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(54) **METHODS AND COMPOSITIONS FOR EVALUATING BREAST CANCER PROGNOSIS**

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(60) Provisional application No. 60/612,073, filed on Sep. 22, 2004, provisional application No. 60/611,965, filed on Sep. 22, 2004.

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(52) **U.S. Cl.** **436/501**

(57) **ABSTRACT**

Methods and compositions for evaluating the prognosis of a breast cancer patient, particularly an early-stage breast cancer patient, are provided. The methods of the invention comprise detecting expression of at least one, more particularly at least two, biomarker(s) in a body sample, wherein overexpression of the biomarker or a combination of biomarkers is indicative of breast cancer prognosis. In some embodiments, the body sample is a breast tissue sample, particularly a primary breast tumor sample. The biomarkers of the invention are proteins and/or genes whose overexpression is indicative of either a good or bad cancer prognosis. Biomarkers of interest include proteins and genes involved in cell cycle regulation, DNA replication, transcription, signal transduction, cell proliferation, invasion, proteolysis, or metastasis. In some aspects of the invention, overexpression of a biomarker of interest is detected at the protein level using biomarker-specific antibodies or at the nucleic acid level using nucleic acid hybridization techniques.

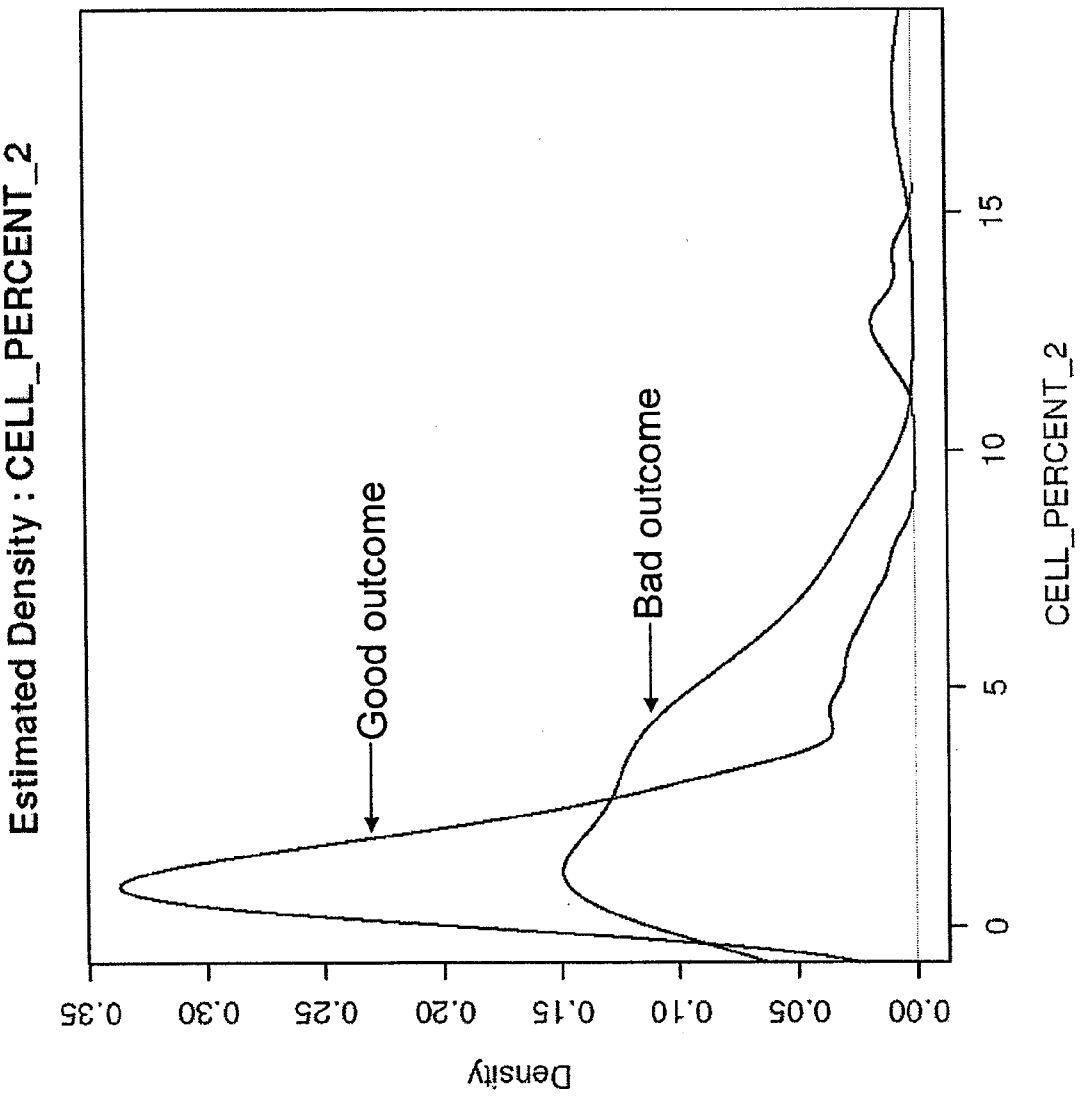

Estimated Density : CELL_PERCENT_2
Good outcome
Bad outcome
Density
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0
5
10
15
CELL_PERCENT_2

FIG. 1

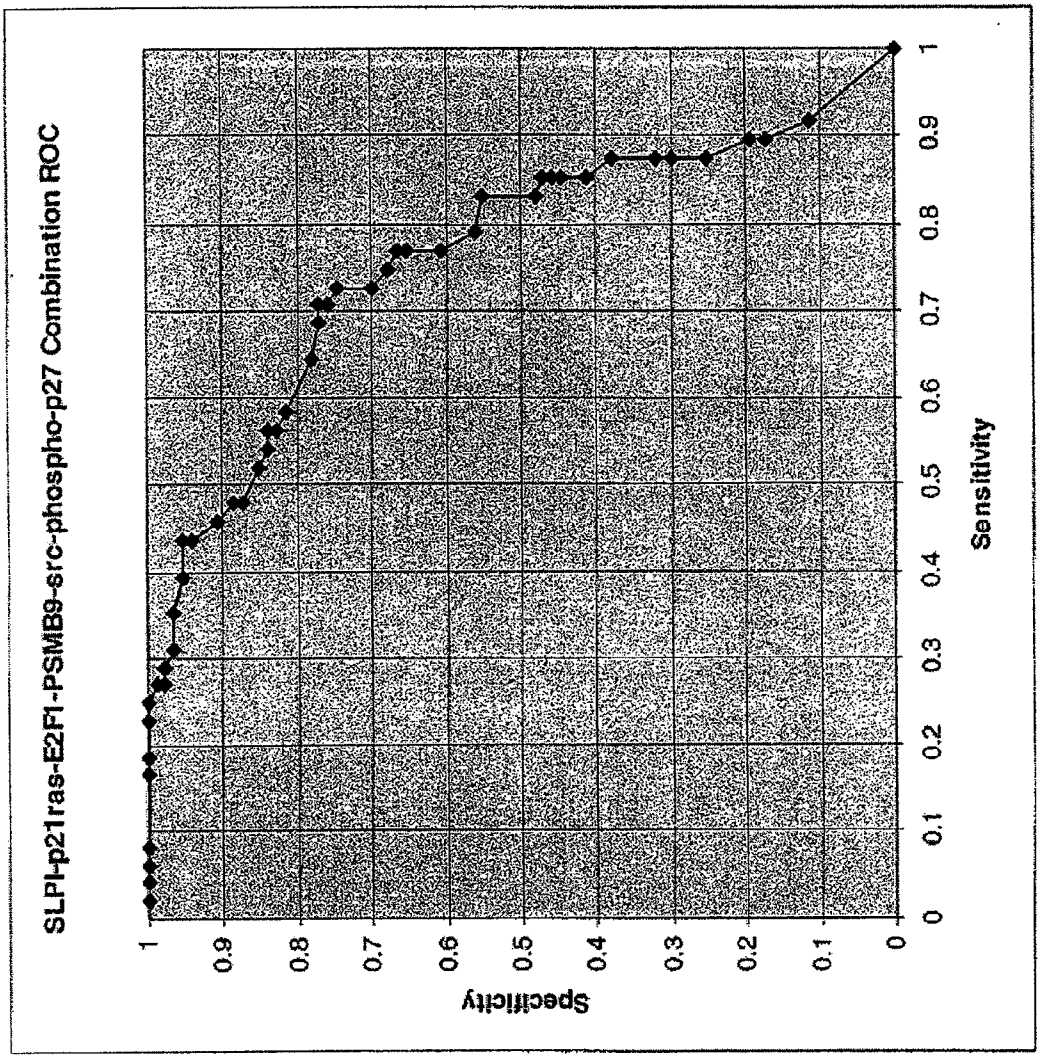

SLPI-p21ras-E2F1-PSMB9-src-phospho-p27 Combination ROC
Specificity
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Sensitivity
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

FIG. 2

FIG. 3

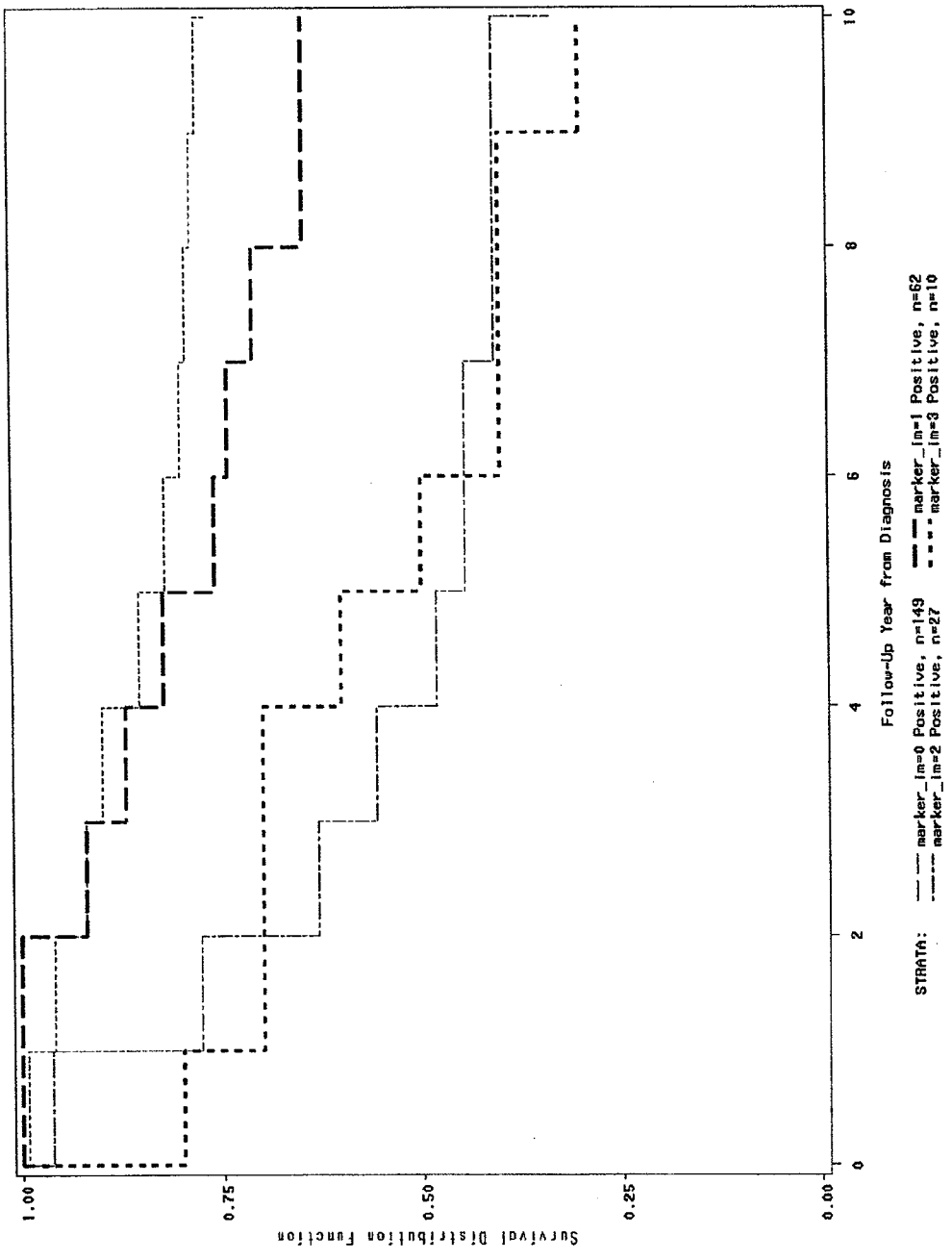

Survival Distribution Function
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
10
Follow-Up Year from Diagnosis
STRATA:
marker_im=0 Positive, n=149
marker_im=2 Positive, n=27
marker_im=1 Positive, n=62
marker_im=3 Positive, n=10

FIG. 4

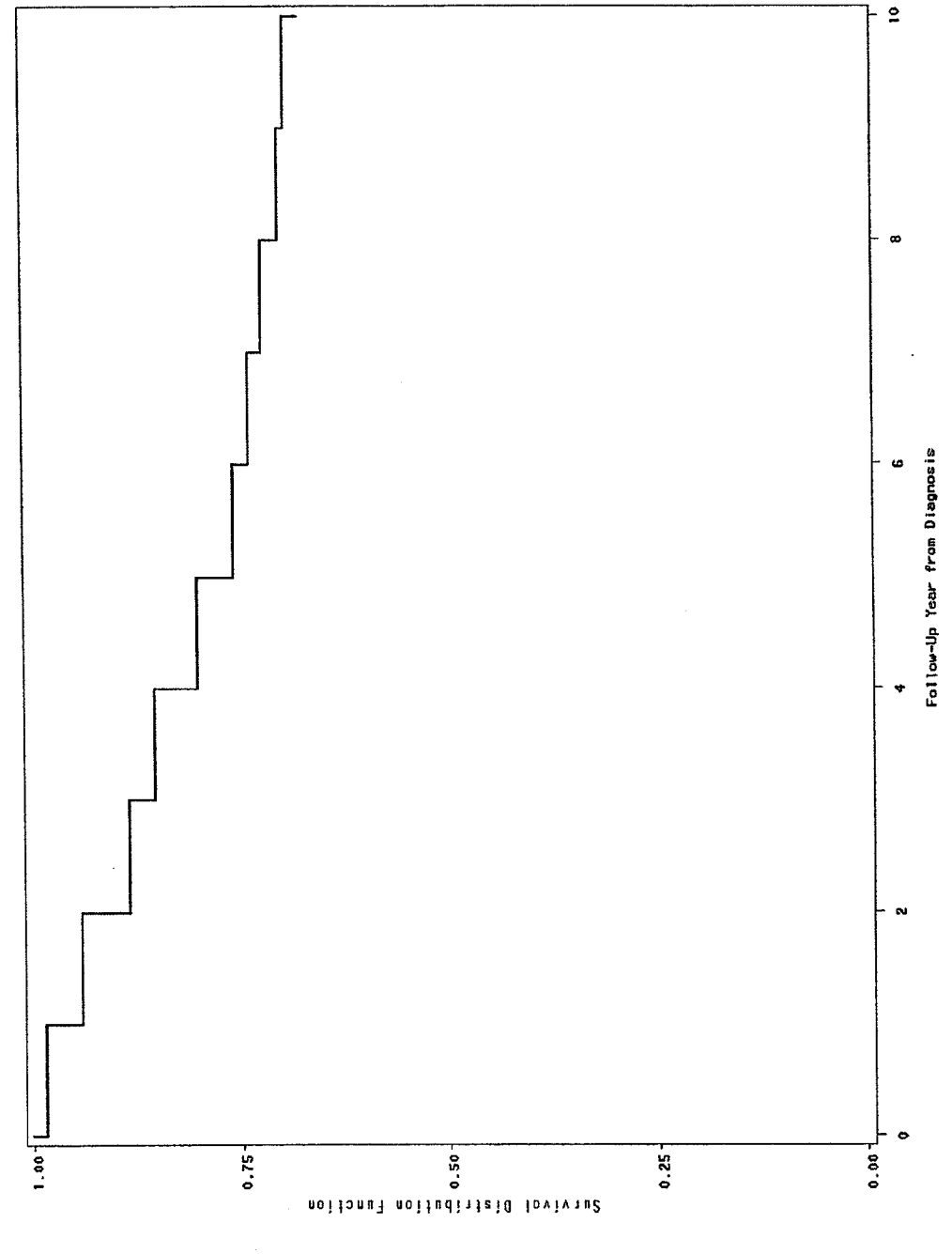
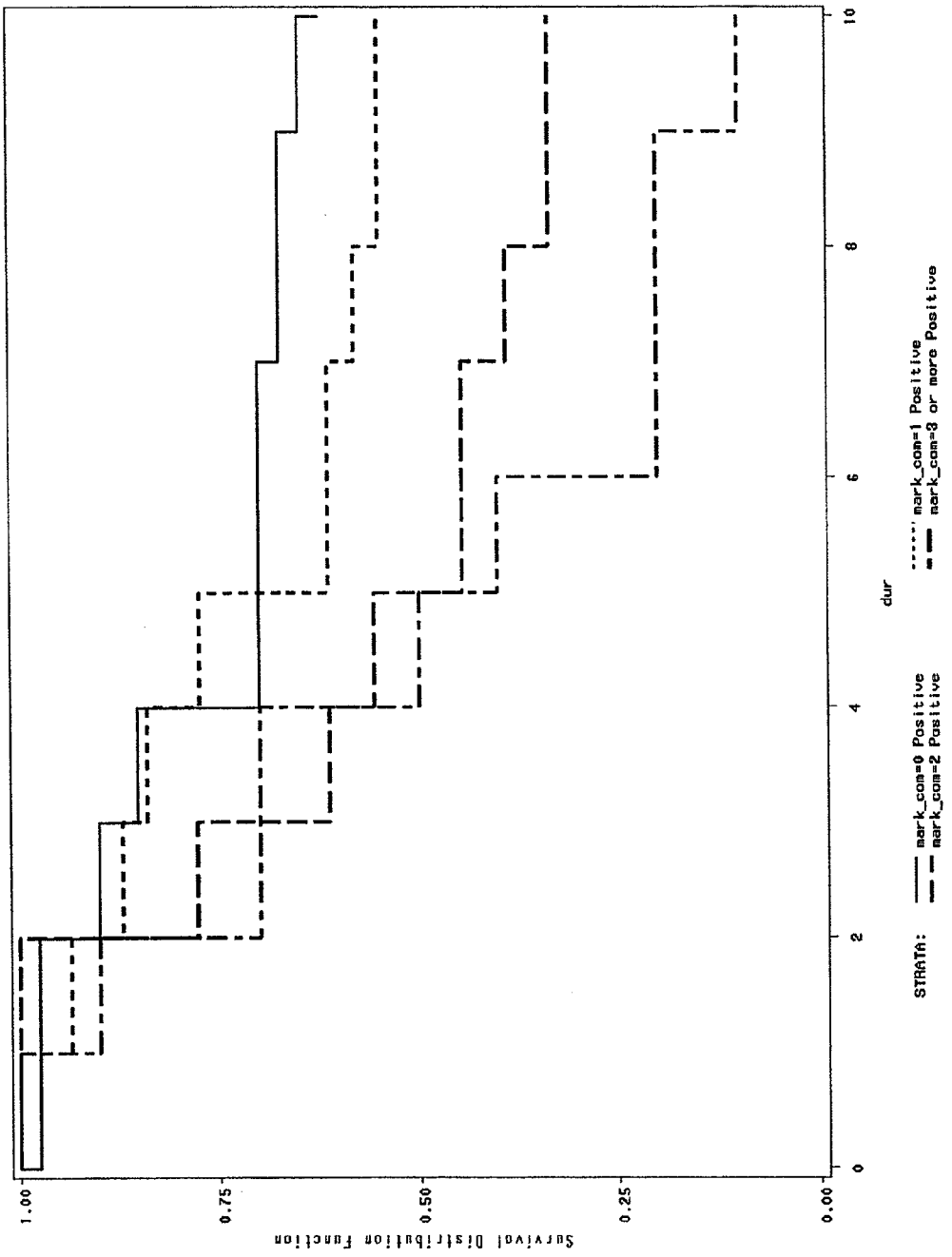

Survival Distribution Function
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
10
Follow-Up Year from Diagnosis

FIG. 5


Survival Distribution Function
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
10
dur
STRATA:
mark_com=0 Positive
mark_com=2 Positive
mark_com=1 Positive
mark_com=3 or more Positive

METHODS AND COMPOSITIONS FOR EVALUATING BREAST CANCER PROGNOSIS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional application of U.S. Utility application Ser. No. 11/233,510, filed Sep. 22, 2005, which claims the benefit of U.S. Provisional Application Ser. No. 60/612,073, filed Sep. 22, 2004, and U.S. Provisional Application Ser. No. 60/611,965, filed Sep. 22, 2004, all of which are incorporated herein by reference in their entirety.

REFERENCE TO A SEQUENCE LISTING SUBMITTED AS A TEXT FILE VIA EFS-WEB

[0002] The official copy of the sequence listing is submitted concurrently with the specification as a text file via EFS-Web, in compliance with the American Standard Code for Information Interchange (ASCII), with a file name of 372587SequenceListing.txt, a creation date of May 14, 2009, and a size of 161 KB. The sequence listing filed via EFS-Web is part of the specification and is hereby incorporated in its entirety by reference herein.

FIELD OF THE INVENTION

[0003] The present invention relates to methods and compositions for evaluating the prognosis of a patient afflicted with breast cancer, particularly early-stage breast cancer.

BACKGROUND OF THE INVENTION

[0004] Breast cancer is the second most common cancer among American women, less frequent only than skin cancer. An American woman has a one in eight chance of developing breast cancer during her lifetime, and the American Cancer Society estimates that more than 250,000 new cases of breast cancer will be reported in the U.S. this year. Breast cancer is the second leading cause of cancer deaths in women, with more than 40,000 Americans expected to die from the disease in 2004.

[0005] Improved detection methods, mass screening, and advances in treatment over the last decade have significantly improved the outlook for woman diagnosed with breast cancer. Today, approximately 80% of breast cancer cases are diagnosed in the early stages of the disease when survival rates are at their highest. As a result, about 85% percent of breast cancer patients are alive at least 5 years after diagnosis.

[0006] Despite these advances, approximately 20% of women diagnosed with early-stage breast cancer have a poor ten-year outcome and will suffer disease recurrence, metastasis, or death within this time period. The remaining 80% of breast cancer patients diagnosed at an early stage, however, have a good 10-year prognosis and are unlikely to need, or benefit from, additional aggressive adjuvant therapy (e.g., chemotherapy). The current clinical consensus is that at least some early-stage, node-negative breast cancer patients should receive adjuvant chemotherapy, but presently there are no widely used assays to risk stratify patients for more aggressive treatment. Since the majority of these early-stage cancer patients enjoy long-term survival following surgery and/or radiation therapy without further treatment, it is likely inappropriate to recommend aggressive adjuvant therapy for all of these patients, particularly in light of the significant side effects associated with cancer chemotherapeutics. Compositions and methods that permit the differentiation of these populations of early-stage breast cancer patients at the time of initial diagnosis into good and bad prognosis groups would assist clinicians in selecting appropriate courses of treatment. Thus, methods for evaluating the prognosis of breast cancer patients, particularly early-stage breast cancer patients, are needed.

[0007] Significant research has focused on identifying methods and factors for assessing breast cancer prognosis and predicting therapeutic response. (See generally, Ross and Hortobagyi, eds. (in press) Molecular Oncology of Breast Cancer (Jones and Bartlett Publishers, Boston, Mass.) and the references cited therein, all of which are herein incorporated by reference in their entirety). Prognostic indicators include more conventional factors, such as tumor size, nodal status, and histological grade, as well as molecular markers that provide some information regarding prognosis and likely response to particular treatments. For example, determination of estrogen (ER) and progesterone (PR) steroid hormone receptor status has become a routine procedure in assessment of breast cancer patients. See, for example, Fitzgibbons et al. (2000) *Arch. Pathol. Lab. Med.* 124:966-978. Tumors that are hormone receptor positive are more likely to respond to hormone therapy and also typically grow less aggressively, thereby resulting in a better prognosis for patients with ER+/PR+tumors.

[0008] Overexpression of human epidermal growth factor receptor 2 (HER-2/neu), a transmembrane tyrosine kinase receptor protein, has been correlated with poor breast cancer prognosis. Ross et al. (2003) *The Oncologist:*307-325. Her2/neu expression levels in breast tumors are currently used to predict response to the anti-Her-2/neu antibody therapeutic trastuzumab (Herceptin®; Genentech). See, for example, Id. and Ross et al., supra. Furthermore, approximately one-third of breast cancers have mutations in the tumor suppressor gene p53, and these mutations have been associated with increased disease aggressiveness and poor prognostic outcome. Fitzgibbons et al., supra. Ki-67 is a non-histone nuclear protein that is expressed during the G1 through M phases of the cell cycle. Studies have shown that overexpression of the cellular proliferation marker Ki-67 also correlates with poor breast cancer prognosis. Id.

[0009] Although current prognostic criteria and molecular markers provide some guidance in predicting patient outcome and selecting appropriate course of treatment, a significant need exists for a specific and sensitive method for evaluating breast cancer prognosis, particularly in early-stage, lymph-node negative patients. Such a method should specifically distinguish breast cancer patients with a poor prognosis from those with a good prognosis and permit the identification of high-risk, early-stage breast cancer patients who are likely to need aggressive adjuvant therapy.

SUMMARY OF THE INVENTION

[0010] Methods and compositions for evaluating the prognosis of a cancer patient, particularly a breast cancer patient, are provided. The methods comprise detecting expression of at least one, more particularly at least two, biomarker(s) in a body sample, wherein the overexpression of a biomarker or combination of biomarkers is indicative of cancer prognosis. Overexpression of the biomarker or combination of biomarkers of the invention is indicative of either a good prognosis (i.e., disease-free survival) or a bad prognosis (i.e., cancer recurrence, metastasis, or death from the underlying cancer).

Thus, the present method permits the differentiation of breast cancer patients with a good prognosis from those patients with a bad prognosis. The methods disclosed herein can be used in combination with assessment of conventional clinical factors (e.g., tumor size, tumor grade, lymph node status, and family history) and/or analysis of the expression level of molecular markers, such as Her2/neu, Ki67, p53, and estrogen and progesterone hormone receptors. In this manner, the methods of the invention permit a more accurate evaluation of breast cancer prognosis.

[0011] The biomarkers of the invention are proteins and/or genes whose overexpression is indicative of cancer prognosis, including those biomarkers involved in cell cycle regulation, DNA replication, transcription, signal transduction, cell proliferation, invasion, or metastasis. The detection of overexpression of the biomarker genes or proteins of the invention permits the evaluation of cancer prognosis and facilitates the separation of breast cancer patients into good and bad prognosis risk groups for the purposes of, for example, treatment selection.

[0012] Biomarker expression can be assessed at the protein or nucleic acid level. In some embodiments, immunohistochemistry techniques are provided that utilize antibodies to detect the expression of biomarker proteins in breast tumor samples. In this aspect of the invention, at least one antibody directed to a specific biomarker of interest is used. Expression can also be detected by nucleic acid-based techniques, including, for example, hybridization and RT-PCR.

[0013] Compositions include monoclonal antibodies capable of binding to biomarker proteins of the invention. Antigen-binding fragments and variants of these monoclonal antibodies, hybridoma cell lines producing these antibodies, and isolated nucleic acid molecules encoding the amino acid sequences of these monoclonal antibodies are also encompassed herein. Kits comprising reagents for practicing the methods of the invention are further provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIG. **1** shows the distribution of percentage of cells staining with an intensity of 2 as a function of actual breast cancer outcome. Experimental details are provided in Example 4.

[0015] FIG. **2** provides the ROC curve obtained using the sequence-based interpretation approach for the SLPI/p21ras/E2F1/PSMB9/src/phospho-p27 combination. Experimental details are provided in Example 5.

[0016] FIG. **3** provides the Kaplan-Meier plot for the prognostic performance of the SLPI, src, PSMB9, p21ras, and E2F1 biomarker panel. Details are provided in Example 8.

[0017] FIG. **4** provides a graphical representation of the long-term survival data for the general breast cancer patient population, independent of analysis of biomarker overexpression. Details are provided in Example 8.

[0018] FIG. **5** provides the Kaplan-Meier plot for the prognostic performance of the SLPI, src, PSMB9, p21ras, E2F1, and MUC-1 biomarker panel. Details are provided in Example 9.

DETAILED DESCRIPTION OF THE INVENTION

[0019] The present invention provides methods and compositions for evaluating the prognosis of a cancer patient, particularly a breast cancer patient, more particularly an early-stage breast cancer patient. The methods comprise detecting the expression of biomarkers in a patient tissue or body fluid sample and determining if said biomarkers are overexpressed. Overexpression of a biomarker or combination of biomarkers used in the practice of the invention is indicative of breast cancer prognosis (i.e., bad or good prognosis). Thus, overexpression of a particular biomarker or combination of biomarkers of interest permits the differentiation of breast cancer patients that are likely to experience disease recurrence (i.e., poor prognosis) from those who are more likely to remain cancer-free (i.e., good prognosis). In some aspects of the invention, the methods involve detecting the overexpression of at least one biomarker in a breast tumor sample that is indicative of a poor breast cancer prognosis and thereby identifying patients who are more likely to suffer a recurrence of the underlying cancer. The methods of the invention can also be used to assist in selecting appropriate courses of treatment and to identify patients that would benefit from more aggressive therapy. In particular embodiments, antibodies and immunohistochemistry techniques are used to detect expression of a biomarker of interest and to evaluate the prognosis of a breast cancer patient. Monoclonal antibodies specific for biomarkers of interest and kits for practicing the methods of the invention are further provided.

[0020] By "breast cancer" is intended, for example, those conditions classified by biopsy as malignant pathology. The clinical delineation of breast cancer diagnoses is well-known in the medical arts. One of skill in the art will appreciate that breast cancer refers to any malignancy of the breast tissue, including, for example, carcinomas and sarcomas. In particular embodiments, the breast cancer is ductal carcinoma in situ (DCIS), lobular carcinoma in situ (LCIS), or mucinous carcinoma. Breast cancer also refers to infiltrating ductal (IDC) or infiltrating lobular carcinoma (ILC). In most embodiments of the invention, the subject of interest is a human patient suspected of or actually diagnosed with breast cancer.

[0021] The American Joint Committee on Cancer (AJCC) has developed a standardized system for breast cancer staging using a "TNM" classification scheme. Patients are assessed for primary tumor size (T), regional lymph node status (N), and the presence/absence of distant metastasis (M) and then classified into stages 0-IV based on this combination of factors. In this system, primary tumor size is categorized on a scale of 0-4 (T0=no evidence of primary tumor; T1=≦2 cm; T2=>2 cm-≦5 cm; T3=>5 cm; T4=tumor of any size with direct spread to chest wall or skin). Lymph node status is classified as N0-N3 (N0=regional lymph nodes are free of metastasis; N1=metastasis to movable, same-side axillary lymph node(s); N2=metastasis to same-side lymph node(s) fixed to one another or to other structures; N3=metastasis to same-side lymph nodes beneath the breastbone). Metastasis is categorized by the absence (M0) or presence of distant metastases (M1). While breast cancer patients at any clinical stage are encompassed by the present invention, breast cancer patients in early-stage breast cancer are of particular interest. By "early-stage breast cancer" is intended stages 0 (in situ breast cancer), I (T1, N0, M0), IIA (T0-1, N1, M0 or T2, N0, M0), and IIB (T2, N1, M0 or T3, N0, M0). Early-stage breast cancer patients exhibit little or no lymph node involvement. As used herein, "lymph node involvement" or "lymph node status" refers to whether the cancer has metastasized to the lymph nodes. Breast cancer patients are classified as "lymph node-positive" or "lymph node-negative" on this basis. Methods of identifying breast cancer patients and staging the disease are well known and may include manual examination,

biopsy, review of patient's and/or family history, and imaging techniques, such as mammography, magnetic resonance imaging (MRI), and positron emission tomography (PET).

[0022] The term "prognosis" is recognized in the art and encompasses predictions about the likely course of disease or disease progression, particularly with respect to likelihood of disease remission, disease relapse, tumor recurrence, metastasis, and death. "Good prognosis" refers to the likelihood that a patient afflicted with cancer, particularly breast cancer, will remain disease-free (i.e., cancer-free). "Poor prognosis" is intended to mean the likelihood of a relapse or recurrence of the underlying cancer or tumor, metastasis, or death. Cancer patients classified as having a "good outcome" remain free of the underlying cancer or tumor. In contrast, "bad outcome" cancer patients experience disease relapse, tumor recurrence, metastasis, or death. In particular embodiments, the time frame for assessing prognosis and outcome is, for example, less than one year, one, two, three, four, five, six, seven, eight, nine, ten, fifteen, twenty or more years. As used herein, the relevant time for assessing prognosis or disease-free survival time begins with the surgical removal of the tumor or suppression, mitigation, or inhibition of tumor growth. Thus, for example, in particular embodiments, a "good prognosis" refers to the likelihood that a breast cancer patient will remain free of the underlying cancer or tumor for a period of at least five, more particularly, a period of at least ten years. In further aspects of the invention, a "bad prognosis" refers to the likelihood that a breast cancer patient will experience disease relapse, tumor recurrence, metastasis, or death within less than five years, more particularly less than ten years. Time frames for assessing prognosis and outcome provided above are illustrative and are not intended to be limiting.

[0023] In some embodiments described herein, prognostic performance of the biomarkers and/or other clinical parameters was assessed utilizing a Cox Proportional Hazards Model Analysis, which is a regression method for survival data that provides an estimate of the hazard ratio and its confidence interval. The Cox model is a well-recognized statistical technique for exploring the relationship between the survival of a patient and particular variables. This statistical method permits estimation of the hazard (i.e., risk) of individuals given their prognostic variables (e.g., overexpression of particular biomarkers, as described herein). Cox model data are commonly presented as Kaplan-Meier curves. The "hazard ratio" is the risk of death at any given time point for patients displaying particular prognostic variables. See generally Spruance et al. (2004) *Antimicrob. Agents & Chemo.* 48:2787-2792. In particular embodiments, the biomarkers of interest are statistically significant for assessment of the likelihood of breast cancer recurrence or death due to the underlying breast cancer. Methods for assessing statistical significance are well known in the art and include, for example, using a log-rank test Cox analysis and Kaplan-Meier curves. In some aspects of the invention, a p-value of less than 0.05 constitutes statistical significance.

[0024] As described herein above, a number of clinical and prognostic breast cancer factors are known in the art and are used to predict treatment outcome and the likelihood of disease recurrence. Such factors include lymph node involvement, tumor size, histologic grade, family history, estrogen and progesterone hormone receptor status, Her 2/neu levels, and tumor ploidy. As used herein, estrogen and progesterone hormone receptor status refers to whether these receptors are expressed in the breast tumor of a particular breast cancer patient. Thus, an "estrogen receptor-positive patient" displays estrogen receptor expression in a breast tumor, whereas an "estrogen receptor-negative patient" does not. Using the methods of the present invention, the prognosis of a breast cancer patient can be determined independent of or in combination with assessment of these or other clinical and prognostic factors. In some embodiments, combining the methods disclosed herein with evaluation of other prognostic factors may permit a more accurate determination of breast cancer prognosis. The methods of the invention may be coupled with analysis of, for example, Her2/neu, Ki67, and/or p53 expression levels. Other factors, such as patient clinical history, family history, and menopausal status, may also be considered when evaluating breast cancer prognosis via the methods of the invention. In some embodiments, patient data obtained via the methods disclosed herein may be coupled with analysis of clinical information and existing tests for breast cancer prognosis to develop a reference laboratory prognostic algorithm. Such algorithms find used in stratifying breast cancer patients, particularly early-stage breast cancer patients, into good and bad prognosis populations. Patients assessed as having a poor prognosis may be upstaged for more aggressive breast cancer treatment.

