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(57) Abrégé/Abstract:
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Abstract

The invention relates to an isolated DNA sequence which codes for an antigen expressed by tumor cells which is recognized by cytotoxic T cells, leading to lysis of the tumor which expresses it. Also described are cells transfected by the DNA sequence, and various therapeutic and diagnostic uses arising out of the properties of the DNA and the antigen for which it codes.

TUMOR REJECTION ANTIGEN PRECURSORS, TUMOR REJECTION ANTIGENS AND USES THEREOF

FIELD OF THE INVENTION

This invention relates in general to the field of immunogenetics as applied to the study of oncology. More specifically, it relates to the study and analysis of mechanisms by which tumors are recognized by the organism's immune system such as through the presentation of so-called tumor rejection antigens, and the expression of what will be referred to herein as "tumor rejection antigen precursors".

BACKGROUND AND PRIOR ART

The study of the recognition or lack of recognition of cancer cells by a host organism has proceeded in many different directions. Understanding of the field presumes some understanding of both basic immunology and oncology.

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Early research on mouse tumors revealed that these displayed molecules which led to rejection of tumor cells when transplanted into syngeneic animals. These molecules are "recognized" by T-cells in the recipient animal, and provoke a cytolytic T-cell response with lysis of the transplanted cells. This evidence was first obtained with tumors induced in vitro by chemical carcinogens, such as methylcholanthrene. The antigens expressed by the tumors and which elicited the T-cell response were found to be different for each tumor. See Prehn, et al., J. Natl. Canc. Inst. 18: 769-778 (1957); Klein et al., Cancer Res. 20: 1561-1572 (1960); Gross, Cancer Res. 3: 326-333 (1943), Basombrio, Cancer Res. 30: 2458-2462 (1970) for general teachings on inducing tumors with chemical carcinogens and differences in cell surface antigens. This class of antigens has come to be known as "tumor specific transplantation antigens" or "TSTAs". Following the observation of the presentation of such antigens when induced by chemical carcinogens, similar results were obtained when tumors were induced in vitro via ultraviolet radiation. See Kripke, J. Natl. Canc. Inst. 53: 333-1336 (1974).

While T-cell mediated immune responses were observed for the types of tumor described supra, spontaneous tumors were thought to be generally non-immunogenic. These were therefore believed not to present antigens which provoked a response to the tumor in the tumor carrying subject. See Hewitt, et al., Brit. J. Cancer 33: 241-259 (1976).

The family of tum$^-$ antigen presenting cell lines are immunogenic variants obtained by mutagenesis of mouse tumor cells or cell lines, as described by Boon et al., J. Exp. Med. 152: 1184-1193 (1980).

To elaborate, tum$^-$ antigens are obtained by mutating tumor cells which do not generate an immune response in syngeneic mice and will form tumors (i.e., "tum$^+$" cells). When these tum$^+$ cells are mutagenized, they are rejected by syngeneic mice, and fail to form tumors (thus "tum$^-$"). See Boon et al., Proc. Natl. Acad. Sci. USA 74: 272 (1977).

Many tumor types have been shown to exhibit this phenomenon. See, e.g., Frost et al., Cancer Res. 43: 125 (1983).

It appears that tum$^-$ variants fail to form progressive tumors because they elicit an immune rejection process. The evidence in favor of this hypothesis includes the ability of "tum$^-$" variants of tumors, i.e., those which do not normally form tumors, to do so in mice with immune systems suppressed by sublethal irradiation, Van Pel et al., Proc. Natl, Acad. Sci. USA 76: 5282-5285 (1979); and the observation that intraperitoneally injected tum$^-$ cells of mastocytoma P815 multiply exponentially for 12-15 days, and then are eliminated in only a few days in the midst of an influx of lymphocytes and macrophages (Uyttenhove et al., J. Exp. Med. 152: 1175-1183 (1980)). Further evidence includes the observation that mice acquire an immune memory

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which permits them to resist subsequent challenge to the same tum$^-$ variant, even when immunosuppressive amounts of radiation are administered with the following challenge of cells (Boon et al., Proc. Natl, Acad. Sci. USA 74: 272-275 (1977); Van Pel et al., supra; Uyttenhove et al., supra).

Later research found that when spontaneous tumors were subjected to mutagenesis, immunogenic variants were produced which did generate a response. Indeed, these variants were able to elicit an immune protective response against the original tumor. See Van Pel et al., J. Exp. Med. 157: 1992-2001 (1983). Thus, it has been shown that it is possible to elicit presentation of a so-called "tumor rejection antigen" in a tumor which is a target for a syngeneic rejection response. Similar results have been obtained when foreign genes have been transfected into spontaneous tumors. See Fearson et al., Cancer Res. 48: 2975-1980 (1988) in this regard.

A class of antigens has been recognized which are presented on the surface of tumor cells and are recognized by cytotoxic T cells, leading to lysis. This class of antigens will be referred to as "tumor rejection antigens" or "TRAs" hereafter. TRAs may or may not elicit antibody responses. The extent to which these antigens have been studied, has been via cytolytic T cell characterization studies, in vitro i.e., the study of the identification of the antigen by a particular cytolytic T cell ("CTL" hereafter) subset. The subset proliferates upon recognition of the presented tumor rejection antigen, and

the cells presenting the antigen are lysed. Characterization studies have identified CTL clones which specifically lyse cells expressing the antigens. Examples of this work may be found in Levy et al., Adv. Cancer Res. 24: 1-59 (1977); Boon et al., J. Exp. Med. 152: 1184-1193 (1980); Brunner et al., J. Immunol. 124: 1627-1634 (1980); Maryanski et al., Eur. J. Immunol. 124: 1627-1634 (1980); Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982); Palladino et al., Canc. Res. 47: 5074-5079 (1987). This type of analysis is required for other types of antigens recognized by CTLs, including minor histocompatibility antigens, the male specific H-Y antigens, and a class of antigens, referred to as "tum-" antigens, and discussed herein.

A tumor exemplary of the subject matter described supra is known as P815. See DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988); Szikora et al., EMBO J 9: 1041-1050 (1990), and Sibille et al., J. Exp. Med. 172: 35-45 (1990).

The P815 tumor is a mastocytoma, induced in a DBA/2 mouse with methylcholanthrene and cultured as both an in vitro tumor and a cell line. The P815 line has generated many tum^- variants following mutagenesis, including variants referred to as P91A (DePlaen, supra), 35B (Szikora, supra), and P198 (Sibille, supra). In contrast to tumor rejection antigens - and this is a key distinction - the tum^- antigens are

only present after the tumor cells are mutagenized. Tumor rejection antigens are present on cells of a given tumor without mutagenesis. Hence, with reference to the literature, a cell line can be tum^+, such as the line referred to as "P1", and can be provoked to produce tum^- variants. Since the tum^- phenotype differs from that of the parent cell line, one expects a difference in the DNA of tum^- cell lines as compared to their tum^+ parental lines, and this difference can be exploited to locate the gene of interest in tum^- cells. As a result, it was found that genes of tum^- variants such as P91A, 35B and P198 differ from their normal alleles by point mutations in the coding regions of the gene. See Szikora and Sibille, supra, and Lurquin et al., Cell 58: 293-303 (1989). This has proved not to be the case with the TRAs of this invention. These papers also demonstrated that peptides derived from the tum^- antigen are presented by the L^d molecule for recognition by CTLs. P91A is presented by L^d, P35 by D^d and P198 by K^d.

It has now been found that the genes which code for the molecules which are processed to form the presentation tumor rejection antigens (referred to as "tumor rejection antigen precursors", "precursor molecules" or "TRAPs" hereafter), are not expressed in most normal adult tissues but are expressed in tumor cells. Genes which code for the TRAPs have now been isolated and cloned, and represent a portion of the invention disclosed herein.

The gene is useful as a source for the isolated and purified tumor rejection antigen precursor and the TRA themselves, either of which can be used as an agent for treating the cancer for which the antigen is a "marker", as well as in various diagnostic and surveillance approaches to oncology, discussed infra. It is known, for example, that tum^- cells can be used to generate CTLs which lyse cells presenting different tum^- antigens as well as tum^+ cells. See, e.g., Maryanski et al., Eur. J. Immunol 12: 401 (1982); and Van den Eynde et al., Modern Trends in Leukemia IX (June 1990).

The tumor rejection antigen precursor may be expressed in cells transfected by the gene, and then used to generate an immune response against a tumor of interest.

In the parallel case of human neoplasms, it has been observed that autologous mixed lymphocyte-tumor cell cultures ("MLTC" hereafter) frequently generate responder lymphocytes which lyse autologous tumor cells and do not lyse natural killer targets, autologous EBV-transformed B cells, or autologous fibroblasts (see Anichini et al., Immunol. Today 8: 385-389 (1987)). This response has been particularly well studied for melanomas, and MLTC have been carried out either with peripheral blood cells or with tumor infiltrating lymphocytes. Examples of the literature in this area including Knuth et al., Proc. Natl. Acad. Sci. USA 86: 2804-2802 (1984); Mukherji et al., J. Exp. Med.

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158: 240 (1983); Hérin et all, Int. J. Canc. 39: 390-396 (1987); Topalian et al, J. Clin. Oncol 6: 839-853 (1988). Stable cytotoxic T cell clones ("CTLs" hereafter) have been derived from MLTC responder cells, and these clones are specific for the tumor cells. See Mukherji et al., supra, Hérin et all, supra, Knuth et al., supra. The antigens recognized on tumor cells by these autologous CTLs do not appear to represent a cultural artifact, since they are found on fresh tumor cells. Topalian et al., supra; Degiovanni et al., Eur. J. Immunol. 20: 1865-1868 (1990). These observations, coupled with the techniques used herein to isolate the genes for specific murine tumor rejection antigen precursors, have led to the isolation of nucleic acid sequences coding for tumor rejection antigen precursors of TRAs presented on human tumors. It is now possible to isolate the nucleic acid sequences which code for tumor rejection antigen precursors, including, but not being limited to those most characteristic of a particular tumor, with ramifications that are described infra. These isolated nucleic acid sequences for human tumor rejection antigen precursors and applications thereof, as described infra, are also the subject of this invention.

These and various other aspects of the invention are elaborated upon in the disclosure which follows.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts detection of transfectants expressing antigen P815A.

Figure 2 shows the sensitivity of clones P1.HTR, PO.HTR, genomic transfectant P1A.T2 and cosmid transfectant P1A.TC3.1 to lysis by various CTLs, as determined by chromium release assays.

Figure 3 is a restriction map of cosmid C1A.3.1.

Figure 4 shows Northern Blot analysis of expression of gene P1A.

Figure 5 sets forth the structure of gene P1A with its restriction sites.

Figure 6 shows the results obtained when cells were transfected with the gene from P1A, either isolated from P815 or normal cells and then tested with CTL lysis.

Figure 7 shows lytic studies using mast cell line L138. 8A.

Figure 8 is a map of subfragments of the 2.4 kb antigen E fragment sequence which also express the antigen.

Figure 9 shows homology of sections of exon 3 from genes mage 1, 2 and 3.

Figure 10 shows the result of Northern blots for MAGE genes on various tissues.

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Figure 11 presents the data of Figure 13 in table form.

Figure 12 shows Southern Blot experiments using the various human melanoma cell lines employed in this application.

Figure 13 is a generalized schematic of the expression of MAGE 1, 2 and 3 genes by tumor and normal tissues.

BRIEF DESCRIPTION OF SEQUENCES

SEQ ID NO: 1 is cDNA for part of gene P1A.

SEQ ID NO: 2 presents coding region of cDNA for gene P1A.

SEQ ID NO: 3 shows non coding DNA for P1A cDNA which is 3' to the coding region of SEQ ID NO: 2.

SEQ ID NO: 4 is the entire sequence of cDNA for P1A.

SEQ ID NO: 5 is the genomic DNA sequence for P1A.

SEQ ID NO: 6 shows the amino acid sequence for the antigenic peptides for P1A TRA. The sequence is for cells which are A^+ B^+, i.e., express both the A and B antigens.

SEQ ID NO: 7 is a nucleic acid sequence coding for antigen E.

SEQ ID NO: 8 is a nucleic acid sequence coding for MAGE-1.

SEQ ID NO: 9 is the gene for MAGE-2.

SEQ ID NO: 10 is the gene for MAGE-21.

SEQ ID NO: 11 is cDNA for MAGE-3.

SEQ ID NO: 12 is the gene for MAGE-31.

SEQ ID NO: 13 is the gene for MAGE-4.

SEQ ID NO: 14 is the gene for MAGE-41.

SEQ ID NO: 15 is cDNA for MAGE-4.

SEQ ID NO: 16 is cDNA for MAGE-5.

SEQ ID NO: 17 is genomic DNA for MAGE-51.

SEQ ID NO: 18 is cDNA for MAGE-6.

SEQ ID NO: 19 is genomic DNA for MAGE-7.

SEQ ID NO: 20 is genomic DNA for MAGE-8.

SEQ ID NO: 21 is genomic DNA for MAGE-9.

SEQ ID NO: 22 is genomic DNA for MAGE-10.

SEQ ID NO: 23 is genomic DNA for MAGE-11.

SEQ ID NO: 24 is genomic DNA for smage-I.

SEQ ID NO: 25 is genomic DNA for smage-II.

SEQ ID NO: 26 is a synthetic peptide derived from Exon 3.1.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Many different "MAGE" genes have been identified, as will be seen from the sequences which follow the application. The protocols described in the following

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examples were used to isolate these genes and cDNA sequences.

"MAGE" as used herein refers to a nucleic acid sequence isolated from human cells. The acronym "smage" is used to describe sequences of murine origin.

When "TRAP" or "TRAs" are discussed herein as being specific to a tumor type, this means that the molecule under consideration is associated with that type of tumor, although not necessarily to the exclusion of other tumor types.

Example 1

In order to identify and isolate the gene coding for antigen P815A, gene transfection was used. This approach requires both a source of the gene, and a recipient cell line. Highly transfectable cell line P1.HTR was the starting material for the recipient, but it could not be used without further treatment, as it presents "antigen A", one of four recognized P815 tumor antigens. See Van Pel et al., Molecular Genetics 11: 467-475 (1985). Thus, screening experiments were carried out to isolate cell lines which did not express the antigen and which nonetheless possessed P1.HTR's desirable qualities.

To do this, P1.HTR was screened with CTLs which were specific for each of tumor antigens A, B, C and D. Such CTLs are described by Uyttenhove et al., J. Exp. Med. 157: 1040-1052 (1983).

To carry out the selection, 10^6 cells of P1.HTR were mixed with 2-4x10^6 cells of the CTL clone in a round bottom tube in 2 ml of medium, and centrifuged for three minutes at 150xg. After four hours at 37°C, the cells were washed and resuspended in 10 ml of medium, following Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982). Additional information on the CTL assay and screening protocol, in general may be found in Boon et al., J. Exp. Med. 152: 1184-1193 (1980), and Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982).

When these selections were carried out, a cell line variant was found which expressed neither antigen A or B. Additional selections with CTLs specific for antigen C then yielded a variant which also lacked antigen C. Please see figure 2 for a summary of the results of these screenings. The variant PO.HTR is negative for antigens A, B and C, and was therefore chosen for the transfection experiments.

The cell line PO.HTR has been deposited in accordance with the Budapest Treaty at the Institute Pasteur Collection Nationale De Cultures De Microorganismes, 28, Rue de Docteur Roux, 75724 Paris France, and has accession number I-1117.

This methodology is adaptable to secure other cell lines which are variants of a cell type which normally presents at least one of the four recognized P815 tumor antigens, i.e., antigens A, B, C and D, where the variants

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present none of antigens A, B and C. P1.HTR is a mastocytoma cell line, so it will be seen that the protocol enables the isolation of biologically pure mastocytoma cell lines which express none of P815 antigens A, B and C, but which are highly transfectable. Other tumor types may also be screened in this fashion to secure desired, biologically pure cell lines. The resulting cell lines should be at least as transfectable with foreign DNA as is P1.HTR, and should be selected so as to not express a specific antigen.

Example 2

Previous work reported by DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988) had shown the efficacy of using cosmid library transfection to recover genes coding for tum^- antigens.

Selective plasmid and genomic DNA of P1.HTR were prepared, following Wölfel et al., Immunogenetics 26: 178-187 (1987). The transfection procedure followed Corsaro et al., Somatic Cell Molec. Genet 7: 603-616 (1981), with some modification. Briefly, 60 μg of cellular DNA and 3 μg of DNA of plasmid pHMR272, described by Bernard et al., Exp. Cell. Biol. 158: 237-243 (1985) were mixed. This plasmid confers hygromycin resistance upon recipient cells, and therefore provides a convenient way to screen for transfectants. The mixed DNA was combined with 940 ul of 1 mM Tris-HCl (pH 7.5), 0.1 mM EDTA; and 310 ul 1M $CaCl_2$.

The solution was added slowly, and under constant agitation to 1.25 ml of 50 mM Hepes, 280 mM NaCl, 1.5 mM Na_2HPO_4, adjusted to pH 7.1 with NaOH. Calcium phosphate - DNA precipitates were allowed to form for 30-45 minutes at room temperature. Following this, fifteen groups of PO.HTR cells ($5x10^6$) per group were centrifuged for 10 minutes at 400 g. Supernatants were removed, and pellets were resuspended directly into the medium containing the DNA precipitates. This mixture was incubated for 20 minutes at 37°C, after which it was added to an 80 cm^2 tissue culture flask containing 22.5 ml DMEM, supplemented with 10% fetal calf serum. After 24 hours, medium was replaced. Forty-eight hours after transfection, cells were collected and counted. Transfected cells were selected in mass culture using culture medium supplemented with hygromycin B (350 ug/ml). This treatment selected cells for hygromycin resistance.

For each group, two flasks were prepared, each containing $8x10^6$ cells in 40 ml of medium. In order to estimate the number of transfectants, $1x10^6$ cells from each group were plated in 5 ml DMEM with 10% fetal calf serum (FCS), 0.4% bactoagar*, and 300 ug/ml hygromycin B. The colonies were then counted 12 days later. Two independent determinations were carried out and the average taken. This was multiplied by 5 to estimate the number of transfectants in the corresponding group. Correction had

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to be made for the cloning efficiency of P815 cells, known to be about 0.3.

Example 3

Eight days after transfection as described in example 2, supra, antibiotic resistant transfectants were separated from dead cells, using density centrifugation with Ficoll*-Paque. These cells were maintained in non-selective medium for 1 or 2 days. The cells were plated in 96 well microplates (round bottom), at 30 cells/microwell in 200 ul of culture medium. Anywhere from 100-400 microwells were prepared, depending on the number of transfectants prepared. Agar colony tests gave estimates of 500-3000. After 5 days, the wells contained about $6x10^4$ cells and replicate plates were prepared by transferring 1/10 of the wells to microplates which were then incubated at 30°C. One day later, master plates were centrifuged, medium removed, and 750 CTLs against P815 antigen A (CTL-P1:5) were added to each well together with 10^6 irradiated syngeneic feeder spleen cells in CTL culture medium containing 40 U/ml recombinant human IL-2, and HAT medium to kill stimulator cells. Six days later, plates were examined visually to identify wells where CTLs had proliferated. Where plates showed proliferating microcultures, aliquots of 100 ul of the wells were transferred to another plate containing ^{51}Cr labeled P1.HTR target cells ($2x10^3$ - $4x10^3$ per well), and chromium release

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was measured after 4 hours. Replicate microcultures corresponding to those showing high CTL activity were expanded and cloned by limited dilution in DMEM with 10% FCS. Five days later, about 200 clones were collected and screened with the CTL.P1:5 cell line, described supra, in a visual lysis assay. See figure 1A for these results.

In these experiments, three of the fifteen groups of transfectants yielded a few positive microcultures. These microcultures were tested for lytic activity against P1.HTR, as described supra. Most of the microcultures where proliferation was observed showed lytic activity. This activity was well above background, as shown in figure 1B. This figure summarizes data wherein two groups of cells (groups "5" and "14"), 400 and 300 microwells were seeded with 30 hygromycin resistant transfected cells. Amplification and duplication of the microcultures was followed by addition of anti-A CTL P1:5. Six days later, lytic activity against P1.HTR was tested. In the figure, each point represents lytic activity of a single microculture.

Duplicate microcultures corresponding to several positive wells were subcloned, and more than 1% of the subclones were found to be lysed by anti-A CTL. Thus, three independent transfectants expressing P815A were obtained from 33,000 hygromycin resistant transfectants. One of these lines, referred to hereafter as P1A.T2 was tested further.

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The relevant antigen profile of P1A.T2 is shown in figure 2, this being obtained via anti-CTL assays of the type described supra.

Example 4

The CTL assays carried out for P1A.T2 demonstrated that it presented antigen A ("P815A"), and therefore had received the gene from P1.HTR. To that end, this cell line was used as a source for the gene for the antigen precursor in the following experiments.

Prior work had shown that genes coding for tum$^-$ antigens could be recovered directly from transfectants obtained with a cosmid library. See DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988). This procedure was followed for recovery of the P815 gene.

Total genomic DNA of P1A.T2 was partially digested with restriction endonuclease Sau 3A1, and fractionated by NaCl density gradient ultracentrifugation to enrich for 35-50 kb DNA fragments, following Grosveld et al., Gene 10: 6715-6732 (1982). These fragments were ligated to cosmid arms of C2RB, described by Bates et al., Gene 26: 137-146 (1983).

These cosmid arms had been obtained by cleavage with SmaI and treatment with calf intestinal phosphatase, followed by digestion with BamHI. Ligated DNA was packaged into lambda phage components, and titrated on E. coli ED 8767, following Grosveld et al., supra. Approximately $9x10^5$

ampicillin resistant colonies were obtained per microgram of DNA insert.

The cosmid groups were amplified by mixing 30,000 independent cosmids with 2 ml of ED 8767 in 10 mM $MgCl_2$, incubated 20 minutes at 37°C, diluted with 20 ml of Luria Bertani ("LB") medium, followed by incubation for one hour. This suspension was titrated and used to inoculate 1 liter of LB medium in the presence of ampicillin (50 ug/ml). At a bacterial concentration of $2x10^8$ cells/ml (OD_{600}=0.8), a 10 ml aliquot was frozen, and 200 ug/ml chloramphenicol was added to the culture for overnight incubation. Total cosmid DNA was isolated by alkaline lysis procedure, and purified on CsCl gradient.

In these experiments, a library of 650,000 cosmids was prepared. The amplification protocol involved the use of 21 groups of approximately 30,000 cosmids.

Example 5

Using the twenty-one groups of cosmids alluded to supra, (60 ug) and 4 ug of pHMR272, described supra, groups of $5x10^6$ PO.HTR cells were used as transfectant hosts. Transfection was carried out in the same manner as described in the preceding experiments. An average of 3000 transfectants per group were tested for antigen presentation, again using CTL assays as described. One group of cosmids repeatedly yielded positive transfectants, at a frequency of about 1/5,000 drug resistant

transfectants. The transfectants, as with P1A.T2, also showed expression of both antigen A and B. The pattern of expression of transfectant P1A.TC3.1 is shown in figure 2.

Example 6

As indicated in Example 5, supra, three independent cosmid transfected cells presenting P815A antigen were isolated. The DNA of these transfectants was isolated and packaged directly with lambda phage extracts, following DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988). The resulting product was titrated on E. coli ED 8767 with ampicillin selection, as in Example 5. Similarly, amplification of the cosmids and transfection followed Example 5, again using PO.HTR.

High frequencies of transfection were observed, as described in Table 1, which follows:

Table 1. Transfer of the expression of antigen P815A by cosmids obtained by direct packaging

Transfectant obtained with the cosmid library	No. of cosmids obtained by direct packaging of 0.5 µg of DNA	No. of transfectants expressing P815A / no. of HmB' transfectants
TC3.1	32	87/192
TC3.2	32000	49/384
TC3.3	44	25/72

The cosmids were analyzed with restriction enzymes and it was found that directly packaged transfectant P1A.TC3.1 contained 32 cosmids, 7 of which were different. Each of these 7 cosmids was transfected into PO.HTR, in the manner described supra, and again, following the protocols described above, transfectants were studied for presentation of P815A. Four of the cosmid transfectants showed P815A presentation and, as with all experiments described herein, P815B was co-expressed.

Of the four cosmids showing presentation of the two antigens, cosmid C1A.3.1 was only 16.7 kilobases long, and was selected for further analysis as described infra.

The cosmid C1A.3.1 was subjected to restriction endonuclease analysis, yielding the map shown in Figure 3.

All EcoRI fragments were transfected, again using the above described protocols, and only the 7.4 kilobase fragment produced a transfectant that anti-A CTLs could lyse. Similar experiments were carried out on the PstI fragments, and only a 4.1 kb fragment fully contained within the 7.4 kb EcoRI fragment produced lysable transfectants.

This fragment (i.e., the 4.1 kb PstI fragment), was digested with SmaI, giving a 2.3 kb fragment which also yielded host cells presenting antigens A and B after transfection. Finally, a fragment 900 bases long, secured with SmaI/XbaI, also transferred expression of the precursors of these two antigens, i.e., the transfected host cell presented both antigen A and antigen B.

Example 7

The 900 base fragment described above was used as a probe to detect the expression of the P815A gene in parent cell line P1.HTR. To accomplish this, total cellular RNA was first isolated using the guanidine-isothiocyanate procedure of Davis et al., Basic Methods In Molecular Biology (Elseview Science Publishing Co, New York) (1986). The same reference was the source of the method used to isolate and purify polyA$^+$ mRNA using oligodT cellulose column chromatography.

Samples were then subjected to Northern Blot analysis. RNA samples were fractionated on 1% agarose gels containing 0.66 M formaldehyde. The gels were treated with 10xSSC (SSC: 0.15 M NaCl; 0.015 M sodium citrate, pH 7.0) for 30 minutes before overnight blotting on nitrocellulose membranes. These were baked for two hours at 80°C, after which the membranes were prehybridized for 15 minutes at 60°C in a solution containing 10% dextran sulfate, 1% SDS and 1M NaCl. Hybridization was then carried out using denatured probe (the 900 base fragment), together with 100 ug/ml salmon sperm DNA.

When this protocol was carried out using P1.HTR poly A$^+$ RNA, a band of 1.2 kb and two fainter bands were identified, as shown in Figure 4, lane 1 (6 ug of the RNA).

