyr Asp
255                 260                 265                 270

Thr Ala Ile Asp Glu Ser Val Gln Ala Arg Leu Thr Phe Ile Glu Pro
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275 280 285

Gly Ile Pro Ser Phe Lys Met Lys Asp Val Gln Val Asp Asp Ala Gly
290 295 300

Leu Tyr Val Val Val Ala Leu Tyr Asn Gly Arg Pro Ser Ala Trp Thr
305 310 315

Tyr Ile Tyr Leu Ser Thr Val Glu Thr Tyr Leu Asn Val Tyr Glu Asn
320 325 330

Tyr His Lys Pro Gly Phe Gly Tyr Lys Ser Phe Leu Gln Asn Ser Ser
335 340 345 350

Ile Val Asp Glu Asn Glu Ala Ser Asp Trp Ser Ser Ser Ser Ile Lys
355 360 365

Arg Arg Asn Asn Gly Thr Ile Ile Tyr Asp Ile Leu Leu Thr Ser Leu
370 375 380

Ser Ile Gly Ala Ile Ile Ile Val Ile Val Gly Gly Val Cys Ile Ala
385 390 395

Ile Leu Ile Arg Arg Arg Arg Arg Arg Arg Thr Arg Gly Leu Phe Asp
400 405 410

Glu Tyr Pro Lys Tyr Met Thr Leu Pro Gly Asn Asp Leu Gly Gly Met
415 420 425 430

Asn Val Pro Tyr Asp Asn Thr Cys Ser Gly Asn Gln Val Glu Tyr Tyr
435 440 445

Gln Glu Lys Ser Ala Lys Met Lys Arg Met Gly Ser Gly Tyr Thr Ala
450 455 460

Trp Leu Lys Asn Asp Met Pro Lys Ile Arg Lys Arg Leu Asp Leu Tyr
465 470 475

His End

WE CLAIM:

-1-

A 2.53 Kb segment of DNA with a gene coding MDV glycoprotein E (gE) precursor, between a 8488 and 9978 bp sequence of Marek's disease herpesvirus DNA and identified as part of SEQ ID No:1, and containing potential promoter sequences up to 400 nucleotides in length 5' of ~~each~~ the gene and subfragments of the DNA which selectively recognize the DNA when in the form of a probe.

-2-

The segment of Claim 1 wherein the glycoprotein encoded contains 497 amino acids.

-3-

The substantially pure glycoprotein gE precursor which comprises:

Met Cys Val Phe Gln Ile Leu Ile Ile Val Thr Thr Ile Lys Val Ala
-15 -10 -5

Gly Thr Ala Asn Ile Asn His Ile Asp Val Pro Ala Gly His Ser Ala
1 5 10

Thr Thr Thr Ile Pro Arg Tyr Pro Pro Val Val Asp Gly Thr Leu Tyr
15 20 25 30

Thr Glu Thr Trp Thr Trp Ile Pro Asn His Cys Asn Glu Thr Ala Thr
35 40 45

Gly Tyr Val Cys Leu Glu Ser Ala His Cys Phe Thr Asp Leu Ile Leu
50 55 60

Gly Val Ser Cys Met Arg Tyr Ala Asp Glu Ile Val Leu Arg Thr Asp
65 70 75

Lys Phe Ile Val Asp Ala Gly Ser Ile Lys Gln Ile Glu Ser Leu Ser
80 85 90

Leu Asn Gly Val Pro Asn Ile Phe Leu Ser Thr Lys Ala Ser Asn Lys
95 100 105 110

Leu Glu Ile Leu Asn Ala Ser Leu Gln Asn Ala Gly Ile Tyr Ile Arg
115 120 125

Tyr Ser Arg Asn Gly Thr Arg Thr Ala Lys Leu Asp Val Val Val Val
130 135 140

Gly Val Leu Gly Gln Ala Arg Asp Arg Leu Pro Gln Met Ser Ser Pro
145 150 155

Met Ile Ser Ser His Ala Asp Ile Lys Leu Ser Leu Lys Asn Phe Lys
160 165 170

Ala Leu Val Tyr His Val Gly Asp Thr Ile Asn Val Ser Thr Ala Val
175 180 185 190

Ile Leu Gly Pro Ser Pro Glu Ile Phe Thr Leu Glu Phe Arg Val Leu
195 200 205

Phe Leu Arg Tyr Asn Pro Thr Cys Lys Phe Val Thr Ile Tyr Glu Pro
210 215 220

Cys Ile Phe His Pro Lys Glu Pro Glu Cys Ile Thr Thr Ala Glu Gln
225 230 235

Ser Val Cys His Phe Ala Ser Asn Ile Asp Ile Leu Gln Ile Ala Ala
240 245 250

Ala Arg Ser Glu Asn Cys Ser Thr Gly Tyr Arg Arg Cys Ile Tyr Asp
255 260 265 270

Thr Ala Ile Asp Glu Ser Val Gln Ala Arg Leu Thr Phe Ile Glu Pro
275 280 285

Gly Ile Pro Ser Phe Lys Met Lys Asp Val Gln Val Asp Asp Ala Gly
290 295 300

Leu Tyr Val Val Val Ala Leu Tyr Asn Gly Arg Pro Ser Ala Trp Thr
305 310 315

Tyr Ile Tyr Leu Ser Thr Val Glu Thr Tyr Leu Asn Val Tyr Glu Asn
320 325 330

Tyr His Lys Pro Gly Phe Gly Tyr Lys Ser Phe Leu Gln Asn Ser Ser
335 340 345 350

Ile Val Asp Glu Asn Glu Ala Ser Asp Trp Ser Ser Ser Ser Ile Lys
355 360 365

Arg Arg Asn Asn Gly Thr Ile Ile Tyr Asp Ile Leu Leu Thr Ser Leu
370 375 380

Ser Ile Gly Ala Ile Ile Ile Val Ile Val Gly Gly Val Cys Ile Ala
385 390 395

Ile Leu Ile Arg Arg Arg Arg Arg Arg Arg Thr Arg Gly Leu Phe Asp
400 405 410

Glu Tyr Pro Lys Tyr Met Thr Leu Pro Gly Asn Asp Leu Gly Gly Met
415 420 425 430

Asn Val Pro Tyr Asp Asn Thr Cys Ser Gly Asn Gln Val Glu Tyr Tyr
435 440 445

Gln Glu Lys Ser Ala Lys Met Lys Arg Met Gly Ser Gly Tyr Thr Ala
450 455 460

Trp Leu Lys Asn Asp Met Pro Lys Ile Arg Lys Arg Leu Asp Leu Tyr
465 470 475

His End

-64-

-4-

A method for reducing pathogenicity or virulence of a Marek's disease herpesvirus whereby a gene which encodes for a glycoprotein selected from glycoproteins I and E is altered.