[0025] The methods of the invention permit the superior assessment of breast cancer prognosis in comparison to analysis of other known prognostic indicators (e.g., lymph node involvement, tumor size, histologic grade, estrogen and progesterone receptor levels, Her 2/neu status, tumor ploidy, and family history). In particular aspects of the invention, the sensitivity and specificity is equal to or greater than that of known cancer prognostic evaluation methods. The endpoint for assessing specificity and sensitivity is comparison of the prognosis or outcome predicted using the methods of the invention (i.e., at or near the time of diagnosis) with the actual clinical outcome (i.e., whether the patient remained cancer-free or suffered a recurrence within a specified time period). As used herein, "specificity" refers to the level at which a method of the invention can accurately identify true negatives. In a clinical study, specificity is calculated by dividing the number of true negatives by the sum of true negatives and false positives. By "sensitivity" is intended the level at which a method of the invention can accurately identify samples that are true positives. Sensitivity is calculated in a clinical study by dividing the number of true positives by the sum of true positives and false negatives. In some embodiments, the sensitivity of the disclosed methods for the evaluation of breast cancer is at least about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more. Furthermore, the specificity of the present methods is preferably at least about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more. In further embodiments, the combined sensitivity and specificity value for the prognostic methods of the invention is assessed. By "combined sensitivity and specificity value" is intended the sum of the individual specificity and sensitivity values, as defined herein above. The combined sensitivity and specificity value of the present methods is preferably at least about 105%, 110%, 115%, 120%, 130%, 140%, 150%, 160% or more.

[0026] As used herein, the definitions of "true" and "false" positives and negatives will be dependent upon whether the biomarker or combination of biomarkers under consideration

are good outcome or bad outcome biomarkers. That is, in the case of good outcome biomarkers (i.e., those indicative of a good prognosis), "true positive" refers to those samples exhibiting overexpression of the biomarker of interest, as determined by the methods of the invention (e.g., positive staining by immunohistochemistry), that have a confirmed good actual clinical outcome. In contrast, "false positives" display overexpression of the good outcome biomarker(s) but have a confirmed bad actual clinical outcome. "True negatives" and "false negatives" with respect to good outcome biomarkers do not display biomarker overexpression (e.g., do not stain positive in immunohistochemistry methods) and have confirmed bad and good actual clinical outcomes, respectively.

[0027] Similarly, in the case of bad outcome biomarkers, "true positives" refers to those samples exhibiting overexpression of the biomarker or combination of biomarkers of interest that have a confirmed bad actual clinical outcome. That is, "true positive" with respect to both good and bad outcome biomarkers refers to samples in which the actual clinical outcome (i.e., good or bad) is accurately predicted. "False positives" display overexpression of the bad outcome biomarker but have a confirmed good actual clinical outcome. "True negatives" and "false negatives" with respect to bad outcome biomarkers do not display biomarker overexpression and have confirmed good and bad actual clinical outcomes, respectively.

[0028] Breast cancer is managed by several alternative strategies that may include, for example, surgery, radiation therapy, hormone therapy, chemotherapy, or some combination thereof. As is known in the art, treatment decisions for individual breast cancer patients can be based on the number of lymph nodes involved, estrogen and progesterone receptor status, size of the primary tumor, and stage of the disease at diagnosis. Analysis of a variety of clinical factors and clinical trials has led to the development of recommendations and treatment guidelines for early-stage breast cancer by the International Consensus Panel of the St. Gallen Conference (2001). See Goldhirsch et al. (2001) *J. Clin. Oncol.* 19:3817-3827, which is herein incorporated by reference in its entirety. The guidelines indicate that treatment for patients with node-negative breast cancer varies substantially according to the baseline prognosis. More aggressive treatment is recommended for patients with a relative high risk of recurrence when compared to patients with a relatively low risk of recurrence. It has been demonstrated that chemotherapy for the high risk population has resulted in a reduction in the risk of relapse. Women with a low risk category are usually treated with radiation and hormonal therapy. Stratification of patients into poor prognosis or good prognosis risk groups at the time of diagnosis using the methods disclosed herein may provide an additional or alternative treatment decision-making factor. The methods of the invention permit the differentiation of breast cancer patients with a good prognosis from those more likely to suffer a recurrence (i.e., patients who might need or benefit from additional aggressive treatment at the time of diagnosis). The methods of the invention find particular use in choosing appropriate treatment for early-stage breast cancer patients. As discussed above, the majority of breast cancer patients diagnosed at an early-stage of the disease enjoy long-term survival following surgery and/or radiation therapy without further adjuvant therapy. A significant percentage (approximately 20%) of these patients, however, will suffer disease recurrence or death, leading to clinical recommendations that some or all early-stage breast cancer patients should receive adjuvant therapy (e.g., chemotherapy). The methods of the present invention find use in identifying this high-risk, poor prognosis population of early-stage breast cancer patients and thereby determining which patients would benefit from continued and/or more aggressive therapy and close monitoring following treatment. For example, early-stage breast cancer patients assessed as having a poor prognosis by the methods disclosed herein may be selected for more aggressive adjuvant therapy, such as chemotherapy, following surgery and/or radiation treatment. In particular embodiments, the methods of the present invention may be used in conjunction with the treatment guidelines established by the St. Gallens Conference to permit physicians to make more informed breast cancer treatment decisions. The present methods for evaluating breast cancer prognosis can also be combined with other prognostic methods and molecular marker analyses known in the art (e.g., Her2/neu, Ki67, and p53 expression levels) for purposes of selecting an appropriate breast cancer treatment. Furthermore, the methods of the invention can be combined with later-developed prognostic methods and molecular marker analyses not currently known in the art.

[0029] The methods disclosed herein also find use in predicting the response of a breast cancer patient to a selected treatment. By "predicting the response of a breast cancer patient to a selected treatment" is intended assessing the likelihood that a patient will experience a positive or negative outcome with a particular treatment. As used herein, "indicative of a positive treatment outcome" refers to an increased likelihood that the patient will experience beneficial results from the selected treatment (e.g., complete or partial remission, reduced tumor size, etc.). By "indicative of a negative treatment outcome" is intended an increased likelihood that the patient will not benefit from the selected treatment with respect to the progression of the underlying breast cancer. In some aspects of the invention, the selected treatment is chemotherapy.

[0030] In certain embodiments, methods for predicting the likelihood of survival of a breast cancer patient are provided. In particular, the methods may be used predict the likelihood of long-term, disease-free survival. By "predicting the likelihood of survival of a breast cancer patient" is intended assessing the risk that a patient will die as a result of the underlying breast cancer. "Long-term, disease-free survival" is intended to mean that the patient does not die from or suffer a recurrence of the underlying breast cancer within a period of at least five years, more particularly at least ten or more years, following initial diagnosis or treatment. Such methods for predicting the likelihood of survival of a breast cancer patient comprise detecting expression of multiple biomarkers in a patient sample, wherein the likelihood of survival, particularly long-term, disease-free survival, decreases as the number of biomarkers determined to be overexpressed in the patient sample increases. For example, in one aspect of the invention, the expression of at least five biomarkers is determined, wherein overexpression of none of the biomarkers is indicative of an increased likelihood of survival, and wherein overexpression of two or more biomarkers is indicative of a decreased likelihood of survival. Likelihood of survival may be assessed in comparison to, for example, breast cancer survival statistics available in the art. In other embodiments, methods for predicting the likelihood of survival of breast cancer patient comprise determining the expression of at least

six biomarkers and assessing the number of these biomarkers that are overexpressed. Biomarkers useful for these methods may be selected from, for example, E2F1, SLPI, MUC-1, src, p21ras, and PSMB9. See generally examples 8 and 9.

[0031] The biomarkers of the invention include genes and proteins. Such biomarkers include DNA comprising the entire or partial sequence of the nucleic acid sequence encoding the biomarker, or the complement of such a sequence. The biomarker nucleic acids also include RNA comprising the entire or partial sequence of any of the nucleic acid sequences of interest. A biomarker protein is a protein encoded by or corresponding to a DNA biomarker of the invention. A biomarker protein comprises the entire or partial amino acid sequence of any of the biomarker proteins or polypeptides. Fragments and variants of biomarker genes and proteins are also encompassed by the present invention. By "fragment" is intended a portion of the polynucleotide or a portion of the amino acid sequence and hence protein encoded thereby. Polynucleotides that are fragments of a biomarker nucleotide sequence generally comprise at least 10, 15, 20, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, or 1,400 contiguous nucleotides, or up to the number of nucleotides present in a full-length biomarker polynucleotide disclosed herein. A fragment of a biomarker polynucleotide will generally encode at least 15, 25, 30, 50, 100, 150, 200, or 250 contiguous amino acids, or up to the total number of amino acids present in a full-length biomarker protein of the invention. "Variant" is intended to mean substantially similar sequences. Generally, variants of a particular biomarker of the invention will have at least about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to that biomarker as determined by sequence alignment programs.

[0032] A "biomarker" is any gene or protein whose level of expression in a tissue or cell is altered compared to that of a normal or healthy cell or tissue. The biomarkers of the present invention are genes and proteins whose overexpression correlates with cancer, particularly breast cancer, prognosis. In particular embodiments, selective overexpression of a biomarker or combination of biomarkers of interest in a patient sample is indicative of a poor cancer prognosis. By "indicative of a poor prognosis" is intended that overexpression of the particular biomarker or combination of biomarkers is associated with an increased likelihood of relapse or recurrence of the underlying cancer or tumor, metastasis, or death, as defined herein above. For example, "indicative of a poor prognosis" may refer to an increased likelihood of relapse or recurrence of the underlying cancer or tumor, metastasis, or death within five years, more particularly ten years. Biomarkers that are indicative of a poor prognosis may be referred to herein as "bad outcome biomarkers." In other aspects of the invention, the absence of overexpression of a biomarker or combination of biomarkers of interest is indicative of a good prognosis. As used herein, "indicative of a good prognosis" refers to an increased likelihood that the patient will remain cancer-free, as defined herein above. In some embodiments, "indicative of a good prognosis" refers to an increased likelihood that the patient will remain cancer-free for at least five, more particularly at least ten years. Such biomarkers may be referred to as "good outcome biomarkers."

[0033] The biomarkers of the present invention include any gene or protein whose overexpression correlates with breast cancer prognosis, as described herein above. Biomarkers include genes and proteins that are indicative of a poor breast cancer prognosis (i.e., bad outcome biomarkers) as well as those that are indicative of a good prognosis (i.e., good outcome biomarkers). Biomarkers of particular interest include genes and proteins that are involved in regulation of cell growth and proliferation, cell cycle control, DNA replication and transcription, apoptosis, signal transduction, angiogenesis/lymphogenesis, or metastasis. In some embodiments, the biomarkers regulate protease systems involved in tissue remodeling, extracellular matrix degradation, and adjacent tissue invasion. Although any biomarker whose overexpression is indicative of breast cancer prognosis can be used to practice the invention, in particular embodiments, biomarkers are selected from the group consisting of SLPI, p21ras, MUC-1, DARPP-32, phospho-p27, src, MGC 14832, myc, TGFβ-3, SERHL, E2F1, PDGFRα, NDRG-1, MCM2, PSMB9, MCM6, and p53. See Table 43. In one embodiment, the biomarkers of interest comprise SLPI, PSMB9, phospho-p27, src, E2F1, p21ras, or p53. In one aspect of the invention, the methods for evaluating breast cancer prognosis comprise detecting the expression of E2F1 and SLPI, wherein overexpression of at least one of these biomarkers is indicative of a poor prognosis. In another embodiment, the methods comprise detecting the expression of E2F1, src, and SLPI, wherein overexpression of at least two of the biomarkers is indicative of a poor breast cancer prognosis. In a further embodiment, the methods of the present invention comprise detecting the expression of E2F1, src, PSMB9, and SLPI, wherein overexpression of at least two of these biomarkers is indicative of a poor breast cancer prognosis. In other aspects of the invention, the expression of E2F1, SLPI, PSMB9, p21ras, and src is detected, and overexpression of at least two of these biomarkers is indicative of a poor prognosis. In yet another embodiment, the methods comprise detecting the expression of SLPI, p21ras, E2F1, PSMB9, phospho-p27, and src in a patient sample, wherein overexpression of at least four of these biomarkers is indicative of a poor prognosis.

[0034] In another embodiment, the biomarkers of interest comprise E2F1, SLPI, MUC-1, src, p21ras, and PSMB9. In one aspect of the invention, the methods for evaluating breast cancer prognosis comprise detecting the expression of E2F1 and SLPI, wherein overexpression of at least one of these biomarkers is indicative of a poor prognosis. In another embodiment, the methods comprise detecting the expression of E2F1, SLPI, and PSMB9, wherein overexpression of at least two of the biomarkers is indicative of a poor breast cancer prognosis. In a further embodiment, the methods of the present invention comprise detecting the expression of E2F1, SLPI, MUC-1, and src, wherein overexpression of at least two of these biomarkers is indicative of a poor breast cancer prognosis. In other aspects of the invention, the expression of E2F1, SLPI, MUC-1, src, and p21ras is detected, and overexpression of at least two of these biomarkers is indicative of a poor prognosis. In yet another embodiment, the methods comprise detecting the expression of E2F1, SLPI, MUC-1, src, p21ras, and PSMB9 in a patient sample, wherein overexpression of at least four of these biomarkers is indicative of a poor prognosis.

[0035] Secretory Leukocyte Protease Inhibitor (SLPI) is a non-specific inhibitor that can inactivate a number of proteases including leukocyte elastase, trypsin, chymotrypsin and the cathepsins (e.g., cathepsin G). SLPI is known to be involved in inflammation and the inflammatory response in

relation to tissue repair. Protease inhibitors have generally been considered to counteract tumor progression and metastasis. However, expression of serine protease inhibitors (SPI's) in tumors is often associated with poor prognosis of cancer patients. Cathepsin G is over expressed in breast cancer and is an indicator of poor prognosis. Its inhibitory effect contributes to the immune response by protecting epithelial surfaces from attack by endogenous proteolytic enzymes. The gene location for SLPI is 20q12, which is a chromosomal region implicated in breast cancer chromosomal alterations and aneuploidy.

[0036] PSMB9 is a member of the proteasome B-type family, also known as the T1B family, that is a 20S core beta subunit. This gene is located in the class II region of the MHC (major histocompatibility complex). Expression of this gene is induced by gamma interferon, and this gene product replaces catalytic subunit 1 (proteasome beta 6 subunit) in the immunoproteasome. Proteolytic processing is required to generate a mature subunit.

[0037] NDRG-1 (N-Myc downstream regulated) is upregulated during cell differentiation, repressed by N-myc and c-myc in embryonic cells, and suppressed in several tumor cells. Overexpression may be related to hypoxia and the subsequent signaling to induce angiogenesis. Hypoxia causes the accumulation of the transcription factor hypoxia-inducible factor 1 (HIF-1), culminating in the expression of hypoxia-inducible genes such as those for vascular endothelial growth factor (VEGF) and NDRG-1. NDRG-1 is found in some breast cancers as an overexpressed mRNA. NDRG-1 is located on chromosome 8q24 adjacent to the c-myc gene.

[0038] MUC1 is a heavily O-glycosylated transmembrane protein expressed on most secretory epithelium, including mammary glands and some hematopoietic cells. It is expressed abundantly in lactating mammary glands and overexpressed in more than 90% of breast carcinomas and metastases. In normal mammary glands, it is expressed on the apical surface of glandular epithelium.

[0039] p27 is a key regulator of the cell cycle and participates in the G1-to-S phase progression. It interacts specifically with the cyclin E/cdk2 complex during G1 phase and also with D-type cyclin-cdks. p27 can be phosphorylated on threonine 187 by Cdks. Phosphorylation of p27 at threonine 187 is also cell-cycle dependent, present in proliferating cells but undetectable in G1 cells. Activation of p27 degradation is seen in proliferating cells and in many types of aggressive human carcinomas. Overexpression of p27 may lead to an inhibition of apoptosis and resistance to some chemotherapy.

[0040] The Src family of protein tyrosine kinases (including Src, Lyn, Fyn, Yes, Lck, Blk, Hck, etc.) is important in the regulation of growth and differentiation of eukaryotic cells. Src activity is regulated by tyrosine phosphorylation at two sites with opposing effects. Phosphorylation of Tyr416 in the activation loop of the kinase domain upregulates the enzyme. Phosphorylation of Tyr527 in the C-terminal tail by Csk renders the enzyme less active.

[0041] E2F1 is a member of a family of transcription factors involved in the regulation of both G1 and S phase cyclins, in particular cyclin D1. These proteins participate in the Rb pathway of cell-cycle regulation and control of DNA synthesis. During the G1 phase of the cell-cycle, the E2F transcription factors are bound in an inactive complex with the Rb tumor suppressor protein. During the G1/S boundary of the cell cycle, the Rb protein is hyperphosphorylated and releases the E2F transcription factor from its inhibitory complex. The E2F transcription factor then activates transcription for those genes responsible for the S-phase of the cell-cycle, predominantly resulting in initiation of DNA synthesis and preparation for mitosis and subsequent cell division. Overexpression of E2F1 has been shown to lead to the induction of apoptosis possibly through the inhibition of cyclinD1-dependent kinase activity coupled with the induction of a p 16 related transcript. In addition, regulation of E2F1 at the level of transcription, E2F1 protein levels are also controlled by the ubiquitin-proteosome dependent degradation pathway. Ubiquitination is blocked by the Rb and E2F1 complex, which directly controls aspects of cell cycle progression.

[0042] p21ras is a member of a large group of cytoplasmic proteins involved in signal transduction. Guanine nucleotide binding proteins (G proteins) comprise a large group of cytoplasmic proteins present in eukaryotic cells that are involved in signal transduction. There are two forms, the large heterotrimeric G proteins and the smaller monomers. The 3 ras oncogenes, H-ras, K-ras, and N-ras are members of the smaller monomeric G proteins and are located on chromosomes 11, 12 and 1 respectively. They encode 21-kD proteins called p21s and contain 188 amino acids. p21 ras proteins are involved in normal cell growth, protease activities, and cell adhesion.

[0043] Collectively, the three forms of p21ras function by linking ligand-mediated extracellular receptor activation with intracellular tyrosine kinase activation and subsequent initiation of a number of cellular processes relevant to breast cancer progression, including DNA replication, proliferation, and anchorage independent growth. The K- and H-ras genes are most often implicated in breast cancer. In both of these ras genes, mutations at codons 12 and 13 are common. These gain-of-function mutations result in constitutive activation that uncouples the normal ligand-induced signal transduction within the ras signaling pathways. Less common in breast cancer is the involvement of N-ras. Two mechanisms have been reported for N-ras associated changes in breast cancer: mutation at codon 61 resulting in constitutive activation of the oncogene, similar to the mutations mentioned above for K- and H-ras, and chromosomal amplification. Moreover, in addition to activation of intracellular signaling pathways, the ras oncogenes have been reported to induce overexpression of proteases important for tissue remodeling and invasion. H-ras has been implicated in matrix metalloprotease-2 (MMP-2) overexpression, and N-ras has been associated with overexpression of MMP-9. See generally Correll and Zoll (1988) *Human Genetics* 79:225-259; Tong et al. (1989) *Nature* 337: 90-93; Watson et al. (1991) *Breast Cancer Res. Treat.* 17:161-169; Dati et al. (1991) *Int. J. Cancer* 47:833-838; Archer et al. (1995) *Br. J. Cancer* 72:1259-1266; Bland et al. (1995) *Ann. Surg.* 221:706-718; Shackney et al. (1998) *Clin. Cancer Res.* 4:913-928; and Gohring et al. (1999) *Tumor Biol.* 20:173-183, all of which are herein incorporated by reference in their entirety. Detection of any form (i.e., H-, K-, N-ras) of the p21ras proteins is encompassed by the present invention.

[0044] Minichromosome maintenance (MCM) proteins play an essential part in eukaryotic DNA replication. Each of the MCM proteins has DNA-dependent ATPase motifs in their highly conserved central domain. Levels of MCM proteins generally increase in a variable manner as normal cells progress from G0 into the G1/S phase of the cell cycle. In the G0 phase, MCM2 and MCM5 proteins are much less abundant than are the MCM7 and MCM3 proteins. MCM6 forms a complex with MCM2, MCM4, and MCM7, which binds

histone H3. In addition, the subcomplex of MCM4, MCM6, and MCM7 has helicase activity, which is mediated by the ATP-binding activity of MCM6 and the DNA-binding activity of MCM4. See, for example, Freeman et al. (1999) *Clin. Cancer Res.* 5:2121-2132; Lei et al. (2001) *J. Cell Sci.* 114: 1447-1454; Ishimi et al. (2003) *Eur. J. Biochem.* 270:1089-1101, all of which are herein incorporated by reference in their entirety.

[0045] DARPP32 is an inhibitor of protein phosphatase 1 whose biological function and inhibitory activity are modulated through specific amino acid residue phosphorylation within the DARPP32 protein. Threonine 34 (T34) phosphorylation renders the DARPP32 protein a specific protein phosphatase 1 inhibitor. However, threonine 75 (T75) phosphorylation renders the DARPP32 an inhibitor of protein kinase A (PKA). The gene location for DARPP32 is 17q21.2, which is known to be adjacent to the her2/neu (c-erb-B2 receptor tyrosine kinase) gene at 17q12. This region has been implicated in breast cancer chromosomal amplifications and resultant poor outcome within 25-35% of breast cancers. Several publications have demonstrated specific transcriptional activation of this 17q12-21 amplicon in breast cancer, with a number of genes located within this amplicon being overexpressed.

[0046] p53 plays multiple roles in cells. Expression of high levels of wild-type, but not mutant, p53 has two outcomes: cell cycle arrest or apoptosis. The observation that DNA-damaging agents induce levels of p53 in cells led to the definition of p53 as a checkpoint factor, akin perhaps to the product of the fad9 gene in yeast. While dispensable for viability, in response to genotoxic stress p53 acts as an "emergency brake" inducing either arrest or apoptosis, protecting the genome from accumulating excess mutations. Consistent with this notion, cells lacking p53 have been shown to be genetically unstable and, thus, more prone to tumors. The p53 protein is located in the nucleus of cells and is very labile. p53 is mutated in roughly 50% of all human tumors, predominantly in the DNA-binding domain codons.

[0047] Although the above biomarkers have been discussed in detail, any biomarker whose overexpression is indicative of breast cancer prognosis can be used to practice the invention, including biomarkers not yet identified in the art. Such biomarkers include genes and proteins that are, for example, involved in cell proliferation, cell cycle control, or the generalized mechanisms of cancer motility and invasion. Biomarkers of potential interest include cyclooxygenase-2 (cox-2), rhoC, c-myc, urokinase plasminogen activator receptor (uPAR), Wilms' tumor suppressor, akt kinase, and osteopontin. See, for example, Perou et al. (2000) *Nature* 406:747-752; Sorlie et al. (2001) *Proc. Natl. Acad. Sci.* 98:10869-10874; Van't Veer et al. (2002) *Nature* 415:530-536; Huang et al. (2003) *Lancet* 361:1590-1596, all of which are herein incorporated by reference in their entirety.

[0048] In particular embodiments, the biomarkers are kinases that are involved in signal transduction pathways, such as PI3K regulatory a, LTk, Ser/thr kinase 15, MAPK8IPI, MAPKAPK2, and PK428, PRKR. Growth factors, extracellular signal transduction proteins, and extracellular matrix proteins are also biomarkers of interest. Such proteins include EGFR, TNF receptor associated factor 4, GFR bound protein 7, ErbB2 (her 2), VEGF, GDF1, IGFBP5, EGF8 ras homolog, MMP 9, MMP 7, SLPI, keratin 5, keratin 17, laminin gamma 2 (laminin V), troponin, and tubulin.

[0049] In some aspects of the invention, the biomarkers comprise genes and proteins that are involved in chromosome condensation and maintenance, such as, for example, Cc related, HMG non-histone chromosomal 11, MMD5, MCM5, MCM6, and Swi/snf related actin. Biomarkers that are associated with centromere and centrosome function, including CENPA, CENPF, CENPE, Bub 1, polo-like kinase, and HsEg5, MCAK, and HSET, can also be used in the methods described herein. The biomarkers of the invention may also comprise transcription factors, particularly those associated with cell cycle regulation. Transcription factors of interest include but are not limited to E2F1, E2F4, NDRG-1, ORC6L, PCNA, nuclear factor 1, EZH2, and TFAP2A. Cyclins, such as CDC20, CDC 25B, cyclin A2, cyclin E, and cyclin F, may also be used to practice the disclosed methods.

[0050] Although the methods of the invention require the detection of at least one, more particularly at least two, biomarker(s) in a patient sample for evaluating breast cancer prognosis, 3, 4, 5, 6, 7, 8, 9, 10 or more biomarkers may be used to practice the present invention. It is recognized that detection of more than one biomarker in a body sample may be used to evaluate cancer, particularly breast cancer, prognosis. Therefore, in some embodiments, two or more biomarkers are used, more preferably, two or more complementary biomarkers. By "complementary" is intended that detection of the combination of biomarkers in a body sample results in the accurate determination of cancer prognosis in a greater percentage of cases than would be identified if only one of the biomarkers was used. Thus, in some cases, a more accurate determination of cancer prognosis can be made by using at least two biomarkers. Accordingly, where at least two biomarker proteins are used, at least two antibodies directed to distinct biomarker proteins will be used to practice the immunohistochemistry methods disclosed herein. The antibodies may be contacted with the body sample simultaneously or successively.

[0051] When a combination of two or more biomarkers is used, the biomarkers will typically be substantially statistically independent of one another. By "statistically independent" biomarkers is intended that the prognoses generated therefrom are independent such that one biomarker does not provide substantially repetitive information with regard to the complementary biomarker. This may ensure, for instance, that a biomarker is not used in conjunction with a first biomarker when the two are not substantially statistically independent. The dependence of the two biomarkers may indicate that they are duplicative and that the addition of a second biomarker adds no additional value to the prognostic power of a given pair of biomarkers. In order to optimize the prognostic power of a given panel of biomarkers it is also desirable to reduce the amount of signal "noise" by minimizing the use of biomarkers that provide duplicative prognostic information when compared to another biomarker in the panel. Methods for determining statistical independence are known in the art. Statistical independence of biomarkers of interest can be assessed using any method, including, for example, the methods disclosed in U.S. Application Publication No. 2006/0078926 entitled "Methods and Computer Programs for Analysis and Optimization of Marker Candidates for Cancer Prognosis," filed Sep. 22, 2005 and incorporated by reference in its entirety. Where independent, prognostic biomarkers are used to practice the present methods, the prognostic value is increased by detecting the expression of 2, 3, 4, 5, 6, 7 or more biomarkers. In such cases, any combination of independent biomarkers can be used.

[0052] One of skill in the art will also recognize that a panel of biomarkers can be used to evaluate the prognosis of a breast cancer patient in accordance with the methods of the invention. In some embodiments, a panel comprising at least two biomarkers selected from the group consisting of SLPI, p21ras, MUC-1, DARPP-32, phospho-p27, src, MGC 14832, myc, TGFβ-3, SERHL, E2F1, PDGFRα, NDRG-1, MCM2, PSMB9, MCM6, and p53 is provided. One particular panel of biomarkers may comprise, for example, all or a subset of E2F1, SLPI, MUC-1, src, p21ras, and PSMB9. A panel of biomarkers may comprise any number or combination of biomarkers of interest. In certain aspects of the invention, a panel comprises at least two statistically independent, prognostic biomarkers.

[0053] In particular embodiments, the methods for evaluating breast cancer prognosis comprise collecting a patient body sample, preferably a breast tissue sample, more preferably a primary breast tumor tissue sample, contacting the sample with at least one antibody specific for a biomarker of interest, detecting antibody binding, and determining if the biomarker is overexpressed. That is, samples are incubated with the biomarker antibody for a time sufficient to permit the formation of antibody-antigen complexes, and antibody binding is detected, for example, by a labeled secondary antibody. Samples that exhibit overexpression of at least one bad outcome biomarker, as determined by antibody binding, are classified as having a poor prognosis. Similarly, patient samples that display overexpression of at least one good outcome biomarker are categorized as having a good prognosis. Furthermore, the overexpression of certain combinations of biomarkers of interest is specifically used to distinguish breast cancer patients with a poor prognosis from those with a good prognosis. In some aspects of the invention, the methods comprise detecting the expression of two or more biomarkers in a patient sample and determining if said biomarkers are overexpressed, wherein overexpression of all or some subset of these biomarkers is indicative of breast cancer prognosis. For example, in one embodiment, the methods comprise detecting the expression of SLPI, p21ras, E2F1, PSMB9, phospho-p27, and src, wherein overexpression of at least four of these biomarkers is indicative of a poor prognosis. In another aspect of the invention, the methods comprise detecting the expression of SLPI, E2F1, and src, wherein overexpression of at least two of these biomarkers is indicative of a poor prognosis. In other embodiments, the methods comprise detecting the expression of E2F1, SLPI, MUC-1, src, p21ras, and PSMB9, wherein overexpression of at least four of these biomarkers is indicative of a poor prognosis. In another aspect of the invention, the methods comprise detecting the expression of SLPI, E2F1, and MUC-1, wherein overexpression of at least two of these biomarkers is indicative of a poor prognosis.

[0054] By "body sample" is intended any sampling of cells, tissues, or bodily fluids in which expression of a biomarker can be detected. Examples of such body samples include but are not limited to blood, lymph, urine, gynecological fluids, biopsies, and smears. Bodily fluids useful in the present invention include blood, urine, saliva, nipple aspirates, or any other bodily secretion or derivative thereof. Blood can include whole blood, plasma, serum, or any derivative of blood. In preferred embodiments, the body sample comprises breast cells, particularly breast tissue from a biopsy, more particularly a breast tumor tissue sample. Body samples may be obtained from a patient by a variety of techniques including, for example, by scraping or swabbing an area, by using a needle to aspirate bodily fluids, or by removing a tissue sample (i.e., biopsy). Methods for collecting various body samples are well known in the art. In some embodiments, a breast tissue sample is obtained by, for example, fine needle aspiration biopsy, core needle biopsy, or excisional biopsy. Fixative and staining solutions may be applied to the cells or tissues for preserving the specimen and for facilitating examination. Body samples, particularly breast tissue samples, may be transferred to a glass slide for viewing under magnification. In preferred embodiments, the body sample is a formalin-fixed, paraffin-embedded breast tissue sample, particularly a primary breast tumor sample.

[0055] Any methods available in the art for detecting expression of biomarkers are encompassed herein. The expression of a biomarker of the invention can be detected on a nucleic acid level or a protein level. By "detecting expression" is intended determining the quantity or presence of a biomarker gene or protein. Thus, "detecting expression" encompasses instances where a biomarker is determined not to be expressed, not to be detectably expressed, expressed at a low level, expressed at a normal level, or overexpressed. In order to determine overexpression, the body sample to be examined may be compared with a corresponding body sample that originates from a healthy person. That is, the "normal" level of expression is the level of expression of the biomarker in, for example, a breast tissue sample from a human subject or patient not afflicted with breast cancer. Such a sample can be present in standardized form. In some embodiments, determination of biomarker overexpression requires no comparison between the body sample and a corresponding body sample that originates from a healthy person. For example, detection of overexpression of a biomarker indicative of a poor prognosis in a breast tumor sample may preclude the need for comparison to a corresponding breast tissue sample that originates from a healthy person. Moreover, in some aspects of the invention, no expression, underexpression, or normal expression (i.e., the absence of overexpression) of a biomarker or combination of biomarkers of interest provides useful information regarding the prognosis of a breast cancer patient.

[0056] Methods for detecting expression of the biomarkers of the invention comprise any methods that determine the quantity or the presence of the biomarkers either at the nucleic acid or protein level. Such methods are well known in the art and include but are not limited to western blots, northern blots, southern blots, ELISA, immunoprecipitation, immunofluorescence, flow cytometry, immunohistochemistry, nucleic acid hybridization techniques, nucleic acid reverse transcription methods, and nucleic acid amplification methods. In particular embodiments, expression of a biomarker is detected on a protein level using, for example, antibodies that are directed against specific biomarker proteins. These antibodies can be used in various methods such as Western blot, ELISA, immunoprecipitation, or immunohistochemistry techniques. Likewise, immunostaining of breast tissue, particularly breast tumor tissue, can be combined with assessment of clinical information, conventional prognostic methods, and expression of molecular markers (e.g., Her2/neu, Ki67, p53, and hormone receptor status) known in the art. In this manner, the disclosed methods may permit the more accurate determination of breast cancer prognosis.

[0057] In one embodiment, antibodies specific for biomarker proteins are utilized to detect the expression of a biomar-

ker protein in a body sample. The method comprises obtaining a body sample from a patient, contacting the body sample with at least one antibody directed to SLPI, p21ras, MUC-1, DARPP-32, phospho-p27, src, MGC 14832, myc, TGFβ-3, SERHL, E2F1, PDGFRα, NDRG-1, MCM2, PSMB9, or MCM6, and detecting antibody binding to determine if the biomarker is overexpressed in the patient sample. Overexpression of the biomarker protein is indicative of prognosis, more particularly, a bad breast cancer prognosis. In other embodiments, the methods of the invention comprise detecting the expression of at least two biomarkers, wherein overexpression of at least one of the biomarkers is indicative of prognosis. Such methods may comprise the detection of multiple biomarkers in a patient sample wherein it is the overexpression of all or a subset of these biomarkers that is indicative of breast cancer prognosis.

[0058] One aspect of the present invention provides an immunohistochemistry technique for evaluating the prognosis of a breast cancer patient. Specifically, this method comprises antibody staining of biomarkers within a breast tissue sample, more particularly a breast tumor sample, that are indicative of prognosis. One of skill in the art will recognize that the immunohistochemistry methods described herein below may be performed manually or in an automated fashion using, for example, the Autostainer Universal Staining System (Dako). One protocol for antibody staining (i.e., immunohistochemistry) of breast tissue samples is provided in Example 1.

[0059] In one immunohistochemistry method, a patient breast tissue sample is collected by, for example, biopsy techniques known in the art. Samples may be frozen for later preparation or immediately placed in a fixative solution. Tissue samples may be fixed by treatment with a reagent such as formalin, gluteraldehyde, methanol, or the like and embedded in paraffin. Methods for preparing slides for immunohistochemical analysis from formalin-fixed, paraffin-embedded tissue samples are well known in the art. In some embodiments, particularly the immunohistochemistry methods of the invention, samples may need to be modified in order to make the biomarker antigens accessible to antibody binding. For example, formalin fixation of tissue samples results in extensive cross-linking of proteins that can lead to the masking or destruction of antigen sites and, subsequently, poor antibody staining. As used herein, "antigen retrieval" or "antigen unmaksing" refers to methods for increasing antigen accessibility or recovering antigenicity in, for example, formalin-fixed, paraffin-embedded tissue samples. Any method for making antigens more accessible for antibody binding may be used in the practice of the invention, including those antigen retrieval methods known in the art. See, for example, Hanausek and Walaszek, eds. (1998) *Tumor Marker Protocols* (Humana Press, Inc., Totowa, N.J.); and Shi et al., eds. (2000) *Antigen Retrieval Techniques: Immunohistochemistry and Molecular Morphology* (Eaton Publishing, Natick, Mass.), both of which are herein incorporated by reference in their entirety.

[0060] Antigen retrieval methods include but are not limited to treatment with proteolytic enzymes (e.g., trypsin, chymoptrypsin, pepsin, pronase, etc.) or antigen retrieval solutions. Antigen retrieval solutions of interest include, for example, citrate buffer, pH 6.0 (Dako), tris buffer, pH 9.5 (Biocare), EDTA, pH 8.0 (Biocare), L.A.B. ("Liberate Antibody Binding Solution;" Polysciences), antigen retrieval Glyca solution (Biogenex), citrate buffer solution, pH 4.0 (Zymed), Dawn® detergent (Proctor & Gamble), deionized water, and 2% glacial acetic acid. In some embodiments, antigen retrieval comprises applying the antigen retrieval solution to a formalin-fixed tissue sample and then heating the sample in an oven (e.g., 60° C.), steamer (e.g., 95° C.), or pressure cooker (e.g., 120° C.) at specified temperatures for defined time periods. In other aspects of the invention, antigen retrieval may be performed at room temperature. Incubation times will vary with the particular antigen retrieval solution selected and with the incubation temperature. For example, an antigen retrieval solution may be applied to a sample for as little as 5, 10, 20, or 30 minutes or up to overnight. The design of assays to determine the appropriate antigen retrieval solution and optimal incubation times and temperatures is standard and well within the routine capabilities of those of ordinary skill in the art.