The same probe was used to screen a cDNA library, prepared from poly-A$^+$ RNA from the cell line. This yielded

a clone with a 1kb insert, suggesting a missing 5′ end. The Northern blots for the cDNA are not shown.

Hybridization experiments in each case were carried out overnight at 60°C. The blots were washed twice at room temperature with 2xSSC and twice at 60°C with 2xSSC supplemented with 1% SDS.

The foregoing experiments delineated the DNA expressing the P815A antigen precursor sufficiently to allow sequencing, using the well known Sanger dideoxy chain termination method. This was carried out on clones generated using a variety of restriction endonucleases and by specific priming with synthetic oligonucleotide primers. The results for exons of the gene are set forth in sequence id no: 4.

Example 8

The Northern analysis described supra suggested that the 5′ end of the cDNA was missing. To obtain this sequence, cDNA was prepared from P1.HTR RNA using a primer corresponding to positions 320-303. The sequence was then amplified using the polymerase chain reaction using a 3′ primer corresponding to positions 286-266 and a 5′ primer described by Frohman et al., Proc. Natl. Acad. Sci. USA 85: 8998-9002 (1988). A band of the expected size (270 bases) was found, which hybridized to the 900 bp SmaI/XbaI fragment described supra on a Southern blot. Following cloning into m13tg 130 λ tg 131, the small, 270 bp fragment was sequenced. The sequence is shown in sequence id no: 1.

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Example 9

Following the procurement of the sequences described in Examples 7 and 8 and depicted in seq id no: 4, a 5.7 kb region of cosmid C1A.3.1 was sequenced. This fragment was known to contain the 900 base fragment which expressed P815A in transfectants. This experiment permitted delineation of introns and exons, since the cosmid is genomic in origin.

The delineated structure of the gene is shown in figure 5. Together with seq id no: 4, these data show that the gene for the antigen precursor, referred to as "P1A" hereafter, is approximately 5 kilobases long and contains 3 exons. An ORF for a protein of 224 amino acids starts in exon 1, ending in exon 2. The 900 base pair fragment which transfers expression of precursors for antigens A and B only contains exon 1. The promoter region contains a CAAT box, as indicated in seq. id no: 1, and an enhancer sequence. This latter feature has been observed in promoters of most MHC class I genes, as observed by Geraghty et al., J. Exp. Med 171: 1-18 (1990); Kimura et al., Cell 44: 261-272 (1986).

A computer homology search was carried out, using program FASTA* with K-triple parameters of 3 and 6, as suggested by Lipman et al., Science 227: 1435-1441 (1985), and using Genbank* database release 65 (October 1990). No homology was found except for a stretch of 95 bases corresponding to part of an acid region coded by exon 1 (positions 524-618), which is similar to sequences coding

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for acidic regions in mouse nucleolar protein NO38/B23, as described by Bourbon et al., Mol. Biol. 200: 627-638 (1988), and Schmidt-Zachmann et al., Chromosoma 96: 417-426 (1988). Fifty six of 95 bases were identical. In order to test whether these homologies were the reason for cross hybridizing, experiments were carried out using a mouse spleen cDNA library screened with the 900 base fragment. cDNA clones corresponding closely to the sizes of the cross hybridizing bands were obtained. These were partially sequenced, and the 2.6 kb cDNA was found to correspond exactly to reported cDNA sequence of mouse nucleolin, while the 1.5 kb cDNA corresponded to mouse nucleolar protein NO38/B23.

Analysis of the nucleotide sequence of the gene, referred to as "P1A" hereafter, suggests that its coded product has a molecular mass of 25 kd. Analysis of the sequence id no: 4 shows a potential nuclear targeting signal at residues 5-9 (Dingwall et al., Ann. Rev. Cell Biol. 2: 367-390 (1986)), as well as a large acidic domain at positions 83-118. As indicated supra, this contains the region of homology between P1A and the two nucleolar proteins. A putative phosphorylation site can be found at position 125 (serine). Also, a second acidic domain is found close to the C-terminus as an uninterrupted stretch of 14 glutamate residues. A similar C-terminal structure has been found by Kessel et al. Proc. Natl. Acad. Sci. USA 84: 5306-5310 (1987), in a murine homeodomain protein having nuclear localization.

In studies comparing the sequence of gene P1A to the sequences for P91A, 35B and P198, no similarities were found, showing that P1A is indicative of a different class of genes and antigens.

Example 10

With the P1A probe and sequence in hand, investigations were carried out to determine whether the gene present in normal tissue was identical to that expressed by the tumor. To do this, phage libraries were prepared, using lambda zapII 10 and genomic DNA of DBA2 murine kidney cells. P1A was used as a probe. Hybridization conditions were as described supra, and a hybridizing clone was found. The clone contained exons one and two of the P1A gene, and corresponded to positions -0.7 to 3.8 of figure 5. Following localization of this sequence, PCR amplification was carried out to obtain the sequence corresponding to 3.8 to 4.5 of figure 5.

Sequence analysis was carried out, and no differences were found between the gene from normal kidneys and the P1A gene as obtained from the P815 tumor cells.

In further experiments, the gene as found in DBA/2 kidney cells was transfected into PO.HTR, as described supra. These experiments, presented pictorially in figure 7, showed that antigens A and B were expressed as efficiently by the kidney gene isolated from normal kidney cells as with the P1A gene isolated from normal kidney cells.

These experiments lead to the conclusion that the gene coding for the tumor rejection antigen precursor is a gene that does not result from a mutation; rather, it would appear that the gene is the same as one present in normal cells, but is not expressed therein. The ramifications of this finding are important, and are discussed infra.

In studies not elaborated upon herein, it was found that variants of the gene were available. Some cells were "P1A^{-}B^{+}", rather than the normal "P1A". The only difference between these is a point mutation in exon 1, with the 18th triplet coding for Ala in the variant instead of Val.

Example 11

Additional experiments were carried out with other cell types. Following the protocols described for Northern blot hybridizations supra, RNA of normal liver and spleen cells was tested to determine if a transcript of the P1A gene could be found. The Northern blot data are presented in figure 4 and, as can be seen, there is no evidence of expression.

The murine P815 cell line from which P1A was isolated is a mastocytoma. Therefore, mast cell lines were studied to determine if they expressed the gene. Mast cell line MC/9, described by Nabel et al., Cell 23: 19-28 (1981), and short term cultures of bone marrow derived mast cells were tested in the manner described supra (Northern blotting), but no transcript was found. In contrast when a Balb/C derived IL-3 dependent cell line L138.8A (Hültner et al.,

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J. Immunol. 142: 3440-3446 (1989)) was tested, a strong signal was found. The mast cell work is shown in figure 4.

It is known that both BALB/C and DBA/2 mice share H-2^d haplotype, and thus it was possible to test sensitivity to lysis using the CTLs described supra. Figure 8 shows these results, which essentially prove that anti-A and anti-B CTLs lysed the cells strongly, whereas anti-C and anti-D lines did not.

Further tests were carried out on other murine tumor cell lines, i.e., teratocarcinoma cell line PCC4 (Boon et al., Proc. Natl. Acad. Sci. USA 74: 272-275 (1977), and leukemias LEC and WEH1-3B. Expression could not be detected in any of these samples.

Example 12

The actual presentation of the P1A antigen by MHC molecules was of interest. To test this, cosmid C1A.3.1 was transfected into fibroblast cell line DAP, which shows phenotype H-2^k. The cell lines were transfected with genes expressing one of the K^d, D^d, and L^d antigen. Following transfection with both the cosmid and the MHC gene, lysis with CTLs was studied, again as described supra. These studies, summarized in Table 2, show that L^d is required for presentation of the P1A antigens A and B.

Table 2. H-2-restriction of antigens P815A and P815B

Recipient cell*	No of clones lysed by the CTL/ no. of H-2^d clones*	
	CTL anti-A	CTL anti-B
DAP (H-2^k)	0/208	0/194
DAP + K^d	0/165	0/162
DAP + D^d	0/157	0/129
DAP + L^d	25/33	15/20

*Cosmid C1A.3.1 containing the entire P1A gene was transfected in DAP cells previously transfected with H-2^d class I genes as indicated.

*Independent drug-resistant colonies were tested for lysis by anti-A or anti-B CTL in a visual assay.

The observation that one may associate presentation of a tumor rejection antigen with a particular MHC molecule was confirmed in experiments with human cells and HLA molecules, as elaborated upon infra.

Example 13

Using the sequence of the P1A gene as well as the amino acid sequence derivable therefrom, antigenic peptides which were A$^+$ B$^+$ (i.e., characteristic of cells which express both the A and B antigens), and those which are A$^-$ B$^+$ were identified. The peptide is presented in Figure 10. This peptide when administered to samples of PO.HTR cells

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in the presence of CTL cell lines specific to cells presenting it, led to lysis of the PO.HTR cells, lending support to the view that peptides based on the product expressed by the gene can be used as vaccines.

Example 14

The human melanoma cell line referred to hereafter as MZ2-MEL is not a clonal cell line. It expresses four stable antigens recognized by autologous CTLs, known as antigens "D, E, F, and A". In addition, two other antigens "B" and "C" are expressed by some sublines of the tumor. CTL clones specific for these six antigens are described by Van den Eynde et al., Int. J. Canc. 44: 634-640 (1989). Among the recognized subclones of MZ2-MEL are MEL.43, MEL3.0 and MEL3.1. (Van den Eynde et al., supra). Cell line MEL3.1 expresses antigen E, as determined by CTL studies as described for P815 variants, supra, so it was chosen as a source for the nucleic acid sequence expressing the antigen precursor.

In isolating the pertinent nucleic acid sequence for a tumor rejection antigen precursor, the techniques developed supra, showed that a recipient cell is needed which fulfills two criteria: (i) the recipient cell must not express the TRAP of interest under normal conditions, and (ii) it must express the relevant class I HLA molecule. Also, the recipient cell must have a high transfection frequency, i.e., it must be a "good" recipient.

In order to secure such a cell line, the clonal subline ME3.1 was subjected to repeated selection with anti-E CTL 82/30 as described by Van den Eynde, supra. The repeated cycles of selection led to isolation of subclone MZ2-MEL-2.2 isc E^-. This subclone is also $HPRT^-$, (i.e., sensitive to HAT medium: 10^{-4} M hypoxanthine, 3.8×10^{-7} aminopterine, 1.6×10^{-5} M 2-deoxythymidine). The subclone is referred to as "MEL-2.2" for simplicity hereafter.

Example 15

The genomic DNA of MEL3.0 was prepared following Wölfel et al., Immunogenetics 26: 178-187 (1987).

The plasmid pSVtkneoß, as described by Nicolas et al., Cold Spring Harb., Conf. Cell Prolif. 10: 469-485 (1983) confers geneticin resistance, so it can be used as a marker for cotransfection, as it was in this experiment.

Following a procedure similar but not identical to that of Corsao et al., Somatic Cell Molec. Genet 7: 603-616 (1981), total genomic DNA and the plasmid were cotransfected. The genomic DNA (60 µg) and plasmid DNA (6 µg) were mixed in 940 µl of 1 mM Tris·HCl (pH 7.5), 0.1 mM EDTA, after which 310 µl of 1M $CaCl_2$ was added. This solution was slowly added, under constant agitation, to 1.25 ml of 2xHBS (50 mM HEPES, 280 mM NaCl 1.5 mM Na_2HPO_4, adjusted to pH 7.1 with NaOH). The calcium phosphate DNA precipitates were allowed to form for 30-45 minutes at room

temperature, after which they were applied to 80 cm^2 tissue culture flasks which had been seeded 24 hours previously with $3x10^6$ MEL2.2 cells, in 22.5 ml of melanoma culture medium (Dulbecco's Modified Eagle's Medium) supplemented with 10% fetal calf serum. After 24 hours, the medium was replaced. Forty eight hours after transfection, the cells were harvested and seeded at $4x10^6$ cells per 80 cm^2 flask in melanoma culture medium supplemented with 2 mg/ml of geneticin. The geneticin serves as a selection marker.

Example 16

Thirteen days after transfection, geneticin-resistant colonies were counted, harvested, and cultured in nonselective medium for 2 or 3 days. Transfected cells were then plated in 96-well microplates at 200 cells/well in 200 ul of culture medium with 20% fetal calf serum (FCS) in order to obtain approximately 30 growing colonies per well. The number of microcultures was aimed at achieving redundancy, i.e., such that every independent transfectant should be represented at least four times.

After 10 days, wells contained approximately $6x10^4$ cells. These cells were detached, and 1/3 of each microculture was transferred to a duplicate plate. After 6 hours, i.e., after readherence, medium was removed and 1500 anti-E CTL (CTL 82/30), were added to each well in 100 µl of CTL culture medium with 35 U/ml of IL-2. One day later, the supernatant (50 µl) was harvested and examined

for TNF concentration, for reasons set forth in the following example.

Example 17

The size of the mammalian genome is $6x10^6$ kb. As the average amount of DNA integrated in each drug-resistant transfectant was expected to be about 200 kb, a minimum of 30,000 transfectants would need to be examined to ascertain whether antigen E had been transfected. Prior work with murine cells had shown that when a CTL stimulation assay was used, groups containing only 3% of cells expressing the antigen of interested could be identified. This should reduce the number of assays by a factor of 30. While an anti-E CTL assay, as described supra, in mixed E^+/E^- cells was helpful, it was not sufficient in that consistent results could not be obtained.

As a result, an alternative test was devised. Stimulation of CTLs was studied by release of tumor necrosis factor ("TNF") using well known methodologies which need not be repeated here. As described in Example 15, 1500 CTL 82/30 cells had been added per well of transfectants. These CTLs were collected 6 days after stimulation. As indicated supra, after 1/3 of the cells in each well had been removed and the remaining 2/3 ($4x10^4$) had readhered, the CTLs and IL-2 were added thereto. The 50 μl of supernatant was removed 24 hours later and transferred to a microplate containing $3x10^4$ W13 (WEHI-164 clone 13;

Espevik et al., J. Immunol. Meth. 95: 99-105 (1986)) cells in 50 µl of W13 culture medium (RPMI-1640, supplemented with L-arginine (116 mg/l), L-asparagine (36 mg/l), L-glutamine (216 mg/l), and 10% FCS supplemented with 2 µg of actinomycin D at 37% in an 8% CO_2 atmosphere. The cell line W13 is a mouse fibrosarcoma cell line sensitive to TNF. Dilutions of recombinant TNF-ß in RPMI 1640 were added to target cell controls.

The W13 cultures were evaluated after 20 hours of incubation, and dead cell percentage was measured using an adaptation of the colorimetric assay of Hansen et al., J. Immunol. Meth. 119: 203-210 (1989). This involved adding 50 ml of (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide at 2.5 mg/ml in PBS, followed by two hours of incubation at 37°C. Dark blue formazan crystals were dissolved by adding 100 µl of lysis solution (1 volume N,N dimethyl formamide mixed at 37°C with two volumes of water containing 30% (w/v) sodium dodecyl sulphate, at pH 4.7 from 1.6% acetic acid and 2.5% 1N HCl). Plates were incubated at 37°C overnight, and ODs were taken at 570 nm using 650 nm as control. Dead cell percentage was determined via the formula:

$$100 \times \left[1 - \frac{100-(OD_{570} \text{ sample well})}{OD_{570} \text{ well + medium}} \right]$$

following Espevik et al., J. Immunol. Meth. 95: 99-105 (1986). The results showed that even when the ratio of E^+/E^- cells was as low as 1/45, significant production of TNF was observed, thus showing active CTLs. This led to the decision to test the drug resistant transfectants in groups of 30.

Example 18

Cells were tested for TNF production as discussed in Example 17, supra. A total of 100 groups of E^- cells ($4x10^6$ cells/group) were tested following transfection, and $7x10^4$ independent geneticin resistant transfectants were obtained, for an average of 700 per group. Only one group of transfected cells led to a microculture which caused anti-E antigen CTL clone 82/30 to produce TNF. Of 300 clones tested, 8 were positive. These clones were then tested for lysis by anti-E CTL, using the standard ^{51}Cr release assay, and were found to be lysed as efficiently as the original E^+ cell line. The transfectant E.T1, discussed herein, had the same lysis pattern as did MEL2.2 for CTLs against antigens B,C,D and F.

The fact that only one transfectant presented the antigen out of 70,000 geneticin resistance transfectants may at first seem very low, but it is not. The work described supra for P815 showed an average frequency of 1/13,000. Human DNA recipient MEL2.2 appears to integrate 5 times less DNA than P1.HTR.

Example 19

Once transfectant E.T1 was found, analysis had to address several questions including whether an E^+ contaminant of the cell population was the cause. The analysis of antigen presentation, described supra, shows that E.T1 is B^- and C^-, just like the recipient cell MEL2.2. It was also found to be $HPRT^-$, using standard selection procedures. All E^+ cells used in the work described herein, however, were $HPRT^+$.

It was also possible that an E^+ revertant of MEL2.2 was the source for E.T1. To test this, the observation by Perucho et al., Cell 22: 309-317 (1980), that cotransfected sequences usually integrate together at a single location of recipient genome was employed. If antigen E in a transfectant results from cotransfec-tion with pSVtkneoß, then sequences should be linked and deletion of the antigen might also delete the neighboring pSVtkneoß sequences. Wölfel et al., supra, has shown this to be true. If a normally E^- cell is transfected with pSVtkneoß, then sequences should be linked and deletion of the antigen might also delete the neighboring pSVtkneoß sequences. If a normally E^+ cell transfected with pSVtkneoß is E.T1, however, "co-deletion" should not take place. To test this, the transfectant E.T1 was subjected to immunoselection with 82/30, as described supra. Two antigen loss variants were obtained, which resisted lysis by this CTL. Neither of these had lost geneticin

resistance; however, Southern blot analysis showed loss of several neo^r sequences in the variants, showing close linkage between the E gene and neo^r gene in E.T1, leading to the conclusion that E.T1 was a transfectant.

Example 20

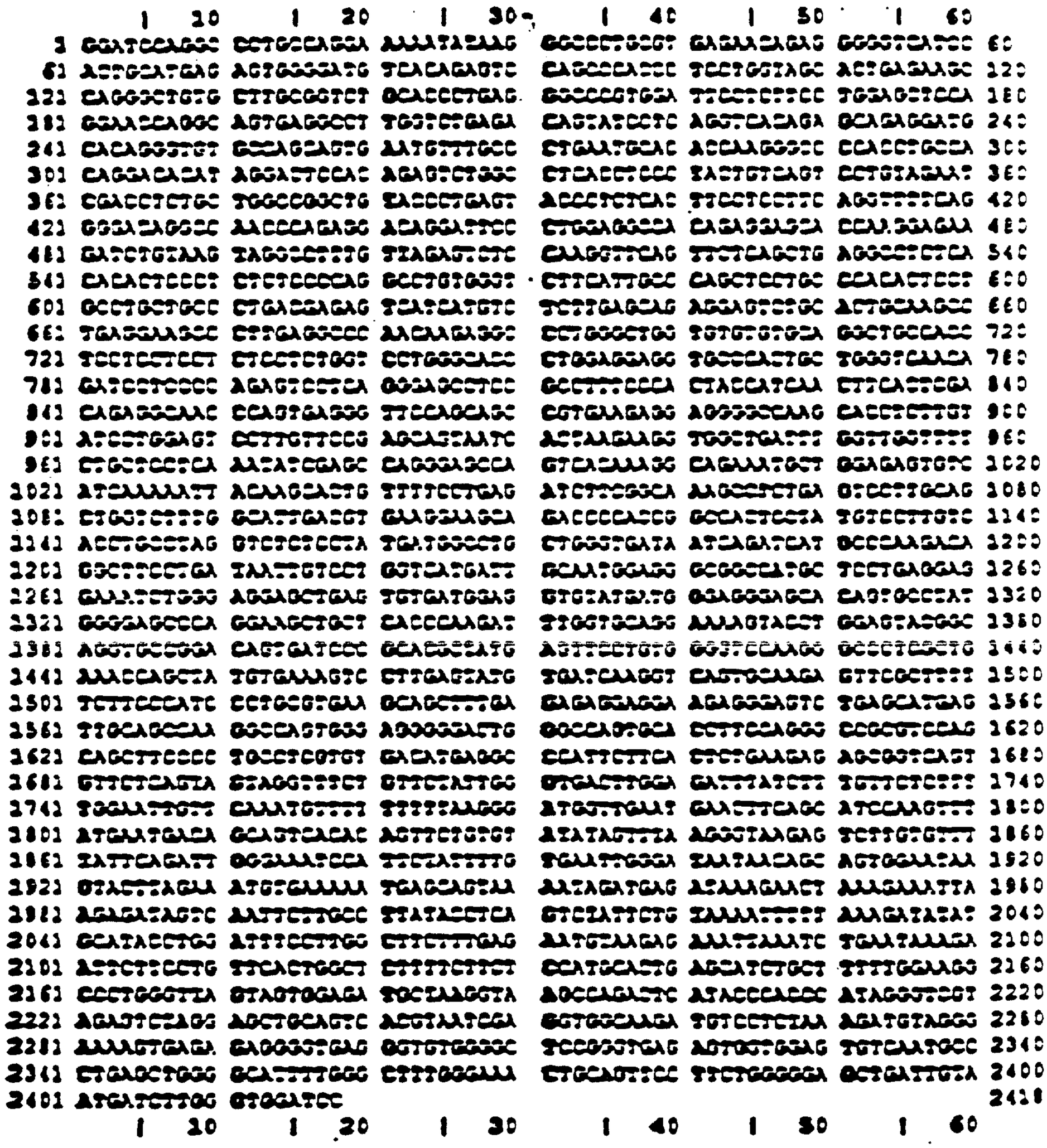
The E^+ subclone MZ2-MEL 4B was used as a source of DNA for preparation of a cosmid library. This library of nearly 700,000 cosmids was transfected into MZ2-MEL 2.2 cells, following the cosmid transfection protocols described supra.

By packaging the DNA of cosmid transfectants directly into lambda phase components, it is sometimes possible to retrieve cosmids that contain the sequences of interest. This procedure was unsuccessful here, so we rescued the transfected sequence by ligating DNA of the transfectant to appropriate restriction fragments of cosmid vector pTL6. This was tried with two transfectants and was successful with one of them. One cosmid, referred to as B3, was recovered from this experiment, and subjected to restriction endonuclease digestion via XmaI, or by BamHI digestion of a large, 12 kb XmaI transfected fragment. The fragments were cloned into vector pTZ 18R, and then transfected into MEL2.2. Again, TNF production was the measure by which successful transfection was determined. The experiments led to the determination of a gene sequence capable of transfecting antigen E on the 12 kb XmaI

fragment, and then on the 2.4 kb fragment of BamHI digestion of the 12 kb segment.

The 2.4 kb fragment hybridizes with a 2.4 kb fragment from MZ2-MEL and with a T cell clone of patient MZ-2, as determined by Southern Blots (BamHI/SmaI digested DNA). The band is absent from E^- antigen loss variants of MZ2-MEL, as seen in Figure 12.

The sequence for the E antigen precursor gene has been determined, and is presented herein:



Example 21

After the 2.4 kb genomic segment had been identified, studies were carried out to determine if an "E^+" subline expressed any homologous DNA. Cell line MZ2-MEL 3.0 was used as a source, and a cDNA library was prepared from its mRNA, using art known techniques. The 2.4 kb segment was used as a probe, and mRNA of about 1.8 kb was identified as homologous, using Northern blot analysis. When cDNA was screened, clones were obtained showing almost complete identity to parts of the 2.4 kb fragment. Two exons were thus identified. An additional exon was located upstream of these, via sequencing segments of cosmid B3 located in front of the 2.4 kb BamHI fragment. The gene extends over about 4.5 kb, as shown in Figure 8. The starting point of the transcribed region was confirmed using PCR for the 5' end of the cDNA. The three exons comprise 65, 73, and 1551 base pairs. An ATG is located at position 66 of exon 3, followed by an 828 base pair reading frame.

Example 22

To determine if smaller segments of the 2.4 kb fragment could transfer the expression of antigen E, smaller pieces corresponding to the larger gene were prepared, using art recognized techniques, and transferred into E^- cells. Figure 8 shows the boundaries of the three segments.

Transfer of antigen expression in this manner indicates that the gene codes for the antigen precursor, rather than coding for a protein which activates the antigen.

Example 23

The probing of cDNA described supra revealed, surprisingly, two different but closely related cDNAs. These cDNAs, when tested, did not transfer expression of antigen E, but they do show substantial homology to the first cDNA segment. The three segments, appear to indicate a newly recognized family of genes, referred to as "MAGE" for "melanoma antigen". In Figure 9, "mage -1" directs expression of the antigen from MZ2 cells. Portions of the third exon of each gene are presented in Figure 9. The second and third sequences are more closely related to each other than the first (18.1 and 18.9% difference compared to the first; 12% with each other). Out of 9 cDNA clones obtained, three of each type were obtained, suggesting equal expression. "MAGE" as used hereafter refers to a family of molecules, and the nucleic acids coding for them. These nucleic acids share a certain degree of homology and are expressed in tumor cells including several types of human tumor cells as well as in human tumors. The family is referred to as "MAGE" because the first members were identified in human melanoma cells. As the experiments which follow indicate, however, the members of the MAGE family are not at all restricted to melanoma tumors;

rather, MAGE refers to a family of tumor rejection antigen precursors and the nucleic acid sequences coding therefore. The antigens resulting therefrom are referred to herein as "MAGE TRAs" or "melanoma antigen tumor rejection antigens"

Example 24

Experiments with mouse tumors have demonstrated that new antigens recognized by T cells can result from point mutations that modify active genes in a region that codes for the new antigenic peptide. New antigens can also arise from the activation of genes that are not expressed in most normal cells. To clarify this issue for antigen MZ2-E, the mage-1 gene present in the melanoma cells was compared to that present in normal cells of patient MZ2.

Amplification by polymerase chain reaction (PCR) of DNA of phytohemagglutinin-activated blood lymphocytes using primers surrounding a 1300 bp stretch covering the first half of the 2.4 kb fragment was carried out. As expected, a PCR product was obtained whereas none was obtained with the DNA of the E^- variant. The sequence of this PCR product proved identical to the corresponding sequence of the gene carried by the E^+ melanoma cells. Moreover, it was found that antigen MZ2-E was expressed by cells transfected with the cloned PCR product. This result suggests that the activation of a gene normally silent is responsible for the appearance of tumor rejection antigen MZ2-E.