-5-

In a method for producing a virus vector vaccine, for use in vivo, or an in vitro expression vector, for a protein which produces antibodies against Marek's disease by providing in the vaccine or vector a segment of DNA from the genome of a Marek's disease herpesvirus that encodes a protein producing an antibody response in birds, the improvement which comprises:

inserting into the vaccine or vector a segment of DNA containing all or part of a gene encoding a glycoprotein selected from the group consisting of gD, gI and gE of the Marek's disease herpesvirus.

-6-

A method for providing foreign genes in a Marek's disease herpesvirus which comprises inserting the foreign gene into a region of DNA of the herpesvirus which encodes a non-essential protein selected from the group consisting of gI and gE.

-7-

A segment of DNA of Marek's disease herpesvirus (MDV) genome with genes encoding multiple glycoproteins between a 1 and 8799 nucleotide sequence and identified as SEQ ID NO:1, and optionally containing regulatory sequences up to 400 nucleotides in length 5' of each gene as shown in Figure 2 and subsegments of the DNA which selectively recognize portions of the segment of the DNA when in the form of a probe.

-8-

An EcoR1 I segment of Marek's disease herpesvirus genome encoding MDV glycoprotein D (gD) precursor and regulatory sequences and subsegments of the DNA which selectively recognize the DNA when in the form of a probe.

-9-

A 1.75 kbp NcoI-SstII segment of DNA of Marek's disease herpesvirus with a gene encoding MDV gD and containing a 5' regulatory region with the gene and subsegments of the DNA which selectively recognize the DNA when in the form of a probe.

-10-

A segment of DNA encoding MDV gD precursor, between a 5964 and 7175 bp nucleotide sequence of Marek's disease herpesvirus and identified as part of SEQ ID No:1, and optionally containing a 5' regulatory region of up to 400 bp in length as shown in Figure 2 and subsegments of the segment of DNA which selectively recognize the DNA when ~~the~~ 10 form of a probe.

-11-

The segment of Claim 10 wherein the glycoprotein encoded contains 403 amino acids.

-12-

A segment of DNA with a gene encoding a glycoprotein I (gI) precursor between a 7282 and 8349 bp DNA sequence of Marek's disease herpesvirus and identified as part of SEQ ID No:1, and optionally containing a 5' regulatory region with the gene of up to 400 bp in length as shown in Figure 2 and subfragments of the segment of DNA which selectively recognize the DNA when in the form of a probe.

-13-

The segment of Claim 12 wherein the glycoprotein encoded contains 355 amino acids.

-14-

A segment of DNA with a gene encoding a part of MDV glycoprotein E (gE) precursor, between a 8488 and 8799 bp DNA sequence of Marek's disease herpesvirus and identified as part of SEQ ID No:1, and optionally containing a 5' regulatory region with the gene of up to 400 bp in length, as shown in Figure 2 and subfragments of the DNA which selectively recognize the DNA when in the form of a probe.

-15-

The segment of Claim 14 wherein the glycoprotein encoded contains 104 amino acids.

-16-

The substantially pure glycoprotein gI precursor which comprises:

```
Met Tyr Val Leu Gln Leu Leu Phe Trp Ile Arg Leu Phe Arg Gly Ile
            -15                 -10                 -5
Trp Ser Ile Val Tyr Thr Gly Thr Ser Val Thr Leu Ser Thr Asp Gln
         1               5                  10
Ser Ala Leu Val Ala Phe Arg Gly Leu Asp Lys Met Val Asn Val Arg
 15                  20                  25                  30
Gly Gln Leu Leu Phe Leu Gly Asp Gln Thr Arg Thr Ser Ser Tyr Thr
                 35                  40                  45
Gly Thr Thr Glu Ile Leu Lys Trp Asp Glu Glu Tyr Lys Cys Tyr Ser
             50                  55                  60
Val Leu His Ala Thr Ser Tyr Met Asp Cys Pro Ala Ile Asp Ala Thr
         65                  70                  75
Val Phe Arg Gly Cys Arg Asp Ala Val Val Tyr Ala Gln Pro His Gly
     80                  85                  90
Arg Val Gln Pro Phe Pro Glu Lys Gly Thr Leu Leu Arg Ile Val Glu
 95                  100                 105                 110
Pro Arg Val Ser Asp Thr Gly Ser Tyr Tyr Ile Arg Val Ser Leu Ala
                115                 120                 125
Gly Arg Asn Met Ser Asp Ile Phe Arg Met Val Val Ile Ile Arg Ser
            130                 135                 140
Ser Lys Ser Trp Ala Cys Asn His Ser Ala Ser Ser Phe Gln Ala His
        145                 150                 155
Lys Cys Ile Arg Tyr Val Asp Arg Met Ala Phe Glu Asn Tyr Leu Ile
    160                 165                 170
Gly His Val Gly Asn Leu Leu Asp Ser Asp Ser Glu Leu His Ala Ile
175                 180                 185                 190
Tyr Asn Ile Thr Pro Gln Ser Ile Ser Thr Asp Ile Asn Ile Val Thr
                195                 200                 205
Thr Pro Phe Tyr Asp Asn Ser Gly Thr Ile Tyr Ser Pro Thr Val Phe
            210                 215                 220
Asn Leu Phe Asn Asn Asn Ser His Val Asp Ala Met Asn Ser Thr Gly
        225                 230                 235
Met Trp Asn Thr Val Leu Lys Tyr Thr Leu Pro Arg Leu Ile Tyr Phe
    240                 245                 250
Ser Thr Met Ile Val Leu Cys Ile Ile Ala Leu Ala Ile Tyr Leu Val
255                 260                 265                 270
Cys Glu Arg Cys Arg Ser Pro His Arg Arg Ile Tyr Ile Gly Glu Pro
                275                 280                 285
Arg Ser Asp Glu Ala Pro Leu Ile Thr Ser Ala Val Asn Glu Ser Phe
            290                 295                 300
Gln Tyr Asp Tyr Asn Val Lys Glu Thr Pro Ser Asp Val Ile Glu Lys
        305                 310                 315
Glu Leu Met Glu Lys Leu Lys Lys Lys Val Glu Leu Leu Glu Arg Glu
    320                 325                 330
Glu Cys Val
335
```

