



(86) Date de dépôt PCT/PCT Filing Date: 2001/08/22
(87) Date publication PCT/PCT Publication Date: 2002/02/28
(45) Date de délivrance/Issue Date: 2013/04/02
(85) Entrée phase nationale/National Entry: 2003/02/20
(86) N° demande PCT/PCT Application No.: US 2001/026276
(87) N° publication PCT/PCT Publication No.: 2002/016593
(30) Priorités/Priorities: 2000/08/22 (US60/227,098);
2001/04/10 (US60/282,739)

(51) Cl.Int./Int.Cl. *C12Q 1/68* (2006.01),
A61K 39/395 (2006.01), *A61K 47/48* (2006.01),
A61P 35/00 (2006.01), *C07K 14/47* (2006.01),
C07K 16/18 (2006.01), *C12N 15/12* (2006.01),
G01N 33/50 (2006.01)

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(54) Titre : ACIDE NUCLEIQUE ET PROTEINE CORRESPONDANTE NOMME 158P1D7 SERVANT DANS LE
TRAITEMENT ET LA DETECTION DU CANCER DE LA VESICULE ET D'AUTRES CANCERS
(54) Title: NUCLEIC ACID AND CORRESPONDING PROTEIN NAMED 158P1D7 USEFUL IN THE TREATMENT AND
DETECTION OF BLADDER AND OTHER CANCERS

(57) **Abrégé/Abstract:**

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
28 February 2002 (28.02.2002)

PCT

(10) International Publication Number
WO 02/016593 A3

(51) International Patent Classification⁷: **C12N 15/12**,
C07K 14/47, C12Q 1/68, A61K 39/00, 38/00

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(21) International Application Number: PCT/US01/26276

(22) International Filing Date: 22 August 2001 (22.08.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/227,098 22 August 2000 (22.08.2000) US
60/282,739 10 April 2001 (10.04.2001) US

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI,
SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA,
ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD,
TG).

Published:

— with international search report

(88) Date of publication of the international search report:
27 December 2002

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

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COMPREND PLUS D'UN TOME.**

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JUMBO APPLICATIONS / PATENTS

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NUCLEIC ACID AND CORRESPONDING PROTEIN NAMED 158P1D7 USEFUL IN THE TREATMENT AND DETECTION OF BLADDER AND OTHER CANCERS

FIELD OF THE INVENTION

The invention described herein relates to a novel nucleic acid sequence and its encoded protein, referred to as 158P1D7, and to diagnostic and therapeutic methods and compositions useful in the management of various cancers that express 158P1D7.

BACKGROUND OF THE INVENTION

Cancer is the second leading cause of human death next to coronary disease. Worldwide, millions of people die from cancer every year. In the United States alone, as reported by the American Cancer Society, cancer causes the death of well over a half-million people annually, with over 1.2 million new cases diagnosed per year. While deaths from heart disease have been declining significantly, those resulting from cancer generally are on the rise. In the early part of the next century, cancer is predicted to become the leading cause of death.

Of all new cases of cancer in the United States, bladder cancer represents approximately 5 percent in men (fifth most common neoplasm) and 3 percent in women (eighth most common neoplasm). The incidence is increasing slowly, concurrent with an increasing older population. In 1998, there was an estimated 54,500 cases, including 39,500 in men and 15,000 in women. The age-adjusted incidence in the United States is 32 per 100,000 for men and 8 per 100,000 in women. The historic male/female ratio of 3:1 may be decreasing related to smoking patterns in women. There were an estimated 11,000 deaths from bladder cancer in 1998 (7,800 in men and 3,900 in women). Bladder cancer incidence and mortality strongly increase with age and will be an increasing problem as the population becomes more elderly.

Bladder cancers comprise a heterogeneous group of diseases. The main determinants of disease control and survival are histology and extent of disease. The main codes for these factors include pathology classification, the International Classification of Diseases-Oncology (ICDO), and staging classification of extent of disease, the TNM classification.(Table XXI). For a general discussion of bladder and other urogenital cancers, see, e.g., Vogelzang, et al, Eds. Comprehensive Textbook of Genitourinary Oncology, (Williams & Wilkins, Baltimore 1996), in particular pages 295-556.

Three primary types of tumors have been reported in the bladder. The most common type of bladder cancer is Transitional cell carcinoma (TCC); this accounts for about 90% of all bladder cancers.

The second form of bladder cancer is squamous cell carcinoma, which accounts for about 8% of all bladder cancers where schistosomiasis is not endemic, and approximately 75% of bladder carcinomas where schistosomiasis is endemic. Squamous cell carcinomas tend to invade deeper layers of the bladder. The third type of bladder cancer is adenocarcinoma, which account for 1%-2% of bladder cancers; these are primarily invasive forms of cancer.

Bladder cancer is commonly detected and diagnosed using cystoscopy and urine cytology. However these methods demonstrate poor sensitivity. Relatively more reliable methods of detection currently used in the clinic include the bladder tumor antigen (BTA) stat test, NMP22 protein assay, telomerase expression and hyaluronic acid and hyaluronidase (HA-HAase) urine test. The advantage of using such markers in the diagnosis of bladder cancer is their relative high sensitivity in earlier tumor stages compared to standard cytology.

For example, the BTA stat test has 60-80% sensitivity and 50-70% specificity for bladder cancer, while the HA-HAase urine test shows 90-92% sensitivity and 80-84% specificity for bladder cancer (J Urol 2001 165:1067). In general, sensitivity for stage Ta tumors was 81% for nuclear matrix protein (NMP22), 70% for telomerase, 32% for bladder tumor antigen (BTA) and 26% for cytology (J Urol 2001 166:470; J Urol 1999, 161:810). Although the telomeric repeat assay which measures telomerase activity is relatively sensitive, instability of telomerase in urine presently renders this detection method unreliable.

Most bladder cancers recur in the bladder. Generally, bladder cancer is managed with a combination of transurethral resection of the bladder (TUR) and intravesical chemotherapy or immunotherapy. The multifocal and recurrent nature of bladder cancer points out the limitations of TUR. Most muscle-invasive cancers are not cured by TUR alone. Radical cystectomy and urinary diversion is the most effective means to eliminate the cancer but carry an undeniable impact on urinary and sexual function.

Intravesical bacilli Calmette-Guerin (BCG) is a common and efficacious immunotherapeutic agent used in the treatment of bladder cancer. BCG is also used as a prophylactic agent to prevent recurrence of bladder cancer. However, 30% of patients fail to respond to BCG therapy and go on to develop invasive and metastatic disease (Catalona et al. J Urol 1987, 137:220-224). BCG-related side effects have been frequently observed such as drug-induced cystitis, risk of bacterial infection, and hematuria, amongst others. Other alternative immunotherapies have been used for the treatment of bladder cancer, such as KLH (Flamm et al. Urologe 1994; 33:138-143) interferons (Bazarbashi et al. J Surg Oncol. 2000; 74:181-4), and MAGE-3 peptide loaded dendritic cells (Nishiyama et al. Clin Cancer Res 2001; 7:23-31). All these approaches are still experimental (Zlotta et al. Eur Urol 2000;37 Suppl 3:10-15). There continues to be a significant need for diagnostic and treatment modalities that are beneficial for bladder cancer patients. Furthermore, from a worldwide standpoint, several cancers stand out as the leading killers. In particular, carcinomas of the lung, prostate, breast, colon, pancreas, and ovary are primary causes of cancer death. These and virtually all other carcinomas share a common lethal feature. With very few exceptions, metastatic disease from a carcinoma is fatal. Moreover, even for those cancer patients who initially survive

their primary cancers, their lives are dramatically altered. Many cancer patients experience strong anxieties driven by the awareness of the potential for recurrence or treatment failure. Many cancer patients experience physical debilitations following treatment. Furthermore, many cancer patients experience a recurrence.

Prostate cancer is the fourth most prevalent cancer in men worldwide. In North America and Northern Europe, it is by far the most common cancer in males and is the second leading cause of cancer death in men. In the United States alone, well over 30,000 men die annually of this disease, second only to lung cancer. Despite the magnitude of these figures, there is still no effective treatment for metastatic prostate cancer. Surgical prostatectomy, radiation therapy, hormone ablation therapy, surgical castration and chemotherapy continue to be the main treatment modalities. Unfortunately, these treatments are ineffective for many and are often associated with undesirable consequences.

On the diagnostic front, the lack of a prostate tumor marker that can accurately detect early-stage, localized tumors remains a significant limitation in the diagnosis and management of this disease. Although the serum prostate specific antigen (PSA) assay has been a very useful tool, however its specificity and general utility is widely regarded as lacking in several important respects. While previously identified markers such as PSA, PSM, PCTA and PSCA have facilitated efforts to diagnose and treat prostate cancer, there is need for the identification of additional markers and therapeutic targets for prostate and related cancers in order to further improve diagnosis and therapy.

Renal cell carcinoma (RCC) accounts for approximately 3 percent of adult malignancies. Once adenomas reach a diameter of 2 to 3 cm, malignant potential exists. In the adult, the two principal malignant renal tumors are renal cell adenocarcinoma and transitional cell carcinoma of the renal pelvis or ureter. The incidence of renal cell adenocarcinoma is estimated at more than 29,000 cases in the United States, and more than 11,600 patients died of this disease in 1998. Transitional cell carcinoma is less frequent, with an incidence of approximately 500 cases per year in the United States.

Surgery has been the primary therapy for renal cell adenocarcinoma for many decades. Until recently, metastatic disease has been refractory to any systemic therapy. With recent developments in systemic therapies, particularly immunotherapies, metastatic renal cell carcinoma may be approached aggressively in appropriate patients with a possibility of durable responses. Nevertheless, there is a remaining need for effective therapies for these patients.

An estimated 130,200 cases of colorectal cancer occurred in 2000 in the United States, including 93,800 cases of colon cancer and 36,400 of rectal cancer. Colorectal cancers are the third most common cancers in men and women. Incidence rates declined significantly during 1992-1996 (-2.1% per year). Research suggests that these declines have been due to increased screening and polyp removal, preventing progression of polyps to invasive cancers. There were an estimated 56,300 deaths (47,700 from colon cancer, 8,600 from rectal cancer) in 2000, accounting for about 11% of all U.S. cancer deaths.

At present, surgery is the most common form of therapy for colorectal cancer, and for cancers that have not spread, it is frequently curative. Chemotherapy, or chemotherapy plus radiation is given before or

after surgery to most patients whose cancer has deeply perforated the bowel wall or has spread to the lymph nodes. A permanent colostomy (creation of an abdominal opening for elimination of body wastes) is occasionally needed for colon cancer and is infrequently required for rectal cancer. There continues to be a need for effective diagnostic and treatment modalities for colorectal cancer.

There were an estimated 164,100 new cases of lung and bronchial cancer in 2000, accounting for 14% of all U.S. cancer diagnoses. The incidence rate of lung and bronchial cancer is declining significantly in men, from a high of 86.5 per 100,000 in 1984 to 70.0 in 1996. In the 1990s, the rate of increase among women began to slow. In 1996, the incidence rate in women was 42.3 per 100,000.

Lung and bronchial cancer caused an estimated 156,900 deaths in 2000, accounting for 28% of all cancer deaths. During 1992–1996, mortality from lung cancer declined significantly among men (-1.7% per year) while rates for women were still significantly increasing (0.9% per year). Since 1987, more women have died each year of lung cancer than breast cancer, which, for over 40 years, was the major cause of cancer death in women. Decreasing lung cancer incidence and mortality rates most likely resulted from decreased smoking rates over the previous 30 years; however, decreasing smoking patterns among women lag behind those of men. Of concern, although the declines in adult tobacco use have slowed, tobacco use in youth is increasing again.

Treatment options for lung and bronchial cancer are determined by the type and stage of the cancer and include surgery, radiation therapy, and chemotherapy. For many localized cancers, surgery is usually the treatment of choice. Because the disease has usually spread by the time it is discovered, radiation therapy and chemotherapy are often needed in combination with surgery. Chemotherapy alone or combined with radiation is the treatment of choice for small cell lung cancer; on this regimen, a large percentage of patients experience remission, which in some cases is long lasting. There is however, an ongoing need for effective treatment and diagnostic approaches for lung and bronchial cancers.

An estimated 182,800 new invasive cases of breast cancer were expected to have occurred among women in the United States during 2000. Additionally, about 1,400 new cases of breast cancer were expected to be diagnosed in men in 2000. After increasing about 4% per year in the 1980s, breast cancer incidence rates in women have leveled off in the 1990s to about 110.6 cases per 100,000.

In the U.S. alone, there were an estimated 41,200 deaths (40,800 women, 400 men) in 2000 due to breast cancer. Breast cancer ranks second among cancer deaths in women. According to the most recent data, mortality rates declined significantly during 1992–1996 with the largest decreases in younger women, both white and black. These decreases were probably the result of earlier detection and improved treatment.

Taking into account the medical circumstances and the patient's preferences, treatment of breast cancer may involve lumpectomy (local removal of the tumor) and removal of the lymph nodes under the arm; mastectomy (surgical removal of the breast) and removal of the lymph nodes under the arm; radiation therapy; chemotherapy; or hormone therapy. Often, two or more methods are used in combination. Numerous studies have shown that, for early stage disease, long-term survival rates after lumpectomy plus

radiotherapy are similar to survival rates after modified radical mastectomy. Significant advances in reconstruction techniques provide several options for breast reconstruction after mastectomy. Recently, such reconstruction has been done at the same time as the mastectomy.

Local excision of ductal carcinoma in situ (DCIS) with adequate amounts of surrounding normal breast tissue may prevent the local recurrence of the DCIS. Radiation to the breast and/or tamoxifen may reduce the chance of DCIS occurring in the remaining breast tissue. This is important because DCIS, if left untreated, may develop into invasive breast cancer. Nevertheless, there are serious side effects or sequelae to these treatments. There is, therefore, a need for efficacious breast cancer treatments.

There were an estimated 23,100 new cases of ovarian cancer in the United States in 2000. It accounts for 4% of all cancers among women and ranks second among gynecologic cancers. During 1992–1996, ovarian cancer incidence rates were significantly declining. Consequent to ovarian cancer, there were an estimated 14,000 deaths in 2000. Ovarian cancer causes more deaths than any other cancer of the female reproductive system.

Surgery, radiation therapy, and chemotherapy are treatment options for ovarian cancer. Surgery usually includes the removal of one or both ovaries, the fallopian tubes (salpingo-oophorectomy), and the uterus (hysterectomy). In some very early tumors, only the involved ovary will be removed, especially in young women who wish to have children. In advanced disease, an attempt is made to remove all intra-abdominal disease to enhance the effect of chemotherapy. There continues to be an important need for effective treatment options for ovarian cancer.

There were an estimated 28,300 new cases of pancreatic cancer in the United States in 2000. Over the past 20 years, rates of pancreatic cancer have declined in men. Rates among women have remained approximately constant but may be beginning to decline. Pancreatic cancer caused an estimated 28,200 deaths in 2000 in the United States. Over the past 20 years, there has been a slight but significant decrease in mortality rates among men (about –0.9% per year) while rates have increased slightly among women.

Surgery, radiation therapy, and chemotherapy are treatment options for pancreatic cancer. These treatment options can extend survival and/or relieve symptoms in many patients but are not likely to produce a cure for most. There is a significant need for additional therapeutic and diagnostic options for pancreatic cancer.

SUMMARY OF THE INVENTION

The present invention relates to a novel nucleic acid sequence and its encoded polypeptide, designated 158P1D7. As used herein, “158P1D7” may refer to the novel polynucleotides or polypeptides or both of the disclosed invention.

Nucleic acids encoding 158P1D7 are over-expressed in the cancer(s) listed in Table I. Northern blot expression analysis of 158P1D7 expression in normal tissues shows a restricted expression pattern in adult tissues. The nucleotide (Figure 2) and amino acid (Figure 2, and Figure 3) sequences of 158P1D7 are provided. The tissue-related profile of 158P1D7 in normal adult tissues, combined with the over-

expression observed in bladder tumors, shows that 158P1D7 is aberrantly over-expressed in at least some cancers. Thus, 158P1D7 nucleic acids and polypeptides serve as a useful diagnostic agent (or indicator) and/or therapeutic target for cancers of the tissues, such as those listed in Table I.

The invention provides polynucleotides corresponding or complementary to all or part of the 158P1D7 nucleic acids, mRNAs, and/or coding sequences, preferably in isolated form, including polynucleotides encoding 158P1D7-related proteins and fragments of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or more than 25 contiguous amino acids; at least about 30, 35, 40, 45, 50, 55, 60, 65, 70, 80, 85, 90, 95, 100 or more than 100 contiguous amino acids of a 158P1D7-related protein, as well as the peptides/proteins themselves; DNA, RNA, DNA/RNA hybrids, and related molecules (such as PNAs), polynucleotides or oligonucleotides complementary or having at least a 90% homology to 158P1D7 nucleic acid sequences or mRNA sequences or parts thereof, and polynucleotides or oligonucleotides that hybridize to the 158P1D7 genes, mRNAs, or to 158P1D7-encoding polynucleotides. Also provided are means for isolating cDNAs and the gene(s) encoding 158P1D7. Recombinant DNA molecules containing 158P1D7 polynucleotides, cells transformed or transduced with such molecules, and host-vector systems for the expression of 158P1D7 gene products are also provided. The invention further provides antibodies that bind to 158P1D7 proteins and polypeptide fragments thereof, including polyclonal and monoclonal antibodies, murine and other mammalian antibodies, chimeric antibodies, humanized and fully human antibodies, and antibodies labeled with a detectable marker. The invention also comprises T cell clones that recognize an epitope of 158P1D7 in the context of a particular HLA molecule.

The invention further provides methods for detecting the presence, amount, and status of 158P1D7 polynucleotides and proteins in various biological samples, as well as methods for identifying cells that express 158P1D7 polynucleotides and polypeptides. A typical embodiment of this invention provides methods for monitoring 158P1D7 polynucleotides and polypeptides in a tissue or hematology sample having or suspected of having some form of growth dysregulation such as cancer.

The invention further provides various immunogenic or therapeutic compositions and strategies for treating cancers that express 158P1D7 such as bladder cancers, including therapies aimed at inhibiting the transcription, translation, processing or function of 158P1D7 as well as cancer vaccines.

This invention further provides use of a vector that comprises a composition of this invention wherein the vector delivers the single chain monoclonal antibody coding sequence to the cancer cells and the encoded single chain antibody is expressed intracellularly therein for treating a patient with a cancer that expresses 158P1D7.

This invention further provides use of a pharmaceutical composition of this invention comprising a ribozyme capable of cleaving a polynucleotide, said polynucleotide having a 158P1D7 coding sequence, for treating a patient with a cancer that expresses 158P1D7.

In a further embodiment there is provided method for detecting expression levels of a 158P1D7 (SEQ ID NO. 1) gene product in a biological sample and a normal sample obtained from a patient who has or who is suspected of having cancer, the method comprising: providing the biological sample and the normal sample obtained from the patient; determining expression levels of the 158P1D7 gene product in the biological sample and the normal sample obtained from the patient; and comparing the expression levels of the 158P1D7 gene product detected in the biological sample and the normal sample obtained from the patient, wherein the 158P1D7 gene product is selected from the group consisting of a 158P1D7 mRNA, a 158P1D7 protein or a protein that is at least 90% identical to 158P1D7.

In a further embodiment the presence of elevated 158P1D7 mRNA or protein expression in the biological sample relative to the normal sample provides an indication of a cancer in the biological sample.

In a further embodiment wherein the cancer occurs in a tissue selected from the group consisting of bladder, prostate, colon, lung, breast, and ovary tissue.

In a further embodiment there is provided a substance that inhibits expression of a 158P1D7 (SEQ ID NO. 1) gene product, wherein the substance is selected from the group consisting of an antibody or epitope binding fragment thereof specific for the 158P1D7 gene product, an antisense polynucleotide that binds specifically to the 158P1D7 gene product, and a ribozyme with enzymatic activity against the 158P1D7 gene product.

In a further embodiment the composition comprises a pharmaceutically acceptable carrier.

In a further embodiment the composition is in a human unit dose form.

In a further embodiment the composition of comprises a protein that is at least 90% identical to 158P1D7 (SEQ ID NO. 1).

In a further embodiment the antibody or fragment thereof that specifically binds to the 158P1D7 gene product binds to a protein that is at least 90% identical to 158P1D7.

In a further embodiment the antibody is a single chain monoclonal antibody that immunospecifically binds to 158P1D7 (SEQ ID NO. 1) or a protein that is at least 90% identical to 158P1D7.

In a further embodiment the composition further comprises a polynucleotide encoding the antibody of claim 9.

In a further embodiment the antisense polynucleotide that binds specifically to the 158P1D7 gene product is complementary to a polynucleotide having a 158P1D7 (SEQ ID NO. 1) coding sequence.

In a further embodiment the ribozyme is capable of enzymatically cleaving a polynucleotide having a 158P1D7 (SEQ ID NO. 1) coding sequence.

In a further embodiment there is provided a method of inhibiting growth of cancer cells that express 158P1D7, the method comprising: administering to the cells the composition of any one of claims 4-12.

In a further embodiment there is provided a method of treating a patient with a cancer that expresses 158P1D7 (SEQ ID NO. 1), the method comprising steps of: administering to said patient a vector that comprises the composition of claim 10, such that the vector delivers the single chain monoclonal antibody coding sequence to the cancer cells and the encoded single chain antibody is expressed intracellularly therein.

In a further embodiment there is provided a method of inhibiting growth of cancer cells that express 158P1D7 (SEQ ID NO. 1), the method comprising steps of: administering to said cells a polynucleotide comprising a 158P1D7 coding sequence that is at least 90% identical to 158P1D7.

In a further embodiment there is provided a method of inhibiting growth of cancer cells that express 158P1D7 (SEQ ID NO. 1), the method comprising steps of: administering to said cells an antisense polynucleotide complementary to a polynucleotide having a 158P1D7 coding sequence.

In a further embodiment there is provided a method of treating a patient with a cancer that expresses 158P1D7 (SEQ ID NO. 1), the method comprising steps of: identifying that the patient has a cancer the cells of which express 158P1D7; administering to the patient a pharmaceutical composition of claim 12 that comprises a ribozyme capable of cleaving a polynucleotide having a 158P1D7 coding sequence.

In a further embodiment there is provided a method of generating a mammalian immune response directed to 158P1D7 (SEQ ID NO. 1), the method comprising: exposing cells of the mammal's immune system to an immunogenic portion of a 158P1D7 coding sequence that is at least 90% identical to 158P1D7 or a nucleotide sequence that encodes said coding sequence, whereby an immune response is generated to 158P1D7.

In a further embodiment there is provided a method of delivering a cytotoxic agent to a cell that expresses 158P1D7 (SEQ ID NO. 1), said method comprising: providing a cytotoxic agent conjugated to an antibody or fragment thereof that specifically binds to 158P1D7; and, exposing the cell to the antibody-agent conjugate.

In a further embodiment there is provided a method of inducing an immune response to a 158P1D7 protein (SEQ ID NO. 1), said method comprising: providing a protein that is at least 90% identical to 158P1D7, that comprises at least one T cell or at least one B cell epitope; contacting the epitope with an immune system T cell or B cell respectively, whereby the immune system T cell or B cell is induced.

In a further embodiment the immune system cell is a B cell, whereby the induced B cell generates antibodies that specifically bind to the protein that is at least 90% identical to 158P1D7.

In a further embodiment the immune system cell is a T cell that is a cytotoxic T cell (CTL), whereby the activated CTL kills an autologous cell that expresses the 158P1D7 protein.

In a further embodiment the immune system cell is a T cell that is a helper T cell (HTL), whereby the activated HTL secretes cytokines that facilitate the cytotoxic activity of a CTL or the antibody producing activity of a B cell.

In a further embodiment there is provided an antibody or fragment thereof that specifically binds to a 158P1D7 (SEQ ID NO. 1) or a protein that is at least 90% identical to 158P1D7.

In a further embodiment the antibody or fragment thereof is monoclonal.

In a further embodiment the antibody or fragment thereof is labeled with a detectable marker.

In a further embodiment the antibody or fragment thereof is a Fab, F (ab')₂, Fv or sFv fragment.

In a further embodiment the antibody is a human antibody.

In a further embodiment there is provided a recombinant protein comprising the antigen-binding region of a monoclonal antibody.

In a further embodiment the recombinant protein is labeled with a detectable marker.

In a further embodiment the recombinant protein comprises murine antigen binding region residues and human constant region residues.

In a further embodiment there is provided a non-human transgenic animal that produces an antibody disclosed.

In a further embodiment there is disclosed a method of producing an antibody using the transgenic animal..

In a further embodiment there is disclosed a hybridoma that produces an antibody.

In a further embodiment there is disclosed a single chain monoclonal antibody that comprises the variable domains of the heavy and light chains of a monoclonal antibody.

In a further embodiment there is disclosed a vector comprising a polynucleotide that encodes a single chain monoclonal antibody that immunospecifically binds to 158P1D7 (SEQ ID NO. 1) or a protein that is at least 90% identical to 158P1D7.

In a further embodiment there is disclosed a an assay for detecting the presence of a 158P1D7 gene product in a biological sample from a patient who has or who is suspected of having cancer, comprising steps of: contacting the biological sample with an antibody or a polynucleotide that specifically binds to the 158P1D7 gene product (SEQ ID NO. 1); and detecting the presence of a complex of the antibody or the polynucleotide and 158P1D7 gene product.

In a further embodiment the assay further comprises obtaining the sample from a patient who has or who is suspected of having cancer.

In a further embodiment the assay comprises contacting the sample with a polynucleotide probe that specifically hybridizes to a polynucleotide encoding a 158P1D7 gene product, wherein the gene product has the amino acid sequence SEQ ID NO. 657 of Figure 2; and detecting the presence of a hybridization complex formed by the hybridization of the probe with 158P1D7 polynucleotide in the sample, wherein the presence of the hybridization complex indicates the presence of 158P1D7 polynucleotide within the sample.

In a further embodiment there is disclosed an assay for detecting the presence of 158P1D7 (SEQ ID NO. 1) mRNA in a biological sample from a patient who has or who is suspected of having cancer, said method comprising: producing cDNA from the sample by reverse transcription using at least one primer; amplifying the cDNA so produced using 158P1D7 polynucleotides as sense and antisense primers, wherein the 158P1D7 polynucleotides used as the sense and antisense primers are capable of amplifying the 158P1D7 cDNA contained within plasmid p158P1D7- Turbo/3PX (SEQ ID NO. 1); and detecting the presence of the amplified 158P1D7 cDNA.

In a further embodiment there is disclosed a composition comprising a polynucleotide from position number 23 through number 2548 SEQ ID NO. 656.

In a further embodiment there is disclosed a composition comprising SEQ ID NO. 656 of Figure 2.

In a further embodiment there is disclosed the composition wherein T is substituted with U.

In a further embodiment there is disclosed a composition comprising a polynucleotide that encodes a protein that is at least 90% homologous to the entire amino acid sequence shown in SEQ ID NO. 657.

In a further embodiment there is disclosed an analog peptide of eight, nine, ten or eleven contiguous amino acids of SEQ ID NO. 657.

In a further embodiment there is disclosed a polynucleotide that encodes an analog peptide.

In a further embodiment there is disclosed composition comprising a polynucleotide that encodes at least one peptide set forth in Tables V-XVIII.

In a further embodiment there is disclosed a composition comprising a polynucleotide that encodes a peptide region of at least 5 amino acids of SEQ ID NO. 657 in any whole

number increment up to 841 that includes an amino acid position selected from: an amino acid position having a value greater than 0.5 in the Hydrophilicity profile of Figure 11, an amino acid position having a value less than 0.5 in the Hydropathicity profile of Figure 12; an amino acid position having a value greater than 0.5 in the Percent Accessible Residues profile of Figure 13; an amino acid position having a value greater than 0.5 in the Average Flexibility profile on Figure 14; or an amino acid position having a value greater than 0.5 in the Beta-turn profile of Figure 15.

In a further embodiment there is disclosed a composition comprising a polynucleotide that is fully complementary to a polynucleotide of any one of other embodiments.

In a further embodiment there is disclosed a composition comprising a polynucleotide that encodes a protein that is at least 90% identical to 158P1D7 whose sequence is encoded by the cDNAs contained in the plasmid designated p158P1D7- Turbo/3PX (SEQ ID NO. 1) deposited with American Type Culture Collection as Accession No. (PTA-3662).

In a further embodiment there is disclosed a composition comprising a polypeptide at least 90% homologous to SEQ ID NO. 657.

In a further embodiment the polypeptide is at least 90% identical to SEQ ID NO. 657.

In a further embodiment the polypeptide comprises SEQ ID NO. 657.

In a further embodiment the composition comprises a CTL polypeptide epitope from SEQ ID NO. 657.

In a further embodiment the CTL epitope comprises a polypeptide selected from Tables V-XVIII.

In a further embodiment there is disclosed a composition comprising a peptide region of at least 5 amino acids of SEQ ID NO. 657 of Figure 2 in any whole number increment up to 841 that includes an amino acid position selected from: an amino acid position having a value greater than 0.5 in the Hydrophilicity profile of Figure 11, an amino acid position having a value less than 0.5 in the Hydropathicity profile of Figure 12; an amino acid position having a value greater than 0.5 in the Percent Accessible Residues profile of Figure 13; an amino acid position having a value greater than 0.5 in the Average Flexibility profile on Figure 14; or an amino acid position having a value greater than 0.5 in the Beta-turn profile of Figure 15.

In a further embodiment there is disclosed a composition comprising a 158P1D7 protein or a protein that is at least 90% identical to 158P1D7 whose sequence is encoded by the cDNAs contained in the plasmid designated p 158P1D7- Turbo/3 PX deposited with American Type Culture Collection as Accession No. (PTA-3662).

In a further embodiment there is disclosed the use of a vector that comprises a composition wherein the vector delivers the single chain monoclonal antibody coding sequence to the cancer cells and the encoded single chain antibody is expressed intracellularly therein for treating a patient with a cancer that expresses 158P1D7 (SEQ ID NO. 1).

In a further embodiment there is disclosed the use of a pharmaceutical composition comprising a ribozyme capable of cleaving a polynucleotide, said polynucleotide having a 158P1D7 coding sequence, for treating a patient with a cancer that expresses 158P1D7.

In a further embodiment there is disclosed the use of the composition as an anti-cancer agent.

In a further embodiment there is disclosed the use of the composition in the preparation of a medicament.

In a further embodiment there is disclosed the use of the composition in the treatment of a patient with a cancer that expresses 158P1D7 (SEQ ID NO. 1).

In a further embodiment there is disclosed the use of the composition in the preparation of a medicament for cancers that express 158P1D7 (SEQ ID NO. 1).

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. 158P1D7 SSH nucleic acid sequence. The 158P1D7 SSH sequence contains 231 bp. (SEQ ID NO. 655)

Figure 2. The cDNA (SEQ ID NO. 656) and amino acid (SEQ ID NO. 657) sequences of 158P1D7. The start methionine is underlined. The open reading frame extends from nucleic acid 23 to 2548 including the stop codon.

Figure 3. Amino acid sequence of 158P1D7 (SEQ ID NO. 657).

Figure 4. Sequence alignment of 158P1D7 (SEQ ID NO. 657) with human hypothetical protein FLJ22774, clone KAIA1575 (SEQ ID NO. 658).

Figure 5a. Amino acid sequence alignment of 158P1D7 with human protein (FLJ227744, SEQ ID. NO. :659).

Figure 5b. Amino acid sequence alignment of 158P1D7 with human protein similar to IGFALS (SEQ ID. NO. :660).

Figure 6. Expression of 158P1D7 by RT-PCR. First strand cDNA was prepared from vital pool 1 (VP1: liver, lung and kidney), vital pool 2 (VP2, pancreas, colon and stomach), prostate xenograft pool (LAPC-4AD, LAPC-4AI, LAPC-9AD, LAPC-9AI), prostate cancer pool, bladder cancer pool, colon cancer pool, lung cancer pool, ovary cancer pool, breast cancer pool, and metastasis pool. Normalization was performed by PCR using primers to actin and GAPDH. Semi-quantitative PCR, using primers to 158P1D7, was performed at 30 cycles of amplification. Strong expression of 158P1D7 is observed in bladder cancer pool and breast cancer pool. Lower levels of expression are observed in VP1, VP2, xenograft pool, prostate cancer pool, colon cancer pool, lung cancer pool, ovary cancer pool, and metastasis pool.

Figure 7. Expression of 158P1D7 in normal human tissues. Two multiple tissue northern blots, with 2 µg of mRNA/lane, were probed with the 158P1D7 fragment. Size standards in kilobases (kb) are indicated on the side. The results show expression of 158P1D7 in prostate, liver, placenta, heart and, to lower levels, in small intestine and colon.

Figure 8A and 8B. Expression of 158P1D7 in bladder cancer patient specimens. RNA was extracted from the bladder cancer cell lines (CL), normal bladder (N), bladder tumors (T) and matched normal adjacent tissue (N_{AT}) isolated from bladder cancer patients. Northern blots with 10 µg of total RNA/lane were probed with the 158P1D7 fragment. Size standards in kilobases (kb) are indicated on the side. The results show expression of 158P1D7 in 1 of 3 bladder cancer cell lines. In patient specimens, 158P1D7 expression is detected in 4 of 6 tumors tested (8A). In another study, 158P1D7 expression is detected in all patient tumors tested (8B). The expression observed in normal adjacent tissues (isolated from diseased tissues) but not in normal tissue, isolated from healthy donors, may indicate that these tissues are not fully normal and that 158P1D7 may be expressed in early stage tumors.

Figure 9. Expression of 158P1D7 in lung cancer patient specimens. RNA was extracted from lung cancer cell lines (CL), lung tumors (T), and their normal adjacent tissues (N_{AT}) isolated from lung cancer patients. Northern blot with 10 µg of total RNA/lane was probed with the 158P1D7 fragment. Size standards in kilobases (kb) are indicated on the side. The results show expression of 158P1D7 in 1 of 3 lung cancer cell lines and in all 3 lung tumors tested, but not in normal lung tissues.

Figure 10. Expression of 158P1D7 in breast cancer patient specimens. RNA was extracted from breast cancer cell lines (CL), normal breast (N), and breast tumors (T) isolated from breast cancer patients. Northern blot with 10 µg of total RNA/lane was probed with the 158P1D7 fragment. Size standards in kilobases (kb) are indicated on the side. The results show expression of 158P1D7 in 2 of 3 breast cancer cell lines and in 2 breast tumors, but not in normal breast tissue.

Figure 11. Hydrophilicity amino acid profile of 158P1D7 determined by computer algorithm sequence analysis using the method of Hopp and Woods (Hopp, T.P., Woods, K.R. 1981, "Prediction of protein antigenic determinants from amino acid sequences." *Proc. Natl. Acad. Sci. USA*, 78: 3824-38280).

Figure 12. Hydropathicity amino acid profile of 158P1D7 determined by computer algorithm sequence analysis using the method of Kyte and Doolittle (Kyte, J., Doolittle, R.F., 1982, "A simple method for displaying the hydropathic character of a protein", *J. Mol. Biol.* 157: 105-132).

Figure 13. Percent accessible residues amino acid profile of 158P1D7 determined by computer algorithm sequence analysis using the method of Janin (Janin J., 1979, "Surface and inside volumes in globular proteins", *Nature* 277, 491-492).

Figure 14. Average flexibility amino acid profile of 158P1D7 determined by computer algorithm sequence analysis using the method of Bhaskaran and Ponnuswamy (Bhaskaran.R. and Ponnuswamy.PK, 1988, "Positional flexibilities of amino acid residues in globular proteins", *Int. J. Pept. Protein Res.*, 32, 242-255).

Figure 15. Beta-turn amino acid profile of 158P1D7 determined by computer algorithm sequence analysis using the method of Deleage and Roux (Deleage, G. and Roux, B., 1987, "An algorithm for protein secondary structure prediction based on class prediction", *Protein Engineering*, vol. 1 no. 4, pp. 289-294).

Figure 16A and 16B. Transmembrane region and orientation prediction for 158P1D7.

DETAILED DESCRIPTION OF THE INVENTION

Outline of Sections

- I.) Definitions
- II.) 158P1D7 Polynucleotides
 - II.A.) Uses of 158P1D7 Polynucleotides
 - II.A.1.) Monitoring of Genetic Abnormalities
 - II.A.2.) Antisense Embodiments
 - II.A.3.) Primers and Primer Pairs
 - II.A.4.) Isolation of 158P1D7-Encoding Nucleic Acid Molecules
 - II.A.5.) Recombinant Nucleic Acid Molecules and Host-Vector Systems
- III.) 158P1D7-related Proteins
 - III.A.) Motif-bearing Protein Embodiments
 - III.B.) Expression of 158P1D7-related Proteins
 - III.C.) Modifications of 158P1D7-related Proteins
 - III.D.) Uses of 158P1D7-related Proteins
- IV.) 158P1D7 Antibodies

- V.) 158P1D7 Cellular Immune Responses
- VI.) 158P1D7 Transgenic Animals
- VII.) Methods for the Detection of 158P1D7
- VIII.) Methods for Monitoring the Status of 158P1D7-related Genes and Their Products
- IX.) Identification of Molecules That Interact With 158P1D7
- X.) Therapeutic Methods and Compositions
 - X.A.) Anti-Cancer Vaccines
 - X.B.) 158P1D7 as a Target for Antibody-Based Therapy
 - X.C.) 158P1D7 as a Target for Cellular Immune Responses
 - X.C.1. Minigene Vaccines
 - X.C.2. Combinations of CTL Peptides with Helper Peptides
 - X.C.3. Combinations of CTL Peptides with T Cell Priming Agents
 - X.C.4. Vaccine Compositions Comprising DC Pulsed with CTL and/or HTL Peptides
 - X.D.) Adoptive Immunotherapy
 - X.E.) Administration of Vaccines for Therapeutic or Prophylactic Purposes
- XI.) Diagnostic and Prognostic Embodiments of 158P1D7.
- XII.) Inhibition of 158P1D7 Protein Function
 - XII.A.) Inhibition of 158P1D7 With Intracellular Antibodies
 - XII.B.) Inhibition of 158P1D7 with Recombinant Proteins
 - XII.C.) Inhibition of 158P1D7 Transcription or Translation
 - XII.D.) General Considerations for Therapeutic Strategies
- XIII.) KITS

I.) Definitions:

Unless otherwise defined, all terms of art, notations and other scientific terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this invention pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. Many of the techniques and procedures described or referenced herein are well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized molecular cloning methodologies described in Sambrook et al., Molecular Cloning: A Laboratory Manual 2nd. edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. As appropriate, procedures involving the use of commercially available kits and reagents are generally carried out in accordance with manufacturer defined protocols and/or parameters unless otherwise noted.

The terms "invasive bladder cancer" means bladder cancers that have extended into the bladder muscle wall, and are meant to include stage stage T2 - T4 and disease under the TNM (tumor; node, metastasis) system. In general, these patients have substantially less favorable outcomes compared to patients having non-invasive cancer. Following cystectomy, 50% or more of the patients with invasive cancer will develop metastasis (Whittmore. Semin Urol 1983; 1:4-10).

"Altering the native glycosylation pattern" is intended for purposes herein to mean deleting one or more carbohydrate moieties found in native sequence 158P1D7 (either by removing the underlying glycosylation site or by deleting the glycosylation by chemical and/or enzymatic means), and/or adding one or more glycosylation sites that are not present in the native sequence 158P1D7. In addition, the phrase includes qualitative changes in the glycosylation of the native proteins, involving a change in the nature and proportions of the various carbohydrate moieties present.

The term "analog" refers to a molecule which is structurally similar or shares similar or corresponding attributes with another molecule (e.g. a 158P1D7-related protein). For example an analog of the 158P1D7 protein can be specifically bound by an antibody or T cell that specifically binds to 158P1D7 protein.

The term "antibody" is used in the broadest sense. Therefore an "antibody" can be naturally occurring or man-made such as monoclonal antibodies produced by conventional hybridoma technology. Anti-158P1D7 antibodies bind 158P1D7 proteins, or a fragment thereof, and comprise monoclonal and polyclonal antibodies as well as fragments containing the antigen-binding domain and/or one or more complementarity determining regions of these antibodies.

An "antibody fragment" is defined as at least a portion of the variable region of the immunoglobulin molecule that binds to its target, i.e., the antigen-binding region. In one embodiment it specifically covers single anti-158P1D7 antibodies and clones thereof (including agonist, antagonist and neutralizing antibodies) and anti-158P1D7 antibody compositions with polyepitopic specificity.

The term "codon optimized sequences" refers to nucleotide sequences that have been optimized for a particular host species by replacing any one or more than one codon having a usage frequency of less than about 20%, more preferably less than about 30% or 40%. A sequence may be "completely optimized" to contain no codon having a usage frequency of less than about 20%, more preferably less than about 30% or 40%. Nucleotide sequences that have been optimized for expression in a given host species by elimination of spurious polyadenylation sequences, elimination of exon/intron splicing signals, elimination of transposon-like repeats and/or optimization of GC content in addition to codon optimization are referred to herein as an "expression enhanced sequences."

The term "cytotoxic agent" refers to a substance that inhibits or prevents one or more than one function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes chemotherapeutic agents, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof. Examples of cytotoxic agents include, but are not limited to maytansinoids, yttrium, bismuth, ricin, ricin A-chain, doxorubicin, daunorubicin, taxol, ethidium bromide, mitomycin, etoposide, tenoposide, vincristine,

vinblastine, colchicine, dihydroxy anthracin dione, actinomycin, diphtheria toxin, Pseudomonas exotoxin (PE) A, PE40, abrin, abrin A chain, modeccin A chain, alpha-sarcin, gelonin, mitogellin, retstrictocin, phenomycin, enomycin, curicin, crotin, calicheamicin, sapaonaria officinalis inhibitor, and glucocorticoid and other chemotherapeutic agents, as well as radioisotopes such as At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³² and radioactive isotopes of Lu. Antibodies may also be conjugated to an anti-cancer pro-drug activating enzyme capable of converting the pro-drug to its active form.

The term "homolog" refers to a molecule which exhibits homology to another molecule, by for example, having sequences of chemical residues that are the same or similar at corresponding positions.

"Human Leukocyte Antigen" or "HLA" is a human class I or class II Major Histocompatibility Complex (MHC) protein (*see, e.g., Stites, et al., IMMUNOLOGY, 8TH ED., Lange Publishing, Los Altos, CA (1994).*

The terms "hybridize", "hybridizing", "hybridizes" and the like, used in the context of polynucleotides, are meant to refer to conventional hybridization conditions, preferably such as hybridization in 50% formamide/6XSSC/0.1% SDS/100 µg/ml ssDNA, in which temperatures for hybridization are above 37 degrees C and temperatures for washing in 0.1XSSC/0.1% SDS are above 55 degrees C.

The phrases "isolated" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany the material as it is found in its native state. Thus, isolated peptides in accordance with the invention preferably do not contain materials normally associated, or present, with the peptides in their *in situ* environment. For example, a polynucleotide is said to be "isolated" when it is substantially separated from contaminant polynucleotides that correspond or are complementary to nucleic acids other than those of 158P1D7 or that encode polypeptides other than 158P1D7 gene product or fragments thereof. A skilled artisan can readily employ nucleic acid isolation procedures to obtain an isolated 158P1D7 polynucleotide. A protein is said to be "isolated," for example, when physical, mechanical and/or chemical methods are employed to remove the 158P1D7 protein from cellular constituents that are normally associated, or present, with the protein. A skilled artisan can readily employ standard purification methods to obtain an isolated 158P1D7 protein. Alternatively, an isolated protein can be prepared by synthetic or chemical means.

The term "mammal" refers to any organism classified as a mammal, including mice, rats, rabbits, dogs, cats, cows, horses and humans. In one embodiment of the invention, the mammal is a mouse. In another embodiment of the invention, the mammal is a human.

The terms "metastatic bladder cancer" and "metastatic disease" mean bladder cancers that have spread to regional lymph nodes or to distant sites, and are meant to stage TxNxM+ under the TNM system. The most common site for bladder cancer metastasis is lymph node. Other common sites for metastasis include lung, bone and liver.

The term "monoclonal antibody" refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the antibodies comprising the population are identical except for possible naturally occurring mutations that are present in minor amounts.

A "motif", as in biological motif of an 158P1D7-related protein, refers to any pattern of amino acids forming part of the primary sequence of a protein, that is associated with a particular function (e.g. protein-protein interaction, protein-DNA interaction, etc) or modification (e.g. that is phosphorylated, glycosylated or amidated), or localization (e.g. secretory sequence, nuclear localization sequence, etc.) or a sequence that is correlated with being immunogenic, either humorally or cellularly. A motif can be either contiguous or capable of being aligned to certain positions that are generally correlated with a certain function or property. In the context of HLA motifs, "motif" refers to the pattern of residues in a peptide of defined length, usually a peptide of from about 8 to about 13 amino acids for a class I HLA motif and from about 6 to about 25 amino acids for a class II HLA motif, which is recognized by a particular HLA molecule. Peptide motifs for HLA binding are typically different for each protein encoded by each human HLA allele and differ in the pattern of the primary and secondary anchor residues.

A "pharmaceutical excipient" comprises a material such as an adjuvant, a carrier, pH-adjusting and buffering agents, tonicity adjusting agents, wetting agents, preservative, and the like.

"Pharmaceutically acceptable" refers to a non-toxic, inert, and/or composition that is physiologically compatible with mammals, such as humans.

The term "polynucleotide" means a polymeric form of nucleotides of at least 3, 4, 5, 6, 7, 8, 9, or 10 bases or base pairs in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide, and is meant to include single and double stranded forms of DNA and/or RNA. In the art, this term is often used interchangeably with "oligonucleotide", although "oligonucleotide" may be used to refer to the subset of polynucleotides less than about 50 nucleotides in length. A polynucleotide can comprise a nucleotide sequence disclosed herein wherein thymidine (T) (as shown for example in SEQ ID NO: 656) can also be uracil (U); this definition pertains to the differences between the chemical structures of DNA and RNA, in particular the observation that one of the four major bases in RNA is uracil (U) instead of thymidine (T).

The term "polypeptide" means a polymer of at least about 4, 5, 6, 7, or 8 amino acids. Throughout the specification, standard three letter or single letter designations for amino acids are used. In the art, this term is often used interchangeably with "peptide" or "protein", thus "peptide" may be used to refer to the subset of polypeptides less than about 50 amino acids in length.

An HLA "primary anchor residue" is an amino acid at a specific position along a peptide sequence which is understood to provide a contact point between the immunogenic peptide and the HLA molecule. One to three, usually two, primary anchor residues within a peptide of defined length generally defines a "motif" for an immunogenic peptide. These residues are understood to fit in close contact with peptide binding groove of an HLA molecule, with their side chains buried in specific pockets of the binding groove. In one embodiment, for example, the primary anchor residues for an HLA class I molecule are

located at position 2 (from the amino terminal position) and at the carboxyl terminal position of a 8, 9, 10, 11, or 12 residue peptide epitope in accordance with the invention. In another embodiment, for example, the primary anchor residues of a peptide that will bind an HLA class II molecule are spaced relative to each other, rather than to the termini of a peptide, where the peptide is generally of at least 9 amino acids in length. The primary anchor positions for each motif and supermotif are set forth in Table IV. For example, analog peptides can be created by altering the presence or absence of particular residues in the primary and/or secondary anchor positions shown in Table IV. Such analogs are used to modulate the binding affinity and/or population coverage of a peptide comprising a particular HLA motif or supermotif.

A "recombinant" DNA or RNA molecule is a DNA or RNA molecule that has been subjected to molecular manipulation *in vitro*.

"Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured nucleic acid sequences to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature that can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel et al., Current Protocols in Molecular Biology, Wiley Interscience Publishers, (1995).

"Stringent conditions" or "high stringency conditions", as defined herein, are identified by, but not limited to, those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% FicollTM/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42 °C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42 °C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55 °C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55 °C. "Moderately stringent conditions" are described by, but not limited to, those in Sambrook et al., Molecular Cloning: A Laboratory Manual, New York: Cold Spring Harbor Press, 1989, and include the use of washing solution and hybridization conditions (e.g., temperature, ionic strength and %SDS) less stringent than those described above. An example of moderately stringent conditions is overnight incubation at 37°C in a solution comprising: 20% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 mg/mL denatured sheared salmon sperm DNA, followed by washing the filters in 1 x SSC at about 37-50°C. The skilled artisan will

recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

An HLA "supermotif" is a peptide binding specificity shared by HLA molecules encoded by two or more HLA alleles.

A "transgenic animal" (e.g., a mouse or rat) is an animal having cells that contain a transgene, which transgene was introduced into the animal or an ancestor of the animal at a prenatal, e.g., an embryonic stage. A "transgene" is a DNA that is integrated into the genome of a cell from which a transgenic animal develops.

As used herein, an HLA or cellular immune response "vaccine" is a composition that contains or encodes one or more peptides of the invention. There are numerous embodiments of such vaccines, such as a cocktail of one or more individual peptides; one or more peptides of the invention comprised by a polyepitopic peptide; or nucleic acids that encode such individual peptides or polypeptides, e.g., a minigene that encodes a polyepitopic peptide. The "one or more peptides" can include any whole unit integer from 1-150 or more, e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, or 150 or more peptides of the invention. The peptides or polypeptides can optionally be modified, such as by lipidation, addition of targeting or other sequences. HLA class I peptides of the invention can be admixed with, or linked to, HLA class II peptides, to facilitate activation of both cytotoxic T lymphocytes and helper T lymphocytes. HLA vaccines can also comprise peptide-pulsed antigen presenting cells, e.g., dendritic cells.

The term "variant" refers to a molecule that exhibits a variation from a described type or norm, such as a protein that has one or more different amino acid residues in the corresponding position(s) of a specifically described protein (e.g. the 158P1D7 protein shown in Figure 2 or Figure 3). An analog is an example of a variant protein.

The 158P1D7-related proteins of the invention include those specifically identified herein, as well as allelic variants, conservative substitution variants, analogs and homologs that can be isolated/generated and characterized without undue experimentation following the methods outlined herein or readily available in the art. Fusion proteins that combine parts of different 158P1D7 proteins or fragments thereof, as well as fusion proteins of a 158P1D7 protein and a heterologous polypeptide are also included. Such 158P1D7 proteins are collectively referred to as the 158P1D7-related proteins, the proteins of the invention, or 158P1D7. The term "158P1D7-related protein" refers to a polypeptide fragment or an 158P1D7 protein sequence of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or more than 25 amino acids; or, at least about 30, 35, 40, 45, 50, 55, 60, 65, 70, 80, 85, 90, 95, 100 or more than 100 amino acids.

II.) 158P1D7 Polynucleotides

One aspect of the invention provides polynucleotides corresponding or complementary to all or part of an 158P1D7 gene, mRNA, and/or coding sequence, preferably in isolated form, including

polynucleotides encoding an 158P1D7-related protein and fragments thereof, DNA, RNA, DNA/RNA hybrid, and related molecules, polynucleotides or oligonucleotides complementary to an 158P1D7 gene or mRNA sequence or a part thereof, and polynucleotides or oligonucleotides that hybridize to an 158P1D7 gene, mRNA, or to an 158P1D7 encoding polynucleotide (collectively, "158P1D7 polynucleotides"). In all instances when referred to in this section, T can also be U in Figure 2.

Embodiments of a 158P1D7 polynucleotide include: a 158P1D7 polynucleotide having the sequence shown in Figure 2, the nucleotide sequence of 158P1D7 as shown in Figure 2, wherein T is U; at least 10 contiguous nucleotides of a polynucleotide having the sequence as shown in Figure 2; or, at least 10 contiguous nucleotides of a polynucleotide having the sequence as shown in Figure 2 where T is U. For example, embodiments of 158P1D7 nucleotides comprise, without limitation:

- (a) a polynucleotide comprising or consisting of the sequence as shown in Figure 2, wherein T can also be U;
- (b) a polynucleotide comprising or consisting of the sequence as shown in Figure 2, from nucleotide residue number 23 through nucleotide residue number 2548, wherein T can also be U;
- (c) a polynucleotide that encodes a 158P1D7-related protein whose sequence is encoded by the cDNAs contained in the plasmid designated p158P1D7- Turbo/3PX deposited with American Type Culture Collection as Accession No. PTA-3662 on 22 August 2001 (sent via Federal Express on 20 August 2001);
- (d) a polynucleotide that encodes an 158P1D7-related protein that is at least 90% homologous to the entire amino acid sequence shown in Figure 2;
- (e) a polynucleotide that encodes an 158P1D7-related protein that is at least 90% identical to the entire amino acid sequence shown in Figure 2;
- (f) a polynucleotide that encodes at least one peptide set forth in Tables V-XVIII;
- (g) a polynucleotide that encodes a peptide region of at least 5 amino acids of Figure 3 in any whole number increment up to 841 that includes an amino acid position having a value greater than 0.5 in the Hydrophilicity profile of Figure 11;
- (h) a polynucleotide that encodes a peptide region of at least 5 amino acids of Figure 3 in any whole number increment up to 841 that includes an amino acid position having a value less than 0.5 in the Hydropathicity profile of Figure 12;

- (i) a polynucleotide that encodes a peptide region of at least 5 amino acids of Figure 3 in any whole number increment up to 841 that includes an amino acid position having a value greater than 0.5 in the Percent Accessible Residues profile of Figure 13;
- (j) a polynucleotide that encodes a peptide region of at least 5 amino acids of Figure 3 in any whole number increment up to 841 that includes an amino acid position having a value greater than 0.5 in the Average Flexibility profile on Figure 14;
- (k) a polynucleotide that encodes a peptide region of at least 5 amino acids of Figure 3 in any whole number increment up to 841 that includes an amino acid position having a value greater than 0.5 in the Beta-turn profile of Figure 15;
- (l) a polynucleotide that is fully complementary to a polynucleotide of any one of (a)-(k);
- (m) a polynucleotide that selectively hybridizes under stringent conditions to a polynucleotide of (a)-(l);
- (n) a peptide that is encoded by any of (a)-(k); and,
- (o) a polynucleotide of any of (a)-(m) or peptide of (n) together with a pharmaceutical excipient and/or in a human unit dose form.

As used herein, a range is understood to specifically disclose all whole unit positions thereof.

Typical embodiments of the invention disclosed herein include 158P1D7 polynucleotides that encode specific portions of the 158P1D7 mRNA sequence (and those which are complementary to such sequences) such as those that encode the protein and fragments thereof, for example of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75; 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 725, 750, 775, 800, 825 or 841 contiguous amino acids.

For example, representative embodiments of the invention disclosed herein include: polynucleotides and their encoded peptides themselves encoding about amino acid 1 to about amino acid 10 of the 158P1D7 protein shown in Figure 2 or Figure 3, polynucleotides encoding about amino acid 10 to about amino acid 20 of the 158P1D7 protein shown in Figure 2, or Figure 3, polynucleotides encoding about amino acid 20 to about amino acid 30 of the 158P1D7 protein shown in Figure 2 or Figure 3, polynucleotides encoding about amino acid 30 to about amino acid 40 of the 158P1D7 protein shown in Figure 2 or Figure 3, polynucleotides encoding about amino acid 40 to about amino acid 50 of the 158P1D7 protein shown in Figure 2 or Figure 3, polynucleotides encoding about amino acid 50 to about amino acid 60 of the 158P1D7 protein shown in Figure 2 or Figure 3, polynucleotides encoding about amino acid 60 to about amino acid 70 of the 158P1D7 protein shown in Figure 2 or Figure 3, polynucleotides encoding

about amino acid 70 to about amino acid 80 of the 158P1D7 protein shown in Figure 2 or Figure 3, polynucleotides encoding about amino acid 80 to about amino acid 90 of the 158P1D7 protein shown in Figure 2 or Figure 3, polynucleotides encoding about amino acid 90 to about amino acid 100 of the 158P1D7 protein shown in Figure 2 or Figure 3, in increments of about 10 amino acids, ending at the carboxyl terminal amino acid set forth in Figure 2 or Figure 3. Accordingly polynucleotides encoding portions of the amino acid sequence (of about 10 amino acids), of amino acids 100 through the carboxyl terminal amino acid of the 158P1D7 protein are embodiments of the invention. Wherein it is understood that each particular amino acid position discloses that position plus or minus five amino acid residues.

Polynucleotides encoding relatively long portions of the 158P1D7 protein are also within the scope of the invention. For example, polynucleotides encoding from about amino acid 1 (or 20 or 30 or 40 etc.) to about amino acid 20, (or 30, or 40 or 50 etc.) of the 158P1D7 protein shown in Figure 2 or Figure 3 can be generated by a variety of techniques well known in the art. These polynucleotide fragments can include any portion of the 158P1D7 sequence as shown in Figure 2 or Figure 3.

Additional illustrative embodiments of the invention disclosed herein include 158P1D7 polynucleotide fragments encoding one or more of the biological motifs contained within the 158P1D7 protein sequence, including one or more of the motif-bearing subsequences of the 158P1D7 protein set forth in Tables V-XVIII. In another embodiment, typical polynucleotide fragments of the invention encode one or more of the regions of 158P1D7 that exhibit homology to a known molecule. In another embodiment of the invention, typical polynucleotide fragments can encode one or more of the 158P1D7 N-glycosylation sites, cAMP and cGMP-dependent protein kinase phosphorylation sites, casein kinase II phosphorylation sites or N-myristoylation site and amidation sites.

II.A.) Uses of 158P1D7 Polynucleotides

II.A.1.) Monitoring of Genetic Abnormalities

The polynucleotides of the preceding paragraphs have a number of different specific uses. The human 158P1D7 gene maps to the chromosomal location set forth in Example 3. For example, because the 158P1D7 gene maps to this chromosome, polynucleotides that encode different regions of the 158P1D7 protein are used to characterize cytogenetic abnormalities of this chromosomal locale, such as abnormalities that are identified as being associated with various cancers. In certain genes, a variety of chromosomal abnormalities including rearrangements have been identified as frequent cytogenetic abnormalities in a number of different cancers (see e.g. Krajcinovic et al., *Mutat. Res.* 382(3-4): 81-83 (1998); Johansson et al., *Blood* 86(10): 3905-3914 (1995) and Finger et al., *P.N.A.S.* 85(23): 9158-9162 (1988)). Thus, polynucleotides encoding specific regions of the 158P1D7 protein provide new tools that can be used to delineate, with greater precision than previously possible, cytogenetic abnormalities in the chromosomal region that encodes 158P1D7 that may contribute to the malignant phenotype. In this context, these polynucleotides satisfy a need in the art for expanding the sensitivity of chromosomal

screening in order to identify more subtle and less common chromosomal abnormalities (see e.g. Evans et al., Am. J. Obstet. Gynecol 171(4): 1055-1057 (1994)).

Furthermore, as 158P1D7 was shown to be highly expressed in bladder and other cancers, 158P1D7 polynucleotides are used in methods assessing the status of 158P1D7 gene products in normal versus cancerous tissues. Typically, polynucleotides that encode specific regions of the 158P1D7 protein are used to assess the presence of perturbations (such as deletions, insertions, point mutations, or alterations resulting in a loss of an antigen etc.) in specific regions of the 158P1D7 gene, such as such regions containing one or more motifs. Exemplary assays include both RT-PCR assays as well as single-strand conformation polymorphism (SSCP) analysis (see, e.g., Marrogi et al., J. Cutan. Pathol. 26(8): 369-378 (1999), both of which utilize polynucleotides encoding specific regions of a protein to examine these regions within the protein.

II.A.2.) Antisense Embodiments

Other specifically contemplated nucleic acid related embodiments of the invention disclosed herein are genomic DNA, cDNAs, ribozymes, and antisense molecules, as well as nucleic acid molecules based on an alternative backbone, or including alternative bases, whether derived from natural sources or synthesized, and include molecules capable of inhibiting the RNA or protein expression of 158P1D7. For example, antisense molecules can be RNAs or other molecules, including peptide nucleic acids (PNAs) or non-nucleic acid molecules such as phosphorothioate derivatives, that specifically bind DNA or RNA in a base pair-dependent manner. A skilled artisan can readily obtain these classes of nucleic acid molecules using the 158P1D7 polynucleotides and polynucleotide sequences disclosed herein.

Antisense technology entails the administration of exogenous oligonucleotides that bind to a target polynucleotide located within the cells. The term "antisense" refers to the fact that such oligonucleotides are complementary to their intracellular targets, e.g., 158P1D7. See for example, Jack Cohen, Oligodeoxynucleotides, Antisense Inhibitors of Gene Expression, CRC Press, 1989; and Synthesis 1:1-5 (1988). The 158P1D7 antisense oligonucleotides of the present invention include derivatives such as S-oligonucleotides (phosphorothioate derivatives or S-oligos, see, Jack Cohen, supra), which exhibit enhanced cancer cell growth inhibitory action. S-oligos (nucleoside phosphorothioates) are isoelectronic analogs of an oligonucleotide (O-oligo) in which a nonbridging oxygen atom of the phosphate group is replaced by a sulfur atom. The S-oligos of the present invention can be prepared by treatment of the corresponding O-oligos with 3H-1,2-benzodithiol-3-one-1,1-dioxide, which is a sulfur transfer reagent. See Iyer, R. P. et al, J. Org. Chem. 55:4693-4698 (1990); and Iyer, R. P. et al., J. Am. Chem. Soc. 112:1253-1254 (1990). Additional 158P1D7 antisense oligonucleotides of the present invention include morpholino antisense oligonucleotides known in the art (see, e.g., Partridge et al., 1996, Antisense & Nucleic Acid Drug Development 6: 169-175).

The 158P1D7 antisense oligonucleotides of the present invention typically can be RNA or DNA that is complementary to and stably hybridizes with the first 100 5' codons or last 100 3' codons of the 158P1D7 genomic sequence or the corresponding mRNA. Absolute complementarity is not required,

although high degrees of complementarity are preferred. Use of an oligonucleotide complementary to this region allows for the selective hybridization to 158P1D7 mRNA and not to mRNA specifying other regulatory subunits of protein kinase. In one embodiment, 158P1D7 antisense oligonucleotides of the present invention are 15 to 30-mer fragments of the antisense DNA molecule that have a sequence that hybridizes to 158P1D7 mRNA. Optionally, 158P1D7 antisense oligonucleotide is a 30-mer oligonucleotide that is complementary to a region in the first 10 5' codons or last 10 3' codons of 158P1D7. Alternatively, the antisense molecules are modified to employ ribozymes in the inhibition of 158P1D7 expression, see, e.g., L. A. Couture & D. T. Stinchcomb; *Trends Genet* 12: 510-515 (1996).

II.A.3.) Primers and Primer Pairs

Further specific embodiments of this nucleotides of the invention include primers and primer pairs, which allow the specific amplification of polynucleotides of the invention or of any specific parts thereof, and probes that selectively or specifically hybridize to nucleic acid molecules of the invention or to any part thereof. Primers may also be used as probes and can be labeled with a detectable marker, such as, for example, a radioisotope, fluorescent compound, bioluminescent compound, a chemiluminescent compound, metal chelator or enzyme. Such probes and primers are used to detect the presence of a 158P1D7 polynucleotide in a sample and as a means for detecting a cell expressing a 158P1D7 protein.

Examples of such probes include polypeptides comprising all or part of the human 158P1D7 cDNA sequence shown in Figure 2. Examples of primer pairs capable of specifically amplifying 158P1D7 mRNAs are also described in the Examples. As will be understood by the skilled artisan, a great many different primers and probes can be prepared based on the sequences provided herein and used effectively to amplify and/or detect a 158P1D7 mRNA. Preferred probes of the invention are polynucleotides of more than about 9, about 12, about 15, about 18, about 20, about 23, about 25, about 30, about 35, about 40, about 45, and about 50 consecutive nucleotides found in 158P1D7 nucleic acids disclosed herein.

The 158P1D7 polynucleotides of the invention are useful for a variety of purposes, including but not limited to their use as probes and primers for the amplification and/or detection of the 158P1D7 gene(s), mRNA(s), or fragments thereof; as reagents for the diagnosis and/or prognosis of bladder cancer and other cancers; as coding sequences capable of directing the expression of 158P1D7 polypeptides; as tools for modulating or inhibiting the expression of the 158P1D7 gene(s) and/or translation of the 158P1D7 transcript(s); and as therapeutic agents.

II.A.4.) Isolation of 158P1D7-Encoding Nucleic Acid Molecules

The 158P1D7 cDNA sequences described herein enable the isolation of other polynucleotides encoding 158P1D7 gene product(s), as well as the isolation of polynucleotides encoding 158P1D7 gene product homologs, alternatively spliced isoforms, allelic variants, and mutant forms of the 158P1D7 gene product as well as polynucleotides that encode analogs of 158P1D7-related proteins. Various molecular cloning methods that can be employed to isolate full length cDNAs encoding an 158P1D7 gene are well known (see, for example, Sambrook, J. et al., *Molecular Cloning: A Laboratory Manual*, 2d edition, Cold Spring Harbor Press, New York, 1989; *Current Protocols in Molecular Biology*. Ausubel et al., Eds., Wiley and Sons, 1995). For

example, lambda phage cloning methodologies can be conveniently employed, using commercially available cloning systems (e.g., Lambda ZAP Express, Stratagene). Phage clones containing 158P1D7 gene cDNAs can be identified by probing with a labeled 158P1D7 cDNA or a fragment thereof. For example, in one embodiment, the 158P1D7 cDNA (Figure 2) or a portion thereof can be synthesized and used as a probe to retrieve overlapping and full-length cDNAs corresponding to a 158P1D7 gene. The 158P1D7 gene itself can be isolated by screening genomic DNA libraries, bacterial artificial chromosome libraries (BACs), yeast artificial chromosome libraries (YACs), and the like, with 158P1D7 DNA probes or primers.

The present invention includes the use of any probe as described herein to identify and isolate a 158P1D7 or 158P1D7 related nucleic acid sequence from a naturally occurring source, such as humans or other mammals, as well as the isolated nucleic acid sequence *per se*, which would comprise all or most of the sequences found in the probe used.

II.A.5.) Recombinant Nucleic Acid Molecules and Host-Vector Systems

The invention also provides recombinant DNA or RNA molecules containing an 158P1D7 polynucleotide, a fragment, analog or homologue thereof, including but not limited to phages, plasmids, phagemids, cosmids, YACs, BACs, as well as various viral and non-viral vectors well known in the art, and cells transformed or transfected with such recombinant DNA or RNA molecules. Methods for generating such molecules are well known (see, for example, Sambrook et al, 1989, *supra*). The invention further provides a host-vector system comprising a recombinant DNA molecule containing a 158P1D7 polynucleotide, fragment, analog or homologue thereof within a suitable prokaryotic or eukaryotic host cell. Examples of suitable eukaryotic host cells include a yeast cell, a plant cell, or an animal cell, such as a mammalian cell or an insect cell (e.g., a baculovirus-infectible cell such as an Sf9 or HighFive cell). Examples of suitable mammalian cells include various bladder cancer cell lines such as SCaBER, UM-UC3, HT1376, RT4, T24, TCC-SUP, J82 and SW780, other transfectable or transducible bladder cancer cell lines, as well as a number of mammalian cells routinely used for the expression of recombinant proteins (e.g., COS, CHO, 293, 293T cells). More particularly, a polynucleotide comprising the coding sequence of 158P1D7 or a fragment, analog or homolog thereof can be used to generate 158P1D7 proteins or fragments thereof using any number of host-vector systems routinely used and widely known in the art.