Example 25

In order to evaluate the expression of gene mage-1 by various normal and tumor cells, Northern blots were hybridized with a probe covering most of the third exon. In contrast with the result observed with human tumor cell line MZ2-MEL 3.0, no band was observed with RNA isolated from a CTL clone of patient MZ2 and phytohemagglutinin-activated blood lymphocytes of the same patient. Also negative were several normal tissues of other individuals (Figure 10 and Figure 11). Fourteen melanoma cell lines of other patients were tested. Eleven were positive with bands of varying intensities. In addition to these culture cell lines, four samples of melanoma tumor tissue were analyzed. Two samples, including a metastasis of patient MZ2 proved positive, excluding the possibility that expression of the gene represented a tissue culture artefact. A few tumors of other histological types, including lung tumors were tested. Most of these tumors were positive (Figures 10 and 11). These results indicated that the MAGE gene family is expressed by many melanomas and also by other tumors. However, they provided no clear indication as to which of genes mage-1, 2 or 3 were expressed by these cells, because the DNA probes corresponding to the three genes cross-hybridized to a considerable extent. To render this analysis more specific, PCR amplification and hybridization with highly specific oligo- nucleotide probes were used. cDNAs were obtained and amplified by PCR using oligonucleotide primers

corresponding to sequences of exon 3 that were identical for the three MAGE genes discussed herein. The PCR products were then tested for their ability to hybridize to three other oligonucleotides that showed complete specificity for one of the three genes (Figure 9). Control experiments carried out by diluting RNA of melanoma MZ2-MEL 3.0 in RNA from negative cells indicated that under the conditions used herein the intensity of the signal decreased proportionally to the dilution and that positive signals could still be detected at a dilution of 1/300. The normal cells (lymphocytes) that were tested by PCR were confirmed to be negative for the expression of the three MAGE genes, suggesting therefore a level of expression of less than $1/300^{th}$ that of the MZ2 melanoma cell line (Figure 11). For the panel of melanoma cell lines, the results clearly showed that some melanomas expressed MAGE genes mage 1, 2 and 3 whereas other expressed only mage-2 and 3 (Figures 11 and 10). Some of the other tumors also expressed all three genes whereas others expressed only mage-2 and 3 or only mage-3. It is impossible to exclude formally that some positive PCR results do not reflect the expression of one of the three characterized MAGE genes but that of yet another closely related gene that would share the sequence of the priming and hybridizing oligo-nucleotides. It can be concluded that the MAGE gene family is expressed by a large array of different tumors and that these genes are silent in the normal cells tested to this point.

Exammple 26

The availability of a sequence that transfects at high efficiency and efficiently expresses a TRAP made it possible to search for the associated major histocompatibility complex (MHC) class I molecule. The class I specificities of patient MZ2 are HLA-A1, A29, B37, B44 and C6. Four other melanomas of patients that had A1 in common with MZ2 were cotransfected with the 2.4 kb fragment and pSVtkneoß. Three of them yielded neo^r transfectants that stimulated TNF release by anti-E CTL clone 82/30, which is CD8+ (Figure 10). No E- transfectant was obtained with four other melanomas, some of which shared A29, B44 or C6 with MZ2. This suggests that the presenting molecule for antigen MZ2-E is HLA-A1. In confirmation, it was found that, out of 6 melanoma cell lines derived from tumors of HLA-A1 patients, two stimulated TNF release by anti-E CTL clone 82/30 of patient MZ2. One of these tumor cell lines, MI13443-MEL also showed high sensitivity to lysis by these anti-E CTL. These two melanomas were those that expressed mage-1 gene (Figure 13). Eight melanomas of patients with HLA haplotypes that did not include A1 were examined for their sensitivity to lysis and for their ability to stimulate TNF release by the CTL. None was found to be positive. The ability of some human anti-tumor CTL to lyse allogeneic tumors sharing an appropriate HLA specificity with the original tumor has been reported previously (Darrow, et al., J. Immunol. 142: 3329 (1989)). It is quite possible that antigenic peptides encoded by genes

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mage 2 and 3 can also be presented to autologous CTL by HLA-A1 or other class I molecules, especially in view of the similar results found with murine tumors, as elaborated upon supra.

Example 27

As indicated supra, melanoma MZ2 expressed antigens F, D and A', in addition to antigen E. Following the isolation of the nucleic acid sequence coding for antigen E, similar experiments were carried out to isolate the nucleic acid sequence coding for antigen F.

To do this, cultures of cell line MZ2-MEL2.2, an E^- cell line described supra, were treated with anti-F CTL clone 76/6, in the same manner described for treatment with anti-E CTL clones. This resulted in the isolation of an F antigen loss variant, which was then subjected to several rounds of selection. The resulting cell line, "MZ2-MEL2.2.5" was completely resistant to lysis by anti-F CTLs, yet proved to be lysed by anti-D CTLs.

Again, following the protocols set forth for isolation of antigen -E precursor DNA, the F^- variant was transfected with genomic DNA from F^+ cell line MZ2-MEL3.0. The experiments yielded 90,000 drug resistant transfectants. These were tested for MZ2-F expression by using pools of 30 cells in the TNF detection assay elaborated upon supra. One pool stimulated TNF release by anti-F CTLs, and was cloned. Five of 145 clones were found to stimulate anti-

F CTLs. Lysis assays, also following protocols described supra, confirmed (i) expression of the gene coding for antigen F, and (ii) presentation of antigen F itself.

Example 28

Following identification of F^+ cell lines, the DNA therefrom was used to transfect cells. To do this, a cosmid library of F^+ cell line MZ2-MEL.43 was prepared, again using the protocols described supra. The library was divided into 14 groups of about 50,000 cosmids, and DNA from each group was transfected into MZ2-MEL2.2.5. Transfectants were then tested for their ability to stimulate TNF release from anti-F CTL clone 76/6. Of 14 groups of cosmids, one produced two independent transfectants expressing antigen F; a yield of two positives out of 17,500 geniticin resistant transfectants.

Example 29

The existence of a gene family was suggested by the pattern observed on the Southern blot (Figure 12). To do this, the 2.4 kb BamHI fragment, which transferred the expression of antigen M22-E, was labelled with 32p and used as a probe on a Southern Blot of BamHI digested DNA of E + cloned subclone M22-MEL2.2. Hybridization conditions included 50 $\mu l/cm^2$ of 3.5xSSC, 1xDenhardt's solution; 25 mM sodium phosphate buffer (pH 7.0), 0.5% SDS, 2mM EDTA, where the 2.4 kb probes had been labelled with [α^{32}p]dCTP (2-3000

Ci/mole), at $3x10^6$ cpm/ml. Hybridization was carried out for 18 hours at 65°C. After this, the membranes were washed at 65°C four times for one hour each in 2xSSC, 0.1% SDS, and finally for 30 minutes in 0.1xSSC, 0.1% SDS. To identify hybridization, membranes were autoradiographed using Kodak X-AR film and Kodak X-Omatic fine intensifying screens.

In the following examples, whenever "hybridization" is referred to, the stringency conditions used were similar to those described supra. "Stringent conditions" as used herein thus refers to the foregoing conditions; subject to routine, art recognized modification.

Example 30

The cDNA coding for mage 4 was identified from a sample of the human sarcoma cell line LB23-SAR. This cell line was found to not express mage 1, 2 or 3, but the mRNA of the cell line did hybridize to the 2.4 kb sequence for mage 1. To study this further, a cDNA library was prepared from total LB23-SAR mRNA, and was then hybridized to the 2.4 kb fragment. A cDNA sequence was identified as hybridizing to this probe, and is identified hereafter as mage 4.

Example 31

Experiments were carried out using PHA-activated lymphocytes from patient "MZ2", the source of the "MZ" cells discussed supra. An oligonucleotide probe which

showed homology to mage 1 but not mage 2 or 3 was hybridized with a cosmid library derived from the PHA activated cells. The size of the hybridizing BamHI cosmid fragment, however, was 4.5 kb, thus indicating that the material was not mage 1; however, on the basis of homology to mage 1-4, the fragment can be referred to as "mage 5". The sequence of MAGE 5 is presented in SEQ ID NO: 16.

Example 32

Melanoma cell line LB-33-MEL was tested. Total mRNA from the cell line was used to prepare cDNA, which was then amplified with oligos CHO9: (ACTCAGCTCCTCCCAGATTT), and CHO10: (GAAGAGGAGGGGCCAAG). These oligos correspond to regions of exon 3 that are common to previously described mage 1, 2 and 3.

To do this, 1 μg of RNA was diluted to a total volume of 20 μl, using 2 μl of 10x PCR buffer, 2 μl of each of 10 mM dNTP, 1.2 μl of 25 mM $MgCl_2$, 1 μl of an 80 mM solution of CHO9, described supra, 20 units of RNAsin, and 200 units of M-MLV reverse transcriptase. This was followed by incubation for 40 minutes at 42°C. PCR amplification followed, using 8 μl of 10x PCR buffer, 4.8 μl of 25 mM $MgCl_2$, 1 μl of CHO10, 2.5 units of Thermus acquaticus ("Taq") polymerase, and water to a total volume of 100 μl. Amplification was then carried out for 30 cycles (1 minute 94°C; 2 minutes at 52°C, 3 minutes at 72°C). Ten μl of each reaction were then size fractionated on agarose gel,

followed by nitrocellulose blotting. The product was found to hybridize with oligonucleotide probe CHO18 (TCTTGTATCCTGGAGTCC). This probe identified mage 1 but not mage 2 or 3. However, the product did not hybridize to probe SEQ 4 (TTGCCAAGATCTCAGGAA). This probe also binds mage 1 but not 2 and 3. This indicated that the PCR product contained a sequence that differed from mage 1, 2 and 3. Sequencing of this fragment also indicated differences with respect to mage 4 and 5. These results indicate a sequence differing from previously identified mage 1, 2, 3, 4 and 5, and is named mage 6.

Example 33

In additional experiments using cosmid libraries from PHA-activated lymphocytes of MZ2, the 2.4 kb mage 1 fragment was used as a probe and isolated a complementary fragment. This clone, however, did not bind to oligonucleotides specific for mage 1, 2, 3 or 4. The sequence obtained shows some homology to exon 3 of mage 1, and differs from mages 1-6. It is referred to as mage 7 hereafter. Additional screenings yielded mage 8-11.

Example 34

The usefulness of the TRAPs, as well as TRAs derived therefrom, was exemplified by the following.

Exon 3 of mage 1 was shown to transfer expression of antigen E. As a result, it was decided to test whether

synthetic peptides derived from this exon 3 could be used to confer sensitivity to anti-E CTL.

To do this, and using standard protocols, cells normally insensitive to anti-E/CTLs were incubated with the synthetic peptides derived from Exon 3.1. Using the CTL lytic assays described supra on P815A, and a peptide concentration of 3 mM, the peptide Glu-Ala-Asp-Pro-Thr-Gly-His-Ser-Tyr was shown to be best. The assay showed lysis of 30%, indicating conferring of sensitivity to the anti-E CTL.

Example 35

Nucleic acid sequences referred to as "smage" were isolated from murine cells. Using the protocols described supra, a cosmid library was prepared from the DNA of normal DBA/2 kidney cells, using cosmid vector C2RB. As a probe, the 2.4 kb BamHI fragment of MAGE-1 was used. The DNA was blotted to nylon filters, and these were washed in 2xSSC at 65°C to identify the smage material.

Example 36

Further tissue samples were tested for the presence of MAGE genes, using the protocols discussed supra. Some of these results follow.

There was no expression of the MAGE genes in brain or kidney tumor tissue. Colon tumor tissue showed expression of MAGE 1, 2, 3 and 4, although not all tumors tested showed expression of all MAGE genes. This is also true for

pancreatic tumor (MAGE 1); non-small cell lung (MAGE 1, 2, 3 and 4), prostate (MAGE 1), sarcomas (MAGE 1, 2, 3 and 4), breast (MAGE 1, 2 and 3), and larynx (MAGE 1 and 4).

The foregoing disclosure, including the examples, places many tools of extreme value in the hands of the skilled artisan. To begin, the examples identify and provide a methodology for isolating nucleic acid molecules which code for tumor rejection antigen precursors as well as the nucleic acid molecules complementary thereto. It is known that DNA exists in double stranded form, and that each of the two strands is complementary to the other. Nucleic acid hybridization technology has developed to the point where, given a strand of DNA, the skilled artisan can isolate its complement, or synthesize it.

"Nucleic acid molecule" as used herein refers to all species of DNA and RNA which possess the properties discussed supra. Genomic and complementary DNA, or "cDNA" both code for particular proteins, and as the examples directed to isolation of MAGE coding sequences show, this disclosure teaches the artisan how to secure both of these.

Similarly, RNA molecules, such as mRNA can be secured. Again, with reference to the skilled artisan, once one has a coding sequence in hand, mRNA can be isolated or synthesized.

Complementary sequences which do not code for TRAP, such as "antisense DNA" or mRNA are useful, e.g., in

probing for the coding sequence as well as in methodologies for blocking its expression.

It will also be clear that the examples show the manufacture of biologically pure cultures of cell lines which have been transfected with nucleic acid sequences which code for or express the TRAP molecules. Such cultures can be used as a source for tumor rejection antigens, e.g., or as therapeutics. This aspect of the invention is discussed infra.

Cells transfected with the TRAP coding sequences may also be transfected with other coding sequences. Examples of other coding sequences include cytokine genes, such as interleukins (e.g., IL-2 or IL-4), or major histocompatibility complex (MHC) or human leukocyte antigen (HLA) molecules. Cytokine gene transfection is of value because expression of these is expected to enhance the therapeutic efficacy of the biologically pure culture of the cells in vivo. The art is well aware of therapies where interleukin transfectants have been administered to subjects for treating cancerous conditions. In a particularly preferred embodiment, cells are transfected with sequences coding for each of (i) a TRAP molecule, (ii) an HLA/MHC molecule, and (iii) a cytokine.

Transfection with an MHC/HLA coding sequence is desirable because certain of the TRAs may be preferentially or specifically presented only by particular MHC/HLA molecules. Thus, where a recipient cell already expresses the MHC/HLA molecule associated with presentation of a TRA,

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additional transfection may not be necessary although further transformation could be used to cause over-expression of the antigen. On the other hand, it may be desirable to transfect with a second sequence when the recipient cell does not normally express the relevant MHC/HLA molecule. It is to be understood, of course, that transfection with one additional sequence does not preclude further transfection with other sequences.

The term "biologically pure" as used in connection with the cell line described herein simply means that these are essentially free of other cells. Strictly speaking, a "cell line" by definition is "biologically pure", but the recitation will establish this fully.

Transfection of cells requires that an appropriate vector be used. Thus, the invention encompasses expression vectors where a coding sequence for the TRAP of interest is operably linked to a promoter. The promoter may be a strong promoter, such as those well known to the art, or a differential promoter, i.e., one which is operative only in specific cell types. The expression vectors may also contain all or a part of a viral or bacterial genome, such as vaccinia virus or BCG. Such vectors are especially useful in preparing vaccines.

The expression vectors may incorporate several coding sequences, as long as the TRAP sequence is contained therein. The cytokine and/or MHC/HLA genes discussed supra may be included in a single vector with the TRAP sequence. Where this is not desired, then an expression system may be

provided, where two or more separate vectors are used where each coding sequence is operably linked to a promoter. Again, the promoter may be a strong or differential promoter. Co-transfection is a well known technique, and the artisan in this field is expected to have this technology available for utilization. The vectors may be constructed so that they code for the TRA molecule directly, rather than the TRAP molecule. This eliminates the need for post-translational processing.

As the foregoing discussion makes clear, the sequences code for "tumor rejection antigen precursors" ("TRAPs") which, in turn, are processed into tumor rejection antigens ("TRAs"). Isolated forms of both of these categories are described herein, including specific examples of each. Perhaps their most noteworthy aspect is as vaccines for treating various cancerous conditions. The evidence points to presentation of TRAs on tumor cells, followed by the development of an immune response and deletion of the cells. The examples show that when various TRAs are administered to cells, a CTL response is mounted and presenting cells are deleted. This is behavior characteristic of vaccines, and hence TRAPs, which are processed into TRAs, and the TRAs themselves may be used, either alone or in pharmaceutically appropriate compositions, as vaccines. Similarly, presenting cells may be used in the same manner, either alone or as combined with ingredients to yield pharmaceutical compositions. Additional materials which may be used as vaccines include

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isolated cells which present the TRA molecule on their surface, as well as TRAP fragments, mutated viruses, especially etiolated forms, and transfected bacteria. "Fragments" as used herein refers to peptides which are smaller than the TRA, but which possess the properties required of a vaccine, as discussed supra. Another vaccine comprises or consists of complexes of TRA and HLA molecule. Vaccines of the type described herein may be used preventively, i.e., via administration to a subject in an amount sufficient to prevent onset of a cancerous condition.

The generation of an immune response, be it T-cell or B-cell related, is characteristic of the effect of the presented tumor rejection antigen. With respect to the B-cell response, this involves, inter alia, the generation of antibodies to the TRA, i.e., which specifically bind thereto. In addition, the TRAP molecules are of sufficient size to render them immunogenic, and antibodies which specifically bind thereto are a part of this invention. These antibodies may be polyclonal or monoclonal, the latter being prepared by any of the well recognized methodologies for their preparation which need not be repeated here. For example, mAbs may be prepared using an animal model, e.g., a Balb/C mouse or in a test tube, using, e.g., EBV transformants. In addition, antiserum may be isolated from a subject afflicted with a cancerous condition where certain cells present a TRA. Such

antibodies may also be generated to epitopes defined by the interaction of TRA and HLA/MHC molecules.

Review of the foregoing disclosure will show that there are a number of facets to the system which may be referred to as "tumor rejection antigen presentation and recognition". Recognition of these phenomena has diagnostic consequences. For example, the existence of specific CTL clones, or antibodies to the TRA makes it possible to diagnose or monitor cancerous conditions (explained infra), by monitoring the CTLs in a sample from a subject, binding of antibodies to TRAs, or the activity of anti-TRA CTLs in connection with subject samples. Similarly, the expression of nucleic acid molecules for TRAPs can be monitored via amplification (e.g., "polymerase chain reaction"), anti-sense hybridization, probe technologies, and so forth. Various subject samples, including body fluids (blood, serum, and other exudates, e.g.), tissues and tumors may be so assayed.

A particular manner of diagnosis is to use an adaptation of the standard "tuberculin test" currently used for diagnosis of tuberculosis. This standard skin test administers a stable form of "purified protein derivative" or "PPD" as a diagnostic aid. In a parallel fashion, TRAs in accordance with this invention may be used in such a skin test as a diagnostic aid or monitoring method.

The term "cancerous condition" is used herein to embrace all physiological events that commence with the initiation of the cancer and result in final clinical

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manifestation. Tumors do not spring up "ab initio" as visible tumors; rather there are various events associated with the transformation of a normal cell to malignancy, followed by development of a growth of biomass, such as a tumor, metastasis, etc. In addition, remission may be conceived of as part of "a cancerous condition" as tumors seldom spontaneously disappear. The diagnostic aspects of this invention include all events involved in carcinogenesis, from the first transformation to malignancy of a single cell, through tumor development and metastasis, as well as remission. All are embraced herein.

Where "subject" is used, the term embraces any species which can be afflicted with a cancerous condition. This includes humans and non-humans, such as domesticated animals, breeding stock, and so forth.

There are therapeutic aspects of this invention as well. The efficacy of administration of effective amounts of TRAPs and TRAs as vaccines has already been discussed supra. Similarly, one may develop the specific CTLs in vitro and then administer these to the subject. Antibodies may be administered, either polyclonal or monoclonal, which specifically bind to cells presenting the TRA of interest. These antibodies may be coupled to specific antitumor agents, including, but not being limited to, methotrexate radio-iodinated compounds, toxins such as ricin, other cytostatic or cytolytic drugs, and so forth. Thus, "targeted" antibody therapy is included herein, as is the

application of deletion of the cancerous cells by the use of CTLs.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

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(1) GENERAL INFORMATION:

(i) APPLICANTS: Boon, Thierry, Van den Eynde, Benoît

(ii) TITLE OF INVENTION: Isolated And Purified DNA Sequence Coding Antigen Expressed By Tumor Cells And Recognized By Cytotoxic T Cells, And Uses Thereof

(iii) NUMBER OF SEQUENCES: 26

(iv) CORRESPONDENCE ADDRESS:
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(F) ZIP: 10022

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette, 5.25 inch, 360 kb storage
(B) COMPUTER: IBM
(C) OPERATING SYSTEM: PC-DOS
(D) SOFTWARE: Wordperfect

(vi) CURRENT APPLICATION DATA:
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(B) FILING DATE:

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(2) INFORMATION FOR SEQUENCE ID NO: 1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 462 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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CAGCCAATGA GCTTACTGTT CTCGTGGGGG GTTTGTGAGC CTTGGGTAGG     150
AAGTTTTGCA AGTTCCGCCT ACAGCTCTAG CTTGTGAATT TGTACCCTTT     200
CACGTAAAAA AGTAGTCCAG AGTTTACTAC ACCCTCCCTC CCCCCTCCCA     250
CCTCGTGCTG TGCTGAGTTT AGAAGTCTTC CTTATAGAAG TCTTCCGTAT     300
AGAACTCTTC CGGAGGAAGG AGGGAGGACC CCCCCCCTTT GCTCTCCCAG     350
CATGCATTGT GTCAACGCCA TTGCACTGAG CTGGTCGAAG AAGTAAGCCG     400
CTAGCTTGCG ACTCTACTCT TATCTTAACT TAGCTCGGCT TCCTGCTGGT     450
ACCCTTTGTG CC                                             462
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(2) INFORMATION FOR SEQUENCE ID NO: 2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 675 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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ATG TCT GAT AAC AAG AAA CCA GAC AAA GCC CAC AGT GGC TCA GGT GGT      48
Met Ser Asp Asn Lys Lys Pro Asp Lys Ala His Ser Gly Ser Gly Gly
                5                  10                  15

GAC GGT GAT GGG AAT AGG TGC AAT TTA TTG CAC CGG TAC TCC CTG GAA      96
Asp Gly Asp Gly Asn Arg Cys Asn Leu Leu His Arg Tyr Ser Leu Glu
            20                  25                  30

GAA ATT CTG CCT TAT CTA GGG TGG CTG GTC TTC GCT GTT GTC ACA ACA     144
Glu Ile Leu Pro Tyr Leu Gly Trp Leu Val Phe Ala Val Val Thr Thr
        35                  40                  45

AGT TTT CTG GCG CTC CAG ATG TTC ATA GAC GCC CTT TAT GAG GAG CAG     192
Ser Phe Leu Ala Leu Gln Met Phe Ile Asp Ala Leu Tyr Glu Glu Gln
    50                  55                  60

TAT GAA AGG GAT GTG GCC TGG ATA GCC AGG CAA AGC AAG CGC ATG TCC     240
Tyr Glu Arg Asp Val Ala Trp Ile Ala Arg Gln Ser Lys Arg Met Ser
65                  70                  75                  80

TCT GTC GAT GAG GAT GAA GAC GAT GAG GAT GAT GAG GAT GAC TAC TAC     288
Ser Val Asp Glu Asp Glu Asp Asp Glu Asp Asp Glu Asp Asp Tyr Tyr
                85                  90                  95

GAC GAC GAG GAC GAC GAC GAC GAT GCC TTC TAT GAT GAT GAG GAT GAT     336
Asp Asp Glu Asp Asp Asp Asp Asp Ala Phe Tyr Asp Asp Glu Asp Asp
            100                 105                 110

GAG GAA GAA GAA TTG GAG AAC CTG ATG GAT GAT GAA TCA GAA GAT GAG     384
Glu Glu Glu Glu Leu Glu Asn Leu Met Asp Asp Glu Ser Glu Asp Glu
        115                 120                 125

GCC GAA GAA GAG ATG AGC GTG GAA ATG GGT GCC GGA GCT GAG GAA ATG     432
Ala Glu Glu Glu Met Ser Val Glu Met Gly Ala Gly Ala Glu Glu Met
    130                 135                 140

GGT GCT GGC GCT AAC TGT GCC TGT GTT CCT GGC CAT CAT TTA AGG AAG     480
Gly Ala Gly Ala Asn Cys Ala Cys Val Pro Gly His His Leu Arg Lys
145                 150                 155                 160

AAT GAA GTG AAG TGT AGG ATG ATT TAT TTC TTC CAC GAC CCT AAT TTC     528
Asn Glu Val Lys Cys Arg Met Ile Tyr Phe Phe His Asp Pro Asn Phe
                165                 170                 175
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CTG GTG TCT ATA CCA GTG AAC CCT AAG GAA CAA ATG GAG TGT AGG TGT    576
Leu Val Ser Ile Pro Val Asn Pro Lys Glu Gln Met Glu Cys Arg Cys
            180                 185                 190

GAA AAT GCT GAT GAA GAG GTT GCA ATG GAA GAG GAA GAA GAA GAA GAG    624
Glu Asn Ala Asp Glu Glu Val Ala Met Glu Glu Glu Glu Glu Glu Glu
        195                 200             210

GAG GAG GAG GAG GAA GAG GAA ATG GGA AAC CCG GAT GGC TTC TCA CCT    672
Glu Glu Glu Glu Glu Glu Glu Met Gly Asn Pro Asp Gly Phe Ser Pro
    220             225             230             235

TAG                                                                675
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(2) INFORMATION FOR SEQUENCE ID NO: 3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 228 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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TCATTATGCA AAGATGTCAC CAACAGACTT CTGACTGCAT GGTGAACTTT CATATGATAC  180
ATAGGATTAC ACTTGTACCT GTTAAAAATA AAAGTTTGAC TTGCATAC               228
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(2) INFORMATION FOR SEQUENCE ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1365 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

