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(54) Title: PROSTATE SPECIFIC GENES AND THE USE THEREOF AS TARGETS FOR PROSTATE CANCER THERAPY AND DIAGNOSIS

(57) Abstract: Genes that are upregulated in human prostate tumor tissues and the corresponding proteins are identified. These genes and the corresponding antigens are suitable targets for the treatment, diagnosis or prophylaxis of prostate cancer.



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**PROSTATE SPECIFIC GENES AND THE
USE THEREOF AS TARGETS FOR PROSTATE CANCER
THERAPY AND DIAGNOSIS**

5 **FIELD OF THE INVENTION**

The present invention relates to the identification of DNA sequences that correspond to alternatively spliced events in genes expressed on the surface of prostate cancer cells. These genes or their corresponding proteins are to be targeted for the treatment, prevention and/or diagnosis of cancers wherein these genes are differentially regulated and/or spliced,
10 particularly in prostate cancer.

BACKGROUND OF THE INVENTION

Genetic detection of human disease states is a rapidly developing field (Taparowsky et al., 1982; Slamon et al., 1989; Sidransky et al., 1992; Miki et al., 1994; Dong et al., 1995;
15 Morahan et al., 1996; Lifton, 1996; Barinaga, 1996). However, some problems exist with this approach. A number of known genetic lesions merely predispose an individual to the development of specific disease states. Individuals carrying the genetic lesion may not develop the disease state, while other individuals may develop the disease state without possessing a particular genetic lesion. In human cancers, genetic defects may potentially occur in a large
20 number of known tumor suppresser genes and proto-oncogenes.

Genetic detection of cancer has a long history. Some of the earliest genetic lesions shown to predispose to cancer were transforming point mutations in the ras oncogenes (Taparowsky et al., 1982). Transforming ras point mutations may be detected in the stool of individuals with benign and malignant colorectal tumors (Sidransky et al., 1992). However,
25 only 50% of such tumors contained a ras mutation (Sidransky et al., 1992). Similar results have been obtained with amplification of HER-2/neu in breast and prostate cancer (Slamon et al., 1989), deletion and mutation of p53 in bladder cancer (Sidransky et al., 1991), deletion of DCC in colorectal cancer (Fearon et al., 1990) and mutation of BRCA1 in breast and prostate cancer (Miki et al., 1994).

30 None of these genetic lesions are capable of predicting a majority of individuals with cancer and most require direct sampling of a suspected tumor, and make screening difficult. Further, none of the markers described above are capable of distinguishing between metastatic

and non-metastatic forms of cancer. In effective management of cancer patients, identification of those individuals whose tumors have already metastasized or are likely to metastasize is critical. Because metastatic cancer kills 560,000 people in the U.S. each year (ACS home page), identification of markers for metastatic prostate cancer would be an important advance.

5 A particular problem in cancer detection and diagnosis occurs with prostate cancer. Carcinoma of the prostate is the most frequently diagnosed cancer among men in the United States (Veltri et al., 1996). Prostate cancer was diagnosed in approximately 189,500 men in 1998 and about 40,000 men succumbed to the malignancy (Landis et al, 1998). Although relatively few prostate tumors progress to clinical significance during the lifetime of the
10 patient, those which are progressive in nature are likely to have metastasized by the time of detection. Survival rates for individuals with metastatic prostate cancer are quite low. Between these extremes are patients with prostate tumors that will metastasize but have not yet done so, for whom surgical prostate removal is curative. Determination of which group a patient falls within is critical in determining optimal treatment and patient survival.

15 The FDA approval of the serum prostate specific antigen (PSA) test in 1984 changed the way that prostate disease was managed (Allhoff et al., 1989; Cooner et al., 1990; Jacobson et al, 1995; Orozco et al., 1998). PSA is widely used as a serum biomarker to detect and monitor therapeutic response in prostate cancer patients (Badalament et al., 1996; O'Dowd et al., 1997). Several modifications in PSA assays (Partin and Oesterling, 1994; Babian et al.,
20 1996; Zlotta et al, 1997) have resulted in earlier diagnoses and improved treatment.

 Although PSA has been widely used as a clinical marker of prostate cancer since 1988 (Partin and Oesterling, 1994), screening programs utilizing PSA alone or in combination with digital rectal examination (DRE) have not been successful in improving the survival rate for men with prostate cancer (Partin and Oesterling, 1994). Although PSA is specific to prostate
25 tissue, it is produced by normal and benign as well as malignant prostatic epithelium, resulting in a high false-positive rate for prostate cancer detection (Partin and Oesterling, 1994).

 While an effective indicator of prostate cancer when serum levels are relatively high, PSA serum levels are more ambiguous indicators of prostate cancer when only modestly elevated, for example when levels are between 2-10 ng/ml. At these modest elevations, serum
30 PSA may have originated from non-cancerous disease states such as BPH (benign prostatic hyperplasia), prostatitis or physical trauma (McCormack et al, 1995). Although application of

the lower 2.0 ng/ml cancer detection cutoff concentration of serum PSA has increased the diagnosis of prostate cancer, especially in younger men with nonpalpable early stage tumors (Stage T1c) (Soh et al., 1997; Carter and Coffey, 1997; Harris et al., 1997; Orozco et al., 1998), the specificity of the PSA assay for prostate cancer detection at low serum PSA levels remains a problem.

Several investigators have sought to improve upon the specificity of serologic detection of prostate cancer by examining a variety of other biomarkers besides serum PSA concentration (Ralph and Veltri, 1997). One of the most heavily investigated of these other biomarkers is the ratio of free versus total PSA (f/t PSA) in a patient's blood. Most PSA in serum is in a molecular form that is bound to other proteins such as alpha1-antichymotrypsin (ACT) or alpha2-macroglobulin (Christensson et al, 1993; Stenman et al., 1991; Lilja et al., 1991). Free PSA is not bound to other proteins. The ratio of free to total PSA (f/tPSA) is usually significantly higher in patients with BPH compared to those with organ confined prostate cancer (Marley et al., 1996; Oesterling et al., 1995; Pettersson et al., 1995). When an appropriate cutoff is determined for the f/tPSA assay, the f/tPSA assay can help distinguish patients with BPH from those with prostate cancer in cases in which serum PSA levels are only modestly elevated (Marley et al., 1996; Partin and Oesterling, 1996). Unfortunately, while f/tPSA may improve on the detection of prostate cancer, information in the f/tPSA ratio is insufficient to improve the sensitivity and specificity of serologic detection of prostate cancer to desirable levels.

Other markers that have been used for prostate cancer detection include prostatic acid phosphatase (PAP) and prostate secreted protein (PSP). PAP is secreted by prostate cells under hormonal control (Brawn et al., 1996). It has less specificity and sensitivity than does PSA. As a result, it is used much less now, although PAP may still have some applications for monitoring metastatic patients that have failed primary treatments. In general, PSP is a more sensitive biomarker than PAP, but is not as sensitive as PSA (Huang et al., 1993). Like PSA, PSP levels are frequently elevated in patients with BPH as well as those with prostate cancer.

Another serum marker associated with prostate disease is prostate specific membrane antigen (PSMA) (Horoszewicz et al., 1987; Carter and Coffey, 1996; Murphy et al., 1996). PSMA is a Type II cell membrane protein and has been identified as Folic Acid Hydrolase (FAH) (Carter and Coffey, 1996). Antibodies against PSMA react with both normal prostate

tissue and prostate cancer tissue (Horoszewicz et al., 1987). Murphy et al. (1995) used ELISA to detect serum PSMA in advanced prostate cancer. As a serum test, PSMA levels are a relatively poor indicator of prostate cancer. However, PSMA may have utility in certain circumstances. PSMA is expressed in metastatic prostate tumor capillary beds (Silver et al.,
5 1997) and is reported to be more abundant in the blood of metastatic cancer patients (Murphy et al., 1996). PSMA messenger RNA (mRNA) is down-regulated 8-10 fold in the LNCaP prostate cancer cell line after exposure to 5-alpha-dihydroxytestosterone (DHT) (Israeli et al., 1994).

Two relatively new potential biomarkers for prostate cancer are human kallekrein 2
10 (HK2) (Piironen et al., 1996) and prostate specific transglutaminase (pTGase) (Dubbink et al., 1996). HK2 is a member of the kallekrein family that is secreted by the prostate gland (Piironen et al., 1996). Prostate specific transglutaminase is a calcium-dependent enzyme expressed in prostate cells that catalyzes post-translational cross-linking of proteins (Dubbink et al., 1996). In theory, serum concentrations of HK2 or pTGase may be of utility in prostate
15 cancer detection or diagnosis, but the usefulness of these markers is still being evaluated.

Interleukin 8 (IL-8) has also been reported as a marker for prostate cancer. (Veltri et al., 1999). Serum IL-8 concentrations were reported to be correlated with increasing stage of prostate cancer and to be capable of differentiating BPH from malignant prostate tumors. (Id.) The wide-scale applicability of this marker for prostate cancer detection and diagnosis is still
20 under investigation.

In addition to these protein markers for prostate cancer, several genetic changes have been reported to be associated with prostate cancer, including: allelic loss (Bova, et al., 1993; Macoska et al., 1994; Carter et al., 1990); DNA hypermethylation (Isaacs et al., 1994); point mutations or deletions of the retinoblastoma (Rb), p53 and KAI1 genes (Bookstein et al.,
25 1990a; Bookstein et al., 1990b; Isaacs et al., 1991; Dong et al., 1995); and aneuploidy and aneusomy of chromosomes detected by fluorescence in situ hybridization (FISH) (Macoska et al., 1994; Visakorpi et al., 1994; Takahashi et al., 1994; Alcaraz et al., 1994). None of these have been reported to exhibit sufficient sensitivity and specificity to be useful as general screening tools for asymptomatic prostate cancer.

30 In current clinical practice, the serum PSA assay and digital rectal exam (DRE) is used to indicate which patients should have a prostate biopsy (Lithrup et al., 1994; Orozco et al.,

1998). Histological examination of the biopsied tissue is used to make the diagnosis of prostate cancer. Based upon the 189,500 cases of diagnosed prostate cancer in 1998 (Landis, 1998) and a known cancer detection rate of about 35% (Parker et al., 1996), it is estimated that in 1998 over one-half million prostate biopsies were performed in the United States (Orozco et al., 1998; Veltri et al., 1998). Clearly, there would be much benefit derived from a serological test that was sensitive enough to detect small and early stage prostate tumors that also had sufficient specificity to exclude a greater portion of patients with noncancerous or clinically insignificant conditions.

There remain deficiencies in the prior art with respect to the identification of the genes linked with the progression of prostate cancer and the development of diagnostic methods to monitor disease progression. Likewise, the identification of genes, which are differentially expressed in prostate cancer, would be of considerable importance in the development of a rapid, inexpensive method to diagnose cancer. Although a few prostate specific genes have been cloned (PSA, PSMA, HK2, pTGase, etc.), these are typically not upregulated in prostate cancer. The identification of a novel, prostate specific gene that is differentially expressed in prostate cancer, compared to non-malignant prostate tissue, would represent a major, unexpected advance for the diagnosis, prognosis and treatment of prostate cancer.

The use of therapeutic antibodies for treatment of cancers that target surface proteins is known. Examples thereof include RITUXAN® that targets CD20 on B cell lymphoma, Campath® that targets a surface antigen CD52 expressed by chronic lymphocytic leukemia, Herceptin® that targets erbB2 on breast and other cancers and Mybtara that targets CD33 surface antigen expressed on leukemia cells. However, to date, a monoclonal antibody for treatment of prostate cancer has not been approved for therapeutic use.

SUMMARY OF THE INVENTION

The present invention relates to the identification of novel nucleic acid and amino acid sequences that are characteristic of prostate cancer cell or tissue, and which represent targets for therapy or diagnosis of such a condition in a subject.

The invention more specifically discloses 159 specific, isolated nucleic acid molecules that encode novel expression sequences. Of these, 122 are expressed sequence tags that are differentially spliced and correspond to SEQ ID NOS 1-65, 74, 80, 85, 102-134, 136, 141, 146,

150-165, 167, 168. In addition, 42 specific isoforms of known genes have been identified corresponding to SEQ ID NOS. 67-72, 75-77, 81-83, 86-90, 92, 93, 95-98, 100, 101, 137-139, 143, 144, 147-149, 169-173, 175, 177, 179, and 181. These novel sequences were found to be differentially expressed between normal prostate and prostate cancer. The expressed sequence
5 tag represent novel exons that are alternatively spliced in prostate cancer, and as such, directly identify distinct isoforms. These sequences and molecules represent targets and valuable information to develop methods and materials for the detection, diagnosis, and treatment of prostate cancer.

10 It is an object of the invention to provide methods and materials for treatment and diagnosis of prostate cancer.

It is a more specific object of the invention to identify novel exons (novel splice variants) that are expressed by prostate cancer tissue which are potential gene targets for treatment and diagnosis of prostate cancer.

15 It is a specific object of the invention to develop novel therapies for treatment of prostate cancer involving the administration or use of anti-sense oligonucleotides corresponding to novel gene targets that are specifically expressed by the prostate cancer.

It is another specific object of the invention to identify exons and the corresponding protein domain encoded by those exons specifically upregulated in prostate cancer cells.

20 It is another specific object of the invention to produce ligands that bind antigens encoded by the exons, expressed as a protein domain by certain prostate cancers, including, but not limited to, monoclonal antibodies.

It is another specific object of the invention to provide novel therapeutic regimens for the treatment of prostate cancer that involve the administration or use of antigens expressed by
25 certain prostate cancers, alone or in combination with adjuvants that elicit an antigen-specific cytotoxic T-cell lymphocyte response against cancer cells that express such antigen.

It is another object of the invention to provide novel therapeutic regimens for the treatment of prostate cancer that involve the administration or use of ligands, especially monoclonal antibodies that specifically bind novel antigens that are expressed by certain
30 prostate cancers.

It is an other object of this invention to provide pharmaceutical compositions comprising a ligand or antigen as defined above, in combination with a pharmaceutically acceptable carrier or excipient and/or an adjuvant.

5 It is another object of the invention to provide a novel method for diagnosis of prostate cancer by using ligands, e.g., monoclonal antibodies, which specifically bind to antigens that are specifically expressed by certain prostate cancers, in order to detect whether a subject has or is at increased risk of developing prostate cancer.

10 It is another object of the invention to provide a novel method of detecting persons having, or at increased risk of developing prostate cancer by use of labeled DNAs that hybridize to novel gene targets expressed by certain prostate cancers.

15 It is yet another object of the invention to provide diagnostic test kits for the detection of persons having or at increased risk of developing prostate cancer that comprise a ligand, e.g., monoclonal antibody that specifically binds to an antigen expressed by prostate cancer cells, and a detectable label, e.g. indicator enzymes, a radiolabels, fluorophores, or paramagnetic particles.

It is another object of the invention to provide diagnostic kits for detection of persons having or at risk of developing prostate cancer that comprise DNA primers or probes specific for novel gene targets specifically expressed by prostate cancer cells, and a detectable label, e.g. indicator enzymes, a radiolabels, fluorophores, or paramagnetic particles.

20 It is another object of this invention to provide methods for selecting, identifying, screening, characterizing or optimizing biologically active compounds, comprising a determination of whether a candidate compound binds, preferably selectively, an antigen or a polynucleotide as disclosed in the present application. Such compounds represent drug candidates or leads for treating cancer diseases, particularly prostate cancer.

25 It is another object of the invention to identify genes that are expressed in altered forms in prostate cancer cells. These forms represent splice variants of the gene, where the DATASTM fragment either 1) indicates the splice event occurring within the gene, or 2) points to a gene that is actively spliced to produce different gene products. These different splice variants or isoforms can be targets for therapeutic intervention.

LEGEND TO THE FIGURES

Figure 1 : Expression of Sequence ID: No. 92 in normal human tissue. Primers were designed to detect the DATAS clone sequence and RT-PCR analysis was performed for 30 cycles. Lane 1, Prostate; lane 2, Heart; lane 3, Lung; lane 4, Kidney; lane 5, Liver; lane 6, Brain; lane 7, Placenta; lane 8, Sk. Muscle; lane 9, Pancreas; lane 10, Spleen; lane 11, Thymus; lane 12, Testis; lane 13, Ovary; lane 14, Sm. Intestine; lane 15, Colon; lane 16 Leukocyte.

Figure 2 : Expression of clone (SEQ ID NO 92) in normal and tumor prostate samples. Primers were designed to detect the DATAS clone and RT-PCR analysis was performed for 40 cycles. Individual RNA samples (normal and tumor) were tested both as pooled and as individual samples. The pooled RNA samples were used to produce cDNA using either an oligo dT approach (dT) or through a random primer protocol (RP). Individual patient cDNA samples (lanes 9-12) were prepared through the random primed protocol. Lane 1, prostate tumor pool 1 (RP cDNA); lane 2, normal prostate pool 1 (RP cDNA); lane 3, prostate tumor pool 2 (RP cDNA); lane 4, normal prostate pool 2 (RP cDNA); lane 5, prostate tumor pool 1 (dT cDNA); lane 6, normal prostate pool 1 (dT cDNA); lane 7, normal prostate pool 2 (dT cDNA); lane 8, NTC; lane 9, Patient 1 (OHK); lane 10, Patient 2 (T523); lane 11, Patient 3 (82B) ; lane 12, Patient 4 (4BK).

Figure 3 : Alignment of the different isoforms isolated from structural analysis of clone (DATAS clone number). The sequences isolated from the DATAS derived events were mapped using Blat against the Human genome to annotate the gene and determine the each unique splicing event. Five events are mapped with AK092666, an EST that closely resembles the five events.

Figure 4 : Western blot analysis for the expression of STEAP2 isoforms. Protein extracts from prostate cancer cell lines were separated on SDS_PAGE gels and transferred to nitrocellulose, and probed with an antibody raised against a peptide sequence present in the N-terminal portion of the wild type STEAP2 protein. Five different cell lines were analyzed: lane 1) LNCaP; 2) 22Rv1 3) MDA-PCa2b; 4) PC3; 5) DU145. The blot was developed using standard chemiluminescence reagents.

DETAILED DESCRIPTION OF THE INVENTION

DATAS (Different Analysis of Transcripts with Alternative Splicing) analyzes structural differences between expressed genes and provides systematic access to alterations in RNA splicing (disclosed in U.S. Patent No.6,251,590, the disclosure of which is incorporated by reference in its entirety). Having access to these spliced sequences, which are critical for cellular homeostasis, represents a useful advance in functional genomics.

The DATAS Technology generates two libraries when comparing two samples, such as normal vs. tumor tissue. Each library specifically contains clones of sequences that are present and more highly expressed in one sample. For example, library A will contain sequences that are present in genes in the normal samples but absent in the tumor samples. These sequences are identified as being removed or spliced out from the genes in the tumor samples. In contrast, library B will contain sequences that are present only in the tumor samples and not present in the normal samples. These represent exons/introns that are alternatively spliced into genes expressed *only* in the tumor samples.

The present invention is based in part on the identification of exons that are isolated using DATAS and then determined to be differentially regulated or expressed in prostate tumor samples. Specifically, 122 expressed sequence tags were identified through DATAS and confirmed to be differentially expressed between normal prostate tissue and prostate tumor tissue. These DATAS fragments (DF) are small sections of genes that are selected for inclusion or exclusion in one sample but not the other. These small sections are part of the expressed gene transcript, and can consist of sequences derived from several different regions of the gene, including, but not limited to, portions of single exons, several exons, sequence from introns, and sequences from exons and introns. This alternative usage of exons in different biological samples produces different gene products from the same gene through a process well known in the art as alternative RNA splicing. In particular, 37 alternatively spliced isoforms have been identified from the DATAS fragment sequences, and produce alternate gene products that fit all the descriptions of targets and gene products below.

Alternatively spliced mRNA's produced from the same gene contain different ribonucleotide sequence, and therefore translate into proteins with different amino acid sequences. Nucleic acid sequences that are alternatively spliced into or out of the gene products can be inserted or deleted in frame or out of frame from the original gene sequence. This leads to the translation of different proteins from each variant. Differences can include simple sequence deletions, or novel sequence information inserted into the gene product. Sequences inserted out of frame can lead to the production of an early stop codon and produce a truncated form of the protein. Alternatively, in-frame insertions of nucleic acid may cause an additional protein domain to be expressed from the mRNA. The end stage target is a novel protein containing either a novel epitope or function. Many variations of known genes have been identified and produce protein variants that can be agonistic or antagonistic with the original biological activity of the protein.

DATAS fragments thus identify genes and proteins which are subject to differential regulation and alternative splicing(s) in prostate cancer cells. DATAS fragments thus allow the definition of target molecules suitable for diagnosis or therapy of prostate cancers, which target molecules comprise all or a portion of genes or RNAs comprising the sequence of a DATAS fragment, or of genes or RNA from which the sequence of a DATAS fragment derives, as well as corresponding polypeptides or proteins, and variants thereof.

A first type of target molecule is a target nucleic acid molecule comprising the sequence of a full gene or RNA molecule comprising the sequence of a DATAS fragment as disclosed in the present application. Indeed, since DATAS identifies genetic deregulations associated with prostate tumor, the whole gene or RNA sequence from which said DATAS fragment derives can be used as a target of therapeutic intervention or diagnosis.

Similarly, another type of target molecule is a target polypeptide molecule comprising the sequence of a full-length protein comprising the amino acid sequence encoded by a DATAS fragment as disclosed in the present application.

A further type of target molecule is a target nucleic acid molecule comprising a fragment of a gene or RNA as disclosed above. Indeed, since DATAS identifies genes and RNAs that are altered in prostate tumor cells, portions of such genes or RNAs, including portions that do not comprise the sequence of a DATAS fragment, can be used as a target for therapeutic intervention or diagnosis. Examples of such portions include : DATAS fragments, portions

thereof, alternative exons or introns of said gene or RNA, exon-exon, exon-intron or intron-intron junction sequences generated by splicing(s) in said RNA, etc. Particular portions comprise a sequence encoding a extra-cellular domain of a polypeptide.

Similarly, another type of target molecule is a fragment of a protein comprising the amino acid sequence encoded by a DATAS fragment as disclosed in the present application. Such fragments may comprise or not the DATAS sequence, and may comprise newly generated amino acid sequence resulting, for instance, from a frame shift, a novel exon-exon or exon-intron junction, the creation of new stop codon, etc.

- 10 These target molecules (including genes, fragments, proteins and their variants) can serve as diagnostic agents and as targets for the development of therapeutics. For example, these therapeutics may modulate biological processes associated with prostate tumor viability. Agents may also be identified that are associated with the induction of apoptosis (cell death) in prostate tumor cells. Other agents can also be developed, such as monoclonal antibodies, that
- 15 bind to the protein or its variant and alter the biological processes important for cell growth. Alternatively, antibodies can deliver a toxin which can inhibit cell growth and lead to cell death.

- Specifically, the invention provides sequences that are expressed in a variant protein
- 20 and are prostate tumor specific or prostate specific. These sequences are portions of genes identified to be in the plasma membrane of the cell through bioinformatic analysis, and the specific sequences of the invention are expressed on the extracellular region of the protein, so that the sequences may be useful in the preparation of prostate tumor vaccines, including prophylatic and therapeutic vaccines.

- 25 Based thereon, it is anticipated that the disclosed genes that are associated with the differentially expressed sequences and the corresponding variant proteins should be suitable targets for prostate cancer therapy, prevention or diagnosis, e.g. for the development of antibodies, small molecular inhibitors, anti-sense therapeutics, and ribozymes. The potential therapies are described in greater detail below.

- 30 Such therapies will include the synthesis of oligonucleotides having sequences in the antisense orientation relative to the subject nucleic acids which appear to be up-regulated in

prostate cancer. Suitable therapeutic antisense oligonucleotides will typically vary in length from two to several hundred nucleotides in length, more typically about 50-70 nucleotides in length or shorter. These antisense oligonucleotides may be administered as naked nucleic acids or in protected forms, e.g., encapsulated in liposomes. The use of liposomal or other
5 protected forms may be advantageous as it may enhance *in vivo* stability and thus facilitate delivery to target sites, i.e., prostate tumor cells.