[0061] Following antigen retrieval, samples are blocked using an appropriate blocking agent, e.g., hydrogen peroxide. An antibody directed to a biomarker of interest is then incubated with the sample for a time sufficient to permit antigen-antibody binding. As noted above, one of skill in the art will appreciate that a more accurate breast cancer prognosis may be obtained in some cases by detecting overexpression of more than one biomarker in a patient sample. Therefore, in particular embodiments, at least two antibodies directed to two distinct biomarkers are used to evaluate the prognosis of a breast cancer patient. Where more than one antibody is used, these antibodies may be added to a single sample sequentially as individual antibody reagents or simultaneously as an antibody cocktail. Alternatively, each individual antibody may be added to a separate tissue section from a single patient sample, and the resulting data pooled.

[0062] Techniques for detecting antibody binding are well known in the art. Antibody binding to a biomarker of interest may be detected through the use of chemical reagents that generate a detectable signal that corresponds to the level of antibody binding and, accordingly, to the level of biomarker protein expression. For example, antibody binding can be detected through the use of a secondary antibody that is conjugated to a labeled polymer. Examples of labeled polymers include but are not limited to polymer-enzyme conjugates. The enzymes in these complexes are typically used to catalyze the deposition of a chromogen at the antigen-antibody binding site, thereby resulting in cell staining that corresponds to expression level of the biomarker of interest. Enzymes of particular interest include horseradish peroxidase (HRP) and alkaline phosphatase (AP). Commercial antibody detection systems, such as, for example the Dako Envision+ system and Biocare Medical's Mach 3 system, may be used to practice the present invention.

[0063] In one immunohistochemistry method of the invention, antibody binding to a biomarker is detected through the use of an HRP-labeled polymer that is conjugated to a secondary antibody. Slides are stained for antibody binding using the chromogen 3,3-diaminobenzidine (DAB) and then counterstained with hematoxylin and, optionally, a bluing agent such as ammonium hydroxide. In some aspects of the invention, slides are reviewed microscopically by a pathologist to assess cell staining (i.e., biomarker overexpression) and to evaluate breast cancer prognosis. Alternatively, samples may be reviewed via automated microscopy or by personnel with the assistance of computer software that facilitates the identification of positive staining cells.

[0064] The terms "antibody" and "antibodies" broadly encompass naturally occurring forms of antibodies and recombinant antibodies such as single-chain antibodies, chimeric and humanized antibodies and multi-specific antibodies as well as fragments and derivatives of all of the foregoing, which fragments and derivatives have at least an antigenic binding site. Antibody derivatives may comprise a protein or chemical moiety conjugated to the antibody.

[0065] "Antibodies" and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to an antigen, immunoglobulins include both antibodies and other antibody-like molecules that lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas.

[0066] The term "antibody" is used in the broadest sense and covers fully assembled antibodies, antibody fragments that can bind antigen (e.g., Fab', $F'(ab)_2$, Fv, single chain antibodies, diabodies), and recombinant peptides comprising the foregoing.

[0067] The term "monoclonal antibody" as used herein refers to an antibody 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.

[0068] "Antibody fragments" comprise a portion of an intact antibody, preferably the antigen-binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')2, and Fv fragments; diabodies; linear antibodies (Zapata et al. (1995) *Protein Eng.* 8(10):1057-1062); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize 35 readily. Pepsin treatment yields an F(ab')2 fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

[0069] "Fv" is the minimum antibody fragment that contains a complete antigen recognition and binding site. In a two-chain Fv species, this region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv species, one heavy- and one light-chain variable domain can be covalently linked by flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that in a two-chain Fv species. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0070] The Fab fragment also contains the constant domain of the light chain and the first constant domain (C_H1) of the heavy chain. Fab fragments differ from Fab' fragments by the addition of a few residues at the carboxy terminus of the heavy-chain C_H1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')2 antibody fragments originally were produced as pairs of Fab' fragments that have hinge cysteines between them.

[0071] Monoclonal antibodies can be prepared using the method of Kohler et al. (1975) *Nature* 256:495-496, or a modification thereof. Typically, a mouse is immunized with a solution containing an antigen. Immunization can be performed by mixing or emulsifying the antigen-containing solution in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally. Any method of immunization known in the art may be used to obtain the monoclonal antibodies of the invention. After immunization of the animal, the spleen (and optionally, several large lymph nodes) are removed and dissociated into single cells. The spleen cells may be screened by applying a cell suspension to a plate or well coated with the antigen of interest. The B cells expressing membrane bound immunoglobulin specific for the antigen bind to the plate and are not rinsed away. Resulting B cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium. The resulting cells are plated by serial dilution and are assayed for the production of antibodies that specifically bind the antigen of interest (and that do not bind to unrelated antigens). The selected monoclonal antibody (mAb)-secreting hybridomas are then cultured either in vitro (e.g., in tissue culture bottles or hollow fiber reactors), or in vivo (as ascites in mice).

[0072] As an alternative to the use of hybridomas, antibody can be produced in a cell line such as a CHO cell line, as disclosed in U.S. Pat. Nos. 5,545,403; 5,545,405; and 5,998,144; incorporated herein by reference. Briefly the cell line is transfected with vectors capable of expressing a light chain and a heavy chain, respectively. By transfecting the two proteins on separate vectors, chimeric antibodies can be produced. Another advantage is the correct glycosylation of the antibody. A monoclonal antibody can also be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with a biomarker protein to thereby isolate immunoglobulin library members that bind the biomarker protein. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage Antibody System, Catalog No. 27-9400-01; and the Stratagene SurfZAP θ Phage Display Kit, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, U.S. Pat. No. 5,223,409; PCT Publication Nos. WO 92/18619; WO 91/17271; WO 92/20791; WO 92/15679; 93/01288; WO 92/01047; 92/09690; and 90/02809; Fuchs et al. (1991) *Bio/Technology* 9:1370-1372; Hay et al. (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse et al. (1989) *Science* 246:1275-1281; Griffiths et al. (1993) *EMBO J.* 12:725-734.

[0073] Polyclonal antibodies can be prepared by immunizing a suitable subject (e.g., rabbit, goat, mouse, or other mammal) with a biomarker protein immunogen. The antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized biomarker protein. At an appropriate time after immunization, e.g., when the antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497, the human B cell hybri-

doma technique (Kozbor et al. (1983) *Immunol. Today* 4:72), the EBV-hybridoma technique (Cole et al. (1985) in *Monoclonal Antibodies and Cancer Therapy*, ed. Reisfeld and Sell (Alan R. Liss, Inc., New York, N.Y.), pp. 77-96) or trioma techniques. The technology for producing hybridomas is well known (see generally Coligan et al., eds. (1994) *Current Protocols in Immunology* (John Wiley & Sons, Inc., New York, N.Y.); Galfre et al. (1977) *Nature* 266:55052; Kenneth (1980) in *Monoclonal Antibodies: A New Dimension In Biological Analyses* (Plenum Publishing Corp., NY; and Lerner (1981) *Yale J. Biol. Med.,* 54:387-402).

[0074] The compositions of the invention further comprise monoclonal antibodies and variants and fragments thereof that specifically bind to biomarker proteins of interest. For example, monoclonal antibodies specific for SLPI (designated clone 5G6.24), DARPP-32 (8G11.20), MGC 14832 (1F3.9 and 2D1.14), NDRG-1 (10A9.34), PSMB9 (3A2.4), and MUC-1 (16E3.3) are provided. The monoclonal antibodies may be labeled with a detectable substance as described below to facilitate biomarker protein detection in the sample. Such antibodies find use in practicing the methods of the invention. Monoclonal antibodies having the binding characteristics of the antibodies disclosed herein are also encompassed by the present invention. Compositions further comprise antigen-binding variants and fragments of the monoclonal antibodies, hybridoma cell lines producing these antibodies, and isolated nucleic acid molecules encoding the amino acid sequences of these monoclonal antibodies.

[0075] Antibodies having the binding characteristics of a monoclonal antibody of the invention are also provided. "Binding characteristics" or "binding specificity" when used in reference to an antibody means that the antibody recognizes the same or similar antigenic epitope as a comparison antibody. Examples of such antibodies include, for example, an antibody that competes with a monoclonal antibody of the invention in a competitive binding assay. One of skill in the art could determine whether an antibody competitively interferes with another antibody using standard methods.

[0076] By "epitope" is intended the part of an antigenic molecule to which an antibody is produced and to which the antibody will bind. Epitopes can comprise linear amino acid residues (i.e., residues within the epitope are arranged sequentially one after another in a linear fashion), nonlinear amino acid residues (referred to herein as "nonlinear epitopes"; these epitopes are not arranged sequentially), or both linear and nonlinear amino acid residues. Typically epitopes are short amino acid sequences, e.g. about five amino acids in length. Systematic techniques for identifying epitopes are known in the art and are described, for example, in U.S. Pat. No. 4,708,871. Briefly, a set of overlapping oligopeptides derived from the antigen may be synthesized and bound to a solid phase array of pins, with a unique oligopeptide on each pin. The array of pins may comprise a 96-well microtiter plate, permitting one to assay all 96 oligopeptides simultaneously, e.g., for binding to a biomarker-specific monoclonal antibody. Alternatively, phage display peptide library kits (New England BioLabs) are currently commercially available for epitope mapping. Using these methods, the binding affinity for every possible subset of consecutive amino acids may be determined in order to identify the epitope that a given antibody binds. Epitopes may also be identified by inference when epitope length peptide sequences are used to immunize animals from which antibodies are obtained.

[0077] Antigen-binding fragments and variants of the monoclonal antibodies disclosed herein are further provided. Such variants will retain the desired binding properties of the parent antibody. Methods for making antibody fragments and variants are generally available in the art. For example, amino acid sequence variants of a monoclonal antibody described herein, can be prepared by mutations in the cloned DNA sequence encoding the antibody of interest. Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Walker and Gaastra, eds. (1983) *Techniques in Molecular Biology* (MacMillan Publishing Company, New York); Kunkel (1985) *Proc. Natl. Acad. Sci. USA* 82:488-492; Kunkel et al. (1987) *Methods Enzymol.* 154:367-382; Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor, N.Y.); U.S. Pat. No. 4,873,192; and the references cited therein; herein incorporated by reference. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the polypeptide of interest may be found in the model of Dayhoff et al. (1978) in *Atlas of Protein Sequence and Structure* (Natl. Biomed. Res. Found., Washington, D.C.), herein incorporated by reference. Conservative substitutions, such as exchanging one amino acid with another having similar properties, may be preferred. Examples of conservative substitutions include, but are not limited to, Gly⇔Ala, Val⇔Ile ⇔Leu, Asp⇔Glu, Lys⇔Arg, Asn⇔Gln, and Phe⇔Trp ⇔Tyr.

[0078] In constructing variants of the antibody polypeptide of interest, modifications are made such that variants continue to possess the desired activity, i.e., similar binding affinity to the biomarker. Obviously, any mutations made in the DNA encoding the variant polypeptide must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. See EP Patent Application Publication No. 75,444.

[0079] Preferably, variants of a reference biomarker antibody have amino acid sequences that have at least 70% or 75% sequence identity, preferably at least 80% or 85% sequence identity, more preferably at least 90%, 91%, 92%, 93%, 94% or 95% sequence identity to the amino acid sequence for the reference antibody molecule, or to a shorter portion of the reference antibody molecule. More preferably, the molecules share at least 96%, 97%, 98% or 99% sequence identity. For purposes of the present invention, percent sequence identity is determined using the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is taught in Smith and Waterman (1981) *Adv. Appl. Math.* 2:482-489. A variant may, for example, differ from the reference antibody by as few as 1 to 15 amino acid residues, as few as 1 to 10 amino acid residues, such as 6-10, as few as 5, as few as 4, 3, 2, or even 1 amino acid residue.

[0080] With respect to optimal alignment of two amino acid sequences, the contiguous segment of the variant amino acid sequence may have additional amino acid residues or deleted amino acid residues with respect to the reference amino acid sequence. The contiguous segment used for comparison to the reference amino acid sequence will include at least 20 contiguous amino acid residues, and may be 30, 40, 50, or more amino acid residues. Corrections for sequence identity associated with conservative residue substitutions or gaps can be made (see Smith-Waterman homology search algorithm).

[0081] The antibodies used to practice the invention are selected to have specificity for the biomarker proteins of interest. Methods for making antibodies and for selecting appropriate antibodies are known in the art. See, for example, Celis, ed. (in press) *Cell Biology & Laboratory Handbook,* 3rd edition (Academic Press, New York), which is herein incorporated in its entirety by reference. In some embodiments, commercial antibodies directed to specific biomarker proteins may be used to practice the invention. The antibodies of the invention may be selected on the basis of desirable staining of histological samples. That is, in preferred embodiments the antibodies are selected with the end sample type (e.g., formalin-fixed, paraffin-embedded breast tumor tissue samples) in mind and for binding specificity.

[0082] In some aspects of the invention, antibodies directed to specific biomarkers of interest are selected and purified via a multi-step screening process. In particular embodiments, polydomas are screened to identify biomarker-specific antibodies that possess the desired traits of specificity and sensitivity. As used herein, "polydoma" refers to multiple hybridomas. The polydomas of the invention are typically provided in multi-well tissue culture plates. In the initial antibody screening step, a set of individual slides or tumor tissue microarrays comprising normal (i.e., non-cancerous) breast tissue and stage I, II, III, and IV breast tumor samples is used. Methods and equipment, such as the Chemicon® Advanced Tissue Arrayer, for generating arrays of multiple tissues on a single slide are known in the art. See, for example, U.S. Pat. No. 4,820,504. Undiluted supernatants from each well containing a polydoma are assayed for positive staining using standard immunohistochemistry techniques. At this initial screening step, background, non-specific binding is essentially ignored. Polydomas producing positive staining are selected and used in the second phase of antibody screening.

[0083] In the second screening step, the positive polydomas are subjected to a limiting dilution process. The resulting unscreened antibodies are assayed via standard immunohistochemistry techniques for positive staining of breast tumor tissue samples with known 5-year outcomes. To do this, tissue microarrays comprising normal breast tissue, early-stage breast tumor samples with known good 5-year outcomes, early-stage breast tumor samples with known bad 5-year outcomes, normal non-breast tissue, and cancerous non-breast tissue are generated. At this stage, background staining is relevant, and the candidate polydomas that stain positive for abnormal cells (i.e., cancer cells) only are selected for further analysis to identify antibodies that differentiate good and bad outcome patient samples.

[0084] Positive-staining cultures are prepared as individual clones in order to select individual candidate monoclonal antibodies. Methods for isolating individual clones and for purifying antibodies through affinity adsorption chromatography are well known in the art. Individual clones are further analyzed to determine the optimized antigen retrieval conditions and working dilution.

[0085] One of skill in the art will recognize that optimization of staining reagents and conditions, for example, antibody titer and detection chemistry parameters, is needed to maximize the signal to noise ratio for a particular antibody. Antibody concentrations that maximize specific binding to the biomarkers of the invention and minimize non-specific binding (or "background") will be determined. In particular embodiments, appropriate antibody titers are determined by initially testing various antibody dilutions on formalin-fixed, paraffin-embedded normal and cancerous breast tissue samples. The design of assays to optimize antibody titer and detection conditions is standard and well within the routine capabilities of those of ordinary skill in the art. Some antibodies require additional optimization to reduce background staining and/or to increase specificity and sensitivity of staining.

[0086] Furthermore, one of skill in the art will recognize that the concentration of a particular antibody used to practice the methods of the invention will vary depending on such factors as time for binding, level of specificity of the antibody for the biomarker protein, and method of body sample preparation. Moreover, when multiple antibodies are used in a single sample, the required concentration may be affected by the order in which the antibodies are applied to the sample, i.e., simultaneously as a cocktail or sequentially as individual antibody reagents. Furthermore, the detection chemistry used to visualize antibody binding to a biomarker of interest must also be optimized to produce the desired signal to noise ratio. One example of optimization of staining reagents and conditions for immunohistochemistry is described in Example 6.

[0087] Detection of antibody binding can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, P-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S, or ^{3}H.

[0088] In regard to detection of antibody staining in the immunohistochemistry methods of the invention, there also exist in the art, video-microscopy and software methods for the quantitative determination of an amount of multiple molecular species (e.g., biomarker proteins) in a biological sample wherein each molecular species present is indicated by a representative dye marker having a specific color. Such methods are also known in the art as a calorimetric analysis methods. In these methods, video-microscopy is used to provide an image of the biological sample after it has been stained to visually indicate the presence of a particular biomarker of interest. Some of these methods, such as those disclosed in U.S. patent application Ser. No. 09/957,446 to Marcelpoil et al. and U.S. patent application Ser. No. 10/057,729 to Marcelpoil et al., incorporated herein by reference, disclose the use of an imaging system and associated software to determine the relative amounts of each molecular species present based on the presence of representative color dye markers as indicated by those color dye markers' optical density or transmittance value, respectively, as determined by an imaging system and associated software. These techniques provide quantitative determinations of the relative amounts of each molecular species in a stained biological sample using a single video image that is "deconstructed" into its component color parts.

[0089] The methods of the invention can be used in conjunction with imaging systems and associated imaging software for the detection of biomarker expression. Biomarkers for use in the methods of the invention can be selected based on methods and computer programs such as those disclosed in U.S. Patent Application Publication No. 2006/0078926 entitled "Methods and Computer Programs for Analysis and Optimization of Marker Candidates for Cancer Prognosis," filed Sep. 22, 2005, and incorporated by reference in its entirety. The methods disclosed therein can be used to develop algorithms for evaluating breast cancer prognosis.

[0090] In other embodiments, the expression of a biomarker of interest is detected at the nucleic acid level. Nucleic acid-based techniques for assessing expression are well known in the art and include, for example, determining the level of biomarker mRNA in a body sample. Many expression detection methods use isolated RNA. Any RNA isolation technique that does not select against the isolation of mRNA can be utilized for the purification of RNA (see, e.g., Ausubel et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, New York 1987-1999). Additionally, large numbers of tissue samples can readily be processed using techniques well known to those of skill in the art, such as, for example, the single-step RNA isolation process of Chomczynski (1989, U.S. Pat. No. 4,843,155).

[0091] The term "probe" refers to any molecule that is capable of selectively binding to a specifically intended target biomolecule, for example, a nucleotide transcript or a protein encoded by or corresponding to a biomarker. Probes can be synthesized by one of skill in the art, or derived from appropriate biological preparations. Probes may be specifically designed to be labeled. Examples of molecules that can be utilized as probes include, but are not limited to, RNA, DNA, proteins, antibodies, and organic molecules.

[0092] Isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction analyses and probe arrays. One method for the detection of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to the mRNA encoded by the gene being detected. The nucleic acid probe can be, for example, a full-length cDNA, or a portion thereof, such as an oligonucleotide of at least 7, 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to an mRNA or genomic DNA encoding a biomarker of the present invention. Hybridization of an mRNA with the probe indicates that the biomarker in question is being expressed.

[0093] In one embodiment, the mRNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the mRNA is contacted with the probe(s), for example, in an Affymetrix gene chip array. A skilled artisan can readily adapt known mRNA detection methods for use in detecting the level of mRNA encoded by the biomarkers of the present invention.

[0094] An alternative method for determining the level of biomarker mRNA in a sample involves the process of nucleic acid amplification, e.g., by RT-PCR (the experimental embodiment set forth in Mullis, 1987, U.S. Pat. No. 4,683,202), ligase chain reaction (Barany, 1991, Proc. Natl. Acad. Sci. USA, 88:189-193), self sustained sequence replication (Guatelli et al., 1990, Proc. Natl. Acad. Sci. USA 87:1874-1878), transcriptional amplification system (Kwoh et al., 1989, Proc. Natl. Acad. Sci. USA 86:1173-1177), Q-Beta Replicase (Lizardi et al., 1988, Bio/Technology 6:1197), rolling circle replication (Lizardi et al., U.S. Pat. No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. In particular aspects of the invention, biomarker expression is assessed by quantitative fluorogenic RT-PCR (i.e., the TaqMan® System).

[0095] Biomarker expression levels of RNA may be monitored using a membrane blot (such as used in hybridization analysis such as Northern, Southern, dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound nucleic acids). See U.S. Pat. Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934, which are incorporated herein by reference. The detection of biomarker expression may also comprise using nucleic acid probes in solution.

[0096] In one embodiment of the invention, microarrays are used to detect biomarker expression. Microarrays are particularly well suited for this purpose because of the reproducibility between different experiments. DNA microarrays provide one method for the simultaneous measurement of the expression levels of large numbers of genes. Each array consists of a reproducible pattern of capture probes attached to a solid support. Labeled RNA or DNA is hybridized to complementary probes on the array and then detected by laser scanning. Hybridization intensities for each probe on the array are determined and converted to a quantitative value representing relative gene expression levels. See, U.S. Pat. Nos. 6,040,138, 5,800,992 and 6,020,135, 6,033,860, and 6,344,316, which are incorporated herein by reference. High-density oligonucleotide arrays are particularly useful for determining the gene expression profile for a large number of RNA's in a sample.

[0097] Techniques for the synthesis of these arrays using mechanical synthesis methods are described in, e.g., U.S. Pat. No. 5,384,261, incorporated herein by reference in its entirety for all purposes. Although a planar array surface is preferred, the array may be fabricated on a surface of virtually any shape or even a multiplicity of surfaces. Arrays may be peptides or nucleic acids on beads, gels, polymeric surfaces, fibers such as fiber optics, glass or any other appropriate substrate, see U.S. Pat. Nos. 5,770,358, 5,789,162, 5,708,153, 6,040,193 and 5,800,992, each of which is hereby incorporated in its entirety for all purposes. Arrays may be packaged in such a manner as to allow for diagnostics or other manipulation of an all-inclusive device. See, for example, U.S. Pat. Nos. 5,856,174 and 5,922,591 herein incorporated by reference.

[0098] In one approach, total mRNA isolated from the sample is converted to labeled cRNA and then hybridized to an oligonucleotide array. Each sample is hybridized to a separate array. Relative transcript levels may be calculated by reference to appropriate controls present on the array and in the sample.

[0099] Kits for practicing the methods of the invention are further provided. By "kit" is intended any manufacture (e.g., a package or a container) comprising at least one reagent, e.g. an antibody, a nucleic acid probe, etc. for specifically detecting the expression of a biomarker of the invention. The kit

may be promoted, distributed, or sold as a unit for performing the methods of the present invention. Additionally, the kits may contain a package insert describing the kit and methods for its use.

[0100] In particular embodiments, kits for practicing the immunohistochemistry methods of the invention are provided. Such kits are compatible with both manual and automated immunohistochemistry techniques (e.g., cell staining) as described herein below in Example 1. These kits comprise at least one antibody directed to a biomarker protein of interest. Chemicals for the detection of antibody binding to the biomarker, a counterstain, and a bluing agent to facilitate identification of positive staining cells are optionally provided. Alternatively, the immunochemistry kits of the present invention are used in conjunction with commercial antibody binding detection systems, such as, for example the Dako Envision+system and Biocare Medical's Mach 3 system. Any chemicals that detect antigen-antibody binding may be used in the practice of the invention. In some embodiments, the detection chemicals comprise a labeled polymer conjugated to a secondary antibody. For example, a secondary antibody that is conjugated to an enzyme that catalyzes the deposition of a chromogen at the antigen-antibody binding site may be provided. Such enzymes and techniques for using them in the detection of antibody binding are well known in the art. In one embodiment, the kit comprises a secondary antibody that is conjugated to an HRP-labeled polymer. Chromogens compatible with the conjugated enzyme (e.g., DAB in the case of an HRP-labeled secondary antibody) and solutions, such as hydrogen peroxide, for blocking non-specific staining may be further provided. The kits of the present invention may also comprise a counterstain, such as, for example, hematoxylin. A bluing agent (e.g., ammonium hydroxide) may be further provided in the kit to facilitate detection of positive staining cells.

[0101] In another embodiment, the immunohistochemistry kits of the invention comprise at least two reagents, e.g., antibodies, for specifically detecting the expression of at least two distinct biomarkers. Each antibody may be provided in the kit as an individual reagent or, alternatively, as an antibody cocktail comprising all of the antibodies directed to the different biomarkers of interest. Furthermore, any or all of the kit reagents may be provided within containers that protect them from the external environment, such as in sealed containers. Positive and/or negative controls may be included in the kits to validate the activity and correct usage of reagents employed in accordance with the invention. Controls may include samples, such as tissue sections, cells fixed on glass slides, etc., known to be either positive or negative for the presence of the biomarker of interest. The design and use of controls is standard and well within the routine capabilities of those of ordinary skill in the art.

[0102] In other embodiments, kits for evaluating the prognosis of a breast cancer patient comprising detecting biomarker overexpression at the nucleic acid level are further provided. Such kits comprise, for example, at least one nucleic acid probe that specifically binds to a biomarker nucleic acid or fragment thereof. In particular embodiments, the kits comprise at least two nucleic acid probes that hybridize with distinct biomarker nucleic acids.

[0103] One of skill in the art will appreciate that any or all steps in the methods of the invention could be implemented by personnel or, alternatively, performed in an automated fashion. Thus, the steps of body sample preparation, sample staining, and detection of biomarker expression may be automated. Moreover, in some embodiments, the immunohistochemical methods of the invention are used in conjunction with computerized imaging equipment and software to facilitate the identification of positive-staining cells by a pathologist. The methods disclosed herein can also be combined with other prognostic methods or analyses (e.g., tumor size, lymph node status, expression levels of Her2/neu, Ki67, and p53). In this manner detection of overexpression of the biomarkers of the invention can permit a more accurate determination of the prognosis of a breast cancer patient.

[0104] The article "a" and "an" are used herein to refer to one or more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one or more element.

[0105] Throughout the specification the word "comprising," or variations such as "comprises" or "comprising," will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

[0106] The following examples are offered by way of illustration and not by way of limitation:

EXPERIMENTAL

Example 1

Detection of Biomarker Overexpression Using Immunohistochemistry

Slide Preparation

[0107] 4 μM sections of formalin-fixed, paraffin-embedded breast tumor tissue samples are cut using a microtome and placed on SuperFrost+ slides (VWR). The slides are baked in a forced air oven for 20 minutes and then contacted with a Histo-Orienter until the paraffin melts. Slides are washed three times with xylene for 5 minutes to remove paraffin and then rinsed three times in absolute alcohol at 2 minutes/rinse.

Pretreatment and Antigen Retrieval

[0108] To prevent non-specific background staining, the slides are incubated in a hydrogen peroxide/methanol block for five minutes at room temperature. Slides are then rinsed thoroughly with several changes of dH_2O.

[0109] In order to make the antigens accessible to antibody binding, slides are incubated in an antigen retrieval solution in a pressure cooker for 5 minutes. Slides are allowed to cool to room temperature for 20 minutes on the bench, and the citrate buffer is gradually replaced with dH_2O, tris buffered saline (TBS), or phosphate buffered saline (PBS) by successive dilutions. The slides are then rinsed three times in TBS at 2 minutes per rinse. To break the surface tension, 750 μl/50 ml of 1% BSA/TBS is added to each slide.

Manual Immunohistochemistry

[0110] To prevent non-specific background staining, slides are not permitted to dry out during the staining procedure. Slides that have been subjected to antigen retrieval are loaded into a humidity chamber filled with water moistened paper towels. A SLPI antibody (clone 5G6.24; 1:100 dilution) is applied to the slide in a volume sufficient to completely cover the tissue section for 1 hour at room temperature. Following incubation with the primary antibody, the slides are rinsed

three times in TBS at 2 minutes per wash. 750 μl/50 ml of 1% BSA/TBS is added to the final wash.

[0111] The Dako Envision+ HRP-labeled polymer secondary antibody is applied to the slide for 30 minutes at room temperature, followed by a TBS rinse. The HRP substrate chromogen DAB is applied for 10 minutes, and then the slides are rinsed for 5 minutes with water. Each slide is counterstained with hematoxylin for 5 seconds and then rinsed with water until clear. Following counterstaining, the slides are "blued" by soaking in ammonia water for 10 seconds and then rinsed with water for 1 minute.

[0112] Samples are dehydrated by immersing the slides in 95% ethanol for 1 minute and then in absolute ethanol for an additional minute. Slides are cleared by rinsing 3 times in xylene for 1 minute per rinse. Slides are then coverslipped with permanent mounting media and incubated at 35° C. to dry. Biomarker staining is visualized using a bright-field microscope. Scoring is performed by a board certified pathologist in a blind manner.

Automated Immunohistochemistry

[0113] The Dako Autostainer Universal Staining system is programmed according to the manufacturer's instructions, and the necessary staining and counterstaining reagents described above for manual immunohistochemistry are loaded onto the machine. The prepared slides are loaded onto the Autostainer, and the program is run. At the end of the run, the slides are removed and rinsed in water for 5 minutes. The slides are dehydrated, cleared, coverslipped, and analyzed as described above.

Example 2

Detection of Overexpression of Individual Biomarkers in Clinical Samples

[0114] Approximately 130 breast tumor tissue samples from patients at various disease stages were collected. The average patient age was 77. Actual clinical outcome data for each patient was known, and each patient was categorized as having a good or bad outcome. In this study, good outcome was defined as remaining cancer-free for at least 5 years; bad outcome was defined as suffering disease relapse, recurrence, or death within 5 years. The following table indicates the number of samples within each diagnosis group analyzed, as well as actual clinical outcome data.

TABLE 1

Clinical Samples Analyzed

Stage	Good Outcome	Bad Outcome	Total
T1N0	50	13	63
T1N1	6	4	10
T2N0	26	19	45
T2N1	9	7	16
T3N0	0	3	3
T3N1	0	1	1

Lymph Node Status	Good Outcome	Bad Outcome
N0	76	35
N1	15	12

[0115] The samples were analyzed by the automated immunohistochemistry described in Example 1 to identify biomarkers whose overexpression is indicative of a bad cancer prognosis. That is, the goal of this clinical study was to identify biomarkers that can distinguish good and bad outcome patient samples. Antibodies were used to detect the overexpression of eight biomarkers of interest: SLPI, PSMB9, NDRG-1, E2F1, p21ras, MUC-1, phospho-p27, and src. For quality control purposes, samples were also analyzed for ER, PR, p53, Ki67, and Her2/neu expression.

[0116] Commercial antibodies or monoclonal antibodies, identified by polydoma screening as described herein, directed to the biomarkers of interest were diluted as indicated in Table 2 and used to detect biomarker overexpression. The antigen retrieval conditions for each biomarker are also listed below.

TABLE 2

Antibody Dilutions and Antigen Retrieval Conditions

Biomarker	Antibody (Dilution)	Antigen Retrieval Conditions
SLPI	Clone 5G6.24 (1:100)	Citrate buffer, pH 6.0/pressure cooker
PSMB9	Clone 3A2.4 (1:500)	Citrate buffer, pH 4.0/steamer
NDRG-1	Zymed (1:200)	Citrate buffer, pH 4.0/steamer
E2F1	Calbiochem (1:50)	Tris, pH 9.5/pressure cooker
p21ras	Dako (1:50)	Citrate buffer, pH 4.0/steamer
MUC-1	Clone 16E3.3 (1:400)	Citrate buffer, pH 4.0/steamer
phospho-p27	Zymed (1:100)	EDTA, pH 8.0/steamer
src	Upstate (1:50)	Citrate buffer, pH 4.0/steamer

Interpretation of Slides

[0117] Each slide was reviewed and scored by a board certified pathologist that was unaware of the actual clinical patient outcomes. Samples were scored for biomarker staining intensity on a scale of 0-3. See, for example, Hanausek and Walaszek, eds. (1998) *Tumor Marker Protocols* (Humana Press, Inc., Totowa, N.J.); and Shi et al, eds. (2000) *Antigen Retrieval Techniques: Immunohistochemistry and Molecular Morphology* (Eaton Publishing, Natick, Mass.), both of which are herein incorporated by reference in their entirety. For each biomarker, a threshold staining intensity was established. Samples exhibiting a staining intensity of less than this threshold value for a particular biomarker were deemed negative for that biomarker. The staining intensity threshold values for the biomarkers of interest were as follows:

[0118] Src: ≧1

[0119] MUC-1: ≧3

[0120] Phospho-p27: ≧0.5

[0121] PSMB9: ≧0.5

[0122] NDRG-1: ≧1

[0123] E2F1: ≧3

[0124] p21ras: ≧0.5

[0125] SLPI: ≧2

[0126] The staining intensity results were compared with the known actual clinical outcome data available for each patient, and each slide was then given a final result of true positive (TP), true negative (TN), false positive (FP), false negative (FN), according to the parameters described below. Sensitivity and specificity values for each biomarker were calculated.

TABLE 3

Slide Classification for Bad Outcome Biomarkers

	Biomarker Staining	Actual Clinical Outcome*
True Positive	Positive	Bad outcome
True Negative	Negative	Good outcome
False Positive	Positive	Good outcome
False Negative	Negative	Bad outcome

*Good clinical outcome = cancer-free survival for at least 5 years
Bad clinical outcome = recurrence or death from the underlying cancer within 5 years

Calculations Used

Sensitivity=TP/(TP+FN)

Specificity=TN/(FP+TN)

Positive Predictive Power (PPP)=TP/(TP+FP)

Negative Predictive Power (NPP)=TN/(FN+TN)

Results

[0127] The results for each biomarker are summarized below.

TABLE 4

Summary of Results with Individual Biomarkers

	Src	MUC-1	Phospho-p27	PSMB9	NDRG-1	E2F1	p21ras	SLPI
TP	8	7	7	13	7	3	10	5
FP	8	4	6	15	14	4	11	7
FN	35	37	44	30	31	34	30	39
TN	59	70	76	54	54	57	60	64
Sensitivity	18.60%	15.91%	13.73%	30.23%	18.42%	8.11%	25.00%	11.36%
Specificity	88.06%	94.59%	92.68%	78.26%	79.41%	93.44%	84.51%	90.14%

Example 3

Detection of Biomarker Overexpression in Clinical Samples Combining Biomarkers

[0128] In order to determine if the sensitivity and specificity of the methods of the invention could be improved if multiple biomarkers were combined, the data from Example 2 was subjected to further analysis. Thus, various combinations of biomarkers were considered, and samples that stained positive for any of the biomarkers in the combination of interest were deemed positive. These results were compared with the known actual clinical outcome data available for each patient, and each slide was then given a final result of true positive (TP), true negative (TN), false positive (FP), false negative (FN) as before. Sensitivity, specificity, positive predictive value (PPV), and negative predictive values (NPV) for each combination of biomarkers were calculated.

Results

[0129] The results for each combination of biomarkers are summarized below.

TABLE 5

SLPI, PSMB9, MUC-1, and phospho-p27	
TP	24
FP	25
FN	23
TN	58
Sensitivity	51.06%
Specificity	69.88%
NPV	71.60%
PPV	48.98%

TABLE 6

SLPI, PSMB9, MUC-1, phospho-p27, and src	
TP	28
FP	28
FN	24
TN	60

TABLE 6-continued

SLPI, PSMB9, MUC-1, phospho-p27, and src	
Sensitivity	53.85%
Specificity	68.18%
NPV	71.43%
PPV	50.00%

TABLE 7

SLPI, PSMB9, MUC-1, phospho-p27, src, p21ras, E2F1, and NDRG-1	
TP	33
FP	41
FN	19

TABLE 7-continued

SLPI, PSMB9, MUC-1, phospho-p27, src, p21ras, E2F1, and NDRG-1	
TN	47
Sensitivity	63.46%
Specificity	53.41%
NPV	71.21%
PPV	44.59%

Example 4

Detection of Overexpression of Individual Biomarkers in Clinical Samples Using Marker Analysis Research System (MARS)

[0130] Over 200 patients were analyzed in this study. As summarized in Table 8 this population of patients was quite heterogeneous and exhibited tumors of different stages ranging from T1N0 to T3N0.

TABLE 8

	Patient Population Analyzed		
Stage	Good	Bad	All
T1N0	60	20	80
T1N1	6	7	13
T2N0	59	39	98
T3N0	6	10	16
Totals	131	76	207

[0131] The targeted characteristic of the patients was their good outcome or bad outcome status. In this study, good outcome patients were those still disease-free after five years; bad outcome patients were defined as patients with recurrence, relapse, or death within five years.