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

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CACGTAAAAA AGTAGTCCAG AGTTTACTAC ACCCTCCCTC CCCCCTCCCA         250
CCTCGTGCTG TGCTGAGTTT AGAAGTCTTC CTTATAGAAG TCTTCCGTAT         300
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CATGCATTGT GTCAACGCCA TTGCACTGAG CTGGTCGAAG AAGTAAGCCG         400
CTAGCTTGCG ACTCTACTCT TATCTTAACT TAGCTCGGCT TCCTGCTGGT         450
ACCCTTTGTG CC                                                 462
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GGT GGT GAC GGT GAT GGG AAT AGG TGC AAT TTA TTG CAC CGG        546
TAC TCC CTG GAA GAA ATT CTG CCT TAT CTA GGG TGG CTG GTC        588
TTC GCT GTT GTC ACA ACA AGT TTT CTG GCG CTC CAG ATG TTC        630
ATA GAC GCC CTT TAT GAG GAG CAG TAT GAA AGG GAT GTG GCC        672
TGG ATA GCC AGG CAA AGC AAG CGC ATG TCC TCT GTC GAT GAG        714
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GAG GAC GAC GAC GAC GAT GCC TTC TAT GAT GAT GAG GAT GAT        798
GAG GAA GAA GAA TTG GAG AAC CTG ATG GAT GAT GAA TCA GAA        840
GAT GAG GCC GAA GAA GAG ATG AGC GTG GAA ATG GGT GCC GGA        882
GCT GAG GAA ATG GGT GCT GGC GCT AAC TGT GCC TGT GTT CCT        924
GGC CAT CAT TTA AGG AAG AAT GAA GTG AAG TGT AGG ATG ATT        966
TAT TTC TTC CAC GAC CCT AAT TTC CTG GTG TCT ATA CCA GTG       1008
AAC CCT AAG GAA CAA ATG GAG TGT AGG TGT GAA AAT GCT GAT       1050
GAA GAG GTT GCA ATG GAA GAG GAA GAA GAA GAA GAG GAG GAG       1092
GAG GAG GAA GAG GAA ATG GGA AAC CCG GAT GGC TTC TCA CCT       1134
TAG                                                          1137
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TTGTTTTTTT TTCCCCTTCA TTAATTTTCT AGTTTTTAGT AATCCAGAAA       1237
ATTTGATTTT GTTCTAAAGT TCATTATGCA AAGATGTCAC CAACAGACTT       1287
CTGACTGCAT GGTGAACTTT CATATGATAC ATAGGATTAC ACTTGTACCT       1337
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(2) INFORMATION FOR SEQUENCE ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4698 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

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

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CCTCGTGCTG TGCTGAGTTT AGAAGTCTTC CTTATAGAAG TCTTCCGTAT         300
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CATGCATTGT GTCAACGCCA TTGCACTGAG CTGGTCGAAG AAGTAAGCCG         400
CTAGCTTGCG ACTCTACTCT TATCTTAACT TAGCTCGGCT TCCTGCTGGT         450
ACCCTTTGTG CC                                                 462
ATG TCT GAT AAC AAG AAA CCA GAC AAA GCC CAC AGT GGC TCA        504
GGT GGT GAC GGT GAT GGG AAT AGG TGC AAT TTA TTG CAC CGG        546
TAC TCC CTG GAA GAA ATT CTG CCT TAT CTA GGG TGG CTG GTC        588
TTC GCT GTT GTC ACA ACA AGT TTT CTG GCG CTC CAG ATG TTC        630
ATA GAC GCC CTT TAT GAG GAG CAG TAT GAA AGG GAT GTG GCC        672
TGG ATA GCC AGG CAA AGC AAG CGC ATG TCC TCT GTC GAT GAG        714
GAT GAA GAC GAT GAG GAT GAT GAG GAT GAC TAC TAC GAC GAC        756
GAG GAC GAC GAC GAC GAT GCC TTC TAT GAT GAT GAG GAT GAT        798
GAG GAA GAA GAA TTG GAG AAC CTG ATG GAT GAT GAA TCA GAA        840
GAT GAG GCC GAA GAA GAG ATG AGC GTG GAA ATG GGT GCC GGA        882
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GCCTTTCTTT TTTCTCCTCT CTGGTCTCCC TAATCCCTTT TCTGCATGTT        1766
AACTCCCCTT TTGGCACCTT TCCTTTACAG GACCCCCTCC CCCTCCCTGT        1816
TTCCCTTCCG GCACCCTTCC TAGCCCTGCT CTGTTCCCTC TCCCTGCTCC        1866
CCTCCCCCTC TTTGCTCGAC TTTTAGCAGC CTTACCTCTC CCTGCTTTCT        1916
GCCCCGTTCC CCTTTTTTGT GCCTTTCCTC CTGGCTCCCC TCCACCTTCC        1966
AGCTCACCTT TTTGTTTGTT TGGTTGTTTG GTTGTTTGGT TTGCTTTTTT        2016
TTTTTTTTTT GCACCTTGTT TTCCAAGATC CCCCTCCCCC TCCGGCTTCC        2066
CCTCTGTGTG CCTTTCCTGT TCCCTCCCCC TCGCTGGCTC CCCCTCCCTT        2116
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TCCTTCTACA GGTGAGAAGT GGAAAAATTG TCACTATGAA GTTCTTTTTA      3266
GGCTAAAGAT ACTTGGAACC ATAGAAGCGT TGTTAAAATA CTGCTTTCTT      3316
TTGCTAAAAT ATTCTTTCTC ACATATTCAT ATTCTCCAG                  3355
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AGG ATG ATT TAT TTC TTC CAC GAC CCT AAT TTC CTG GTG TCT      3438
ATA CCA GTG AAC CCT AAG GAA CAA ATG GAG TGT AGG TGT GAA      3480
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2109727

(2) INFORMATION FOR SEQUENCE ID NO: 6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Leu Pro Tyr Leu Gly Trp Leu Val Phe
5

(2) INFORMATION FOR SEQUENCE ID NO: 7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2418 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

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(2) INFORMATION FOR SEQUENCE ID NO: 8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5724 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-1 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

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2109727

(2) INFORMATION FOR SEQUENCE ID NO: 9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4157 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-2 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

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SUBSTITUTE SHEET

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GGC CTC TCC TAC GAT GGC CTG CTG GGC GAC AAT CAG GTC ATG      3185
CCC AAG ACA GGC CTC CTG ATA ATC GTC CTG GCC ATA ATC GCA      3227
ATA GAG GGC GAC TGT GCC CCT GAG GAG AAA ATC TGG GAG GAG      3269
CTG AGT ATG TTG GAG GTG TTT GAG GGG AGG GAG GAC AGT GTC      3311
TTC GCA CAT CCC AGG AAG CTG CTC ATG CAA GAT CTG GTG CAG      3353
GAA AAC TAC CTG GAG TAC CGG CAG GTG CCC GGC AGT GAT CCT      3395
GCA TGC TAC GAG TTC CTG TGG GGT CCA AGG GCC CTC ATT GAA      3437
ACC AGC TAT GTG AAA GTC CTG CAC CAT ACA CTA AAG ATC GGT      3479
GGA GAA CCT CAC ATT TCC TAC CCA CCC CTG CAT GAA CGG GCT      3521
TTG AGA GAG GGA GAA GAG TGA                                 3542
GTCTCAGCAC ATGTTGCAGC CAGGGCCAGT GGGAGGGGGT CTGGGCCAGT      3592
GCACCTTCCA GGGCCCCATC CATTAGCTTC CACTGCCTCG TGTGATATGA      3642
GGCCCATTCC TGCCTCTTTG AAGAGAGCAG TCAGCATTCT TAGCAGTGAG      3692
TTTCTGTTCT GTTGGATGAC TTTGAGATTT ATCTTTCTTT CCTGTTGGAA      3742
TTGTTCAAAT GTTCCTTTTA ACAAATGGTT GGATGAACTT CAGCATCCAA      3792
GTTTATGAAT GACAGTAGTC ACACATAGTG CTGTTTATAT AGTTTAGGGG      3842
TAAGAGTCCT GTTTTTTATT CAGATTGGGA AATCCATTCC ATTTTGTGAG      3892
TTGTCACATA ATAACAGCAG TGGAATATGT ATTTGCCTAT ATTGTGAACG      3942
AATTAGCAGT AAAATACATG ATACAAGGAA CTCAAAAGAT AGTTAATTCT      3992
TGCCTTATAC CTCAGTCTAT TATGTAAAAT TAAAAATATG TGTATGTTTT      4042
TGCTTCTTTG AGAATGCAAA AGAAATTAAA TCTGAATAAA TTCTTCCTGT      4092
TCACTGGCTC ATTTCTTTAC CATTCACTCA GCATCTGCTC TGTGGAAGGC      4142
CCTGGTAGTA GTGGG                                           4157
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(2) INFORMATION FOR SEQUENCE ID NO: 10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 662 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-21 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

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GGATCCCCAT GGATCCAGGA AGAATCCAGT TCCACCCCTG CTGTGAACCC      50
AGGGAAGTCA CGGGGCCGGA TGTGACGCCA CTGACTTGCG CGTTGGAGGT     100
CAGAGAACAG CGAGATTCTC GCCCTGAGCA ACGGCCTGAC GTCGGCGGAG     150
GGAAGCAGGC GCAGGCTCCG TGAGGAGGCA AGGTAAGATG CCGAGGGAGG     200
ACTGAGGCGG GCCTCACCCC AGACAGAGGG CCCCCAATAA TCCAGCGCTG     250
CCTCTGCTGC CAGGCCTGGA CCACCCTGCA GGGGAAGACT TCTCAGGCTC     300
AGTCGCCACC ACCTCACCCC GCCACCCCCC GCCGCTTTAA CCGCAGGGAA     350
CTCTGGTGTA AGAGCTTTGT GTGACCAGGG CAGGGCTGGT TAGAAGTGCT     400
CAGGGCCCAG ACTCAGCCAG GAATCAAGGT CAGGACCCCA AGAGGGGACT     450
GAGGGTAACC CCCCCGCACC CCCACCACCA TTCCCATCCC CCAACACCAA     500
CCCCACCCCC ATCCCCCAAC ACCAAACCCA CCACCATCGC TCAAACATCA     550
ACGGCACCCC CAAACCCCGA TTCCCATCCC CACCCATCCT GGCAGAATCG     600
GAGCTTTGCC CCTGCAATCA ACCCACGGAA GCTCCGGGAA TGGCGGCCAA     650
GCACGCGGAT CC                                             662
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(2) INFORMATION FOR SEQUENCE ID NO: 11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1640 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA to mRNA
(ix) FEATURE:
(A) NAME/KEY: cDNA MAGE-3
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

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GCCGCGAGGG AAGCCGGCCC AGGCTCGGTG AGGAGGCAAG GTTCTGAGGG          50
GACAGGCTGA CCTGGAGGAC CAGAGGCCCC CGGAGGAGCA CTGAAGGAGA         100
AGATCTGCCA GTGGGTCTCC ATTGCCCAGC TCCTGCCCAC ACTCCCGCCT         150
GTTGCCCTGA CCAGAGTCAT C                                        171
ATG CCT CTT GAG CAG AGG AGT CAG CAC TGC AAG CCT GAA GAA         213
GGC CTT GAG GCC CGA GGA GAG GCC CTG GGC CTG GTG GGT GCG         255
CAG GCT CCT GCT ACT GAG GAG CAG GAG GCT GCC TCC TCC TCT         297
TCT ACT CTA GTT GAA GTC ACC CTG GGG GAG GTG CCT GCT GCC         339
GAG TCA CCA GAT CCT CCC CAG AGT CCT CAG GGA GCC TCC AGC         381
CTC CCC ACT ACC ATG AAC TAC CCT CTC TGG AGC CAA TCC TAT         423
GAG GAC TCC AGC AAC CAA GAA GAG GAG GGG CCA AGC ACC TTC         465
CCT GAC CTG GAG TCC GAG TTC CAA GCA GCA CTC AGT AGG AAG         507
GTG GCC GAG TTG GTT CAT TTT CTG CTC CTC AAG TAT CGA GCC         549
AGG GAG CCG GTC ACA AAG GCA GAA ATG CTG GGG AGT GTC GTC         591
GGA AAT TGG CAG TAT TTC TTT CCT GTG ATC TTC AGC AAA GCT         633
TCC AGT TCC TTG CAG CTG GTC TTT GGC ATC GAG CTG ATG GAA         675
GTG GAC CCC ATC GGC CAC TTG TAC ATC TTT GCC ACC TGC CTG         717
GGC CTC TCC TAC GAT GGC CTG CTG GGT GAC AAT CAG ATC ATG         759
CCC AAG GCA GGC CTC CTG ATA ATC GTC CTG GCC ATA ATC GCA         801
AGA GAG GGC GAC TGT GCC CCT GAG GAG AAA ATC TGG GAG GAG         843
CTG AGT GTG TTA GAG GTG TTT GAG GGG AGG GAA GAC AGT ATG         885
TTG GGG GAT CCC AAG AAG CTG CTC ACC CAA CAT TTC GTG CAG         927
GAA AAC TAC CTG GAG TAC CGG CAG GTC CCC GGC AGT GAT CCT         969
GCA TGT TAT GAA TTC CTG TGG GGT CCA AGG GCC CTC GTT GAA        1011
ACC AGC TAT GTG AAA GTC CTG CAC CAT ATG GTA AAG ATC AGT        1053
GGA GGA CCT CAC ATT TCC TAC CCA CCC CTG CAT GAG TGG GTT        1095
TTG AGA GAG GGG GAA GAG TGA                                   1116
GTCTGAGCAC GAGTTGCAGC CAGGGCCAGT GGGAGGGGGT CTGGGCCAGT        1166
GCACCTTCCG GGGCCGCATC CCTTAGTTTC CACTGCCTCC TGTGACGTGA        1216
GGCCCATTCT TCACTCTTTG AAGCGAGCAG TCAGCATTCT TAGTAGTGGG        1266
TTTCTGTTCT GTTGGATGAC TTTGAGATTA TTCTTTGTTT CCTGTTGGAG        1316
TTGTTCAAAT GTTCCTTTTA ACGGATGGTT GAATGAGCGT CAGCATCCAG        1366
GTTTATGAAT GACAGTAGTC ACACATAGTG CTGTTTATAT AGTTTAGGAG        1416
TAAGAGTCTT GttTTTTACT CAAATTgGGA AATCCATTCC ATTTTGTGAA        1466
TTGTGACATA ATAATAGCAG TGGTAAAAGT ATTTGCTTAA AATTGTGAGC        1516
GAATTAGCAA TAACATACAT GAGATAACTC AAGAAATCAA AAGATAGTTG        1566
ATTCTTGCCT TGTACCTCAA TCTATTCTGT AAAATTAAAC AAATATGCAA        1616
ACCAGGATTT CCTTGACTTC TTTG                                    1640
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(2) INFORMATION FOR SEQUENCE ID NO: 12:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 943 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-31 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GGATCCTCCA CCCCAGTAGA GTGGGGACCT CACAGAGTCT GGCCAACCCT 50
CCTGACAGTT CTGGGAATCC GTGGCTGCGT TTGCTGTCTG CACATTGGGG 100
GCCCGTGGAT TCCTCTCCCA GGAATCAGGA GCTCCAGGAA CAAGGCAGTG 150
AGGACTTGGT CTGAGGCAGT GTCCTCAGGT CACAGAGTAG AGGGGgCTCA 200
GATAGTGCCA ACGGTGAAGG TTTGCCTTGG ATTCAAACCA AGGGCCCCAC 250
CTGCCCCAGA ACACATGGAC TCCAGAGCGC CTGGCCTCAC CCTCAATACT 300
TTCAGTCCTG CAGCCTCAGC ATGCGCTGGC CGGATGTACC CTGAGGTGCC 350
CTCTCACTTC CTCCTTCAGG TTCTGAGGGG ACAGGCTGAC CTGGAGGACC 400
AGAGGCCCCC GGAGGAGCAC TGAAGGAGAA GATCTGTAAG TAAGCCTTTG 450
TTAGAGCCTC CAAGGTTCCA TTCAGTACTC AGCTGAGGTC TCTCACATGC 500
TCCCTCTCTC CCCAGGCCAG TGGGTCTCCA TTGCCCAGCT CCTGCCCACA 550
CTCCCGCCTG TTGCCCTGAC CAGAGTCATC 580
ATG CCT CTT GAG CAG AGG AGT CAG CAC TGC AAG CCT GAA GAA 622
GGC CTT GAG GCC CGA GGA GAg GCC CTG GGC CTG GTG GGT GCG 664
CAG GCT CCT GCT ACT GAG GAG CAG GAG GCT GCC TCC TCC TCT 706
TCT AGT GTA GTT GAA GTC ACC CTG GGG GAG GTG CCT GCT GCC 748
GAG TCA CCA GAT CCT CCC CAG AGT CCT CAG GGA GCC TCC AGC 790
CTC CCC ACT ACC ATG AAC TAC CCT CTC TGG AGC CAA TCC TAT 832
GAG GAC TCC AGC AAC CAA GAA GAG GAG GGG CCA AGC ACC TTC 874
CCT GAC CTG GAG TCT GAG TTC CAA GCA GCA CTC AGT AGG AAG 916
GTG GCC AAG TTG GTT CAT TTT CTG CTC 943

(2) INFORMATION FOR SEQUENCE ID NO: 13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2531 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-4 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

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GGATCCAGGC CCTGCCTGGA GAAATGTGAG GGCCCTGAGT GAACACAGTG          50
GGGATCATCC ACTCCATGAG AGTGGGGACC TCACAGAGTC CAGCCTACCC         100
TCTTGATGGC ACTGAGGGAC CGGGGCTGTG CTTACAGTCT GCACCCTAAG         150
GGCCCATGGA TTCCTCTCCT AGGAGCTCCA GGAACAAGGC AGTGAGGCCT         200
TGGTCTGAGA CAGTGTCCTC AGGTTACAGA GCAGAGGATG CACAGGCTGT         250
GCCAGCAGTG AATGTTTGCC CTGAATGCAC ACCAAGGGCC CCACCTGCCA         300
CAAGACACAT AGGACTCCAA AGAGTCTGGC CTCACCTCCC TACCATCAAT         350
CCTGCAGAAT CGACCTCTGC TGGCCGGCTA TACCCTGAGG TGCTCTCTCA         400
CTTCCTCCTT CAGGTTCTGA GCAGACAGGC CAACCGGAGA CAGGATTCCC         450
TGGAGGCCAC AGAGGAGCAC CAAGGAGAAG ATCTGTAAGT AAGCCTTTGT         500
TAGAGCCTCT AAGATTTGGT TCTCAGCTGA GGTCTCTCAC ATGCTCCCTC         550
TCTCCGTAGG CCTGTGGGTC CCCATTGCCC AGCTTTTGCC TGCACTCTTG         600
CCTGCTGCCC TGACCAGAGT CATC                                     624
ATG TCT TCT GAG CAG AAG AGT CAG CAC TGC AAG CCT GAG GAA         666
GGC GTT GAG GCC CAA GAA GAG GCC CTG GGC CTG GTG GGT GCA         708
CAG GCT CCT ACT ACT GAG GAG CAG GAG GCT GCT GTC TCC TCC         750
TCC TCT CCT CTG GTC CCT GGC ACC CTG GAG GAA GTG CCT GCT         792
GCT GAG TCA GCA GGT CCT CCC CAG AGT CCT CAG GGA GCC TCT         834
GCC TTA CCC ACT ACC ATC AGC TTC ACT TGC TGG AGG CAA CCC         876
AAT GAG GGT TCC AGC AGC CAA GAA GAG GAG GGG CCA AGC ACC         918
TCG CCT GAC GCA GAG TCC TTG TTC CGA GAA GCA CTC AGT AAC         960
AAG GTG GAT GAG TTG GCT CAT TTT CTG CTC CGC AAG TAT CGA        1002
GCC AAG GAG CTG GTC ACA AAG GCA GAA ATG CTG GAG AGA GTC        1044
ATC AAA AAT TAC AAG CGC TGC TTT CCT GTG ATC TTC GGC AAA        1086
GCC TCC GAG TCC CTG AAG ATG ATC TTT GGC ATT GAC GTG AAG        1128
GAA GTG GAC CCC GCC AGC AAC ACC TAC ACC CTT GTC ACC TGC        1170
CTG GGC CTT TCC TAT GAT GGC CTG CTG GGT AAT AAT CAG ATC        1212
TTT CCC AAG ACA GGC CTT CTG ATA ATC GTC CTG GGC ACA ATT        1254
GCA ATG GAG GGC GAC AGC GCC TCT GAG GAG GAA ATC TGG GAG        1296
GAG CTG GGT GTG ATG GGG GTG TAT GAT GGG AGG GAG CAC ACT        1338
GTC TAT GGG GAG CCC AGG AAA CTG CTC ACC CAA GAT TGG GTG        1380
CAG GAA AAC TAC CTG GAG TAC CGG CAG GTA CCC GGC AGT AAT        1422
CCT GCG CGC TAT GAG TTC CTG TGG GGT CCA AGG GCT CTG GCT        1464
GAA ACC AGC TAT GTG AAA GTC CTG GAG CAT GTG GTC AGG GTC        1506
AAT GCA AGA GTT CGC ATT GCC TAC CCA TCC CTG CGT GAA GCA        1548
GCT TTG TTA GAG GAG GAA GAG GGA GTC TGA                       1578
GCATGAGTTG CAGCCAGGGC TGTGGGGAAG GGGCAGGGCT GGGCCAGTGC        1628
ATCTAACAGC CCTGTGCAGC AGCTTCCCTT GCCTCGTGTA ACATGAGGCC        1678
CATTCTTCAC TCTGTTTGAA GAAAATAGTC AGTGTTCTTA GTAGTGGGTT        1728
TCTATTTTGT TGGATGACTT GGAGATTTAT CTCTGTTTCC TTTTACAATT        1778
GTTGAAATGT TCCTTTTAAT GGATGGTTGA ATTAACTTCA GCATCCAAGT        1828
TTATGAATCG TAGTTAACGT ATATTGCTGT TAATATAGTT TAGGAGTAAG        1878
AGTCTTGTTT TTTATTCAGA TTGGGAAATC CGTTCTATTT TGTGAATTTG        1928
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GGACATAATA ACAGCAGTGG AGTAAGTATT TAGAAGTGTG AATTCACCGT 1978
GAAATAGGTG AGATAAATTA AAAGATACTT AATTCCCGCC TTATGCCTCA 2028
GTCTATTCTG TAAAATTTAA AAATATATAT GCATACCTGG ATTTCCTTGG 2078
CTTCGTGAAT GTAAGAGAAA TTAAATCTGA ATAAATAATT CTTTCTGTTA 2128
ACTGGCTCAT TTCTTCTCTA TGCACTGAGC ATCTGCTCTG TGGAAGGCCC 2178
AGGATTAGTA GTGGAGATAC TAGGGTAAGC CAGACACACA CCTACCGATA 2228
GGGTATTAAG AGTCTAGGAG CGCGGTCATA TAATTAAGGT GACAAGATGT 2278
CCTCTAAGAT GTAGGGGAAA AGTAACGAGT GTGGGTATGG GGCTCCAGGT 2328
GAGAGTGGTC GGGTGTAAAT TCCCTGTGTG GGGCCTTTTG GGCTTTGGGA 2378
AACTGCATTT TCTTCTGAGG GATCTGATTC TAATGAAGCT TGGTGGGTCC 2428
AGGGCCAGAT TCTCAGAGGG AGAGGGAAAA GCCCAGATTG GAAAAGTTGC 2478
TCTGAGCAGT TCCTTTGTGA CAATGGATGA ACAGAGAGGA GCCTCTACCT 2528
GGG 2531

(2) INFORMATION FOR SEQUENCE ID NO: 14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2531 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-41 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

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GGATCCAGGC CCTGCCTGGA GAAATGTGAG GGCCCTGAGT GAACACAGTG          50
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TCTTGATGGC ACTGAGGGAC CGGGGCTGTG CTTACAGTCT GCACCCTAAG         150
GGCCCATGGA TTCCTCTCCT AGGAGCTCCA GGAACAAGGC AGTGAGGCCT         200
TGGTCTGAGA CAGTGTCCTC AGGTTACAGA GCAGAGGATG CACAGGCTGT         250
GCCAGCAGTG AATGTTTGCC CTGAATGCAC ACCAAGGGCC CCACCTGCCA         300
CAAGACACAT AGGACTCCAA AGAGTCTGGC CTCACCTCCC TACCATCAAT         350
CCTGCAGAAT CGACCTCTGC TGGCCGGCTA TACCCTGAGG TGCTCTCTCA         400
CTTCCTCCTT CAGGTTCTGA GCAGACAGGC CAACCGGAGA CAGGATTCCC         450
TGGAGGCCAC AGAGGAGCAC CAAGGAGAAG ATCTGTAAGT AAGCCTTTGT         500
TAGAGCCTCT AAGATTTGGT TCTCAGCTGA GGTCTCTCAC ATGCTCCCTC         550
TCTCCGTAGG CCTGTGGGTC CCCATTGCCC AGCTTTTGCC TGCACTCTTG         600
CCTGCTGCCC TGAGCAGAGT CATC                                     624
ATG TCT TCT GAG CAG AAG AGT CAG CAC TGC AAG CCT GAG GAA         666
GGC GTT GAG GCC CAA GAA GAG GCC CTG GGC CTG GTG GGT GCG         708
CAG GCT CCT ACT ACT GAG GAG CAG GAG GCT GCT GTC TCC TCC         750
TCC TCT CCT CTG GTC CCT GGC ACC CTG GAG GAA GTG CCT GCT         792
GCT GAG TCA GCA GGT CCT CCC CAG AGT CCT CAG GGA GCC TCT         834
GCC TTA CCC ACT ACC ATC AGC TTC ACT TGC TGG AGG CAA CCC         876
AAT GAG GGT TCC AGC AGC CAA GAA GAG GAG GGG CCA AGC ACC         918
TCG CCT GAC GCA GAG TCC TTG TTC CGA GAA GCA CTC AGT AAC         960
AAG GTG GAT GAG TTG GCT CAT TTT CTG CTC CGC AAG TAT CGA        1002
GCC AAG GAG CTG GTC ACA AAG GCA GAA ATG CTG GAG AGA GTC        1044
ATC AAA AAT TAC AAG CGC TGC TTT CCT GTG ATC TTC GGC AAA        1086
GCC TCC GAG TCC CTG AAG ATG ATC TTT GGC ATT GAC GTG AAG        1128
GAA GTG GAC CCC ACC AGC AAC ACC TAC ACC CTT GTC ACC TGC        1170
CTG GGC CTT TCC TAT GAT GGC CTG CTG GGT AAT AAT CAG ATC        1212
TTT CCC AAG ACA GGC CTT CTG ATA ATC GTC CTG GGC ACA ATT        1254
GCA ATG GAG GGC GAC AGC GCC TCT GAG GAG GAA ATC TGG GAG        1296
GAG CTG GGT GTG ATG GGG GTG TAT GAT GGG AGG GAG CAC ACT        1338
GTC TAT GGG GAG CCC AGG AAA CTG CTC ACC CAA GAT TGG GTG        1380
CAG GAA AAC TAC CTG GAG TAC CGG CAG GTA CCC GGC AGT AAT        1422
CCT GCG CGC TAT GAG TTC CTG TGG GGT CCA AGG GCT CTG GCT        1464
GAA ACC AGC TAT GTG AAA GTC CTG GAG CAT GTG GTC AGG GTC        1506
AAT GCA AGA GTT CGC ATT GCC TAC CCA TCC CTG CGT GAA GCA        1548
GCT TTG TTA GAG GAG GAA GAG GGA GTC TGA                        1578
GCATGAGTTG CAGCCAGGGC TGTGGGGAAG GGGCAGGGCT GGGCCAGTGC        1628
ATCTAACAGC CCTGTGCAGC AGCTTCCCTT GCCTCGTGTA ACATGAGGCC        1678
CATTCTTCAC TCTGTTTGAA GAAAATAGTC AGTGTTCTTA GTAGTGGGTT        1728
TCTATTTTGT TGGATGACTT GGAGATTTAT CTCTGTTTCC TTTTACAATT        1778
GTTGAAATGT TCCTTTTAAT GGATGGTTGA ATTAACTTCA GCATCCAAGT        1828
TTATGAATCG TAGTTAACGT ATATTGCTGT TAATATAGTT TAGGAGTAAG        1878
AGTCTTGTTT TTTATTCAGA TTGGGAAATC CGTTCTATTT TGTGAATTTG        1928
GGACATAATA ACAGCAGTGG AGTAAGTATT TAGAAGTGTG AATTCACCGT        1978
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2109727 82