-17-

The substantially pure glycoprotein gD partial precursor which comprises:

```
Met Asn Arg Tyr Arg Tyr Glu Ser Ile Phe Phe Arg Tyr Ile Ser Ser
-30                 -25                 -20                 -15
Thr Arg Met Ile Leu Ile Ile Cys Leu Leu Leu Gly Thr Gly Asp Met
                -10                 -5                  1
Ser Ala Met Gly Leu Lys Lys Asp Asn Ser Pro Ile Ile Pro Thr Leu
            5                   10                  15
His Pro Lys Gly Asn Glu Asn Leu Arg Ala Thr Leu Asn Glu Tyr Lys
        20                  25                  30
Ile Pro Ser Pro Leu Phe Asp Thr Leu Asp Asn Ser Tyr Glu Thr Lys
    35                  40                  45                  50
His Val Ile Tyr Thr Asp Asn Cys Ser Phe Ala Val Leu Asn Pro Phe
                    55                  60                  65
Gly Asp Pro Lys Tyr Thr Leu Leu Ser Leu Leu Leu Met Gly Arg Arg
                70                  75                  80
Lys Tyr Asp Ala Leu Val Ala Trp Phe Val Leu Gly Arg Ala Cys Gly
            85                  90                  95
Arg Pro Ile Tyr Leu Arg Glu Tyr Ala Asn Cys Ser Thr Asn Glu Pro
        100                 105                 110
Phe Gly Thr Cys Lys Leu Lys Ser Leu Gly Trp Trp Asp Arg Arg Tyr
115                 120                 125                 130
Ala Met Thr Ser Tyr Ile Asp Arg Asp Glu Leu Lys Leu Ile Ile Ala
                135                 140                 145
Ala Pro Ser Arg Glu Leu Ser Gly Leu Tyr Thr Arg Leu Ile Ile Ile
            150                 155                 160
Asn Gly Glu Pro Ile Ser Ser Asp Ile Leu Leu Thr Val Lys Gly Thr
        165                 170                 175
Cys Ser Phe Ser Arg Arg Gly Ile Lys Asp Asn Lys Leu Cys Lys Pro
    180                 185                 190
Phe Ser Phe Phe Val Asn Gly Thr Thr Arg Leu Leu Asp Met Val Arg
195                 200                 205                 210
Thr Gly Thr Pro Arg Ala His Glu Glu Asn Val Lys Gln Trp Leu Glu
                215                 220                 225
Arg Asn Gly Gly Lys His Leu Pro Ile Val Val Glu Thr Ser Met Gln
            230                 235                 240
Gln Val Ser Asn Leu Pro Arg Ser Phe Arg Asp Ser Tyr Leu Lys Ser
        245                 250                 255
Pro Asp Asp Asp Lys Tyr Asn Asp Val Lys Met Thr Ser Ala Thr Thr
    260                 265                 270
Asn Asn Ile Thr Thr Ser Val Asp Gly Tyr Thr Gly Leu Thr Asn Arg
275                 280                 285                 290
Pro Glu Asp Phe Glu Lys Ala Pro Tyr Ile Thr Lys Arg Pro Ile Ile
                295                 300                 305
Ser Val Glu Glu Ala Ser Ser Gln Ser Pro Lys Ile Ser Thr Glu Lys
            310                 315                 320
Lys Ser Arg Thr Gln Ile Ile Ile Ser Leu Val Val Leu Cys Val Met
        325                 330                 335
Phe Cys Phe Ile Val Ile Gly Ser Gly Ile Trp Ile Leu Arg Lys His
    340                 345                 350
Arg Lys Thr Val Met Tyr Asp Arg Arg Arg Pro Ser Arg Arg Ala Tyr
355                 360                 365                 370
Ser Arg Leu
```

-18-

The substantially pure glycoprotein gE precursor which comprises:

```
Met Cys Val Phe Gln Ile Leu Ile Ile Val Thr Thr Ile Lys Val Ala
               -15                 -10                  -5
Gly Thr Ala Asn Ile Asn His Ile Asp Val Pro Arg Gly His Ser Ala
          1               5                  10
Thr Thr Thr Ile Pro Arg Tyr Pro Pro Val Val Asp Gly Thr Leu Tyr
 15                  20                  25                  30
Thr Glu Thr Trp Thr Trp Ile Pro Asn His Cys Asn Glu Thr Ala Thr
                35                  40                  45
Gly Tyr Val Cys Leu Glu Ser Ala His Cys Phe Thr Asp Leu Ile Leu
            50                  55                  60
Gly Val Ser Cys Met Arg Tyr Ala Asp Glu Ile Val Leu Arg Thr Asp
        65                  70                  75
Lys Phe Ile Val Asp Ala Gly Ser
     80                  85
```

-19-

A 5' regulatory region for glycoprotein gD between 5664 and 5963 nucleotide sequence as shown in Figure 2.

-20-

A 5' regulatory region for glycoprotein gE between 8088 and 8407 nucleotide sequence as shown in Figure 2.

-21-

A regulatory region for glycoprotein gI between 6882 and 7281 nucleotide sequence as shown in Figure 2.