Biomarker Selection

[0132] The paradigm used for biomarker selection was that biomarker overexpression would capture some of the bad outcome patients and show a very high specificity. Combining different markers would therefore ensure high specificity and gain sensitivity to reach, for example, an 80% sensitivity and 80% specificity. After a multi-step selection process, nine biomarkers were selected for the current study. These markers are shown in Table 9, along with their respective subcellular localization.

TABLE 9

	Biomarkers Analyzed
Marker Name	Localization
E2F1	Nucleus
MUC-1 (IF3.9)	Membrane
NDRG-1 (ZYMED CAP43)	Cytoplasm (Nucleus + Membrane)
$p21^{ras}$	Cytoplasm
p53	Nucleus
Phospho p27	Cytoplasm (Nucleus)
PSMB9 (3A2.4)	Cytoplasm
SLPI (5G6.24)	Cytoplasm
src	Cytoplasm

Automated Immunohistochemistry

[0133] The patient samples were analyzed by automated immunohistochemistry, essentially as described in Example 1, to identify biomarkers whose overexpression is indicative of a bad cancer prognosis. That is, the goal of this clinical study was to identify biomarkers that can distinguish good and bad outcome patient samples. Antibodies were used to detect the overexpression of the nine biomarkers of interest: SLPI, PSMB9, NDRG-1, E2F1, p21ras, p53, MUC-1, phospho-p27, and src. Samples were also analyzed for ER, PR, Ki67, and Her2/neu (CerbB2) expression.

[0134] Slides were prepared as described in Example 1 and subjected to antigen retrieval. Specifically, prepared slides were immersed in an antigen retrieval solution and then placed in a pressure cooker (120-125° C. at 17-23 psi) for 5 minutes. The antigen retrieval solutions for each biomarker are listed below in Table 10.

TABLE 10

	Antigen Retrieval Solutions
Biomarker	Antigen Retrieval Solution
SLPI	Citrate pH 6.0 (Dako #S1699)
PSMB9	EDTA (Biocare #CB917L)
NDRG-1	Citrate pH 6.0 (Dako #S1699)
E2F1	EDTA (Biocare #CB917L)
p21ras	citrate buffer pH 6.0 (Dako #S1699
MUC-1	Citrate pH 6.0 (Dako #S1699)
phospho-p27	deionized water
src	Tris pH 9.5 (Biocare CB911M)

[0135] Slides were gradually returned to room temperature deionized water. The slides were rinsed 3 times in TBS/tween-20 at 2 minutes per wash. 200 pt of a biomarker-specific antibody was added to each slide and incubated at room temperature for one hour. Commercial antibodies or monoclonal antibodies, identified by polydoma screening as described herein, directed to the biomarkers of interest were used to detect biomarker overexpression.

[0136] Following incubation with the primary antibody, slides were rinsed twice with TBS/tween-20. 200 μl of labeled polymer (Dako Envision+HRP-labeled polymer secondary antibody) was then added for 30 minutes at room temperature. Slides were again rinsed 3 times with TBS/tween-20 prior to the addition of 200 μl of DAB solution for five minutes at room temperature. The slides were then rinsed three times with TBS/tween-20 and one time with deionized water. 200 μl of hematoxylin was added for 5 minutes. The slides were then rinsed 3 times with deionized water, one time with TBS/tween-20, and 2 additional times with deionized water. The slides were dehydrated, cleared, and coverslipped as described in Example 1.

Pathologist Evaluation

[0137] A board certified pathologist manually scored the slides. p53 expression was scored for staining intensity using a scale of 0, 0.5, 1, 2, or 3, percentage of labeled cells, and a clinical diagnostic score. SLPI and PSMB9 were scored for staining intensity using a scale of 0, 0.5, 1, 2, or 3 and percentage of labeled cells. The pathologist also denoted on the slide the tumor area (ROI) used in making the determination. Up to ten individual 20× fields of view from within the selected regions for each tumor, organized in a single focus,

were obtained using MARS. The actual number of images obtained from each sample was dependant on the size of the individual tumor. An Excel spreadsheet containing all of the above scoring information along with the patient outcome, lymph node status, and tumor size was generated. The data was subjected to further analysis as described below.

Data Extraction

[0138] Using MARS, the following steps were systematically performed for every file:

[0139] Chromogen separation was optimized for each biomarker using the available slide that showed the best quality stain.

[0140] Segmentation set up was customized for each biomarker according to its subcellular localization (nucleus, cytoplasm or membrane).

[0141] Features were extracted at cell, field of view (FOV), and focus level, within the defined ROI and exported to an output file (XML format).

Data Analysis

[0142] A specific program named Multi Marker Analyzer was developed in order to integrate new analysis algorithms and meet the heavy computation needs for this analysis. This software provided a means to load all or a portion of either TMAs or tissue section XML files generated with MARS, to merge data contained in these files using XML files describing the TMA keys (in the case of a TMA analysis) or Excel files giving patient clinical status and patient evaluation (in the case of a tissue section analysis), and all the further analyzes. This merge process included the association of the parameters measured by MARS for each core (or patient) with the information kept in the TMA key (or the Excel file) about the patient: identification number and medical status (good or bad outcome) and the pathologist evaluation if not included in the XML formatted MARS file.

[0143] Because some of the samples did not go through the complete experimental process, the number of analyzed patients was smaller than the number of patients reported in Table 8 above and varies from one biomarker to another. The number of tissue sections analyzed for each biomarker is listed below in Table 11. The number of tissue samples analyzed for the conventional breast cancer markers (i.e., ER, PR, Ki67, and Her2/neu (CerbB2)) is in Table 12.

TABLE 11

Number of Tissue Sections Analyzed for Biomarker Overexpression

Marker	Bad	Good	Total
E2F1	66	106	172
MUC-1	65	108	173
NDRG1 (CAP 43)	75	115	190
p21ras	72	109	181
p53	71	121	192
Phospho-p27	70	115	185

TABLE 11-continued

Number of Tissue Sections Analyzed for Biomarker Overexpression

Marker	Bad	Good	Total
PSMB9	74	118	192
SLPI	75	118	193
src	66	108	174

TABLE 12

Number of Tissue Sections Analyzed for Conventional Breast Cancer Markers

Marker	Bad	Good	Total
CerbB2	69	122	191
ER	70	123	193
Ki67	69	124	193
PR	69	123	192

Segmentation and Dispatchers Setup

[0144] In order to bring MARS analysis closer to the pathologist manner of characterizing slides, only cells considered as being at least 1+ were selected. Table 13 summarizes the segmentation setup used in MARS for this analysis. This segmentation setup lead to the detection of the most stained cells. Segmentation and dispatchers transmittance thresholds were based upon cytologists input. The segmentation setup was pixel-based using 20× images captured with a Dage camera on the computer TPO-RDLAB5.

TABLE 13

Main segmentation setup parameters

Size (pixels)	Cell	68
	Nucleus	32
Hematoxylin Contribution	Nucleus	80%
	Cytoplasm	100%
	Membrane	0%
Hematoxylin Max. Transmittance	Nucleus	80%
	Cytoplasm	100%
	Membrane	100%
DAB Contribution	Nucleus	30%
	Cytoplasm	100%
	Membrane	0%
DAB Max. Transmittance	Nucleus	90%
	Cytoplasm	100%
	Membrane	100%

[0145] In order to assign the selected cells to categories based upon the biomarker staining intensity in the targeted cellular compartment, valid cells resulting from segmentation were dispatched into 3 categories: 1 (in MARS: NegRef), 2 (in MARS: Test) and 3 (in MARS: PosRef). Table 14 provides MARS features and their values used to perform this dispatch, as a function of the cellular localization of the marker.

TABLE 14

Dispatcher Settings Resulting in the Assignment of Selected Cells into Category 1, 2 or 3

Marker Targeted Cell Compartment	Dispatch Step	If MARS Feature	Is	Value (Transmittance)	Cell(s)	Is
Nucleus	1	NUCL_DYE2_OD_MEAN	>	0.161151 (69%)	All	(2 or 3) otherwise 1
Cytoplasmic	2	NUCL_DYE2_OD_MEAN	>	0.29243 (51%)	2 and 3	3 otherwise 2
	1	CYTO_DYE2_OD_MEAN	>	0.173925 (67%)	All	(2 or 3) otherwise 1
Membrane	2	CYTO_DYE2_OD_MEAN	>	0.29243 (51%)	2 and 3	3 otherwise 2
		CYTO_DYE2_OD_MEAN	>	0.06048 (87%)		
	1	MEMB_DYE2_OD_MEAN	>	0.200659 (63%)	All	(2 or 3) otherwise 1
		MEMB_AREA	>	150 pix.		
		CYTO_DYE2_OD_MEAN	>	0.173925 (67%)		
	2	MEMB_DYE2_OD_MEAN	>	0.29243 (51%)	2 and 3	3 otherwise 2
		MEMB_AREA	>	150 pix.		

[0146] An evaluation of category 0 (corresponding to the "expected number of non-stained cells") was performed. The approximate number of these cells was computed using the average tumor cell area (1100 pixels as estimated from the MARS feature called CELL_AREA) obtained from the area of cells with a staining intensity of 1, 2 and 3 cells:

$$N_1 = N_{NegRef}$$

$$N_2 = N_{Test}$$

$$N_3 = N_{PosRef}$$

$$N_{Total} = \max\left(N_1 + N_2 + N_3 \cdot \frac{\text{FOCUS\_AREA}}{1100}\right)$$

$$N_0 = \max(0, N_{Total} - N_1 - N_2 - N_3)$$

[0147] Using N_0, N_1, N_2 and N_3, the percentage of cells staining 0, 1, 2 and 3 cells were computed. Table 15 gives the name of these new features.

TABLE 15

Percentage Summary Features

Percentage of cells from categories	Feature Name
0	CELL_PERCENT_0
1	CELL_PERCENT_1
2	CELL_PERCENT_2
3	CELL_PERCENT_3
0 and 1	CELL_PERCENT_01
2 and 3	CELL_PERCENT_23
0, 1 and 2	CELL_PERCENT_012
1, 2 and 3	CELL_PERCENT_123

[0148] These features were computed as a simple percentage, e.g. for CELL_PERCENT_0:

$$\text{CELL\_PERCENT\_0} = \frac{N_0}{N_{Total}} \times 100$$

[0149] This study was run with MARS features, these new summary features, and the pathologist scores. USER_TYPE is the name of the MARS feature for pathologist scoring only.

Multiple Biomarker Analysis

[0150] In order to obtain an improved sensitivity/specificity couple, data from multiple biomarkers was combined and analyzed. The specificity target for each biomarker was dependent on the number of biomarkers combined. As an example, a combination of 3 biomarkers will reach 80% specificity if each individual marker specificity is at least of 0.81/3=93%. Table 16 provides the list of required specificity values based on the number of biomarkers in the combination, from 1 to 9.

TABLE 16

Minimum specificity required per biomarker when an overall specificity of 0.8 is targeted for a given combination of up to 9 biomarkers

Marker Number in combination	Specificity Required Per Marker
1	0.8
2	0.694427
3	0.926318
4	0.945742
5	0.956352
6	0.963492
7	0.968625
8	0.972492
9	0.975511

Data Interpretation

[0151] As used herein, the term "marker performance" encompasses the complete experimental performance that relates to the true biological discriminative power of the marker, as well as to the origin and storage of the biological samples, the staining protocols, the scanning process, the imaging and data mining procedures.

Results

1. Per Biomarker

A. Pathologist Scoring

[0152] The threshold giving the best sensitivity/specificity couple was computed when considering only the pathologist scores (USER_TYPE in MARS). The most significant results are summarized in Table 17 when a specificity of 0.75 was targeted.

TABLE 17

Best Sensitivity and Specificity Couple for Biomarkers (Pathologist Scoring)

Marker	Threshold	Sensitivity	Specificity
E2F1	1.75	0.30	0.69
MUC-1	0.75	0.21	0.61
NDRG1	2.5	0.28	0.74
p21^{ras}	0.25	0.05	0.98
p53	0.5	0.29	0.74
Phospho-p27	1.25	0.17	0.73
PSMB9	0.75	0.10	0.94
SLPI	2.5	0.18	0.63
src	2.5	0.10	0.67

[0153] The threshold giving the best sensitivity/specificity couple was also computed when considering only the pathologist evaluation for conventional markers of the breast panel (i.e., ER, PR, Ki67, and Her2/neu (CerbB2)). The most significant results are summarized in Table 18 when a specificity of 0.75 was targeted.

TABLE 18

Best Sensitivity and Specificity Couple for Conventional Markers (Pathologist Scoring)

Marker	Threshold	Sensitivity	Specificity
CerbB2	2.5	0.17	0.85
ER	0.5	0.31	0.72
Ki67	0.25	0.14	0.89
PR	2.5	0.23	0.69

[0154] For every biomarker and conventional breast cancer marker (i.e., ER, PR, Ki67, and Her2/neu (CerbB2)), the feature and threshold giving the best sensitivity/specificity couple was computed for the pathologist evaluation alone (USER_TYPE). Corresponding receiver operating characteristics (ROC) curves were prepared (data not shown).

B. Single-Feature Analysis

[0155] For every biomarker, the feature and threshold giving the best sensitivity/specificity couple was computed when considering every MARS features defined as being meaningful in respect to the analyzed biomarker. Corresponding ROCs were prepared (data not shown). The feature and threshold giving the best result for each biomarker are summarized in Table 19 when a specificity of 0.75 was targeted.

TABLE 19

Best Sensitivity and Specificity Couple for Each Biomarker Obtained from MARS Features (Single Feature Algorithm)

Marker	Feature	Threshold	Sens.	Spec.	Rule
E2F1	CELL_PERCENT_01	97.20165	0.575758	0.716981	8
MUC-1	CELL_PERCENT_1	21.4664	0.415385	0.685185	1
NDRG1	CELL_PERCENT_1	16.97263	0.386667	0.713043	8
p21ras	CELL_PERCENT_123	61.04522	0.402776	0.724771	1
p53	CELL_PERCENT_3	0.08369	0.422535	0.702479	8
phospho-p27	CELL_PERCENT_1	0.442341	0.528571	0.643478	8
PSMB9	CELL_PERCENT_123	30.42549	0.391892	0.711864	1
SLPI	CELL_PERCENT_123	0.610623	0.493333	0.694915	1
src	CELL_PERCENT_1	36.80664	0.409091	0.731481	1

*A decision rule of 1 means that patients above the threshold are considered as being positive (i.e. TRUE POSITIVE if bad actual clinical outcome), whereas a decision rule of 8 means that patients above the threshold are considered as being negative (i.e. FALSE NEGATIVE if bad actual clinical outcome).

C. Multiple Feature Analysis

[0156] Every percent summary feature was combined two-by-two, and thresholds giving the best sensitivity/specificity couple were computed. The most significant results for each biomarker are provided in Table 20 for a target specificity of 0.75.

TABLE 20

Best Sensitivity and Specificity Couple for Each Biomarker Obtained from MARS features (Multiple Feature Algorithm)

Marker	Feature 1	Feature 2	Threshold 1	Threshold 2	Sensitivity	Specificity	Rule
E2F1	CELL_PERCENT_2	CELL_PERCENT_3	2.386008	1.275799	0.575758	0.745283	7
MUC-1	CELL_PERCENT_1	CELL_PERCENT_3	21.26747	0.311046	0.507892	0.835135	9
NDRG1	CELL_PERCENT_1	CELL_PERCENT_23	32.96842	0.125389	0.48	0.713043	9
p21ras	CELL_PERCENT_3	CELL_PERCENT_01	0.1695	99.97016	0.458333	0.715596	6
p53	CELL_PERCENT_1	CELL_PERCENT_123	1.667596	17.22644	0.492958	0.710744	2
phospho-p27	CELL_PERCENT_1	CELL_PERCENT_01	0.456608	100	0.5	0.695652	8
PSMB9	CELL_PERCENT_1	CELL_PERCENT_123	47.63697	20.25946	0.466486	0.720339	4

TABLE 20-continued

Best Sensitivity and Specificity Couple for Each Biomarker Obtained from MARS features (Multiple Feature Algorithm)

Marker	Feature 1	Feature 2	Threshold 1	Threshold 2	Sensitivity	Specificity	Rule
SLPI	CELL__PERCENT__0	CELL__PERCENT__1	62.11591	0.414484	0.573333	0.728814	1
src	CELL__PERCENT__2	CELL__PERCENT__3	16.31145	0.082021	0.545455	0.712963	4

*Decision rules correspond to quadrant affection in the 2 features space.

2. Combinations of Biomarkers

[0157] The complete set of possible combinations of 1 to 9 markers was investigated using successively: the pathologist scoring, one MARS feature, and two MARS features per marker. The sensitivity and specificity were computed according to an FDA-like and a sequence-based interpretation method. "FDA-like" means that any marker ON (1) leads to a bad outcome decision. That is, a combination of markers is considered positive if at least one marker is positive. The sequence-based interpretation relies on sensitivity/specificity of each specific ON/OFF combination. The results obtained with pathologist scoring (Table 21) and percentage features evaluation (Table 22) are presented below.

TABLE 21

Best Sensitivity/Specificity Couples for Biomarker Combinations Using Different Targeted Specificities (75% and 95%) and Different Interpretation Algorithms (Pathologist Scoring)

	Target Spec	Markers	1 Marker	2 Markers	3 Markers	4 Markers	5 Markers	6 Markers	7 Markers	8 Markers	9 Markers
FDA	0.75	spec	0.74	0.52							
		sens	0.29	0.58							
SEQUENCE		spec		0.84	0.84	0.80	0.79	0.80	0.82	0.82	0.77
		sens		0.24	0.26	0.31	0.34	0.34	0.31	0.32	0.30
FDA	0.95	spec	0.90	0.86	0.88	0.84					
		sens	0.13	0.23	0.25	0.30					
SEQUENCE		spec					0.86	0.85	0.85	0.86	0.85
		sens					0.30	0.31	0.31	0.30	0.30

*Each patient is characterized by the pathologist score.

TABLE 22

Best Sensitivity/Specificity Couples for Biomarker Combinations Using Different Targeted Specificities (75% and 95%) and Different Interpretation Algorithms (Percentage Features)

All Povs % Features		Target Spec	Markers	1 Marker	2 Markers	3 Markers	4 Markers	5 Markers	6 Markers	7 Markers	8 Markers	9 Markers
1	FDA	0.75	spec	0.71	0.53							
			sens	0.57	0.80							
	SEQUENCE		spec		0.96	0.88	0.80	0.81	0.81	0.81	0.80	0.80
			sens		0.33	0.47	0.60	0.58	0.55	0.62	0.58	0.59
	FDA	0.95	spec	0.93	0.87	0.83	0.81					
			sens	0.18	0.34	0.44	0.50					
	SEQUENCE		spec					0.82	0.81	0.81	0.81	0.82
			sens					0.48	0.49	0.49	0.49	0.46
2	FDA	0.75	spec	0.74	0.55							
			sens	0.57	0.80							
	SEQUENCE		spec		0.97	0.85	0.82	0.86	0.84	0.84	0.86	0.83
			sens		0.39	0.61	0.66	0.65	0.69	0.71	0.73	0.71
	FDA	0.95	spec	0.94	0.90	0.83	0.82	0.81				
			sens	0.30	0.50	0.63	0.72	0.76				
	SEQUENCE		spec					0.83	0.81	0.80	0.81	0.80
			sens					0.73	0.70	0.70	0.69	0.69

*Each patient is characterized by the percentage of 1, 2 and 3 staining cells.

[0158] Specific examples for combinations of four and six biomarkers are provided in Examples 5.

Analysis without Data from Infiltrating Lobular Cancer (ILC) Patients

[0159] The patient population described in Table 8 was further subdivided based on diagnosis. Specifically, data from patients with infiltrating lobular carcinoma (ILC) was excluded, and the above analysis was performed on the resulting data set. Details of the patient population analyzed in this study are provided in Table 23.

TABLE 23

Patient Population Analyzed (Without ILC Patients)

Stage	Good	Bad	All
T1N0	56	19	75
T1N1	6	7	13
T2N0	54	33	87
T3N0	6	7	13
Totals	122	66	188

Results

1. Per Biomarker

A. Pathologist Scoring

[0160]

TABLE 24

Best Sensitivity and Specificity Couple for Biomarkers without ILC Patient Data (Pathologist Scoring)

Marker	Threshold	Sensitivity	Specificity
E2F1	1.75	0.29	0.69
MUC-1	0.75	0.26	0.80
NDRG-1	2.5	0.26	0.72
$p21^{ras}$	0.25	0.03	0.98
p53	0.5	0.29	0.75
Phospho-p27	1.25	0.16	0.71
PSMB9	0.75	0.12	0.93
SLPI	2.5	0.22	0.81
src	2.5	0.10	0.86

TABLE 25

Best Sensitivity and Specificity Couple for Conventional Markers without ILC Patient Data (Pathologist Scoring)

Marker	Threshold	Sensitivity	Specificity
CerbB2	2.5	0.16	0.85
ER	0.5	0.38	0.71
Ki67	0.25	0.14	0.88
PR	2.5	0.23	0.69

B. Single-Feature Analysis

[0161]

TABLE 26

Best Sensitivity and Specificity Couple for Each Biomarker Obtained from MARS Features without ILC Patient Data (Single Feature Algorithm)

Marker	Feature	Threshold	Sens.	Spec.	Rule
E2F1	CELL_PERCENT_23	3.19079	0.58182	0.73469	1
MUC-1	CELL_PERCENT_23	8.437	0.38462	0.71717	8
NDRG1	CELL_PERCENT_123	26.13234	0.39683	0.69811	8
p21ras	CELL_PERCENT_123	61.04522	0.45763	0.72277	1
p53	CELL_PERCENT_3	0.08289	0.41379	0.71171	8
phospho-p27	CELL_PERCENT_123	0.44587	0.49153	0.64486	8
PSMB9	CELL_PERCENT_123	30.42545	0.40323	0.71560	1
SLPI	CELL_PERCENT_123	0.57594	0.53226	0.70370	1
src	CELL_PERCENT_23	13.08501	0.43636	0.66000	8

*A decision rule of 1 means that patients above the threshold are considered as being positive (i.e., TRUE POSITIVE if bad actual clinical outcome) whereas a decision rule of 8 means that patients above the threshold are considered as being negative (i.e., FALSE NEGATIVE if bad actual clinical outcome).

C. Multiple Feature Analysis

[0162]

TABLE 27

Best Sensitivity and Specificity Couple for Each Biomarker Obtained from MARS Features without ILC Patient Data (Multiple Feature Algorithm)

Marker	Feature 1	Feature 2	Threshold 1	Threshold 2	Sensitivity	Specificity	Rule
E2F1	CELL_PERCENT_2	CELL_PERCENT_3	2.47761	1.2758	0.61818	0.7449	7
MUC-1	CELL_PERCENT_1	CELL_PERCENT_2	9.6658	13.2244	0.51923	0.68687	9
NDRG1	CELL_PERCENT_0	CELL_PERCENT_123	28.32391	16.95268	0.49206	0.70755	6
p21ras	CELL_PERCENT_3	CELL_PERCENT_01	0.1695	99.97219	0.49153	0.72277	6
p53	CELL_PERCENT_0	CELL_PERCENT_3	48.61018	0.07805	0.46552	0.71171	6
phospho-p27	CELL_PERCENT_1	CELL_PERCENT_01	0.50369	100	0.49153	0.69159	8
PSMB9	CELL_PERCENT_123	CELL_PERCENT_01	30.42545	99.09092	0.45161	0.7156	11
SLPI	CELL_PERCENT_0	CELL_PERCENT_123	62.11591	0.40094	0.58065	0.75	1
src	CELL PERCENT 2	CELL PERCENT 3	16.31145	0.08202	0.52727	0.72	4

*Decision rules correspond to quadrant affection in the 2 features space.

D. Variations Between Analyses: All Patients v. Without ILC Patients

[0163] Variations in the sensitivity and specificity values obtained on a per biomarker basis with the analysis of the complete patient population (Table 8) and the population without ILC patients (Table 23) was determined. The results are presented below in Table 28. The sum column (d^2) gives the difference of quadratic distance on an ROC curve, i.e., the overall gain in sensitivity and specificity.

TABLE 28

Variations in Sensitivity and Specificity Obtained with the Complete Patient Population and Without ILC Patients (Per Biomarker)

	Pathologist Scoring			Single-Feature			Multi-Features		
Marker	Sens.	Spec.	d^1	Sens.	Spec.	d^2	Sens.	Spec.	d^2
E2F1	↓	—	−0.004	↑	↑	0.018	↑	↓	0.026
MUC-1	↑	↓	0.004	↓	↑	0.013	↑	↑	0.006
NDRG1	↓	↓	−0.026	↑	↓	−0.006	↑	↓	0.002
p21ras	↓	—	−0.001	↑	↓	0.026	↑	↑	0.024
p53	—	↑	0.009	↓	↑	0.003	↓	↑	−0.015
phospho-p27	↓	↓	−0.022	↓	↑	−0.022	↓	↓	−0.008
PSMB9	↑	↓	−0.008	↑	↑	0.000	↓	↓	−0.023
SLPI	↑	↓	−0.010	↑	↑	0.030	↑	↑	0.021
src	—	↓	−0.010	↓	↓	−0.047	↓	↑	−0.005

2. Combinations of Biomarkers

A. Pathologist Scoring

[0164]

TABLE 29

Best Sensitivity/Specificity Couples for Biomarker Combinations without ILC Patient Data Using Different Targeted Specificities (75% and 95%) and Different Interpretation Algorithms (Pathologist Scoring)

	Target Spec.	Markers	1 Marker	2 Markers	3 Markers	4 Markers	5 Markers	6 Markers	7 Markers	8 Markers	9 Markers
FDA	0.75	spec	0.75	0.63							
SEQUENCE		sens	0.20	0.67							
		spec		0.88	0.83	0.84	0.84	0.90	0.85	0.83	0.77
		sens		0.24	0.35	0.37	0.35	0.36	0.35	0.37	0.27

*Each patient is characterized by the pathologist score.

B. Percentage Features Analysis

[0165]

TABLE 30

Best Sensitivity/Specificity Couples for Biomarker Combinations without ILC Patients Using Different Targeted Specificities (75% and 95%) and Different Interpretation Algorithms (Percentage Features)

All Povs % Features		Target Spec	Markers	1 Marker	2 Markers	3 Markers	4 Markers	5 Markers	6 Markers	7 Markers	8 Markers	9 Markers
1	FDA	0.79	spec	0.73	0.54							
	SEQUENCE		sens	0.58	0.22							
			spec		0.95	0.86	0.81	0.68	0.85	0.82	0.92	0.78
			sens		0.35	0.46	0.56	0.58	0.38	0.82	0.57	0.50
2	FDA	0.35	spec	0.74	0.55							
	SEQUENCE		sens	0.80	0.63							
			spec		0.36	0.67	0.64	0.71	0.70	0.72	0.85	0.70
			sens		0.35	0.67	0.94	0.75	0.70	0.72	0.56	0.70

*Each patient is characterized by the percentage of 1, 2 and 3 staining cells.

[0166] Table 30 shows an increase in specificity (0.88 compared to 0.81, see Table 28) when considering a 5 biomarker combination excluding ILC patients with a single percent feature. An increase in sensitivity was observed when using 2 features (0.71 vs. 0.65, see Table 28) for a 5 biomarker sequence analysis when excluding ILC patients from the study.

C. Variations Between Analyses: All Patients v. without ILC Patients

[0167] Variations in the sensitivity and specificity values obtained for biomarker combinations with the analysis of the complete patient population and the population without ILC patients was determined. The results are presented below in Table 31. The sum column (d^2) gives the difference of quadratic distance on a ROC curve, i.e., the overall gain in sensitivity and specificity. A slight gain in performance for a 5 biomarker sequence analysis using one or two percentage features was observed when ILC patients were excluded from the study.

and decision rule defined in Table 32. A 60% sensitivity and an 80% specificity was obtained using the rule: if E2F1 was ON (i.e. 1) and not the only biomarker to be ON, then the patient was considered bad outcome; otherwise, considered good outcome. FIG. **1** shows the distribution of the percentage feature as a function of bad and good outcome patients for E2F1. Using a threshold of 2.46% sensitivity and specificity values of 0.54 and 0.75, respectively, were obtained.

TABLE 32

Percentage Summary Features for Four Biomarker Analysis

Marker	Feature	Threshold	Rule (1 if)
SLPI	CELL_PERCENT_01	99.887874	<
p21ras	CELL_PERCENT_0	35.642851	<
E2F1	CELL_PERCENT_2	2.463659	>
src	CELL_PERCENT_1	37.624326	>

TABLE 31

Variations in Sensitivity and Specificity Obtained with Complete Patient Population and Without ILC Patients (Biomarker Combinations)

All Povs % Features		Target Spec	Markers	1 Marker	2 Markers	3 Markers	4 Markers	5 Markers	6 Markers	7 Markers	8 Markers	9 Markers
1	FDA	0.75	d2	0.02	0.02							
	SEQUENCE		d2		0.00	0.01	0.00	0.06	0.04	0.01	0.01	−0.07
2	FDA	0.75	d2	0.02	0.02							
	SEQUENCE		d2		−0.01	0.00	0.02	0.02	−0.01	−0.01	−0.06	−0.04

Example 5

Specific Biomarker Combinations

[0168] The data obtained in the study described above in example 4 were further analyzed, and specific biomarker combinations were considered. The results obtained with a combination of four (SLPI/p21ras/E2F1/src) and six (SLPI/p21ras/PSMB9/E2F1/src/phospho-p27) biomarkers are presented below.

Four Biomarker Combination: SLPI/p21ras/E2F1/src

[0169] Analysis was performed using only one percentage feature for SLPI, p21ras, E2F1, and src with the thresholds

[0170] A sequence-based interpretation approach was used to analyze the four biomarker combination. The sequence-based decision rule used was: if E2F1 was ON (i.e. 1) and not the only biomarker to be ON, then the patient was considered bad outcome; otherwise, considered good outcome. The sensitivity and specificity values for all of the possible combinations of the four biomarkers are provided in Table 33. The ROC curve obtained using the sequence interpretation approach for the SLPI/p21ras/E2F1/src combination was prepared (data not shown).

TABLE 33

Sensitivity and Specificity Couples Using Sequence-based Interpretation Approach for SLPI, p21ras, E2F1 and SRC Combination SLPI-p21ras-E2F1-src

Sequence	CumulBad	CumulGood	Sensitivity	Specificity
S1111	4	0	0.069	1
S1011	7	0	0.1207	1
S1110	12	0	0.2069	1
S0111	14	8	0.2414	0.9184
S1010	22	12	0.3793	0.8776
S1101	26	14	0.4483	0.8571
S0011	31	16	0.5345	0.8367
S0110	35	19	0.6034	0.8061
S1001	37	24	0.6379	0.7551
S1100	37	26	0.6379	0.7347
S0010	39	37	0.6724	0.6224
S0101	41	40	0.7069	0.5918
S1000	46	56	0.7931	0.4286
S0001	49	63	0.8448	0.3571
S0100	52	71	0.8966	0.2755
S0000	58	98	1	0

*A sequence S0110 is read as follows: SLPI = OFF/p21ras = ON/E2F1 = ON/src = OFF.

[0171] An interpretation based on E2F1 alone gave a sensitivity and specificity of 54% and 75%, respectively. A specificity and sensitivity of 60% and 80%, respectively, was obtained using the sequence-based algorithm defined above (i.e., if E2F1 was ON (i.e. 1) and not the only biomarker to be ON, then the patient was considered bad outcome; otherwise, considered good outcome).

Six Biomarker Combination: SLPI/p21ras/E2F1/src/PSMB9/phospho-p27

[0172] Analysis was performed using only one percentage feature for a six biomarker combination of SLPI, p21ras, E2F1, src, PSMB9, and phospho-p27 with the thresholds and decision rules defined in Table 34.

TABLE 34

Percentage Summary Features for Six Biomarker Analysis

MarkerName	Feature	Threshold	Sensitivity	Specificity	Rule (1 if)
SLPI	CELL_PERCENT_123	0.576	53.2%	70.4%	>
p21ras	CELL_PERCENT_123	61.045	45.8%	72.3%	>
E2F1	CELL_PERCENT_23	3.191	58.2%	73.5%	>
PSMB9	CELL_PERCENT_123	30.425	40.3%	71.6%	>
src	CELL_PERCENT_23	13.085	43.6%	66.0%	<
phospho-p27	CELL_PERCENT_123	0.446	49.2%	64.5%	<

[0173] A sequence-based interpretation approach was used to analyze the six biomarker combination. The sequence-based decision rule used was: If E2F1 was ON (i.e. 1) and either SLPI or 21ras, or E2F1 and any 2 biomarkers, or SLPI and any 2 biomarkers, or any 4 biomarkers or more were ON, then the patient was considered bad outcome; otherwise considered good outcome. The sensitivity and specificity values for all of the possible combinations of the six biomarkers of interest are provided in Table 35. The ROC curve obtained using the sequence interpretation approach for the SLPI/p21ras/E2F1/PSMB9/src/phospho-p27 combination are shown in FIG. **2**.

TABLE 35

Sensitivity and Specificity Couples Using Sequence-based Interpretation Approach for SLPI, p21ras, E2F1, PSMB9, SRC, and Phospho-p27 Combination SLPI-p21ras-E2F1-PSMB9-src-phospho-p27

Sequence	CumulBad	CumulGood	Sensitivity	Specificity
S111111	1	0	0.0208	1
S111101	2	0	0.0417	1
S111011	2	0	0.0417	1
S111110	2	0	0.0417	1
S101111	3	0	0.0625	1
S111001	4	0	0.0833	1
S111100	8	0	0.1667	1
S011111	8	0	0.1667	1
S111010	9	0	0.1875	1
S101101	11	0	0.2292	1
S101011	12	0	0.25	1
S110111	12	0	0.25	1
S101110	12	0	0.25	1
S011101	12	0	0.25	1
S111000	12	0	0.25	1
S011011	13	1	0.2708	0.9885
S011110	13	1	0.2708	0.9885
S101001	13	2	0.2708	0.977
S110101	14	2	0.2917	0.977
S101100	15	3	0.3125	0.9655
S001111	17	3	0.3542	0.9655
S110011	19	4	0.3958	0.954
S101010	21	4	0.4375	0.954
S110110	21	4	0.4375	0.954
S011001	21	5	0.4375	0.9425
S011100	22	8	0.4583	0.908
S011010	23	10	0.4792	0.8851
S100111	23	10	0.4792	0.8851
S001101	23	11	0.4792	0.8736
S110001	23	11	0.4792	0.8736
S101000	25	13	0.5208	0.8506
S001011	26	14	0.5417	0.8391
S110100	27	14	0.5625	0.8391

TABLE 35-continued

Sensitivity and Specificity Couples Using Sequence-based Interpretation Approach for SLPI, p21ras, E2F1, PSMB9, SRC, and Phospho-p27 Combination SLPI-p21ras-E2F1-PSMB9-src-phospho-p27

Sequence	CumulBad	CumulGood	Sensitivity	Specificity
S010111	27	15	0.5625	0.8276
S001110	28	16	0.5833	0.8161
S110010	28	16	0.5833	0.8161
S011000	31	19	0.6458	0.7816

TABLE 35-continued

Sensitivity and Specificity Couples Using Sequence-based Interpretation Approach for SLPI, p21ras, E2F1, PSMB9, SRC, and Phospho-p27 Combination
SLPI-p21ras-E2F1-PSMB9-src-phospho-p27

Sequence	CumulBad	CumulGood	Sensitivity	Specificity
S100101	31	19	0.6458	0.7816
S100011	33	20	0.6875	0.7701
S100110	34	20	0.7083	0.7701
S001001	34	21	0.7083	0.7586
S010101	35	22	0.7292	0.7471
S001100	35	26	0.7292	0.7011
S110000	36	28	0.75	0.6782
S010011	37	29	0.7708	0.6667
S001010	37	30	0.7708	0.6552
S010110	37	30	0.7708	0.6552
S100001	37	34	0.7708	0.6092
S100100	38	38	0.7917	0.5632
S000111	40	39	0.8333	0.5517
S100010	40	45	0.8333	0.4828
S010001	41	46	0.8542	0.4713
S001000	41	46	0.8542	0.4713
S010100	41	47	0.8542	0.4598
S010010	41	48	0.8542	0.4483
S000101	41	51	0.8542	0.4138
S100000	42	54	0.875	0.3793
S000011	42	59	0.875	0.3218
S000110	42	61	0.875	0.2989
S010000	42	65	0.875	0.2529
S000001	43	70	0.8958	0.1954
S000100	43	72	0.8958	0.1724
S000010	44	77	0.9167	0.1149
S000000	48	87	1	0

[0174] A specificity and sensitivity of 70% and 77%, respectively, was obtained using the sequence-based algorithm defined above.