GAAATAGGTG AGATAAATTA AAAGATACTT AATTCCCGCC TTATGCCTCA 2028
GTCTATTCTG TAAAATTTAA AAATATATAT GCATACCTGG ATTTCCTTGG 2078
CTTCGTGAAT GTAAGAGAAA TTAAATCTGA ATAAATAATT CTTTCTGTTA 2128
ACTGGCTCAT TTCTTCTCTA TGCACTGAGC ATCTGCTCTG TGGAAGGCCC 2178
AGGATTAGTA GTGGAGATAC TAGGGTAAGC CAGACACACA CCTACCGATA 2228
GGGTATTAAG AGTCTAGGAG CGCGGTCATA TAATTAAGGT GACAAGATGT 2278
CCTCTAAGAT GTAGGGGAAA AGTAACGAGT GTGGGTATGG GGCTCCAGGT 2328
GAGAGTGGTC GGGTGTAAAT TCCCTGTGTG GGGCCTTTTG GGCTTTGGGA 2378
AACTCCATTT TCTTCTGAGG GATCTGATTC TAATGAAGCT TGGTGGGTCC 2428
AGGGCCAGAT TCTCAGAGGG AGAGGGAAAA GCCCAGATTG GAAAAGTTGC 2478
TCTGAGCGGT TCCTTTGTGA CAATGGATGA ACAGAGAGGA GCCTCTACCT 2528
GGG 2531

(2) INFORMATION FOR SEQUENCE ID NO: 15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1068 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA to mRNA
(ix) FEATURE:
(A) NAME/KEY: cDNA MAGE-4
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

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GAA GCA CTC AGT AAC AAG GTG GAT GAG TTG GCT CAT TTT CTG        82
CTC CGC AAG TAT CGA GCC AAG GAG CTG GTC ACA AAG GCA GAA       124
ATG CTG GAG AGA GTC ATC AAA AAT TAC AAG CGC TGC TTT CCT       166
GTG ATC TTC GGC AAA GCC TCC GAG TCC CTG AAG ATG ATC TTT       208
GGC ATT GAC GTG AAG GAA GTG GAC CCC GCC AGC AAC ACC TAC       250
ACC CTT GTC ACC TGC CTG GGC CTT TCC TAT GAT GGC CTG CTG       292
GGT AAT AAT CAG ATC TTT CCC AAG ACA GGC CTT CTG ATA ATC       334
GTC CTG GGC ACA ATT GCA ATG GAG GGC GAC AGC GCC TCT GAG       376
GAG GAA ATC TGG GAG GAG CTG GGT GTG ATG GGG GTG TAT GAT       418
GGG AGG GAG CAC ACT GTC TAT GGG GAG CCC AGG AAA CTG CTC       460
ACC CAA GAT TGG GTG CAG GAA AAC TAC CTG GAG TAC CGG CAG       502
GTA CCC GGC AGT AAT CCT GCG CGC TAT GAG TTC CTG TGG GGT       544
CCA AGG GCT CTG GCT GAA ACC AGC TAT GTG AAA GTC CTG GAG       586
CAT GTG GTC AGG GTC AAT GCA AGA GTT CGC ATT GCC TAC CCA       628
TCC CTG CGT GAA GCA GCT TTG TTA GAG GAG GAA GAG GGA GTC       670
TGAGCATGAG TTGCAGCCAG GGCTGTGGGG AAGGGGCAGG GCTGGGCCAG       720
TGCATCTAAC AGCCCTGTGC AGCAGCTTCC CTTGCCTCGT GTAACATGAG       770
GCCCATTCTT CACTCTGTTT GAAGAAAATA GTCAGTGTTC TTAGTAGTGG       820
GTTTCTATTT TGTTGGATGA CTTGGAGATT TATCTCTGTT TCCTTTTACA       870
ATTGTTGAAA TGTTCCTTTT AATGGATGGT TGAATTAACT TCAGCATCCA       920
AGTTTATGAA TCGTAGTTAA CGTATATTGC TGTTAATATA GTTTAGGAGT       970
AAGAGTCTTG TTTTTTATTC AGATTGGGAA ATCCGTTCTA TTTTGTGAAT      1020
TTGGGACATA ATAACAGCAG TGGAGTAAGT ATTTAGAAGT GTGAATTC        1068
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2109727

(2) INFORMATION FOR SEQUENCE ID NO: 16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2226 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-5 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

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GGATCCAGGC CTTGCCAGGA GAAAGGTGAG GGCCCTGTGT GAGCACAGAG          50
GGGACCATTC ACCCCAAGAG GGTGGAGACC TCACAGATTC CAGCCTACCC         100
TCCTGTTAGC ACTGGGGGCC TGAGGCTGTG CTTGCAGTCT GCACCCTGAG         150
GGCCCATGCA TTCCTCTTCC AGGAGCTCCA GGAAACAGAC ACTGAGGCCT         200
TGGTCTGAGG CCGTGCCCTC AGGTCACAGA GCAGAGGAGA TGCAGACGTC         250
TAGTGCCAGC AGTGAACGTT TGCCTTGAAT GCACACTAAT GGCCCCCATC         300
GCCCCAGAAC ATATGGGACT CCAGAGCACC TGGCCTCACC CTCTCTACTG         350
TCAGTCCTGC AGAATCAGCC TCTGCTTGCT TGTGTACCCT GAGGTGCCCT         400
CTCACTTTTT CCTTCAGGTT CTCAGGGGAC AGGCTGACCA GGATCACCAG         450
GAAGCTCCAG AGGATCCCCA GGAGGCCCTA GAGGAGCACC AAAGGAGAAG         500
ATCTGTAAGT AAGCCTTTGT TAGAGCCTCC AAGGTTCAGT TTTTAGCTGA         550
GGCTTCTCAC ATGCTCCCTC TCTCTCCAGG CCAGTGGGTC TCCATTGCCC         600
AGCTCCTGCC CACACTCCTG CCTGTTGCGG TGACCAGAGT CGTC               644
ATG TCT CTT GAG CAG AAG AGT CAG CAC TGC AAG CCT GAG GAA         684
CTC CTC TGG TCC CAG GCA CCC TGG GGG AGG TGC CTG CTG CTG         728
GGT CAC CAG GTC CTC TCA AGA GTC CTC AGG GAG CCT CCG CCA         770
TCC CCA CTG CCA TCG ATT TCA CTC TAT GGA GGC AAT CCA TTA         812
AGG GCT CCA GCA ACC AAG AAG AGG AGG GGC CAA GCA CCT CCC         854
CTG ACC CAG AGT CTG TGT TCC GAG CAG CAC TCA GTA AGA AGG         896
TGG CTG ACT TGA                                               908
TTCATTTTCT GCTCCTCAAG TATTAAGTCA AGGAGCTGGT CACAAAGGCA         958
GAAATGCTGG AGAGCGTCAT CAAAAATTAC AAGCGCTGCT TTCCTGAGAT        1008
CTTCGGCAAA GCCTCCGAGT CCTTGCAGCT GGTCTTTGGC ATTGACGTGA        1058
AGGAAGCGGA CCCCACCAGC AACACCTACA CCCTTGTCAC CTGCCTGGGA        1108
CTCCTATGAT GGCCTGCTGG TTGATAATAA TCAGATCATG CCCAAGACGG        1158
GCCTCCTGAT AATCGTCTTG GGCATGATTG CAATGGAGGG CAAATGCGTC        1208
CCTGAGGAGA AAATCTGGGA GGAGCTGAGT GTGATGAAGG TGTATGTTGG        1258
GAGGGAGCAC AGTGTCTGTG GGGAGCCCAG GAAGCTGCTC ACCCAAGATT        1308
TGGTGCAGGA AAACTACCTG GAGTACCGGC AGGTGCCCAG CAGTGATCCC        1358
ATATGCTATG AGTTACTGTG GGGTCCAAGG GCACTCGCTG CTTGAAAGTA        1408
CTGGAGCACG TGGTCAGGGT CAATGCAAGA GTTCTCATTT CCTACCCATC        1458
CCTGCGTGAA GCAGCTTTGA GAGAGGAGGA AGAGGGAGTC TGAGCATGAG        1508
CTGCAGCCAG GGCCACTGCG AGGGGGGCTG GGCCAGTGCA CCTTCCAGGG        1558
CTCCGTCCAG TAGTTTCCCC TGCCTTAATG TGACATGAGG CCCATTCTTC        1608
TCTCTTTGAA GAGAGCAGTC AACATTCTTA GTAGTGGGTT TCTGTTCTAT        1658
TGGATGACTT TGAGATTTGT CTTTGTTTCC TTTTGGAATT GTTCAAATGT        1708
TTCTTTTAAT GGGTGGTTGA ATGAACTTCA GCATTCAAAT TTATGAATGA        1758
CAGTAGTCAC ACATAGTGCT GTTTATATAG TTTAGGAGTA AGAGTCTTGT        1808
TTTTTATTCA GATTGGGAAA TCCATTCCAT TTTGTGAATT GGGACATAGT        1858
TACAGCAGTG GAATAAGTAT TCATTTAGAA ATGTGAATGA GCAGTAAAAC        1908
TGATGACATA AAGAAATTAA AAGATATTTA ATTCTTGCTT ATACTCAGTC        1958
TATTCGGTAA AATTTTTTTT AAAAAATGTG CATACCTGGA TTTCCTTGGC        2008
TTCTTTGAGA ATGTAAGACA AATTAAATCT GAATAAATCA TTCTCCCTGT        2058
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TCACTGGCTC ATTTATTCTC TATGCACTGA GCATTTGCTC TGTGGAAGGC      2108
CCTGGGTTAA TAGTGGAGAT GCTAAGGTAA GCCAGACTCA CCCCTACCCA      2158
CAGGGTAGTA AAGTCTAGGA GCAGCAGTCA TATAATTAAG GTGGAGAGAT      2208
GCCCTCTAAG ATGTAGAG                                         2226
```

2109727

(2) INFORMATION FOR SEQUENCE ID NO: 17:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2305 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-51 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GGATCCAGGC CTTGCCAGGA GAAAGGTGAG GGCCCTGTGT GAGCACAGAG 50
GGGACCATTC ACCCCAAGAG GGTGGAGACC TCACAGATTC CAGCCTACCC 100
TCCTGTTAGC ACTGGGGGCC TGAGGCTGTG CTTGCAGTCT GCACCCTGAG 150
GGCCCATGCA TTCCTCTTCC AGGAGCTCCA GGAAACAGAC ACTGAGGCCT 200
TGGTCTGAGG CCGTGCCCTC AGGTCACAGA GCAGAGGAGA TGCAGACGTC 250
TAGTGCCAGC AGTGAACGTT TGCCTTGAAT GCACACTAAT GGCCCCCATC 300
GCCCCAGAAC ATATGGGACT CCAGAGCACC TGGCCTCACC CTCTCTACTG 350
TCAGTCCTGC AGAATCAGCC TCTGCTTGCT TGTGTACCCT GAGGTGCCCT 400
CTCACTTTTT CCTTCAGGTT CTCAGGGGAC AGGCTGACCA GGATCACCAG 450
GAAGCTCCAG AGGATCCCCA GGAGGCCCTA GAGGAGCACC AAAGGAGAAG 500
ATCTGTAAGT AAGCCTTTGT TAGAGCCTCC AAGGTTCAGT TTTTAGCTGA 550
GGCTTCTCAC ATGCTCCCTC TCTCTCCAGG CCAGTGGGTC TCCATTGCCC 600
AGCTCCTGCC CACACTCCTG CCTGTTGCGG TGACCAGAGT CGTC 644
ATG TCT CTT GAG CAG AAG AGT CAG CAC TGC AAG CCT GAG GAA 686
GGC CTT GAC ACC CAA GAA GAG CCC TGG GCC TGG TGG GTG TGC 728
AGG CTG CCA CTA CTG AGG AGC AGG AGG CTG TGT CCT CCT CCT 770
CTC CTC TGG TCC CAG GCA CCC TGG GGG AGG TGC CTG CTG CTG 812
GGT CAC CAG GTC CTC TCA AGA GTC CTC AGG GAG CCT CCG CCA 854
TCC CCA CTG CCA TCG ATT TCA CTC TAT GGA GGC AAT CCA TTA 896
AGG GCT CCA GCA ACC AAG AAG AGG AGG GGC CAA GCA CCT CCC 938
CTG ACC CAG AGT CTG TGT TCC GAG CAG CAC TCA GTA AGA AGG 980
TGG CTG ACT TGA 992
TTCATTTTCT GCTCCTCAAG TATTAAGTCA AGGAGCCGGT CACAAAGGCA 1042
GAAATGCTGG AGAGCGTCAT CAAAAATTAC AAGCGCTGCT TTCCTGAGAT 1092
CTTCGGCAAA GCCTCCGAGT CCTTGCAGCT GGTCTTTGGC ATTGACGTGA 1142
AGGAAGCGGA CCCCACCAGC AACACCTACA CCCTTGTCAC CTGCCTGGGA 1192
CTCCTATGAT GGCCTGGTGG TTTAATCAGA TCATGCCCAA GACGGGCCTC 1242
CTGATAATCG TCTTGGGCAT GATTGCAATG GAGGGCAAAT GCGTCCCTGA 1292
GGAGAAAATC TGGGAGGAGC TGGGTGTGAT GAAGGTGTAT GTTGGGAGGG 1342
AGCACAGTGT CTGTGGGGAG CCCAGGAAGC TGCTCACCCA AGATTTGGTG 1392
CAGGAAAACT ACCTGGAGTA CCGCAGGTGC CCAGCAGTGA TCCCATATGC 1442
TATGAGTTAC TGTGGGGTCC AAGGGCACTC GCTGCTTGAA AGTACTGGAG 1492
CACGTGGTCA GGGTCAATGC AAGAGTTCTC ATTTCCTACC CATCCCTGCA 1542
TGAAGCAGCT TTGAGAGAGG AGGAAGAGGG AGTCTGAGCA TGAGCTGCAG 1592
CCAGGGCCAC TGCGAGGGGG GCTGGGCCAG TGCACCTTCC AGGGCTCCGT 1642
CCAGTAGTTT CCCCTGCCTT AATGTGACAT GAGGCCCATT CTTCTCTCTT 1692
TGAAGAGAGC AGTCAACATT CTTAGTAGTG GGTTTCTGTT CTATTGGATG 1742
ACTTTGAGAT TTGTCTTTGT TTCCTTTTGG AATTGTTCAA ATGTTCCTTT 1792
TAATGGGTGG TTGAATGAAC TTCAGCATTC AAATTTATGA ATGACAGTAG 1842
TCACACATAG TGCTGTTTAT ATAGTTTAGG AGTAAGAGTC TTGTTTTTTA 1892
TTCAGATTGG GAAATCCATT CCATTTTGTG AATTGGGACA TAGTTACAGC 1942
AGTGGAATAA GTATTCATTT AGAAATGTGA ATGAGCAGTA AAACTGATGA 1992
GATAAAGAAA TTAAAAGATA TTTAATTCTT GCCTTATACT CAGTCTATTC 2042

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GGTAAAATTT TTTTTTAAAA ATGTGCATAC CTGGATTTCC TTGGCTTCTT      2092
TGAGAATGTA AGACAAATTA AATCTGAATA AATCATTCTC CCTGTTCACT      2142
GGCTCATTTA TTCTCTATGC ACTGAGCATT TGCTCTGTGG AAGGCCCTGG      2192
GTTAATAGTG GAGATGCTAA GGTAAGCCAG ACTCACCCCT ACCCACAGGG      2242
TAGTAAAGTC TAGGAGCAGC AGTCATATAA TTAAGGTGGA GAGATGCCCT      2292
CTAAGATGTA GAG                                             2305
```

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(2) INFORMATION FOR SEQUENCE ID NO: 18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 225 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-6 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

```
TAT TTC TTT CCT GTG ATC TTC AGC AAA GCT TCC GAT TCC TTG        42
CAG CTG GTC TTT GGC ATC GAG CTG ATG GAA GTG GAC CCC ATC        84
GGC CAC GTG TAC ATC TTT GCC ACC TGC CTG GGC CTC TCC TAC       126
GAT GGC CTG CTG GGT GAC AAT CAG ATC ATG CCC AGG ACA GGC       168
TTC CTG ATA ATC ATC CTG GCC ATA ATC GCA AGA GAG GGC GAC       210
TGT GCC CCT GAG GAG                                          225
```

(2) INFORMATION FOR SEQUENCE ID NO: 19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1947 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-7 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

```
TGAATGGACA ACAAGGGCCC CACACTCCCC AGAACACAAG GGACTCCAGA          50
GAGCCCAGCC TCACCTTCCC TACTGTCAGT CCTGCAGCCT CAGCCTCTGC         100
TGGCCGGCTG TACCCTGAGG TGCCCTCTCA CTTCCTCCTT CAGGTTCTCA         150
GCGGACAGGC CGGCCAGGAG GTCAGAAGCC CCAGGAGGCC CCAGAGGAGC         200
ACCGAAGGAG AAGATCTGTA AGTAGGCCTT TGTTAGGGCC TCCAGGGCGT         250
GGTTCACAAA TGAGGCCCCT CACAAGCTCC TTCTCTCCCC AGATCTGTGG         300
GTTCCTCCCC ATCGCCCAGC TGCTGCCCGC ACTCCAGCCT GCTGCCCTGA         350
CCAGAGTCAT CATGTCTTCT GAGCAGAGGA GTCAGCACTG CAAGCCTGAG         400
GATGCCTTGA GGCCCAAGGA CAGGAGGCTC TGGGCCTGGT GGGTGCGCAG         450
GCTCCCGCCA CCGAGGAGCA CGAGGCTGCC TCCTCCTTCA CTCTGATTGA         500
AGGCACCCTG GAGGAGGTGC CTGCTGCTGG GTCCCCCAGT CCTCCCCTGA         550
GTCTCAGGGT TCCTCCTTTT CCCTGACCAT CAGCAACAAC ACTCTATGGA         600
GCCAATCCAG TGAGGGCACC AGCAGCCGGG AAGAGGAGGG GCCAACCACC         650
TAGACACACC CCGCTCACCT GGCGTCCTTG TTCCA                         685
ATG GGA AGG TGG CTG AGT TGG TTC GCT TCC TGC TGC ACA AGT         727
ATC GAG TCA AGG AGC TGG TCA CAA AGG CAG AAA TGC TGG ACA         769
GTG TCA TCA AAA ATT ACA AGC ACT AGT TTC CTT GTG ATC TAT         811
GGC AAA GCC TCA GAG TGC ATG CAG GTG ATG TTT GGC ATT GAC         853
ATG AAG GAA GTG GAC CCC GCG GCC ACT CCT ACG TCC TTG TCA         895
CCT GCT TGG GCC TCT CCT ACA ATG GCC TGC TGG GTG ATG ATC         937
AGA GCA TGC CCG AGA CCG GCC TTC TGA                            964
TTATGGTCTT GACCATGATC TTAATGGAGG GCCACTGTGC CCCTGAGGAG        1014
GCAATCTGGG AAGCGTTGAG TGTAATGGTG TATGATGGGA TGGAGCAGTT        1064
TCTTTGGGCA GCTGAGGAAG CTGCTCACCC AAGATTGGGT GCAGGAAAAC        1114
TACCTGCAAT ACCGCCAGGT GCCCAGCAGT GATCCCCCGT GCTACCAGTT        1164
CCTGTGGGGT CCAAGGGCCC TCATTGAAAC CAGCTATGTG AAAGTCCTGG        1214
AGTATGCAGC CAGGGTCAGT ACTAAAGAGA GCATTTCCTA CCCATCCCTG        1264
CATGAAGAGG CTTTGGGAGA GGAGGAAGAG GGAGTCTGAG CAGAAGTTGC        1314
AGCCAGGGCC AGTGGGGCAG ATTGGGGGAG GGCCTGGGCA GTGCACGTTC        1364
CACACATCCA CCACCTTCCC TGTCCTGTTA CATGAGGCCC ATTCTTCACT        1414
CTGTGTTTGA AGAGAGCAGT CAATGTTCTC AGTAGCGGGG AGTGTGTTGG        1464
GTGTGAGGGA ATACAAGGTG GACCATCTCT CAGTTCCTGT TCTCTTGGGC        1514
GATTTGGAGG TTTATCTTTG TTTCCTTTTG CAGTCGTTCA AATGTTCCTT        1564
TTAATGGATG GTGTAATGAA CTTCAACATT CATTTCATGT ATGACAGTAG        1614
GCAGACTTAC TGTTTTTTAT ATAGTTAAAA GTAAGTGCAT TGTTTTTTAT        1664
TTATGTAAGA AAATCTATGT TATTTCTTGA ATTGGGACAA CATAACATAG        1714
CAGAGGATTA AGTACCTTTT ATAATGTGAA AGAACAAAGC GGTAAAATGG        1764
GTGAGATAAA GAAATAAAGA AATTAAATTG GCTGGGCACG GTGGCTCACG        1814
CCTGTAATCC CAGCACTTTA GGAGGCAGAG GCACGGGGAT CACGAGGTCA        1864
GGAGATCGAG ACCATTCTGG CTAACACAGT GAAACACCAT CTCTATTAAA        1914
AATACAAAAC TTAGCCGGGC GTGGTGGCGG GTG                          1947
```

(2) INFORMATION FOR SEQUENCE ID NO: 20:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1810 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-8 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

```
GAGCTCCAGG AACCAGGCTG TGAGGTCTTG GTCTGAGGCA GTATCTTCAA          50
TCACAGAGCA TAAGAGGCCC AGGCAGTAGT AGCAGTCAAG CTGAGGTGGT         100
GTTTCCCCTG TATGTATACC AGAGGCCCCT CTGGCATCAG AACAGCAGGA         150
ACCCCACAGT TCCTGGCCCT ACCAGCCCTT TTGTCAGTCC TGGAGCCTTG         200
GCCTTTGCCA GGAGGCTGCA CCCTGAGATG CCCTCTCAAT TTCTCCTTCA         250
GGTTCGCAGA GAACAGGCCA GCCAGGAGGT CAGGAGGCCC CAGAGAAGCA         300
CTGAAGAAGA CCTGTAAGTA GACCTTTGTT AGGGCATCCA GGGTGTAGTA         350
CCCAGCTGAG GCCTCTCACA CGCTTCCTCT CTCCCCAGGC CTGTGGGTCT         400
CAATTGCCCA GCTCCGGCCC ACACTCTCCT GCTGCCCTGA CCTGAGTCAT         450
C                                                             451
ATG CTT CTT GGG CAG AAG AGT CAG CGC TAC AAG GCT GAG GAA        493
GGC CTT CAG GCC CAA GGA GAG GCA CCA GGG CTT ATG GAT GTG        535
CAG ATT CCC ACA GCT GAG GAG CAG AAG GCT GCA TCC TCC TCC        577
TCT ACT CTG ATC ATG GGA ACC CTT GAG GAG GTG ACT GAT TCT        619
GGG TCA CCA AGT CCT CCC CAG AGT CCT GAG GGT GCC TCC TCT        661
TCC CTG ACT GTC ACC GAC AGC ACT CTG TGG AGC CAA TCC GAT        703
GAG GGT TCC AGC AGC AAT GAA GAG GAG GGG CCA AGC ACC TCC        745
CCG GAC CCA GCT CAC CTG GAG TCC CTG TTC CGG GAA GCA CTT        787
GAT GAG AAA GTG GCT GAG TTA GTT CGT TTC CTG CTC CGC AAA        829
TAT CAA ATT AAG GAG CCG GTC ACA AAG GCA GAA ATG CTT GAG        871
AGT GTC ATC AAA AAT TAC AAG AAC CAC TTT CCT GAT ATC TTC        913
AGC AAA GCC TCT GAG TGC ATG CAG GTG ATC TTT GGC ATT GAT        955
GTG AAG GAA GTG GAC CCT GCC GGC CAC TCC TAC ATC CTT GTC        997
ACC TGC CTG GGC CTC TCC TAT GAT GGC CTG CTG GGT GAT GAT       1039
CAG AGT ACG CCC AAG ACC GGC CTC CTG ATA ATC GTC CTG GGC       1081
ATG ATC TTA ATG GAG GGC AGC CGC GCC CCG GAG GAG GCA ATC       1123
TGG GAA GCA TTG AGT GTG ATG GGG GCT GTA TGA                   1156
TGGGAGGGAG CACAGTGTCT ATTGGAAGCT CAGGAAGCTG CTCACCCAAG        1206
AGTGGGTGCA GGAGAACTAC CTGGAGTACC GCCAGGCGCC CGGCAGTGAT        1256
CCTGTGCGCT ACGAGTTCCT GTGGGGTCCA AGGGCCCTTG CTGAAACCAG        1306
CTATGTGAAA GTCCTGGAGC ATGTGGTCAG GGTCAATGCA AGAGTTCGCA        1356
TTTCCTACCC ATCCCTGCAT GAAGAGGCTT TGGGAGAGGA GAAAGGAGTT        1406
TGAGCAGGAG TTGCAGCTAG GGCCAGTGGG GCAGGTTGTG GGAGGGCCTG        1456
GGCCAGTGCA CGTTCCAGGG CCACATCCAC CACTTTCCCT GCTCTGTTAC        1506
ATGAGGCCCA TTCTTCACTC TGTGTTTGAA GAGAGCAGTC ACAGTTCTCA        1556
GTAGTGGGGA GCATGTTGGG TGTGAGGGAA CACAGTGTGG ACCATCTCTC        1606
AGTTCCTGTT CTATTGGGCG ATTTGGAGGT TTATCTTTGT TTCCTTTTGG        1656
AATTGTTCCA ATGTTCCTTC TAATGGATGG TGTAATGAAC TTCAACATTC        1706
ATTTTATGTA TGACAGTAGA CAGACTTACT GCTTTTTATA TAGTTTAGGA        1756
GTAAGAGTCT TGCTTTTCAT TTATACTGGG AAACCCATGT TATTTCTTGA        1806
ATTC                                                         1810
```