Fig. 1A A. MDV genome structure and BamHI map

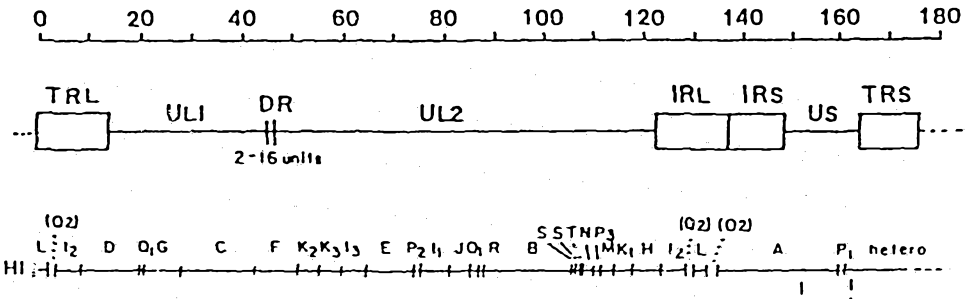


Fig. 1B B. Map location and sequencing strategy

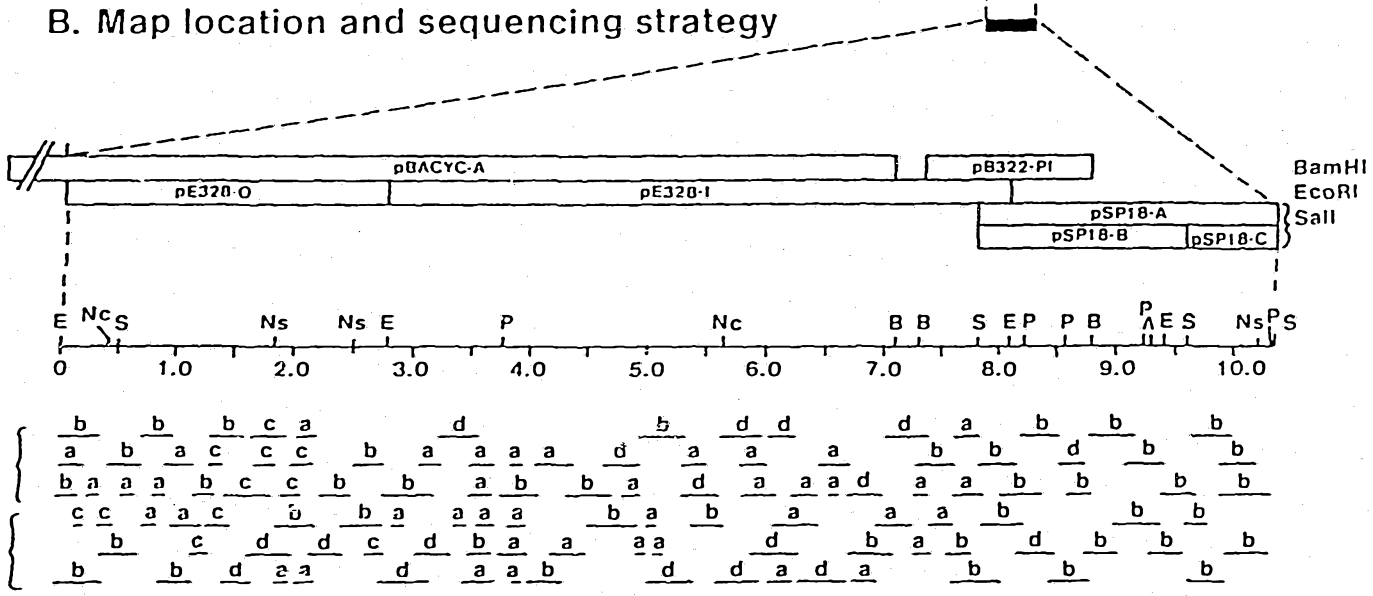


Fig. 1C C. Organization of MDV Us ORFs