Also, the subject novel genes may be used to design novel ribozymes that target the cleavage of the corresponding mRNAs in prostate tumor cells. Similarly, these ribozymes may be administered in free (naked) form or by the use of delivery systems that enhance stability
10 and/or targeting, e.g., liposomes.

Also, the present invention embraces the administration of use of nucleic acids that hybridize to the novel nucleic acid targets identified *infra*, attached to therapeutic effector moieties, e.g., radiolabels, (e.g., ^{90}Y , ^{131}I) cytotoxins, cytotoxic enzymes, and the like in order to selectively target and kill cells that express these nucleic acids, i.e., prostate tumor cells.

Also, the present invention embraces the treatment and/or diagnosis of prostate cancer
15 by targeting altered genes or the corresponding altered protein particularly splice variants that are expressed in altered form in prostate tumor cells. These methods will provide for the selective detection of cells and/or eradication of cells that express such altered forms thereby minimizing adverse effects to normal cells.

Still further, the present invention encompasses non-nucleic acid based therapies. For
20 example, the invention encompasses the use of a DNA containing one of the novel cDNAs corresponding to novel antigen identified herein. It is anticipated that the antigens so encoded may be used as therapeutic or prophylactic anti-tumor vaccines. For example, a particular contemplated application of these antigens involves their administration with adjuvants that
25 induce a cytotoxic T lymphocyte response.

Administration of the subject novel antigens in combination with an adjuvant may result in a humoral immune response against such antigens, thereby delaying or preventing the development of prostate cancer.

These embodiments of the invention will comprise administration of one or more of
30 the subject novel prostate cancer antigens, ideally in combination with an adjuvant, e.g., PROVAX™ (as disclosed U.S. Patents Nos. 5,709,860, 5,695,770, and 5,585,103, which

comprises a microfluidized adjuvant containing Squalene, Tween and Pluronic), ISCOM'S[®], DETOX[®], SAF, Freund's adjuvant, Alum[®], Saponin[®], among others. This composition will be administered in an amount sufficient to be therapeutically or prophylactically effective, e.g. on the order of 50 to 20,000 mg/kg body weight, 100 to 5000 mg/kg body weight.

5 Yet another embodiment of the invention will comprise the preparation of monoclonal antibodies against the antigens encoded by the novel genes containing the nucleic acid sequences disclosed infra. Such monoclonal antibodies may be produced by conventional methods and include human monoclonal antibodies, humanized monoclonal antibodies, chimeric monoclonal antibodies, single chain antibodies, e.g., scFv's and antigen-binding
10 antibody fragments such as Fab and Fab' fragments. Methods for the preparation of monoclonal antibodies are known in the art. In general, preparation of monoclonal antibodies will comprise immunization of an appropriate (non-homologous) host with the subject prostate cancer antigens, isolation of immune cells therefrom, use of such immune cells to isolate monoclonal antibodies and screening for monoclonal antibodies that specifically bind to either
15 of such antigens. Antibody fragments may be prepared by known methods, e.g., enzymatic change of monoclonal antibodies.

These monoclonal antibodies and fragments will be useful for passive anti-tumor immunotherapy, or may be attached to therapeutic effector moieties, e.g., radiolabels, cytotoxins, therapeutic enzymes, agents that induce apoptosis, and the like in order to provide
20 for targeted cytotoxicity, i.e., killing of human prostate tumor cells. Given the fact that the subject genes are apparently not significantly expressed by many normal tissues this should not result in significant adverse side effects (toxicity to non-target tissues).

In one embodiment, of the present invention such antibodies or fragments will be administered in labeled or unlabeled form, alone or in conjunction with other therapeutics,
25 e.g., chemotherapeutics such as cisplatin, methotrexate, adriamycin, and the like suitable for prostate cancer therapy. The administered composition will also typically include a pharmaceutically acceptable carrier, and optionally adjuvants, stabilizers, etc., used in antibody compositions for therapeutic use.

Preferably, the subject monoclonal antibodies will bind the target antigens with high
30 affinity, e.g., possess a binding affinity (Kd) on the order of 10^{-6} to 10^{-12} M.

As noted, the present invention also embraces diagnostic applications that provide for detection of the expression of prostate specific splice variants disclosed herein. This will comprise detecting the expression of one or more of these genes at the RNA level and/or at the protein level.

5 For nucleic acids, expression of the subject genes will be detected by known nucleic acid detection methods, e.g., Northern blot hybridization, strand displacement amplification (SDA), catalytic hybridization amplification (CHA), and other known nucleic acid detection methods. Preferably, a cDNA library will be made from prostate cells obtained from a subject to be tested for prostate cancer by PCR using primers corresponding to the novel isoforms
10 disclosed in this application.

The presence or absence of prostate cancer can be determined based on whether PCR products are obtained, and the level of expression. The levels of expression of such PCR product may be quantified in order to determine the prognosis of a particular prostate cancer patient (as the levels of expression of the PCR product often will increase or decrease
15 significantly as the disease progresses.) This may provide a method for monitoring the status of a prostate cancer patient.

Alternatively, the status of a subject to be tested for prostate cancer may be evaluated by testing biological fluids, e.g., blood, urine, lymph, and the like with an antibody or antibodies or fragment that specifically binds to the novel prostate tumor antigens disclosed
20 herein.

Methods for using antibodies to detect antigen expression are well known and include ELISA, competitive binding assays, and the like. In general, such assays use an antibody or antibody fragment that specifically binds the target antigen directly or indirectly bound to a label that provides for detection, e.g. indicator enzymes, a radiolabels, fluorophores, or
25 paramagnetic particles.

Patients which test positive for the enhanced presence of the antigen on prostate cells will be diagnosed as having or being at increased risk of developing prostate cancer. Additionally, the levels of antigen expression may be useful in determining patient status, i.e., how far disease has advanced (stage of prostate cancer).

30 As noted, the present invention provides novel splice variants that encode antigens that correlate to human prostate cancer. The present invention also embraces variants thereof. As

used herein "variants" means sequences that are at least about 75% identical thereto, more preferably at least about 85% identical, and most preferably at least 90% identical and still more preferably at least about 95-99% identified when these DNA sequences are compared to a nucleic acid sequence encoding the subject DNAs or a fragment thereof having a size of at least about 50 nucleotides. This includes allelic and splice variants of the subject genes. The present invention also encompasses nucleic acid sequences that hybridize to the subject splice variants under high, moderate or low stringency conditions e.g., as described infra.

Also, the present invention provides for primer pairs that result in the amplification of DNAs encoding the subject novel genes or a portion thereof in an mRNA library obtained from a desired cell source, typically human prostate cell or tissue sample. Typically, such primers will be on the order of 12 to 50 nucleotides in length, and will be constructed such that they provide for amplification of the entire or most of the target gene.

Also, the invention embraces the antigens encoded by the subject DNAs or fragments thereof that bind to or elicits antibodies specific to the full-length antigens. Typically, such fragments will be at least 10 amino acids in length, more typically at least 25 amino acids in length.

As noted, the subject DNA fragments are expressed in a majority of prostate tumor samples tested. The invention further contemplates the identification of other cancers that express such genes and the use thereof to detect and treat such cancers. For example, the subject DNA fragments or variants thereof may be expressed on other cancers, e.g., breast, ovary, pancreas, lung or prostate cancers. Essentially, the present invention embraces the detection of any cancer wherein the expression of the subject novel genes or variants thereof correlate to a cancer or an increased likelihood of cancer. To facilitate under-study of the invention, the following definitions are provided.

"Isolated tumor antigen or tumor protein" refers to any protein that is not in its normal cellular environment. This includes by way of example compositions comprising recombinant proteins encoded by the genes disclosed infra, pharmaceutical compositions comprising such purified proteins, diagnostic compositions comprising such purified proteins, and isolated protein compositions comprising such proteins. In preferred embodiments, an isolated prostate tumor protein according to the invention will comprise a substantially pure protein, in that it is substantially free of other proteins, preferably that is at least 90% pure, that comprises the

amino acid sequence contained herein or natural homologues or mutants having essentially the same sequence. A naturally occurring mutant might be found, for instance, in tumor cells expressing a gene encoding a mutated protein according to the invention.

“Native tumor antigen or tumor protein” refers to a protein that is a non-human primate
5 homologue of the protein having the amino acid sequence contained infra.

“Isolated prostate tumor gene or nucleic acid sequence” refers to a nucleic acid molecule that encodes a tumor antigen according to the invention which is not in its normal human cellular environment, e.g., is not comprised in the human or non-human primate chromosomal DNA. This includes by way of example vectors that comprise a gene according
10 to the invention, a probe that comprises a gene according to the invention, and a nucleic acid sequence directly or indirectly attached to a detectable moiety, e.g. a fluorescent or radioactive label, or a DNA fusion that comprises a nucleic acid molecule encoding a gene according to the invention fused at its 5’ or 3’ end to a different DNA, e.g. a promoter or a DNA encoding a detectable marker or effector moiety. Also included are natural homologues or mutants having
15 substantially the same sequence. Naturally occurring homologues that are degenerate would encode the same protein including nucleotide differences that do not change the corresponding amino acid sequence. Naturally occurring mutants might be found in tumor cells, wherein such nucleotide differences may result in a mutant tumor antigen. Naturally occurring homologues containing conservative substitutions are also encompassed.

“Variant of prostate tumor antigen or tumor protein” refers to a protein possessing an amino acid sequence that possess at least 90% sequence identity, more preferably at least 91% sequence identity, even more preferably at least 92% sequence identity, still more preferably at least 93% sequence identity, still more preferably at least 94% sequence identity, even more preferably at least 95% sequence identity, still more preferably at least 96% sequence identity,
25 even more preferably at least 97% sequence identity, still more preferably at least 98% sequence identity, and most preferably at least 99% sequence identity, to the corresponding native tumor antigen wherein sequence identity is as defined infra. Preferably, this variant will possess at least one biological property in common with the native protein.

“Variant of prostate tumor gene or nucleic acid molecule or sequence” refers to a
30 nucleic acid sequence that possesses at least 90% sequence identity, more preferably at least 91%, more preferably at least 92%, even more preferably at least 93%, still more preferably at

least 94%, even more preferably at least 95%, still more preferably at least 96%, even more preferably at least 97%, even more preferably at least 98% sequence identity, and most preferably at least 99% sequence identity, to the corresponding native human nucleic acid sequence, wherein "sequence identity" is as defined infra.

5 "Fragment of prostate antigen encoding nucleic acid molecule or sequence" refers to a nucleic acid sequence corresponding to a portion of the native human gene wherein said portion is at least about 50 nucleotides in length, or 100, more preferably at least 150 nucleotides in length.

 "Antigenic fragments of prostate tumor antigen" refer to polypeptides corresponding to
10 a fragment of a prostate protein or a variant or homologue thereof that when used itself or attached to an immunogenic carrier elicits antibodies that specifically bind the protein. Typically such antigenic fragments will be at least 8-15 amino acids in length, and may be much longer.

 Sequence identity or percent identity is intended to mean the percentage of the same
15 residues shared between two sequences, referenced to human protein A or protein B or gene A or gene B, when the two sequences are aligned using the Clustal method [Higgins et al, Cabios 8:189-191 (1992)] of multiple sequence alignment in the Lasergene biocomputing software (DNASTAR, INC, Madison, WI), or alignment programs available from the Genetics Computer Group (GCG Wisconsin package, Accelrys, San Diego, CA). In this method,
20 multiple alignments are carried out in a progressive manner, in which larger and larger alignment groups are assembled using similarity scores calculated from a series of pairwise alignments. Optimal sequence alignments are obtained by finding the maximum alignment score, which is the average of all scores between the separate residues in the alignment, determined from a residue weight table representing the probability of a given amino acid
25 change occurring in two related proteins over a given evolutionary interval. Penalties for opening and lengthening gaps in the alignment contribute to the score. The default parameters used with this program are as follows: gap penalty for multiple alignment=10; gap length penalty for multiple alignment=10; k-tuple value in pairwise alignment=1; gap penalty in pairwise alignment=3; window value in pairwise alignment=5; diagonals saved in pairwise
30 alignment=5. The residue weight table used for the alignment program is PAM250

[Dayhoff et al., in Atlas of Protein Sequence and Structure, Dayhoff, Ed., NDRF, Washington, Vol. 5, suppl. 3, p. 345, (1978)].

Percent conservation is calculated from the above alignment by adding the percentage of identical residues to the percentage of positions at which the two residues represent a conservative substitution (defined as having a log odds value of greater than or equal to 0.3 in the PAM250 residue weight table). Conservation is referenced to human Gene A or gene B when determining percent conservation with non-human Gene A or gene B, e.g. mgene A or gene B, when determining percent conservation. Conservative amino acid changes satisfying this requirement include: R-K; E-D, Y-F, L-M; V-I, Q-H.

Polypeptide Fragments

The invention provides polypeptide fragments of the disclosed proteins. Polypeptide fragments of the invention can comprise at least 8, more preferably at least 25, still more preferably at least 50 amino acid residues of the protein or an analogue thereof. More particularly such fragment will comprise at least 75, 100, 125, 150, 175, 200, 225, 250, 275 residues of the polypeptide encoded by the corresponding gene. Even more preferably, the protein fragment will comprise the majority of the native protein, e.g. about 100 contiguous residues of the native protein.

Biologically Active Variants

The invention also encompasses mutants of the novel prostate proteins disclosed infra which comprise an amino acid sequence that is at least 80%, more preferably 90%, still more preferably 95-99% similar to the native protein.

Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity can be found using computer programs well known in the art, such as DNASTAR or software from the Genectics Computer Group (GCG). Preferably, amino acid changes in protein variants are conservative amino acid changes, *i.e.*, substitutions of similarly charged or uncharged amino acids. A conservative amino acid change involves substitution of one of a family of amino acids which are related in their side chains. Naturally occurring amino acids are generally divided into four families: acidic (aspartate, glutamate), basic (lysine, arginine, histidine), non-polar (alanine, valine,

leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), and uncharged polar (glycine, asparagine, glutamine, cystine, serine, threonine, tyrosine) amino acids. Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids.

5 A subset of mutants, called muteins, is a group of polypeptides in which neutral amino acids, such as serines, are substituted for cysteine residues which do not participate in disulfide bonds. These mutants may be stable over a broader temperature range than native secreted proteins. See Mark *et al.*, U.S. Patent 4,959,314.

10 It is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the biological properties of the resulting secreted protein or polypeptide variant.

15 Protein variants include glycosylated forms, aggregative conjugates with other molecules, and covalent conjugates with unrelated chemical moieties. Also, protein variants also include allelic variants, species variants, and muteins. Truncations or deletions of regions which do not affect the differential expression of the gene are also variants. Covalent variants can be prepared by linking functionalities to groups which are found in the amino acid chain or at the N- or C-terminal residue, as is known in the art.

20 It will be recognized in the art that some amino acid sequence of the prostate proteins of the invention can be varied without significant effect on the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there are critical areas on the protein which determine activity. In general, it is possible to replace residues that form the tertiary structure, provided that residues performing a similar function are used. In other instances, the type of residue may be completely unimportant if the
25 alteration occurs at a non-critical region of the protein. The replacement of amino acids can also change the selectivity of binding to cell surface receptors. Ostade et al., *Nature* 361:266-268 (1993) describes certain mutations resulting in selective binding of TNF-alpha to only one of the two known types of TNF receptors. Thus, the polypeptides of the present invention may include one or more amino acid substitutions, deletions or additions, either from natural
30 mutations or human manipulation.

The invention further includes variations of the prostate proteins disclosed infra which show comparable expression patterns or which include antigenic regions. Such mutants include deletions, insertions, inversions, repeats, and site substitutions. Guidance concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie, J.U., et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science* 247:1306-1310 (1990).

Of particular interest are substitutions of charged amino acids with another charged amino acid and with neutral or negatively charged amino acids. The latter results in proteins with reduced positive charge to improve the characteristics of the disclosed protein. The prevention of aggregation is highly desirable. Aggregation of proteins not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard et al., *Clin. Exp. Immunol.* 2:331-340 (1967); Robbins et al., *Diabetes* 36:838-845 (1987); Cleland et al., *Crit. Rev. Therapeutic Drug Carrier Systems* 10:307-377 (1993)).

Amino acids in the polypeptides of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science* 244: 1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as binding to a natural or synthetic binding partner. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith et al., *J Mol. Biol.* 224:899-904 (1992) and de Vos et al. *Science* 255: 306-312 (1992)).

As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein. Of course, the number of amino acid substitutions a skilled artisan would make depends on many factors, including those described above. Generally speaking, the number of substitutions for any given polypeptide will not be more than 50, 40, 30, 25, 20, 15, 10, 5 or 3.

Fusion Proteins

Fusion proteins comprising proteins or polypeptide fragments of the subject prostate tumor antigen can also be constructed. Fusion proteins are useful for generating antibodies against amino acid sequences and for use in various assay systems. For example, fusion proteins can be used to identify proteins which interact with a protein of the invention or which interfere with its biological function. Physical methods, such as protein affinity chromatography, or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can also be used for this purpose. Such methods are well known in the art and can also be used as drug screens. Fusion proteins comprising a signal sequence and/or a transmembrane domain of a protein according to the invention or a fragment thereof can be used to target other protein domains to cellular locations in which the domains are not normally found, such as bound to a cellular membrane or secreted extracellularly.

A fusion protein comprises two protein segments fused together by means of a peptide bond. As noted, these fragments may range in size from about 8 amino acids up to the full length of the protein.

The second protein segment can be a full-length protein or a polypeptide fragment. Proteins commonly used in fusion protein construction include β -galactosidase, β -glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags can be used in fusion protein constructions, including histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex a DNA binding domain (DBD) fusions, GAL4 DNA binding domain fusions, and herpes simplex virus (HSV) BP 16 protein fusions.

These fusions can be made, for example, by covalently linking two protein segments or by standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which comprises a coding sequence encoding a possible antigen according to the invention or a fragment thereof in proper reading frame with a nucleotide encoding the second protein segment and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion

proteins are available from companies that supply research labs with tools for experiments, including, for example, Promega Corporation (Madison, WI), Stratagene (La Jolla, CA), Clontech (Mountain View, CA), Santa Cruz Biotechnology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

Proteins, fusion proteins, or polypeptides of the invention can be produced by recombinant DNA methods. For production of recombinant proteins, fusion proteins, or polypeptides, a sequence encoding the protein can be expressed in prokaryotic or eukaryotic host cells using expression systems known in the art. These expression systems include bacterial, yeast, insect, and mammalian cells.

The resulting expressed protein can then be purified from the culture medium or from extracts of the cultured cells using purification procedures known in the art. For example, for proteins fully secreted into the culture medium, cell-free medium can be diluted with sodium acetate and contacted with a cation exchange resin, followed by hydrophobic interaction chromatography. Using this method, the desired protein or polypeptide is typically greater than 95% pure. Further purification can be undertaken, using, for example, any of the techniques listed above.

It may be necessary to modify a protein produced in yeast or bacteria, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain a functional protein. Such covalent attachments can be made using known chemical or enzymatic methods.

A protein or polypeptide of the invention can also be expressed in cultured host cells in a form which will facilitate purification. For example, a protein or polypeptide can be expressed as a fusion protein comprising, for example, maltose binding protein, glutathione-S-transferase, or thioredoxin, and purified using a commercially available kit. Kits for expression and purification of such fusion proteins are available from companies such as New England BioLabs, Pharmacia, and Invitrogen. Proteins, fusion proteins, or polypeptides can also be tagged with an epitope, such as a "Flag" epitope (Kodak), and purified using an antibody which specifically binds to that epitope.

The coding sequence of the protein variants identified through the sequences disclosed herein can also be used to construct transgenic animals, such as mice, rats, guinea pigs, cows, goats, pigs, or sheep. Female transgenic animals can then produce proteins, polypeptides, or

fusion proteins of the invention in their milk. Methods for constructing such animals are known and widely used in the art.

Alternatively, synthetic chemical methods, such as solid phase peptide synthesis, can be used to synthesize a secreted protein or polypeptide. General means for the production of peptides, analogs or derivatives are outlined in Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins -- A Survey of Recent Developments, B. Weinstein, ed. (1983). Substitution of D-amino acids for the normal L-stereoisomer can be carried out to increase the half-life of the molecule.

Typically, homologous polynucleotide sequences can be confirmed by hybridization under stringent conditions, as is known in the art. For example, using the following wash conditions: 2 x SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2 x SSC, 0.1% SDS, 50 °C once, 30 minutes; then 2 x SSC, room temperature twice, 10 minutes each, homologous sequences can be identified which contain at most about 25-30% basepair mismatches. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches.

The invention also provides polynucleotide probes which can be used to detect complementary nucleotide sequences, for example, in hybridization protocols such as Northern or Southern blotting or *in situ* hybridizations. Polynucleotide probes of the invention comprise at least 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, or 40 or more contiguous nucleotides of the nucleic acid sequences provided herein. Polynucleotide probes of the invention can comprise a detectable label, such as a radioisotopic, fluorescent, enzymatic, or chemiluminescent label.

Isolated genes corresponding to the cDNA sequences disclosed herein are also provided. Standard molecular biology methods can be used to isolate the corresponding genes using the cDNA sequences provided herein. These methods include preparation of probes or primers from the nucleotide sequence disclosed herein for use in identifying or amplifying the genes from mammalian, including human, genomic libraries or other sources of human genomic DNA.

Polynucleotide molecules of the invention can also be used as primers to obtain additional copies of the polynucleotides, using polynucleotide amplification methods. Polynucleotide molecules can be propagated in vectors and cell lines using techniques well

known in the art. Polynucleotide molecules can be on linear or circular molecules. They can be on autonomously replicating molecules or on molecules without replication sequences. They can be regulated by their own or by other regulatory sequences, as is known in the art.

5 *Polynucleotide Constructs*

Polynucleotide molecules comprising the coding sequences of the gene variants identified through the sequences disclosed herein can be used in a polynucleotide construct, such as a DNA or RNA construct. Polynucleotide molecules of the invention can be used, for example, in an expression construct to express all or a portion of a protein, variant, fusion
10 protein, or single-chain antibody in a host cell. An expression construct comprises a promoter which is functional in a chosen host cell. The skilled artisan can readily select an appropriate promoter from the large number of cell type-specific promoters known and used in the art. The expression construct can also contain a transcription terminator which is functional in the host cell. The expression construct comprises a polynucleotide segment which encodes all or a
15 portion of the desired protein. The polynucleotide segment is located downstream from the promoter. Transcription of the polynucleotide segment initiates at the promoter. The expression construct can be linear or circular and can contain sequences, if desired, for autonomous replication.

Also included are polynucleotide molecules comprising the promoter and UTR
20 sequences of the subject novel genes, operably linked to the associated protein coding sequence and/or other sequences encoding a detectable or selectable marker. Such promoter and/or UTR-based constructs are useful for studying the transcriptional and translational regulation of protein expression, and for identifying activating and/or inhibitory regulatory proteins.

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Host Cells

An expression construct can be introduced into a host cell. The host cell comprising the expression construct can be any suitable prokaryotic or eukaryotic cell. Expression systems in bacteria include those described in Chang *et al.*, *Nature* 275:615 (1978); Goeddel *et al.*, *Nature* 281: 544 (1979); Goeddel *et al.*, *Nucleic Acids Res.* 8:4057 (1980); EP 36,776;
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U.S. 4,551,433; deBoer *et al.*, *Proc. Natl. Acad. Sci. USA* 80: 21-25 (1983); and Siebenlist *et al.*, *Cell* 20: 269 (1980).

