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(54) **IDENTIFICATION DES GENES MODIFIES DANS LE MYELOME MULTIPLE**

(54) **IDENTIFICATION OF GENES ALTERED IN MULTIPLE MYELOMA**

(57) Cette invention concerne un procédé permettant d'établir le point de cassure chromosomique chez un sujet atteint de myélome multiple. Ledit procédé consiste (a) à prélever un échantillon d'ADN chez un sujet atteint de myélome multiple, (b) à déterminer s'il existe une disjonction J et C dans le gène des chaînes lourdes des immunoglobulines dudit échantillon, (c) à produire une banque génomique ayant des clones contenant des fragments d'ADN génomiques issus de l'échantillon d'ADN qui présente une disjonction J et C positive, (d) à sélectionner et à isoler des clones de ladite banque qui présentent une hybridation moléculaire positive au moyen d'une sonde capable de s'hybrider avec la région C et non avec la région J du gène des chaînes lourdes d'immunoglobulines, (e) à préparer des sondes fluorescentes à partir des fragments d'ADN génomiques des clones isolés au cours de l'étape (d), (f) à hybrider lesdites sondes fluorescentes à des chromosomes en métaphase, et (g) à établir l'identité des chromosomes qui sont capables de s'hybrider auxdites sondes fluorescentes, l'identification d'un chromosome autre qu'un chromosome 14 indiquant que le point de cassure chromosomique est compris entre le chromosome 14 et le chromosome identifié, ce qui permet d'établir le point de cassure chromosomique chez le sujet atteint de myélome multiple. Cette invention a également trait au gène identifié modifié par un point de cassure chromosomique ainsi qu'à diverses utilisations de ce gène.

(57) This invention provides a method of determining a chromosomal breakpoint in a subject suffering from multiple myeloma which comprises steps of: (a) obtaining a DNA sample from the subject suffering from multiple myeloma; (b) determining whether there is J and C disjunction in the immunoglobulin heavy chain gene in the obtained DNA sample; (c) obtaining a genomic library having clones which contain genomic DNA fragments from the DNA sample which shows positive J and C disjunction; (d) selecting and isolating clones of the obtained library which show positive hybridization with a probe which is capable of specifically hybridizing with the C but not the J region of the immunoglobulin heavy chain gene; (e) preparing fluorescent probes from the genomic DNA fragments of the isolated clones from step (d); (f) hybridizing said fluorescent probes with metaphase chromosomes; and (g) determining the identity of the chromosomes which are capable of hybridizing to said fluorescent probes, wherein the identification of a chromosome other than chromosome 14 would indicate that the chromosomal breakpoint is between chromosome 14 and the identified chromosome, thereby determining a chromosomal breakpoint in a subject suffering from multiple myeloma. This invention also provides the identified gene altered by a chromosomal breakpoint and various uses thereof.

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(54) Title: IDENTIFICATION OF GENES ALTERED IN MULTIPLE MYELOMA

(57) Abstract

This invention provides a method of determining a chromosomal breakpoint in a subject suffering from multiple myeloma which comprises steps of: (a) obtaining a DNA sample from the subject suffering from multiple myeloma; (b) determining whether there is J and C disjunction in the immunoglobulin heavy chain gene in the obtained DNA sample; (c) obtaining a genomic library having clones which contain genomic DNA fragments from the DNA sample which shows positive J and C disjunction; (d) selecting and isolating clones of the obtained library which show positive hybridization with a probe which is capable of specifically hybridizing with the C but not the J region of the immunoglobulin heavy chain gene; (e) preparing fluorescent probes from the genomic DNA fragments of the isolated clones from step (d); (f) hybridizing said fluorescent probes with metaphase chromosomes; and (g) determining the identity of the chromosomes which are capable of hybridizing to said fluorescent probes, wherein the identification of a chromosome other than chromosome 14 would indicate that the chromosomal breakpoint is between chromosome 14 and the identified chromosome, thereby determining a chromosomal breakpoint in a subject suffering from multiple myeloma. This invention also provides the identified gene altered by a chromosomal breakpoint and various uses thereof.

IDENTIFICATION OF GENES ALTERED IN MULTIPLE MYELOMA

The invention disclosed herein was made with Government support under NIH Grant No. CA 44025, CA-44029, and CA-34775. Accordingly, the U.S. Government has certain rights in this invention.

Background of the Invention

Throughout this application, various references are referred to within parentheses. Disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains. Full bibliographic citation for these references may be found at the end of this application, preceding the claims.

Multiple myeloma (MM) is an incurable B cell tumor affecting B cell end-stage differentiation. Clinically, the course of MM is similar to end-stage plasma cell leukemia (PCL), i.e., there is an uncontrollable proliferation of myeloma cells accompanied by numerous complications, including hyperviscosity syndromes, hypercalcemia, infections, multiple bone fractures, and organ failure.

Non-random chromosomal translocation is known to play a crucial role in the tumorigenesis of hematologic malignancies (1). In B-cell lymphomas, many important proto-oncogenes deregulated by juxtaposition to immunoglobulin (Ig) gene locus have been identified. Each proto-oncogene is associated with a specific subtype of lymphoma, such as c-MYC in Burkitt's lymphoma, Cyclin DI IBCLI in mantle cell lymphoma, BCL-2 in follicular lymphoma and BCL-6 in diffuse large cell lymphoma (2-8). In

contrast, little is known about molecular alterations of human MM/PCL, due to the difficulty in cytogenetic analysis. However, previous cytogenetic reports have shown a 14q+ chromosome, suggesting the existence of a chromosomal translocation involving the Ig heavy chain (IgH) locus, is observed in 20 ~ 30 % of the MM/PCL cases and it is the most frequent consistent abnormality (9-12). Even in such cases, most cytogenetic data have failed to identify donor chromosomes other than 11q13, 8q24, and 18q21, where proto-oncogenes Cyclin DIIBCL-IIPRADI, c-MYC and BCL-2 are located, respectively. Among them, the 11q13 locus has been demonstrated to be involved in nearly 5~10% of the cases and also in 62% of the established cell lines (13). The t(11;14)(q13;q32) translocation is also accompanied by a corresponding overexpression of the Cyclin D1 gene, which raises a strong possibility of the involvement of this gene, although the breakpoints at 11q13 do not cluster like those of the lymphoma cases (14-16). Recent advances in fluorescence in situ hybridization (FISH) have made it possible to clarify both the frequency of the 14q+ chromosomes and the partner chromosomes of the IgH loci. One such report revealed an intriguing result, i.e., that numerous chromosomal loci are able to translocate to IgH locus, including 6p21, 1q21, 3p11, 7q11, 11q23 (17). This has prompted a search for the proto-oncogenes deregulated by the regulatory elements of the IgH gene for a further understanding of the molecular mechanisms of MM/PCL. In the present study, one candidate proto-oncogene, MUM1 (multiple myeloma oncogene 1), was found juxtaposed to the IgH gene as a result of t(6;14)(p25; q32) translocation in human myeloma cell line, SKMM-1. Over expression of the MUM1 mRNA was observed in this cell line. A second gene, called MUM-2

was found translocated in proximity to the IgH gene on chromosome 14q32 in human myeloma cell line, U-266.

The method of analysis of 14q+ chromosomal translocations and identification of the genes altered in multiple myeloma of this invention are useful since 1) no method is currently available to determine the chromosomal sequences involved in 14q+ translocations, the most important cytogenetic lesions associated with MM pathogenesis; 2) no specific gene lesion is currently known for MM; 3) no diagnostic method based on gene/DNA lesion is currently available for MM and 4) there are no therapeutic approaches aimed at counteracting the action of abnormal gene products in MM.

Summary of the Invention

This invention provides a method of determining a chromosomal breakpoint in a subject suffering from multiple myeloma which comprises steps of: (a) obtaining a DNA sample from the subject suffering from multiple myeloma; (b) determining whether there is J and C disjunction in the immunoglobulin heavy chain gene in the obtained DNA sample; (c) obtaining a genomic library having clones which contain genomic DNA fragments from the DNA sample which shows positive J and C disjunction; (d) selecting and isolating clones of the obtained library which show positive hybridization with a probe which is capable of specifically hybridizing with the C but not the J region of the immunoglobulin heavy chain gene; (e) preparing fluorescent probes from the genomic DNA fragments of the isolated clones from step (d); (f) hybridizing said fluorescent probes with metaphase chromosomes; and (g) determining the identity of the chromosomes which are capable of hybridizing to said fluorescent probes, wherein the identification of a chromosome other than chromosome 14 would indicate that the chromosomal breakpoint is between chromosome 14 and the identified chromosome, thereby determining a chromosomal breakpoint in a subject suffering from multiple myeloma.

This invention provides a method to identify a gene other than the immunoglobulin gene which is located in chromosome 14, altered by a chromosomal breakpoint detected in a subject suffering from multiple myeloma which comprises steps of: a) selecting a probe having a sequence of a chromosome other than chromosome 14, identified at the chromosomal breakpoint detected in a subject suffering from multiple myeloma, wherein said probe is capable of hybridizing to the unique sequence of the gene other than

the immunoglobulin gene altered by a chromosomal breakpoint detected in a subject suffering from multiple myeloma; b) contacting said probe with mRNA isolated from a cell under conditions permitting formation of a complex between said probe and the mRNA; c) isolating the complex resulting from step (b); d) determining the sequence of the mRNA in the isolated complex, thereby determining the identity of the gene.

This invention provides a gene designated *MUM-1*. This invention provides a gene designated *MUM-2*. This invention provides an isolated nucleic acid molecule encoding a MUM protein. This invention provides a DNA encoding a MUM protein. This invention provides a cDNA encoding a MUM protein. This invention provides a genomic DNA molecule encoding a MUM protein. This invention provides a RNA molecule encoding a MUM protein. This invention provides an isolated nucleic acid molecule encoding a human MUM-1 protein. This invention provides an isolated nucleic acid molecule encoding a human MUM-2 protein. This invention provides an isolated nucleic acid molecule encoding a MUM protein operatively linked to a promoter of RNA transcription. This invention provides a vector comprising the an isolated cDNA encoding a MUM protein. This invention provides a vector which comprises an isolated cDNA encoding a MUM protein. This invention provides a vector which comprises an isolated cDNA encoding a MUM protein, wherein the vector is a plasmid. This invention provides a host cell for the vector which comprises an isolated cDNA encoding a MUM protein.

This invention provides a nucleic acid probe comprising a

nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a MUM protein. This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding a MUM protein.

This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding a MUM protein which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

This invention provides a nucleic acid probe comprising a the sequence of a nucleic acid molecule encoding a MUM-1 protein which is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14. This invention provides a nucleic acid probe comprising a the sequence of a nucleic acid molecule encoding a MUM-2 protein which is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14.

This invention provides a method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-1 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-1 protein. This invention provides a method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-2 protein in a sample from a subject which comprises detecting in a sample from the

subject a rearrangement of nucleic acid encoding MUM-2 protein.

This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding a human MUM-1 protein so as to prevent overexpression of the mRNA molecule. This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding a human MUM-2 protein so as to prevent overexpression of the mRNA molecule.

This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to an isolated cDNA molecule encoding a MUM protein. This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to the isolated genomic DNA molecule encoding a MUM protein. This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to an isolated RNA molecule encoding a MUM protein.

This invention provides a purified MUM protein. This invention provides a purified MUM-1 protein. This invention provides an antibody directed to a purified MUM-1 protein. This invention provides an antibody capable of specifically recognizing MUM-1 protein. This invention provides a purified MUM-2 protein. This invention provides an antibody directed to a purified MUM-2 protein. This invention provides an antibody capable of specifically recognizing a MUM-2 protein.

This invention provides a pharmaceutical composition

comprising an amount of an oligonucleotide effective to prevent overexpression of a human MUM-1 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane. This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide effective to prevent overexpression of a human MUM-2 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

Brief Description of the Figures

Figure 1. JH-Cμ dissociation in *Bam*HI digested DNA of the 14q+ SK-MM-1 cell line. A 10 μ g of the high molecular weight DNA was completely digested with *Bam*HI, loaded on each lane and blotted. The same filter was sequentially hybridized with JH, Cμ, Cγ2, and 0.7B/H probes. JH probe detects two rearranged bands of 12.0 kb and 9.7kb. The 9.7 kb band is comigrated with that probed with Cγ2 probe, suggesting it to be a physiological rearrangement. On the other hand, one allele of the Cμ locus is deleted and another is rearranged (6.5 kb) without being comigrated with rearranged bands of JH. Therefore, 12.0 kb and 6.5 kb bands detected by JH and Cμ (shown by arrowheads) might represent unknown derivative chromosome and derivative 14 chromosome, respectively. As expected, 0.7B/H probe (Fig. 2A) detected the rearranged band comigrated with 6.5kb band of Cμ. Dashed lines show the comigration. Size markers of λ/*Hin*dIII are shown on the left.

Figures 2A-B. Molecular cloning of the breakpoints of the t(6;14) translocation and germline walking at MUM1 locus. (A) Restriction maps of λSKB-4a and λSKS-3 clones representing derivative 6 and 14 are shown, together with germline maps of IgH locus at 14q32 and MUM1

locus at 6p25. Arrows indicate the chromosomal breakpoints. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII. (B) Comparison of the nucleotide sequences around the breakpoints on derivative 6 (SEQ ID NO: 16) and derivative 14 (SEQ ID NO: 17) chromosome. Homologous regions are indicated by dashes. The arrow indicates the breakpoint (SEQ ID NO: 15). Nucleotide (SEQ ID NO: 17) numbers shown below are the same as in the Sμ sequence reported by Sun, et al. (18).

Figure 3. Mapping of the MUM1 locus to chromosome 6p25. λMUM-3 genomic clone (Figure 2A) was used as a probe for *in situ* hybridization. The white arrow indicates the fluorescence signal on chromosome 6 band p25. Right panel shows the G-banding picture stained with DAPI.

Figures 4A-C. Expression of the MUM1 gene in hematopoietic lineage. A 10 ug aliquot of total RNA was loaded on each lane and Northern blot analysis was performed using the 2.1H probe (Figure 2A). GAPDH or β-actin probes were used to control for amount of RNA loaded. (A) MUM1 RNA expression in various hematopoietic cell lines. MUM1 RNA is detected in B cell and mature T cell lines as a single 6kb transcript. HELA, epithelial lineage; LCL, Epstein-Barr virus-transformed lymphoblastoid cell line; RAMOS and SK-MM-1,

B-cell lineage; HUT-78 and MOLT-4, T-cell lineage; HL-60 and U937, myelomonocytic lineage; K562, erythroid lineage. Dashes indicate 28S and 18S. (B) Expression in B cell lines derived from various stages of B cell differentiation. MUM1 RNA is seen throughout the B cell development except for BJAB cell line. 697, pre-B cell stage; RAMOS and BJA-B, Burkitt cell line representing mature-B cell stage; RPMI-8226 and U-266, plasma cell stage. (C) Comparison of the expression level among myeloma cell lines. MUM1 RNA is overexpressed in SK-MM-1 cell line carrying t(6;14). Overexpression of the MUM1 is also demonstrated in XG-4, XG-7, and XG-10 cell lines. RPMI-8226, U-266, EJM, and SKMM-1 are IL-6 (interleukin-6) independent lines, whereas XG-1, XG-2, XG-4, XG-5, XG-6, XG-7, and XG-10 are IL-6 dependent lines.

Figures 5A-B-3. Sequence of MUM1 cDNA and structure of its predicted protein product. (A) Restriction map of the MUM1 cDNA and the position of the open reading frame (box). The solid box indicates approximate position of the DNA binding domain. Sc, *Sac*II; A, *Apa*I; P, *Pst*I; H, *Hin*dIII; S, *Sac*I (B) Nucleotide sequence of the MUM1 cDNA (SEQ ID NO: 13) and corresponding amino acid sequence (SEQ ID NO: 14). Putative

translation initiation codons and preceding stop codons appearing in frame are underlined. The asterisk indicates the translation stop codon.

Figures 6A-B. Homology between MUM1 (SEQ ID NO: 1) and other IRF family proteins (SEQ ID NOS: 2-7). (A) Similarity at N-terminal DNA binding domain. Black background indicates identical residues found more than four times. Gray indicates conserved residues that appear in at least four sequences at a given position. Conserved tryptophan residues in DNA binding domain among IRF family members are indicated by closed circles. (B) Similarity at C-terminal region between human MUM1 (SEQ ID NO: 8), Mouse LSIRF/Pip (SEQ ID NO: 9), Human ICSBP (SEQ ID NO: 10), Human ISGF3γ (SEQ ID NO: 11), and Human IRF-3 (SEQ ID NO: 12). Black and gray background are as in (A).

Figure 7. Genomic organization of the MUM1 gene and location of the chromosomal breakpoints in multiple myeloma. Filled boxes indicate the coding regions and empty boxes indicate the noncoding regions. The position, and the size of each exon of the MUM1 gene are approximate and have been determined by the hybridizations. One exon in each restriction fragment may consist of more than two exons. Translation initiation

codon (ATG) and stop codon (TGA) are indicated. Genomic probes used for further investigations are shown as solid bars below the map. Arrows indicate the chromosomal breakpoints of SKMM-1 cell line and case 10. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII.

Figure 8. Scheme of the t(6;14)(p25;q32) translocation involving the MUM1 and the immunoglobulin heavy chain (IgH) gene loci. VH-D-J-CH indicates variable-diversity joining-constant region of the IgH gene. Direction of the MUM1 gene on the chromosome 6 is tentatively drawn.

Figure 9A-B. Demonstration of JH-Ca disjunction in U-266 cells and cloning of normal and 14q+ chromosomal breakpoints. (A) The panel shows the results of Southern blot analysis of BamHI digested U-266 and normal control (placenta) genomic DNA using the indicated JH and Cα probes. The arrowheads indicate two DNA fragments containing Cα sequences not linked to JH sequences, suggesting the presence of a chromosomal breakpoint in 14q32. (B) The panel provides a schematic representation of the phage clones isolated from a library constructed from U-266 DNA and screened with a Cα probe. Based on restriction enzyme analysis, the three cloned regions represent a normal Cα region (14q32 germ-line), and two rearranged

regions (der.14 and 14q32) containing unknown sequences linked to Cα sequences. The 2.5BE probe used for Northern blot analysis of MUM2 transcripts (Fig. 10) is also shown.

Figure 10. Identification of MUM2 RNA transcripts. The figure shows the results of a Northern blot analysis of RNA extracted from various MM/PCL cell lines using the 2.5BE probe (see Fig. 9) or GAPDH probe (as a control for RNA loading). A 1.9 Kb RNA transcript is detectable in some cell lines including U-266, indicating that the 2.5BE fragments represents part of a gene, MUM2.

Figure 11A-B. Schematic representation of IgH DNA rearrangements in normal B cells and in tumors carrying chromosomal translocations breaking the S region of the IgH locus. Note that in physiological IgH rearrangements (panel 11A) JH sequences and C sequences (Cμ before and Cγ after switch recombination, respectively) are consistently found within the same BamHI restriction fragment. Conversely, JH and C sequences are not linked, and are present on two different chromosomes [derivative X and derivative 14(14q+)] in cells carrying a chromosomal translocation breaking the switch region (panel 11B)

Figure 12A-B. MUM1 cDNA. cDNA insert is cloned into EcoRI/BamHI site of the pBluescript KS+. Bacteria strain used is DH5α cells. pcMUM1.16a contains full length open reading frame of nt. 217-1572.

Figure 13. Breakpoint Cloning of the U-266 Cell Line. pMUM2-8 has a 22.0 KB insert in BamHI site of pBluescript KS+.

Fig. 14A-B. Molecular cloning of the 14q+ chromosome in SK-MM-1 cell line. A. Southern blot analysis indicating illegitimate rearrangement in *IgH* locus. A 10 µg of high molecular weight DNA was completely digested with *Bam*HI, loaded on each lane and blotted. The same filter was sequentially hybridized with JH, Cµ Cγ2, and 0.7B/H (Fig. 14B) probes. Illegitimately rearranged bands detected by JH and Cµ probes are shown by arrowheads. 0.7B/H probe detected the rearranged band comigrated with 6.5kb band detected with Cµ probe. Dashed lines show the comigration. Size markers of λ/*Hin*dIII are shown on the left. B. SK-MM-1 cells carry cryptic t(6;14)(p25;q32). Restriction maps of λSKB-4a and λSKS-3 clones representing der (6) and der (14) are shown, together with germline maps of *IgH* locus at 14q32 and *MUM1* locus at 6p25. Genomic organization of the *MUM1* gene is shown. Filled boxes indicate the coding regions and empty boxes indicate

the noncoding regions. The position, and the size of each exon of the *MUM1* are approximate and have been determined by the hybridizations. One exon in each restriction fragment may consist of more than two exons. Translation initiation codon (ATG) and stop codon (TGA) are indicated. Genomic probes applied for Southern analyses are shown as solid bars below the map. Arrows indicate the chromosomal breakpoint of the SK-MM-1 cell line. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII.

Fig. 15A-D. A-B. Mapping *MUM1* locus to chromosome 6p25 in a metaphase spread of normal lymphocytes by 360kb non-chimeric YAC, y927E3 DNA. The arrowheads indicate the fluorescence signals on chromosome 6 band p25. C-D. Splitting of y927E3 DNA signal to chromosome 6p25 and 14q32 in a metaphase spread of XG-7 cell line. The arrowheads indicate positive signals. Right panel shows the corresponding DAPI image showing a G-banding like staining.

Fig. 16A-E Expression of *MUM1*. A 10 μg aliquot of total RNA was loaded on each lane and Northern blot analysis was performed using 2.1H probe (Fig. 14B). *GAPDH* or *β-ACTIN* probes were used to control for amount of RNA loaded. A. Left panel shows the distribution in various hematopoietic cell lines. B. Right panel shows expression in B-cell lines derived from various stages of differentiation. HELA,

epithelial lineage; LCL, Epstein-Barr virus-transformed lymphoblastoid cell line; HUT-78 and MOLT-4, T-cell lineage; HL-60 and U937, myelomonocytic lineage; K562, erythroid lineage; 697, pre-B cell stage; RAMOS and BJA-B, Burkitt lymphoma derived cell lines representing mature-B cell stage. Dashes indicate 28S and 18S. C-D. Comparison of the expression level among MM cell lines. D. indicates a densitometric quantitation of the *MUM1* mRNA corrected by *β-ACTIN* level. Mean values of black dots were indicated by horizontal bars. The expression level in SK-MM-1 and XG-7 cell line was indicated by open dot. IL-6(-); IL-6 independent MM cell lines, IL-6(+); IL-6 dependent MM cell lines. E. Identification of DNA-binding complexes containing MUM1 in nuclear extracts from MM cell lines. 1 μg of the nuclear extract from MM cell lines was assayed for binding to GBP-ISRE probe by EMSA. Arrows indicate MUM1-containing supershifted complexes performed by using an anti-MUM1/ICSAT antiserum. An anti-BCL6 antiserum was used as a control for specificity. Cold cognate oligomer competition was performed using a 100-fold excess of the GBP-ISRE probe.

Fig. 17A-B. A. Western blot analysis of Rat-1 cell clones with anti-HA (12CA5) MoAb. The 52 kD MUM1-HA product is shown by an arrowhead. B. Anchorage independent growth of Rat-1 cells expressing MUM1 protein. The number of the colonies in soft agar assay of Rat-1 cell clones expressing MUM1-HA and those

transfected with CMV control vector are shown. Error bar indicates +1SD.

5

Detailed Description of the Invention

The following standard abbreviations are used throughout the specification to indicate specific nucleotides:

C=cytosine	A=adenosine
T=thymidine	G=guanosine

This invention provides a method of determining a chromosomal breakpoint in a subject suffering from multiple myeloma which comprises steps of: (a) obtaining a DNA sample from the subject suffering from multiple myeloma; (b) determining whether there is J and C disjunction in the immunoglobulin heavy chain gene in the obtained DNA sample; (c) obtaining a genomic library having clones which contain genomic DNA fragments from the DNA sample which shows positive J and C disjunction; (d) selecting and isolating clones of the obtained library which show positive hybridization with a probe which is capable of specifically hybridizing with the C but not the J region of the immunoglobulin heavy chain gene; (e) preparing fluorescent probes from the genomic DNA fragments of the isolated clones from step (d); (f) hybridizing said fluorescent probes with metaphase chromosomes; and (g) determining the identity of the chromosomes which are capable of hybridizing to said fluorescent probes, wherein the identification of a chromosome other than chromosome 14 would indicate that the chromosomal breakpoint is between chromosome 14 and the identified chromosome, thereby determining a chromosomal breakpoint in a subject suffering from multiple myeloma.

In an embodiment, step (b) of the above described method of this invention is performed by Southern blotting. In another embodiment, step (b) of the above method of this

invention is performed by polymerase chain reaction (PCR) with appropriate probes. Polymerase chain reaction is well known in the art. Since the sequences of both the C and J regions of an immunoglobulin heavy chain gene are known, appropriate probes for PCR may routinely be designed.

In an embodiment, the genomic library is a phage vector library. In another embodiment, the genomic DNA fragments are generated by cleaving genomic DNA from cells of the subject with an appropriate restriction enzyme. In a further embodiment, the restriction enzyme is *Bam*HI. In an embodiment, the restriction enzyme is *Sau*3AI. In another embodiment, the probe of step (d) is a human IgH J region JH probe. In a further embodiment, the probe of step (d) is a human IgH Cμ probe. In an embodiment, the probe of step (d) is a human IgH Cγ2 probe. In another embodiment, the chromosomal breakpoint identified is a t(6;14)(p25;q32) translocation. In an embodiment, the chromosomal breakpoint identified is a t(1;14) translocation.

This invention provides a method to identify a gene other than the immunoglobulin gene which is located in chromosome 14, altered by a chromosomal breakpoint detected in a subject suffering from multiple myeloma which comprises steps of: a) selecting a probe having a sequence of a chromosome other than chromosome 14, identified at the chromosomal breakpoint detected in a subject suffering from multiple myeloma, wherein said probe is capable of hybridizing to the unique sequence of the gene other than the immunoglobulin gene altered by a chromosomal breakpoint detected in a subject suffering from multiple myeloma; b) contacting said probe with mRNA isolated from a cell under conditions permitting formation of a complex between said probe and the mRNA; c) isolating the complex resulting from step (b); and d) determining the sequence of the mRNA in the

isolated complex, thereby determining the identity of the gene.

In an embodiment, step (d) of the method to identify a gene other than the immunoglobulin gene which is located in chromosome 14, altered by a chromosomal breakpoint detected in a subject suffering from multiple myeloma comprises steps of: i) synthesizing complementary DNA to the mRNA; and ii) performing sequence analysis of the complementary DNA to determine the sequence of the mRNA.

This invention provides a gene identified by the method to identify a gene other than the immunoglobulin gene which is located in chromosome 14, altered by a chromosomal breakpoint detected in a subject suffering from multiple myeloma.