(2) INFORMATION FOR SEQUENCE ID NO: 21:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1412 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-9 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

```
TCTGAGACAG TGTCCTCAGG TCGCAGAGCA GAGGAGACCC AGGCAGTGTC          50
AGCAGTGAAG GTGAAGTGTT CACCCTGAAT GTGCACCAAG GGCCCCACCT         100
GCCCCAGCAC ACATGGGACC CCATAGCACC TGGCCCCATT CCCCCTACTG         150
TCACTCATAG AGCCTTGATC TCTGCAGGCT AGCTGCACGC TGAGTAGCCC         200
TCTCACTTCC TCCCTCAGGT TCTCGGGACA GGCTAACCAG GAGGACAGGA         250
GCCCCAAGAG GCCCCAGAGC AGCACTGACG AAGACCTGTA AGTCAGCCTT         300
TGTTAGAACC TCCAAGGTTC GGTTCTCAGC TGAAGTCTCT CACACACTCC         350
CTCTCTCCCC AGGCCTGTGG GTCTCCATCG CCCAGCTCCT GCCCACGCTC         400
CTGACTGCTG CCCTGACCAG AGTCATC                                  427
ATG TCT CTC GAG CAG AGG AGT CCG CAC TGC AAG CCT GAT GAA         469
GAC CTT GAA GCC CAA GGA GAG GAC TTG GGC CTG ATG GGT GCA         511
CAG GAA CCC ACA GGC GAG GAG GAG GAG ACT ACC TCC TCC TCT         553
GAC AGC AAG GAG GAG GAG GTG TCT GCT GCT GGG TCA TCA AGT         595
CCT CCC CAG AGT CCT CAG GGA GGC GCT TCC TCC TCC ATT TCC         637
GTC TAC TAC ACT TTA TGG AGC CAA TTC GAT GAG GGC TCC AGC         679
AGT CAA GAA GAG GAA GAG CCA AGC TCC TCG GTC GAC CCA GCT         721
CAG CTG GAG TTC ATG TTC CAA GAA GCA CTG AAA TTG AAG GTG         763
GCT GAG TTG GTT CAT TTC CTG CTC CAC AAA TAT CGA GTC AAG         805
GAG CCG GTC ACA AAG GCA GAA ATG CTG GAG AGC GTC ATC AAA         847
AAT TAC AAG CGC TAC TTT CCT GTG ATC TTC GGC AAA GCC TCC         889
GAG TTC ATG CAG GTG ATC TTT GGC ACT GAT GTG AAG GAG GTG         931
GAC CCC GCC GGC CAC TCC TAC ATC CTT GTC ACT GCT CTT GGC         973
CTC TCG TGC GAT AGC ATG CTG GGT GAT GGT CAT AGC ATG CCC        1015
AAG GCC GCC CTC CTG ATC ATT GTC CTG GGT GTG ATC CTA ACC        1057
AAA GAC AAC TGC GCC CCT GAA GAG GTT ATC TGG GAA GCG TTG        1099
AGT GTG ATG GGG GTG TAT GTT GGG AAG GAG CAC ATG TTC TAC        1141
GGG GAG CCC AGG AAG CTG CTC ACC CAA GAT TGG GTG CAG GAA        1183
AAC TAC CTG GAG TAC CGG CAG GTG CCC GGC AGT GAT CCT GCG        1225
CAC TAC GAG TTC CTG TGG GGT TCC AAG GCC CAC GCT GAA ACC        1267
AGC TAT GAG AAG GTC ATA AAT TAT TTG GTC ATG CTC AAT GCA        1309
AGA GAG CCC ATC TGC TAC CCA TCC CTT TAT GAA GAG GTT TTG        1351
GGA GAG GAG CAA GAG GGA GTC TGA                               1375
GCACCAGCCG CAGCCGGGGC CAAAGTTTGT GGGGTCA                      1412
```

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(2) INFORMATION FOR SEQUENCE ID NO: 22:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 920 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-10 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

```
ACCTGCTCCA GGACAAAGTG GACCCCACTG CATCAGCTCC ACCTACCCTA          50
CTGTCAGTCC TGGAGCCTTG GCCTCTGCCG GCTGCATCCT GAGGAGCCAT         100
CTCTCACTTC CTTCTTCAGG TTCTCAGGGG ACAGGGAGAG CAAGAGGTCA         150
AGAGCTGTGG GACACCACAG AGCAGCACTG AAGGAGAAGA CCTGTAAGTT         200
GGCCTTTGTT AGAACCTCCA GGGTGTGGTT CTCAGCTGTG GCCACTTACA         250
CCCTCCCTCT CTCCCCAGGC CTGTGGGTCC CCATCGCCCA AGTCCTGCCC         300
ACACTCCCAC CTGCTACCCT GATCAGAGTC ATC                           333
ATG CCT CGA GCT CCA AAG CGT CAG CGC TGC ATG CCT GAA GAA         375
GAT CTT CAA TCC CAA AGT GAG ACA CAG GGC CTC GAG GGT GCA         417
CAG GCT CCC CTG GCT GTG GAG GAG GAT GCT TCA TCA TCC ACT         459
TCC ACC AGC TCC TCT TTT CCA TCC TCT TTT CCC TCC TCC TCC         501
TCT TCC TCC TCC TCC TCC TGC TAT CCT CTA ATA CCA AGC ACC         543
CCA GAG GAG GTT TCT GCT GAT GAT GAG ACA CCA AAT CCT CCC         585
CAG AGT GCT CAG ATA GCC TGC TCC TCC CCC TCG GTC GTT GCT         627
TCC CTT CCA TTA GAT CAA TCT GAT GAG GGC TCC AGC AGC CAA         669
AAG GAG GAG AGT CCA AGC ACC CTA CAG GTC CTG CCA GAC AGT         711
GAG TCT TTA CCC AGA AGT GAG ATA GAT GAA AAG GTG ACT GAT         753
TTG GTG CAG TTT CTG CTC TTC AAG TAT CAA ATG AAG GAG CCG         795
ATC ACA AAG GCA GAA ATA CTG GAG AGT GTC ATA AAA AAT TAT         837
GAA GAC CAC TTC CCT TTG TTG TTT AGT GAA GCC TCC GAG TGC         879
ATG CTG CTG GTC TTT GGC ATT GAT GTA AAG GAA GTG GAT CC          920
```

(2) INFORMATION FOR SEQUENCE ID NO: 23:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1107 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-11 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

```
AGAGAACAGG CCAACCTGGA GGACAGGAGT CCCAGGAGAA CCCAGAGGAT          50
CACTGGAGGA GAACAAGTGT AAGTAGGCCT TTGTTAGATT CTCCATGGTT         100
CATATCTCAT CTGAGTCTGT TCTCACGCTC CCTCTCTCCC CAGGCTGTGG         150
GGCCCCATCA CCCAGATATT TCCCACAGTT CGGCCTGCTG ACCTAACCAG         200
AGTCATCATG CCTCTTGAGC AAAGAAGTCA GCACTGCAAG CCTGAGGAAG         250
CCTTCAGGCC CAAGAAGAAG ACCTGGGCCT GGTGGGTGCA CAGGCTCTCC         300
AAGCTGAGGA GCAGGAGGCT GCCTTCTTCT CCTCTACTCT GAATGTGGGC         350
ACTCTAGAGG AGTTGCCTGC TGCTGAGTCA CCAAGTCCTC CCCAGAGTCC         400
TCAGGAAGAG TCCTTCTCTC CCACTGCCAT GGATGCCATC TTTGGGAGCC         450
TATCTGATGA GGGCTCTGGC AGCCAAGAAA AGGAGGGGCC AAGTACCTCG         500
CCTGACCTGA TAGACCCTGA GTCCTTTTCC CAAGATATAC TACATGACAA         550
GATAATTGAT TTGGTTCATT TATTCTCCGC AAGTATCGAG TCAAGGGGCT         600
GATCACAAAG GCAGAA                                             616
ATG CTG GGG AGT GTC ATC AAA AAT TAT GAG GAC TAC TTT CCT        658
GAG ATA TTT AGG GAA GCC TCT GTA TGC ATG CAA CTG CTC TTT        700
GGC ATT GAT GTG AAG GAA GTG GAC CCC ACT AGC CAC TCC TAT        742
GTC CTT GTC ACC TCC CTC AAC CTC TCT TAT GAT GGC ATA CAG        784
TGT AAT GAG CAG AGC ATG CCC AAG TCT GGC CTC CTG ATA ATA        826
GTC CTG GGT GTA ATC TTC ATG GAG GGG AAC TGC ATC CCT GAA        868
GAG GTT ATG TGG GAA GTC CTG AGC ATT ATG GGG GTG TAT GCT        910
GGA AGG GAG CAC TTC CTC TTT GGG GAG CCC AAG AGG CTC CTT        952
ACC CAA AAT TGG GTG CAG GAA AAG TAC CTG GTG TAC CGG CAG        994
GTG CCC GGC ACT GAT CCT GCA TGC TAT GAG TTC CTG TGG GGT       1036
CCA AGG GCC CAC GCT GAG ACC AGC AAG ATG AAA GTT CTT GAG       1078
TAC ATA GCC AAT GCC AAT GGG AGG GAT CC                        1107
```