A wide range of host-vector systems suitable for the expression of 158P1D7 proteins or fragments thereof are available, see for example, Sambrook et al., 1989, *supra*; Current Protocols in Molecular Biology, 1995, *supra*). Preferred vectors for mammalian expression include but are not limited to pcDNA 3.1 myc-His-tag (Invitrogen) and the retroviral vector pSRotkneo (Muller et al., 1991, MCB 11:1785). Using these expression vectors, 158P1D7 can be expressed in several bladder cancer and non-bladder cell lines, including for example SCaBER, UM-UC3, HT1376, RT4, T24, TCC-SUP, J82 and SW780. The host-vector systems of the invention are useful for the production of a 158P1D7 protein or fragment thereof. Such host-vector systems can be employed to study the functional properties of 158P1D7 and 158P1D7 mutations or analogs.

Recombinant human 158P1D7 protein or an analog or homolog or fragment thereof can be produced by mammalian cells transfected with a construct encoding a 158P1D7-related nucleotide. For example, 293T cells can be transfected with an expression plasmid encoding 158P1D7 or fragment, analog or homolog thereof, the 158P1D7 or related protein is expressed in the 293T cells, and the recombinant 158P1D7 protein is isolated using standard purification methods (e.g., affinity purification using anti-158P1D7 antibodies). In another embodiment, a 158P1D7 coding sequence is subcloned into the retroviral vector pSR α MSVtkneo and used to infect various mammalian cell lines, such as NIH 3T3, TsuPr1, 293 and rat-1 in order to establish 158P1D7 expressing cell lines. Various other expression systems well known in the art can also be employed. Expression constructs encoding a leader peptide joined in frame to the 158P1D7 coding sequence can be used for the generation of a secreted form of recombinant 158P1D7 protein.

As discussed herein, redundancy in the genetic code permits variation in 158P1D7 gene sequences. In particular, it is known in the art that specific host species often have specific codon preferences, and thus one can adapt the disclosed sequence as preferred for a desired host. For example, preferred analog codon sequences typically have rare codons (i.e., codons having a usage frequency of less than about 20% in known sequences of the desired host) replaced with higher frequency codons. Codon preferences for a specific species are calculated, for example, by utilizing codon usage tables available on the INTERNET.

Additional sequence modifications are known to enhance protein expression in a cellular host. These include elimination of sequences encoding spurious polyadenylation signals, exon/intron splice site signals, transposon-like repeats, and/or other such well-characterized sequences that are deleterious to gene expression. The GC content of the sequence is adjusted to levels average for a given cellular host, as calculated by reference to known genes expressed in the host cell. Where possible, the sequence is modified to avoid predicted hairpin secondary mRNA structures. Other useful modifications include the addition of a translational initiation consensus sequence at the start of the open reading frame, as described in Kozak M., 1989, "Context effects and (inefficient) initiation at non-AUG codons in eucaryotic cell-free translation systems", *Mol. Cell. Biol.*, 9:5073-5080. Skilled artisans understand that the general rule that eukaryotic ribosomes initiate translation exclusively at 5' proximal AUG codon is abrogated only under rare conditions (see e.g. Kozak M., 1995, "Adherence to the first-AUG rule when a second AUG codon follows closely upon the first", *Proc Natl. Acad. Sci. USA*, 92:2662-2666 and Kozak M., 1987, "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs", *Nucleic Acids Res.*, 15:8125-8148).

III.) 158P1D7-related Proteins

Another aspect of the present invention provides 158P1D7-related proteins. Specific embodiments of 158P1D7 proteins comprise a polypeptide having all or part of the amino acid sequence of human 158P1D7 as shown in Figure 2 or Figure 3. Alternatively, embodiments of 158P1D7 proteins comprise variant, homolog or analog polypeptides that have alterations in the amino acid sequence of 158P1D7 shown in Figure 2 or Figure 3.

In general, naturally occurring allelic variants of human 158P1D7 share a high degree of structural identity and homology (e.g., 90% or more homology). Typically, allelic variants of the 158P1D7 protein contain conservative amino acid substitutions within the 158P1D7 sequences described herein or contain a substitution of an amino acid from a corresponding position in a homologue of 158P1D7. One class of 158P1D7 allelic variants are proteins that share a high degree of homology with at least a small region of a particular 158P1D7 amino acid sequence, but further contain a radical departure from the sequence, such as a non-conservative substitution, truncation, insertion or frame shift. In comparisons of protein sequences, the terms, similarity, identity, and homology each have a distinct meaning as appreciated in the field of genetics. Moreover, orthology and paralogy can be important concepts describing the relationship of members of a given protein family in one organism to the members of the same family in other organisms.

Amino acid abbreviations are provided in Table II. Conservative amino acid substitutions can frequently be made in a protein without altering either the conformation or the function of the protein. Proteins of the invention can comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more conservative substitutions. Such changes include substituting any of isoleucine (I), valine (V), and leucine (L) for any other of these hydrophobic amino acids; aspartic acid (D) for glutamic acid (E) and vice versa; glutamine (Q) for asparagine (N) and vice versa; and serine (S) for threonine (T) and vice versa. Other substitutions can also be considered conservative, depending on the environment of the particular amino acid and its role in the three-dimensional structure of the protein. For example, glycine (G) and alanine (A) can frequently be interchangeable, as can alanine (A) and valine (V). Methionine (M), which is relatively hydrophobic, can frequently be interchanged with leucine and isoleucine, and sometimes with valine. Lysine (K) and arginine (R) are frequently interchangeable in locations in which the significant feature of the amino acid residue is its charge and the differing pK's of these two amino acid residues are not significant. Still other changes can be considered "conservative" in particular environments (see, e.g. Table III herein; pages 13-15 "Biochemistry" 2nd ED. Lubert Stryer ed (Stanford University); Henikoff et al., PNAS 1992 Vol 89 10915-10919; Lei et al., J Biol Chem 1995 May 19; 270(20):11882-6).

Embodiments of the invention disclosed herein include a wide variety of art-accepted variants or analogs of 158P1D7 proteins such as polypeptides having amino acid insertions, deletions and substitutions. 158P1D7 variants can be made using methods known in the art such as site-directed mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis (Carter et al., *Nucl. Acids Res.*, 13:4331 (1986); Zoller et al., *Nucl. Acids Res.*, 10:6487 (1987)), cassette mutagenesis (Wells et al., *Gene*, 34:315 (1985)), restriction selection mutagenesis (Wells et al., *Philos. Trans. R. Soc. London SerA*, 317:415 (1986)) or other known techniques can be performed on the cloned DNA to produce the 158P1D7 variant DNA.

Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence that is involved in a specific biological activity such as a protein-protein interaction. Among the preferred scanning amino acids are relatively small, neutral amino acids. Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically a preferred scanning amino acid among

this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the main-chain conformation of the variant. Alanine is also typically preferred because it is the most common amino acid. Further, it is frequently found in both buried and exposed positions (Creighton, *The Proteins*, (W.H. Freeman & Co., N.Y.); Chothia, *J. Mol. Biol.*, 150:1 (1976)). If alanine substitution does not yield adequate amounts of variant, an isosteric amino acid can be used.

As defined herein, 158P1D7 variants, analogs or homologs, have the distinguishing attribute of having at least one epitope that is "cross reactive" with a 158P1D7 protein having the amino acid sequence of SEQ ID NO: 657. As used in this sentence, "cross reactive" means that an antibody or T cell that specifically binds to an 158P1D7 variant also specifically binds to the 158P1D7 protein having the amino acid sequence of SEQ ID NO: 657. A polypeptide ceases to be a variant of the protein shown in SEQ ID NO: 657 when it no longer contains any epitope capable of being recognized by an antibody or T cell that specifically binds to the 158P1D7 protein. Those skilled in the art understand that antibodies that recognize proteins bind to epitopes of varying size, and a grouping of the order of about four or five amino acids, contiguous or not, is regarded as a typical number of amino acids in a minimal epitope. See, e.g., Nair et al., *J. Immunol* 2000 165(12): 6949-6955; Hebbes et al., *Mol Immunol* (1989) 26(9):865-73; Schwartz et al., *J Immunol* (1985) 135(4):2598-608.

Another class of 158P1D7-related protein variants share 70%, 75%, 80%, 85% or 90% or more similarity with the amino acid sequence of SEQ ID NO: 657 or a fragment thereof. Another specific class of 158P1D7 protein variants or analogs comprise one or more of the 158P1D7 biological motifs described herein or presently known in the art. Thus, encompassed by the present invention are analogs of 158P1D7 fragments (nucleic or amino acid) that have altered functional (e.g. immunogenic) properties relative to the starting fragment. It is to be appreciated that motifs now or which become part of the art are to be applied to the nucleic or amino acid sequences of Figure 2 or Figure 3.

As discussed herein, embodiments of the claimed invention include polypeptides containing less than the full amino acid sequence of the 158P1D7 protein shown in Figure 2 or Figure 3. For example, representative embodiments of the invention comprise peptides/proteins having any 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more contiguous amino acids of the 158P1D7 protein shown in Figure 2 or Figure 3.

Moreover, representative embodiments of the invention disclosed herein include polypeptides consisting of about amino acid 1 to about amino acid 10 of the 158P1D7 protein shown in Figure 2 or Figure 3, polypeptides consisting of about amino acid 10 to about amino acid 20 of the 158P1D7 protein shown in Figure 2 or Figure 3, polypeptides consisting of about amino acid 20 to about amino acid 30 of the 158P1D7 protein shown in Figure 2 or Figure 3, polypeptides consisting of about amino acid 30 to about amino acid 40 of the 158P1D7 protein shown in Figure 2 or Figure 3, polypeptides consisting of about amino acid 40 to about amino acid 50 of the 158P1D7 protein shown in Figure 2 or Figure 3, polypeptides consisting of about amino acid 50 to about amino acid 60 of the 158P1D7 protein shown in Figure 2 or Figure 3, polypeptides consisting of about amino acid 60 to about amino acid 70 of the 158P1D7 protein shown in Figure 2 or Figure 3, polypeptides consisting of about amino acid 70 to about

amino acid 80 of the 158P1D7 protein shown in Figure 2 or Figure 3, polypeptides consisting of about amino acid 80 to about amino acid 90 of the 158P1D7 protein shown in Figure 2 or Figure 3, polypeptides consisting of about amino acid 90 to about amino acid 100 of the 158P1D7 protein shown in Figure 2 or Figure 3, etc. throughout the entirety of the 158P1D7 amino acid sequence. Moreover, polypeptides consisting of about amino acid 1 (or 20 or 30 or 40 etc.) to about amino acid 20, (or 130, or 140 or 150 etc.) of the 158P1D7 protein shown in Figure 2 or Figure 3 are embodiments of the invention. It is to be appreciated that the starting and stopping positions in this paragraph refer to the specified position as well as that position plus or minus 5 residues.

158P1D7-related proteins are generated using standard peptide synthesis technology or using chemical cleavage methods well known in the art. Alternatively, recombinant methods can be used to generate nucleic acid molecules that encode a 158P1D7-related protein. In one embodiment, nucleic acid molecules provide a means to generate defined fragments of the 158P1D7 protein (or variants, homologs or analogs thereof).

III.A.) Motif-bearing Protein Embodiments

Additional illustrative embodiments of the invention disclosed herein include 158P1D7 polypeptides comprising the amino acid residues of one or more of the biological motifs contained within the 158P1D7 polypeptide sequence set forth in Figure 2 or Figure 3.

Motif bearing subsequences of the 158P1D7 protein are set forth and identified in Table XIX.

Table XX sets forth several frequently occurring motifs based on pfam searches.

The columns of Table XX list (1) motif name abbreviation, (2) percent identity found amongst the different member of the motif family, (3) motif name or description and (4) most common function; location information is included if the motif is relevant for location.

Polypeptides comprising one or more of the 158P1D7 motifs discussed above are useful in elucidating the specific characteristics of a malignant phenotype in view of the observation that the 158P1D7 motifs discussed above are associated with growth dysregulation and because 158P1D7 is overexpressed in certain cancers (See, e.g., Table I). Casein kinase II, cAMP and camp-dependent protein kinase, and Protein Kinase C, for example, are enzymes known to be associated with the development of the malignant phenotype (see e.g. Chen et al., 1998, "Up-regulation of the cAMP/PKA pathway inhibits proliferation, induces differentiation, and leads to apoptosis in malignant gliomas", *Lab. Invest.*, 78(2):165-174; Gaiddon et al., 1995, "Transcriptional effects in GH3 cells of Gs alpha mutants associated with human pituitary tumors: stimulation of adenosine 3',5'-monophosphate response element-binding protein-mediated transcription and of prolactin and growth hormone promoter activity via protein kinase A", *Endocrinology*, 136(10):4331-4338; Hall et al., 1996, "Phosphorylation of p53 at the casein kinase II site selectively regulates p53-dependent transcriptional repression but not transactivation", *Nucleic Acid Research*, 24(6):1119-1126; Peterziel et al., 1999, "Rapid signalling by androgen receptor in prostate cancer cells", *Oncogene*, 18(46):6322-6329 and O'Brian, 1998, "Protein kinase C-alpha: a novel target for the therapy of androgen-independent prostate cancer? (Review-hypothesis)", *Oncol. Rep.*, 5(2):305-309). Moreover, both glycosylation and myristoylation are protein modifications also associated with cancer and cancer progression (see e.g. Dennis et al., 1999, "Glycoprotein glycosylation and cancer progression", *Biochem. Biophys Acta*, 1473(1):21-34; Raju et al., 1997, "N-Myristoyltransferase overexpression in

human colorectal adenocarcinomas”, *Exp. Cell Res.*, 235(1):145-154). Amidation is another protein modification also associated with cancer and cancer progression (see e.g. Treston et. al., 1992, “Control of tumor cell biology through regulation of peptide hormone processing”, *J. Natl. Cancer Inst. Monogr.* (13):169-175).

In another embodiment, proteins of the invention comprise one or more of the immunoreactive epitopes identified in accordance with art-accepted methods, such as the peptides set forth in Tables V- XVIII. CTL epitopes can be determined using specific algorithms to identify peptides within an 158P1D7 protein that are capable of optimally binding to specified HLA alleles (e. g., Table IV ; EpimatrixTM and EpimerTM, Brown University; and BIMAS). Moreover, processes for identifying peptides that have sufficient binding affinity for HLA molecules and which are correlated with being immunogenic epitopes, are well known in the art, and are carried out without undue experimentation. In addition, processes for identifying peptides that are immunogenic epitopes, are well known in the art, and are carried out without undue experimentation either in vitro or in vivo.

Also known in the art are principles for creating analogs of such epitopes in order to modulate immunogenicity. For example, one begins with an epitope that bears a CTL or HTL motif (see, e. g., the HLA Class I and HLA Class II motifs/super motifs of Table IV). The epitope is analoged by substituting out an amino acid at one of the specified positions, and replacing it with another amino acid specified for that position. For example, one can substitute out a deleterious residue in favor of any other residue, such as a preferred residue as defined in Table IV; substitute a less-preferred residue with a preferred residue as defined in Table IV; or substitute an originally-occurring preferred residue with another preferred residue as defined in Table IV. Substitutions can occur at primary anchor positions or at other positions in a peptide; see, e. g., Table IV.

A variety of references reflect the art regarding the identification and generation of epitopes in a protein of interest as well as analogs thereof. See, for example, International Application WO 9733602 (Chesnut et al.); Sette, “Nine major HLA class I supertypes account for the vast preponderance of HLA-A and -B polymorphism”, 1999, *Immunogenetics*, 50 (3-4):201-212; Sette et al., “Overcoming T Cell Tolerance to the Hepatitis B Virus Surface Antigen in Hepatitis B Virus-Transgenic Mice”, *The Journal of Immunology*, 2001, 166 (2): 1389-1397; Sidney et al., “The HLA-A*0207 peptide binding repertoire is limited to a subset of the A*0201 repertoire”, 1997, *Hum. Immunol.* 58 (1):12-20; Kondo et al., “Two distinct *HLA-A* *0101 -specific submotifs illustrate alternative peptide binding modes”, *Immunogenetics*, 1997 45 (4): 249-258; Sidney et al., “Specificity and degeneracy in peptide binding to HLA-B7-like class I molecules”, *J. Immunol.*, 1996, 157 (8): 3480-90; and Falk et al., “Allele-specific motifs revealed by sequencing of selfpeptides eluted from MHC molecules”, 1991, *Nature* 351: 290-6; Hunt et al., “Characterization of peptides bound to the class I MHC molecule HLA-A2.1 by mass spectrometry”, 1992, *Science* 255: 1261-3; Parker et al., “Sequence motifs important for peptide binding to the human MHC class I molecule, HLA-A2”, 1992, *J. Immunol.* 149: 3580-7; Parker et al., “Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains.”, 1994, *J. Immunol.* 152: 163-75; Kast et al., “Role of HLA-A motifs in identification of potential CTL epitopes in human papillomavirus type 16, E6 and E7 proteins”, 1994 152 (8): 3904-12; Borrás-Cuesta et al., “Specific and general HLA-DR binding motifs: comparison of algorithms”, *Hum. Immunol.* 2000 61 (3): 266-278; Alexander et al., “Linear PADRE T Helper Epitope and Carbohydrate B Cell Epitope Conjugates Induce Specific High Titer IgG Antibody Responses”, *J. Immunol.*, 2000, 164(3):1625-1633; Alexander et al., “Development of high potency universal DR-restricted helper epitopes by modification of high affinity DR-blocking peptides”, *Immunity.*, 1994, 1(9):751-761, PMID: 7895164, UI:95202582; O’Sullivan et al., J., “On the interaction of promiscuous antigenic peptides with different DR alleles: identification of common structural motifs”, *Immunol.* 1991 147 (8): 2663-2669; and Alexander et al., “The optimization of helper T lymphocyte (HTL) function in vaccine development”, *Immunol. Res.* 1998 18 (2): 79-92.

Related embodiments of the inventions include polypeptides comprising combinations of the different motifs set forth in Table XIX, and/or, one or more of the predicted CTL epitopes of Table V through Table XVIII, and/or, one or more of the T cell binding motifs known in the art. Preferred embodiments contain no insertions, deletions or substitutions either within the motifs or the intervening sequences of the polypeptides. In addition, embodiments which include a number of either N-terminal and/or C-terminal amino acid residues on either side of these motifs may be desirable (to, for example, include a greater portion of the polypeptide architecture in which the motif is located). Typically the number of N-terminal and/or C-terminal amino acid residues on either side of a motif is between about 1 to about 100 amino acid residues, preferably 5 to about 50 amino acid residues.

158PID7-related proteins are embodied in many forms, preferably in isolated form. A purified 158PID7 protein molecule will be substantially free of other proteins or molecules that impair the binding of 158PID7 to antibody, T cell or other ligand. The nature and degree of isolation and purification will depend on the intended use. Embodiments of a 158PID7-related proteins include purified 158PID7-related proteins and functional, soluble 158PID7-related proteins. In one embodiment, a functional, soluble 158PID7 protein or fragment thereof retains the ability to be bound by antibody, T cell or other ligand.

The invention also provides 158PID7 proteins comprising biologically active fragments of the 158PID7 amino acid sequence shown in Figure 2 or Figure 3. Such proteins exhibit properties of the 158PID7 protein, such as the ability to elicit the generation of antibodies that specifically bind an epitope associated with the 158PID7 protein; to be bound by such antibodies ; to elicit the activation of HTL or CTL; and/or, to be recognized by HTL or CTL.

158PID7-related polypeptides that contain particularly interesting structures can be predicted and/or identified using various analytical techniques well known in the art, including, for example, the methods of Chou-Fasman, Gamier-Robson, Kyte-Doolittle, Eisenberg, Karplus-Schultz or Jameson-Wolf analysis, or on the basis of immunogenicity. Fragments that contain such structures are particularly useful in generating subunit-specific anti-158PID7 antibodies, or T cells or in identifying cellular factors that bind to 158PID7.

CTL epitopes can be determined using specific algorithms to identify peptides within an 158PID7 protein that are capable of optimally binding to specified HLA alleles (e. g., by using the SYFPEITHI site at World Wide Web URL syfpeithi.bmi-heidelberg.com/ ; the listings in Table IV (A)- (E); EpimatrixTM and EpimerTM, Brown University; and BIMAS). Illustrating this, peptide epitopes from 158PID7 that are presented in the context of human MHC class I molecules HLA-A1, A2, A3, All, A24, B7 and B35 were predicted (Tables V-XVIII). Specifically, the complete amino acid sequence of the 158PID7 protein was entered into the HLA Peptide Motif Search algorithm found in the Bioinformatics and Molecular Analysis Section (BIMAS) web site listed above. The HLA peptide motif search algorithm was developed by Dr. Ken Parker based on binding of specific peptide sequences in the groove of HLA Class I molecules, in particular HLA-A2 (see, e. g., Falk et al., "Allele-specific motifs revealed by sequencing of selfpeptides eluted from MHC molecules", 1991, Nature 351: 290-6; Hunt et al., "Characterization of peptides bound to the class I MHC molecule HLA-A2.1 by mass spectrometry", 1992, Science 255: 1261-3; Parker et al., "Sequence motifs important for peptide binding to the human MHC class I molecule, HLA-A2", 1992, J. Immunol. 149: 3580-7; Parker et al., "Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains.", 1994, J. Immunol. 152: 163-75). This algorithm allows location and ranking of 8-mer, 9-mer, and 10-mer peptides from a complete protein sequence for predicted binding to HLA-A2 as well as numerous other HLA Class I molecules. Many HLA class I binding peptides are 8-, 9-, 10 or 11-mers. For example, for class I HLA-A2, the epitopes preferably contain a leucine (L) or methionine (M) at position 2 and a valine (V) or leucine (L) at the C-terminus (see, e. g., Parker et al., J. Immunol. 149 : 3580-7 (1992)). Selected results of 158PID7 predicted binding peptides are shown in Tables V-XVIII herein. In Tables V-XVIII, the top 50 ranking candidates, 9-mers and 10-

mers, for each family member are shown along with their location, the amino acid sequence of each specific peptide, and an estimated binding score. The binding score corresponds to the estimated half time of dissociation of complexes containing the peptide at 37°C at pH 6.5. Peptides with the highest binding score are predicted to be the most tightly bound to HLA Class I on the cell surface for the greatest period of time and thus represent the best immunogenic targets for T-cell recognition.

Actual binding of peptides to an HLA allele can be evaluated by stabilization of HLA expression on the antigen-processing defective cell line T2 (see, e.g., Xue et al., Prostate 30:73-8 (1997) and Peshwa et al., Prostate 36:129-38 (1998)). Immunogenicity of specific peptides can be evaluated *in vitro* by stimulation of CD8+ cytotoxic T lymphocytes (CTL) in the presence of antigen presenting cells such as dendritic cells.

It is to be appreciated that every epitope predicted by the BIMAS site, Epimer™ and Epimatrix™ sites, or specified by the HLA class I or class II motifs available in the art or which become part of the art such as set forth in Table IV (or determined using World Wide Web site URL syfpeithi.bmi-heidelberg.com/) are to be “applied” to the 158P1D7 protein. As used in this context “applied” means that the 158P1D7 protein is evaluated, e.g., visually or by computer-based patterns finding methods, as appreciated by those of skill in the relevant art. Every subsequence of the 158P1D7 of 8, 9, 10, or 11 amino acid residues that bears an HLA Class I motif, or a subsequence of 9 or more amino acid residues that bear an HLA Class II motif are within the scope of the invention.

III.B.) Expression of 158P1D7-related Proteins

In an embodiment described in the examples that follow, 158P1D7 can be conveniently expressed in cells (such as 293T cells) transfected with a commercially available expression vector such as a CMV-driven expression vector encoding 158P1D7 with a C-terminal 6XHis and MYC tag (pcDNA3.1/mycHis, Invitrogen or Tag5, GenHunter Corporation, Nashville TN). The Tag5 vector provides an IgGK secretion signal that can be used to facilitate the production of a secreted 158P1D7 protein in transfected cells. The secreted HIS-tagged 158P1D7 in the culture media can be purified, e.g., using a nickel column using standard techniques.

III.C.) Modifications of 158P1D7-related Proteins

Modifications of 158P1D7-related proteins such as covalent modifications are included within the scope of this invention. One type of covalent modification includes reacting targeted amino acid residues of a 158P1D7 polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C- terminal residues of the 158P1D7. Another type of covalent modification of the 158P1D7 polypeptide included within the scope of this invention comprises altering the native glycosylation pattern of a protein of the invention. Another type of covalent modification of 158P1D7 comprises linking the 158P1D7 polypeptide to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol (PEG), polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

The 158P1D7-related proteins of the present invention can also be modified to form a chimeric molecule comprising 158P1D7 fused to another, heterologous polypeptide or amino acid sequence. Such a chimeric molecule can be synthesized chemically or recombinantly. A chimeric molecule can have a protein of the invention fused to another tumor-associated antigen or fragment thereof. Alternatively, a protein in accordance with the invention can comprise a fusion of fragments of the 158P1D7 sequence (amino or nucleic acid) such that a molecule is created that is not, through its length, directly homologous to the amino or nucleic acid sequences shown in Figure 2 or Figure 3. Such a chimeric molecule can comprise multiples of the same subsequence of 158P1D7. A chimeric molecule can comprise a fusion of a 158P1D7-related protein with a polyhistidine epitope tag, which provides an epitope to which immobilized nickel can selectively bind, with cytokines or with growth factors. The epitope tag is generally placed at the amino- or carboxyl- terminus of the 158P1D7. In an alternative embodiment, the chimeric molecule can comprise a fusion of a 158P1D7-related protein with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of the chimeric molecule (also referred to as an "immunoadhesin"), such a fusion could be to the Fc region of an IgG molecule. The Ig fusions preferably include the substitution of a soluble (transmembrane domain deleted or inactivated) form of a 158P1D7 polypeptide in place of at least one variable region within an Ig molecule. In a preferred embodiment, the immunoglobulin fusion includes the hinge, CH2 and CH3, or the hinge, CH1, CH2 and CH3 regions of an IgG1 molecule. For the production of immunoglobulin fusions see, e.g., U.S. Patent No. 5,428,130 issued June 27, 1995.

III.D.) Uses of 158P1D7-related Proteins

The proteins of the invention have a number of different uses. As 158P1D7 is highly expressed in bladder and other cancers, 158P1D7-related proteins are used in methods that assess the status of 158P1D7 gene products in normal versus cancerous tissues, thereby elucidating the malignant phenotype. Typically, polypeptides from specific regions of the 158P1D7 protein are used to assess the presence of perturbations (such as deletions, insertions, point mutations etc.) in those regions (such as regions containing one or more motifs). Exemplary assays utilize antibodies or T cells targeting 158P1D7-related proteins comprising the amino acid residues of one or more of the biological motifs contained within the 158P1D7 polypeptide sequence in order to evaluate the characteristics of this region in normal versus cancerous tissues or to elicit an immune response to the epitope. Alternatively, 158P1D7-related proteins that contain the amino acid residues of one or more of the biological motifs in the 158P1D7 protein are used to screen for factors that interact with that region of 158P1D7.

158P1D7 protein fragments/subsequences are particularly useful in generating and characterizing domain-specific antibodies (e.g., antibodies recognizing an extracellular or intracellular epitope of an 158P1D7 protein), for identifying agents or cellular factors that bind to 158P1D7 or a particular structural domain thereof, and in various therapeutic and diagnostic contexts, including but not limited to diagnostic assays, cancer vaccines and methods of preparing such vaccines.

Proteins encoded by the 158P1D7 genes, or by analogs, homologs or fragments thereof, have a variety of uses, including but not limited to generating antibodies and in methods for identifying ligands and other agents and cellular constituents that bind to an 158P1D7 gene product. Antibodies raised against an 158P1D7 protein or fragment thereof are useful in diagnostic and prognostic assays, and imaging methodologies in the management of human cancers characterized by expression of 158P1D7 protein, such as those listed in Table I. Such antibodies can be expressed intracellularly and used in methods of treating patients with such cancers. 158P1D7-related nucleic acids or proteins are also used in generating HTL or CTL responses.

Various immunological assays useful for the detection of 158P1D7 proteins are used, including but not limited to various types of radioimmunoassays, enzyme-linked immunosorbent assays (ELISA), enzyme-linked immunofluorescent assays (ELIFA), immunocytochemical methods, and the like. Antibodies can be labeled and used as immunological imaging reagents capable of detecting 158P1D7-expressing cells (e.g., in radioscintigraphic imaging methods). 158P1D7 proteins are also particularly useful in generating cancer vaccines, as further described herein.

IV.) 158P1D7 Antibodies

Another aspect of the invention provides antibodies that bind to 158P1D7-related proteins. Preferred antibodies specifically bind to a 158P1D7-related protein and do not bind (or bind weakly) to peptides or proteins that are not 158P1D7-related proteins. For example, antibodies bind 158P1D7 can bind 158P1D7-related proteins such as the homologs or analogs thereof.

158P1D7 antibodies of the invention are particularly useful in bladder cancer diagnostic and prognostic assays, and imaging methodologies. Similarly, such antibodies are useful in the treatment, diagnosis, and/or prognosis of other cancers, to the extent 158P1D7 is also expressed or overexpressed in these other cancers. Moreover, intracellularly expressed antibodies (e.g., single chain antibodies) are therapeutically useful in treating cancers in which the expression of 158P1D7 is involved, such as advanced or metastatic bladder cancers.

The invention also provides various immunological assays useful for the detection and quantification of 158P1D7 and mutant 158P1D7-related proteins. Such assays can comprise one or more 158P1D7 antibodies capable of recognizing and binding a 158P1D7-related protein, as appropriate. These assays are performed within various immunological assay formats well known in the art, including but not limited to various types of radioimmunoassays, enzyme-linked immunosorbent assays (ELISA), enzyme-linked immunofluorescent assays (ELIFA), and the like.

Immunological non-antibody assays of the invention also comprise T cell immunogenicity assays (inhibitory or stimulatory) as well as major histocompatibility complex (MHC) binding assays.

In addition, immunological imaging methods capable of detecting bladder cancer and other cancers expressing 158P1D7 are also provided by the invention, including but not limited to radioscintigraphic imaging methods using labeled 158P1D7 antibodies. Such assays are clinically useful in the detection, monitoring, and prognosis of 158P1D7 expressing cancers such as bladder cancer.

158P1D7 antibodies are also used in methods for purifying a 158P1D7-related protein and for isolating 158P1D7 homologues and related molecules. For example, a method of purifying a 158P1D7-related protein comprises incubating an 158P1D7 antibody, which has been coupled to a solid matrix, with a lysate or other solution containing a 158P1D7-related protein under conditions that permit the 158P1D7 antibody to bind to the 158P1D7-related protein; washing the solid matrix to eliminate impurities; and eluting the 158P1D7-related protein from the coupled antibody. Other uses of the 158P1D7 antibodies of the invention include generating anti-idiotypic antibodies that mimic the 158P1D7 protein.

Various methods for the preparation of antibodies are well known in the art. For example, antibodies can be prepared by immunizing a suitable mammalian host using a 158P1D7-related protein, peptide, or fragment, in isolated or immunoconjugated form (Antibodies: A Laboratory Manual, CSH Press, Eds., Harlow, and Lane (1988); Harlow, Antibodies, Cold Spring Harbor Press, NY (1989)). In addition, fusion proteins of 158P1D7 can also be used, such as a 158P1D7 GST-fusion protein. In a particular embodiment, a GST fusion protein comprising all or most of the amino acid sequence of Figure 2 or Figure 3 is produced, then used as an immunogen to generate appropriate antibodies. In another embodiment, a 158P1D7-related protein is synthesized and used as an immunogen.

In addition, naked DNA immunization techniques known in the art are used (with or without purified 158P1D7-related protein or 158P1D7 expressing cells) to generate an immune response to the encoded immunogen (for review, see Donnelly et al., 1997, Ann. Rev. Immunol. 15: 617-648).

The amino acid sequence of 158P1D7 as shown in Figure 2 or Figure 3 can be analyzed to select specific regions of the 158P1D7 protein for generating antibodies. For example, hydrophobicity and hydrophilicity analyses of the 158P1D7 amino acid sequence are used to identify hydrophilic regions in the 158P1D7 structure (see, e. g., the Example entitled "Antigenicity profiles"). Regions of the 158P1D7 protein that show immunogenic structure, as well as other regions and domains, can readily be identified using various other methods known in the art, such as Chou-Fasman, Hopp and Woods, Kyte-Doolittle, Janin, Bhaskaran and Ponnuswamy, Deleage and Roux, Garnier-Robson, Eisenberg, Karplus-Schultz, or Jameson-Wolf analysis. Thus, each region identified by any of these programs or methods is within the scope of the present invention. Methods for the generation of 158P1D7 antibodies are further illustrated by way of the examples provided herein. Methods for preparing a protein or polypeptide for use as an immunogen are well known in the art. Also well known in the art are methods for preparing immunogenic conjugates of a protein with a carrier, such as BSA, KLH or other carrier protein. In some circumstances, direct conjugation using, for example, carbodiimide reagents are used; in other instances linking reagents such as those supplied by Pierce Chemical Co., Rockford, IL, are effective. Administration of a 158P1D7 immunogen is often conducted by injection over a suitable time period and with use of a suitable adjuvant, as is understood in the art. During the immunization schedule, titers of antibodies can be taken to determine adequacy of antibody formation.

158P1D7 monoclonal antibodies can be produced by various means well known in the art. For example, immortalized cell lines that secrete a desired monoclonal antibody are prepared using the standard hybridoma technology of Kohler and Milstein or modifications that immortalize antibody-producing B cells, as

is generally known. Immortalized cell lines that secrete the desired antibodies are screened by immunoassay in which the antigen is a 158P1D7-related protein. When the appropriate immortalized cell culture is identified, the cells can be expanded and antibodies produced either from *in vitro* cultures or from ascites fluid.

The antibodies or fragments of the invention can also be produced, by recombinant means. Regions that bind specifically to the desired regions of the 158P1D7 protein can also be produced in the context of chimeric or complementarity determining region (CDR) grafted antibodies of multiple species origin. Humanized or human 158P1D7 antibodies can also be produced, and are preferred for use in therapeutic contexts. Methods for humanizing murine and other non-human antibodies, by substituting one or more of the non-human antibody CDRs for corresponding human antibody sequences, are well known (see for example, Jones et al., 1986, Nature 321: 522-525; Riechmann et al., 1988, Nature 332: 323-327; Verhoeyen et al., 1988, Science 239: 1534-1536). See also, Carter et al., 1993, Proc. Natl. Acad. Sci. USA 89: 4285 and Sims et al., 1993, J. Immunol. 151: 2296.

Methods for producing fully human monoclonal antibodies include phage display and transgenic methods (for review, see Vaughan et al., 1998, Nature Biotechnology 16: 535-539). Fully human 158P1D7 monoclonal antibodies can be generated using cloning technologies employing large human Ig gene combinatorial libraries (i.e., phage display) (Griffiths and Hoogenboom, Building an *in vitro* immune system: human antibodies from phage display libraries. In: Protein Engineering of Antibody Molecules for Prophylactic and Therapeutic Applications in Man, Clark, M. (Ed.), Nottingham Academic, pp 45-64 (1993); Burton and Barbas, Human Antibodies from combinatorial libraries. *Id.*, pp 65-82). Fully human 158P1D7 monoclonal antibodies can also be produced using transgenic mice engineered to contain human immunoglobulin gene loci as described in PCT Patent Application WO98/24893, Kucherlapati and Jakobovits et al., published December 3, 1997 (see also, Jakobovits, 1998, Exp. Opin. Invest. Drugs 7(4): 607-614; U.S. patents 6,162,963 issued 19 December 2000; 6,150,584 issued 12 November 2000; and, 6,114,598 issued 5 September 2000). This method avoids the *in vitro* manipulation required with phage display technology and efficiently produces high affinity authentic human antibodies.

Reactivity of 158P1D7 antibodies with an 158P1D7-related protein can be established by a number of well known means, including Western blot, immunoprecipitation, ELISA, and FACS analyses using, as appropriate, 158P1D7-related proteins, 158P1D7-expressing cells or extracts thereof. A 158P1D7 antibody or fragment thereof can be labeled with a detectable marker or conjugated to a second molecule. Suitable detectable markers include, but are not limited to, a radioisotope, a fluorescent compound, a bioluminescent compound, chemiluminescent compound, a metal chelator or an enzyme. Further, bi-specific antibodies specific for two or more 158P1D7 epitopes are generated using methods generally known in the art. Homodimeric antibodies can also be generated by cross-linking techniques known in the art (e.g., Wolff et al., Cancer Res. 53: 2560-2565).

V.) 158P1D7 Cellular Immune Responses

The mechanism by which T cells recognize antigens has been delineated. Efficacious peptide epitope vaccine compositions of the invention induce a therapeutic or prophylactic immune responses in very broad segments of the world-wide population. For an understanding of the value and efficacy of compositions of the invention that induce cellular immune responses, a brief review of immunology-related technology is provided.

A complex of an HLA molecule and a peptidic antigen acts as the ligand recognized by HLA-restricted T cells (Buus, S. *et al.*, *Cell* 47:1071, 1986; Babbitt, B. P. *et al.*, *Nature* 317:359, 1985; Townsend, A. and Bodmer, H., *Annu. Rev. Immunol.* 7:601, 1989; Germain, R. N., *Annu. Rev. Immunol.* 11:403, 1993). Through the study of single amino acid substituted antigen analogs and the sequencing of endogenously bound, naturally processed peptides, critical residues that correspond to motifs required for specific binding to HLA antigen molecules have been identified and are set forth in Table IV (*see also, e.g.*, Southwood, *et al.*, *J. Immunol.* 160:3363, 1998; Rammensee, *et al.*, *Immunogenetics* 41:178, 1995; Rammensee *et al.*, SYFPEITHI, access via World Wide Web at URL syfpeithi.bmi-heidelberg.com/; Sette, A. and Sidney, J. *Curr. Opin. Immunol.* 10:478, 1998; Engelhard, V. H., *Curr. Opin. Immunol.* 6:13, 1994; Sette, A. and Grey, H. M., *Curr. Opin. Immunol.* 4:79, 1992; Sinigaglia, F. and Hammer, J. *Curr. Biol.* 6:52, 1994; Ruppert *et al.*, *Cell* 74:929-937, 1993; Kondo *et al.*, *J. Immunol.* 155:4307-4312, 1995; Sidney *et al.*, *J. Immunol.* 157:3480-3490, 1996; Sidney *et al.*, *Human Immunol.* 45:79-93, 1996; Sette, A. and Sidney, J. *Immunogenetics* 1999 Nov; 50(3-4):201-12, Review).

Furthermore, x-ray crystallographic analyses of HLA-peptide complexes have revealed pockets within the peptide binding cleft/groove of HLA molecules which accommodate, in an allele-specific mode, residues borne by peptide ligands; these residues in turn determine the HLA binding capacity of the peptides in which they are present. (*See, e.g.*, Madden, D.R. *Annu. Rev. Immunol.* 13:587, 1995; Smith, *et al.*, *Immunity* 4:203, 1996; Fremont *et al.*, *Immunity* 8:305, 1998; Stern *et al.*, *Structure* 2:245, 1994; Jones, E.Y. *Curr. Opin. Immunol.* 9:75, 1997; Brown, J. H. *et al.*, *Nature* 364:33, 1993; Guo, H. C. *et al.*, *Proc. Natl. Acad. Sci. USA* 90:8053, 1993; Guo, H. C. *et al.*, *Nature* 360:364, 1992; Silver, M. L. *et al.*, *Nature* 360:367, 1992; Matsumura, M. *et al.*, *Science* 257:927, 1992; Madden *et al.*, *Cell* 70:1035, 1992; Fremont, D. H. *et al.*, *Science* 257:919, 1992; Saper, M. A., Bjorkman, P. J. and Wiley, D. C., *J. Mol. Biol.* 219:277, 1991.)

Accordingly, the definition of class I and class II allele-specific HLA binding motifs, or class I or class II supermotifs allows identification of regions within a protein that are correlated with binding to particular HLA antigen(s).

Thus, by a process of HLA motif identification, candidates for epitope-based vaccines have been identified; such candidates can be further evaluated by HLA-peptide binding assays to determine binding affinity and/or the time period of association of the epitope and its corresponding HLA molecule. Additional confirmatory work can be performed to select, amongst these vaccine candidates, epitopes with preferred characteristics in terms of population coverage, and/or immunogenicity.

Various strategies can be utilized to evaluate cellular immunogenicity, including:

1) Evaluation of primary T cell cultures from normal individuals (*see, e.g.,* Wentworth, P. A. *et al., Mol. Immunol.* 32:603, 1995; Celis, E. *et al., Proc. Natl. Acad. Sci. USA* 91:2105, 1994; Tsai, V. *et al., J. Immunol.* 158:1796, 1997; Kawashima, I. *et al., Human Immunol.* 59:1, 1998). This procedure involves the stimulation of peripheral blood lymphocytes (PBL) from normal subjects with a test peptide in the presence of antigen presenting cells *in vitro* over a period of several weeks. T cells specific for the peptide become activated during this time and are detected using, *e.g.,* a lymphokine- or ^{51}Cr -release assay involving peptide sensitized target cells.

2) Immunization of HLA transgenic mice (*see, e.g.,* Wentworth, P. A. *et al., J. Immunol.* 26:97, 1996; Wentworth, P. A. *et al., Int. Immunol.* 8:651, 1996; Alexander, J. *et al., J. Immunol.* 159:4753, 1997). For example, in such methods peptides in incomplete Freund's adjuvant are administered subcutaneously to HLA transgenic mice. Several weeks following immunization, splenocytes are removed and cultured *in vitro* in the presence of test peptide for approximately one week. Peptide-specific T cells are detected using, *e.g.,* a ^{51}Cr -release assay involving peptide sensitized target cells and target cells expressing endogenously generated antigen.

3) Demonstration of recall T cell responses from immune individuals who have been either effectively vaccinated and/or from chronically ill patients (*see, e.g.,* Rehermann, B. *et al., J. Exp. Med.* 181:1047, 1995; Doolan, D. L. *et al., Immunity* 7:97, 1997; Bertoni, R. *et al., J. Clin. Invest.* 100:503, 1997; Threlkeld, S. C. *et al., J. Immunol.* 159:1648, 1997; Diepolder, H. M. *et al., J. Virol.* 71:6011, 1997). Accordingly, recall responses are detected by culturing PBL from subjects that have been exposed to the antigen due to disease and thus have generated an immune response "naturally", or from patients who were vaccinated against the antigen. PBL from subjects are cultured *in vitro* for 1-2 weeks in the presence of test peptide plus antigen presenting cells (APC) to allow activation of "memory" T cells, as compared to "naive" T cells. At the end of the culture period, T cell activity is detected using assays including ^{51}Cr release involving peptide-sensitized targets, T cell proliferation, or lymphokine release.

VI.) 158P1D7 Transgenic Animals

Nucleic acids that encode a 158P1D7-related protein can also be used to generate either transgenic animals or "knock out" animals which, in turn, are useful in the development and screening of therapeutically useful reagents. In accordance with established techniques, cDNA encoding 158P1D7 can be used to clone genomic DNA that encodes 158P1D7. The cloned genomic sequences can then be used to generate transgenic animals containing cells that express DNA that encode 158P1D7. Methods for generating transgenic animals, particularly animals such as mice or rats, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 issued 12 April 1988, and 4,870,009 issued 26 September 1989. Typically, particular cells would be targeted for 158P1D7 transgene incorporation with tissue-specific enhancers.

Transgenic animals that include a copy of a transgene encoding 158P1D7 can be used to examine the effect of increased expression of DNA that encodes 158P1D7. Such animals can be used as tester

animals for reagents thought to confer protection from, for example, pathological conditions associated with its overexpression. In accordance with this aspect of the invention, an animal is treated with a reagent and a reduced incidence of a pathological condition, compared to untreated animals that bear the transgene, would indicate a potential therapeutic intervention for the pathological condition.

Alternatively, non-human homologues of 158P1D7 can be used to construct a 158P1D7 "knock out" animal that has a defective or altered gene encoding 158P1D7 as a result of homologous recombination between the endogenous gene encoding 158P1D7 and altered genomic DNA encoding 158P1D7 introduced into an embryonic cell of the animal. For example, cDNA that encodes 158P1D7 can be used to clone genomic DNA encoding 158P1D7 in accordance with established techniques. A portion of the genomic DNA encoding 158P1D7 can be deleted or replaced with another gene, such as a gene encoding a selectable marker that can be used to monitor integration. Typically, several kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector (see, e.g., Thomas and Capecchi, *Cell*, 51:503 (1987) for a description of homologous recombination vectors). The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced DNA has homologously recombined with the endogenous DNA are selected (see, e.g., Li et al., *Cell*, 69:915 (1992)). The selected cells are then injected into a blastocyst of an animal (e.g., a mouse or rat) to form aggregation chimeras (see, e.g., Bradley, in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. J. Robertson, ed. (IRL, Oxford, 1987), pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal, and the embryo brought to term to create a "knock out" animal. Progeny harboring the homologously recombined DNA in their germ cells can be identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knock out animals can be characterized, for example, for their ability to defend against certain pathological conditions or for their development of pathological conditions due to absence of the 158P1D7 polypeptide.

VII.) Methods for the Detection of 158P1D7

Another aspect of the present invention relates to methods for detecting 158P1D7 polynucleotides and polypeptides and 158P1D7-related proteins, as well as methods for identifying a cell that expresses 158P1D7. The expression profile of 158P1D7 makes it a diagnostic marker for metastasized disease. Accordingly, the status of 158P1D7 gene products provides information useful for predicting a variety of factors including susceptibility to advanced stage disease, rate of progression, and/or tumor aggressiveness. As discussed in detail herein, the status of 158P1D7 gene products in patient samples can be analyzed by a variety of protocols that are well known in the art including immunohistochemical analysis, the variety of Northern blotting techniques including *in situ* hybridization, RT-PCR analysis (for example on laser capture micro-dissected samples), Western blot analysis and tissue array analysis.

More particularly, the invention provides assays for the detection of 158P1D7 polynucleotides in a biological sample, such as urine, serum, bone, prostatic fluid, tissues, semen, cell preparations, and the like.

Detectable 158P1D7 polynucleotides include, for example, a 158P1D7 gene or fragment thereof, 158P1D7 mRNA, alternative splice variant 158P1D7 mRNAs, and recombinant DNA or RNA molecules that contain a 158P1D7 polynucleotide. A number of methods for amplifying and/or detecting the presence of 158P1D7 polynucleotides are well known in the art and can be employed in the practice of this aspect of the invention.

In one embodiment, a method for detecting an 158P1D7 mRNA in a biological sample comprises producing cDNA from the sample by reverse transcription using at least one primer; amplifying the cDNA so produced using an 158P1D7 polynucleotides as sense and antisense primers to amplify 158P1D7 cDNAs therein; and detecting the presence of the amplified 158P1D7 cDNA. Optionally, the sequence of the amplified 158P1D7 cDNA can be determined.

In another embodiment, a method of detecting a 158P1D7 gene in a biological sample comprises first isolating genomic DNA from the sample; amplifying the isolated genomic DNA using 158P1D7 polynucleotides as sense and antisense primers; and detecting the presence of the amplified 158P1D7 gene. Any number of appropriate sense and antisense probe combinations can be designed from the nucleotide sequence provided for the 158P1D7 (Figure 2) and used for this purpose.

The invention also provides assays for detecting the presence of an 158P1D7 protein in a tissue or other biological sample such as urine, serum, semen, bone, prostate, cell preparations, and the like. Methods for detecting a 158P1D7-related protein are also well known and include, for example, immunoprecipitation, immunohistochemical analysis, Western blot analysis, molecular binding assays, ELISA, ELIFA and the like. For example, a method of detecting the presence of a 158P1D7-related protein in a biological sample comprises first contacting the sample with a 158P1D7 antibody, a 158P1D7-reactive fragment thereof, or a recombinant protein containing an antigen binding region of a 158P1D7 antibody; and then detecting the binding of 158P1D7-related protein in the sample.

Methods for identifying a cell that expresses 158P1D7 are also within the scope of the invention. In one embodiment, an assay for identifying a cell that expresses a 158P1D7 gene comprises detecting the presence of 158P1D7 mRNA in the cell. Methods for the detection of particular mRNAs in cells are well known and include, for example, hybridization assays using complementary DNA probes (such as *in situ* hybridization using labeled 158P1D7 riboprobes, Northern blot and related techniques) and various nucleic acid amplification assays (such as RT-PCR using complementary primers specific for 158P1D7, and other amplification type detection methods, such as, for example, branched DNA, SISBA, TMA and the like). Alternatively, an assay for identifying a cell that expresses a 158P1D7 gene comprises detecting the presence of 158P1D7-related protein in the cell or secreted by the cell. Various methods for the detection of proteins are well known in the art and are employed for the detection of 158P1D7-related proteins and cells that express 158P1D7-related proteins.

158P1D7 expression analysis is also useful as a tool for identifying and evaluating agents that modulate 158P1D7 gene expression. For example, 158P1D7 expression is significantly upregulated in bladder cancer, and is expressed in cancers of the tissues listed in Table I. Identification of a molecule or biological agent that inhibits 158P1D7 expression or over-expression in cancer cells is of therapeutic value.

For example, such an agent can be identified by using a screen that quantifies 158P1D7 expression by RT-PCR, nucleic acid hybridization or antibody binding.

VIII.) Methods for Monitoring the Status of 158P1D7-related Genes and Their Products

Oncogenesis is known to be a multistep process where cellular growth becomes progressively dysregulated and cells progress from a normal physiological state to precancerous and then cancerous states (see, e.g., Alers et al., *Lab Invest.* 77(5): 437-438 (1997) and Isaacs et al., *Cancer Surv.* 23: 19-32 (1995)). In this context, examining a biological sample for evidence of dysregulated cell growth (such as aberrant 158P1D7 expression in cancers) allows for early detection of such aberrant physiology, before a pathologic state such as cancer has progressed to a stage that therapeutic options are more limited and or the prognosis is worse. In such examinations, the status of 158P1D7 in a biological sample of interest can be compared, for example, to the status of 158P1D7 in a corresponding normal sample (e.g. a sample from that individual or alternatively another individual that is not affected by a pathology). An alteration in the status of 158P1D7 in the biological sample (as compared to the normal sample) provides evidence of dysregulated cellular growth. In addition to using a biological sample that is not affected by a pathology as a normal sample, one can also use a predetermined normative value such as a predetermined normal level of mRNA expression (see, e.g., Grever et al., *J. Comp. Neurol.* 1996 Dec 9;376(2):306-14 and U.S. Patent No. 5,837,501) to compare 158P1D7 status in a sample.

The term "status" in this context is used according to its art accepted meaning and refers to the condition or state of a gene and its products. Typically, skilled artisans use a number of parameters to evaluate the condition or state of a gene and its products. These include, but are not limited to the location of expressed gene products (including the location of 158P1D7 expressing cells) as well as the level, and biological activity of expressed gene products (such as 158P1D7 mRNA, polynucleotides and polypeptides). Typically, an alteration in the status of 158P1D7 comprises a change in the location of 158P1D7 and/or 158P1D7 expressing cells and/or an increase in 158P1D7 mRNA and/or protein expression.

158P1D7 status in a sample can be analyzed by a number of means well known in the art, including without limitation, immunohistochemical analysis, *in situ* hybridization, RT-PCR analysis on laser capture micro-dissected samples, Western blot analysis, and tissue array analysis. Typical protocols for evaluating the status of the 158P1D7 gene and gene products are found, for example in Ausubel et al. eds., 1995, *Current Protocols In Molecular Biology*, Units 2 (Northern Blotting), 4 (Southern Blotting), 15 (Immunoblotting) and 18 (PCR Analysis). Thus, the status of 158P1D7 in a biological sample is evaluated by various methods utilized by skilled artisans including, but not limited to genomic Southern analysis (to examine, for example perturbations in the 158P1D7 gene), Northern analysis and/or PCR analysis of 158P1D7 mRNA (to examine, for example alterations in the polynucleotide sequences or expression levels of 158P1D7 mRNAs), and, Western and/or immunohistochemical analysis (to examine, for example alterations in polypeptide sequences, alterations in polypeptide localization within a sample, alterations in expression levels of 158P1D7 proteins and/or associations of 158P1D7 proteins with polypeptide binding

partners). Detectable 158P1D7 polynucleotides include, for example, a 158P1D7 gene or fragment thereof, 158P1D7 mRNA, alternative splice variants, 158P1D7 mRNAs, and recombinant DNA or RNA molecules containing a 158P1D7 polynucleotide.

The expression profile of 158P1D7 makes it a diagnostic marker for local and/or metastasized disease, and provides information on the growth or oncogenic potential of a biological sample. In particular, the status of 158P1D7 provides information useful for predicting susceptibility to particular disease stages, progression, and/or tumor aggressiveness. The invention provides methods and assays for determining 158P1D7 status and diagnosing cancers that express 158P1D7, such as cancers of the tissues listed in Table I. For example, because 158P1D7 mRNA is so highly expressed in bladder and other cancers relative to normal bladder tissue, assays that evaluate the levels of 158P1D7 mRNA transcripts or proteins in a biological sample can be used to diagnose a disease associated with 158P1D7 dysregulation, and can provide prognostic information useful in defining appropriate therapeutic options.

The expression status of 158P1D7 provides information including the presence, stage and location of dysplastic, precancerous and cancerous cells, predicting susceptibility to various stages of disease, and/or for gauging tumor aggressiveness. Moreover, the expression profile makes it useful as an imaging reagent for metastasized disease. Consequently, an aspect of the invention is directed to the various molecular prognostic and diagnostic methods for examining the status of 158P1D7 in biological samples such as those from individuals suffering from, or suspected of suffering from a pathology characterized by dysregulated cellular growth, such as cancer.

As described above, the status of 158P1D7 in a biological sample can be examined by a number of well-known procedures in the art. For example, the status of 158P1D7 in a biological sample taken from a specific location in the body can be examined by evaluating the sample for the presence or absence of 158P1D7 expressing cells (e.g. those that express 158P1D7 mRNAs or proteins). This examination can provide evidence of dysregulated cellular growth, for example, when 158P1D7-expressing cells are found in a biological sample that does not normally contain such cells (such as a lymph node), because such alterations in the status of 158P1D7 in a biological sample are often associated with dysregulated cellular growth. Specifically, one indicator of dysregulated cellular growth is the metastases of cancer cells from an organ of origin (such as the bladder) to a different area of the body (such as a lymph node). By example, evidence of dysregulated cellular growth is important because occult lymph node metastases can be detected in a substantial proportion of patients with prostate cancer, and such metastases are associated with known predictors of disease progression (see, e.g., Murphy et al., Prostate 42(4): 315-317 (2000); Su et al., Semin. Surg. Oncol. 18(1): 17-28 (2000) and Freeman et al., J Urol 1995 Aug 154(2 Pt 1):474-8).

In one aspect, the invention provides methods for monitoring 158P1D7 gene products by determining the status of 158P1D7 gene products expressed by cells from an individual suspected of having a disease associated with dysregulated cell growth (such as hyperplasia or cancer) and then comparing the status so determined to the status of 158P1D7 gene products in a corresponding normal sample. The

presence of aberrant 158P1D7 gene products in the test sample relative to the normal sample provides an indication of the presence of dysregulated cell growth within the cells of the individual.

In another aspect, the invention provides assays useful in determining the presence of cancer in an individual, comprising detecting a significant increase in 158P1D7 mRNA or protein expression in a test cell or tissue sample relative to expression levels in the corresponding normal cell or tissue. The presence of 158P1D7 mRNA can, for example, be evaluated in tissue samples including but not limited to those listed in Table I. The presence of significant 158P1D7 expression in any of these tissues is useful to indicate the emergence, presence and/or severity of a cancer, since the corresponding normal tissues do not express 158P1D7 mRNA or express it at lower levels.

In a related embodiment, 158P1D7 status is determined at the protein level rather than at the nucleic acid level. For example, such a method comprises determining the level of 158P1D7 protein expressed by cells in a test tissue sample and comparing the level so determined to the level of 158P1D7 expressed in a corresponding normal sample. In one embodiment, the presence of 158P1D7 protein is evaluated, for example, using immunohistochemical methods. 158P1D7 antibodies or binding partners capable of detecting 158P1D7 protein expression are used in a variety of assay formats well known in the art for this purpose.

In a further embodiment, one can evaluate the status of 158P1D7 nucleotide and amino acid sequences in a biological sample in order to identify perturbations in the structure of these molecules. These perturbations can include insertions, deletions, substitutions and the like. Such evaluations are useful because perturbations in the nucleotide and amino acid sequences are observed in a large number of proteins associated with a growth dysregulated phenotype (see, e.g., Marrogi et al., 1999, *J. Cutan. Pathol.* 26(8):369-378). For example, a mutation in the sequence of 158P1D7 may be indicative of the presence or promotion of a tumor. Such assays therefore have diagnostic and predictive value where a mutation in 158P1D7 indicates a potential loss of function or increase in tumor growth.

A wide variety of assays for observing perturbations in nucleotide and amino acid sequences are well known in the art. For example, the size and structure of nucleic acid or amino acid sequences of 158P1D7 gene products are observed by the Northern, Southern, Western, PCR and DNA sequencing protocols discussed herein. In addition, other methods for observing perturbations in nucleotide and amino acid sequences such as single strand conformation polymorphism analysis are well known in the art (see, e.g., U.S. Patent Nos. 5,382,510 issued 7 September 1999, and 5,952,170 issued 17 January 1995).

Additionally, one can examine the methylation status of the 158P1D7 gene in a biological sample. Aberrant demethylation and/or hypermethylation of CpG islands in gene 5' regulatory regions frequently occurs in immortalized and transformed cells, and can result in altered expression of various genes. For example, promoter hypermethylation of the DBCCR1, PAX6 and APC genes have been detected in bladder cancers leading to aberrant expression of the genes (Esteller et al., *Cancer Res* 2001; 61:3225-3229). A variety of assays for examining methylation status of a gene are well known in the art. For example, one can utilize, in Southern hybridization approaches, methylation-sensitive restriction enzymes which cannot cleave sequences that contain methylated CpG sites to assess the methylation status of CpG islands. In addition, MSP

(methylation specific PCR) can rapidly profile the methylation status of all the CpG sites present in a CpG island of a given gene. This procedure involves initial modification of DNA by sodium bisulfite (which will convert all unmethylated cytosines to uracil) followed by amplification using primers specific for methylated versus unmethylated DNA. Protocols involving methylation interference can also be found for example in Current Protocols In Molecular Biology, Unit 12, Frederick M. Ausubel et al. eds., 1995.

Gene amplification is an additional method for assessing the status of 158P1D7. Gene amplification is measured in a sample directly, for example, by conventional Southern blotting or Northern blotting to quantitate the transcription of mRNA (Thomas, 1980, Proc. Natl. Acad. Sci. USA, 77:5201-5205), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies are employed that recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn are labeled and the assay carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Biopsied tissue or peripheral blood can be conveniently assayed for the presence of cancer cells using for example, Northern, dot blot or RT-PCR analysis to detect 158P1D7 expression. The presence of RT-PCR amplifiable 158P1D7 mRNA provides an indication of the presence of cancer. RT-PCR assays are well known in the art. RT-PCR detection assays for tumor cells in peripheral blood are currently being evaluated for use in the diagnosis and management of a number of human solid tumors.

A further aspect of the invention is an assessment of the susceptibility that an individual has for developing cancer. In one embodiment, a method for predicting susceptibility to cancer comprises detecting 158P1D7 mRNA or 158P1D7 protein in a tissue sample, its presence indicating susceptibility to cancer, wherein the degree of 158P1D7 mRNA expression correlates to the degree of susceptibility. In a specific embodiment, the presence of 158P1D7 in bladder or other tissue is examined, with the presence of 158P1D7 in the sample providing an indication of bladder cancer susceptibility (or the emergence or existence of a bladder tumor). Similarly, one can evaluate the integrity 158P1D7 nucleotide and amino acid sequences in a biological sample, in order to identify perturbations in the structure of these molecules such as insertions, deletions, substitutions and the like. The presence of one or more perturbations in 158P1D7 gene products in the sample is an indication of cancer susceptibility (or the emergence or existence of a tumor).

The invention also comprises methods for gauging tumor aggressiveness. In one embodiment, a method for gauging aggressiveness of a tumor comprises determining the level of 158P1D7 mRNA or 158P1D7 protein expressed by tumor cells, comparing the level so determined to the level of 158P1D7 mRNA or 158P1D7 protein expressed in a corresponding normal tissue taken from the same individual or a normal tissue reference sample, wherein the degree of 158P1D7 mRNA or 158P1D7 protein expression in the tumor sample relative to the normal sample indicates the degree of aggressiveness. In a specific embodiment, aggressiveness of a tumor is evaluated by determining the extent to which 158P1D7 is expressed in the tumor cells, with higher expression levels indicating more aggressive tumors. Another embodiment is the evaluation

of the integrity of 158P1D7 nucleotide and amino acid sequences in a biological sample, in order to identify perturbations in the structure of these molecules such as insertions, deletions, substitutions and the like. The presence of one or more perturbations indicates more aggressive tumors.

Another embodiment of the invention is directed to methods for observing the progression of a malignancy in an individual over time. In one embodiment, methods for observing the progression of a malignancy in an individual over time comprise determining the level of 158P1D7 mRNA or 158P1D7 protein expressed by cells in a sample of the tumor, comparing the level so determined to the level of 158P1D7 mRNA or 158P1D7 protein expressed in an equivalent tissue sample taken from the same individual at a different time, wherein the degree of 158P1D7 mRNA or 158P1D7 protein expression in the tumor sample over time provides information on the progression of the cancer. In a specific embodiment, the progression of a cancer is evaluated by determining 158P1D7 expression in the tumor cells over time, where increased expression over time indicates a progression of the cancer. Also, one can evaluate the integrity 158P1D7 nucleotide and amino acid sequences in a biological sample in order to identify perturbations in the structure of these molecules such as insertions, deletions, substitutions and the like, where the presence of one or more perturbations indicates a progression of the cancer.

The above diagnostic approaches can be combined with any one of a wide variety of prognostic and diagnostic protocols known in the art. For example, another embodiment of the invention is directed to methods for observing a coincidence between the expression of 158P1D7 gene and 158P1D7 gene products (or perturbations in 158P1D7 gene and 158P1D7 gene products) and a factor that is associated with malignancy, as a means for diagnosing and prognosticating the status of a tissue sample. A wide variety of factors associated with malignancy can be utilized, such as the expression of genes associated with malignancy (e.g. PSCA, H-ras and p53 expression etc.) as well as gross cytological observations (see, e.g., Bocking et al., 1984, *Anal. Quant. Cytol.* 6(2):74-88; Epstein, 1995, *Hum. Pathol.* 26(2):223-9; Thorson et al., 1998, *Mod. Pathol.* 11(6):543-51; Baisden et al., 1999, *Am. J. Surg. Pathol.* 23(8):918-24). Methods for observing a coincidence between the expression of 158P1D7 gene and 158P1D7 gene products (or perturbations in 158P1D7 gene and 158P1D7 gene products) and another factor that is associated with malignancy are useful, for example, because the presence of a set of specific factors that coincide with disease provides information crucial for diagnosing and prognosticating the status of a tissue sample.

In one embodiment, methods for observing a coincidence between the expression of 158P1D7 gene and 158P1D7 gene products (or perturbations in 158P1D7 gene and 158P1D7 gene products) and another factor associated with malignancy entails detecting the overexpression of 158P1D7 mRNA or protein in a tissue sample, detecting the overexpression of BLCA-4A mRNA or protein in a tissue sample (or PSCA expression), and observing a coincidence of 158P1D7 mRNA or protein and BLCA-4 mRNA or protein overexpression (or PSCA expression) (Amara et al., 2001, *Cancer Res* 61:4660-4665; Konety et al., *Clin Cancer Res*, 2000, 6(7):2618-2625). In a specific embodiment, the expression of 158P1D7 and BLCA-4 mRNA in bladder tissue is examined, where the coincidence of 158P1D7 and BLCA-4 mRNA overexpression

in the sample indicates the existence of bladder cancer, bladder cancer susceptibility or the emergence or status of a bladder tumor.

Methods for detecting and quantifying the expression of 158P1D7 mRNA or protein are described herein, and standard nucleic acid and protein detection and quantification technologies are well known in the art. Standard methods for the detection and quantification of 158P1D7 mRNA include *in situ* hybridization using labeled 158P1D7 riboprobes, Northern blot and related techniques using 158P1D7 polynucleotide probes, RT-PCR analysis using primers specific for 158P1D7, and other amplification type detection methods, such as, for example, branched DNA, SISBA, TMA and the like. In a specific embodiment, semi-quantitative RT-PCR is used to detect and quantify 158P1D7 mRNA expression. Any number of primers capable of amplifying 158P1D7 can be used for this purpose, including but not limited to the various primer sets specifically described herein. In a specific embodiment, polyclonal or monoclonal antibodies specifically reactive with the wild-type 158P1D7 protein can be used in an immunohistochemical assay of biopsied tissue.

IX.) Identification of Molecules That Interact With 158P1D7

The 158P1D7 protein and nucleic acid sequences disclosed herein allow a skilled artisan to identify proteins, small molecules and other agents that interact with 158P1D7, as well as pathways activated by 158P1D7 via any one of a variety of art accepted protocols. For example, one can utilize one of the so-called interaction trap systems (also referred to as the "two-hybrid assay"). In such systems, molecules interact and reconstitute a transcription factor which directs expression of a reporter gene, whereupon the expression of the reporter gene is assayed. Other systems identify protein-protein interactions *in vivo* through reconstitution of a eukaryotic transcriptional activator, see, e.g., U.S. Patent Nos. 5,955,280 issued 21 September 1999, 5,925,523 issued 20 July 1999, 5,846,722 issued 8 December 1998 and 6,004,746 issued 21 December 1999. Algorithms are also available in the art for genome-based predictions of protein function (see, e.g., Marcotte, et al., Nature 402: 4 November 1999, 83-86).

Alternatively one can screen peptide libraries to identify molecules that interact with 158P1D7 protein sequences. In such methods, peptides that bind to 158P1D7 are identified by screening libraries that encode a random or controlled collection of amino acids. Peptides encoded by the libraries are expressed as fusion proteins of bacteriophage coat proteins, the bacteriophage particles are then screened against the 158P1D7 protein.

Accordingly, peptides having a wide variety of uses, such as therapeutic, prognostic or diagnostic reagents, are thus identified without any prior information on the structure of the expected ligand or receptor molecule. Typical peptide libraries and screening methods that can be used to identify molecules that interact with 158P1D7 protein sequences are disclosed for example in U.S. Patent Nos. 5,723,286 issued 3 March 1998 and 5,733,731 issued 31 March 1998.