As used herein, "MUM" means any gene rearranged in 14q+ chromosomal abnormalities associated with multiple myeloma.

This invention provides a gene identified by the above method designated *MUM-1*. This invention provides a gene identified by the above method designated *MUM-2*.

This invention provides a gene identified by the above method, wherein the gene identified comprises a nucleic acid encoding a MUM protein. In an embodiment, the gene identified by the above method comprises a nucleic acid encoding a MUM-1 protein. In another embodiment, the gene identified by the above method comprises a nucleic acid encoding a MUM-2 protein.

This invention provides an isolated nucleic acid molecule encoding a MUM protein. In an embodiment, the isolated

nucleic acid molecule encoding a MUM protein is a DNA molecule. In another embodiment, the isolated nucleic acid molecule encoding a MUM protein is a cDNA molecule.

In an embodiment, a cDNA nucleic acid molecule encoding a MUM-1 protein is cloned into a pBluescript KS+ and the resulting plasmid is designated as pcMUM1-1.6a (ATCC Accession No. 97579). Plasmid pcMUM1-1.6a was deposited on May 28, 1996 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. Plasmid pcMUM1-1.6a was accorded ATCC Accession Number 97579.

In another embodiment, a partial cDNA nucleic acid molecule encoding a MUM-1 protein is cloned into a pBluescript KS+ and the resulting plasmid is designated as pMUM1-2.4B/N (ATCC Accession No. 97578). Plasmid pMUM1-2.4B/N was deposited on May 28, 1996 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. Plasmid pMUM1-2.4B/N was accorded ATCC Accession Number 97578.

In another embodiment, a partial cDNA nucleic acid molecule encoding a MUM-1 protein is cloned into a pBluescript KS+ and the resulting plasmid is designated as pMUM1-7.7B (ATCC Accession No. 97577). Plasmid pMUM1-7.7B was deposited on May 28, 1996 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852,

U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. Plasmid pMUM1-7.7B was accorded ATCC Accession Number 97577.

In another embodiment, a partial cDNA of the nucleic acid molecule encoding a MUM-2 protein is cloned into a pBluescript KS+ and the resulting plasmid is designated as pMUM2-8 (ATCC Accession No. 97580). Plasmid pMUM2-8 was deposited on May 28, 1996 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. Plasmid pMUM2-8 was accorded ATCC Accession Number 97580.

In an embodiment, the isolated DNA molecule encoding a MUM protein is a cDNA molecule having the nucleotide sequence shown in Figure 5B-1-5B-3 (SEQ. ID NO 13).

In an embodiment, the isolated DNA molecule encoding a MUM protein is genomic DNA molecule. In an embodiment, the isolated nucleic acid molecule encoding a MUM protein is an RNA molecule.

In an embodiment, the isolated nucleic acid encodes a human MUM-1 protein. In another embodiment, the isolated nucleic acid molecule encodes a human MUM-2 protein.

In an embodiment, isolated nucleic molecule encodes the a human MUM-1 protein having substantially the same amino acid sequence as shown in Figure 5B-1-5B-2 (SEQ. ID NO 14). In

an embodiment, isolated nucleic molecule encodes a human MUM-1 protein having the same amino acid sequence as shown in Figure 5B-1-B-2 (SEQ. ID NO 14). In an embodiment, the isolated nucleic acid molecule encoding a MUM protein is operatively linked to a promoter of RNA transcription.

This invention provides a vector comprising a cDNA molecule encoding a MUM protein. In an embodiment, a vector comprising cDNA encoding for MUM-1 is designated pcMUM1.6a. In an embodiment, a vector comprising partial cDNA encoding for MUM-1 is designated pMUM1.2.4B/N. In an embodiment, a vector comprising partial cDNA encoding for MUM-1 is designated pMUM1-7.7B. In an embodiment, a vector comprising partial cDNA encoding for MUM-2 is designated pMUM2-8. In an embodiment, a vector comprises genomic DNA encoding for MUM. In an embodiment, the vector is a plasmid. In an embodiment, a host cell comprises the vector comprising cDNA encoding for MUM. In an embodiment, a host cell comprises the vector comprising genomic DNA encoding for MUM. In a further embodiment, the host cell comprising vectors comprising cDNA encoding for MUM or comprising genomic DNA encoding for MUM is selected from a group consisting of a bacterial cell, a plant cell, and insect cell and a mammalian cell.

This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a MUM protein. This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding a MUM protein.

As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs.

In an embodiment, the nucleic acid probe specifically hybridizes with nucleic acid encoding MUM-1. In an embodiment, the nucleic acid probe is complementary to nucleic acid encoding MUM-1. In an embodiment, the nucleic acid probe specifically hybridizes with nucleic acid encoding MUM-2. In an embodiment, the nucleic acid probe is complementary to nucleic acid encoding MUM-2.

In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-1 is a DNA probe. In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-2 is a DNA probe.

In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-1 is a RNA probe. In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-2 is a RNA probe.

In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-1 is a genomic DNA probe. In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-2 is a genomic DNA probe.

In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-1 is labeled with a detectable marker. In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid

encoding MUM-2 is labeled with a detectable marker.

In an embodiment, the detectable marker is selected from the group consisting of a radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-1 is linked to a nucleic acid sequence capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule of human chromosome 14. In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-2 is linked to a nucleic acid sequence capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule of human chromosome 14.

This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding a MUM protein which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

In an embodiment, the nucleic acid probe comprises a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding a MUM-1 protein which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

In an embodiment, the nucleic acid probe comprises a nucleic acid molecule of at least 15 nucleotides which is

complementary to a sequence of the isolated nucleic acid molecule encoding a MUM-2 protein which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

In an embodiment, the nucleic acid probe comprises a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding a MUM-1 protein which is linked at a specific break point to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

In an embodiment, the nucleic acid probe comprises a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding a MUM-2 protein which is linked at a specific break point to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

In an embodiment, the specific break point of the nucleic acid probe comprises a portion of the t(6;14)(p25;q32) translocation. In an embodiment, the specific break point of the nucleic acid probe comprises a portion of a t(1;14) translocation. In an embodiment, the nucleic acid probe comprising a portion of the t(6;14)(p25;q32) translocation is labeled with a detectable marker. In an embodiment, the nucleic acid probe comprising a portion of a t(1;14) translocation is labeled with a detectable marker. In an embodiment, the nucleic acid probe comprising a portion of the t(6;14)(p25;q32) or comprising a portion of a t(1;14) translocation of claim 60, has a detectable marker selected

from the group consisting of a radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

This invention provides a method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-1 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-1 protein.

This invention provides a method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-2 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-2 protein.

In an embodiment, the rearrangement of nucleic acid encoding MUM-1 protein is detected by contacting the nucleic acid from the sample with a MUM-1 probe under conditions permitting the MUM-1 probe to hybridize with the nucleic acid encoding MUM-1 protein from the sample, thereby detecting the rearrangement of nucleic acid encoding MUM-1 protein in the sample.

In an embodiment, the rearrangement of nucleic acid encoding MUM-2 protein is detected by contacting the nucleic acid from the sample with a MUM-2 probe under conditions permitting the MUM-2 probe to hybridize with the nucleic acid encoding MUM-2 protein from the sample, thereby detecting the rearrangement of nucleic acid encoding MUM-2 protein in the sample.

In an embodiment, the rearrangement of nucleic acid encoding MUM-1 protein is detected by a MUM-1 probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding MUM-1 protein which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

In an embodiment, the rearrangement of nucleic acid encoding MUM-2 protein is detected by a the MUM-2 probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding MUM-2 protein which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 1.

In an embodiment, the MUM-1 probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding MUM-1 protein is linked at a specific break point to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

In an embodiment, the MUM-2 probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding MUM-2 protein is linked at a specific break point to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 1.

In an embodiment, the MUM-1 probe comprises a specific break point comprising a portion of the t(6;14)(p25;q32) translocation. In an embodiment, the MUM-2 probe comprises a specific break point comprising a portion of a t(1;14) translocation.

In an embodiment, the method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-1 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-1 protein comprises: a) obtaining DNA from the sample of the subject suffering from multiple myeloma; b) performing a restriction digest of the DNA with a panel of restriction enzymes; c) separating the resulting DNA fragments by size fractionation; d) contacting the resulting DNA fragments with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-1 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-1 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 and labeled with a detectable marker; e) detecting labeled bands which have hybridized to the nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-1 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-1 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 to create a unique band pattern specific to the DNA of subjects suffering from multiple myeloma; f) preparing DNA obtained from a sample of a subject for diagnosis by steps (a-e); and g) comparing the detected band pattern specific to the DNA obtained from a sample of subjects suffering from multiple myeloma from step (e) and the DNA obtained from a sample of the subject for diagnosis from step (f) to determine whether the patterns are the same or different and to diagnose thereby predisposition to multiple myeloma if the patterns are the same.

In an embodiment, the method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-2 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-2 protein comprises: a) obtaining DNA from the sample of the subject suffering from multiple myeloma; b) performing a restriction digest of the DNA with a panel of restriction enzymes; c) separating the resulting DNA fragments by size fractionation; d) contacting the resulting DNA fragments with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-2 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-2 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 and labeled with a detectable marker; e) detecting labeled bands which have hybridized to the nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-2 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-2 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 to create a unique band pattern specific to the DNA of subjects suffering from multiple myeloma; f) preparing DNA obtained from a sample of a subject for diagnosis by steps (a-e); and g) comparing the detected band pattern specific to the DNA obtained from a sample of subjects suffering from multiple myeloma from step (e) and the DNA obtained from a sample of the subject for diagnosis from step (f) to determine whether the patterns are the same or different and to diagnose thereby predisposition to multiple myeloma if the patterns are the same.

In an embodiment, the size fractionation in step (c) is effected by a polyacrylamide or agarose gel. In an embodiment, the detectable marker is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

In an embodiment, the method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-1 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-1 protein comprises: a) obtaining RNA from the sample of the subject suffering from multiple myeloma; b) separating the RNA sample by size fractionation; c) contacting the resulting RNA species with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-1 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-1 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 and labeled with a detectable marker; d) detecting labeled bands which have hybridized to the RNA species to create a unique band pattern specific to the RNA of subjects suffering from multiple myeloma; e) preparing RNA obtained from a sample of a subject for diagnosis by steps (a-d); and f) comparing the detected band pattern specific to the RNA obtained from a sample of subjects suffering from multiple myeloma from step (d) and the RNA obtained from a sample of the subject for diagnosis from step (f) to determine whether the patterns are the same or different and to diagnose thereby predisposition to multiple myeloma if the patterns are the same.

In an embodiment, the method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-2 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-2 protein comprises: a) obtaining RNA from the sample of the subject suffering from multiple myeloma; b) separating the RNA sample by size fractionation; c) contacting the resulting RNA species with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-2 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-2 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 15 and labeled with a detectable marker; d) detecting labeled bands which have hybridized to the RNA species to create a unique band pattern specific to the RNA of subjects suffering from multiple myeloma; e) preparing RNA obtained from a sample of a subject for diagnosis by steps (a-d); and f) comparing the detected band pattern specific to the RNA obtained from a sample of subjects suffering from multiple myeloma from step (d) and the RNA obtained from a sample of the subject for diagnosis from step (f) to determine whether the patterns are the same or different and to diagnose thereby predisposition to multiple myeloma if the patterns are the same.

In an embodiment, the size fractionation in step (b) is effected by a polyacrylamide or agarose gel. In an embodiment, the detectable marker is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

In an embodiment, multiple myeloma associated with the

expression of a specific human MUM-1 is diagnosed by the method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-1 protein in a DNA or RNA sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-1 protein.

In an embodiment, multiple myeloma associated with the expression of a specific human MUM-2 is diagnosed by the method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-12 protein in a DNA or RNA sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-2 protein.

This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding a human MUM-1 protein so as to prevent overexpression of the mRNA molecule. This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding a human MUM-2 protein so as to prevent overexpression of the mRNA molecule.

This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to the cDNA molecule encoding a MUM protein. This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to the genomic DNA molecule encoding a MUM protein. This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to the RNA molecule encoding a MUM protein.

This invention provides a purified MUM protein. This invention provides a purified MUM-1 protein. This invention provides a purified human MUM-1 protein. This invention provides an antibody directed to a purified MUM-1 protein. This invention provides an antibody capable of specifically recognizing MUM-1 protein. In an embodiment, the antibody capable of specifically recognizing MUM-1 protein is a human MUM-1 protein.

This invention provides a purified MUM-2 protein. This invention provides a purified human MUM-2 protein. This invention provides an antibody directed to a purified MUM-2 protein. This invention provides an antibody capable of specifically recognizing MUM-2 protein. In an embodiment, the antibody capable of specifically recognizing MUM-2 protein is a human MUM-2 protein.

In an embodiment, the antibody directed to a purified MUM-1 protein is a monoclonal antibody. In an embodiment, the antibody capable of specifically recognizing MUM-1 protein is a monoclonal antibody. In an embodiment, the antibody capable of specifically recognizing MUM-1 protein is a human MUM-1 protein.

In an embodiment, the antibody directed to a purified MUM-2 protein is a monoclonal antibody. In an embodiment, the antibody capable of specifically recognizing MUM-2 protein is a monoclonal antibody. In an embodiment, the antibody capable of specifically recognizing MUM-2 protein is a human MUM-2 protein.

This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide having a

sequence capable of specifically hybridizing to an mRNA molecule encoding a human MUM-1 protein so as to prevent overexpression of the mRNA molecule effective to prevent overexpression of a human MUM-1 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide having a sequence capable of specifically hybridizing to a cDNA molecule encoding a MUM protein effective to prevent overexpression of a human MUM-1 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide having a sequence capable of specifically hybridizing to a genomic DNA molecule effective to prevent overexpression of a human MUM-1 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding a human MUM-2 protein so as to prevent overexpression of the mRNA molecule effective to prevent overexpression of a human MUM-2 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide having a

sequence capable of specifically hybridizing to a cDNA molecule encoding a MUM protein effective to prevent overexpression of a human MUM-2 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide having a sequence capable of specifically hybridizing to a genomic DNA molecule effective to prevent overexpression of a human MUM-2 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

EXPERIMENTAL DETAILS

Materials and Methods

Cell lines. The following myeloma cell lines were used in the present study: SK-MM-1, RPMI-8226, U266, EJM, XG-1, XG-2, XG-4, XG-5, XG-6, XG-7, and XG-10. The RPMI-8226 cell line was obtained through the American Type Culture Collection (ATCC, Rockville, MD). SKMM-1 and U-266 cell lines were gifts from Dr. A. N. Houghton and Dr. K. Nilsson, respectively (18; 12). Characterization of these cell lines were previously reported. Six XG cell lines were gifts from Dr. B. Klein and were cultured in RPMI 1640 containing 10% fetal calf serum (FCS), Sx10-smol/L 2-ME, and rIL-6(1ng/mL)(13;19). Other myeloma cell lines used were all IL-6 independent. The SK-MM-1 cell line was used to isolate the chromosomal breakpoint carrying the 14q+ chromosome without any information on the donor chromosome. XG-1, XG-2, XG-6, XG-8 cell lines are reported to carry the t(11;14)(q13;q32) translocation. XG-5 cells also share both t(11;14) and t(8;14)(q24;q32).

Southern and Northern blot analyses. Southern blot analysis was performed as previously described (21). Briefly, ten micrograms of high molecular-weight DNA extracted from each cell line was digested to completion with *Bam*HI and *Hind*III restriction enzymes, size- fractionated on 0.7% agarose gel, and transferred onto Duralose nitrocellulose membrane (Stratagene) according to the manufacturer's instructions. Blots were hybridized with a random-primed DNA probe and washed at 60°C in 0.2 x SSC and 0.1 % SDS for 5 minutes. Genomic probes used in this study were as follows; human IgH

J region JH probe (6.6kb *Bam*HI-*Hin*dIII fragment) was provided by Dr. J. V. Ravetch, human IgH Cμ probe (1.3 kb *Eco*RI fragment) was provided by Dr. S.J. Korsmeyer. Human IgH region Cγ2 probe was provided by Dr. C. Croce.

Northern blot analysis was performed as described previously (21). Briefly, a 10 μg aliquot of total RNA was loaded on each lane and probed with a 2.1H probe of the MUMI gene (Figure 2A). GAPDH or β-actin probes were used as controls for amount of total RNA.

Genomic library. High molecular-weight DNA of SK-MM-1 cell line was digested completely with *Bam*HI and partially with *Sau*3AI, and size-fractionated by using a low-melting point agarose gel. DNA ranging from 10kb to 23kb were purified and ligated into the *Bam*HI sites of λ-DASH II phage vector (Stratagene, La Jolla, CA). After packaging, 3 x 10^5 and 6 x 10^5 recombinant clones of the *Bam*HI digested library and partially digested library were screened with JH and Cμ probes, respectively. To isolate the germline region of the 6p25 locus, a commercially available human placental library (Stratagene) was screened. Positive clones were mapped with restriction enzymes by partial digestion of the phage DNAs followed by probing with T7 and T3 primers labeled with T4 polynucleokinase and 32p-γATP.

cDNA library. A phage library constructed by oligo-dT and random-priming normal human spleen RNA (Clontech) was screened by 2.1H probe (Figure 2A) to isolate initial MUMl cDNA clones. After the first round of screening, positive clones were used as probes to walk to the 5' side using the same library. Positive clones were subcloned into

pBluescript and analyzed for mapping and sequencing.

DNA sequencing. DNA sequences were determined by the dideoxy chain termination method and analyzed by an ABI(Applied Biosystems) autosequencer. Deletion mutants for sequencing were prepared using exonuclease III and mung bean nuclease. cDNA sequences were analyzed with the Genetics Computer Group (GCG) programs. Sequence homology searches were carried out through the BLAST E-mail server at the National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD.

Fluorescence in situ hybridization (FISH). Metaphase chromosome from human lymphocytes were prepared. A biotin-labeled probe was prepared by nick-translation using Bio-16-dUTP. Conditions for hybridization and washing were described previously (22).

Experimental Results

IgH gene rearrangement of the SK-MM-I cell line. In *Bam*HI digestion, the JH probe detects two rearranged bands of the size of 12.0 kb and 9.7kb (Fig 1). The 9.7 kb band is comigrated with that probed with Cγ2 probe, suggesting it to be a physiological rearrangement, although this cell line secretes only λ chain. One allele of the Cμ locus is deleted and another is rearranged (6.5 kb) without being comigrated with rearranged bands of JH. Hybridization with a Cα probe showed only the germline band (data not shown). These results suggested the possibility of the chromosomal breakpoint between JH and Cμ locus. Hence, the 12.0 kb and the 6.5 kb bands detected by JH and Cμ were considered to

represent unknown derivative chromosome and derivative 14 chromosome, respectively.

Molecular cloning of the t(6;14)(p25;q32) breakpoint. A genomic library constructed with *Bam*HI complete digestion was screened with a JH probe to isolate the 12.0 kb *Bam*HI band. Another library constructed with *Sau*3AI partial digestion was screened with a Cμ probe to isolate phage clones containing the 6.5 kb *Bam*HI fragment. Two phage clones, λSKB-4a and λ SKS-3, considered to represent the unknown derivative and derivative 14 chromosomes respectively, were obtained (Fig 2A). A 0.7 kb *Bam*HI-*Hin*dIII probe (0.7B/H) of the λSKS-3 was used to confirm the comigration with the rearranged 6.5 kb Cμ band by Southern analysis (Fig 1). The chromosomal origin of the centromeric side of the λSKB-4a and telomeric side of the λSKS-3 were confirmed by hybridization to a somatic cell hybrid DNA panel with a 4.5 kb ApaI fragment(4.5A) and 2.1 kb HindIII(2.1H) probes. Both probes showed positive signals in hybrid cell DNA containing a human chromosome 6 (data not shown). These probes were also used to isolate the germline chromosome 6 region by screening the human placental genomic library. One of the phage clone DNA (λMUM-3) was used as a probe for FISH analysis. It identified the localization of this region to be chromosome 6 short arm p25 (Fig 3). To investigate the precise breakpoint within the IgH gene, a 1.5 kb *Hin*dIII-*Eco*RI fragment of the λSKS-3, containing the breakpoint on derivative 14 chromosome was sequenced. The breakpoint was confirmed to be just 3' to the switch μ(S μ) repetitive sequences [SEQ ID NO: 15] (Fig 2B). Nucleotide sequencing of the region around the breakpoints of chromosome 6 and

derivative 6 chromosome showed that the chromosomal translocation was reciprocal with minimum deletion of both the IgH and 6p25 sequences.

Transcriptional unit in the vicinity of the 6p25 breakpoint. An attempt to find a functional transcriptional unit in the vicinity of the breakpoints was made. Although a 4.5A probe on derivative 6 chromosome could not detect any transcripts, a 2.1H probe on derivative 14 chromosome detected a single 6 kb transcript in the SK-MM-1 cell line. Accordingly, this gene was designated as *MUM1* (multiple myeloma oncogene 1). The same probe was used to study the expression of the MUM1 gene in various hematopoietic cell lines. The 6 kb message was expressed at high levels in most B cell lines and at low levels in peripheral T cell lines (Fig 4A). Cell lines derived from immature T cells, the myelomonocytic lineage, and erythroid lineage do not seem to express *MUM1*. In B cells, MUM1 appears to be expressed throughout the development from the preB cell stage to the plasma cell stage (Fig 4B). However, some of the Burkitt's lymphoma derived cell lines such as BJA-B did not express this gene (data not shown). The expression level of the MUM1 transcript in myeloma cell lines was also examined (Fig 4C). The SK-MM-1 cell line showed a 7.5-fold overexpression when compared with the other three IL-6 independent cell lines, suggesting a deregulated expression of the translocated allele. It is of interest that the IL-6 dependent XG-4, XG-7, and XG-10 cell lines are also expressing at high levels. Particularly, expression in the XG-7 cell line is 19.9 times the average of the aforementioned control cell lines.

MUMI cDNA cloning, sequencing, and homology search. Human spleen cDNA library was initially screened with a 2.1H probe followed by three times walking to 5' side using cDNA probes. A 5.5kb cDNA, approximately corresponding to the size detected by Northern analysis was isolated. This cDNA contained a 1,353 base pair open reading frame (ORF) and a long 3' untranslated region (Fig 5A). The ORF encodes for a protein of 451 amino acids with a predicted molecular weight of 50 kD (Fig 5B-1-B-2; SEQ ID NO:14). The putative ATG initiation codon at position 217 has G at the -3 position which corresponds to the Kozak consensus sequence (23). The ORF is preceded by two in-frame stop codons. A database search demonstrated a significant similarity between MUM-1 ORF and the interferon regulatory factor (IRF) family proteins. The NH_2-terminal of the MUM-1 ORF (SEQ ID NO:1) shares a high homology with all of the IRF family proteins (SEQ ID NOS: 2-7) which share a characteristic DNA binding motif consisting of the conserved 5 tryptophan residues (Fig 6A). The COOH-terminal (SEQ ID NO:8) also has a high homology with ICSBP (SEQ ID NO:10)(interferon consensus sequence binding protein)(21), ISGF3γ (SEQ ID NO:11)(interferon-stimulated gene factor-3 gamma)(22), and IRF-3 protein (SEQ ID NO:12)(23)(Fig 6B), although it did not have any homologous regions with IRF-1 and IRF-2 protein. The highest similarity (95.1%) and identity (91.8%) were found with a possible mouse homolog, LSIRF (SEQ ID NOS:2 and 9)(lymphoid specific interferon regulatory factor)/Pip (PU-1 cofactor protein-1)(24,25). A high similarity was found with ICSBP (63.98%), ISGF3γ (55.8%), and IRF3 (50.1%) among the human IRF family protein members. A gene sequence encoding a nearly identical protein was recently deposited in GenBank. This gene, termed

ICSAT (interferon consensus sequence binding protein in adult T-cell leukemia cell lines or activated T cells) is likely to be the same gene as MUM1 (26).

Breakpoints at MUM1 locus in multiple myeloma.

In order to analyze the exact location of the SK-MM-1 breakpoint at the 6p25 locus and to explore the frequency of the MUM1 gene involvement in myeloma cases, we walked nearly 55kb in a human placental genomic phage library around the MUM1 gene and determined the rough exon-intron structure as shown in Figure 7 (Fig. 7). The SK-MM-1 breakpoint was located 3' to the last exon, containing **a** poly A additional signal, consistent with an unaltered size of the MUM1 transcript of this cell line in Northern analysis. Seven repeat-free genomic probes shown in Figure 7 have been used to investigate the rearrangement in Southern analyses of the 11 MM cell lines and 18 MM cases. One case (case 10) displayed rearranged bands in *Bam*HI and *Xba*I digests when analyzed using a 0.9A probe located at 3' to the MUM1 gene.

Cloning of the MUM2 locus from the U-266 multiple myeloma cell line.

Using an experimental strategy analogous to the one described for the cloning of the MUM1 gene from the SK-MM-1 cell line, a second genetic locus altered in multiple myeloma (MUM2) was identified by analyzing the U-266 multiple myeloma cell line. Briefly, Southern blot analysis using BamH restiction digestion and various Ig probes showed that U-266 DNA contained two rearranged fragments (shown by arrowheads in Fig. 9) containing Cα sequences and lacking J sequences. These two fragments (der 14 and 14q32 in Fig. 9) were cloned from a genomic library constructed from U-266

DNA along with a normal 14q 32 locus (14q32 germline in Fig. 9). In order to determine whether a gene was located in proximity to the chromosomal breakpoints in der 14, the 2.5 BE restriction fragment (see Fig. 9), which was at the opposite side of the Ig Ca sequences, was used to probe a Northern blot carrying RNA from various MM cell lines. The results (Fig. 10) showed that a 1.9 kb mRNA was detectable in some of these cell lines including U-266. This result showed that a gene, called MUM2, normally not present within the Ig locus on chromosome 14q32, had been translocated in proximity of the Ig locus in U-266 cells. Since the Ig locus contains strong transcriptional regulatory elements, it is likely that the expression of this gene is deregulated in these cells. The structure of the MUM2 gene and its protein are currently under investigation. The 2.5 BE probe and other probes derived from the der 14 phage can be used to screen MM cases for MUM2 rearrangements as shown for MUM1 (Fig. 7).