Expression systems in yeast include those described in Hinneken *et al.*, *Proc. Natl. Acad. Sci. USA* 75: 1929 (1978); Ito *et al.*, *J Bacteriol* 153: 163 (1983); Kurtz *et al.*, *Mol. Cell. Biol.* 6: 142 (1986); Kunze *et al.*, *J Basic Microbiol.* 25: 141 (1985); Gleeson *et al.*, *J. Gen. Microbiol.* 132: 3459 (1986), Roggenkamp *et al.*, *Mol. Gen. Genet.* 202: 302 (1986)); Das *et al.*, *J Bacteriol.* 158: 1165 (1984); De Louvencourt *et al.*, *J Bacteriol.* 154:737 (1983), Van den Berg *et al.*, *Bio/Technology* 8: 135 (1990); Kunze *et al.*, *J. Basic Microbiol.* 25: 141 (1985); Cregg *et al.*, *Mol. Cell. Biol.* 5: 3376 (1985); U.S. 4,837,148; U.S. 4,929,555; Beach and Nurse, *Nature* 300: 706 (1981); Davidow *et al.*, *Curr. Genet.* 10: 380 (1985); Gaillardin *et al.*, *Curr. Genet.* 10: 49 (1985); Ballance *et al.*, *Biochem. Biophys. Res. Commun.* 112: 284-289 (1983); Tilburn *et al.*, *Gene* 26: 205-22 (1983); Yelton *et al.*, *Proc. Natl. Acad. Sci. USA* 81: 1470-1474 (1984); Kelly and Hynes, *EMBO J.* 4: 475479 (1985); EP 244,234; and WO 91/00357.

Expression of heterologous genes in insects can be accomplished as described in U.S. 4,745,051; Friesen *et al.* (1986) "The Regulation of Baculovirus Gene Expression" in: THE MOLECULAR BIOLOGY OF BACULOVIRUSES (W. Doerfler, ed.); EP 127,839; EP 155,476; Vlak *et al.*, *J. Gen. Virol.* 69: 765-776 (1988); Miller *et al.*, *Ann. Rev. Microbiol.* 42: 177 (1988); Carbonell *et al.*, *Gene* 73: 409 (1988); Maeda *et al.*, *Nature* 315: 592-594 (1985); Lebacqz-Verheyden *et al.*, *Mol. Cell Biol.* 8: 3129 (1988); Smith *et al.*, *Proc. Natl. Acad. Sci. USA* 82: 8404 (1985); Miyajima *et al.*, *Gene* 58: 273 (1987); and Martin *et al.*, *DNA* 7:99 (1988). Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts are described in Luckow *et al.*, *Bio/Technology* (1988) 6: 47-55, Miller *et al.*, in GENETIC ENGINEERING (Setlow, J.K. *et al.* eds.), Vol. 8, pp. 277-279 (Plenum Publishing, 1986); and Maeda *et al.*, *Nature*, 315: 592-594 (1985).

Mammalian expression can be accomplished as described in Dijkema *et al.*, *EMBO J.* 4: 761(1985); Gorman *et al.*, *Proc. Natl. Acad. Sci. USA* 79: 6777 (1982b); Boshart *et al.*, *Cell* 41: 521 (1985); and U.S. 4,399,216. Other features of mammalian expression can be facilitated as described in Ham and Wallace, *Meth Enz.* 58: 44 (1979);

Expression constructs can be introduced into host cells using any technique known in the art. These techniques include transferrin-polycation-mediated DNA transfer, transfection

with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, "gene gun," and calcium phosphate-mediated transfection.

5 The invention can also include hybrid and modified forms thereof including fusion proteins, fragments and hybrid and modified forms in which certain amino acids have been deleted or replaced, modifications such as where one or more amino acids have been changed to a modified amino acid or unusual amino acid.

Also included within the meaning of substantially homologous is any human or non-human primate protein which may be isolated by virtue of cross-reactivity with antibodies to
10 proteins encoded by a gene described herein or whose encoding nucleotide sequences including genomic DNA, mRNA or cDNA may be isolated through hybridization with the complementary sequence of genomic or subgenomic nucleotide sequences or cDNA of a gene herein or fragments thereof. It will also be appreciated by one skilled in the art that degenerate DNA sequences can encode a tumor protein according to the invention and these are also
15 intended to be included within the present invention as are allelic variants of the subject genes.

Preferred is a prostate protein according to the invention prepared by recombinant DNA technology. By "pure form" or "purified form" or "substantially purified form" it is meant that a protein composition is substantially free of other proteins which are not the desired protein.

20 The present invention also includes therapeutic or pharmaceutical compositions comprising a protein according to the invention in an effective amount for treating patients with disease, and a method comprising administering a therapeutically effective amount of the protein. These compositions and methods are useful for treating cancers associated with the subject proteins, e.g. prostate cancer. One skilled in the art can readily use a variety of assays
25 known in the art to determine whether the protein would be useful in promoting survival or functioning in a particular cell type.

Anti-Prostate Antigen Antibodies

As noted, the invention includes the preparation and use of anti-prostate antigen antibodies and fragments for use as diagnostics and therapeutics. These antibodies may be
30 polyclonal or monoclonal. Polyclonal antibodies can be prepared by immunizing rabbits or other animals by injecting antigen followed by subsequent boosts at appropriate intervals. The

animals are bled and sera assayed against purified protein usually by ELISA or by bioassay based upon the ability to block the action of the corresponding gene. When using avian species, e.g., chicken, turkey and the like, the antibody can be isolated from the yolk of the egg. Monoclonal antibodies can be prepared after the method of Milstein and Kohler by fusing splenocytes from immunized mice with continuously replicating tumor cells such as myeloma or lymphoma cells. [Milstein and Kohler, *Nature* 256:495-497 (1975); Gutfre and Milstein, *Methods in Enzymology: Immunochemical Techniques* 73:1-46, Langone and Banatis eds., Academic Press, (1981) which are incorporated by reference]. The hybridoma cells so formed are then cloned by limiting dilution methods and supernates assayed for antibody production by ELISA, RIA or bioassay.

The unique ability of antibodies to recognize and specifically bind to target proteins provides an approach for treating an overexpression of the protein. Thus, another aspect of the present invention provides for a method for preventing or treating diseases involving overexpression of the protein by treatment of a patient with specific antibodies to the protein.

Specific antibodies, either polyclonal or monoclonal, to the protein can be produced by any suitable method known in the art as discussed above. For example, by recombinant methods, preferably in eukaryotic cells murine or human monoclonal antibodies can be produced by hybridoma technology or, alternatively, the protein, or an immunologically active fragment thereof, or an anti-idiotypic antibody, or fragment thereof can be administered to an animal to elicit the production of antibodies capable of recognizing and binding to the protein. Such antibodies can be from any class of antibodies including, but not limited to IgG, IgA, IgM, IgD, and IgE or in the case of avian species, IgY and from any subclass of antibodies.

The availability of isolated protein allows for the identification of small molecules and low molecular weight compounds that inhibit the binding of protein to binding partners, through routine application of high-throughput screening methods (HTS). HTS methods generally refer to technologies that permit the rapid assaying of lead compounds for therapeutic potential. HTS techniques employ robotic handling of test materials, detection of positive signals, and interpretation of data. Lead compounds may be identified via the incorporation of radioactivity or through optical assays that rely on absorbance, fluorescence or luminescence as read-outs. [Gonzalez, J.E. *et al.*, *Curr. Opin. Biotech.* 9:624-631 (1998)].

Model systems are available that can be adapted for use in high throughput screening for compounds that inhibit the interaction of protein with its ligand, for example by competing with protein for ligand binding. Sarubbi *et al.*, *Anal. Biochem.* 237:70-75 (1996) describe cell-free, non-isotopic assays for discovering molecules that compete with natural ligands for binding to the active site of IL-1 receptor. Martens, C. *et al.*, *Anal. Biochem.* 273:20-31 (1999) describe a generic particle-based nonradioactive method in which a labeled ligand binds to its receptor immobilized on a particle; label on the particle decreases in the presence of a molecule that competes with the labeled ligand for receptor binding.

Antibody Preparation

10 (i) Starting Materials and Methods

Immunoglobulins (Ig) and certain variants thereof are known and many have been prepared in recombinant cell culture. For example, see U.S. Pat. No. 4,745,055; EP 256,654; EP 120,694; EP 125,023; EP 255,694; EP 266,663; WO 30 88/03559; Faulkner *et al.*, *Nature*, 298: 286 (1982); Morrison, *J. Immunol.*, 123: 793 (1979); Koehler *et al.*, *Proc. Natl. Acad. Sci. USA*, 77: 2197 (1980); Raso *et al.*, *Cancer Res.*, 41: 2073 (1981); Morrison *et al.*, *Ann. Rev. Immunol.*, 2: 239 (1984); Morrison, *Science*, 229: 1202 (1985); and Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81: 6851 (1984). Reassorted immunoglobulin chains are also known. See, for example, U.S. Pat. No. 4,444,878; WO 88/03565; and EP 68,763 and references cited therein. The immunoglobulin moiety in the chimeras of the present invention may be obtained from IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA, IgE, IgD, or IgM, but preferably from IgG-1 or IgG-3.

(ii) Polyclonal Antibodies

Polyclonal antibodies to the subject prostate antigens are generally raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the antigen and an adjuvant. It may be useful to conjugate the antigen or a fragment containing the target amino acid sequence to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues),

glutaraldehyde or succinic anhydride.

Animals are immunized against the polypeptide or fragment, immunogenic conjugates, or derivatives by combining about 1 mg or 1 μ g of the peptide or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer to the antigen or a fragment thereof. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same polypeptide or fragment thereof, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

(iii) Monoclonal Antibodies

Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies.

For example, monoclonal antibodies using for practicing this invention may be made using the hybridoma method first described by Kohler and Milstein, *Nature*, 256: 495 (1975), or may be made by recombinant DNA methods (Cabilly et al., *supra*).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the antigen or fragment thereof used for immunization. Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 [Academic Press, 1986]).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, Calif. USA, and SP-2 cells available from the American Type Culture Collection, Rockville, Md. USA.

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the prostate antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., 107: 220 (1980).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, supra). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxyapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DNA encoding the monoclonal antibodies of the invention is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are

capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra et al., *Curr. Opin. in Immunol.*, 5: 256-262 (1993) and Pluckthun, *Immunol. Revs.*, 130: 151-188 (1992).

The DNA also may be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous murine sequences (Morrison, et al., *Proc. Natl. Acad. Sci. USA*, 81: 6851 [1984]), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. In that manner, "chimeric" or "hybrid" antibodies are prepared that have the binding specificity of an anti-prostate antigen monoclonal antibody herein.

Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody of the invention, or they are substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for prostate antigen according to the invention and another antigen-combining site having specificity for a different antigen.

Chimeric or hybrid antibodies also may be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide-exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

(iv) Humanized Antibodies

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be

essentially performed following the method of Winter and co-workers (Jones et al., *Nature* 321, 522-525 [1986]; Riechmann et al., *Nature* 332, 323-327 [1988]; Verhoeyen et al., *Science* 239, 1534-1536 [1988]), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (Cabilly et al., *supra*), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody (Sims et al., *J. Immunol.*, 151: 2296 [1993]; Chothia and Lesk, *J. Mol. Biol.*, 196: 901 [1987]). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter et al., *Proc. Natl. Acad. Sci. USA*, 89: 4285 [1992]; Presta et al., *J. Immunol.*, 151: 2623 [1993]).

It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity for the target

antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

(v) Human Antibodies

Human monoclonal antibodies can be made by the hybridoma method. Human
5 myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described, for example, by Kozbor, J. Immunol. 133, 3001 (1984); Brodeur, et al., Monoclonal Antibody Production Techniques and Applications, pp.51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., J. Immunol., 147: 86-95 (1991).

10 It is now possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production.
15 Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al., Proc. Natl. Acad. Sci. USA, 90: 2551 (1993); Jakobovits et al., Nature, 362: 255-258 (1993); Bruggermann et al., Year in Immuno., 7: 33 (1993).

Alternatively, the phage display technology (McCafferty et al., Nature, 348: 552-553
20 [1990]) can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from non-immunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous
25 particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats; for their review see, e.g., Johnson and Chiswell, Curr. Op. Struct. Biol., 3: 564-571 (1993). Several sources of V-gene segments can
30 be used for phage display. Clackson et al., Nature, 352: 624-628 (1991) isolated a diverse array

of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from non-immunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks et al., J. Mol. Biol., 222: 581-597 (1991), or Griffith et al., EMBO J., 12: 725-734 (1993).

In a natural immune response, antibody genes accumulate mutations at a high rate (somatic hypermutation). Some of the changes introduced will confer higher affinity, and B cells displaying high-affinity surface immunoglobulin are preferentially replicated and differentiated during subsequent antigen challenge. This natural process can be mimicked by employing the technique known as "chain shuffling" (Marks et al., Bio/Technology, 10: 779-783 [1992]). In this method, the affinity of "primary" human antibodies obtained by phage display can be improved by sequentially replacing the heavy and light chain V region genes with repertoires of naturally occurring variants (repertoires) of V domain genes obtained from non-immunized donors. This technique allows the production of antibodies and antibody fragments with affinities in the nM range. A strategy for making very large phage antibody repertoires has been described by Waterhouse et al., Nucl. Acids Res., 21: 2265-2266 (1993).

Gene shuffling can also be used to derive human antibodies from rodent antibodies, where the human antibody has similar affinities and specificities to the starting rodent antibody. According to this method, which is also referred to as "epitope imprinting", the heavy or light chain V domain gene of rodent antibodies obtained by phage display technique is replaced with a repertoire of human V domain genes, creating rodent-human chimeras. Selection on antigen results in isolation of human variable capable of restoring a functional antigen-binding site, i.e., the epitope governs (imprints) the choice of partner. When the process is repeated in order to replace the remaining rodent V domain, a human antibody is obtained (see PCT WO 93/06213, published Apr. 1, 1993). Unlike traditional humanization of rodent antibodies by CDR grafting, this technique provides completely human antibodies, which have no framework or CDR residues of rodent origin.

(vi) Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the

binding specificities will be to a prostate antigen according to the invention. Methods for making bispecific antibodies are known in the art.

Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains
5 have different specificities (Milstein and Cuello, *Nature*, 305: 537-539 [1983]). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar
10 procedures are disclosed in WO 93/08829 published May 13, 1993, and in Traunecker et al., *EMBO J.*, 10: 3655-3659 (1991).

According to a different and more preferred approach, antibody-variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant-domain sequences. The fusion preferably is with an
15 immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1), containing the site necessary for light-chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism.
20 This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the production of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of
25 no particular significance. In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin
30 chain combinations, as the presence of an immunoglobulin light chain in only one half of the

bispecific molecule provides for a facile way of separation.

For further details of generating bispecific antibodies, see, for example, Suresh et al., *Methods in Enzymology*, 121: 210 (1986).

(vii) Heteroconjugate Antibodies

5 Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Pat. No. 4,676,980), and for treatment of HIV infection (WO 91/00360; WO 92/00373; and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods.
10 Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Pat. No. 4,676,980, along with a number of cross-linking techniques.

The polynucleotides and polypeptides of the present invention may be utilized in gene delivery vehicles. The gene delivery vehicle may be of viral or non-viral origin (*see generally*, Jolly, *Cancer Gene Therapy* 1:51-64 (1994); Kimura, *Human Gene Therapy* 5:845-852
15 (1994); Connelly, *Human Gene Therapy* 1:185-193 (1995); and Kaplitt, *Nature Genetics* 6:148-153 (1994)). Gene therapy vehicles for delivery of constructs including a coding sequence of a therapeutic according to the invention can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches. Expression of such coding sequences can be induced using endogenous mammalian or heterologous
20 promoters. Expression of the coding sequence can be either constitutive or regulated. Preferred vehicles for gene therapy include retroviral and adeno-viral vectors.

Representative examples of adenoviral vectors include those described by Berkner, *Biotechniques* 6:616-627 (Biotechniques); Rosenfeld *et al.*, *Science* 252:431-434 (1991); WO 93/19191; Kolls *et al.*, *P.N.A.S.* 215-219 (1994); Kass-Bisleret *et al.*, *P.N.A.S.* 90: 11498-11502
25 (1993); Guzman *et al.*, *Circulation* 88: 2838-2848 (1993); Guzman *et al.*, *Cir. Res.* 73: 1202-1207 (1993); Zabner *et al.*, *Cell* 75: 207-216 (1993); Li *et al.*, *Hum. Gene Ther.* 4: 403-409 (1993); Cailaud *et al.*, *Eur. J. Neurosci.* 5: 1287-1291 (1993); Vincent *et al.*, *Nat. Genet.* 5: 130-134 (1993); Jaffe *et al.*, *Nat. Genet.* 1: 372-378 (1992); and Levrero *et al.*, *Gene* 101: 195-202 (1992). Exemplary adenoviral gene therapy vectors employable in this invention also
30 include those described in WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO

95/11984 and WO 95/00655. Administration of DNA linked to kill adenovirus as described in Curiel, *Hum. Gene Ther.* 3: 147-154 (1992) may be employed.

Other gene delivery vehicles and methods may be employed; including polycationic condensed DNA linked or unlinked to kill adenovirus alone, for example Curiel, *Hum. Gene Ther.* 3: 147-154 (1992); ligand-linked DNA, for example see Wu, *J. Biol. Chem.* 264: 16985-16987 (1989); eukaryotic cell delivery vehicles cells, for example see U.S. Serial No. 08/240,030, filed May 9, 1994, and U.S. Serial No. 08/404,796; deposition of photopolymerized hydrogel materials; hand-held gene transfer particle gun, as described in U.S. Patent No. 5,149,655; ionizing radiation as described in U.S. Patent No. 5,206,152 and in
10 WO 92/11033; nucleic charge neutralization or fusion with cell membranes. Additional approaches are described in Philip, *Mol. Cell Biol.* 14:2411-2418 (1994), and in Woffendin, *Proc. Natl. Acad. Sci.* 91:1581-1585 (1994).

Naked DNA may also be employed. Exemplary naked DNA introduction methods are described in WO 90/11092 and U.S. Patent No. 5,580,859. Uptake efficiency may be
15 improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved further by treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm. Liposomes that can act as gene delivery vehicles are described in U.S. Patent No. 5,422,120, PCT Patent Publication Nos. WO 95/13
20 796, WO 94/23697, and WO 91/14445, and EP No. 0 524 968.

Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin et al., *Proc. Natl. Acad. Sci. USA* 91(24): 11581-11585 (1994). Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional
25 methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun, as described in U.S. Patent No. 5,149,655; use of ionizing radiation for activating transferred gene, as described in U.S. Patent No. 5,206,152 and PCT Patent Publication No. WO 92/11033.

The subject antibodies or antibody fragments may be conjugated directly or indirectly
30 to effective moieties, e.g., radionuclides, toxins, chemotherapeutic agents, prodrugs, cytoslatic agents, enzymes and the like. In a preferred embodiment the antibody or fragment will be

attached to a therapeutic or diagnostic radiolabel directly or by use of a chelating agent. Examples of suitable radiolabels are well known and include ^{90}Y , ^{125}I , ^{131}I , ^{111}I , ^{105}Rh , ^{153}Sm , ^{67}Cu , ^{67}Ga , ^{166}Ho , ^{177}Lu , ^{186}Re and ^{188}Re .

5 Examples of suitable drugs that may be coupled to antibodies include methotrexate, adriamycine and lymphokines such as interferons, interleukins and the like. Suitable toxins which may be coupled include ricin, cholera and diphtheria toxin.

In a preferred embodiment, the subject antibodies will be attached to a therapeutic radiolabel and used for radioimmunotherapy.

Anti-sense Oligonucleotides

10 In certain circumstances, it may be desirable to modulate or decrease the amount of the protein expressed by a prostate cell. Thus, in another aspect of the present invention, anti-sense oligonucleotides can be made and a method utilized for diminishing the level of expression a prostate antigen according to the invention by a cell comprising administering one or more anti-sense oligonucleotides. By anti-sense oligonucleotides reference is made to
15 oligonucleotides that have a nucleotide sequence that interacts through base pairing with a specific complementary nucleic acid sequence involved in the expression of the target such that the expression of the gene is reduced. Preferably, the specific nucleic acid sequence involved in the expression of the gene is a genomic DNA molecule or mRNA molecule that encodes the gene. This genomic DNA molecule can comprise regulatory regions of the gene,
20 or the coding sequence for the mature gene.

The term complementary to a nucleotide sequence in the context of antisense oligonucleotides and methods therefor means sufficiently complementary to such a sequence as to allow hybridization to that sequence in a cell, *i.e.*, under physiological conditions. Antisense oligonucleotides preferably comprise a sequence containing from about 8 to about
25 100 nucleotides and more preferably the antisense oligonucleotides comprise from about 15 to about 30 nucleotides. Antisense oligonucleotides can also contain a variety of modifications that confer resistance to nucleolytic degradation such as, for example, modified internucleoside linkages [Uhlmann and Peyman, *Chemical Reviews* 90:543-548 (1990); Schneider and Banner, *Tetrahedron Lett.* 31:335, (1990) which are incorporated by reference],
30 modified nucleic acid bases as disclosed in 5,958,773 and patents disclosed therein, and/or sugars and the like.

Any modifications or variations of the antisense molecule which are known in the art to be broadly applicable to antisense technology are included within the scope of the invention.

Such modifications include preparation of phosphorus-containing linkages as disclosed in U.S. Patents 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361, 5,625,050 and
5 5,958,773.

The antisense compounds of the invention can include modified bases. The antisense oligonucleotides of the invention can also be modified by chemically linking the oligonucleotide to one or more moieties or conjugates to enhance the activity, cellular distribution, or cellular uptake of the antisense oligonucleotide. Such moieties or conjugates
10 include lipids such as cholesterol, cholic acid, thioether, aliphatic chains, phospholipids, polyamines, polyethylene glycol (PEG), palmityl moieties, and others as disclosed in, for example, U.S. Patents 5,514,758, 5,565,552, 5,567,810, 5,574,142, 5,585,481, 5,587,371, 5,597,696 and 5,958,773.

Chimeric antisense oligonucleotides are also within the scope of the invention, and can
15 be prepared from the present inventive oligonucleotides using the methods described in, for example, U.S. Patents 5,013,830, 5,149,797, 5,403,711, 5,491,133, 5,565,350, 5,652,355, 5,700,922 and 5,958,773.

In the antisense art a certain degree of routine experimentation is required to select optimal antisense molecules for particular targets. To be effective, the antisense molecule
20 preferably is targeted to an accessible, or exposed, portion of the target RNA molecule. Although in some cases information is available about the structure of target mRNA molecules, the current approach to inhibition using antisense is via experimentation. mRNA levels in the cell can be measured routinely in treated and control cells by reverse transcription of the mRNA and assaying the cDNA levels. The biological effect can be determined
25 routinely by measuring cell growth or viability as is known in the art.

Measuring the specificity of antisense activity by assaying and analyzing cDNA levels is an art-recognized method of validating antisense results. It has been suggested that RNA from treated and control cells should be reverse-transcribed and the resulting cDNA populations analyzed. [Branch, A. D., *T.I.B.S.* 23:45-50 (1998)].

30 The therapeutic or pharmaceutical compositions of the present invention can be administered by any suitable route known in the art including for example intravenous,

subcutaneous, intramuscular, transdermal, intrathecal or intracerebral. Administration can be either rapid as by injection or over a period of time as by slow infusion or administration of slow release formulation.

Additionally, the subject prostate tumor proteins can also be linked or conjugated with agents that provide desirable pharmaceutical or pharmacodynamic properties. For example, the protein can be coupled to any substance known in the art to promote penetration or transport across the blood-brain barrier such as an antibody to the transferrin receptor, and administered by intravenous injection (see, for example, Friden et al., *Science* 259:373-377 (1993) which is incorporated by reference). Furthermore, the subject protein A or protein B can be stably linked to a polymer such as polyethylene glycol to obtain desirable properties of solubility, stability, half-life and other pharmaceutically advantageous properties. [See, for example, Davis et al., *Enzyme Eng.* 4:169-73 (1978); Buruham, *Am. J. Hosp. Pharm.* 51:210-218 (1994) which are incorporated by reference].

The compositions are usually employed in the form of pharmaceutical preparations. Such preparations are made in a manner well known in the pharmaceutical art. See, e.g. Remington Pharmaceutical Science, 18th Ed., Merck Publishing Co. Eastern PA, (1990). One preferred preparation utilizes a vehicle of physiological saline solution, but it is contemplated that other pharmaceutically acceptable carriers such as physiological concentrations of other non-toxic salts, five percent aqueous glucose solution, sterile water or the like may also be used. It may also be desirable that a suitable buffer be present in the composition. Such solutions can, if desired, be lyophilized and stored in a sterile ampoule ready for reconstitution by the addition of sterile water for ready injection. The primary solvent can be aqueous or alternatively non-aqueous. The subject prostate tumor antigens, fragments or variants thereof can also be incorporated into a solid or semi-solid biologically compatible matrix which can be implanted into tissues requiring treatment.