Alternatively, cell lines that express 158P1D7 are used to identify protein-protein interactions mediated by 158P1D7. Such interactions can be examined using immunoprecipitation techniques (see, e.g., Hamilton BJ, et al. Biochem. Biophys. Res. Commun. 1999, 261:646-51). 158P1D7 protein can be

immunoprecipitated from 158P1D7-expressing cell lines using anti-158P1D7 antibodies. Alternatively, antibodies against His-tag can be used in a cell line engineered to express fusions of 158P1D7 and a His-tag (vectors mentioned above). The immunoprecipitated complex can be examined for protein association by procedures such as Western blotting, ³⁵S-methionine labeling of proteins, protein microsequencing, silver staining and two-dimensional gel electrophoresis.

Small molecules and ligands that interact with 158P1D7 can be identified through related embodiments of such screening assays. For example, small molecules can be identified that interfere with protein function, including molecules that interfere with 158P1D7's ability to mediate phosphorylation and de-phosphorylation, interaction with DNA or RNA molecules as an indication of regulation of cell cycles, second messenger signaling or tumorigenesis. Similarly, small molecules that modulate 158P1D7 related ion channel, protein pump, or cell communication functions 158P1D7 are identified and used to treat patients that have a cancer that expresses 158P1D7 (see, e.g., Hille, B., *Ionic Channels of Excitable Membranes* 2nd Ed., Sinauer Assoc., Sunderland, MA, 1992). Moreover, ligands that regulate 158P1D7 function can be identified based on their ability to bind 158P1D7 and activate a reporter construct. Typical methods are discussed for example in U.S. Patent No. 5,928,868 issued 27 July 1999, and include methods for forming hybrid ligands in which at least one ligand is a small molecule. In an illustrative embodiment, cells engineered to express a fusion protein of 158P1D7 and a DNA-binding protein are used to co-express a fusion protein of a hybrid ligand/small molecule and a cDNA library transcriptional activator protein. The cells further contain a reporter gene, the expression of which is conditioned on the proximity of the first and second fusion proteins to each other, an event that occurs only if the hybrid ligand binds to target sites on both hybrid proteins. Those cells that express the reporter gene are selected and the unknown small molecule or the unknown ligand is identified. This method provides a means of identifying modulators which activate or inhibit 158P1D7.

An embodiment of this invention comprises a method of screening for a molecule that interacts with an 158P1D7 amino acid sequence shown in Figure 2 or Figure 3, comprising the steps of contacting a population of molecules with the 158P1D7 amino acid sequence, allowing the population of molecules and the 158P1D7 amino acid sequence to interact under conditions that facilitate an interaction, determining the presence of a molecule that interacts with the 158P1D7 amino acid sequence, and then separating molecules that do not interact with the 158P1D7 amino acid sequence from molecules that do. In a specific embodiment, the method further comprises purifying, characterizing and identifying a molecule that interacts with the 158P1D7 amino acid sequence. The identified molecule can be used to modulate a function performed by 158P1D7. In a preferred embodiment, the 158P1D7 amino acid sequence is contacted with a library of peptides.

X.) Therapeutic Methods and Compositions

The identification of 158P1D7 as a protein that is normally expressed in a restricted set of tissues, but which is also expressed in bladder and other cancers, opens a number of therapeutic approaches to the

treatment of such cancers. As contemplated herein, 158P1D7 functions as a transcription factor involved in activating tumor-promoting genes or repressing genes that block tumorigenesis.

Accordingly, therapeutic approaches that inhibit the activity of the 158P1D7 protein are useful for patients suffering from a cancer that expresses 158P1D7. These therapeutic approaches generally fall into two classes. One class comprises various methods for inhibiting the binding or association of the 158P1D7 protein with its binding partner or with other proteins. Another class comprises a variety of methods for inhibiting the transcription of the 158P1D7 gene or translation of 158P1D7 mRNA.

X.A.) Anti-Cancer Vaccines

The invention provides cancer vaccines comprising a 158P1D7-related protein or 158P1D7-related nucleic acid. In view of the expression of 158P1D7, cancer vaccines prevent and/or treat 158P1D7-expressing cancers with minimal or no effects on non-target tissues. The use of a tumor antigen in a vaccine that generates humoral and/or cell-mediated immune responses as anti-cancer therapy is well known in the art (see, e.g., Hodge et al., 1995, *Int. J. Cancer* 63:231-237; Fong et al., 1997, *J. Immunol.* 159:3113-3117).

Such methods can be readily practiced by employing a 158P1D7-related protein, or a 158P1D7-encoding nucleic acid molecule and recombinant vectors capable of expressing and presenting the 158P1D7 immunogen (which typically comprises a number of antibody or T cell epitopes). Skilled artisans understand that a wide variety of vaccine systems for delivery of immunoreactive epitopes are known in the art (see, e.g., Heryln et al., *Ann Med* 1999 Feb 31(1):66-78; Maruyama et al., *Cancer Immunol Immunother* 2000 Jun 49(3):123-32). Briefly, such methods of generating an immune response (e.g. humoral and/or cell-mediated) in a mammal, comprise the steps of: exposing the mammal's immune system to an immunoreactive epitope (e.g. an epitope present in the 158P1D7 protein shown in SEQ ID NO: 657 or analog or homolog thereof) so that the mammal generates an immune response that is specific for that epitope (e.g. generates antibodies that specifically recognize that epitope). In a preferred method, the 158P1D7 immunogen contains a biological motif, see e.g., Tables V-XVIII, or a peptide of a size range from 158P1D7 indicated in Figure 9, Figure 10, Figure 11, Figure 12, and Figure 13.

The entire 158P1D7 protein, immunogenic regions or epitopes thereof can be combined and delivered by various means. Such vaccine compositions can include, for example, lipopeptides (e.g., Vitiello, A. et al., *J. Clin. Invest.* 95:341, 1995), peptide compositions encapsulated in poly(DL-lactide-co-glycolide) ("PLG") microspheres (see, e.g., Eldridge, et al., *Molec. Immunol.* 28:287-294, 1991; Alonso et al., *Vaccine* 12:299-306, 1994; Jones et al., *Vaccine* 13:675-681, 1995), peptide compositions contained in immune stimulating complexes (ISCOMS) (see, e.g., Takahashi et al., *Nature* 344:873-875, 1990; Hu et al., *Clin Exp Immunol.* 113:235-243, 1998), multiple antigen peptide systems (MAPs) (see e.g., Tam, J. P., *Proc. Natl. Acad. Sci. U.S.A.* 85:5409-5413, 1988; Tam, J.P., *J. Immunol. Methods* 196:17-32, 1996), peptides formulated as multivalent peptides; peptides for use in ballistic delivery systems, typically crystallized peptides, viral delivery vectors (Perkus, M. E. et al., In: *Concepts in vaccine development*, Kaufmann, S. H. E., ed., p. 379, 1996; Chakrabarti, S. et al., *Nature* 320:535, 1986; Hu, S. L. et al., *Nature* 320:537, 1986; Kieny, M.-P. et al., *AIDS Bio/Technology* 4:790, 1986; Top, F. H. et al., *J. Infect. Dis.*

124:148, 1971; Chanda, P. K. *et al.*, *Virology* 175:535, 1990), particles of viral or synthetic origin (*e.g.*, Kofler, N. *et al.*, *J. Immunol. Methods* 192:25, 1996; Eldridge, J. H. *et al.*, *Sem. Hematol.* 30:16, 1993; Falo, L. D., Jr. *et al.*, *Nature Med.* 7:649, 1995), adjuvants (Warren, H. S., Vogel, F. R., and Chedid, L. A. *Annu. Rev. Immunol.* 4:369, 1986; Gupta, R. K. *et al.*, *Vaccine* 11:293, 1993), liposomes (Reddy, R. *et al.*, *J. Immunol.* 148:1585, 1992; Rock, K. L., *Immunol. Today* 17:131, 1996), or, naked or particle absorbed cDNA (Ulmer, J. B. *et al.*, *Science* 259:1745, 1993; Robinson, H. L., Hunt, L. A., and Webster, R. G., *Vaccine* 11:957, 1993; Shiver, J. W. *et al.*, In: *Concepts in vaccine development*, Kaufmann, S. H. E., ed., p. 423, 1996; Cease, K. B., and Berzofsky, J. A., *Annu. Rev. Immunol.* 12:923, 1994 and Eldridge, J. H. *et al.*, *Sem. Hematol.* 30:16, 1993). Toxin-targeted delivery technologies, also known as receptor mediated targeting, such as those of Avant Immunotherapeutics, Inc. (Needham, Massachusetts) may also be used.

In patients with 158P1D7-associated cancer, the vaccine compositions of the invention can also be used in conjunction with other treatments used for cancer, *e.g.*, surgery, chemotherapy, drug therapies, radiation therapies, *etc.* including use in combination with immune adjuvants such as IL-2, IL-12, GM-CSF, and the like.

Cellular Vaccines:

CTL epitopes can be determined using specific algorithms to identify peptides within 158P1D7 protein that bind corresponding HLA alleles (see *e.g.*, Table IV; Epimer™ and Epimatrix™, Brown University (URL www.brown.edu/Research/TB-HIV_Lab/epimatrix/epimatrix.html); and, BIMAS, (URL bimas.dcrt.nih.gov/; SYFPEITHI at URL syfpeithi.bmi-heidelberg.com/). In a preferred embodiment, the 158P1D7 immunogen contains one or more amino acid sequences identified using techniques well known in the art, such as the sequences shown in Tables V-XVIII or a peptide of 8, 9, 10 or 11 amino acids specified by an HLA Class I motif/supermotif (*e.g.*, Table IV (A), Table IV (D), or Table IV (E)) and/or a peptide of at least 9 amino acids that comprises an HLA Class II motif/supermotif (*e.g.*, Table IV (B) or Table IV (C)). As is appreciated in the art, the HLA Class I binding groove is essentially closed ended so that peptides of only a particular size range can fit into the groove and be bound, generally HLA Class I epitopes are 8, 9, 10, or 11 amino acids long. In contrast, the HLA Class II binding groove is essentially open ended; therefore a peptide of about 9 or more amino acids can be bound by an HLA Class II molecule. Due to the binding groove differences between HLA Class I and II, HLA Class I motifs are length specific, *i.e.*, position two of a Class I motif is the second amino acid in an amino to carboxyl direction of the peptide. The amino acid positions in a Class II motif are relative only to each other, not the overall peptide, *i.e.*, additional amino acids can be attached to the amino and/or carboxyl termini of a motif-bearing sequence. HLA Class II epitopes are often 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 amino acids long, or longer than 25 amino acids.

Antibody-based Vaccines

A wide variety of methods for generating an immune response in a mammal are known in the art (for example as the first step in the generation of hybridomas). Methods of generating an immune response in a mammal comprise exposing the mammal's immune system to an immunogenic epitope on a protein

(e.g. the 158P1D7 protein) so that an immune response is generated. A typical embodiment consists of a method for generating an immune response to 158P1D7 in a host, by contacting the host with a sufficient amount of at least one 158P1D7 B cell or cytotoxic T-cell epitope or analog thereof; and at least one periodic interval thereafter re-contacting the host with the 158P1D7 B cell or cytotoxic T-cell epitope or analog thereof. A specific embodiment consists of a method of generating an immune response against a 158P1D7-related protein or a man-made multiepitopic peptide comprising: administering 158P1D7 immunogen (e.g. the 158P1D7 protein or a peptide fragment thereof, an 158P1D7 fusion protein or analog etc.) in a vaccine preparation to a human or another mammal. Typically, such vaccine preparations further contain a suitable adjuvant (see, e.g., U.S. Patent No. 6,146,635) or a universal helper epitope such as a PADRETM peptide (Epimmune Inc., San Diego, CA; see, e.g., Alexander et al., J. Immunol. 2000 164(3): 164(3): 1625-1633; Alexander et al., Immunity 1994 1(9): 751-761 and Alexander et al., Immunol. Res. 1998 18(2): 79-92). An alternative method comprises generating an immune response in an individual against a 158P1D7 immunogen by: administering *in vivo* to muscle or skin of the individual's body a DNA molecule that comprises a DNA sequence that encodes an 158P1D7 immunogen, the DNA sequence operatively linked to regulatory sequences which control the expression of the DNA sequence; wherein the DNA molecule is taken up by cells, the DNA sequence is expressed in the cells and an immune response is generated against the immunogen (see, e.g., U.S. Patent No. 5,962,428). Optionally a genetic vaccine facilitator such as anionic lipids; saponins; lectins; estrogenic compounds; hydroxylated lower alkyls; dimethyl sulfoxide; and urea is also administered.

Nucleic Acid Vaccines:

Vaccine compositions of the invention include nucleic acid-mediated modalities. DNA or RNA that encode protein(s) of the invention can be administered to a patient. Genetic immunization methods can be employed to generate prophylactic or therapeutic humoral and cellular immune responses directed against cancer cells expressing 158P1D7. Constructs comprising DNA encoding a 158P1D7-related protein/immunogen and appropriate regulatory sequences can be injected directly into muscle or skin of an individual, such that the cells of the muscle or skin take-up the construct and express the encoded 158P1D7 protein/immunogen. Alternatively, a vaccine comprises a 158P1D7-related protein. Expression of the 158P1D7-related protein immunogen results in the generation of prophylactic or therapeutic humoral and cellular immunity against cells that bear 158P1D7 protein. Various prophylactic and therapeutic genetic immunization techniques known in the art can be used (for review, see information and references published at Internet address www.genweb.com). Nucleic acid-based delivery is described, for instance, in Wolff *et. al.*, *Science* 247:1465 (1990) as well as U.S. Patent Nos. 5,580,859; 5,589,466; 5,804,566; 5,739,118; 5,736,524; 5,679,647; WO 98/04720. Examples of DNA-based delivery technologies include "naked DNA", facilitated (bupivacaine, polymers, peptide-mediated) delivery, cationic lipid complexes, and particle-mediated ("gene gun") or pressure-mediated delivery (see, e.g., U.S. Patent No. 5,922,687).

For therapeutic or prophylactic immunization purposes, proteins of the invention can be expressed via viral or bacterial vectors. Various viral gene delivery systems that can be used in the practice of the

invention include, but are not limited to, vaccinia, fowlpox, canarypox, adenovirus, influenza, poliovirus, adeno-associated virus, lentivirus, and sindbis virus (see, e.g., Restifo, 1996, *Curr. Opin. Immunol.* 8:658-663; Tsang et al. *J. Natl. Cancer Inst.* 87:982-990 (1995)). Non-viral delivery systems can also be employed by introducing naked DNA encoding a 158P1D7-related protein into the patient (e.g., intramuscularly or intradermally) to induce an anti-tumor response.

Vaccinia virus is used, for example, as a vector to express nucleotide sequences that encode the peptides of the invention. Upon introduction into a host, the recombinant vaccinia virus expresses the protein immunogenic peptide, and thereby elicits a host immune response. Vaccinia vectors and methods useful in immunization protocols are described in, e.g., U.S. Patent No. 4,722,848. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover *et al.*, *Nature* 351:456-460 (1991). A wide variety of other vectors useful for therapeutic administration or immunization of the peptides of the invention, e.g. adeno and adeno-associated virus vectors, retroviral vectors, *Salmonella typhi* vectors, detoxified anthrax toxin vectors, and the like, will be apparent to those skilled in the art from the description herein.

Thus, gene delivery systems are used to deliver a 158P1D7-related nucleic acid molecule. In one embodiment, the full-length human 158P1D7 cDNA is employed. In another embodiment, 158P1D7 nucleic acid molecules encoding specific cytotoxic T lymphocyte (CTL) and/or antibody epitopes are employed.

Ex Vivo Vaccines

Various *ex vivo* strategies can also be employed to generate an immune response. One approach involves the use of antigen presenting cells (APCs) such as dendritic cells (DC) to present 158P1D7 antigen to a patient's immune system. Dendritic cells express MHC class I and II molecules, B7 co-stimulator, and IL-12, and are thus highly specialized antigen presenting cells. In bladder cancer, autologous dendritic cells pulsed with peptides of the MAGE-3 antigen are being used in a Phase I clinical trial to stimulate bladder cancer patients' immune systems (Nishiyama et al., 2001, *Clin Cancer Res.* 7(1):23-31). Thus, dendritic cells can be used to present 158P1D7 peptides to T cells in the context of MHC class I or II molecules. In one embodiment, autologous dendritic cells are pulsed with 158P1D7 peptides capable of binding to MHC class I and/or class II molecules. In another embodiment, dendritic cells are pulsed with the complete 158P1D7 protein. Yet another embodiment involves engineering the overexpression of the 158P1D7 gene in dendritic cells using various implementing vectors known in the art, such as adenovirus (Arthur et al., 1997, *Cancer Gene Ther.* 4:17-25), retrovirus (Henderson et al., 1996, *Cancer Res.* 56:3763-3770), lentivirus, adeno-associated virus, DNA transfection (Ribas et al., 1997, *Cancer Res.* 57:2865-2869), or tumor-derived RNA transfection (Ashley et al., 1997, *J. Exp. Med.* 186:1177-1182). Cells that express 158P1D7 can also be engineered to express immune modulators, such as GM-CSF, and used as immunizing agents.

X.B.) 158P1D7 as a Target for Antibody-based Therapy

158P1D7 is an attractive target for antibody-based therapeutic strategies. A number of antibody strategies are known in the art for targeting both extracellular and intracellular molecules (see, e.g.,

complement and ADCC mediated killing as well as the use of intrabodies). Because 158P1D7 is expressed by cancer cells of various lineages relative to corresponding normal cells, systemic administration of 158P1D7-immunoreactive compositions are prepared that exhibit excellent sensitivity without toxic, non-specific and/or non-target effects caused by binding of the immunoreactive composition to non-target organs and tissues. Antibodies specifically reactive with domains of 158P1D7 are useful to treat 158P1D7-expressing cancers systemically, either as conjugates with a toxin or therapeutic agent, or as naked antibodies capable of inhibiting cell proliferation or function.

158P1D7 antibodies can be introduced into a patient such that the antibody binds to 158P1D7 and modulates a function, such as an interaction with a binding partner, and consequently mediates destruction of the tumor cells and/or inhibits the growth of the tumor cells. Mechanisms by which such antibodies exert a therapeutic effect can include complement-mediated cytotoxicity, antibody-dependent cellular cytotoxicity, modulation of the physiological function of 158P1D7, inhibition of ligand binding or signal transduction pathways, modulation of tumor cell differentiation, alteration of tumor angiogenesis factor profiles, and/or apoptosis.

Those skilled in the art understand that antibodies can be used to specifically target and bind immunogenic molecules such as an immunogenic region of the 158P1D7 sequence shown in Figure 2 or Figure 3. In addition, skilled artisans understand that it is routine to conjugate antibodies to cytotoxic agents (see, e.g., Slevers et al. *Blood* 93:11 3678-3684 (June 1, 1999)). When cytotoxic and/or therapeutic agents are delivered directly to cells, such as by conjugating them to antibodies specific for a molecule expressed by that cell (e.g. 158P1D7), the cytotoxic agent will exert its known biological effect (i.e. cytotoxicity) on those cells.

A wide variety of compositions and methods for using antibody-cytotoxic agent conjugates to kill cells are known in the art. In the context of cancers, typical methods entail administering to an animal having a tumor a biologically effective amount of a conjugate comprising a selected cytotoxic and/or therapeutic agent linked to a targeting agent (e.g. an anti-158P1D7 antibody) that binds to a marker (e.g. 158P1D7) expressed, accessible to binding or localized on the cell surfaces. A typical embodiment is a method of delivering a cytotoxic and/or therapeutic agent to a cell expressing 158P1D7, comprising conjugating the cytotoxic agent to an antibody that immunospecifically binds to a 158P1D7 epitope, and, exposing the cell to the antibody-agent conjugate. Another illustrative embodiment is a method of treating an individual suspected of suffering from metastasized cancer, comprising a step of administering parenterally to said individual a pharmaceutical composition comprising a therapeutically effective amount of an antibody conjugated to a cytotoxic and/or therapeutic agent.

Cancer immunotherapy using anti-158P1D7 antibodies can be done in accordance with various approaches that have been successfully employed in the treatment of other types of cancer, including but not limited to colon cancer (Arlen et al., 1998, *Crit. Rev. Immunol.* 18:133-138), multiple myeloma (Ozaki et al., 1997, *Blood* 90:3179-3186, Tsunenari et al., 1997, *Blood* 90:2437-2444), gastric cancer (Kasprzyk et al., 1992, *Cancer Res.* 52:2771-2776), B-cell lymphoma (Funakoshi et al., 1996, *J. Immunother. Emphasis*

Tumor Immunol. 19:93-101), leukemia (Zhong et al., 1996, Leuk. Res. 20:581-589), colorectal cancer (Moun et al., 1994, Cancer Res. 54:6160-6166; Velders et al., 1995, Cancer Res. 55:4398-4403), and breast cancer (Shepard et al., 1991, J. Clin. Immunol. 11:117-127). Some therapeutic approaches involve conjugation of naked antibody to a toxin, such as the conjugation of Y⁹¹ or I¹³¹ to anti-CD20 antibodies (e.g., ZevalinTM, IDEC Pharmaceuticals Corp. or BexxarTM, Coulter Pharmaceuticals), while others involve co-administration of antibodies and other therapeutic agents, such as HerceptinTM (trastuzumab) with paclitaxel (Genentech, Inc.). To treat bladder cancer, for example, 158P1D7 antibodies can be administered in conjunction with radiation, chemotherapy or hormone ablation.

Although 158P1D7 antibody therapy is useful for all stages of cancer, antibody therapy can be particularly appropriate in advanced or metastatic cancers. Treatment with the antibody therapy of the invention is indicated for patients who have received one or more rounds of chemotherapy. Alternatively, antibody therapy of the invention is combined with a chemotherapeutic or radiation regimen for patients who have not received chemotherapeutic treatment. Additionally, antibody therapy can enable the use of reduced dosages of concomitant chemotherapy, particularly for patients who do not tolerate the toxicity of the chemotherapeutic agent very well.

Cancer patients can be evaluated for the presence and level of 158P1D7 expression, preferably using immunohistochemical assessments of tumor tissue, quantitative 158P1D7 imaging, or other techniques that reliably indicate the presence and degree of 158P1D7 expression. Immunohistochemical analysis of tumor biopsies or surgical specimens is preferred for this purpose. Methods for immunohistochemical analysis of tumor tissues are well known in the art.

Anti-158P1D7 monoclonal antibodies that treat bladder and other cancers include those that initiate a potent immune response against the tumor or those that are directly cytotoxic. In this regard, anti-158P1D7 monoclonal antibodies (mAbs) can elicit tumor cell lysis by either complement-mediated or antibody-dependent cell cytotoxicity (ADCC) mechanisms, both of which require an intact Fc portion of the immunoglobulin molecule for interaction with effector cell Fc receptor sites on complement proteins. In addition, anti-158P1D7 mAbs that exert a direct biological effect on tumor growth are useful to treat cancers that express 158P1D7. Mechanisms by which directly cytotoxic mAbs act include: inhibition of cell growth, modulation of cellular differentiation, modulation of tumor angiogenesis factor profiles, and the induction of apoptosis. The mechanism(s) by which a particular anti-158P1D7 mAb exerts an anti-tumor effect is evaluated using any number of *in vitro* assays that evaluate cell death such as ADCC, ADMMC, complement-mediated cell lysis, and so forth, as is generally known in the art.

In some patients, the use of murine or other non-human monoclonal antibodies, or human/mouse chimeric mAbs can induce moderate to strong immune responses against the non-human antibody. This can result in clearance of the antibody from circulation and reduced efficacy. In the most severe cases, such an immune response can lead to the extensive formation of immune complexes which, potentially, can cause renal failure. Accordingly, preferred monoclonal antibodies used in the therapeutic methods of the

invention are those that are either fully human or humanized and that bind specifically to the target 158P1D7 antigen with high affinity but exhibit low or no antigenicity in the patient.

Therapeutic methods of the invention contemplate the administration of single anti-158P1D7 mAbs as well as combinations, or cocktails, of different mAbs. Such mAb cocktails can have certain advantages inasmuch as they contain mAbs that target different epitopes, exploit different effector mechanisms or combine directly cytotoxic mAbs with mAbs that rely on immune effector functionality. Such mAbs in combination can exhibit synergistic therapeutic effects. In addition, anti-158P1D7 mAbs can be administered concomitantly with other therapeutic modalities, including but not limited to various chemotherapeutic agents, androgen-blockers, immune modulators (e.g., IL-2, GM-CSF), surgery or radiation. The anti-158P1D7 mAbs are administered in their "naked" or unconjugated form, or can have a therapeutic agent(s) conjugated to them.

Anti-158P1D7 antibody formulations are administered via any route capable of delivering the antibodies to a tumor cell. Routes of administration include, but are not limited to, intravenous, intraperitoneal, intramuscular, intratumor, intradermal, and the like. Treatment generally involves repeated administration of the anti-158P1D7 antibody preparation, via an acceptable route of administration such as intravenous injection (IV), typically at a dose in the range of about 0.1 to about 10 mg/kg body weight. In general, doses in the range of 10-500 mg mAb per week are effective and well tolerated.

Based on clinical experience with the Herceptin mAb in the treatment of metastatic breast cancer, an initial loading dose of approximately 4 mg/kg patient body weight IV, followed by weekly doses of about 2 mg/kg IV of the anti- 158P1D7 mAb preparation represents an acceptable dosing regimen. Preferably, the initial loading dose is administered as a 90 minute or longer infusion. The periodic maintenance dose is administered as a 30 minute or longer infusion, provided the initial dose was well tolerated. As appreciated by those of skill in the art, various factors can influence the ideal dose regimen in a particular case. Such factors include, for example, the binding affinity and half life of the Ab or mAbs used, the degree of 158P1D7 expression in the patient, the extent of circulating shed 158P1D7 antigen, the desired steady-state antibody concentration level, frequency of treatment, and the influence of chemotherapeutic or other agents used in combination with the treatment method of the invention, as well as the health status of a particular patient.

Optionally, patients should be evaluated for the levels of 158P1D7 in a given sample (e.g. the levels of circulating 158P1D7 antigen and/or 158P1D7 expressing cells) in order to assist in the determination of the most effective dosing regimen, etc. Such evaluations are also used for monitoring purposes throughout therapy, and are useful to gauge therapeutic success in combination with the evaluation of other parameters (for example, urine cytology and/or ImmunoCyt levels in bladder cancer therapy, or by analogy, serum PSA levels in prostate cancer therapy).

Anti-idiotypic anti-158P1D7 antibodies can also be used in anti-cancer therapy as a vaccine for inducing an immune response to cells expressing a 158P1D7-related protein. In particular, the generation of anti-idiotypic antibodies is well known in the art; this methodology can readily be adapted to generate

anti-idiotypic anti-158P1D7 antibodies that mimic an epitope on a 158P1D7-related protein (see, for example, Wagner et al., 1997, Hybridoma 16: 33-40; Foon et al., 1995, J. Clin. Invest. 96:334-342; Herlyn et al., 1996, Cancer Immunol. Immunother. 43:65-76). Such an anti-idiotypic antibody can be used in cancer vaccine strategies.

X.C.) 158P1D7 as a Target for Cellular Immune Responses

Vaccines and methods of preparing vaccines that contain an immunogenically effective amount of one or more HLA-binding peptides as described herein are further embodiments of the invention. Furthermore, vaccines in accordance with the invention encompass compositions of one or more of the claimed peptides. A peptide can be present in a vaccine individually. Alternatively, the peptide can exist as a homopolymer comprising multiple copies of the same peptide, or as a heteropolymer of various peptides. Polymers have the advantage of increased immunological reaction and, where different peptide epitopes are used to make up the polymer, the additional ability to induce antibodies and/or CTLs that react with different antigenic determinants of the pathogenic organism or tumor-related peptide targeted for an immune response. The composition can be a naturally occurring region of an antigen or can be prepared, *e.g.*, recombinantly or by chemical synthesis.

Carriers that can be used with vaccines of the invention are well known in the art, and include, *e.g.*, thyroglobulin, albumins such as human serum albumin, tetanus toxoid, polyamino acids such as poly L-lysine, poly L-glutamic acid, influenza, hepatitis B virus core protein, and the like. The vaccines can contain a physiologically tolerable (*i.e.*, acceptable) diluent such as water, or saline, preferably phosphate buffered saline. The vaccines also typically include an adjuvant. Adjuvants such as incomplete Freund's adjuvant, aluminum phosphate, aluminum hydroxide, or alum are examples of materials well known in the art. Additionally, as disclosed herein, CTL responses can be primed by conjugating peptides of the invention to lipids, such as tripalmitoyl-S-glycerylcysteinylserine (P₃CSS). Moreover, an adjuvant such as a synthetic cytosine-phosphorothiolated-guanine-containing (CpG) oligonucleotides has been found to increase CTL responses 10- to 100-fold. (see, *e.g.* Davila and Celis *J. Immunol.* 165:539-547 (2000))

Upon immunization with a peptide composition in accordance with the invention, via injection, aerosol, oral, transdermal, transmucosal, intrapleural, intrathecal, or other suitable routes, the immune system of the host responds to the vaccine by producing large amounts of CTLs and/or HTLs specific for the desired antigen. Consequently, the host becomes at least partially immune to later development of cells that express or overexpress 158P1D7 antigen, or derives at least some therapeutic benefit when the antigen was tumor-associated.

In some embodiments, it may be desirable to combine the class I peptide components with components that induce or facilitate neutralizing antibody and or helper T cell responses directed to the target antigen. A preferred embodiment of such a composition comprises class I and class II epitopes in accordance with the invention. An alternative embodiment of such a composition comprises a class I and/or class II epitope in accordance with the invention, along with a cross reactive HTL epitope such as PADRE™ (Epimmune, San Diego, CA) molecule (described *e.g.*, in U.S. Patent Number 5,736,142).

A vaccine of the invention can also include antigen-presenting cells (APC), such as dendritic cells (DC), as a vehicle to present peptides of the invention. Vaccine compositions can be created *in vitro*, following dendritic cell mobilization and harvesting, whereby loading of dendritic cells occurs *in vitro*. For example, dendritic cells are transfected, *e.g.*, with a minigene in accordance with the invention, or are pulsed with peptides. The dendritic cell can then be administered to a patient to elicit immune responses *in vivo*. Vaccine compositions, either DNA- or peptide-based, can also be administered *in vivo* in combination with dendritic cell mobilization whereby loading of dendritic cells occurs *in vivo*.

Preferably, the following principles are utilized when selecting an array of epitopes for inclusion in a polyepitopic composition for use in a vaccine, or for selecting discrete epitopes to be included in a vaccine and/or to be encoded by nucleic acids such as a minigene. It is preferred that each of the following principles be balanced in order to make the selection. The multiple epitopes to be incorporated in a given vaccine composition may be, but need not be, contiguous in sequence in the native antigen from which the epitopes are derived.

1.) Epitopes are selected which, upon administration, mimic immune responses that have been observed to be correlated with tumor clearance. For HLA Class I this includes 3-4 epitopes that come from at least one tumor associated antigen (TAA). For HLA Class II a similar rationale is employed; again 3-4 epitopes are selected from at least one TAA (*see, e.g., Rosenberg et al., Science 278:1447-1450*). Epitopes from one TAA may be used in combination with epitopes from one or more additional TAAs to produce a vaccine that targets tumors with varying expression patterns of frequently-expressed TAAs.

2.) Epitopes are selected that have the requisite binding affinity established to be correlated with immunogenicity: for HLA Class I an IC_{50} of 500 nM or less, often 200 nM or less; and for Class II an IC_{50} of 1000 nM or less.

3.) Sufficient supermotif bearing-peptides, or a sufficient array of allele-specific motif-bearing peptides, are selected to give broad population coverage. For example, it is preferable to have at least 80% population coverage. A Monte Carlo analysis, a statistical evaluation known in the art, can be employed to assess the breadth, or redundancy of, population coverage.

4.) When selecting epitopes from cancer-related antigens it is often useful to select analogs because the patient may have developed tolerance to the native epitope.

5.) Of particular relevance are epitopes referred to as "nested epitopes." Nested epitopes occur where at least two epitopes overlap in a given peptide sequence. A nested peptide sequence can comprise B cell, HLA class I and/or HLA class II epitopes. When providing nested epitopes, a general objective is to provide the greatest number of epitopes per sequence. Thus, an aspect is to avoid providing a peptide that is any longer than the amino terminus of the amino terminal epitope and the carboxyl terminus of the carboxyl terminal epitope in the peptide. When providing a multi-epitopic sequence, such as a sequence comprising nested epitopes, it is generally important to screen the sequence in order to insure that it does not have pathological or other deleterious biological properties.

6.) If a polyepitopic protein is created, or when creating a minigene, an objective is to generate the smallest peptide that encompasses the epitopes of interest. This principle is similar, if not the same as that employed when selecting a peptide comprising nested epitopes. However, with an artificial polyepitopic peptide, the size minimization objective is balanced against the need to integrate any spacer sequences between epitopes in the polyepitopic protein. Spacer amino acid residues can, for example, be introduced to avoid junctional epitopes (an epitope recognized by the immune system, not present in the target antigen, and only created by the man-made juxtaposition of epitopes), or to facilitate cleavage between epitopes and thereby enhance epitope presentation. Junctional epitopes are generally to be avoided because the recipient may generate an immune response to that non-native epitope. Of particular concern is a junctional epitope that is a "dominant epitope." A dominant epitope may lead to such a zealous response that immune responses to other epitopes are diminished or suppressed.

7.) Where the sequences of multiple variants of the same target protein are present, potential peptide epitopes can also be selected on the basis of their conservancy. For example, a criterion for conservancy may define that the entire sequence of an HLA class I binding peptide or the entire 9-mer core of a class II binding peptide be conserved in a designated percentage of the sequences evaluated for a specific protein antigen.

X.C.1. Minigene Vaccines

A number of different approaches are available which allow simultaneous delivery of multiple epitopes. Nucleic acids encoding the peptides of the invention are a particularly useful embodiment of the invention. Epitopes for inclusion in a minigene are preferably selected according to the guidelines set forth in the previous section. A preferred means of administering nucleic acids encoding the peptides of the invention uses minigene constructs encoding a peptide comprising one or multiple epitopes of the invention.

The use of multi-epitope minigenes is described below and in, Ishioka *et al.*, *J. Immunol.* 162:3915-3925, 1999; An, L. and Whitton, J. L., *J. Virol.* 71:2292, 1997; Thomson, S. A. *et al.*, *J. Immunol.* 157:822, 1996; Whitton, J. L. *et al.*, *J. Virol.* 67:348, 1993; Hanke, R. *et al.*, *Vaccine* 16:426, 1998. For example, a multi-epitope DNA plasmid encoding supermotif- and/or motif-bearing epitopes derived 158P1D7, the PADRE® universal helper T cell epitope (or multiple HTL epitopes from 158P1D7), and an endoplasmic reticulum-translocating signal sequence can be engineered. A vaccine may also comprise epitopes that are derived from other TAAs.

The immunogenicity of a multi-epitopic minigene can be confirmed in transgenic mice to evaluate the magnitude of CTL induction responses against the epitopes tested. Further, the immunogenicity of DNA-encoded epitopes *in vivo* can be correlated with the *in vitro* responses of specific CTL lines against target cells transfected with the DNA plasmid. Thus, these experiments can show that the minigene serves to both: 1.) generate a CTL response and 2.) that the induced CTLs recognized cells expressing the encoded epitopes.

For example, to create a DNA sequence encoding the selected epitopes (minigene) for expression in human cells, the amino acid sequences of the epitopes may be reverse translated. A human codon usage table can be used to guide the codon choice for each amino acid. These epitope-encoding DNA sequences may be directly adjoined, so that when translated, a continuous polypeptide sequence is created. To optimize expression and/or immunogenicity, additional elements can be incorporated into the minigene design. Examples of amino acid sequences that can be reverse translated and included in the minigene sequence include: HLA class I epitopes, HLA class II epitopes, antibody epitopes, a ubiquitination signal sequence, and/or an endoplasmic reticulum targeting signal. In addition, HLA presentation of CTL and HTL epitopes may be improved by including synthetic (*e.g.* poly-alanine) or naturally-occurring flanking sequences adjacent to the CTL or HTL epitopes; these larger peptides comprising the epitope(s) are within the scope of the invention.

The minigene sequence may be converted to DNA by assembling oligonucleotides that encode the plus and minus strands of the minigene. Overlapping oligonucleotides (30-100 bases long) may be synthesized, phosphorylated, purified and annealed under appropriate conditions using well known techniques. The ends of the oligonucleotides can be joined, for example, using T4 DNA ligase. This synthetic minigene, encoding the epitope polypeptide, can then be cloned into a desired expression vector.

Standard regulatory sequences well known to those of skill in the art are preferably included in the vector to ensure expression in the target cells. Several vector elements are desirable: a promoter with a down-stream cloning site for minigene insertion; a polyadenylation signal for efficient transcription termination; an *E. coli* origin of replication; and an *E. coli* selectable marker (*e.g.* ampicillin or kanamycin resistance). Numerous promoters can be used for this purpose, *e.g.*, the human cytomegalovirus (hCMV) promoter. See, *e.g.*, U.S. Patent Nos. 5,580,859 and 5,589,466 for other suitable promoter sequences.

Additional vector modifications may be desired to optimize minigene expression and immunogenicity. In some cases, introns are required for efficient gene expression, and one or more synthetic or naturally-occurring introns could be incorporated into the transcribed region of the minigene. The inclusion of mRNA stabilization sequences and sequences for replication in mammalian cells may also be considered for increasing minigene expression.

Once an expression vector is selected, the minigene is cloned into the polylinker region downstream of the promoter. This plasmid is transformed into an appropriate *E. coli* strain, and DNA is prepared using standard techniques. The orientation and DNA sequence of the minigene, as well as all other elements included in the vector, are confirmed using restriction mapping and DNA sequence analysis. Bacterial cells harboring the correct plasmid can be stored as a master cell bank and a working cell bank.

In addition, immunostimulatory sequences (ISSs or CpGs) appear to play a role in the immunogenicity of DNA vaccines. These sequences may be included in the vector, outside the minigene coding sequence, if desired to enhance immunogenicity.

In some embodiments, a bi-cistronic expression vector which allows production of both the minigene-encoded epitopes and a second protein (included to enhance or decrease immunogenicity) can be

used. Examples of proteins or polypeptides that could beneficially enhance the immune response if co-expressed include cytokines (e.g., IL-2, IL-12, GM-CSF), cytokine-inducing molecules (e.g., LeIF), costimulatory molecules, or for HTL responses, pan-DR binding proteins (PADRE™, Epimmune, San Diego, CA). Helper (HTL) epitopes can be joined to intracellular targeting signals and expressed separately from expressed CTL epitopes; this allows direction of the HTL epitopes to a cell compartment different than that of the CTL epitopes. If required, this could facilitate more efficient entry of HTL epitopes into the HLA class II pathway, thereby improving HTL induction. In contrast to HTL or CTL induction, specifically decreasing the immune response by co-expression of immunosuppressive molecules (e.g. TGF- β) may be beneficial in certain diseases.

Therapeutic quantities of plasmid DNA can be produced for example, by fermentation in *E. coli*, followed by purification. Aliquots from the working cell bank are used to inoculate growth medium, and grown to saturation in shaker flasks or a bioreactor according to well-known techniques. Plasmid DNA can be purified using standard bioseparation technologies such as solid phase anion-exchange resins supplied by QIAGEN, Inc. (Valencia, California). If required, supercoiled DNA can be isolated from the open circular and linear forms using gel electrophoresis or other methods.

Purified plasmid DNA can be prepared for injection using a variety of formulations. The simplest of these is reconstitution of lyophilized DNA in sterile phosphate-buffer saline (PBS). This approach, known as "naked DNA," is currently being used for intramuscular (IM) administration in clinical trials. To maximize the immunotherapeutic effects of minigene DNA vaccines, an alternative method for formulating purified plasmid DNA may be desirable. A variety of methods have been described, and new techniques may become available. Cationic lipids, glycolipids, and fusogenic liposomes can also be used in the formulation (see, e.g., as described by WO 93/24640; Mannino & Gould-Fogerite, *BioTechniques* 6(7): 682 (1988); U.S. Pat No. 5,279,833; WO 91/06309; and Felgner, *et al.*, *Proc. Nat'l Acad. Sci. USA* 84:7413 (1987). In addition, peptides and compounds referred to collectively as protective, interactive, non-condensing compounds (PINC) could also be complexed to purified plasmid DNA to influence variables such as stability, intramuscular dispersion, or trafficking to specific organs or cell types.

Target cell sensitization can be used as a functional assay for expression and HLA class I presentation of minigene-encoded CTL epitopes. For example, the plasmid DNA is introduced into a mammalian cell line that is suitable as a target for standard CTL chromium release assays. The transfection method used will be dependent on the final formulation. Electroporation can be used for "naked" DNA, whereas cationic lipids allow direct *in vitro* transfection. A plasmid expressing green fluorescent protein (GFP) can be co-transfected to allow enrichment of transfected cells using fluorescence activated cell sorting (FACS). These cells are then chromium-51 (^{51}Cr) labeled and used as target cells for epitope-specific CTL lines; cytolysis, detected by ^{51}Cr release, indicates both production of, and HLA presentation of, minigene-encoded CTL epitopes. Expression of HTL epitopes may be evaluated in an analogous manner using assays to assess HTL activity.

In vivo immunogenicity is a second approach for functional testing of minigene DNA formulations. Transgenic mice expressing appropriate human HLA proteins are immunized with the DNA product. The dose and route of administration are formulation dependent (*e.g.*, IM for DNA in PBS, intraperitoneal (i.p.) for lipid-complexed DNA). Twenty-one days after immunization, splenocytes are harvested and restimulated for one week in the presence of peptides encoding each epitope being tested. Thereafter, for CTL effector cells, assays are conducted for cytolysis of peptide-loaded, ⁵¹Cr-labeled target cells using standard techniques. Lysis of target cells that were sensitized by HLA loaded with peptide epitopes, corresponding to minigene-encoded epitopes, demonstrates DNA vaccine function for *in vivo* induction of CTLs. Immunogenicity of HTL epitopes is confirmed in transgenic mice in an analogous manner.

Alternatively, the nucleic acids can be administered using ballistic delivery as described, for instance, in U.S. Patent No. 5,204,253. Using this technique, particles comprised solely of DNA are administered. In a further alternative embodiment, DNA can be adhered to particles, such as gold particles.

Minigenes can also be delivered using other bacterial or viral delivery systems well known in the art, *e.g.*, an expression construct encoding epitopes of the invention can be incorporated into a viral vector such as vaccinia.

X.C.2. Combinations of CTL Peptides with Helper Peptides

Vaccine compositions comprising CTL peptides of the invention can be modified, *e.g.*, analoged, to provide desired attributes, such as improved serum half life, broadened population coverage or enhanced immunogenicity.

For instance, the ability of a peptide to induce CTL activity can be enhanced by linking the peptide to a sequence which contains at least one epitope that is capable of inducing a T helper cell response. Although a CTL peptide can be directly linked to a T helper peptide, often CTL epitope/HTL epitope conjugates are linked by a spacer molecule. The spacer is typically comprised of relatively small, neutral molecules, such as amino acids or amino acid mimetics, which are substantially uncharged under physiological conditions. The spacers are typically selected from, *e.g.*, Ala, Gly, or other neutral spacers of nonpolar amino acids or neutral polar amino acids. It will be understood that the optionally present spacer need not be comprised of the same residues and thus may be a hetero- or homo-oligomer. When present, the spacer will usually be at least one or two residues, more usually three to six residues and sometimes 10 or more residues. The CTL peptide epitope can be linked to the T helper peptide epitope either directly or via a spacer either at the amino or carboxy terminus of the CTL peptide. The amino terminus of either the immunogenic peptide or the T helper peptide may be acylated.

In certain embodiments, the T helper peptide is one that is recognized by T helper cells present in a majority of a genetically diverse population. This can be accomplished by selecting peptides that bind to many, most, or all of the HLA class II molecules. Examples of such amino acid bind many HLA Class II molecules include sequences from antigens such as tetanus toxoid at positions 830-843 (QYIKANSKFIGITE; SEQ ID NO: 651), *Plasmodium falciparum* circumsporozoite (CS) protein at

positions 378-398 (DIEKKIAKMEKASSVFNVVNS; SEQ ID NO: 652), and *Streptococcus* 18kD protein at positions 116-131 (GAVDSILGGVATYGAA; SEQ ID NO: 653). Other examples include peptides bearing a DR 1-4-7 supermotif, or either of the DR3 motifs.

Alternatively, it is possible to prepare synthetic peptides capable of stimulating T helper lymphocytes, in a loosely HLA-restricted fashion, using amino acid sequences not found in nature (*see, e.g.,* PCT publication WO 95/07707). These synthetic compounds called Pan-DR-binding epitopes (*e.g.,* PADRE™, Epimmune, Inc., San Diego, CA) are designed to most preferably bind most HLA-DR (human HLA class II) molecules. For instance, a pan-DR-binding epitope peptide having the formula: aKXVAAWTLKAAa (SEQ ID NO: 654), where "X" is either cyclohexylalanine, phenylalanine, or tyrosine, and a is either D-alanine or L-alanine, has been found to bind to most HLA-DR alleles, and to stimulate the response of T helper lymphocytes from most individuals, regardless of their HLA type. An alternative of a pan-DR binding epitope comprises all "L" natural amino acids and can be provided in the form of nucleic acids that encode the epitope.

HTL peptide epitopes can also be modified to alter their biological properties. For example, they can be modified to include D-amino acids to increase their resistance to proteases and thus extend their serum half life, or they can be conjugated to other molecules such as lipids, proteins, carbohydrates, and the like to increase their biological activity. For example, a T helper peptide can be conjugated to one or more palmitic acid chains at either the amino or carboxyl termini.

X.C.3. Combinations of CTL Peptides with T Cell Priming Agents

In some embodiments it may be desirable to include in the pharmaceutical compositions of the invention at least one component which primes B lymphocytes or T lymphocytes. Lipids have been identified as agents capable of priming CTL *in vivo*. For example, palmitic acid residues can be attached to the ϵ - and α - amino groups of a lysine residue and then linked, *e.g.,* via one or more linking residues such as Gly, Gly-Gly-, Ser, Ser-Ser, or the like, to an immunogenic peptide. The lipidated peptide can then be administered either directly in a micelle or particle, incorporated into a liposome, or emulsified in an adjuvant, *e.g.,* incomplete Freund's adjuvant. In a preferred embodiment, a particularly effective immunogenic composition comprises palmitic acid attached to ϵ - and α - amino groups of Lys, which is attached via linkage, *e.g.,* Ser-Ser, to the amino terminus of the immunogenic peptide.

As another example of lipid priming of CTL responses, *E. coli* lipoproteins, such as tripalmitoyl-S-glycerylcysteinylserine (P₃CSS) can be used to prime virus specific CTL when covalently attached to an appropriate peptide (*see, e.g.,* Deres, *et al., Nature* 342:561, 1989). Peptides of the invention can be coupled to P₃CSS, for example, and the lipopeptide administered to an individual to specifically prime an immune response to the target antigen. Moreover, because the induction of neutralizing antibodies can also be primed with P₃CSS-conjugated epitopes, two such compositions can be combined to more effectively elicit both humoral and cell-mediated responses.

X.C.4. Vaccine Compositions Comprising DC Pulsed with CTL and/or HTL Peptides

An embodiment of a vaccine composition in accordance with the invention comprises *ex vivo* administration of a cocktail of epitope-bearing peptides to PBMC, or isolated DC therefrom, from the patient's blood. A pharmaceutical to facilitate harvesting of DC can be used, such as ProgenipoiectinTM (Pharmacia-Monsanto, St. Louis, MO) or GM-CSF/IL-4. After pulsing the DC with peptides and prior to reinfusion into patients, the DC are washed to remove unbound peptides. In this embodiment, a vaccine comprises peptide-pulsed DCs which present the pulsed peptide epitopes complexed with HLA molecules on their surfaces.

The DC can be pulsed *ex vivo* with a cocktail of peptides, some of which stimulate CTL responses to 158P1D7. Optionally, a helper T cell (HTL) peptide, such as a natural or artificial loosely restricted HLA Class II peptide, can be included to facilitate the CTL response. Thus, a vaccine in accordance with the invention is used to treat a cancer which expresses or overexpresses 158P1D7.

X.D. Adoptive Immunotherapy

Antigenic 158P1D7-related peptides are used to elicit a CTL and/or HTL response *ex vivo*, as well. The resulting CTL or HTL cells, can be used to treat tumors in patients that do not respond to other conventional forms of therapy, or will not respond to a therapeutic vaccine peptide or nucleic acid in accordance with the invention. *Ex vivo* CTL or HTL responses to a particular antigen are induced by incubating in tissue culture the patient's, or genetically compatible, CTL or HTL precursor cells together with a source of antigen-presenting cells (APC), such as dendritic cells, and the appropriate immunogenic peptide. After an appropriate incubation time (typically about 7-28 days), in which the precursor cells are activated and expanded into effector cells, the cells are infused back into the patient, where they will destroy (CTL) or facilitate destruction (HTL) of their specific target cell (e.g., a tumor cell). Transfected dendritic cells may also be used as antigen presenting cells.

X.E. Administration of Vaccines for Therapeutic or Prophylactic Purposes

Pharmaceutical and vaccine compositions of the invention are typically used to treat and/or prevent a cancer that expresses or overexpresses 158P1D7. In therapeutic applications, peptide and/or nucleic acid compositions are administered to a patient in an amount sufficient to elicit an effective B cell, CTL and/or HTL response to the antigen and to cure or at least partially arrest or slow symptoms and/or complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on, e.g., the particular composition administered, the manner of administration, the stage and severity of the disease being treated, the weight and general state of health of the patient, and the judgment of the prescribing physician.

For pharmaceutical compositions, the immunogenic peptides of the invention, or DNA encoding them, are generally administered to an individual already bearing a tumor that expresses 158P1D7. The peptides or DNA encoding them can be administered individually or as fusions of one or more peptide sequences. Patients can be treated with the immunogenic peptides separately or in conjunction with other treatments, such as surgery, as appropriate.

For therapeutic use, administration should generally begin at the first diagnosis of 158P1D7-associated cancer. This is followed by boosting doses until at least symptoms are substantially abated and for a period thereafter. The embodiment of the vaccine composition (*i.e.*, including, but not limited to embodiments such as peptide cocktails, polyepitopic polypeptides, minigenes, or TAA-specific CTLs or pulsed dendritic cells) delivered to the patient may vary according to the stage of the disease or the patient's health status. For example, in a patient with a tumor that expresses 158P1D7, a vaccine comprising 158P1D7-specific CTL may be more efficacious in killing tumor cells in patient with advanced disease than alternative embodiments.

It is generally important to provide an amount of the peptide epitope delivered by a mode of administration sufficient to effectively stimulate a cytotoxic T cell response; compositions which stimulate helper T cell responses can also be given in accordance with this embodiment of the invention.

The dosage for an initial therapeutic immunization generally occurs in a unit dosage range where the lower value is about 1, 5, 50, 500, or 1,000 μg and the higher value is about 10,000; 20,000; 30,000; or 50,000 μg . Dosage values for a human typically range from about 500 μg to about 50,000 μg per 70 kilogram patient. Boosting dosages of between about 1.0 μg to about 50,000 μg of peptide pursuant to a boosting regimen over weeks to months may be administered depending upon the patient's response and condition as determined by measuring the specific activity of CTL and HTL obtained from the patient's blood. Administration should continue until at least clinical symptoms or laboratory tests indicate that the neoplasia, has been eliminated or reduced and for a period thereafter. The dosages, routes of administration, and dose schedules are adjusted in accordance with methodologies known in the art.

In certain embodiments, the peptides and compositions of the present invention are employed in serious disease states, that is, life-threatening or potentially life threatening situations. In such cases, as a result of the minimal amounts of extraneous substances and the relative nontoxic nature of the peptides in preferred compositions of the invention, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these peptide compositions relative to these stated dosage amounts.

The vaccine compositions of the invention can also be used purely as prophylactic agents. Generally the dosage for an initial prophylactic immunization generally occurs in a unit dosage range where the lower value is about 1, 5, 50, 500, or 1000 μg and the higher value is about 10,000; 20,000; 30,000; or 50,000 μg . Dosage values for a human typically range from about 500 μg to about 50,000 μg per 70 kilogram patient. This is followed by boosting dosages of between about 1.0 μg to about 50,000 μg of peptide administered at defined intervals from about four weeks to six months after the initial administration of vaccine. The immunogenicity of the vaccine can be assessed by measuring the specific activity of CTL and HTL obtained from a sample of the patient's blood.

The pharmaceutical compositions for therapeutic treatment are intended for parenteral, topical, oral, nasal, intrathecal, or local (*e.g.* as a cream or topical ointment) administration. Preferably, the pharmaceutical compositions are administered parentally, *e.g.*, intravenously, subcutaneously, intradermally, or intramuscularly. Thus, the invention provides compositions for parenteral administration

which comprise a solution of the immunogenic peptides dissolved or suspended in an acceptable carrier, preferably an aqueous carrier.

A variety of aqueous carriers may be used, *e.g.*, water, buffered water, 0.8% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilized by conventional, well-known sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration.

The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH-adjusting and buffering agents, tonicity adjusting agents, wetting agents, preservatives, and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, *etc.*

The concentration of peptides of the invention in the pharmaceutical formulations can vary widely, *i.e.*, from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, *etc.*, in accordance with the particular mode of administration selected.

A human unit dose form of the peptide composition is typically included in a pharmaceutical composition that comprises a human unit dose of an acceptable carrier, preferably an aqueous carrier, and is administered in a volume of fluid that is known by those of skill in the art to be used for administration of such compositions to humans (*see, e.g.*, Remington's Pharmaceutical Sciences, 17th Edition, A. Gennaro, Editor, Mack Publishing Co., Easton, Pennsylvania, 1985).

Proteins(s) of the invention, and/or nucleic acids encoding the protein(s), can also be administered via liposomes, which may also serve to: 1) target the proteins(s) to a particular tissue, such as lymphoid tissue; 2) to target selectively to diseases cells; or, 3) to increase the half-life of the peptide composition. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. In these preparations, the peptide to be delivered is incorporated as part of a liposome, alone or in conjunction with a molecule which binds to a receptor prevalent among lymphoid cells, such as monoclonal antibodies which bind to the CD45 antigen, or with other therapeutic or immunogenic compositions. Thus, liposomes either filled or decorated with a desired peptide of the invention can be directed to the site of lymphoid cells, where the liposomes then deliver the peptide compositions. Liposomes for use in accordance with the invention are formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of, *e.g.*, liposome size, acid lability and stability of the liposomes in the blood stream. A variety of methods are available for preparing liposomes, as described in, *e.g.*, Szoka, *et al.*, *Ann. Rev. Biophys. Bioeng.* 9:467 (1980), and U.S. Patent Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369.

For targeting cells of the immune system, a ligand to be incorporated into the liposome can include, *e.g.*, antibodies or fragments thereof specific for cell surface determinants of the desired immune

system cells. A liposome suspension containing a peptide may be administered intravenously, locally, topically, *etc.* in a dose which varies according to, *inter alia*, the manner of administration, the peptide being delivered, and the stage of the disease being treated.

For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient, that is, one or more peptides of the invention, and more preferably at a concentration of 25%-75%.

For aerosol administration, immunogenic peptides are preferably supplied in finely divided form along with a surfactant and propellant. Typical percentages of peptides are about 0.01%-20% by weight, preferably about 1%-10%. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from about 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. The surfactant may constitute about 0.1%-20% by weight of the composition, preferably about 0.25-5%. The balance of the composition is ordinarily propellant. A carrier can also be included, as desired, as with, *e.g.*, lecithin for intranasal delivery.

XI.) Diagnostic and Prognostic Embodiments of 158P1D7.

As disclosed herein, 158P1D7 polynucleotides, polypeptides, reactive cytotoxic T cells (CTL), reactive helper T cells (HTL) and anti-polypeptide antibodies are used in well known diagnostic, prognostic and therapeutic assays that examine conditions associated with dysregulated cell growth such as cancer, in particular the cancers listed in Table I (see, *e.g.*, both its specific pattern of tissue expression as well as its overexpression in certain cancers as described for example in Example 4).

158P1D7 can be used in a manner analogous to, or as complementary to, the bladder associated antigen combination, mucins and CEA, represented in a diagnostic kit called ImmunoCytTM. ImmunoCyt is a commercially available assay to identify and monitor the presence of bladder cancer (see Fradet et al., 1997, Can J Urol, 4(3):400-405). A variety of other diagnostic markers are also used in similar contexts including p53 and H-ras (see, *e.g.*, Tulchinsky et al., Int J Mol Med 1999 Jul 4(1):99-102 and Minimoto et al., Cancer Detect Prev 2000;24(1):1-12). Therefore, this disclosure of the 158P1D7 polynucleotides and polypeptides (as well as the 158P1D7 polynucleotide probes and anti-158P1D7 antibodies used to identify the presence of these molecules) and their properties allows skilled artisans to utilize these molecules in methods that are analogous to those used, for example, in a variety of diagnostic assays directed to examining conditions associated with cancer.

Typical embodiments of diagnostic methods which utilize the 158P1D7 polynucleotides, polypeptides, reactive T cells and antibodies are analogous to those methods from well-established

diagnostic assays which employ, e.g., PSA polynucleotides, polypeptides, reactive T cells and antibodies. For example, just as PSA polynucleotides are used as probes (for example in Northern analysis, see, e.g., Sharief et al., *Biochem. Mol. Biol. Int.* 33(3):567-74(1994)) and primers (for example in PCR analysis, see, e.g., Okegawa et al., *J. Urol.* 163(4): 1189-1190 (2000)) to observe the presence and/or the level of PSA mRNAs in methods of monitoring PSA overexpression or the metastasis of prostate cancers, the 158P1D7 polynucleotides described herein can be utilized to detect 158P1D7 overexpression or the metastasis of bladder and other cancers expressing this gene. Alternatively, just as PSA polypeptides are used to generate antibodies specific for PSA which can then be used to observe the presence and/or the level of PSA proteins in methods to monitor PSA protein overexpression (see, e.g., Stephan et al., *Urology* 55(4):560-3 (2000)) or the metastasis of prostate cells (see, e.g., Alanen et al., *Pathol. Res. Pract.* 192(3):233-7 (1996)), the 158P1D7 polypeptides described herein can be utilized to generate antibodies for use in detecting 158P1D7 overexpression or the metastasis of bladder cells and cells of other cancers expressing this gene.

Specifically, because metastases involves the movement of cancer cells from an organ of origin (such as the lung or bladder etc.) to a different area of the body (such as a lymph node), assays which examine a biological sample for the presence of cells expressing 158P1D7 polynucleotides and/or polypeptides can be used to provide evidence of metastasis. For example, when a biological sample from tissue that does not normally contain 158P1D7-expressing cells (lymph node) is found to contain 158P1D7-expressing cells such as the 158P1D7 expression seen in LAPC4 and LAPC9, xenografts isolated from lymph node and bone metastasis, respectively, this finding is indicative of metastasis.

Alternatively 158P1D7 polynucleotides and/or polypeptides can be used to provide evidence of cancer, for example, when cells in a biological sample that do not normally express 158P1D7 or express 158P1D7 at a different level are found to express 158P1D7 or have an increased expression of 158P1D7 (see, e.g., the 158P1D7 expression in the cancers listed in Table I and in patient samples etc. shown in the accompanying Figures). In such assays, artisans may further wish to generate supplementary evidence of metastasis by testing the biological sample for the presence of a second tissue restricted marker (in addition to 158P1D7) such as ImmunoCyt™, PSCA etc. (see, e.g., Fradet et al., 1997, *Can J Urol*, 4(3):400-405; Amara et al., 2001, *Cancer Res* 61:4660-4665). Just as PSA polynucleotide fragments and polynucleotide variants are employed by skilled artisans for use in methods of monitoring PSA, 158P1D7 polynucleotide fragments and polynucleotide variants are used in an analogous manner. In particular, typical PSA polynucleotides used in methods of monitoring PSA are probes or primers which consist of fragments of the PSA cDNA sequence. Illustrating this, primers used to PCR amplify a PSA polynucleotide must include less than the whole PSA sequence to function in the polymerase chain reaction. In the context of such PCR reactions, skilled artisans generally create a variety of different polynucleotide fragments that can be used as primers in order to amplify different portions of a polynucleotide of interest or to optimize amplification reactions (see, e.g., Caetano-Anolles, G. *Biotechniques* 25(3): 472-476, 478-480 (1998); Robertson et al., *Methods Mol. Biol.* 98:121-154 (1998)). An additional illustration of the use of such

fragments is provided in Example 4, where a 158P1D7 polynucleotide fragment is used as a probe to show the expression of 158P1D7 RNAs in cancer cells. In addition, variant polynucleotide sequences are typically used as primers and probes for the corresponding mRNAs in PCR and Northern analyses (see, e.g., Sawai et al., Fetal Diagn. Ther. 1996 Nov-Dec 11(6):407-13 and Current Protocols In Molecular Biology, Volume 2, Unit 2, Frederick M. Ausubel et al. eds., 1995)). Polynucleotide fragments and variants are useful in this context where they are capable of binding to a target polynucleotide sequence (e.g. the 158P1D7 polynucleotide shown in SEQ ID NO: 655) under conditions of high stringency.

Furthermore, PSA polypeptides which contain an epitope that can be recognized by an antibody or T cell that specifically binds to that epitope are used in methods of monitoring PSA. 158P1D7 polypeptide fragments and polypeptide analogs or variants can also be used in an analogous manner. This practice of using polypeptide fragments or polypeptide variants to generate antibodies (such as anti-PSA antibodies or T cells) is typical in the art with a wide variety of systems such as fusion proteins being used by practitioners (see, e.g., Current Protocols In Molecular Biology, Volume 2, Unit 16, Frederick M. Ausubel et al. eds., 1995). In this context, each epitope(s) functions to provide the architecture with which an antibody or T cell is reactive. Typically, skilled artisans create a variety of different polypeptide fragments that can be used in order to generate immune responses specific for different portions of a polypeptide of interest (see, e.g., U.S. Patent No. 5,840,501 and U.S. Patent No. 5,939,533). For example it may be preferable to utilize a polypeptide comprising one of the 158P1D7 biological motifs discussed herein or a motif-bearing subsequence which is readily identified by one of skill in the art based on motifs available in the art. Polypeptide fragments, variants or analogs are typically useful in this context as long as they comprise an epitope capable of generating an antibody or T cell specific for a target polypeptide sequence (e.g. the 158P1D7 polypeptide shown in SEQ ID NO: 657).

As shown herein, the 158P1D7 polynucleotides and polypeptides (as well as the 158P1D7 polynucleotide probes and anti-158P1D7 antibodies or T cells used to identify the presence of these molecules) exhibit specific properties that make them useful in diagnosing cancers such as those listed in Table I. Diagnostic assays that measure the presence of 158P1D7 gene products, in order to evaluate the presence or onset of a disease condition described herein, such as bladder cancer, are used to identify patients for preventive measures or further monitoring, as has been done so successfully with PSA for monitoring prostate cancer. Materials such as 158P1D7 polynucleotides and polypeptides (as well as the 158P1D7 polynucleotide probes and anti-158P1D7 antibodies used to identify the presence of these molecules) satisfy a need in the art for molecules having similar or complementary characteristics to PSA in situations of bladder cancer. Finally, in addition to their use in diagnostic assays, the 158P1D7 polynucleotides disclosed herein have a number of other utilities such as their use in the identification of oncogenetic associated chromosomal abnormalities in the chromosomal region to which the 158P1D7 gene maps (see Example 3 below). Moreover, in addition to their use in diagnostic assays, the 158P1D7-related proteins and polynucleotides disclosed herein have other utilities such as their use in the forensic analysis of tissues of unknown origin (see, e.g., Takahama K Forensic Sci Int 1996 Jun 28;80(1-2): 63-9).

Additionally, 158P1D7-related proteins or polynucleotides of the invention can be used to treat a pathologic condition characterized by the over-expression of 158P1D7. For example, the amino acid or nucleic acid sequence of Figure 2 or Figure 3, or fragments of either, can be used to generate an immune response to the 158P1D7 antigen. Antibodies or other molecules that react with 158P1D7 can be used to modulate the function of this molecule, and thereby provide a therapeutic benefit.

XII.) Inhibition of 158P1D7 Protein Function

The invention includes various methods and compositions for inhibiting the binding of 158P1D7 to its binding partner or its association with other protein(s) as well as methods for inhibiting 158P1D7 function.

XII.A.) Inhibition of 158P1D7 With Intracellular Antibodies

In one approach, a recombinant vector that encodes single chain antibodies that specifically bind to 158P1D7 are introduced into 158P1D7 expressing cells via gene transfer technologies. Accordingly, the encoded single chain anti-158P1D7 antibody is expressed intracellularly, binds to 158P1D7 protein, and thereby inhibits its function. Methods for engineering such intracellular single chain antibodies are well known. Such intracellular antibodies, also known as "intrabodies", are specifically targeted to a particular compartment within the cell, providing control over where the inhibitory activity of the treatment is focused. This technology has been successfully applied in the art (for review, see Richardson and Marasco, 1995, TIBTECH vol. 13). Intrabodies have been shown to virtually eliminate the expression of otherwise abundant cell surface receptors (see, e.g., Richardson et al., 1995, Proc. Natl. Acad. Sci. USA 92: 3137-3141; Beerli et al., 1994, J. Biol. Chem. 269: 23931-23936; Deshane et al., 1994, Gene Ther. 1: 332-337).

Single chain antibodies comprise the variable domains of the heavy and light chain joined by a flexible linker polypeptide, and are expressed as a single polypeptide. Optionally, single chain antibodies are expressed as a single chain variable region fragment joined to the light chain constant region. Well-known intracellular trafficking signals are engineered into recombinant polynucleotide vectors encoding such single chain antibodies in order to precisely target the intrabody to the desired intracellular compartment. For example, intrabodies targeted to the endoplasmic reticulum (ER) are engineered to incorporate a leader peptide and, optionally, a C-terminal ER retention signal, such as the KDEL amino acid motif. Intrabodies intended to exert activity in the nucleus are engineered to include a nuclear localization signal. Lipid moieties are joined to intrabodies in order to tether the intrabody to the cytosolic side of the plasma membrane. Intrabodies can also be targeted to exert function in the cytosol. For example, cytosolic intrabodies are used to sequester factors within the cytosol, thereby preventing them from being transported to their natural cellular destination.