Experimental Discussion

Using the experimental strategies used for the identification of the MUM1 and MUM2 genes in the SK-MM-1 and U-266 cell lines, respectively, it is possible to analyze most MM cases and isolate the corresponding genes. The scheme shown in Fig. 11 shows that the physiological IgH gene rearrangements (Fig. 11A) typically maintain linkage of C and J sequences and this linkage becomes detectable by using an appropriate restriction enzyme digestion (BamHI in the example in Fig. 11). Conversely, chromosomal translocations (14q+) affecting the IgH locus on 14q32 lead to breakage of the C-J linkage and the two sets of sequences

appear on distinct restriction fragments. (Fig. 11B) Table 1 shows the application of this analysis to a panel of MM cell lines and biopsies. The results show that at least 65% of cases show breakage of the C-J linkage within Ig J or switch regions. The restiction fragments containing either C or J sequences (R in Table 1) can be cloned as shown for the SK-MM-1 and U-266 cell lines and the genes flanking the chromosomal breakpoints can be used as probes to screen additional MM cases for similar rearrangements, whereas the sequence of the genes can be used to understand the consequences of these genetic lesions in multiple myeloma. Cloning of the chromosomal breakpoints and corresponding genes is currently ongoing for all of the MM cases shown in Table 1.

The method of analysis of 14q+ chromosomal translocations and identification of the genes altered in multiple myeloma of this invention will allow 1) the determination of chromosomal sequences involved in 14q+ translocations, the most important cytogenetic lesion associated with MM pathogenesis elucidation; 2) elucidation of specific gene lesions for MM; 3) a diagnostic method based on gene/DNA lesion and 4) a therapeutic approach aimed at counteracting the action of abnormal gene products.

Table 1. Summary of JH-C breakage analysis in MM cell lines and biopsies (cases). Rearrangement (R) involving physiologic Ig recombinations, i.e. retaining JH-C linkage are marked as R*; rearrangements lacking JH-C linkage, and therefore suggesting a 14q+ chromosomal breakpoint, are marked as R. The latter represents candidates for cloning an further analysis.

Cell Line/Case	sIg	JH	Cμ	Cα	Sγ3'	possible breakpoint locus
RPMI-8226		λ	D/D	D/D	G	R/G	Sγ
U-266		Eλ	R/D	D/D	R/R/G	G	Sα
EJM		Gλ	R/R*	D/D	G	R*/G	JH~Sμ
XG-1		Aκ	R*/D	D/D	R*	G	ND
XG-2		Gλ	R*/D	D/D	G	R*/G	ND
XG-4		Gκ	R*/R	D/D	G	R*/G	J H ~ S μ
XG-5		λ	R/D	D/D	G	G	JH~Sμ
XG-6		Gλ	R*/R	D/D	G	R*/G	JH~Sμ
XG-7		Aκ	R/D	D/D	R/G	R/D	Sγ
XG-10	G	R*/R	D/D	G	R*/G	JH~Sμ	
SK-MM-1	κ	R*/R	R/D	G	R*/G	JH~Sμ	
CASE125		R*	G	G	R*	ND
CASE33		R/R*	G	G	R/R*	Sγ
CASE34		R*	G	R*	G	ND
CASE93		R*	G	R*	G	ND
CASE91		R*	R*	R	G	Sα
CASE128		R*	G	R*	G	ND

R*, comigrated bands with JH; R, target bands to isolate; ND, not determined

Possible breakage in switch regions:

Cell Lines	4/11 (**36%**)	
Cases	2/6 (**33%**)	Total 6/17 (**35%**)

Possible breakage in JH ~ switch regions:

Cell Lines	9/11 (**82%**)	
Cases	2/6 (**33%**)	Total 11/17 (**65%**)

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Second Series of Experiments

The pathogenesis of multiple myeloma (MM), an incurable tumor affecting terminally differentiated B cells, is unknown (1). Chromosomal translocation (14q+) affecting 14q32 and unidentified partner chromosome(s) are common in this tumor (2,3), suggesting that they may cause the activation of novel oncogenes. By cloning and mapping the chromosomal breakpoints in a MM cell line, this study shows that the 14q+ translocation represents a t(6;14)(p25;q32) and that this aberration is recurrent in MM since it was found in 2 of 11 MM cell lines. The translocation juxtaposes the immunoglobulin heavy-chain (*IgH*) locus to the *MUM1* (*mu*ltiple *m*yeloma oncogene *1*)/*IRF4* gene, a member of the interferon regulatory factor (IRF) family involved in the control of B cell proliferation and differentiation. As a result, the *MUM1/IRF4* gene is overexpressed, an event that may contribute to tumorigenesis since we show that *MUM1/IRF4* has oncogenic activity in vitro. These findings identify a novel genetic alteration associated with MM with implications for the pathogenesis and diagnostics of this tumor.

Analysis of MM karyotypes by fluorescence *in situ* hybridization (FISH) using probes from the *IgH* locus have shown that chromosomal translocations involving the *IgH* locus on band 14q32 (14q+) are common in MM (62% of cases) (4). A recent study has shown that most 14q+ chromosomes represent translocations of a variety of loci into the *IgH* switch region (5), but except for a fraction of cases involving the *BCL-1* locus on chromosome 11q13 (6), the genes involved in the partner chromosomes have not been identified. In order to investigate the nature of the sequences linked to the *IgH* locus in 14q+ chromosomes, a number of cell lines were screened for the presence of

abnormally rearranged *IgH* loci which could reflect the presence of chromosomal translocations. A Southern blot hybridization assay was used aimed at distinguishing rearranged *IgH* alleles containing *IgH* J regions (JH) linked to C regions (representing physiologically rearranged *IgH* loci) from those in which JH and C sequences are not linked, as these alleles reflect illegitimate *IgH* switch recombinations often representing chromosomal translocations. Using this assay, 9 of 11 MM lines tested showed evidence of illegitimate switch recombinations (not shown).

Among these cell lines, the pattern of *IgH* gene rearrangements detectable in the SK-MM-1 strongly suggested the presence of a chromosomal translocation (Fig. 14A). *Bam*HI digestion of SK-MM-1 genomic DNA and hybridization with JH probe showed two rearranged restriction fragments (12.0 and 9.7kb, Fig. 14A). One (9.7 kb) was hybridized also with a Cγ2 probe, suggesting it to be derivation from a physiological rearrangement, while the second (12.0 kb) was not hybridized with any C probe. Conversely, hybridization with a Cμ probe led to the identification of a 6.5 kb fragment which did not contain any J sequences. Hence, the 12.0 kb and 6.5 kb bands detected by JH and Cμ respectively were considered to represent potential chromosomal breakpoints and cloned from a phage library constructed from SK-MM-1 genomic DNA. Two phage clones (λSKB-4a; λSKS-3; Fig. 14B) were representative of the two rearranged fragments detected in SK-MM-1 DNA in that they displayed *Bam*HI restriction fragments containing JH and Cμ sequences not reciprocally linked as well as sequences which based on their restriction map could not derive from the *IgH* locus. A 0.7B/H probe from the λSKS-3 phage insert representing non-*IgH* sequences (Fig. 14B) hybridized to a *Bam*HI fragment comigrating with that containing Cμ sequences

in SK-MM-1 DNA, indicating its derivation from the abnormally rearranged Cμ fragment (Fig. 14A). The same probe was used to clone its corresponding normal locus from a library constructed from normal human DNA (Fig. 14B). This locus was assigned the name *MUM1* (multiple myeloma oncogene 1) by the Nomenclature committee of the Genome Data Base for the gene locus in humans.

The *MUM1* locus was mapped to chromosome 6 by hybridization of the 2.1H and 4.5A probes to a somatic cell hybrid panel representative of individual human chromosomes (not shown). Using a 360kb non-chimeric YAC (y927E3) spanning the *MUM1* region, the *MUM1* locus was further submapped to 6p25 by FISH analysis (Fig. 15A-B). The same YAC probe hybridized to the 14q+ chromosome as well as to a der(6) chromosome (as well as to normal chromosomes 6) in SK-MM-1 cells (not shown) as well in a second MM cell line (XG-7) (Fig. 15C-D), while no abnormality was detectable in additional 9 MM lines. Taken together, these observations indicated that the 14q+ chromosome present in SK-MM-1 and XG-7 cells was part of a reciprocal t(6;14)(p25;q32) translocation. This abnormality is recurrent in MM since it was detectable in 2 of 11 cases tested.

Next it was investigated whether the *MUM1* locus adjacent to the chromosomal breakpoints contained a transcriptional unit. Probe 2.1H detected a major 6 kb RNA in B-cell lines representative of various stages of B cell differentiation (from preB cells through to plasma cells), as well as in lines displaying a mature T cell phenotype (Fig. 16A-B). This result indicates that 6p25 sequences immediately adjacent to the chromosomal breakpoints are part of a gene (*MUM1*) which is transcribed in lymphoid tissues.

The corresponding cDNA was cloned from a cDNA library derived from normal human spleen library. Nucleotide sequence analysis of clones spanning 5.5 kb *MUM1* cDNA sequences showed an open reading frame (ORF) encoding for 451 amino acids with a predicted molecular weight of 50 kD (data not shown). An homology search in the human gene data bank revealed that *MUM1* was virtually identical to the *IRF4* gene (7-10), a member of IRF family of transcription factors.

The *MUM1/IRF4* cDNA was used to determine the exon-intron organization of the corresponding genomic locus (Fig. 14B). This allowed to determine that the chromosomal breakpoint was located 3' to the *MUM1/IRF4* gene in SK-MM-1 cells (Fig. 14B). Probes spanning 55kb of the *MUM1* locus including the entire *MUM1/IRF4* gene failed to detect rearrangements in 11 MM cell lines and 18 MM cases studied (not shown), including the XG-7 lines which was shown to contain a t(6;14)(p25;q32) by FISH analysis. These results indicate that, analogous to other translocations involving *Ig* genes, the breakpoints of t(6;14)(p25;q32) can scatter along 6p25 at variable distance from the *MUM1* locus.

To investigate the consequences of chromosomal translocation on *MUM1/IRF4* expression, comparisons were made of the levels of *MUM1/IRF4* RNA in MM cell lines carrying t(6;14)(p25;q32) (SK-MM-1, XG-7) or lacking detectable 6p25 abnormalities. In general, the levels of *MUM1/IRF4* RNA expression tended to be higher (3.4-fold) in interleukin-6 (IL-6) dependent than in IL-6 independent MM cell lines (Fig. 16C-D). However, SK-MM-1 and the XG-7 cell line displayed the highest levels of *MUM1/IRF4* expression, a 7.5- and a 6-fold overexpression compared to the average of other MM cell lines (Fig. 16C-D). To study whether in the same cell lines increased *MUM1/IRF4* RNA expression was associated with increased levels of

protein expression, analysis was made of the levels of MUM1/IRF4 specifically bound to DNA by electrophoretic mobility shift analysis (EMSA) of nuclear extracts using a IRF binding site as a probe (GBP-ISRE). GBP-IRSE-bound MUM1/IRF4 could not be detected by EMSA in any of the cell lines tested as it is occasionally the case with other transcription factors which form unstable complexes with DNA in vitro; however, these complexes could be stabilized by the addition of anti- MUM-1/IRF-4 antibodies (but not by control antibodies) and became visible as "supershift" bands (Fig. 16E). Both cell lines carrying alterations of the *MUM1* locus (SK-MM-1 and XG-7) showed higher amounts (3 to 3.5-fold) of DNA-bound MUM1/IRF4 than control MM lines, suggesting that overexpression of the *MUM1/IRF4* gene was associated with increased amounts of functional MUM1/IRF4 protein.

To investigate whether *MUM1/IRF4* overexpression could contribute to malignant transformation, it was tested whether transfection of a *MUM1/IRF4* expression vector in Rat-1 fibroblasts could increase their clonogenic properties in agar, a typical sign of malignant transformation. The oncogenic properties on cellular growth was studied. Rat-1 cells were transfected with an expression vector (CMV-MUM1-HA) in which a CMV promoter drives the expression of MUM1/IRF4 tagged by a hemagglutinin (HA) epitope recognizable by a specific monoclonal antibody. Fig. 17 shows that CMV-MUM1-HA transfected Rat-1 clones and expressing detectable levels of exogenous MUM1-HA protein (Fig. 17A), but not control (CMV) transfected clones, acquired anchorage independent growth in soft agar (Fig. 17A-B). These results indicate that *MUM1/IRF4* can behave as an oncogene in vitro and support the notion that it may contribute to oncogenesis in vivo.

Chromosomal translocations involving the *MUM1/IRF4* gene represent the first lesion specifically associated with MM. Several studies have shown that gene alterations commonly associated with most human tumors, such as inactivation of the *p53* tumor suppressor gene and *RAS* oncogene mutations, can be found in MM (11,12). However, these lesions are found at low frequency and in association with advanced stages of the disease, suggesting that their role may be limited to tumor progression. The association with 14q+, a cytogenetic aberration typical of lymphoid malignancies and often detectable at diagnosis (13), suggest that *MUM1/IRF4* deregulation may be specifically associated with early stages of MM development.

A role of *MUM1/IRF4* deregulation in oncogenesis is supported by its oncogenic activity in vitro (Fig. 17) as well as by a number of observations on the biological function of IRFs (14). Various members of this family of transcription factors have been shown to be involved in the control of cell proliferation, differentiation and apoptosis in response to cytokines such as interferon (IFN) and IL-6 (15-18). Some members of the IRF family of genes have been directly implicated in the pathogenesis of various tumors, including *ICSBP*, whose loss is associated with chronic myelogeneous leukemia (CML)(19-21). Targeted disruption in the mouse germ-line has shown that *IRF4* itself controls the differentiation of B cells into plasma cells (22). Since its function does not appear to be regulated by IFN (7,8), MUM1/IRF4 may act as an effector of other cytokines which regulate B cell differentiation. Interestingly, *MUM1/IRF4* gene expression appears to be regulated by IL-6 (Fig. 16 and data not shown), suggesting that its deregulated expression may contribute to the IL-6 autocrine loop that has been shown to support MM cell growth (23).

The observation that the *MUM1* locus is involved in a subset, but not all 14q+ alterations underscores the biological heterogeneity of MM. This heterogeneity may correlate with the heterogeneous clinical behaviour of MM. FISH analysis of MM cases using probes for the *MUM1* locus may be useful to investigate the frequency of *MUM1* alterations in large panels of MM cases and to determine whether this lesion identifies a prognostically distinct subset of tumors.

EXPERIMENTAL DETAILS

Methods

Cell lines and patient samples. The MM cell lines used in this study are as follows: SK-MM-1, RPMI-8226, U-266, EJM, XG-1, XG-2, XG-4, XG-5, XG-6, XG-7, and XG-10. RPMI-8226 cell line was obtained through American Type Culture Collection (ATCC, Rockville, MD). SK-MM-1 (24) and U-266 (25) cell lines are gifts from Dr. A. N. Houghton (Memorial Sloan-Kettering Cancer Center, New York, NY) and Dr. K. Nilsson (University of Uppsala, Uppsala, Sweden), respectively. Six XG cell lines were cultured in the presence of 1ng/mL recombinant IL-6 (rIL-6) as described previously (26).

Southern and Northern blot analyses. Southern and Northern blot analyses were performed as described previously (27). Genomic probes used in this study were as follows; human *IgH* JH probe (6.6kb *Bam*HI-*Hin*dIII) and *IgH* Cμ probe (1.3 kb *Eco*RI) were provided by Dr. S. J. Korsmeyer (Washington University, St Louis, MO). Human *IgH* Cγ2 probe (4.0 kb *Hin*dIII-*Bam*HI) was provided by Dr. V. Bertness (National Cancer Institute, Bethesda, MD).

Cloning breakpoints, cDNA library screening and DNA

sequencing. Genomic libraries from SK-MM-1 and human placental DNAs were constructed as described previously (27). Oligo-dT and random-primed human spleen library (Clontech, Palo Alto, CA) was screened by 2.1H probe (Fig. 14B) to isolate initial *MUM1* cDNA clones. After the first round of screening, positive clones were used as probes for walking. Positive clones were subcloned into pBluescript and analyzed for mapping and sequencing. DNA sequences were performed as described previously (26). Sequence homology searches were carried out through the BLAST E-mail server at the National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD.

FISH and isolation of *MUM1* YAC. Preparation of metaphase spreads and detailed procedure of FISH and Yeast Artificial Chromosome (YAC) DNA extraction were reported previously (27). PCR primers (sense 5'-TACTCGCACCTCTTGGCT-3'; antisense 5'-CTGGAGAGCAATGAACGG-3') derived from the 6p25 sequence within the last exon of the *MUM1* gene were used to screen a human CEPH YAC DNA pools (Research Genetics, Huntsville, AL).

Electrophoretic mobility shift assay (EMSA). Preparation of nuclear extract, EMSA and antibody-mediated supershift assays were performed as described (28). GBP-ISRE sequence used as a probe is 5'-AAGTACTTTCAGTTTCATATT-3' (7). Binding buffer used in EMSA was 10mM Tris-HCl (pH 7.5), 50mM KCl, 1mM dithiothreitol, 1mM EDTA, 0.1% Triton X-100 and 12.5% glycerol. Antiserum against ICSAT/MUM1 was a gift from Drs. T. Yamagata and H. Hirai (Tokyo University, Tokyo, Japan).

Expression constructs. The *MUM1* cDNA (nt.1~1,461) encoding a full length ORF was connected in-frame to the COOH-terminal HA (MAYPYDVPDYASLGPGP) tag, blunt-ended by

Klenow enzyme, and cloned into blunt-ended *Not* I site of pHeBo-CMV eukaryotic expression vector.

Western blot analysis. Cell pellets prepared from MM and Rat-1 cells were resuspended in 1 x Laemmli sample buffer and boiled for 5 minutes. Protein lysates derived from 10^6 cells were fractionated on an SDS 12.5% acrylamide gel and transferred to a nitrocellulose membrane (Schleicher & Schuell, Keene, NH). The filter blocked with 5% milk in Tris-buffered saline (TBS)-0.2% Tween was incubated for overnight at 4°C with a 1/500 anti-HA (12CA5; Boehringer Mannheim, Indianapolis, IN) in TBS-0.1% Tween with 3% bovine serum albumin followed by incubation with a 1/3,000 antimouse IgG, horseradish peroxidase-linked antibody (Amersham, Arlington Heights, IL) in TBS-0.1% Tween with 5% milk. Reactive bands were detected using an ECL system (Amersham).

Rat-1 cell transfection and soft agar assay. Rat-1 cells plated at 1 x 10^6 per 100-mm dish in Dulbecco modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) were transfected with 3pmol of either pHeBo-CMV or pHeBo-CMV-*MUM1*-HA by modified calcium phosphate method (29). Forty-eight hours after transfection, cells were reseeded into five 100-mm dishes and cultured in DMEM supplemented with 10% FBS containing 600μg/ml of G418. G418-resistant colonies were isolated after 10 days of selection. Analysis of clonogenecity was performed as described previously (29). In brief, Rat-1 cells were plated in triplicate agar plates (0.3% in DMEM plus 10% FBS) at 2.5 x 10^3, 5 x 10^3, and 1 x 10^4 cells per dish onto 35-mm agar plates containing DMEM, 10% FBS, and 0.5% agar. After incubation for 2 weeks at 37°C, 5% CO_2, colonies larger than 0.25mm were counted.

GenBank accession number. *MUM1*cDNA: U63738; 5' promoter:

U63739.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK

(ii) TITLE OF INVENTION: IDENTIFICATION OF GENES ALTERED IN MULTIPLE MYELOMA

(iii) NUMBER OF SEQUENCES: 22

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 2,256,455
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

```
Lys Leu Arg Gln Trp Leu Ile Asp Gln Ile Asp Ser Gly Lys Tyr Pro
1               5                  10                  15

Gly Leu Val Trp Glu Asn Glu Glu Lys Ser Ile Phe Arg Ile Pro Trp
            20                  25                  30

Lys His Ala Gly Lys Gln Asp Tyr Asn Arg Glu Glu Asp Ala Ala Leu
        35                  40                  45

Phe Lys Ala Trp Ala Leu Phe Lys Gly Lys Phe Arg Glu Gly Ile Asp
    50                  55                  60

Lys Pro Asp Pro Pro Thr Trp Lys Thr Arg Leu Arg Cys Ala Leu Asn
65                  70                  75                  80

Lys Ser Asn Asp Phe Glu Glu Leu Val Glu Arg Ser Gln Leu Asp Ile
                85                  90                  95

Ser Asp Pro Tyr Lys Val Tyr Arg Ile Val Pro Glu
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

```
Lys Leu Arg Gln Trp Leu Ile Asp Gln Ile Asp Ser Gly Lys Tyr Pro
1               5                  10                  15

Gly Leu Val Trp Glu Asn Glu Glu Lys Ser Val Phe Arg Ile Pro Trp
            20                  25                  30

Lys His Ala Gly Lys Gln Asp Tyr Asn Arg Glu Glu Asp Ala Ala Leu
        35                  40                  45
```

Phe Lys Ala Trp Ala Leu Phe Lys Gly Lys Phe Arg Glu Gly Ile Asp
50 55 60

Lys Pro Asp Pro Pro Thr Trp Lys Thr Arg Leu Arg Cys Ala Leu Asn
65 70 75 80

Lys Ser Asn Asp Phe Glu Glu Leu Val Glu Arg Ser Gln Leu Asp Ile
85 90 95

Ser Asp Pro Tyr Lys Val Tyr Arg Ile Val Pro Glu
100 105

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

Arg Met Arg Pro Trp Leu Glu Met Gln Ile Asn Ser Asn Gln Ile Pro
1 5 10 15

Gly Leu Ile Trp Ile Asn Lys Glu Glu Met Ile Phe Gln Ile Pro Trp
20 25 30

Lys His Ala Ala Lys His Gly Trp Asp Ile Asn Lys Asp Ala Cys Leu
35 40 45

Phe Arg Ser Trp Ala Ile His Thr Gly Arg Tyr Lys Ala Gly Glu Lys
50 55 60

Glu Pro Asp Pro Lys Thr Trp Lys Ala Asn Phe Arg Cys Ala Met Asn
65 70 75 80

Ser Leu Pro Asp Ile Glu Glu Val Lys Asp Gln Ser Arg Asn Lys Gly
85 90 95

Ser Ser Ala Val Arg Val Tyr Arg Met Leu Pro Pro
100 105

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

```
Arg Met Arg Pro Trp Leu Glu Glu Gln Ile Asn Ser Asn Thr Ile Pro
1               5                  10                  15

Gly Leu Lys Trp Leu Asn Lys Glu Lys Lys Ile Phe Gln Ile Pro Trp
            20                  25                  30

Met His Ala Ala Arg His Gly Trp Asp Val Glu Lys Asp Ala Pro Leu
        35                  40                  45

Phe Arg Asn Trp Ala Ile His Thr Gly Lys His Gln Pro Gly Val Asp
    50                  55                  60

Lys Pro Asp Pro Lys Thr Trp Lys Ala Asn Phe Arg Cys Ala Met Asn
65                  70                  75                  80

Ser Leu Pro Asp Ile Glu Glu Val Lys Asp Lys Ser Ile Lys Lys Gly
                85                  90                  95

Asn Asn Ala Phe Arg Val Tyr Arg Met Leu Pro Leu
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

```
Arg Leu Arg Gln Trp Leu Ile Glu Gln Ile Asp Ser Ser Met Tyr Pro
1               5                  10                  15

Gly Leu Ile Trp Glu Asn Glu Glu Lys Ser Met Phe Arg Ile Pro Trp
            20                  25                  30
```

Lys His Ala Gly Lys Gln Asp Tyr Asn Gln Glu Val Asp Ala Ser Ile
35 40 45

Phe Lys Ala Trp Ala Val Phe Lys Gly Lys Phe Lys Glu Gly Asp Lys
50 55 60

Ala Glu Pro Ala Thr Trp Lys Thr Arg Leu Arg Cys Ala Leu Asn Lys
65 70 75 80

Ser Pro Asp Phe Glu Glu Val Thr Asp Arg Ser Gln Leu Asp Ile Ser
85 90 95

Glu Pro Tyr Lys Val Tyr Arg Ile Val Pro Glu
100 105

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

Lys Leu Arg Asn Trp Val Val Glu Gln Val Glu Ser Gly Gln Phe Pro
1 5 10 15

Gly Val Cys Trp Asp Asp Thr Ala Lys Thr Met Phe Arg Ile Pro Trp
20 25 30

Lys His Ala Gly Lys Gln Asp Phe Arg Glu Asp Gln Asp Ala Ala Phe
35 40 45

Phe Lys Ala Trp Ala Ile Phe Lys Gly Lys Tyr Lys Glu Gly Asp Thr
50 55 60

Gly Gly Pro Ala Val Trp Lys Thr Arg Leu Arg Cys Ala Leu Asn Lys
65 70 75 80

Ser Ser Glu Phe Lys Glu Val Pro Glu Arg Gly Arg Met Asp Val Ala
85 90 95

Glu Pro Tyr Lys Val Tyr Gln Leu Leu Pro Pro
100 105

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 106 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

```
Arg Ile Leu Pro Trp Leu Val Ser Gln Leu Asp Leu Gly Gln Leu Glu
1               5                  10                  15

Gly Val Ala Trp Val Asn Lys Ser Arg Thr Arg Phe Arg Ile Pro Trp
            20                  25                  30

Lys His Gly Leu Arg Gln Asp Ala Gln Gln Glu Asp Phe Gly Ile Phe
        35                  40                  45

Gln Ala Trp Ala Glu Ala Thr Gly Ala Tyr Val Pro Gly Arg Asp Lys
    50                  55                  60

Pro Asp Leu Pro Thr Trp Lys Arg Asn Phe Arg Ser Ala Leu Asn Arg
65                  70                  75                  80

Lys Glu Gly Leu Arg Leu Ala Glu Asp Arg Ser Lys Asp Pro His Asp
                85                  90                  95

Pro His Lys Ile Tyr Glu Phe Val Asn Ser
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 95 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

```
Lys Arg Leu Cys Gln Ser Thr Ile Tyr Trp Asp Gly Pro Leu Ala Leu
1               5                  10                  15

Cys Asn Asp Arg Pro Asn Lys Leu Glu Arg Asp Gln Thr Cys Lys Leu
            20                  25                  30
```

```
Phe Asp Thr Gln Gln Phe Leu Ser Glu Leu Gln Ala Phe Ala His His
        35                  40                  45

Gly Arg Ser Leu Pro Arg Phe Gln Val Thr Leu Cys Phe Gly Glu Glu
    50                  55                  60

Phe Pro Asp Pro Gln Arg Gln Arg Lys Leu Ile Thr Ala His Val Glu
65                  70                  75                  80

Pro Leu Leu Ala Arg Gln Leu Tyr Tyr Phe Ala Gln Gln Asn Ser
                85                  90                  95
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 95 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

```
Lys Arg Leu Cys Gln Ser Arg Ile Tyr Trp Asp Gly Pro Leu Ala Leu
1               5                   10                  15

Cys Ser Asp Arg Pro Asn Lys Leu Glu Arg Asp Gln Thr Cys Lys Leu
            20                  25                  30

Phe Asp Thr Gln Gln Phe Leu Ser Glu Leu Gln Val Phe Ala His His
        35                  40                  45

Gly Arg Pro Ala Pro Arg Phe Gln Val Thr Leu Cys Phe Gly Glu Glu
    50                  55                  60

Phe Pro Asp Pro Gln Arg Gln Arg Lys Leu Ile Thr Ala His Val Glu
65                  70                  75                  80

Pro Leu Leu Ala Arg Gln Leu Tyr Tyr Phe Ala Gln Gln Asn Thr
                85                  90                  95
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 96 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