The carrier can also contain other pharmaceutically-acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation. Similarly, the carrier may contain still other pharmaceutically-acceptable excipients for modifying or maintaining release or absorption or penetration across the blood-brain barrier. Such excipients are those substances usually and customarily employed to formulate dosages for parental administration in either unit dosage or

multi-dose form or for direct infusion into the cerebrospinal fluid by continuous or periodic infusion.

Dose administration can be repeated depending upon the pharmacokinetic parameters of the dosage formulation and the route of administration used.

5 It is also contemplated that certain formulations containing the subject antibody or nucleic acid antagonists are to be administered orally. Such formulations are preferably encapsulated and formulated with suitable carriers in solid dosage forms. Some examples of suitable carriers, excipients, and diluents include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, calcium silicate, microcrystalline cellulose, 10 polyvinylpyrrolidone, cellulose, gelatin, syrup, methyl cellulose, methyl- and propylhydroxybenzoates, talc, magnesium, stearate, water, mineral oil, and the like. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions may be formulated so as to provide rapid, sustained, or delayed release of the 15 active ingredients after administration to the patient by employing procedures well known in the art. The formulations can also contain substances that diminish proteolytic degradation and promote absorption such as, for example, surface active agents.

The specific dose is calculated according to the approximate body weight or body surface area of the patient or the volume of body space to be occupied. The dose will also be 20 calculated dependent upon the particular route of administration selected. Further refinement of the calculations necessary to determine the appropriate dosage for treatment is routinely made by those of ordinary skill in the art. Such calculations can be made without undue experimentation by one skilled in the art in light of the activity disclosed herein in assay preparations of target cells. Exact dosages are determined in conjunction with standard dose- 25 response studies. It will be understood that the amount of the composition actually administered will be determined by a practitioner, in the light of the relevant circumstances including the condition or conditions to be treated, the choice of composition to be administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the chosen route of administration.

30 In one embodiment of this invention, the protein may be therapeutically administered by implanting into patients vectors or cells capable of producing a biologically-active form of

the protein or a precursor of protein, *i.e.*, a molecule that can be readily converted to a biological-active form of the protein by the body. In one approach, cells that secrete the protein may be encapsulated into semipermeable membranes for implantation into a patient. The cells can be cells that normally express the protein or a precursor thereof or the cells can
5 be transformed to express the protein or a precursor thereof. It is preferred that the cell be of human origin and that the protein be a human protein when the patient is human. However, it is anticipated that non-human primate homologues of the protein discussed *infra* may also be effective.

Detection of Subject Prostate Proteins or Nucleic Acids

10 In a number of circumstances it would be desirable to determine the levels of protein or corresponding mRNA in a patient. Evidence disclosed *infra* suggests the subject prostate proteins may be expressed at different levels during some diseases, e.g., cancers, provides the basis for the conclusion that the presence of these proteins serves a normal physiological function related to cell growth and survival. Endogenously produced protein according to the
15 invention may also play a role in certain disease conditions.

The term "detection" as used herein in the context of detecting the presence of protein in a patient is intended to include the determining of the amount of protein or the ability to express an amount of protein in a patient, the estimation of prognosis in terms of probable outcome of a disease and prospect for recovery, the monitoring of the protein levels over a
20 period of time as a measure of status of the condition, and the monitoring of protein levels for determining a preferred therapeutic regimen for the patient, e.g. one with prostate cancer.

To detect the presence of a prostate protein according to the invention in a patient, a sample is obtained from the patient. The sample can be a tissue biopsy sample or a sample of blood, plasma, serum, CSF, urine or the like. It has been found that the subject proteins are
25 expressed at high levels in some cancers. Samples for detecting protein can be taken from prostate tissues. When assessing peripheral levels of protein, it is preferred that the sample be a sample of blood, plasma or serum. When assessing the levels of protein in the central nervous system a preferred sample is a sample obtained from cerebrospinal fluid or neural tissue. The sample may be obtained by non-invasive methods, such as from tissue collection(s)
30 or culture(s), or using directly available tissue material (urine, saliva, stools, hair, etc.).

In some instances, it is desirable to determine whether the gene is intact in the patient or in a tissue or cell line within the patient. By an intact gene, it is meant that there are no alterations in the gene such as point mutations, deletions, insertions, chromosomal breakage, chromosomal rearrangements and the like wherein such alteration might alter production of the corresponding protein or alter its biological activity, stability or the like to lead to disease processes. Thus, in one embodiment of the present invention a method is provided for detecting and characterizing any alterations in the gene. The method comprises providing an oligonucleotide that contains the gene, genomic DNA or a fragment thereof or a derivative thereof. By a derivative of an oligonucleotide, it is meant that the derived oligonucleotide is substantially the same as the sequence from which it is derived in that the derived sequence has sufficient sequence complementarity to the sequence from which it is derived to hybridize specifically to the gene. The derived nucleotide sequence is not necessarily physically derived from the nucleotide sequence, but may be generated in any manner including for example, chemical synthesis or DNA replication or reverse transcription or transcription.

Typically, patient genomic DNA is isolated from a cell sample from the patient and digested with one or more restriction endonucleases such as, for example, TaqI and AluI. Using the Southern blot protocol, which is well known in the art, this assay determines whether a patient or a particular tissue in a patient has an intact prostate gene according to the invention or a gene abnormality.

Hybridization to a gene would involve denaturing the chromosomal DNA to obtain a single-stranded DNA; contacting the single-stranded DNA with a gene probe associated with the gene sequence; and identifying the hybridized DNA-probe to detect chromosomal DNA containing at least a portion of a gene.

The term "probe" as used herein refers to a structure comprised of a polynucleotide that forms a hybrid structure with a target sequence, due to complementarity of probe sequence with a sequence in the target region. Oligomers suitable for use as probes may contain a minimum of about 8-12 contiguous nucleotides which are complementary to the targeted sequence and preferably a minimum of about 20.

A gene according to the present invention can be DNA or RNA oligonucleotides and can be made by any method known in the art such as, for example, excision, transcription or chemical synthesis. Probes may be labeled with any detectable label known in the art such as,

for example, radioactive or fluorescent labels or enzymatic marker. Labeling of the probe can be accomplished by any method known in the art such as by PCR, random priming, end labeling, nick translation or the like. One skilled in the art will also recognize that other methods not employing a labeled probe can be used to determine the hybridization. Examples
5 of methods that can be used for detecting hybridization include Southern blotting, fluorescence in situ hybridization, and single-strand conformation polymorphism with PCR amplification.

Hybridization is typically carried out at 25° - 45° C, more preferably at 32° -40° C and more preferably at 37° - 38° C. The time required for hybridization is from about 0.25 to about 96 hours, more preferably from about one to about 72 hours, and most preferably from about 4
10 to about 24 hours.

Gene abnormalities can also be detected by using the PCR method and primers that flank or lie within the gene. The PCR method is well known in the art. Briefly, this method is performed using two oligonucleotide primers which are capable of hybridizing to the nucleic acid sequences flanking a target sequence that lies within a gene and amplifying the target
15 sequence. The terms "oligonucleotide primer" as used herein refers to a short strand of DNA or RNA ranging in length from about 8 to about 30 bases. The upstream and downstream primers are typically from about 20 to about 30 base pairs in length and hybridize to the flanking regions for replication of the nucleotide sequence. The polymerization is catalyzed by a DNA-polymerase in the presence of deoxynucleotide triphosphates or nucleotide analogs to
20 produce double-stranded DNA molecules. The double strands are then separated by any denaturing method including physical, chemical or enzymatic. Commonly, a method of physical denaturation is used involving heating the nucleic acid, typically to temperatures from about 80°C to 105°C for times ranging from about 1 to about 10 minutes. The process is repeated for the desired number of cycles.

25 The primers are selected to be substantially complementary to the strand of DNA being amplified. Therefore, the primers need not reflect the exact sequence of the template, but must be sufficiently complementary to selectively hybridize with the strand being amplified.

After PCR amplification, the DNA sequence comprising the gene or a fragment thereof is then directly sequenced and analyzed by comparison of the sequence with the sequences
30 disclosed herein to identify alterations which might change activity or expression levels or the like.

In another embodiment, a method for detecting a tumor protein according to the invention is provided based upon an analysis of tissue expressing the gene. Certain tissues such as prostate tissues have been found to overexpress the subject gene. The method comprises hybridizing a polynucleotide to mRNA from a sample of tissue that normally
5 expresses the gene. The sample is obtained from a patient suspected of having an abnormality in the gene.

To detect the presence of mRNA encoding the protein, a sample is obtained from a patient. The sample can be from blood or from a tissue biopsy sample. The sample may be treated to extract the nucleic acids contained therein. The resulting nucleic acid from the
10 sample is subjected to gel electrophoresis or other size separation techniques.

The mRNA of the sample is contacted with a DNA sequence serving as a probe to form hybrid duplexes. The use of a labeled probes as discussed above allows detection of the resulting duplex.

When using the cDNA encoding the protein or a derivative of the cDNA as a probe,
15 high stringency conditions can be used in order to prevent false positives, that is the hybridization and apparent detection of the gene nucleotide sequence when in fact an intact and functioning gene is not present. When using sequences derived from the gene cDNA, less stringent conditions could be used, however, this would be a less preferred approach because of the likelihood of false positives. The stringency of hybridization is determined by a number
20 of factors during hybridization and during the washing procedure, including temperature, ionic strength, length of time and concentration of formamide. These factors are outlined in, for example, Sambrook et al. [Sambrook et al. (1989), *supra*].

In order to increase the sensitivity of the detection in a sample of mRNA encoding the protein A or protein B, the technique of reverse transcription/ polymerization chain reaction
25 (RT/PCR) can be used to amplify cDNA transcribed from mRNA encoding the prostate tumor antigen. The method of RT/PCR is well known in the art, and can be performed as follows. Total cellular RNA is isolated by, for example, the standard guanidium isothiocyanate method and the total RNA is reverse transcribed. The reverse transcription method involves synthesis of DNA on a template of RNA using a reverse transcriptase enzyme and a 3' end primer.
30 Typically, the primer contains an oligo(dT) sequence. The cDNA thus produced is then amplified using the PCR method and gene A or gene B specific primers. [Belyavsky et al.,

Nucl. Acid Res. 17:2919-2932 (1989); Krug and Berger, *Methods in Enzymology*, 152:316-325, Academic Press, NY (1987) which are incorporated by reference].

The polymerase chain reaction method is performed as described above using two oligonucleotide primers that are substantially complementary to the two flanking regions of the DNA segment to be amplified. Following amplification, the PCR product is then electrophoresed and detected by ethidium bromide staining or by phosphoimaging.

The present invention further provides for methods to detect the presence of the protein in a sample obtained from a patient. Any method known in the art for detecting proteins can be used. Such methods include, but are not limited to immunodiffusion, immunoelectrophoresis, immunochemical methods, binder-ligand assays, immunohistochemical techniques, agglutination and complement assays. [*Basic and Clinical Immunology*, 217-262, Sites and Terr, eds., Appleton & Lange, Norwalk, CT, (1991), which is incorporated by reference]. Preferred are binder-ligand immunoassay methods including reacting antibodies with an epitope or epitopes of the prostate tumor antigen protein and competitively displacing a labeled prostate antigen according to the invention or derivative thereof.

As used herein, a derivative of the subject prostate tumor antigen is intended to include a polypeptide in which certain amino acids have been deleted or replaced or changed to modified or unusual amino acids wherein the derivative is biologically equivalent to gene and wherein the polypeptide derivative cross-reacts with antibodies raised against the protein. By cross-reaction it is meant that an antibody reacts with an antigen other than the one that induced its formation.

Numerous competitive and non-competitive protein binding immunoassays are well known in the art. Antibodies employed in such assays may be unlabeled, for example as used in agglutination tests, or labeled for use in a wide variety of assay methods. Labels that can be used include radionuclides, enzymes, fluorescers, chemiluminescers, enzyme substrates or co-factors, enzyme inhibitors, particles, dyes and the like for use in radioimmunoassay (RIA), enzyme immunoassays, e.g., enzyme-linked immunosorbent assay (ELISA), fluorescent immunoassays and the like.

A further aspect of this invention relates to a method for selecting, identifying, screening, characterizing or optimizing biologically active compounds, comprising a determination of whether a candidate compound binds, preferably selectively, a target molecule as disclosed above. Such target molecules include nucleic acid sequences, polypeptides and fragments thereof, typically prostate-specific antigens, even more preferably extracellular portions thereof. Binding may be assessed in vitro or in vivo, typically in vitro, in cell based or acellular systems. Typically, the target molecule is contacted with the candidate compound in any appropriate device, and the formation of a complex is determined. The target molecule and/or the candidate compound may be immobilized on a support. The compounds identified or selected represent drug candidates or leads for treating cancer diseases, particularly prostate cancer.

While the invention has been described supra, including preferred embodiments, the following examples are provided to further illustrate the invention.

EXAMPLE

Tissue Sources:

Appropriate patient samples were procured for evaluation of research protocol. Samples were provided with relevant clinical parameters, and patient consent. Histological assessment was performed on all samples and diagnosis by pathology confirmed the presence and/or absence of malignancy within each sample. Clinical data generally included patient history, physiopathology, and parameters relating to prostate cancer physiology. Ten normal and ten malignant samples were procured along with available clinical information. In addition, ten samples from organs other than normal prostate and prostate cancer were procured to determine the tissue specific expression profile of epitopes. RNA derived from normal tissue samples was obtained from known commercial sources.

Generation of the DATAS Library

Samples were pooled based on their pathological diagnosis (normal vs. tumor). Samples were pooled based on equivalent amounts of total RNA to produce total pooled RNA samples of 100ug. DATAS libraries were constructed as previously disclosed in U.S. Patent

No. 6,251,590, the disclosure of which is incorporated by reference in its entirety. Briefly, total RNA was isolated from the normal and tumor pooled samples and mRNA was subsequently purified from the total RNA for each pooled sample. Synthesis of cDNA was performed using a biotinylated oligo (dT) primer. The biotinylated cDNA was hybridized with the mRNA of the opposite sample to form heteroduplexes between the cDNA and the mRNA. For example, the biotinylated cDNA of the pooled normal prostate sample was hybridized with prostate tumor mRNA. Similarly, prostate tumor biotinylated cDNA was hybridized with prostate normal RNA to generate the second DATAS library. Streptavidin coated beads were used to purify the complexes by binding the biotin present on the cDNA. The heteroduplexes were digested with RNase H to degrade the RNA that was complementary to the cDNA. All mRNA sequences that were different from the cDNA remained intact. These single stranded RNA fragments or "loops" were subsequently amplified with degenerate primers and cloned into either pGEM-Tor pCR II TOPO vector (Company source) to produce the DATAS library.

15 **Clone sequencing and Bioinformatics Analysis:**

The DATAS library was used to transform *E. coli* so that individual clones could be isolated using standard molecular biology techniques. From these libraries, 10,665 individual clones were isolated and sequenced using an automated Applied Biosystems 3100 sequencer. The nucleotide sequences that were obtained were submitted to the bioinformatics pipeline for analysis. As the DATAS library is prepared with PCR amplified DNA, many copies of the same sequence are present in the clones isolated from the libraries. Therefore it is important to reduce the redundancy of the clones to identify the number of unique, nonrepeating sequences that are isolated. From this large set of DATAS fragments, 1699 unique, nonredundant sequences were identified and each DATAS fragment was annotated with a candidate gene. The annotation was performed by aligning the DATAS fragment to the human genome sequence by two methods; 1) a publicly available alignment and genome viewer tool, Blat (Kent et al., 2002); and 2) a commercially available genomic alignment and viewer tool, Prophecy (Doubletwise). Each DATAS fragment sequence was annotated with a corresponding gene that overlapped the genomic sequence containing the DATAS fragment. Genes were annotated with either the RefSeq accession number, or a hypothetical gene prediction from different algorithms, for example, Genscan, Twinscan, or Fgenesh++.

- Identified genes were either matched to the sequence of the DATAS fragment (in case of exon to fragment match), or overlapped with the DATAS fragment (in case of intron to fragment match), and the full length sequence of the gene was identified. These sequences were further analyzed to detect all potential membrane spanning proteins. Membrane proteins were predicted through the use of different algorithms publicly available. For example, TMHMM (CBS) was used to identify membrane-spanning domains present within the amino acid sequence of the candidate gene. DATAS fragments were located within the sequence in an attempt to determine whether the spliced event affected intracellular or extracellular domains. Genes associated with the sequence were ranked in order to maximize the identification of successful therapeutic targets. The highest priority genes had characteristics where the gene was a known membrane protein, the function of the gene was known, and the DATAS fragment mapped to an intron on the extracellular domain of the protein, indicating that the DATAS fragment would be presented outside the cell, and available for therapeutic intervention by monoclonal antibodies.
- Based on the bioinformatic analysis, clones were prioritized in three groups:
- A) Known transmembrane genes with DATAS fragments located in introns on the extracellular domain.
 - B) Known and predicted transmembrane genes with DATAS fragments located in exons in either the extracellular or intracellular domain.
 - C) DATAS fragments that did not match the genome

Expression Monitoring:

- A valid epitope target for prostate cancer requires that the expression of the epitope be limited to prostate tissue, or preferably to prostate tumors. Assessment of the expression profile for each prioritized sequence was performed by RT-PCR, a procedure well known in the art. A protocol known as touchdown PCR was used, described in the user's manual for the GeneAmp PCR system 9700, Applied Biosystems. Briefly, PCR primers were designed to the DATAS fragment and used for end point RT-PCR analysis. Each RT reaction contained 5 µg of total RNA and was performed in a 100 µl volume using Archive RT Kit (Applied Biosystems). The RT reactions were diluted 1:50 with water and 4 µl of the diluted stock was used in a 50 µl PCR reaction consisting of one cycle at 94°C for 3 min, 5 cycles at 94°C for 30

seconds, 60°C for 30 seconds and 72°C for 45 seconds, with each cycle reducing the annealing temperature by 0.5 degree. This was followed by 30 cycles at 94°C for 30 seconds, 55°C for 30 seconds, and 72 °C for 45 seconds. 15 µl was removed from each reaction for analysis and the reactions were allowed to proceed for an additional 10 cycles. This produced reactions for analysis at 30 and 40 cycles, and allowed the detection of differences in expression where the 40 cycle reactions had saturated. The level of expression profile of the DATAS fragment was determined in normal and tumor prostate total RNA, as well as total RNA from normal samples of brain, heart, liver, lung, kidney, colon, bone marrow, muscle, spleen, and testis. Expression profiles were prioritized accordingly for specific expression in prostate tumor and low expression found in normal tissues, including normal prostate.

Verification of RNA Structure:

DATAS identifies sequences that are altered between the experimental samples. However, the exact sequence of the junctions or borders that the DATAS fragment represents can not be determined directly from the isolated DATAS fragment sequence. The DATAS fragment was used, however, to design experiments that elucidate the sequence of each transcript present in each sample. Primers were designed to amplify a region of the gene larger than the proposed DATAS fragment sequence. These amplicons were subsequently cloned and sequenced for the identification of the exact junctions of all exons and introns. This required partial cloning of the isoforms from an identified sample to verify the primary structure (sequence) of the isoforms. All twenty samples (10 normal and 10 tumor samples) initially used to generate the DATAS libraries were used for the verification of the mRNA structure of the prioritized genes.

Isolation of full-length clones of isoforms:

Isolation of the full-length clones containing both isoforms was accomplished utilizing the information and DNA fragments generated during the structure validation process. Several methods are applicable to isolation of the full length clone. Where full sequence information regarding the coding sequence is available, gene specific primers were designed from the sequence and used to amplify the coding sequence directly from the total RNA of the tissue samples. An RT-PCR reaction was set up using these gene specific primers. The RT reaction

was performed as described infra, using oligo dT to prime for cDNA. Second strand was produced by standard methods to produce double stranded cDNA. PCR amplification of the gene was accomplished using gene specific primers. PCR consisted of 30 cycles at 94°C for 30 seconds, 55°C for 30 seconds, and 72 °C for 45 seconds. The reaction products were
5 analyzed on 1% agarose gels and the amplicons were ligated into prepared vectors with A overhangs for amplicon cloning. 1 µl of the ligation mixture was used to transform E. coli for cloning and isolation of the amplicon. Once purified, the plasmid containing the amplicon was sequenced on an ABI 3100 automated sequencer.

10 Where limited sequence information was available, the oligo pulling method was utilized. Briefly, a gene-specific oligonucleotide was designed based on the DATAS fragment. The oligonucleotide was labeled with biotin and used to hybridize with a single stranded plasmid DNA library prepared from either normal prostate tissue or prostate tumor tissue following the procedures of Sambrook et al (1989). The hybridized cDNA was separated by streptavidin
15 conjugated beads and eluted by heating. The eluted cDNA was converted to double strand plasmid DNA and used to transform E. coli cells and the longest cDNA clone was subjected to DNA sequencing.

RESULTS

20 Using methods described above, 1699 DNA fragments have been identified that putatively correspond to exons (novel splice variants) expressed exclusively or at an increased level in prostate tumor tissue when compared to matched normal prostate tissue.

These sequences were used to search public databases containing human genomic
25 sequences to identify related genes. This search identified 122 fragments that correspond to exons of either known or potential cell surface proteins.

Additionally, thirty seven distinct alternatively spliced isoforms were identified from the initial sequence tags that appear to contain novel sequence information of cell surface proteins.

30 These DNA sequences are disclosed in the Sequence Listing as well as in Table 1, and correspond to the nucleic acid sequences having SEQ ID NOS: 1-173, 175, 177, 179, and 181. Oligonucleotide primers were designed to each DATAS fragment to determine the specific

expression of the mRNA in a panel of normal human tissue. An example is shown in figure 1, where the clone corresponding to Sequence ID: No. 92 displays specific expression in prostate with very low levels detected in kidney (lane 4) and pancreas (lane 9). All clones that were found to be either specifically expressed in prostate or highly expressed in prostate compared to other tissues were analyzed for expression in tumor samples.

Figure 2 illustrates the expression profile of one DATAS clone in normal and tumor prostate tissue. Expression of this clone is upregulated in two of the three tumor pooled samples and is highly expressed in three of the four individual tumor samples. The high expression of this splice event in tumor samples as compared to normal prostate, and the low expression in other normal human tissues is an example of one candidate that has utility for development as a novel epitope for prostate cancer.

The splice events for DATAS clones that displayed a specific expression profile for prostate and a high differential expression profile for prostate tumors were isolated and the sequences for each event was determined. An example is shown in figure 3, where the sequence of the isolated event was mapped to the genome in Blat, and genomic viewer developed by the bioinformatics department at UCSC (Kent et al., 2002). Five distinct clones were isolated that mapped to the gene locus for STEAP2. One expressed sequence tag (EST), AK092666, contained many similar domains as the splice events that were isolated using DATAS. The sequences and predicted protein translations for all five clones are described in SEQ NOS. 173 – 182 and are graphically illustrated in figure 3. The length of the open reading frame and the predicted protein size for each isoform is described in Table 2. The EST, AK092666 contains a large deletion in exon 5, the terminal 3' exon of STEAP2, with two novel exons in the 5' region of the transcript. The nomenclature for the DATAS derived events was based on AK092666 because of higher similarity when compared to the RefSeq sequence for STEAP2. The first isoform identified, AK092666_01 (SEQ ID NO 173), contains a novel C-terminal exon when compared to AK092666, and therefore generates a novel junction, and a novel sequence for translation and generates a unique amino acid sequence (SEQ ID NO 183). The same novel sequence was generated by isoform AK092666_03 (SEQ ID NO 177), which contains the same novel exon with an additional splicing event of an in frame truncation of exon 4, and by isoform AK092666_05 (SEQ ID NO 181), which contains a single codon deletion from AK092666_01. AK092666_02 (SEQ ID

NO 175) skipped exon 6 of AK092666 and generated the novel amino acid sequence in SEQ ID NO 184. AK092666_04 (SEQ ID NO 179) contains a short out of frame truncation of exon 4, which results in the creation of 8 novel amino acids before encountering a premature stop codon (SEQ ID NO 185).