In one embodiment, intrabodies are used to capture 158P1D7 in the nucleus, thereby preventing its activity within the nucleus. Nuclear targeting signals are engineered into such 158P1D7 intrabodies in order to achieve the desired targeting. Such 158P1D7 intrabodies are designed to bind specifically to a particular 158P1D7 domain. In another embodiment, cytosolic intrabodies that specifically bind to the

158P1D7 protein are used to prevent 158P1D7 from gaining access to the nucleus, thereby preventing it from exerting any biological activity within the nucleus (e.g., preventing 158P1D7 from forming transcription complexes with other factors).

In order to specifically direct the expression of such intrabodies to particular cells, the transcription of the intrabody is placed under the regulatory control of an appropriate tumor-specific promoter and/or enhancer. In order to target intrabody expression specifically to bladder, for example, the PSCA promoter and/or promoter/enhancer can be utilized (See, for example, U.S. Patent No. 5,919,652 issued 6 July 1999 and Lin et al. PNAS, USA 92(3):679-683 (1995)).

XII.B.) Inhibition of 158P1D7 with Recombinant Proteins

In another approach, recombinant molecules bind to 158P1D7 and thereby inhibit 158P1D7 function. For example, these recombinant molecules prevent or inhibit 158P1D7 from accessing/binding to its binding partner(s) or associating with other protein(s). Such recombinant molecules can, for example, contain the reactive part(s) of a 158P1D7 specific antibody molecule. In a particular embodiment, the 158P1D7 binding domain of a 158P1D7 binding partner is engineered into a dimeric fusion protein, whereby the fusion protein comprises two 158P1D7 ligand binding domains linked to the Fc portion of a human IgG, such as human IgG1. Such IgG portion can contain, for example, the C_H2 and C_H3 domains and the hinge region, but not the C_H1 domain. Such dimeric fusion proteins are administered in soluble form to patients suffering from a cancer associated with the expression of 158P1D7, whereby the dimeric fusion protein specifically binds to 158P1D7 and blocks 158P1D7 interaction with a binding partner. Such dimeric fusion proteins are further combined into multimeric proteins using known antibody linking technologies.

XII.C.) Inhibition of 158P1D7 Transcription or Translation

The present invention also comprises various methods and compositions for inhibiting the transcription of the 158P1D7 gene. Similarly, the invention also provides methods and compositions for inhibiting the translation of 158P1D7 mRNA into protein.

In one approach, a method of inhibiting the transcription of the 158P1D7 gene comprises contacting the 158P1D7 gene with a 158P1D7 antisense polynucleotide. In another approach, a method of inhibiting 158P1D7 mRNA translation comprises contacting the 158P1D7 mRNA with an antisense polynucleotide. In another approach, a 158P1D7 specific ribozyme is used to cleave the 158P1D7 message, thereby inhibiting translation. Such antisense and ribozyme based methods can also be directed to the regulatory regions of the 158P1D7 gene, such as the 158P1D7 promoter and/or enhancer elements. Similarly, proteins capable of inhibiting a 158P1D7 gene transcription factor are used to inhibit 158P1D7 mRNA transcription. The various polynucleotides and compositions useful in the aforementioned methods have been described above. The use of antisense and ribozyme molecules to inhibit transcription and translation is well known in the art.

Other factors that inhibit the transcription of 158P1D7 by interfering with 158P1D7 transcriptional activation are also useful to treat cancers expressing 158P1D7. Similarly, factors that interfere with

158P1D7 processing are useful to treat cancers that express 158P1D7. Cancer treatment methods utilizing such factors are also within the scope of the invention.

XII.D.) General Considerations for Therapeutic Strategies

Gene transfer and gene therapy technologies can be used to deliver therapeutic polynucleotide molecules to tumor cells synthesizing 158P1D7 (i.e., antisense, ribozyme, polynucleotides encoding intrabodies and other 158P1D7 inhibitory molecules). A number of gene therapy approaches are known in the art. Recombinant vectors encoding 158P1D7 antisense polynucleotides, ribozymes, factors capable of interfering with 158P1D7 transcription, and so forth, can be delivered to target tumor cells using such gene therapy approaches.

The above therapeutic approaches can be combined with any one of a wide variety of surgical, chemotherapy or radiation therapy regimens. The therapeutic approaches of the invention can enable the use of reduced dosages of chemotherapy (or other therapies) and/or less frequent administration, an advantage for all patients and particularly for those that do not tolerate the toxicity of the chemotherapeutic agent well.

The anti-tumor activity of a particular composition (e.g., antisense, ribozyme, intrabody), or a combination of such compositions, can be evaluated using various *in vitro* and *in vivo* assay systems. *In vitro* assays that evaluate therapeutic activity include cell growth assays, soft agar assays and other assays indicative of tumor promoting activity, binding assays capable of determining the extent to which a therapeutic composition will inhibit the binding of 158P1D7 to a binding partner, etc.

In vivo, the effect of a 158P1D7 therapeutic composition can be evaluated in a suitable animal model. For example, xenogenic bladder cancer models can be used, wherein human bladder cancer explants or passaged xenograft tissues are introduced into immune compromised animals, such as nude or SCID mice (Shibayama et al., 1991, J Urol., 146(4):1136-7; Beecken et al., 2000, Urology, 56(3):521-526). Efficacy can be predicted using assays that measure inhibition of tumor formation, tumor regression or metastasis, and the like.

In vivo assays that evaluate the promotion of apoptosis are useful in evaluating therapeutic compositions. In one embodiment, xenografts from tumor bearing mice treated with the therapeutic composition can be examined for the presence of apoptotic foci and compared to untreated control xenograft-bearing mice. The extent to which apoptotic foci are found in the tumors of the treated mice provides an indication of the therapeutic efficacy of the composition.

The therapeutic compositions used in the practice of the foregoing methods can be formulated into pharmaceutical compositions comprising a carrier suitable for the desired delivery method. Suitable carriers include any material that when combined with the therapeutic composition retains the anti-tumor function of the therapeutic composition and is generally non-reactive with the patient's immune system. Examples include, but are not limited to, any of a number of standard pharmaceutical carriers such as sterile phosphate buffered saline solutions, bacteriostatic water, and the like (see, generally, Remington's Pharmaceutical Sciences 16th Edition, A. Osal., Ed., 1980).

Therapeutic formulations can be solubilized and administered via any route capable of delivering the therapeutic composition to the tumor site. Potentially effective routes of administration include, but are not limited to, intravenous, parenteral, intraperitoneal, intramuscular, intratumor, intradermal, intraorgan, orthotopic, and the like. A preferred formulation for intravenous injection comprises the therapeutic composition in a solution of preserved bacteriostatic water, sterile unpreserved water, and/or diluted in polyvinylchloride or polyethylene bags containing 0.9% sterile Sodium Chloride for Injection, USP. Therapeutic protein preparations can be lyophilized and stored as sterile powders, preferably under vacuum, and then reconstituted in bacteriostatic water (containing for example, benzyl alcohol preservative) or in sterile water prior to injection.

Dosages and administration protocols for the treatment of cancers using the foregoing methods will vary with the method and the target cancer, and will generally depend on a number of other factors appreciated in the art.

XIII.) Kits

For use in the diagnostic and therapeutic applications described herein, kits are also within the scope of the invention. Such kits can comprise a carrier, package or container that is compartmentalized to receive one or more containers such as vials, tubes, and the like, each of the container(s) comprising one of the separate elements to be used in the method. For example, the container(s) can comprise a probe that is or can be detectably labeled. Such probe can be an antibody or polynucleotide specific for a 158P1D7-related protein or a 158P1D7 gene or message, respectively. Where the method utilizes nucleic acid hybridization to detect the target nucleic acid, the kit can also have containers containing nucleotide(s) for amplification of the target nucleic acid sequence and/or a container comprising a reporter-means, such as a biotin-binding protein, such as avidin or streptavidin, bound to a reporter molecule, such as an enzymatic, florescent, or radioisotope label. The kit can include all or part of the amino acid sequence of Figure 2 or Figure 3 or analogs thereof, or a nucleic acid molecules that encodes such amino acid sequences.

The kit of the invention will typically comprise the container described above and one or more other containers comprising materials desirable from a commercial and user standpoint, including buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

A label can be present on the container to indicate that the composition is used for a specific therapy or non-therapeutic application, and can also indicate directions for either *in vivo* or *in vitro* use, such as those described above. Directions and or other information can also be included on a label or on an insert which is included with the kit.

EXAMPLES

Various aspects of the invention are further described and illustrated by way of the several examples that follow, none of which are intended to limit the scope of the invention.

Example 1: SSH-Generated Isolation of a cDNA Fragment of the 158P1D7 Gene

To isolate genes that are over-expressed in bladder cancer we used the Suppression Subtractive Hybridization (SSH) procedure using cDNA derived from bladder cancer tissues, including invasive transitional cell carcinoma. The 158P1D7 SSH cDNA sequence was derived from a bladder cancer pool minus normal bladder cDNA subtraction. Included in the driver were also cDNAs derived from 9 other normal tissues. The 158P1D7 cDNA was identified as highly expressed in the bladder cancer tissue pool, with lower expression seen in a restricted set of normal tissues.

The SSH DNA sequence of 231 bp (Figure 1) has high homology (230/231 identity) to a hypothetical protein FLJ22774 (GenBank accession XM_033183) derived from a chromosome 13 genomic clone. A 158P1D7 cDNA clone (TurboScript3PX) of 2,555 bp was isolated from bladder cancer cDNA, revealing an ORF of 841 amino acids (Figure 2 and Figure 3).

The 158P1D7 protein has a signal sequence and a transmembrane domain and is predicted to be localized to the cell surface using the the PSORT-I program.

Amino acid sequence analysis of 158P1D7 reveals 100% identity over 798 amino acid region to a human hypothetical protein FLJ22774 (GenBank Accession XP_033182, Figure 4).

Materials and Methods**Human Tissues:**

The bladder cancer patient tissues were purchased from several sources such as from the NDRI (Philadelphia, PA). mRNA for some normal tissues were purchased from Clontech, Palo Alto, CA.

RNA Isolation:

Tissues were homogenized in Trizol reagent (Life Technologies, Gibco BRL) using 10 ml/ g tissue isolate total RNA. Poly A RNA was purified from total RNA using Qiagen's Oligotex mRNA Mini and Midi kits. Total and mRNA were quantified by spectrophotometric analysis (O.D. 260/280 nm) and analyzed by gel electrophoresis.

Oligonucleotides:

The following HPLC purified oligonucleotides were used.

DPNCDN (cDNA synthesis primer):

5'TTTTGATCAAGCTT₃₀3' (SEQ ID NO: 661)

Adaptor 1:

5'CTAATACGACTCACTATAGGGCTCGAGCGGCCGCCCGGGCAG3' (SEQ ID NO: 662)

3'GGCCCGTCCTAG5' (SEQ ID NO: 663)

Adaptor 2:

5'GTAATACGACTCACTATAGGGCAGCGTGGTCGCGGCCGAG3' (SEQ ID NO:664)
 3'CGGCTCCTAG5' (SEQ ID NO: 665)

PCR primer 1:

5'CTAATACGACTCACTATAGGGC3' (SEQ ID NO: 666)

Nested primer (NP)1:

5'TCGAGCGGCCCGCCCGGGCAGGA3' (SEQ ID NO: 667)

Nested primer (NP)2:

5'AGCGTGGTCGCGGCCGAGGA3' (SEQ ID NO: 668)

Suppression Subtractive Hybridization:

Suppression Subtractive Hybridization (SSH) was used to identify cDNAs corresponding to genes that may be differentially expressed in bladder cancer. The SSH reaction utilized cDNA from bladder cancer and normal tissues.

The gene 158P1D7 sequence was derived from a bladder cancer pool minus normal bladder cDNA subtraction. The SSH DNA sequence (Figure 1) was identified.

The cDNA derived from of pool of normal bladder tissues was used as the source of the "driver" cDNA, while the cDNA from a pool of bladder cancer tissues was used as the source of the "tester" cDNA. Double stranded cDNAs corresponding to tester and driver cDNAs were synthesized from 2 µg of poly(A)⁺ RNA isolated from the relevant xenograft tissue, as described above, using CLONTECH's PCR-Select cDNA Subtraction Kit and 1 ng of oligonucleotide DPNCDN as primer. First- and second-strand synthesis were carried out as described in the Kit's user manual protocol (CLONTECH Protocol No. PT1117-1, Catalog No. K1804-1). The resulting cDNA was digested with Dpn II for 3 hrs at 37°C. Digested cDNA was extracted with phenol/chloroform (1:1) and ethanol precipitated.

Driver cDNA was generated by combining in a 1:1 ratio Dpn II digested cDNA from the relevant tissue source (see above) with a mix of digested cDNAs derived from the nine normal tissues: stomach, skeletal muscle, lung, brain, liver, kidney, pancreas, small intestine, and heart.

Tester cDNA was generated by diluting 1 µl of Dpn II digested cDNA from the relevant tissue source (see above) (400 ng) in 5 µl of water. The diluted cDNA (2 µl, 160 ng) was then ligated to 2 µl of Adaptor 1 and Adaptor 2 (10 µM), in separate ligation reactions, in a total volume of 10 µl at 16°C overnight, using 400 u of T4 DNA ligase (CLONTECH). Ligation was terminated with 1 µl of 0.2 M EDTA and heating at 72°C for 5 min.

The first hybridization was performed by adding 1.5 µl (600 ng) of driver cDNA to each of two tubes containing 1.5 µl (20 ng) Adaptor 1- and Adaptor 2- ligated tester cDNA. In a final volume of 4 µl, the samples were overlaid with mineral oil, denatured in an MJ Research thermal cycler at 98°C for 1.5

minutes, and then were allowed to hybridize for 8 hrs at 68°C. The two hybridizations were then mixed together with an additional 1 µl of fresh denatured driver cDNA and were allowed to hybridize overnight at 68°C. The second hybridization was then diluted in 200 µl of 20 mM Hepes, pH 8.3, 50 mM NaCl, 0.2 mM EDTA, heated at 70°C for 7 min. and stored at -20°C.

PCR Amplification, Cloning and Sequencing of Gene Fragments Generated from SSH:

To amplify gene fragments resulting from SSH reactions, two PCR amplifications were performed. In the primary PCR reaction 1 µl of the diluted final hybridization mix was added to 1 µl of PCR primer 1 (10 µM), 0.5 µl dNTP mix (10 µM), 2.5 µl 10 x reaction buffer (CLONTECH) and 0.5 µl 50 x Advantage cDNA polymerase Mix (CLONTECH) in a final volume of 25 µl. PCR 1 was conducted using the following conditions: 75°C for 5 min., 94°C for 25 sec., then 27 cycles of 94°C for 10 sec, 66°C for 30 sec, 72°C for 1.5 min. Five separate primary PCR reactions were performed for each experiment. The products were pooled and diluted 1:10 with water. For the secondary PCR reaction, 1 µl from the pooled and diluted primary PCR reaction was added to the same reaction mix as used for PCR 1, except that primers NP1 and NP2 (10 µM) were used instead of PCR primer 1. PCR 2 was performed using 10-12 cycles of 94°C for 10 sec, 68°C for 30 sec, and 72°C for 1.5 minutes. The PCR products were analyzed using 2% agarose gel electrophoresis.

The PCR products were inserted into pCR2.1 using the T/A vector cloning kit (Invitrogen). Transformed *E. coli* were subjected to blue/white and ampicillin selection. White colonies were picked and arrayed into 96 well plates and were grown in liquid culture overnight. To identify inserts, PCR amplification was performed on 1 ml of bacterial culture using the conditions of PCR1 and NP1 and NP2 as primers. PCR products were analyzed using 2% agarose gel electrophoresis.

Bacterial clones were stored in 20% glycerol in a 96 well format. Plasmid DNA was prepared, sequenced, and subjected to nucleic acid homology searches of the GenBank, dBest, and NCI-CGAP databases.

RT-PCR Expression Analysis:

First strand cDNAs can be generated from 1 µg of mRNA with oligo (dT)12-18 priming using the Gibco-BRL Superscript Preamplification system. The manufacturer's protocol was used which included an incubation for 50 min at 42°C with reverse transcriptase followed by RNase H treatment at 37°C for 20 min. After completing the reaction, the volume can be increased to 200 µl with water prior to normalization. First strand cDNAs from 16 different normal human tissues can be obtained from Clontech.

Normalization of the first strand cDNAs from multiple tissues was performed by using the primers 5'atctgcccgcgctcgtcgtcgacaa3' (SEQ ID NO: 669) and 5'agccacacgcagctcattgtagaagg 3' (SEQ ID NO: 670) to amplify β-actin. First strand cDNA (5 µl) were amplified in a total volume of 50 µl containing 0.4 µM primers, 0.2 µM each dNTPs, 1XPCR buffer (Clontech, 10 mM Tris-HCL, 1.5 mM MgCl₂, 50 mM KCl, pH8.3) and 1X KlenTaq DNA polymerase (Clontech). Five µl of the PCR reaction can be removed at 18, 20, and 22 cycles and used for agarose gel electrophoresis. PCR was performed using an MJ Research thermal cycler under the following conditions: Initial denaturation can be at 94°C for 15 sec, followed by a

18, 20, and 22 cycles of 94°C for 15, 65°C for 2 min, 72°C for 5 sec. A final extension at 72°C was carried out for 2 min. After agarose gel electrophoresis, the band intensities of the 283 b.p. β -actin bands from multiple tissues were compared by visual inspection. Dilution factors for the first strand cDNAs were calculated to result in equal β -actin band intensities in all tissues after 22 cycles of PCR. Three rounds of normalization can be required to achieve equal band intensities in all tissues after 22 cycles of PCR.

To determine expression levels of the 158P1D7 gene, 5 μ l of normalized first strand cDNA were analyzed by PCR using 26, and 30 cycles of amplification. Semi-quantitative expression analysis can be achieved by comparing the PCR products at cycle numbers that give light band intensities. The primers used for RT-PCR were designed using the 158P1D7 SSH sequence and are listed below:

158P1D7.1 5' ATAAGCTTTCAATGTTGCGCTCCT 3' (SEQ ID NO: 671)

158P1D7.2 5' TGTCAACTAAGACCACGTCCATTC3' (SEQ ID NO: 672)

A typical RT-PCR expression analysis is shown in Figure 6. RT-PCR expression analysis was performed on first strand cDNAs generated using pools of tissues from multiple samples. The cDNAs were shown to be normalized using beta-actin PCR. Expression of 158P1D7 was observed in bladder cancer pool.

Example 2: Full Length Cloning of 158P1D7

The 158P1D7 SSH cDNA sequence was derived from a bladder cancer pool minus normal bladder cDNA subtraction. The SSH cDNA sequence (Figure 1) was designated 158P1D7. The full-length cDNA clone 158P1D7-clone TurboScript3PX (Figure 2) was cloned from bladder cancer pool cDNA.

158P1D7 clone cDNA was deposited under the terms of the Budapest Treaty on 22 August 2001, with the American Type Culture Collection (ATCC; 10801 University Blvd., Manassas, VA 20110-2209 USA) as plasmid p158P1D7- Turbo/3PX, and has been assigned Accession No. PTA-3662.

Example 3: Chromosomal Mapping of 158P1D7

Chromosomal localization can implicate genes in disease pathogenesis. Several chromosome mapping approaches are available including fluorescent *in situ* hybridization (FISH), human/hamster radiation hybrid (RH) panels (Walter et al., 1994; Nature Genetics 7:22; Research Genetics, Huntsville AL), human-rodent somatic cell hybrid panels such as is available from the Coriell Institute (Camden, New Jersey), and genomic viewers utilizing BLAST homologies to sequenced and mapped genomic clones (NCBI, Bethesda, Maryland).

158P1D7 maps to chromosome 13, using 158P1D7 sequence and the NCBI BLAST. This is a region of frequent amplification in bladder cancer (Prat et al., "Detection of chromosomal imbalances in papillary bladder tumors by comparative genomic hybridization", *Urology*, 2001, 57(5):986-92) and is associated with rapid tumor cell proliferation in advanced bladder cancer (Tomovska et al., "Molecular cytogenetic alterations associated with rapid tumor cell proliferation in advanced urinary bladder cancer", *Int. J Oncol.* 2001; 18 (6):1239-44).

Example 4: Expression analysis of 158P1D7 in normal tissues and patient specimens

Analysis of 158P1D7 by RT-PCR is shown in Figure 6. Strong expression of 158P1D7 is observed in bladder cancer pool and breast cancer pool. Lower levels of expression are observed in VP1, VP2, xenograft pool, prostate cancer pool, colon cancer pool, lung cancer pool, ovary cancer pool, and metastasis pool.

Extensive northern blot analysis of 158P1D7 in 16 human normal tissues confirms the expression observed by RT-PCR (Figure 7). Two transcripts of approximately 4.6 and 4.2 kb are detected in prostate and, to lower levels, in heart, placenta, liver, small intestine and colon.

Northern blot analysis on patient tumor specimens shows expression of 158P1D7 in most bladder tumor tissues tested and in the bladder cancer cell line SCaBER (Figure 8A and 8B). The expression detected in normal adjacent tissues (isolated from patients) but not in normal tissues (isolated from a healthy donor) may indicate that these tissues are not fully normal and that 158P1D7 may be expressed in early stage tumors. Expression of 158P1D7 is also detected in 2 of 4 lung cancer cell lines, and in all 3 lung cancer tissues tested (Figure 9). In breast cancer samples, 158P1D7 expression is observed in the MCF7 and CAMA-1 breast cancer cell lines, in breast tumor tissues isolated from breast cancer patients, but not in normal breast tissues (Figure 10).

The restricted expression of 158P1D7 in normal tissues and the expression detected in prostate cancer, bladder cancer, colon cancer, lung cancer, ovarian cancer, and breast cancer suggest that 158P1D7 is a potential therapeutic target and a diagnostic marker for human cancers.

Example 5: Production of Recombinant 158P1D7 in Prokaryotic Systems

A. *In vitro* transcription and translation constructs:

pCRII: To generate 158P1D7 sense and anti-sense RNA probes for RNA *in situ* investigations, pCRII constructs (Invitrogen, Carlsbad CA) are generated encoding either all or fragments of the 158P1D7 cDNA. The pCRII vector has Sp6 and T7 promoters flanking the insert to drive the transcription of 158P1D7 RNA for use as probes in RNA *in situ* hybridization experiments. These probes are used to analyze the cell and tissue expression of 158P1D7 at the RNA level. Transcribed 158P1D7 RNA representing the cDNA amino acid coding region of the 158P1D7 gene is used in *in vitro* translation systems such as the TnTTM Coupled Reticulolysate System (Promega, Corp., Madison, WI) to synthesize 158P1D7 protein.

B. Bacterial Constructs:

pGEX Constructs: To generate recombinant 158P1D7 proteins in bacteria that are fused to the Glutathione S-transferase (GST) protein, all or parts of the 158P1D7 cDNA protein coding sequence are fused to the GST gene by cloning into pGEX-6P-1 or any other GST- fusion vector of the pGEX family (Amersham Pharmacia Biotech, Piscataway, NJ). These constructs allow controlled expression of recombinant 158P1D7 protein sequences with GST fused at the amino-terminus and a six histidine epitope (6X His) at the carboxyl-terminus. The GST and 6X His tags permit purification of the recombinant fusion protein from induced bacteria with the appropriate affinity matrix and allow recognition of the fusion protein with anti-GST and His antibodies. The 6X His tag is generated by adding 6 histidine codons to the cloning primer at the 3' end of the open reading frame (ORF). A proteolytic cleavage site, such as the PreScissionTM recognition site in pGEX-6P-1, may be employed such that it permits cleavage of the GST tag from 158P1D7-related protein. The ampicillin resistance gene and pBR322 origin permits selection and maintenance of the pGEX plasmids in E. coli. For example, constructs are made utilizing pGEX-6P-1 such that the following regions of 158P1D7 are expressed as an amino-terminal fusions to GST: amino acids 1 to 841; or any 8, 9, 10, 11, 12,13, 14,15, or more contiguous amino acids from 158P1D7 or analogs thereof.

pMAL Constructs: To generate recombinant 158P1D7 proteins that are fused to maltose-binding protein (MBP) in bacterial cells, all or parts of the 158P1D7 cDNA protein coding sequence are fused to the MBP gene by cloning into the pMAL-c2X and pMAL-p2X vectors (New England Biolabs, Beverly, MA). These constructs allow controlled expression of recombinant 158P1D7 protein sequences with MBP fused at the amino-terminus and a 6X His epitope at the carboxyl-terminus. The MBP and 6X His tags permit purification of the recombinant protein from induced bacteria with the appropriate affinity matrix and allow recognition of the fusion protein with anti-MBP and anti-His antibodies. The 6X His is generated by adding the histidine codons to the 3' cloning primer. A Factor Xa recognition site permits cleavage of the pMAL tag from 158P1D7. The pMAL-c2X and pMAL-p2X vectors are optimized to express the recombinant protein in the cytoplasm or periplasm respectively. Periplasm expression enhances folding of proteins with disulfide bonds. For example, constructs are made utilizing pMAL-c2X and pMAL-p2X such that the following regions of the 158P1D7 protein are expressed as amino-terminal fusions to MBP: amino acids 1 to 841; or any 8, 9, 10, 11, 12,13, 14, 15, or more contiguous amino acids from 158P1D7 or analogs thereof.

pET Constructs: To express 158P1D7 in bacterial cells, all or parts of the 158P1D7 cDNA protein coding sequence are cloned into the pET family of vectors (Novagen, Madison, WI). These vectors allow tightly controlled expression of recombinant 158P1D7 protein in bacteria with and without fusion to proteins that enhance solubility, such as NusA and thioredoxin (Trx), and epitope tags, such as 6X His and S-TagTM that aid purification and detection of the recombinant protein. For example, constructs are made utilizing pET NusA fusion system 43.1 such that the following regions of the 158P1D7 protein are expressed as amino-terminal fusions to NusA : amino acids 1 to 841; or any 8, 9, 10, 11, 12,13, 14, 15, or more contiguous amino acids from 158P1D7 or analogs thereof.

B. Yeast Constructs:

pESC Constructs: To express 158P1D7 in the yeast species *Saccharomyces cerevisiae* for generation of recombinant protein and functional studies, all or parts of the 158P1D7 cDNA protein coding sequence are cloned into the pESC family of vectors each of which contain 1 of 4 selectable markers, HIS3, TRP1, LEU2, and URA3 (Stratagene, La Jolla, CA). These vectors allow controlled expression from the same plasmid of up to 2 different genes or cloned sequences containing either FlagTM or Myc epitope tags in the same yeast cell. This system is useful to conform protein-protein interactions of 158P1D7. In addition, expression in yeast yields similar post-translational modifications, such as glycosylations and phosphorylations, that are found when expressed in eukaryotic cells. For example, constructs are made utilizing pESC-HIS such that the following regions of the 158P1D7 protein are expressed: amino acids 1 to 841; or any 8, 9, 10, 11, 12,13, 14, 15, or more contiguous amino acids from 158P1D7 or analogs thereof.

pESP Constructs: To express 158P1D7 in the yeast species *Saccharomyces pombe*, all or parts of the 158P1D7 cDNA protein coding sequence are cloned into the pESP family of vectors. These vectors allow controlled high level of expression of a 158P1D7 protein sequence that is fused at either the amino terminus or at the carboxyl terminus to GST which aids purification of the recombinant protein. A FlagTM epitope tag allows detection of the recombinant protein with anti-FlagTM antibody. For example, constructs are made utilizing pESP-1 vector such that the following regions of the 158P1D7 protein are expressed as amino-terminal fusions to GST: amino acids 1 to 841; or any 8, 9, 10, 11, 12,13, 14, 15, or more contiguous amino acids from 158P1D7 or analogs thereof.

Example 6: Production of Recombinant 158P1D7 in Eukaryotic Systems

A. Mammalian Constructs:

To express recombinant 158P1D7 in eukaryotic cells, the full or partial length 158P1D7 cDNA sequences can be cloned into any one of a variety of expression vectors known in the art. The constructs can be transfected into any one of a wide variety of mammalian cells such as 293T cells. Transfected 293T cell lysates can be probed with the anti-158P1D7 polyclonal serum, described above.

pcDNA4/HisMax Constructs: To express 158P1D7 in mammalian cells, the 158P1D7 ORF is cloned into pcDNA4/HisMax Version A (Invitrogen, Carlsbad, CA). Protein expression is driven from the cytomegalovirus (CMV) promoter and the SP163 translational enhancer. The recombinant protein has XpressTM and six histidine epitopes fused to the N-terminus. The pcDNA4/HisMax vector also contains the bovine growth hormone (BGH) polyadenylation signal and transcription termination sequence to enhance mRNA stability along with the SV40 origin for episomal replication and simple vector rescue in cell lines expressing the large T antigen. The Zeocin resistance gene allows for selection of mammalian cells expressing the protein and the ampicillin resistance gene and ColE1 origin permits selection and maintenance of the plasmid in *E. coli*. The following regions of 158P1D7 are expressed in this construct, amino acids 1 to 841; or any 8, 9, 10, 11, 12,13, 14,15, or more contiguous amino acids from 158P1D7, variants, or analogs thereof.

pcDNA3.1/MycHis Constructs: To express 158P1D7 in mammalian cells, the ORFs with consensus Kozak translation initiation site were cloned into pcDNA3.1/MycHis Version A (Invitrogen, Carlsbad, CA). Protein expression is driven from the cytomegalovirus (CMV) promoter. The recombinant proteins have the myc epitope and six histidines fused to the C-terminus. The pcDNA3.1/MycHis vector also contains the bovine growth hormone (BGH) polyadenylation signal and transcription termination sequence to enhance mRNA stability, along with the SV40 origin for episomal replication and simple vector rescue in cell lines expressing the large T antigen. The Neomycin resistance gene can be used, as it allows for selection of mammalian cells expressing the protein and the ampicillin resistance gene and ColE1 origin permits selection and maintenance of the plasmid in *E. coli*. The following regions of 158P1D7 are expressed in this construct, amino acids 1 to 841; or any 8, 9, 10, 11, 12,13, 14,15, or more contiguous amino acids from 158P1D7, variants, or analogs thereof.

pcDNA3.1/CT-GFP-TOPO Construct: To express 158P1D7 in mammalian cells and to allow detection of the recombinant proteins using fluorescence, the ORFs with consensus Kozak translation initiation site are cloned into pcDNA3.1CT-GFP-TOPO (Invitrogen, CA). Protein expression is driven from the cytomegalovirus (CMV) promoter. The recombinant proteins have the Green Fluorescent Protein (GFP) fused to the C-terminus facilitating non-invasive, in vivo detection and cell biology studies. The pcDNA3.1CT-GFP-TOPO vector also contains the bovine growth hormone (BGH) polyadenylation signal and transcription termination sequence to enhance mRNA stability along with the SV40 origin for episomal replication and simple vector rescue in cell lines expressing the large T antigen. The Neomycin resistance gene allows for selection of mammalian cells that express the protein, and the ampicillin resistance gene and ColE1 origin permits selection and maintenance of the plasmid in *E. coli*. An additional construct with a N-terminal GFP fusion is made in pcDNA3.1/NT-GFP-TOPO spanning the entire length of the 158P1D7 protein. The following regions of 158P1D7 are expressed in this construct, amino acids 1 to 841; or any 8, 9, 10, 11, 12,13, 14,15, or more contiguous amino acids from 158P1D7, variants, or analogs thereof.

PAPtag: The 158P1D7 ORFs are cloned into pAPtag-5 (GenHunter Corp. Nashville, TN). This construct generates an alkaline phosphatase fusion at the C-terminus of the 158P1D7 proteins while fusing the IgGκ signal sequence to N-terminus. The resulting recombinant 158P1D7 proteins are optimized for secretion into the media of transfected mammalian cells and can be used to identify proteins such as ligands or receptors that interact with the 158P1D7 proteins. Protein expression is driven from the CMV promoter and the recombinant proteins also contain myc and six histidines fused to the C-terminus of alkaline phosphatase. The Zeocin resistance gene allows for selection of mammalian cells expressing the protein and the ampicillin resistance gene permits selection of the plasmid in *E. coli*. The following regions of 158P1D7 are expressed in this construct, amino acids 1 to 841; or any 8, 9, 10, 11, 12,13, 14,15, or more contiguous amino acids from 158P1D7, variants, or analogs thereof.

ptag5: The 158P1D7 ORFs are also cloned into pTag-5. This vector is similar to pAPtag but without the alkaline phosphatase fusion. This construct generates an immunoglobulin G1 Fc fusion at the

C-terminus of the 158P1D7 protein while fusing the IgGK signal sequence to the N-terminus. The resulting recombinant 158P1D7 proteins are optimized for secretion into the media of transfected mammalian cells, and can be used to identify proteins such as ligands or receptors that interact with the 158P1D7 proteins. Protein expression is driven from the CMV promoter and the recombinant protein also contains myc and six histidines fused to the C-terminus of alkaline phosphatase. The Zeocin resistance gene allows for selection of mammalian cells expressing the protein, and the ampicillin resistance gene permits selection of the plasmid in *E. coli*. The following regions of 158P1D7 are expressed in this construct, amino acids 1 to 841; or any 8, 9, 10, 11, 12,13, 14,15, or more contiguous amino acids from 158P1D7, variants, or analogs thereof.

PsecFc: The 158P1D7 ORFs are also cloned into psecFc. The psecFc vector was assembled by cloning immunoglobulin G1 Fc (hinge, CH2, CH3 regions) into pSecTag2 (Invitrogen, California). This construct generates an immunoglobulin G1 Fc fusion at the C-terminus of the 158P1D7 proteins, while fusing the IgG-kappa signal sequence to N-terminus. The resulting recombinant 158P1D7 protein is optimized for secretion into the media of transfected mammalian cells, and can be used to identify proteins such as ligands or receptors that interact with the 158P1D7 protein. Protein expression is driven from the CMV promoter and the recombinant protein also contain myc and six histidines fused to the C-terminus of alkaline phosphatase. The Zeocin resistance gene allows for selection of mammalian cells that express the protein, and the ampicillin resistance gene permits selection of the plasmid in *E. coli*. The following regions of 158P1D7 are expressed in this construct, amino acids 1 to 841; or any 8, 9, 10, 11, 12,13, 14,15, or more contiguous amino acids from 158P1D7, variants, or analogs thereof.

pSR α Constructs: To generate mammalian cell lines that express 158P1D7 constitutively, the ORFs are cloned into pSR α constructs. Amphotropic and ecotropic retroviruses are generated by transfection of pSR α constructs into the 293T-10A1 packaging line or co-transfection of pSR α and a helper plasmid (containing deleted packaging sequences) into the 293 cells, respectively. The retrovirus can be used to infect a variety of mammalian cell lines, resulting in the integration of the cloned gene, 158P1D7, into the host cell-lines. Protein expression is driven from a long terminal repeat (LTR). The Neomycin resistance gene allows for selection of mammalian cells that express the protein, and the ampicillin resistance gene and ColE1 origin permit selection and maintenance of the plasmid in *E. coli*. The retroviral vectors can thereafter be used for infection and generation of various cell lines using, for example, SCaBER, NIH 3T3, TsuPr1, 293 or rat-1 cells.

Additional pSR α constructs are made that fuse an epitope tag such as the FLAG tag to the C-terminus of 158P1D7 sequences to allow detection using anti-epitope tag antibodies. For example, the FLAG sequence 5' gat tac aag gat gac gac gat aag 3' is added to cloning primer at the 3' end of the ORF. Additional pSR α constructs are made to produce both N-terminal and C-terminal GFP and myc/6 HIS fusion proteins of the full-length 158P1D7 proteins. The following regions of 158P1D7 are expressed in such constructs, amino acids 1 to 841; or any 8, 9, 10, 11, 12,13, 14,15, or more contiguous amino acids from 158P1D7, variants, or analogs thereof.

Additional pSR α constructs are made to produce both N-terminal and C-terminal GFP and myc/6 HIS (SEQ ID NO: 748) fusion proteins of the full-length 158P1D7 proteins. The following regions of 158P1D7 are expressed in such constructs, amino acids 1 to 841; or any 8, 9, 10, 11, 12,13, 14,15, or more contiguous amino acids from 158P1D7, variants, or analogs thereof.

Additional Viral Vectors: Additional constructs are made for viral-mediated delivery and expression of 158P1D7. High virus titer leading to high level expression of 158P1D7 is achieved in viral delivery systems such as adenoviral vectors and herpes amplicon vectors. The 158P1D7 coding sequences or fragments thereof are amplified by PCR and subcloned into the AdEasy shuttle vector (Stratagene). Recombination and virus packaging are performed according to the manufacturer's instructions to generate adenoviral vectors. Alternatively, 158P1D7 coding sequences or fragments thereof are cloned into the HSV-1 vector (Imgenex) to generate herpes viral vectors. The viral vectors are thereafter used for infection of various cell lines such as SCaBER, NIH 3T3, 293 or rat-1 cells. The following regions of 158P1D7 are expressed in this construct, amino acids 1 to 841; or any 8, 9, 10, 11, 12,13, 14,15, or more contiguous amino acids from 158P1D7, variants, or analogs thereof.

Regulated Expression Systems: To control expression of 158P1D7 in mammalian cells, coding sequences of 158P1D7 are cloned into regulated mammalian expression systems such as the T-Rex System (Invitrogen), the GeneSwitch System (Invitrogen) and the tightly-regulated Ecdysone System (Stratagene). These systems allow the study of the temporal and concentration dependent effects of recombinant 158P1D7. These vectors are thereafter used to control expression of 158P1D7 in various cell lines such as SCaBER, NIH 3T3, 293 or rat-1 cells. The following regions of 158P1D7 are expressed in these constructs, amino acids 1 to 841; or any 8, 9, 10, 11, 12,13, 14,15, or more contiguous amino acids from 158P1D7, variants, or analogs thereof.

B. Baculovirus Expression Systems

To generate recombinant 158P1D7 proteins in a baculovirus expression system, 158P1D7 ORFs are cloned into the baculovirus transfer vector pBlueBac 4.5 (Invitrogen), which provides a His-tag at the N-terminus. Specifically, pBlueBac-158P1D7 is co-transfected with helper plasmid pBac-N-Blue (Invitrogen) into SF9 (*Spodoptera frugiperda*) insect cells to generate recombinant baculovirus (see Invitrogen instruction manual for details). Baculovirus is then collected from cell supernatant and purified by plaque assay.

Recombinant 158P1D7 protein is then generated by infection of HighFive insect cells (Invitrogen) with purified baculovirus. Recombinant 158P1D7 protein can be detected using anti-158P1D7 or anti-His-tag antibody. 158P1D7 protein can be purified and used in various cell-based assays or as immunogen to generate polyclonal and monoclonal antibodies specific for 158P1D7.

The following regions of 158P1D7 are expressed in this construct, amino acids 1 to 841; or any 8, 9, 10, 11, 12,13, 14,15, or more contiguous amino acids from 158P1D7, variants, or analogs thereof.

Example 7 Antigenicity Profiles

These profiles: Figure 11, Hydrophilicity, (Hopp T.P., Woods K.R., 1981. *Proc. Natl. Acad. Sci. U.S.A.* 78:3824-3828); Figure 12, Hydropathicity, (Kyte J., Doolittle R.F., 1982. *J. Mol. Biol.* 157:105-132); Figure 13, Percentage Accessible Residues (Janin J., 1979 *Nature* 277:491-492); Figure 14, Average Flexibility, (Bhaskaran R., and Ponnuswamy P.K., 1988. *Int. J. Pept. Protein Res.* 32:242-255); Figure 15, Beta-turn (Deleage, G., Roux B. 1987 *Protein Engineering* 1:289-294); and optionally others available in the art, such as on the ProtScale website, were used to identify antigenic regions of the 158P1D7 protein. Each of the above amino acid profiles of 158P1D7 were generated using the following ProtScale parameters for analysis: 1) A window size of 9; 2) 100% weight of the window edges compared to the window center; and, 3) amino acid profile values normalized to lie between 0 and 1.

Hydrophilicity (Figure 11), Hydropathicity (Figure 12) and Percentage Accessible Residues (Figure 13) profiles were used to determine stretches of hydrophilic amino acids (i.e., values greater than 0.5 on the Hydrophilicity and Percentage Accessible Residues profile, and values less than 0.5 on the Hydropathicity profile). Such regions are likely to be exposed to the aqueous environment, be present on the surface of the protein, and thus available for immune recognition, such as by antibodies.

Average Flexibility (Figure 14) and Beta-turn (Figure 15) profiles determine stretches of amino acids (i.e., values greater than 0.5 on the Beta-turn profile and the Average Flexibility profile) that are not constrained in secondary structures such as beta sheets and alpha helices. Such regions are also more likely to be exposed on the protein and thus accessible to immune recognition, such as by antibodies.

Antigenic sequences of the 158P1D7 protein indicated, e.g., by the profiles set forth in Figure 11, Figure 12, Figure 13, Figure 14, or Figure 15 are used to prepare immunogens, either peptides or nucleic acids that encode them, to generate therapeutic and diagnostic anti-158P1D7 antibodies. The immunogen can be any 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 25, 30, 35, 40, 45, 50 or more than 50 contiguous amino acids, or the corresponding nucleic acids that encode them, from the 158P1D7 protein. In particular, peptide immunogens of the invention can comprise, a peptide region of at least 5 amino acids of Figure 2 in any whole number increment up to 841 that includes an amino acid position having a value greater than 0.5 in the Hydrophilicity profile of Figure 11; a peptide region of at least 5 amino acids of Figure 2 in any whole number increment up to 841 that includes an amino acid position having a value less than 0.5 in the Hydropathicity profile of Figure 12; a peptide region of at least 5 amino acids of Figure 2 in any whole number increment up to 841 that includes an amino acid position having a value greater than 0.5 in the Percent Accessible Residues profile of Figure 13; a peptide region of at least 5 amino acids of Figure 2 in any whole number increment up to 841 that includes an amino acid position having a value greater than 0.5 in the Average Flexibility profile on Figure 14; and, a peptide region of at least 5 amino acids of Figure 2 in any whole number increment up to 841 that includes an amino acid position having a value greater than 0.5 in the Beta-turn profile of Figure 15. Peptide immunogens of the invention can also comprise nucleic acids that encode any of the forgoing. All immunogens of the invention, peptide or nucleic acid, can be embodied in human unit dose form, or comprised by a composition that includes a pharmaceutical excipient compatible with human physiology.

Example 8: Generation of 158P1D7 Polyclonal Antibodies

Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. In addition to immunizing with the full length 158P1D7 protein, computer algorithms are employed in design of immunogens that, based on amino acid sequence analysis contain characteristics of being antigenic and available for recognition by the immune system of the immunized host (see the Example entitled "Antigenicity Profiles"). Such regions would be predicted to be hydrophilic, flexible, in beta-turn conformations, and be exposed on the surface of the protein (see, e.g., Figure 11, Figure 12, Figure 13, Figure 14, or Figure 15 for amino acid profiles that indicate such regions of 158P1D7).

For example, 158P1D7 recombinant bacterial fusion proteins or peptides encoding hydrophilic, flexible, beta-turn regions of the 158P1D7 sequence, such as amino acids 25-45 and 250-385 are used as antigens to generate polyclonal antibodies in New Zealand White rabbits. It is useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include, but are not limited to, keyhole limpet hemocyanin (KLH), serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. In one embodiment, a peptide encoding amino acids 25-45 of 158P1D7 is conjugated to KLH and used to immunize the rabbit. Alternatively the immunizing agent may include all or portions of the 158P1D7 protein, analogs or fusion proteins thereof. For example, the 158P1D7 amino acid sequence can be fused using recombinant DNA techniques to any one of a variety of fusion protein partners that are well known in the art, such as glutathione-S-transferase (GST) and HIS tagged fusion proteins. Such fusion proteins are purified from induced bacteria using the appropriate affinity matrix. In one embodiment, a GST-fusion protein encoding amino acids 250-385 of 158P1D7 is produced and purified, and a cleavage product is generated in which GST sequences are removed by proteolytic cleavage. This cleaved 158P1D7 protein is used as immunogen. Other recombinant bacterial fusion proteins that may be employed include maltose binding protein, LacZ, thioredoxin, NusA, or an immunoglobulin constant region (see the section entitled "Production of 158P1D7 in Prokaryotic Systems" and Current Protocols In Molecular Biology, Volume 2, Unit 16, Frederick M. Ausubul et al. eds., 1995; Linsley, P.S., Brady, W., Urnes, M., Grosmaire, L., Damle, N., and Ledbetter, L.(1991) J.Exp. Med. 174, 561-566).

In addition to bacterial derived fusion proteins, mammalian expressed protein antigens are also used. These antigens are expressed from mammalian expression vectors such as the Tag5 and Fc-fusion vectors (see the section entitled "Production of Recombinant 158P1D7 in Eukaryotic Systems"), and retain post-translational modifications such as glycosylations found in native 158P1D7 protein. In one embodiment, the predicted extracellular domain of 158P1D7, amino acids 1-614, is cloned into the Tag5 mammalian secretion vector. The recombinant protein is purified by metal chelate chromatography from

tissue culture supernatants of 293T cells stably expressing the recombinant vector. The purified Tag5 158P1D7 extracellular domain is then used as immunogen.

During the immunization protocol, it is useful to mix or emulsify the antigen in adjuvants that enhance the immune response of the host animal. Examples of adjuvants include, but are not limited to, complete Freund's adjuvant (CFA) and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate).

In a typical immunization protocol, rabbits are initially injected subcutaneously with up to 200 µg, typically 100-200 µg, of fusion protein or peptide conjugated to KLH mixed in complete Freund's adjuvant (CFA). Rabbits are then injected subcutaneously every two weeks with up to 200 µg, typically 100-200 µg, of the immunogen in incomplete Freund's adjuvant (IFA). Test bleeds are taken approximately 7-10 days following each immunization and used to monitor the titer of the antiserum by ELISA.

To test serum, such as rabbit serum, for reactivity with 158P1D7 proteins, the full-length 158P1D7 cDNA can be cloned into an expression vector such as one that provides a 6X His tag at the carboxyl-terminus (pCDNA 3.1 myc-his, Invitrogen, see the Example entitled "Production of Recombinant 158P1D7 in Eukaryotic Systems"). After transfection of the constructs into 293T cells, cell lysates are probed with the anti-158P1D7 serum and with anti-His antibody (Santa Cruz Biotechnologies, Santa Cruz, CA) to determine specific reactivity to denatured 158P1D7 protein using the Western blot technique. In addition, recognition of native protein by the antiserum can be determined by immunoprecipitation and flow cytometric analyses of 293T and other recombinant 158P1D7-expressing cells. Alternatively, specificity of the antiserum is tested by Western blot, immunoprecipitation, fluorescent microscopy, and flow cytometric techniques using cells that endogenously express 158P1D7.

Sera from rabbits immunized with fusion proteins, such as GST and MBP fusion proteins, are purified by depletion of antibodies reactive to GST, MBP, or other fusion partner sequence by passage over an affinity column containing the fusion partner either alone or in the context of an irrelevant fusion protein. Sera from His-tagged protein and peptide immunized rabbits as well as fusion partner depleted sera are further purified by passage over an affinity column composed of the original protein immunogen or free peptide coupled to Affigel matrix (BioRad).

Example 9: Generation of 158P1D7 Monoclonal Antibodies (mAbs)

In one embodiment, therapeutic mAbs to 158P1D7 comprise those that react with epitopes of the protein that would disrupt or modulate the biological function of 158P1D7, for example those that would disrupt its interaction with ligands or proteins that mediate or are involved in its biological activity. Therapeutic mAbs also comprise those which specifically bind epitopes of 158P1D7 exposed on the cell surface and thus are useful in targeting mAb-toxin conjugates. Immunogens for generation of such mAbs include those designed to encode or contain the entire 158P1D7 protein or regions of the 158P1D7 protein predicted to be antigenic from computer analysis of the amino acid sequence (see, e.g., Figure 11, Figure 12, Figure 13, Figure 14, or Figure 15, and the Example entitled "Antigenicity Profiles"). Immunogens include peptides, recombinant bacterial proteins, and mammalian expressed Tag 5 proteins and human and

murine IgG FC fusion proteins. In addition, cells expressing high levels of 158P1D7, such as 293T-158P1D7 cells, are used to immunize mice.

To generate mAbs to 158P1D7, mice are first immunized intraperitoneally (IP) with, typically, 10-50 µg of protein immunogen or 10^7 158P1D7-expressing cells mixed in complete Freund's adjuvant. Mice are then subsequently immunized IP every 2-4 weeks with, typically, 10-50 µg of protein immunogen or 10^7 cells mixed in incomplete Freund's adjuvant. Alternatively, MPL-TDM adjuvant is used in immunizations. In addition to the above protein and cell-based immunization strategies, a DNA-based immunization protocol is employed in which a mammalian expression vector encoding 158P1D7 sequence is used to immunize mice by direct injection of the plasmid DNA. For example, the extracellular domain of 158P1D7, amino acids 1-614, is cloned into the Tag5 mammalian secretion vector and the recombinant vector is used as immunogen. In another example, the nucleic acid sequence encoding amino acids 250-385 of 158P1D7 (predicted to be antigenic from sequence analysis, see, e.g., Figure 11, Figure 12, Figure 13, Figure 14 or Figure 15) is cloned into an Fc-fusion secretion vector in which the 158P1D7 sequence is fused at the amino-terminus to an IgK leader sequence and at the carboxyl-terminus to the coding sequence of the murine or human IgG Fc region. This recombinant vector is then used as immunogen. The plasmid immunization protocols are used in combination with purified proteins expressed from the same vector and with cells expressing 158P1D7.

During the immunization protocol, test bleeds are taken 7-10 days following an injection to monitor titer and specificity of the immune response. Once appropriate reactivity and specificity is obtained as determined by ELISA, Western blotting, immunoprecipitation, fluorescence microscopy, and flow cytometric analyses, fusion and hybridoma generation is then carried out with established procedures well known in the art (see, e.g., Harlow and Lane, 1988).

In one embodiment for generating 158P1D7 monoclonal antibodies, a glutathione-S-transferase (GST) fusion protein encoding amino acids 250-385 of 158P1D7 protein is expressed and purified. A cleavage fragment encoding 158P1D7 specific amino acids is then used as immunogen in which GST is removed by site-specific proteolysis. Balb C mice are initially immunized intraperitoneally with 25 µg of the 158P1D7 cleavage protein mixed in complete Freund's adjuvant. Mice are subsequently immunized every two weeks with 25 µg of 158P1D7 cleavage protein mixed in incomplete Freund's adjuvant for a total of three immunizations. The titer of serum from immunized mice is determined by ELISA using the full length GST-fusion protein and the cleaved immunogen. Reactivity and specificity of serum to full length 158P1D7 protein is monitored by Western blotting, immunoprecipitation and flow cytometry using 293T cells transfected with an expression vector encoding the 158P1D7 cDNA (see e.g., the Example entitled "Production of Recombinant 158P1D7 in Eukaryotic Systems"). Other recombinant 158P1D7-expressing cells or cells endogenously expressing 158P1D7 are also used. Mice showing the strongest reactivity are rested and given a final injection of 158P1D7 cleavage protein in PBS and then sacrificed four days later. The spleens of the sacrificed mice are harvested and fused to SPO/2 myeloma cells using standard procedures (Harlow and Lane, 1988). Supernatants from growth wells following HAT selection

are screened by ELISA, Western blot, immunoprecipitation, fluorescent microscopy, and flow cytometry to identify 158P1D7 specific antibody-producing clones.

The binding affinity of a 158P1D7 monoclonal antibody is determined using standard technologies. Affinity measurements quantify the strength of antibody to epitope binding and are used to help define which 158P1D7 monoclonal antibodies preferred for diagnostic or therapeutic use, as appreciated by one of skill in the art. The BIAcore system (Uppsala, Sweden) is a preferred method for determining binding affinity. The BIAcore system uses surface plasmon resonance (SPR, Welford K. 1991, Opt. Quant. Elect. 23:1; Morton and Myszk, 1998, Methods in Enzymology 295: 268) to monitor biomolecular interactions in real time. BIAcore analysis conveniently generates association rate constants, dissociation rate constants, equilibrium dissociation constants, and affinity constants.

Example 10: HLA Class I and Class II Binding Assays

HLA class I and class II binding assays using purified HLA molecules are performed in accordance with disclosed protocols (e.g., PCT publications WO 94/20127 and WO 94/03205; Sidney *et al.*, *Current Protocols in Immunology* 18.3.1 (1998); Sidney, *et al.*, *J. Immunol.* 154:247 (1995); Sette, *et al.*, *Mol. Immunol.* 31:813 (1994)). Briefly, purified MHC molecules (5 to 500 nM) are incubated with various unlabeled peptide inhibitors and 1-10 nM ¹²⁵I-radiolabeled probe peptides as described. Following incubation, MHC-peptide complexes are separated from free peptide by gel filtration and the fraction of peptide bound is determined. Typically, in preliminary experiments, each MHC preparation is titrated in the presence of fixed amounts of radiolabeled peptides to determine the concentration of HLA molecules necessary to bind 10-20% of the total radioactivity. All subsequent inhibition and direct binding assays are performed using these HLA concentrations.

Since under these conditions [label]<[HLA] and IC₅₀≥[HLA], the measured IC₅₀ values are reasonable approximations of the true K_D values. Peptide inhibitors are typically tested at concentrations ranging from 120 µg/ml to 1.2 ng/ml, and are tested in two to four completely independent experiments. To allow comparison of the data obtained in different experiments, a relative binding figure is calculated for each peptide by dividing the IC₅₀ of a positive control for inhibition by the IC₅₀ for each tested peptide (typically unlabeled versions of the radiolabeled probe peptide). For database purposes, and inter-experiment comparisons, relative binding values are compiled. These values can subsequently be converted back into IC₅₀ nM values by dividing the IC₅₀ nM of the positive controls for inhibition by the relative binding of the peptide of interest. This method of data compilation is accurate and consistent for comparing peptides that have been tested on different days, or with different lots of purified MHC.

Binding assays as outlined above may be used to analyze HLA supermotif and/or HLA motif-bearing peptides.

Example 11: Identification of HLA Supermotif- and Motif-Bearing CTL Candidate Epitopes

HLA vaccine compositions of the invention can include multiple epitopes. The multiple epitopes can comprise multiple HLA supermotifs or motifs to achieve broad population coverage. This example illustrates the identification and confirmation of supermotif- and motif-bearing epitopes for the inclusion in such a vaccine composition. Calculation of population coverage is performed using the strategy described below.

Computer searches and algorithms for identification of supermotif and/or motif-bearing epitopes

The searches performed to identify the motif-bearing peptide sequences in the Example entitled "Antigenicity Profiles" and Tables V-XVIII employ the protein sequence data from the gene product of 158P1D7 set forth in Figures 2 and 3.

Computer searches for epitopes bearing HLA Class I or Class II supermotifs or motifs are performed as follows. All translated 158P1D7 protein sequences are analyzed using a text string search software program to identify potential peptide sequences containing appropriate HLA binding motifs; such programs are readily produced in accordance with information in the art in view of known motif/supermotif disclosures. Furthermore, such calculations can be made mentally.

Identified A2-, A3-, and DR-supermotif sequences are scored using polynomial algorithms to predict their capacity to bind to specific HLA-Class I or Class II molecules. These polynomial algorithms account for the impact of different amino acids at different positions, and are essentially based on the premise that the overall affinity (or ΔG) of peptide-HLA molecule interactions can be approximated as a linear polynomial function of the type:

$$"\Delta G" = a_{1i} \times a_{2i} \times a_{3i} \dots \times a_{ni}$$

where a_{ji} is a coefficient which represents the effect of the presence of a given amino acid (j) at a given position (i) along the sequence of a peptide of n amino acids. The crucial assumption of this method is that the effects at each position are essentially independent of each other (i.e., independent binding of individual side-chains). When residue j occurs at position i in the peptide, it is assumed to contribute a constant amount j_i to the free energy of binding of the peptide irrespective of the sequence of the rest of the peptide.

The method of derivation of specific algorithm coefficients has been described in Gulukota *et al.*, *J. Mol. Biol.* 267:1258-126, 1997; (see also Sidney *et al.*, *Human Immunol.* 45:79-93, 1996; and Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998). Briefly, for all i positions, anchor and non-anchor alike, the geometric mean of the average relative binding (ARB) of all peptides carrying j is calculated relative to the remainder of the group, and used as the estimate of j_i . For Class II peptides, if multiple alignments are possible, only the highest scoring alignment is utilized, following an iterative procedure. To calculate an algorithm score of a given peptide in a test set, the ARB values corresponding to the sequence of the peptide are multiplied. If this product exceeds a chosen threshold, the peptide is predicted to bind. Appropriate thresholds are chosen as a function of the degree of stringency of prediction desired.

Selection of HLA-A2 supertype cross-reactive peptides

Complete protein sequences from 158P1D7 are scanned utilizing motif identification software, to identify 8-, 9- 10- and 11-mer sequences containing the HLA-A2-supermotif main anchor specificity.

Typically, these sequences are then scored using the protocol described above and the peptides corresponding to the positive-scoring sequences are synthesized and tested for their capacity to bind purified HLA-A*0201 molecules *in vitro* (HLA-A*0201 is considered a prototype A2 supertype molecule).

These peptides are then tested for the capacity to bind to additional A2-supertype molecules (A*0202, A*0203, A*0206, and A*6802). Peptides that bind to at least three of the five A2-supertype alleles tested are typically deemed A2-supertype cross-reactive binders. Preferred peptides bind at an affinity equal to or less than 500 nM to three or more HLA-A2 supertype molecules.

Selection of HLA-A3 supermotif-bearing epitopes

The 158P1D7 protein sequence scanned above is also examined for the presence of peptides with the HLA-A3-supermotif primary anchors. Peptides corresponding to the HLA A3 supermotif-bearing sequences are then synthesized and tested for binding to HLA-A*0301 and HLA-A*1101 molecules, the molecules encoded by the two most prevalent A3-supertype alleles. The peptides that bind at least one of the two alleles with binding affinities of ≤ 500 nM, often ≤ 200 nM, are then tested for binding cross-reactivity to the other common A3-supertype alleles (e.g., A*3101, A*3301, and A*6801) to identify those that can bind at least three of the five HLA-A3-supertype molecules tested.

Selection of HLA-B7 supermotif bearing epitopes

The 158P1D7 protein is also analyzed for the presence of 8-, 9-, 10-, or 11-mer peptides with the HLA-B7-supermotif. Corresponding peptides are synthesized and tested for binding to HLA-B*0702, the molecule encoded by the most common B7-supertype allele (*i.e.*, the prototype B7 supertype allele). Peptides binding B*0702 with IC_{50} of ≤ 500 nM are identified using standard methods. These peptides are then tested for binding to other common B7-supertype molecules (e.g., B*3501, B*5101, B*5301, and B*5401). Peptides capable of binding to three or more of the five B7-supertype alleles tested are thereby identified.

Selection of A1 and A24 motif-bearing epitopes

To further increase population coverage, HLA-A1 and -A24 epitopes can also be incorporated into vaccine compositions. An analysis of the 158P1D7 protein can also be performed to identify HLA-A1- and A24-motif-containing sequences.

High affinity and/or cross-reactive binding epitopes that bear other motif and/or supermotifs are identified using analogous methodology.

Example 12: Confirmation of Immunogenicity

Cross-reactive candidate CTL A2-supermotif-bearing peptides that are identified as described herein are selected to confirm *in vitro* immunogenicity. Confirmation is performed using the following methodology:

Target Cell Lines for Cellular Screening:

The .221A2.1 cell line, produced by transferring the HLA-A2.1 gene into the HLA-A, -B, -C null mutant human B-lymphoblastoid cell line 721.221, is used as the peptide-loaded target to measure activity

of HLA-A2.1-restricted CTL. This cell line is grown in RPMI-1640 medium supplemented with antibiotics, sodium pyruvate, nonessential amino acids and 10% (v/v) heat inactivated FCS. Cells that express an antigen of interest, or transfectants comprising the gene encoding the antigen of interest, can be used as target cells to confirm the ability of peptide-specific CTLs to recognize endogenous antigen.

Primary CTL Induction Cultures:

Generation of Dendritic Cells (DC): PBMCs are thawed in RPMI with 30 µg/ml DNase, washed twice and resuspended in complete medium (RPMI-1640 plus 5% AB human serum, non-essential amino acids, sodium pyruvate, L-glutamine and penicillin/streptomycin). The monocytes are purified by plating 10×10^6 PBMC/well in a 6-well plate. After 2 hours at 37°C, the non-adherent cells are removed by gently shaking the plates and aspirating the supernatants. The wells are washed a total of three times with 3 ml RPMI to remove most of the non-adherent and loosely adherent cells. Three ml of complete medium containing 50 ng/ml of GM-CSF and 1,000 U/ml of IL-4 are then added to each well. TNFα is added to the DCs on day 6 at 75 ng/ml and the cells are used for CTL induction cultures on day 7.

Induction of CTL with DC and Peptide: CD8⁺ T-cells are isolated by positive selection with Dynal immunomagnetic beads (Dynabeads® M-450) and the detacha-bead® reagent. Typically about 200-250x10⁶ PBMC are processed to obtain 24x10⁶ CD8⁺ T-cells (enough for a 48-well plate culture). Briefly, the PBMCs are thawed in RPMI with 30µg/ml DNase, washed once with PBS containing 1% human AB serum and resuspended in PBS/1% AB serum at a concentration of 20x10⁶ cells/ml. The magnetic beads are washed 3 times with PBS/AB serum, added to the cells (140µl beads/20x10⁶ cells) and incubated for 1 hour at 4°C with continuous mixing. The beads and cells are washed 4x with PBS/AB serum to remove the nonadherent cells and resuspended at 100x10⁶ cells/ml (based on the original cell number) in PBS/AB serum containing 100µl/ml detacha-bead® reagent and 30 µg/ml DNase. The mixture is incubated for 1 hour at room temperature with continuous mixing. The beads are washed again with PBS/AB/DNase to collect the CD8⁺ T-cells. The DC are collected and centrifuged at 1300 rpm for 5-7 minutes, washed once with PBS with 1% BSA, counted and pulsed with 40µg/ml of peptide at a cell concentration of 1-2x10⁶/ml in the presence of 3µg/ml β₂- microglobulin for 4 hours at 20°C. The DC are then irradiated (4,200 rads), washed 1 time with medium and counted again.

Setting up induction cultures: 0.25 ml cytokine-generated DC (at 1x10⁵ cells/ml) are co-cultured with 0.25ml of CD8⁺ T-cells (at 2x10⁶ cell/ml) in each well of a 48-well plate in the presence of 10 ng/ml of IL-7. Recombinant human IL-10 is added the next day at a final concentration of 10 ng/ml and rhuman IL-2 is added 48 hours later at 10 IU/ml.

Restimulation of the induction cultures with peptide-pulsed adherent cells: Seven and fourteen days after the primary induction, the cells are restimulated with peptide-pulsed adherent cells. The PBMCs are thawed and washed twice with RPMI and DNase. The cells are resuspended at 5x10⁶ cells/ml and irradiated at ~4200 rads. The PBMCs are plated at 2x10⁶ in 0.5 ml complete medium per well and incubated for 2 hours at 37°C. The plates are washed twice with RPMI by tapping the plate gently to remove the nonadherent cells and the adherent cells pulsed with 10µg/ml of peptide in the presence of

3 µg/ml B₂ microglobulin in 0.25ml RPMI/5%AB per well for 2 hours at 37°C. Peptide solution from each well is aspirated and the wells are washed once with RPMI. Most of the media is aspirated from the induction cultures (CD8+ cells) and brought to 0.5 ml with fresh media. The cells are then transferred to the wells containing the peptide-pulsed adherent cells. Twenty four hours later recombinant human IL-10 is added at a final concentration of 10 ng/ml and recombinant human IL2 is added the next day and again 2-3 days later at 50IU/ml (Tsai *et al.*, *Critical Reviews in Immunology* 18(1-2):65-75, 1998). Seven days later, the cultures are assayed for CTL activity in a ⁵¹Cr release assay. In some experiments the cultures are assayed for peptide-specific recognition in the *in situ* IFNγ ELISA at the time of the second restimulation followed by assay of endogenous recognition 7 days later. After expansion, activity is measured in both assays for a side-by-side comparison.

Measurement of CTL lytic activity by ⁵¹Cr release.

Seven days after the second restimulation, cytotoxicity is determined in a standard (5 hr) ⁵¹Cr release assay by assaying individual wells at a single E:T. Peptide-pulsed targets are prepared by incubating the cells with 10µg/ml peptide overnight at 37°C.

Adherent target cells are removed from culture flasks with trypsin-EDTA. Target cells are labelled with 200µCi of ⁵¹Cr sodium chromate (Dupont, Wilmington, DE) for 1 hour at 37°C. Labelled target cells are resuspended at 10⁶ per ml and diluted 1:10 with K562 cells at a concentration of 3.3x10⁶/ml (an NK-sensitive erythroblastoma cell line used to reduce non-specific lysis). Target cells (100 µl) and effectors (100µl) are plated in 96 well round-bottom plates and incubated for 5 hours at 37°C. At that time, 100 µl of supernatant are collected from each well and percent lysis is determined according to the formula: [(cpm of the test sample- cpm of the spontaneous ⁵¹Cr release sample)/(cpm of the maximal ⁵¹Cr release sample- cpm of the spontaneous ⁵¹Cr release sample)] x 100.

Maximum and spontaneous release are determined by incubating the labelled targets with 1% TritonTM X-100 and media alone, respectively. A positive culture is defined as one in which the specific lysis (sample- background) is 10% or higher in the case of individual wells and is 15% or more at the two highest E:T ratios when expanded cultures are assayed.

In situ Measurement of Human IFNγ Production as an Indicator of Peptide-specific and Endogenous Recognition

Immulon 2 plates are coated with mouse anti-human IFNγ monoclonal antibody (4 µg/ml 0.1M NaHCO₃, pH8.2) overnight at 4°C. The plates are washed with Ca²⁺, Mg²⁺-free PBS/0.05% TweenTM 20 and blocked with PBS/10% FCS for two hours, after which the CTLs (100 µl/well) and targets (100 µl/well) are added to each well, leaving empty wells for the standards and blanks (which received media only). The target cells, either peptide-pulsed or endogenous targets, are used at a concentration of 1x10⁶ cells/ml. The plates are incubated for 48 hours at 37°C with 5% CO₂.

Recombinant human IFN-gamma is added to the standard wells starting at 400 pg or 1200pg/100 microliter/well and the plate incubated for two hours at 37°C. The plates are washed and 100 µl of biotinylated mouse anti-human IFN-gamma monoclonal antibody (2 microgram/ml in PBS/3%FCS/0.05%

Tween 20) are added and incubated for 2 hours at room temperature. After washing again, 100 microliter HRP-streptavidin (1:4000) are added and the plates incubated for one hour at room temperature. The plates are then washed 6x with wash buffer, 100 microliter/well developing solution (TMB 1:1) are added, and the plates allowed to develop for 5-15 minutes. The reaction is stopped with 50 microliter/well 1M H₃PO₄ and read at OD450. A culture is considered positive if it measured at least 50 pg of IFN-gamma/well above background and is twice the background level of expression.

CTL Expansion.