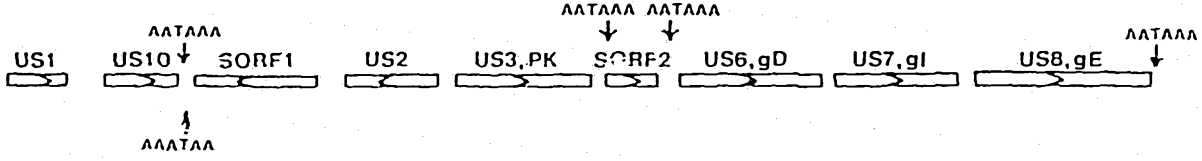


Fig. 2

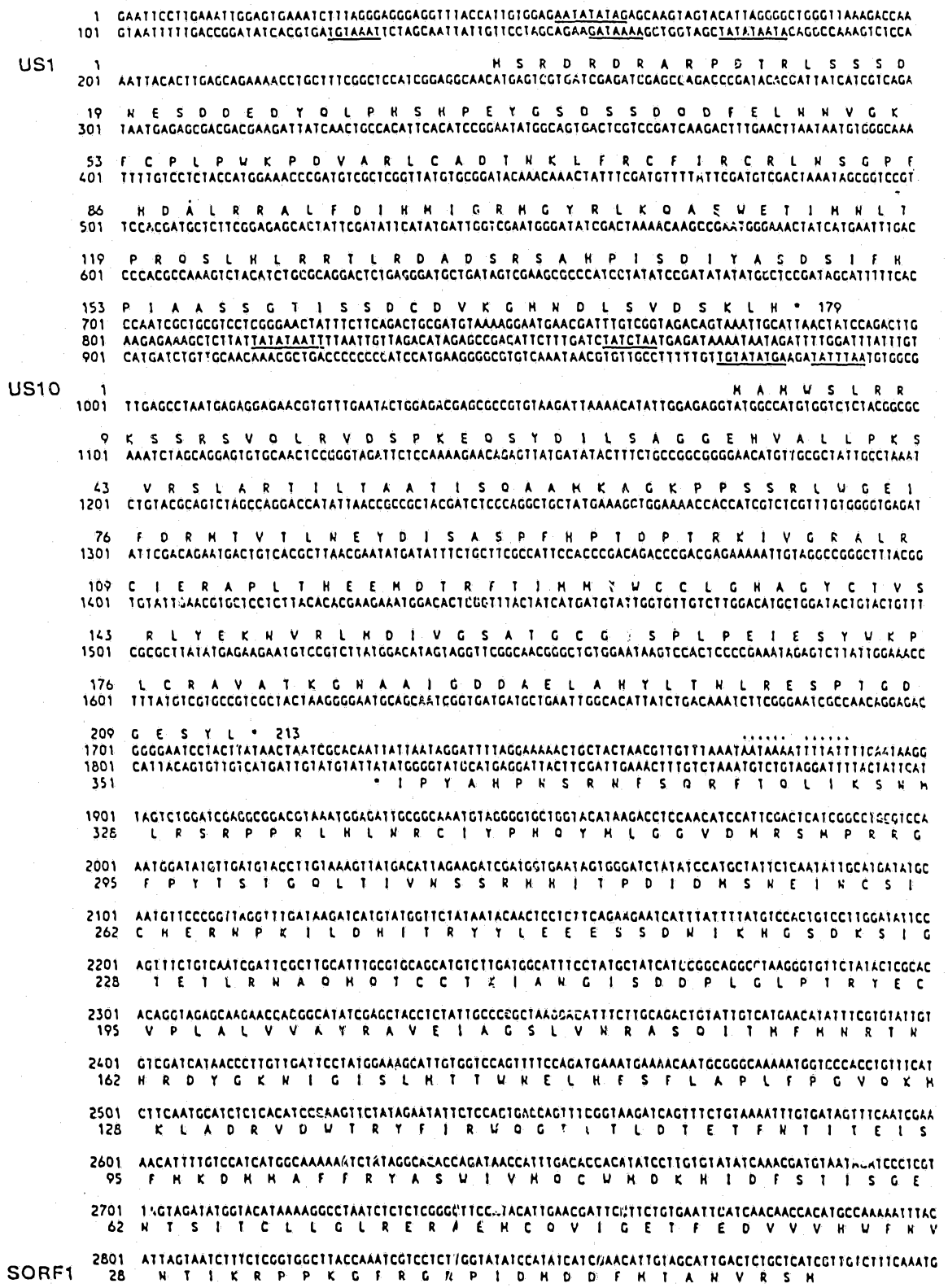


Fig. 2 Continued

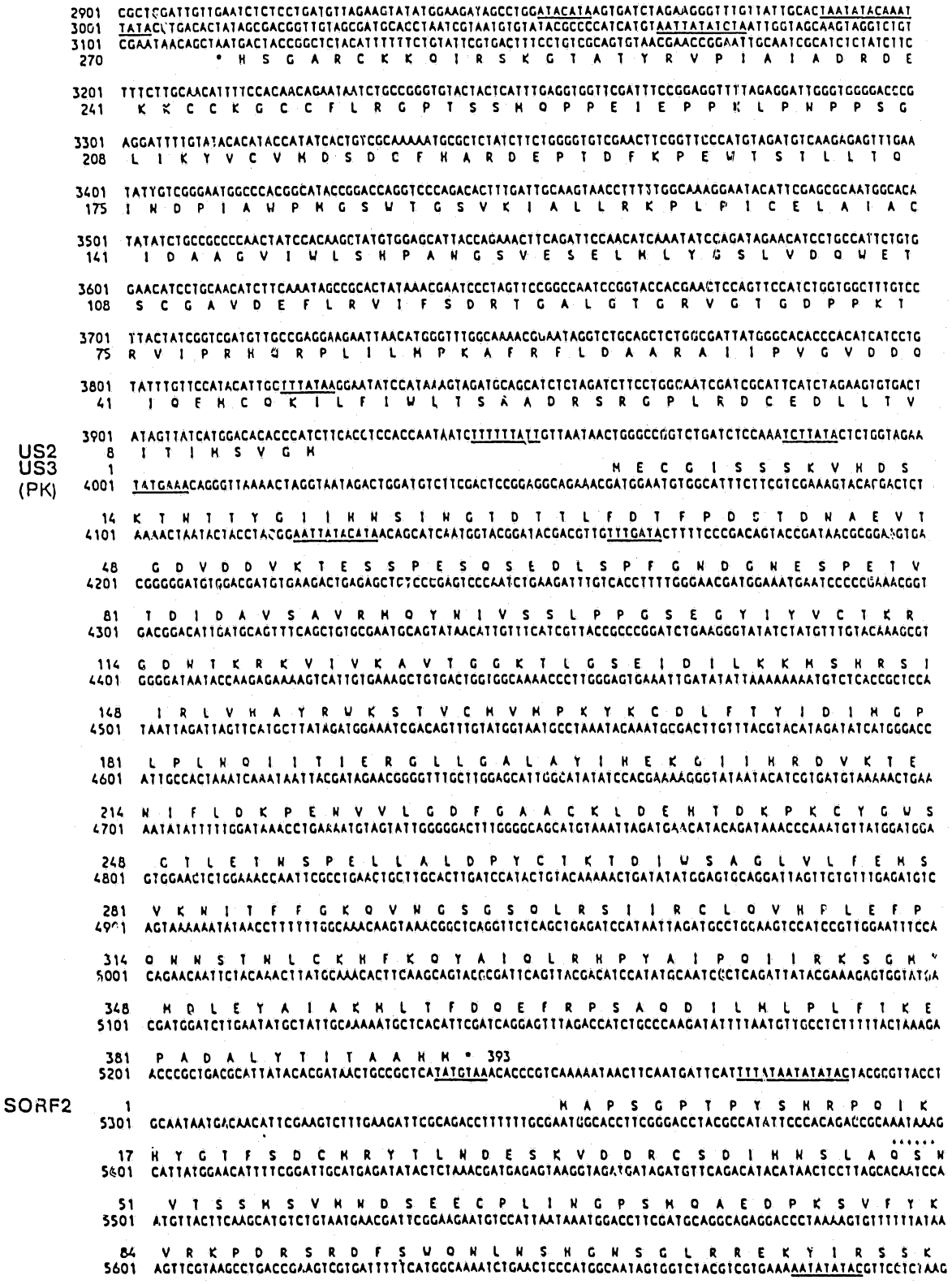


Fig. 2 Continued

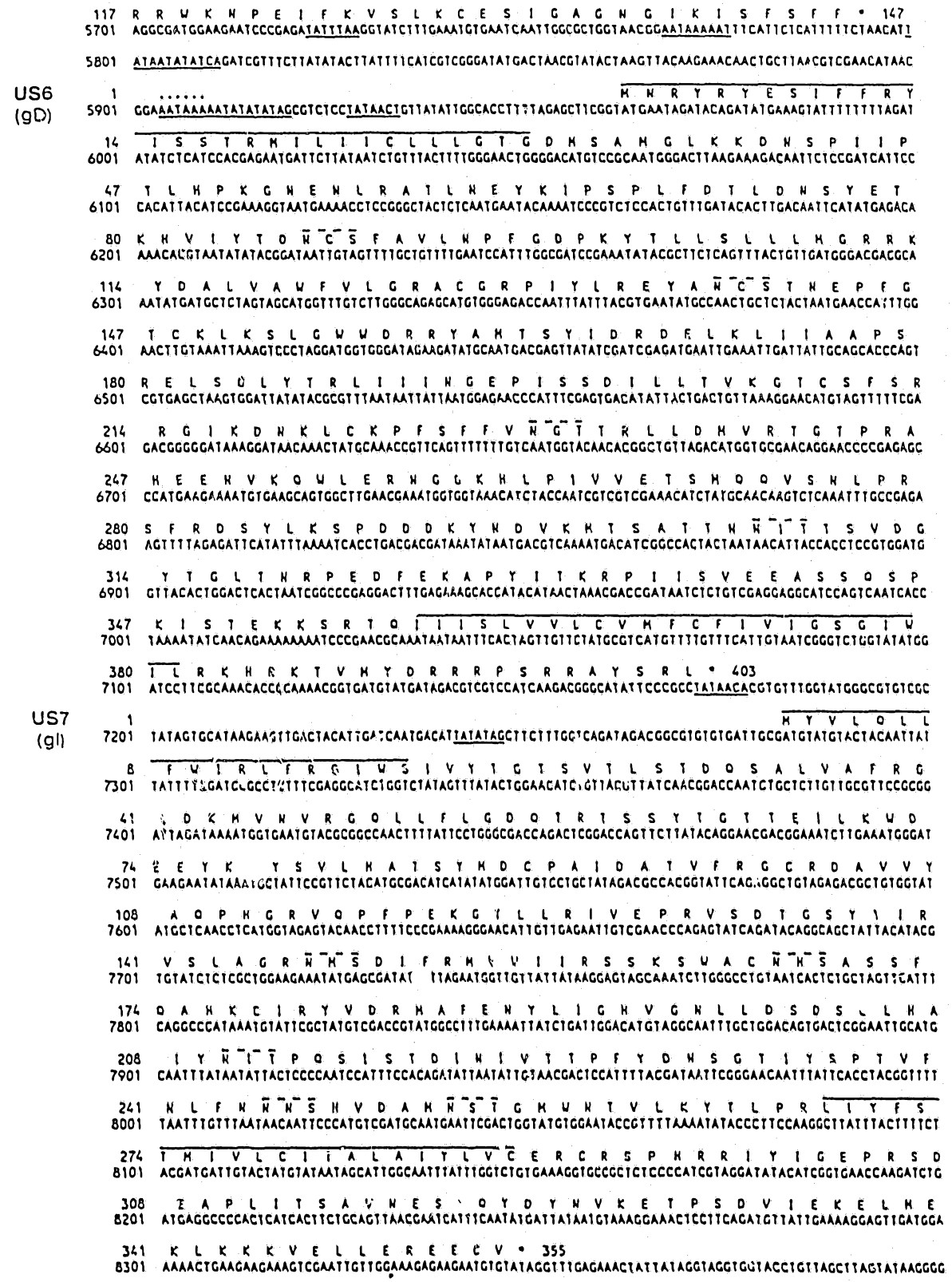


Fig. 2 Continued