Example 6

Optimization of Reagents and Staining Conditions for Immunohistochemistry

[0175] In order to maximize the signal to noise ratio for detection of expression of a particular biomarker using the immunohistochemistry methods disclosed herein, experiments to select the optimal antigen retrieval solution and conditions, antibody concentration and diluent formulation, and detection chemistry parameters were performed. For each set of experiments, biomarker-specific tissue microarrays (TMAs) were constructed by obtaining cylindrical tissue specimens from regular paraffin blocks, assembling them into a single block, and preparing sections containing multiple tissue specimens. TMAs with 2-3 pre-selected known positive and negative tumors for each breast biomarker were used. Slides were prepared and automated immunohistochemistry was performed essentially as described in Example 1. The following control reagents were used during all of the optimization experiments:

[0176] For the negative control, the application of the primary antibody was replaced with a ready to use universal negative reagent, either non-specific mouse or rabbit IgG.

[0177] EF1-α was used as a positive control.

[0178] A positive marker control slide was run following the optimized labeling parameters established during feasibility for each antibody being tested.

[0179] A biomarker specific TMA containing both positive and negative tumors was used in the testing of each breast marker antibody.

1. Optimization of Antigen Retrieval

A. Antigen Retrieval Solutions

[0180] Each antigen retrieval solution listed below was tested using each of the biomarker antibodies of interest. The time and temperatures used here were standard accepted values as defined below.

TABLE 36

Antigen Retrieval Solutions Tested

Solution	Time	Temperature	Device
Citrate Buffer pH 6.0 (Dako)	5 minutes	120° C.	Pressure Cooker
Tris Buffer pH 9.5 (Biocare)	5 minutes	120° C.	Pressure Cooker
EDTA pH 8.0 (Biocare)	20 minutes	95° C.	Steamer
L.A.B. (Polysciences)	20 minutes	20° C. and 60° C.	None/oven
Antigen Retrieval Glyca Solution (Biogenex)	5 minutes	120° C.	Pressure Cooker
Citrate Buffer Solution, pH 4.0 (Zymed)	20 minutes	95° C.	Steamer
diH_20	20 minutes	120° C.	Pressure Cooker
Dawn (Protor & Gamble)	3 minutes	120° C.	Pressure Cooker
2% Glacial Acetic Acid	10 minutes	95° C.	Steamer

[0181] The slides were scored by a pathologist, and the best performing antigen retrieval solution were determined by comparing the labeling specificity and intensity between positive and negative tumors. If the results were essentially negative, alternative antigen retrieval solutions were screened. If results were positive, i.e. labeling more intense than no antigen retrieval, the top (1-3) solutions were identified and used for antigen retrieval time and temperature testing. The activity of the selected antigen retrieval solutions was verified by labeling a representative sample of positive and negative whole tissue sections.

B. Antigen Retrieval Conditions—Time and Temperature

[0182] The best-performing antigen retrieval solutions were tested using the following time and temperature criteria:

TABLE 37

Antigen Retrieval Time and Temperature Conditions Tested

Temp	3 minutes	5 minutes	10 minutes	20 minutes	30 minutes	4 hours	Overnight
2-8° C.						*	*
25° C.						*	*
37° C.						*	*
60° C.						*	*
95° C./ ST			*	*	*		
120° C./ PC	*	*	*				

[0183] The slides were scored by a pathologist, and the best-performing antigen retrieval time and temperature combinations were determined by comparing the labeling specificity and intensity between positive and negative tumors. The activity of the selected antigen retrieval solutions and time and temperature combinations was verified by labeling a representative sample of positive and negative whole tissue sections utilizing the controls listed above.

2. Optimization of Antibody Dilution and Diluent Formulations

A. Antibody Dilution

[0184] Each breast cancer biomarker antibody was tested over a range of antibody dilutions. Table 38 provides an example of antibody dilutions tested for the SLPI 5G6.24 antibody. All other breast biomarker antibodies were tested in a similar manner.

TABLE 38

Antibody Dilutions Tested

Antibody	IgG concentration	µg/slide (200 µl/slide)	Dilution
SLPI 5G6.24	3.5 mg/ml (3.5 µg/ul)	3.5	1:200
		1.75	1:400
		1.17	1:600
		0.88	1:800
		0.7	1:1000
		0.47	1:1500

[0185] The slides were scored by a pathologist, and the labeling intensities between controls, known positive, and known negative tumors were assessed. The labeling data was analyzed to determine both the upper and lower limits of the antibody dilutions that maintained the desired labeling intensity and the width of the utility range for each antibody. If the initial dilution range tested did not result in the identification of the upper and lower limits, additional antibody dilutions were tested.

B. Antibody Diluent Formulation

[0186] Various antibody diluents were tested using each of the breast biomarker antibodies of interest. The table below provides a description of the diluent parameters that were tested.

TABLE 39

Antibody Diluents Tested

PBS pH 7.4			
PBS pH 7.4	0.1% tween 20		
PBS pH 7.4		1% BSA	
PBS pH 7.4			0.05% NaN_3
PBS pH 7.4	0.1% tween 20	1% BSA	
PBS pH 7.4	0.1% tween 20		0.05% NaN_3
PBS pH 7.4		1% BSA	0.05% NaN_3
PBS pH 7.4	0.1% tween 20	1% BSA	0.05% NaN_3

[0187] The slides were scored by a pathologist for labeling intensity. The effectiveness of the diluent formulation was determined by comparing the labeling grade of the biomarker control slide to the experimental slides. Those that resulted in the most specific and highest signal to noise ratio by comparing the labeling of positive and negative tumors were carried forward. The diluent formulations (approximately one to three) that resulted in the optimal labeling intensity were carried forward into further optimization and stability studies. The activity of the selected diluents was verified by labeling a representative sample of positive and negative whole breast cancer tissue sections.

3. Optimization of Detection Chemistry

[0188] Each of the breast biomarker antibodies was tested utilizing the DAKO Envision+ detection kit over the range of times and concentrations listed below.

TABLE 40

Detection Chemistry Time and Concentration Conditions Tested

	Time		
Concentration	10 minutes	30 minutes	60 minutes
1.0X Concentration			
0.75X Concentration			
0.5X Concentration			

[0189] The slides were scored by a pathologist, and the labeling intensities between controls, known positive, and known negative tumors were assessed. The activity of the selected detection chemistry time and concentration combinations was verified by labeling a representative sample of positive and negative whole breast cancer tissue sections.

Results

[0190] A significantly improved signal to noise ratio was observed with optimized staining reagent conditions (data not shown).

Example 7

Real-Time PCR Detection of Biomarkers in Clinical Samples

[0191] TaqMan® real-time PCR was performed with the ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, Calif.). The primers and probes were designed with the aid of the Primer Express™ program, version 1.5 (Applied Biosystems, Foster City, Calif.), for specific amplification of the targeted breast staging markers (e.g., DARPP32 and NDRG-1) in this study. The sequence information on primers and probes is shown below:

[0192] DARPP32:

(SEQ ID NO: 33)
Forward Primer Name: DARPP32_t1-F
Sequence: TACACACCACCTTCGCTGAAAG

(SEQ ID NO: 34)
Reverse Primer Name: DARPP32_t1-R
Sequence: GGCCTGGTTCTCATTCAAATTG

(SEQ ID NO: 35)
TaqMan Probe Name: DARPP32_t1-Probe
Sequence: CGCATTGCTGAGTCTCACCTGCAGTC

(SEQ ID NO: 36)
Forward Primer Name: DARPP32_t2-F
Sequence: CAGCCTTACAGAGACTGGAAAAGAA

-continued

(SEQ ID NO: 37)
Reverse Primer Name: DARPP32_t2-R
Sequence: GAGGCTCAGGGACCCAAAG

(SEQ ID NO: 38)
TaqMan Probe Name: DARPP32_t2-Probe
Sequence: CCAAACCAAGGCCCCCAGAGAGGT

[0193] NDRG-1:

Forward Primer Name: NDRG-1-F
Sequence: CCTACCGCCAGCACATTGT (SEQ ID NO: 39)

Reverse Primer Name: NDRG-1-R
Sequence: GCTGTTGTAGGCATTGATGAACA (SEQ ID NO: 40)

TaqMan Probe Name: NDRG-1-Probe
Sequence: AATGACATGAACCCCGGCAACCTG (SEQ ID NO: 41)

[0194] The probes were labeled with a fluorescent dye FAM (6-carboxyfluorescein) on the 5' base, and a quenching dye TAMRA (6-carboxytetramethylrhodamine) on the 3' base. The sizes of the amplicons were around 100 bp. 18S ribosomal RNA was applied as endogenous control. 18S rRNA probe was labeled with a fluorescent dye VIC. Pre-Developed 18S rRNA primer/probe mixture was purchased from Applied Biosystems (P/N: 4310893E). 20 frozen breast tissues (i.e., 6 tumors with bad outcome, 12 tumors with good outcome, and 2 normal tissues) were analyzed in this study. In this study, good outcome was defined as remaining cancer-free for at least 5 years; bad outcome was defined as suffering disease relapse, recurrence, or death within 5 years. 5 μg of total RNA extracted from the frozen breast tissues was quantitatively converted into the single stranded cDNA form with random hexamers (not with oligo-dT) by using the High-Capacity cDNA Archive Kit (Applied Biosystems, P/N: 4322171). The following reaction reagents were prepared:

20X Master Mix of Primers/Probe (in 200 μl)	
180 μM Forward primer	20 μl
180 μM Reverse primer	20 μl
100 μM TaqMan probe	10 μl
H_2O	150 μl
Final Reaction Mix (25 μl/well)	
20X master mix of primers/probe	1.25 μl
2X TaqMan Universal PCR master mix (P/N: 4304437)	12.5 μl
cDNA template	5.0 μl
H_2O	6.25 μl

[0195] 20× TaqMan Universal PCR Master Mix was purchased from Applied Biosystems (P/N: 430-4437). The final primer and probe concentrations, in a total volume of 25 μl, were 0.9 μM and 0.25 μM, respectively. 10 ng of total RNA was applied to each well of the reaction. The amplification conditions were 2 min at 50° C., 10 min at 95° C., and a two-step cycle of 95° C. for 15 seconds and 60° C. for 60 seconds for a total of 40 cycles. At least three no-template control reaction mixtures were included in each run. All experiments were performed in triplicate.

[0196] At the end of each reaction, the recorded fluorescence intensity was used for the following calculations: Rn^+ is the Rn value of a reaction containing all components, Rn^- is the Rn value of an unreacted sample (baseline value or the value detected in NTC). ΔRn is the difference between Rn^+ and Rn^-. It is an indicator of the magnitude of the signal generated by the PCR. Expression level of a target gene was computed by comparative CT method. This method uses no known amount of standard but compares the relative amount of the target sequence to the reference values chosen (18S rRNA was selected as a reference in this study). See the Applied Biosystems' *TaqMan Human Endogenous Control Plate Protocol* that contains detailed instructions regarding MS Excel based data analysis.

Results

[0197] The results obtained with each biomarker and with the specific primers are listed below in tabular form. Results obtained with normal breast tissue samples are designated N; those obtained with breast cancer samples are labeled T.

TABLE 41

DARPP32 TaqMan ® Results

Samples	t1	t2	t1t2
2T	**0.18**	**0.5**	**0.54**
7T	**5.7**	**23.5**	**62.5**
12T	**73.5**	**16.9**	**84.2**
13T	**1.2**	**1.1**	**2.2**
21T	**5.8**	**6.1**	**16.1**
24T	**4.2**	**2.9**	**7.9**
26T	0.6	0.3	1.9
1T	0.02	0.2	0.1
3T	0.4	0.04	0.8
4T	2.5	1	4.8
5T	1.2	0.5	3.7
6T	0.9	0.6	2.6
9T	0.3	0.6	0.5
10T	0.1	0.2	0.3
11T	0.7	0.1	0.9
19T	0.8	0.3	1.6
22T	0.6	0.6	1.6
23T	0.5	0.4	1.2
25T	0.2	0.1	0.3
1N	1.1	1.3	2
8N	0.7	0.3	1.3
Bad Mean:	15.10	8.50	28.91
Good Mean	0.69	0.39	1.53
t-test P =	0.046	0.004	0.007

[0198] DARPP32 has two transcripts: t1 and t2. TaqMan® data showed that both t1 and t2 were overexpressed in the breast tumors with bad outcomes (in bold) as compared with those with good outcomes.

TABLE 42

NDRG-1 TaqMan ® Results

Samples	NDRG-1
2T	**2.8**
7T	**12.8**
12T	**5.5**
13T	**6.4**
21T	**2.4**
24T	**6.7**
26T	2.3
1T	4.1
3T	4.2
4T	2.8

TABLE 42-continued

NDRG-1 TaqMan ® Results

Samples	NDRG-1
5T	3.2
6T	1.3
9T	3.1
10T	3.7
11T	1.6
19T	3.4
22T	5.5
23T	1.6
25T	3.1
1N	0.9
8N	0.5
Bad Mean:	6.10
Good Mean:	3.13
t-test P =	0.021

[0199] NDRG-1 has one transcript. TaqMan data showed that NDRG-1 was overexpressed in the breast tumors with bad outcomes (in bold) as compared with those with good outcomes.

Example 8

Detection of Biomarker Overexpression in a Chemo-Naïve Patient Population with 10-Year Clinical Follow-Up (Five Biomarker Panel)

[0200] Breast tumor tissue samples collected at or near the time of initial diagnosis from 255 early-stage breast cancer patients were analyzed for biomarker overexpression in this study. Ten-year clinical follow-up data was available for all patients in the study. None of the patients received cytotoxic chemotherapy at any time during their treatment for breast cancer. The clinical demographics, distribution, and standard histopathological parameters (e.g., ER/PR hormone receptor status, histological grade, etc.) for the patient population are summarized below in Table 43.

TABLE 43

Clinical Characteristics of Chemo-Naïve Patient Population

Characteristics	Overall
Age at diagnosis (years)	n = 255
Mean (std)	64.0 (10.6)
Range	30-85
Age group distribution	
<40	6 (2.4%)
40-<50	23 (9.0%)
50-<60	48 (18.8%)
60-<70	87 (34.1%)
>=70	91 (35.7%)
Tumor size (cm)	n = 255
Mean (std)	2.1 (1.19)
Range	0.3-11.0
Tumor size group	
<1.0	16 (6.3%)
1.0-<2.0	104 (40.8%)
2.0-<4.0	122 (47.8%)
>=4.0	13 (5.1%)
Lymph node status	n = 255
Negative	232 (91.0%)

TABLE 43-continued

Clinical Characteristics of Chemo-Naïve Patient Population

Characteristics	Overall
Positive	23 (9.0%)
Histological Grade	n = 244
1	38 (15.6%)
2	135 (55.3%
3	71 (29.1%)
ER Status	n = 249
Negative	64 (25.7%)
Positive	185 (74.3%)
Her2/neu status	n = 249
Negative	176 (70.7%)
Positive	73 (29.3%)

[0201] Detection of expression of a five biomarker panel comprising SLPI, src, PSMB9, p21ras, and E2F1 was performed essentially as described above. That is, breast tumor samples were prepared and stained for biomarker expression using the Dako Autostainer, as described above in Example 1. Biomarker overexpression was determined using the imaging analysis described in Example 4.

[0202] The prognostic performance of the 5 biomarker panel was assessed utilizing a Cox Proportional Hazards Model analysis. See, for example Spruance et al., supra. The prognostic value of each biomarker and/or histological characteristic to identify the patients who suffered disease recurrence or death within ten years over the patients disease-free after ten years was calculated. In the analysis without the biomarker panel, age and tumor size were found to be independent prognostic factors with a p value<0.05. When the biomarkers were added to this analysis, they exhibited the highest statistically significant independent prognostic utility with a p value of <0.0001. The results of the Cox Proportional Hazard analysis are summarized below in Table 44.

TABLE 44

Results of Cox Proportional Hazard Analysis with Chemo-Naïve Patient Population (SLPI, src, PSMB9, p21ras, and E2F1 Biomarker Panel)

Variable	P Value	Hazard Ratio	(95% CI)
Analysis (without Biomarkers)			
Age at Diagnosis	0.0002	1.05	(1.02, 1.08)
Tumor Size	0.0066	1.28	(1.07, 1.53)
ER	0.2506	1.40	(0.79, 2.50)
Total Grade	0.0674	1.39	(0.98, 1.99)
Analysis (with Biomarkers)*			
Age at Diagnosis	0.0004	1.05	(1.02, 1.08)
Tumor Size	0.0318	1.21	(1.02, 1.44)
ER	0.0134	2.20	(1.18, 4.12)
Total Grade	0.0845	1.37	(0.96, 1.96)
TPO Marker	<0.0001	1.92	(1.47, 2.50)

Age at diagnosis was continuous variable and the biomarker was ordinary variable with 0 or 1, 2, 3, 4 (0 = none positive marker, 1 = one positive marker, or 2, 3, 4 positive marker).

[0203] The prognostic performance of the SLPI, src, PSMB9, p21ras, and E2F1 biomarker panel is graphically presented in the Kaplan-Meier plot of FIG. **3**. The x-axis represents years from initial diagnosis, and the y axis is the percentage of disease-free survival. The corresponding graph for the general breast cancer population independent of biom-

arker analysis is presented in FIG. **4**. These plot demonstrate the ability of this biomarker panel to risk stratify this early stage breast cancer patient population for disease recurrence and/or death due to primary disease. The risk of reoccurrence and/or death due to primary disease increases as the number of biomarkers that are overexpressed in the patient samples increases. The disease-free survival rates of the patient subgroups identified by the number of overexpressed biomarkers are statistically significant from each other with a p value of <0.001, as determined by log-rank test for comparison of 0 positive, 1 positive, 2 positive, 3 or more positive biomarker groups. A biomarker that is classified as overexpressed by the imaging analyses described herein is deemed "positive."

[0204] Because one of the most important clinical features of a breast cancer patient's diagnosis relates to estrogen receptor (ER) status, the prognostic performance of the SLPI, src, PSMB9, p21ras, and E2F1 biomarker panel was further assessed using the Cox Proportional Hazard analysis in the ER-positive and -negative patient subgroups. Clinical management and prognosis of these two subgroups is different because ER-positive patients are candidates for tamoxifen therapy whereas ER negative patients are not. The results of the analysis are summarized below in Table 45. The data indicate that the five biomarkers of interest have prognostic utility in both the ER positive and negative breast cancer patient subgroups. Therefore, while the biomakers SLPI, src, PSMB9, p21ras, and E2F1 are indicative of prognosis independent of the patient's ER status, these biomarkers also correlate with ER status.

TABLE 45

Results of Cox Proportional Hazard Analysis with Chemo-Naïve Patient Population (SLPI, src, PSMB9, p21ras, and E2F1 Biomarker Panel in ER Positive and Negative Patient Subgroups)

	Variable	P Value	Hazard Ratio	(95% CI)
ER Positive	Analysis without Biomarker			
	Age at Diagnosis	0.0012	1.05	(1.02, 1.09)
	Tumor Size	0.0237	1.25	(1.03, 1.51)
	HER2	0.5732	1.18	(0.66, 2.13)
	Total Grade	0.0566	1.47	(0.99, 2.20)
	Analysis with Biomarker*			
	Age at Diagnosis	0.0009	1.06	(1.03, 1.10)
	Tumor Size	0.0753	1.19	(0.98, 1.43)
	HER2	0.8523	1.06	(0.58, 1.93)
	Total Grade	0.0440	1.50	(1.01, 2.23)
	TPO Marker	<0.0001	1.98	(1.46, 2.69)
ER Negative	Analysis without Biomarker			
	Age at Diagnosis	0.0771	1.04	(1.00, 1.09)
	Tumor Size	0.1527	1.51	(0.86, 2.64)
	HER2	0.2562	0.55	(0.19, 1.55)
	Total Grade	0.9883	1.01	(0.45, 2.24)
	Analysis with Biomarker*			
	Age at Diagnosis	0.3467	1.03	(0.97, 1.08)
	Tumor Size	0.1854	1.44	(0.84, 2.48)
	HER2	0.6577	0.78	(0.27, 2.30)
	Total Grade	0.7327	0.86	(0.38, 1.99)
	TPO Marker	0.0089	1.91	(1.18, 3.09)

Age at diagnosis was continuous variable and the TPO marker was ordinary variable with 0 or 1, 2, 3, 4 (0 = none positive marker, 1 = one positive marker, or 2, 3, 4 positive marker).

Example 9

Detection of Biomarker Overexpression in a Chemo-Naïve Patient Population with 10-Year Clinical Follow-up (Six Biomarker Panel)

[0205] Breast tumor tissue samples from 100 patients (50 good outcome; 50 bad outcome patients) from the chemo-naïve patient population described in Example 8 were analyzed for biomarker overexpression of six biomarkers of interest (SLPI, src, PSMB9, $p21^{ras}$, E2F1, and MUC-1). Detection of expression of the six biomarker panel was performed by automated immunohistochemistry essentially as described above except that an alternate staining platform, the Ventana BenchMark XT, was used in place of the Dako Autostainer. A standard manual for operating the Ventana BenchMark XT is readily available from the manufacturer. Additional modifications to the immunohistochemistry parameters used with the Ventana BenchMark XT staining platform are summarized in Table 46 below. Biomarker overexpression was determined as before using the imaging analysis described in Example 4.

TABLE 46

Immunohistochemistry Parameters for Biomarker Staining with the Ventana BenchMark XT Staining Platform

Biomarker	Antibody Concentration (ug/ml)	Antigen Retrieval Solution	Antigen Retrieval Time	Antibody Incubation Temp	Antibody Incubation Time	Block and Amplification
SLPI	3.6	CC1	Extended	RT	1 hr	None
E2F1	2.0	CC1	Extended	37° C.	16 min	Pro & Biotin Amp
SRC	40	CC2	Standard	37° C.	1 hr	None
$p21^{ras}$	13.7	CC1	Short	37° C.	12 min	None
PSMB9	6.5	CC2	Standard	RT	1 hr	None
MUC1	5.0	CC1	Extended	37° C.	1 hr	None

CC1 and CC2 refer to cell conditioning reagents commercially available from Ventana. With respect to antigen retrieval times: short = 30 min; standard = 60 min; and extended = 90 min.

[0206] The prognostic performance of the 6 biomarker panel was assessed utilizing a Cox Proportional Hazards Model analysis, as above. The prognostic value of each biomarker and/or histological characteristic to identify the patients who suffered disease recurrence or death within ten years over the patients disease-free after 10 years was calculated. The biomarkers of interest (SLPI, src, PSMB9, $p21^{ras}$, E2F1, and MUC-1) exhibited statistically significant prognostic utility with a p value of 0.0220. The results of the Cox Proportional Hazard analysis are summarized below in Table 47.

TABLE 47

Results of Cox Proportional Hazard Analysis with Chemo-Naïve Patient Population (SLPI, src, PSMB9, p21ras, E2F1, and MUC-1 Biomarker Panel)

Variable	P Value	Hazard Ratio	95% Hazard Ratio Confidence Limits	
Age at Diagnosis	0.0523	1.032	1.000	1.066
Tumor Size	0.0180	1.319	1.049	1.658
Her2	0.2619	0.640	0.293	1.396
ER	0.4539	1.359	0.609	3.035
Total Grade	0.7693	1.075	0.661	1.749

TABLE 47-continued

Results of Cox Proportional Hazard Analysis with Chemo-Naïve Patient Population (SLPI, src, PSMB9, p21ras, E2F1, and MUC-1 Biomarker Panel)

Variable	P Value	Hazard Ratio	95% Hazard Ratio Confidence Limits	
Biomarkers (SLPI, src, PSMB9, p21ras, and E2F1 Biomarker Panel)	0.0220	1.335	1.042	1.709

[0207] The prognostic performance of the SLPI, src, PSMB9, p21ras, E2F1, and MUC-1 biomarker panel is graphically presented in the Kaplan-Meier plot of FIG. **5**. The x-axis represents years from initial diagnosis, and the y axis is the percentage of disease-free survival. This plot demonstrates the ability of this biomarker panel to risk stratify this early stage breast cancer patient population for disease recurrence and/or death due to primary disease. The risk of reoccurrence and/or death due to primary disease increases as the number of biomarkers that are overexpressed in the patient samples increases. The disease-free survival rates of the patient subgroups identified by the number of overexpressed biomarkers are statistically significant from each other with a p value of <0.0065, as determined by log-rank test for comparison of 0 positive, 1 positive, 2 positive, 3 or more positive biomarker groups. As described above, a biomarker that is classified as overexpressed by the imaging analyses described herein is deemed "positive."

TABLE 48

Biomarker Nucleotide and Amino Acid Sequence Information

Biomarker Name	Nucleotide Sequence Accession No.	Nucleotide Sequence Identifier	Amino Acid Sequence Accession No.	Amino Acid Sequence Identifier
SLPI	NM_003064	SEQ ID NO: 1	NP_003055	SEQ ID NO: 2
DARPP-32	NM_032192	SEQ ID NO: 3	NP_115568	SEQ ID NO: 4
MGC14832	NM_032339	SEQ ID NO: 5	NP_115715	SEQ ID NO: 6
NDRG-1	NM_006096	SEQ ID NO: 7	NP_006087	SEQ ID NO: 8
PSMB9	NM_002800	SEQ ID NO: 9	NP_002791	SEQ ID NO: 10
p27	NM_004064	SEQ ID NO: 11	NP_004055	SEQ ID NO: 12
E2F1	NM_005225	SEQ ID NO: 13	NP_005216	SEQ ID NO: 14
MCM6	NM_005915	SEQ ID NO: 15	NP_005906	SEQ ID NO: 16
MCM2	D83987	SEQ ID NO: 17	BAA12177	SEQ ID NO: 18
MUC-1	NM_182741	SEQ ID NO: 19	NP_877418	SEQ ID NO: 20
p21ras	NM_005343	SEQ ID NO: 21	NP_005334	SEQ ID NO: 22
Src	NM_005417	SEQ ID NO: 23	NP_005408	SEQ ID NO: 24
TGF-beta3	BC018503	SEQ ID NO: 25	AAH18503	SEQ ID NO: 26
PDGFRalpha	M21574	SEQ ID NO: 27	AAA96715	SEQ ID NO: 28
Myc	V00568	SEQ ID NO: 29	CAA23831	SEQ ID NO: 30
SERHL	NM_014509	SEQ ID NO: 31	NP_055324	SEQ ID NO: 32

[0208] All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

[0209] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

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35 40 45

Pro Glu Cys Gln Ser Asp Trp Gln Cys Pro Gly Lys Lys Arg Cys Cys
50 55 60

Pro Asp Thr Cys Gly Ile Lys Cys Leu Asp Pro Val Asp Thr Pro Asn
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Pro Thr Arg Arg Lys Pro Gly Lys Cys Pro Val Thr Tyr Gly Gln Cys
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Ser Pro Ser Glu Pro Gly Thr  *
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ggt gcc ttt gag ata gag ata aat gga cag ctg gtg ttc tcc aag ctg      241
Gly Ala Phe Glu Ile Glu Ile Asn Gly Gln Leu Val Phe Ser Lys Leu
65                  70                  75

gag aat ggg ggc ttt ccc tat gag aaa gat ctc att gag gcc atc cga      289
Glu Asn Gly Gly Phe Pro Tyr Glu Lys Asp Leu Ile Glu Ala Ile Arg
80                  85                  90

aga gcc agt aat gga gaa acc cta gaa aag atc acc aac agc cgt cct      337
Arg Ala Ser Asn Gly Glu Thr Leu Glu Lys Ile Thr Asn Ser Arg Pro
95                 100                 105                 110

ccc tgc gtc atc ctg tga ctgcacagga ctctgggttc ctgctctgtt             385
Pro Cys Val Ile Leu  *
115

ctggggtcca aaccttggtc tccctttggt cctgctggga gctccccctg cctctttccc    445

ctacttagct ccttagcaaa gagaccctgg cctccacttt gccctttggg tacaaagaag    505

gaatagaaga ttccgtggcc ttgggggcag gagagagaca ctctccatga acacttctcc    565

agccacctca taccccttc ccagggtaag tgcccacgaa agcccagtcc actcttcgcc    625

tcggtaatac ctgtctgatg ccacagattt tatttattct cccctaaccc agggcaatgt    685

cagctattgg cagtaaagtg gcgctacaaa cactaaaaaa aaaaaaaaaa aaaaaaaaaa    745

aaa                                                                  748
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<210> SEQ ID NO 6
<211> LENGTH: 115
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

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```
Met Ser Gly Glu Pro Gly Gln Thr Ser Val Ala Pro Pro Pro Glu Glu
1               5                   10                  15

Val Glu Pro Gly Ser Gly Val Arg Ile Val Val Glu Tyr Cys Glu Pro
20                  25                  30

Cys Gly Phe Glu Ala Thr Tyr Leu Glu Leu Ala Ser Ala Val Lys Glu
35                  40                  45

Gln Tyr Pro Gly Ile Glu Ile Glu Ser Arg Leu Gly Gly Thr Gly Ala
50                  55                  60

Phe Glu Ile Glu Ile Asn Gly Gln Leu Val Phe Ser Lys Leu Glu Asn
65                  70                  75                  80

Gly Gly Phe Pro Tyr Glu Lys Asp Leu Ile Glu Ala Ile Arg Arg Ala
85                  90                  95

Ser Asn Gly Glu Thr Leu Glu Lys Ile Thr Asn Ser Arg Pro Pro Cys
100                 105                 110

Val Ile Leu
115


<210> SEQ ID NO 7
<211> LENGTH: 3020
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (111)...(1295)

<400> SEQUENCE: 7

tgaagctcgt cagttcacca tccgccctcg gcttccgcgg ggcgctgggc cgccagcctc     60

ggcaccgtcc tttcctttct ccctcgcgtt aggcaggtga cagcagggac atg tct       116
Met Ser
1

cgg gag atg cag gat gta gac ctc gct gag gtg aag cct ttg gtg gag      164
Arg Glu Met Gln Asp Val Asp Leu Ala Glu Val Lys Pro Leu Val Glu
5                   10                  15

aaa ggg gag acc atc acc ggc ctc ctg caa gag ttt gat gtc cag gag      212
Lys Gly Glu Thr Ile Thr Gly Leu Leu Gln Glu Phe Asp Val Gln Glu
20                  25                  30

cag gac atc gag act tta cat ggc tct gtt cac gtc acg ctg tgt ggg      260
Gln Asp Ile Glu Thr Leu His Gly Ser Val His Val Thr Leu Cys Gly
35                  40                  45                  50

act ccc aag gga aac cgg cct gtc atc ctc acc tac cat gac atc ggc      308
Thr Pro Lys Gly Asn Arg Pro Val Ile Leu Thr Tyr His Asp Ile Gly
55                  60                  65

atg aac cac aaa acc tgc tac aac ccc ctc ttc aac tac gag gac atg      356
Met Asn His Lys Thr Cys Tyr Asn Pro Leu Phe Asn Tyr Glu Asp Met
70                  75                  80

cag gag atc acc cag cac ttt gcc gtc tgc cac gtg gac gcc cct ggc      404
Gln Glu Ile Thr Gln His Phe Ala Val Cys His Val Asp Ala Pro Gly
85                  90                  95

cag cag gac ggc gca gcc tcc ttc ccc gca ggg tac atg tac ccc tcc      452
Gln Gln Asp Gly Ala Ala Ser Phe Pro Ala Gly Tyr Met Tyr Pro Ser
100                 105                 110

atg gat cag ctg gct gaa atg ctt cct gga gtc ctt caa cag ttt ggg      500
Met Asp Gln Leu Ala Glu Met Leu Pro Gly Val Leu Gln Gln Phe Gly
115                 120                 125                 130

ctg aaa agc att att ggc atg gga aca gga gca ggc gcc tac acc cta      548
Leu Lys Ser Ile Ile Gly Met Gly Thr Gly Ala Gly Ala Tyr Thr Leu
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135                 140                 145

act cga ttt gct cta aac aac cct gag atg gtg gag ggc ctt gtc ctt      596
Thr Arg Phe Ala Leu Asn Asn Pro Glu Met Val Glu Gly Leu Val Leu
150                 155                 160

atc aac gtg aac cct tgt gcg gaa ggc tgg atg gac tgg gcc gcc tcc      644
Ile Asn Val Asn Pro Cys Ala Glu Gly Trp Met Asp Trp Ala Ala Ser
165                 170                 175

aag atc tca gga tgg acc caa gct ctg ccg gac atg gtg gtg tcc cac      692
Lys Ile Ser Gly Trp Thr Gln Ala Leu Pro Asp Met Val Val Ser His
180                 185                 190

ctt ttt ggg aag gaa gaa atg cag agt aac gtg gaa gtg gtc cac acc      740
Leu Phe Gly Lys Glu Glu Met Gln Ser Asn Val Glu Val Val His Thr
195                 200                 205                 210

tac cgc cag cac att gtg aat gac atg aac ccc ggc aac ctg cac ctg      788
Tyr Arg Gln His Ile Val Asn Asp Met Asn Pro Gly Asn Leu His Leu
215                 220                 225

ttc atc aat gcc tac aac agc cgg cgc gac ctg gag att gag cga cca      836
Phe Ile Asn Ala Tyr Asn Ser Arg Arg Asp Leu Glu Ile Glu Arg Pro
230                 235                 240

atg ccg gga acc cac aca gtc acc ctg cag tgc cct gct ctg ttg gtg      884
Met Pro Gly Thr His Thr Val Thr Leu Gln Cys Pro Ala Leu Leu Val
245                 250                 255

gtt ggg gac agc tcg cct gca gtg gat gcc gtg gtg gag tgc aac tca      932
Val Gly Asp Ser Ser Pro Ala Val Asp Ala Val Val Glu Cys Asn Ser
260                 265                 270

aaa ttg gac cca aca aag acc act ctc ctc aag atg gcg gac tgt ggc      980
Lys Leu Asp Pro Thr Lys Thr Thr Leu Leu Lys Met Ala Asp Cys Gly
275                 280                 285                 290

ggc ctc ccg cag atc tcc cag ccg gcc aag ctc gct gag gcc ttc aag     1028
Gly Leu Pro Gln Ile Ser Gln Pro Ala Lys Leu Ala Glu Ala Phe Lys
295                 300                 305

tac ttc gtg cag ggc atg gga tac atg ccc tcg gct agc atg acc cgc     1076
Tyr Phe Val Gln Gly Met Gly Tyr Met Pro Ser Ala Ser Met Thr Arg
310                 315                 320

ctg atg cgg tcc cgc aca gcc tct ggt tcc agc gtc act tct ctg gat     1124
Leu Met Arg Ser Arg Thr Ala Ser Gly Ser Ser Val Thr Ser Leu Asp
325                 330                 335

ggc acc cgc agc cgc tcc cac acc agc gag ggc acc cga agc cgc tcc     1172
Gly Thr Arg Ser Arg Ser His Thr Ser Glu Gly Thr Arg Ser Arg Ser
340                 345                 350

cac acc agc gag ggc acc cgc agc cgc tcg cac acc agc gag ggg gcc     1220
His Thr Ser Glu Gly Thr Arg Ser Arg Ser His Thr Ser Glu Gly Ala
355                 360                 365                 370

cac ctg gac atc acc ccc aac tcg ggt gct gct ggg aac agc gcc ggg     1268
His Leu Asp Ile Thr Pro Asn Ser Gly Ala Ala Gly Asn Ser Ala Gly
375                 380                 385

ccc aag tcc atg gag gtc tcc tgc tag gcggcctgcc cagctgccgc           1315
Pro Lys Ser Met Glu Val Ser Cys  *
390

ccccggactc tgatctctgt agtggccccc tcctccccgg ccccttttcg ccccctgcct   1375

gccatactgc gcctaactcg gtattaatcc aaagcttatt ttgtaagagt gagctctggt   1435

ggagacaaat gaggtctatt acgtgggtgc cctctccaaa ggcggggtgg cggtggacca   1495

aaggaaggaa gcaagcatct ccgcatcgca tcctcttcca ttaaccagtg gccggttgcc   1555

actctcctcc cctccctcag agacaccaaa ctgccaaaaa caagacgcgt agcagcacac   1615
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acttcacaaa gccaagccta ggccgccctg agcatcctgg ttcaaacggg tgcctggtca   1675

gaaggccagc cgcccacttc ccgtttcctc tttaactgag gagaagctga tccagctttc   1735

cggaaacaaa atccttttct tcatttgggg agggggtaa tagtgacatg caggcacctc   1795

ttttaaacag gcaaaacagg aaggggaaa aggtgggatt catgtcgagg ctagaggcat   1855

ttggaacaac aaatctacgt agttaacttg aagaaaccga tttttaaagt tggtgcatct   1915

agaaagcttt gaatgcagaa gcaaacaagc ttgatttttc tagcatcctc ttaatgtgca   1975

gcaaaagcag gcaacaaaat ctcctggctt tacagacaaa aatatttcag caaacgttgg   2035

gcatcatggt ttttgaaggc tttagttctg ctttctgcct ctcctccaca gccccaacct   2095

cccacccctg atacatgagc cagtgattat tcttgttcag ggagaagatc atttagattt   2155

gttttgcatt ccttagaatg gagggcaaca ttccacagct gccctggctg tgatgagtgt   2215

ccttgcaggg gccggagtag gagcactggg gtgggggcgg aattggggtt actcgatgta   2275

agggattcct tgttgttgtg ttgagatcca gtgcagttgt gatttctgtg gatcccagct   2335

tggtccagga attttgagag attggcttaa atccagtttt caatcttcga cagctgggct   2395

ggaacgtgaa ctcagtagct gaacctgtct gacccggtca cgttcttgga tcctcagaac   2455

tctttgctct tgtcggggtg ggggtgggaa ctcacgtggg gagcggtggc tgagaaaatg   2515

taaggattct ggaatacata ttccatggac tttccttccc tctcctgctt cctcttttcc   2575

tgctccctaa cctttcgccg aatggggcag acaaacactg acgtttctgg gtggccagtg   2635

cggctgccag gttcctgtac tactgccttg tacttttcat tttggctcac cgtggatttt   2695

ctcataggaa gtttggtcag agtgaattga atattgtaag tcagccactg ggacccgagg   2755

atttctggga ccccgcagtt gggaggagga agtagtccag ccttccaggt gggcgtgaga   2815

ggcaatgact cgttacctgc cgcccatcac cttggaggcc ttccctggcc ttgagtagaa   2875

aagtcgggga tcggggcaag agaggctgag tacggatggg aaactattgt gcacaagtct   2935

ttccagagga gtttcttaat gagatatttg tatttatttc cagaccaata aatttgtaac   2995

tttgcaaaaa aaaaaaaaaa aaaaa                                         3020
```

<210> SEQ ID NO 8
<211> LENGTH: 394
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