Those cultures that demonstrate specific lytic activity against peptide-pulsed targets and/or tumor targets are expanded over a two week period with anti-CD3. Briefly, 5x10⁴ CD8⁺ cells are added to a T25 flask containing the following: 1x10⁶ irradiated (4,200 rad) PBMC (autologous or allogeneic) per ml, 2x10⁵ irradiated (8,000 rad) EBV- transformed cells per ml, and OKT3 (anti-CD3) at 30ng per ml in RPMI-1640 containing 10% (v/v) human AB serum, non-essential amino acids, sodium pyruvate, 25μM 2-mercaptoethanol, L-glutamine and penicillin/streptomycin. Recombinant human IL2 is added 24 hours later at a final concentration of 200IU/ml and every three days thereafter with fresh media at 50IU/ml. The cells are split if the cell concentration exceeds 1x10⁶/ml and the cultures are assayed between days 13 and 15 at E:T ratios of 30, 10, 3 and 1:1 in the ⁵¹Cr release assay or at 1x10⁶/ml in the *in situ* IFNγ assay using the same targets as before the expansion.

Cultures are expanded in the absence of anti-CD3⁺ as follows. Those cultures that demonstrate specific lytic activity against peptide and endogenous targets are selected and 5x10⁴ CD8⁺ cells are added to a T25 flask containing the following: 1x10⁶ autologous PBMC per ml which have been peptide-pulsed with 10 μg/ml peptide for two hours at 37°C and irradiated (4,200 rad); 2x10⁵ irradiated (8,000 rad) EBV-transformed cells per ml RPMI-1640 containing 10%(v/v) human AB serum, non-essential AA, sodium pyruvate, 25mM 2-ME, L-glutamine and gentamicin.

Immunogenicity of A2 supermotif-bearing peptides

A2-supermotif cross-reactive binding peptides are tested in the cellular assay for the ability to induce peptide-specific CTL in normal individuals. In this analysis, a peptide is typically considered to be an epitope if it induces peptide-specific CTLs in at least individuals, and preferably, also recognizes the endogenously expressed peptide.

Immunogenicity can also be confirmed using PBMCs isolated from patients bearing a tumor that expresses 158P1D7. Briefly, PBMCs are isolated from patients, re-stimulated with peptide-pulsed monocytes and assayed for the ability to recognize peptide-pulsed target cells as well as transfected cells endogenously expressing the antigen.

Evaluation of A*03/A11 immunogenicity

HLA-A3 supermotif-bearing cross-reactive binding peptides are also evaluated for immunogenicity using methodology analogous for that used to evaluate the immunogenicity of the HLA-A2 supermotif peptides.

Evaluation of B7 immunogenicity

Immunogenicity screening of the B7-supertype cross-reactive binding peptides identified as set forth herein are confirmed in a manner analogous to the confirmation of A2-and A3-supermotif-bearing peptides.

Peptides bearing other supermotifs/motifs, *e.g.*, HLA-A1, HLA-A24 *etc.* are also confirmed using similar methodology

Example 13: Implementation of the Extended Supermotif to Improve the Binding Capacity of Native Epitopes by Creating Analogs

HLA motifs and supermotifs (comprising primary and/or secondary residues) are useful in the identification and preparation of highly cross-reactive native peptides, as demonstrated herein. Moreover, the definition of HLA motifs and supermotifs also allows one to engineer highly cross-reactive epitopes by identifying residues within a native peptide sequence which can be analoged to confer upon the peptide certain characteristics, *e.g.* greater cross-reactivity within the group of HLA molecules that comprise a supertype, and/or greater binding affinity for some or all of those HLA molecules. Examples of analoging peptides to exhibit modulated binding affinity are set forth in this example.

Analoging at Primary Anchor Residues

Peptide engineering strategies are implemented to further increase the cross-reactivity of the epitopes. For example, the main anchors of A2-supermotif-bearing peptides are altered, for example, to introduce a preferred L, I, V, or M at position 2, and I or V at the C-terminus.

To analyze the cross-reactivity of the analog peptides, each engineered analog is initially tested for binding to the prototype A2 supertype allele A*0201, then, if A*0201 binding capacity is maintained, for A2-supertype cross-reactivity.

Alternatively, a peptide is confirmed as binding one or all supertype members and then analoged to modulate binding affinity to any one (or more) of the supertype members to add population coverage.

The selection of analogs for immunogenicity in a cellular screening analysis is typically further restricted by the capacity of the parent wild type (WT) peptide to bind at least weakly, *i.e.*, bind at an IC₅₀ of 5000nM or less, to three or more A2 supertype alleles. The rationale for this requirement is that the WT peptides must be present endogenously in sufficient quantity to be biologically relevant. Analoged peptides have been shown to have increased immunogenicity and cross-reactivity by T cells specific for the parent epitope (*see, e.g.*, Parkhurst *et al.*, *J. Immunol.* 157:2539, 1996; and Pogue *et al.*, *Proc. Natl. Acad. Sci. USA* 92:8166, 1995).

In the cellular screening of these peptide analogs, it is important to confirm that analog-specific CTLs are also able to recognize the wild-type peptide and, when possible, target cells that endogenously express the epitope.

Analoging of HLA-A3 and B7-supermotif-bearing peptides

Analogues of HLA-A3 supermotif-bearing epitopes are generated using strategies similar to those employed in analoging HLA-A2 supermotif-bearing peptides. For example, peptides binding to 3/5 of the

A3-supertype molecules are engineered at primary anchor residues to possess a preferred residue (V, S, M, or A) at position 2.

The analog peptides are then tested for the ability to bind A*03 and A*11 (prototype A3 supertype alleles). Those peptides that demonstrate ≤ 500 nM binding capacity are then confirmed as having A3-supertype cross-reactivity.

Similarly to the A2- and A3- motif bearing peptides, peptides binding 3 or more B7-supertype alleles can be improved, where possible, to achieve increased cross-reactive binding or greater binding affinity or binding half life. B7 supermotif-bearing peptides are, for example, engineered to possess a preferred residue (V, I, L, or F) at the C-terminal primary anchor position, as demonstrated by Sidney *et al.* (*J. Immunol.* 157:3480-3490, 1996).

Analoging at primary anchor residues of other motif and/or supermotif-bearing epitopes is performed in a like manner.

The analog peptides are then be confirmed for immunogenicity, typically in a cellular screening assay. Again, it is generally important to demonstrate that analog-specific CTLs are also able to recognize the wild-type peptide and, when possible, targets that endogenously express the epitope.

Analoging at Secondary Anchor Residues

Moreover, HLA supermotifs are of value in engineering highly cross-reactive peptides and/or peptides that bind HLA molecules with increased affinity by identifying particular residues at secondary anchor positions that are associated with such properties. For example, the binding capacity of a B7 supermotif-bearing peptide with an F residue at position 1 is analyzed. The peptide is then analoged to, for example, substitute L for F at position 1. The analoged peptide is evaluated for increased binding affinity, binding half life and/or increased cross-reactivity. Such a procedure identifies analoged peptides with enhanced properties.

Engineered analogs with sufficiently improved binding capacity or cross-reactivity can also be tested for immunogenicity in HLA-B7-transgenic mice, following for example, IFA immunization or lipopeptide immunization. Analogued peptides are additionally tested for the ability to stimulate a recall response using PBMC from patients with 158P1D7-expressing tumors.

Other analoguing strategies

Another form of peptide analoguing, unrelated to anchor positions, involves the substitution of a cysteine with α -amino butyric acid. Due to its chemical nature, cysteine has the propensity to form disulfide bridges and sufficiently alter the peptide structurally so as to reduce binding capacity. Substitution of α -amino butyric acid for cysteine not only alleviates this problem, but has been shown to improve binding and crossbinding capabilities in some instances (*see, e.g.,* the review by Sette *et al.*, In: Persistent Viral Infections, Eds. R. Ahmed and I. Chen, John Wiley & Sons, England, 1999).

Thus, by the use of single amino acid substitutions, the binding properties and/or cross-reactivity of peptide ligands for HLA supertype molecules can be modulated.

Example 14. Identification and confirmation of 158P1D7-derived sequences with HLA-DR binding motifs

Peptide epitopes bearing an HLA class II supermotif or motif are identified and confirmed as outlined below using methodology similar to that described for HLA Class I peptides.

Selection of HLA-DR-supermotif-bearing epitopes.

To identify 158P1D7-derived, HLA class II HTL epitopes, the 158P1D7 antigen is analyzed for the presence of sequences bearing an HLA-DR-motif or supermotif. Specifically, 15-mer sequences are selected comprising a DR-supermotif, comprising a 9-mer core, and three-residue N- and C-terminal flanking regions (15 amino acids total).

Protocols for predicting peptide binding to DR molecules have been developed (Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998). These protocols, specific for individual DR molecules, allow the scoring, and ranking, of 9-mer core regions. Each protocol not only scores peptide sequences for the presence of DR-supermotif primary anchors (i.e., at position 1 and position 6) within a 9-mer core, but additionally evaluates sequences for the presence of secondary anchors. Using allele-specific selection tables (see, *e.g.*, Southwood *et al.*, *ibid.*), it has been found that these protocols efficiently select peptide sequences with a high probability of binding a particular DR molecule. Additionally, it has been found that performing these protocols in tandem, specifically those for DR1, DR4w4, and DR7, can efficiently select DR cross-reactive peptides.

The 158P1D7-derived peptides identified above are tested for their binding capacity for various common HLA-DR molecules. All peptides are initially tested for binding to the DR molecules in the primary panel: DR1, DR4w4, and DR7. Peptides binding at least two of these three DR molecules are then tested for binding to DR2w2 β 1, DR2w2 β 2, DR6w19, and DR9 molecules in secondary assays. Finally, peptides binding at least two of the four secondary panel DR molecules, and thus cumulatively at least four of seven different DR molecules, are screened for binding to DR4w15, DR5w11, and DR8w2 molecules in tertiary assays. Peptides binding at least seven of the ten DR molecules comprising the primary, secondary, and tertiary screening assays are considered cross-reactive DR binders. 158P1D7-derived peptides found to bind common HLA-DR alleles are of particular interest.

Selection of DR3 motif peptides

Because HLA-DR3 is an allele that is prevalent in Caucasian, Black, and Hispanic populations, DR3 binding capacity is a relevant criterion in the selection of HTL epitopes. Thus, peptides shown to be candidates may also be assayed for their DR3 binding capacity. However, in view of the binding specificity of the DR3 motif, peptides binding only to DR3 can also be considered as candidates for inclusion in a vaccine formulation.

To efficiently identify peptides that bind DR3, target 158P1D7 antigens are analyzed for sequences carrying one of the two DR3-specific binding motifs reported by Geluk *et al.* (*J. Immunol.* 152:5742-5748, 1994). The corresponding peptides are then synthesized and confirmed as having the

ability to bind DR3 with an affinity of 1 μ M or better, i.e., less than 1 μ M. Peptides are found that meet this binding criterion and qualify as HLA class II high affinity binders.

DR3 binding epitopes identified in this manner are included in vaccine compositions with DR supermotif-bearing peptide epitopes.

Similarly to the case of HLA class I motif-bearing peptides, the class II motif-bearing peptides are analogized to improve affinity or cross-reactivity. For example, aspartic acid at position 4 of the 9-mer core sequence is an optimal residue for DR3 binding, and substitution for that residue often improves DR 3 binding.

Example 15: Immunogenicity of 158P1D7-derived HTL epitopes

This example determines immunogenic DR supermotif- and DR3 motif-bearing epitopes among those identified using the methodology set forth herein.

Immunogenicity of HTL epitopes are confirmed in a manner analogous to the determination of immunogenicity of CTL epitopes, by assessing the ability to stimulate HTL responses and/or by using appropriate transgenic mouse models. Immunogenicity is determined by screening for: 1.) *in vitro* primary induction using normal PBMC or 2.) recall responses from patients who have 158P1D7-expressing tumors.

Example 16: Calculation of phenotypic frequencies of HLA-supertypes in various ethnic backgrounds to determine breadth of population coverage

This example illustrates the assessment of the breadth of population coverage of a vaccine composition comprised of multiple epitopes comprising multiple supermotifs and/or motifs.

In order to analyze population coverage, gene frequencies of HLA alleles are determined. Gene frequencies for each HLA allele are calculated from antigen or allele frequencies utilizing the binomial distribution formulae $gf=1-(\text{SQRT}(1-af))$ (see, *e.g.*, Sidney *et al.*, *Human Immunol.* 45:79-93, 1996). To obtain overall phenotypic frequencies, cumulative gene frequencies are calculated, and the cumulative antigen frequencies derived by the use of the inverse formula $[af=1-(1-Cgf)^2]$.

Where frequency data is not available at the level of DNA typing, correspondence to the serologically defined antigen frequencies is assumed. To obtain total potential supertype population coverage no linkage disequilibrium is assumed, and only alleles confirmed to belong to each of the supertypes are included (minimal estimates). Estimates of total potential coverage achieved by inter-loci combinations are made by adding to the A coverage the proportion of the non-A covered population that could be expected to be covered by the B alleles considered (*e.g.*, $\text{total}=A+B*(1-A)$). Confirmed members of the A3-like supertype are A3, A11, A31, A*3301, and A*6801. Although the A3-like supertype may also include A34, A66, and A*7401, these alleles were not included in overall frequency calculations. Likewise, confirmed members of the A2-like supertype family are A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*6802, and A*6901. Finally, the B7-like supertype-confirmed alleles are: B7,

B*3501-03, B51, B*5301, B*5401, B*5501-2, B*5601, B*6701, and B*7801 (potentially also B*1401, B*3504-06, B*4201, and B*5602).

Population coverage achieved by combining the A2-, A3- and B7-supertypes is approximately 86% in five major ethnic groups. Coverage may be extended by including peptides bearing the A1 and A24 motifs. On average, A1 is present in 12% and A24 in 29% of the population across five different major ethnic groups (Caucasian, North American Black, Chinese, Japanese, and Hispanic). Together, these alleles are represented with an average frequency of 39% in these same ethnic populations. The total coverage across the major ethnicities when A1 and A24 are combined with the coverage of the A2-, A3- and B7-supertype alleles is >95%. An analogous approach can be used to estimate population coverage achieved with combinations of class II motif-bearing epitopes.

Immunogenicity studies in humans (*e.g.*, Bertoni *et al.*, *J. Clin. Invest.* 100:503, 1997; Doolan *et al.*, *Immunity* 7:97, 1997; and Threlkeld *et al.*, *J. Immunol.* 159:1648, 1997) have shown that highly cross-reactive binding peptides are almost always recognized as epitopes. The use of highly cross-reactive binding peptides is an important selection criterion in identifying candidate epitopes for inclusion in a vaccine that is immunogenic in a diverse population.

With a sufficient number of epitopes (as disclosed herein and from the art), an average population coverage is predicted to be greater than 95% in each of five major ethnic populations. The game theory Monte Carlo simulation analysis, which is known in the art (see *e.g.*, Osborne, M.J. and Rubinstein, A. "A course in game theory" MIT Press, 1994), can be used to estimate what percentage of the individuals in a population comprised of the Caucasian, North American Black, Japanese, Chinese, and Hispanic ethnic groups would recognize the vaccine epitopes described herein. A preferred percentage is 90%. A more preferred percentage is 95%.

Example 17: CTL Recognition Of Endogenously Processed Antigens After Priming

This example confirms that CTL induced by native or analoged peptide epitopes identified and selected as described herein recognize endogenously synthesized, *i.e.*, native antigens.

Effector cells isolated from transgenic mice that are immunized with peptide epitopes, for example HLA-A2 supermotif-bearing epitopes, are re-stimulated *in vitro* using peptide-coated stimulator cells. Six days later, effector cells are assayed for cytotoxicity and the cell lines that contain peptide-specific cytotoxic activity are further re-stimulated. An additional six days later, these cell lines are tested for cytotoxic activity on ⁵¹Cr labeled Jurkat-A2.1/K^b target cells in the absence or presence of peptide, and also tested on ⁵¹Cr labeled target cells bearing the endogenously synthesized antigen, *i.e.* cells that are stably transfected with 158P1D7 expression vectors.

The results demonstrate that CTL lines obtained from animals primed with peptide epitope recognize endogenously synthesized 158P1D7 antigen. The choice of transgenic mouse model to be used for such an analysis depends upon the epitope(s) that are being evaluated. In addition to HLA-A*0201/K^b transgenic mice, several other transgenic mouse models including mice with human A11, which may also

be used to evaluate A3 epitopes, and B7 alleles have been characterized and others (e.g., transgenic mice for HLA-A1 and A24) are being developed. HLA-DR1 and HLA-DR3 mouse models have also been developed, which may be used to evaluate HTL epitopes.

Example 18: Activity Of CTL-HTL Conjugated Epitopes In Transgenic Mice

This example illustrates the induction of CTLs and HTLs in transgenic mice, by use of a 158P1D7-derived CTL and HTL peptide vaccine compositions. The vaccine composition used herein comprise peptides to be administered to a patient with a 158P1D7-expressing tumor. The peptide composition can comprise multiple CTL and/or HTL epitopes. The epitopes are identified using methodology as described herein. This example also illustrates that enhanced immunogenicity can be achieved by inclusion of one or more HTL epitopes in a CTL vaccine composition; such a peptide composition can comprise an HTL epitope conjugated to a CTL epitope. The CTL epitope can be one that binds to multiple HLA family members at an affinity of 500 nM or less, or analogs of that epitope. The peptides may be lipidated, if desired.

Immunization procedures: Immunization of transgenic mice is performed as described (Alexander *et al.*, *J. Immunol.* 159:4753-4761, 1997). For example, A2/K^b mice, which are transgenic for the human HLA A2.1 allele and are used to confirm the immunogenicity of HLA-A*0201 motif- or HLA-A2 supermotif-bearing epitopes, and are primed subcutaneously (base of the tail) with a 0.1 ml of peptide in Incomplete Freund's Adjuvant, or if the peptide composition is a lipidated CTL/HTL conjugate, in DMSO/saline, or if the peptide composition is a polypeptide, in PBS or Incomplete Freund's Adjuvant. Seven days after priming, splenocytes obtained from these animals are restimulated with syngenic irradiated LPS-activated lymphoblasts coated with peptide.

Cell lines: Target cells for peptide-specific cytotoxicity assays are Jurkat cells transfected with the HLA-A2.1/K^b chimeric gene (e.g., Vitiello *et al.*, *J. Exp. Med.* 173:1007, 1991)

In vitro CTL activation: One week after priming, spleen cells (30x10⁶ cells/flask) are co-cultured at 37°C with syngeneic, irradiated (3000 rads), peptide coated lymphoblasts (10x10⁶ cells/flask) in 10 ml of culture medium/T25 flask. After six days, effector cells are harvested and assayed for cytotoxic activity.

Assay for cytotoxic activity: Target cells (1.0 to 1.5x10⁶) are incubated at 37°C in the presence of 200 µl of ⁵¹Cr. After 60 minutes, cells are washed three times and resuspended in R10 medium. Peptide is added where required at a concentration of 1 µg/ml. For the assay, 10⁴ ⁵¹Cr-labeled target cells are added to different concentrations of effector cells (final volume of 200 µl) in U-bottom 96-well plates. After a six hour incubation period at 37°C, a 0.1 ml aliquot of supernatant is removed from each well and radioactivity is determined in a Micromedic automatic gamma counter. The percent specific lysis is determined by the formula: percent specific release = 100 x (experimental release - spontaneous release)/(maximum release - spontaneous release). To facilitate comparison between separate CTL assays run under the same conditions, % ⁵¹Cr release data is expressed as lytic units/10⁶ cells. One lytic unit is arbitrarily defined as the number of effector cells required to achieve 30% lysis of 10,000 target cells in a six hour ⁵¹Cr release

assay. To obtain specific lytic units/ 10^6 , the lytic units/ 10^6 obtained in the absence of peptide is subtracted from the lytic units/ 10^6 obtained in the presence of peptide. For example, if 30% ^{51}Cr release is obtained at the effector (E): target (T) ratio of 50:1 (i.e., 5×10^5 effector cells for 10,000 targets) in the absence of peptide and 5:1 (i.e., 5×10^4 effector cells for 10,000 targets) in the presence of peptide, the specific lytic units would be: $[(1/50,000) - (1/500,000)] \times 10^6 = 18 \text{ LU}$.

The results are analyzed to assess the magnitude of the CTL responses of animals injected with the immunogenic CTL/HTL conjugate vaccine preparation and are compared to the magnitude of the CTL response achieved using, for example, CTL epitopes as outlined above in the Example entitled "Confirmation of Immunogenicity". Analyses similar to this may be performed to confirm the immunogenicity of peptide conjugates containing multiple CTL epitopes and/or multiple HTL epitopes. In accordance with these procedures, it is found that a CTL response is induced, and concomitantly that an HTL response is induced upon administration of such compositions.

Example 19: Selection of CTL and HTL epitopes for inclusion in an 158P1D7-specific vaccine.

This example illustrates a procedure for selecting peptide epitopes for vaccine compositions of the invention. The peptides in the composition can be in the form of a nucleic acid sequence, either single or one or more sequences (i.e., minigene) that encodes peptide(s), or can be single and/or polyepitopic peptides.

The following principles are utilized when selecting a plurality of epitopes for inclusion in a vaccine composition. Each of the following principles is balanced in order to make the selection.

Epitopes are selected which, upon administration, mimic immune responses that are correlated with 158P1D7 clearance. The number of epitopes used depends on observations of patients who spontaneously clear 158P1D7. For example, if it has been observed that patients who spontaneously clear 158P1D7 generate an immune response to at least three (3) from 158P1D7 antigen, then three or four (3-4) epitopes should be included for HLA class I. A similar rationale is used to determine HLA class II epitopes.

Epitopes are often selected that have a binding affinity of an IC_{50} of 500 nM or less for an HLA class I molecule, or for class II, an IC_{50} of 1000 nM or less; or HLA Class I peptides with high binding scores from the BIMAS web site.

In order to achieve broad coverage of the vaccine through out a diverse population, sufficient supermotif bearing peptides, or a sufficient array of allele-specific motif bearing peptides, are selected to give broad population coverage. In one embodiment, epitopes are selected to provide at least 80% population coverage. A Monte Carlo analysis, a statistical evaluation known in the art, can be employed to assess breadth, or redundancy, of population coverage.

When creating polyepitopic compositions, or a minigene that encodes same, it is typically desirable to generate the smallest peptide possible that encompasses the epitopes of interest. The principles

employed are similar, if not the same, as those employed when selecting a peptide comprising nested epitopes. For example, a protein sequence for the vaccine composition is selected because it has maximal number of epitopes contained within the sequence, *i.e.*, it has a high concentration of epitopes. Epitopes may be nested or overlapping (*i.e.*, frame shifted relative to one another). For example, with overlapping epitopes, two 9-mer epitopes and one 10-mer epitope can be present in a 10 amino acid peptide. Each epitope can be exposed and bound by an HLA molecule upon administration of such a peptide. A multi-epitopic peptide can be generated synthetically, recombinantly, or via cleavage from the native source. Alternatively, an analog can be made of this native sequence, whereby one or more of the epitopes comprise substitutions that alter the cross-reactivity and/or binding affinity properties of the polypeptidic peptide. Such a vaccine composition is administered for therapeutic or prophylactic purposes. This embodiment provides for the possibility that an as yet undiscovered aspect of immune system processing will apply to the native nested sequence and thereby facilitate the production of therapeutic or prophylactic immune response-inducing vaccine compositions. Additionally such an embodiment provides for the possibility of motif-bearing epitopes for an HLA makeup that is presently unknown. Furthermore, this embodiment (absent the creating of any analogs) directs the immune response to multiple peptide sequences that are actually present in 158P1D7, thus avoiding the need to evaluate any junctional epitopes. Lastly, the embodiment provides an economy of scale when producing nucleic acid vaccine compositions. Related to this embodiment, computer programs can be derived in accordance with principles in the art, which identify in a target sequence, the greatest number of epitopes per sequence length.

A vaccine composition comprised of selected peptides, when administered, is safe, efficacious, and elicits an immune response similar in magnitude to an immune response that controls or clears cells that bear or overexpress 158P1D7.

Example 20: Construction of "Minigene" Multi-Epitope DNA Plasmids

This example discusses the construction of a minigene expression plasmid. Minigene plasmids may, of course, contain various configurations of B cell, CTL and/or HTL epitopes or epitope analogs as described herein.

A minigene expression plasmid typically includes multiple CTL and HTL peptide epitopes. In the present example, HLA-A2, -A3, -B7 supermotif-bearing peptide epitopes and HLA-A1 and -A24 motif-bearing peptide epitopes are used in conjunction with DR supermotif-bearing epitopes and/or DR3 epitopes. HLA class I supermotif or motif-bearing peptide epitopes derived 158P1D7, are selected such that multiple supermotifs/motifs are represented to ensure broad population coverage. Similarly, HLA class II epitopes are selected from 158P1D7 to provide broad population coverage, *i.e.* both HLA DR-1-4-7 supermotif-bearing epitopes and HLA DR-3 motif-bearing epitopes are selected for inclusion in the minigene construct. The selected CTL and HTL epitopes are then incorporated into a minigene for expression in an expression vector.

Such a construct may additionally include sequences that direct the HTL epitopes to the endoplasmic reticulum. For example, the Ii protein may be fused to one or more HTL epitopes as described in the art, wherein the CLIP sequence of the Ii protein is removed and replaced with an HLA class II epitope sequence so that HLA class II epitope is directed to the endoplasmic reticulum, where the epitope binds to an HLA class II molecules.

This example illustrates the methods to be used for construction of a minigene-bearing expression plasmid. Other expression vectors that may be used for minigene compositions are available and known to those of skill in the art.

The minigene DNA plasmid of this example contains a consensus Kozak sequence and a consensus murine kappa Ig-light chain signal sequence followed by CTL and/or HTL epitopes selected in accordance with principles disclosed herein. The sequence encodes an open reading frame fused to the Myc and His antibody epitope tag coded for by the pcDNA 3.1 Myc-His vector.

Overlapping oligonucleotides that can, for example, average about 70 nucleotides in length with 15 nucleotide overlaps, are synthesized and HPLC-purified. The oligonucleotides encode the selected peptide epitopes as well as appropriate linker nucleotides, Kozak sequence, and signal sequence. The final multiepitope minigene is assembled by extending the overlapping oligonucleotides in three sets of reactions using PCR. A Perkin/Elmer 9600 PCR machine is used and a total of 30 cycles are performed using the following conditions: 95°C for 15 sec, annealing temperature (5° below the lowest calculated T_m of each primer pair) for 30 sec, and 72°C for 1 min.

For example, a minigene is prepared as follows. For a first PCR reaction, 5 µg of each of two oligonucleotides are annealed and extended: In an example using eight oligonucleotides, i.e., four pairs of primers, oligonucleotides 1+2, 3+4, 5+6, and 7+8 are combined in 100 µl reactions containing *Pfu* polymerase buffer (1x= 10 mM KCL, 10 mM (NH₄)₂SO₄, 20 mM Tris-chloride, pH 8.75, 2 mM MgSO₄, 0.1% Triton X-100, 100 µg/ml BSA), 0.25 mM each dNTP, and 2.5 U of *Pfu* polymerase. The full-length dimer products are gel-purified, and two reactions containing the product of 1+2 and 3+4, and the product of 5+6 and 7+8 are mixed, annealed, and extended for 10 cycles. Half of the two reactions are then mixed, and 5 cycles of annealing and extension carried out before flanking primers are added to amplify the full length product. The full-length product is gel-purified and cloned into pCR-blunt (Invitrogen) and individual clones are screened by sequencing.

Example 21: The Plasmid Construct and the Degree to Which It Induces Immunogenicity.

The degree to which a plasmid construct, for example a plasmid constructed in accordance with the previous Example, is able to induce immunogenicity is confirmed *in vitro* by determining epitope presentation by APC following transduction or transfection of the APC with an epitope-expressing nucleic acid construct. Such a study determines “antigenicity” and allows the use of human APC. The assay determines the ability of the epitope to be presented by the APC in a context that is recognized by a T cell by quantifying the density of epitope-HLA class I complexes on the cell surface. Quantitation can be

performed by directly measuring the amount of peptide eluted from the APC (*see, e.g.,* Sijts *et al.*, *J. Immunol.* 156:683-692, 1996; Demotz *et al.*, *Nature* 342:682-684, 1989); or the number of peptide-HLA class I complexes can be estimated by measuring the amount of lysis or lymphokine release induced by diseased or transfected target cells, and then determining the concentration of peptide necessary to obtain equivalent levels of lysis or lymphokine release (*see, e.g.,* Kageyama *et al.*, *J. Immunol.* 154:567-576, 1995).

Alternatively, immunogenicity is confirmed through *in vivo* injections into mice and subsequent *in vitro* assessment of CTL and HTL activity, which are analyzed using cytotoxicity and proliferation assays, respectively, as detailed *e.g.,* in Alexander *et al.*, *Immunity* 1:751-761, 1994.

For example, to confirm the capacity of a DNA minigene construct containing at least one HLA-A2 supermotif peptide to induce CTLs *in vivo*, HLA-A2.1/K^b transgenic mice, for example, are immunized intramuscularly with 100 µg of naked cDNA. As a means of comparing the level of CTLs induced by cDNA immunization, a control group of animals is also immunized with an actual peptide composition that comprises multiple epitopes synthesized as a single polypeptide as they would be encoded by the minigene.

Splenocytes from immunized animals are stimulated twice with each of the respective compositions (peptide epitopes encoded in the minigene or the polyepitopic peptide), then assayed for peptide-specific cytotoxic activity in a ⁵¹Cr release assay. The results indicate the magnitude of the CTL response directed against the A2-restricted epitope, thus indicating the *in vivo* immunogenicity of the minigene vaccine and polyepitopic vaccine.

It is, therefore, found that the minigene elicits immune responses directed toward the HLA-A2 supermotif peptide epitopes as does the polyepitopic peptide vaccine. A similar analysis is also performed using other HLA-A3 and HLA-B7 transgenic mouse models to assess CTL induction by HLA-A3 and HLA-B7 motif or supermotif epitopes, whereby it is also found that the minigene elicits appropriate immune responses directed toward the provided epitopes.

To confirm the capacity of a class II epitope-encoding minigene to induce HTLs *in vivo*, DR transgenic mice, or for those epitopes that cross react with the appropriate mouse MHC molecule, I-A^b-restricted mice, for example, are immunized intramuscularly with 100 µg of plasmid DNA. As a means of comparing the level of HTLs induced by DNA immunization, a group of control animals is also immunized with an actual peptide composition emulsified in complete Freund's adjuvant. CD4⁺ T cells, *i.e.* HTLs, are purified from splenocytes of immunized animals and stimulated with each of the respective compositions (peptides encoded in the minigene). The HTL response is measured using a ³H-thymidine incorporation proliferation assay, (*see, e.g.,* Alexander *et al.* *Immunity* 1:751-761, 1994). The results indicate the magnitude of the HTL response, thus demonstrating the *in vivo* immunogenicity of the minigene.

DNA minigenes, constructed as described in the previous Example, can also be confirmed as a vaccine in combination with a boosting agent using a prime boost protocol. The boosting agent can consist of recombinant protein (*e.g.,* Barnett *et al.*, *Aids Res. and Human Retroviruses* 14, Supplement 3:S299-S309, 1998) or recombinant vaccinia, for example, expressing a minigene or DNA encoding the complete

protein of interest (*see, e.g., Hanke et al., Vaccine* 16:439-445, 1998; Sedegah *et al., Proc. Natl. Acad. Sci USA* 95:7648-53, 1998; Hanke and McMichael, *Immunol. Letters* 66:177-181, 1999; and Robinson *et al., Nature Med.* 5:526-34, 1999).

For example, the efficacy of the DNA minigene used in a prime boost protocol is initially evaluated in transgenic mice. In this example, A2.1/K^b transgenic mice are immunized IM with 100 µg of a DNA minigene encoding the immunogenic peptides including at least one HLA-A2 supermotif-bearing peptide. After an incubation period (ranging from 3-9 weeks), the mice are boosted IP with 10⁷ pfu/mouse of a recombinant vaccinia virus expressing the same sequence encoded by the DNA minigene. Control mice are immunized with 100 µg of DNA or recombinant vaccinia without the minigene sequence, or with DNA encoding the minigene, but without the vaccinia boost. After an additional incubation period of two weeks, splenocytes from the mice are immediately assayed for peptide-specific activity in an ELISPOT assay. Additionally, splenocytes are stimulated *in vitro* with the A2-restricted peptide epitopes encoded in the minigene and recombinant vaccinia, then assayed for peptide-specific activity in an alpha, beta and/or gamma IFN ELISA.

It is found that the minigene utilized in a prime-boost protocol elicits greater immune responses toward the HLA-A2 supermotif peptides than with DNA alone. Such an analysis can also be performed using HLA-A11 or HLA-B7 transgenic mouse models to assess CTL induction by HLA-A3 or HLA-B7 motif or supermotif epitopes. The use of prime boost protocols in humans is described below in the Example entitled "Induction of CTL Responses Using a Prime Boost Protocol."

Example 22: Peptide Composition for Prophylactic Uses

Vaccine compositions of the present invention can be used to prevent 158P1D7 expression in persons who are at risk for tumors that bear this antigen. For example, a polyepitopic peptide epitope composition (or a nucleic acid comprising the same) containing multiple CTL and HTL epitopes such as those selected in the above Examples, which are also selected to target greater than 80% of the population, is administered to individuals at risk for a 158P1D7-associated tumor.

For example, a peptide-based composition is provided as a single polypeptide that encompasses multiple epitopes. The vaccine is typically administered in a physiological solution that comprises an adjuvant, such as Incomplete Freund's Adjuvant. The dose of peptide for the initial immunization is from about 1 to about 50,000 µg, generally 100-5,000 µg, for a 70 kg patient. The initial administration of vaccine is followed by booster dosages at 4 weeks followed by evaluation of the magnitude of the immune response in the patient, by techniques that determine the presence of epitope-specific CTL populations in a PBMC sample. Additional booster doses are administered as required. The composition is found to be both safe and efficacious as a prophylaxis against 158P1D7-associated disease.

Alternatively, a composition typically comprising transfecting agents is used for the administration of a nucleic acid-based vaccine in accordance with methodologies known in the art and disclosed herein.

Example 23: Polyepitopic Vaccine Compositions Derived from Native 158P1D7 Sequences

A native 158P1D7 polyprotein sequence is analyzed, preferably using computer algorithms defined for each class I and/or class II supermotif or motif, to identify “relatively short” regions of the polyprotein that comprise multiple epitopes. The “relatively short” regions are preferably less in length than an entire native antigen. This relatively short sequence that contains multiple distinct or overlapping, “nested” epitopes is selected; it can be used to generate a minigene construct. The construct is engineered to express the peptide, which corresponds to the native protein sequence. The “relatively short” peptide is generally less than 250 amino acids in length, often less than 100 amino acids in length, preferably less than 75 amino acids in length, and more preferably less than 50 amino acids in length. The protein sequence of the vaccine composition is selected because it has maximal number of epitopes contained within the sequence, *i.e.*, it has a high concentration of epitopes. As noted herein, epitope motifs may be nested or overlapping (*i.e.*, frame shifted relative to one another). For example, with overlapping epitopes, two 9-mer epitopes and one 10-mer epitope can be present in a 10 amino acid peptide. Such a vaccine composition is administered for therapeutic or prophylactic purposes.

The vaccine composition will include, for example, multiple CTL epitopes from 158P1D7 antigen and at least one HTL epitope. This polyepitopic native sequence is administered either as a peptide or as a nucleic acid sequence which encodes the peptide. Alternatively, an analog can be made of this native sequence, whereby one or more of the epitopes comprise substitutions that alter the cross-reactivity and/or binding affinity properties of the polyepitopic peptide.

The embodiment of this example provides for the possibility that an as yet undiscovered aspect of immune system processing will apply to the native nested sequence and thereby facilitate the production of therapeutic or prophylactic immune response-inducing vaccine compositions. Additionally such an embodiment provides for the possibility of motif-bearing epitopes for an HLA makeup that is presently unknown. Furthermore, this embodiment (excluding an analoged embodiment) directs the immune response to multiple peptide sequences that are actually present in native 158P1D7, thus avoiding the need to evaluate any junctional epitopes. Lastly, the embodiment provides an economy of scale when producing peptide or nucleic acid vaccine compositions.

Related to this embodiment, computer programs are available in the art which can be used to identify in a target sequence, the greatest number of epitopes per sequence length.

Example 24: Polyepitopic Vaccine Compositions From Multiple Antigens

The 158P1D7 peptide epitopes of the present invention are used in conjunction with epitopes from other target tumor-associated antigens, to create a vaccine composition that is useful for the prevention or treatment of cancer that expresses 158P1D7 and such other antigens. For example, a vaccine composition can be provided as a single polypeptide that incorporates multiple epitopes from 158P1D7 as well as tumor-associated antigens that are often expressed with a target cancer associated with 158P1D7

expression, or can be administered as a composition comprising a cocktail of one or more discrete epitopes. Alternatively, the vaccine can be administered as a minigene construct or as dendritic cells which have been loaded with the peptide epitopes *in vitro*.

Example 25: Use of peptides to evaluate an immune response

Peptides of the invention may be used to analyze an immune response for the presence of specific antibodies, CTL or HTL directed to 158P1D7. Such an analysis can be performed in a manner described by Ogg *et al.*, *Science* 279:2103-2106, 1998. In this Example, peptides in accordance with the invention are used as a reagent for diagnostic or prognostic purposes, not as an immunogen.

In this example highly sensitive human leukocyte antigen tetrameric complexes ("tetramers") are used for a cross-sectional analysis of, for example, 158P1D7 HLA-A*0201-specific CTL frequencies from HLA A*0201-positive individuals at different stages of disease or following immunization comprising an 158P1D7 peptide containing an A*0201 motif. Tetrameric complexes are synthesized as described (Musey *et al.*, *N. Engl. J. Med.* 337:1267, 1997). Briefly, purified HLA heavy chain (A*0201 in this example) and β 2-microglobulin are synthesized by means of a prokaryotic expression system. The heavy chain is modified by deletion of the transmembrane-cytosolic tail and COOH-terminal addition of a sequence containing a BirA enzymatic biotinylation site. The heavy chain, β 2-microglobulin, and peptide are refolded by dilution. The 45-kD refolded product is isolated by fast protein liquid chromatography and then biotinylated by BirA in the presence of biotin (Sigma, St. Louis, Missouri), adenosine 5' triphosphate and magnesium. Streptavidin-phycoerythrin conjugate is added in a 1:4 molar ratio, and the tetrameric product is concentrated to 1 mg/ml. The resulting product is referred to as tetramer-phycoerythrin.

For the analysis of patient blood samples, approximately one million PBMCs are centrifuged at 300g for 5 minutes and resuspended in 50 μ l of cold phosphate-buffered saline. Tri-color analysis is performed with the tetramer-phycoerythrin, along with anti-CD8-Tricolor, and anti-CD38. The PBMCs are incubated with tetramer and antibodies on ice for 30 to 60 min and then washed twice before formaldehyde fixation. Gates are applied to contain >99.98% of control samples. Controls for the tetramers include both A*0201-negative individuals and A*0201-positive non-diseased donors. The percentage of cells stained with the tetramer is then determined by flow cytometry. The results indicate the number of cells in the PBMC sample that contain epitope-restricted CTLs, thereby readily indicating the extent of immune response to the 158P1D7 epitope, and thus the status of exposure to 158P1D7, or exposure to a vaccine that elicits a protective or therapeutic response.

Example 26: Use of Peptide Epitopes to Evaluate Recall Responses

The peptide epitopes of the invention are used as reagents to evaluate T cell responses, such as acute or recall responses, in patients. Such an analysis may be performed on patients who have recovered from 158P1D7-associated disease or who have been vaccinated with an 158P1D7 vaccine.

For example, the class I restricted CTL response of persons who have been vaccinated may be analyzed. The vaccine may be any 158P1D7 vaccine. PBMC are collected from vaccinated individuals and HLA typed. Appropriate peptide epitopes of the invention that, optimally, bear supermotifs to provide cross-reactivity with multiple HLA supertype family members, are then used for analysis of samples derived from individuals who bear that HLA type.

PBMC from vaccinated individuals are separated on Ficoll-Histopaque density gradients (Sigma Chemical Co., St. Louis, MO), washed three times in HBSS (GIBCO Laboratories), resuspended in RPMI-1640 (GIBCO Laboratories) supplemented with L-glutamine (2mM), penicillin (50U/ml), streptomycin (50 µg/ml), and Hepes (10mM) containing 10% heat-inactivated human AB serum (complete RPMI) and plated using microculture formats. A synthetic peptide comprising an epitope of the invention is added at 10 µg/ml to each well and HBV core 128-140 epitope is added at 1 µg/ml to each well as a source of T cell help during the first week of stimulation.

In the microculture format, 4×10^5 PBMC are stimulated with peptide in 8 replicate cultures in 96-well round bottom plate in 100 µl/well of complete RPMI. On days 3 and 10, 100 ul of complete RPMI and 20 U/ml final concentration of rIL-2 are added to each well. On day 7 the cultures are transferred into a 96-well flat-bottom plate and restimulated with peptide, rIL-2 and 10^5 irradiated (3,000 rad) autologous feeder cells. The cultures are tested for cytotoxic activity on day 14. A positive CTL response requires two or more of the eight replicate cultures to display greater than 10% specific ^{51}Cr release, based on comparison with non-diseased control subjects as previously described (Rehermann, *et al.*, *Nature Med.* 2:1104,1108, 1996; Rehermann *et al.*, *J. Clin. Invest.* 97:1655-1665, 1996; and Rehermann *et al.* *J. Clin. Invest.* 98:1432-1440, 1996).

Target cell lines are autologous and allogeneic EBV-transformed B-LCL that are either purchased from the American Society for Histocompatibility and Immunogenetics (ASHI, Boston, MA) or established from the pool of patients as described (Guilhot, *et al.* *J. Virol.* 66:2670-2678, 1992).

Cytotoxicity assays are performed in the following manner. Target cells consist of either allogeneic HLA-matched or autologous EBV-transformed B lymphoblastoid cell line that are incubated overnight with the synthetic peptide epitope of the invention at 10 µM, and labeled with 100 µCi of ^{51}Cr (Amersham Corp., Arlington Heights, IL) for 1 hour after which they are washed four times with HBSS.

Cytolytic activity is determined in a standard 4-h, split well ^{51}Cr release assay using U-bottomed 96 well plates containing 3,000 targets/well. Stimulated PBMC are tested at effector/target (E/T) ratios of 20-50:1 on day 14. Percent cytotoxicity is determined from the formula: $100 \times [(\text{experimental release} - \text{spontaneous release}) / (\text{maximum release} - \text{spontaneous release})]$. Maximum release is determined by lysis of targets by detergent (2% Triton X-100; Sigma Chemical Co., St. Louis, MO). Spontaneous release is <25% of maximum release for all experiments.

The results of such an analysis indicate the extent to which HLA-restricted CTL populations have been stimulated by previous exposure to 158P1D7 or an 158P1D7 vaccine.

Similarly, Class II restricted HTL responses may also be analyzed. Purified PBMC are cultured in a 96-well flat bottom plate at a density of 1.5×10^5 cells/well and are stimulated with 10 $\mu\text{g/ml}$ synthetic peptide of the invention, whole 158P1D7 antigen, or PHA. Cells are routinely plated in replicates of 4-6 wells for each condition. After seven days of culture, the medium is removed and replaced with fresh medium containing 10U/ml IL-2. Two days later, 1 μCi ^3H -thymidine is added to each well and incubation is continued for an additional 18 hours. Cellular DNA is then harvested on glass fiber mats and analyzed for ^3H -thymidine incorporation. Antigen-specific T cell proliferation is calculated as the ratio of ^3H -thymidine incorporation in the presence of antigen divided by the ^3H -thymidine incorporation in the absence of antigen.

Example 27: Induction Of Specific CTL Response In Humans

A human clinical trial for an immunogenic composition comprising CTL and HTL epitopes of the invention is set up as an IND Phase I, dose escalation study and carried out as a randomized, double-blind, placebo-controlled trial. Such a trial is designed, for example, as follows:

A total of about 27 individuals are enrolled and divided into 3 groups:

Group I: 3 subjects are injected with placebo and 6 subjects are injected with 5 μg of peptide composition;

Group II: 3 subjects are injected with placebo and 6 subjects are injected with 50 μg peptide composition;

Group III: 3 subjects are injected with placebo and 6 subjects are injected with 500 μg of peptide composition.

After 4 weeks following the first injection, all subjects receive a booster inoculation at the same dosage.

The endpoints measured in this study relate to the safety and tolerability of the peptide composition as well as its immunogenicity. Cellular immune responses to the peptide composition are an index of the intrinsic activity of this the peptide composition, and can therefore be viewed as a measure of biological efficacy. The following summarize the clinical and laboratory data that relate to safety and efficacy endpoints.

Safety: The incidence of adverse events is monitored in the placebo and drug treatment group and assessed in terms of degree and reversibility.

Evaluation of Vaccine Efficacy: For evaluation of vaccine efficacy, subjects are bled before and after injection. Peripheral blood mononuclear cells are isolated from fresh heparinized blood by Ficoll-Hypaque density gradient centrifugation, aliquoted in freezing media and stored frozen. Samples are assayed for CTL and HTL activity.

The vaccine is found to be both safe and efficacious.

Example 28: Phase II Trials In Patients Expressing 158P1D7

Phase II trials are performed to study the effect of administering the CTL-HTL peptide compositions to patients having cancer that expresses 158P1D7. The main objectives of the trial are to determine an effective dose and regimen for inducing CTLs in cancer patients that express 158P1D7, to establish the safety of inducing a CTL and HTL response in these patients, and to see to what extent activation of CTLs improves the clinical picture of these patients, as manifested, e.g., by the reduction and/or shrinking of lesions. Such a study is designed, for example, as follows:

The studies are performed in multiple centers. The trial design is an open-label, uncontrolled, dose escalation protocol wherein the peptide composition is administered as a single dose followed six weeks later by a single booster shot of the same dose. The dosages are 50, 500 and 5,000 micrograms per injection. Drug-associated adverse effects (severity and reversibility) are recorded.

There are three patient groupings. The first group is injected with 50 micrograms of the peptide composition and the second and third groups with 500 and 5,000 micrograms of peptide composition, respectively. The patients within each group range in age from 21-65 and represent diverse ethnic backgrounds. All of them have a tumor that expresses 158P1D7.

Clinical manifestations or antigen-specific T-cell responses are monitored to assess the effects of administering the peptide compositions. The vaccine composition is found to be both safe and efficacious in the treatment of 158P1D7-associated disease.

Example 29: Induction of CTL Responses Using a Prime Boost Protocol

A prime boost protocol similar in its underlying principle to that used to confirm the efficacy of a DNA vaccine in transgenic mice, such as described above in the Example entitled "The Plasmid Construct and the Degree to Which It Induces Immunogenicity," can also be used for the administration of the vaccine to humans. Such a vaccine regimen can include an initial administration of, for example, naked DNA followed by a boost using recombinant virus encoding the vaccine, or recombinant protein/polypeptide or a peptide mixture administered in an adjuvant.

For example, the initial immunization may be performed using an expression vector, such as that constructed in the Example entitled "Construction of 'Minigene' Multi-Epitope DNA Plasmids" in the form of naked nucleic acid administered IM (or SC or ID) in the amounts of 0.5-5 mg at multiple sites. The nucleic acid (0.1 to 1000 µg) can also be administered using a gene gun. Following an incubation period of 3-4 weeks, a booster dose is then administered. The booster can be recombinant fowlpox virus administered at a dose of $5 \cdot 10^7$ to $5 \cdot 10^9$ pfu. An alternative recombinant virus, such as an MVA, canarypox, adenovirus, or adeno-associated virus, can also be used for the booster, or the polypeptidic protein or a mixture of the peptides can be administered. For evaluation of vaccine efficacy, patient blood samples are obtained before immunization as well as at intervals following administration of the initial vaccine and booster doses of the vaccine. Peripheral blood mononuclear cells are isolated from fresh heparinized blood by Ficoll-Hypaque density gradient centrifugation, aliquoted in freezing media and stored frozen. Samples are assayed for CTL and HTL activity.

Analysis of the results indicates that a magnitude of response sufficient to achieve a therapeutic or protective immunity against 158P1D7 is generated.

Example 30: Administration of Vaccine Compositions Using Dendritic Cells (DC)

Vaccines comprising peptide epitopes of the invention can be administered using APCs, or "professional" APCs such as DC. In this example, peptide-pulsed DC are administered to a patient to stimulate a CTL response *in vivo*. In this method, dendritic cells are isolated, expanded, and pulsed with a vaccine comprising peptide CTL and HTL epitopes of the invention. The dendritic cells are infused back into the patient to elicit CTL and HTL responses *in vivo*. The induced CTL and HTL then destroy or facilitate destruction, respectively, of the target cells that bear the 158P1D7 protein from which the epitopes in the vaccine are derived.

For example, a cocktail of epitope-comprising peptides is administered *ex vivo* to PBMC, or isolated DC therefrom. A pharmaceutical to facilitate harvesting of DC can be used, such as Progenipoietin™ (Monsanto, St. Louis, MO) or GM-CSF/IL-4. After pulsing the DC with peptides, and prior to reinfusion into patients, the DC are washed to remove unbound peptides.

As appreciated clinically, and readily determined by one of skill based on clinical outcomes, the number of DC reinfused into the patient can vary (*see, e.g., Nature Med.* 4:328, 1998; *Nature Med.* 2:52, 1996 and *Prostate* 32:272, 1997). Although $2-50 \times 10^6$ DC per patient are typically administered, larger number of DC, such as 10^7 or 10^8 can also be provided. Such cell populations typically contain between 50-90% DC.

In some embodiments, peptide-loaded PBMC are injected into patients without purification of the DC. For example, PBMC generated after treatment with an agent such as Progenipoietin™ are injected into patients without purification of the DC. The total number of PBMC that are administered often ranges from 10^8 to 10^{10} . Generally, the cell doses injected into patients is based on the percentage of DC in the blood of each patient, as determined, for example, by immunofluorescence analysis with specific anti-DC antibodies. Thus, for example, if Progenipoietin™ mobilizes 2% DC in the peripheral blood of a given patient, and that patient is to receive 5×10^6 DC, then the patient will be injected with a total of 2.5×10^8 peptide-loaded PBMC. The percent DC mobilized by an agent such as Progenipoietin™ is typically estimated to be between 2-10%, but can vary as appreciated by one of skill in the art.

Ex vivo activation of CTL/HTL responses

Alternatively, *ex vivo* CTL or HTL responses to 158P1D7 antigens can be induced by incubating, in tissue culture, the patient's, or genetically compatible, CTL or HTL precursor cells together with a source of APC, such as DC, and immunogenic peptides. After an appropriate incubation time (typically about 7-28 days), in which the precursor cells are activated and expanded into effector cells, the cells are infused into the patient, where they will destroy (CTL) or facilitate destruction (HTL) of their specific target cells, *i.e.*, tumor cells.

Example 31: An Alternative Method of Identifying and Confirming Motif-Bearing Peptides

Another method of identifying and confirming motif-bearing peptides is to elute them from cells bearing defined MHC molecules. For example, EBV transformed B cell lines used for tissue typing have been extensively characterized to determine which HLA molecules they express. In certain cases these cells express only a single type of HLA molecule. These cells can be transfected with nucleic acids that express the antigen of interest, *e.g.* 158P1D7. Peptides produced by endogenous antigen processing of peptides produced as a result of transfection will then bind to HLA molecules within the cell and be transported and displayed on the cell's surface. Peptides are then eluted from the HLA molecules by exposure to mild acid conditions and their amino acid sequence determined, *e.g.*, by mass spectral analysis (*e.g.*, Kubo *et al.*, *J. Immunol.* 152:3913, 1994). Because the majority of peptides that bind a particular HLA molecule are motif-bearing, this is an alternative modality for obtaining the motif-bearing peptides correlated with the particular HLA molecule expressed on the cell.

Alternatively, cell lines that do not express endogenous HLA molecules can be transfected with an expression construct encoding a single HLA allele. These cells can then be used as described, *i.e.*, they can then be transfected with nucleic acids that encode 158P1D7 to isolate peptides corresponding to 158P1D7 that have been presented on the cell surface. Peptides obtained from such an analysis will bear motif(s) that correspond to binding to the single HLA allele that is expressed in the cell.

As appreciated by one in the art, one can perform a similar analysis on a cell bearing more than one HLA allele and subsequently determine peptides specific for each HLA allele expressed. Moreover, one of skill would also recognize that means other than transfection, such as loading with a protein antigen, can be used to provide a source of antigen to the cell.

Example 32: Complementary Polynucleotides

Sequences complementary to the 158P1D7-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring 158P1D7. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using, *e.g.*, OLIGO 4.06 software (National Biosciences) and the coding sequence of 158P1D7. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the 158P1D7-encoding transcript.

Example 33: Purification of Naturally-occurring or Recombinant 158P1D7 Using 158P1D7 Specific Antibodies

Naturally occurring or recombinant 158P1D7 is substantially purified by immunoaffinity chromatography using antibodies specific for 158P1D7. An immunoaffinity column is constructed by covalently coupling anti-158P1D7 antibody to an activated chromatographic resin, such as CNBr-activated

SEPHAROSETM (Amersham Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing 158P1D7 are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of 158P1D7 (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/158P1D7 binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and GCRP is collected.

Example 34: Identification of Molecules Which Interact with 158P1D7

158P1D7, or biologically active fragments thereof, are labeled with ¹²⁵I Bolton-Hunter reagent. (See, e.g., Bolton et al. (1973) Biochem. J. 133:529.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled 158P1D7, washed, and any wells with labeled 158P1D7 complex are assayed. Data obtained using different concentrations of 158P1D7 are used to calculate values for the number, affinity, and association of 158P1D7 with the candidate molecules. Throughout this application, various website data content, publications, applications and patents are referenced. (Websites are referenced by their Uniform Resource Locator, or URL, addresses on the World Wide Web.) The disclosures of each of these items of information are hereby incorporated by reference herein in their entireties.

Example 35: *In Vivo* Assay for 158P1D7 Tumor Growth Promotion

The effect of the 158P1D7 protein on tumor cell growth can be confirmed *in vivo* by gene overexpression in bladder cancer cells. For example, SCID mice can be injected SQ on each flank with 1 x 10⁶ bladder cancer cells (such as SCaBER, UM-UC-3, HT1376, RT4, T24, TCC-SUP, J82 and SW780 cells) containing tkNeo empty vector or 158P1D7.

At least two strategies may be used: (1) Constitutive 158P1D7 expression under regulation of a promoter such as a constitutive promoter obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), or from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, provided such promoters are compatible with the host cell systems. (2) Regulated expression under control of an inducible vector system, such as ecdysone, tet, etc., can be used provided such promoters are compatible with the host cell systems. Tumor volume is then monitored at the appearance of palpable tumors and is followed over time to determine if 158P1D7-expressing cells grow at a faster rate and whether tumors produced by 158P1D7-expressing cells demonstrate characteristics of altered aggressiveness (e.g. enhanced metastasis, vascularization, reduced responsiveness to chemotherapeutic drugs). Additionally, mice can be implanted with the same cells orthotopically to determine if 158P1D7 has an effect on local growth in the bladder or on the ability of the cells to metastasize, specifically to lungs

or lymph nodes (Fu, X., et al., "Extensive multi-organ metastasis following orthotopic onplantation of histologically intact human bladder carcinoma tissue in nude mice", *Int. J. Cancer*, 1991, 49:938-939; Chang, S., et al., "Antimetastatic activity of the new platinum analog {Pt(cis-dach)(DPPE) $\cdot$ 2NO $_3$ } in a metastatic model of human bladder", *Anticancer Res.*, 1997, 17: p.3239-3242; Peralta, E. A., et al., "Immunotherapy of bladder cancer: targeting p53 in a SCID mouse model", *J. Urol.*, 1999, 162:1806-1811). Furthermore, this assay is useful to confirm the 158P1D7 inhibitory effect of candidate therapeutic compositions, such as for example, 158P1D7 antibodies or intrabodies, and 158P1D7 antisense molecules or ribozymes.

Example 36: 158P1D7 Monoclonal Antibody-mediated Inhibition of Bladder Tumors *In Vivo*

The significant expression of 158P1D7 in cancer tissues, together with its restricted expression in normal tissues, makes 158P1D7 an excellent target for antibody therapy. In cases where the monoclonal antibody target is a cell surface protein, antibodies have been shown to be efficacious at inhibiting tumor growth.

In cases where the target is not on the cell surface, such as PSA and PAP in prostate cancer, antibodies have still been shown to recognize and inhibit growth of cells expressing those proteins.

As with any cellular protein with a restricted expression profile, 158P1D7 is a target for T cell-based immunotherapy.

Accordingly, the therapeutic efficacy of anti-158P1D7 mAbs in human bladder cancer mouse models is modeled in 158P1D7-expressing bladder cancer xenografts or bladder cancer cell lines, such as those described in Example (the Example entitled "*In Vivo* Assay for 158P1D7 Tumor Growth Promotion", that have been engineered to express 158P1D7.

Antibody efficacy on tumor growth and metastasis formation is confirmed, e.g., in a mouse orthotopic bladder cancer xenograft model. The antibodies can be unconjugated, as discussed in this Example, or can be conjugated to a therapeutic modality, as appreciated in the art. It is confirmed that anti-158P1D7 mAbs inhibit formation of 158P1D7-expressing bladder tumors. Anti-158P1D7 mAbs also retard the growth of established orthotopic tumors and prolong survival of tumor-bearing mice. These results indicate the utility of anti-158P1D7 mAbs in the treatment of local and advanced stages of bladder cancer.

Administration of anti-158P1D7 mAbs retard established orthotopic tumor growth and inhibit metastasis to distant sites, resulting in a significant prolongation in the survival of tumor-bearing mice. These studies indicate that 158P1D7 is an attractive target for immunotherapy and demonstrate the therapeutic potential of anti-158P1D7 mAbs for the treatment of local and metastatic bladder cancer.

This example demonstrates that unconjugated 158P1D7 monoclonal antibodies effectively to inhibit the growth of human bladder tumors grown in SCID mice; accordingly a combination of such efficacious monoclonal antibodies is also effective.

Tumor inhibition using multiple unconjugated 158P1D7 mAbs

Materials and Methods

158P1D7 Monoclonal Antibodies:

Monoclonal antibodies are raised against 158P1D7 as described in the Example entitled "Generation of 158P1D7 Monoclonal Antibodies (mAbs)." The antibodies are characterized by ELISA, Western blot, FACS, and immunoprecipitation, in accordance with techniques known in the art, for their capacity to bind 158P1D7. Epitope mapping data for the anti-158P1D7 mAbs, as determined by ELISA and Western analysis, recognize epitopes on the 158P1D7 protein. Immunohistochemical analysis of bladder cancer tissues and cells with these antibodies is performed.

The monoclonal antibodies are purified from ascites or hybridoma tissue culture supernatants by Protein-G SepharoseTM chromatography, dialyzed against PBS, filter sterilized, and stored at -20°C. Protein determinations are performed by a Bradford assay (Bio-Rad, Hercules, CA). A therapeutic monoclonal antibody or a cocktail comprising a mixture of individual monoclonal antibodies is prepared and used for the treatment of mice receiving subcutaneous or orthotopic injections of bladder tumor xenografts.

Bladder Cancer Cell Lines

Bladder cancer cell lines (Scaber, J82, UM-UC-3, HT1376, RT4, T24, TCC-SUP, J82 and SW780) expressing 158P1D7 are generated by retroviral gene transfer as described in Hubert, R.S., et al., STEAP: a prostate-specific cell-surface antigen highly expressed in human prostate tumors. *Proc Natl Acad Sci U S A*, 1999. 96(25):14523-8. Anti-158P1D7 staining is detected by using an FITC-conjugated goat anti-mouse antibody (Southern Biotechnology Associates) followed by analysis on a Coulter EpicsTM-XL flow cytometer.

In Vivo Mouse Models.

Subcutaneous (s. c.) tumors are generated by injection of 1×10^6 158P1D7-expressing bladder cancer cells mixed at a 1:1 dilution with Matrigel (Collaborative Research) in the right flank of male SCID mice. To test antibody efficacy on tumor formation, i. p. antibody injections are started on the same day as tumor-cell injections. As a control, mice are injected with either purified mouse IgG (ICN) or PBS; or a purified monoclonal antibody that recognizes an irrelevant antigen not expressed in human cells. In preliminary studies, no difference is found between mouse IgG or PBS on tumor growth. Tumor sizes are determined by vernier caliper measurements, and the tumor volume is calculated as length x width x height. Mice with s. c. tumors greater than 1.5 cm in diameter are sacrificed. Circulating levels of anti-158P1D7 mAbs are determined by a capture ELISA kit (Bethyl Laboratories, Montgomery, TX).

Orthotopic injections are performed, for example, in two alternative embodiments, under anesthesia by, for example, use of ketamine/xylazine. In a first embodiment, an intravesicular injection of bladder cancer cells is administered directly through the urethra and into the bladder (Peralta, E. A., et al., "Immunotherapy of bladder cancer: targeting p53 in a SCID mouse model", *J. Urol.*, 1999, 162:1806-1811). In a second embodiment, an incision is made through the abdominal wall, the bladder is exposed, and bladder tumor tissue pieces (1-2 mm in size) derived from a s. c. tumor are surgically glued onto the exterior wall of the bladder, termed "onplantation" (Fu, X., et al., "Extensive multi-organ metastasis following orthotopic onplantation of histologically intact human bladder carcinoma tissue in nude mice", *Int. J. Cancer*, 1991, 49:938-939; Chang, S., et al., "Antimetastatic activity of the new platinum analog {Pt(cis-dach) (DPPE)·2NO₃} in a metastatic model of human bladder", *Anticancer Res.*, 1997, 17: p.3239-3242). Antibodies can be

administered to groups of mice at the time of tumor injection or onplantation, or after 1-2 weeks to allow tumor establishment.

Anti-158P1D7 mAbs Inhibit Growth of 158P1D7-Expressing Bladder Cancer Tumors

In one embodiment, the effect of anti-158P1D7 mAbs on tumor formation is tested by using the bladder onplantation orthotopic model. As compared with the s.c. tumor model, the orthotopic model, which requires surgical attachment of tumor tissue directly on the bladder, results in a local tumor growth, development of metastasis in distal sites, and subsequent death (Fu, X., *et al.*, *Int. J. Cancer*, 1991. 49: p. 938-939; Chang, S., *et al.*, *Anticancer Res.*, 1997. 17: p. 3239-3242). This features make the orthotopic model more representative of human disease progression and allows one to follow the therapeutic effect of mAbs, as well as other therapeutic modalities, on clinically relevant end points.

Accordingly, 158P1D7-expressing tumor cells are onplanted orthotopically, and 2 days later, the mice are segregated into two groups and treated with either: a) 50-2000 μ g, usually 200-500 μ g, of anti-158P1D7 Ab, or b) PBS, three times per week for two to five weeks. Mice are monitored weekly for indications of tumor growth.

As noted, a major advantage of the orthotopic bladder cancer model is the ability to study the development of metastases. Formation of metastasis in mice bearing established orthotopic tumors is studied by histological analysis of tissue sections, including lung and lymph nodes (Fu, X., *et al.*, *Int. J. Cancer*, 1991. 49:938-939; Chang, S., *et al.*, *Anticancer Res.*, 1997. 17:3239-3242). Additionally, IHC analysis using anti-158P1D7 antibodies can be performed on the tissue sections.

Mice bearing established orthotopic 158P1D7-expressing bladder tumors are administered 1000 μ g injections of either anti-158P1D7 mAb or PBS over a 4-week period. Mice in both groups are allowed to establish a high tumor burden (1-2 weeks growth), to ensure a high frequency of metastasis formation in mouse lungs and lymph nodes. Mice are then sacrificed and their local bladder tumor and lung and lymph node tissue are analyzed for the presence of tumor cells by histology and IHC analysis.

These studies demonstrate a broad anti-tumor efficacy of anti-158P1D7 antibodies on initiation and progression of bladder cancer in mouse models. Anti-158P1D7 antibodies inhibit tumor formation and retard the growth of already established tumors and prolong the survival of treated mice. Moreover, anti-158P1D7 mAbs demonstrate a dramatic inhibitory effect on the spread of local bladder tumor to distal sites, even in the presence of a large tumor burden. Thus, anti-158P1D7 mAbs are efficacious on major clinically relevant end points including lessened tumor growth, lessened metastasis, and prolongation of survival.

Example 37: Homology Comparison of 158P1D7 to Known Sequences

The 158P1D7 protein of Figure 3 has 841 amino acids with calculated molecular weight of 95.1 kDa, and pI of 6.07. 158P1D7 is predicted to be a nuclear protein (65% by PSORT)

with a possibility of it being a plasma membrane protein (0.46 PSORT).

158P1D7 has a potential cleavage site between aa 626 and 627 and a potential signal site at aa 3-25.

By use of the PubMed website of the N. C. B. I. it was found at the protein level that 158P1D7 shows best homology to the hypothetical protein FLJ22774 (PubMed record: gi 14149932) of unknown function, with 97% identity and 97% homology. The 158P1D7 protein demonstrates some homology to a human protein similar to IGFBP3 (insulin-like growth factor binding protein, acid labile subunit) (PubMed record: gi 6691962) with 36% identity and 52% homology and to mouse Slit 1 protein (PubMed record: gi 5532493) with 24% identity and 37% homology (Figures 5a and 5b).

Insulin-like growth factors (IGF) have been shown to play an important role in tumor growth including prostate, breast, brain and ovarian cancer (Wang J et al., "Activation of the IGF-IR system contributes to malignant growth of human and mouse medulloblastomas", *Oncogene*, 2001, 20:3857; Helle S. et al, "Plasma insulin-like growth factor binding protein-3 proteolysis is increased in primary breast cancer", *Br J Cancer*, 2001, 85:74). IGFs produce their oncogenic effect by binding to specific cell surface receptors and activating survival as well as mitogenic pathways (Babajko S et al, "IGFBPs are involved in xenograft development in nude mice", *Med Pediatr Oncol*. 2001,36: 154; Scalia P et al, "Regulation of the Akt/Glycogen synthase kinase-3 axis by insulin-like growth factor-II via activation of the human insulin receptor isoform-A", *J Cell Biochem*. 2001,82: 610). The activity of insulin-like growth factors is regulated by IGF binding proteins (IGF-BP) and the acid labile subunit (ALS) of IGF-BP (Zeslawski W et al, "The interaction of insulin-like growth factor-I with the N-terminal domain of IGFBP-5. ", *EMBO J*. 2001,20: 3638; Jones JL and Clemmons DR., "Insulin-like growth factors and their binding proteins: biological actions.", *Endocr. Rev.* 1995, 16: 3). In the plasma, most IGFs exist as a ternary complex containing IGF-BP and ALS (Jones JL and Clemmons DR., "Insulin-like growth factors and their binding proteins: biological actions." *Endocr. Rev.* 1995, 16: 3). Association with ALS allows the retention of the ternary complex in the vasculature and extends its lifespan (Ueki I et al, "Inactivation of the acid labile subunit gene in mice results in mild retardation of postnatal growth despite profound disruptions in the circulating insulin-like growth factor system ", *Proc Natl Acad Sci U S A*, 2000, 97:6868). Studies in mice demonstrate the contribution of ALS to cell growth by showing that mice carrying mutant ALS exhibit a growth deficit (Ueki I et al, "Inactivation of the acid labile subunit gene in mice results in mild retardation of postnatal growth despite profound disruptions in the circulating insulin-like growth factor system", *Proc Natl Acad Sci U S A*, 2000, 97:6868), indicating that ALS plays a critical role in the growth of tumor cells.