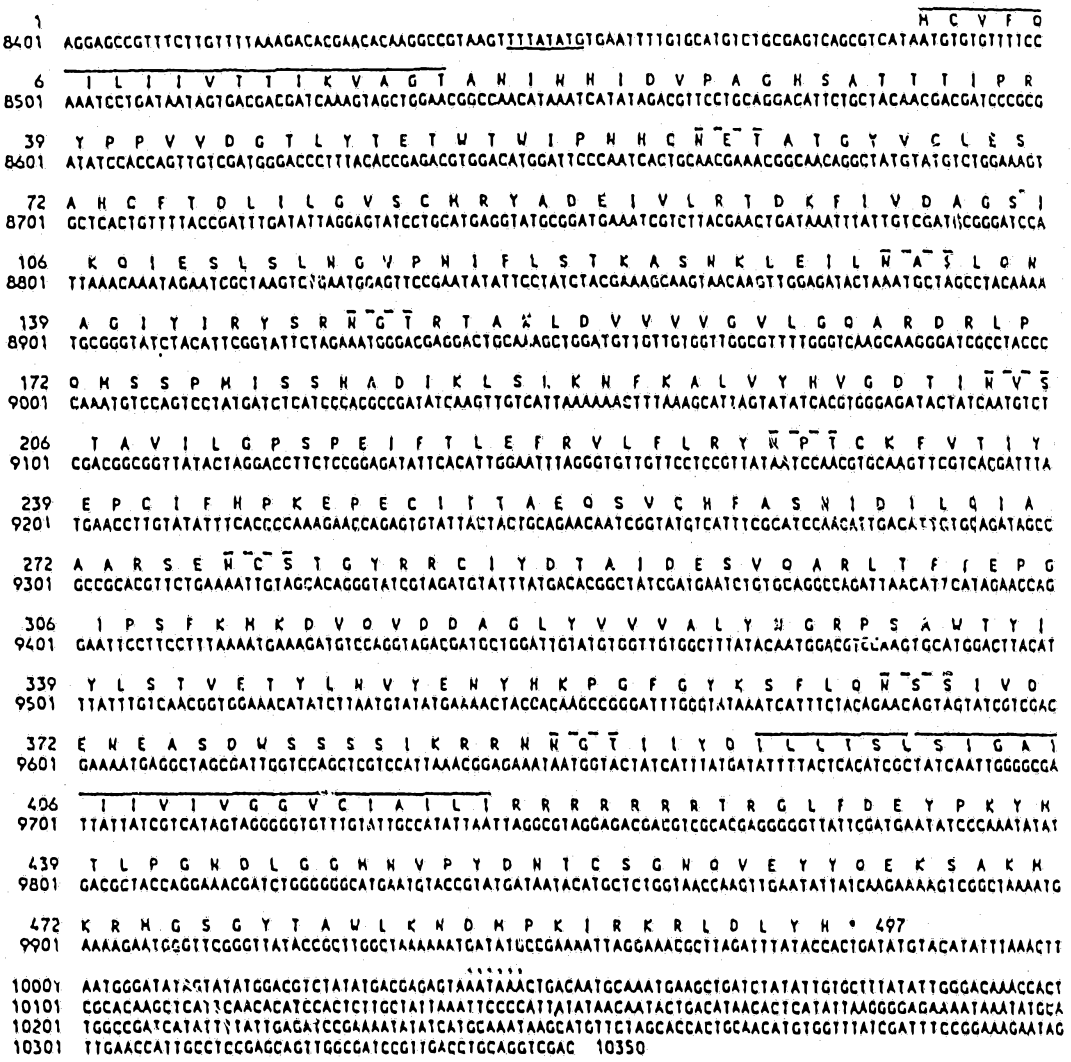


Fig. 3A A. Multiple alignments displaying regions of maximum amino acid conservation.

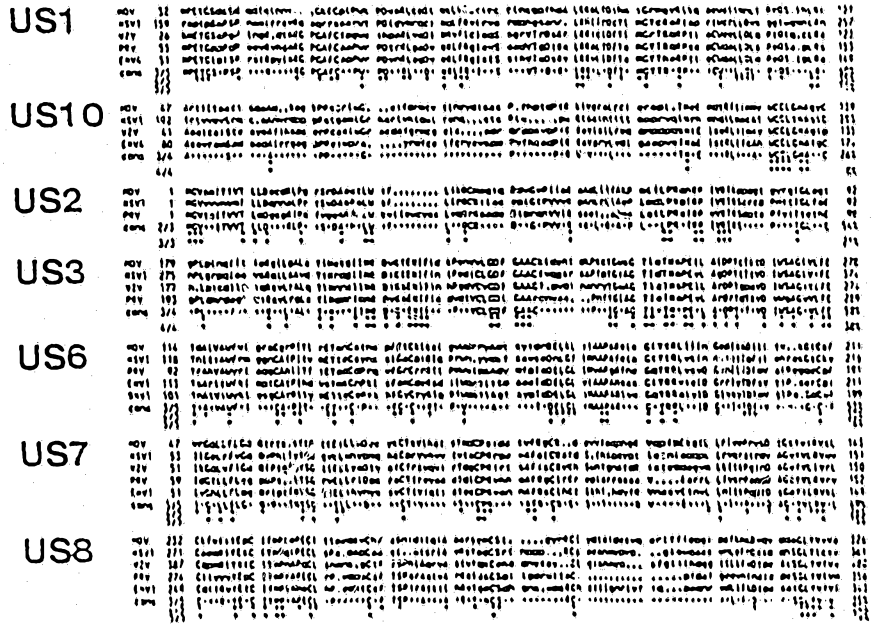


Fig. 3B B. Dot matrix analyses depicting overall homologies.