5

Table 2. Length of the open reading frame and the predicted protein size for each novel isoform.

Clone Name	ORF length (bp)	Protein size (KD)
STEAP2	1473	56
AK092666	1365	51.7
AK092666_01	1389	52.7
AK092666_02	1260	47.8
AK092666_03	900	34.1
AK092666_04	705	26.7
AK092666_05	1386	52.5

10 The novel amino acids found in SEQ ID NOS 183 and 184 represent novel epitopes that are specifically expressed in prostate cancer in a membrane protein. These epitopes are targets for monoclonal antibody immunotherapy for the treatment of prostate cancer. To illustrate the different isoforms present, an antibody was generated from the invariant sequence present in the 5' region (or the amino terminal portion of the protein) that recognizes all the
15 different isoforms.

An antibody was generated against an amino acid sequence that was common to all five isoforms, as well as present in STEAP2 and AK092666. Prostate cancer cell lines were analyzed by western blot to determine what different isoforms would be expressed at the protein level. Figure 4 illustrates two bands that were specifically detected by the antibody.
20 Band A potentially represents the glycosylated, wild type STEAP2 and band B indicates isoforms AK092666, AK092666_01, or AK092666_05, which is unresolvable in the gel analysis. In addition, multiple bands of the proper size were detected suggesting that isoforms of the STEAP2 locus are expressed and represent targets for the immunotherapy in prostate cancer.

25

Table 1. Sequence information of the DATAS fragments and the alternatively spliced isoforms.

- 5 Sequence ID: No. 1
 Accession #: NM_005656
 Genomic sequence: chr21:39407238-39450894
 Sequence definition: transmembrane protease serine 2
- 10 Sequence ID: No. 2
 Accession #: NM_001423
 Genomic sequence: chr12:13265134-13265266
 Sequence definition: Homo sapiens epithelial membrane protein 1 EMP1
- 15 Sequence ID: No. 3
 Accession #: NM_000484
 Genomic sequence: chr21:23832850-24123073
 Sequence definition: beta amyloid A4
- 20 Sequence ID: No. 4
 Accession #: NM_002841
 Genomic sequence: chr3:62548596-63240788_1
 Sequence definition: protein tyrosine phosphatase G-type
- 25 Sequence ID: No. 5
 Accession #: NM_022124
 Genomic sequence: chr10:74968313-75112962
 Sequence definition: cadherin related 23 isoform 1 precursor
- 30 Sequence ID: No. 6
 Accession #: NM_033056
 Genomic sequence: chr10:55940286-56920530_02
 Sequence definition: protocadherin 15 precursor
- 35 Sequence ID: No. 7
 Accession #: NM_002847
 Genomic sequence: chr7:158586667-159621018_01
 Sequence definition: protein tyrosine phosphatase receptor type N
- 40 Sequence ID: No. 8
 Accession #: NM_002222
 Genomic sequence: chr3:5000696-5354641_1
 Sequence definition: ITPR inositol 145-triphosphate receptor type 1
- 45 Sequence ID: No. 9
 Accession #: AC078864.20
 Genomic sequence: chr12:52201280-52201714
 Sequence definition: Genscan prediction
- 50 Sequence ID: No. 10
 Accession #: NM_014554; NM_001844; NT_009785.3
 Genomic sequence: chr12:45785273-45856561
 Sequence definition: chr12_498 potential fusion of SENP1 and Collagen 2A;
 also overlaps GS prediction
- 55 Sequence ID: No. 11
 Accession #: AB064665
 Genomic sequence: chrM:9411-9524
 Sequence definition: Homo sapiens mRNA for OK/SW-CL.16

- Sequence ID: No. 12
Accession #: NM_024029
Genomic sequence: chr19:10880041-10883719
5 Sequence definition: hypothetical protein MGC3262
- Sequence ID: No. 13
Accession #: NT_008748.79
Genomic sequence: chr10:80881918-80882092
10 Sequence definition: Genscan prediction
- Sequence ID: No. 14
Accession #: AB002360
Genomic sequence: chr13:112761227-112761344
15 Sequence definition: KIAA0362
- Sequence ID: No. 15
Accession #: AK057572
Genomic sequence: chr16:14547315-14547422
20 Sequence definition: FLJ33010
- Sequence ID: No. 16
Accession #: NT_034410.56/NM_033102.1
Genomic sequence: chr1:203503646-203554883/chr1:192169879-192474008
25 Sequence definition: Genscan - Elk4/LOC85414 - Homo sapiens prostein protein LOC85414
- Sequence ID: No. 17
Accession #: NT_019696.29
Genomic sequence: chrX:64173951-64275396
30 Sequence definition: Genscan prediction
- Sequence ID: No. 18
Accession #: NT_007834.17
Genomic sequence: chr7:71656530-71727938
35 Sequence definition: Genscan prediction
- Sequence ID: No. 19
Accession #: NT_005403.1000
Genomic sequence: chr2:208067141-208067324
40 Sequence definition: Genscan prediction
- Sequence ID: No. 20
Accession #: NT_009654.19
Genomic sequence: chr12:116716120-116840364
45 Sequence definition: Genscan prediction
- Sequence ID: No. 21
Accession #: AC126564.7
Genomic sequence: chr12:131440407-131440735
50 Sequence definition: genomic match
- Sequence ID: No. 22
Accession #: NT_006171.64
Genomic sequence: chr4:172269202-172299375
55 Sequence definition: Genscan prediction
- Sequence ID: No. 23
Accession #: NM_025149.1
Genomic sequence: chr17:61361324-61409903
60 Sequence definition: FLJ20920

- Sequence ID: No. 24
Accession #: NT_026437.145
Genomic sequence: chr14:72272372-72462407
Sequence definition: Genscan prediction
- 5
Sequence ID: No. 25
Accession #: NT_030059.13
Genomic sequence: chr10:103933731-103955924
Sequence definition: Genscan prediction
- 10
Sequence ID: No. 26
Accession #: AK058112
Genomic sequence: chr19:1815692-1822319
Sequence definition: FLJ25383
- 15
Sequence ID: No. 27
Accession #: NM_002205.1
Genomic sequence: chr12:55541534-55565494
Sequence definition: Homo sapiens integrin alpha 5 fibronectin receptor
alpha polypeptide
- 20
Sequence ID: No. 28
Accession #: NM_004716.1
Genomic sequence: chr11:117114115-117114448
Sequence definition: Homo sapiens proprotein convertase subtilisin/kexin
type 7 PCSK7 mRNA
- 25
Sequence ID: No. 29
Accession #: NM_030774
Genomic sequence: chr11:5003431-5021099
Sequence definition: prostate specific G-protein coupled receptor [Homo
sapiens]
- 30
Sequence ID: No. 30
Accession #: AB007932
Genomic sequence: chr1:204846394-204846755
Sequence definition: Homo sapiens plexin A2 PLXNA2 mRNA
- 35
Sequence ID: No. 31
Accession #: AB023177
Genomic sequence: chr7:11157196-11157402
Sequence definition: Homo sapiens mRNA for KIAA0960 protein
- 40
Sequence ID: No. 32
Accession #: NT_004858.23
Genomic sequence: chr1:147688399-147725025
Sequence definition: Genscan prediction
- 45
Sequence ID: No. 33
Accession #: NT_004873.61
Genomic sequence: chr1:14678698-14732191
Sequence definition: Genscan prediction
- 50
Sequence ID: No. 34
Accession #: NT_029860.99
Genomic sequence: chr1:110751286-110854188
Sequence definition: Genscan prediction
- 55
Sequence ID: No. 35
Accession #: NM_032385.1
Genomic sequence: chr5:170086201-170251515
Sequence definition: Homo sapiens chromosome 5 open reading frame 4
- 60

C5orf4

- Sequence ID: No. 36
Accession #: NM_014752.1
5 Genomic sequence: chr11:73182947-73211393
Sequence definition: KIAA0102
- Sequence ID: No. 37
Accession #: NP_000295
10 Genomic sequence: chr17:15500091-15500332
Sequence definition: Homo sapiens peripheral myelin protein 22
- Sequence ID: No. 38
Accession #: NM_020433
15 Genomic sequence: chr20:42528457-42528759
Sequence definition: Homo sapiens junctophilin 2
- Sequence ID: No. 39
Accession #: NT_999999.2
20 Genomic sequence: chrM:9411-9524
Sequence definition: Genscan Gene Predictions
- Sequence ID: No. 40
Accession #: NT_004754.1
25 Genomic sequence: chr1:117988850-117989247
Sequence definition: Genscan Gene Predictions
- Sequence ID: No. 41
Accession #: NT_011568.108
30 Genomic sequence: chrX:47583156-47583796
Sequence definition: Acembly Gene Predictions/Genscan Gene Predictions
- Sequence ID: No. 42
Accession #: NP_061116
35 Genomic sequence: chr7:140900079-140900876
Sequence definition: transient receptor potential cation channel
- Sequence ID: No. 43
Accession #: NT_011295.163
40 Genomic sequence: chr19:19799239-19804450
Sequence definition: Genscan prediction
- Sequence ID: No. 44
Accession #: NP_056051
45 Genomic sequence: chr4:62284401-62284770
Sequence definition: lectomedin-3
- Sequence ID: No. 45
Accession #: NT_033927.57
50 Genomic sequence: chr11:75518014-75562375
Sequence definition: Genscan prediction
- Sequence ID: No. 46
Accession #: NM_030774
55 Genomic sequence: chr11:5003431-5021099
Sequence definition: prostate specific G-protein coupled receptor Homo sapiens
- Sequence ID: No. 47
60 Accession #: NM_022119
Genomic sequence: chr16:2939532-2939842
Sequence definition: protease serine 22

- Sequence ID: No. 48
Accession #: NP_000155
Genomic sequence: chr19:46824678-46824801
5 Sequence definition: Homo sapiens gastric inhibitory polypeptide receptor
- Sequence ID: No. 49
Accession #: NM_001627
Genomic sequence: chr3:104784804-104787209
10 Sequence definition: activated leukocyte cell adhesion molecule
- Sequence ID: No. 50
Accession #: NP_056343
Genomic sequence: chr17:5263335-5263632
15 Sequence definition: Homo sapiens DKFZP566H073 protein
- Sequence ID: No. 51
Accession #: NT_033275.9
Genomic sequence: chr15:19767754-19767842
20 Sequence definition: Acembly Gene Predictions/Genscan Gene Predictions
- Sequence ID: No. 52
Accession #: NT_004511.105
Genomic sequence: chr1:37657082-37657508
25 Sequence definition: Genscan Gene Predictions
- Sequence ID: No. 53
Accession #: NT_007819.76
Genomic sequence: chr7:2293638-2293859
30 Sequence definition: Acembly Gene Predictions/Genscan Gene Predictions
- Sequence ID: No. 54
Accession #: NT_008046.179
Genomic sequence: chr8:101509107-101509191
35 Sequence definition: Acembly Gene Predictions/Genscan Gene Predictions
- Sequence ID: No. 55
Accession #: NP_001668
Genomic sequence: chr1:166712801-166712951
40 Sequence definition: ATPase Na⁺/K⁺ transporting beta 1 polypeptide
- Sequence ID: No. 56
Accession #: NP_061332
Genomic sequence: chr7:105724807-105753208
45 Sequence definition: B-cell receptor-associated protein BAP29
- Sequence ID: No. 57
Accession #: NT_008251.42
Genomic sequence: chr8:36531104-36531405
50 Sequence definition: Acembly Gene Predictions/Genscan Gene Predictions
- Sequence ID: No. 58
Accession #: NT_008984.116
Genomic sequence: chr11:97792879-97792961
55 Sequence definition: Genscan Gene Predictions
- Sequence ID: No. 59
Accession #: NT_011176.84
Genomic sequence: chr19:11151260-11154382
60 Sequence definition: Acembly Gene Predictions/Genscan Gene Predictions
- Sequence ID: No. 60

- Accession #: ENST00000255124
Genomic sequence: chr20:46047371-46047445
Sequence definition: Acembly Gene Predictions/Genscan Gene Predictions
- 5 Sequence ID: No. 61
 Accession #: ENST00000262657
 Genomic sequence: chr20:29935469-29937596
 Sequence definition: Acembly Gene Predictions/Genscan Gene Predictions
- 10 Sequence ID: No. 62
 Accession #: NT_033903.44
 Genomic sequence: chr11:58671001-58671164
 Sequence definition: Genscan Gene Predictions
- 15 Sequence ID: No. 63
 Accession #: NP_000360
 Genomic sequence: chr14:78989775-78989913
 Sequence definition: Homo sapiens thyroid stimulating hormone receptor
- 20 Sequence ID: No. 64
 Accession #: NP_005219
 Genomic sequence: chr7:54724858-54725037
 Sequence definition: Homo sapiens epidermal growth factor receptor
 erythroblastic leukemia viral v-erb-b oncogene homolog avian
- 25 Sequence ID: No. 65
 Accession #: NP_149093
 Genomic sequence: chr1:203548697-203549088
 Sequence definition: Homo sapiens prostatein protein
- 30 Sequence ID: No. 66
 Accession #: NM_030774
 Genomic sequence: chr11:5003431-5021099
 Sequence definition: prostate specific G-protein coupled receptor Homo
- 35 sapiens
 Sequence ID: No. 67
 Accession #: NM_030774
 Genomic sequence: chr11:5004995-5010301
- 40 Sequence definition: prostate specific G-protein coupled receptor Homo
 sapiens
 Sequence ID: No. 68
 Accession #: NM_030774
- 45 Genomic sequence: chr11:5004983-5010305
 Sequence definition: prostate specific G-protein coupled receptor Homo
 sapiens
- 50 Sequence ID: No. 69
 Accession #: NM_030774
 Genomic sequence: chr11:5004983-5010305
 Sequence definition: prostate specific G-protein coupled receptor Homo
 sapiens
- 55 Sequence ID: No. 70
 Accession #: NM_030774
 Genomic sequence: chr11:4667240-4678100
 Sequence definition: prostate specific G-protein coupled receptor Homo
 sapiens
- 60 Sequence ID: No. 71
 Accession #: NM_030774

Genomic sequence: chr11:4677792-4677987
Sequence definition: prostate specific G-protein coupled receptor Homo sapiens

- 5 Sequence ID: No. 72
Accession #: NM_030774
Genomic sequence: chr11:5003430-5007773
Sequence definition: prostate specific G-protein coupled receptor Homo sapiens
- 10 Sequence ID: No. 73
Accession #: AK075546
Genomic sequence: chr11:36643617-36930167
Sequence definition: predicted protein
- 15 Sequence ID: No. 74
Accession #: AK075546
Genomic sequence: chr11:36643626-36931023
Sequence definition: predicted protein
- 20 Sequence ID: No. 75
Accession #: AK075546
Genomic sequence: chr11:36643617-36929351
Sequence definition: predicted protein
- 25 Sequence ID: No. 76
Accession #: AK075546
Genomic sequence: chr11:36643617-36929351
Sequence definition: predicted protein
- 30 Sequence ID: No. 77
Accession #: AK075546
Genomic sequence: chr11:36643617-36929351
Sequence definition: predicted protein
- 35 Sequence ID: No. 78
Accession #: NT_033927.57
Genomic sequence: chr11:75518014-75562375
Sequence definition: Genscan prediction
- 40 Sequence ID: No. 79
Accession #: NM_000300
Genomic sequence: chr1:19337078-19342056
Sequence definition: phospholipase A2 group IIA platelets synovial
- 45 Sequence ID: No. 80
Accession #: NM_000300
Genomic sequence: chr1:19337078-19342056
Sequence definition: phospholipase A2 group IIA platelets synovial
- 50 Sequence ID: No. 81
Accession #: NM_000300
Genomic sequence: chr1:19337078-19342056
Sequence definition: phospholipase A2 group IIA platelets synovial
- 55 Sequence ID: No. 82
Accession #: NM_000300
Genomic sequence: chr1:19337078-19342056
Sequence definition: phospholipase A2 group IIA platelets synovial
- 60 Sequence ID: No. 83
Accession #: NM_000300

Genomic sequence: chr1:19337078-19342056
Sequence definition: phospholipase A2 group IIA platelets synovial

- Sequence ID: No. 84
5 Accession #: NM_032323
Genomic sequence: chr1:152017962-152027457
Sequence definition: hypothetical protein MGC13102 - refseq
- Sequence ID: No. 85
10 Accession #: NM_032323
Genomic sequence: chr1:152017962-152027457
Sequence definition: hypothetical protein MGC13102
- Sequence ID: No. 86
15 Accession #: NM_032323
Genomic sequence: chr1:152017962-152027457
Sequence definition: hypothetical protein MGC13102
- Sequence ID: No. 87
20 Accession #: NM_032323
Genomic sequence: chr1:152017962-152027457
Sequence definition: hypothetical protein MGC13102
- Sequence ID: No. 88
25 Accession #: NM_032323
Genomic sequence: chr1:152017962-152027457
Sequence definition: hypothetical protein MGC13102
- Sequence ID: No. 89
30 Accession #: NM_032323
Genomic sequence: chr1:152017962-152027457
Sequence definition: hypothetical protein MGC13102
- Sequence ID: No. 90
35 Accession #: NM_032323
Genomic sequence: chr1:152017962-152027457
Sequence definition: hypothetical protein MGC13102
- Sequence ID: No. 91
40 Accession #: AK092666
Genomic sequence: chr7:88376306-88402240
Sequence definition: STEAP2/AK092666
- Sequence ID: No. 92
45 Accession #: AK092666
Genomic sequence: chr7:88376306-88402240
Sequence definition: STEAP2/AK092666
- Sequence ID: No. 93
50 Accession #: AK092666
Genomic sequence: chr7:88376306-88402240
Sequence definition: STEAP2/AK092666
- Sequence ID: No. 94
55 Accession #: NM_005656
Genomic sequence: chr21:39493446-39537043
Sequence definition: TMPRSS2
- Sequence ID: No. 95
60 Accession #: NM_005656
Genomic sequence: chr21:39493446-39537043
Sequence definition: TMPRSS2

- Sequence ID: No. 96
Accession #: NM_005656
Genomic sequence: chr21:39493446-39537043
5 Sequence definition: TMPRSS2
- Sequence ID: No. 97
Accession #: NM_005656
Genomic sequence: chr21:39493446-39537043
10 Sequence definition: TMPRSS2
- Sequence ID: No. 98
Accession #: NM_005656
Genomic sequence: chr21:39493446-39537043
15 Sequence definition: TMPRSS2
- Sequence ID: No. 99
Accession #: NM_004476
Genomic sequence: chr11:50361918-50423952
20 Sequence definition: PSMA/FOLH1
- Sequence ID: No. 100
Accession #: NM_004476
Genomic sequence: chr11:50361918-50423952
25 Sequence definition: PSMA/FOLH1
- Sequence ID: No. 101
Accession #: NM_004476
Genomic sequence: chr11:50361918-50423952
30 Sequence definition: PSMA/FOLH1
- Sequence ID: No. 102
Accession #: no match to index
Genomic sequence: No match BLAT
35 Sequence definition: No match BLAT
- Sequence ID: No. 103
Accession #: AC105101.8
Genomic sequence: chr18:45441503-45442177
40 Sequence definition: genomic match
- Sequence ID: No. 104
Accession #: BC043509
Genomic sequence: chr2:7566735-7567210
45 Sequence definition: genomic match
- Sequence ID: No. 105
Accession #: no match to index
Genomic sequence: No match BLAT
50 Sequence definition: No match BLAT
- Sequence ID: No. 106
Accession #: NT_007914.345
Genomic sequence: chr7:150965224-150965948
55 Sequence definition: Genscan prediction
- Sequence ID: No. 107
Accession #: NM_002474
Genomic sequence: chr16:15123743-15124024
60 Sequence definition: smooth muscle myosin heavy chain 11 isoform
- Sequence ID: No. 108

- Accession #: no match to index
Genomic sequence: No match BLAT
Sequence definition: No match BLAT
- 5 Sequence ID: No. 109
 Accession #: AL450472.14
 Genomic sequence: chrX:132596913-132597349
 Sequence definition: genomic match
- 10 Sequence ID: No. 110
 Accession #: no match to index
 Genomic sequence: No match BLAT
 Sequence definition: No match BLAT
- 15 Sequence ID: No. 111
 Accession #: NM_024490
 Genomic sequence: chr15:18676827-18681314
 Sequence definition: ATPase Class V type 10A
- 20 Sequence ID: No. 112
 Accession #: NT_007741.24
 Genomic sequence: chr7:154483727-154484200
 Sequence definition: Genscan prediction
- 25 Sequence ID: No. 113
 Accession #: NT_010168.1
 Genomic sequence: chr14:100136759-100137109
 Sequence definition: Genscan prediction
- 30 Sequence ID: No. 114
 Accession #: AK074158
 Genomic sequence: chr7:2347770-2347996
 Sequence definition: Homo sapiens mRNA for FLJ00231 protein
- 35 Sequence ID: No. 115
 Accession #: no match to index
 Genomic sequence: No match BLAT
 Sequence definition: No match BLAT
- 40 Sequence ID: No. 116
 Accession #: AL549429
 Genomic sequence: chr11:9027915-9028089
 Sequence definition: genomic match
- 45 Sequence ID: No. 117
 Accession #: NM_015541
 Genomic sequence: chr3:65899978-65900329
 Sequence definition: leucine-rich repeats and immunoglobulin-like
- 50 Sequence ID: No. 118
 Accession #: NM_024897
 Genomic sequence: chr1:151978744-151978881
 Sequence definition: hypothetical protein FLJ22672
- 55 Sequence ID: No. 119
 Accession #: NM_006598
 Genomic sequence: chr5:1165896-1168793
 Sequence definition: solute carrier family 12 potassium/chloride
- 60 Sequence ID: No. 120
 Accession #: NM_021569
 Genomic sequence: chr9:131740238-131740388

Sequence definition: NMDA receptor 1 isoform NR1-2 precursor

Sequence ID: No. 121

Accession #: AL445467.6

5 Genomic sequence: chrX:15985515-15985779

Sequence definition: genomic match

Sequence ID: No. 122

Accession #: BM976799

10 Genomic sequence: chr1:54049149-54049432

Sequence definition: genomic/EST match

Sequence ID: No. 123

Accession #: no match to index

15 Genomic sequence: No match BLAT

Sequence definition: No match BLAT

Sequence ID: No. 124

Accession #: NT_007933.414

20 Genomic sequence: chr7:98285605-98286140

Sequence definition: Genscan prediction

Sequence ID: No. 125

Accession #: NM_020428

25 Genomic sequence: chr19:10964586-10965036

Sequence definition: Homo sapiens CTL2 gene CTL2 mRNA

Sequence ID: No. 126

Accession #: no match to index

30 Genomic sequence: No match BLAT

Sequence definition: No match BLAT

Sequence ID: No. 127

Accession #: NM_006292

35 Genomic sequence: chr11:19444265-19444422

Sequence definition: Homo sapiens tumor susceptibility gene 101 TSG101 mRNA

Sequence ID: No. 128

Accession #: NM_052932

40 Genomic sequence: chr11:102306433-102306907

Sequence definition: Homo sapiens pro-oncosis receptor inducing membrane injury gene PORIMIN mRNA

45 Sequence ID: No. 129

Accession #: NM_000014

Genomic sequence: chr12:9416444-9416720

Sequence definition: Homo sapiens alpha-2-macroglobulin A2M mRNA

50 Sequence ID: No. 130

Accession #: NM_002337

Genomic sequence: chr4:3426547-3433294

Sequence definition: low density lipoprotein-related

55 Sequence ID: No. 131

Accession #: AL834445

Genomic sequence: chr20:23304135-23304477

Sequence definition: Homo sapiens mRNA; cDNA DKFZp761J109

60 Sequence ID: No. 132

Accession #: NM_004986

Genomic sequence: chr14:49879277-49880762

Sequence definition: kinectin 1

Sequence ID: No. 133

Accession #: NM_024295

5 Genomic sequence: chr8:124092754-124095061

Sequence definition: hypothetical protein MGC3067

Sequence ID: No. 134

Accession #: AC018457.14

10 Genomic sequence: chr3:165236534-165236724

Sequence definition: genomic match

Sequence ID: No. 135

Accession #: NM_004753

15 Genomic sequence: chr1:12208898-12258427

Sequence definition: Homo sapiens short-chain dehydrogenase/reductase 1
SDR1 mRNA