Slit proteins were first identified in *Drosophila* as secreted proteins that regulate axon guidance and orientation (Rajagopalan S et al, "Selecting a longitudinal pathway: Robo receptors specify the lateral position of axons in the *Drosophila* CNS.", *Cell*, 2000, 103:1033; Chen J et al, "The N-terminal Leucine-Rich Regions in Slit Are Sufficient To Repel Olfactory Bulb Axons and Subventricular Zone Neurons ", *J Neurosci*, 2001, 21:1548). Mammalian homologs were cloned in mice and humans, where they are shown to regulate migration and chemotaxis (Wu J et al, "The neuronal repellent Slit inhibits leukocyte chemotaxis induced by chemotactic factors", *Nature*, 2001, 410:948; Brose K and Tessier M, "Slit proteins: key regulators of axon guidance, axonal branching, and cell migration", *Curr Opin Neurobiol*, 2001, 10:95). Slit proteins localize at two distinct subcellular sites within epithelial cells depending on cell stage, with Slit 3 predominantly localizing in the mitochondria and targeting to the cell surface in more confluent cells (Little MH et al, "Dual trafficking of Slit3 to mitochondria and cell surface demonstrates novel localization for Slit protein", *Am J*

Physiol Cell Physiol. 2001;281:C486). The differential Slit localization suggests that Slit may function differently whether it is secreted, associated with the cell surface or retained in the mitochondria.

The disclosure of the present invention that 158P 1D7 is highly expressed in several cancers while showing a restricted expression pattern in normal tissues indicates that the 158P1D7 gene plays an important role in various cancers, including cancers of the bladder. It is provided by the present invention that 158P1D7 controls tumor growth and progression by regulating proliferation, survival, migration, gene expression as well as cell surface availability. Accordingly, when 158P1D7 functions as a regulator of cell growth and apoptosis, or expression, 158P1D7 is used for therapeutic, diagnostic, prognostic or preventative purposes.

Additionally, Figure 16A and 16B set forth a transmembrane region and orientation prediction for 158P 1 D7. Figure 16A is a schematic representation of the probability of the existence of transmembrane regions and the extracellular and intracellular orientation of 158P1D7 based on the algorithm of Sonnhammer, von Heijne, and Krogh (Erik L. L. Sonnhammer, Gunnar von Heijne, and Anders Krogh: A hidden Markov model for predicting transmembrane helices in protein sequences. In Proc. of Sixth Int. Conf. on Intelligent Systems for Molecular Biology, p 175-182 Ed J. Glasgow, T. Littlejohn, F. Major, R. Lathrop, D. Sankoff, and C. Sensen Menlo Park, CA: AAAI Press, 1998). The method predicts that 158P1D7 contains a single transmembrane region from amino acids 611-633 with high probability that the amino-terminus resides outside, consistent with the topology of a Type 1 transmembrane protein. Also visualized is a short hydrophobic stretch from amino acids 3-25, consistent with the existence of an amino- terminal signal peptide. Figure 16B is a schematic representation of the probability of existence of transmembrane regions and orientation of 158P1D7 based on the TMPred algorithm of Hofmann and Stoffel which utilizes TMBASE (K. Hofmann, W. Stoffel. TMBASE-A database of membrane spanning protein segments Biol. Chem. Hoppe-Seyler 374:166, 1993). The method predicts that 158P1D7 contains a primary transmembrane region from amino acids 609-633 and a secondary transmembrane region from amino acids 3-25 (contiguous amino acids with values greater than 0 on the plot have high probability of being transmembrane regions) with an orientation in which the amino terminus resides inside and the carboxyl terminus outside. An alternative model is also predicted, consistent with Figure 16A, that 158P1D7 is a Type 1 transmembrane protein in which the amino-terminus resides outside and the protein contains a secondary transmembrane domain signal peptide from amino acids 3-25 and a primary transmembrane domain from AA 615-633. The transmembrane prediction algorithms for Figure 16A and Figure 16B are accessed through the ExPasy molecular biology server.

Example 38: Identification and Confirmation of Signal Transduction Pathways

Many mammalian proteins have been reported to interact with signaling molecules and to participate in regulating signaling pathways. (Abe, Kazuho and Saito, Hiroshi, "Possible linkage between glutamate transporter and mitogen-activated protein kinase cascade in cultured rat cortical astrocytes", *J Neurochem.* 2001, 76:217-223). In particular, IGF and IGF-BP have been shown to regulate mitogenic and survival pathways (Babajko S et al, "IGFBPs are involved in xenograft development in nude mice", *Med Pediatr Oncol.*, 2001, 36: 154; Scalia P et al, "Regulation of the Akt/Glycogen synthase kinase-3 axis by insulin-like growth factor-II via activation of the human insulin receptor isoform-A", *J Cell Biochem.* 2001,82: 610). Using immunoprecipitation and Western blotting techniques, proteins are identified that associate with 158P1D7 and mediate signaling events. Several pathways known to play a role in cancer biology are regulated

by 158P1D7, including phospholipid pathways such as PI3K, AKT, etc, adhesion and migration pathways, including FAK, Rho, Rac-1, etc, as well as mitogenic/survival cascades such as ERK, p38, etc. (Mirza AM et al., "Oncogenic transformation of cells by a conditionally active form of the protein kinase Akt/PKB", *Cell Growth Differ.*, 2000, 11:279; "Janulis M., et al., "Role of mitogen-activated protein kinases and c-Jun/AP-1 trans-activating activity in the regulation of protease mRNAs and the malignant phenotype in NIH 3T3 fibroblasts.", *J Biol Chem.*, 1999, 274: 801; Ivanov VN, Ronai Z., "p38 protects human melanoma cells from UV-induced apoptosis through down-regulation of NF-kappaB activity and Fas expression.", *Oncogene.*, 2000, 19: 3003; Machesky, LM, Hall, A. "Role of actin polymerization and adhesion to extracellular matrix in Rac- and Rho-induced cytoskeletal reorganization.", *J. Cell Biol.*, 1997, 138: 913). Bioinformatic analysis revealed that 158P1D7 can become phosphorylated by serine/threonine as well as tyrosine kinases. Thus, the phosphorylation of 158P1D7 is provided by the present invention to lead to activation of the above listed pathways.

Using, e.g., Western blotting techniques, the ability of 158P1D7 to regulate these pathways is confirmed. Cells expressing or lacking 158P1D7 are either left untreated or stimulated with cytokines, hormones and anti-integrin antibodies. Cell lysates are analyzed using anti-phospho-specific antibodies (Cell Signaling, Santa Cruz Biotechnology) in order to detect phosphorylation and regulation of ERK, p38, AKT, PI3K, PLC and other signaling molecules. When 158P1D7 plays a role in the regulation of signaling pathways, whether individually or communally, it is used as a target for diagnostic, prognostic, preventative and therapeutic purposes.

To confirm that 158P1D7 directly or indirectly activates known signal transduction pathways in cells, luciferase (luc) based transcriptional reporter assays are carried out in cells expressing individual genes. These transcriptional reporters contain consensus-binding sites for known transcription factors that lie downstream of well-characterized signal transduction pathways. The reporters and examples of these associated transcription factors, signal transduction pathways, and activation stimuli are listed below:

1. NFkB-luc, NFkB/Rel; Ik-kinase/SAPK; growth/apoptosis/stress
2. SRE-luc, SRF/TCF/ELK1; MAPK/SAPK; growth/differentiation
3. AP-1-luc, FOS/JUN; MAPK/SAPK/PKC; growth/apoptosis/stress
4. ARE-luc, androgen receptor; steroids/MAPK; growth/differentiation/apoptosis
5. p53-luc, p53; SAPK; growth/differentiation/apoptosis
6. CRE-luc, CREB/ATF2; PKA/p38; growth/apoptosis/stress

Gene-mediated effects are assayed in cells showing mRNA expression. Luciferase reporter plasmids are introduced by lipid-mediated transfection (TFX-50, Promega). Luciferase activity, an indicator of relative transcriptional activity, is measured by incubation of cell extracts with luciferin substrate and luminescence of the reaction is monitored in a luminometer.

Signaling pathways activated by 158P1D7 are mapped and used for the identification and validation of therapeutic targets. When 158P1D7 is involved in cell signaling, it is used as target for diagnostic, prognostic, preventative and therapeutic purposes.

Example 39: Involvement in Tumor Progression

The 158P1D7 gene can contribute to the growth of cancer cells. The role of 158P1D7 in tumor growth is confirmed in a variety of primary and transfected cell lines including prostate, colon, bladder and kidney cell lines as well as NIH 3T3 cells engineered to stably express 158P1D7. Parental cells lacking 158P1D7 and cells expressing 158P1D7 are evaluated for cell growth using a well-documented proliferation assay (see, e.g., Fraser SP, Grimes JA, Djamgoz MB. Prostate. 2000;44:61, Johnson DE, Ochieng J, Evans SL. Anticancer Drugs. 1996, 7:288).

To confirm the role of 158P1D7 in the transformation process, its effect in colony forming assays is investigated. Parental NIH3T3 cells lacking 158P1D7 are compared to NHI-3T3 cells expressing

158P1D7, using a soft agar assay under stringent and more permissive conditions (Song Z. et al. Cancer Res. 2000, 60:6730).

To confirm the role of 158P1D7 in invasion and metastasis of cancer cells, a well-established assay is used, e.g., a Transwell Insert System assay (Becton Dickinson) (Cancer Res. 1999, 59:6010). Control cells, including prostate, colon, bladder and kidney cell lines lacking 158P1D7 are compared to cells expressing 158P1D7, respectively. Cells are loaded with the fluorescent dye, calcein, and plated in the top well of the Transwell insert coated with a basement membrane analog. Invasion is determined by fluorescence of cells in the lower chamber relative to the fluorescence of the entire cell population.

158P1D7 can also play a role in cell cycle and apoptosis. Parental cells and cells expressing 158P1D7 are compared for differences in cell cycle regulation using a well-established BrdU assay (Abdel-Malek ZA. J Cell Physiol. 1988, 136:247). In short, cells are grown under both optimal (full serum) and limiting (low serum) conditions are labeled with BrdU and stained with anti-BrdU Ab and propidium iodide. Cells are analyzed for entry into the G1, S, and G2M phases of the cell cycle. Alternatively, the effect of stress on apoptosis is evaluated in control parental cells and cells expressing 158P1D7, including normal and tumor bladder cells. Engineered and parental cells are treated with various chemotherapeutic agents, such as paclitaxel, gemcitabine, etc, and protein synthesis inhibitors, such as cycloheximide. Cells are stained with annexin V-FITC and cell death is measured by FACS analysis. The modulation of cell death by 158P1D7 can play a critical role in regulating tumor progression and tumor load.

When 158P1D7 plays a role in cell growth, transformation, invasion or apoptosis, it is used as a target for diagnostic, prognostic, preventative and therapeutic purposes.

Example 40: Involvement in Angiogenesis

Angiogenesis or new capillary blood vessel formation is necessary for tumor growth (Hanahan D, Folkman J. Cell. 1996, 86:353; Folkman J. Endocrinology. 1998 139:441). Several assays have been developed to measure angiogenesis *in vitro* and *in vivo*, such as the tissue culture assays, endothelial cell tube formation, and endothelial cell proliferation. Using these assays as well as *in vitro* neo-vascularization, the effect of 158P1D7 on angiogenesis is confirmed. For example, endothelial cells engineered to express 158P1D7 are evaluated using tube formation and proliferation assays. The effect of 158P1D7 is also confirmed in animal models *in vivo*. For example, cells either expressing or lacking 158P1D7 are implanted subcutaneously in immunocompromised mice. Endothelial cell migration and angiogenesis are evaluated 5-15 days later using immunohistochemistry techniques. When 158P1D7 affects angiogenesis, it is used as a target for diagnostic, prognostic, preventative and therapeutic purposes.

Example 41: Regulation of Transcription

The above-indicated localization of 158P1D7 to the nucleus and its similarity to IGF-BP which has been found to activate signalling pathways and to regulate essential cellular functions, support the present invention use of 158P1D7 based on its role in the transcriptional regulation of eukaryotic genes.

Regulation of gene expression is confirmed, e.g., by studying gene expression in cells expressing or lacking 158P1D7. For this purpose, two types of experiments are performed.

In the first set of experiments, RNA from parental and 158P1D7-expressing cells are extracted and hybridized to commercially available gene arrays (Clontech) (Smid-Koopman E et al. Br J Cancer. 2000. 83:246). Resting cells as well as cells treated with FBS or androgen are compared. Differentially expressed genes are identified in accordance with procedures known in the art. The differentially expressed genes are then mapped to biological pathways (Chen K et al., Thyroid. 2001. 11:41.).

In the second set of experiments, specific transcriptional pathway activation is evaluated using commercially available (e.g., Stratagene) luciferase reporter constructs including: NFkB-luc, SRE-luc, ELK1-luc, ARE-luc, p53-luc, and CRE-luc. These transcriptional reporters contain consensus binding sites for known transcription factors that lie downstream of well-characterized signal transduction pathways, and represent a good tool to ascertain pathway activation and screen for positive and negative modulators of pathway activation.

When 158P1D7 plays a role in gene regulation, it is used as a target for diagnostic, prognostic, preventative and therapeutic purposes.

Example 42: Subcellular Localization of 158P1D7

The cellular location of 158P1D7 is assessed using subcellular fractionation techniques widely used in cellular biology (Storrie B, et al. Methods Enzymol. 1990;182:203-25). A variety of cell lines, including prostate, kidney and bladder cell lines as well as cell lines engineered to express 158P1D7 are separated into nuclear, cytosolic and membrane fractions. Gene expression and location in nuclei, heavy membranes (lysosomes, peroxisomes, and mitochondria), light membranes (plasma membrane and endoplasmic reticulum), and soluble protein fractions are tested using Western blotting techniques.

Alternatively, 293T cells are transfected with an expression vector encoding individual genes, HIS-tagged (PCDNA 3.1 MYC/HIS, Invitrogen) and the subcellular localization of these genes is determined as described above. In short, the transfected cells are harvested and subjected to a differential subcellular fractionation protocol (Pemberton, P.A. et al, 1997, J of Histochemistry and Cytochemistry, 45:1697-1706). Location of the HIS-tagged genes is followed by Western blotting.

Using 158P1D7 antibodies, it is possible to demonstrate cellular localization by immunofluorescence and immunohistochemistry. For example, cells expressing or lacking 158P1D7 are adhered to a microscope slide and stained with anti-158P1D7 specific Ab. Cells are incubated with an FITC-coupled secondary anti-species Ab, and analyzed by fluorescent microscopy. Alternatively, cells and tissues lacking or expressing 158P1D7 are analyzed by IHC as described herein.

When 158P1D7 is localized to specific cell compartments, it is used as a target for diagnostic, preventative and therapeutic purposes.

Example 43: Involvement of 158P1D7 in Protein Trafficking.

Due to its similarity to Slit proteins, 158P1D7 can regulate intracellular trafficking and retention into mitochondrial and/or nuclear compartments. Its role in the trafficking of proteins can be confirmed using well-established methods (Valetti C. et al. Mol Biol Cell. 1999, 10:4107). For example, FITC-conjugated α 2-macroglobulin is incubated with 158P1D7-expressing and 158P1D7-negative cells. The location and uptake of FITC- α 2-macroglobulin is visualized using a fluorescent microscope. In another approach, the co-localization of 158P1D7 with vesicular proteins is confirmed by co-precipitation and Western blotting techniques and fluorescent microscopy.

Alternatively, 158P1D7-expressing and 158P1D7-lacking cells are compared using bodipy-ceramide labeled bovine serum albumine (Huber L et al. Mol. Cell. Biol. 1995, 15:918). Briefly, cells are allowed to take up the labeled BSA and are placed intermittently at 4°C and 18°C to allow for trafficking to take place. Cells are examined under fluorescent microscopy, at different time points, for the presence of labeled BSA in specific vesicular compartments, including Golgi, endoplasmic reticulum, etc.

In another embodiment, the effect of 158P1D7 on membrane transport is examined using biotin-avidin complexes. Cells either expressing or lacking 158P1D7 are transiently incubated with biotin. The cells are placed at 4°C or transiently warmed to 37°C for various periods of time. The cells are fractionated and examined by avidin affinity precipitation for the presence of biotin in specific cellular compartments. Using such assay systems, proteins, antibodies and small molecules are identified that modify the effect of 158P1D7 on vesicular transport. When 158P1D7 plays a role in intracellular trafficking, 158P1D7 is a target for diagnostic, prognostic, preventative and therapeutic purposes

Example 44: Protein-Protein Association

IGF and IGF-BP proteins have been shown to interact with other proteins, thereby forming protein complexes that can regulate protein localization, biological activity, gene transcription, and cell transformation (Zeslawski W et al, EMBO J. 2001, 20:3638; Yu H, Rohan T, J Natl Cancer Inst. 2000, 92:1472). Using immunoprecipitation techniques as well as two yeast hybrid systems, proteins are identified that associate with 158P1D7. Immunoprecipitates from cells expressing 158P1D7 and cells lacking 158P1D7 are compared for specific protein-protein associations.

Studies are performed to determine the extent of the association of 158P1D7 with receptors, such as the EGF and IGF receptors, and with intracellular proteins, such as IGF-BP, cytoskeletal proteins etc. Studies comparing 158P1D7 positive and 158P1D7 negative cells, as well as studies comparing unstimulated/resting cells and cells treated with epithelial cell activators, such as cytokines, growth factors and anti-integrin Ab reveal unique protein-protein interactions.

In addition, protein-protein interactions are confirmed using two yeast hybrid methodology (Curr Opin Chem Biol. 1999, 3:64). A vector carrying a library of proteins fused to the activation domain of a transcription factor is introduced into yeast expressing a 158P1D7-DNA-binding domain fusion protein and a reporter construct. Protein-protein interaction is detected by colorimetric reporter activity. Specific

association with surface receptors and effector molecules directs one of skill to the mode of action of 158P1D7, and thus identifies therapeutic, prognostic, preventative and/or diagnostic targets for cancer. This and similar assays are also used to identify and screen for small molecules that interact with 158P1D7.

When 158P1D7 associates with proteins or small molecules it is used as a target for diagnostic, prognostic, preventative and therapeutic purposes.

The present invention is not to be limited in scope by the embodiments disclosed herein, which are intended as single illustrations of individual aspects of the invention, and any that are functionally equivalent are within the scope of the invention. Various modifications to the models and methods of the invention, in addition to those described herein, will become apparent to those skilled in the art from the foregoing description and teachings, and are similarly intended to fall within the scope of the invention. Such modifications or other embodiments can be practiced without departing from the true scope and spirit of the invention.

TABLES**TABLE I: Tissues that Express 158P1D7 When Malignant**

Bladder, Prostate, Colon, Lung, Breast, Ovary

TABLE II: AMINO ACID ABBREVIATIONS

SINGLE LETTER	THREE LETTER	FULL NAME
F	Phe	phenylalanine
L	Leu	leucine
S	Ser	serine
Y	Tyr	tyrosine
C	Cys	cysteine
W	Trp	tryptophan
P	Pro	proline
H	His	histidine
Q	Gln	glutamine
R	Arg	arginine
I	Ile	isoleucine
M	Met	methionine
T	Thr	threonine
N	Asn	asparagine
K	Lys	lysine
V	Val	valine
A	Ala	alanine
D	Asp	aspartic acid
E	Glu	glutamic acid
G	Gly	glycine

TABLE III: AMINO ACID SUBSTITUTION MATRIX

Adapted from the GCG Software 9.0 BLOSUM62 amino acid substitution matrix (block substitution matrix). The higher the value, the more likely a substitution is found in related, natural proteins. (See URL www.ikp.unibe.ch/manual/blosum62.html)

A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	.
4	0	-2	-1	-2	0	-2	-1	-1	-1	-1	-2	-1	-1	-1	1	0	0	-3	-2	A
	9	-3	-4	-2	-3	-3	-1	-3	-1	-1	-3	-3	-3	-3	-1	-1	-1	-2	-2	C
		6	2	-3	-1	-1	-3	-1	-4	-3	1	-1	0	-2	0	-1	-3	-4	-3	D
			5	-3	-2	0	-3	1	-3	-2	0	-1	2	0	0	-1	-2	-3	-2	E
				6	-3	-1	0	-3	0	0	-3	-4	-3	-3	-2	-2	-1	1	3	F
					6	-2	-4	-2	-4	-3	0	-2	-2	-2	0	-2	-3	-2	-3	G
						8	-3	-1	-3	-2	1	-2	0	0	-1	-2	-3	-2	2	H
							4	-3	2	1	-3	-3	-3	-3	-2	-1	3	-3	-1	I
								5	-2	-1	0	-1	1	2	0	-1	-2	-3	-2	K
									4	2	-3	-3	-2	-2	-2	-1	1	-2	-1	L
										5	-2	-2	0	-1	-1	-1	1	-1	-1	M
											6	-2	0	0	1	0	-3	-4	-2	N
												7	-1	-2	-1	-1	-2	-4	-3	P
													5	1	0	-1	-2	-2	-1	Q
														5	-1	-1	-3	-3	-2	R
															4	1	-2	-3	-2	S
																5	0	-2	-2	T
																	4	-3	-1	V
																		11	2	W
																			7	Y

TABLE IV A

SUPERMOTIFS	POSITION 2 (Primary Anchor)	POSITION 3 (Primary Anchor)	POSITION C Terminus (Primary Anchor)
A1	T <i>ILVMS</i>		FWY
A2	L <i>IVMATQ</i>		I <i>VMATL</i>
A3	V <i>SMATLI</i>		RK
A24	Y <i>FWIVLMT</i>		F <i>IYWLM</i>
B7	P		V <i>ILFMWYA</i>
B27	RHK		F <i>YLWMIVA</i>
B44	E <i>D</i>		F <i>WYLMVA</i>
B58	A <i>TS</i>		F <i>WYLVMA</i>
B62	Q <i>LIVMP</i>		F <i>WYMIVLA</i>
MOTIFS			
A1	TSM		Y
A1		DEAS	Y
A2.1	L <i>MVQIAT</i>		V <i>LIMAT</i>
A3	L <i>MVISATFCGD</i>		K <i>YRHFA</i>
A11	V <i>TMLISAGNCDF</i>		K <i>RYH</i>
A24	Y <i>FWM</i>		F <i>LIW</i>
A*3101	M <i>VTALIS</i>		RK
A*3301	M <i>VALFIST</i>		RK
A*6801	A <i>VTMSLI</i>		RK
B*0702	P		L <i>MFWYAIV</i>
B*3501	P		L <i>MFWYIVA</i>
B51	P		L <i>IVFWYAM</i>
B*5301	P		I <i>MFWYALV</i>
B*5401	P		A <i>TIVLMFWY</i>

Bolded residues are preferred, italicized residues are less preferred: A peptide is considered motif-bearing if it has primary anchors at each primary anchor position for a motif or supermotif as specified in the above table.

TABLE IV (B): HLA CLASS II SUPERMOTIF

1	6	9
W, F, Y, V, .I, L	A, V, I, L, P, C, S, T	A, V, I, L, C, S, T, M, Y

TABLE IV C

MOTIFS	1° anchor 1	2	3	4	5	1° anchor 6	7	8	9
DR4	preferred <i>FMYLIVW</i>	M	T		I	<i>VSTCPALIM</i>	MH		MH
	deleterious			W			R		WDE
DR1	preferred <i>MFLIVWY</i>			PAMQ		<i>VMATSPLIC</i>	M		<i>AVM</i>
	deleterious	C	CH	FD	CWD		GDE	D	
DR7	preferred <i>MFLIVWY</i>	M	W	A		<i>IVMSACTPL</i>	M		IV
	deleterious	C		G			GRD	N	G
<u>DR3</u>	<u>MOTIFS</u>	1° anchor 1	2	3	1° anchor 4	5	1° anchor 6		
motif a preferred	<i>LIVMFY</i>				D				
motif b preferred	<i>LIVMFAY</i>				DNQUEST		KRH		
DR Supermotif	<i>MFLIVWY</i>						<i>VMSTACPLI</i>		

Italicized residues indicate less preferred or “tolerated” residues.

TABLE IV D

POSITION								
	1	2	3	4	5	6	7	8
								C-terminus
<u>SUPERMOTIFS</u>								
A1		<u>1° Anchor</u> TILVMS						<u>1° Anchor</u> FWY
A2		<u>1° Anchor</u> LIVMATQ						<u>1° Anchor</u> LIVMAT
A3	preferred	<u>1° Anchor</u> VSMATLI	YFW(4/5)		YFW (3/5)	YFW (4/5)	P (4/5)	<u>1° Anchor</u> RK
	deleterious		DE (3/5); P (5/5)		DE (4/5)			
A24		<u>1° Anchor</u> YFWIVLMT						<u>1° Anchor</u> FIYWLM
B7	preferred	FWY (5/5) LIVM (3/5)	FWY(4/5)				FWY (3/5)	<u>1° Anchor</u> VILFMWYA
	deleterious	DE (3/5); P(5/5); G(4/5); A(3/5); QN(3/5)			DE(3/5)	G(4/5)	QN(4/5)	DE(4/5)
B27		<u>1° Anchor</u> RHK						<u>1° Anchor</u> FYLWMIVA

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B44	<u>1° Anchor</u> ED	<u>1° Anchor</u> FWYLMVA
B58	<u>1° Anchor</u> ATS	<u>1° Anchor</u> FWYLI/MA
B62	<u>1° Anchor</u> QL/MP	<u>1° Anchor</u> FWYMI/VA

TABLE IV E

	1	2	3	4	5	6	7	8	9	C-terminus
										or C-terminus
A1 9-mer	preferred	GFYW	DEA	YFW		P	DEQN	YFW	<u>1°Anchor</u> Y	
	deleterious	DE	RHKLIVMP	A	G	A				
A1 9-mer	preferred	GRHK	ASTCLIVM	GSTC		ASTC	LIVM	DE	<u>1°Anchor</u> Y	
	deleterious	A	RHKDEPY FW	DE	PQN	RHK	PG	GP		
A1 10-mer	preferred	YFW	<u>1°Anchor</u> STM	A	YFWQN		PASTC	GDE	P	<u>1°Anchor</u> Y
	deleterious	GP		DE	RHK	QNA	RHKYFW	RHK	A	
A1 10-mer	preferred	YFW	STCLIVM	A	YFW		PG	G	YFW	<u>1°Anchor</u> Y
	deleterious	RHK	RHKDEPY FW		P	G		PRHK	QN	
A2.1 9-mer	preferred	YFW	<u>1°Anchor</u> LMIVQAT	STC	YFW		A	P	<u>1°Anchor</u> VLIMAT	
	deleterious	DEP				RKH	DERKH			
A2.1 10-mer	preferred	AYFW	<u>1°Anchor</u> LMIVQAT	G		G		FYWL VIM		<u>1°Anchor</u> VLIMAT

	1	2	3	4	5	6	7	8	9	C-terminus
	deleterious	DEP	DE	RKHA	P		RKH	DERKH	RKH	
3	preferred	RHK	YFW	PRHKYFW	A	YFW		P	1°Anchor KYRHHFA	
	deleterious	DEP	DE							
11	preferred	A	YFW	YFW	A	YFW	YFW	P	1°Anchor KRYH	
	deleterious	DEP					A	G		
24	preferred	YFWRHK		STC			YFW	YFW	1°Anchor FLIW	
	deleterious	DEG	DE	G	QNP	DERHK	G	AQN		
24	preferred			P	YFWP		P		1°Anchor FLIW	
	deleterious		GDE	QN	RHK	DE	A	QN	DEA	
101	preferred	RHK	YFW	P		YFW	YFW	AP	1°Anchor RK	
	deleterious	DEP	DE		ADE	DE	DE	DE		
301	preferred		YFW				AYFW		1°Anchor RK	
	deleterious	GP	DE							

	1	2	3	4	5	6	7	8	9	C-terminus
A6801	preferred	YFWSTC	<u>1°Anchor</u> AVTMSLI		YFWLIVM		YFW	P	<u>1°Anchor</u> RK	
	deleterious	GP	DEG		RHK			A		
B0702	preferred	RHKFWY	<u>1°Anchor</u> P		RHK	RHK	RHK	PA	<u>1°Anchor</u> LMFWYAIIV	
	deleterious	DEQNP	DEP	DE	DE	GDE	QN	DE		
B3501	preferred	FWYLIVM	<u>1°Anchor</u> P		FWY		FWY		<u>1°Anchor</u> LMFWYIIV A	
	deleterious	AGP			G	G				
B51	preferred	LIVMFWY	<u>1°Anchor</u> P		FWY		G	FWY	<u>1°Anchor</u> LIVFWYAM	
	deleterious	AGPDERH KSTC			DE	G	DEQN	GDE		
B5301	preferred	LIVMFWY	<u>1°Anchor</u> P		FWY		LIVMFWY	FWY	<u>1°Anchor</u> IMFWYAL V	
	deleterious	AGPQN				G	RHKQN	DE		
B5401	preferred	FWY	<u>1°Anchor</u> P		LIVM		ALIVM	FWYAP	<u>1°Anchor</u> ATTVLMF WY	

	1	2	3	4	5	6	7	8	9	C-terminus
deleterious	GPQNDE		GDESTC		RHKDE	DE	QNDGE	DE		

Italicized residues indicate less preferred or “tolerated” residues. The information in this Table is specific for 9-mers unless otherwise specified.

TABLE V				
HLA Peptide Scoring Results – 158P1D7 – A1, 9-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
1	150	VIEPSAFSK	900.000	1.
2	436	NLEYLYLEY	225.000	2.
3	812	LVEQTKNEY	45.000	3.
4	828	HAEPDYLEV	45.000	4.
5	711	GSDAKHLQR	37.500	5.
6	546	CTSPGHLDK	25.000	6.
7	265	SICPTPPVY	10.000	7.
8	351	NIESLSDLR	9.000	8.
9	799	LMETLMYSR	9.000	9.
10	173	ESLPPNIFR	7.500	10.
11	650	DNSPVHLQY	6.250	11.
12	601	LTDAVPLSV	6.250	12.
13	174	SLPPNIFRF	5.000	13.
14	100	IADIEIGAF	5.000	14.
15	682	MVSPMVHVY	5.000	15.
16	102	DIEIGAFNG	4.500	16.
17	134	GLENLEFLQ	4.500	17.
18	47	NCEAKGIKM	4.500	18.
19	383	LVEYFTLEM	4.500	19.
20	401	VLEEGSFMN	4.500	20.
21	388	TLEMLHLGN	4.500	21.
22	749	FQDASSLYR	3.750	22.
23	56	VSEISVPPS	2.700	23.
24	561	NSEILCPGL	2.700	24.
25	431	FLGLHNLEY	2.500	25.
26	291	INDSRMSTK	2.500	26.
27	142	QADNNFITV	2.500	27.
28	502	ILDDL DLLT	2.500	28.
29	522	SCDLVGLQQ	2.500	29.
30	223	NCDLLQLKT	2.500	30.

TABLE V				
HLA Peptide Scoring Results – 158P1D7 – A1, 9-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
31	771	ITEYLRKNI	2.250	31.
32	232	WLENMPPQS	1.800	32.
33	171	AIESLPPNI	1.800	33.
34	137	NLEFLQADN	1.800	34.
35	355	LSDLRPPPQ	1.500	35.
36	380	KSDLVEYFT	1.500	36.
37	59	ISVPPSRPF	1.500	37.
38	255	GSILSRLKK	1.500	38.
39	540	VTDDILCTS	1.250	39.
40	308	TKAPGLIPY	1.250	40.
41	817	KNEYFELKA	1.125	41.
42	743	STEFLSFQD	1.125	42.
43	359	RPPQNP RK	1.000	43.
44	246	VCNSPPFFK	1.000	44.
45	417	YLNGNHLTK	1.000	45.
46	433	GLHNLEYLY	1.000	46.
47	785	DMEAHYPGA	0.900	47.
48	398	RIEVLEEGS	0.900	48.
49	701	EEEEERNEK	0.900	49.
50	833	YLEVLEQQT	0.900	50.

TABLE VI				
HLA Peptide Scoring Results – 158P1D7 – A1, 10-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
1	56	VSEISVPPSR	27.000	51.
2	669	TTERPSASLY	11.250	52.
3	210	ILDQLQLEDNK	10.000	53.
4	781	QLQPDMEAHY	10.000	54.
5	150	VIEPSAFSKL	9.000	55.
6	171	AIESLPPNIF	9.000	56.
7	828	HAEPDYLEV L	9.000	57.

TABLE VI

HLA Peptide Scoring Results – 158P1D7 – A1, 10-mers

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
8	123	SLEILKEDTF	9.000	58.
9	398	RIEVLEEGSF	9.000	59.
10	812	LVEQTKNEYF	9.000	60.
11	173	ESLPPNIFRF	7.500	61.
12	546	CTSPGHLDKK	5.000	62.
13	134	GLENLEFLQA	4.500	63.
14	401	VLEEGSFMNL	4.500	64.
15	380	KSDLVEYFTL	3.750	65.
16	456	NPMPKLKVLY	2.500	66.
17	505	DLDLLTQIDL	2.500	67.
18	502	ILDDLDLLTQ	2.500	68.
19	743	STEFLSFQDA	2.250	69.
20	771	ITEYLRKNIA	2.250	70.
21	682	MVSPMVHVYR	2.000	71.
22	214	QLEDNKWACN	1.800	72.
23	355	LSDLRPPPQN	1.500	73.
24	264	ESICPTPPVY	1.500	74.
25	753	SSLYRNILEK	1.500	75.
26	561	NSEILCPGLV	1.350	76.
27	601	LTDAVPLSVL	1.250	77.
28	276	HEDPSGSLHL	1.250	78.
29	590	TTNTADTILR	1.250	79.
30	149	TVIEPSAFSK	1.000	80.
31	106	GAFNGLGLLK	1.000	81.
32	801	ETLMYSRPRK	1.000	82.
33	545	LCTSPGHLDK	1.000	83.
34	824	KANLHAEPDY	1.000	84.
35	525	LVGLQQWIQK	1.000	85.
36	300	TTSILKLPTK	1.000	86.
37	477	HIFSGVPLTK	1.000	87.
38	100	IADIEIGAFN	1.000	88.
39	768	QLGITEYLRK	1.000	89.

TABLE VI

HLA Peptide Scoring Results – 158P1D7 – A1, 10-mers

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
40	245	VVCNSPPFFK	1.000	90.
41	721	LLEQENHSPL	0.900	91.
42	700	LEEEERNEK	0.900	92.
43	102	DIEIGAFNGL	0.900	93.
44	441	YLEYNAIKEI	0.900	94.
45	436	NLEYLYLEYN	0.900	95.
46	36	NCEEKDGTML	0.900	96.
47	513	DLEDNPWDCS	0.900	97.
48	383	LVEYFTLEML	0.900	98.
49	388	TLEMLHLGNN	0.900	99.
50	137	NLEFLQADNN	0.900	100.

TABLE VII

HLA Peptide Scoring Results – 158P1D7 – A2, 9-mers

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
1	465	YLNNNLLQV	735.860	101.
2	614	LLIMFITIV	423.695	102.
3	193	NQLQTLPYV	330.059	103.
4	616	IMFITIVFC	285.492	104.
5	140	FLQADNNFI	263.950	105.
6	415	KLYLNGNHL	239.259	106.
7	439	YLYLEYNAI	230.356	107.
8	611	ILGLLIMFI	224.357	108.
9	2	KLWIHLFYS	158.832	109.
10	429	GMFLGLHNL	131.296	110.
11	581	YLMVTTPAT	126.833	111.
12	463	VLYLNNNLL	116.211	112.
13	574	SMPTQTSYL	84.856	113.
14	71	LLNNGLTML	83.527	114.
15	4	WIHLFYSSL	77.017	115.
16	305	KLPTKAPGL	74.768	116.

TABLE VII				
HLA Peptide Scoring Results – 158P1D7 – A2, 9-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
17	613	GLLIMFITI	73.343	117.
18	213	LQLEDNKWA	71.445	118.
19	826	NLHAEPDYL	57.572	119.
20	803	LMYSRPRKV	54.652	120.
21	501	NILDDL DLL	50.218	121.
22	798	KLMETLMYS	50.051	122.
23	527	GLQQWIKL	49.134	123.
24	158	KLNRLK VLI	36.515	124.
25	178	NIFRFVPLT	33.135	125.
26	225	DLLQLKTWL	32.604	126.
27	462	KVLYLNNNL	24.206	127.
28	767	QQLGITEYL	21.597	128.
29	116	QLHINHNSL	21.362	129.
30	68	QLSLLNGL	21.362	130.
31	502	ILDDL DLLT	20.776	131.
32	70	SLLNGLTM	18.382	132.
33	470	LLQVLPHI	17.736	133.
34	391	MLHLGNNRI	17.736	134.
35	164	VLILNDNAI	17.736	135.
36	337	VLSPSGLLI	17.736	136.
37	774	YLRKNIAQL	17.177	137.
38	450	ILPGTFNPM	16.047	138.
39	323	QLPGPYCPI	15.649	139.
40	367	KLILAGNII	14.971	140.
41	316	YITKPSTQL	13.512	141.
42	141	LQADNNFIT	12.523	142.
43	214	QLEDNKWAC	9.777	143.
44	582	LMVTTPATT	9.149	144.
45	758	NILEKEREL	8.912	145.
46	17	SLHSQTPVL	8.759	146.
47	182	FVPLTHLDL	8.598	147.
48	609	VLILGLLIM	7.964	148.

TABLE VII				
HLA Peptide Scoring Results – 158P1D7 – A2, 9-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
49	295	RMSTKTTSI	7.535	149.
50	309	KAPGLIPYI	6.415	150.

TABLE VIII				
HLA Peptide Scoring Results – 158P1D7 – A2, 10-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
1	613	GLLMFITIV	922.161	151.
2	431	FLGLHNLEYL	609.108	152.
3	616	IMFITIVFCA	301.064	153.
4	600	SLTDAVPLSV	285.163	154.
5	417	YLNGNHLTKL	226.014	155.
6	473	VLPPHIFSGV	224.653	156.
7	70	SLLNNGLTML	181.794	157.
8	433	GLHNLEYLYL	176.240	158.
9	166	ILNDNAIESL	167.806	159.
10	407	FMNLTRLQKL	163.232	160.
11	174	SLPPNIFRFV	145.364	161.
12	425	KLSKGMFLGL	142.060	162.
13	581	YLMVTTPATT	126.833	163.
14	409	NLTRLQKLYL	117.493	164.
15	610	LILGLLIMFI	114.142	165.
16	746	FLSFQDASSL	98.267	166.
17	213	LQLEDNKWAC	97.424	167.
18	141	LQADNNFITV	93.387	168.
19	465	YLNNNLLQVL	92.666	169.
20	369	ILAGNIIHSL	83.527	170.
21	415	KLYLNGNHLT	83.462	171.
22	140	FLQADNNFIT	81.516	172.
23	158	KLNRLKVLIL	70.507	173.
24	611	ILGLLIMFIT	69.289	174.
25	78	MLHTNDFSGL	69.001	175.

TABLE VIII				
HLA Peptide Scoring Results – 158P1D7 – A2, 10-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
26	615	LIMFITIVFC	54.353	176.
27	802	TLMYSRPRKV	51.468	177.
28	531	WIQKLSKNTV	43.992	178.
29	469	NLLQVLPPHI	38.601	179.
30	67	FQLSLLNNGI	36.864	180.
31	803	LMYSRPRKVL	34.412	181.
32	115	KQLHINHNSL	28.049	182.
33	462	KVLYLNNNLL	24.206	183.
34	86	GLTNAISHL	21.362	184.
35	401	VLEEGSFMNL	18.106	185.
36	44	MLINCEAKGI	17.736	186.
37	596	TILRSLTDAV	17.338	187.
38	621	IVFCAAGIVV	15.695	188.
39	501	NILDDLDTLT	15.544	189.
40	4	WIHLFYSSLL	13.512	190.
41	486	KVNLKTNQFT	12.552	191.
42	163	KVLILNDNAI	11.822	192.
43	336	KVLSPSGLLI	11.822	193.
44	60	SVPPSRPFQL	10.841	194.
45	282	SLHLAATSSI	10.433	195.
46	110	GLGLLKQLHI	10.433	196.
47	766	LQQLGITEYL	9.923	197.
48	126	ILKEDTFHGL	9.902	198.
49	15	CISLHSQTPV	9.563	199.
50	582	LMVTTPATTT	9.149	200.

TABLE IX				
HLA Peptide Scoring Results - 158P1D7 - A3, 9-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
1	754	SLYRNILEK	300.000	201.
2	417	YLNGNHLTK	60.000	202.

TABLE IX				
HLA Peptide Scoring Results - 158P1D7 - A3, 9-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
3	407	FMNLTRLQK	40.000	203.
4	433	GLHNLEYLY	36.000	204.
5	802	TLMYSRPRK	30.000	205.
6	43	TMLINCEAK	30.000	206.
7	342	GLLIHCQER	18.000	207.
8	799	LMETLMYSR	18.000	208.
9	613	GLLIMFITI	16.200	209.
10	429	GMFLGLHNL	13.500	210.
11	174	SLPPNIFRF	13.500	211.
12	768	QLGITEYLR	12.000	212.
13	627	GIVVLVLHR	10.800	213.
14	150	VIEPSAFSK	9.000	214.
15	415	KLYLNGNHL	9.000	215.
16	527	GLQQWIKL	8.100	216.
17	436	NLEYLYLEY	8.000	217.
18	431	FLGLHNLEY	8.000	218.
19	378	LMKSDLVEY	6.000	219.
20	529	QQWIKLSK	6.000	220.
21	546	CTSPGHLDK	3.000	221.
22	463	VLYLNNNLL	3.000	222.
23	439	YLYLEYNAI	3.000	223.
24	2	KLWIHLFYS	2.700	224.
25	367	KLILAGNII	2.700	225.
26	297	STKTTSLK	2.000	226.
27	6	HLFYSSLLA	2.000	227.
28	632	VLHRRRRYK	2.000	228.
29	409	NLTRLQKLY	2.000	229.
30	611	ILGLLIMFI	1.800	230.
31	337	VLSPSGLLI	1.800	231.
32	305	KLPTKAPGL	1.800	232.
33	390	EMLHLGNNR	1.800	233.
34	158	KLNRKVLII	1.800	234.

TABLE IX				
HLA Peptide Scoring Results - 158P1D7 - A3, 9-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
35	682	MVSPMVHVY	1.800	235.
36	616	IMFITIVFC	1.500	236.
37	659	SMYGHKTTH	1.500	237.
38	628	IVVLVLHRR	1.350	238.
39	614	LLIMFITIV	1.350	239.
40	323	QLPGPYCPI	1.350	240.
41	610	LILGLLIMF	1.350	241.
42	729	PLTGSNMKY	1.200	242.
43	453	GTFNPMPKL	1.012	243.
44	228	QLKTWLENM	0.900	244.
45	450	ILPGTFNPM	0.900	245.
46	615	LIMFITIVF	0.900	246.
47	609	VLILGLLIM	0.900	247.
48	255	GSILSRLKK	0.900	248.
49	482	VPLTKVNLK	0.900	249.
50	774	YLRKNIAQL	0.900	250.

TABLE X				
HLA Peptide Scoring Results - 158P1D7 - A3, 10-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
1	439	YLYLEYNAIK	300.000	251.
2	798	KLMETLMYSR	121.500	252.
3	632	VLHRRRRYKK	60.000	253.
4	768	QLGITEYLRK	40.000	254.
5	477	HIFSGVPLTK	30.000	255.
6	210	ILDQLQLEDNK	20.000	256.
7	481	GVPLTKVNLK	18.000	257.
8	681	HMVSPMVHVY	18.000	258.
9	616	IMFITIVFCA	13.500	259.
10	149	TVIEPSAFSK	13.500	260.
11	158	KLNRLKVLIL	10.800	261.

TABLE X				
HLA Peptide Scoring Results – 158P1D7 – A3, 10-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
12	425	KLSKGMFLGL	10.800	262.
13	815	QTKNEYFELK	9.000	263.
14	609	VLILGLLIMF	9.000	264.
15	245	VVCNSPPFFK	9.000	265.
16	614	LLIMFITIVF	9.000	266.
17	811	VLVEQTKNEY	9.000	267.
18	377	SLMKSDLVEY	9.000	268.
19	453	GTFNPMPKLK	7.500	269.
20	781	QLQPDMEAHY	6.000	270.
21	655	HLQYSMYGHK	6.000	271.
22	378	LMKSDLVEYF	6.000	272.
23	75	GLTMLHTNDF	6.000	273.
24	106	GAFNGLGLLK	6.000	274.
25	2	KLWIHLFYSS	5.400	275.
26	86	GLTNAISHL	5.400	276.
27	401	VLEEGSFMNL	5.400	277.
28	42	GTMLINCEAK	4.500	278.
29	613	GLLIMFITIV	4.050	279.
30	627	GIVVLVLHRR	4.050	280.
31	525	LVGLQQWIQK	4.000	281.
32	134	GLENLEFLQA	3.600	282.
33	433	GLHNLEYLYL	3.600	283.
34	110	GLGLLKQLHI	3.600	284.
35	6	HLFYSSLLAC	3.000	285.
36	470	LLQVLPPHIF	3.000	286.
37	194	QLQTLPYVGF	3.000	287.
38	290	SINDSRMSTK	3.000	288.
39	126	ILKEDTFHGL	2.700	289.
40	357	DLRPPPQNPR	2.700	290.
41	796	ELKLMETLMY	2.400	291.
42	546	CTSPGHLDKK	2.250	292.
43	803	LMYSRPRKVL	2.250	293.

TABLE X				
HLA Peptide Scoring Results – 158P1D7 – A3, 10-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
44	729	PLTGSNMKYK	2.250	294.
45	369	ILAGNIIHSL	2.025	295.
46	123	SLEILKEDTF	2.000	296.
47	765	ELQQLGITEY	1.800	297.
48	112	GLLKQLHINH	1.800	298.
49	367	KLILAGNIIH	1.800	299.
50	78	MLHTNDFSGL	1.800	300.

TABLE XI				
HLA Peptide Scoring Results – 158P1D7 – A11, 9-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
1	529	QQWIKLSK	2.400	301.
2	297	STKTTSLK	2.000	302.
3	546	CTSPGHLDK	2.000	303.
4	754	SLYRNILEK	1.600	304.
5	656	LQYSMYGHK	1.200	305.
6	150	VIEPSAFSK	1.200	306.
7	407	FMNLTRLQK	0.800	307.
8	802	TLMYSRPRK	0.800	308.
9	417	YLNGNHLTK	0.800	309.
10	627	GIVVLVLHR	0.720	310.
11	628	IVVLVLHRR	0.600	311.
12	440	LYLEYNAIK	0.600	312.
13	246	VCNSPPFFK	0.600	313.
14	359	RPPPQNPRK	0.600	314.
15	664	KTTHHTTER	0.600	315.
16	43	TMLINCEAK	0.600	316.
17	730	LTGSNMKYK	0.500	317.
18	478	IFSGVPLTK	0.400	318.
19	107	AFNGLGLLK	0.400	319.
20	372	GNIHSLMK	0.360	320.

TABLE XI				
HLA Peptide Scoring Results – 158PID7 – A11, 9-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
21	342	GLLIHCQER	0.360	321.
22	482	VPLTKVNLK	0.300	322.
23	728	SPLTGSNMK	0.300	323.
24	420	GNHLTKLSK	0.240	324.
25	749	FQDASSLYR	0.240	325.
26	287	ATSSINDSR	0.200	326.
27	790	YPGAHEELK	0.200	327.
28	328	YCPIPCNCK	0.200	328.
29	255	GSILSRLKK	0.180	329.
30	799	LMETLMYSR	0.160	330.
31	768	QLGITEYLR	0.160	331.
32	20	SQTPVLSSR	0.120	332.
33	454	TFNPMPKLK	0.100	333.
34	550	GHLDDKELK	0.090	334.
35	809	RKVLVEQTK	0.090	335.
36	336	KVLSPSGLL	0.090	336.
37	462	KVLYLNNNL	0.090	337.
38	163	KVLILNDNA	0.090	338.
39	252	FFKGSILSR	0.080	339.
40	351	NIESLSDLR	0.080	340.
41	769	LGITEYLRK	0.060	341.
42	526	VGLQQWQK	0.060	342.
43	453	GTFNPMPKL	0.060	343.
44	42	GTMLINCEA	0.060	344.
45	629	VVLVLHRRR	0.060	345.
46	608	SVLILGLLI	0.060	346.
47	183	VPLTHLDIR	0.060	347.
48	486	KVNLKTNQF	0.060	348.
49	481	GVPLTKVNL	0.060	349.
50	707	NEKEGSDAK	0.060	350.

TABLE XII				
HLA Peptide Scoring Results – 158P1D7 – A11, 10-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
1	149	TVIEPSAFSK	9.000	351.
2	245	VVCNSPPFFK	6.000	352.
3	42	GTMLINCEAK	6.000	353.
4	481	GVPLTKVNLK	6.000	354.
5	525	LVGLQQWIQK	4.000	355.
6	453	GTFNPMPKLK	3.000	356.
7	106	GAFNGLGLLK	2.400	357.
8	477	HIFSGVPLTK	1.600	358.
9	416	LYLNGNHLTK	1.200	359.
10	528	LQQWIKLSK	1.200	360.
11	815	QTKNEYFELK	1.000	361.
12	300	TTSILKLPTK	1.000	362.
13	546	CTSPGHLDKK	1.000	363.
14	798	KLMETLMYSR	0.960	364.
15	200	YVGFEHIGR	0.800	365.
16	406	SFMNLTRLQK	0.800	366.
17	439	YLYLEYNAIK	0.800	367.
18	768	QLGITEYLRK	0.800	368.
19	632	VLHRRRRYKK	0.800	369.
20	801	ETLMYSRPRK	0.450	370.
21	310	APGLIPYITK	0.400	371.
22	789	HYPGAHEELK	0.400	372.
23	655	HLQYSMYGHK	0.400	373.
24	451	LPGTNPMPK	0.400	374.
25	689	VYRSPSFGPK	0.400	375.
26	545	LCTSPGHLDK	0.400	376.
27	210	ILDQLLEDNK	0.400	377.
28	590	TTNTADTILR	0.400	378.
29	290	SINDSRMSTK	0.400	379.
30	45	LINCEAKGIK	0.400	380.
31	682	MVSPMVHVYR	0.400	381.
32	182	FVPLTHDLR	0.400	382.

TABLE XII

HLA Peptide Scoring Results – 158P1D7 – A11, 10-mers

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
33	767	QQLGITEYLR	0.360	383.
34	627	GIVVLVLHRR	0.360	384.
35	631	LVLHRRRRYK	0.300	385.
36	221	ACNCDLLQLK	0.200	386.
37	336	KVLSPSGLLI	0.180	387.
38	706	RNEKEGSDAK	0.120	388.
39	254	KGSILSRLKK	0.120	389.
40	462	KVLYLNNNLL	0.090	390.
41	163	KVLILNDNAI	0.090	391.
42	621	IVFCAAGIVV	0.080	392.
43	748	SFQDASSLYR	0.080	393.
44	119	INHNSLEILK	0.080	394.
45	753	SSLYRNILEK	0.060	395.
46	60	SVPPSRPFQL	0.060	396.
47	490	KTNQFTHLPV	0.060	397.
48	700	LEEEERNEK	0.060	398.
49	628	IVVLVLHRRR	0.060	399.
50	608	SVLILGLLIM	0.060	400.

TABLE XIII

HLA Peptide Scoring Results – 158P1D7 – A24, 9-mers

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
1	443	EYNAIKEIL	420.000	1
2	789	HYPGAHEEL	330.000	2
3	819	EYFELKANL	288.000	3
4	804	MYSRPRKVL	200.000	4
5	8	FYSSLLACI	60.000	5
6	386	YFTLEMLHL	20.000	6
7	139	EFLQADNNF	18.000	7
8	462	KVLYLNNNL	17.280	8
9	350	RNIESLSDL	14.400	9
10	599	RSLTDAVPL	12.000	10
11	336	KVLSPSGLL	12.000	11
12	305	KLPTKAPGL	12.000	12
13	736	KYKTTNQST	12.000	13

TABLE XIII				
HLA Peptide Scoring Results – 158P1D7 – A24, 9-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
14	580	SYLMVTTPA	10.500	14
15	415	KLYLNGNHL	9.600	15
16	272	VYEEHEDPS	9.000	16
17	202	GFLEHIGRI	9.000	17
18	438	EYLYLEYNA	9.000	18
19	466	LNNNLLQVL	8.640	19
20	767	QQLGITEYL	8.400	20
21	203	FLEHIGRI	8.400	21
22	607	LSVLILGLL	8.400	22
23	87	LTNAISHL	8.400	23
24	537	KNTVTDDIL	8.000	24
25	219	KWACNCDLL	8.000	25
26	758	NILEKEREL	7.920	26
27	408	MNLTRLQKL	7.920	27
28	527	GLQQWIKL	7.920	28
29	416	LYLNGNHLT	7.500	29
30	199	PYVGLEHI	7.500	30
31	486	KVNLKTNQF	7.200	31
32	109	NGLGLLKQL	7.200	32
33	196	QTLPLYVGL	7.200	33
34	133	HGLENLEFL	7.200	34
35	225	DLLQLKTWL	7.200	35
36	83	DFSGLTNAI	7.200	36
37	456	NPMPKLKVL	7.200	37
38	561	NSEILCPGL	7.200	38
39	501	NILDDLDL	7.200	39
40	500	SNILDDLDL	6.000	40
41	221	ACNCDLLQL	6.000	41
42	71	LLNNGLTML	6.000	42
43	604	AVPLSVLIL	6.000	43
44	182	FVPLTHLDL	6.000	44
45	347	CQERNIESL	6.000	45
46	669	TTERPSASL	6.000	46
47	10	SSLACISL	6.000	47
48	590	TTNTADTIL	6.000	48
49	481	GVPLTKVNL	6.000	49
50	432	LGLHNLEYL	6.000	50

TABLE XIV				
HLA Peptide Scoring Results – 158P1D7 – A24, 10-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
1	773	EYLRKNIAQL	300.000	401.

TABLE XIV				
HLA Peptide Scoring Results – 158P1D7 – A24, 10-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
2	385	EYFTLEMLHL	200.000	402.
3	438	EYLYLEYNAI	90.000	403.
4	181	RFVPLTHLDL	72.000	404.
5	202	GFLEHIGRIL	50.400	405.
6	677	LYEQHMOVSPM	37.500	406.
7	315	PYITKPSTQL	30.000	407.
8	252	FFKGSILSRL	28.000	408.
9	622	VFCAAGIVVL	20.000	409.
10	179	IFRFVPLTHL	20.000	410.
11	359	RPPPQNPRKL	15.840	411.
12	462	KVLYLNNNLL	14.400	412.
13	115	KQLHINHNSL	14.400	413.
14	757	RNILEKEREL	13.200	414.
15	832	DYLEVLEQQT	12.960	415.
16	691	RSPSFGPKHL	12.000	416.
17	428	KGMFLGLHNL	12.000	417.
18	158	KLNRLKVLIL	12.000	418.
19	131	TFHGLENLEF	11.000	419.
20	425	KLSKGMFLGL	9.600	420.
21	150	VIEPSAFSKL	9.504	421.
22	139	EFLQADNNFI	9.000	422.
23	102	DIEIGAFNGL	8.640	423.
24	465	YLNNNLLQVL	8.640	424.
25	67	FQLSLLNNGI	8.640	425.
26	401	VLEEGSFMNL	8.640	426.
27	497	LPVSNILDDL	8.400	427.
28	766	LQQLGITEYL	8.400	428.
29	96	GFNNIADIEI	8.250	429.
30	738	KTTNQSTEFL	8.000	430.
31	380	KSDLVEYFTL	8.000	431.
32	295	RMSTKTTSIL	8.000	432.
33	526	VGLQQWQIKL	7.920	433.

TABLE XIV				
HLA Peptide Scoring Results – 158P1D7 – A24, 10-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
34	407	FMNLTRLQKL	7.920	434.
35	580	SYLMVTTPAT	7.500	435.
36	464	LYLNNNLLQV	7.500	436.
37	828	HAEPDYLEVL	7.200	437.
38	329	CPIPCNCKVL	7.200	438.
39	36	NCEEKDGTM	7.200	439.
40	346	HCQERNIESL	7.200	440.
41	166	ILNDNAIESL	7.200	441.
42	60	SVPPSRPFQL	7.200	442.
43	605	VPLSVLILGL	7.200	443.
44	480	SGVPLTKVNL	7.200	444.
45	603	DAVPLSVLIL	7.200	445.
46	494	FTHLPVSNIL	6.720	446.
47	592	NTADTILRSL	6.720	447.
48	417	YLNGNHLTKL	6.600	448.
49	118	HINHNSLEIL	6.000	449.
50	500	SNILDDL DLL	6.000	450.

TABLE XV				
HLA Peptide Scoring Results – 158P1D7 – B7, 9-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
1	456	NPMPKLKVL	240.000	451.
2	458	MPKLKVLYL	80.000	452.
3	692	SPSFGPKHL	80.000	453.
4	61	VPPSRPFQL	80.000	454.
5	517	NPWDCSCDL	80.000	455.
6	604	AVPLSVLIL	60.000	456.
7	26	SSRGSCDSL	40.000	457.
8	207	IGRILDLQL	40.000	458.
9	410	LTRLQKLYL	40.000	459.
10	159	LNRLKVLIL	40.000	460.

TABLE XV				
HLA Peptide Scoring Results – 158P1D7 – B7, 9-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
11	774	YLRKNIAQL	40.000	461.
12	625	AAGIVVLVL	36.000	462.
13	336	KVLSPSGLL	30.000	463.
14	481	GVPLTKVNL	20.000	464.
15	182	FVPLTHLDL	20.000	465.
16	462	KVLYLNNNL	20.000	466.
17	652	SPVHLQYSM	20.000	467.
18	575	MPTQTSYLM	20.000	468.
19	752	ASSLYRNIL	18.000	469.
20	370	LAGNIIHSL	12.000	470.
21	154	SAFSKLNRL	12.000	471.
22	713	DAKHLQRSL	12.000	472.
23	221	ACNCDLLQL	12.000	473.
24	106	GAFNGLGLL	12.000	474.
25	249	SPPFFKGSIL	8.000	475.
26	306	LPTKAPGLI	8.000	476.
27	250	PPFFKGSIL	8.000	477.
28	360	PPPQNPRKL	8.000	478.
29	453	GTFNPMPKL	6.000	479.
30	310	APGLIPYIT	6.000	480.
31	316	YITKPSTQL	6.000	481.
32	400	EVLEEGSFM	5.000	482.
33	429	GMFLGLHNL	4.000	483.
34	418	LNGNHLTKL	4.000	484.
35	544	ILCTSPGHL	4.000	485.
36	826	NLHAEPDYL	4.000	486.
37	350	RNIESLSDL	4.000	487.
38	4	WIHLFYSSL	4.000	488.
39	501	NILDDLDLL	4.000	489.
40	109	NGLGLLKQL	4.000	490.
41	607	LSVLILGLL	4.000	491.
42	71	LLNNGLTML	4.000	492.

TABLE XV				
HLA Peptide Scoring Results – 158P1D7 – B7, 9-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
43	599	RSLTDAVPL	4.000	493.
44	739	TTNQSTEFL	4.000	494.
45	87	LTNAISIHL	4.000	495.
46	130	DTFHGLENL	4.000	496.
47	415	KLYLNGNHL	4.000	497.
48	175	LPPNIFRFV	4.000	498.
49	105	IGAFNGLGL	4.000	499.
50	296	MSTKTTSIL	4.000	500.

TABLE XVI				
HLA Peptide Scoring Results – 158P1D7 – B7, 10-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
1	249	SPPFFKGSIL	80.000	501.
2	548	SPGHLDKKEL	80.000	502.
3	497	LPVSNILDDL	80.000	503.
4	329	CPIPCNCKVL	80.000	504.
5	790	YPGAHEELKL	80.000	505.
6	605	VPLSVLILGL	80.000	506.
7	359	RPPPQNPRKL	80.000	507.
8	189	DLRGNQLQTL	40.000	508.
9	647	QMRDNSPVHL	40.000	509.
10	566	CPGLVNNPSM	20.000	510.
11	807	RPRKVLVEQT	20.000	511.
12	462	KVLYLNNNLL	20.000	512.
13	60	SVPPSRPFQL	20.000	513.
14	713	DAKHLQRSLL	18.000	514.
15	751	DASSLYRNIL	18.000	515.
16	603	DAVPLSVLIL	12.000	516.
17	624	CAAGIVVLVL	12.000	517.
18	428	KGMFLGLHNL	12.000	518.
19	825	ANLHAEPDYL	12.000	519.

TABLE XVI

HLA Peptide Scoring Results – 158PID7 – B7, 10-mers

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
20	220	WACNCDLLQL	12.000	520.
21	803	LMYSRPRKVL	9.000	521.
22	198	LPYVGFLHI	8.000	522.
23	361	PPQNPRKLIL	8.000	523.
24	176	PPNIFRFVPL	8.000	524.
25	475	PPHIFSGVPL	8.000	525.
26	62	PPSRPFQLSL	8.000	526.
27	179	IFRFVPLTHL	6.000	527.
28	668	HTTERPSASL	6.000	528.
29	383	LVEYFTLEML	6.000	529.
30	608	SVLILGLLIM	5.000	530.
31	393	HLGNNRIEVL	4.000	531.
32	589	TTTNTADTIL	4.000	532.
33	738	KTNQSTEFLL	4.000	533.
34	78	MLHTNDFSGL	4.000	534.
35	16	ISLHSQTPVL	4.000	535.
36	9	YSSLLACISL	4.000	536.
37	814	EQTKNEYFEL	4.000	537.
38	407	FMNLTRLQKL	4.000	538.
39	575	MPTQTSYLMV	4.000	539.
40	4	WIHLFYSSLL	4.000	540.
41	417	YLNGNHLTKL	4.000	541.
42	63	PSRPFQLSLL	4.000	542.
43	757	RNILEKEREL	4.000	543.
44	108	FNGLGLLKQL	4.000	544.
45	409	NLTRLQKLYL	4.000	545.
46	556	ELKALNSEIL	4.000	546.
47	166	ILNDNAIESL	4.000	547.
48	217	DNKWACNCDL	4.000	548.
49	364	NPRKLILAGN	4.000	549.
50	295	RMSTKTTSIL	4.000	550.

TABLE XVII				
HLA Peptide Scoring Results – 158P1D7 – B35, 9-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
1	458	MPKLKVLYL	60.000	551.
2	652	SPVHLQYSM	40.000	552.
3	575	MPTQTSYLM	40.000	553.
4	517	NPWDCSCDL	40.000	554.
5	456	NPMPKLKVL	20.000	555.
6	692	SPSFGPKHL	20.000	556.
7	61	VPPSRPFQL	20.000	557.
8	792	GAHEELKLM	18.000	558.
9	26	SSRGSCDSL	15.000	559.
10	426	LSKGMFLGL	15.000	560.
11	599	RSLTDAVPL	15.000	561.
12	727	HSPLTGSNM	10.000	562.
13	288	TSSINDSRM	10.000	563.
14	713	DAKHLQRSL	9.000	564.
15	378	LMKSDLVEY	9.000	565.
16	306	LPTKAPGLI	8.000	566.
17	249	SPPFFKGS	8.000	567.
18	747	LSFQDASSL	7.500	568.
19	228	QLKTWLENM	6.000	569.
20	674	SASLYEQHM	6.000	570.
21	400	EVLEEGSFM	6.000	571.
22	258	LSRLKKESI	6.000	572.
23	796	ELKLMETLM	6.000	573.
24	752	ASSLYRNIL	5.000	574.
25	607	LSVLILGLL	5.000	575.
26	10	SSLLACISL	5.000	576.
27	59	ISVPPSRPF	5.000	577.
28	296	MSTKTTSIL	5.000	578.
29	405	GSFMNLTRL	5.000	579.
30	815	QTKNEYFEL	4.500	580.
31	350	RNIESLSDL	4.000	581.
32	329	CPIPCNCKV	4.000	582.

TABLE XVII				
HLA Peptide Scoring Results – 158P1D7 – B35, 9-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
33	474	LPPHIFSGV	4.000	583.
34	782	LQPDMEAHY	4.000	584.
35	65	RPFQLSLLN	4.000	585.
36	175	LPPNIFRFV	4.000	586.
37	805	YSRPRKVLV	3.000	587.
38	774	YLRKNIAQL	3.000	588.
39	154	SAFSKLNRL	3.000	589.
40	410	LTRLQKLYL	3.000	590.
41	207	IGRILDLQL	3.000	591.
42	370	LAGNIIHSL	3.000	592.
43	106	GAFNGLGLL	3.000	593.
44	156	FSKLNRLKV	3.000	594.
45	501	NILDDDLL	3.000	595.
46	423	LTKLSKGMF	3.000	596.
47	625	AAGIVVLVL	3.000	597.
48	159	LNRLKVLIL	3.000	598.
49	89	NAISHLGF	3.000	599.
50	309	KAPGLIPYI	2.400	600.

TABLE XVIII				
HLA Peptide Scoring Results – 158P1D7 – B35, 10-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
1	319	KPSTQLPGPY	80.000	601.
2	566	CPGLVNNPSM	40.000	602.
3	728	SPLTGSNMKY	40.000	603.
4	572	NPSMPTQTSY	40.000	604.
5	652	SPVHLQYSMY	40.000	605.
6	359	RPPPQNPRKL	40.000	606.
7	456	NPMPKLKVLV	40.000	607.
8	548	SPGHLDKKEL	30.000	608.
9	790	YPGAHEELKL	30.000	609.