```
Lys Arg Leu Cys Gln Gly Arg Val Phe Cys Ser Gly Asn Ala Val Val
1               5                  10                  15

Cys Lys Gly Arg Pro Asn Lys Leu Glu Arg Asp Glu Val Val Gln Val
            20                  25                  30

Phe Asp Thr Ser Gln Phe Phe Arg Glu Leu Gln Gln Phe Tyr Asn Ser
        35                  40                  45

Gln Gly Arg Leu Pro Asp Gly Arg Val Val Leu Cys Phe Gly Glu Glu
    50                  55                  60

Phe Pro Asp Met Ala Pro Leu Arg Ser Lys Leu Ile Leu Val Gln Ile
65                  70                  75                  80

Glu Gln Leu Tyr Val Arg Gln Leu Ala Glu Glu Ala Gly Lys Ser Cys
                85                  90                  95
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 96 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

```
Gln Arg Leu Cys Pro Ile Pro Ile Ser Trp Asn Ala Pro Gln Ala Pro
1               5                  10                  15

Pro Gly Pro Gly Pro His Leu Leu Pro Ser Asn Glu Cys Val Glu Leu
            20                  25                  30

Phe Arg Thr Ala Tyr Phe Cys Arg Asp Leu Val Arg Tyr Phe Gln Gly
        35                  40                  45

Leu Gly Pro Pro Pro Lys Phe Gln Val Thr Leu Asn Phe Trp Glu Glu
    50                  55                  60
```

Ser His Gly Ser Ser His Thr Pro Gln Asn Leu Ile Thr Val Lys Met
65 70 75 80

Glu Gln Ala Phe Ala Arg Tyr Leu Leu Glu Gln Thr Pro Glu Gln Gln
85 90 95

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 100 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

Gln Arg Leu Gly His Cys His Thr Tyr Trp Ala Val Ser Glu Glu Leu
1 5 10 15

Leu Pro Asn Ser Gly His Gly Pro Asp Gly Glu Val Pro Lys Asp Lys
20 25 30

Glu Gly Gly Val Phe Asp Leu Gly Pro Phe Ile Val Asp Leu Ile Thr
35 40 45

Phe Thr Glu Gly Ser Gly Arg Ser Pro Arg Tyr Ala Leu Trp Phe Cys
50 55 60

Val Gly Glu Ser Trp Pro Gln Asp Gln Pro Trp Thr Lys Arg Leu Val
65 70 75 80

Met Val Lys Val Val Pro Thr Cys Leu Arg Ala Leu Val Glu Met Ala
85 90 95

Arg Val Gly Gly
100

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5176 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 217..1569

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

```
GCCTGACCAA CATGGTAAAA CCCCATCTCT GCTAAAACTA CAAAAAATTA GCTGGATGTG      60

GTGGCAGGGA ACCTGTCATC CCAGCTAGTT GGGAGACTGA GGCAGGAGAA TCGCTCGATC     120

TTGGGACCCA CCGCTGCCCT CAGCTCCGAG TCCAGGGCGA GTGCAGAGCA CAGCGGGCGG     180

AGGACCCCGG GCGCGGGCGC GGACGGCACG CGGGGC ATG AAC CTG GAG GGC GGC       234
                                        Met Asn Leu Glu Gly Gly
                                          1               5

GGC CGA GGC GGA GAG TTC GGC ATG AGC GCG GTG AGC TGC GGC AAC GGG       282
Gly Arg Gly Gly Glu Phe Gly Met Ser Ala Val Ser Cys Gly Asn Gly
             10                  15                  20

AAG CTC CGC CAG TGG CTG ATC GAC CAG ATC GAC AGC GGC AAG TAC CCC       330
Lys Leu Arg Gln Trp Leu Ile Asp Gln Ile Asp Ser Gly Lys Tyr Pro
         25                  30                  35

GGG CTG GTG TGG GAG AAC GAG GAG AAG AGC ATC TTC CGC ATC CCC TGG       378
Gly Leu Val Trp Glu Asn Glu Glu Lys Ser Ile Phe Arg Ile Pro Trp
     40                  45                  50

AAG CAC GCG GGC AAG CAG GAC TAC AAC CGC GAG GAG GAC GCC GCG CTC       426
Lys His Ala Gly Lys Gln Asp Tyr Asn Arg Glu Glu Asp Ala Ala Leu
 55                  60                  65                  70

TTC AAG GCT TGG GCA CTG TTT AAA GGA AAG TTC CGA GAA GGC ATC GAC       474
Phe Lys Ala Trp Ala Leu Phe Lys Gly Lys Phe Arg Glu Gly Ile Asp
                 75                  80                  85

AAG CCG GAC CCT CCC ACC TGG AAG ACG CGC CTG CGG TGC GCT TTG AAC       522
Lys Pro Asp Pro Pro Thr Trp Lys Thr Arg Leu Arg Cys Ala Leu Asn
             90                  95                 100

AAG AGC AAT GAC TTT GAG GAA CTG GTT GAG CGG AGC CAG CTG GAC ATC       570
Lys Ser Asn Asp Phe Glu Glu Leu Val Glu Arg Ser Gln Leu Asp Ile
         105                 110                 115

TCA GAC CCG TAC AAA GTG TAC AGG ATT GTT CCT GAG GGA GCC AAA AAA       618
Ser Asp Pro Tyr Lys Val Tyr Arg Ile Val Pro Glu Gly Ala Lys Lys
     120                 125                 130

GGA GCC AAG CAG CTC ACC CTG GAG GAC CCG CAG ATG TCC ATG AGC CAC       666
Gly Ala Lys Gln Leu Thr Leu Glu Asp Pro Gln Met Ser Met Ser His
135                 140                 145                 150
```

```
CCC TAC ACC ATG ACA ACG CCT TAC CCT TCG CTC CCA GCC CAG CAG GTT      714
Pro Tyr Thr Met Thr Thr Pro Tyr Pro Ser Leu Pro Ala Gln Gln Val
                155                 160                 165

CAC AAC TAC ATG ATG CCA CCC CTC GAC CGA AGC TGG AGG GAC TAC GTC      762
His Asn Tyr Met Met Pro Pro Leu Asp Arg Ser Trp Arg Asp Tyr Val
            170                 175                 180

CCG GAT CAG CCA CAC CCG GAA ATC CCG TAC CAA TGT CCC ATG ACG TTT      810
Pro Asp Gln Pro His Pro Glu Ile Pro Tyr Gln Cys Pro Met Thr Phe
        185                 190                 195

GGA CCC CGC GGC CAC CAC TGG CAA GGC CCA GCT TGT GAA AAT GGT TGC      858
Gly Pro Arg Gly His His Trp Gln Gly Pro Ala Cys Glu Asn Gly Cys
    200                 205                 210

CAG GTG ACA GGA ACC TTT TAT GCT TGT GCC CCA CCT GAG TCC CAG GCT      906
Gln Val Thr Gly Thr Phe Tyr Ala Cys Ala Pro Pro Glu Ser Gln Ala
215                 220                 225                 230

CCC GGA GTC CCC ACA GAG CCA AGC ATA AGG TCT GCC GAA GCC TTG GCG      954
Pro Gly Val Pro Thr Glu Pro Ser Ile Arg Ser Ala Glu Ala Leu Ala
                235                 240                 245

TTC TCA GAC TGC CGG CTG CAC ATC TGC CTG TAC TAC CGG GAA ATC CTC     1002
Phe Ser Asp Cys Arg Leu His Ile Cys Leu Tyr Tyr Arg Glu Ile Leu
            250                 255                 260

GTG AAG GAG CTG ACC ACG TCC AGC CCC GAG GGC TGC CGG ATC TCC CAT     1050
Val Lys Glu Leu Thr Thr Ser Ser Pro Glu Gly Cys Arg Ile Ser His
        265                 270                 275

GGA CAT ACG TAT GAC GCC AGC AAC CTG GAC CAG GTC CTG TTC CCC TAC     1098
Gly His Thr Tyr Asp Ala Ser Asn Leu Asp Gln Val Leu Phe Pro Tyr
    280                 285                 290

CCA GAG GAC AAT GGC CAC AGG AAA AAC ATT GAG AAC CTG CTG AGC CAC     1146
Pro Glu Asp Asn Gly His Arg Lys Asn Ile Glu Asn Leu Leu Ser His
295                 300                 305                 310

CTG GAG AGG GGC GTG GTC CTC TGG ATG GCC CCC GAC GGG CTC TAT GCG     1194
Leu Glu Arg Gly Val Val Leu Trp Met Ala Pro Asp Gly Leu Tyr Ala
                315                 320                 325

AAA AGA CTG TGC CAG AGC ACG ATC TAC TGG GAC GGG CCC CTG GCG CTG     1242
Lys Arg Leu Cys Gln Ser Thr Ile Tyr Trp Asp Gly Pro Leu Ala Leu
            330                 335                 340

TGC AAC GAC CGG CCC AAC AAA CTG GAG AGA GAC CAG ACC TGC AAG CTC     1290
Cys Asn Asp Arg Pro Asn Lys Leu Glu Arg Asp Gln Thr Cys Lys Leu
        345                 350                 355

TTT GAC ACA CAG CAG TTC TTG TCA GAG CTG CAA GCG TTT GCT CAC CAC     1338
Phe Asp Thr Gln Gln Phe Leu Ser Glu Leu Gln Ala Phe Ala His His
    360                 365                 370
```

```
GGC CGC TCC CTG CCA AGA TTC CAG GTG ACT CTA TGC TTT GGA GAG GAG     1386
Gly Arg Ser Leu Pro Arg Phe Gln Val Thr Leu Cys Phe Gly Glu Glu
375                 380                 385                 390

TTT CCA GAC CCT CAG AGG CAA AGA AAG CTC ATC ACA GCT CAC GTA GAA     1434
Phe Pro Asp Pro Gln Arg Gln Arg Lys Leu Ile Thr Ala His Val Glu
                395                 400                 405

CCT CTG CTA GCC AGA CAA CTA TAT TAT TTT GCT CAA CAA AAC AGT GGA     1482
Pro Leu Leu Ala Arg Gln Leu Tyr Tyr Phe Ala Gln Gln Asn Ser Gly
            410                 415                 420

CAT TTC CTG AGG GGC TAC GAT TTA CCA GAA CAC ATC AGC AAT CCA GAA     1530
His Phe Leu Arg Gly Tyr Asp Leu Pro Glu His Ile Ser Asn Pro Glu
        425                 430                 435

GAT TAC CAC AGA TCT ATC CGC CAT TCC TCT ATT CAA GAA TGAAAAATGT      1579
Asp Tyr His Arg Ser Ile Arg His Ser Ser Ile Gln Glu
    440                 445                 450

CAAGATGAGT GGTTTTCTTT TTCCTTTTTT TTTTTTTTTT TTTTGATACG GAGATACGGG   1639

GTCTTGCTCT GTCTCCCAGG CTGGAGTGCA GTGACACAAT CTCAGCTCAC TGTGACCTCC   1699

GCCTCCTGGG TTCAAGAGAC TCTCCTGCCT CAGCCTCCCT GGTAGCTGGG ATTACAGGTG   1759

TGAGCCACTG CACCCACCCA AGACAAGTGA TTTTCATTGT AAATATTTGA CTTTAGTGAA   1819

AGCGTCCAAT TGACTGCCCT CTTACTGTTT TGAGGAACTC AGAAGTGGAG ATTTCAGTTC   1879

AGCGGTTGAG GAGAATTGCG GCGAGACAAG CATGGAAAAT CAGTGACATC TGATTGGCAG   1939

ATGAGCTTAT TTCAAAAGGA AGGGTGGCTT TGCATTTTCT TGTGTTCTGT AGACTGCCAT   1999

CATTGATGAT CACTGTGAAA ATTGACCAAG TGATGTGTTT ACATTTACTG AAATGCGCTC   2059

TTTAATTTGT TGTAGATTAG GTCTTGCTGG AAGACAGAGA AAACTTGCCT TTCAGTATTG   2119

ACACTGACTA GAGTGATGAC TGCTTGTAGG TATGTCTGTG CCATTTCTCA GGGAAGTAAG   2179

ATGTAAATTG AAGAAGCCTC ACACGTAAAA GAAATGTATT AATGTATGTA GGAGCTGCAG   2239

TTCTTGTGGA AGACACTTGC TGAGTGAAGG AAATGAATCT TTGACTGAAG CCGTGCCTGT   2299

AGCCTTGGGG AGGCCCATCC CCCACCTGCC AGCGGTTTCC TGGTGTGGGT CCCTCTGCCC   2359

CACCCTCCTT CCCATTGGCT TTCTCTCCTT GGCCTTTCCT GGAAGCCAGT TAGTAAACTT   2419

CCTATTTTCT TGAGTCAAAA AACATGAGCG CTACTCTTGG ATGGGACATT TTTGTCTGTC   2479

CTACAATCTA GTAATGTCTA AGTAATGGTT AAGTTTTCTT GTTTCTGCAT CTTTTTGACC   2539

CTCATTCTTT AGAGATGCTA AAATTCTTCG CATAAAGAAG AAGAAATTAA GGAACATAAA   2599

TCTTAATACT TGAACTGTTG CCCTTCTGTC CAAGTACTTA ACTATCTGTT CCCTTCCTCT   2659
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GTGCCACGCT CCTCTGTTTG TTTGGCTGTC CAGCGATCAG CCATGGCGAC ACTAAAGGAG 2719

GAGGAGCCGG GGACTCCCAG GCTGGAGAGC ACTGCCAGGA CCCACCACTG GAAGCAGGAT 2779

GGAGCTGACT ACGGAACTGC ACACTCAGTG GGCTGTTTCT GCTTATTTCA TCTGTTCTAT 2839

GCTTCCTCGT GCCAATTATA GTTTGACAGG GCCTTAAAAT TACTTGGCTT TTTCCAAATG 2899

CTTCTATTTA TAGAAATCCC AAAGACCTCC ACTTGCTTAA GTATACCTAT CACTTACATT 2959

TTTGTGGTTT TGAGAAAGTA CAGCAGTAGA CTGGGGCGTC ACCTCCAGGC CGTTTCTCAT 3019

ACTACAGGAT ATTTACTATT ACTCCCAGGA TTCAGCAGAA GATTGCGTTA GCTCTCAAAT 3079

GTGTGTTCCT GCTTTTCTAA TGGATATTTT AAATTCATTC AACAAGCACC TAGTAAGTGC 3139

CTGCTGTATC CCTACATTAC ACAGTTCAGC CTTTATCAAG CTTAGTGAGC AGTGAGCACT 3199

GAAACATTAT TTTTTAATGT TTAAAAAGTT TCTAATATTA AAGTCAGAAT ATTAATACAA 3259

TTAATATTAA TATTAACTAC AGAAAAGACA AACAGTAGAG AACAGCAAAA AAATAAAAAG 3319

GATCTCCTTT TTTCCCAGCC CAAATTCTCC TCTCTAAAAG TGTCCACAAG AAGGGGTGTT 3379

TATTCTTCCA ACACATTTCA CTTTTCTGTA AATATACATA AACTTAAAAA GAAAACCTCA 3439

TGGAGTCATC TTGCACACAC TTTTCATGCA GTGCTCTTTG TAGCTAAACA GTGAAGATTT 3499

ACCTCGTTCT GCTCAGAGGC CTTGCTGTGG AGCTCCACTG CCATGTACCC AGTAGGGTTT 3559

GACATTTCAT TAGCCATGCA ACATGGATAT GTATTGGGCA GCAGACTGTG TTTCGTGAAC 3619

TGCAGTGATG TATACATCTT ATAGATGCAA AGTATTTTGG GGTATATTAT CCTAAGGGAA 3679

GATAAAGATG ATATTAAGAA CTGCTGTTTC ACGGGGCCCT TACCTGTGAC CCTCTTTGCT 3739

GAAGAATATT TAACCCCACA CAGCACTTCA AAGAAGCTGT CTTGGAAGTC TGTCTCAGGA 3799

GCACCCTGTC TTCTTAATTC TCCAAGCGGA TGCTCCATTT CAATTGCTTT GTGACTTCTT 3859

CTTCTTTGTT TTTTTAAATA TTATGCTGCT TTAACAGTGG AGCTGAATTT TCTGGAAAAT 3919

GCTTCTTGGC TGGGGCCACT ACCTCCTTTC CTATCTTTAC ATCTATGTGT ATGTTGACTT 3979

TTTAAAATTC TGAGTGATCC AGGGTATGAC CTAGGGAATG AACTAGCTAT GGAAATAACT 4039

CAGGGTTAGG AATCCTAGCA CTTGTCTCAG GACTCTGAAA AGGAACGGCT TCCTCATTCC 4099

TTGTCTTGAT AAAGTGGAAT TGGCAAACTA GAATTTAGTT TGTACTCAGT GGACAGTGCT 4159

GTTGAAGATT TGAGGACTTG TTAAAGAGCA CTGGGTCATA TGGAAAAAAT GTATGTGTCT 4219

CCCCAGGTGC ATTTTCTTGG TTTATGTCTT GTTCTTGAGA TTTTGTATAT TTAGGAAAAC 4279

CTCAAGCAGT AATTAATATC TCCTGGAACA CTATAGAGAA CCAAGTGACC GACTCATTTA 4339

CAACTGAAAC CTAGGAAGCC CCTGAGTCCT GAGCGAAAAC AGGAGAGTTA GTCGCCCTAC 4399

AGAAAACCCA GCTAGACTAT TGGGTATGAA CTAAAAAGAG ACTGTGCCAT GGTGAGAAAA 4459

ATGTAAAATC CTACAGTGGA ATGAGCAGCC CTTACAGTGT TGTTACCACC AAGGGCAGGT 4519

AGGTATTAGT GTTTGAAAAA GCTGGTCTTT GAGCGAGGGC ATAAATACAG CTAGCCCCAG 4579

GGGTGGAACA ACTGTGGGAG TCTTGGGTAC TCGCACCTCT TGGCTTTGTT GATGCTCCGC 4639

CAGGAAGGCC ACTTGTGTGT GCGTGTCAGT TACTTTTTTA GTAACAATTC AGATCCAGTG 4699

TAAACTTCCG TTCATTGCTC TCCAGTCACA TGCCCCCACT TCCCCACAGG TGAAAGTTTT 4759

TCTGAAGTGT TGGGATTGGT TAAGGTCTTT ATTTGTATTA CGTATCTCCC CAAGTCCTCT 4819

GTGGCCAGCT GCATCTGTCT GAATGGTGCG TGAAGGCTCT CAGACCTTAC ACACCATTTT 4879

GTAAGTTATG TTTTACATGC CCCGTTTTTG AGACTGATCT CGATGCAGGT GGATCTCCTT 4939

GAGATCCTGA TAGCCTGTTA CAGGAATGAA GTAAAGGTCA GTTTTTTTTG TATTGATTTT 4999

CACAGCTTTG AGGAACATGC ATAAGAAATG TAGCTGAAGT AGAGGGGACG TGAGAGAAGG 5059

GCCAGGCCGG CAGGCCAACC CTCCTCCAAT GGAAATTCCC GTGTTGCTTC AAACTGAGAC 5119

AGATGGGACT TAACAGGCAA TGGGGTCCAC TTCCCCCTCT TCAGCATCCC CCGTACC 5176

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 451 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

```
Met Asn Leu Glu Gly Gly Gly Arg Gly Gly Glu Phe Gly Met Ser Ala
 1               5                  10                  15

Val Ser Cys Gly Asn Gly Lys Leu Arg Gln Trp Leu Ile Asp Gln Ile
            20                  25                  30

Asp Ser Gly Lys Tyr Pro Gly Leu Val Trp Glu Asn Glu Glu Lys Ser
        35                  40                  45

Ile Phe Arg Ile Pro Trp Lys His Ala Gly Lys Gln Asp Tyr Asn Arg
    50                  55                  60

Glu Glu Asp Ala Ala Leu Phe Lys Ala Trp Ala Leu Phe Lys Gly Lys
65                  70                  75                  80
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Phe Arg Glu Gly Ile Asp Lys Pro Asp Pro Pro Thr Trp Lys Thr Arg
                  85                  90                  95

Leu Arg Cys Ala Leu Asn Lys Ser Asn Asp Phe Glu Glu Leu Val Glu
            100                 105                 110

Arg Ser Gln Leu Asp Ile Ser Asp Pro Tyr Lys Val Tyr Arg Ile Val
        115                 120                 125

Pro Glu Gly Ala Lys Lys Gly Ala Lys Gln Leu Thr Leu Glu Asp Pro
    130                 135                 140

Gln Met Ser Met Ser His Pro Tyr Thr Met Thr Thr Pro Tyr Pro Ser
145                 150                 155                 160

Leu Pro Ala Gln Gln Val His Asn Tyr Met Met Pro Pro Leu Asp Arg
                165                 170                 175

Ser Trp Arg Asp Tyr Val Pro Asp Gln Pro His Pro Glu Ile Pro Tyr
            180                 185                 190

Gln Cys Pro Met Thr Phe Gly Pro Arg Gly His His Trp Gln Gly Pro
        195                 200                 205

Ala Cys Glu Asn Gly Cys Gln Val Thr Gly Thr Phe Tyr Ala Cys Ala
    210                 215                 220

Pro Pro Glu Ser Gln Ala Pro Gly Val Pro Thr Glu Pro Ser Ile Arg
225                 230                 235                 240

Ser Ala Glu Ala Leu Ala Phe Ser Asp Cys Arg Leu His Ile Cys Leu
                245                 250                 255

Tyr Tyr Arg Glu Ile Leu Val Lys Glu Leu Thr Thr Ser Ser Pro Glu
            260                 265                 270

Gly Cys Arg Ile Ser His Gly His Thr Tyr Asp Ala Ser Asn Leu Asp
        275                 280                 285

Gln Val Leu Phe Pro Tyr Pro Glu Asp Asn Gly His Arg Lys Asn Ile
    290                 295                 300

Glu Asn Leu Leu Ser His Leu Glu Arg Gly Val Val Leu Trp Met Ala
305                 310                 315                 320

Pro Asp Gly Leu Tyr Ala Lys Arg Leu Cys Gln Ser Thr Ile Tyr Trp
                325                 330                 335

Asp Gly Pro Leu Ala Leu Cys Asn Asp Arg Pro Asn Lys Leu Glu Arg
            340                 345                 350

Asp Gln Thr Cys Lys Leu Phe Asp Thr Gln Gln Phe Leu Ser Glu Leu
        355                 360                 365
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Gln Ala Phe Ala His His Gly Arg Ser Leu Pro Arg Phe Gln Val Thr
    370                 375                 380

Leu Cys Phe Gly Glu Glu Phe Pro Asp Pro Gln Arg Gln Arg Lys Leu
385                 390                 395                 400

Ile Thr Ala His Val Glu Pro Leu Leu Ala Arg Gln Leu Tyr Tyr Phe
                405                 410                 415

Ala Gln Gln Asn Ser Gly His Phe Leu Arg Gly Tyr Asp Leu Pro Glu
            420                 425                 430

His Ile Ser Asn Pro Glu Asp Tyr His Arg Ser Ile Arg His Ser Ser
        435                 440                 445