Sequence ID: No. 136

20 Accession #: NM_004753

Genomic sequence: chr1:12221576-12258383

Sequence definition: Homo sapiens short-chain dehydrogenase/reductase 1
SDR1 mRNA

25 Sequence ID: No. 137

Accession #: NM_004753

Genomic sequence: chr1:12221576-12258383

Sequence definition: Homo sapiens short-chain dehydrogenase/reductase 1
SDR1 mRNA

30

Sequence ID: No. 138

Accession #: NM_004753

Genomic sequence: chr1:12221576-12258383

35 Sequence definition: Homo sapiens short-chain dehydrogenase/reductase 1
SDR1 mRNA

Sequence ID: No. 139

Accession #: NM_004753

Genomic sequence: chr1:12221576-12258383

40 Sequence definition: Homo sapiens short-chain dehydrogenase/reductase 1
SDR1 mRNA

Sequence ID: No. 140

Accession #: D87438

45 Genomic sequence: chr16:14996279-15058862

Sequence definition: Human mRNA for KIAA0251 gene partial cds

Sequence ID: No. 141

Accession #: D87438

50 Genomic sequence: chr16:15018972-15027737

Sequence definition: Human mRNA for KIAA0251 gene partial cds

Sequence ID: No. 142

Accession #: AB007932

55 Genomic sequence: chr1:204843635-205060532

Sequence definition: Homo sapiens plexin A2 long form PLXNA2 mRNA

Sequence ID: No. 143

Accession #: AB007932

60 Genomic sequence: chr1:204843635-205060532

Sequence definition: Homo sapiens plexin A2 long form PLXNA2 mRNA

- Sequence ID: No. 144
Accession #: AB007932
Genomic sequence: chr1:204843635-205060532
Sequence definition: Homo sapiens plexin A2 long form PLXNA2 mRNA
- 5
Sequence ID: No. 145
Accession #: AB037745
Genomic sequence: chr1:108833848-108851509
Sequence definition: Homo sapiens mRNA for KIAA1324 protein partial cds
- 10
Sequence ID: No. 146
Accession #: AB037745
Genomic sequence: chr1:108851126-108851424
Sequence definition: Homo sapiens mRNA for KIAA1324 protein partial cds
- 15
Sequence ID: No. 147
Accession #: AB037745
Genomic sequence: chr1:108851126-108851424
Sequence definition: Homo sapiens mRNA for KIAA1324 protein partial cds
- 20
Sequence ID: No. 148
Accession #: AB037745
Genomic sequence: chr1:108851126-108851424
Sequence definition: Homo sapiens mRNA for KIAA1324 protein partial cds
- 25
Sequence ID: No. 149
Accession #: AB037745
Genomic sequence: chr1:108851126-108851424
Sequence definition: Homo sapiens mRNA for KIAA1324 protein partial cds
- 30
Sequence ID: No. 150
Accession #: NM_002253
Genomic sequence: chr4:55795152-55795458
Sequence definition: Homo sapiens kinase insert domain receptor a type
35 III receptor tyrosine kinase KDR mRNA
- Sequence ID: No. 151
Accession #: NM_004879
Genomic sequence: chr11:125479160-125481382
40 Sequence definition: Homo sapiens etoposide induced 2.4 mRNA EI24 mRNA
- Sequence ID: No. 152
Accession #: BC041788
Genomic sequence: chr8:144841449-144841809
45 Sequence definition: Homo sapiens Similar to RIKEN cDNA 1110025J15 gene
clone MGC:32881 IMAGE:4738372 mRNA complete cds
- Sequence ID: No. 153
Accession #: AB033073
50 Genomic sequence: chr20:46925235-46925516
Sequence definition: Homo sapiens mRNA for KIAA1247 protein partial cds
- Sequence ID: No. 154
Accession #: NT_011520.136
55 Genomic sequence: chr22:21548074-21562329
Sequence definition: Genscan prediction
- Sequence ID: No. 155
Accession #: NM_005581
60 Genomic sequence: chr19:49998069-49998792
Sequence definition: Homo sapiens Lutheran blood group Auberger b antigen
included LU mRNA

- Sequence ID: No. 156
Accession #: NM_004355
Genomic sequence: chr5:149769000-149775442
5 Sequence definition: Homo sapiens CD74 antigen invariant polypeptide of major histocompatibility complex class II antigen-associated CD74 mRNA
- Sequence ID: No. 157
Accession #: NM_000484
10 Genomic sequence: chr21:26174980-26175131
Sequence definition: Homo sapiens amyloid beta A4 precursor protein protease nexin-II Alzheimer disease APP mRNA
- Sequence ID: No. 158
Accession #: NM_005745
15 Genomic sequence: chrX:150566783-150575554
Sequence definition: Homo sapiens accessory protein BAP31 BCAP31 mRNA
- Sequence ID: No. 159
Accession #: NM_005570
20 Genomic sequence: chr18:56780509-56781078
Sequence definition: Homo sapiens lectin mannose-binding 1 LMAN1 mRNA
- Sequence ID: No. 160
Accession #: NT_029218.14
25 Genomic sequence: chr1:19080562-19080917
Sequence definition: Genscan prediction
- Sequence ID: No. 161
Accession #: NT_011387.8
30 Genomic sequence: chr20:410654-410816
Sequence definition: Genscan prediction
- Sequence ID: No. 162
Accession #: NM_002587
35 Genomic sequence: chr5:141227996-141231527
Sequence definition: Homo sapiens protocadherin 1 PDCH1
- Sequence ID: No. 163
Accession #: NT_035036.5
40 Genomic sequence: chr10:51263955-51274232
Sequence definition: Genscan prediction
- Sequence ID: No. 164
Accession #: NM_007176
45 Genomic sequence: chr14:74107662-74107815
Sequence definition: Homo sapien Chr 14 open reading frame
- Sequence ID: No. 165
Accession #: AP000531.1
50 Genomic sequence: chr22:14703272-14703359
Sequence definition: poor genomic match to repeat
- Sequence ID: No. 166
Accession #: NM_020182
55 Genomic sequence: chr20:56850452-56936716
Sequence definition: Homo sapiens transmembrane prostate androgen induced RNA TMEPAI mRNA
- Sequence ID: No. 167
Accession #: NM_020182
60 Genomic sequence: chr20:56850452-56936716

Sequence definition: Homo sapiens transmembrane prostate androgen induced RNA TMEPAI mRNA

Sequence ID: No. 168

5 Accession #: NM_020182

Genomic sequence: chr20:56850452-56936716

Sequence definition: Homo sapiens transmembrane prostate androgen induced RNA TMEPAI mRNA

10 Sequence ID: No. 169

Accession #: NM_020182

Genomic sequence: chr20:56850452-56936716

Sequence definition: Homo sapiens transmembrane prostate androgen induced RNA TMEPAI mRNA

15

Sequence ID: No. 170

Accession #: NM_020182

Genomic sequence: chr20:56850452-56936716

20 Sequence definition: Homo sapiens transmembrane prostate androgen induced RNA TMEPAI mRNA

Sequence ID: No. 171

Accession #: NM_020182

Genomic sequence: chr20:56850452-56936716

25 Sequence definition: Homo sapiens transmembrane prostate androgen induced RNA TMEPAI mRNA

Sequence ID: No. 172

Accession #: NM_020182

30 Genomic sequence: chr20:56850452-56936716

Sequence definition: Homo sapiens transmembrane prostate androgen induced RNA TMEPAI mRNA

35 Sequence ID: No. 173

Accession #: AK092666_01

Sequence definition: Novel spliced isoform of STEAP2

Sequence ID: No. 174

Accession #: AK092666_01

40 Sequence definition: Protein translation of novel spliced isoform of STEAP2

Sequence ID: No. 175

Accession #: AK092666_02

45 Sequence definition: Novel spliced isoform of STEAP2

Sequence ID: No. 176

Accession #: AK092666_02

50 Sequence definition: Protein translation of novel spliced isoform of STEAP2

Sequence ID: No. 177

Accession #: AK092666_03

55 Sequence definition: Novel spliced isoform of STEAP2

Sequence ID: No. 178

Accession #: AK092666_03

60 Sequence definition: Protein translation of novel spliced isoform of STEAP2

Sequence ID: No. 179

Accession #: AK092666_04

Sequence definition: Novel spliced isoform of STEAP2

Sequence ID: No. 180

Accession #: AK092666_04

5 Sequence definition: Protein translation of novel spliced isoform of STEAP2

Sequence ID: No. 181

Accession #: AK092666_05

10 Sequence definition: Novel spliced isoform of STEAP2

Sequence ID: No. 182

Accession #: AK092666_05

15 Sequence definition: Novel spliced isoform of STEAP2

Sequence ID: No. 183

Accession #: AK092666_01aa

Sequence definition: Novel amino acids generated by spliced isoforms
AK092666_01, AK092666_03, AK092666_05

20

Sequence ID: No. 184

Accession #: AK092666_02aa

Sequence definition: Novel amino acids generated by spliced isoform
AK092666_02

25

Sequence ID: No. 185

Accession #: AK092666_04aa

Sequence definition: Novel amino acids generated by spliced isoform
AK092666_04

30

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- 40 U.S. Pat. No. 4,683,195
- U.S. Pat. No. 4,683,202
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- 45

WHAT IS CLAIMED IS:

1. An isolated nucleic acid sequence that is expressed by human prostate cancer
5 cells, selected from the group consisting of:
 - (i) the nucleic acid sequence contained in SEQ ID NOS.: 1 to 173, 175, 177, 179, 181;
 - (ii) variants thereof, wherein such variants have a nucleic acid sequence that is at least 70% identical to the sequence of (i) when aligned without allowing for
10 gaps; and
 - (iii) fragments of (i) or (ii) having a size of at least 20 nucleotides in length.
2. The nucleic acid sequence of Claim 1 which comprises the nucleic acid
15 sequence contained in any one of SEQ ID NOS.: 1 to 173, 175, 177, 179, 181 or a fragment thereof.
3. A primer mixture that comprises primers that result in the specific amplification of one of the nucleic acid sequences of Claim 1.
- 20 4. A method of detecting prostate cancer comprising determining whether a human prostate cell sample expresses a target nucleic acid molecule, wherein said target nucleic acid molecule comprises the sequence of a gene or RNA comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOS.: 1 to 173, 175, 177, 179, 181 or of a fragment of said gene or RNA having a size of at least 20 nucleotides in length.
25
5. The method of Claim 4, wherein said method comprises detecting the expression of said target nucleic acid molecule using a nucleic acid sequence that specifically hybridizes thereto.
- 30 6. The method of Claim 5, wherein said method comprises detecting the expression of said target nucleic acid molecule using primers that result in the amplification thereof.

7. The method of Claim 5, wherein the expression of said target nucleic acid molecule is detected by assaying for the antigen encoded by said nucleic acid.

5 8. The method of Claim 7, wherein said assay involves the use of a monoclonal antibody or fragment that specifically binds to said antigen.

9. The method of Claim 8, wherein said assay comprises an ELISA or competitive binding assay.

10

10. An antigen expressed by human prostate cancer cells, wherein said antigen is selected from the group consisting of:

- (i) the antigen encoded by a nucleic acid sequence having at least 90% sequence identity in SEQ ID NOS.: 1 to 173, 175, 177, 179, 181;
- 15 (ii) an antigen derived from a protein comprising a sequences having at least 90% identity in SEQ ID NOS. 174, 176, 178, 180, 182-185; and
- (iii) an antigenic fragment of (i) or (ii).

11. A prostate antigen comprising (i) the amino acid sequence encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOS.: 1 to 173, 175, 177, 179, 181 or (ii) an amino acid sequence selected from SEQ ID NOS. : 174, 176, 178, 180, and 182-185, or (iii) an antigenic fragment of (i) or (ii).

12. A monoclonal antibody or antigen-binding fragment thereof that specifically binds to a target polypeptide molecule selected from:

- (i) a polypeptide encoded by a nucleic acid molecule comprising the sequence of a gene or RNA comprising a sequence selected from the group consisting of SEQ ID NOS.: 1 to 173, 175, 177, 179, 181, or by a fragment of said gene or RNA having a size of at least 20 nucleotides in length, or a polypeptide derived from SEQ ID NOS. :174, 176, 178, 180, and 182 - 185
- 30 (ii) an antigen according to Claim 10 or 11, and

- (iii) an antigenic fragment of (i) or (ii).

13. A monoclonal antibody or fragment thereof that specifically binds the antigen of Claim 11.

5

14. The antigen of Claim 10 or 11 which is attached directly or indirectly to a detectable label.

15. The antibody of Claim 12 or 13 which is attached directly or indirectly to a detectable label.

10

16. A diagnostic kit for detection of prostate cancer which comprises a DNA according to Claim 1 and a detectable label.

17. A diagnostic kit for detection of prostate cancer which comprises primers according to Claim 3 and a diagnostically acceptable carrier.

15

18. A diagnostic kit for detection of prostate cancer which comprises a monoclonal antibody according to Claim 12 or 13 and a detectable label.

20

19. A method for treating prostate cancer, which comprises administering to a subject a therapeutically effective amount of a ligand which specifically binds a target molecule selected from (i) a gene or RNA comprising a sequence selected from the group consisting of SEQ ID NOS.: 1 to 173, 175, 177, 179, 181, a variant thereof or a fragment of said gene or RNA having a size of at least 20 nucleotides in length, and (ii) a protein or polypeptide encoded by a gene or RNA comprising a sequence selected from the group consisting of SEQ ID NOS.: 1 to 173, 175, 177, 179, 181, a variant thereof or a fragment of said gene or RNA having a size of at least 20 nucleotides in length, or a polypeptide derived from SEQ ID NOS. :174, 176, 178, 180, and 182 - 185.

25

30

20. The method of claim 19, wherein the ligand is a ribozyme or antisense

oligonucleotide that inhibits the expression of a gene having a DNA sequence selected from the group consisting of SEQ ID NOS.: 1 to 173, 175, 177, 179, 181 or a fragment, or variant thereof, or a polypeptide derived from SEQ ID NOS. :174, 176, 178, 180, and 182 - 185.

5 21. The method of claim 19 or 20, wherein the ligand is directly or indirectly attached to an effector moiety.

 22. The method of Claim 21, wherein said effector moiety is a therapeutic radiolabel, enzyme, cytotoxin, growth factor, or drug.

10

 23. A method for treating prostate cancer comprising administering to a subject a therapeutically effective amount of an antigen according to Claim 10 or 11, and optionally an adjuvant that elicits a humoral or cytotoxic T-lymphocyte response to said antigen.

15 24. A method for treating prostate cancer comprising administering to a subject a therapeutically effective amount of a ligand which specifically binds to a protein encoded by a gene or RNA comprising a sequence selected from the group consisting of SEQ ID NOS.: 1 to 173, 175, 177, 179, 181 or a fragment, or variant thereof, or a polypeptide derived from SEQ ID NOS. :174, 176, 178, 180, and 182 - 185 optionally directly or indirectly attached to a
20 therapeutic effector moiety.

 25. The method of Claim 24, wherein said effector moiety is a radiolabel, enzyme, cytotoxin, growth factor, or drug.

25 26. The method of Claim 25 wherein the radiolabel is yttrium.

 27. The method of Claim 25 wherein the radiolabel is indium.

 28. The method of claim 24 wherein said ligand is a monoclonal antibody or
30 fragment thereof.

29. The method of claim 24 wherein said ligand is a small molecule.

30. The method of claim 24 wherein said ligand is a peptide.

5 31. The method of claim 24, wherein said ligand binds an extracellular domain of said protein.

32. A molecule, selected from:

- 10 (i) a polypeptide comprising the sequence of an extracellular domain of a protein encoded by a gene or RNA comprising a sequence selected from the group consisting of SEQ ID NOS.: 1 to 185; and
- (ii) a nucleic acid molecule encoding a polypeptide of (i).

15 33. The molecule of claim 32, wherein said polypeptide has 8 to 100 amino acids in length.

34. A method for selecting, identifying, screening, characterizing or optimizing biologically active compounds, comprising contacting a candidate compound with a target molecule and determining whether the candidate compound binds said target molecule, 20 wherein said target molecule is selected from (i) a nucleic acid molecule comprising the sequence of a gene or RNA comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOS.: 1 to 173, 175, 177, 179, 181, (ii) a fragment of said gene or RNA having a size of at least 20 nucleotides in length, and (iii) a polypeptide encoded by (i) or (ii) or a polypeptide derived from SEQ ID NOS.: 174, 176, 178, 180, and 182 - 185.

1/2

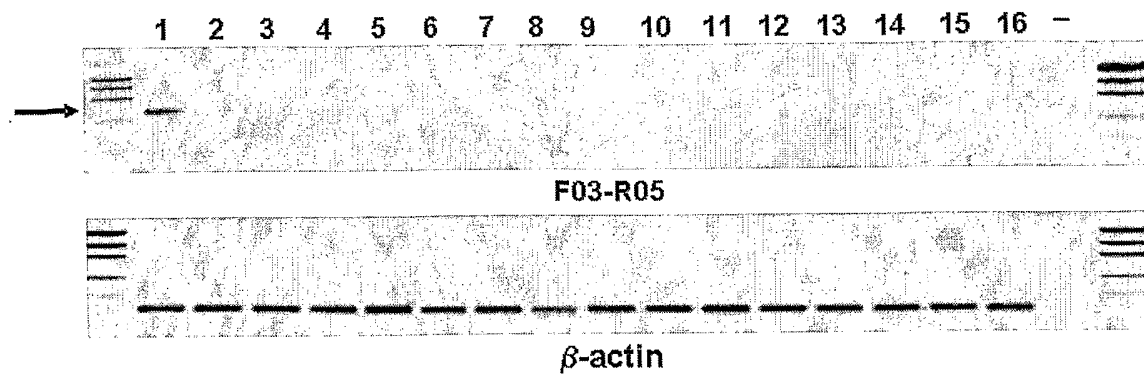


Figure 1

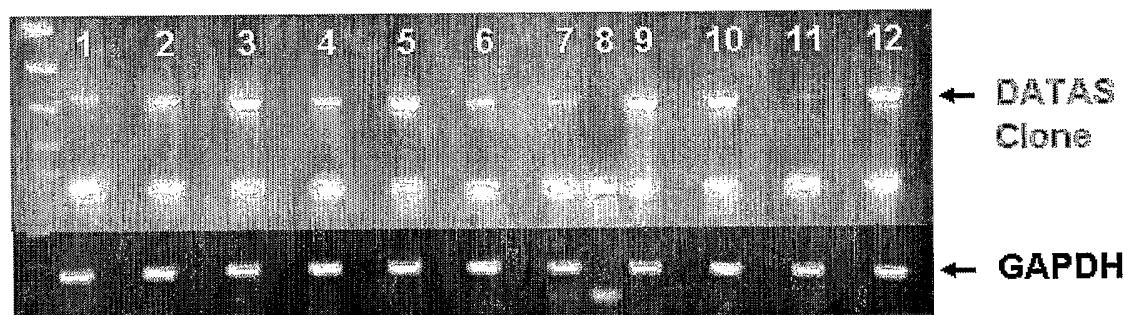


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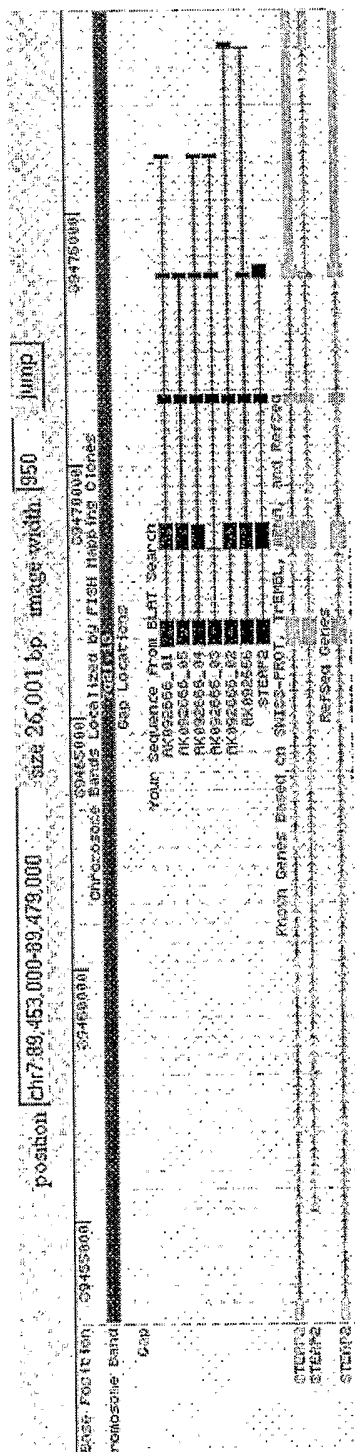


Figure 3

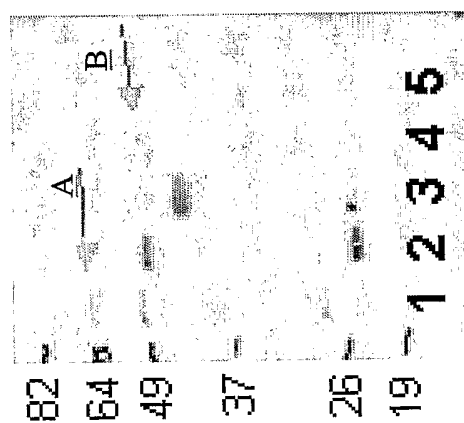


Figure 4

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 caggtcctgg accggatcaa aggctcactg ctgccccggc ctggccacaa ctttgtgctg 180
 caccatctgc ggaatcggcc ggatctgtat ggcccccttct ggatctgtgc cacgttggcc 240
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<210> 13

<211> 183

<212> DNA

<213> Homo sapiens

<400> 13
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 aaccacactg tggggtgggg aagcagagag gcgggaccag ccacttccag caggaagttt 120
 ccaacctgga ctgggttggg acggtgaggg gatagtcac tgccatcagt ttacataggt 180
 ggt 183

<210> 14

<211> 124

<212> DNA

<213> Homo sapiens

<400> 14
 cggcaggcat cacacaagag ggtgtcccta agatgcatca cgtccgtgga gaaaacaaag 60

B0213WO seq list.ST25.txt

caggacacac gtgtgagtcg tcacaccctt ggtcaccgg cttggccgtc acacatgcct 120
ccct 124

<210> 15

<211> 132

<212> DNA

<213> Homo sapiens

<400> 15
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ggacgaccga tttgcacgtc aggaccgcta cggacctcca ccagagtttc ctctggcttc 120
gccctggggc cg 132

<210> 16

<211> 124

<212> DNA

<213> Homo sapiens

<400> 16
cacaccttgg ggacaggcat gaggaacaga ttaatgtgag atattctaga ggtacatgca 60
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tgag 124

<210> 17

<211> 489

<212> DNA

<213> Homo sapiens

<400> 17
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gacactttgt gggaaattca cagaccatca aagataaggg aaaaacctca tgggctacag 180
cagaaaagag acccattcta cacaaagaac aagttcacaa tatgagacag agccacgcac 240
agaggatgac tgctatgacc tgacgacggg gaagtgtctt gttctctggg aagccgcctc 300
taaagatggc caaagacatg gtttttccta ggtttgaggt gcactacttc aggggttcctg 360
cttacctcct gtcgtttctc tggccctcat tgtgaccatg cttcactctc ccacctgtg 420
ctgggacaac catttttctt tccttattgc tgctactgca gagtgaggta gggctgtggc 480

B0213WO seq list.ST25.txt

ctcgcaccg

489

<210> 18

<211> 244

<212> DNA

<213> Homo sapiens

<400> 18

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ccatgggagg caaaaataca aattccatca ccaagagggc acgccatgcg gtgttgttcc	120
ataaggggtga gcaaagctgc caggcccaca ggagagagag cccacaggag ccctggatcc	180
tgtgggactt tggatcctac acagtgagt atctcagaac ttgcaaggc tgaggcaggg	240
agac	244