TABLE XVIII

HLA Peptide Scoring Results – 158P1D7 – B35, 10-mers

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
10	329	CPIPCNCKVL	20.000	610.
11	249	SPPFFKGSIL	20.000	611.
12	605	VPLSVLILGL	20.000	612.
13	497	LPVSNILDDL	20.000	613.
14	156	FSKLNRLKVL	15.000	614.
15	824	KANLHAEPDY	12.000	615.
16	807	RPRKVLVEQT	12.000	616.
17	747	LSFQDASSLY	10.000	617.
18	691	RSPSFGPKHL	10.000	618.
19	264	ESICPTPPVY	10.000	619.
20	651	NSPVHLQYSM	10.000	620.
21	69	LSLLNNGLTM	10.000	621.
22	796	ELKLMETLMY	9.000	622.
23	713	DAKHLQRSLL	9.000	623.
24	198	LPYVGFLEHI	8.000	624.
25	517	NPWDCSCDLV	8.000	625.
26	499	VSNILDDL DL	7.500	626.
27	126	ILKEDTFHGL	6.000	627.
28	370	LAGNIIHSLM	6.000	628.
29	458	MPKLKVLYLN	6.000	629.
30	364	NPRKLILAGN	6.000	630.
31	647	QMRDNSPVHL	6.000	631.
32	446	AIKEILPGTF	6.000	632.
33	535	LSKNTVTDDI	6.000	633.
34	25	LSSRGSCDSL	5.000	634.
35	9	YSSLLACISL	5.000	635.
36	173	ESLPPNIFRF	5.000	636.
37	16	ISLHSQTPVL	5.000	637.
38	380	KSDLVEYFTL	4.500	638.
39	220	WACNCDLLQL	4.500	639.
40	435	HNLEYLYLEY	4.000	640.
41	236	MPPQSIIGDV	4.000	641.

TABLE XVIII				
HLA Peptide Scoring Results – 158P1D7 – B35, 10-mers				
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)	Seq.ID#
42	382	DLVEYFTLEM	4.000	642.
43	35	CNCEEKDGTM	4.000	643.
44	575	MPTQTSYLMV	4.000	644.
45	777	KNIAQLQPDM	4.000	645.
46	191	RGNQLQTLPY	4.000	646.
47	65	RPFQLSLLNN	4.000	647.
48	811	VLVEQTKNEY	4.000	648.
49	46	INCEAKGIKM	4.000	649.
50	556	ELKALNSEIL	3.000	650.

Table XIX: Motif-bearing Subsequences of the 158P1D7 Protein**Protein Motifs of 158P1D7**

N-glycosylation site

Number of matches: 3

- 1 292-295 NDSR
- 2 409-412 NLTR
- 3 741-744 NQST

cAMP- and cGMP-dependent protein kinase phosphorylation site

262-265 KKES

Protein kinase C phosphorylation site

Number of matches: 3

- 1 26-28 SSR
- 2 297-299 STK
- 3 670-672 TER

Casein kinase II phosphorylation site

Number of matches: 12

- 1 149-152 TVIE
- 2 186-189 THLD
- 3 231-234 TWLE
- 4 290-293 SIND
- 5 354-357 SLSD
- 6 510-513 TQID
- 7 539-542 TVTD
- 8 600-603 SLTD
- 9 676-679 SLYE
- 10 720-723 SLLE
- 11 748-751 SFQD
- 12 816-819 TKNE

Tyrosine kinase phosphorylation site

798-805 KLMETLMY

N-myristoylation site

Number of matches: 8

- 1 29-34 GSCDSL
- 2 86-91 GLTNAI
- 3 106-111 GAFNGL
- 4 255-260 GSILSR
- 5 405-410 GSFMNL
- 6 420-425 GNHLTK
- 7 429-434 GMFLGL
- 8 481-486 GVPLTK

Two Protein Motifs were predicted by Pfam

1-Archaeal-ATPase at aa 441-451

2-Leucine rich repeat C-terminal at aa 218-268 and aa 517-567

Table XX: Frequently Occurring Motifs			
Name	avrg. % identity	Description	Potential Function
<u>zf-C2H2</u>	34%	Zinc finger, C2H2 type	Nucleic acid-binding protein functions as transcription factor, nuclear location probable
<u>cytochrome b N</u>	68%	Cytochrome b(N-terminal)/b6/petB	membrane bound oxidase, generate superoxide
<u>ig</u>	19%	Immunoglobulin domain	domains are one hundred amino acids long and include a conserved intradomain disulfide bond.
<u>WD40</u>	18%	WD domain, G-beta repeat	tandem repeats of about 40 residues, each containing a Trp-Asp motif. Function in signal transduction and protein interaction
<u>PDZ</u>	23%	PDZ domain	may function in targeting signaling molecules to sub-membranous sites
<u>LRR</u>	28%	Leucine Rich Repeat	short sequence motifs involved in protein-protein interactions
<u>pkinese</u>	23%	Protein kinase domain	conserved catalytic core common to both serine/threonine and tyrosine protein kinases containing an ATP binding site and a catalytic site
<u>PH</u>	16%	PH domain	pleckstrin homology involved in intracellular signaling or as constituents of the cytoskeleton
<u>EGF</u>	34%	EGF-like domain	30-40 amino-acid long found in the extracellular domain of membrane-bound proteins or in secreted proteins
<u>rvt</u>	49%	Reverse transcriptase (RNA-dependent DNA polymerase)	
<u>ank</u>	25%	Ank repeat	Cytoplasmic protein, associates integral membrane proteins to the cytoskeleton

<u>oxidored_q1</u>	32%	NADH- Ubiquinone/plastoquinone (complex I), various chains	membrane associated. Involved in proton translocation across the membrane
<u>efhand</u>	24%	EF hand	calcium-binding domain, consists of a 12 residue loop flanked on both sides by a 12 residue alpha-helical domain
<u>rvp</u>	79%	Retroviral aspartyl protease	Aspartyl or acid proteases, centered on a catalytic aspartyl residue
<u>Collagen</u>	42%	Collagen triple helix repeat (20 copies)	extracellular structural proteins involved in formation of connective tissue. The sequence consists of the G-X-Y and the polypeptide chains forms a triple helix.
<u>fn3</u>	20%	Fibronectin type III domain	Located in the extracellular ligand-binding region of receptors and is about 200 amino acid residues long with two pairs of cysteines involved in disulfide bonds
<u>7tm_1</u>	19%	7 transmembrane receptor (rhodopsin family)	seven hydrophobic transmembrane regions, with the N-terminus located extracellularly while the C-terminus is cytoplasmic. Signal through G proteins

Table VII: TNM CLASSIFICATION OF BLADDER TUMORS**Primary tumor (T)**

The suffix (m) should be added to the appropriate T category to indicate multiple tumors. The suffix (is) may be added to any T to indicate the presence of associated carcinoma *in situ*.

TX	Primary tumor cannot be assessed
TO	No evidence of primary tumor
Ta	Noninvasive papillary carcinoma
Tis	Carcinoma <i>in situ</i> : "flat tumor"
T1	Tumor invades sub-epithelial connective tissue
T2	Tumor invades superficial muscle (inner half)
T3	Tumor invades deep muscle or perivesical fat
T3a	Tumor invades deep muscle (outer half)
T3b	Tumor invades perivesical fat
	i. microscopically
	ii. macroscopically (extravesical mass)
T4	Tumor invades any of the following: prostate, uterus, vagina, pelvic wall, or abdominal wall
T4a	Tumor invades the prostate, uterus, vagina
T4b	Tumor invades the pelvic wall or abdominal wall or both

Regional lymph nodes (N)

Regional lymph nodes are those within the true pelvis: all others are distant nodes

NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single lymph node, 2 cm or less in greatest dimension
N2	Metastasis in a single lymph node, more than 2 cm but not more than 5 cm in greatest dimension, or multiple lymph nodes, none more than 5 cm in greatest dimension
N3	Metastasis in a lymph node more than 5 cm in greatest dimension

Distant metastasis (M)

MX	Presence of distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

Stage grouping

Stage	0 _a	Ta	N0	M0
	0 _{is}	Tis	N0	M0
I		T1	N0	M0
II		T2	N0	M0
		T3a	N0	M0
III		T3b	N0	M0
		T4a	N0	M0
IV		T4b	N0	M0
		Any T	N1-3	M0
		Any T	Any N	M1

SEQUENCE LISTING

<110> AGENSYS, INC.

<120> NUCLEIC ACID AND CORRESPONDING PROTEIN NAMED 158P1D7
USEFUL IN THE TREATMENT AND DETECTION OF BLADDER AND
OTHER CANCERS

<130> 49324-233

<140> CA 2,420,543

<141> 2001-08-22

<150> US 60/227,098

<151> 2000-08-22

<150> US 60/282,739

<151> 2001-04-10

<160> 750

<170> PatentIn Ver. 2.1

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1				5				

<210> 2

<211> 9

<212> PRT

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Peptide motif

<400> 2

Asn	Leu	Glu	Tyr	Leu	Tyr	Leu	Glu	Tyr
1				5				

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<400> 3

Leu	Val	Glu	Gln	Thr	Lys	Asn	Glu	Tyr
1				5				

<210> 4
<211> 9
<212> PRT
<213> Artificial Sequence

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His Ala Glu Pro Asp Tyr Leu Glu Val
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<210> 5
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 5
Gly Ser Asp Ala Lys His Leu Gln Arg
1 5

<210> 6
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
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<400> 6
Cys Thr Ser Pro Gly His Leu Asp Lys
1 5

<210> 7
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<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 7
Ser Ile Cys Pro Thr Pro Pro Val Tyr
1 5

<210> 8
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<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 8

Asn Ile Glu Ser Leu Ser Asp Leu Arg

1

5

<210> 9

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 9

Leu Met Glu Thr Leu Met Tyr Ser Arg

1

5

<210> 10

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 10

Glu Ser Leu Pro Pro Asn Ile Phe Arg

1

5

<210> 11

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 11

Asp Asn Ser Pro Val His Leu Gln Tyr

1

5

<210> 12

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 12

Leu Thr Asp Ala Val Pro Leu Ser Val

1

5

<210> 13

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 13

Ser Leu Pro Pro Asn Ile Phe Arg Phe
1 5

<210> 14

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 14

Ile Ala Asp Ile Glu Ile Gly Ala Phe
1 5

<210> 15

<211> 9

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<213> Artificial Sequence

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<223> Description of Artificial Sequence: Peptide motif

<400> 15

Met Val Ser Pro Met Val His Val Tyr
1 5

<210> 16

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 16

Asp Ile Glu Ile Gly Ala Phe Asn Gly
1 5

<210> 17

<211> 9

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<223> Description of Artificial Sequence: Peptide motif

<400> 17

Gly Leu Glu Asn Leu Glu Phe Leu Gln
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<210> 18
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<400> 18
Asn Cys Glu Ala Lys Gly Ile Lys Met
1 5

<210> 19
<211> 9
<212> PRT
<213> Artificial Sequence

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<400> 19
Leu Val Glu Tyr Phe Thr Leu Glu Met
1 5

<210> 20
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
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<400> 20
Val Leu Glu Glu Gly Ser Phe Met Asn
1 5

<210> 21
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
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<400> 21
Thr Leu Glu Met Leu His Leu Gly Asn
1 5

<210> 22
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
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Phe Gln Asp Ala Ser Ser Leu Tyr Arg
1 5

<210> 23
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
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<400> 23
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1 5

<210> 24
<211> 9
<212> PRT
<213> Artificial Sequence

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<400> 24
Asn Ser Glu Ile Leu Cys Pro Gly Leu
1 5

<210> 25
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
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<400> 25
Phe Leu Gly Leu His Asn Leu Glu Tyr
1 5

<210> 26
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 26
Ile Asn Asp Ser Arg Met Ser Thr Lys
1 5

<210> 27
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<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 27

Gln Ala Asp Asn Asn Phe Ile Thr Val
 1 5

<210> 28

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 28

Ile Leu Asp Asp Leu Asp Leu Leu Thr
 1 5

<210> 29

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 29

Ser Cys Asp Leu Val Gly Leu Gln Gln
 1 5

<210> 30

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 30

Asn Cys Asp Leu Leu Gln Leu Lys Thr
 1 5

<210> 31

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 31

Ile Thr Glu Tyr Leu Arg Lys Asn Ile
 1 5

<210> 32
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 32
Trp Leu Glu Asn Met Pro Pro Gln Ser
1 5

<210> 33
<211> 9
<212> PRT
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<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 33
Ala Ile Glu Ser Leu Pro Pro Asn Ile
1 5

<210> 34
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 34
Asn Leu Glu Phe Leu Gln Ala Asp Asn
1 5

<210> 35
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 35
Leu Ser Asp Leu Arg Pro Pro Pro Gln
1 5

<210> 36
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 36

Lys Ser Asp Leu Val Glu Tyr Phe Thr
 1 5

<210> 37

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 37

Ile Ser Val Pro Pro Ser Arg Pro Phe
 1 5

<210> 38

<211> 9

<212> PRT

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Peptide motif

<400> 38

Gly Ser Ile Leu Ser Arg Leu Lys Lys
 1 5

<210> 39

<211> 9

<212> PRT

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<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 39

Val Thr Asp Asp Ile Leu Cys Thr Ser
 1 5

<210> 40

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 40

Thr Lys Ala Pro Gly Leu Ile Pro Tyr
 1 5

<210> 41

<211> 9

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<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 41
Lys Asn Glu Tyr Phe Glu Leu Lys Ala
1 5

<210> 42
<211> 9
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<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 42
Ser Thr Glu Phe Leu Ser Phe Gln Asp
1 5

<210> 43
<211> 9
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<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 43
Arg Pro Pro Pro Gln Asn Pro Arg Lys
1 5

<210> 44
<211> 9
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<223> Description of Artificial Sequence: Peptide motif

<400> 44
Val Cys Asn Ser Pro Pro Phe Phe Lys
1 5

<210> 45
<211> 9
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 45
Tyr Leu Asn Gly Asn His Leu Thr Lys
1 5

<210> 46
<211> 9
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<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 46
Gly Leu His Asn Leu Glu Tyr Leu Tyr
1 5

<210> 47
<211> 9
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<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 47
Asp Met Glu Ala His Tyr Pro Gly Ala
1 5

<210> 48
<211> 9
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 48
Arg Ile Glu Val Leu Glu Glu Gly Ser
1 5

<210> 49
<211> 9
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<213> Artificial Sequence

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<223> Description of Artificial Sequence: Peptide motif

<400> 49
Glu Glu Glu Glu Glu Arg Asn Glu Lys
1 5

<210> 50
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 50

Tyr Leu Glu Val Leu Glu Gln Gln Thr
1 5

<210> 51

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 51

Val Ser Glu Ile Ser Val Pro Pro Ser Arg
1 5 10

<210> 52

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 52

Thr Thr Glu Arg Pro Ser Ala Ser Leu Tyr
1 5 10

<210> 53

<211> 10

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<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 53

Ile Leu Asp Leu Gln Leu Glu Asp Asn Lys
1 5 10

<210> 54

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 54

Gln Leu Gln Pro Asp Met Glu Ala His Tyr
1 5 10

<210> 55

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 55

Val	Ile	Glu	Pro	Ser	Ala	Phe	Ser	Lys	Leu
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<210> 56

<211> 10

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<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 56

Ala	Ile	Glu	Ser	Leu	Pro	Pro	Asn	Ile	Phe
1				5					10

<210> 57

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 57

His	Ala	Glu	Pro	Asp	Tyr	Leu	Glu	Val	Leu
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<210> 58

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 58

Ser	Leu	Glu	Ile	Leu	Lys	Glu	Asp	Thr	Phe
1				5					10

<210> 59

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 59

Arg	Ile	Glu	Val	Leu	Glu	Glu	Gly	Ser	Phe
1				5					10

<210> 60
<211> 10
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<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 60
Leu Val Glu Gln Thr Lys Asn Glu Tyr Phe
1 5 10

<210> 61
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 61
Glu Ser Leu Pro Pro Asn Ile Phe Arg Phe
1 5 10

<210> 62
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 62
Cys Thr Ser Pro Gly His Leu Asp Lys Lys
1 5 10

<210> 63
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 63
Gly Leu Glu Asn Leu Glu Phe Leu Gln Ala
1 5 10

<210> 64
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 64

Val	Leu	Glu	Glu	Gly	Ser	Phe	Met	Asn	Leu
1				5					10

<210> 65

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 65

Lys	Ser	Asp	Leu	Val	Glu	Tyr	Phe	Thr	Leu
1				5					10

<210> 66

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 66

Asn	Pro	Met	Pro	Lys	Leu	Lys	Val	Leu	Tyr
1				5					10

<210> 67

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 67

Asp	Leu	Asp	Leu	Leu	Thr	Gln	Ile	Asp	Leu
1				5					10

<210> 68

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 68

Ile	Leu	Asp	Asp	Leu	Asp	Leu	Leu	Thr	Gln
1				5					10

<210> 69

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 69

Ser	Thr	Glu	Phe	Leu	Ser	Phe	Gln	Asp	Ala
1				5					10

<210> 70

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 70

Ile	Thr	Glu	Tyr	Leu	Arg	Lys	Asn	Ile	Ala
1				5					10

<210> 71

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 71

Met	Val	Ser	Pro	Met	Val	His	Val	Tyr	Arg
1				5					10

<210> 72

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 72

Gln	Leu	Glu	Asp	Asn	Lys	Trp	Ala	Cys	Asn
1				5					10

<210> 73

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 73

Leu	Ser	Asp	Leu	Arg	Pro	Pro	Pro	Gln	Asn
1				5					10

<210> 74
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 74
Glu Ser Ile Cys Pro Thr Pro Pro Val Tyr
1 5 10

<210> 75
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 75
Ser Ser Leu Tyr Arg Asn Ile Leu Glu Lys
1 5 10

<210> 76
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 76
Asn Ser Glu Ile Leu Cys Pro Gly Leu Val
1 5 10

<210> 77
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 77
Leu Thr Asp Ala Val Pro Leu Ser Val Leu
1 5 10

<210> 78
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 78

His	Glu	Asp	Pro	Ser	Gly	Ser	Leu	His	Leu
1				5					10

<210> 79

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 79

Thr	Thr	Asn	Thr	Ala	Asp	Thr	Ile	Leu	Arg
1				5					10

<210> 80

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 80

Thr	Val	Ile	Glu	Pro	Ser	Ala	Phe	Ser	Lys
1				5					10

<210> 81

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 81

Gly	Ala	Phe	Asn	Gly	Leu	Gly	Leu	Leu	Lys
1				5					10

<210> 82

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 82

Glu	Thr	Leu	Met	Tyr	Ser	Arg	Pro	Arg	Lys
1				5					10

<210> 83

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 83

Leu	Cys	Thr	Ser	Pro	Gly	His	Leu	Asp	Lys
1				5					10

<210> 84

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 84

Lys	Ala	Asn	Leu	His	Ala	Glu	Pro	Asp	Tyr
1				5					10

<210> 85

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 85

Leu	Val	Gly	Leu	Gln	Gln	Trp	Ile	Gln	Lys
1				5					10

<210> 86

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 86

Thr	Thr	Ser	Ile	Leu	Lys	Leu	Pro	Thr	Lys
1				5					10

<210> 87

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 87

His	Ile	Phe	Ser	Gly	Val	Pro	Leu	Thr	Lys
1				5					10

<210> 88
 <211> 10
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<220>
 <223> Description of Artificial Sequence: Peptide motif

<400> 88
 Ile Ala Asp Ile Glu Ile Gly Ala Phe Asn
 1 5 10

<210> 89
 <211> 10
 <212> PRT
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<220>
 <223> Description of Artificial Sequence: Peptide motif

<400> 89
 Gln Leu Gly Ile Thr Glu Tyr Leu Arg Lys
 1 5 10

<210> 90
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Peptide motif

<400> 90
 Val Val Cys Asn Ser Pro Pro Phe Phe Lys
 1 5 10

<210> 91
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Peptide motif

<400> 91
 Leu Leu Glu Gln Glu Asn His Ser Pro Leu
 1 5 10

<210> 92
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Peptide motif

<400> 92

Leu Glu Glu Glu Glu Glu Arg Asn Glu Lys
1 5 10

<210> 93

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 93

Asp Ile Glu Ile Gly Ala Phe Asn Gly Leu
1 5 10

<210> 94

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 94

Tyr Leu Glu Tyr Asn Ala Ile Lys Glu Ile
1 5 10

<210> 95

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 95

Asn Leu Glu Tyr Leu Tyr Leu Glu Tyr Asn
1 5 10

<210> 96

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 96

Asn Cys Glu Glu Lys Asp Gly Thr Met Leu
1 5 10

<210> 97

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 97

Asp	Leu	Glu	Asp	Asn	Pro	Trp	Asp	Cys	Ser
1				5					10

<210> 98

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 98

Leu	Val	Glu	Tyr	Phe	Thr	Leu	Glu	Met	Leu
1				5					10

<210> 99

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 99

Thr	Leu	Glu	Met	Leu	His	Leu	Gly	Asn	Asn
1				5					10

<210> 100

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 100

Asn	Leu	Glu	Phe	Leu	Gln	Ala	Asp	Asn	Asn
1				5					10

<210> 101

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 101

Tyr	Leu	Asn	Asn	Asn	Leu	Leu	Gln	Val
1				5				

<210> 102
<211> 9
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<213> Artificial Sequence

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<223> Description of Artificial Sequence: Peptide motif

<400> 102
Leu Leu Ile Met Phe Ile Thr Ile Val
1 5

<210> 103
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 103
Asn Gln Leu Gln Thr Leu Pro Tyr Val
1 5

<210> 104
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 104
Ile Met Phe Ile Thr Ile Val Phe Cys
1 5

<210> 105
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 105
Phe Leu Gln Ala Asp Asn Asn Phe Ile
1 5

<210> 106
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 106

Lys Leu Tyr Leu Asn Gly Asn His Leu
1 5

<210> 107

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 107

Tyr Leu Tyr Leu Glu Tyr Asn Ala Ile
1 5

<210> 108

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 108

Ile Leu Gly Leu Leu Ile Met Phe Ile
1 5

<210> 109

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 109

Lys Leu Trp Ile His Leu Phe Tyr Ser
1 5

<210> 110

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 110

Gly Met Phe Leu Gly Leu His Asn Leu
1 5

<210> 111

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 111

Tyr Leu Met Val Thr Thr Pro Ala Thr
 1 5

<210> 112

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 112

Val Leu Tyr Leu Asn Asn Asn Leu Leu
 1 5

<210> 113

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 113

Ser Met Pro Thr Gln Thr Ser Tyr Leu
 1 5

<210> 114

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 114

Leu Leu Asn Asn Gly Leu Thr Met Leu
 1 5

<210> 115

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 115

Trp Ile His Leu Phe Tyr Ser Ser Leu
 1 5

<210> 116
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 116
Lys Leu Pro Thr Lys Ala Pro Gly Leu
1 5

<210> 117
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 117
Gly Leu Leu Ile Met Phe Ile Thr Ile
1 5

<210> 118
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 118
Leu Gln Leu Glu Asp Asn Lys Trp Ala
1 5

<210> 119
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 119
Asn Leu His Ala Glu Pro Asp Tyr Leu
1 5

<210> 120
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 120

Leu Met Tyr Ser Arg Pro Arg Lys Val
1 5

<210> 121

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 121

Asn Ile Leu Asp Asp Leu Asp Leu Leu
1 5

<210> 122

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 122

Lys Leu Met Glu Thr Leu Met Tyr Ser
1 5

<210> 123

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 123

Gly Leu Gln Gln Trp Ile Gln Lys Leu
1 5

<210> 124

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 124

Lys Leu Asn Arg Leu Lys Val Leu Ile
1 5

<210> 125

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 125

Asn Ile Phe Arg Phe Val Pro Leu Thr
1 5

<210> 126

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 126

Asp Leu Leu Gln Leu Lys Thr Trp Leu
1 5

<210> 127

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 127

Lys Val Leu Tyr Leu Asn Asn Asn Leu
1 5

<210> 128

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 128

Gln Gln Leu Gly Ile Thr Glu Tyr Leu
1 5

<210> 129

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 129

Gln Leu His Ile Asn His Asn Ser Leu
1 5

<210> 130
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 130
Gln Leu Ser Leu Leu Asn Asn Gly Leu
1 5

<210> 131
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 131
Ile Leu Asp Asp Leu Asp Leu Leu Thr
1 5

<210> 132
<211> 9
<212> PRT
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<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 132
Ser Leu Leu Asn Asn Gly Leu Thr Met
1 5

<210> 133
<211> 9
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<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 133
Leu Leu Gln Val Leu Pro Pro His Ile
1 5

<210> 134
<211> 9
<212> PRT
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<223> Description of Artificial Sequence: Peptide motif

<400> 134

Met Leu His Leu Gly Asn Asn Arg Ile
1 5

<210> 135

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 135

Val Leu Ile Leu Asn Asp Asn Ala Ile
1 5

<210> 136

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 136

Val Leu Ser Pro Ser Gly Leu Leu Ile
1 5

<210> 137

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 137

Tyr Leu Arg Lys Asn Ile Ala Gln Leu
1 5

<210> 138

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 138

Ile Leu Pro Gly Thr Phe Asn Pro Met
1 5

<210> 139

<211> 9

<212> PRT

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 139
Gln Leu Pro Gly Pro Tyr Cys Pro Ile
1 5

<210> 140
<211> 9
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 140
Lys Leu Ile Leu Ala Gly Asn Ile Ile
1 5

<210> 141
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 141
Tyr Ile Thr Lys Pro Ser Thr Gln Leu
1 5

<210> 142
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 142
Leu Gln Ala Asp Asn Asn Phe Ile Thr
1 5

<210> 143
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 143
Gln Leu Glu Asp Asn Lys Trp Ala Cys
1 5

<210> 144
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 144
Leu Met Val Thr Thr Pro Ala Thr Thr
1 5

<210> 145
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 145
Asn Ile Leu Glu Lys Glu Arg Glu Leu
1 5

<210> 146
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 146
Ser Leu His Ser Gln Thr Pro Val Leu
1 5

<210> 147
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 147
Phe Val Pro Leu Thr His Leu Asp Leu
1 5

<210> 148
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 148

Val Leu Ile Leu Gly Leu Leu Ile Met
 1 5

<210> 149

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 149

Arg Met Ser Thr Lys Thr Thr Ser Ile
 1 5

<210> 150

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 150

Lys Ala Pro Gly Leu Ile Pro Tyr Ile
 1 5

<210> 151

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 151

Gly Leu Leu Ile Met Phe Ile Thr Ile Val
 1 5 10

<210> 152

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 152

Phe Leu Gly Leu His Asn Leu Glu Tyr Leu
 1 5 10

<210> 153

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 153

Ile	Met	Phe	Ile	Thr	Ile	Val	Phe	Cys	Ala
1				5					10

<210> 154

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 154

Ser	Leu	Thr	Asp	Ala	Val	Pro	Leu	Ser	Val
1				5					10

<210> 155

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 155

Tyr	Leu	Asn	Gly	Asn	His	Leu	Thr	Lys	Leu
1				5					10

<210> 156

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 156

Val	Leu	Pro	Pro	His	Ile	Phe	Ser	Gly	Val
1				5					10

<210> 157

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 157

Ser	Leu	Leu	Asn	Asn	Gly	Leu	Thr	Met	Leu
1				5					10

<210> 158
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<220>
 <223> Description of Artificial Sequence: Peptide motif

<400> 158
 Gly Leu His Asn Leu Glu Tyr Leu Tyr Leu
 1 5 10

<210> 159
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Peptide motif

<400> 159
 Ile Leu Asn Asp Asn Ala Ile Glu Ser Leu
 1 5 10

<210> 160
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Peptide motif

<400> 160
 Phe Met Asn Leu Thr Arg Leu Gln Lys Leu
 1 5 10

<210> 161
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Peptide motif

<400> 161
 Ser Leu Pro Pro Asn Ile Phe Arg Phe Val
 1 5 10

<210> 162
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Peptide motif

<400> 162

Lys Leu Ser Lys Gly Met Phe Leu Gly Leu
1 5 10

<210> 163

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 163

Tyr Leu Met Val Thr Thr Pro Ala Thr Thr
1 5 10

<210> 164

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 164

Asn Leu Thr Arg Leu Gln Lys Leu Tyr Leu
1 5 10

<210> 165

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 165

Leu Ile Leu Gly Leu Leu Ile Met Phe Ile
1 5 10

<210> 166

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 166

Phe Leu Ser Phe Gln Asp Ala Ser Ser Leu
1 5 10

<210> 167

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 167

Leu	Gln	Leu	Glu	Asp	Asn	Lys	Trp	Ala	Cys
1				5					10

<210> 168

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 168

Leu	Gln	Ala	Asp	Asn	Asn	Phe	Ile	Thr	Val
1				5					10

<210> 169

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 169

Tyr	Leu	Asn	Asn	Asn	Leu	Leu	Gln	Val	Leu
1				5					10

<210> 170

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 170

Ile	Leu	Ala	Gly	Asn	Ile	Ile	His	Ser	Leu
1				5					10

<210> 171

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 171

Lys	Leu	Tyr	Leu	Asn	Gly	Asn	His	Leu	Thr
1				5					10

<210> 172
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 172
Phe Leu Gln Ala Asp Asn Asn Phe Ile Thr
1 5 10

<210> 173
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 173
Lys Leu Asn Arg Leu Lys Val Leu Ile Leu
1 5 10

<210> 174
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 174
Ile Leu Gly Leu Leu Ile Met Phe Ile Thr
1 5 10

<210> 175
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 175
Met Leu His Thr Asn Asp Phe Ser Gly Leu
1 5 10

<210> 176
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 176

Leu	Ile	Met	Phe	Ile	Thr	Ile	Val	Phe	Cys
1				5					10

<210> 177

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 177

Thr	Leu	Met	Tyr	Ser	Arg	Pro	Arg	Lys	Val
1				5					10

<210> 178

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 178

Trp	Ile	Gln	Lys	Leu	Ser	Lys	Asn	Thr	Val
1				5					10

<210> 179

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 179

Asn	Leu	Leu	Gln	Val	Leu	Pro	Pro	His	Ile
1				5					10

<210> 180

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 180

Phe	Gln	Leu	Ser	Leu	Leu	Asn	Asn	Gly	Leu
1				5					10

<210> 181

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 181

Leu	Met	Tyr	Ser	Arg	Pro	Arg	Lys	Val	Leu
1				5					10

<210> 182

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 182

Lys	Gln	Leu	His	Ile	Asn	His	Asn	Ser	Leu
1				5					10

<210> 183

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 183

Lys	Val	Leu	Tyr	Leu	Asn	Asn	Asn	Leu	Leu
1				5					10

<210> 184

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 184

Gly	Leu	Thr	Asn	Ala	Ile	Ser	Ile	His	Leu
1				5					10

<210> 185

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 185

Val	Leu	Glu	Glu	Gly	Ser	Phe	Met	Asn	Leu
1				5					10

<210> 186
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<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 186
Met Leu Ile Asn Cys Glu Ala Lys Gly Ile
1 5 10

<210> 187
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 187
Thr Ile Leu Arg Ser Leu Thr Asp Ala Val
1 5 10

<210> 188
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 188
Ile Val Phe Cys Ala Ala Gly Ile Val Val
1 5 10

<210> 189
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 189
Asn Ile Leu Asp Asp Leu Asp Leu Leu Thr
1 5 10

<210> 190
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 190

Trp Ile His Leu Phe Tyr Ser Ser Leu Leu
 1 5 10

<210> 191

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 191

Lys Val Asn Leu Lys Thr Asn Gln Phe Thr
 1 5 10

<210> 192

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 192

Lys Val Leu Ile Leu Asn Asp Asn Ala Ile
 1 5 10

<210> 193

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 193

Lys Val Leu Ser Pro Ser Gly Leu Leu Ile
 1 5 10

<210> 194

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 194

Ser Val Pro Pro Ser Arg Pro Phe Gln Leu
 1 5 10

<210> 195

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 195

Ser	Leu	His	Leu	Ala	Ala	Thr	Ser	Ser	Ile
1				5					10

<210> 196

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 196

Gly	Leu	Gly	Leu	Leu	Lys	Gln	Leu	His	Ile
1				5					10

<210> 197

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 197

Leu	Gln	Gln	Leu	Gly	Ile	Thr	Glu	Tyr	Leu
1				5					10

<210> 198

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 198

Ile	Leu	Lys	Glu	Asp	Thr	Phe	His	Gly	Leu
1				5					10

<210> 199

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 199

Cys	Ile	Ser	Leu	His	Ser	Gln	Thr	Pro	Val
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<210> 200
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 200
Leu Met Val Thr Thr Pro Ala Thr Thr Thr
1 5 10

<210> 201
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 201
Ser Leu Tyr Arg Asn Ile Leu Glu Lys
1 5

<210> 202
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 202
Tyr Leu Asn Gly Asn His Leu Thr Lys
1 5

<210> 203
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 203
Phe Met Asn Leu Thr Arg Leu Gln Lys
1 5

<210> 204
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 204

Gly Leu His Asn Leu Glu Tyr Leu Tyr
1 5

<210> 205

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 205

Thr Leu Met Tyr Ser Arg Pro Arg Lys
1 5

<210> 206

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 206

Thr Met Leu Ile Asn Cys Glu Ala Lys
1 5

<210> 207

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 207

Gly Leu Leu Ile His Cys Gln Glu Arg
1 5

<210> 208

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 208

Leu Met Glu Thr Leu Met Tyr Ser Arg
1 5

<210> 209

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 209

Gly Leu Leu Ile Met Phe Ile Thr Ile
1 5

<210> 210

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 210

Gly Met Phe Leu Gly Leu His Asn Leu
1 5

<210> 211

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 211

Ser Leu Pro Pro Asn Ile Phe Arg Phe
1 5

<210> 212

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 212

Gln Leu Gly Ile Thr Glu Tyr Leu Arg
1 5

<210> 213

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 213

Gly Ile Val Val Leu Val Leu His Arg
1 5

<210> 214
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 214
Val Ile Glu Pro Ser Ala Phe Ser Lys
1 5

<210> 215
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 215
Lys Leu Tyr Leu Asn Gly Asn His Leu
1 5

<210> 216
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 216
Gly Leu Gln Gln Trp Ile Gln Lys Leu
1 5

<210> 217
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 217
Asn Leu Glu Tyr Leu Tyr Leu Glu Tyr
1 5

<210> 218
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 218

Phe Leu Gly Leu His Asn Leu Glu Tyr
1 5

<210> 219

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 219

Leu Met Lys Ser Asp Leu Val Glu Tyr
1 5

<210> 220

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 220

Gln Gln Trp Ile Gln Lys Leu Ser Lys
1 5

<210> 221

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 221

Cys Thr Ser Pro Gly His Leu Asp Lys
1 5

<210> 222

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 222

Val Leu Tyr Leu Asn Asn Asn Leu Leu
1 5

<210> 223

<211> 9

<212> PRT

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 223
Tyr Leu Tyr Leu Glu Tyr Asn Ala Ile
1 5

<210> 224
<211> 9
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 224
Lys Leu Trp Ile His Leu Phe Tyr Ser
1 5

<210> 225
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 225
Lys Leu Ile Leu Ala Gly Asn Ile Ile
1 5

<210> 226
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 226
Ser Thr Lys Thr Thr Ser Ile Leu Lys
1 5

<210> 227
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 227
His Leu Phe Tyr Ser Ser Leu Leu Ala
1 5

<210> 228
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 228
Val Leu His Arg Arg Arg Arg Tyr Lys
1 5

<210> 229
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 229
Asn Leu Thr Arg Leu Gln Lys Leu Tyr
1 5

<210> 230
<211> 9
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<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 230
Ile Leu Gly Leu Leu Ile Met Phe Ile
1 5

<210> 231
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 231
Val Leu Ser Pro Ser Gly Leu Leu Ile
1 5

<210> 232
<211> 9
<212> PRT
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<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 232
Lys Leu Pro Thr Lys Ala Pro Gly Leu
1 5

<210> 233
<211> 9
<212> PRT
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<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 233
Glu Met Leu His Leu Gly Asn Asn Arg
1 5

<210> 234
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<220>
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<400> 234
Lys Leu Asn Arg Leu Lys Val Leu Ile
1 5

<210> 235
<211> 9
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 235
Met Val Ser Pro Met Val His Val Tyr
1 5

<210> 236
<211> 9
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 236
Ile Met Phe Ile Thr Ile Val Phe Cys
1 5

<210> 237
<211> 9
<212> PRT
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<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 237

Ser Met Tyr Gly His Lys Thr Thr His
1 5

<210> 238

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 238

Ile Val Val Leu Val Leu His Arg Arg
1 5

<210> 239

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 239

Leu Leu Ile Met Phe Ile Thr Ile Val
1 5

<210> 240

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 240

Gln Leu Pro Gly Pro Tyr Cys Pro Ile
1 5

<210> 241

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 241

Leu Ile Leu Gly Leu Leu Ile Met Phe
1 5

<210> 242
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 242
Pro Leu Thr Gly Ser Asn Met Lys Tyr
1 5

<210> 243
<211> 9
<212> PRT
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<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 243
Gly Thr Phe Asn Pro Met Pro Lys Leu
1 5

<210> 244
<211> 9
<212> PRT
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<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 244
Gln Leu Lys Thr Trp Leu Glu Asn Met
1 5

<210> 245
<211> 9
<212> PRT
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<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 245
Ile Leu Pro Gly Thr Phe Asn Pro Met
1 5

<210> 246
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 246

Leu Ile Met Phe Ile Thr Ile Val Phe
1 5

<210> 247

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 247

Val Leu Ile Leu Gly Leu Leu Ile Met
1 5

<210> 248

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 248

Gly Ser Ile Leu Ser Arg Leu Lys Lys
1 5

<210> 249

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 249

Val Pro Leu Thr Lys Val Asn Leu Lys
1 5

<210> 250

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 250

Tyr Leu Arg Lys Asn Ile Ala Gln Leu
1 5

<210> 251

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 251

Tyr	Leu	Tyr	Leu	Glu	Tyr	Asn	Ala	Ile	Lys
1				5					10

<210> 252

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 252

Lys	Leu	Met	Glu	Thr	Leu	Met	Tyr	Ser	Arg
1				5					10

<210> 253

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 253

Val	Leu	His	Arg	Arg	Arg	Arg	Tyr	Lys	Lys
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<210> 254

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 254

Gln	Leu	Gly	Ile	Thr	Glu	Tyr	Leu	Arg	Lys
1				5					10

<210> 255

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 255

His	Ile	Phe	Ser	Gly	Val	Pro	Leu	Thr	Lys
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<210> 256
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 256
Ile Leu Asp Leu Gln Leu Glu Asp Asn Lys
1 5 10

<210> 257
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 257
Gly Val Pro Leu Thr Lys Val Asn Leu Lys
1 5 10

<210> 258
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 258
His Met Val Ser Pro Met Val His Val Tyr
1 5 10

<210> 259
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 259
Ile Met Phe Ile Thr Ile Val Phe Cys Ala
1 5 10

<210> 260
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 260

Thr Val Ile Glu Pro Ser Ala Phe Ser Lys
 1 5 10

<210> 261

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 261

Lys Leu Asn Arg Leu Lys Val Leu Ile Leu
 1 5 10

<210> 262

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 262

Lys Leu Ser Lys Gly Met Phe Leu Gly Leu
 1 5 10

<210> 263

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 263

Gln Thr Lys Asn Glu Tyr Phe Glu Leu Lys
 1 5 10

<210> 264

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 264

Val Leu Ile Leu Gly Leu Leu Ile Met Phe
 1 5 10

<210> 265

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 265

Val	Val	Cys	Asn	Ser	Pro	Pro	Phe	Phe	Lys
1				5					10

<210> 266

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 266

Leu	Leu	Ile	Met	Phe	Ile	Thr	Ile	Val	Phe
1				5					10

<210> 267

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 267

Val	Leu	Val	Glu	Gln	Thr	Lys	Asn	Glu	Tyr
1				5					10

<210> 268

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 268

Ser	Leu	Met	Lys	Ser	Asp	Leu	Val	Glu	Tyr
1				5					10

<210> 269

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 269

Gly	Thr	Phe	Asn	Pro	Met	Pro	Lys	Leu	Lys
1				5					10

<210> 270
 <211> 10
 <212> PRT
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<220>
 <223> Description of Artificial Sequence: Peptide motif

<400> 270
 Gln Leu Gln Pro Asp Met Glu Ala His Tyr
 1 5 10

<210> 271
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Peptide motif

<400> 271
 His Leu Gln Tyr Ser Met Tyr Gly His Lys
 1 5 10

<210> 272
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Peptide motif

<400> 272
 Leu Met Lys Ser Asp Leu Val Glu Tyr Phe
 1 5 10

<210> 273
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Peptide motif

<400> 273
 Gly Leu Thr Met Leu His Thr Asn Asp Phe
 1 5 10

<210> 274
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Peptide motif

<400> 274

Gly	Ala	Phe	Asn	Gly	Leu	Gly	Leu	Leu	Lys
1				5					10

<210> 275

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 275

Lys	Leu	Trp	Ile	His	Leu	Phe	Tyr	Ser	Ser
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<210> 276

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 276

Gly	Leu	Thr	Asn	Ala	Ile	Ser	Ile	His	Leu
1				5					10

<210> 277

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 277

Val	Leu	Glu	Glu	Gly	Ser	Phe	Met	Asn	Leu
1				5					10

<210> 278

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 278

Gly	Thr	Met	Leu	Ile	Asn	Cys	Glu	Ala	Lys
1				5					10

<210> 279

<211> 10

<212> PRT

<213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Peptide motif

<400> 279
 Gly Leu Leu Ile Met Phe Ile Thr Ile Val
 1 5 10

<210> 280
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Peptide motif

<400> 280
 Gly Ile Val Val Leu Val Leu His Arg Arg
 1 5 10

<210> 281
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Peptide motif

<400> 281
 Leu Val Gly Leu Gln Gln Trp Ile Gln Lys
 1 5 10

<210> 282
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Peptide motif

<400> 282
 Gly Leu Glu Asn Leu Glu Phe Leu Gln Ala
 1 5 10

<210> 283
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Peptide motif

<400> 283
 Gly Leu His Asn Leu Glu Tyr Leu Tyr Leu
 1 5 10

<210> 284
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 284
Gly Leu Gly Leu Leu Lys Gln Leu His Ile
1 5 10

<210> 285
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 285
His Leu Phe Tyr Ser Ser Leu Leu Ala Cys
1 5 10

<210> 286
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 286
Leu Leu Gln Val Leu Pro Pro His Ile Phe
1 5 10

<210> 287
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 287
Gln Leu Gln Thr Leu Pro Tyr Val Gly Phe
1 5 10

<210> 288
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 288

Ser Ile Asn Asp Ser Arg Met Ser Thr Lys
1 5 10

<210> 289

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 289

Ile Leu Lys Glu Asp Thr Phe His Gly Leu
1 5 10

<210> 290

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 290

Asp Leu Arg Pro Pro Pro Gln Asn Pro Arg
1 5 10

<210> 291

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 291

Glu Leu Lys Leu Met Glu Thr Leu Met Tyr
1 5 10

<210> 292

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 292

Cys Thr Ser Pro Gly His Leu Asp Lys Lys
1 5 10

<210> 293

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 293

Leu	Met	Tyr	Ser	Arg	Pro	Arg	Lys	Val	Leu
1				5					10

<210> 294

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 294

Pro	Leu	Thr	Gly	Ser	Asn	Met	Lys	Tyr	Lys
1				5					10

<210> 295

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 295

Ile	Leu	Ala	Gly	Asn	Ile	Ile	His	Ser	Leu
1				5					10

<210> 296

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 296

Ser	Leu	Glu	Ile	Leu	Lys	Glu	Asp	Thr	Phe
1				5					10

<210> 297

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 297

Glu	Leu	Gln	Gln	Leu	Gly	Ile	Thr	Glu	Tyr
1				5					10

<210> 298
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 298
Gly Leu Leu Lys Gln Leu His Ile Asn His
1 5 10

<210> 299
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 299
Lys Leu Ile Leu Ala Gly Asn Ile Ile His
1 5 10

<210> 300
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 300
Met Leu His Thr Asn Asp Phe Ser Gly Leu
1 5 10

<210> 301
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 301
Gln Gln Trp Ile Gln Lys Leu Ser Lys
1 5

<210> 302
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 302
Ser Thr Lys Thr Thr Ser Ile Leu Lys
1 5

<210> 303
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 303
Cys Thr Ser Pro Gly His Leu Asp Lys
1 5

<210> 304
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 304
Ser Leu Tyr Arg Asn Ile Leu Glu Lys
1 5

<210> 305
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 305
Leu Gln Tyr Ser Met Tyr Gly His Lys
1 5

<210> 306
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 306
Val Ile Glu Pro Ser Ala Phe Ser Lys
1 5

<210> 307
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 307
Phe Met Asn Leu Thr Arg Leu Gln Lys
1 5

<210> 308
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 308
Thr Leu Met Tyr Ser Arg Pro Arg Lys
1 5

<210> 309
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 309
Tyr Leu Asn Gly Asn His Leu Thr Lys
1 5

<210> 310
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 310
Gly Ile Val Val Leu Val Leu His Arg
1 5

<210> 311
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 311
Ile Val Val Leu Val Leu His Arg Arg
1 5

<210> 312
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 312
Leu Tyr Leu Glu Tyr Asn Ala Ile Lys
1 5

<210> 313
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 313
Val Cys Asn Ser Pro Pro Phe Phe Lys
1 5

<210> 314
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 314
Arg Pro Pro Pro Gln Asn Pro Arg Lys
1 5

<210> 315
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 315
Lys Thr Thr His His Thr Thr Glu Arg
1 5

<210> 316
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 316
Thr Met Leu Ile Asn Cys Glu Ala Lys
1 5

<210> 317
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 317
Leu Thr Gly Ser Asn Met Lys Tyr Lys
1 5

<210> 318
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 318
Ile Phe Ser Gly Val Pro Leu Thr Lys
1 5

<210> 319
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 319
Ala Phe Asn Gly Leu Gly Leu Leu Lys
1 5

<210> 320
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 320
Gly Asn Ile Ile His Ser Leu Met Lys
1 5

<210> 321
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 321
Gly Leu Leu Ile His Cys Gln Glu Arg
1 5

<210> 322
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 322
Val Pro Leu Thr Lys Val Asn Leu Lys
1 5

<210> 323
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 323
Ser Pro Leu Thr Gly Ser Asn Met Lys
1 5

<210> 324
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<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 324
Gly Asn His Leu Thr Lys Leu Ser Lys
1 5

<210> 325
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 325
Phe Gln Asp Ala Ser Ser Leu Tyr Arg
1 5

<210> 326
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 326
Ala Thr Ser Ser Ile Asn Asp Ser Arg
1 5

<210> 327
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 327
Tyr Pro Gly Ala His Glu Glu Leu Lys
1 5

<210> 328
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 328
Tyr Cys Pro Ile Pro Cys Asn Cys Lys
1 5

<210> 329
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 329
Gly Ser Ile Leu Ser Arg Leu Lys Lys
1 5

<210> 330
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 330

Leu Met Glu Thr Leu Met Tyr Ser Arg
1 5

<210> 331

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 331

Gln Leu Gly Ile Thr Glu Tyr Leu Arg
1 5

<210> 332

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 332

Ser Gln Thr Pro Val Leu Ser Ser Arg
1 5

<210> 333

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 333

Thr Phe Asn Pro Met Pro Lys Leu Lys
1 5

<210> 334

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 334

Gly His Leu Asp Lys Lys Glu Leu Lys
1 5

<210> 335

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 335

Arg Lys Val Leu Val Glu Gln Thr Lys
1 5

<210> 336

<211> 9

<212> PRT

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Peptide motif

<400> 336

Lys Val Leu Ser Pro Ser Gly Leu Leu
1 5

<210> 337

<211> 9

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<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 337

Lys Val Leu Tyr Leu Asn Asn Asn Leu
1 5

<210> 338

<211> 9

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<213> Artificial Sequence

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<223> Description of Artificial Sequence: Peptide motif

<400> 338

Lys Val Leu Ile Leu Asn Asp Asn Ala
1 5

<210> 339

<211> 9

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<213> Artificial Sequence

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<223> Description of Artificial Sequence: Peptide motif

<400> 339

Phe Phe Lys Gly Ser Ile Leu Ser Arg
1 5

<210> 340
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<400> 340
Asn Ile Glu Ser Leu Ser Asp Leu Arg
1 5

<210> 341
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<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 341
Leu Gly Ile Thr Glu Tyr Leu Arg Lys
1 5

<210> 342
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<223> Description of Artificial Sequence: Peptide motif

<400> 342
Val Gly Leu Gln Gln Trp Ile Gln Lys
1 5

<210> 343
<211> 9
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<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 343
Gly Thr Phe Asn Pro Met Pro Lys Leu
1 5

<210> 344
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 344

Gly Thr Met Leu Ile Asn Cys Glu Ala
1 5

<210> 345

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 345

Val Val Leu Val Leu His Arg Arg Arg
1 5

<210> 346

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 346

Ser Val Leu Ile Leu Gly Leu Leu Ile
1 5

<210> 347

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 347

Val Pro Leu Thr His Leu Asp Leu Arg
1 5

<210> 348

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 348

Lys Val Asn Leu Lys Thr Asn Gln Phe
1 5

<210> 349

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 349

Gly	Val	Pro	Leu	Thr	Lys	Val	Asn	Leu
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<210> 350

<211> 9

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<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 350

Asn	Glu	Lys	Glu	Gly	Ser	Asp	Ala	Lys
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<210> 351

<211> 10

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<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 351

Thr	Val	Ile	Glu	Pro	Ser	Ala	Phe	Ser	Lys
1				5					10

<210> 352

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<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 352

Val	Val	Cys	Asn	Ser	Pro	Pro	Phe	Phe	Lys
1				5					10

<210> 353

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 353

Gly	Thr	Met	Leu	Ile	Asn	Cys	Glu	Ala	Lys
1				5					10

<210> 354
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<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 354
Gly Val Pro Leu Thr Lys Val Asn Leu Lys
1 5 10

<210> 355
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide motif

<400> 355
Leu Val Gly Leu Gln Gln Trp Ile Gln Lys
1 5 10

<210> 356
<211> 10
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<211> 10
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<223> Description of Artificial Sequence: Peptide motif

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<211> 10

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<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Peptide motif

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Thr Thr Ser Ile Leu Lys Leu Pro Thr Lys
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<223> Description of Artificial Sequence: Peptide motif

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Cys	Thr	Ser	Pro	Gly	His	Leu	Asp	Lys	Lys
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<223> Description of Artificial Sequence: Peptide motif

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<400> 368
Gln Leu Gly Ile Thr Glu Tyr Leu Arg Lys
1 5 10

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<223> Description of Artificial Sequence: Peptide motif

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Val Leu His Arg Arg Arg Arg Tyr Lys Lys
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Glu Thr Leu Met Tyr Ser Arg Pro Arg Lys
1 5 10

<210> 371
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<223> Description of Artificial Sequence: Peptide motif

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Ala Pro Gly Leu Ile Pro Tyr Ile Thr Lys
1 5 10

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<223> Description of Artificial Sequence: Peptide motif

<400> 372

His	Tyr	Pro	Gly	Ala	His	Glu	Glu	Leu	Lys
1				5					10

<210> 373

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<213> Artificial Sequence

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<223> Description of Artificial Sequence: Peptide motif

<400> 373

His	Leu	Gln	Tyr	Ser	Met	Tyr	Gly	His	Lys
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<210> 374

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<223> Description of Artificial Sequence: Peptide motif

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<223> Description of Artificial Sequence: Peptide motif

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<223> Description of Artificial Sequence: Peptide motif

<400> 376

Leu	Cys	Thr	Ser	Pro	Gly	His	Leu	Asp	Lys
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<223> Description of Artificial Sequence: Peptide motif

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Ile	Leu	Asp	Leu	Gln	Leu	Glu	Asp	Asn	Lys
1				5					10

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Thr	Thr	Asn	Thr	Ala	Asp	Thr	Ile	Leu	Arg
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<210> 379

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<223> Description of Artificial Sequence: Peptide motif

<400> 379

Ser	Ile	Asn	Asp	Ser	Arg	Met	Ser	Thr	Lys
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Leu	Ile	Asn	Cys	Glu	Ala	Lys	Gly	Ile	Lys
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<400> 382
Phe Val Pro Leu Thr His Leu Asp Leu Arg
1 5 10

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<223> Description of Artificial Sequence: Peptide motif

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Gln Gln Leu Gly Ile Thr Glu Tyr Leu Arg
1 5 10

<210> 384
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<213> Artificial Sequence

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<223> Description of Artificial Sequence: Peptide motif

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Gly Ile Val Val Leu Val Leu His Arg Arg
1 5 10

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<400> 385
Leu Val Leu His Arg Arg Arg Arg Tyr Lys
1 5 10

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<400> 386

Ala Cys Asn Cys Asp Leu Leu Gln Leu Lys
1 5 10

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<400> 387

Lys Val Leu Ser Pro Ser Gly Leu Leu Ile
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<400> 388

Arg Asn Glu Lys Glu Gly Ser Asp Ala Lys
1 5 10

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<220>

<223> Description of Artificial Sequence: Peptide motif

<400> 389

Lys Gly Ser Ile Leu Ser Arg Leu Lys Lys
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<210> 390

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<223> Description of Artificial Sequence: Peptide motif

<400> 390

Lys Val Leu Tyr Leu Asn Asn Asn Leu Leu
1 5 10

<210> 391

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<223> Description of Artificial Sequence: Peptide motif

<400> 391

Lys	Val	Leu	Ile	Leu	Asn	Asp	Asn	Ala	Ile
1				5					10

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<223> Description of Artificial Sequence: Peptide motif

<400> 392

Ile	Val	Phe	Cys	Ala	Ala	Gly	Ile	Val	Val
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<223> Description of Artificial Sequence: Peptide motif

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<223> Description of Artificial Sequence: Peptide motif

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Ile	Asn	His	Asn	Ser	Leu	Glu	Ile	Leu	Lys
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<223> Description of Artificial Sequence: Peptide motif

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Ser	Ser	Leu	Tyr	Arg	Asn	Ile	Leu	Glu	Lys
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 <213> Artificial Sequence

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 <223> Description of Artificial Sequence: Peptide motif

<400> 396
 Ser Val Pro Pro Ser Arg Pro Phe Gln Leu
 1 5 10

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 Lys Thr Asn Gln Phe Thr His Leu Pro Val
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 <223> Description of Artificial Sequence: Peptide motif

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 Leu Glu Glu Glu Glu Glu Arg Asn Glu Lys
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 Ile Val Val Leu Val Leu His Arg Arg Arg
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 Glu Tyr Leu Arg Lys Asn Ile Ala Gln Leu
 1 5 10

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 Glu Tyr Leu Tyr Leu Glu Tyr Asn Ala Ile
 1 5 10

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 <223> Description of Artificial Sequence: Peptide motif

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<400> 405

Gly	Phe	Leu	Glu	His	Ile	Gly	Arg	Ile	Leu
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<400> 406

Leu	Tyr	Glu	Gln	His	Met	Val	Ser	Pro	Met
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<210> 407

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<210> 408

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<212> PRT

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<223> Description of Artificial Sequence: Peptide motif

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<223> Description of Artificial Sequence: Peptide motif

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Val	Phe	Cys	Ala	Ala	Gly	Ile	Val	Val	Leu
1				5					10

DEMANDES OU BREVETS VOLUMINEUX

**LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVETS
COMPREND PLUS D'UN TOME.**

CECI EST LE TOME __1__ DE __2__

NOTE: Pour les tomes additionels, veuillez contacter le Bureau Canadien des Brevets.

JUMBO APPLICATIONS / PATENTS

**THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE
THAN ONE VOLUME.**

THIS IS VOLUME __1__ OF __2__

NOTE: For additional volumes please contact the Canadian Patent Office.

Claims:

1. An in vitro method for measuring expression levels of 158P1D7 gene products in a sample, comprising:

determining an expression level of a 158P1D7 gene product expressed by cells in a suspected cancerous test sample and a normal sample, wherein the samples are taken from bladder or breast tissue;

comparing the expression levels of the test sample and the normal sample; and

identifying the presence of an elevated 158P1D7 gene product expression level in the test sample relative to the normal sample, wherein the 158P1D7 gene product comprises a 158P1D7 mRNA encoding the amino acid sequence of SEQ ID NO:657, or the amino acid sequence of SEQ ID NO:657.

2. The method of claim 1 whereby the presence of elevated 158P1D7 mRNA or protein expression in the test sample relative to the normal tissue sample provides an indication of the presence of a cancer.

3. Use of an antibody or antigen binding fragment thereof that specifically binds to the amino acid sequence of SEQ ID NO:657 for the preparation of a medicament to provide the antibody or antigen binding fragment thereof to a bladder cancer cell or a breast cancer cell that expresses the protein.

4. The use of claim 3, wherein the antibody or antigen binding fragment thereof is conjugated to a cytotoxic agent.

5. Use of an antibody or antigen binding fragment thereof that specifically binds to the amino acid sequence of SEQ ID NO:657 for killing a bladder cancer cell or a breast cancer cell that expresses the protein, wherein the antibody or antigen binding fragment thereof is conjugated to a cytotoxic agent.

6. The use of claims 3, 4 or 5, wherein the antibody or antigen binding fragment thereof is an Fab, F(ab')₂, Fv or sFv fragment.

7. The use of claims 3, 4 or 5, wherein the antibody or antigen binding fragment thereof is a monoclonal antibody.

8. The use of claims 3, 4 or 5, wherein the antibody or antigen binding fragment thereof is a human antibody.

9. An assay for detecting the presence of a 158P1D7 gene product, comprising:
contacting a suspected cancerous test sample and a normal sample with a binding agent that specifically binds to the 158P1D7 gene product, wherein the samples are taken from bladder or breast tissue; and

determining the presence of a binding agent-158P1D7 gene product complex in the sample, wherein the 158P1D7 gene product is a 158P1D7 mRNA encoding the amino acid sequence of SEQ ID NO:657 or the amino acid sequence of SEQ ID NO:657, wherein the binding agent is an antibody that binds specifically to said protein or a probe that binds specifically to the 158P1D7 mRNA and wherein detection of the binding agent - 158P1D7 gene product complex is an indication of a bladder or breast cancer cell in the test sample.

10. The assay of claim 9, further comprising:

(a) producing cDNA from the test and normal sample by reverse transcription using at least one primer;

(b) amplifying a 158P1D7 cDNA from a 158P1D7 mRNA so produced using 158P1D7 polynucleotides as sense and antisense primers; and

(c) detecting the presence of the amplified 158P1D7 cDNA, wherein the 158P1D7 cDNA encodes the amino acid sequence of SEQ ID NO:657 .

Figure 1. 158P1D7 SSH sequence (SEQ ID NO:655).

```
1 GATCTGATAA GCTTTCAATG TTGCGCTCCT GACAATGTAT TAGAAGTCCT GATGGGGATA
61 GGACTTTGCA GTTACAAGGA ATAGGGCAGA AAGGTCCTGG AAGTTGAGTG GATGGCTTTG
121 TAATATAAGG TATCAAACCT GGTGCTTTGG TGGGTAGTTT TAGAATGGAC GTGGTCTTAG
181 TTGACATGCG ACTATCATTT ATTGAAGATG TTGCTGCCAG ATGTAATGAT C
```

Figure 2. 158P1D7 cDNA clone TurboScript3PX and open reading frame (ORF)

```

1               M K L W I H L F Y S S L L
1 tcggatttcatcacatgacaacATGAAGCTGTGGATTCATCTCTTTTATTCATCTCTCCT
14  A C I S L H S Q T P V L S S R G S C D S
61 TGCCTGTATATCTTTACACTCCCAAACCTCCAGTGCTCTCATCCAGAGGCTCTTGTGATTC
34  L C N C E E K D G T M L I N C E A K G I
121 TCTTTGCAATTGTGAGGAAAAAGATGGCACAATGCTAATAAATTGTGAAGCAAAAGGTAT
54  K M V S E I S V P P S R P F Q L S L L N
181 CAAGATGGTATCTGAAATAAGTGTGCCACCATCACGACCTTTCCAACCTAAGCTTATTAAA
74  N G L T M L H T N D F S G L T N A I S I
241 TAACGGCTTGACGATGCTTCACACAAATGACTTTTCTGGGCTTACCAATGCTATTTCAAT
94  H L G F N N I A D I E I G A F N G L G L
301 ACACCTTGGATTTAACAATATTGCAGATATTGAGATAGGTGCATTTAATGGCCTTGGCCT
114 L K Q L H I N H N S L E I L K E D T F H
361 CCTGAAACAACCTTCATATCAATCACAATTCTTTAGAAATTCTTAAAGAGGATACTTTCCA
134 G L E N L E F L Q A D N N F I T V I E P
421 TGGACTGGAAAACCTGGAATTCCTGCAAGCAGATAACAATTTTATCACAGTGATTGAACC
154 S A F S K L N R L K V L I L N D N A I E
481 AAGTGCCTTTAGCAAGCTCAACAGACTCAAAGTGTTAATTTTAAATGACAATGCTATTGA
174 S L P P N I F R F V P L T H L D L R G N
541 GAGTCTTCCTCCAAACATCTTCCGATTTGTTCTTTAACCCATCTAGATCTTCGTGGAAA
194 Q L Q T L P Y V G F L E H I G R I L D L
601 TCAATTACAAACATTGCCTTATGTTGGTTTTCTCGAACACATTGGCCGAATATTGGATCT
214 Q L E D N K W A C N C D L L Q L K T W L
661 TCAGTTGGAGGACAACAAATGGGCCTGCAATTGTGACTTATTGCAGTTAAAAACTTGGTT
234 E N M P P Q S I I G D V V C N S P P F F
721 GGAGAACATGCCTCCACAGTCTATAATTGGTGATGTTGTCTGCAACAGCCCTCCATTTT
254 K G S I L S R L K K E S I C P T P P V Y
781 TAAAGGAAGTATACTCAGTAGACTAAAGAAGGAATCTATTTGCCCTACTCCACCAGTGTA
274 E E H E D P S G S L H L A A T S S I N D
841 TGAAGAACATGAGGATCCTTCAGGATCATTACATCTGGCAGCAACATCTTCAATAAATGA
294 S R M S T K T T S I L K L P T K A P G L
901 TAGTCGCATGTCAACTAAGACCACGTCCATTCTAAAACCTACCCACCAAAGCACCAGGTTT
314 I P Y I T K P S T Q L P G P Y C P I P C
961 GATACCTTATATTACAAAGCCATCCACTCAACTTCCAGGACCTTACTGCCCTATTCCTTG
334 N C K V L S P S G L L I H C Q E R N I E
1021 TAACTGCAAAGTCCTATCCCCATCAGGACTTCTAATACATTGTCAGGAGCGCAACATTGA
354 S L S D L R P P P Q N P R K L I L A G N
1081 AAGCTTATCAGATCTGAGACCTCCTCCGCAAAATCCTAGAAAGCTCATTCTAGCGGGAAA
374 I I H S L M K S D L V E Y F T L E M L H
1141 TATTATTCACAGTTTAATGAAGTCTGATCTAGTGGAATATTTCACTTTGGAAATGCTTCA
394 L G N N R I E V L E E G S F M N L T R L
1201 CTTGGGAAACAATCGTATTGAAGTTCTTGAAGAAGGATCGTTTATGAACCTAACGAGATT
414 Q K L Y L N G N H L T K L S K G M F L G
1261 ACAAAAACCTCTATCTAAATGGTAACCACCTGACCAAATTAAGTAAAGGCATGTTCTTGG
434 L H N L E Y L Y L E Y N A I K E I L P G
1321 TCTCCATAATCTTGAATACTTATATCTTGAATACAATGCCATTAAGGAAATACTGCCAGG
454 T F N P M P K L K V L Y L N N N L L Q V
1381 AACCTTTAATCCAATGCCTAAACTTAAAGTCCTGTATTTAAATAACAACCTCCTCCAAGT
474 L P P H I F S G V P L T K V N L K T N Q
1441 TTTACCACCACATATTTTTTTCAGGGGTTCTCTAACTAAGGTAAATCTTAAACAAACCA
494 F T H L P V S N I L D D L D L L T Q I D
1501 GTTTACCCATCTACCTGTAAGTAATATTTTGGATGATCTTGATTTACTAACCCAGATTGA
514 L E D N P W D C S C D L V G L Q Q W I Q

```

1561 CCTTGAGGATAACCCCTGGGACTGCTCCTGTGACCTGGTTGGACTGCAGCAATGGATACA
534 K L S K N T V T D D I L C T S P G H L D
1621 AAAGTTAAGCAAGAACACAGTGACAGATGACATCCTCTGCACTTCCCCCGGGCATCTCGA
554 K K E L K A L N S E I L C P G L V N N P
1681 CAAAAAGGAATTGAAAGCCCTAAATAGTGAAATTCTCTGTCCAGGTTTAGTAAATAACCC
574 S M P T Q T S Y L M V T T P A T T T N T
1741 ATCCATGCCAACACAGACTAGTTACCTTATGGTCACCACTCCTGCAACAACAACAATAAC
594 A D T I L R S L T D A V P L S V L I L G
1801 GGCTGATACTATTTTACGATCTCTTACGGACGCTGTGCCACTGTCTGTTCTAATATTGGG
614 L L I M F I T I V F C A A G I V V L V L
1861 ACTTCTGATTATGTTTCATCACTATTGTTTTCTGTGCTGCAGGGATAGTGTTCTTGTCT
634 H R R R R Y K K K Q V D E Q M R D N S P
1921 TCACCGCAGGAGAAGATACAAAAAGAAACAAGTAGATGAGCAAATGAGAGACAACAGTCC
654 V H L Q Y S M Y G H K T T H H T T E R P
1981 TGTGCATCTTCAGTACAGCATGTATGGCCATAAAACCACTCATCACTACTGAAAGACC
674 S A S L Y E Q H M V S P M V H V Y R S P
2041 CTCTGCCTCACTCTATGAACAGCACATGGTGAGCCCCATGGTTCATGTCTATAGAAGTCC
694 S F G P K H L E E E E E R N E K E G S D
2101 ATCCTTTGGTCCAAAGCATCTGGAAGAGGAAGAAGAGAGGAATGAGAAAGAAGGAAGTGA
714 A K H L Q R S L L E Q E N H S P L T G S
2161 TGCAAAACATCTCCAAAGAAGTCTTTTGGAAACAGGAAAATCATTCACTCAACAGGGTC
734 N M K Y K T T N Q S T E F L S F Q D A S
2221 AAATATGAAATACAAAACCACGAACCAATCAACAGAATTTTATCCTTCCAAGATGCCAG
754 S L Y R N I L E K E R E L Q Q L G I T E
2281 CTCATTGTACAGAAACATTTTAGAAAAAGAAAGGGAACCTCAGCAACTGGGAATCACAGA
774 Y L R K N I A Q L Q P D M E A H Y P G A
2341 ATACCTAAGGAAAAACATTGCTCAGCTCCAGCCTGATATGGAGGCACATTATCCTGGAGC
794 H E E L K L M E T L M Y S R P R K V L V
2401 CCACGAAGAGCTGAAGTTAATGGAAACATTAATGTACTCACGTCCAAGGAAGGTATTAGT
814 E Q T K N E Y F E L K A N L H A E P D Y
2461 GGAACAGACAAAAAATGAGTATTTTGAACCTTAAAGCTAATTTACATGCTGAACCTGACTA
834 L E V L E Q Q T * (SEQ ID NO:657)
2521 TTTAGAAGTCCTGGAGCAGCAAACATAGatggaga (SEQ ID NO:656)

Figure 3. 158P1D7 amino acid sequence.

```
1 MKLWIHLFYS SLLACISLHS QTPVLSSRGS CDSLNCCEEK DGTMLINCEA KGIKMOVSEIS
61 VPPSRPFQLS LLNNGLTMLH TNDFSGLTNA ISIH LGFN NI ADIEIGAFNG LGLLKQLHIN
121 HNSLEILKED TFHGLENL EF LQADNNFITV IEPSAFSKLN RLKVLILNDN AIESLPPNIF
181 RFVPLTHLDL RGNQLQTL PY VGFLEHIGRI LDLQLEDNKW ACNCDLLQLK TWLENMPPQS
241 IIGDVVCNSP PFFKGSILSR LKKE SICPTP PVYEEHEDPS GSLHLAATSS INDSRMSTKT
301 TSILKLPTKA PGLIPYITKP STQLPGPYCP IPCNCKVLSP SGLLIHCQER NIESLSDLRP
361 PPQNPRKLIL AGNIIHSLMK SDLVEYFTLE MLHLGNNRIE VLEEGSFMNL TRLOKLYLNG
421 NHLTKLSKGM FLGLHNLEYL YLEYNAIKEI LPGT FNPMPK LKVL YLNNNL LQVLPPHIFS
481 GVPLTKVNLK TNQFTHLPVS NILDDL DLT QIDLEDNPWD CSCDLVGLQQ WIQKLSKNTV
541 TDDILCTSPG HLDKKELKAL NSEILCPGLV NNPSMPTQTS YLMVTTPATT TNTADTILRS
601 LTDAVPLSVL ILGLLIMFIT IVFCAAGIVV LVLHRRRRYK KKQVDEQMRD NSPVHLQYSM
661 YGHKTTHHTT ERPSASLYEQ HMVSPMVH VY RSPSF GPKHL EEEEEERNEKE GSDAKHLQRS
721 LLEQENHSPL TGSNMKYKTT NQSTEF LSFQ DASSLYRNIL EKERELQQLG ITEYLRKNIA
781 QLQPDMEAHY PGAHEELKLM ETLMYSRPRK VLVEQTKNEY FELKANLHAE PDYLEVLEQQ
841 T* (SEQ ID NO:657)
```

Figure 4. 158P1D7 amino acid BLAST homology to hypothetical protein FLJ22774.