(2) INFORMATION FOR SEQUENCE ID NO: 24:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2150 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: smage-I
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

```
TCTGTCTGCA TATGCCTCCA CTTGTGTGTA GCAGTCTCAA ATGGATCTCT          50
CTCTACAGAC CTCTGTCTGT GTCTGGCACC CTAAGTGGCT TTGCATGGGC         100
ACAGGTTTCT GCCCCTGCAT GGAGCTTAAA TAGATCTTTC TCCACAGGCC         150
TATACCCCTG CATTGTAAGT TTAAGTGGCT TTATGTGGAT ACAGGTCTCT         200
GCCCTTGTAT GCAGGCCTAA GTTTTTCTGT CTGCTTAACC CCTCCAAGTG         250
AAGCTAGTGA AAGATCTAAC CCACTTTTGG AAGTCTGAAA CTAGACTTTT         300
ATGCAGTGGC CTAACAAGTT TTAATTTCTT CCACAGGGTT TGCAGAAAAG         350
AGCTTGATCC ACGAGTTCAG AAGTCCTGGT ATGTTCCTAG AAAG               394
ATG TTC TCC TGG AAA GCT TCA AAA GCC AGG TCT CCA TTA AGT         436
CCA AGG TAT TCT CTA CCT GGT AGT ACA GAG GTA CTT ACA GGT         478
TGT CAT TCT TAT CCT TCC AGA TTC CTG TCT GCC AGC TCT TTT         520
ACT TCA GCC CTG AGC ACA GTC AAC ATG CCT AGG GGT CAA AAG         565
AGT AAG ACC CGC TCC CGT GCA AAA CGA CAG CAG TCA CGC AGG         604
GAG GTT CCA GTA GTT CAG CCC ACT GCA GAG GAA GCA GGG TCT         646
TCT CCT GTT GAC CAG AGT GCT GGG TCC AGC TTC CCT GGT GGT         688
TCT GCT CCT CAG GGT GTG AAA ACC CCT GGA TCT TTT GGT GCA         730
GGT GTA TCC TGC ACA GGC TCT GGT ATA GGT GGT AGA AAT GCT         772
GCT GTC CTG CCT GAT ACA AAA AGT TCA GAT GGC ACC CAG GCA         814
GGG ACT TCC ATT CAG CAC ACA CTG AAA GAT CCT ATC ATG AGG         856
AAG GCT AGT GTG CTG ATA GAA TTC CTG CTA GAT AAA TTT AAG         898
ATG AAA GAA GCA GTT ACA AGG AGT GAA ATG CTG GCA GTA GTT         940
AAC AAG AAG TAT AAG GAG CAA TTC CCT GAG ATC CTC AGG AGA         982
ACT TCT GCA CGC CTA GAA TTA GTC TTT GGT CTT GAG TTG AAG        1024
GAA ATT GAT CCC AGC ACT CAT TCC TAT TTG CTG GTA GGC AAA        1066
CTG GGT CTT TCC ACT GAG GGA AGT TTG AGT AGT AAC TGG GGG        1108
TTG CCT AGG ACA GGT CTC CTA ATG TCT GTC CTA GGT GTG ATC        1150
TTC ATG AAG GGT AAC CGT GCC ACT GAG CAA GAG GTC TGG CAA        1192
TTT CTG CAT GGA GTG GGG GTA TAT GCT GGG AAG AAG CAC TTG        1234
ATC TTT GGC GAG CCT GAG GAG TTT ATA AGA GAT GTA GTG CGG        1276
GAA AAT TAC CTG GAG TAC CGC CAG GTA CCT GGC AGT GAT CCC        1314
CCA AGC TAT GAG TTC CTG TGG GGA CCC AGA GCC CAT GCT GAA        1360
ACA ACC AAG ATG AAA GTC CTG GAA GTT TTA GCT AAA GTC AAT        1402
GGC ACA GTC CCT AGT GCC TTC CCT AAT CTC TAC CAG TTG GCT        1444
CTT AGA GAT CAG GCA GGA GGG GTG CCA AGA AGG AGA GTT CAA        1486
GGC AAG GGT GTT CAT TCC AAG GCC CCA TCC CAA AAG TCC TCT        1528
AAC ATG TAG                                                  1537
TTGAGTCTGT TCTGTTGTGT TTGAAAAACA GTCAGGCTCC TAATCAGTAG        1587
AGAGTTCATA GCCTACCAGA ACCAACATGC ATCCATTCTT GGCCTGTTAT        1637
ACATTAGTAG AATGGAGGCT ATTTTTGTTA CTTTTCAAAT GTTTGTTTAA        1687
CTAAACAGTG CTTTTTGCCA TGCTTCTTGT TAACTGCATA AAGAGGTAAC        1737
TGTCACTTGT CAGATTAGGA CTTGTTTTGT TATTTGCAAC AAACTGGAAA        1787
```

```
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GATTGTCATG GCAATGTGAT ATCATACAGT GGTGAAACAA CAGTGAAGTG      1887
GGAAAGTTTA TATTGTTAAT TTTGAAAATT TTATGAGTGT GATTGCTGTA      1937
TACTTTTTTC TTTTTTGTAT AATGCTAAGT GAAATAAAGT TGGATTTGAT      1987
GACTTTACTC AAATTCATTA GAAAGTAAAT CGTAAAACTC TATTACTTTA      2037
TTATTTTCTT CAATTATGAA TTAAGCATTG GTTATCTGGA AGTTTCTCCA      2087
GTAGCACAGG ATCTAGTATG AAATGTATCT AGTATAGGCA CTGACAGTGA      2137
GTTATCAGAG TCT                                             2150
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(2) INFORMATION FOR SEQUENCE ID NO: 25:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2099 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: smage-II
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

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CTCCACAGGC CTATACCCCT GCATTGTAAG TTTAAGTGGC TTTATGTGGA     200
TACAGGTCTC TGCCCTTGTA TGCAGGCCTA AGTTTTTCTG TCTGCTTAGC     250
CCCTCCAAGT GAAGCTAGTG AAAGATCTAA CCCACTTTTG GAAGTCTGAA     300
ACTAGACTTT TATGCAGTGG CCTAACAAGT TTTAATTTCT TCCACAGGGT     350
TTGCAGAAAA GAGCTTGATC CACGAGTTCG GAAGTCCTGG TATGTTCCTA     400
GAAAGATGTT CTCCTGGAAA GCTTCAAAAG CCAGGTCTCC ATTAAGTCCA     450
AGGTATTCTC TACCTGGTAG TACAGAGGTA CTTACAGGTT GTCATTCTTA     500
TCTTTCCAGA TTCCTGTCTG CCAGCTCTTT TACTTCAGCC CTGAGCACAG     550
TCAACATGCC TAGGGGTCAA AAGAGTAAGA CCCGCTCCCG TGCAAAACGA     600
CAGCAGTCAC GCAGGGAGGT TCCAGTAGTT CAGCCCACTG CAGAGGAAGC     650
AGGGTCTTCT CCTGTTGACC AGAGTGCTGG GTCCAGCTTC CCTGGTGGTT     700
CTGCTCCTCA GGGTGTGAAA ACCCCTGGAT CTTTTGGTGC AGGTGTATCC     750
TGCACAGGCT CTGGTATAGG TGGTAGAAAT GCTGCTGTCC TGCCTGATAC     800
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CAATTTCTGC ATGGAGTGGG GGTATATGCT GGGAAGAAGC ACTTGATCTT    1250
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TGGCTCTTAG AGATCAGGCA GGAGGGGTGC CAAGAAGGAG AGTTCAAGGC    1500
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AAAGTTTATA TTGTTAGTTT TGAAAATTTT ATGAGTGTGA TTGCTGTATA    1950
CTTTTTTCTT TTTTGTATAA TGCTAAGTGA AATAAAGTTG GATTTGATGA    2000
CTTTACTCAA ATTCATTAGA AAGTAAATCA TAAAACTCTA TTACTTTATT    2050
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(2) INFORMATION FOR SEQUENCE ID NO: 26:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acids
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Glu Ala Asp Pro Thr Gly His Ser Tyr
5

WHAT IS CLAIMED:

1. A vaccine comprising a tumor rejection antigen precursor for a non tum⁻ antigen, which provokes an immune response when administered to a subject and a pharmaceutically appropriate carrier; wherein said tumor rejection antigen precursor is encoded by a nucleic acid molecule, the complementary sequence of which hybridizes to SEQ ID NO: 8 under stringent conditions, defined as 65°C, 2xSSC, 0.1%xSDS, followed by a wash for 30 minutes in 0.1xSSC, 0.1%xSDS.

2. A vaccine comprising a peptide fragment derived from a tumor rejection antigen precursor for a non tum⁻ antigen and a pharmaceutically appropriate carrier, wherein said fragment is larger than the tumor rejection antigen derived from said tumor rejection antigen precursor and smaller than said tumor rejection antigen precursor and which provokes an immune response when administered to a subject; wherein said tumor rejection antigen precursor is encoded by a nucleic acid molecule, the complementary sequence of which hybridizes to SEQ ID NO: 8 under stringent conditions, defined as 65°C, 2xSSC, 0.1%xSDS, followed by a wash for 30 minutes in 0.1xSSC, 0.1%xSDS.

3. The vaccine of claim 1, wherein said tumor rejection antigen precursor is a human tumor rejection antigen precursor.

4. The vaccine of claim 1, wherein said tumor rejection antigen precursor is mage-1.

5. The vaccine of claim 3, wherein said tumor rejection antigen precursor is antigen F precursor.

6. A vaccine comprising a tumor rejection antigen encoded by an isolated nucleic acid molecule which codes for a tumor rejection antigen precursor, which provokes an immune response when administered to a subject and a pharmaceutically acceptable carrier; wherein said tumor rejection antigen precursor is encoded by a nucleic acid molecule, the complementary sequence of which hybridizes to SEQ ID NO: 8 under stringent conditions, defined as 65°C, 2xSSC, 0.1%xSDS, followed by a wash for 30 minutes in 0.1xSSC, 0.1%xSDS.

7. The vaccine of claim 6, wherein said tumor rejection antigen has an amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO: 4.

8. The vaccine of claim 6, wherein said tumor rejection antigen is antigen E.

9. The vaccine of claim 6, wherein said tumor rejection antigen is antigen F.

10. The vaccine of claim 1, wherein said tumor rejection antigen precursor is the expression product of an expression vector containing a viral genome or portion thereof.

11. A vaccine comprising a transfected bacteria containing a nucleic acid molecule which codes for a tumor rejection antigen precursor and a pharmaceutically acceptable adjuvant; wherein said tumor rejection antigen precursor is encoded by a nucleic acid molecule, the complementary sequence of which hybridizes to SEQ ID NO: 8 under stringent conditions, defined as 65°C, 2xSSC, 0.1%xSDS, followed by a wash for 30 minutes in 0.1xSSC, 0.1%xSDS.

12. A vaccine comprising a mutated virus containing a nucleic acid molecule which codes for a tumor rejection antigen precursor, and a pharmacologically acceptable adjuvant; wherein said tumor rejection antigen precursor is encoded by a nucleic acid molecule, the complementary sequence of which hybridizes to SEQ ID NO: 8 under stringent conditions, defined as 65°C, 2xSSC, 0.1%xSDS, followed by a wash for 30 minutes in 0.1xSSC, 0.1%xSDS.

13. A vaccine comprising a complex of a tumor rejection antigen encoded by a nucleic acid molecule, the complementary sequence which hybrizes to SEQ ID NO: 8 under stringent conditions, defined as 65°C, 2xSSC, 0.1%xSDS, followed by a wash for 30 minutes in 0.1xSSC, 0.1%xSDS, which is not a tum⁻ antigen and an HLA molecule.

14. An isolated peptide for use in treating a subject afflicted with a cancerous condition, said peptide having the amino acid of SEQ ID NO: 26.

15. A vaccine comprising an isolated cell line transfected with a nucleic acid molecule which codes for a tumor rejection antigen precursor and a pharmacologically acceptable adjuvant; wherein said tumor rejection antigen precursor is encoded by a nucleic acid molecule, the complementary sequence of which hybridizes to SEQ ID NO: 8 under stringent conditions, defined as 65°C, 2xSSC, 0.1%xSDS, followed by a wash for 30 minutes in 0.1xSSC, 0.1%xSDS.

16. A composition of matter comprising a non proliferative cell line having expressed on its surface a tumor rejection antigen precursor for a non tum⁻ antigen specific for a tumor characteristic of a cancerous condition, and a pharmaceutically acceptable carrier; wherein said tumor rejection antigen precursor is encoded by a nucleic acid molecule, the complementary sequence of which hybridizes to SEQ ID NO: 8 under stringent conditions, defined as 65°C, 2xSSC, 0.1%xSDS, followed by a wash for 30 minutes in 0.1xSSC, 0.1%xSDS.

17. A composition of matter of claim 16, wherein said cell line is a human cell line.

18. A composition of matter of claim 16, wherein said pharmaceutically acceptable carrier is a liposome.

19. A composition of matter comprising a non proliferative cell line having expressed on its surface a tumor rejection antigen precursor which is not a tum⁻ antigen specific for a tumor characteristic of a cancerous condition, and a pharmaceutically acceptable carrier; wherein said tumor rejection antigen precursor is encoded by a nucleic acid molecule, the complementary sequence of which hybridizes to SEQ ID NO: 8 under stringent conditions, defined as 65°C, 2xSSC, 0.1%xSDS, followed by a wash for 30 minutes in 0.1xSSC, 0.1%xSDS.

20. A composition of a matter of claim 19, wherein said cell line is a human cell line.

21. A composition of matter of claim 19, wherein said pharmaceutically acceptable carrier is a liposome.