Ile Gln Glu
    450
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 154 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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

```
TTTTCTCTCT ACAGTCACCT CCCTGTTTAC CAAAGATAAT CACAATAAGT CCAGTTTACT    60

TACAAAACAA GTTTAGTTAT TAGAGGAAAC TAAAACTTCA GGATTCAGTC CAGATAATTT   120

TTAAAAACTC TAAAACAATG GACAGGGCTA GAAT                               154
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 75 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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

TGGGCTCGGC CTGGTGGGGC AGCCACAGCG GGACGCAGTA GTGAAAGTCC AGTTTACTTA 60

CAAAACAAGT TTAGT 75

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 122 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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

TGGGCTCGGC CTTGGTGGGG CAGCCACAGC GGGACGCAAG TAGTGAGGGC ACTCAGAACG 60

CCACTCAGCC CCGACAGGCA GGGCACGAGG AGGCAGCTCC TCACCCTCCC TTTCTCTTTT 120

GT 122

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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

TATTAGAGGA AACTAAAACT TCAGGATTCA GCAGGGCATG AGGAGGCAGC TCCTCACCCT 60

CCCTTTCTCT TTTGTAC 77

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: NO

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

TACTCGCACC TCTTGGCT 18

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: YES

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

CTGGAGAGCA ATGAACGG 18

(2) INFORMATION FOR SEQ ID NO:21:

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

(ii) MOLECULE TYPE: DNA (genomic)

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

AGGTACTTTC AGTTTCATAT T 21

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

Met Ala Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Ser Leu Gly Pro Gly
1 5 10 15

Pro

What is claimed is:

1. A method of determining a chromosomal breakpoint in a subject suffering from multiple myeloma which comprises steps of:
   (a) obtaining a DNA sample from the subject suffering from multiple myeloma;
   (b) determining whether there is J and C disjunction in the immunoglobulin heavy chain gene in the obtained DNA sample;
   (c) obtaining a genomic library having clones which contain genomic DNA fragments from the DNA sample which shows positive J and C disjunction;
   (d) selecting and isolating clones of the obtained library which show positive hybridization with a probe which is capable of specifically hybridizing with the C but not the J region of the immunoglobulin heavy chain gene;
   (e) preparing fluorescent probes from the genomic DNA fragments of the isolated clones from step (d);
   (f) hybridizing said fluorescent probes with metaphase chromosomes; and
   (g) determining the identity of the chromosomes which are capable of hybridizing to said fluorescent probes, wherein the identification of a chromosome other than chromosome 14 would indicate that the chromosomal breakpoint is between chromosome 14 and the identified chromosome, thereby determining a chromosomal breakpoint in a subject suffering from multiple myeloma.

2. The method of claim 1, wherein step (b) is performed by Southern blotting.

3. The method of claim 1, wherein step (b) is performed by

polymerase chain reaction with appropriate probes.

4. The method of claim 1, wherein the genomic library is a phage vector library.

5. The method of claim 4, wherein the genomic DNA fragments are generated by cleaving genomic DNA from cells of the subject with an appropriate restriction enzyme.

6. The method of claim 5, wherein the restriction enzyme is *Bam*HI.

7. The method of claim 5, wherein the restriction enzyme is *Sau*3AI.

8. The method of claim 1, wherein the probe of step (d) is a human IgH J region JH probe.

9. The method of claim 1, wherein the probe of step (d) is a human IgH Cμ probe.

10. The method of claim 1, wherein the probe of step (d) is a human IgH Cγ2 probe.

11. The method of claim 1, wherein the chromosomal breakpoint identified is a t(6;14)(p25;q32) translocation.

12. The method of claim 1, wherein the chromosomal breakpoint identified is a t(1;14) translocation.

13. A method to identify a gene other than the immunoglobulin gene which is located in chromosome 14, altered by a chromosomal breakpoint detected in

a subject suffering from multiple myeloma which comprises steps of:

a) selecting a probe having a sequence of a chromosome other than chromosome 14, identified at the chromosomal breakpoint detected in a subject suffering from multiple myeloma, wherein said probe is capable of hybridizing to the unique sequence of the gene other than the immunoglobulin gene altered by a chromosomal breakpoint detected in a subject suffering from multiple myeloma;

b) contacting said probe with mRNA isolated from a cell under conditions permitting formation of a complex between said probe and the mRNA;

c) isolating the complex resulting from step (b);

d) determining the sequence of the mRNA in the isolated complex, thereby determining the identity of the gene.

14. The method of claim 13, wherein step (d) comprises steps of:

i) synthesizing complementary DNA to the mRNA; and

ii) performing sequence analysis of the complementary DNA to determine the sequence of the mRNA.

15. A gene identified by the method of claim 13.

16. The gene of claim 15 designated *MUM-1*.

17. The gene of claim 15 designated *MUM-2*.

18. The method of claim 13, wherein the gene identified comprises a nucleic acid encoding a MUM protein.

19. The method of claim 18, wherein the MUM protein is MUM-1.

20. The method of claim 18, wherein the MUM protein is MUM-2.

21. An isolated nucleic acid molecule encoding a MUM protein.

22. An isolated nucleic acid molecule of claim 21, wherein the nucleic acid molecule is a DNA molecule.

23. The isolated DNA molecule of claim 22, wherein the DNA molecule is a cDNA molecule.

24. The isolated DNA molecule of claim 21, wherein the DNA molecule is a cDNA molecule having the nucleotide sequence shown in Figure 5B (SEQ. ID NO 13).

25. The isolated DNA molecule of claim 22, wherein the DNA molecule is genomic DNA molecule.

26. The isolated nucleic acid molecule of claim 21, wherein the nucleic acid molecule is an RNA molecule.

27. An isolated nucleic acid molecule of claim 21, wherein the nucleic acid molecule encodes a human MUM-1 protein.

28. An isolated nucleic acid molecule of claim 21, wherein the nucleic acid molecule encodes a human MUM-2 protein.

29. An isolated nucleic molecule of claim 27, wherein the human MUM-1 protein has substantially the same amino acid sequence as shown in Figure 5B (SEQ. ID NO 14).

30. An isolated nucleic molecule of claim 27, wherein the human MUM-1 protein has the amino acid sequence as shown in Figure 5B (SEQ. ID NO 14).

31. An isolated nucleic acid molecule of claim 21 operatively linked to a promoter of RNA transcription.

32. A vector comprising the nucleic acid molecule of claim 21.

33. A vector comprising the nucleic acid molecule of claim 23.

34. A vector comprising the nucleic acid molecule of claim 25.

35. A vector of claim 33, wherein the vector is a plasmid.

36. The plasmid of claim 35, designated pcMUM1-1.6a (ATCC Accession No. 97579).

37. The plasmid of claim 35, designated pMUM1-2.4B/N (ATCC Accession No. 97578).

38. The plasmid of claim 35, designated pMUM1-7.7B (ATCC Accession No. 97577).

39. The plasmid of claim 35, designated pcMUM2-8 (ATCC

Accession No. 97580).

40. A host cell comprising the vector of claims 32.

41. The host cell of claim 40, wherein the cell is selected from a group consisting of a bacterial cell, a plant cell, and insect cell and a mammalian cell.

42. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a MUM protein.

43. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding a MUM protein.

44. The nucleic acid probe of either of claims 42 or 43, wherein the MUM protein is MUM-1.

45. The nucleic acid probe of either of claim 42 or 43, wherein the MUM protein is MUM-2.

46. A DNA probe of claim 44 or 45.

47. A RNA probe of claim 44 or 45.

48. A genomic DNA probe of claim 44 or 45.

49. A nucleic acid probe of claim 44 or 45 labeled with a detectable marker.

50. The nucleic acid probe of claim 49, wherein the detectable marker is selected from the group consisting of a radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

51. A nucleic acid probe of claim 44, wherein the sequence of a nucleic acid molecule encoding a MUM-1 protein is linked to a nucleic acid sequence capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule of human chromosome 14.

52. A nucleic acid probe of claim 45, wherein the sequence of a nucleic acid molecule encoding a MUM-2 protein is linked to a nucleic acid sequence capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule of human chromosome 14.

53. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule of claim 21 which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

54. The nucleic acid probe of claim 53, wherein the isolated nucleic acid molecule encodes MUM-1.

55. The nucleic acid probe of claim 53, wherein the isolated nucleic acid molecule encodes MUM-2.

56. The nucleic acid probe of claim 54, wherein the sequence of a nucleic acid molecule encoding a MUM-1

protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14.

57. The nucleic acid probe of claim 55, wherein the sequence of a nucleic acid molecule encoding a MUM-2 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14.

58. The nucleic acid probe of claim 56, wherein the specific break point comprises a portion of the t(6;14)(p25;q32) translocation.

59. The nucleic acid probe of claim 57, wherein the specific break point comprises a portion of a t(1;14) translocation.

60. The nucleic acid probe of either of claims 58 or 59 labeled with a detectable marker.

61. The nucleic acid probe of claim 60, wherein the detectable marker is selected from the group consisting of a radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

62. A method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-1 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-1 protein.

63. A method for detecting a predisposition to multiple

myeloma associated with the expression of a human MUM-2 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-2 protein.

64. The method of claim 62, wherein the rearrangement of nucleic acid encoding MUM-1 protein is detected by contacting the nucleic acid from the sample with a MUM-1 probe under conditions permitting the MUM-1 probe to hybridize with the nucleic acid encoding MUM-1 protein from the sample, thereby detecting the rearrangement of nucleic acid encoding MUM-1 protein in the sample.

65. The method of claim 63, wherein the rearrangement of nucleic acid encoding MUM-2 protein is detected by contacting the nucleic acid from the sample with a MUM-2 probe under conditions permitting the MUM-2 probe to hybridize with the nucleic acid encoding MUM-2 protein from the sample, thereby detecting the rearrangement of nucleic acid encoding MUM-2 protein in the sample.

66. The method of claim 64, wherein the MUM-1 probe comprises a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding MUM-1 protein which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

67. The method of claim 65, wherein the MUM-2 probe comprises a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of

the isolated nucleic acid molecule encoding MUM-2 protein which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 1.

68. The method of claim 66, wherein the nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding MUM-1 protein is linked at a specific break point to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

69. The method of claim 67, wherein the nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding MUM-2 protein is linked at a specific break point to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 1.

70. The method of claim 68, wherein the specific break point comprises a portion of the t(6;14)(p25;q32) translocation.

71. The method of claim 69, wherein the specific break point comprises a portion of a t(1;14) translocation.

72. The method of claim 62, which comprises:

a. obtaining DNA from the sample of the subject suffering from multiple myeloma;

b. performing a restriction digest of the DNA with a panel of restriction enzymes;

c. separating the resulting DNA fragments by size fractionation;

d. contacting the resulting DNA fragments with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-1 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-1 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 and labeled with a detectable marker;

e. detecting labeled bands which have hybridized to the nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-1 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-1 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 to create a unique band pattern specific to the DNA of subjects suffering from multiple myeloma;

f. preparing DNA obtained from a sample of a subject for diagnosis by steps (a-e); and

g. comparing the detected band pattern specific to the DNA obtained from a sample of subjects suffering from multiple myeloma from step (e) and the DNA obtained from a sample of the subject for diagnosis from step (f) to determine whether the patterns are the same or

different and to diagnose thereby predisposition to multiple myeloma if the patterns are the same.

73. The method of claim 63, which comprises:

a. obtaining DNA from the sample of the subject suffering from multiple myeloma;

b. performing a restriction digest of the DNA with a panel of restriction enzymes;

c. separating the resulting DNA fragments by size fractionation;

d. contacting the resulting DNA fragments with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-2 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-2 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 and labeled with a detectable marker;

e. detecting labeled bands which have hybridized to the nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-2 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-2 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 to create

a unique band pattern specific to the DNA of subjects suffering from multiple myeloma;

f. preparing DNA obtained from a sample of a subject for diagnosis by steps (a-e); and

g. comparing the detected band pattern specific to the DNA obtained from a sample of subjects suffering from multiple myeloma from step (e) and the DNA obtained from a sample of the subject for diagnosis from step (f) to determine whether the patterns are the same or different and to diagnose thereby predisposition to multiple myeloma if the patterns are the same.

74. The method of claim 72 or 73, wherein the size fractionation in step (c) is effected by a polyacrylamide or agarose gel.

75. The method of claim 72 or 73, wherein the detectable marker is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

76. A method of claim 62 which comprises:

a. obtaining RNA from the sample of the subject suffering from multiple myeloma;

b. separating the RNA sample by size fractionation;

c. contacting the resulting RNA species with a nucleic acid probe capable of specifically

hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-1 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-1 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 and labeled with a detectable marker;

d. detecting labeled bands which have hybridized to the RNA species to create a unique band pattern specific to the RNA of subjects suffering from multiple myeloma;

f. preparing RNA obtained from a sample of a subject for diagnosis by steps (a-d); and

g. comparing the detected band pattern specific to the RNA obtained from a sample of subjects suffering from multiple myeloma from step (d) and the RNA obtained from a sample of the subject for diagnosis from step (f) to determine whether the patterns are the same or different and to diagnose thereby predisposition to multiple myeloma if the patterns are the same.

77. A method of claim 63 which comprises:

a. obtaining RNA from the sample of the subject suffering from multiple myeloma;

b. separating the RNA sample by size fractionation;

c. contacting the resulting RNA species with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-2 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-2 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 1 and labeled with a detectable marker;

d. detecting labeled bands which have hybridized to the RNA species to create a unique band pattern specific to the RNA of subjects suffering from multiple myeloma;

f. preparing RNA obtained from a sample of a subject for diagnosis by steps (a-d); and

g. comparing the detected band pattern specific to the RNA obtained from a sample of subjects suffering from multiple myeloma from step (d) and the RNA obtained from a sample of the subject for diagnosis from step (f) to determine whether the patterns are the same or different and to diagnose thereby predisposition to multiple myeloma if the patterns are the same.

78. The method of claim 76 or 77, wherein the size fractionation in step (c) is effected by a polyacrylamide or agarose gel.

79. The method of claim 76 or 77, wherein the detectable marker is radioactive isotope, enzyme, dye, biotin,

a fluorescent label or a chemiluminescent label.

80. The method of either of claim 72 or 76, wherein multiple myeloma associated with the expression of a specific human MUM-1 is diagnosed.

81. The method of either of claim 73 or 77, wherein multiple myeloma associated with the expression of a specific human MUM-2 is diagnosed.

82. An antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding a human MUM-1 protein so as to prevent overexpression of the mRNA molecule.

83. An antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding a human MUM-2 protein so as to prevent overexpression of the mRNA molecule.

84. An antisense oligonucleotide having a sequence capable of specifically hybridizing to the cDNA molecule of claim 23.

85. An antisense oligonucleotide having a sequence capable of specifically hybridizing to the genomic DNA molecule of claim 29.

86. An antisense oligonucleotide having a sequence capable of specifically hybridizing to the RNA molecule of claim 30.

87. An purified MUM protein.

88. A purified MUM protein, wherein the MUM protein is

MUM-1 protein.

89. A purified human MUM-1 protein of claim 88.

90. An antibody directed to a purified MUM-1 protein.

91. An antibody capable of specifically recognizing MUM-1 protein.

92. An antibody of claim 91, wherein the MUM-1 protein is a human MUM-1 protein.

93. A purified MUM protein, wherein the MUM protein is MUM-2 protein.

94. A purified human MUM-2 protein of claim 93.

95. An antibody directed to a purified MUM-2 protein.

96. An antibody capable of specifically recognizing a MUM-2 protein.

97. An antibody of claim 96, wherein the MUM-2 protein is a human MUM-2 protein.

98. An monoclonal antibody of any one of claims 90, 91 and 92.

99. An monoclonal antibody of any one of claims 95, 96, and 97.

100. A pharmaceutical composition comprising an amount of the oligonucleotide of any one of claims 82, 84, 85 and 81 effective to prevent overexpression of a human MUM-1 protein and a pharmaceutically

acceptable carrier capable of passing through a cell membrane.

101. A pharmaceutical composition comprising an amount of the oligonucleotide of any one of claims 83, 84, 85 and 81 effective to prevent overexpression of a human MUM-2 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

FIG. 1

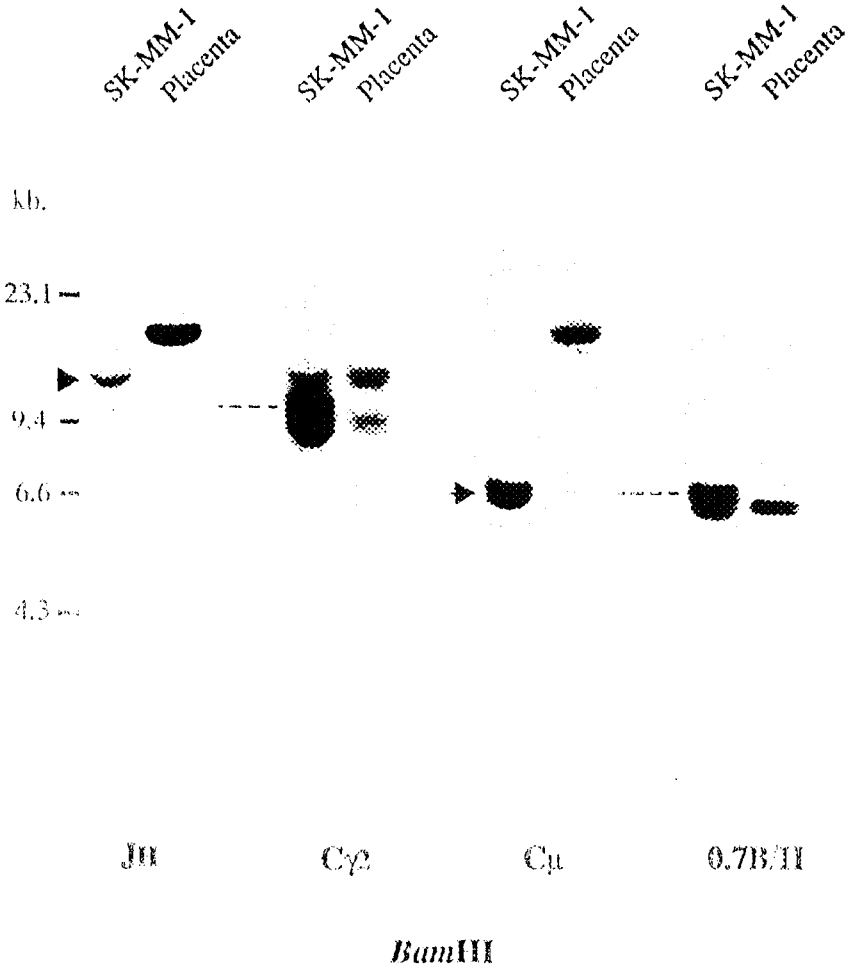

SK-MM-1
Placenta
SK-MM-1
Placenta
SK-MM-1
Placenta
SK-MM-1
Placenta
kb.
23.1
9.4
6.6
4.3
Jα
Cγ2
Cμ
0.7B/H
BamHI

FIG. 2A

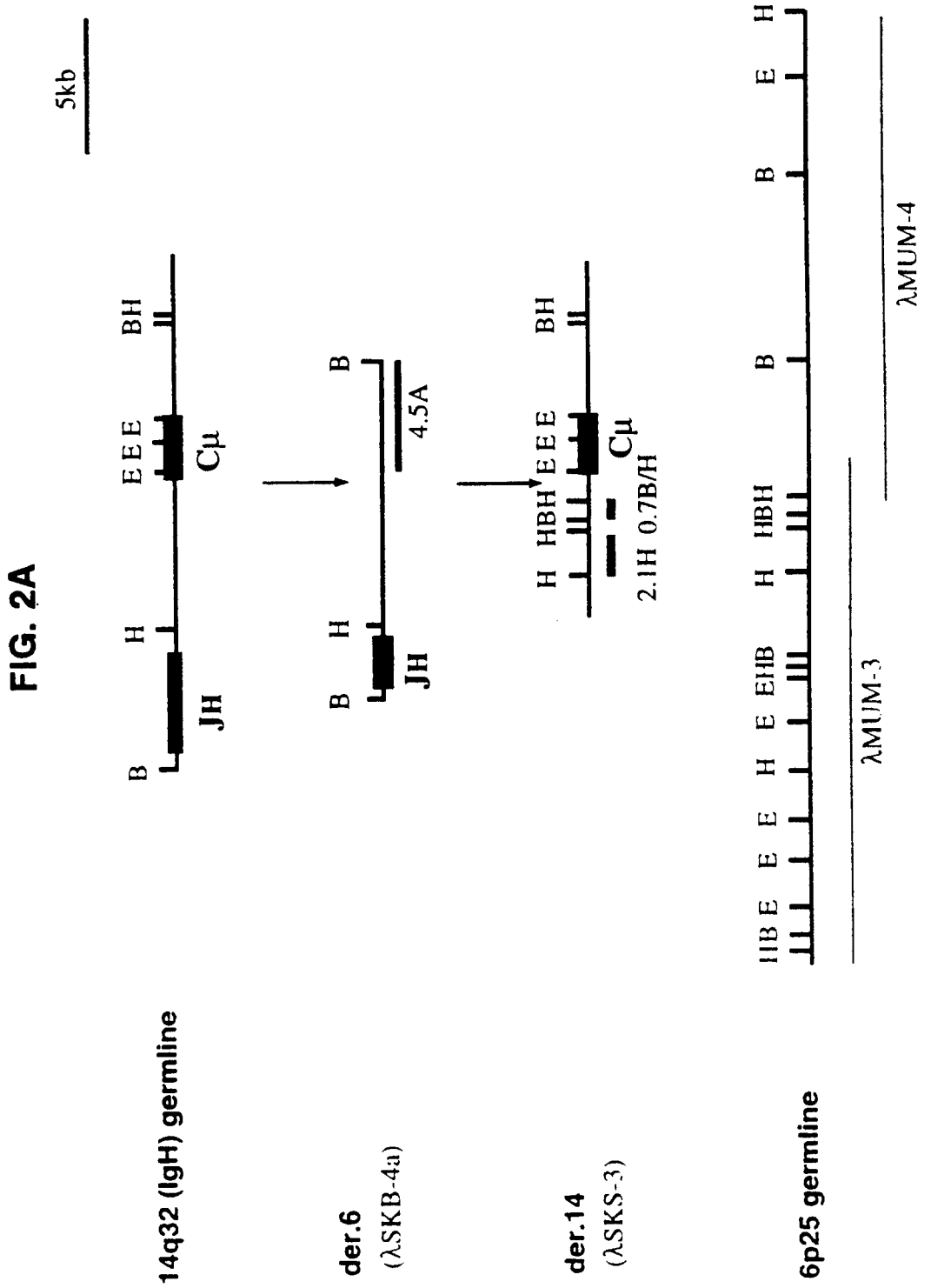

5kb
14q32 (IgH) germline
B
H
JH
E E E
BH
Cμ
der.6
(λSKB-4a)
B
H
JH
B
4.5A
der.14
(λSKS-3)
H HBH
E E E
BH
Cμ
2.1H 0.7B/H
6p25 germline
IIB E E H E EHB H HBH B B E H
λMUM-3
λMUM-4

FIG. 2B

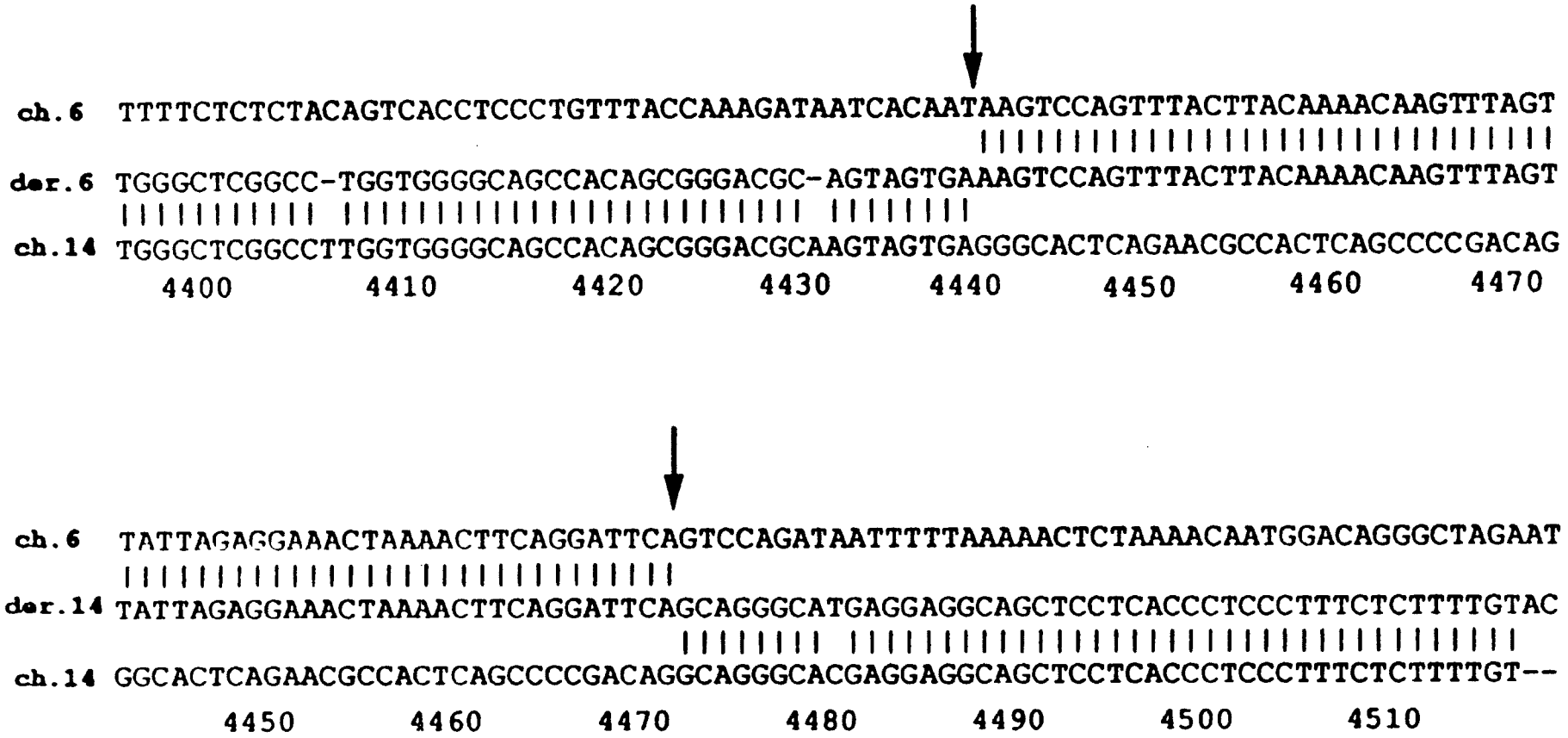
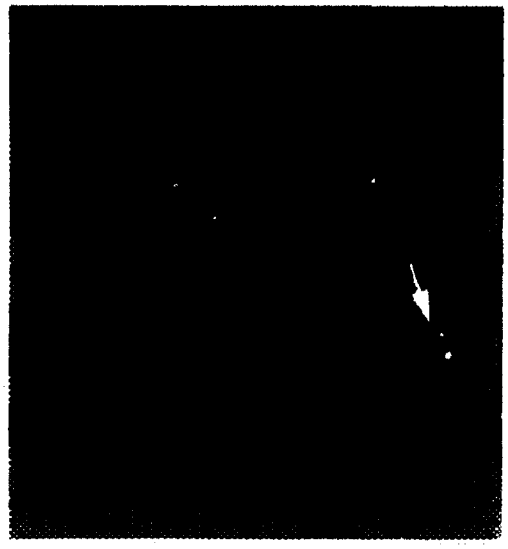

ch.6 TTTTCTCTCTACAGTCACCTCCCTGTTTACCAAAGATAATCACAATAAGTCCAGTTTACTTACAAAACAAGTTTAGT
der.6 TGGGCTCGGCC-TGGTGGGGCAGCCACAGCGGGACGC-AGTAGTGAAAGTCCAGTTTACTTACAAAACAAGTTTAGT
ch.14 TGGGCTCGGCCTTGGTGGGGCAGCCACAGCGGGACGCAAGTAGTGAGGGCACTCAGAACGCCACTCAGCCCCGACAG
4400 4410 4420 4430 4440 4450 4460 4470
ch.6 TATTAGAGGAAACTAAAACTTCAGGATTCAGTCCAGATAATTTTTAAAAACTCTAAAACAATGGACAGGGCTAGAAT
der.14 TATTAGAGGAAACTAAAACTTCAGGATTCAGCAGGGCATGAGGAGGCAGCTCCTCACCCTCCCTTTCTCTTTTGTAC
ch.14 GGCACTCAGAACGCCACTCAGCCCCGACAGGCAGGGCACGAGGAGGCAGCTCCTCACCCTCCCTTTCTCTTTTGT--
4450 4460 4470 4480 4490 4500 4510

FIG. 3A



λ MUM-3

FIG. 3B

λ MUM-3

FIG. 4A

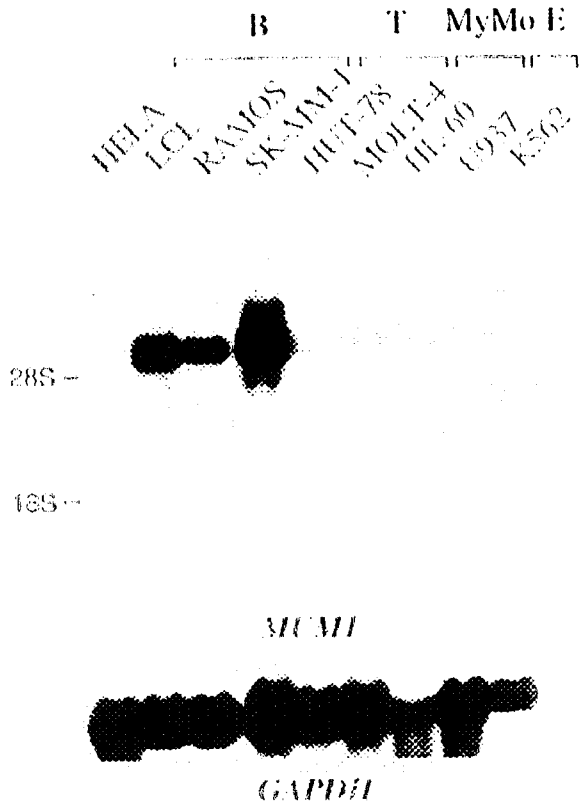

B
T
MyMo E
HELA
LCL
RAMOS
SK-MM-1
HUT-78
MOLT-4
HL-60
U937
K562
28S
18S
MCMI
GAPDH

FIG. 4B

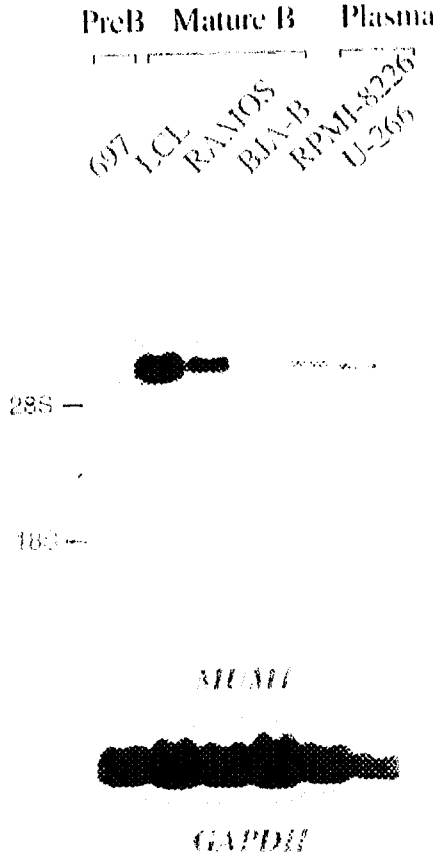

PreB
Mature B
Plasma
697
LCL
RAMOS
BJA-B
RPMI-8226
U-266
28S
18S
MUM1
GAPDH

FIG. 4C

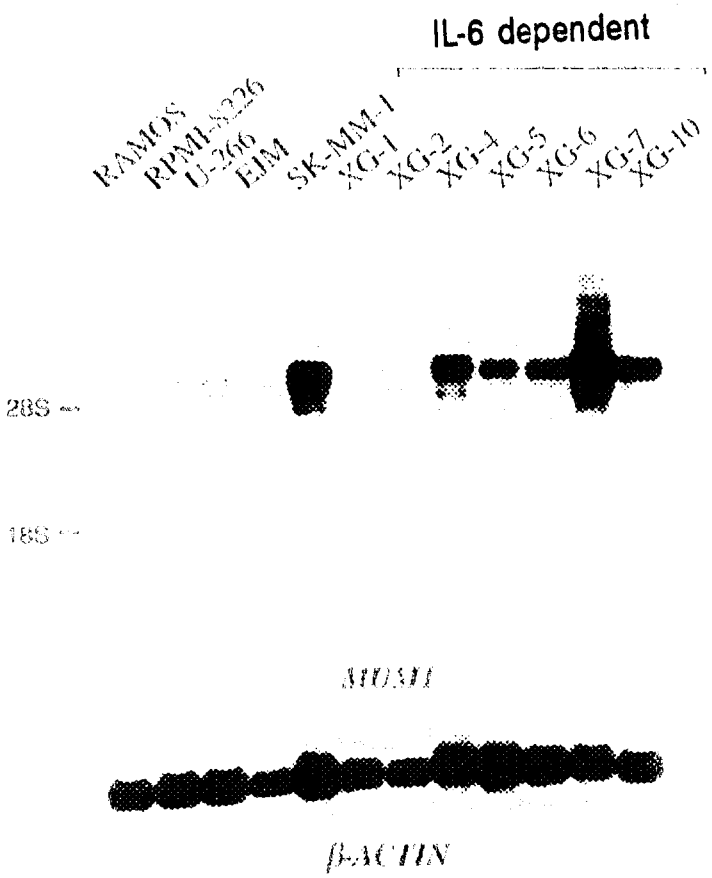

IL-6 dependent
RAMOS
RPMI-8226
U-266
EJM
SK-MM-1
XG-1
XG-2
XG-4
XG-5
XG-6
XG-7
XG-10
28S
18S
MDM1
β-ACTIN

FIG. 5A

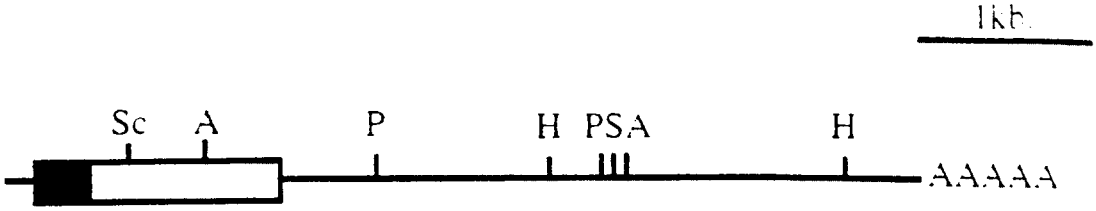


FIG. 5B-1