```
Met Ser Arg Glu Met Gln Asp Val Asp Leu Ala Glu Val Lys Pro Leu
1               5                   10                  15

Val Glu Lys Gly Glu Thr Ile Thr Gly Leu Leu Gln Glu Phe Asp Val
20                  25                  30

Gln Glu Gln Asp Ile Glu Thr Leu His Gly Ser Val His Val Thr Leu
35                  40                  45

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

Ile Gly Met Asn His Lys Thr Cys Tyr Asn Pro Leu Phe Asn Tyr Glu
65                  70                  75                  80

Asp Met Gln Glu Ile Thr Gln His Phe Ala Val Cys His Val Asp Ala
85                  90                  95

Pro Gly Gln Gln Asp Gly Ala Ala Ser Phe Pro Ala Gly Tyr Met Tyr
100                 105                 110
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Pro Ser Met Asp Gln Leu Ala Glu Met Leu Pro Gly Val Leu Gln Gln
115                 120                 125

Phe Gly Leu Lys Ser Ile Ile Gly Met Gly Thr Gly Ala Gly Ala Tyr
130                 135                 140

Thr Leu Thr Arg Phe Ala Leu Asn Asn Pro Glu Met Val Glu Gly Leu
145                 150                 155                 160

Val Leu Ile Asn Val Asn Pro Cys Ala Glu Gly Trp Met Asp Trp Ala
165                 170                 175

Ala Ser Lys Ile Ser Gly Trp Thr Gln Ala Leu Pro Asp Met Val Val
180                 185                 190

Ser His Leu Phe Gly Lys Glu Glu Met Gln Ser Asn Val Glu Val Val
195                 200                 205

His Thr Tyr Arg Gln His Ile Val Asn Asp Met Asn Pro Gly Asn Leu
210                 215                 220

His Leu Phe Ile Asn Ala Tyr Asn Ser Arg Arg Asp Leu Glu Ile Glu
225                 230                 235                 240

Arg Pro Met Pro Gly Thr His Thr Val Thr Leu Gln Cys Pro Ala Leu
245                 250                 255

Leu Val Val Gly Asp Ser Ser Pro Ala Val Asp Ala Val Val Glu Cys
260                 265                 270

Asn Ser Lys Leu Asp Pro Thr Lys Thr Thr Leu Leu Lys Met Ala Asp
275                 280                 285

Cys Gly Gly Leu Pro Gln Ile Ser Gln Pro Ala Lys Leu Ala Glu Ala
290                 295                 300

Phe Lys Tyr Phe Val Gln Gly Met Gly Tyr Met Pro Ser Ala Ser Met
305                 310                 315                 320

Thr Arg Leu Met Arg Ser Arg Thr Ala Ser Gly Ser Ser Val Thr Ser
325                 330                 335

Leu Asp Gly Thr Arg Ser Arg Ser His Thr Ser Glu Gly Thr Arg Ser
340                 345                 350

Arg Ser His Thr Ser Glu Gly Thr Arg Ser Arg Ser His Thr Ser Glu
355                 360                 365

Gly Ala His Leu Asp Ile Thr Pro Asn Ser Gly Ala Ala Gly Asn Ser
370                 375                 380

Ala Gly Pro Lys Ser Met Glu Val Ser Cys
385                 390


<210> SEQ ID NO 9
<211> LENGTH: 778
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (52)...(711)

<400> SEQUENCE: 9

caggttggaa accagtgccc caggcggcga ggagagcggt gccttgcagg g atg ctg      57
Met Leu
1

cgg gcg gga gca cca acc ggg gac tta ccc cgg gcg gga gaa gtc cac     105
Arg Ala Gly Ala Pro Thr Gly Asp Leu Pro Arg Ala Gly Glu Val His
5                   10                  15

acc ggg acc acc atc atg gca gtg gag ttt gac ggg ggc gtt gtg atg     153
Thr Gly Thr Thr Ile Met Ala Val Glu Phe Asp Gly Gly Val Val Met
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20                  25                  30

ggt tct gat tcc cga gtg tct gca ggc gag gcg gtg gtg aac cga gtg     201
Gly Ser Asp Ser Arg Val Ser Ala Gly Glu Ala Val Val Asn Arg Val
35                  40                  45                  50

ttt gac aag ctg tcc ccg ctg cac gag cgc atc tac tgt gca ctc tct     249
Phe Asp Lys Leu Ser Pro Leu His Glu Arg Ile Tyr Cys Ala Leu Ser
55                  60                  65

ggt tca gct gct gat gcc caa gcc gtg gcc gac atg gcc gcc tac cag     297
Gly Ser Ala Ala Asp Ala Gln Ala Val Ala Asp Met Ala Ala Tyr Gln
70                  75                  80

ctg gag ctc cat ggg ata gaa ctg gag gaa cct cca ctt gtt ttg gct     345
Leu Glu Leu His Gly Ile Glu Leu Glu Glu Pro Pro Leu Val Leu Ala
85                  90                  95

gct gca aat gtg gtg aga aat atc agc tat aaa tat cga gag gac ttg     393
Ala Ala Asn Val Val Arg Asn Ile Ser Tyr Lys Tyr Arg Glu Asp Leu
100                 105                 110

tct gca cat ctc atg gta gct ggc tgg gac caa cgt gaa gga ggt cag     441
Ser Ala His Leu Met Val Ala Gly Trp Asp Gln Arg Glu Gly Gly Gln
115                 120                 125                 130

gta tat gga acc ctg gga gga atg ctg act cga cag cct ttt gcc att     489
Val Tyr Gly Thr Leu Gly Gly Met Leu Thr Arg Gln Pro Phe Ala Ile
135                 140                 145

ggt ggc tcc ggc agc acc ttt atc tat ggt tat gtg gat gca gca tat     537
Gly Gly Ser Gly Ser Thr Phe Ile Tyr Gly Tyr Val Asp Ala Ala Tyr
150                 155                 160

aag cca ggc atg tct ccc gag gag tgc agg cgc ttc acc aca gac gct     585
Lys Pro Gly Met Ser Pro Glu Glu Cys Arg Arg Phe Thr Thr Asp Ala
165                 170                 175

att gct ctg gcc atg agc cgg gat ggc tca agc ggg ggt gtc atc tac     633
Ile Ala Leu Ala Met Ser Arg Asp Gly Ser Ser Gly Gly Val Ile Tyr
180                 185                 190

ctg gtc act att aca gct gcc ggt gtg gac cat cga gtc atc ttg ggc     681
Leu Val Thr Ile Thr Ala Ala Gly Val Asp His Arg Val Ile Leu Gly
195                 200                 205                 210

aat gaa ctg cca aaa ttc tat gat gag tga accttcccca gacttctctt       731
Asn Glu Leu Pro Lys Phe Tyr Asp Glu  *
215

tcttattttg taataaactc tctagggcca aaaaaaaaaa aaaaaaa                 778


<210> SEQ ID NO 10
<211> LENGTH: 219
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

Met Leu Arg Ala Gly Ala Pro Thr Gly Asp Leu Pro Arg Ala Gly Glu
1               5                   10                  15

Val His Thr Gly Thr Thr Ile Met Ala Val Glu Phe Asp Gly Gly Val
20                  25                  30

Val Met Gly Ser Asp Ser Arg Val Ser Ala Gly Glu Ala Val Val Asn
35                  40                  45

Arg Val Phe Asp Lys Leu Ser Pro Leu His Glu Arg Ile Tyr Cys Ala
50                  55                  60

Leu Ser Gly Ser Ala Ala Asp Ala Gln Ala Val Ala Asp Met Ala Ala
65                  70                  75                  80

Tyr Gln Leu Glu Leu His Gly Ile Glu Leu Glu Glu Pro Pro Leu Val
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85                  90                  95

Leu Ala Ala Ala Asn Val Val Arg Asn Ile Ser Tyr Lys Tyr Arg Glu
100                 105                 110

Asp Leu Ser Ala His Leu Met Val Ala Gly Trp Asp Gln Arg Glu Gly
115                 120                 125

Gly Gln Val Tyr Gly Thr Leu Gly Gly Met Leu Thr Arg Gln Pro Phe
130                 135                 140

Ala Ile Gly Gly Ser Gly Ser Thr Phe Ile Tyr Gly Tyr Val Asp Ala
145                 150                 155                 160

Ala Tyr Lys Pro Gly Met Ser Pro Glu Glu Cys Arg Arg Phe Thr Thr
165                 170                 175

Asp Ala Ile Ala Leu Ala Met Ser Arg Asp Gly Ser Ser Gly Gly Val
180                 185                 190

Ile Tyr Leu Val Thr Ile Thr Ala Ala Gly Val Asp His Arg Val Ile
195                 200                 205

Leu Gly Asn Glu Leu Pro Lys Phe Tyr Asp Glu
210                 215


<210> SEQ ID NO 11
<211> LENGTH: 2422
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (466)...(1062)

<400> SEQUENCE: 11

gtcagcctcc cttccaccgc catattgggc cactaaaaaa agggggctcg tcttttcggg     60

gtgtttttct ccccctcccc tgtccccgct tgctcacggc tctgcgactc cgacgccggc    120

aaggtttgga gagcggctgg gttcgcggga cccgcgggct tgcacccgcc cagactcgga    180

cgggctttgc caccctctcc gcttgcctgg tcccctctcc tctccgccct cccgctcgcc    240

agtccatttg atcagcggag actcggcggc cgggccgggg cttccccgca gcccctgcgc    300

gctcctagag ctcgggccgt ggctcgtcgg ggtctgtgtc ttttggctcc gagggcagtc    360

gctgggcttc cgagaggggt tcgggccgcg taggggcgct ttgttttgtt cggttttgtt    420

tttttgagag tgcgagagag gcggtcgtgc agacccggga gaaag atg tca aac gtg    477
Met Ser Asn Val
1

cga gtg tct aac ggg agc cct agc ctg gag cgg atg gac gcc agg cag      525
Arg Val Ser Asn Gly Ser Pro Ser Leu Glu Arg Met Asp Ala Arg Gln
5                   10                  15                  20

gcg gag cac ccc aag ccc tcg gcc tgc agg aac ctc ttc ggc ccg gtg      573
Ala Glu His Pro Lys Pro Ser Ala Cys Arg Asn Leu Phe Gly Pro Val
25                  30                  35

gac cac gaa gag tta acc cgg gac ttg gag aag cac tgc aga gac atg      621
Asp His Glu Glu Leu Thr Arg Asp Leu Glu Lys His Cys Arg Asp Met
40                  45                  50

gaa gag gcg agc cag cgc aag tgg aat ttc gat ttt cag aat cac aaa      669
Glu Glu Ala Ser Gln Arg Lys Trp Asn Phe Asp Phe Gln Asn His Lys
55                  60                  65

ccc cta gag ggc aag tac gag tgg caa gag gtg gag aag ggc agc ttg      717
Pro Leu Glu Gly Lys Tyr Glu Trp Gln Glu Val Glu Lys Gly Ser Leu
70                  75                  80

ccc gag ttc tac tac aga ccc ccg cgg ccc ccc aaa ggt gcc tgc aag      765
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Pro Glu Phe Tyr Tyr Arg Pro Pro Arg Pro Pro Lys Gly Ala Cys Lys
85                  90                  95                 100

gtg ccg gcg cag gag agc cag gat gtc agc ggg agc cgc ccg gcg gcg      813
Val Pro Ala Gln Glu Ser Gln Asp Val Ser Gly Ser Arg Pro Ala Ala
105                 110                 115

cct tta att ggg gct ccg gct aac tct gag gac acg cat ttg gtg gac      861
Pro Leu Ile Gly Ala Pro Ala Asn Ser Glu Asp Thr His Leu Val Asp
120                 125                 130

cca aag act gat ccg tcg gac agc cag acg ggg tta gcg gag caa tgc      909
Pro Lys Thr Asp Pro Ser Asp Ser Gln Thr Gly Leu Ala Glu Gln Cys
135                 140                 145

gca gga ata agg aag cga cct gca acc gac gat tct tct act caa aac      957
Ala Gly Ile Arg Lys Arg Pro Ala Thr Asp Asp Ser Ser Thr Gln Asn
150                 155                 160

aaa aga gcc aac aga aca gaa gaa aat gtt tca gac ggt tcc cca aat     1005
Lys Arg Ala Asn Arg Thr Glu Glu Asn Val Ser Asp Gly Ser Pro Asn
165                 170                 175                 180

gcc ggt tct gtg gag cag acg ccc aag aag cct ggc ctc aga aga cgt     1053
Ala Gly Ser Val Glu Gln Thr Pro Lys Lys Pro Gly Leu Arg Arg Arg
185                 190                 195

caa acg taa acagctcgaa ttaagaatat gtttccttgt ttatcagata             1102
Gln Thr  *

catcactgct tgatgaagca aggaagatat acatgaaaat tttaaaaata catatcgctg   1162

acttcatgga atggacatcc tgtataagca ctgaaaaaca acaacacaat aacactaaaa   1222

ttttaggcac tcttaaatga tctgcctcta aaagcgttgg atgtagcatt atgcaattag   1282

gtttttcctt atttgcttca ttgtactacc tgtgtatata gtttttacct tttatgtagc   1342

acataaactt tggggaaggg agggcagggt ggggctgagg aactgacgtg gagcggggta   1402

tgaagagctt gctttgattt acagcaagta gataaatatt tgacttgcat gaagagaagc   1462

aattttgggg aagggtttga attgttttct ttaaagatgt aatgtccctt tcagagacag   1522

ctgatacttc atttaaaaaa atcacaaaaa tttgaacact ggctaaagat aattgctatt   1582

tatttttaca agaagtttat tctcatttgg gagatctggt gatctcccaa gctatctaaa   1642

gtttgttaga tagctgcatg tggctttttt aaaaaagcaa cagaaaccta tcctcactgc   1702

cctccccagt ctctcttaaa gttggaattt accagttaat tactcagcag aatggtgatc   1762

actccaggta gtttggggca aaaatccgag gtgcttggga gttttgaatg ttaagaattg   1822

accatctgct tttattaaat ttgttgacaa aattttctca ttttcttttc acttcgggct   1882

gtgtaaacac agtcaaaata attctaaatc cctcgatatt tttaaagatc tgtaagtaac   1942

ttcacattaa aaaatgaaat atttttttaat ttaaagctta ctctgtccat ttatccacag   2002

gaaagtgtta tttttaaagg aaggttcatg tagagaaaag cacacttgta ggataagtga   2062

aatggatact acatctttaa acagtatttc attgcctgtg tatggaaaaa ccatttgaag   2122

tgtacctgtg tacataactc tgtaaaaaca ctgaaaaatt atactaactt atttatgtta   2182

aaagattttt tttaatctag acaatataca agccaaagtg gcatgttttg tgcatttgta   2242

aatgctgtgt tgggtagaat aggttttccc ctcttttgtt aaataatatg gctatgctta   2302

aaaggttgca tactgagcca agtataattt tttgtaatgt gtgaaaaaga tgccaattat   2362

tgttacacat taagtaatca ataaagaaaa cttccatagc taaaaaaaaa aaaaaaaaaa   2422
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<210> SEQ ID NO 12

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<211> LENGTH: 198
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

Met Ser Asn Val Arg Val Ser Asn Gly Ser Pro Ser Leu Glu Arg Met
1 5 10 15

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

Phe Gly Pro Val Asp His Glu Glu Leu Thr Arg Asp Leu Glu Lys His
35 40 45

Cys Arg Asp Met Glu Glu Ala Ser Gln Arg Lys Trp Asn Phe Asp Phe
50 55 60

Gln Asn His Lys Pro Leu Glu Gly Lys Tyr Glu Trp Gln Glu Val Glu
65 70 75 80

Lys Gly Ser Leu Pro Glu Phe Tyr Tyr Arg Pro Pro Arg Pro Pro Lys
85 90 95

Gly Ala Cys Lys Val Pro Ala Gln Glu Ser Gln Asp Val Ser Gly Ser
100 105 110

Arg Pro Ala Ala Pro Leu Ile Gly Ala Pro Ala Asn Ser Glu Asp Thr
115 120 125

His Leu Val Asp Pro Lys Thr Asp Pro Ser Asp Ser Gln Thr Gly Leu
130 135 140

Ala Glu Gln Cys Ala Gly Ile Arg Lys Arg Pro Ala Thr Asp Asp Ser
145 150 155 160

Ser Thr Gln Asn Lys Arg Ala Asn Arg Thr Glu Glu Asn Val Ser Asp
165 170 175

Gly Ser Pro Asn Ala Gly Ser Val Glu Gln Thr Pro Lys Lys Pro Gly
180 185 190

Leu Arg Arg Arg Gln Thr
195

<210> SEQ ID NO 13
<211> LENGTH: 2486
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (124)...(1437)

<400> SEQUENCE: 13

ccgggacttt gcaggcagcg gcggccgggg gcggagcggg atcgagccct cgccgaggcc 60

tgccgccatg ggcccgcgcc gccgccgccg cctgtcaccc gggccgcgcg ggccgtgagc 120

gtc atg gcc ttg gcc ggg gcc cct gcg ggc ggc cca tgc gcg ccg gcg 168
Met Ala Leu Ala Gly Ala Pro Ala Gly Gly Pro Cys Ala Pro Ala
1 5 10 15

ctg gag gcc ctg ctc ggg gcc ggc gcg ctg cgg ctg ctc gac tcc tcg 216
Leu Glu Ala Leu Leu Gly Ala Gly Ala Leu Arg Leu Leu Asp Ser Ser
20 25 30

cag atc gtc atc atc tcc gcc gcg cag gac gcc agc gcc ccg ccg gct 264
Gln Ile Val Ile Ile Ser Ala Ala Gln Asp Ala Ser Ala Pro Pro Ala
35 40 45

ccc acc ggc ccc gcg gcg ccc gcc gcc ggc ccc tgc gac cct gac ctg 312
Pro Thr Gly Pro Ala Ala Pro Ala Ala Gly Pro Cys Asp Pro Asp Leu
50 55 60

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ctg ctc ttc gcc aca ccg cag gcg ccc cgg ccc aca ccc agt gcg ccg      360
Leu Leu Phe Ala Thr Pro Gln Ala Pro Arg Pro Thr Pro Ser Ala Pro
65                  70                  75

cgg ccc gcg ctc ggc cgc ccg ccg gtg aag cgg agg ctg gac ctg gaa      408
Arg Pro Ala Leu Gly Arg Pro Pro Val Lys Arg Arg Leu Asp Leu Glu
80                  85                  90                  95

act gac cat cag tac ctg gcc gag agc agt ggg cca gct cgg ggc aga      456
Thr Asp His Gln Tyr Leu Ala Glu Ser Ser Gly Pro Ala Arg Gly Arg
100                 105                 110

ggc cgc cat cca gga aaa ggt gtg aaa tcc ccg ggg gag aag tca cgc      504
Gly Arg His Pro Gly Lys Gly Val Lys Ser Pro Gly Glu Lys Ser Arg
115                 120                 125

tat gag acc tca ctg aat ctg acc acc aag cgc ttc ctg gag ctg ctg      552
Tyr Glu Thr Ser Leu Asn Leu Thr Thr Lys Arg Phe Leu Glu Leu Leu
130                 135                 140

agc cac tcg gct gac ggt gtc gtc gac ctg aac tgg gct gcc gag gtg      600
Ser His Ser Ala Asp Gly Val Val Asp Leu Asn Trp Ala Ala Glu Val
145                 150                 155

ctg aag gtg cag aag cgg cgc atc tat gac atc acc aac gtc ctt gag      648
Leu Lys Val Gln Lys Arg Arg Ile Tyr Asp Ile Thr Asn Val Leu Glu
160                 165                 170                 175

ggc atc cag ctc att gcc aag aag tcc aag aac cac atc cag tgg ctg      696
Gly Ile Gln Leu Ile Ala Lys Lys Ser Lys Asn His Ile Gln Trp Leu
180                 185                 190

ggc agc cac acc aca gtg ggc gtc ggc gga cgg ctt gag ggg ttg acc      744
Gly Ser His Thr Thr Val Gly Val Gly Gly Arg Leu Glu Gly Leu Thr
195                 200                 205

cag gac ctc cga cag ctg cag gag agc gag cag cag ctg gac cac ctg      792
Gln Asp Leu Arg Gln Leu Gln Glu Ser Glu Gln Gln Leu Asp His Leu
210                 215                 220

atg aat atc tgt act acg cag ctg cgc ctg ctc tcc gag gac act gac      840
Met Asn Ile Cys Thr Thr Gln Leu Arg Leu Leu Ser Glu Asp Thr Asp
225                 230                 235

agc cag cgc ctg gcc tac gtg acg tgt cag gac ctt cgt agc att gca      888
Ser Gln Arg Leu Ala Tyr Val Thr Cys Gln Asp Leu Arg Ser Ile Ala
240                 245                 250                 255

gac cct gca gag cag atg gtt atg gtg atc aaa gcc cct cct gag acc      936
Asp Pro Ala Glu Gln Met Val Met Val Ile Lys Ala Pro Pro Glu Thr
260                 265                 270

cag ctc caa gcc gtg gac tct tcg gag aac ttt cag atc tcc ctt aag      984
Gln Leu Gln Ala Val Asp Ser Ser Glu Asn Phe Gln Ile Ser Leu Lys
275                 280                 285

agc aaa caa ggc ccg atc gat gtt ttc ctg tgc cct gag gag acc gta     1032
Ser Lys Gln Gly Pro Ile Asp Val Phe Leu Cys Pro Glu Glu Thr Val
290                 295                 300

ggt ggg atc agc cct ggg aag acc cca tcc cag gag gtc act tct gag     1080
Gly Gly Ile Ser Pro Gly Lys Thr Pro Ser Gln Glu Val Thr Ser Glu
305                 310                 315

gag gag aac agg gcc act gac tct gcc acc ata gtg tca cca cca cca     1128
Glu Glu Asn Arg Ala Thr Asp Ser Ala Thr Ile Val Ser Pro Pro Pro
320                 325                 330                 335

tca tct ccc ccc tca tcc ctc acc aca gat ccc agc cag tct cta ctc     1176
Ser Ser Pro Pro Ser Ser Leu Thr Thr Asp Pro Ser Gln Ser Leu Leu
340                 345                 350

agc ctg gag caa gaa ccg ctg ttg tcc cgg atg ggc agc ctg cgg gct     1224
Ser Leu Glu Gln Glu Pro Leu Leu Ser Arg Met Gly Ser Leu Arg Ala
355                 360                 365
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ccc gtg gac gag gac cgc ctg tcc ccg ctg gtg gcg gcc gac tcg ctc     1272
Pro Val Asp Glu Asp Arg Leu Ser Pro Leu Val Ala Ala Asp Ser Leu
370                 375                 380

ctg gag cat gtg cgg gag gac ttc tcc ggc ctc ctc cct gag gag ttc     1320
Leu Glu His Val Arg Glu Asp Phe Ser Gly Leu Leu Pro Glu Glu Phe
385                 390                 395

atc agc ctt tcc cca ccc cac gag gcc ctc gac tac cac ttc ggc ctc     1368
Ile Ser Leu Ser Pro Pro His Glu Ala Leu Asp Tyr His Phe Gly Leu
400                 405                 410                 415

gag gag ggc gag ggc atc aga gac ctc ttc gac tgt gac ttt ggg gac     1416
Glu Glu Gly Glu Gly Ile Arg Asp Leu Phe Asp Cys Asp Phe Gly Asp
420                 425                 430

ctc acc ccc ctg gat ttc tga cagggcttgg agggaccagg gtttccagag        1467
Leu Thr Pro Leu Asp Phe  *
435

tagctcacct tgtctctgca gccctggagc cccctgtccc tggccgtcct cccagcctgt   1527

ttggaaacat ttaatttata cccctctcct ctgtctccag aagcttctag ctctggggtc   1587

tggctaccgc taggaggctg agcaagccag gaagggaagg agtctgtgtg gtgtgtatgt   1647

gcatgcagcc tacacccaca cgtgtgtacc gggggtgaat gtgtgtgagc atgtgtgtgt   1707

gcatgtaccg gggaatgaag gtgaacatac acctctgtgt gtgcactgca gacacgcccc   1767

agtgtgtcca catgtgtgtg catgagtcca tctctgcgcg tgggggggct ctaactgcac   1827

tttcggccct tttgctcgtg gggtcccaca aggcccaggg cagtgcctgc tcccagaatc   1887

tggtgctctg accaggccag gtggggaggc tttggctggc tgggcgtgta ggacggtgag   1947

agcacttctg tcttaaaggt tttttctgat tgaagcttta atggagcgtt atttatttat   2007

cgaggcctct ttggtgagcc tggggaatca gcaaaagggg aggaggggtg tggggttgat   2067

accccaactc cctctaccct tgagcaaggg caggggtccc tgagctgttc ttctgcccca   2127

tactgaagga actgaggcct gggtgattta tttattggga aagtgaggga gggagacaga   2187

ctgactgaca gccatgggtg gtcagatggt ggggtgggcc ctctccaggg ggccagttca   2247

gggcccagct gccccccagg atggatatga gatgggagag gtgagtgggg gaccttcact   2307

gatgtgggca ggaggggtgg tgaaggcctc ccccagccca gaccctgtgg tccctcctgc   2367

agtgtctgaa gcgcctgcct ccccactgct ctgccccacc ctccaatctg cactttgatt   2427

tgcttcctaa cagctctgtt ccctcctgct ttggttttaa taaatatttt gatgacgtt    2486
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<210> SEQ ID NO 14
<211> LENGTH: 437
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 14

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Met Ala Leu Ala Gly Ala Pro Ala Gly Gly Pro Cys Ala Pro Ala Leu
1               5                   10                  15

Glu Ala Leu Leu Gly Ala Gly Ala Leu Arg Leu Leu Asp Ser Ser Gln
20                  25                  30

Ile Val Ile Ile Ser Ala Ala Gln Asp Ala Ser Ala Pro Pro Ala Pro
35                  40                  45

Thr Gly Pro Ala Ala Pro Ala Ala Gly Pro Cys Asp Pro Asp Leu Leu
50                  55                  60

Leu Phe Ala Thr Pro Gln Ala Pro Arg Pro Thr Pro Ser Ala Pro Arg
65                  70                  75                  80
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Pro Ala Leu Gly Arg Pro Pro Val Lys Arg Arg Leu Asp Leu Glu Thr
85                  90                  95

Asp His Gln Tyr Leu Ala Glu Ser Ser Gly Pro Ala Arg Gly Arg Gly
100                 105                 110

Arg His Pro Gly Lys Gly Val Lys Ser Pro Gly Glu Lys Ser Arg Tyr
115                 120                 125

Glu Thr Ser Leu Asn Leu Thr Thr Lys Arg Phe Leu Glu Leu Leu Ser
130                 135                 140

His Ser Ala Asp Gly Val Val Asp Leu Asn Trp Ala Ala Glu Val Leu
145                 150                 155                 160

Lys Val Gln Lys Arg Arg Ile Tyr Asp Ile Thr Asn Val Leu Glu Gly
165                 170                 175

Ile Gln Leu Ile Ala Lys Lys Ser Lys Asn His Ile Gln Trp Leu Gly
180                 185                 190

Ser His Thr Thr Val Gly Val Gly Gly Arg Leu Glu Gly Leu Thr Gln
195                 200                 205

Asp Leu Arg Gln Leu Gln Glu Ser Glu Gln Gln Leu Asp His Leu Met
210                 215                 220

Asn Ile Cys Thr Thr Gln Leu Arg Leu Leu Ser Glu Asp Thr Asp Ser
225                 230                 235                 240

Gln Arg Leu Ala Tyr Val Thr Cys Gln Asp Leu Arg Ser Ile Ala Asp
245                 250                 255

Pro Ala Glu Gln Met Val Met Val Ile Lys Ala Pro Pro Glu Thr Gln
260                 265                 270

Leu Gln Ala Val Asp Ser Ser Glu Asn Phe Gln Ile Ser Leu Lys Ser
275                 280                 285

Lys Gln Gly Pro Ile Asp Val Phe Leu Cys Pro Glu Glu Thr Val Gly
290                 295                 300

Gly Ile Ser Pro Gly Lys Thr Pro Ser Gln Glu Val Thr Ser Glu Glu
305                 310                 315                 320

Glu Asn Arg Ala Thr Asp Ser Ala Thr Ile Val Ser Pro Pro Pro Ser
325                 330                 335

Ser Pro Pro Ser Ser Leu Thr Thr Asp Pro Ser Gln Ser Leu Leu Ser
340                 345                 350

Leu Glu Gln Glu Pro Leu Leu Ser Arg Met Gly Ser Leu Arg Ala Pro
355                 360                 365

Val Asp Glu Asp Arg Leu Ser Pro Leu Val Ala Ala Asp Ser Leu Leu
370                 375                 380

Glu His Val Arg Glu Asp Phe Ser Gly Leu Leu Pro Glu Glu Phe Ile
385                 390                 395                 400

Ser Leu Ser Pro Pro His Glu Ala Leu Asp Tyr His Phe Gly Leu Glu
405                 410                 415

Glu Gly Glu Gly Ile Arg Asp Leu Phe Asp Cys Asp Phe Gly Asp Leu
420                 425                 430

Thr Pro Leu Asp Phe
435


<210> SEQ ID NO 15
<211> LENGTH: 3744
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
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<221> NAME/KEY: CDS
<222> LOCATION: (56)...(2521)