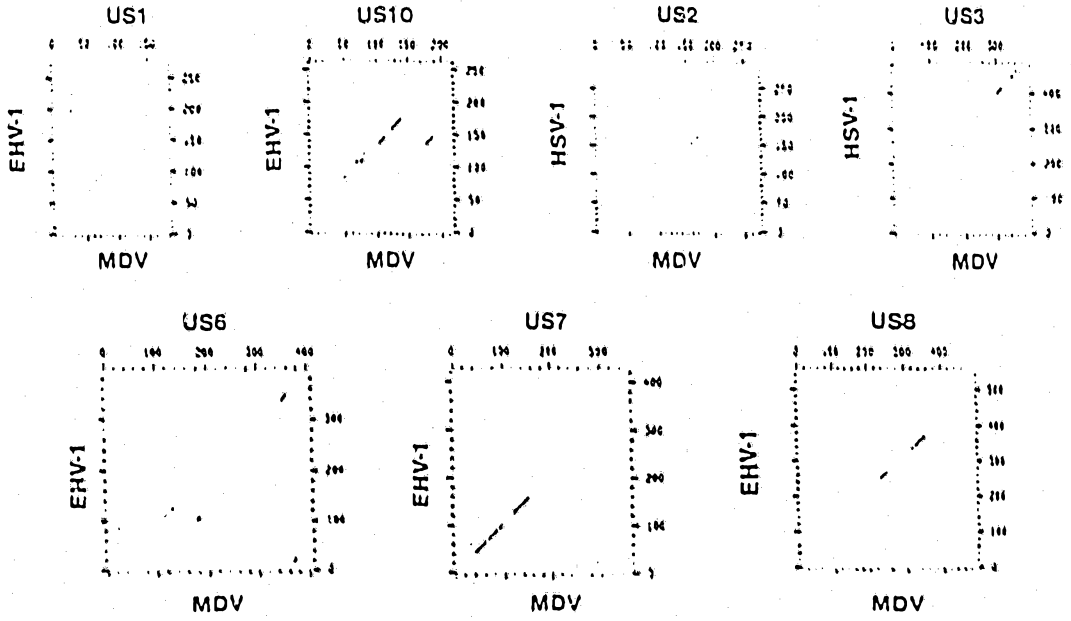
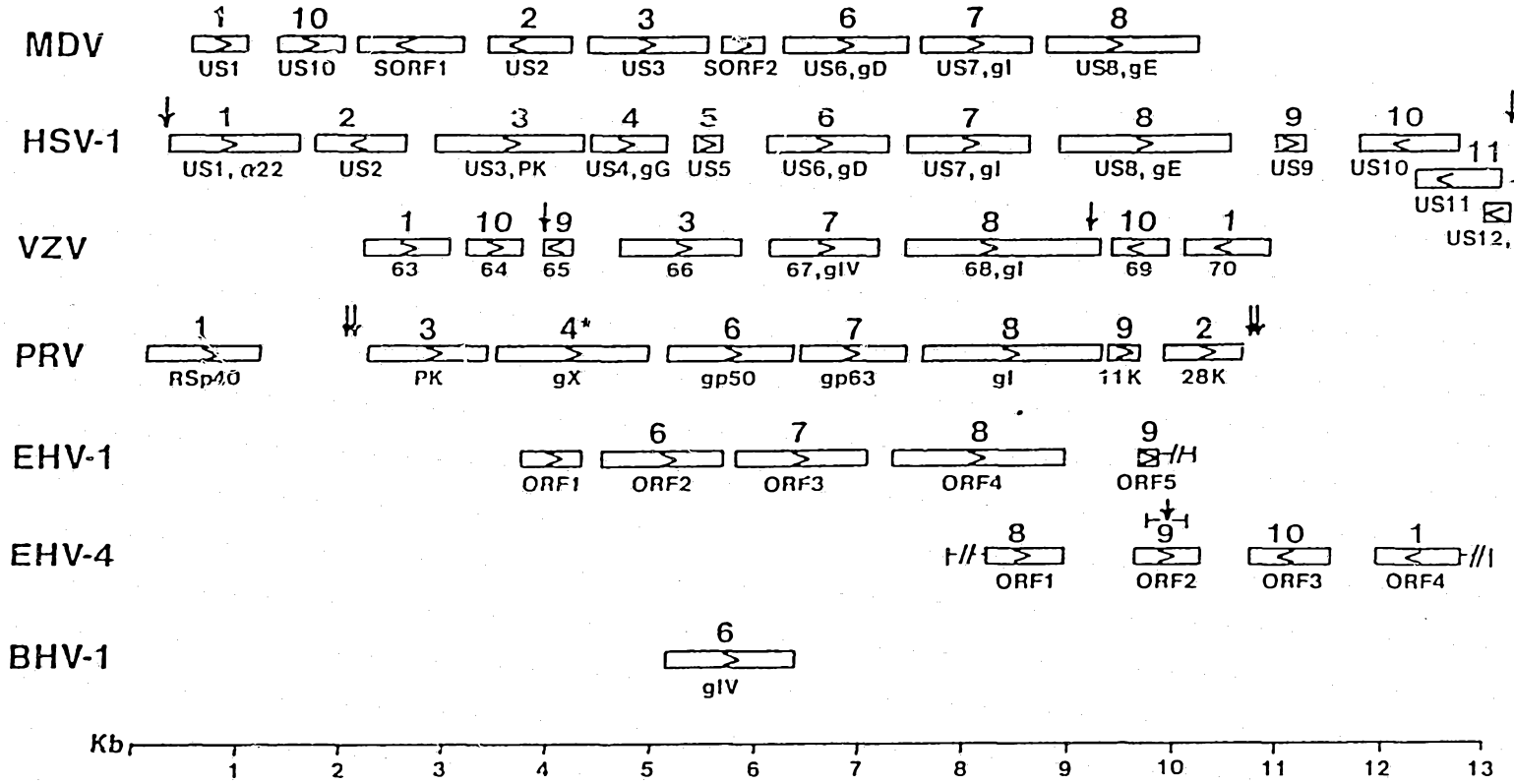