<210> 19

<211> 195

<212> DNA

<213> Homo sapiens

<400> 19

gggcgggacc agtgagagaa aggatagagc gtgtgagatg aaagggctgg tcttattaag	60
ccctacaata ctctgggggc caagcaatcc tgctgtggga ccctgctaag tgaacataat	120
gccgaggaag aacagctctc ctctttttgc caagcctgc caaggtgtca aggcttgaga	180
aagagtgggtg gccta	195

<210> 20

<211> 346

<212> DNA

<213> Homo sapiens

<400> 20

ccggtgcagg cattgtgata atagttggaa tgcagaggtg aattaaagag tatgggcttg	60
tctatataaa taaataaata tatatgtata tgggtgtgat aaccctatta tatatattat	120
gtattaggtg gtgttgata tataatgttta tatatgtgtg tgtttatgta taagtatata	180
tgtgataaga gtttataatc ctatatagag acagatgtat taaaatggat tttttttttt	240
tgagacagag tcttactctg tcatcaaagc tggagtgcag tgggtgcaatc taggctcaca	300
gtgacctaca cctcccatgt tcaaacgatt ctctgcctc ggccgg	346

B0213WO seq list.ST25.txt

<210> 21

<211> 168

<212> DNA

<213> Homo sapiens

<400> 21

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ttctggatcc tggaattgag ttgacactat cggtagtatg aaagtgcag acaccaggat	120
gaagtcactc ttgtcagacc cagaggaaac agggtcagga ggcctggg	168

<210> 22

<211> 435

<212> DNA

<213> Homo sapiens

<400> 22

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aagttatagc ctcggtttct gatcttgctg tggtagact ggatgtagca ttcaaggagt	120
aaatggagaa tccacaaaat aactttccca aggattataa ccgtctgaac tttcaatggg	180
tttgtgtaat ttcctgggca cttgtcctca tttggattag gataagaaca aagcacacct	240
gttaaaaatg ctaaaacaac aaacacgaga tgaataaacc acagaagatt cactatgatg	300
actgtaggaa gaggatggaa tcgggggtcta aagtgcgctt gtaatgagtg gtgtgggaga	360
agctgggcat ccagaagtgg gtcgtcttca atactctggg tgatatccaa ggaaccgtcc	420
tccatcctgc cgcgg	435

<210> 23

<211> 199

<212> DNA

<213> Homo sapiens

<400> 23

cggggaaagg acaggcctgc tgctttattc acagatttag atgtcgttcc atctgctctc	60
gaagtttgaa tttctggatc tttcctgaaa tggtagggg gtagtttgatg acaaacacga	120
tgtacttcgg aatcttgaag tgagagatct tcccttgca gaaagctttt atctcctcca	180
ccgtggtctc ctctcggcg	199

<210> 24

B0213WO seq list.ST25.txt

<211> 258

<212> DNA

<213> Homo sapiens

<400> 24

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gggcgcaggc tatggaggag accgtggtgg tggcagtggc tacggtggag accgaagtgg      60
aggctatgga ggagacagga gtggtggcgg ctatggagga gaccgagggtg ggggctacgg     120
aggagaccga ggtggctatg gaggcaaat gggaggaaga aacgactaca gaaatgatca      180
gcgcaaccga ccatactgat gactgttttg aatgttcctt tgtctctgac atgatccata      240
gtgaaattgc cagagttt                                     258

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<210> 25

<211> 171

<212> DNA

<213> Homo sapiens

<400> 25

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ggttcaggca ggtcagcaag gaacacaaag gcatctgcc cctgagcaag tatkgaacac      60
catcacctgc atccttcaag ggttccagg agatctccag cctctttag gagagaacca      120
agttgaaaag gtcaaacgct ggggacttgg taggaaaagg tggagactcc a              171

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<210> 26

<211> 223

<212> DNA

<213> Homo sapiens

<400> 26

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cagtgcgagg agccgtcact ctgctaagcc tgtatctgct gttcggctac ggagcgtctc      60
tgctgtgcaa tctcatcgga tttgtgtacc ccgcatatgc ctcaatcaaa gctatcgaga      120
gccaagcaa ggacgacgac actgtgtggc tcacctactg ggtgggtgtac gccctgtttg      180
ggctggccga gttcttcagc gatctactcc tgcctgccc ccc                        223

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<210> 27

<211> 249

<212> DNA

<213> Homo sapiens

B0213WO seq list.ST25.txt

<400> 27
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 tacagtgcct tctagctgtg gtagtctgtg tctccaagga ccaacccttc catttctgag 180
 gcttcagaat aaattctggc agttatttct tctctcagac tctatttcag aaaagtgtac 240
 ctgccccca 249

<210> 28
 <211> 334
 <212> DNA
 <213> Homo sapiens

<400> 28
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 cgatcctccc acctcaccct ccccggtagt gggaccacag gtgcacacca ccacacctgg 120
 ctatatgctt cttttgagat tgcttttttc actcacataa tttgcttgaa atttatccac 180
 cagcattttt taaaaattaa ctgtgcatca tcttcagtga gatgtgtgca tttcgtcttt 240
 gttcatgcct tttccactgc ctagaatgcc ctctaccaac cctgtctacc gatctgtatt 300
 catttccttc agtgtgtctc actgctgtgt gcct 334

<210> 29
 <211> 226
 <212> DNA
 <213> Homo sapiens

<400> 29
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 gtttatttgt ttctgtacac tactactaca aaggatagca aatagagctg aaggaaaagg 120
 atggagatac tcaaagtcct aaaaatggaa aggagaaaag ggaatgtcaa cctcaaggac 180
 aactgagatg ttcacagaac ttctgcagat tcttgctccc cacctc 226

<210> 30
 <211> 372
 <212> DNA
 <213> Homo sapiens

<400> 30

B0213WO seq list.ST25.txt

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agcctgcgac aggaccggtt ccacagagga gtcaaaagag ttaaagccca gaaggcagct    120
ggaagagaag gcaaagctgg aaaaggaagt aaaagcagca gctgttctga ttttgaagga    180
gagtaaattg gcttatttgc tttatggtct aaggagcaga gaaaactcat ttcctcttgg    240
ttataaattc ccgagttgca gaaggaagtc tcctaactac tgatcggttc ctcttggggt    300
gggaaaatct ctgagctaca gtgagtcatt cccaggatgc caacaatggc ctccacttct    360
ccctctgccc ct                                                    372

```

<210> 31

<211> 207

<212> DNA

<213> Homo sapiens

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<400> 31
cctctgttct tccagcttgg cttggagaag aactggcaga tgaacacgtc ctgcatggtg    60
gaatgccctg tgaactgtca gctttctgat tggctctcctt ggtcagaatg ttctcaaaca    120
tgtggcctca caggtttggt tgtaccataa cttatattag gctcctgggtc aaggaatatg    180
aaataaaata tccctcttgc tttaacc                                                    207

```

<210> 32

<211> 179

<212> DNA

<213> Homo sapiens

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<400> 32
tggtagggagg tcaactgcttg aaggagtcac atagacgtgg tgtgtgacac ttgtgcccac    60
ttcctgtgcc tgatgtgtag caaagaaagg ttgcatgctc ccttgctccc tgttctcttc    120
cagaccgtct atgaccagta tttcatcacc ctgtataaca tcgtgtacac ctaacgccc    179

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<210> 33

<211> 484

<212> DNA

<213> Homo sapiens

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<400> 33
tgaggagggtg aaggaccgga tcctggagaa catctcgctg tcggtgaaga agttgcagag    60
ctattttgct gcatgtgagg atgagacccc tgccatccgg aaccatgaca aggtcctaca    120

```

B0213WO seq list.ST25.txt

gcgtctgtgt gagcacctgg accacgccct gctgtacgga ctgcaagacc tctcctctgg	180
ctactgggtg ctcgtggtgc attttactcg gagagaggcc atcaagcaga tcgagggtgct	240
gcagcacgtg gccaccaacc tggggcgag ccgtgcctgg ctgtacctgg ccctcaacga	300
gaactccttg gagagctacc tgcggttggt ccaggagaac ctgggcctgc tgcataagta	360
ctacgtcaag aatgccctgg tctgcagcca cgatcacctg acgctcttcc tgaccttgggt	420
gtccgggcta gagttcattc gtttcgagct ggatctggat gccccttacc tagacctccc	480
cacg	484

<210> 34

<211> 443

<212> DNA

<213> Homo sapiens

<400> 34	
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ctgacaggct gtgaggaaac cccggccttg tagcctgtca cctctggggg gatgatgact	120
gcctggcaga cgtaggctgt gatagatttg gagaaccctg actcacctc aggaatccgg	180
aggtcagtga cattgtcggg gcacacagac attttcctac cctgggttcc acagagactg	240
agggtaaagt gatggaagta tttcaaccct ttggaagtga agcttggccc tccagcaaga	300
gtgacggtgt ttgcaaagc ggagaagttg tagttgaaag tcctgggttg agtggtgcgt	360
gagaagggtgc aatcattgta gcacagagag tggatcttgt tgttcttgggt ccctggacca	420
cagggcacac agacctcgcc gca	443

<210> 35

<211> 272

<212> DNA

<213> Homo sapiens

<400> 35	
ggacaggggt ctctccacca tttgaggaag ggatagagga agaccacat ggggaaagat	60
atcatgcact ggttgaaaag aactgtgcgg atagactggc gcagtttcac aggatccaca	120
ggttcattct tgccgacctg aattcggtag cgagagatga agttaggttt tcctgttgtg	180
tcaaccacca atagaagccc attgaagctc cagaagaaga gacaaggcac ttggatggca	240
cctataaaga agaggatcca ctccttcctt ac	272

<210> 36

<211> 224

B0213WO seq list.ST25.txt

<212> DNA

<213> Homo sapiens

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<400> 36
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gcggcttggt ggataagtgg aagatagatg ataagcctgt aaaaattgac aagtgggatg    120
gatcagctgt gaaaaactct ttggatgatt ctgccaaaaa ggtacttctg gaaaaataca    180
aatatgtgga gaattttggt ctaattgatg gtcgccttgt aacc                      224

```

<210> 37

<211> 342

<212> DNA

<213> Homo sapiens

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<400> 37
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tagccaagtt gcaatgcaac cgcgaactga ttttctcttt ctttctccag gtctccttgg    120
tcttcctaaa caatcaacag caacccccac ctccactgct ttctgtttgg ttggtttga    180
gtttgggatt ttgggctagc tcttttttct ttgtctgctt tctggtttcc cttcctccct    240
tccctatgta cgctcagagc ctccagacaga ccgtctgggc gcctcattcg cgtgagaagg    300
gccaggggga aggccaccag gccaggatgt aggcgaaacc gt                      342

```

<210> 38

<211> 315

<212> DNA

<213> Homo sapiens

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<400> 38
tcgggggagg tgggacctga gcatgctgtg ttccagaaaa ggctggagca cagactcaga    60
gagagagaga gtgggggtga cgaggctgga gagattggca aagcccagat tatgaggggt    120
ttgtgggtca cagtgaggag cttgaacttc atccttctag tagattctag gctacccctt    180
cagacactcc ttaggaccag ggacacattc cccgagctgc caggagtgtt agcagctgac    240
agatcctggc tgggtatttc tctccaacaa agggagctgc ctccttcaca tctaatgact    300
gtaatgacct accac                      315

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<210> 39

<211> 127

B0213WO seq list.ST25.txt

<212> DNA

<213> Homo sapiens

<400> 39
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 ctggccc 127

<210> 40

<211> 408

<212> DNA

<213> Homo sapiens

<400> 40
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 acaaccccg c gaaacaaaag ctataaacac acacaagtca gaggatctat aaaccagtgg 120
 gagaaaaaaaa attagatgaa ggttaaccat taaaaagctg cagttgggaa aacacacact 180
 cgattgttac atcagaaagt gccgtgggga gaagagccgt gtgctggtaa acatgtccgc 240
 gctcagaact tgacatgcag aaaagagaga gcgccaagtc ccacctgaga ttagagagga 300
 ctgggttttta gtgtaacaca ctttgtttta aaatatcact gtcctcttct tgccccaatt 360
 gctcctagaa cgtccctctg tcactcccct ccggggccag cctctccg 408

<210> 41

<211> 314

<212> DNA

<213> Homo sapiens

<400> 41
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 ttggtctgaa tgactgggat gatgatgaga tcctagcttc ggtgctggca gtgtcccaac 180
 aggaatacct agacagtatg aagaaaaaca aagtgcacag agaccgccc ccagacaaga 240
 gttgatggag acccagggat tggacaccat ctcccaaccc cagtactcct gctctccggt 300
 gccacctcac cgcc 314

<210> 42

<211> 352

B0213WO seq list.ST25.txt

<212> DNA

<213> Homo sapiens

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<400> 42
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ggcccggatg tcagctccat gctcaatgag cagccgcacg atctcctcac tgttcacaca 180
ggcagcaaag gacaaagggt gtccccaaa gtagatgagg ttgcagggaac tacggcggaa 240
ggcagtgcct gtggctctgg cagagacact ggccctgcgg gcaagcaggg ctcgcaccag 300
gttcatgttc tggttcaca cagcgatgtg cagtgcagtc tgacctccat cc 352

```

<210> 43

<211> 292

<212> DNA

<213> Homo sapiens

```

<400> 43
ggctgggggt aaggaccctg acagggcctc aggtggggac ttgcgcttca ggccgggatc 60
aggtgctgtg aagctgcctg tggagctggc cttggctttc cggaactcct ccagcttctg 120
tcggtagtac ttgtaccctt ggctattggg ctcatacaga aagctgaatg cctggttctc 180
acggttgttc tggaggggcaa tggtttccac ctcggggacc ccgtccgcta tgaacctggc 240
caacttttct gcaaggttct tgacctcttc gtcctctggg ggtgaaactt tc 292

```

<210> 44

<211> 372

<212> DNA

<213> Homo sapiens

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<400> 44
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caagtgaggt tcaattaagc tgagatgtag ttgaaatatt tgagcaccag aatcaccttc 120
tgaactccca atcatctggg gcttaggtgt agttacctcc caggaacaga tctcccagcc 180
caaggtccca cattgtacct tgatgtttct ttactgccta tcaaaatgag ccccaggtag 240
ctacgttatt actgggtttt gctctccaat ttccttattt tcccttgctc ccaaccacat 300
caacagaaca acaacaaaaa gaatccttta tcttcatgca aactacagt agtacagagc 360
aaagaactgg tg 372

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B0213W0 seq list.ST25.txt

<210> 45

<211> 158

<212> DNA

<213> Homo sapiens

<400> 45

aggcctgaga gggaagtttc tggagttcag atactctctg ttgggaacag gacatctcaa 60

cagtctcagg ttcgatcagt gggctctttg gcactttgaa ccttgaccac agggaccaag 120

aagtggcaat gaggacacct gcaggagggg ctgacctt 158

<210> 46

<211> 157

<212> DNA

<213> Homo sapiens

<400> 46

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tgcatgtggt agcatgggca aaaggcatta agaaagtaaa tatacgtccc tgtgttcccc 120

ttgctctccc tccatgcctg ctcttttctt caccctg 157

<210> 47

<211> 310

<212> DNA

<213> Homo sapiens

<400> 47

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actgcgcagg ttctctgctc accagccgct ggggtgatcac tgctgcccac tgtttcaagg 120

agtatgtaca gccggcctgg ggcacttgat cttctaaggc cctgggcttt gtgccccagg 180

ccctgggctc cctttacagg ctctgcttcc gggcctctgt tcaatcttgg tgccccctga 240

ttctacctag aagcctctcc tgccttcagg ggccttcaca cccatgctgt tccccagggt 300

acctcctccc 310

<210> 48

<211> 124

<212> DNA

<213> Homo sapiens

B0213WO seq list.ST25.txt

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 caccaagctc atttggtcgg tagggcccg cccaccggact tgagaacaca cacattctct 120
 aagg 124

<210> 49

<211> 191

<212> DNA

<213> Homo sapiens

<400> 49
 aatctcctaa tattaatggc aggtattata gtaaaattat catttcccct gaagagaatg 60
 ttacattaac ttgcacagca gaaaaccaac tggagagaac agtaaactcc ttgaatgtct 120
 ctgctaataa aaacagagaa aagggtgaatg accaggcaaa actaattgtg ggaatcgttg 180
 ttggtctcct c 191

<210> 50

<211> 305

<212> DNA

<213> Homo sapiens

<400> 50
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 cagaaaagga tacatttagt gcctcccacc cagctccact aaacgggttg gatattctcat 120
 tctttgagtt ggtgttcctt ccccggcgcc cccatgtagc tgggaagtgg gacctggggg 180
 tgggtggacc cctgggatcc taaaggaggg gcagggaggg cgcagaactc cgcttctgct 240
 ccttgctacc aggacgcgcg gcctcctcag cctctttcct cccgctgcc a tgcaccctac 300
 gctcg 305

<210> 51

<211> 108

<212> DNA

<213> Homo sapiens

<400> 51
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B0213WO seq list.ST25.txt

caaaacagac atcatgctgg tgagtgccac gttactcccc tggggtgc 108

<210> 52

<211> 439

<212> DNA

<213> Homo sapiens

<400> 52
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 ctgcaggtga gggcctgtca ccatcaagcc atcagtggga caaaaaactt gggccctcaa 120
 cccaccagca ctgctctgca ttctctgaag ccctggagat aagctgggga ccatggcccc 180
 cacactctca gacacagatg acacaagtgg gaccattctg agcctaagag attttaccaa 240
 aaatacctca atatggtttg gctacgtccc caccxaaatc ttatcttgaa ttgtaatcgc 300
 cgtaatcccc acgtgttgag ggagggatcc agtggaaggt gattaggtca tggaggtggt 360
 tttcccatg ctgttctcat gatagtgagg gagttttcac aagatctgat ggttttataa 420
 gccagtttcc cctccggcc 439

<210> 53

<211> 234

<212> DNA

<213> Homo sapiens

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<212> DNA

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<211> 1982

<212> DNA

<213> Homo sapiens

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<211> 322

<212> DNA

<213> Homo sapiens

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<212> DNA

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<212> DNA

<213> Homo sapiens

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<210> 77

<211> 3024

<212> DNA

<213> Homo sapiens

<400> 77

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<210> 78

<211> 1668

<212> DNA

<213> Homo sapiens

<400> 78

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<210> 79

<211> 997

<212> DNA

<213> Homo sapiens

<400> 79

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<210> 80

<211> 260

<212> DNA

<213> Homo sapiens

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<210> 81

<211> 201

<212> DNA

<213> Homo sapiens

<400> 81
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<210> 82

<211> 272

<212> DNA

<213> Homo sapiens

<400> 82
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B0213WO seq list.ST25.txt

<210> 83

<211> 258

<212> DNA

<213> Homo sapiens

<400> 83

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<210> 84

<211> 2226

<212> DNA

<213> Homo sapiens

<400> 84

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<210> 85

<211> 328

<212> DNA

<213> Homo sapiens

<400> 85

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<210> 86

B0213WO seq list.ST25.txt

<211> 1469

<212> DNA

<213> Homo sapiens

<400> 86

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<210> 87

<211> 180

<212> DNA

<213> Homo sapiens

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<210> 88

<211> 477

<212> DNA

<213> Homo sapiens

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<210> 89

<211> 1013

<212> DNA

<213> Homo sapiens

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<210> 90

<211> 737

<212> DNA

<213> Homo sapiens

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<212> DNA

<213> Homo sapiens

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B0213WO seq list.ST25.txt

<212> DNA

<213> Homo sapiens

<400> 100

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B0213WO seq list.ST25.txt

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<211> 212

<212> DNA

<213> Homo sapiens

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<210> 103

<211> 675

<212> DNA

<213> Homo sapiens

<400> 103

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B0213WO seq list.ST25.txt

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gtcccaaatt gaaaagagat ggccaggcat tgataaacct gatatttaga gacagatagt	420
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<211> 265

<212> DNA

<213> Homo sapiens

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<212> DNA

<213> Homo sapiens

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B0213WO seq list.ST25.txt

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<211> 253

<212> DNA

<213> Homo sapiens

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<210> 108

<211> 448

<212> DNA

<213> Homo sapiens

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<210> 109

<211> 437

B0213WO seq list.ST25.txt

<212> DNA

<213> Homo sapiens

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<211> 244

<212> DNA

<213> Homo sapiens

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<211> 272

<212> DNA

<213> Homo sapiens

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B0213WO seq list.ST25.txt

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<210> 113
 <211> 541
 <212> DNA
 <213> Homo sapiens

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 <211> 226
 <212> DNA
 <213> Homo sapiens

B0213WO seq list.ST25.txt

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<210> 115

<211> 439

<212> DNA

<213> Homo sapiens

<400> 115
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<210> 116

<211> 175

<212> DNA

<213> Homo sapiens

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<210> 117

<211> 521

<212> DNA

<213> Homo sapiens

<400> 117

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<210> 118

<211> 131

<212> DNA

<213> Homo sapiens

<400> 118

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<210> 119

<211> 212

<212> DNA

<213> Homo sapiens

<400> 119

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<210> 120

<211> 137

<212> DNA

<213> Homo sapiens

<400> 120

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<210> 121

<211> 265

<212> DNA

<213> Homo sapiens

<400> 121
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<210> 122

<211> 285

<212> DNA

<213> Homo sapiens

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<212> DNA

<213> Homo sapiens

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<211> 203

<212> DNA

<213> Homo sapiens

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<211> 239

<212> DNA

<213> Homo sapiens

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<212> DNA

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<210> 127

<211> 284

<212> DNA

<213> Homo sapiens

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<211> 252

<212> DNA

<213> Homo sapiens

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<211> 305

<212> DNA

<213> Homo sapiens

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<210> 131

<211> 337

<212> DNA

<213> Homo sapiens

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<210> 132

<211> 174

B0213WO seq list.ST25.txt

<212> DNA

<213> Homo sapiens

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<210> 133

<211> 113

<212> DNA

<213> Homo sapiens

<400> 133

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<210> 134

<211> 191

<212> DNA

<213> Homo sapiens

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<210> 135

<211> 1481

<212> DNA

<213> Homo sapiens

<400> 135

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B0213WO seq list.ST25.txt

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<211> 344

<212> DNA

<213> Homo sapiens

<400> 136

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<212> DNA

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<210> 138

<211> 399

<212> DNA

<213> Homo sapiens

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<211> 745

<212> DNA

<213> Homo sapiens

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<211> 3875

<212> DNA

<213> Homo sapiens

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<211> 493

<212> DNA

<213> Homo sapiens

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<211> 6263

<212> DNA

<213> Homo sapiens

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B0213WO seq list.ST25.txt

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<211> 299

<212> DNA

<213> Homo sapiens

<400> 146

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<210> 147

<211> 1326

<212> DNA

<213> Homo sapiens

<400> 147

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<211> 698

<212> DNA

<213> Homo sapiens

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<210> 149

<211> 478

<212> DNA

<213> Homo sapiens

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B0213WO seq list.ST25.txt

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 caccgactgc ggaggtctaa atcctggcac aaccagagtg agaatcatga agtcattgtc 360
 tgaggctcca gtagctgtgt aaatgtgatc accagccacc tcccagcctg tcatgccctt 420
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<210> 150

<211> 315

<212> DNA

<213> Homo sapiens

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<210> 151

<211> 219

<212> DNA

<213> Homo sapiens

<400> 151
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<210> 152

<211> 351

<212> DNA

<213> Homo sapiens

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<210> 153

<211> 286

<212> DNA

<213> Homo sapiens

<400> 153

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 tcctggctaa tagcacaggt ctgctggaaa tcaccacat ggtttttcat tgcctgtgca 240
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<210> 154

<211> 419

<212> DNA

<213> Homo sapiens

<400> 154

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 agcagctacc tgagcctgtc cataataacg cttctcaatc actagtgcgg ccgcctgcag 360
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<210> 155

<211> 424

<212> DNA

<213> Homo sapiens

B0213WO seq list.ST25.txt

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gggc 424

<210> 156

<211> 396

<212> DNA

<213> Homo sapiens

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<210> 157

<211> 161

<212> DNA

<213> Homo sapiens

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caattttctg caggatgatt gtacagaatc attgcttatg acatgatcgc tttctacact 120
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<210> 158

<211> 382

<212> DNA

B0213WO seq list.ST25.txt

<213> Homo sapiens

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<400> 158
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<210> 159