<400> SEQUENCE: 15

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ccacgcgtcc ggtggcggtc gagcgtggcg taggcgaatc ctcggcacta agcat atg      58
Met
1

gac ctc gcg gcg gca gcg gag ccg ggc gcc ggc agc cag cac ctg gag      106
Asp Leu Ala Ala Ala Ala Glu Pro Gly Ala Gly Ser Gln His Leu Glu
5                   10                  15

gtc cgc gac gag gtg gcc gag aag tgc cag aaa ctg ttc ctg gac ttc      154
Val Arg Asp Glu Val Ala Glu Lys Cys Gln Lys Leu Phe Leu Asp Phe
20                  25                  30

ttg gag gag ttt cag agc agc gat gga gaa att aaa tac ttg caa tta      202
Leu Glu Glu Phe Gln Ser Ser Asp Gly Glu Ile Lys Tyr Leu Gln Leu
35                  40                  45

gca gag gaa ctg att cgt cct gag aga aac aca ttg gtt gtg agt ttt      250
Ala Glu Glu Leu Ile Arg Pro Glu Arg Asn Thr Leu Val Val Ser Phe
50                  55                  60                  65

gtg gac ctg gaa caa ttt aac cag caa ctt tcc acc acc att caa gag      298
Val Asp Leu Glu Gln Phe Asn Gln Gln Leu Ser Thr Thr Ile Gln Glu
70                  75                  80

gag ttc tat aga gtt tac cct tac ctg tgt cgg gcc ttg aaa aca ttc      346
Glu Phe Tyr Arg Val Tyr Pro Tyr Leu Cys Arg Ala Leu Lys Thr Phe
85                  90                  95

gtc aaa gac cgt aaa gag atc cct ctt gcc aag gat ttt tat gtt gca      394
Val Lys Asp Arg Lys Glu Ile Pro Leu Ala Lys Asp Phe Tyr Val Ala
100                 105                 110

ttc caa gac ctg cct acc aga cac aag att cga gag ctc acc tca tcc      442
Phe Gln Asp Leu Pro Thr Arg His Lys Ile Arg Glu Leu Thr Ser Ser
115                 120                 125

aga att ggt ttg ctc act cgc atc agt ggg cag gtg gtg cgg act cac      490
Arg Ile Gly Leu Leu Thr Arg Ile Ser Gly Gln Val Val Arg Thr His
130                 135                 140                 145

cca gtt cac cca gag ctt gtg agc gga act ttt ctg tgc ttg gac tgt      538
Pro Val His Pro Glu Leu Val Ser Gly Thr Phe Leu Cys Leu Asp Cys
150                 155                 160

cag aca gtg atc agg gat gta gaa cag cag ttc aaa tac aca cag cca      586
Gln Thr Val Ile Arg Asp Val Glu Gln Gln Phe Lys Tyr Thr Gln Pro
165                 170                 175

aac atc tgc cga aat cca gtt tgt gcc aac agg agg aga ttc tta ctg      634
Asn Ile Cys Arg Asn Pro Val Cys Ala Asn Arg Arg Arg Phe Leu Leu
180                 185                 190

gat aca aat aaa tca aga ttt gtt gat ttt caa aag gtt cgt att caa      682
Asp Thr Asn Lys Ser Arg Phe Val Asp Phe Gln Lys Val Arg Ile Gln
195                 200                 205

gag acc caa gct gag ctt cct cga ggg agt atc ccc cgc agt tta gaa      730
Glu Thr Gln Ala Glu Leu Pro Arg Gly Ser Ile Pro Arg Ser Leu Glu
210                 215                 220                 225

gta att tta agg gct gaa gct gtg gaa tca gct caa gct ggt gac aag      778
Val Ile Leu Arg Ala Glu Ala Val Glu Ser Ala Gln Ala Gly Asp Lys
230                 235                 240

tgt gac ttt aca ggg aca ctg att gtt gtg cct gac gtc tcc aag ctt      826
Cys Asp Phe Thr Gly Thr Leu Ile Val Val Pro Asp Val Ser Lys Leu
245                 250                 255

agc aca cca gga gca cgt gca gaa act aat tcc cgt gtc agt ggt gtt      874
Ser Thr Pro Gly Ala Arg Ala Glu Thr Asn Ser Arg Val Ser Gly Val
260                 265                 270
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gat gga tat gag aca gaa ggc att cga gga ctc cgg gcc ctt ggt gtt      922
Asp Gly Tyr Glu Thr Glu Gly Ile Arg Gly Leu Arg Ala Leu Gly Val
275                 280                 285

agg gac ctt tct tat agg ctg gtc ttt ctt gcc tgc tgt gtt gcg cca      970
Arg Asp Leu Ser Tyr Arg Leu Val Phe Leu Ala Cys Cys Val Ala Pro
290                 295                 300                 305

acc aac cca agg ttt ggg ggg aaa gag ctc aga gat gag gaa cag aca     1018
Thr Asn Pro Arg Phe Gly Gly Lys Glu Leu Arg Asp Glu Glu Gln Thr
310                 315                 320

gct gag agc att aag aac caa atg act gtg aaa gaa tgg gag aaa gtg     1066
Ala Glu Ser Ile Lys Asn Gln Met Thr Val Lys Glu Trp Glu Lys Val
325                 330                 335

ttt gag atg agt caa gat aaa aat cta tac cac aat ctt tgt acc agc     1114
Phe Glu Met Ser Gln Asp Lys Asn Leu Tyr His Asn Leu Cys Thr Ser
340                 345                 350

ctg ttc cct act ata cat ggc aat gat gaa gta aaa cgg ggt gtc ctg     1162
Leu Phe Pro Thr Ile His Gly Asn Asp Glu Val Lys Arg Gly Val Leu
355                 360                 365

ctg atg ctc ttt ggt ggc gtt cca aag aca aca gga gaa ggg acc tct     1210
Leu Met Leu Phe Gly Gly Val Pro Lys Thr Thr Gly Glu Gly Thr Ser
370                 375                 380                 385

ctt cga ggg gac ata aat gtt tgc att gtt ggt gac cca agt aca gct     1258
Leu Arg Gly Asp Ile Asn Val Cys Ile Val Gly Asp Pro Ser Thr Ala
390                 395                 400

aag agc caa ttt ctc aag cac gtg gag gag ttc agc ccc aga gct gtc     1306
Lys Ser Gln Phe Leu Lys His Val Glu Glu Phe Ser Pro Arg Ala Val
405                 410                 415

tac acc agt ggt aaa gcg tcc agt gct gct ggc tta aca gca gct gtt     1354
Tyr Thr Ser Gly Lys Ala Ser Ser Ala Ala Gly Leu Thr Ala Ala Val
420                 425                 430

gtg aga gat gaa gaa tct cat gag ttt gtc att gag gct gga gct ttg     1402
Val Arg Asp Glu Glu Ser His Glu Phe Val Ile Glu Ala Gly Ala Leu
435                 440                 445

atg ttg gct gat aat ggt gtg tgt tgt att gat gaa ttt gat aag atg     1450
Met Leu Ala Asp Asn Gly Val Cys Cys Ile Asp Glu Phe Asp Lys Met
450                 455                 460                 465

gac gtg cgg gat caa gtt gct att cat gaa gct atg gaa cag cag acc     1498
Asp Val Arg Asp Gln Val Ala Ile His Glu Ala Met Glu Gln Gln Thr
470                 475                 480

ata tcc atc act aaa gca gga gtg aag gct act ctg aac gcc cgg acg     1546
Ile Ser Ile Thr Lys Ala Gly Val Lys Ala Thr Leu Asn Ala Arg Thr
485                 490                 495

tcc att ttg gca gca gca aac cca atc agt gga cac tat gac aga tca     1594
Ser Ile Leu Ala Ala Ala Asn Pro Ile Ser Gly His Tyr Asp Arg Ser
500                 505                 510

aaa tca ttg aaa cag aat ata aat ttg tca gct ccc atc atg tcc cga     1642
Lys Ser Leu Lys Gln Asn Ile Asn Leu Ser Ala Pro Ile Met Ser Arg
515                 520                 525

ttc gat ctc ttc ttt atc ctt gtg gat gaa tgt aat gag gtt aca gat     1690
Phe Asp Leu Phe Phe Ile Leu Val Asp Glu Cys Asn Glu Val Thr Asp
530                 535                 540                 545

tat gcc att gcc agg cgc ata gta gat ttg cat tca aga att gag gaa     1738
Tyr Ala Ile Ala Arg Arg Ile Val Asp Leu His Ser Arg Ile Glu Glu
550                 555                 560

tca att gat cgt gtc tat tcc ctc gat gat atc aga aga tat ctt ctc     1786
Ser Ile Asp Arg Val Tyr Ser Leu Asp Asp Ile Arg Arg Tyr Leu Leu
565                 570                 575
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ttt gca aga cag ttt aaa ccc aag att tcc aaa gag tca gag gac ttc     1834
Phe Ala Arg Gln Phe Lys Pro Lys Ile Ser Lys Glu Ser Glu Asp Phe
580                 585                 590

att gtg gag caa tat aaa cat ctc cgc cag aga gat ggt tct gga gtg     1882
Ile Val Glu Gln Tyr Lys His Leu Arg Gln Arg Asp Gly Ser Gly Val
595                 600                 605

acc aag tct tca tgg agg att aca gtg cga cag ctt gag agc atg att     1930
Thr Lys Ser Ser Trp Arg Ile Thr Val Arg Gln Leu Glu Ser Met Ile
610                 615                 620                 625

cgt ctc tct gaa gct atg gct cgg atg cac tgc tgt gat gag gtc caa     1978
Arg Leu Ser Glu Ala Met Ala Arg Met His Cys Cys Asp Glu Val Gln
630                 635                 640

cct aaa cat gtg aag gaa gct ttc cgg tta ctg aat aaa tca atc atc     2026
Pro Lys His Val Lys Glu Ala Phe Arg Leu Leu Asn Lys Ser Ile Ile
645                 650                 655

cgt gtg gaa aca cct gat gtc aat cta gat caa gag gaa gag atc cag     2074
Arg Val Glu Thr Pro Asp Val Asn Leu Asp Gln Glu Glu Glu Ile Gln
660                 665                 670

atg gag gta gat gag ggt gcc ggt ggc atc aat ggt cat gct gac agc     2122
Met Glu Val Asp Glu Gly Ala Gly Gly Ile Asn Gly His Ala Asp Ser
675                 680                 685

cct gct cct gtg aac ggg atc aat ggc tac aat gaa gac ata aat caa     2170
Pro Ala Pro Val Asn Gly Ile Asn Gly Tyr Asn Glu Asp Ile Asn Gln
690                 695                 700                 705

gag tct gct ccc aaa gcc tcc tta agg ctg ggc ttc tct gag tac tgc     2218
Glu Ser Ala Pro Lys Ala Ser Leu Arg Leu Gly Phe Ser Glu Tyr Cys
710                 715                 720

cga atc tct aac ctt att gtg ctt cac ctc aga aag gtg gaa gaa gaa     2266
Arg Ile Ser Asn Leu Ile Val Leu His Leu Arg Lys Val Glu Glu Glu
725                 730                 735

gag gac gag tca gca tta aag agg agc gag ctt gtt aac tgg tac ttg     2314
Glu Asp Glu Ser Ala Leu Lys Arg Ser Glu Leu Val Asn Trp Tyr Leu
740                 745                 750

aag gaa atc gaa tca gag ata gac tct gaa gaa gaa ctt ata aat aaa     2362
Lys Glu Ile Glu Ser Glu Ile Asp Ser Glu Glu Glu Leu Ile Asn Lys
755                 760                 765

aaa aga atc ata gag aaa gtt att cat cga ctc aca cac tat gat cat     2410
Lys Arg Ile Ile Glu Lys Val Ile His Arg Leu Thr His Tyr Asp His
770                 775                 780                 785

gtt cta att gag ctc acc cag gct gga ttg aaa ggc tcc aca gag gga     2458
Val Leu Ile Glu Leu Thr Gln Ala Gly Leu Lys Gly Ser Thr Glu Gly
790                 795                 800

agt gag agc tat gaa gaa gat ccc tac ttg gta gtt aac cct aac tac     2506
Ser Glu Ser Tyr Glu Glu Asp Pro Tyr Leu Val Val Asn Pro Asn Tyr
805                 810                 815

ttg ctc gaa gat tga gatagtgaaa gtaactgacc agagctgagg aactgtggca     2561
Leu Leu Glu Asp  *
820

cagcacctcg tggcctggag cctggctgga gctctgctag ggacagaagt gtttctggaa   2621

gtgatgcttc caggatttgt tttcagaaac aagaattgag ttgatggtcc tatgtgtcac   2681

attcatcaca ggtttcatac caacacaggc ttcagcactt cctttggtgt gtttcctgtc   2741

ccagtgaagt tggaaccaaa taatgtgtag tctctataac caatacctttt gttttcatgt   2801

gtaagaaaag gcccattact tttaaggtat gtgctgtcct attgagcaaa taacttttttt   2861

tcaattgcca gctactgctt ttattcatca aaataaaata acttgttctg aagttgtcta   2921
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ttggatttct ttctactgta ccctgattat tacttccatc tacttctgaa tgtgagactt   2981

tccctttttg cttaacctgg agtgaagagg tagaactgtg gtattatgga tgaggtttct   3041

atgagaagga gtcattagag aactcatatg aaagctagag gccttagaga tgactttcca   3101

aggttaattc cagtttttt ttttttaag tttataaaag tttattatac ttttttaaaa   3161

ttactcttta gtaatttatt ttacttctgt gtcctaaggg taatttctca ggattgtttt   3221

caaattgctt ttttagggga aataggtcat ttgctatatt acaagcaatc cccaaatttt   3281

atggtcttcc aggaaaagtt attaccgttt atgatactaa cagttcctga gacttagcta   3341

tgatcagtat gttcatgagg tggagcagtt cctgtgttgc agcttttaac aacagatggc   3401

attcattaaa tcacaaagta tgttaaaggt cacaaaagca aaataactgt ctgaggctaa   3461

ggcccacgtg ggacagtcta atacccatga gtactcaact tgccttgatg tctgagcttt   3521

ccagtgcaat gtgaatttga gcagccagaa atctattagt agaaagcaag acagattaat   3581

ataggttaaa acaatgattt aaatatgttt ctcccaataa ttatctcttt ccctggaatc   3641

aacttgtatg aaaccttgtc aaaatgtact ccacaagtat gtacaattaa gtattttaaa   3701

aataaatggc aaacattaaa aaaaaaaaaa aaaaaaaaaa aaa                     3744
```

<210> SEQ ID NO 16
<211> LENGTH: 821
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

```
Met Asp Leu Ala Ala Ala Ala Glu Pro Gly Ala Gly Ser Gln His Leu
1               5                   10                  15

Glu Val Arg Asp Glu Val Ala Glu Lys Cys Gln Lys Leu Phe Leu Asp
20                  25                  30

Phe Leu Glu Glu Phe Gln Ser Ser Asp Gly Glu Ile Lys Tyr Leu Gln
35                  40                  45

Leu Ala Glu Glu Leu Ile Arg Pro Glu Arg Asn Thr Leu Val Val Ser
50                  55                  60

Phe Val Asp Leu Glu Gln Phe Asn Gln Gln Leu Ser Thr Thr Ile Gln
65                  70                  75                  80

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

Phe Val Lys Asp Arg Lys Glu Ile Pro Leu Ala Lys Asp Phe Tyr Val
100                 105                 110

Ala Phe Gln Asp Leu Pro Thr Arg His Lys Ile Arg Glu Leu Thr Ser
115                 120                 125

Ser Arg Ile Gly Leu Leu Thr Arg Ile Ser Gly Gln Val Val Arg Thr
130                 135                 140

His Pro Val His Pro Glu Leu Val Ser Gly Thr Phe Leu Cys Leu Asp
145                 150                 155                 160

Cys Gln Thr Val Ile Arg Asp Val Glu Gln Gln Phe Lys Tyr Thr Gln
165                 170                 175

Pro Asn Ile Cys Arg Asn Pro Val Cys Ala Asn Arg Arg Arg Phe Leu
180                 185                 190

Leu Asp Thr Asn Lys Ser Arg Phe Val Asp Phe Gln Lys Val Arg Ile
195                 200                 205
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Gln Glu Thr Gln Ala Glu Leu Pro Arg Gly Ser Ile Pro Arg Ser Leu
210                 215                 220

Glu Val Ile Leu Arg Ala Glu Ala Val Glu Ser Ala Gln Ala Gly Asp
225                 230                 235                 240

Lys Cys Asp Phe Thr Gly Thr Leu Ile Val Val Pro Asp Val Ser Lys
245                 250                 255

Leu Ser Thr Pro Gly Ala Arg Ala Glu Thr Asn Ser Arg Val Ser Gly
260                 265                 270

Val Asp Gly Tyr Glu Thr Glu Gly Ile Arg Gly Leu Arg Ala Leu Gly
275                 280                 285

Val Arg Asp Leu Ser Tyr Arg Leu Val Phe Leu Ala Cys Cys Val Ala
290                 295                 300

Pro Thr Asn Pro Arg Phe Gly Gly Lys Glu Leu Arg Asp Glu Glu Gln
305                 310                 315                 320

Thr Ala Glu Ser Ile Lys Asn Gln Met Thr Val Lys Glu Trp Glu Lys
325                 330                 335

Val Phe Glu Met Ser Gln Asp Lys Asn Leu Tyr His Asn Leu Cys Thr
340                 345                 350

Ser Leu Phe Pro Thr Ile His Gly Asn Asp Glu Val Lys Arg Gly Val
355                 360                 365

Leu Leu Met Leu Phe Gly Gly Val Pro Lys Thr Thr Gly Glu Gly Thr
370                 375                 380

Ser Leu Arg Gly Asp Ile Asn Val Cys Ile Val Gly Asp Pro Ser Thr
385                 390                 395                 400

Ala Lys Ser Gln Phe Leu Lys His Val Glu Glu Phe Ser Pro Arg Ala
405                 410                 415

Val Tyr Thr Ser Gly Lys Ala Ser Ser Ala Ala Gly Leu Thr Ala Ala
420                 425                 430

Val Val Arg Asp Glu Glu Ser His Glu Phe Val Ile Glu Ala Gly Ala
435                 440                 445

Leu Met Leu Ala Asp Asn Gly Val Cys Cys Ile Asp Glu Phe Asp Lys
450                 455                 460

Met Asp Val Arg Asp Gln Val Ala Ile His Glu Ala Met Glu Gln Gln
465                 470                 475                 480

Thr Ile Ser Ile Thr Lys Ala Gly Val Lys Ala Thr Leu Asn Ala Arg
485                 490                 495

Thr Ser Ile Leu Ala Ala Ala Asn Pro Ile Ser Gly His Tyr Asp Arg
500                 505                 510

Ser Lys Ser Leu Lys Gln Asn Ile Asn Leu Ser Ala Pro Ile Met Ser
515                 520                 525

Arg Phe Asp Leu Phe Phe Ile Leu Val Asp Glu Cys Asn Glu Val Thr
530                 535                 540

Asp Tyr Ala Ile Ala Arg Arg Ile Val Asp Leu His Ser Arg Ile Glu
545                 550                 555                 560

Glu Ser Ile Asp Arg Val Tyr Ser Leu Asp Asp Ile Arg Arg Tyr Leu
565                 570                 575

Leu Phe Ala Arg Gln Phe Lys Pro Lys Ile Ser Lys Glu Ser Glu Asp
580                 585                 590

Phe Ile Val Glu Gln Tyr Lys His Leu Arg Gln Arg Asp Gly Ser Gly
595                 600                 605

Val Thr Lys Ser Ser Trp Arg Ile Thr Val Arg Gln Leu Glu Ser Met
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610                 615                 620

Ile Arg Leu Ser Glu Ala Met Ala Arg Met His Cys Cys Asp Glu Val
625                 630                 635                 640

Gln Pro Lys His Val Lys Glu Ala Phe Arg Leu Leu Asn Lys Ser Ile
645                 650                 655

Ile Arg Val Glu Thr Pro Asp Val Asn Leu Asp Gln Glu Glu Glu Ile
660                 665                 670

Gln Met Glu Val Asp Glu Gly Ala Gly Gly Ile Asn Gly His Ala Asp
675                 680                 685

Ser Pro Ala Pro Val Asn Gly Ile Asn Gly Tyr Asn Glu Asp Ile Asn
690                 695                 700

Gln Glu Ser Ala Pro Lys Ala Ser Leu Arg Leu Gly Phe Ser Glu Tyr
705                 710                 715                 720

Cys Arg Ile Ser Asn Leu Ile Val Leu His Leu Arg Lys Val Glu Glu
725                 730                 735

Glu Glu Asp Glu Ser Ala Leu Lys Arg Ser Glu Leu Val Asn Trp Tyr
740                 745                 750

Leu Lys Glu Ile Glu Ser Glu Ile Asp Ser Glu Glu Glu Leu Ile Asn
755                 760                 765

Lys Lys Arg Ile Ile Glu Lys Val Ile His Arg Leu Thr His Tyr Asp
770                 775                 780

His Val Leu Ile Glu Leu Thr Gln Ala Gly Leu Lys Gly Ser Thr Glu
785                 790                 795                 800

Gly Ser Glu Ser Tyr Glu Glu Asp Pro Tyr Leu Val Val Asn Pro Asn
805                 810                 815

Tyr Leu Leu Glu Asp
820


<210> SEQ ID NO 17
<211> LENGTH: 3371
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (25)...(2712)

<400> SEQUENCE: 17

gcggaatcat cggaatcctt cacc atg gca tcc agc ccg gcc cag cgt cgg        51
Met Ala Ser Ser Pro Ala Gln Arg Arg
1               5

cga ggc aat gat cct ctc acc tcc agc cct ggc cga agc tcc cgg cgt       99
Arg Gly Asn Asp Pro Leu Thr Ser Ser Pro Gly Arg Ser Ser Arg Arg
10                  15                  20                  25

act gat gcc ctc acc tcc agc cct ggc cgt gac ctt cca cca ttt gag      147
Thr Asp Ala Leu Thr Ser Ser Pro Gly Arg Asp Leu Pro Pro Phe Glu
30                  35                  40

gat gag tcc gag ggg ctc cta ggc aca gag ggg ccc ctg gag gaa gaa      195
Asp Glu Ser Glu Gly Leu Leu Gly Thr Glu Gly Pro Leu Glu Glu Glu
45                  50                  55

gag gat gga gag gag ctc att gga gat ggc atg gaa agg gac tac cgc      243
Glu Asp Gly Glu Glu Leu Ile Gly Asp Gly Met Glu Arg Asp Tyr Arg
60                  65                  70

gcc atc cca gag ctg gac gcc tat gag gcc gag gga ctg gct ctg gat      291
Ala Ile Pro Glu Leu Asp Ala Tyr Glu Ala Glu Gly Leu Ala Leu Asp
75                  80                  85
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gat gag gac gta gag gag ctg acg gcc agt cag agg gag gca gca gag      339
Asp Glu Asp Val Glu Glu Leu Thr Ala Ser Gln Arg Glu Ala Ala Glu
90                  95                 100                 105

cgg gcc atg cgg cag cgt gac cgg gag gct ggc cgg ggc ctg ggc cgc      387
Arg Ala Met Arg Gln Arg Asp Arg Glu Ala Gly Arg Gly Leu Gly Arg
110                 115                 120

atg cgc cgt ggg ctc ctg tat gac agc gat gag gag gac gag gag cgc      435
Met Arg Arg Gly Leu Leu Tyr Asp Ser Asp Glu Glu Asp Glu Glu Arg
125                 130                 135

cct gcc cgc aag cgc cgc cag gtg gag cgg gcc acg gag gac ggc gag      483
Pro Ala Arg Lys Arg Arg Gln Val Glu Arg Ala Thr Glu Asp Gly Glu
140                 145                 150

gag gac gag gag atg att gag agc atc gag aac ctg gag gat ctc aaa      531
Glu Asp Glu Glu Met Ile Glu Ser Ile Glu Asn Leu Glu Asp Leu Lys
155                 160                 165

ggc cac tct gtg cgc gag tgg gtg agc atg gcg ggc ccc cgg ctg gag      579
Gly His Ser Val Arg Glu Trp Val Ser Met Ala Gly Pro Arg Leu Glu
170                 175                 180                 185

atc cac cac cgc ttc aag aac ttc ctg cgc act cac gtc gac agc cac      627
Ile His His Arg Phe Lys Asn Phe Leu Arg Thr His Val Asp Ser His
190                 195                 200

ggc cac aac gtc ttc aag gag cgc atc agc gac atg tgc aaa gag aac      675
Gly His Asn Val Phe Lys Glu Arg Ile Ser Asp Met Cys Lys Glu Asn
205                 210                 215

cgt gag agc ctg gtg gtg aac tat gag gac ttg gca gcc agg gag cac      723
Arg Glu Ser Leu Val Val Asn Tyr Glu Asp Leu Ala Ala Arg Glu His
220                 225                 230

gtg ctg gcc tac ttc ctg cct gag gca ccg gcg gag ctg ctg cag atc      771
Val Leu Ala Tyr Phe Leu Pro Glu Ala Pro Ala Glu Leu Leu Gln Ile
235                 240                 245

ttt gat gag gct gcc ctg gag gtg gta ctg gcc atg tac ccc aag tac      819
Phe Asp Glu Ala Ala Leu Glu Val Val Leu Ala Met Tyr Pro Lys Tyr
250                 255                 260                 265

gac cgc atc acc aac cac atc cat gtc cgc atc tcc cac ctg cct ctg      867
Asp Arg Ile Thr Asn His Ile His Val Arg Ile Ser His Leu Pro Leu
270                 275                 280

gtg gag gag ctg cgc tcg ctg agg cag ctg cat ctg aac cag ctg atc      915
Val Glu Glu Leu Arg Ser Leu Arg Gln Leu His Leu Asn Gln Leu Ile
285                 290                 295

cgc acc agt ggg gtg gtg acc agc tgc act ggc gtc ctg ccc cag ctc      963
Arg Thr Ser Gly Val Val Thr Ser Cys Thr Gly Val Leu Pro Gln Leu
300                 305                 310

agc atg gtc aag tac aac tgc aac aag tgc aat ttc gtc ctg ggt cct     1011
Ser Met Val Lys Tyr Asn Cys Asn Lys Cys Asn Phe Val Leu Gly Pro
315                 320                 325

ttc tgc cag tcc cag aac cag gag gtg aaa cca ggc tcc tgt cct gag     1059
Phe Cys Gln Ser Gln Asn Gln Glu Val Lys Pro Gly Ser Cys Pro Glu
330                 335                 340                 345

tgc cag tcg gcc ggc ccc ttt gag gtc aac atg gag gag acc atc tat     1107
Cys Gln Ser Ala Gly Pro Phe Glu Val Asn Met Glu Glu Thr Ile Tyr
350                 355                 360

cag aac tac cag cgt atc cga atc cag gag agt cca ggc aaa gtg gcg     1155
Gln Asn Tyr Gln Arg Ile Arg Ile Gln Glu Ser Pro Gly Lys Val Ala
365                 370                 375

gct ggc cgg ctg ccc cgc tcc aag gac gcc att ctc ctc gca gat ctg     1203
Ala Gly Arg Leu Pro Arg Ser Lys Asp Ala Ile Leu Leu Ala Asp Leu
380                 385                 390
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gtg gac agc tgc aac gca gga gac gag ata gag ctg act ggc atc tat     1251
Val Asp Ser Cys Asn Ala Gly Asp Glu Ile Glu Leu Thr Gly Ile Tyr
395                 400                 405

cac aac aac tat gat ggc tcc ctc aac act gcc aat ggc ttc cct gtc     1299
His Asn Asn Tyr Asp Gly Ser Leu Asn Thr Ala Asn Gly Phe Pro Val
410                 415                 420                 425

ttt gcc act gtc atc cta gcc aac cac gtg gcc aag aag gac aac aag     1347
Phe Ala Thr Val Ile Leu Ala Asn His Val Ala Lys Lys Asp Asn Lys
430                 435                 440

gtt gct gta ggg gaa ctg acc gat gaa gat gtg aag atg atc act agc     1395
Val Ala Val Gly Glu Leu Thr Asp Glu Asp Val Lys Met Ile Thr Ser
445                 450                 455

ctc tcc aag gat cag cag atc gga gag aag atc ttt gcc agc att gct     1443
Leu Ser Lys Asp Gln Gln Ile Gly Glu Lys Ile Phe Ala Ser Ile Ala
460                 465                 470

cct tcc atc tat ggt cat gaa gac atc aag aga ggc ctg gct ctg gcc     1491
Pro Ser Ile Tyr Gly His Glu Asp Ile Lys Arg Gly Leu Ala Leu Ala
475                 480                 485

ctg ttc gga ggg gag ccc aaa aac cca ggt ggc aag cac aag gta cgt     1539
Leu Phe Gly Gly Glu Pro Lys Asn Pro Gly Gly Lys His Lys Val Arg
490                 495                 500                 505

ggt gat atc aac gtg ctc ttg tgc gga gac cct ggc aca gcg aag tcg     1587
Gly Asp Ile Asn Val Leu Leu Cys Gly Asp Pro Gly Thr Ala Lys Ser
510                 515                 520

cag ttt ctc aag tat att gag aaa gtg tcc agc cga gcc atc ttc acc     1635
Gln Phe Leu Lys Tyr Ile Glu Lys Val Ser Ser Arg Ala Ile Phe Thr
525                 530                 535

act ggc cag ggg gcg tcg gct gtg ggc ctc acg gcg tat gtc cag cgg     1683
Thr Gly Gln Gly Ala Ser Ala Val Gly Leu Thr Ala Tyr Val Gln Arg
540                 545                 550

cac cct gtc agc agg gag tgg acc ttg gag gct ggg gcc ctg gtt ctg     1731
His Pro Val Ser Arg Glu Trp Thr Leu Glu Ala Gly Ala Leu Val Leu
555                 560                 565

gct gac cga gga gtg tgt ctc att gat gaa ttt gac aag atg aat gac     1779
Ala Asp Arg Gly Val Cys Leu Ile Asp Glu Phe Asp Lys Met Asn Asp
570                 575                 580                 585

cag gac aga acc agc atc cat gag gcc atg gag caa cag agc atc tcc     1827
Gln Asp Arg Thr Ser Ile His Glu Ala Met Glu Gln Gln Ser Ile Ser
590                 595                 600

atc tcg aag gct ggc atc gtc acc tcc ctg cag gct cgc tgc acg gtc     1875
Ile Ser Lys Ala Gly Ile Val Thr Ser Leu Gln Ala Arg Cys Thr Val
605                 610                 615

att gct gcc gcc aac ccc ata gga ggg cgc tac gac ccc tcg ctg act     1923
Ile Ala Ala Ala Asn Pro Ile Gly Gly Arg Tyr Asp Pro Ser Leu Thr
620                 625                 630

ttc tct gag aac gtg gac ctc aca gag ccc atc atc tca cgc ttt gac     1971
Phe Ser Glu Asn Val Asp Leu Thr Glu Pro Ile Ile Ser Arg Phe Asp
635                 640                 645

atc ctg tgt gtg gtg agg gac acc gtg gac cca gtc cag gac gag atg     2019
Ile Leu Cys Val Val Arg Asp Thr Val Asp Pro Val Gln Asp Glu Met
650                 655                 660                 665

ctg gcc cgc ttc gtg gtg ggc agc cac gtc aga cac cac ccc agc aac     2067
Leu Ala Arg Phe Val Val Gly Ser His Val Arg His His Pro Ser Asn
670                 675                 680

aag gag gag gag ggg ctg gcc aat ggc agc gct gct gag ccc gcc atg     2115
Lys Glu Glu Glu Gly Leu Ala Asn Gly Ser Ala Ala Glu Pro Ala Met
685                 690                 695
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ccc aac acg tat ggc gtg gag ccc ctg ccc cag gag gtc ctg aag aag     2163
Pro Asn Thr Tyr Gly Val Glu Pro Leu Pro Gln Glu Val Leu Lys Lys
700                 705                 710

tac atc atc tac gcc aag gag agg gtc cac ccg aag ctc aac cag atg     2211
Tyr Ile Ile Tyr Ala Lys Glu Arg Val His Pro Lys Leu Asn Gln Met
715                 720                 725

gac cag gac aag gtg gcc aag atg tac agt gac ctg agg aaa gaa tct     2259
Asp Gln Asp Lys Val Ala Lys Met Tyr Ser Asp Leu Arg Lys Glu Ser
730                 735                 740                 745

atg gcg aca ggc agc atc ccc att acg gtg cgg cac atc gag tcc atg     2307
Met Ala Thr Gly Ser Ile Pro Ile Thr Val Arg His Ile Glu Ser Met
750                 755                 760

atc cgc atg gcg gag gcc cac gcg cgc atc cat ctg cgg gac tat gtg     2355
Ile Arg Met Ala Glu Ala His Ala Arg Ile His Leu Arg Asp Tyr Val
765                 770                 775

atc gaa gac gac gtc aac atg gcc atc cgc gtg atg ctg gag agc ttc     2403
Ile Glu Asp Asp Val Asn Met Ala Ile Arg Val Met Leu Glu Ser Phe
780                 785                 790

ata gac aca cag aag ttc agc gtc atg cgc agc atg cgc aag act ttt     2451
Ile Asp Thr Gln Lys Phe Ser Val Met Arg Ser Met Arg Lys Thr Phe
795                 800                 805

gcc cgc tac ctt tca ttc cgg cgt gac aac aat gag ctg ttg ctc ttc     2499
Ala Arg Tyr Leu Ser Phe Arg Arg Asp Asn Asn Glu Leu Leu Leu Phe
810                 815                 820                 825

ata ctg aag cag tta gtg gca gag cag gtg aca tat cag cgc aac cgc     2547
Ile Leu Lys Gln Leu Val Ala Glu Gln Val Thr Tyr Gln Arg Asn Arg
830                 835                 840

ttt ggg gcc cag cag gac act att gag gtc cct gag aag gac ttg gtg     2595
Phe Gly Ala Gln Gln Asp Thr Ile Glu Val Pro Glu Lys Asp Leu Val
845                 850                 855

gat aag gct cgt cag atc aac atc cac aac ctc tct gca ttt tat gac     2643
Asp Lys Ala Arg Gln Ile Asn Ile His Asn Leu Ser Ala Phe Tyr Asp
860                 865                 870

agt gag ctc ttc agg atg aac aag ttc agc cac gac ctg aaa agg aaa     2691
Ser Glu Leu Phe Arg Met Asn Lys Phe Ser His Asp Leu Lys Arg Lys
875                 880                 885

atg atc ctg cag cag ttc tga ggccctatgc catccataag gattccttgg        2742
Met Ile Leu Gln Gln Phe  *
890                 895

gattctggtt tggggtggtc agtgccctct gtgctttatg gacacaaaac cagagcactt   2802

gatgaactcg gggtactagg gtcagggctt atagcaggat gtctggctgc acctggcatg   2862

actgtttgtt tctccaagcc tgctttgtgc ttctcacctt tgggtgggat gccttgccag   2922

tgtgtcttac ttggttgctg aacatcttgc cacctccgag tgctttgtct ccactcagta   2982

ccttggatca gagctgctga gttcaggatg cctgcgtgtg gtttaggtgt tagccttctt   3042

acatggatgt caggagagct gctgccctct tggcgtgagt tgcgtattca ggctgctttt   3102

gctgcctttg gccagagagc tggttgaaga tgtttgtaat cgttttcagt ctcctgcagg   3162

tttctgtgcc cctgtggtgg aagaggcacg acagtgccag cgcagcgttc tgggctcctc   3222

agtcgcaggg gtgggatgtg agtcatgcgg attatccact cgccacagtt atcagctgcc   3282

attgctccct gtctgtttcc ccactctctt atttgtgcat tcggtttggt ttctgtagtt   3342

ttaattttta ataaagttga ataaaatat                                     3371
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<210> SEQ ID NO 18

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<211> LENGTH: 895
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

Met Ala Ser Ser Pro Ala Gln Arg Arg Arg Gly Asn Asp Pro Leu Thr
1 5 10 15

Ser Ser Pro Gly Arg Ser Ser Arg Arg Thr Asp Ala Leu Thr Ser Ser
20 25 30

Pro Gly Arg Asp Leu Pro Pro Phe Glu Asp Glu Ser Glu Gly Leu Leu
35 40 45

Gly Thr Glu Gly Pro Leu Glu Glu Glu Glu Asp Gly Glu Glu Leu Ile
50 55 60

Gly Asp Gly Met Glu Arg Asp Tyr Arg Ala Ile Pro Glu Leu Asp Ala
65 70 75 80

Tyr Glu Ala Glu Gly Leu Ala Leu Asp Asp Glu Asp Val Glu Glu Leu
85 90 95

Thr Ala Ser Gln Arg Glu Ala Ala Glu Arg Ala Met Arg Gln Arg Asp
100 105 110

Arg Glu Ala Gly Arg Gly Leu Gly Arg Met Arg Arg Gly Leu Leu Tyr
115 120 125

Asp Ser Asp Glu Glu Asp Glu Glu Arg Pro Ala Arg Lys Arg Arg Gln
130 135 140

Val Glu Arg Ala Thr Glu Asp Gly Glu Glu Asp Glu Glu Met Ile Glu
145 150 155 160

Ser Ile Glu Asn Leu Glu Asp Leu Lys Gly His Ser Val Arg Glu Trp
165 170 175

Val Ser Met Ala Gly Pro Arg Leu Glu Ile His His Arg Phe Lys Asn
180 185 190

Phe Leu Arg Thr His Val Asp Ser His Gly His Asn Val Phe Lys Glu
195 200 205

Arg Ile Ser Asp Met Cys Lys Glu Asn Arg Glu Ser Leu Val Val Asn
210 215 220

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

Glu Ala Pro Ala Glu Leu Leu Gln Ile Phe Asp Glu Ala Ala Leu Glu
245 250 255

Val Val Leu Ala Met Tyr Pro Lys Tyr Asp Arg Ile Thr Asn His Ile
260 265 270

His Val Arg Ile Ser His Leu Pro Leu Val Glu Glu Leu Arg Ser Leu
275 280 285

Arg Gln Leu His Leu Asn Gln Leu Ile Arg Thr Ser Gly Val Val Thr
290 295 300

Ser Cys Thr Gly Val Leu Pro Gln Leu Ser Met Val Lys Tyr Asn Cys
305 310 315 320

Asn Lys Cys Asn Phe Val Leu Gly Pro Phe Cys Gln Ser Gln Asn Gln
325 330 335

Glu Val Lys Pro Gly Ser Cys Pro Glu Cys Gln Ser Ala Gly Pro Phe
340 345 350

Glu Val Asn Met Glu Glu Thr Ile Tyr Gln Asn Tyr Gln Arg Ile Arg
355 360 365

Ile Gln Glu Ser Pro Gly Lys Val Ala Ala Gly Arg Leu Pro Arg Ser

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370                 375                 380

Lys Asp Ala Ile Leu Leu Ala Asp Leu Val Asp Ser Cys Asn Ala Gly
385                 390                 395                 400

Asp Glu Ile Glu Leu Thr Gly Ile Tyr His Asn Asn Tyr Asp Gly Ser
405                 410                 415

Leu Asn Thr Ala Asn Gly Phe Pro Val Phe Ala Thr Val Ile Leu Ala
420                 425                 430

Asn His Val Ala Lys Lys Asp Asn Lys Val Ala Val Gly Glu Leu Thr
435                 440                 445

Asp Glu Asp Val Lys Met Ile Thr Ser Leu Ser Lys Asp Gln Gln Ile
450                 455                 460

Gly Glu Lys Ile Phe Ala Ser Ile Ala Pro Ser Ile Tyr Gly His Glu
465                 470                 475                 480

Asp Ile Lys Arg Gly Leu Ala Leu Ala Leu Phe Gly Gly Glu Pro Lys
485                 490                 495

Asn Pro Gly Gly Lys His Lys Val Arg Gly Asp Ile Asn Val Leu Leu
500                 505                 510

Cys Gly Asp Pro Gly Thr Ala Lys Ser Gln Phe Leu Lys Tyr Ile Glu
515                 520                 525