```
   1  GCCTGACCAA CATGGTAAAA CCCCATCTCT GCTAAAACTA CAAAAAATTA
  51  GCTGGATGTG GTGGCAGGGA ACCTGTCATC CCAGCTAGTT GGGAGACTGA
 101  GGCAGGAGAA TCGCTCGATC TTGGGACCCA CCGCTGCCCT CAGCTCCGAG
 151  TCCAGGGCGA GTGCAGAGCA CAGCGGGCGG AGGACCCCGG GCGCGGGCGC
 201  GGACGGCACG CGGGGCATGA ACCTGGAGGG CGGCGGCCGA GGCGGAGAGT
                        M  N   L  E  G   G  G  R   G  G  E  F
 251  TCGGCATGAG CGCGGTGAGC TGCGGCAACG GGAAGCTCCG CCAGTGGCTG
        G  M  S   A  V  S   C  G  N  G   K  L  R   Q  W  L
 301  ATCGACCAGA TCGACAGCGG CAAGTACCCC GGGCTGGTGT GGGAGAACGA
      I  D  Q  I   D  S  G   K  Y  P   G  L  V  W   E  N  E
 351  GGAGAAGAGC ATCTTCCGCA TCCCCTGGAA GCACGCGGGC AAGCAGGACT
        E  K  S   I  F  R  I   P  W  K   H  A  G   K  Q  D  Y
 401  ACAACCGCGA GGAGGACGCC GCGCTCTTCA AGGCTTGGGC ACTGTTTAAA
        N  R  E   E  D  A   A  L  F  K   A  W  A   L  F  K
 451  GGAAAGTTCC GAGAAGGCAT CGACAAGCCG GACCCTCCCA CCTGGAAGAC
      G  K  F  R   E  G  I   D  K  P   D  P  P  T   W  K  T
 501  GCGCCTGCGG TGCGCTTTGA ACAAGAGCAA TGACTTTGAG GAACTGGTTG
        R  L  R   C  A  L  N   K  S  N   D  F  E   E  L  V  E
 551  AGCGGAGCCA GCTGGACATC TCAGACCCGT ACAAAGTGTA CAGGATTGTT
        R  S  Q   L  D  I   S  D  P  Y   K  V  Y   R  I  V
 601  CCTGAGGGAG CCAAAAAAGG AGCCAAGCAG CTCACCCTGG AGGACCCGCA
      P  E  G  A   K  K  G   A  K  Q   L  T  L  E   D  P  Q
 651  GATGTCCATG AGCCACCCCT ACACCATGAC AACGCCTTAC CCTTCGCTCC
        M  S  M   S  H  P  Y   T  M  T   T  P  Y   P  S  L  P
 701  CAGCCCAGCA GGTTCACAAC TACATGATGC CACCCCTCGA CCGAAGCTGG
        A  Q  Q   V  H  N   Y  M  M  P   P  L  D   R  S  W
 751  AGGGACTACG TCCCGGATCA GCCACACCCG GAAATCCCGT ACCAATGTCC
      R  D  Y  V   P  D  Q   P  H  P   E  I  P  Y   Q  C  P
 801  CATGACGTTT GGACCCCGCG GCCACCACTG GCAAGGCCCA GCTTGTGAAA
        M  T  F   G  P  R  G   H  H  W   Q  G  P   A  C  E  N
 851  ATGGTTGCCA GGTGACAGGA ACCTTTTATG CTTGTGCCCC ACCTGAGTCC
        G  C  Q   V  T  G   T  F  Y  A   C  A  P   P  E  S
 901  CAGGCTCCCG GAGTCCCCAC AGAGCCAAGC ATAAGGTCTG CCGAAGCCTT
      Q  A  P  G   V  P  T   E  P  S   I  R  S  A   E  A  L
 951  GGCGTTCTCA GACTGCCGGC TGCACATCTG CCTGTACTAC CGGGAAATCC
        A  F  S   D  C  R  L   H  I  C   L  Y  Y   R  E  I  L
1001  TCGTGAAGGA GCTGACCACG TCCAGCCCCG AGGGCTGCCG GATCTCCCAT
        V  K  E   L  T  T   S  S  P  E   G  C  R   I  S  H
1051  GGACATACGT ATGACGCCAG CAACCTGGAC CAGGTCCTGT TCCCCTACCC
      G  H  T  Y   D  A  S   N  L  D   Q  V  L  F   P  Y  P
1101  AGAGGACAAT GGCCACAGGA AAAACATTGA GAACCTGCTG AGCCACCTGG
        E  D  N   G  H  R  K   N  I  E   N  L  L   S  H  L  E
```

FIG. 5B-2

```
1151  AGAGGGGCGT GGTCCTCTGG ATGGCCCCCG ACGGGCTCTA TGCGAAAAGA
        R  G  V    V  L  W    M  A  P  D    G  L  Y    A  K  R
1201  CTGTGCCAGA GCACGATCTA CTGGGACGGG CCCCTGGCGC TGTGCAACGA
      L  C  Q  S    T  I  Y    W  D  G    P  L  A  L    C  N  D
1251  CCGGCCCAAC AAACTGGAGA GAGACCAGAC CTGCAAGCTC TTTGACACAC
        R  P  N    K  L  E  R    D  Q  T    C  K  L    F  D  T  Q
1301  AGCAGTTCTT GTCAGAGCTG CAAGCGTTTG CTCACCACGG CCGCTCCCTG
        Q  F  L    S  E  L    Q  A  F  A    H  H  G    R  S  L
1351  CCAAGATTCC AGGTGACTCT ATGCTTTGGA GAGGAGTTTC CAGACCCTCA
        P  R  F  Q    V  T  L    C  F  G    E  E  F  P    D  P  Q
1401  GAGGCAAAGA AAGCTCATCA CAGCTCACGT AGAACCTCTG CTAGCCAGAC
        R  Q  R    K  L  I  T    A  H  V    E  P  L    L  A  R  Q
1451  AACTATATTA TTTTGCTCAA CAAAACAGTG GACATTTCCT GAGGGGCTAC
        L  Y  Y    F  A  Q    Q  N  S  G    H  F  L    R  G  Y
1501  GATTTACCAG AACACATCAG CAATCCAGAA GATTACCACA GATCTATCCG
      D  L  P  E    H  I  S    N  P  E    D  Y  H  R    S  I  R
1551  CCATTCCTCT ATTCAAGAAT GAAAAATGTC AAGATGAGTG GTTTTCTTTT
        H  S  S    I  Q  E  *
1601  TCCTTTTTTT TTTTTTTTTT TTTGATACGG AGATACGGGG TCTTGCTCTG
1651  TCTCCCAGGC TGGAGTGCAG TGACACAATC TCAGCTCACT GTGACCTCCG
1701  CCTCCTGGGT TCAAGAGACT CTCCTGCCTC AGCCTCCCTG GTAGCTGGGA
1751  TTACAGGTGT GAGCCACTGC ACCCACCCAA GACAAGTGAT TTTCATTGTA
1801  AATATTTGAC TTTAGTGAAA GCGTCCAATT GACTGCCCTC TTACTGTTTT
1851  GAGGAACTCA GAAGTGGAGA TTTCAGTTCA GCGGTTGAGG AGAATTGCGG
1901  CGAGACAAGC ATGGAAAATC AGTGACATCT GATTGGCAGA TGAGCTTATT
1951  TCAAAAGGAA GGGTGGCTTT GCATTTTCTT GTGTTCTGTA GACTGCCATC
2001  ATTGATGATC ACTGTGAAAA TTGACCAAGT GATGTGTTTA CATTTACTGA
2051  AATGCGCTCT TTAATTTGTT GTAGATTAGG TCTTGCTGGA AGACAGAGAA
2101  AACTTGCCTT TCAGTATTGA CACTGACTAG AGTGATGACT GCTTGTAGGT
2151  ATGTCTGTGC CATTTCTCAG GGAAGTAAGA TGTAAATTGA AGAAGCCTCA
2201  CACGTAAAAG AAATGTATTA ATGTATGTAG GAGCTGCAGT TCTTGTGGAA
2251  GACACTTGCT GAGTGAAGGA AATGAATCTT TGACTGAAGC CGTGCCTGTA
2301  GCCTTGGGGA GGCCCATCCC CCACCTGCCA GCGGTTTCCT GGTGTGGGTC
2351  CCTCTGCCCC ACCCTCCTTC CCATTGGCTT TCTCTCCTTG GCCTTTCCTG
2401  GAAGCCAGTT AGTAAACTTC CTATTTTCTT GAGTCAAAAA ACATGAGCGC
2451  TACTCTTGGA TGGGACATTT TTGTCTGTCC TACAATCTAG TAATGTCTAA
2501  GTAATGGTTA AGTTTTCTTG TTTCTGCATC TTTTTGACCC TCATTCTTTA
2551  GAGATGCTAA AATTCTTCGC ATAAAGAAGA AGAAATTAAG GAACATAAAT
2601  CTTAATACTT GAACTGTTGC CCTTCTGTCC AAGTACTTAA CTATCTGTTC
2651  CCTTCCTCTG TGCCACGCTC CTCTGTTTGT TTGGCTGTCC AGCGATCAGC
2701  CATGGCGACA CTAAAGGAGG AGGAGCCGGG GACTCCCAGG CTGGAGAGCA
2751  CTGCCAGGAC CCACCACTGG AAGCAGGATG GAGCTGACTA CGGAACTGCA
2801  CACTCAGTGG GCTGTTTCTG CTTATTTCAT CTGTTCTATG CTTCCTCGTG
2851  CCAATTATAG TTTGACAGGG CCTTAAAATT ACTTGGCTTT TTCCAAATGC
2901  TTCTATTTAT AGAAATCCCA AAGACCTCCA CTTGCTTAAG TATACCTATC
2951  ACTTACATTT TTGTGGTTTT GAGAAAGTAC AGCAGTAGAC TGGGGCGTCA
3001  CCTCCAGGCC GTTTCTCATA CTACAGGATA TTTACTATTA CTCCCAGGAT
```

FIG. 5B-3