Identities = 798/798 (100%)

Query: 44 MLINCEAKGIKMVSEISVPPSRPFQLSLLNNGLTMLHTNDFSGLTNAISIH LGFN NIADI 103
 MLINCEAKGIKMVSEISVPPSRPFQLSLLNNGLTMLHTNDFSGLTNAISIH LGFN NIADI
 Sbjct: 1 MLINCEAKGIKMVSEISVPPSRPFQLSLLNNGLTMLHTNDFSGLTNAISIH LGFN NIADI 60

Query: 104 EIGAFNGLGLLKQLHINHNSLEILKEDTFHGLENLEFLQADNNFITVIEPSAFSKLNRLK 163
 EIGAFNGLGLLKQLHINHNSLEILKEDTFHGLENLEFLQADNNFITVIEPSAFSKLNRLK
 Sbjct: 61 EIGAFNGLGLLKQLHINHNSLEILKEDTFHGLENLEFLQADNNFITVIEPSAFSKLNRLK 120

Query: 164 VLILNDNAIESLPPNIFRFVPLTHLDLRGNQLQTLPHYVGFLHIGRILDQLQLEDNKWACN 223
 VLILNDNAIESLPPNIFRFVPLTHLDLRGNQLQTLPHYVGFLHIGRILDQLQLEDNKWACN
 Sbjct: 121 VLILNDNAIESLPPNIFRFVPLTHLDLRGNQLQTLPHYVGFLHIGRILDQLQLEDNKWACN 180

Query: 224 CDLLQLKTWLENMPPQSIIGDVVCNSPPFFKGSILSRLKKESICPTPPVYEEHEDPSGSL 283
 CDLLQLKTWLENMPPQSIIGDVVCNSPPFFKGSILSRLKKESICPTPPVYEEHEDPSGSL
 Sbjct: 181 CDLLQLKTWLENMPPQSIIGDVVCNSPPFFKGSILSRLKKESICPTPPVYEEHEDPSGSL 240

Query: 284 HLAATSSINDSRMSTKTT SILKLPTKAPGLIPYITKPSTQLPGPYCPIPCNCKVLSPSGL 343
 HLAATSSINDSRMSTKTT SILKLPTKAPGLIPYITKPSTQLPGPYCPIPCNCKVLSPSGL
 Sbjct: 241 HLAATSSINDSRMSTKTT SILKLPTKAPGLIPYITKPSTQLPGPYCPIPCNCKVLSPSGL 300

Query: 344 LIHCQERNIESLSDLRPPPQNPRKLILAGNIIHSLMKSDLVEYFTLEMLHLGNNRIEVLE 403
 LIHCQERNIESLSDLRPPPQNPRKLILAGNIIHSLMKSDLVEYFTLEMLHLGNNRIEVLE
 Sbjct: 301 LIHCQERNIESLSDLRPPPQNPRKLILAGNIIHSLMKSDLVEYFTLEMLHLGNNRIEVLE 360

Query: 404 EGSFMNLTRLQKLYLNGNHLTKLSKGMFLGLHNLEYLYLEYNAIKEILPGTFNPMPKLKV 463
 EGSFMNLTRLQKLYLNGNHLTKLSKGMFLGLHNLEYLYLEYNAIKEILPGTFNPMPKLKV
 Sbjct: 361 EGSFMNLTRLQKLYLNGNHLTKLSKGMFLGLHNLEYLYLEYNAIKEILPGTFNPMPKLKV 420

Query: 464 LYLNNNLLQVLPPHIFSGVPLTKVNLKTNQFTHLPVSNILDDLDLLTQIDLEDNPWDCSC 523
 LYLNNNLLQVLPPHIFSGVPLTKVNLKTNQFTHLPVSNILDDLDLLTQIDLEDNPWDCSC
 Sbjct: 421 LYLNNNLLQVLPPHIFSGVPLTKVNLKTNQFTHLPVSNILDDLDLLTQIDLEDNPWDCSC 480

Query: 524 DLVGLQQW IQKLSKNTVTDDILCTSPGHLDDKKELKALNSEILCPGLVNNPSMPTQTSYLM 583
 DLVGLQQW IQKLSKNTVTDDILCTSPGHLDDKKELKALNSEILCPGLVNNPSMPTQTSYLM
 Sbjct: 481 DLVGLQQW IQKLSKNTVTDDILCTSPGHLDDKKELKALNSEILCPGLVNNPSMPTQTSYLM 540

Query: 584 VTTPATTTNTADTILRSLTDAVPLSVLILGLLIMFITIVFCAAGIVVLVLHRRRRYKKKQ 643
 VTTPATTTNTADTILRSLTDAVPLSVLILGLLIMFITIVFCAAGIVVLVLHRRRRYKKKQ
 Sbjct: 541 VTTPATTTNTADTILRSLTDAVPLSVLILGLLIMFITIVFCAAGIVVLVLHRRRRYKKKQ 600

Query: 644 VDEQMRDN SPVHLQYS MYGHKTTHHTTERPSASLYEQH MVSPMVHVYRSPSFGPKHLEEE 703
 VDEQMRDN SPVHLQYS MYGHKTTHHTTERPSASLYEQH MVSPMVHVYRSPSFGPKHLEEE
 Sbjct: 601 VDEQMRDN SPVHLQYS MYGHKTTHHTTERPSASLYEQH MVSPMVHVYRSPSFGPKHLEEE 660

Query: 704 EERNEKEGSDAKHLQRSLL EQENHSPLTGSNMKYKTTNQSTEFLSFQDASSLYRNILEKE 763
 EERNEKEGSDAKHLQRSLL EQENHSPLTGSNMKYKTTNQSTEFLSFQDASSLYRNILEKE
 Sbjct: 661 EERNEKEGSDAKHLQRSLL EQENHSPLTGSNMKYKTTNQSTEFLSFQDASSLYRNILEKE 720

Query: 764 RELQQLGITEYLRKNIAQLQPDMEAHYPGAHEELKLMETLMYSRPRKVLVEQTKNEYFEL 823
 RELQQLGITEYLRKNIAQLQPDMEAHYPGAHEELKLMETLMYSRPRKVLVEQTKNEYFEL
 Sbjct: 721 RELQQLGITEYLRKNIAQLQPDMEAHYPGAHEELKLMETLMYSRPRKVLVEQTKNEYFEL 780

WO 02/16593

PCT/US01/26276

6/20

Query: 824 KANLHAEPDYLEVLEQQT 841
KANLHAEPDYLEVLEQQT

Sbjct: 781 KANLHAEPDYLEVLEQQT 798 (SEQ ID NO:658)

Figure 5a: Alignment of 158P1D7 with human FLJ22774, CLONE KAIA1575. [Homo sapiens]

Identities = 405/415 (97%), Positives = 405/415 (97%)

```

158P1D7: 44 MLINCEAKGIKVMSEISVPPSRPFQLSLLNGLTMLHTNDFSGLTNAISIH LGFN NIADI 103
              MLINCEAKGIKVMSEISVPPSRPFQLSLLNGLTMLHTNDFSGLTNAISIH LGFN NIADI
Sbjct: 1      MLINCEAKGIKVMSEISVPPSRPFQLSLLNGLTMLHTNDFSGLTNAISIH LGFN NIADI 60

158P1D7:104 EIGAFNGLGLLKQLHINHNSLEILKEDTFHGLENLEFLQADNNFITVIEPSAFSKLNRLK 163
              EIGAFNGLGLLKQLHINHNSLEILKEDTFHGLENLEFLQADNNFITVIEPSAFSKLNRLK
Sbjct: 61     EIGAFNGLGLLKQLHINHNSLEILKEDTFHGLENLEFLQADNNFITVIEPSAFSKLNRLK 120

158P1D7:164 VLILNDNAIESLPPNIFRFVPLTHLDLRGNQLQTLPLYVGFL EHGRI LD LQLEDNKWACN 223
              VLILNDNAIESLPPNIFRFVPLTHLDLRGNQLQTLPLYVGFL EHGRI LD LQLEDNKWACN
Sbjct: 121    VLILNDNAIESLPPNIFRFVPLTHLDLRGNQLQTLPLYVGFL EHGRI LD LQLEDNKWACN 180

158P1D7:224 CDLLQLKTWLENMPPQSIIGDVVCNSPPFFKGSILSRLKKESICPTPPVYEEHEDPSGSL 283
              CDLLQLKTWLENMPPQSIIGDVVCNSPPFFKGSILSRLKKESICPTPPVYEEHEDPSGSL
Sbjct: 181    CDLLQLKTWLENMPPQSIIGDVVCNSPPFFKGSILSRLKKESICPTPPVYEEHEDPSGSL 240

158P1D7:284 HLAATSSINDSRMSTKTTTILKLP TKAPGLIPYITKPSTQLPGPYCPIPCNCKVLSPSGL 343
              HLAATSSINDSRMSTKTTTILKLP TKAPGLIPYITKPSTQLPGPYCPIPCNCKVLSPSGL
Sbjct: 241    HLAATSSINDSRMSTKTTTILKLP TKAPGLIPYITKPSTQLPGPYCPIPCNCKVLSPSGL 300

158P1D7:344 LIHCQERNIESLSDLRPPPQNPRKLILAGNIIHSLMKSDLVEYFTLEMLHLGNNRIEVLE 403
              LIHCQERNIESLSDLRPPPQNPRKLILAGNIIHSLMKSDLVEYFTLEMLHLGNNRIEVLE
Sbjct: 301    LIHCQERNIESLSDLRPPPQNPRKLILAGNIIHSLMKSDLVEYFTLEMLHLGNNRIEVLE 360

158P1D7:404 EGSFMNLTRLQKLYLNGNHLTKLSKGMFLGLHXXXXXXXXXXXXXAIKEILPGTFNPM 458
              EGSFMNLTRLQKLYLNGNHLTKLSKGMFLGLH                AIKEILPGTFNPM
Sbjct: 361    EGSFMNLTRLQKLYLNGNHLTKLSKGMFLGLHNLEYLYLEYNAIKEILPGTFNPM 415 (SEQ ID
NO:659)

```

Figure 5b: Alignment of 158P1D7 protein with a human protein similar to IGFALS

Identities = 316/864 (36%), Positives = 459/864 (52%)

```

158P1D7:1  MKLWIHLFYSSLLACISLHSQTPVLSSRGSCDSLNCCEKDGTMLINCEAKGIKVMSEIS 60
              M LW+ L S+L++ + S V          ++C+C  + + +NCE  +   +++
Sbjct: 17   MFLWLFLILSALISSTNADSDISV-----EICNVCSVCVSENVLYVNCEKVS VYRPNQLK 71

158P1D7:61  VPPSRPFQLSLLNGLTMLHTNDFSGLTNAISIH LGFN NIADIEIGAFNGLGLLKQLHIN 120
              P S  + L+  NN L +L+ N F  ++A+S+HLG N + +IE GAF GL  LKQLH+N
Sbjct: 72   PPWSNFYHLNFQNNFLNILYPNTFLNFSHAVSLHLGNNKLQNI EGGAFLGLSALKQLHLN 131

158P1D7:121 HNSLEILKEDTFHGLENLEFLQADNNFITVIEPSAFSKLNRLKVLILNDNAIESLPPNIF 180
              +N L+IL+ DTF G+ENLE+LQAD N I  IE  AF+KL++LKV LILNDN I  LP NIF
Sbjct: 132  NNELKILRADTFLGIENLEYLQADYNLIK YIERGAFNKLHKLKV LILNDNLISFLPDNIF 191

158P1D7:181 RFVPLTHLDLRGNQLQTLPLYVGFL EHGRI LD LQLEDNKWACNCDLLQLKTWLENMPPQS 240
              RF  LTHLD+RGN++Q LPY+G LEHIGR+++LQLEDN W C+CDLL LK WLENMP
Sbjct: 192  RFASLTHLDIRGNRIQKLPYIGVLEHIGRVVELQLEDNPWNCSCDLLPLKAWLENMPYNI 251

158P1D7:241 IIGDVVCNSPPFFKGSILSRLKKESICP-----TPPVYEEHEDPSGSLHLAATS 289
              IG+ +C +P   G +L   K+ +CP                PP  E+  + + H   TS
Sbjct: 252  YIGEAI CETPSDLYGRLLKETNKQELCPMGTGSDFDVRILPPSQLENGYTTPNGHTTQTS 311

158P1D7:290 SINDSRMSTKTTTILKLP TKAPGLI-----PYITKPSTQLPG-PYCPIPCNCKV- 337
              KTT+   P+K  G++                I   T++P   CP PC CK
Sbjct: 312  LHRLVTKPPKTTN----PSKISGIVAGKALSNRNLSQIVSYQTRVPPLTPCPAPCFCKTH 367

```

158P1D7:338 LSPSGLLIHCQERNIESLSDLRPPPQNPRKLILAGNIIHSLMKSDLVEYFTLEMLHLGNN 397
 S GL ++CQE+NI+S+S+L P P N +KL + GN I + SD ++ L++LHLG+N
 Sbjct: 368 PSDLGLSVNCQEKNIQSMSELIPKPLNAKKLHVNGNSIKDVDVSDFTDFEGLDLLHLGNSN 427

158P1D7:398 RIEVLEEGSFMNLTRLQKLYLNGNHLTKLSKGMFLGLHXXXXXXXXXXXXXAIKEILPGTFNP 457
 +I V++ F NLT L++LYLNGN + +L +F GLH IKEI GTF+
 Sbjct: 428 QITVIKGDVFNLTNLRLRLYLNGNQIERLYPEIFSGLHNLQYLYLEYNLIKEISAGTFDS 487

158P1D7:458 MXXXXXXXXXXXXXXXXXXXXHIFSGVPLTKVNLKTNQFTHLPVSNIXXXXXXXXXXXXXXXXXN 517
 M +IFSG PL ++NL+ N+F +LPVS + N
 Sbjct: 488 MPNLQLLYLNNNLLKSLPVYIFSGAPLARLNLRRNNKFMVLPVSGVLDQLQSLTQIDLEGN 547

158P1D7:518 PWDCSCDLVGLQQWIKLSKNTVTDDILCTSPGHLDKELKALNSEILCPGLVNNPSMPT 577
 PWDC+CDLV L+ W++KLS V ++ C +P ELK+L +EILCP L+N PS P
 Sbjct: 548 PWDCTCDLVALKLWVEKLSDGIVVKELKCETPVQFANIELKSLKNEILCPKLLNKPSAP- 606

158P1D7:578 QTSYLMVXXXXXXXXXXXXXILRSLTDAVPLSVLILGLLIMFITIVFCAAGIVVLVLR 637
 + I VPLS+LIL +L++ I VF A ++V VL R +
 Sbjct: 607 ---FTSPAPAITFTTPLGPIRSPPGGPVPLSILILSVLILTVFVAFCLLVFVLRNK 663

158P1D7:638 RYKKKQVDEQMRDNSPVHLQYSMYGHKTTHHTTERPSASLYEQHMVSPMVHVYRSPSFGP 697
 + K D + LQ + HK T + E + + +S + G
 Sbjct: 664 KPTVKHEGLGNPDCGSMQLQLRKHDHK-----TNKKDGLSTEAFFIPQTIEQMSKSHTCGL 718

158P1D7:698 KHLXXXXXXXXXXGSDAKHLQRSLLLEQENHSPLTGSNMKYKTTNQSTEFLSFQDASSLYR 757
 K G K + R++ ++E + + T ++ E +D++ +
 Sbjct: 719 KESETGFMFSDPPGQ--KVMNRNVADKEKDLLHVDTRKRLSTIDELDELFPDRDSNVFIQ 776

158P1D7:758 NILEKERELQQLGITEYLRKNIAQLQPDMEAHYPGAHEELKLMETLMYSRPRKVLVEQTK 817
 N LE ++E +G++ + E YP + K ++L+ K++VEQ K
 Sbjct: 777 NFLESKKEYNSIGVSGF-----EIRYPEKQPDKKSKKSLIGGNHSHKIVVEQRK 824

158P1D7:818 NEYFELKANLHAEPDYLEVLEQQT 841
 +EYFELKA L + PDYL+VLE+QT
 Sbjct: 825 SEYFELKAKLQSSPDYLQVLEEQT 848 (SEQ ID NO:660)

Figure 6. Expression of 158P1D7 by RT-PCR

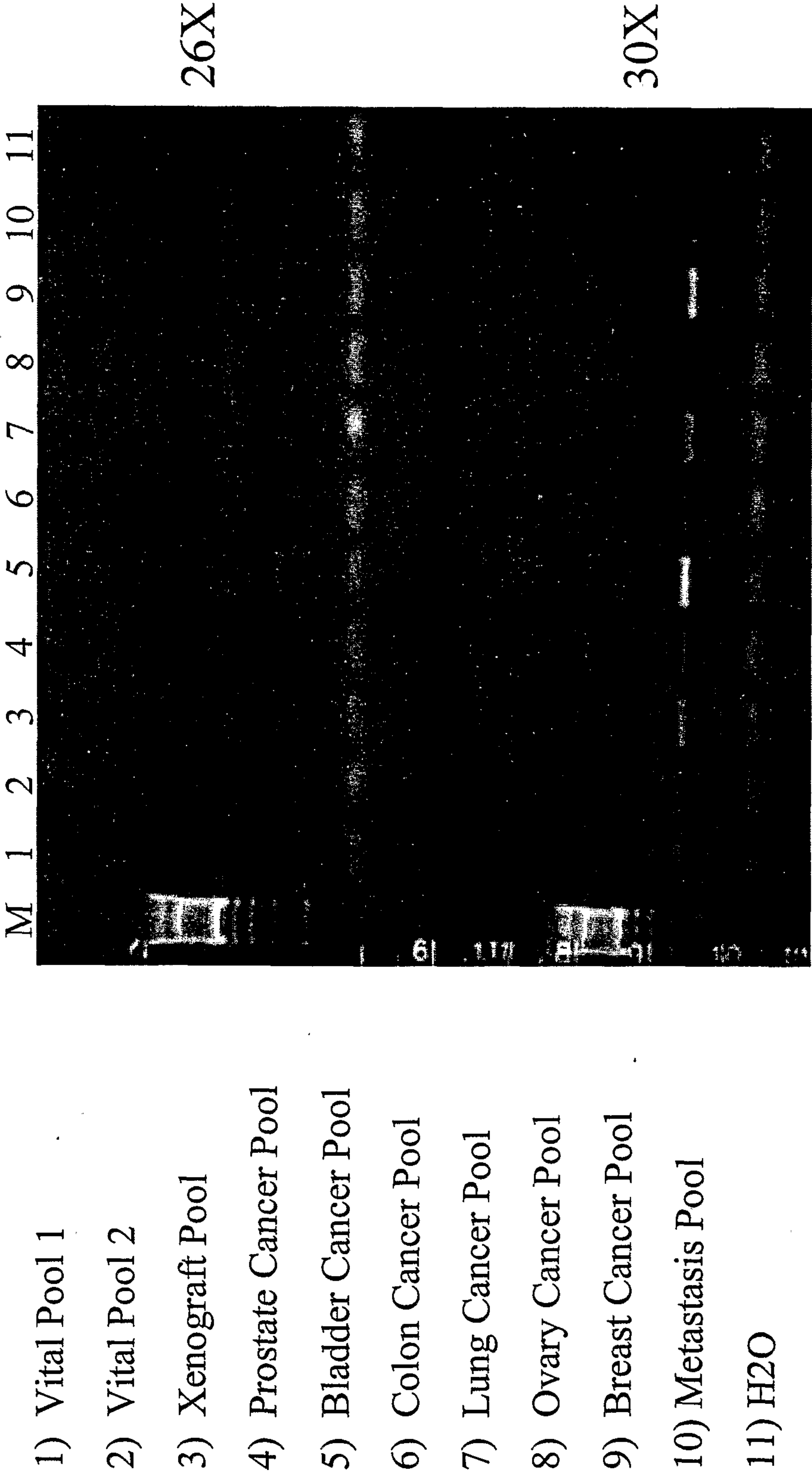


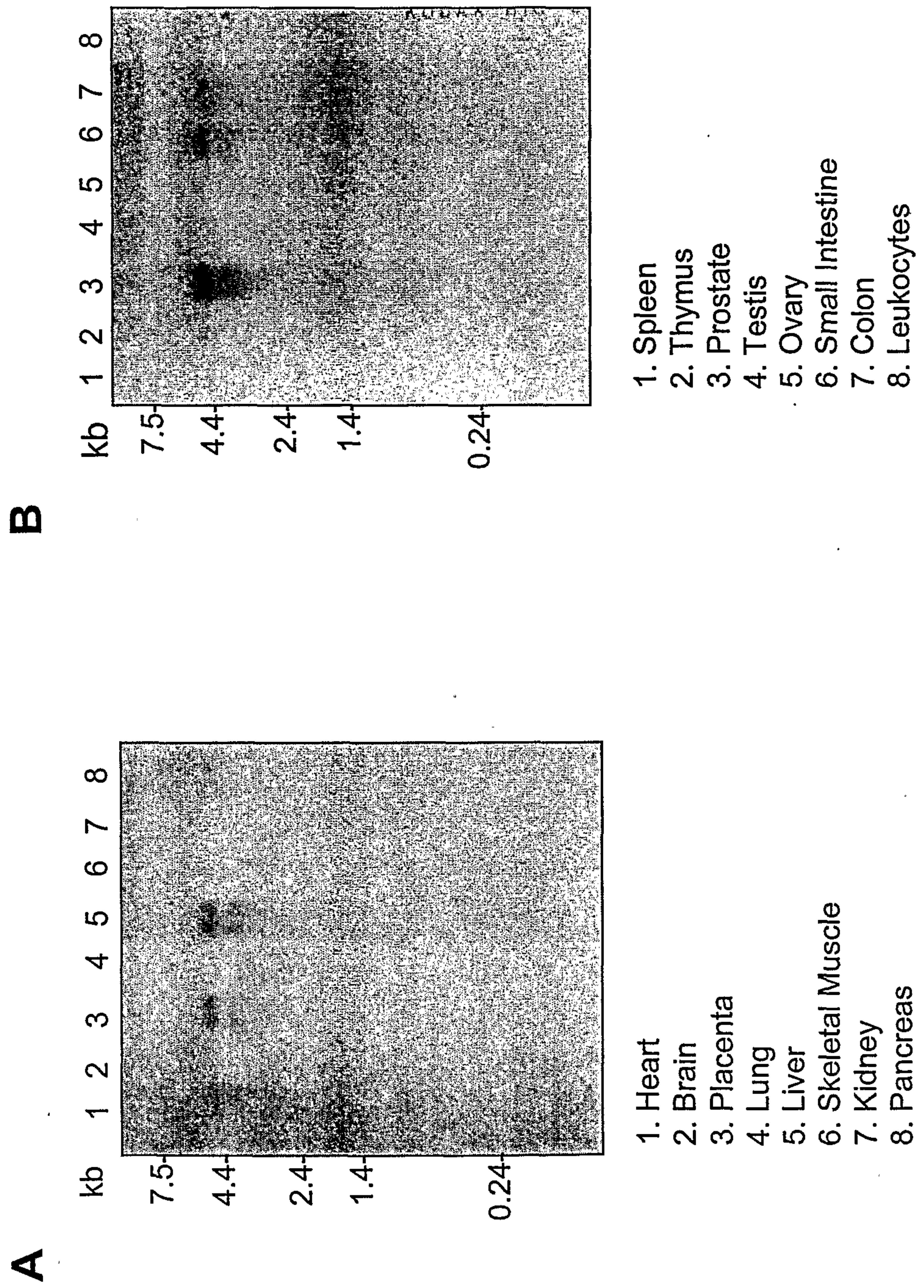
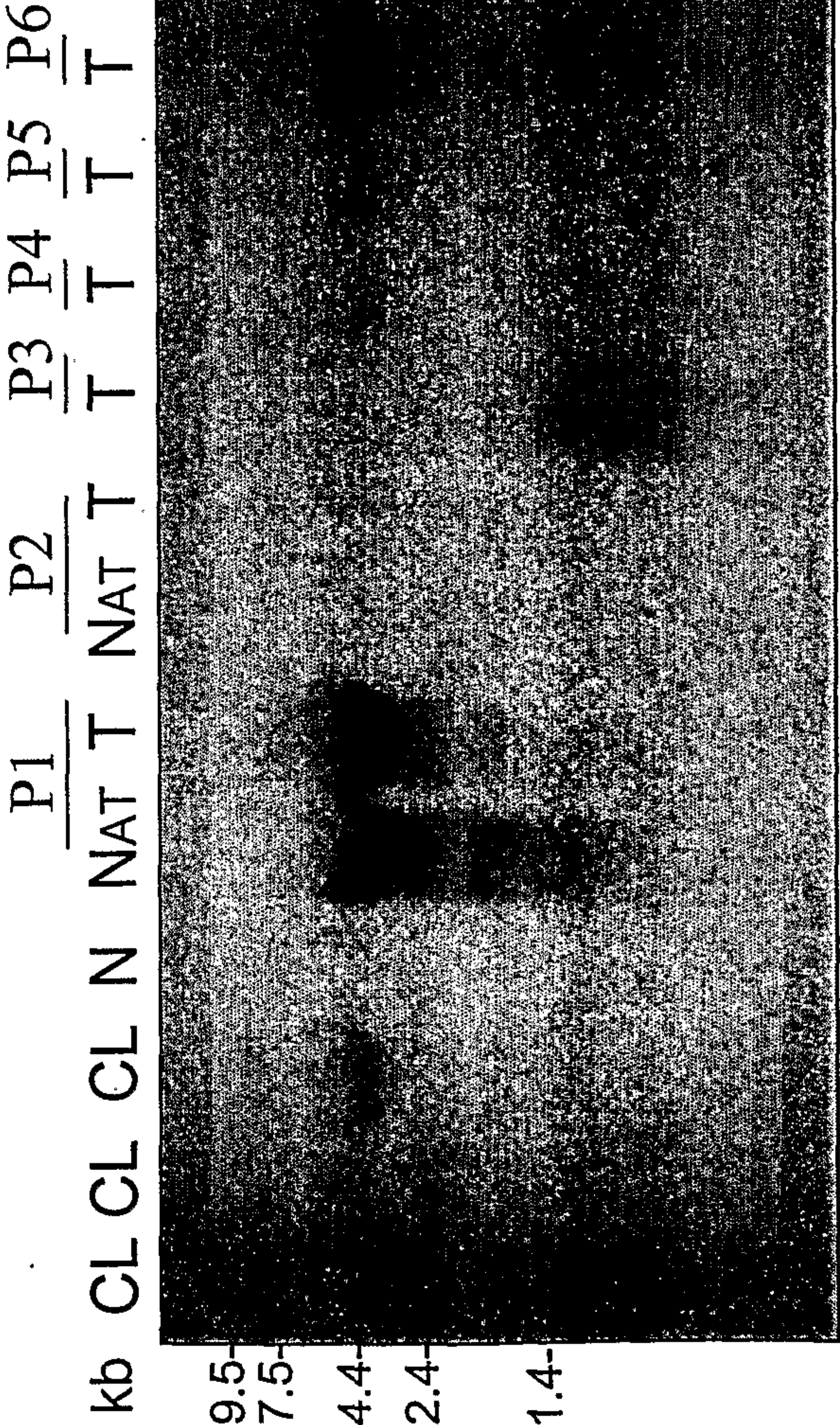
Figure 7. Expression of 158P1D7 in Normal Tissues

Figure 8A. Expression of 158P1D7 in Bladder Cancer Patient Specimens



P1 - Transitional, grade 4
P2 - Squamous inv.
P3 - Transitional, grade 3
P4 - Papillary non -inv, grade 1/3
P5 - Papillary, grade 3/3
P6 - Transitional, grade 3/2

CL = Cell lines (listed in order): UM-UC-3, J82, SCaBER
P = Patient
N = Normal Bladder
NAT = Normal adjacent tissue
T = Tumor

Figure 8B. Expression of 158P1D7 in Bladder Cancer Patient Specimens

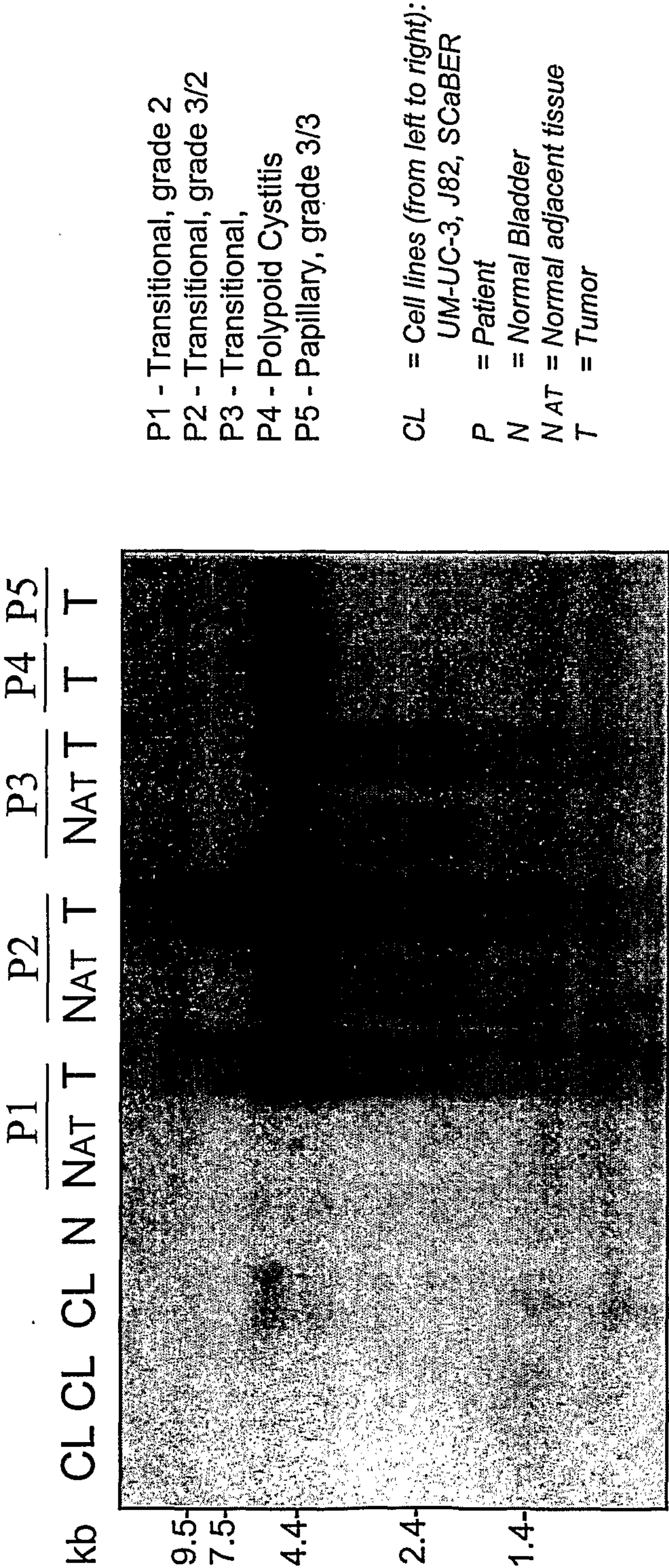


Figure 9. Expression of 158P1D7 in Lung Cancer Patient Specimens

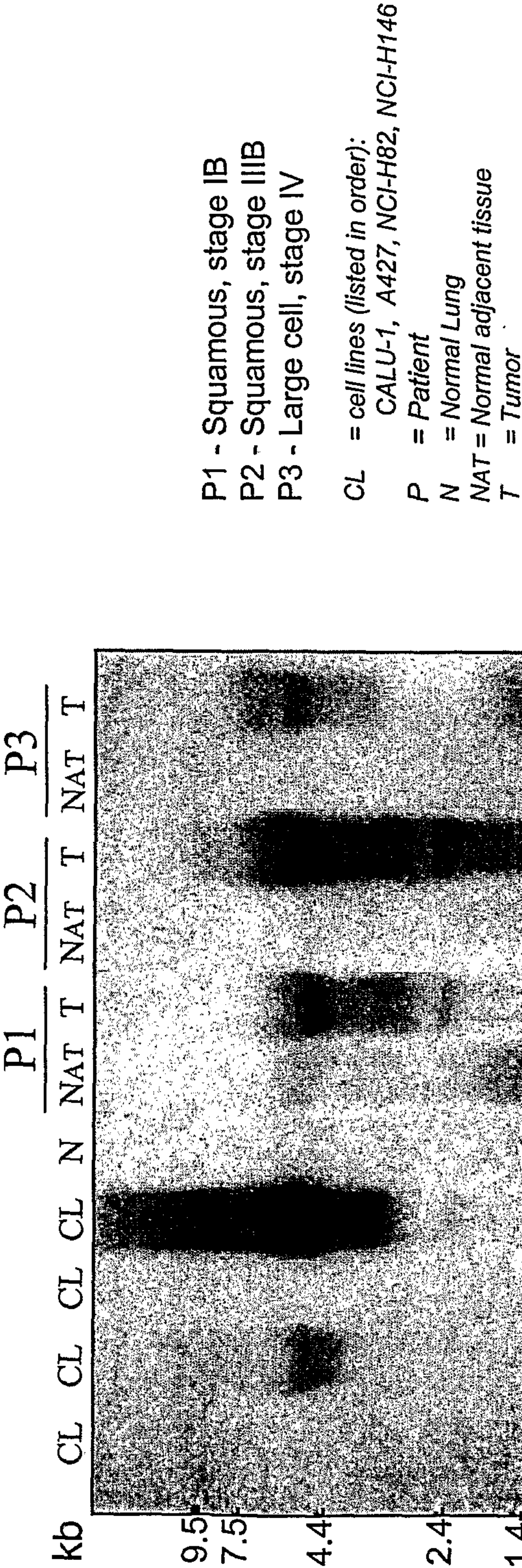


Figure 10. Expression of 158P1D7 in Breast Cancer Patient Specimens

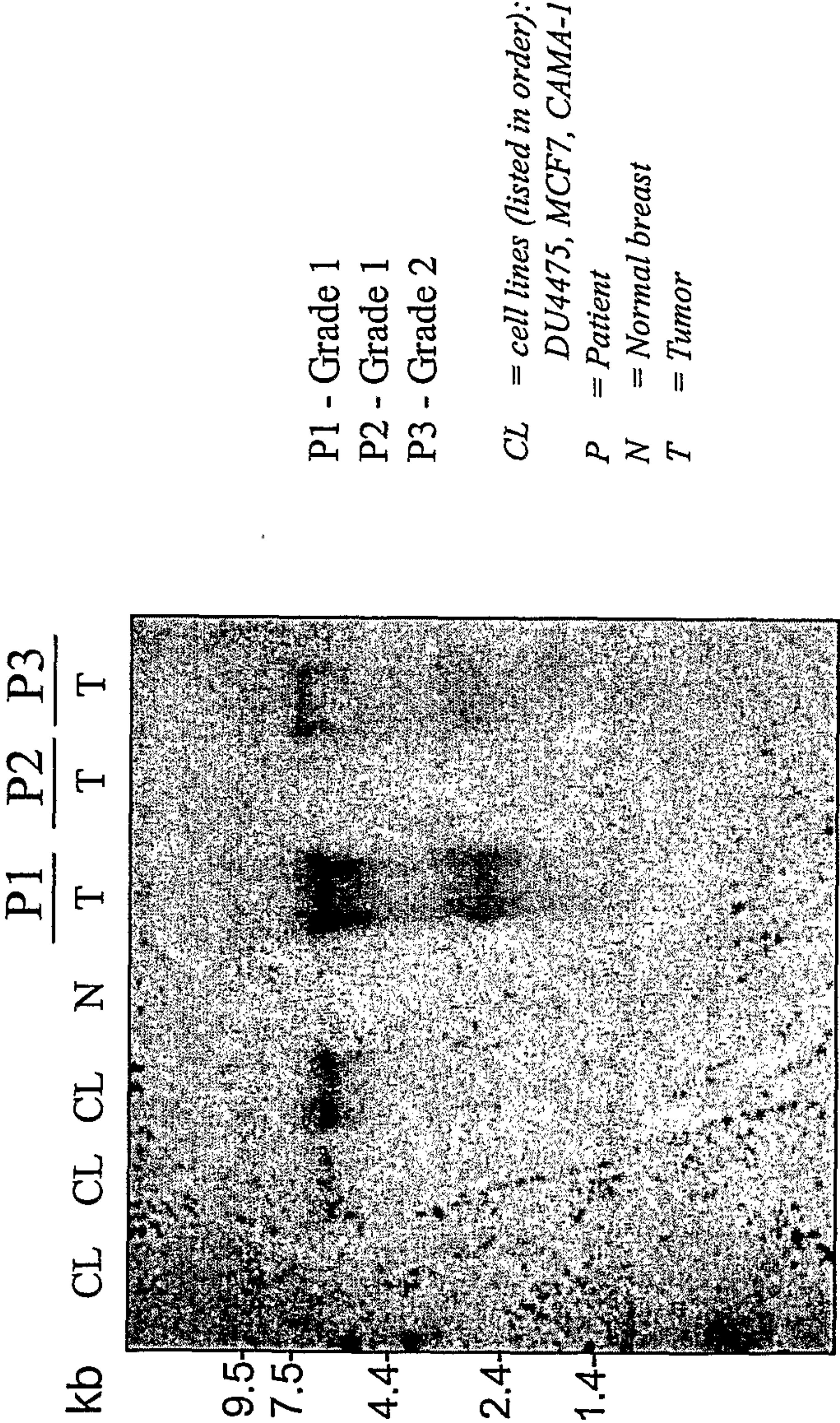


Figure 11. 158P1D7 Hydrophilicity profile
(Hopp T.P., Woods K.R., 1981. Proc. Natl. Acad. Sci. U.S.A. 78:3824-3828)

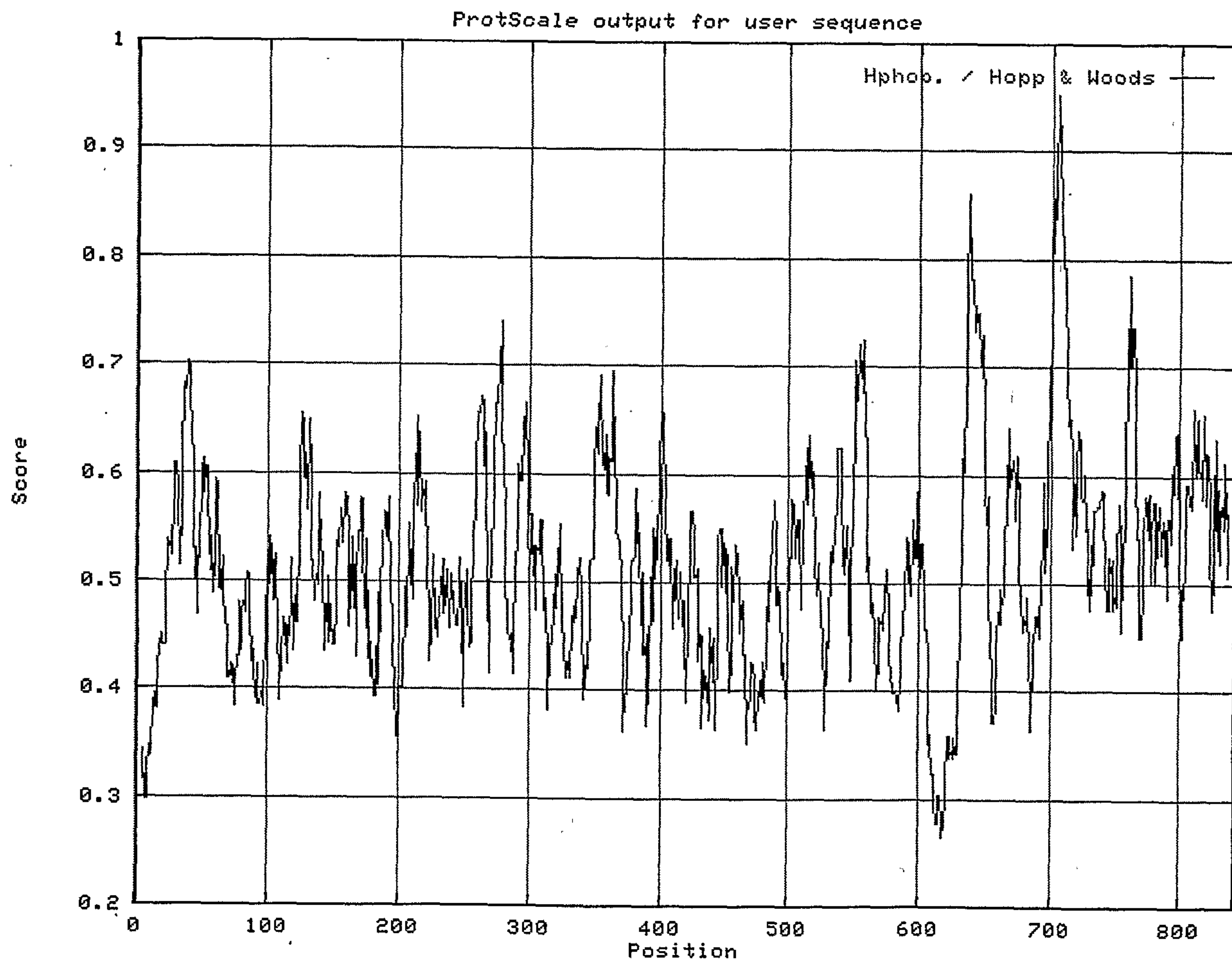


Figure 12. 158P1D7 Hydropathicity Profile
(Kyte J., Doolittle R.F., 1982. J. Mol. Biol. 157:105-132)

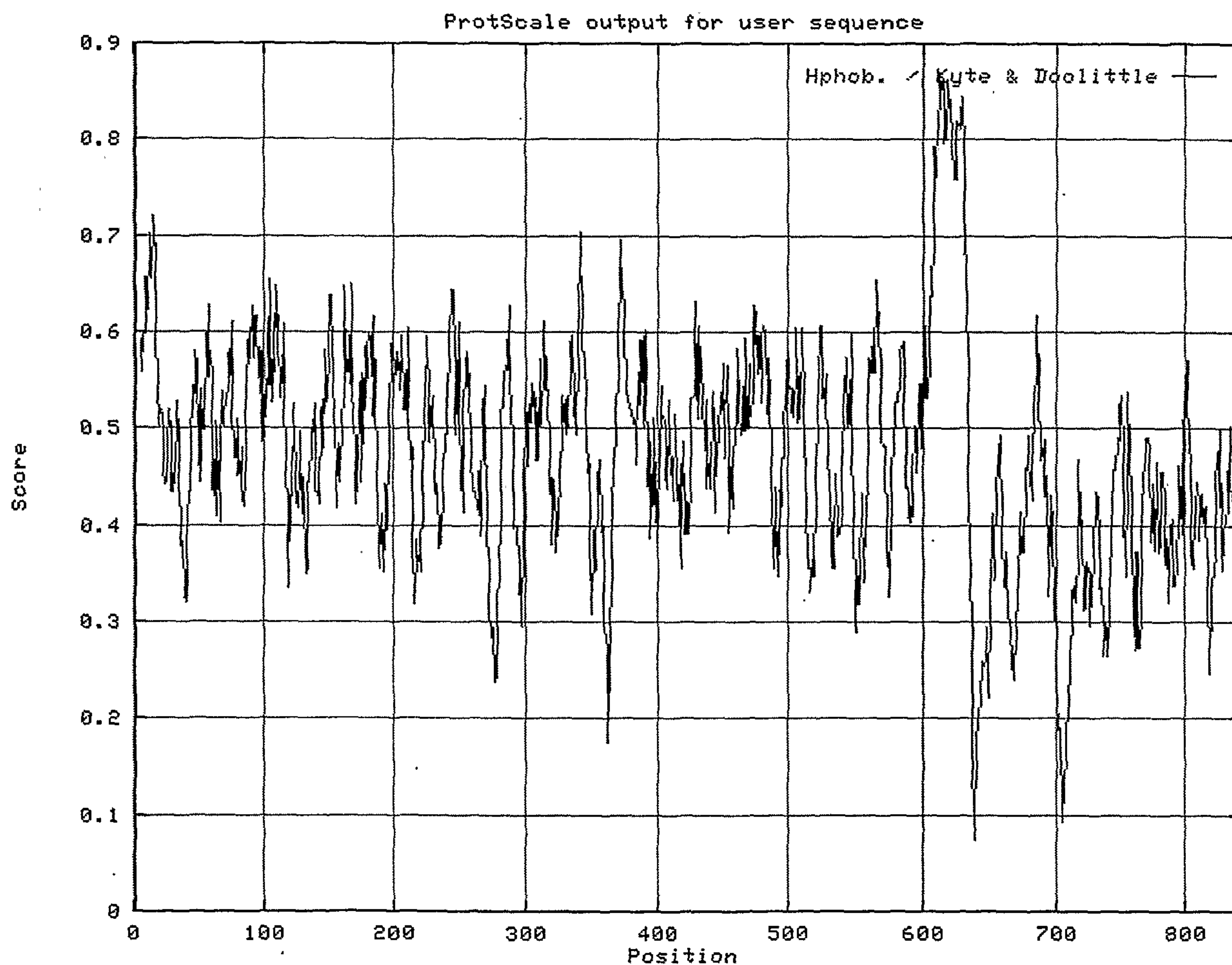


Figure 13. 158P1D7 % Accessible Residues Profile
(Janin J., 1979. Nature 277:491-492)

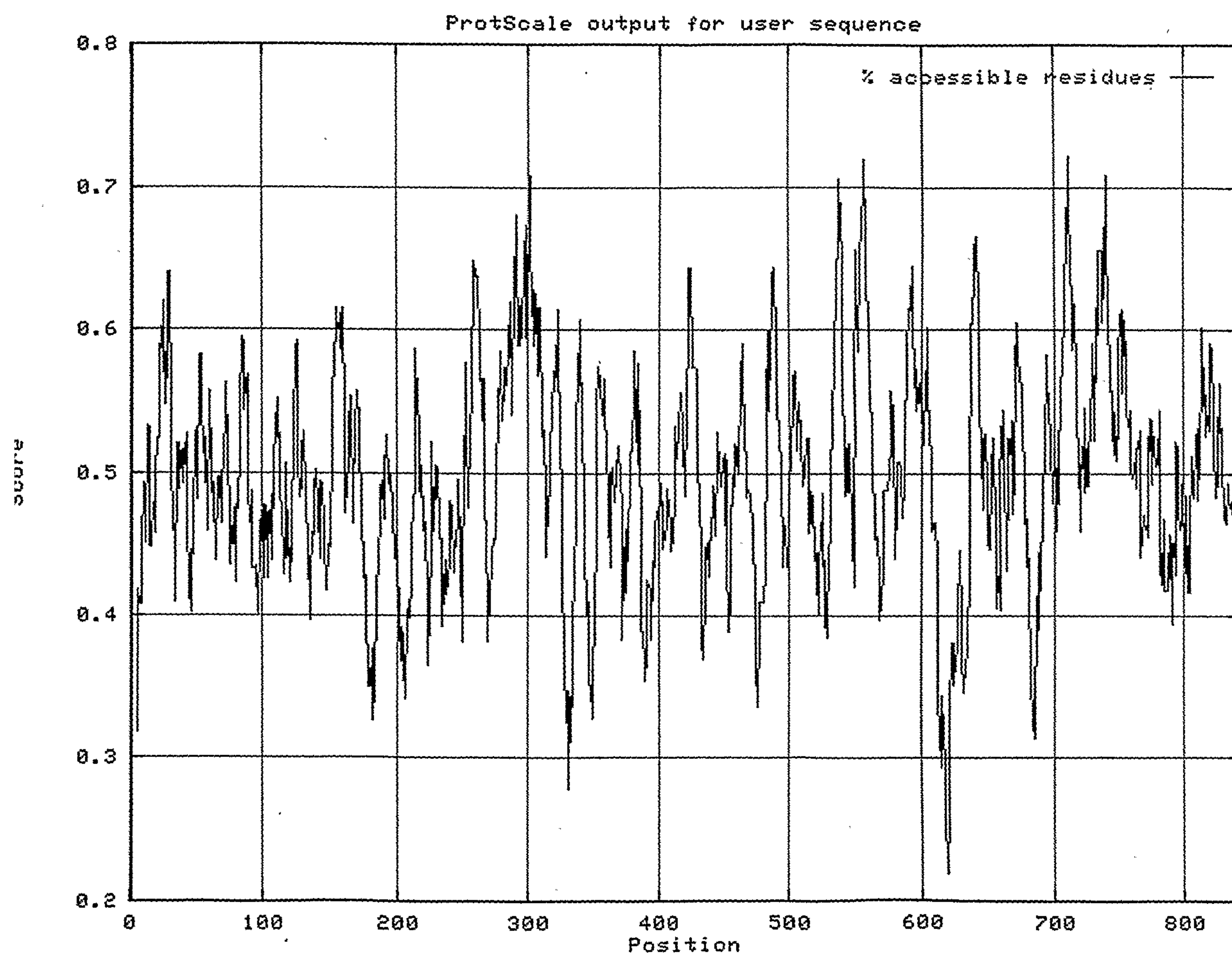


Figure 14. 158P1D7 Average Flexibility Profile
(Bhaskaran R., Ponnuswamy P.K., 1988.
Int. J. Pept. Protein Res. 32:242-255)

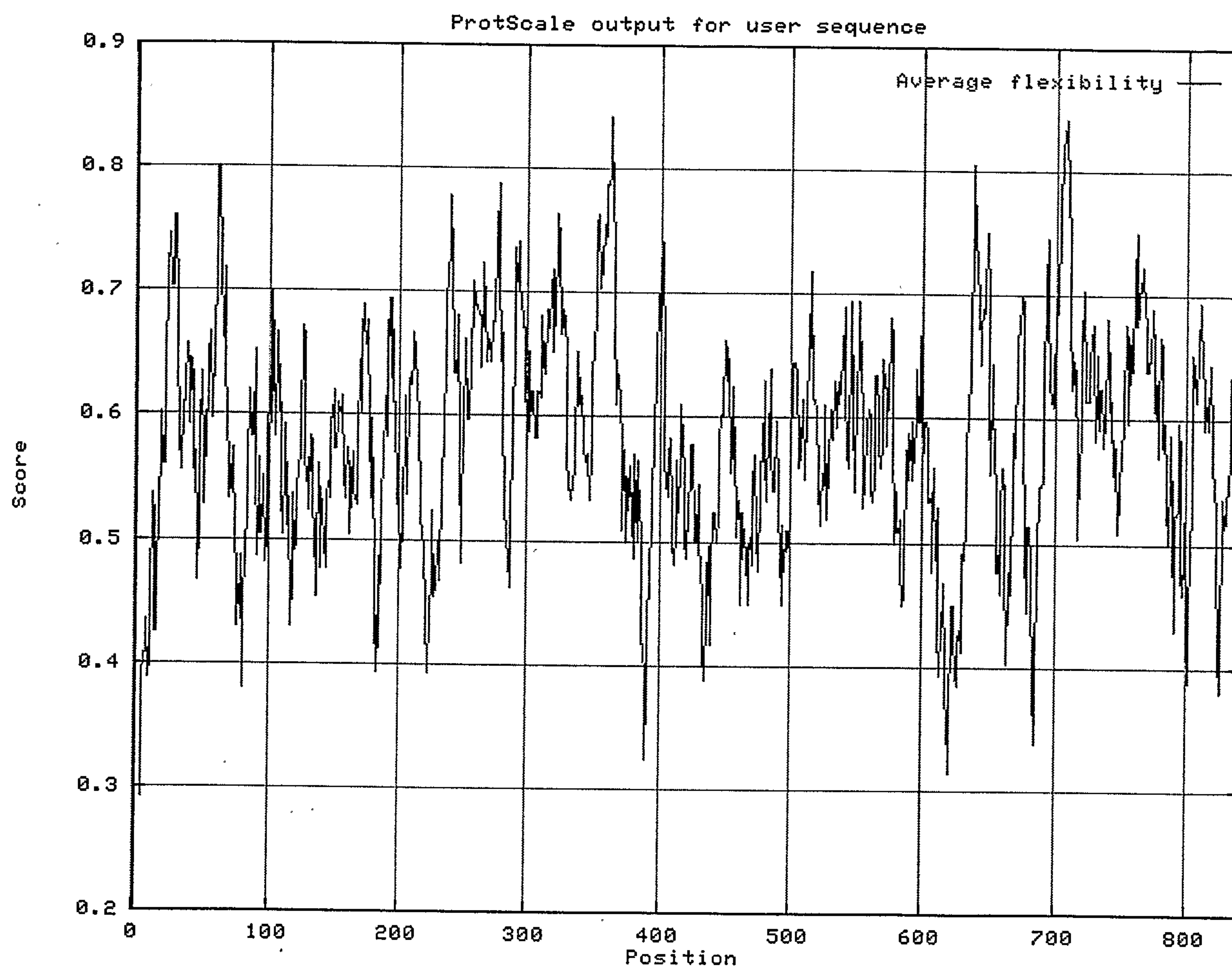
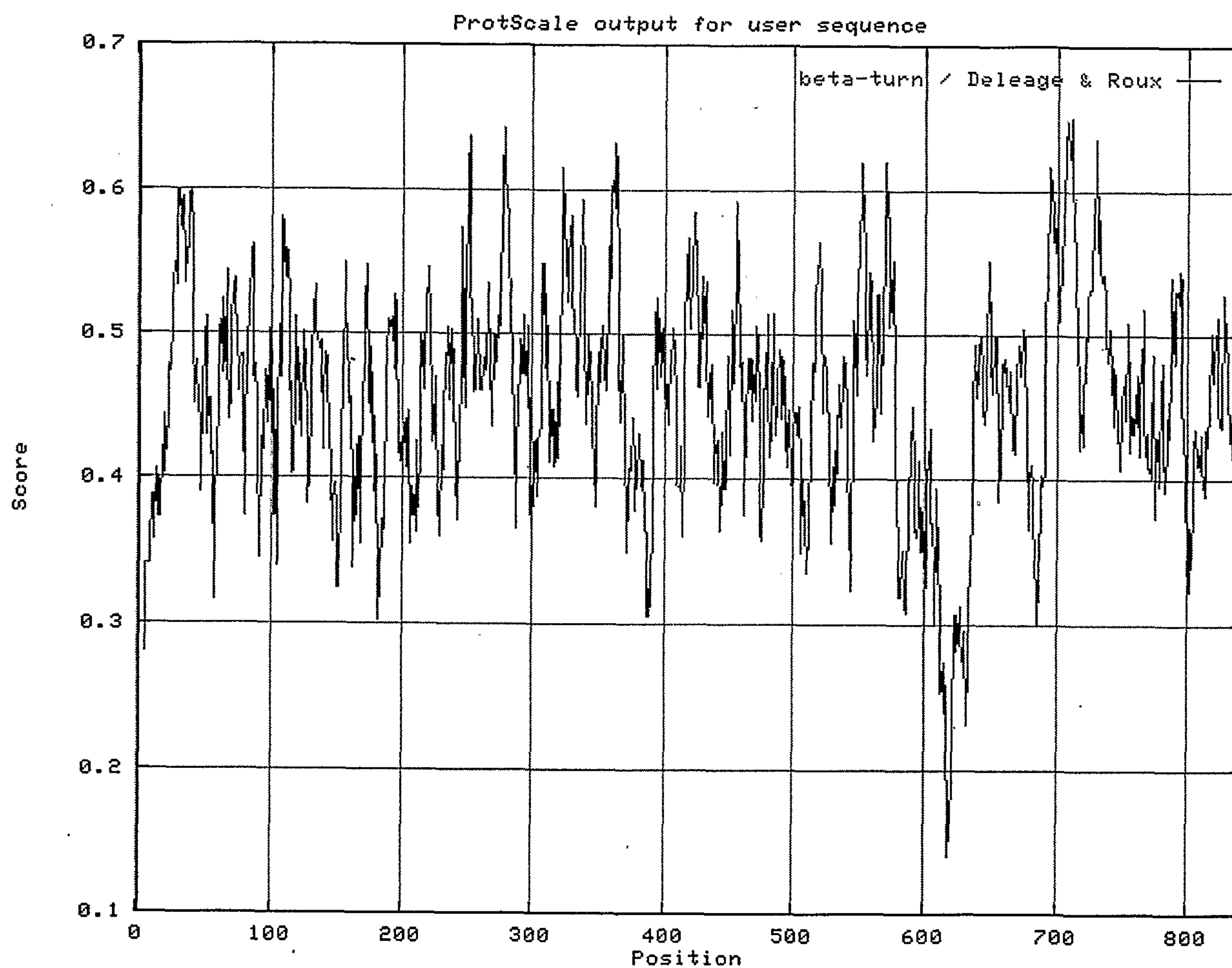
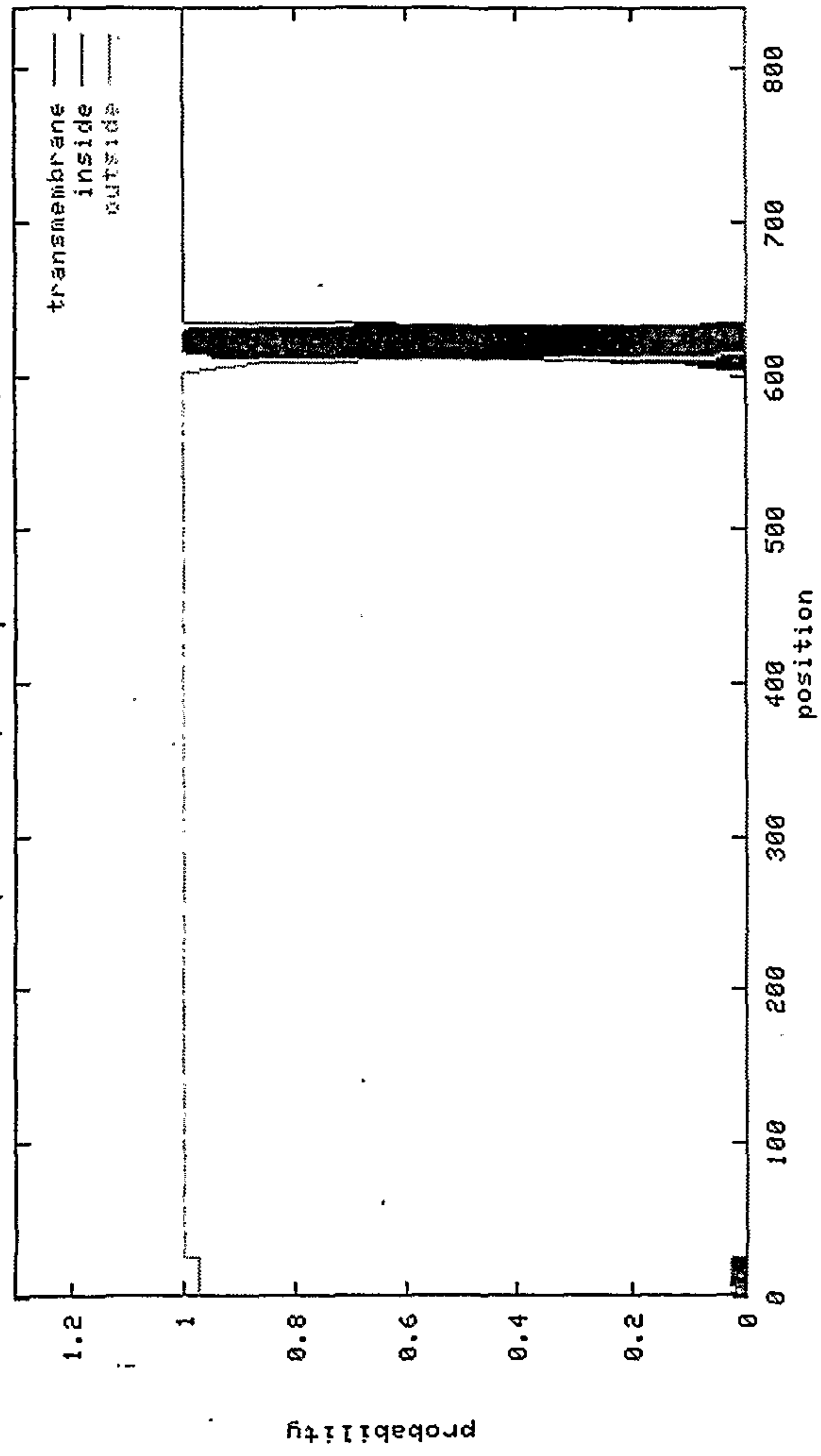


Figure 15. 158P1D7 Beta-turn Profile
(Deleage, G., Roux B. 1987. Protein Engineering 1:289-294)

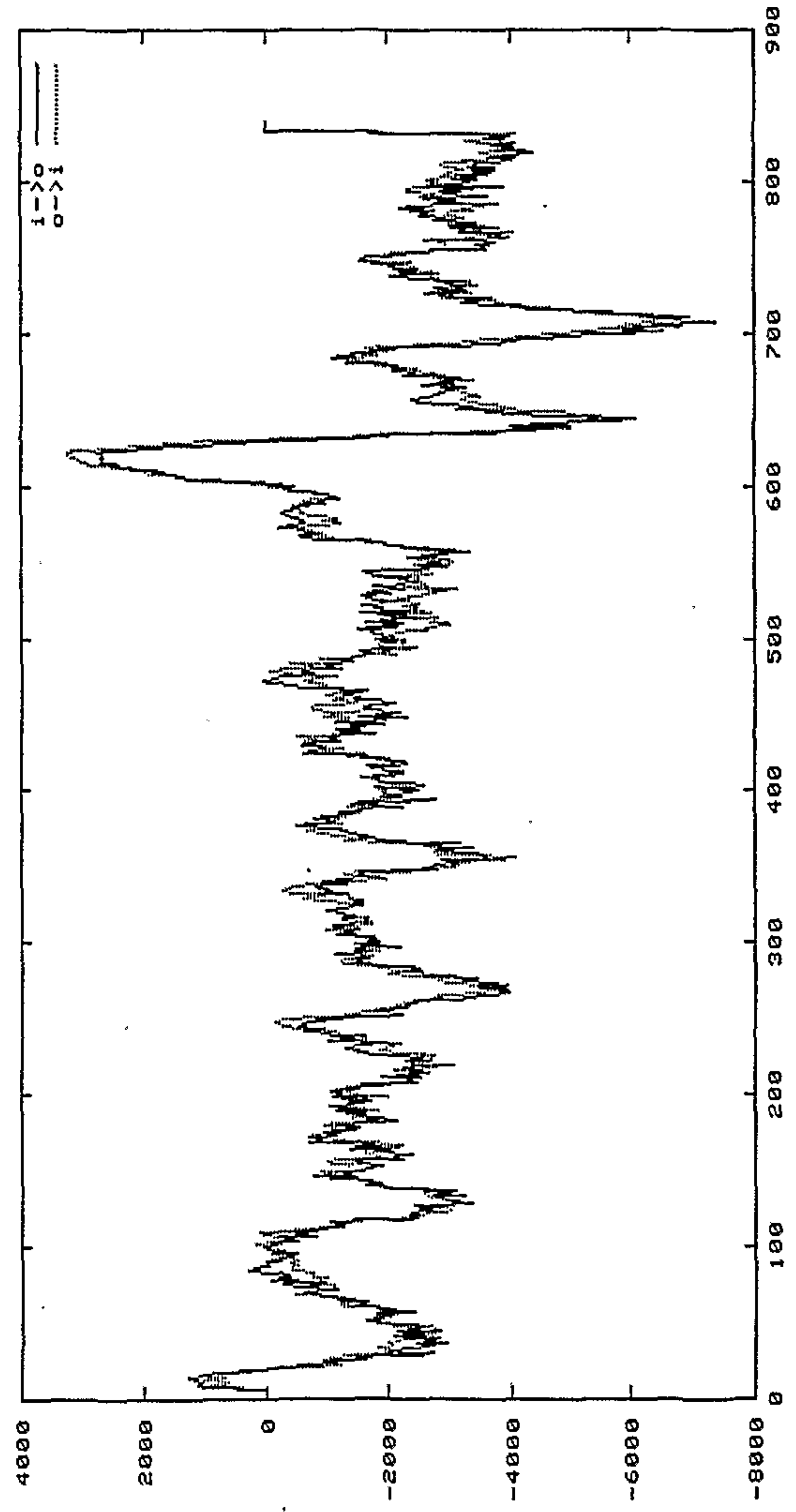


TMHMM transmembrane prediction for 158P1D7



16A

TMpred transmembrane prediction for 158P1D7



16B