Fig. 4



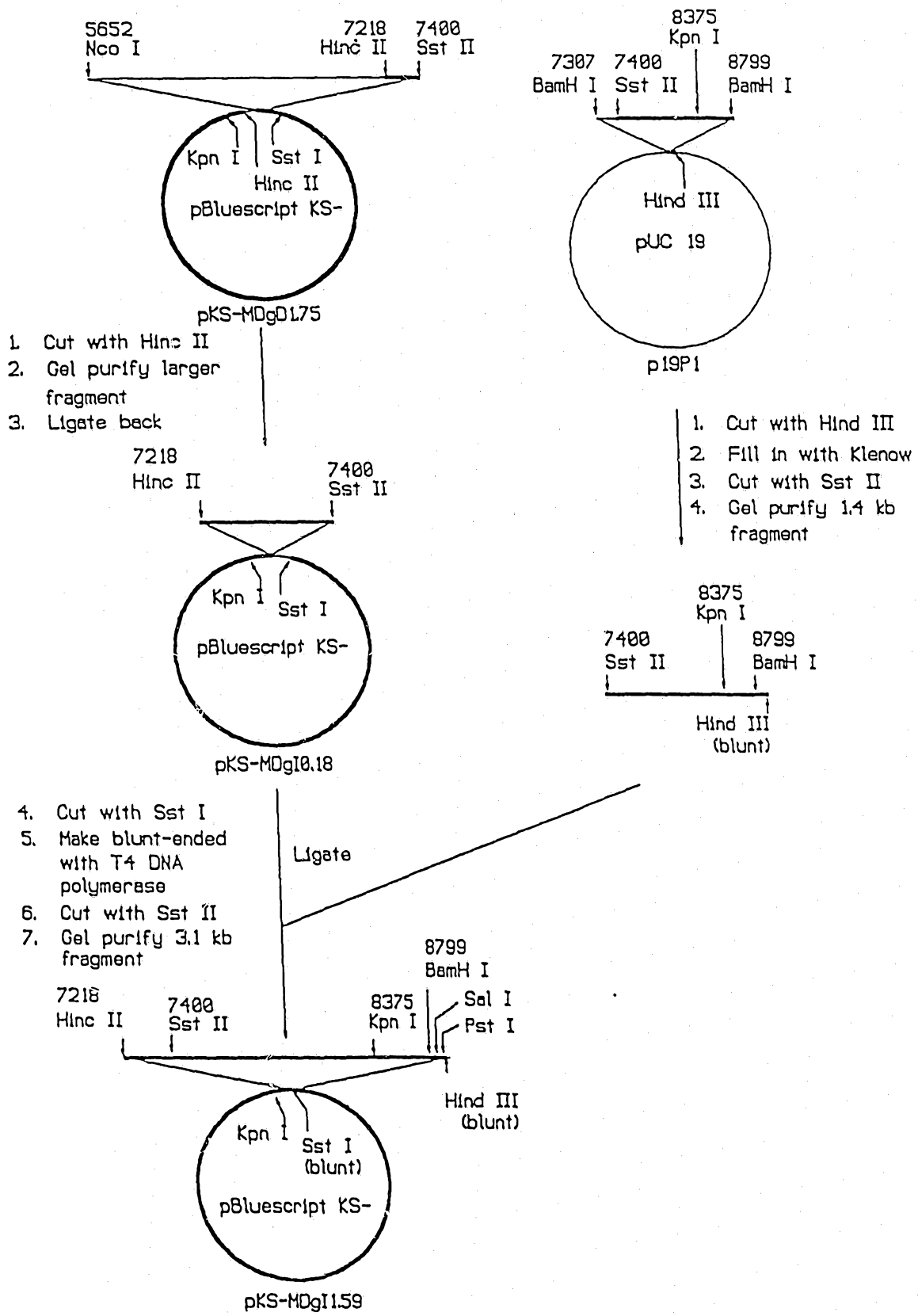
Numbers above boxes identify homologs of HSV-1 U_S genes; designations unique to each virus are presented below boxes.

↓ *repeat-unique region junction.*

• homologous to HSV-2 US4, rather than HSV-1 US4.

Fig. 5

MOLECULAR CLONING OF A CONSTRUCT CONTAINING THE DNA ENCODING MDV gI AND PART OF MDV gE



INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/05870

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): C12N 15/00, 15/38; C07K 13/00
US CL : 536/27;435/172.3;530/403,826

II. FIELDS SEARCHED

Minimum Documentation Searched[4]

Classification System	Classification Symbols
U.S.	536/27;435/172.3;530/403,826

Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched[5]

Biosis, Inpadoc

III. DOCUMENTS CONSIDERED TO BE RELEVANT[14]

Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No.[18]
Y	Journal of General Virology, Vol. 69, issued 1988, Buckmaster et al, "Gene Sequence and Mapping from Marek's Disease Virus and Herpevirus of Turkeys: Implications for Herpesvirus Classification",pages 2033-2042, see entire document.	1-21
Y	Journal of Virology, Vol. 51, issued July 1988, Fukuchi et al, "Structure of Marek's Disease Virus DNA: Detailed Restriction Enzyme Map," pages 102-109, see entire document.	1-21

* Special categories of cited documents:[15]

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search[2]	Date of Mailing of this International Search Report[2]
21 November 1991	19 DEC 1991
International Searching Authority[1]	**Signature of Authorized Officer[20]**
ISA/US	Sharon Nolan

Form PCT/ISA/210 (second sheet)(May 1986) 8

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE[1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _, because they relate to subject matter (1) not required to be searched by this Authority, namely:

2. ☐ Claim numbers _, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out (1), specifically:

3. ☐ Claim numbers _, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING[2]

This International Searching Authority found multiple inventions in this international application as follows:

I. Claims 1,2,4-15 drawn to a DNA, classified in class 536, subclass 27.
II.Claims 3,16-21 drawn to glycoprotein, classified in class 530, subclass 375.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Search Authority did not invite payment of any additional fee.

Remark on protest

☐ The additional search fees were accompanied by applicant's protest.

☒ No protest accompanied the payment of additional search fees.