22. A composition of matter comprising (i) a tumor rejection antigen which is not a tum⁻ antigen or a tumor rejection antigen precursor for a non tum⁻ antigen, (ii) an MHC or HLA molecule, and (iii) a pharmaceutically acceptable carrier; wherein said tumor rejection antigen precursor is encoded by a nucleic acid molecule, the complementary sequence of which hybridizes to SEQ ID NO: 8 under stringent conditions, defined as 65°C, 2xSSC, 0.1%xSDS, followed by a wash for 30 minutes in 0.1xSSC, 0.1%xSDS.

23. The composition of a matter of claim 22, wherein said pharmaceutically acceptable carrier is a liposome.

24. An antibody which specifically binds to a tumor rejection antigen precursor for a non tum⁻ antigen; wherein said tumor rejection antigen precursor is encoded by a nucleic acid molecule, the complementary sequence of which hybridizes to SEQ ID NO: 8 under stringent conditions, defined as 65°C, 2xSSC, 0.1%xSDS, followed by a wash for 30 minutes in 0.1xSSC, 0.1%xSDS.

25. The antibody of claim 24, wherein said tumor rejection antigen precursor is mage-1.

26. The antibody of claim 24, wherein said tumor rejection antigen precursor is antigen F precursor.

27. The antibody of claim 24, wherein said tumor rejection antigen precursor is a MAGE precursor.

28. The antibody of claim 27, wherein said MAGE precursor is mage 1, mage 2, mage 3, mage 4, mage 5, mage 6, mage 7, mage 8, mage 9, mage 10, mage 11, smage I and smage II.

29. An antibody which specifically binds to a tumor rejection antigen; wherein said tumor rejection antigen consists of a peptide fragment derived from a tumor rejection antigen precurser for a non tum$^-$ antigen; wherein said tumor rejection antigen precursor is encoded by a nucleic acid molecule, the complementary sequence of which hybridizes to SEQ ID NO: 8 under stringent conditions, defined as 65°C, 2xSSC, 0.1%xSDS, followed by a wash for 30 minutes in 0.1xSSC, 0.1%xSDS.

30. The antibody of claim 29, wherein said tumor rejection antigen has an amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO: 4.

31. The antibody of claim 29, wherein said tumor rejection antigen is antigen E.

32. The antibody of claim 29, wherein said tumor rejection antigen is antigen F.

33. An antibody which specifically binds to a complex of (i) tumor rejection antigen which is not a tum$^-$ antigen and (ii) HLA molecule, but does not bind to (i) or (ii) alone; wherein said tumor rejection antigen consists of a peptide fragment derived from a tumor rejection antigen precurser for a non tum$^-$ antigen; wherein said tumor rejection antigen

precursor is encoded by a nucleic acid molecule, the complementary sequence of which hybridizes to SEQ ID NO: 8 under stringent conditions, defined as 65°C, 2xSSC, 0.1%xSDS, followed by a wash for 30 minutes in 0.1xSSC, 0.1%xSDS.

34. A method for determining regression, progression or onset of a cancerous condition comprising monitoring a sample from a patient with said cancerous condition for a parameter selected from the group consisting of (i) tumor rejection antigen precursor for a non tum$^-$ antigen, wherein said tumor rejection antigen precurser is encoded by a nucleic acid molecule, the complementary sequence of which hybridizes to SEQ ID NO: 8 under stringent conditions, defined as 65°C, 2xSSC, 0.1%xSDS, followed by a wash for 30 minutes in 0.1xSSC, 0.1%xSDS, (ii) tumor rejection antigen encoded by an isolated nucleic acid molecule which codes for a tumor rejection antigen precursor; wherein said tumor rejection antigen precursor is encoded by a nucleic acid molecule, the complementary sequence of which hybridizes to SEQ ID NO: 8 under stringent conditions, defined as 65°C, 2xSSC, 0.1%xSDS, followed by a wash for 30 minutes in 0.1xSSC, 0.1%xSDS, and (iii) cytolytic T cells specific for a tumor rejection antigen which is not a tum$^-$ antigen associated with said cancerous condition, wherein amount of said parameter is indicative of progression or regression or onset of said cancerous condition comprising intradermally using an amount of a tumor rejection antigen which is not a tum$^-$ antigen sufficient to generate a delayed type response in a subject.

35. A use of an effective amount of the vaccine of claim 1 for preventing onset of a cancerous condition in a subject.

36. A use of an effective amount of the vaccine of claim 2 for preventing onset of a cancerous condition in a subject.

37. A use of an effective amount of the vaccine of claim 6 for preventing onset of a cancerous condition in a subject.

38. A use of an effective amount of the vaccine of claim 10 for preventing onset of a cancerous condition in a subject.

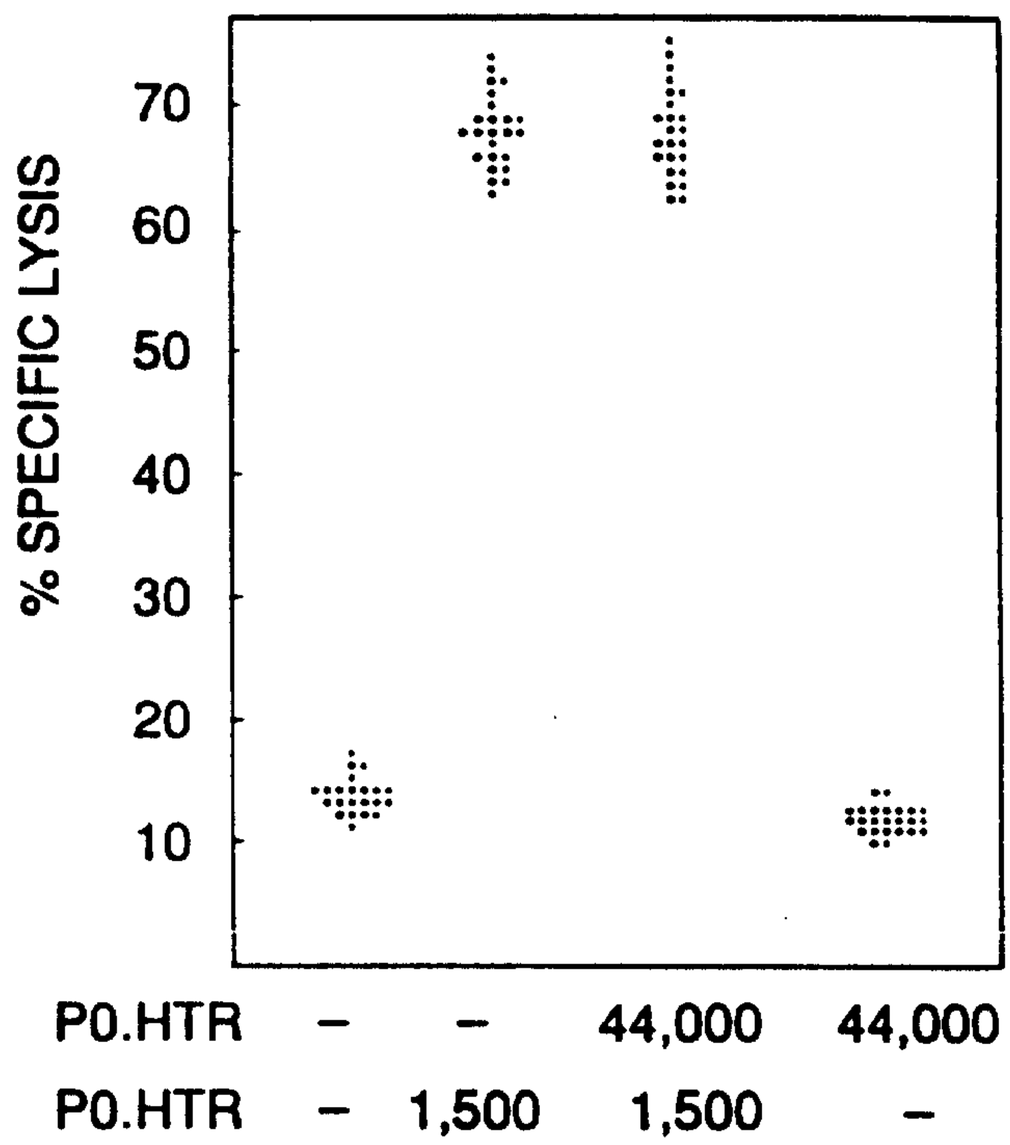
39. A use of an effective amount of the vaccine of claim 11 for preventing onset of a cancerous condition in a subject.

40. A use of an effective amount of the vaccine of claim 12 for preventing onset of a cancerous condition in a subject.

41. A use of an effective amount of the vaccine of claim 13 for preventing onset of a cancerous condition in a subject.

42. A use of an effective amount of the vaccine of claim 15 for preventing onset of a cancerous condition in a subject.

FIG. 1A


% SPECIFIC LYSIS
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60
50
40
30
20
10
P0.HTR – – 44,000 44,000
P0.HTR – 1,500 1,500 –

FIG. 1B

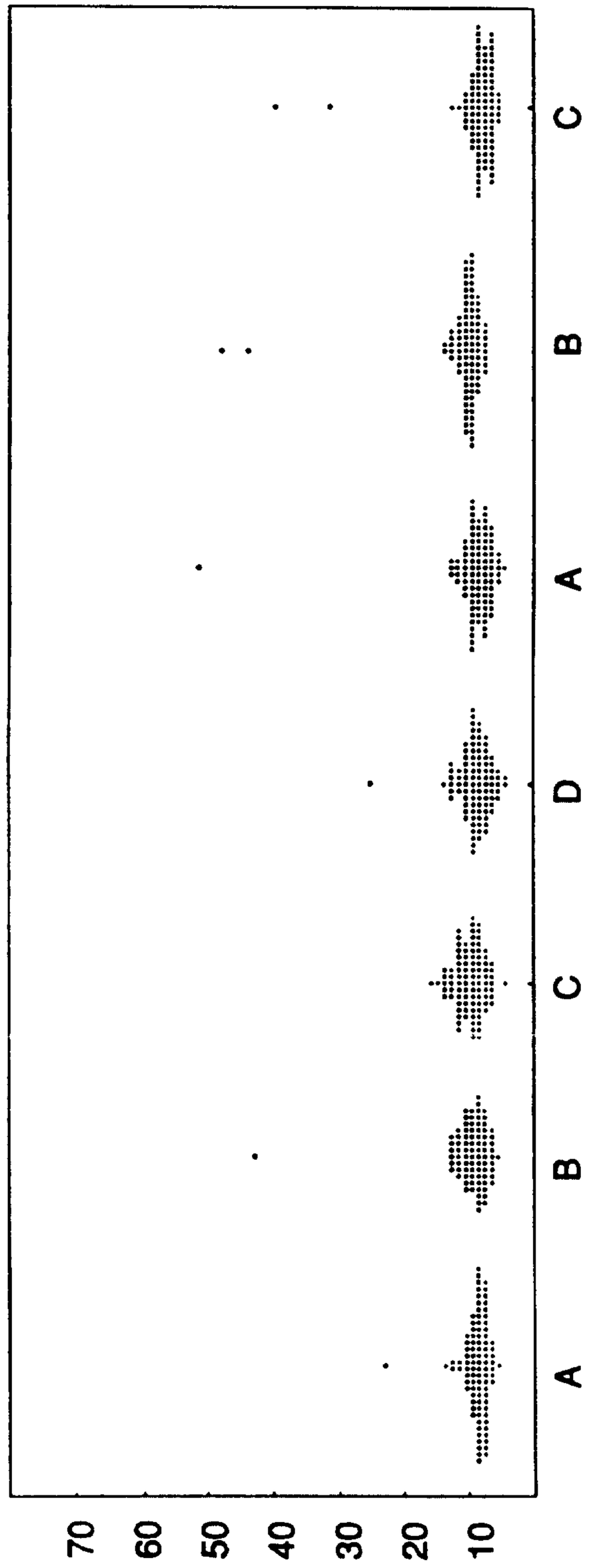

FIG. 2

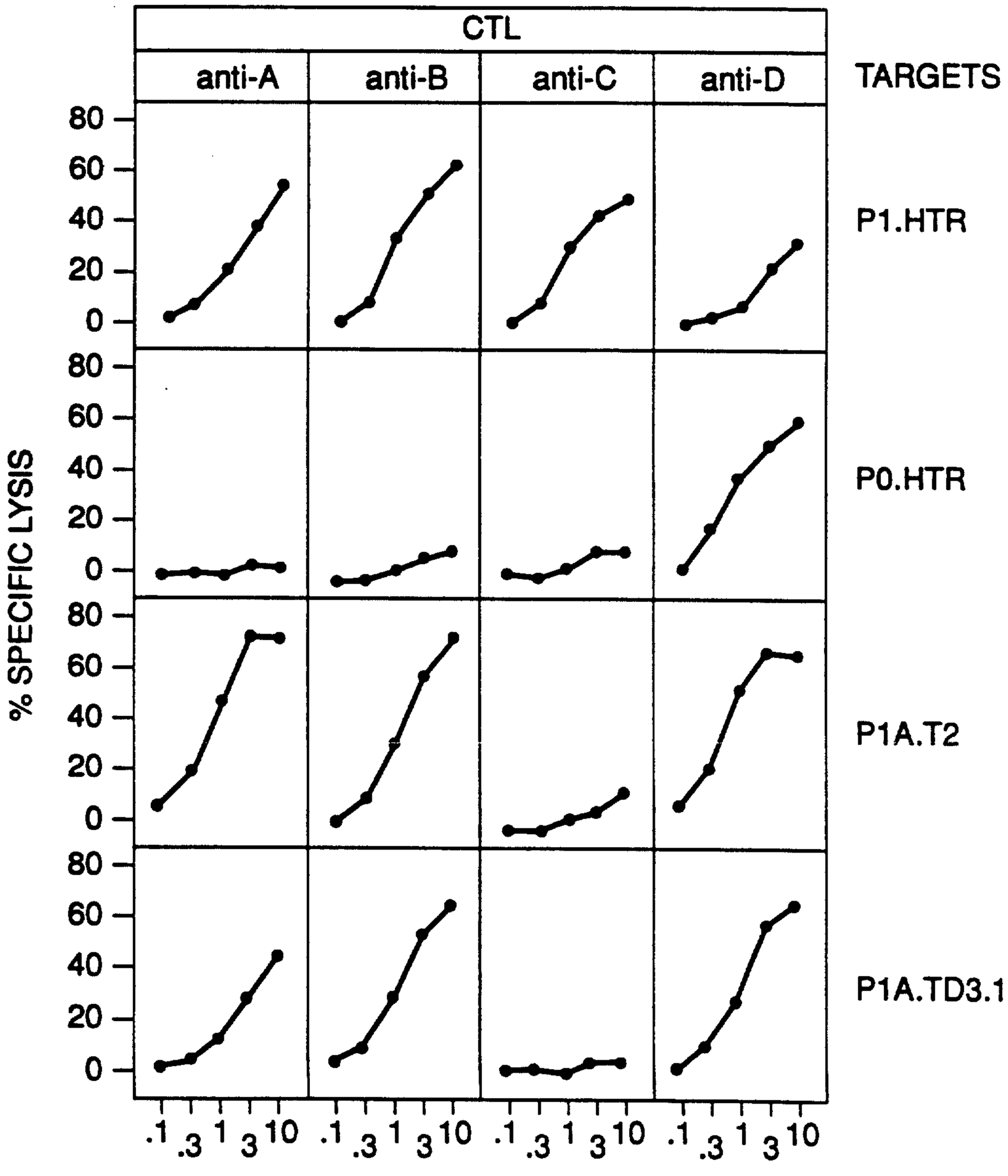

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FIG. 3

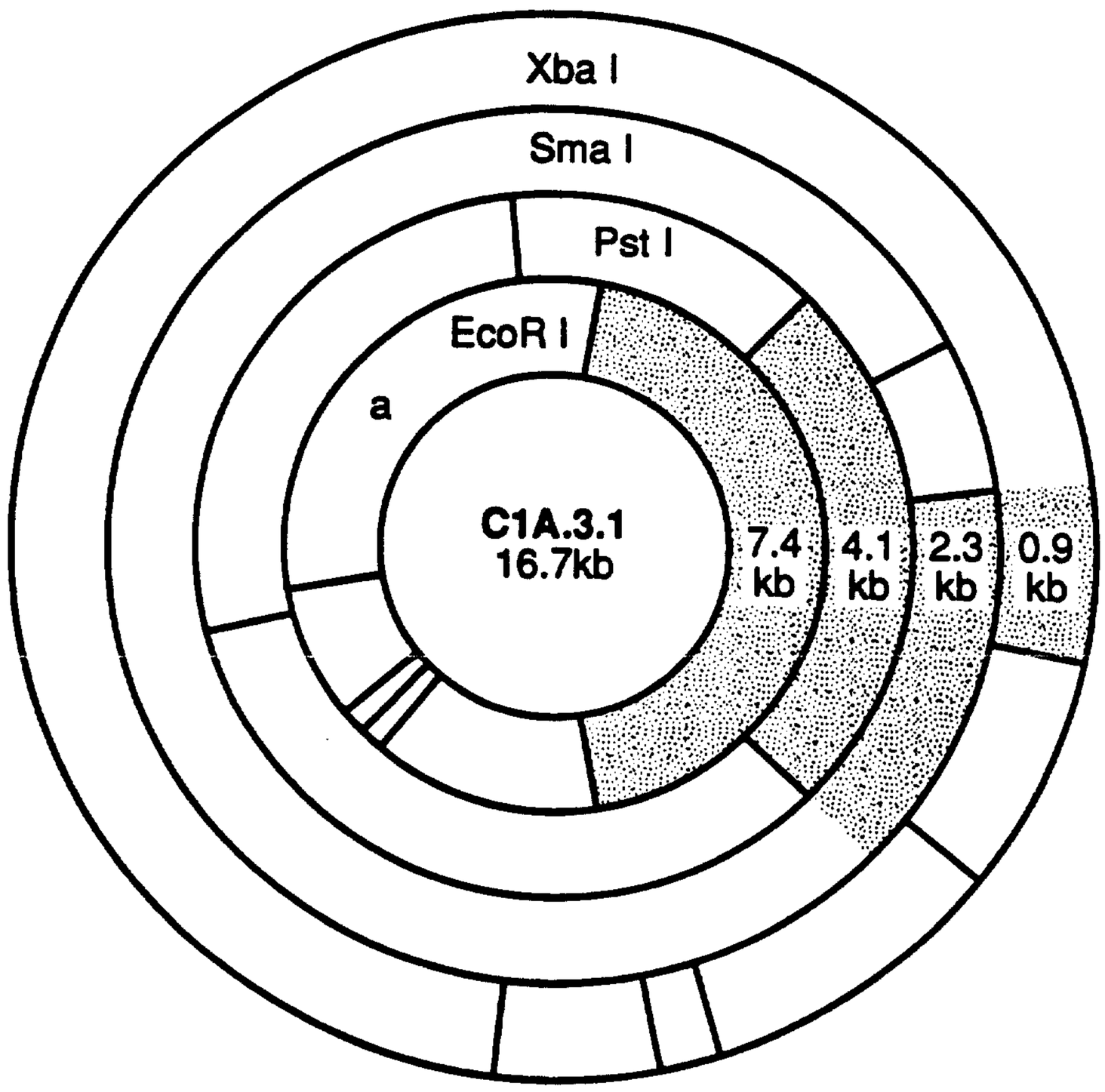

FIG. 4

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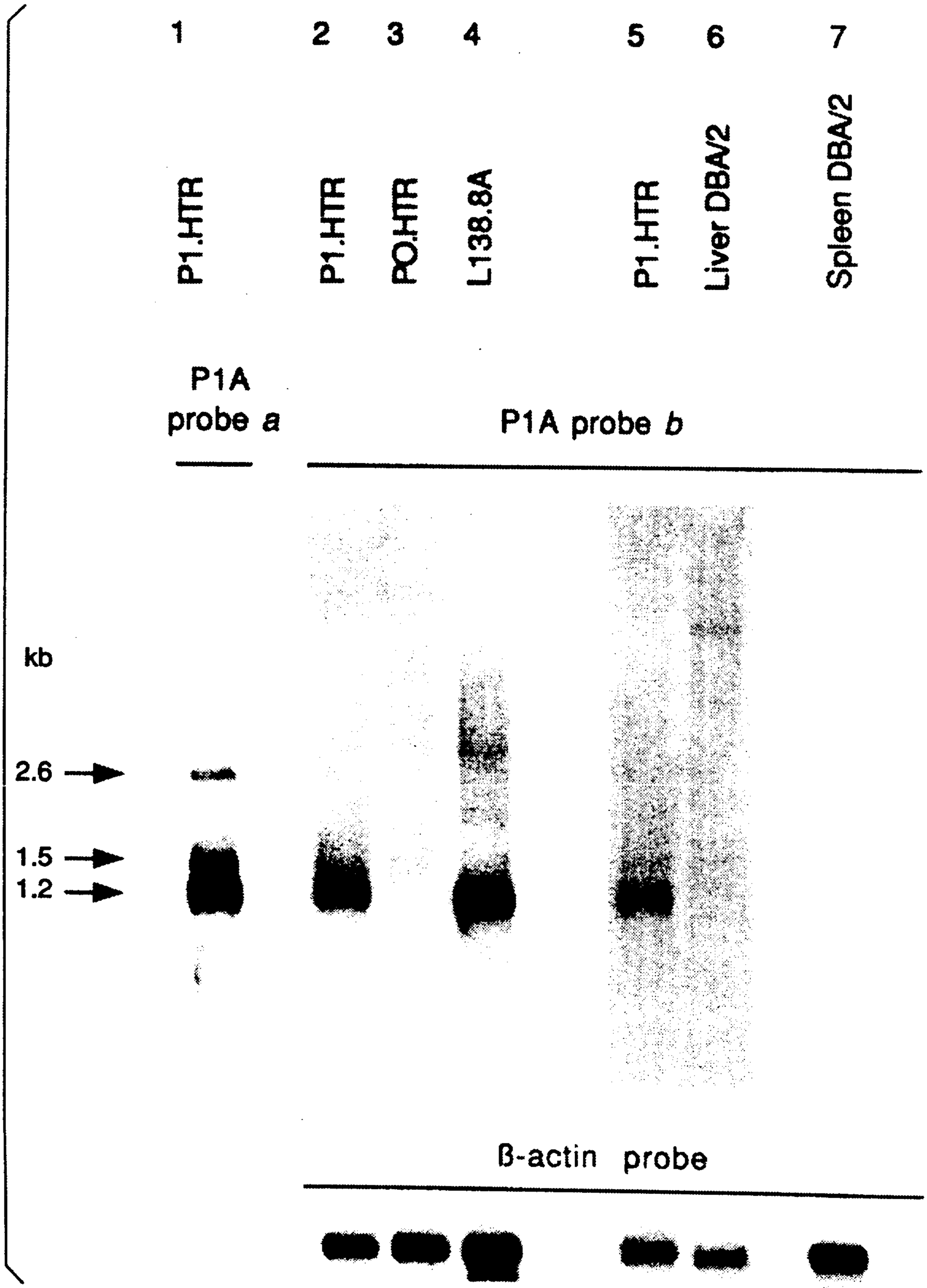

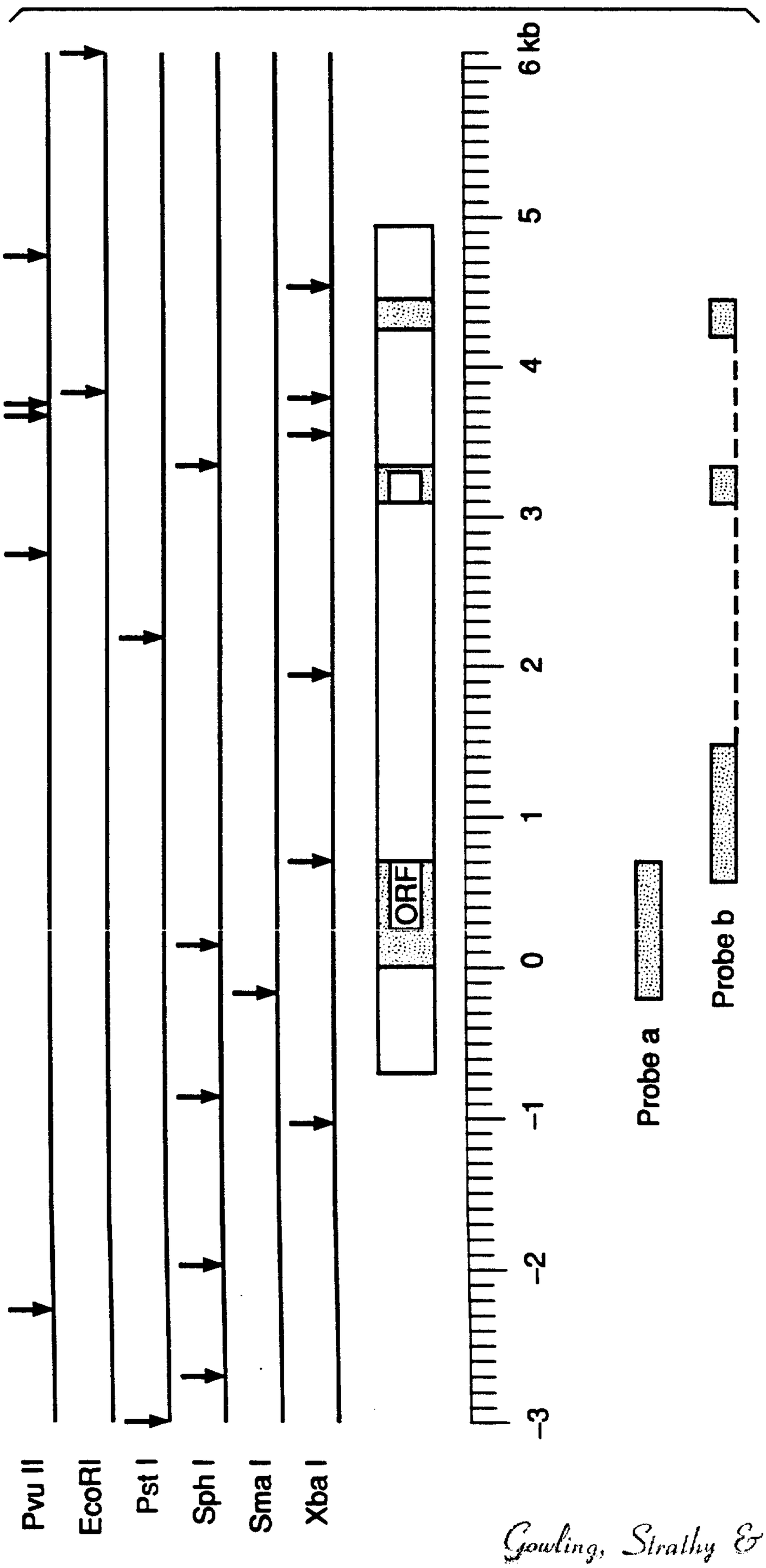
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4
3
2
1
0
−1
−2
−3
ORF
Probe a
Probe b
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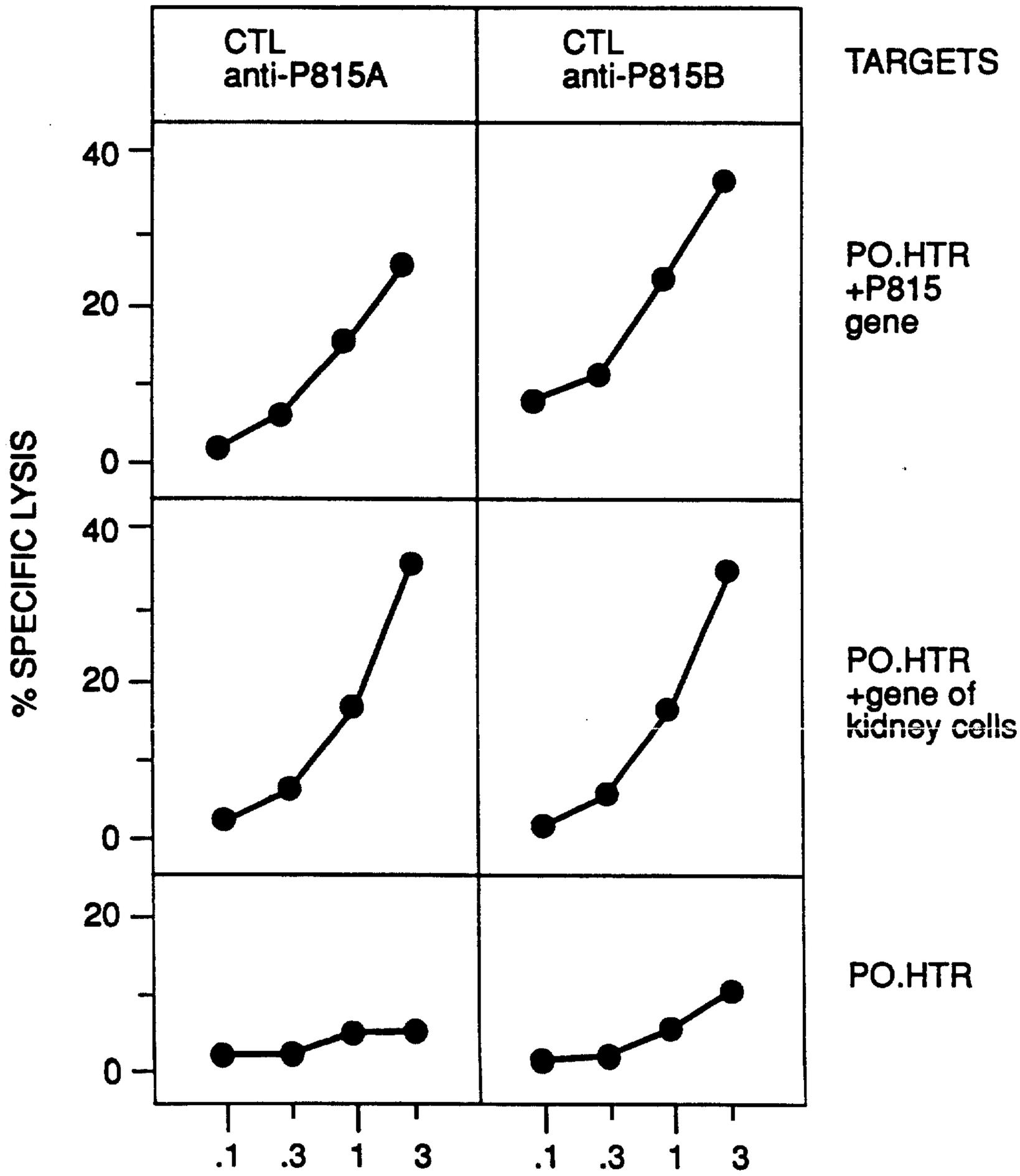
Pst I
Sph I
Sma I
Xba I

FIG. 5

FIG. 6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CTL anti-P815B
TARGETS
PO.HTR +P815 gene
PO.HTR +gene of kidney cells
PO.HTR
% SPECIFIC LYSIS
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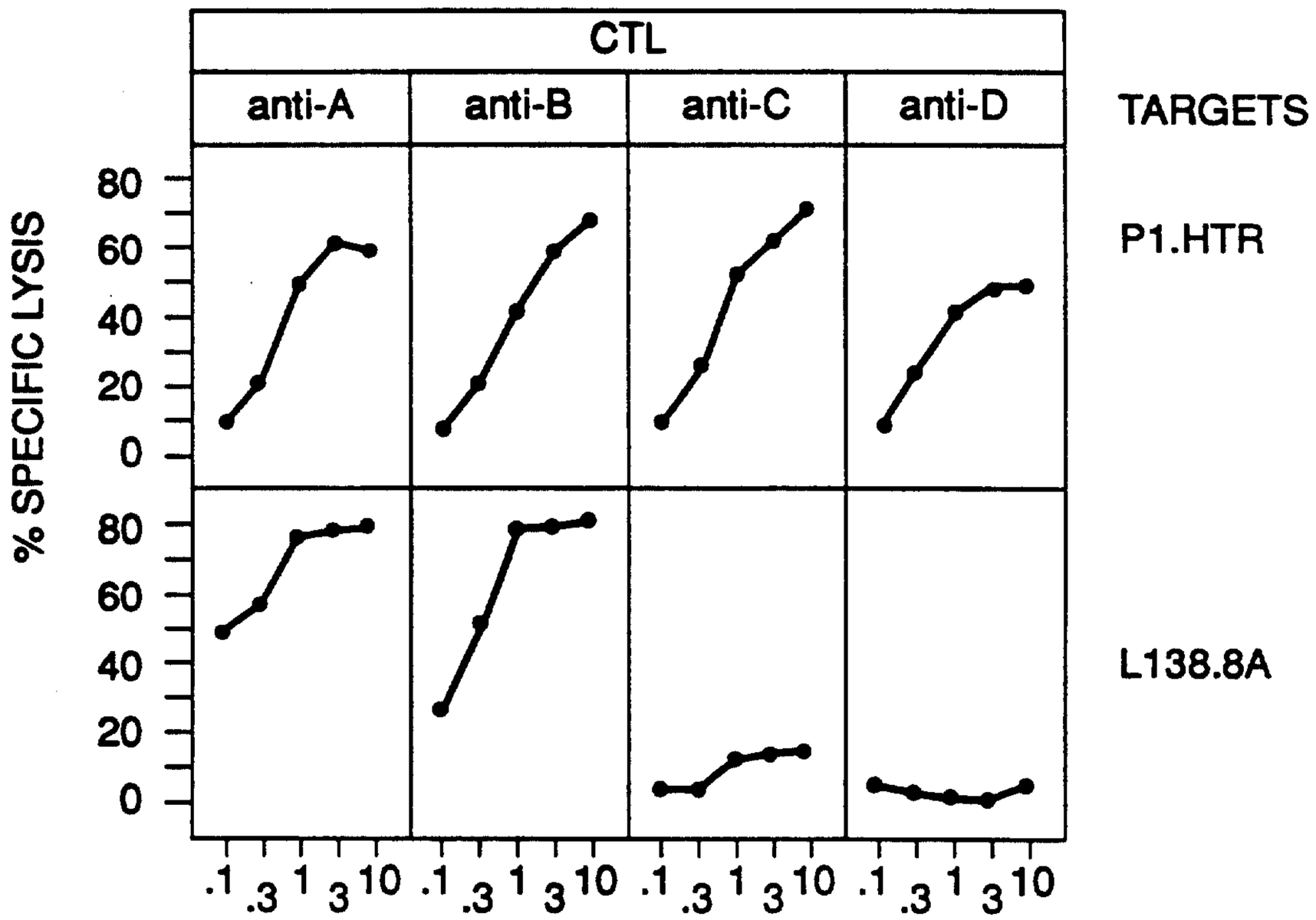
20
0
40
20
0
20
0
.1 .3 1 3 .1 .3 1 3
EFFECTOR / TARGET RATIO

FIG. 7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anti-B
anti-C
anti-D
TARGETS
P1.HTR
L138.8A
% SPECIFIC LYSIS
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60
40
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0
.1 .3 1 3 10 .1 .3 1 3 10 .1 .3 1 3 10 .1 .3 1 3 10
EFFECTOR / TARGET RATIO

FIG. 8

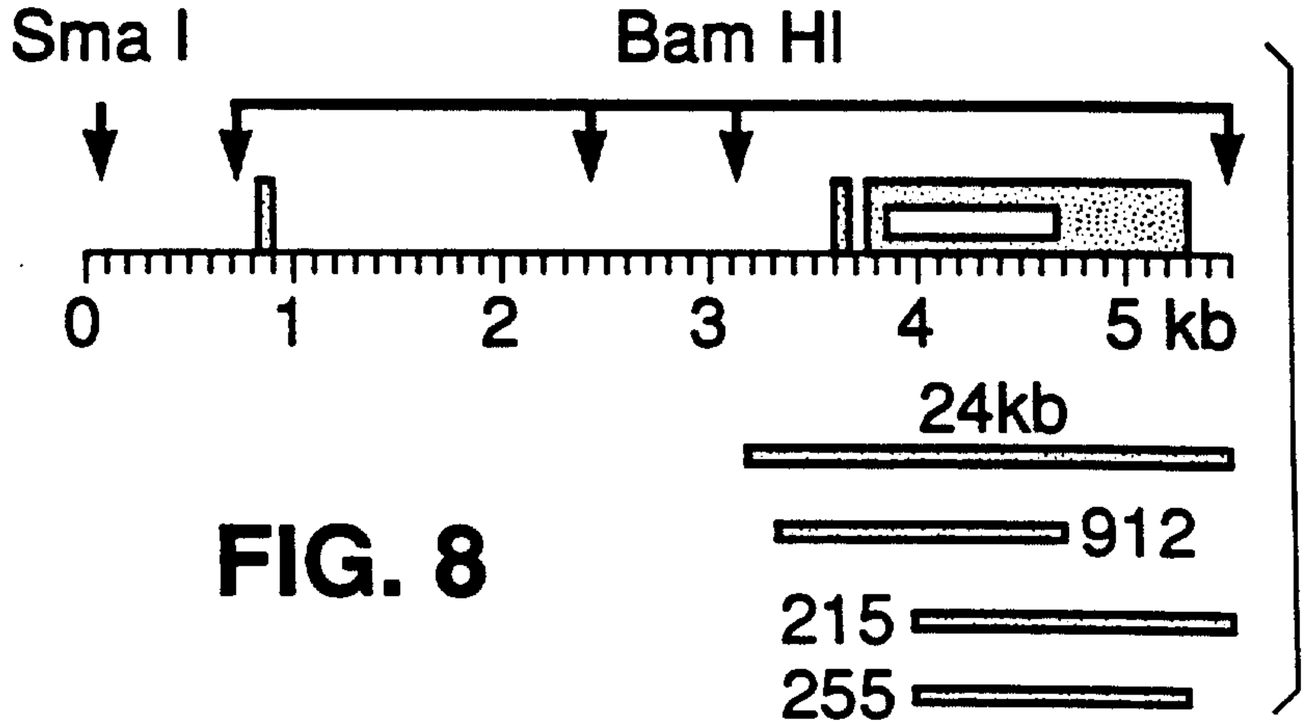
Sma I
Bam HI
0
1
2
3
4
5 kb
24kb
912
215
255

FIG. 9

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MAGE-3 III  CCTCCCCAGAGTCCTCAGGGAGCCTCCagCcTcCCCACTACCATgAACTaCcCTCtctgGAGcCAAtCCtaTGAGGacTCCAGCAaCCaaGAAGAGGAGG
                                                                                  <-- CHO-3
MAGE-2 II   CCTCCCCAcAGTCCTCAGGGAGCCTCCagCTTctCgACTACCATCAACTaCACTCtttgGAGaCAAtCCgaTGAGGGcTCCAGCAaCCaaGAAGAGGAGG
                                                                                  <-- CHO-2
MAGE-1 I    CCTCCCCAGAGTCCTCAGGGAGCCTCCGCCTTTCCCACTACCATCAACTTCACTCGACAGAGGCAACCCAGTGAGGGTTCCAGCAGCCGTGAAGAGGAGG
            225  CHO-8 -->

            III GGCCAAGCACCTtcccTgaCC-TGGAGTCCgaGTTCCaAGCAGcAcTCAgTAgGAAGGTGGCcGAgTTGGTTcaTTTTCTGCTCCTCAAgTATCGAGCCA
            II  GGCCAAGaAtgTtTcccgaCCtTGGAGTCCGAGTTCCaAGCAGcAATCAgTAgGAAGaTGGtTGAgTTGGTTcaTTTTCTGCTCCTCAAgTATCGAGCCA
            I   GGCCAAGCACCTCTTGTATCC-TGGAGTCCTTGTTCCGAGCAGTAATCACTAAGAAGGTGGCTGATTTGGTTGGTTTTCTGCTCCTCAAATATCGAGCCA
            325

            III GGGAGCCgGTCACAAAGGCAGAAATGCTGGgGAGTGTCgTCggAAATTggcAGtAtTtcTTTCCTGtGATCTTCaGCAAAGCtTCcagtTCCTTGCAGCT
            II  GGGAGCCgGTCACAAAGGCAGAAATGCTGGAGAGTGTCcTCAgAAATTgCcAGgACTtcTTTCCcGtGATCTTCaGCAAAGCCTCcGAGTaCTTGCAGCT
            I   GGGAGCCAGTCACAAAGGCAGAAATGCTGGAGAGTGTCATCAAAAATTACAAGCACTGTTTTCCTGAGATCTTCGGCAAAGCCTCTGAGTCCTTGCAGCT
            425                                                                   <-- SEQ-4

            III GGTCTTTGGCATcGAgcTGAtGGAAGtgGACCCCAtCGGCCACTtgTAcaTCtTTGcCACCTGCCTgGGcCTCTCCTAcGATGGCCTGCTGGGTGAcAAT
            II  GGTCTTTGGCATcGAgGTGgtGGAAGtgGtCCCCAtCaGCCACTtgTAcaTCCTTGTCACCTGCCTgGGcCTCTCCTAcGATGGCCTGCTGGGcGAcAAT
            I   GGTCTTTGGCATTGACGTGAAGGAAGCAGACCCCACCGGCCACTCCTATGTCCTTGTCACCTGCCTAGGTCTCTCCTATGATGGCCTGCTGGGTGATAAT
            525

            III CAGATCATGCCCAAGgCAGGCcTCCTGATAATcGTCCTGGcCATaATcGCAAgaGAGGGCGaCtgTGCcCCTGAGGAGaAAATCTGGGAGGAGCTGAGTG
            II  CAGgTCATGCCCAAGACAGGCcTCCTGATAATcGTC-TGGcCATaATcGCAATaGAGGGCGaCtgTGCcCCTGAGGAGaAAATCTGGGAGGAGCTGAGTa
            I   CAGATCATGCCCAAGACAGGCTTCCTGATAATTGTCCTGGTCATGATTGCAATGGAGGGCGGCCATGCTCCTGAGGAGGAAATCTGGGAGGAGCTGAGTG
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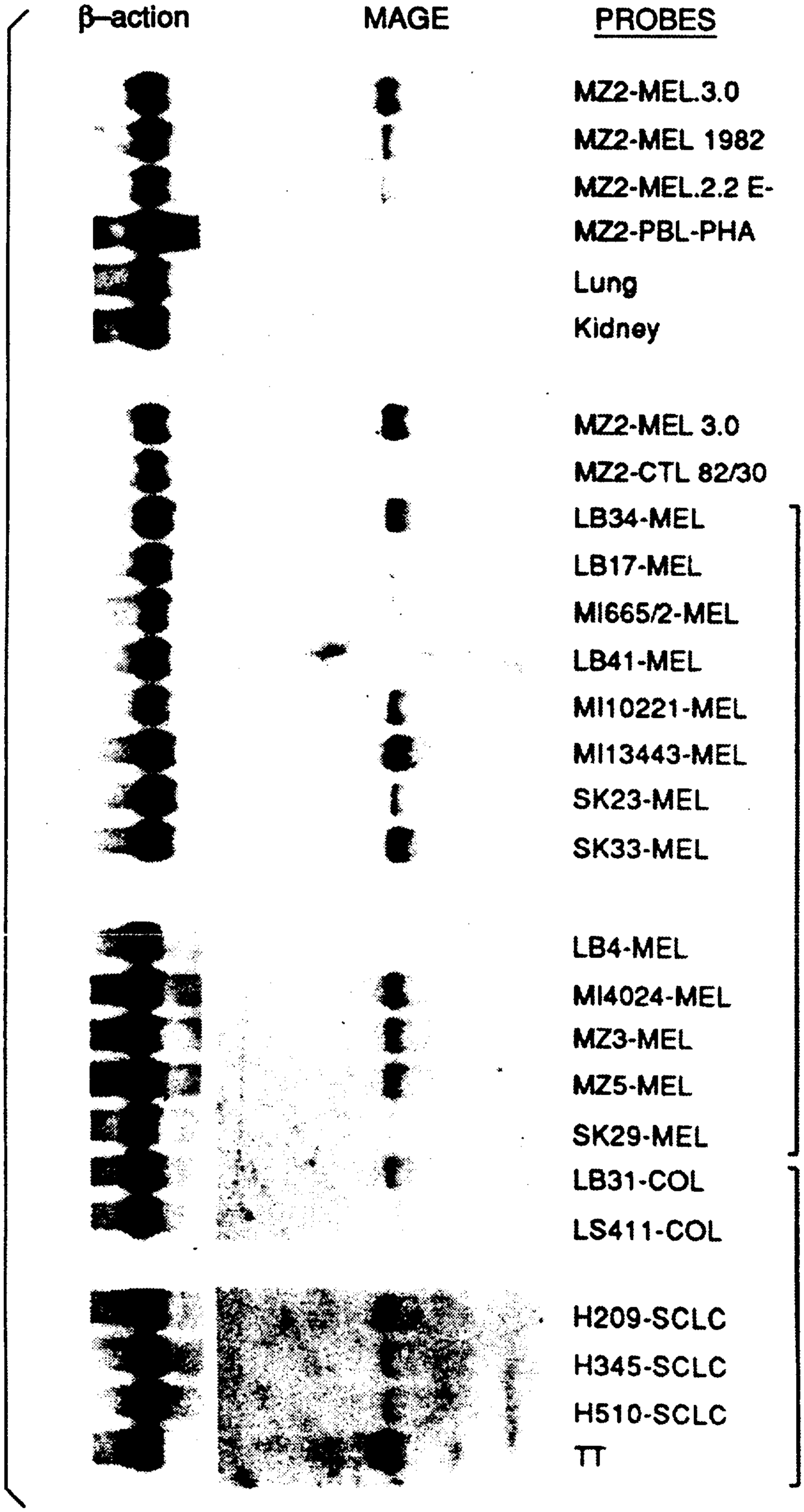

β–action
MAGE
PROBES
MZ2-MEL.3.0
MZ2-MEL 1982
MZ2-MEL.2.2 E-
MZ2-PBL-PHA
Lung
Kidney
MZ2-MEL 3.0
MZ2-CTL 82/30
LB34-MEL
LB17-MEL
MI665/2-MEL
LB41-MEL
MI10221-MEL
MI13443-MEL
SK23-MEL
SK33-MEL
LB4-MEL
MI4024-MEL
MZ3-MEL
MZ5-MEL
SK29-MEL
LB31-COL
LS411-COL
H209-SCLC
H345-SCLC
H510-SCLC
TT
Other melanomas
Other tumors

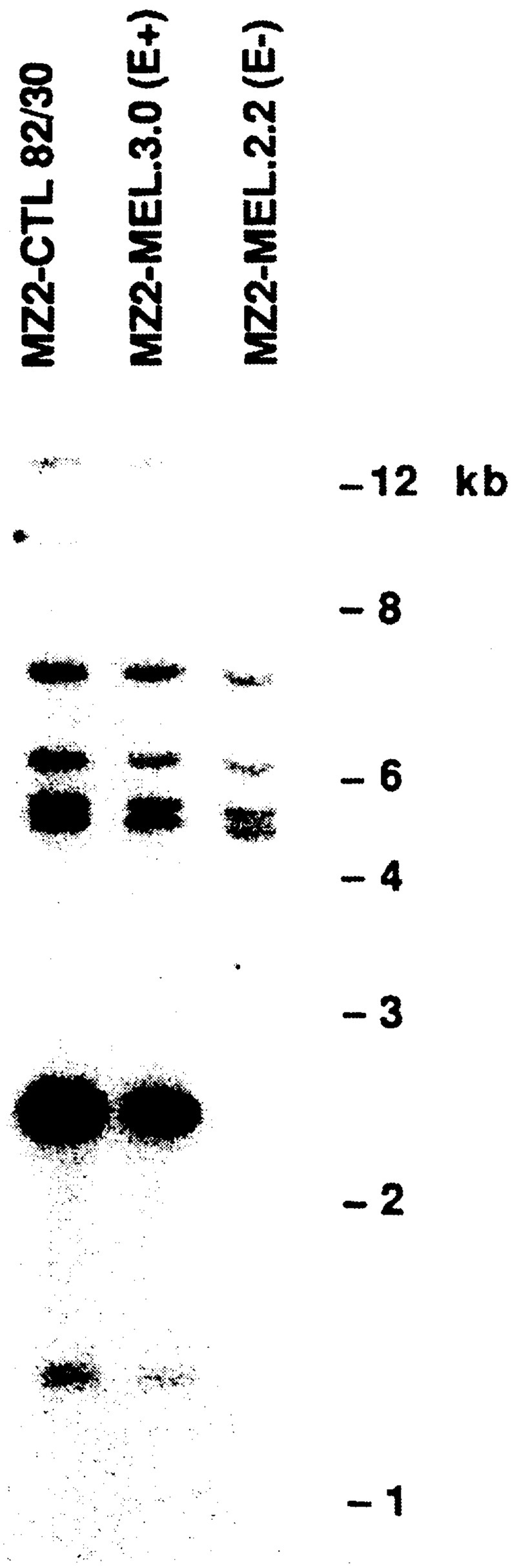
FIG. 10

FIG. 11

		EXPRSSION OF MAGE GENE FAMILY				RECOGNITIN BY ANI-E CTL		Expression of antigen MZ2-E after transaction**
		Northern blot probed with cross-reactive MAGE-1 probe*	cDNA-PCR product probed with oligonucleotide specific for: MAGE-1	MAGE-2	MAGE-3†	tested by: TNF release‡	Lysis§	
Cells of patient MZ2	melanoma cell line MZ2-MEL.3.0	+	+++	+++	+++	+	+	
	tumor sample MZ2 (1982)	+	+++	+++	+++			
	antigen-loss variant MZ2-MEL.2.2	+	−	+++	+++	−	−	
	CTL clone MZ2-CTL.82/30	−	−	−	−			
	PHA-activated blood lymphocytes	−	−	−	−			
Normal tissues	Liver	−	−	−	−			
	Muscle	−	−	−	−			
	Skin	−	−	−	−			
	Lung	−	−	−	−			
	Brain	−	−	−	−			
	Kidney	−	−	−	−			
Melanoma cell lines of HLA-A1 patients	LB34-MEL	+	++	+++	+++	+	+−	
	MI665/2-MEL	−	−	−	−	−	−	+
	MI10221-MEL	+	−	++	+++	−	−	+
	MI13443-MEL	+	+++	+++	+++	+	+	
	SK33-MEL	+	−	+++	+++	−	−	−
	SK23-MEL	+	−	+++	+++	−	−	+
Melanoma cell lines of other patients	LB17-MEL	+	+	+++	+++	−	−	−
	LB33-MEL	+	−	+++	+++	−	−	−
	LB4-MEL	−	−	−	−	−	−	
	LB41-MEL	−	−	−	−	−	−	
	MI4024-MEL	+	+++	+++	+++	−	−	
	SK29-MEL	−	−	−	−	−	−	
	MZ3-MEL	+	+	+++	+++	−	−	
	MZ5-MEL	+	−	+++	+++	−	−	
Melanoma tumor sample	BB5-MEL	+	+++	++	+++			
Other tumor cell lines	small cell lung cancer H209	+	−	+++	+++			
	small cell lung cancer H345	+	−	+++	+++			
	small cell lung cancer H510	+	−	+++	+++			
	small cell lung cancer LB11	+	+	+++	+++			
	bronchial squamous cell carcinoma LB37	+	−	−	+++			
	thyroid medullary carcinoma TT	+	++++	+++	++++			
	colon carcinoma LB31	+	−	+++	++++	−		
	colon carcinoma LS411	−	−	−	−			
Other tumor samples	chronic myeloid leukemia LLC5	−	−	−	−			
	acute myeloid leukemia TA	−	−	−	−			

* Data obtained in the conditions of figure 5.
† Data obtained as described in figure 6.
‡ TNF release by CTL 82/30 after stimulation with the tumor cells as described in (11).
§ Lysis of 51 Cr labelled target by CTL 82/30 in the conditions of figure 1.
** Cells transfected with the 2.4 kb fragment of gene MAGE-1 were tested for their ability to stimulate TNF release by CTL 82/30

FIG. 12



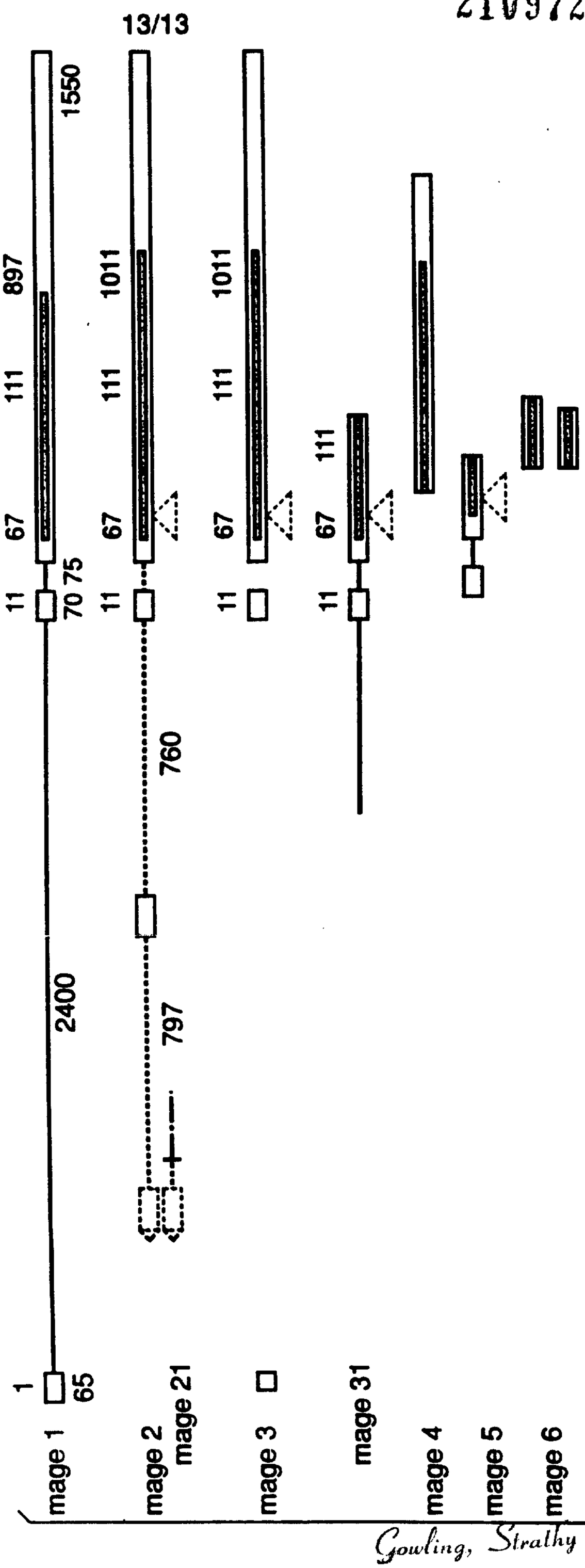

1550
11
67
111
897
70 75
11
67
111
1011
760
11
67
111
1011
11
67
111
2400
797
1
65
mage 1
mage 2
mage 21
mage 3
mage 31
mage 4
mage 5
mage 6
mage 7

FIG. 13