<211> 292

<212> DNA

<213> Homo sapiens

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<210> 160

<211> 447

<212> DNA

<213> Homo sapiens

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gacctccacc caataacgct tctcaatcac tagtgcggcc gcctgcaggt cgaccatatg    420

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B0213WO seq list.ST25.txt

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447

<210> 161

<211> 239

<212> DNA

<213> Homo sapiens

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<210> 162

<211> 410

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cagtcgaaga agacgcctca caacttcggg cgcttggtgg aatgtgtatt cgatttctgc 120

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<210> 163

<211> 327

<212> DNA

<213> Homo sapiens

<400> 163

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gggttttatt agctccggtg ggtaaaataa actcatctgt atcctgcacg aggtatcgtg 180

gatccacatt taaataagga gacagagggg tcataccagt tagcgggacg ccagccaaat 240

B0213WO seq list.ST25.txt

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<210> 164

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<212> DNA

<213> Homo sapiens

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<210> 165

<211> 351

<212> DNA

<213> Homo sapiens

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 caccgcctcc ccgtaataac gcttctcaat cactagtgcg gccgcctgca ggtcgaccat 300
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<210> 166

<211> 4839

<212> DNA

<213> Homo sapiens

B0213W0 seq list.ST25.txt

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B0213WO seq list.ST25.txt

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B0213WO seq list.ST25.txt

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<210> 167

<211> 200

<212> DNA

<213> Homo sapiens

<400> 167

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gagcccacag cctattccgt cctccctcaa gtgctgggga gagtgcggt gcctggacat	180
tccagaaggc accagccctg	200

<210> 168

<211> 355

<212> DNA

<213> Homo sapiens

<400> 168

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ggcagaagca tccactctgc agggacagtg gcccctcggg aaagcccgcc gtcctgtggc	120
aaggcctctc tgcagactcc acgggggctc accctctgcc gtcaggcgac tctgaaattc	180

B0213WO seq list.ST25.txt

cgacatttct cccttaaagt ctcaacagac acaagagaag tttccatcaa gcaagcactg 240
 acatatttat attaaaaaat agtgcaaaat ctcaacattt atataaataa ctctaaaccc 300
 ctgctttgta atttttttct ttacaaggta atacacactt tctgacttgg cactc 355

<210> 169

<211> 579

<212> DNA

<213> Homo sapiens

<400> 169

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 atgaatttgc ctgagtgtca gtggttaaga atacaaaag ggggtcccctt ctctcccaa 180
 ctgctccctc cctgggcccc taagaagggt ctgtgaccgt ctgcccagc ctttgcccag 240
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 atggtggtgg tgatcacgtg cctgctgagc cactacaagc tgtctgcacg gtccttcac 480
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 ccctcggaga gcacagtgtc aggcaacgga atcccagag 579

<210> 170

<211> 310

<212> DNA

<213> Homo sapiens

<400> 170

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 gatcatcatc atcgtggtgg tgatgatggg gatggtggtg gtgatcacgt gcctgctgag 180
 ccactacaag ctgtctgcac ggtccttcac cagccggcac agccagggg ggaggagaga 240
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<210> 171

<211> 301

<212> DNA

B0213WO seq list.ST25.txt

<213> Homo sapiens

<400> 171
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 gatgatgatg atctgaacaa actccagctc cgctagacca gagcgaattc atcctgaaga 180
 actcagagaa agccggtgca ggaagtgggt tcccgtcttc cctgcacagg cacagtgatg 240
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<210> 172

<211> 529

<212> DNA

<213> Homo sapiens

<400> 172
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 cttgtagtgg ctccagcaggc acgtgatcac caccaccatc accatcatca ccaccacgat 180
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 tactgggaat ccatgacaac ctagaccaga gcgaattcat cctgaagaac tcagagaaag 420
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<210> 173

<211> 1429

<212> DNA

<213> Homo sapiens

<400> 173
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 tgattggaag tggagatttt gccaaatcct tgaccattcg acttattaga tgcggctatc 180
 atgtgggtcat aggaagtaga aatcctaagt ttgcttctga attttttcct catgtggtag 240

B0213WO seq list.ST25.txt

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atgtgagcaa taacatgagg ataaaccagt acccagaatc caatgctgaa tatttggtctt 420
cattattccc agattctttg attgtcaaag gatttaaatgt tgtctcagct tgggcacttc 480
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<210> 174

<211> 462

<212> PRT

<213> Homo sapiens

<400> 174

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Met Glu Ser Ile Ser Met Met Gly Ser Pro Lys Ser Leu Ser Glu Thr
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Cys Leu Pro Asn Gly Ile Asn Gly Ile Lys Asp Ala Arg Lys Val Thr
20          25          30

```

```

Val Gly Val Ile Gly Ser Gly Asp Phe Ala Lys Ser Leu Thr Ile Arg
35          40          45

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```

Leu Ile Arg Cys Gly Tyr His Val Val Ile Gly Ser Arg Asn Pro Lys
50          55          60

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B0213WO seq list.ST25.txt

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

B0213WO seq list.ST25.txt

Ala Tyr Gln Gln Val His Ala Asn Ile Glu Asn Ser Trp Asn Glu Glu
 340 345 350

Glu Val Trp Arg Ile Glu Met Tyr Ile Ser Phe Gly Ile Met Ser Leu
 355 360 365

Gly Leu Leu Ser Leu Leu Ala Val Thr Ser Ile Pro Ser Val Ser Asn
 370 375 380

Ala Leu Asn Trp Arg Glu Phe Ser Phe Ile Gln Ser Thr Leu Gly Tyr
 385 390 395 400

Val Ala Leu Leu Ile Ser Thr Phe His Val Leu Ile Tyr Gly Trp Lys
 405 410 415

Arg Ala Phe Glu Glu Glu Tyr Tyr Arg Phe Tyr Thr Pro Pro Asn Phe
 420 425 430

Val Leu Ala Leu Val Leu Pro Ser Ile Val Ile Leu Val Glu Thr Glu
 435 440 445

Phe His Arg Val Ser Gln Asp Gly Leu Asp Leu Leu Thr Ser
 450 455 460

<210> 175

<211> 1329

<212> DNA

<213> Homo sapiens

<400> 175
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 aggataaacc agtaccaga atccaatgct gaatatttgg cttcattatt ccagattct 420
 ttgattgtca aaggatttaa tgttgtctca gcttgggcac ttcagttagg acctaaggat 480
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 attgaaaatt taccctacg actctttact ctctggagag ggccagtggg ggtagctata 660
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B0213WO seq list.ST25.txt

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caactttatt acggcaccaa gtataggaga tttccacctt gggtggaaac ctggttacag 900
tgtagaaaac agcttggatt actaagtttt ttcttcgcta tgggtccatgt tgcctacagc 960
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gcagataccc agactgagct ggaactggaa tttgtcttcc tattgactct acttctttaa 1260
aagcggctgc ccattacatt cctcagctgt ccttgcagtt aggtgtacat gtgactgagt 1320
gttggccag 1329

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<210> 176

<211> 419

<212> PRT

<213> Homo sapiens

<400> 176

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Met Glu Ser Ile Ser Met Met Gly Ser Pro Lys Ser Leu Ser Glu Thr
1      5      10     15

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Phe Leu Pro Asn Gly Ile Asn Gly Ile Lys Asp Ala Arg Lys Val Thr
20     25     30

```

```

Val Gly Val Ile Gly Ser Gly Asp Phe Ala Lys Ser Leu Thr Ile Arg
35     40     45

```

```

Leu Ile Arg Cys Gly Tyr His Val Val Ile Gly Ser Arg Asn Pro Lys
50     55     60

```

```

Phe Ala Ser Glu Phe Phe Pro His Val Val Asp Val Thr His His Glu
65     70     75     80

```

```

Asp Ala Leu Thr Lys Thr Asn Ile Ile Phe Val Ala Ile His Arg Glu
85     90     95

```

```

His Tyr Thr Ser Leu Trp Asp Leu Arg His Leu Leu Val Gly Lys Ile
100    105    110

```

```

Leu Ile Asp Val Ser Asn Asn Met Arg Ile Asn Gln Tyr Pro Glu Ser
115    120    125

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```

Asn Ala Glu Tyr Leu Ala Ser Leu Phe Pro Asp Ser Leu Ile Val Lys

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B0213WO seq list.ST25.txt

130

135

140

Gly Phe Asn Val Val Ser Ala Trp Ala Leu Gln Leu Gly Pro Lys Asp
 145 150 155 160

Ala Ser Arg Gln Val Tyr Ile Cys Ser Asn Asn Ile Gln Ala Arg Gln
 165 170 175

Gln Val Ile Glu Leu Ala Arg Gln Leu Asn Phe Ile Pro Ile Asp Leu
 180 185 190

Gly Ser Leu Ser Ser Ala Arg Glu Ile Asp Asn Leu Pro Leu Arg Leu
 195 200 205

Phe Thr Leu Trp Arg Gly Pro Val Val Val Ala Ile Ser Leu Ala Thr
 210 215 220

Phe Phe Phe Leu Tyr Ser Phe Val Arg Asp Val Ile His Pro Tyr Ala
 225 230 235 240

Arg Asn Gln Gln Ser Asp Phe Tyr Lys Ile Pro Ile Glu Ile Val Asn
 245 250 255

Lys Thr Leu Pro Ile Val Ala Ile Thr Leu Leu Ser Leu Val Tyr Leu
 260 265 270

Ala Gly Leu Leu Ala Ala Ala Tyr Gln Leu Tyr Tyr Gly Thr Lys Tyr
 275 280 285

Arg Arg Phe Pro Pro Trp Leu Glu Thr Trp Leu Gln Cys Arg Lys Gln
 290 295 300

Leu Gly Leu Leu Ser Phe Phe Phe Ala Met Val His Val Ala Tyr Ser
 305 310 315 320

Leu Cys Leu Pro Met Arg Arg Ser Glu Arg Tyr Leu Phe Leu Asn Met
 325 330 335

Ala Tyr Gln Gln Val His Ala Asn Ile Glu Asn Ser Trp Asn Glu Glu
 340 345 350

Glu Val Trp Arg Ile Glu Met Tyr Ile Ser Phe Gly Ile Met Ser Leu
 355 360 365

Gly Leu Leu Ser Leu Leu Ala Val Thr Ser Ile Pro Ser Val Ser Asn
 370 375 380

Ala Leu Asn Trp Arg Glu Phe Ser Phe Ile Gln Ile Phe Cys Ser Phe
 385 390 395 400

Ala Asp Thr Gln Thr Glu Leu Glu Leu Glu Phe Val Phe Leu Leu Thr

405 B0213WO seq list.ST25.txt 415
410

Leu Leu Leu

<210> 177

<211> 940

<212> DNA

<213> Homo sapiens

<400> 177

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gattggaagt ggagattttg ccaaatacctt gaccattcga cttattagat gcggctatca      180
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agaacattat acctccctgt gggacctgag acatctgctt gtgggtaaaa tcctgattga      360
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acaacagggt catgcaaata ttgaaaactc ttggaatgag gaagaagttt ggagaattga      600
aatgtatatc tcctttggca taatgagcct tggcttactt tccctcctgg cagtcacttc      660
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ttttgaggaa gagtactaca gattttatac accaccaaac tttgttcttg ctcttgtttt      840
gccctcaatt gtaattctgg tagagacgga gtttcaccgt gttagccagg atggtctcga      900
tctcctgacc tcgtgatccg cccgccttgg cctccaaagt      940

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<210> 178

<211> 299

<212> PRT

<213> Homo sapiens

<400> 178

Met Glu Ser Ile Ser Met Met Gly Ser Pro Lys Ser Leu Ser Glu Thr
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Cys Leu Pro Asn Gly Ile Asn Gly Ile Lys Asp Ala Arg Lys Val Thr

20

B0213WO seq list.ST25.txt
25 30

Val Gly Val Ile Gly Ser Gly Asp Phe Ala Lys Ser Leu Thr Ile Arg
 35 40 45
 Leu Ile Arg Cys Gly Tyr His Val Val Ile Gly Ser Arg Asn Pro Lys
 50 55 60
 Phe Ala Ser Glu Phe Phe Pro His Val Val Asp Val Thr His His Glu
 65 70 75 80
 Asp Ala Leu Thr Lys Thr Asn Ile Ile Phe Val Ala Ile His Arg Glu
 85 90 95
 His Tyr Thr Ser Leu Trp Asp Leu Arg His Leu Leu Val Gly Lys Ile
 100 105 110
 Leu Ile Asp Val Ser Asn Asn Met Arg Ile Asn Gln Tyr Pro Glu Ser
 115 120 125
 Asn Ala Glu Tyr Leu Ala Ser Leu Phe Pro Asp Ser Leu Ile Val Lys
 130 135 140
 Gly Phe Asn Val Val Ser Ala Trp Ala Leu Gln Leu Gly Pro Lys Asp
 145 150 155 160
 Ala Ser Arg Gln Val Tyr Ile Cys Ser Asn Asn Ile Gln Ala Arg Gln
 165 170 175
 Gln Val His Ala Asn Ile Glu Asn Ser Trp Asn Glu Glu Glu Val Trp
 180 185 190
 Arg Ile Glu Met Tyr Ile Ser Phe Gly Ile Met Ser Leu Gly Leu Leu
 195 200 205
 Ser Leu Leu Ala Val Thr Ser Ile Pro Ser Val Ser Asn Ala Leu Asn
 210 215 220
 Trp Arg Glu Phe Ser Phe Ile Gln Ser Thr Leu Gly Tyr Val Ala Leu
 225 230 235 240
 Leu Ile Ser Thr Phe His Val Leu Ile Tyr Gly Trp Lys Arg Ala Phe
 245 250 255
 Glu Glu Glu Tyr Tyr Arg Phe Tyr Thr Pro Pro Asn Phe Val Leu Ala
 260 265 270
 Leu Val Leu Pro Ser Ile Val Ile Leu Val Glu Thr Glu Phe His Arg
 275 280 285
 Val Ser Gln Asp Gly Leu Asp Leu Leu Thr Ser

290

B0213WO seq list.ST25.txt
295

<210> 179

<211> 1388

<212> DNA

<213> Homo sapiens

<400> 179

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gattggaagt ggagattttg ccaaattcctt gaccattcga cttattagat gcggctatca	180
tgtgggcata ggaagtagaa atcctaagtt tgcttctgaa ttttttcctc atgtggtaga	240
tgtcactcat catgaagatg ctctcacaaa acaaatata atatttggtg ctatacacag	300
agaacattat acctccctgt gggacctgag acatctgctt gtgggtaaaa tcctgattga	360
tgtgagcaat aacatgagga taaaccagta cccagaatcc aatgctgaat atttggcttc	420
attattccca gattctttga ttgtcaaagg atttaatgtt gtctcagctt gggcacttca	480
gttaggacct aaggatgccg gccggcaggt ttatatatgc agcaacaata ttcaagcgcg	540
acaacagggtt attgaacttg cccgccagtt gaatttcatt cccattgact tgggatcctt	600
atcatcagcc agagagattg aaaatttacc cctacgactc tttactctct ggagagggcc	660
agtggtggtg gctataagct tggccacatt tttttccttt attcctttgt cagagatgtg	720
attcatccat atgctagaaa ccaacagagt gacttttaca aaattcctat agagattgtg	780
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gaaacctggt tacagtgtag aaaacagctt ggattactaa gttttttctt cgctatggtc	960
catgttgcct acagcctctg cttaccgatg agaagggtca tgcaaattatt gaaaactctt	1020
ggaatgagga agaagtttgg agaattgaaa tgtatatctc ctttggcata atgagccttg	1080
gcttactttc cctcctggca gtcacttcta tcccgtcagt gagcaatgct tttaaactgga	1140
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atgttttaat ttatggatgg aaacgagctt ttgaggaaga gtactacaga ttttatacac	1260
caccaaactt tgttcttgct cttgttttgc cctcaattgt aattctggta gagacggagt	1320
ttcaccgtgt tagccaggat ggtctcgatc tcctgacctc gtgatccgcc cgccttggcc	1380
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<210> 180

<211> 234

<212> PRT

B0213WO seq list.ST25.txt

<213> Homo sapiens

<400> 180

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 Cys Leu Pro Asn Gly Ile Asn Gly Ile Lys Asp Ala Arg Lys Val Thr
 20 25 30
 Val Gly Val Ile Gly Ser Gly Asp Phe Ala Lys Ser Leu Thr Ile Arg
 35 40 45
 Leu Ile Arg Cys Gly Tyr His Val Val Ile Gly Ser Arg Asn Pro Lys
 50 55 60
 Phe Ala Ser Glu Phe Phe Pro His Val Val Asp Val Thr His His Glu
 65 70 75 80
 Asp Ala Leu Thr Lys Thr Asn Ile Ile Phe Val Ala Ile His Arg Glu
 85 90 95
 His Tyr Thr Ser Leu Trp Asp Leu Arg His Leu Leu Val Gly Lys Ile
 100 105 110
 Leu Ile Asp Val Ser Asn Asn Met Arg Ile Asn Gln Tyr Pro Glu Ser
 115 120 125
 Asn Ala Glu Tyr Leu Ala Ser Leu Phe Pro Asp Ser Leu Ile Val Lys
 130 135 140
 Gly Phe Asn Val Val Ser Ala Trp Ala Leu Gln Leu Gly Pro Lys Asp
 145 150 155 160
 Ala Ser Arg Gln Val Tyr Ile Cys Ser Asn Asn Ile Gln Ala Arg Gln
 165 170 175
 Gln Val Ile Glu Leu Ala Arg Gln Leu Asn Phe Ile Pro Ile Asp Leu
 180 185 190
 Gly Ser Leu Ser Ser Ala Arg Glu Ile Glu Asn Leu Pro Leu Arg Leu
 195 200 205
 Phe Thr Leu Trp Arg Gly Pro Val Val Val Ala Ile Ser Leu Ala Thr
 210 215 220
 Phe Phe Ser Phe Ile Pro Leu Ser Glu Met
 225 230

<210> 181

B0213WO seq list.ST25.txt

<211> 1425

<212> DNA

<213> Homo sapiens

<400> 181

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gattggaagt ggagattttg ccaaatacctt gaccattcga cttattagat gcggctatca      180
tgtggtcata ggaagtagaa atcctaagtt tgcttctgaa ttttttcctc atgtggtaga      240
tgtcactcat catgaagatg ctctcacaaa aacaaatata atatttggtg ctatacacag      300
agaacattat acctccctgt gggacctgag acatctgctt gtgggtaaaa tcctgattga      360
tgtgagcaat aacatgagga taaaccagta ccagaatcc aatgctgaat atttggcttc      420
attattccca gattctttga ttgtcaaagg atttaatggt gtctcagctt gggcacttca      480
gttaggacct aaggatgcca gccggcaggt ttatatatgc agcaacaata ttcaagcgcg      540
acaacagggt attgaacttg cccgccagtt gaatttcatt ccattgact tgggatcctt      600
atcatcagcc agagagattg aaaatttacc cctacgactc tttactctct ggagagggcc      660
agtggtggtg gctataagct tggccacatt ttttttcctt tttcctttg tcagagatgt      720
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gaataaaacc ttacctatag ttgccattac ttgtctctcc ctagtatacc ttgcaggctc      840
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ccatgttgcc tacagcctct gcttaccgat gagaagggtc gagagatatt tgtttctcaa     1020
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gagaattgaa atgtatatct cttttggcat aatgagcctt ggcttacttt ccctcctggc     1140
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gtctacactt ggatatgtcg ctctgctcat aagtactttc catgttttaa tttatggatg     1260
gaaacgagct tttgaggaag agtactacag attttataca ccaccaaact ttgttcttgc     1320
tcttgttttg ccctcaattg taattctgga gacggagttt caccgtgtta gccaggatgg     1380
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<210> 182

<211> 461

<212> PRT

<213> Homo sapiens

B0213W0 seq list.ST25.txt

<400> 182

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Cys Leu Pro Asn Gly Ile Asn Gly Ile Lys Asp Ala Arg Lys Val Thr
 20 25 30

Val Gly Val Ile Gly Ser Gly Asp Phe Ala Lys Ser Leu Thr Ile Arg
 35 40 45

Leu Ile Arg Cys Gly Tyr His Val Val Ile Gly Ser Arg Asn Pro Lys
 50 55 60

Phe Ala Ser Glu Phe Phe Pro His Val Val Asp Val Thr His His Glu
 65 70 75 80

Asp Ala Leu Thr Lys Thr Asn Ile Ile Phe Val Ala Ile His Arg Glu
 85 90 95

His Tyr Thr Ser Leu Trp Asp Leu Arg His Leu Leu Val Gly Lys Ile
 100 105 110

Leu Ile Asp Val Ser Asn Asn Met Arg Ile Asn Gln Tyr Pro Glu Ser
 115 120 125

Asn Ala Glu Tyr Leu Ala Ser Leu Phe Pro Asp Ser Leu Ile Val Lys
 130 135 140

Gly Phe Asn Val Val Ser Ala Trp Ala Leu Gln Leu Gly Pro Lys Asp
 145 150 155 160

Ala Ser Arg Gln Val Tyr Ile Cys Ser Asn Asn Ile Gln Ala Arg Gln
 165 170 175

Gln Val Ile Glu Leu Ala Arg Gln Leu Asn Phe Ile Pro Ile Asp Leu
 180 185 190

Gly Ser Leu Ser Ser Ala Arg Glu Ile Glu Asn Leu Pro Leu Arg Leu
 195 200 205

Phe Thr Leu Trp Arg Gly Pro Val Val Val Ala Ile Ser Leu Ala Thr
 210 215 220

Phe Phe Phe Leu Tyr Ser Phe Val Arg Asp Val Ile His Pro Tyr Ala
 225 230 235 240

Arg Asn Gln Gln Ser Asp Phe Tyr Lys Ile Pro Ile Glu Ile Val Asn
 245 250 255

Lys Thr Leu Pro Ile Val Ala Ile Thr Leu Leu Ser Leu Val Tyr Leu

B0213WO seq list.ST25.txt
260 265 270Ala Gly Leu Leu Ala Ala Ala Tyr Gln Leu Tyr Tyr Gly Thr Lys Tyr
275 280 285Arg Arg Phe Pro Pro Trp Leu Glu Thr Trp Leu Gln Cys Arg Lys Gln
290 295 300Leu Gly Leu Leu Ser Phe Phe Phe Ala Met Val His Val Ala Tyr Ser
305 310 315 320Leu Cys Leu Pro Met Arg Arg Ser Glu Arg Tyr Leu Phe Leu Asn Met
325 330 335Ala Tyr Gln Gln Val His Ala Asn Ile Glu Asn Ser Trp Asn Glu Glu
340 345 350Glu Val Trp Arg Ile Glu Met Tyr Ile Ser Phe Gly Ile Met Ser Leu
355 360 365Gly Leu Leu Ser Leu Leu Ala Val Thr Ser Ile Pro Ser Val Ser Asn
370 375 380Ala Leu Asn Trp Arg Glu Phe Ser Phe Ile Gln Ser Thr Leu Gly Tyr
385 390 395 400Val Ala Leu Leu Ile Ser Thr Phe His Val Leu Ile Tyr Gly Trp Lys
405 410 415Arg Ala Phe Glu Glu Glu Tyr Tyr Arg Phe Tyr Thr Pro Pro Asn Phe
420 425 430Val Leu Ala Leu Val Leu Pro Ser Ile Val Ile Leu Glu Thr Glu Phe
435 440 445His Arg Val Ser Gln Asp Gly Leu Asp Leu Leu Thr Ser
450 455 460

<210> 183

<211> 18

<212> PRT

<213> Homo sapiens

<400> 183

Val Glu Thr Glu Phe His Arg Val Ser Gln Asp Gly Leu Asp Leu Leu
1 5 10 15

Thr Ser

B0213WO seq list.ST25.txt

<210> 184

<211> 24

<212> PRT

<213> Homo sapiens

<400> 184

Ile Phe Cys Ser Phe Ala Asp Thr Gln Thr Glu Leu Glu Leu Glu Phe
1 5 10 15

Val Phe Leu Leu Thr Leu Leu Leu
20

<210> 185

<211> 8

<212> PRT

<213> Homo sapiens

<400> 185

Ser Phe Ile Pro Leu Ser Glu Met
1 5