```
3051  TCAGCAGAAG ATTGCGTTAG CTCTCAAATG TGTGTTCCTG CTTTTCTAAT
3101  GGATATTTTA AATTCATTCA ACAAGCACCT AGTAAGTGCC TGCTGTATCC
3151  CTACATTACA CAGTTCAGCC TTTATCAAGC TTAGTGAGCA GTGAGCACTG
3201  AAACATTATT TTTTAATGTT TAAAAAGTTT CTAATATTAA AGTCAGAATA
3251  TTAATACAAT TAATATTAAT ATTAACTACA GAAAAGACAA ACAGTAGAGA
3301  ACAGCAAAAA AATAAAAAGG ATCTCCTTTT TTCCCAGCCC AAATTCTCCT
3351  CTCTAAAAGT GTCCACAAGA AGGGGTGTTT ATTCTTCCAA CACATTTCAC
3401  TTTTCTGTAA ATATACATAA ACTTAAAAAG AAAACCTCAT GGAGTCATCT
3451  TGCACACACT TTTCATGCAG TGCTCTTTGT AGCTAAACAG TGAAGATTTA
3501  CCTCGTTCTG CTCAGAGGCC TTGCTGTGGA GCTCCACTGC CATGTACCCA
3551  GTAGGGTTTG ACATTTCATT AGCCATGCAA CATGGATATG TATTGGGCAG
3601  CAGACTGTGT TTCGTGAACT GCAGTGATGT ATACATCTTA TAGATGCAAA
3651  GTATTTTGGG GTATATTATC CTAAGGGAAG ATAAAGATGA TATTAAGAAC
3701  TGCTGTTTCA CGGGGCCCTT ACCTGTGACC CTCTTTGCTG AAGAATATTT
3751  AACCCCACAC AGCACTTCAA AGAAGCTGTC TTGGAAGTCT GTCTCAGGAG
3801  CACCCTGTCT TCTTAATTCT CCAAGCGGAT GCTCCATTTC AATTGCTTTG
3851  TGACTTCTTC TTCTTTGTTT TTTTAAATAT TATGCTGCTT TAACAGTGGA
3901  GCTGAATTTT CTGGAAAATG CTTCTTGGCT GGGGCCACTA CCTCCTTTCC
3951  TATCTTTACA TCTATGTGTA TGTTGACTTT TTAAAATTCT GAGTGATCCA
4001  GGGTATGACC TAGGGAATGA ACTAGCTATG GAAATAACTC AGGGTTAGGA
4051  ATCCTAGCAC TTGTCTCAGG ACTCTGAAAA GGAACGGCTT CCTCATTCCT
4101  TGTCTTGATA AAGTGGAATT GGCAAACTAG AATTTAGTTT GTACTCAGTG
4151  GACAGTGCTG TTGAAGATTT GAGGACTTGT TAAAGAGCAC TGGGTCATAT
4201  GGAAAAAATG TATGTGTCTC CCCAGGTGCA TTTTCTTGGT TTATGTCTTG
4251  TTCTTGAGAT TTTGTATATT TAGGAAAACC TCAAGCAGTA ATTAATATCT
4301  CCTGGAACAC TATAGAGAAC CAAGTGACCG ACTCATTTAC AACTGAAACC
4351  TAGGAAGCCC CTGAGTCCTG AGCGAAAACA GGAGAGTTAG TCGCCCTACA
4401  GAAAACCCAG CTAGACTATT GGGTATGAAC TAAAAAGAGA CTGTGCCATG
4451  GTGAGAAAAA TGTAAAATCC TACAGTGGAA TGAGCAGCCC TTACAGTGTT
4501  GTTACCACCA AGGGCAGGTA GGTATTAGTG TTTGAAAAAG CTGGTCTTTG
4551  AGCGAGGGCA TAAATACAGC TAGCCCCAGG GGTGGAACAA CTGTGGGAGT
4601  CTTGGGTACT CGCACCTCTT GGCTTTGTTG ATGCTCCGCC AGGAAGGCCA
4651  CTTGTGTGTG CGTGTCAGTT ACTTTTTTAG TAACAATTCA GATCCAGTGT
4701  AAACTTCCGT TCATTGCTCT CCAGTCACAT GCCCCCACTT CCCCACAGGT
4751  GAAAGTTTTT CTGAAGTGTT GGGATTGGTT AAGGTCTTTA TTTGTATTAC
4801  GTATCTCCCC AAGTCCTCTG TGGCCAGCTG CATCTGTCTG AATGGTGCGT
4851  GAAGGCTCTC AGACCTTACA CACCATTTTG TAAGTTATGT TTTACATGCC
4901  CCGTTTTTGA GACTGATCTC GATGCAGGTG GATCTCCTTG AGATCCTGAT
4951  AGCCTGTTAC AGGAATGAAG TAAAGGTCAG TTTTTTTTGT ATTGATTTTC
5001  ACAGCTTTGA GGAACATGCA TAAGAAATGT AGCTGAAGTA GAGGGGACGT
5051  GAGAGAAGGG CCAGGCCGGC AGGCCAACCC TCCTCCAATG GAAATTCCCG
5101  TGTTGCTTCA AACTGAGACA GATGGGACTT AACAGGCAAT GGGGTCCACT
5151  TCCCCCTCTT CAGCATCCCC CGTACC
```

FIG. 6A

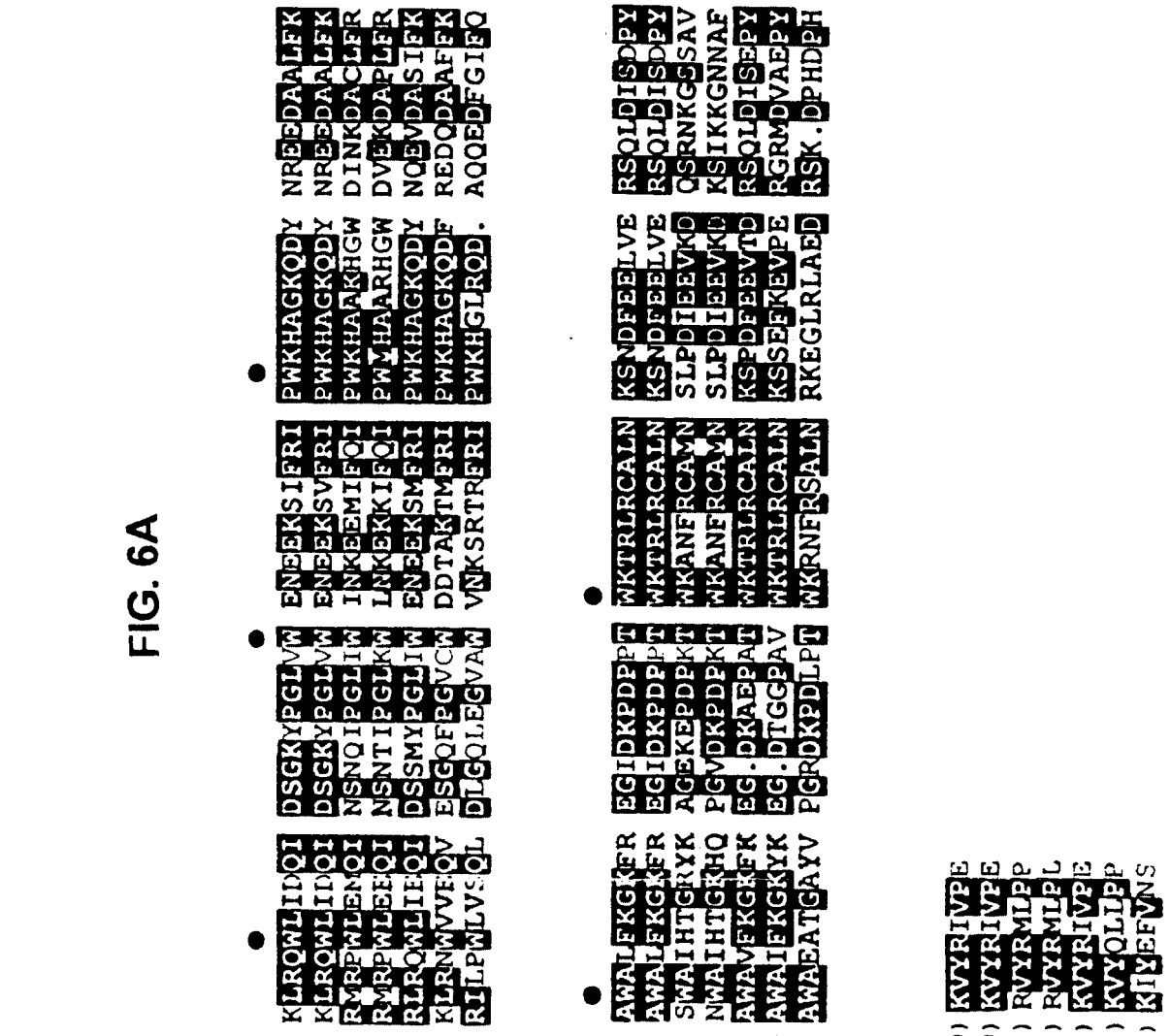

Hum MUM-1 (23-72) KLRQWLIDQI DSGKYPGLVW ENEEKSIFRI PWKHAGKQDY
Mus LSIRF (23-72) KLRQWLIDQI DSGKYPGLVW ENEEKSVFRI PWKHAGKQDY
Hum IRF-1 (7-56) RMRPWLENQI NSNQIPGLIW INKEEMIFQI PWKHAAKHGW
Hum IRF-2 (7-56) RMRPWLEEQI NSNTIPGLKW LNKEKKIFQI PWTHAARHGW
Hum ICSBP (9-60) RLRQWLIEQI DSSMYPGLIW ENEEKSMFRI PWKHAGKQDY
Hum ISGF3γ (11-60) KLRNWVVEQV ESGQFPGVCW DDTAKTMFRI PWKHAGKQDF
Hum IRF-3 (7-55) RILPWLVSQL DLGQLEGVAW VNKSRTRFRI PWKHGLRQD.
Hum MUM-1 (73-122) AWALFKGKFR EGIDKPDPPT WKTRLRCALN KSNDFEELVE RSQLDISDPY
Mus LSIRF (73-122) AWALFKGKFR EGIDKPDPPT WKTRLRCALN KSNDFEELVE RSQLDISDPY
Hum IRF-1 (57-106) SWAIHTGRYK AGEKEPDPKT WKANFRCAYN SLPDIEEVKD QSRNKGNSAV
Hum IRF-2 (57-106) NWAIHTGKHQ PGVDKPDPKT WKANFRCAYN SLPDIEEVKD KSIKKGNNAF
Hum ICSBP (59-107) AWAVFKGKFK EG.DKAEPAT WKTRLRCALN KSPDFEEVTD RSQLDISEPY
Hum ISGF3γ (61-109) AWAIFKGKYK EG.DTGGPAV WKTRLRCALN KSSEFKEVPE RGRMDVAEPY
Hum IRF-3 (56-104) AWAEATGAYV PGRDKPDLPT WKRNFRSALN RKEGLRLAED RSK.DPHDPH
Hum MUM-1 (123-130) KVYRIVPE
Mus LSIRF (123-130) KVYRIVPE
Hum IRF-1 (107-114) RVYRMLPP
Hum IRF-2 (107-114) RVYRMLPL
Hum ICSBP (108-115) KVYRIVPE
Hum ISGF3γ (110-117) KVYQLLPP
Hum IRF-3 (105-112) KIYEFVNS

FIG. 6B

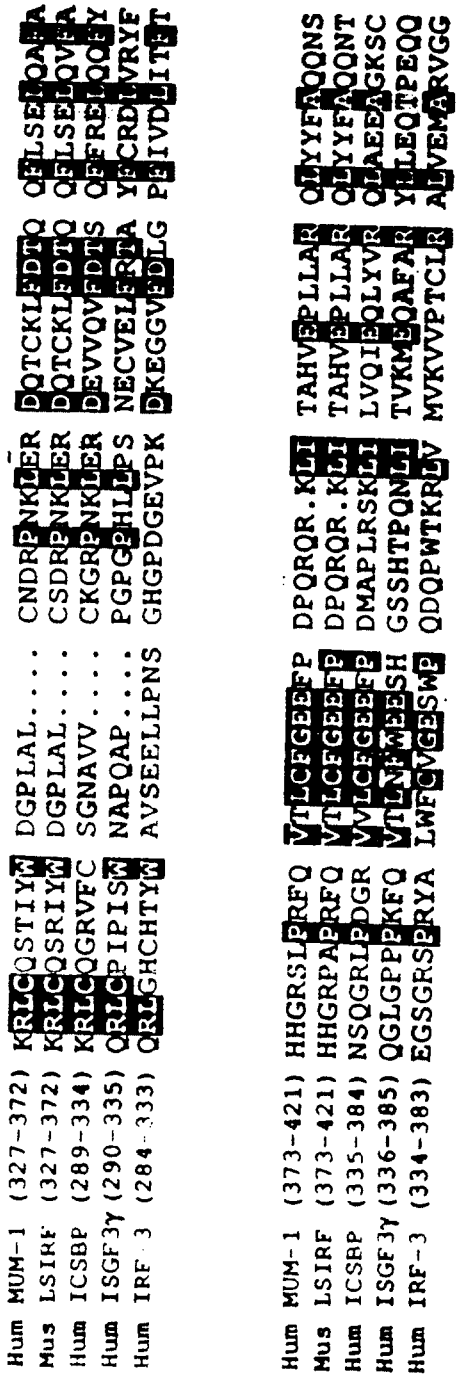

Hum MUM-1 (327-372) KRLCQSTIYW DGPLAL.... CNDRPNKLER DQTCKLFDTQ QFLSELQAEA
Mus LSIRF (327-372) KRLCQSRIYW DGPLAL.... CSDRPNKLER DQTCKLFDTQ QFLSELQVEA
Hum ICSBP (289-334) KRLCQGRVFC SGNAVV.... CKGRPNKLER DEVVQVFDTS QFFRELQQEY
Hum ISGF3γ (290-335) QRLCPIPISW NAPQAP.... PGPGPHLLPS NECVELERTA YECRDLVRYF
Hum IRF-3 (284-333) QRLGHCHTYW AVSEELLPNS GHGPDGEVPK DKEGGVEDLG PEIVDLITFT
Hum MUM-1 (373-421) HHGRSLPRFQ VTLCFGEEFP DPQRQR.KLI TAHVEPLLAR QLYYFAQQNS
Mus LSIRF (373-421) HHGRPAPRFQ VTLCFGEEFP DPQRQR.KLI TAHVEPLLAR QLYYFAQQNT
Hum ICSBP (335-384) NSQGRLPDGR VMLCFGEEFP DMAPLRSKLI LVQIEQLYVR QLAEEAGKSC
Hum ISGF3γ (336-385) QGLGPPPKFQ VTLNFEESH GSSHTPQNLI TVKMEQAFAR YLLEQTPEQQ
Hum IRF-3 (334-383) EGSGRSPRYA LWFCVGESWP QDQPWTKRLV MVKVVPTCLR ALVEMARVGG

FIG. 7

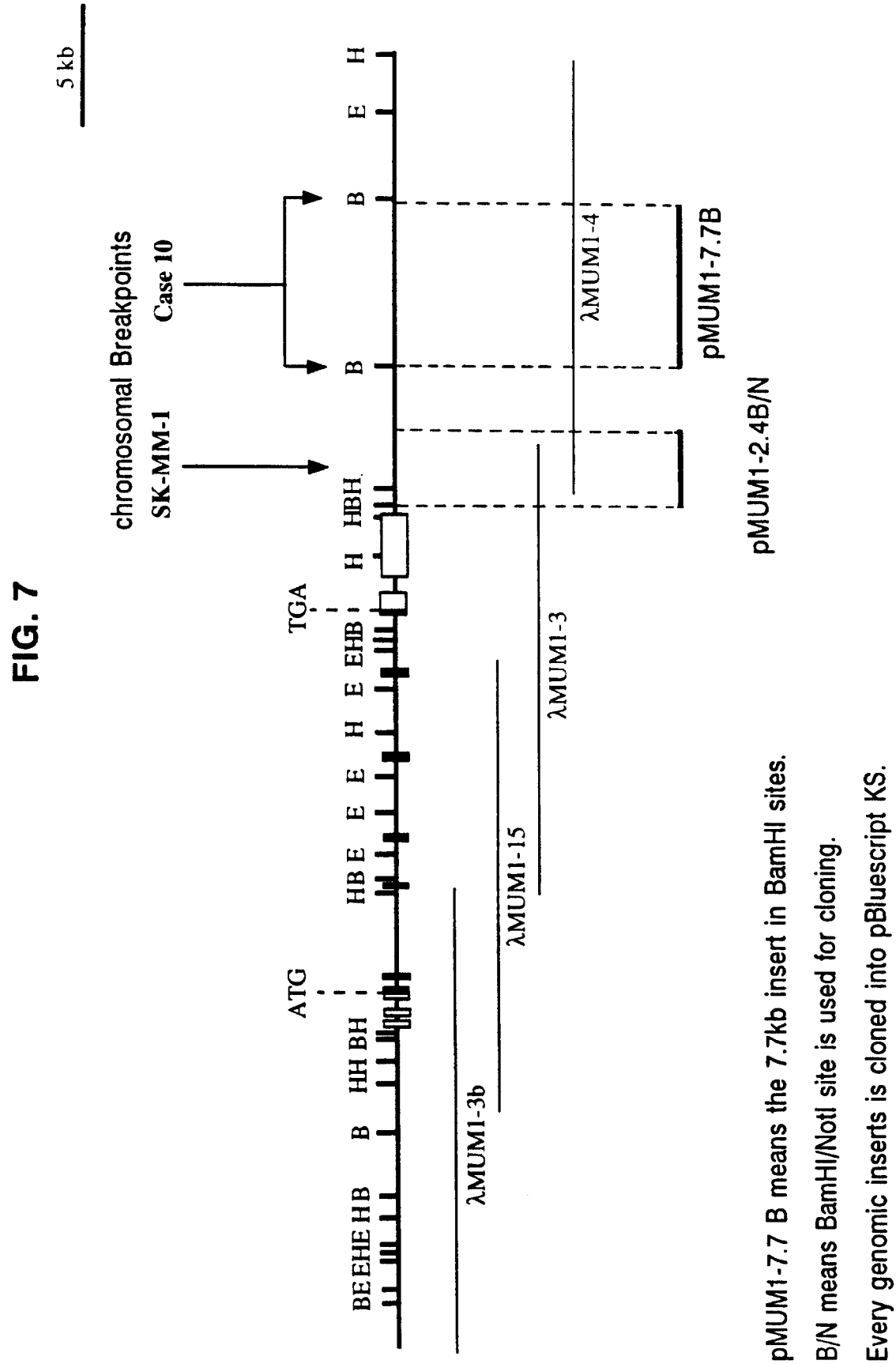

5 kb
chromosomal Breakpoints
SK-MM-1
Case 10
BE EHE HB B HH BH ATG HB E E E H E EHB TGA H HBH B B E H
λMUM1-3b
λMUM1-15
λMUM1-3
λMUM1-4
pMUM1-7.7B
pMUM1-2.4B/N
pMUM1-7.7 B means the 7.7kb insert in BamHI sites.
B/N means BamHI/NotI site is used for cloning.
Every genomic inserts is cloned into pBluescript KS.

FIG. 8

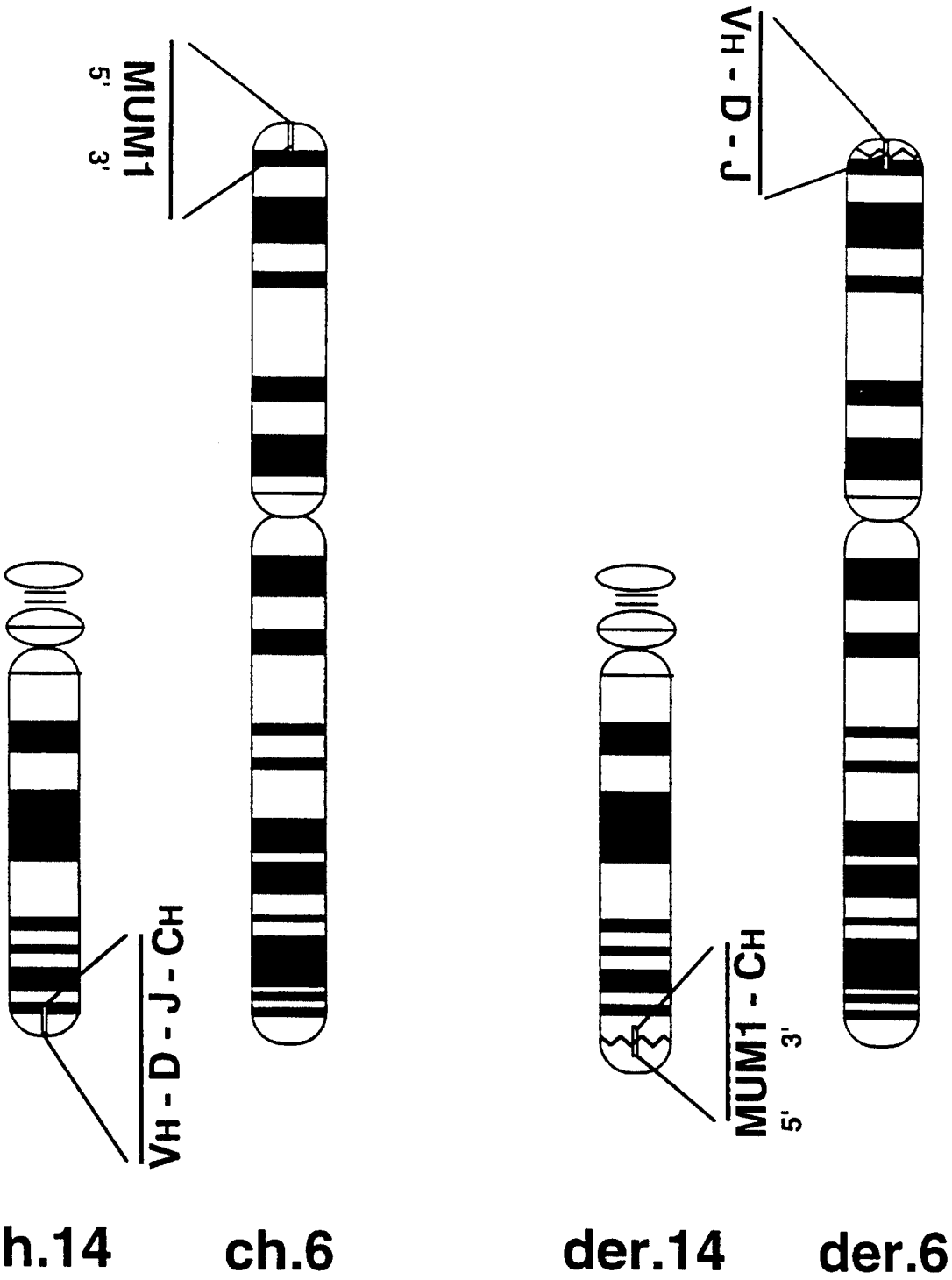


FIG. 9A

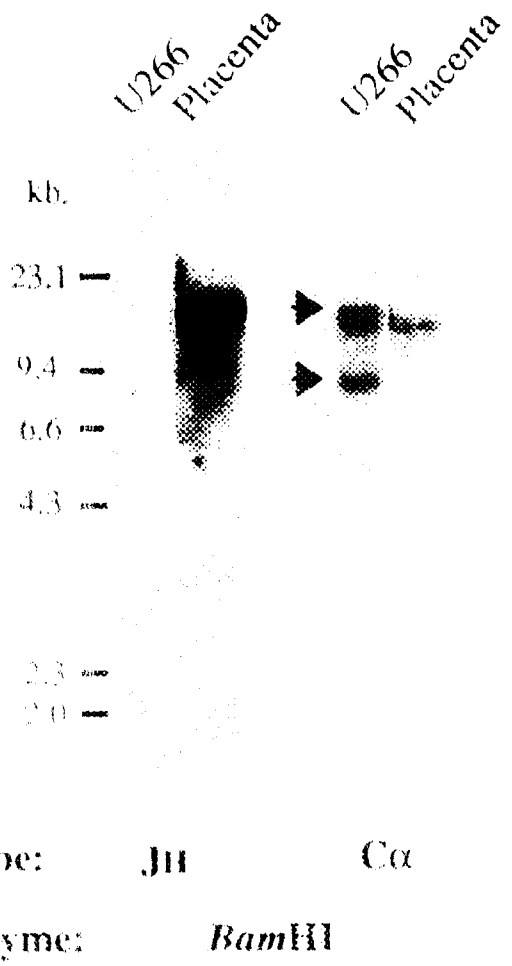
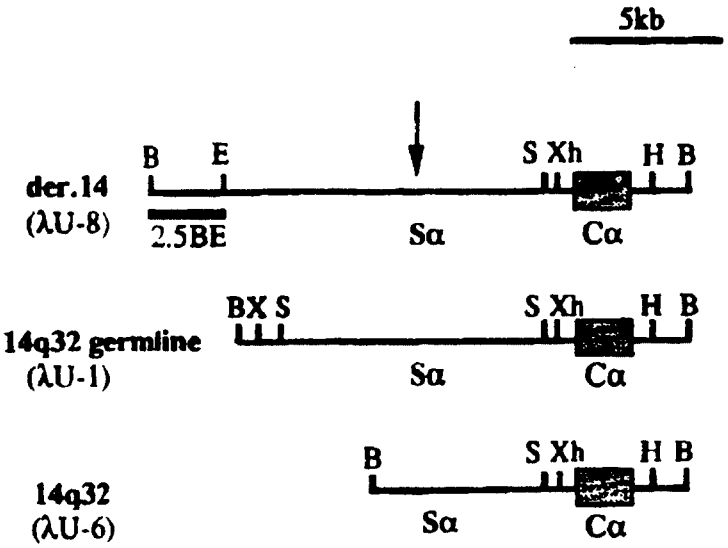

U266
Placenta
U266
Placenta
kb.
23.1
9.4
6.6
4.3
2.3
2.0
Probe: JH Cα
Enzyme: BamHI

FIG. 9B



B, *Bam*HI; H, *Hin*dIII; S, *Sac*II; X, *Xba*I; Xh, *Xho*I

chromosomal breakpoint

FIG. 10

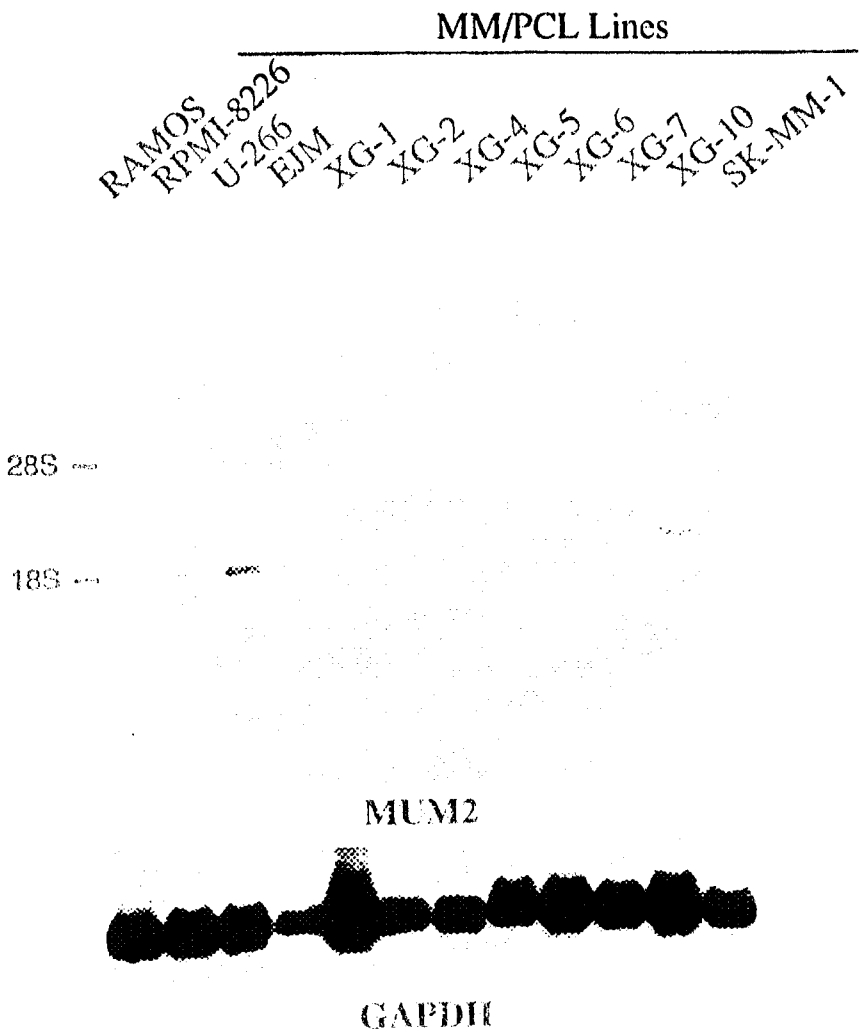

MM/PCL Lines
RAMOS
RPMI-8226
U-266
EJM
XG-1
XG-2
XG-4
XG-5
XG-6
XG-7
XG-10
SK-MM-1
28S
18S
MUM2
GAPDH

FIG. 11A

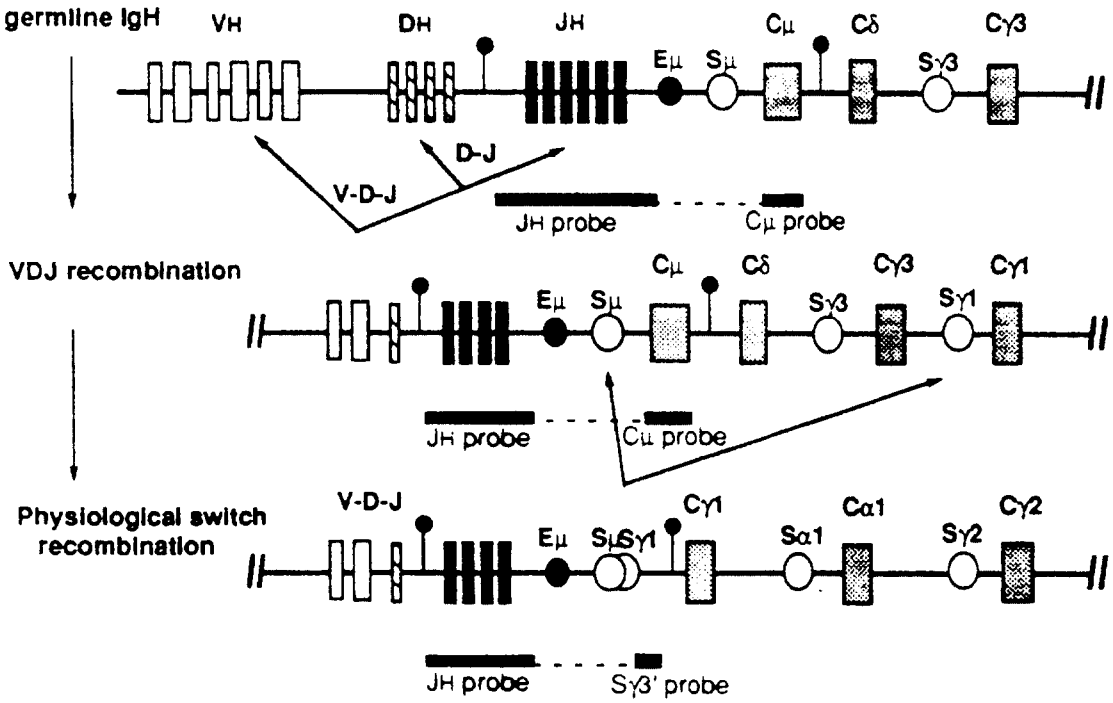


FIG. 11B

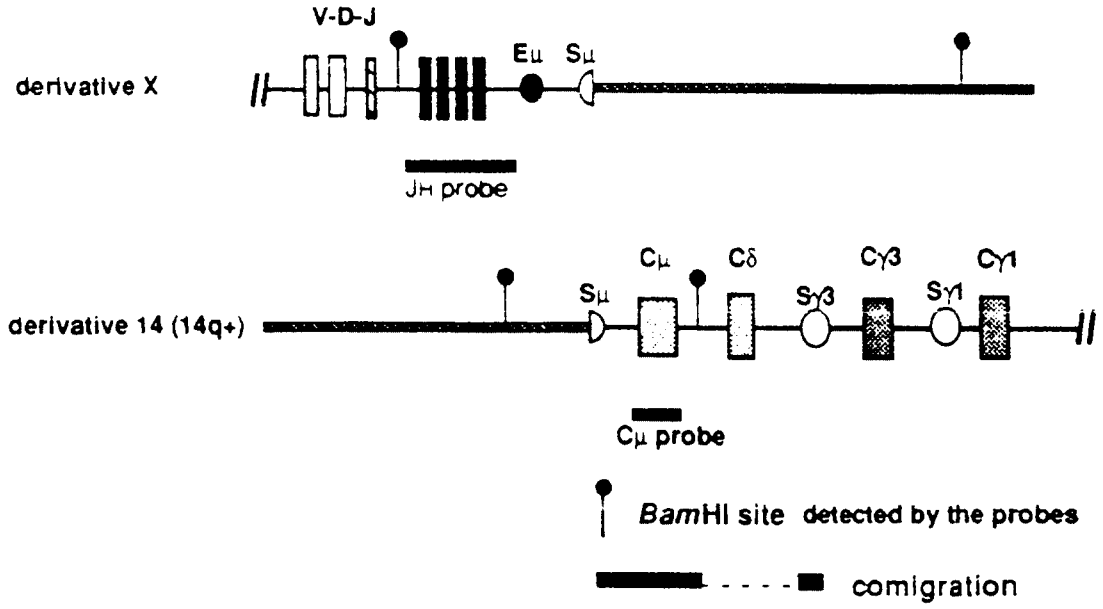
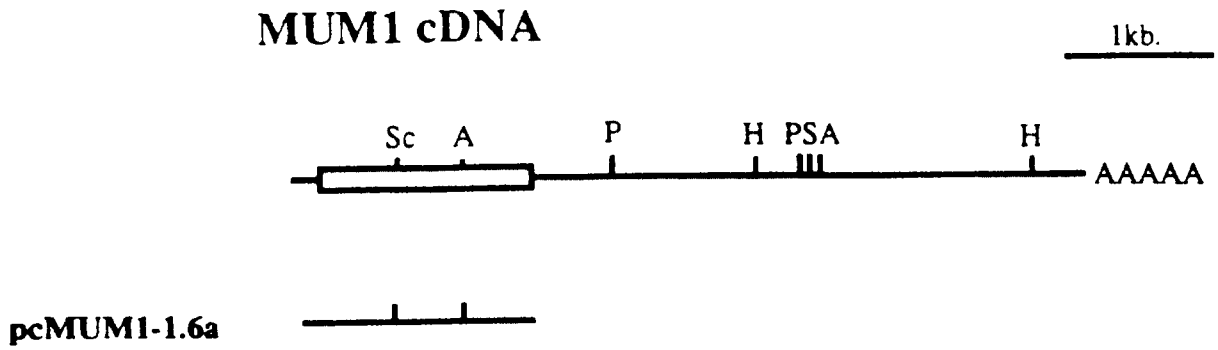


FIG. 12A



Sc; SacII, P;*Pst*I, H;*Hin*dIII, S;*Sac*I, A;*Apa*I

FIG. 12B

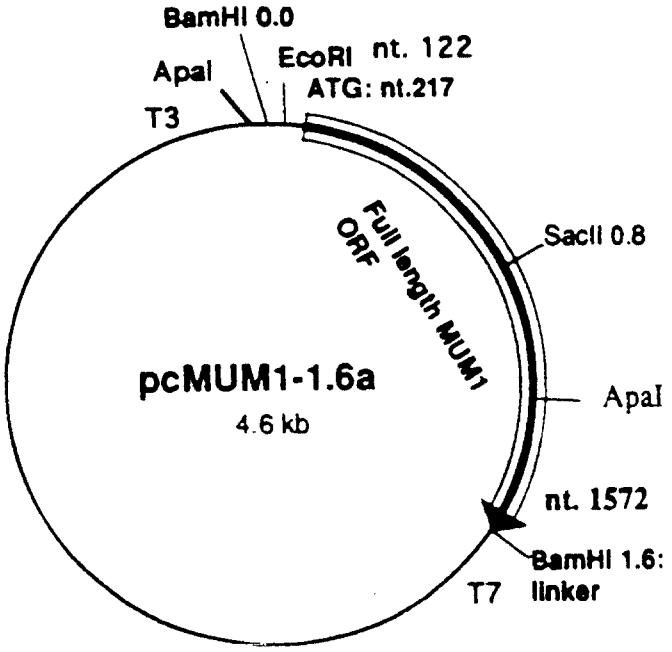
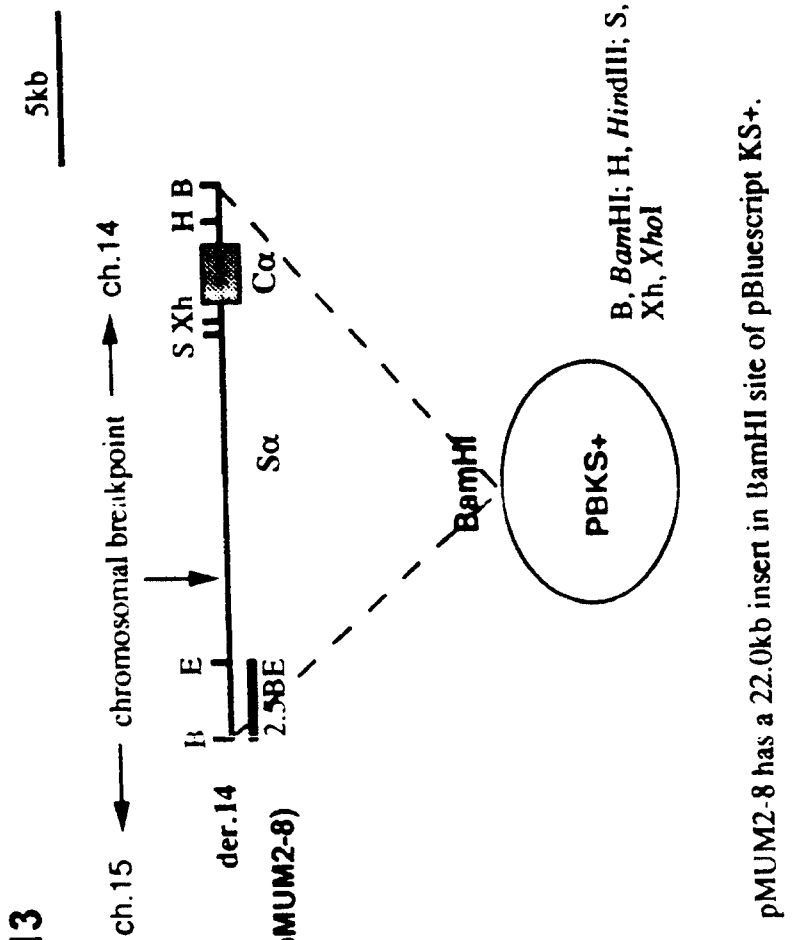


FIG. 13



pMUM2-8 has a 22.0kb insert in BamHI site of pBluescript KS+.

FIG. 14A

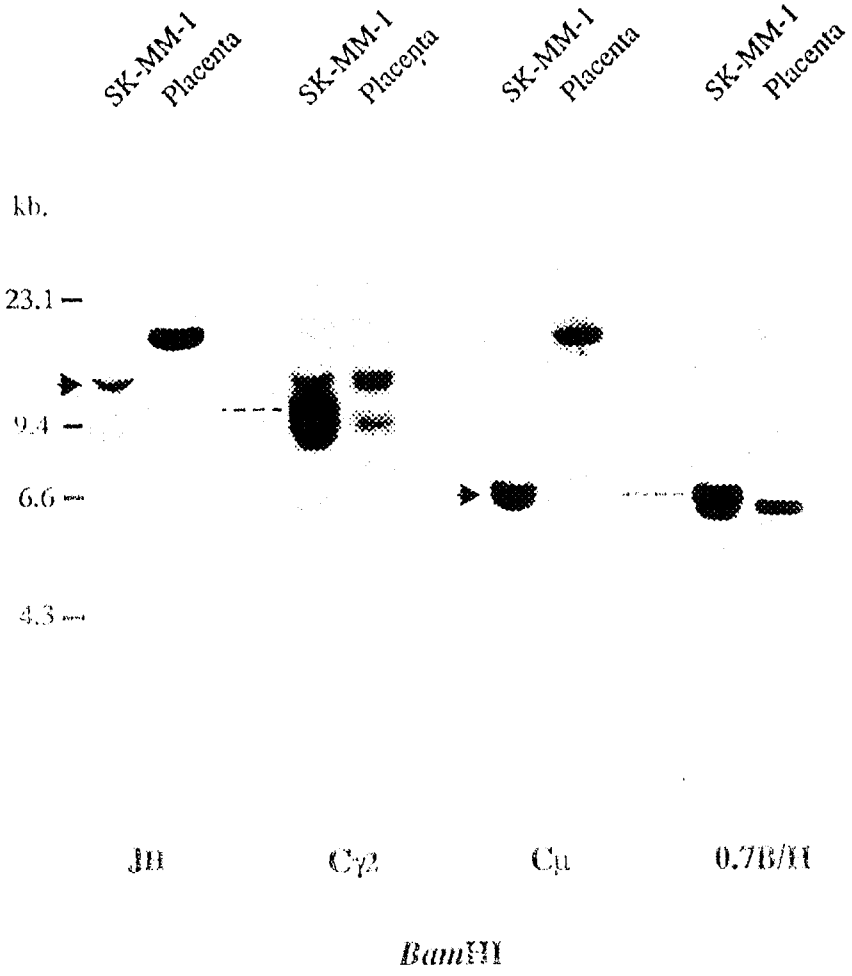

SK-MM-1
Placenta
SK-MM-1
Placenta
SK-MM-1
Placenta
SK-MM-1
Placenta
kb.
23.1
9.4
6.6
4.3
JH
Cγ2
Cμ
0.7B/H
BamHI

FIG. 14B

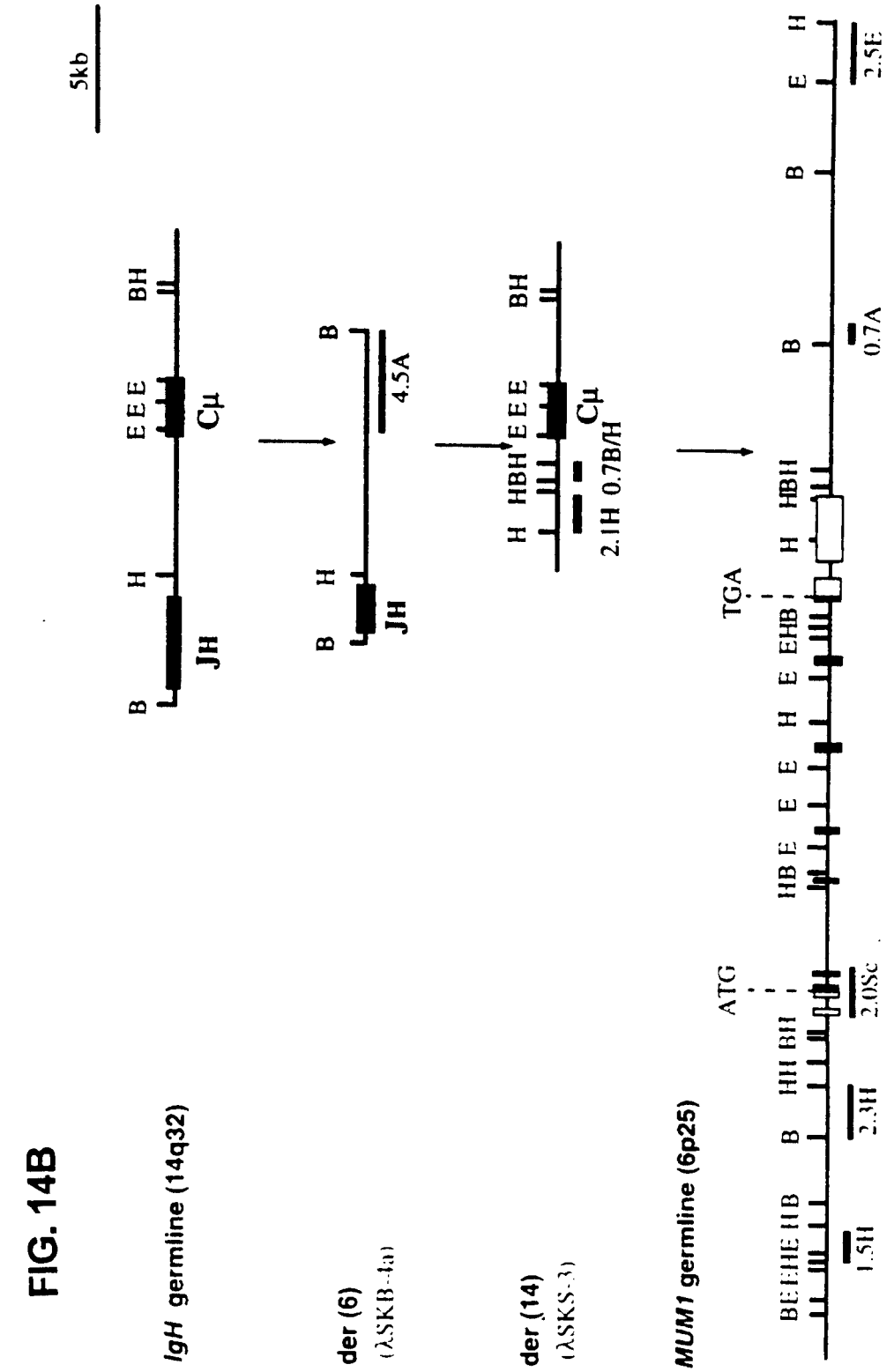
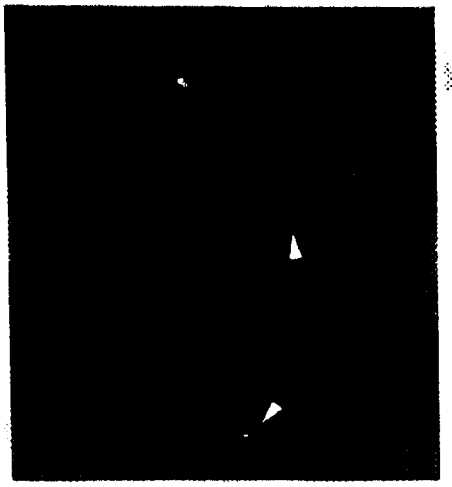

5kb
IgH germline (14q32)
der (6)
der (14)
MUM1 germline (6p25)
JH
Cμ
4.5A
2.1H
0.7B/H
ATG
TGA
0.7A
1.5H
2.3H
2.0Sc
2.5E

FIG. 15A

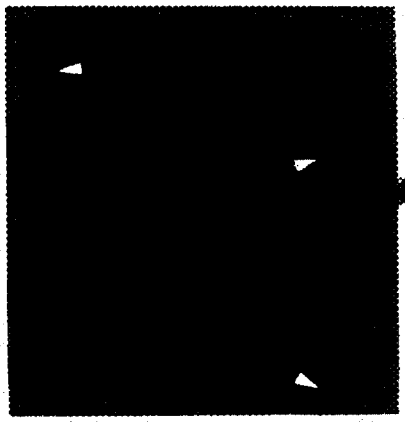


normal lymphocyte

FIG. 15B

normal lymphocyte

FIG. 15C



XG-7

FIG. 15D

XG-7

FIG. 16A

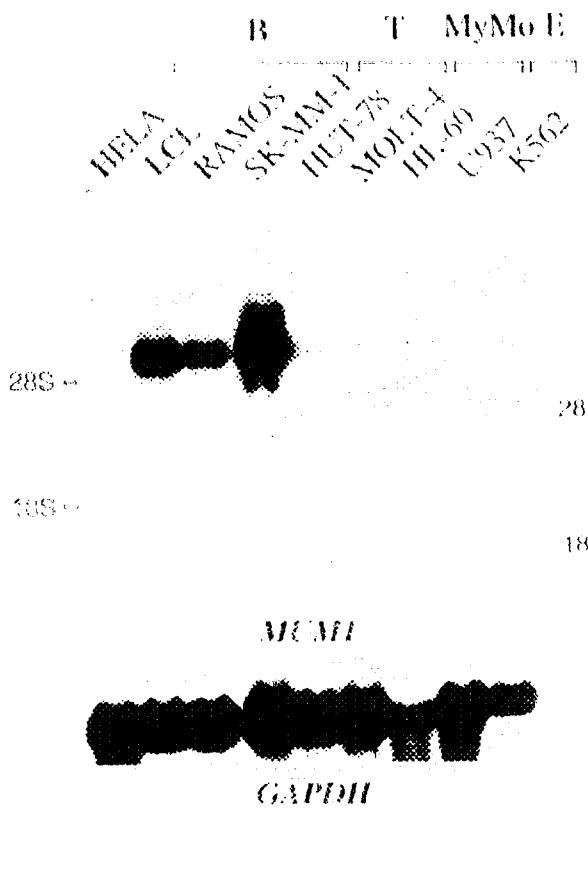


FIG. 16B

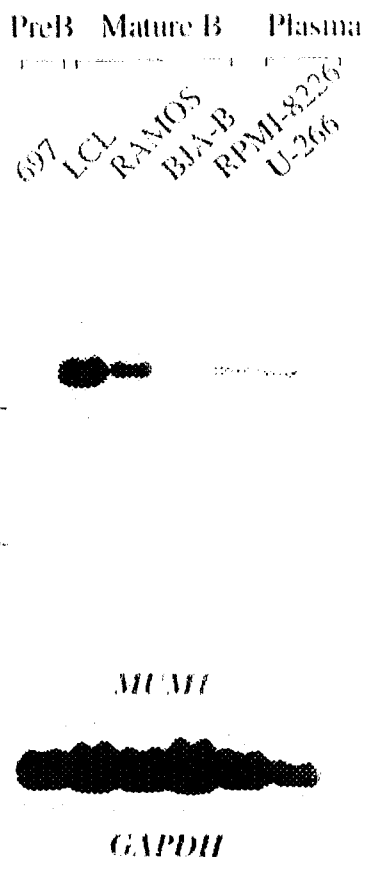


FIG. 16C

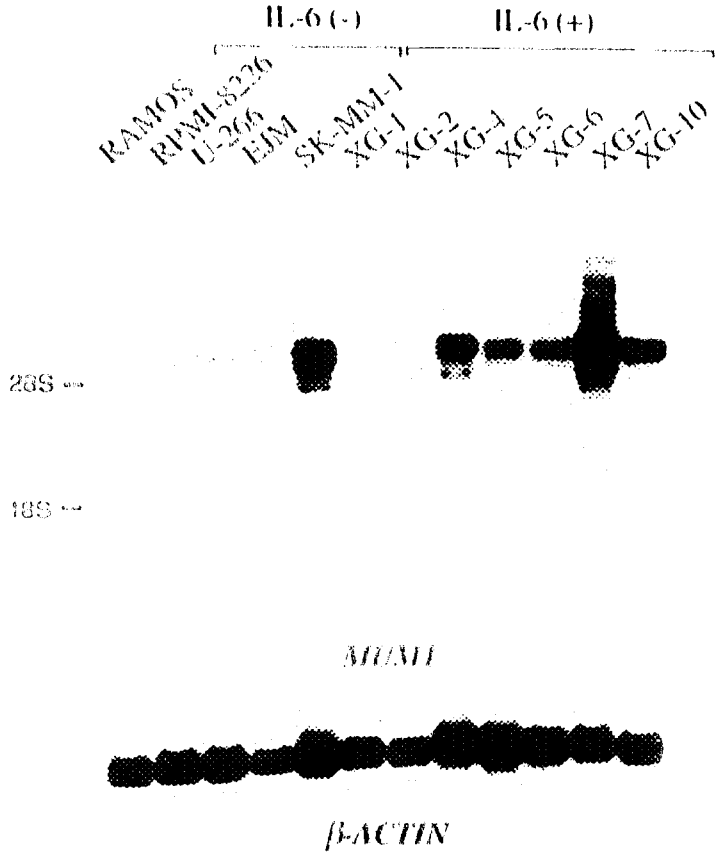

IL-6 (-)
IL-6 (+)
RAMOS
RPMI-8226
U-266
EJM
SK-MM-1
XG-1
XG-2
XG-3
XG-5
XG-6
XG-7
XG-10
28S
18S
MUC1
β-ACTIN

FIG. 16D

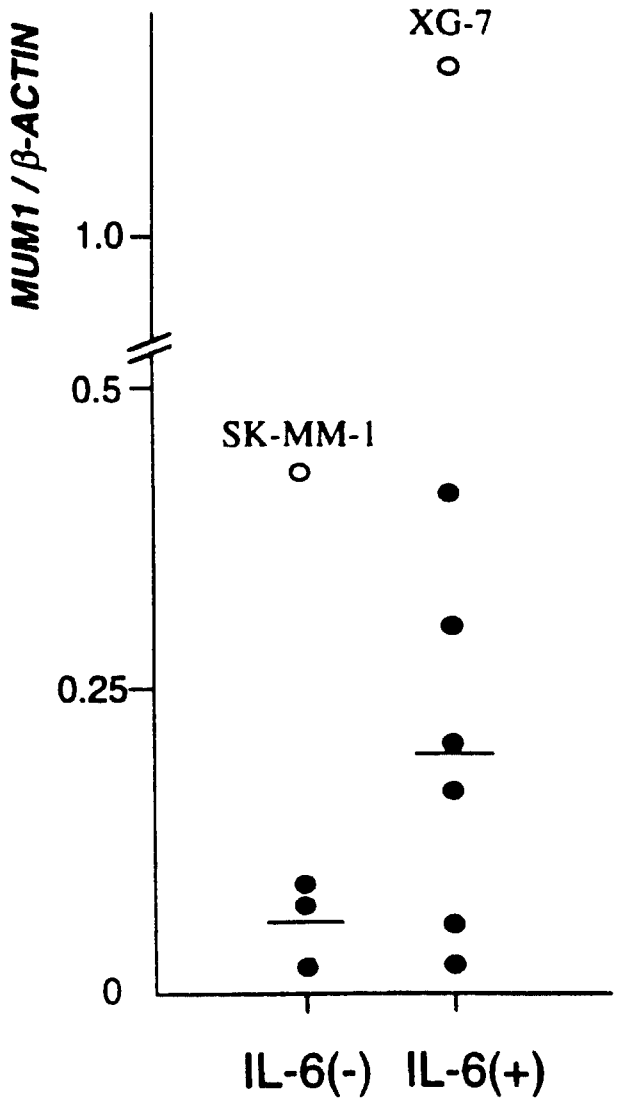
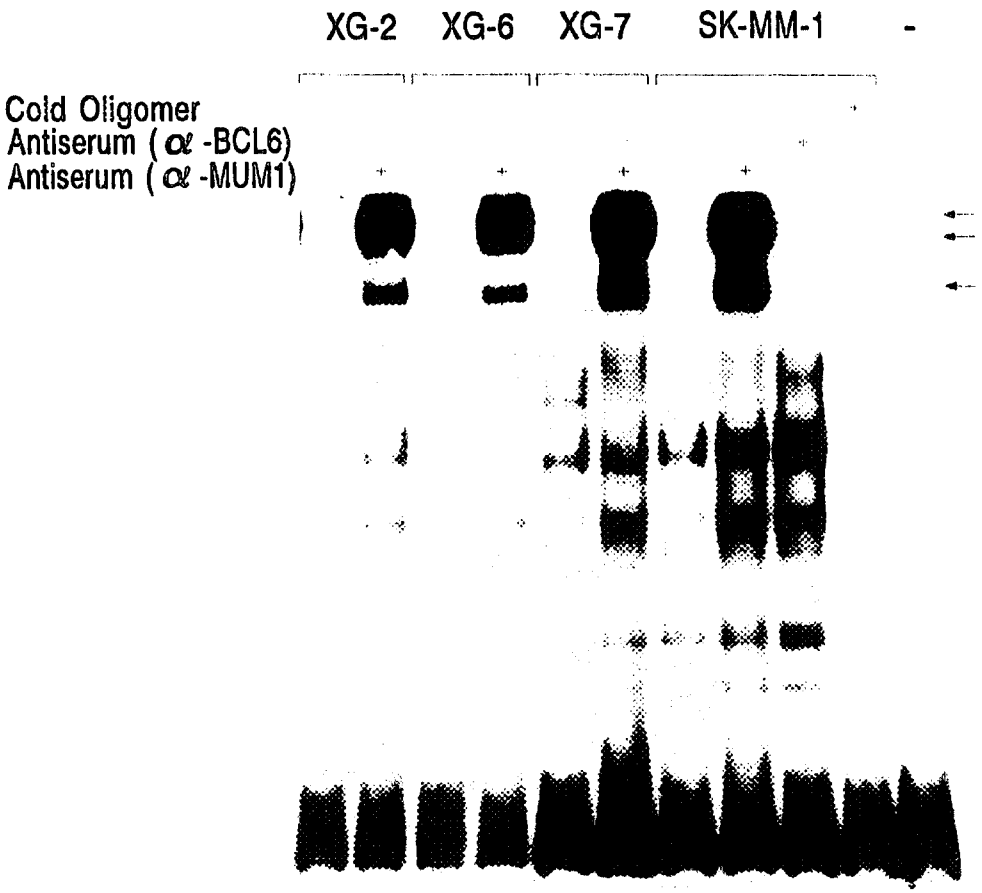


FIG. 16E


XG-2
XG-6
XG-7
SK-MM-1
-
Cold Oligomer
Antiserum (α-BCL6)
Antiserum (α-MUM1)
+
+
+
+

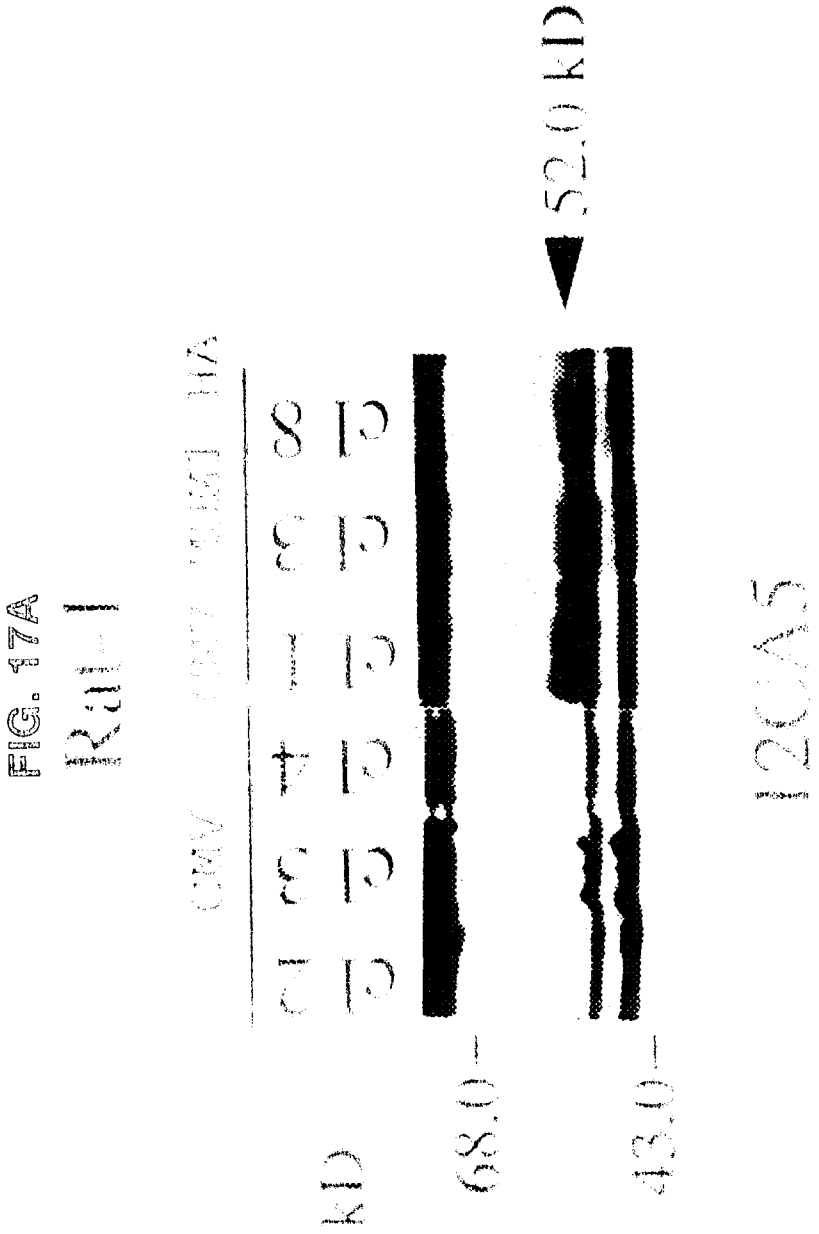


FIG. 17A
Raji

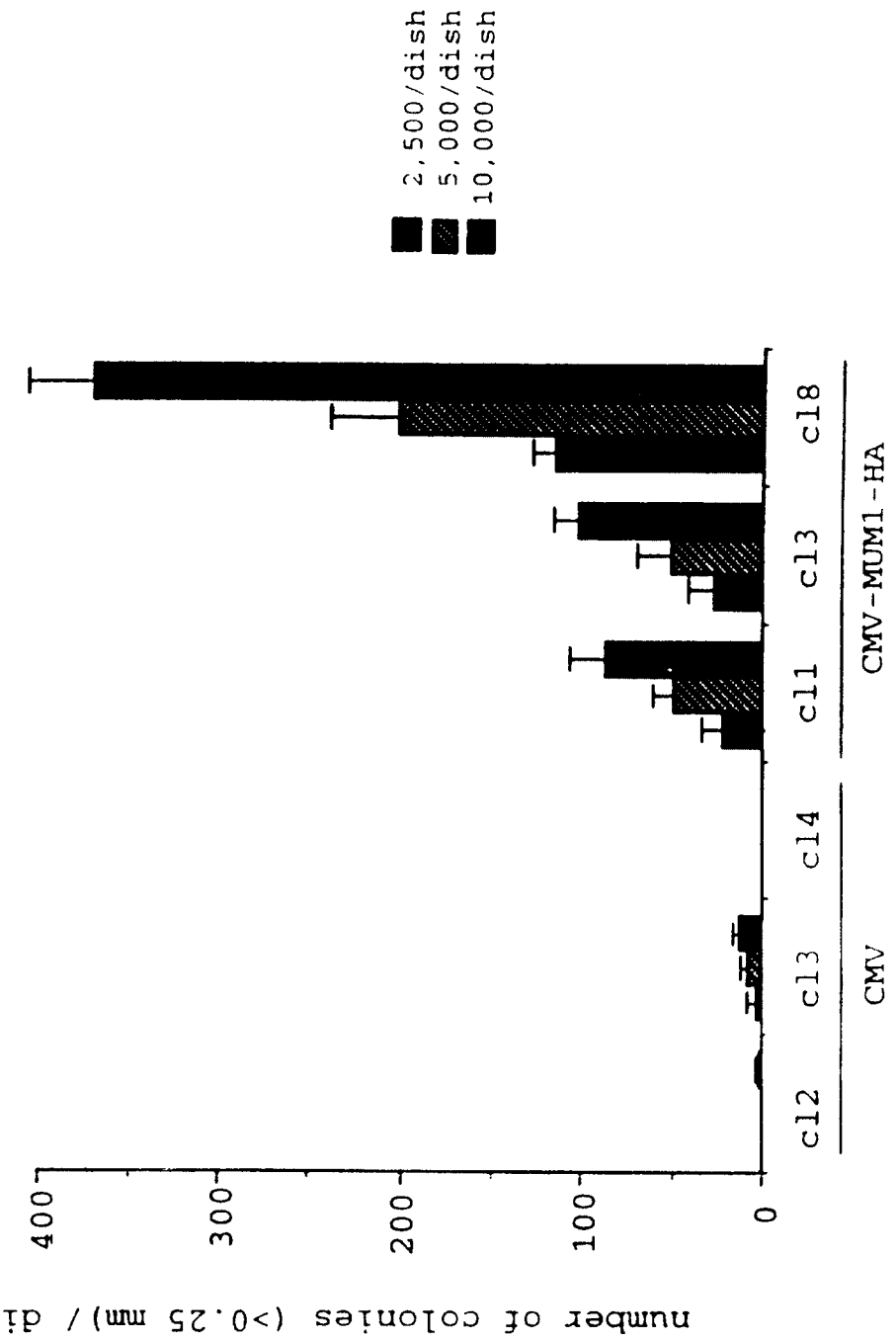

2,500/dish
5,000/dish
10,000/dish
number of colonies (>0.25 mm)/ dish
400
300
200
100
0
c12
c13
c14
c11
c13
c18
CMV
CMV-MUM1-HA

FIG. 17B