Lys Val Ser Ser Arg Ala Ile Phe Thr Thr Gly Gln Gly Ala Ser Ala
530                 535                 540

Val Gly Leu Thr Ala Tyr Val Gln Arg His Pro Val Ser Arg Glu Trp
545                 550                 555                 560

Thr Leu Glu Ala Gly Ala Leu Val Leu Ala Asp Arg Gly Val Cys Leu
565                 570                 575

Ile Asp Glu Phe Asp Lys Met Asn Asp Gln Asp Arg Thr Ser Ile His
580                 585                 590

Glu Ala Met Glu Gln Gln Ser Ile Ser Ile Ser Lys Ala Gly Ile Val
595                 600                 605

Thr Ser Leu Gln Ala Arg Cys Thr Val Ile Ala Ala Ala Asn Pro Ile
610                 615                 620

Gly Gly Arg Tyr Asp Pro Ser Leu Thr Phe Ser Glu Asn Val Asp Leu
625                 630                 635                 640

Thr Glu Pro Ile Ile Ser Arg Phe Asp Ile Leu Cys Val Val Arg Asp
645                 650                 655

Thr Val Asp Pro Val Gln Asp Glu Met Leu Ala Arg Phe Val Val Gly
660                 665                 670

Ser His Val Arg His His Pro Ser Asn Lys Glu Glu Glu Gly Leu Ala
675                 680                 685

Asn Gly Ser Ala Ala Glu Pro Ala Met Pro Asn Thr Tyr Gly Val Glu
690                 695                 700

Pro Leu Pro Gln Glu Val Leu Lys Lys Tyr Ile Ile Tyr Ala Lys Glu
705                 710                 715                 720

Arg Val His Pro Lys Leu Asn Gln Met Asp Gln Asp Lys Val Ala Lys
725                 730                 735

Met Tyr Ser Asp Leu Arg Lys Glu Ser Met Ala Thr Gly Ser Ile Pro
740                 745                 750

Ile Thr Val Arg His Ile Glu Ser Met Ile Arg Met Ala Glu Ala His
755                 760                 765

Ala Arg Ile His Leu Arg Asp Tyr Val Ile Glu Asp Asp Val Asn Met
770                 775                 780
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Ala Ile Arg Val Met Leu Glu Ser Phe Ile Asp Thr Gln Lys Phe Ser
785                 790                 795                 800

Val Met Arg Ser Met Arg Lys Thr Phe Ala Arg Tyr Leu Ser Phe Arg
805                 810                 815

Arg Asp Asn Asn Glu Leu Leu Leu Phe Ile Leu Lys Gln Leu Val Ala
820                 825                 830

Glu Gln Val Thr Tyr Gln Arg Asn Arg Phe Gly Ala Gln Gln Asp Thr
835                 840                 845

Ile Glu Val Pro Glu Lys Asp Leu Val Asp Lys Ala Arg Gln Ile Asn
850                 855                 860

Ile His Asn Leu Ser Ala Phe Tyr Asp Ser Glu Leu Phe Arg Met Asn
865                 870                 875                 880

Lys Phe Ser His Asp Leu Lys Arg Lys Met Ile Leu Gln Gln Phe
885                 890                 895


<210> SEQ ID NO 19
<211> LENGTH: 1721
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (58)...(1605)

<400> SEQUENCE: 19

gaattccctg gctgcttgaa tctgttctgc cccctcccca cccatttcac caccacc atg    60
Met
1

aca ccg ggc acc cag tct cct ttc ttc ctg ctg ctg ctc ctc aca gtg      108
Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Leu Thr Val
5                   10                  15

ctt aca gtt gtt aca ggt tct ggt cat gca agc tct acc cca ggt gga      156
Leu Thr Val Val Thr Gly Ser Gly His Ala Ser Ser Thr Pro Gly Gly
20                  25                  30

gaa aag gag act tcg gct acc cag aga agt tca gtg ccc agc tct act      204
Glu Lys Glu Thr Ser Ala Thr Gln Arg Ser Ser Val Pro Ser Ser Thr
35                  40                  45

gag aag aat gct gtg agt atg acc agc agc gta ctc tcc agc cac agc      252
Glu Lys Asn Ala Val Ser Met Thr Ser Ser Val Leu Ser Ser His Ser
50                  55                  60                  65

ccc ggt tca ggc tcc tcc acc act cag gga cag gat gtc act ctg gcc      300
Pro Gly Ser Gly Ser Ser Thr Thr Gln Gly Gln Asp Val Thr Leu Ala
70                  75                  80

ccg gcc acg gaa cca gct tca ggt tca gct gcc acc tgg gga cag gat      348
Pro Ala Thr Glu Pro Ala Ser Gly Ser Ala Ala Thr Trp Gly Gln Asp
85                  90                  95

gtc acc tcg gtc cca gtc acc agg cca gcc ctg ggc tcc acc acc ccg      396
Val Thr Ser Val Pro Val Thr Arg Pro Ala Leu Gly Ser Thr Thr Pro
100                 105                 110

cca gcc cac gat gtc acc tca gcc ccg gac aac aag cca gcc ccg ggc      444
Pro Ala His Asp Val Thr Ser Ala Pro Asp Asn Lys Pro Ala Pro Gly
115                 120                 125

tcc acc gcc ccc cca gcc cac ggt gtc acc tcg gcc ccg gac acc agg      492
Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr Arg
130                 135                 140                 145

ccg ccc ccg ggc tcc acc gcc ccc cca gcc cac ggt gtc acc tcg gcc      540
Pro Pro Pro Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser Ala
150                 155                 160
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ccg gac acc agg ccg ccc ccg ggc tcc acc gcg ccc gca gcc cac ggt      588
Pro Asp Thr Arg Pro Pro Pro Gly Ser Thr Ala Pro Ala Ala His Gly
165                 170                 175

gtc acc tcg gcc ccg gac acc agg ccg gcc ccg ggc tcc acc gcc ccc      636
Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro Gly Ser Thr Ala Pro
180                 185                 190

cca gcc cat ggt gtc acc tcg gcc ccg gac aac agg ccc gcc ttg gcg      684
Pro Ala His Gly Val Thr Ser Ala Pro Asp Asn Arg Pro Ala Leu Ala
195                 200                 205

tcc acc gcc cct cca gtc cac aat gtc acc tcg gcc tca ggc tct gca      732
Ser Thr Ala Pro Pro Val His Asn Val Thr Ser Ala Ser Gly Ser Ala
210                 215                 220                 225

tca ggc tca gct tct act ctg gtg cac aac ggc acc tct gcc agg gct      780
Ser Gly Ser Ala Ser Thr Leu Val His Asn Gly Thr Ser Ala Arg Ala
230                 235                 240

acc aca acc cca gcc agc aag agc act cca ttc tca att ccc agc cac      828
Thr Thr Thr Pro Ala Ser Lys Ser Thr Pro Phe Ser Ile Pro Ser His
245                 250                 255

cac tct gat act cct acc acc ctt gcc agc cat agc acc aag act gat      876
His Ser Asp Thr Pro Thr Thr Leu Ala Ser His Ser Thr Lys Thr Asp
260                 265                 270

gcc agt agc act cac cat agc acg gta cct cct ctc acc tcc tcc aat      924
Ala Ser Ser Thr His His Ser Thr Val Pro Pro Leu Thr Ser Ser Asn
275                 280                 285

cac agc act tct ccc cag ttg tct act ggg gtc tct ttc ttt ttc ctg      972
His Ser Thr Ser Pro Gln Leu Ser Thr Gly Val Ser Phe Phe Phe Leu
290                 295                 300                 305

tct ttt cac att tca aac ctc cag ttt aat tcc tct ctg gaa gat ccc     1020
Ser Phe His Ile Ser Asn Leu Gln Phe Asn Ser Ser Leu Glu Asp Pro
310                 315                 320

agc acc gac tac tac caa gag ctg cag aga gac att tct gaa atg ttt     1068
Ser Thr Asp Tyr Tyr Gln Glu Leu Gln Arg Asp Ile Ser Glu Met Phe
325                 330                 335

ttg cag att tat aaa caa ggg ggt ttt ctg ggc ctc tcc aat att aag     1116
Leu Gln Ile Tyr Lys Gln Gly Gly Phe Leu Gly Leu Ser Asn Ile Lys
340                 345                 350

ttc agg cca gga tct gtg gtg gta caa ttg act ctg gcc ttc cga gaa     1164
Phe Arg Pro Gly Ser Val Val Val Gln Leu Thr Leu Ala Phe Arg Glu
355                 360                 365

ggt acc atc aat gtc cac gac gtg gag aca cag ttc aat cag tat aaa     1212
Gly Thr Ile Asn Val His Asp Val Glu Thr Gln Phe Asn Gln Tyr Lys
370                 375                 380                 385

acg gaa gca gcc tct cga tat aac ctg acg atc tca gac gtc agc gtg     1260
Thr Glu Ala Ala Ser Arg Tyr Asn Leu Thr Ile Ser Asp Val Ser Val
390                 395                 400

agt gat gtg cca ttt cct ttc tct gcc cag tct ggg gct ggg gtg cca     1308
Ser Asp Val Pro Phe Pro Phe Ser Ala Gln Ser Gly Ala Gly Val Pro
405                 410                 415

ggc tgg ggc atc gcg ctg ctg gtg ctg gtc tgt gtt ctg gtt gcg ctg     1356
Gly Trp Gly Ile Ala Leu Leu Val Leu Val Cys Val Leu Val Ala Leu
420                 425                 430

gcc att gtc tat ctc att gcc ttg gct gtc tgt cag tgc cgc cga aag     1404
Ala Ile Val Tyr Leu Ile Ala Leu Ala Val Cys Gln Cys Arg Arg Lys
435                 440                 445

aac tac ggg cag ctg gac atc ttt cca gcc cgg gat acc tac cat cct     1452
Asn Tyr Gly Gln Leu Asp Ile Phe Pro Ala Arg Asp Thr Tyr His Pro
450                 455                 460                 465
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atg agc gag tac ccc acc tac cac acc cat ggg cgc tat gtg ccc cct     1500
Met Ser Glu Tyr Pro Thr Tyr His Thr His Gly Arg Tyr Val Pro Pro
470                 475                 480

agc agt acc gat cgt agc ccc tat gag aag gtt tct gca ggt aat ggt     1548
Ser Ser Thr Asp Arg Ser Pro Tyr Glu Lys Val Ser Ala Gly Asn Gly
485                 490                 495

ggc agc agc ctc tct tac aca aac cca gca gtg gca gcc act tct gcc     1596
Gly Ser Ser Leu Ser Tyr Thr Asn Pro Ala Val Ala Ala Thr Ser Ala
500                 505                 510

aac ttg tag gggcacgtcg ccctctgagc tgagtggcca gccagtgcca             1645
Asn Leu  *
515

ttccactcca ctcagggctc tctgggccag tcctcctggg agcccccacc acaacacttc   1705

ccaggcatgg aattcc                                                   1721


<210> SEQ ID NO 20
<211> LENGTH: 515
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 20

Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Leu Thr
1               5                   10                  15

Val Leu Thr Val Val Thr Gly Ser Gly His Ala Ser Ser Thr Pro Gly
20                  25                  30

Gly Glu Lys Glu Thr Ser Ala Thr Gln Arg Ser Ser Val Pro Ser Ser
35                  40                  45

Thr Glu Lys Asn Ala Val Ser Met Thr Ser Ser Val Leu Ser Ser His
50                  55                  60

Ser Pro Gly Ser Gly Ser Ser Thr Thr Gln Gly Gln Asp Val Thr Leu
65                  70                  75                  80

Ala Pro Ala Thr Glu Pro Ala Ser Gly Ser Ala Ala Thr Trp Gly Gln
85                  90                  95

Asp Val Thr Ser Val Pro Val Thr Arg Pro Ala Leu Gly Ser Thr Thr
100                 105                 110

Pro Pro Ala His Asp Val Thr Ser Ala Pro Asp Asn Lys Pro Ala Pro
115                 120                 125

Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr
130                 135                 140

Arg Pro Pro Pro Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser
145                 150                 155                 160

Ala Pro Asp Thr Arg Pro Pro Pro Gly Ser Thr Ala Pro Ala Ala His
165                 170                 175

Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro Gly Ser Thr Ala
180                 185                 190

Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Asn Arg Pro Ala Leu
195                 200                 205

Ala Ser Thr Ala Pro Pro Val His Asn Val Thr Ser Ala Ser Gly Ser
210                 215                 220

Ala Ser Gly Ser Ala Ser Thr Leu Val His Asn Gly Thr Ser Ala Arg
225                 230                 235                 240

Ala Thr Thr Thr Pro Ala Ser Lys Ser Thr Pro Phe Ser Ile Pro Ser
245                 250                 255
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His His Ser Asp Thr Pro Thr Thr Leu Ala Ser His Ser Thr Lys Thr
260                 265                 270

Asp Ala Ser Ser Thr His His Ser Thr Val Pro Pro Leu Thr Ser Ser
275                 280                 285

Asn His Ser Thr Ser Pro Gln Leu Ser Thr Gly Val Ser Phe Phe Phe
290                 295                 300

Leu Ser Phe His Ile Ser Asn Leu Gln Phe Asn Ser Ser Leu Glu Asp
305                 310                 315                 320

Pro Ser Thr Asp Tyr Tyr Gln Glu Leu Gln Arg Asp Ile Ser Glu Met
325                 330                 335

Phe Leu Gln Ile Tyr Lys Gln Gly Gly Phe Leu Gly Leu Ser Asn Ile
340                 345                 350

Lys Phe Arg Pro Gly Ser Val Val Val Gln Leu Thr Leu Ala Phe Arg
355                 360                 365

Glu Gly Thr Ile Asn Val His Asp Val Glu Thr Gln Phe Asn Gln Tyr
370                 375                 380

Lys Thr Glu Ala Ala Ser Arg Tyr Asn Leu Thr Ile Ser Asp Val Ser
385                 390                 395                 400

Val Ser Asp Val Pro Phe Pro Phe Ser Ala Gln Ser Gly Ala Gly Val
405                 410                 415

Pro Gly Trp Gly Ile Ala Leu Leu Val Leu Val Cys Val Leu Val Ala
420                 425                 430

Leu Ala Ile Val Tyr Leu Ile Ala Leu Ala Val Cys Gln Cys Arg Arg
435                 440                 445

Lys Asn Tyr Gly Gln Leu Asp Ile Phe Pro Ala Arg Asp Thr Tyr His
450                 455                 460

Pro Met Ser Glu Tyr Pro Thr Tyr His Thr His Gly Arg Tyr Val Pro
465                 470                 475                 480

Pro Ser Ser Thr Asp Arg Ser Pro Tyr Glu Lys Val Ser Ala Gly Asn
485                 490                 495

Gly Gly Ser Ser Leu Ser Tyr Thr Asn Pro Ala Val Ala Ala Thr Ser
500                 505                 510

Ala Asn Leu
515


<210> SEQ ID NO 21
<211> LENGTH: 1061
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (189)...(758)

<400> SEQUENCE: 21

tgccctgcgc ccgcaacccg agccgcaccc gccgcggacg gagcccatgc gcggggcgaa     60

ccgcgcgccc ccgcccccgc cccgccccgg cctcggcccc ggccctggcc ccgggggcag    120

tcgcgcctgt gaacggtggg gcaggagacc ctgtaggagg accccgggcc gcaggcccct    180

gaggagcg atg acg gaa tat aag ctg gtg gtg gtg ggc gcc ggc ggt gtg     230
Met Thr Glu Tyr Lys Leu Val Val Val Gly Ala Gly Gly Val
1               5                   10

ggc aag agt gcg ctg acc atc cag ctg atc cag aac cat ttt gtg gac      278
Gly Lys Ser Ala Leu Thr Ile Gln Leu Ile Gln Asn His Phe Val Asp
15                  20                  25                  30
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gaa tac gac ccc act ata gag gat tcc tac cgg aag cag gtg gtc att      326
Glu Tyr Asp Pro Thr Ile Glu Asp Ser Tyr Arg Lys Gln Val Val Ile
35                  40                  45

gat ggg gag acg tgc ctg ttg gac atc ctg gat acc gcc ggc cag gag      374
Asp Gly Glu Thr Cys Leu Leu Asp Ile Leu Asp Thr Ala Gly Gln Glu
50                  55                  60

gag tac agc gcc atg cgg gac cag tac atg cgc acc ggg gag ggc ttc      422
Glu Tyr Ser Ala Met Arg Asp Gln Tyr Met Arg Thr Gly Glu Gly Phe
65                  70                  75

ctg tgt gtg ttt gcc atc aac aac acc aag tct ttt gag gac atc cac      470
Leu Cys Val Phe Ala Ile Asn Asn Thr Lys Ser Phe Glu Asp Ile His
80                  85                  90

cag tac agg gag cag atc aaa cgg gtg aag gac tcg gat gac gtg ccc      518
Gln Tyr Arg Glu Gln Ile Lys Arg Val Lys Asp Ser Asp Asp Val Pro
95                 100                 105                 110

atg gtg ctg gtg ggg aac aag tgt gac ctg gct gca cgc act gtg gaa      566
Met Val Leu Val Gly Asn Lys Cys Asp Leu Ala Ala Arg Thr Val Glu
115                 120                 125

tct cgg cag gct cag gac ctc gcc cga agc tac ggc atc ccc tac atc      614
Ser Arg Gln Ala Gln Asp Leu Ala Arg Ser Tyr Gly Ile Pro Tyr Ile
130                 135                 140

gag acc tcg gcc aag acc cgg cag gga gtg gag gat gcc ttc tac acg      662
Glu Thr Ser Ala Lys Thr Arg Gln Gly Val Glu Asp Ala Phe Tyr Thr
145                 150                 155

ttg gtg cgt gag atc cgg cag cac aag ctg cgg aag ctg aac cct cct      710
Leu Val Arg Glu Ile Arg Gln His Lys Leu Arg Lys Leu Asn Pro Pro
160                 165                 170

gat gag agt ggc ccc ggc tgc atg agc tgc aag tgt gtg ctc tcc tga      758
Asp Glu Ser Gly Pro Gly Cys Met Ser Cys Lys Cys Val Leu Ser  *
175                 180                 185

cgcagcacaa gctcaggaca tggaggtgcc ggatgcagga aggaggtgca gacggaagga    818

ggaggaagga aggacggaag caaggaagga aggaagggct gctggagccc agtcaccccg    878

ggaccgtggg ccgaggtgac tgcagaccct cccagggagg ctgtgcacag actgtcttga    938

acatcccaaa tgccaccgga accccagccc ttagctcccc tcccaggcct ctgtgggccc    998

ttgtcgggca cagatgggat cacagtaaat tattggatgg tcttgaaaaa aaaaaaaaaa   1058

aaa                                                                 1061
```

<210> SEQ ID NO 22
<211> LENGTH: 189
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 22

```
Met Thr Glu Tyr Lys Leu Val Val Val Gly Ala Gly Gly Val Gly Lys
1               5                   10                  15

Ser Ala Leu Thr Ile Gln Leu Ile Gln Asn His Phe Val Asp Glu Tyr
20                  25                  30

Asp Pro Thr Ile Glu Asp Ser Tyr Arg Lys Gln Val Val Ile Asp Gly
35                  40                  45

Glu Thr Cys Leu Leu Asp Ile Leu Asp Thr Ala Gly Gln Glu Glu Tyr
50                  55                  60

Ser Ala Met Arg Asp Gln Tyr Met Arg Thr Gly Glu Gly Phe Leu Cys
65                  70                  75                  80
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Val Phe Ala Ile Asn Asn Thr Lys Ser Phe Glu Asp Ile His Gln Tyr
85                  90                  95

Arg Glu Gln Ile Lys Arg Val Lys Asp Ser Asp Asp Val Pro Met Val
100                 105                 110

Leu Val Gly Asn Lys Cys Asp Leu Ala Ala Arg Thr Val Glu Ser Arg
115                 120                 125

Gln Ala Gln Asp Leu Ala Arg Ser Tyr Gly Ile Pro Tyr Ile Glu Thr
130                 135                 140

Ser Ala Lys Thr Arg Gln Gly Val Glu Asp Ala Phe Tyr Thr Leu Val
145                 150                 155                 160

Arg Glu Ile Arg Gln His Lys Leu Arg Lys Leu Asn Pro Pro Asp Glu
165                 170                 175

Ser Gly Pro Gly Cys Met Ser Cys Lys Cys Val Leu Ser
180                 185


<210> SEQ ID NO 23
<211> LENGTH: 4145
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (450)...(2060)

<400> SEQUENCE: 23

caaacaagtg cggccatttc accagcccag gctggcttct gctgttgact ggctgtggca     60

cctcaagcag ccccttccc ctctagcctc agtttatcac cgcaagagct accattcatc    120

tagcacaacc tgaccatcct cacactggtc agttccaacc ttcccaggaa tcttctgtgg    180

ccatgttcac tccggtttta cagaacagag aacagaagct cagagaagtg aagcaacttg    240

cccagctatg agagacagag ccaggatttg aaaccagatg aggacgctga ggcccagaga    300

gggaaagcca cttgcctagg gacacacagc ggggagaggt ggagcagggc ctctatttcg    360

agacccctga ctccacacct ggtgtttgtg ccaagacccc aggctgcctc ccaggtcctc    420

tgggacagcc cctgccttct accaggacc atg ggt agc aac aag agc aag ccc      473
Met Gly Ser Asn Lys Ser Lys Pro
1               5

aag gat gcc agc cag cgg cgc cgc agc ctg gag ccc gcc gag aac gtg      521
Lys Asp Ala Ser Gln Arg Arg Arg Ser Leu Glu Pro Ala Glu Asn Val
10                  15                  20

cac ggc gct ggc ggg ggc gct ttc ccc gcc tcg cag acc ccc agc aag      569
His Gly Ala Gly Gly Gly Ala Phe Pro Ala Ser Gln Thr Pro Ser Lys
25                  30                  35                  40

cca gcc tcg gcc gac ggc cac cgc ggc ccc agc gcg gcc ttc gcc ccc      617
Pro Ala Ser Ala Asp Gly His Arg Gly Pro Ser Ala Ala Phe Ala Pro
45                  50                  55

gcg gcc gcc gag ccc aag ctg ttc gga ggc ttc aac tcc tcg gac acc      665
Ala Ala Ala Glu Pro Lys Leu Phe Gly Gly Phe Asn Ser Ser Asp Thr
60                  65                  70

gtc acc tcc ccg cag agg gcg ggc ccg ctg gcc ggt gga gtg acc acc      713
Val Thr Ser Pro Gln Arg Ala Gly Pro Leu Ala Gly Gly Val Thr Thr
75                  80                  85

ttt gtg gcc ctc tat gac tat gag tct agg acg gag aca gac ctg tcc      761
Phe Val Ala Leu Tyr Asp Tyr Glu Ser Arg Thr Glu Thr Asp Leu Ser
90                  95                 100

ttc aag aaa ggc gag cgg ctc cag att gtc aac aac aca gag gga gac      809
Phe Lys Lys Gly Glu Arg Leu Gln Ile Val Asn Asn Thr Glu Gly Asp
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105                 110                 115                 120

tgg tgg ctg gcc cac tcg ctc agc aca gga cag aca ggc tac atc ccc      857
Trp Trp Leu Ala His Ser Leu Ser Thr Gly Gln Thr Gly Tyr Ile Pro
125                 130                 135

agc aac tac gtg gcg ccc tcc gac tcc atc cag gct gag gag tgg tat      905
Ser Asn Tyr Val Ala Pro Ser Asp Ser Ile Gln Ala Glu Glu Trp Tyr
140                 145                 150

ttt ggc aag atc acc aga cgg gag tca gag cgg tta ctg ctc aat gca      953
Phe Gly Lys Ile Thr Arg Arg Glu Ser Glu Arg Leu Leu Leu Asn Ala
155                 160                 165

gag aac ccg aga ggg acc ttc ctc gtg cga gaa agt gag acc acg aaa     1001
Glu Asn Pro Arg Gly Thr Phe Leu Val Arg Glu Ser Glu Thr Thr Lys
170                 175                 180

ggt gcc tac tgc ctc tca gtg tct gac ttc gac aac gcc aag ggc ctc     1049
Gly Ala Tyr Cys Leu Ser Val Ser Asp Phe Asp Asn Ala Lys Gly Leu
185                 190                 195                 200

aac gtg aag cac tac aag atc cgc aag ctg gac agc ggc ggc ttc tac     1097
Asn Val Lys His Tyr Lys Ile Arg Lys Leu Asp Ser Gly Gly Phe Tyr
205                 210                 215

atc acc tcc cgc acc cag ttc aac agc ctg cag cag ctg gtg gcc tac     1145
Ile Thr Ser Arg Thr Gln Phe Asn Ser Leu Gln Gln Leu Val Ala Tyr
220                 225                 230

tac tcc aaa cac gcc gat ggc ctg tgc cac cgc ctc acc acc gtg tgc     1193
Tyr Ser Lys His Ala Asp Gly Leu Cys His Arg Leu Thr Thr Val Cys
235                 240                 245

ccc acg tcc aag ccg cag act cag ggc ctg gcc aag gat gcc tgg gag     1241
Pro Thr Ser Lys Pro Gln Thr Gln Gly Leu Ala Lys Asp Ala Trp Glu
250                 255                 260

atc cct cgg gag tcg ctg cgg ctg gag gtc aag ctg ggc cag ggc tgc     1289
Ile Pro Arg Glu Ser Leu Arg Leu Glu Val Lys Leu Gly Gln Gly Cys
265                 270                 275                 280

ttt ggc gag gtg tgg atg ggg acc tgg aac ggt acc acc agg gtg gcc     1337
Phe Gly Glu Val Trp Met Gly Thr Trp Asn Gly Thr Thr Arg Val Ala
285                 290                 295

atc aaa acc ctg aag cct ggc acg atg tct cca gag gcc ttc ctg cag     1385
Ile Lys Thr Leu Lys Pro Gly Thr Met Ser Pro Glu Ala Phe Leu Gln
300                 305                 310

gag gcc cag gtc atg aag aag ctg agg cat gag aag ctg gtg cag ttg     1433
Glu Ala Gln Val Met Lys Lys Leu Arg His Glu Lys Leu Val Gln Leu
315                 320                 325

tat gct gtg gtt tca gag gag ccc att tac atc gtc acg gag tac atg     1481
Tyr Ala Val Val Ser Glu Glu Pro Ile Tyr Ile Val Thr Glu Tyr Met
330                 335                 340

agc aag ggg agt ttg ctg gac ttt ctc aag ggg gag aca ggc aag tac     1529
Ser Lys Gly Ser Leu Leu Asp Phe Leu Lys Gly Glu Thr Gly Lys Tyr
345                 350                 355                 360

ctg cgg ctg cct cag ctg gtg gac atg gct gct cag atc gcc tca ggc     1577
Leu Arg Leu Pro Gln Leu Val Asp Met Ala Ala Gln Ile Ala Ser Gly
365                 370                 375

atg gcg tac gtg gag cgg atg aac tac gtc cac cgg gac ctt cgt gca     1625
Met Ala Tyr Val Glu Arg Met Asn Tyr Val His Arg Asp Leu Arg Ala
380                 385                 390

gcc aac atc ctg gtg gga gag aac ctg gtg tgc aaa gtg gcc gac ttt     1673
Ala Asn Ile Leu Val Gly Glu Asn Leu Val Cys Lys Val Ala Asp Phe
395                 400                 405

ggg ctg gct cgg ctc att gaa gac aat gag tac acg gcg cgg caa ggt     1721
Gly Leu Ala Arg Leu Ile Glu Asp Asn Glu Tyr Thr Ala Arg Gln Gly
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410                 415                 420

gcc aaa ttc ccc atc aag tgg acg gct cca gaa gct gcc ctc tat ggc     1769
Ala Lys Phe Pro Ile Lys Trp Thr Ala Pro Glu Ala Ala Leu Tyr Gly
425                 430                 435                 440

cgc ttc acc atc aag tcg gac gtg tgg tcc ttc ggg atc ctg ctg act     1817
Arg Phe Thr Ile Lys Ser Asp Val Trp Ser Phe Gly Ile Leu Leu Thr
445                 450                 455

gag ctc acc aca aag gga cgg gtg ccc tac cct ggg atg gtg aac cgc     1865
Glu Leu Thr Thr Lys Gly Arg Val Pro Tyr Pro Gly Met Val Asn Arg
460                 465                 470

gag gtg ctg gac cag gtg gag cgg ggc tac cgg atg ccc tgc ccg ccg     1913
Glu Val Leu Asp Gln Val Glu Arg Gly Tyr Arg Met Pro Cys Pro Pro
475                 480                 485

gag tgt ccc gag tcc ctg cac gac ctc atg tgc cag tgc tgg cgg aag     1961
Glu Cys Pro Glu Ser Leu His Asp Leu Met Cys Gln Cys Trp Arg Lys
490                 495                 500

gag cct gag gag cgg ccc acc ttc gag tac ctg cag gcc ttc ctg gag     2009
Glu Pro Glu Glu Arg Pro Thr Phe Glu Tyr Leu Gln Ala Phe Leu Glu
505                 510                 515                 520

gac tac ttc acg tcc acc gag ccc cag tac cag ccc ggg gag aac ctc     2057
Asp Tyr Phe Thr Ser Thr Glu Pro Gln Tyr Gln Pro Gly Glu Asn Leu
525                 530                 535

tag gcacaggcgg gcccagaccg gcttctcggc ttggatcctg ggctgggtgg          2110
*

cccctgtctc ggggcttgcc ccactctgcc tgcctgctgt tggtcctctc tctgtggggc   2170

tgaattgcca ggggcgaggc ccttcctctt tggtggcatg gaaggggctt ctggacctag   2230

ggtggcctga gagggcggtg ggtatgcgag accagcacgg tgactctgtc cagctcccgc   2290

tgtggccgca cgcctctccc tgcactccct cctggagctc tgtgggtctc tggaagagga   2350

accaggagaa gggctggggc cggggctgag ggtgcccttt tccagcctca gcctactccg   2410

ctcactgaac tccttcccca cttctgtgcc acccccggtc tatgtcgaga gctggccaaa   2470

gagcctttcc aaagaggagc gatgggcccc tggccccgcc tgcctgccac cctgcccctt   2530

gccatccatt ctggaaacac ctgtaggcag aggctgccga gacagaccct ctgccgctgc   2590

ttccaggctg ggcagcacaa ggccttgcct ggcctgatga tggtgggtgg gtgggatgag   2650

taccccctca aaccctgccc tccttagacc tgagggaccc ttcgagatca tcacttcctt   2710

gcccccattt cacccatggg gagacagttg agagcgggga tgtgacatgc ccaaggccac   2770

ggagcagttc agagtggagg cgggcttgga acccggtgct ccctctgtca tcctcaggaa   2830

ccaacaattc gtcggaggca tcatggaaag actgggacag cccaggaaac aaggggtctg   2890

aggatgcatt cgagatggca gattcccact gccgctgccc gctcagccca gctgttggga   2950

acagcatgga ggcagatgtg gggctgagct ggggaatcag ggtaaaaggt gcaggtgtgg   3010

agagagaggc ttcaatcggc ttgtgggtga tgtttgacct tcagagccag ccggctatga   3070

aagggagcga gcccctcggc tctggaggca atcaagcaga catagaagag ccaagagtcc   3130

aggaggccct ggtcctggcc tccttccccg tactttgtcc cgtggcattt caattcctgg   3190

ccctgttctc ctccccaagt cggcaccctt taactcatga ggagggaaaa gagtgcctaa   3250

gcgggggtga aagaggacgt gttacccact gccatgcacc aggactggct gtgtaacctt   3310

gggtggcccc tgctgtctct ctgggctgca gagtctgccc cacatgtggc catggcctct   3370

gcaactgctc agctctggtc caggccctgt ggcaggacac acatggtgag cctagccctg   3430
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ggacatcagg agactgggct ctggctctgt tcggcctttg ggtgtgtggt ggattctccc   3490

tgggcctcag tgtgcccatc tgtaaagggg cagctgacag tttgtggcat cttgccaagg   3550

gtccctgtgt gtgtgtatgt gtgtgcatgt gtgcgtgtct ccatgtgcgt ccatatttaa   3610

catgtaaaaa tgtccccccc gctccgtccc ccaaacatgt tgtacatttc accatggccc   3670

cctcatcata gcaataacat tcccactgcc aggggttctt gagccagcca ggccctgcca   3730

gtggggaagg aggccaagca gtgcctgcct atgaaatttc aacttttcct ttcatacgtc   3790

tttattaccc aagtcttctc ccgtccattc cagtcaaatc tgggctcact caccccagcg   3850

agctctcaaa tccctctcca actgcctaag gccctttgtg taaggtgtct taatactgtc   3910

ctttttttttt ttttaacagt gttttgtaga tttcagatga ctatgcagag gcctgggggga   3970

cccctggctc tgggccgggc ctggggctcc gaaattccaa ggcccagact tgcgggggggt   4030

ggggggggtat ccagaattgg ttgtaaatac tttgcatatt gtctgattaa acacaaacag   4090

acctcagaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaa        4145
```

<210> SEQ ID NO 24
<211> LENGTH: 536
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 24

```
Met Gly Ser Asn Lys Ser Lys Pro Lys Asp Ala Ser Gln Arg Arg Arg
1               5                   10                  15

Ser Leu Glu Pro Ala Glu Asn Val His Gly Ala Gly Gly Gly Ala Phe
20                  25                  30

Pro Ala Ser Gln Thr Pro Ser Lys Pro Ala Ser Ala Asp Gly His Arg
35                  40                  45

Gly Pro Ser Ala Ala Phe Ala Pro Ala Ala Ala Glu Pro Lys Leu Phe
50                  55                  60

Gly Gly Phe Asn Ser Ser Asp Thr Val Thr Ser Pro Gln Arg Ala Gly
65                  70                  75                  80

Pro Leu Ala Gly Gly Val Thr Thr Phe Val Ala Leu Tyr Asp Tyr Glu
85                  90                  95

Ser Arg Thr Glu Thr Asp Leu Ser Phe Lys Lys Gly Glu Arg Leu Gln
100                 105                 110

Ile Val Asn Asn Thr Glu Gly Asp Trp Trp Leu Ala His Ser Leu Ser
115                 120                 125

Thr Gly Gln Thr Gly Tyr Ile Pro Ser Asn Tyr Val Ala Pro Ser Asp
130                 135                 140

Ser Ile Gln Ala Glu Glu Trp Tyr Phe Gly Lys Ile Thr Arg Arg Glu
145                 150                 155                 160

Ser Glu Arg Leu Leu Leu Asn Ala Glu Asn Pro Arg Gly Thr Phe Leu
165                 170                 175

Val Arg Glu Ser Glu Thr Thr Lys Gly Ala Tyr Cys Leu Ser Val Ser
180                 185                 190

Asp Phe Asp Asn Ala Lys Gly Leu Asn Val Lys His Tyr Lys Ile Arg
195                 200                 205

Lys Leu Asp Ser Gly Gly Phe Tyr Ile Thr Ser Arg Thr Gln Phe Asn
210                 215                 220

Ser Leu Gln Gln Leu Val Ala Tyr Tyr Ser Lys His Ala Asp Gly Leu
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225                 230                 235                 240

Cys His Arg Leu Thr Thr Val Cys Pro Thr Ser Lys Pro Gln Thr Gln
245                 250                 255

Gly Leu Ala Lys Asp Ala Trp Glu Ile Pro Arg Glu Ser Leu Arg Leu
260                 265                 270

Glu Val Lys Leu Gly Gln Gly Cys Phe Gly Glu Val Trp Met Gly Thr
275                 280                 285

Trp Asn Gly Thr Thr Arg Val Ala Ile Lys Thr Leu Lys Pro Gly Thr
290                 295                 300

Met Ser Pro Glu Ala Phe Leu Gln Glu Ala Gln Val Met Lys Lys Leu
305                 310                 315                 320

Arg His Glu Lys Leu Val Gln Leu Tyr Ala Val Val Ser Glu Glu Pro
325                 330                 335

Ile Tyr Ile Val Thr Glu Tyr Met Ser Lys Gly Ser Leu Leu Asp Phe
340                 345                 350

Leu Lys Gly Glu Thr Gly Lys Tyr Leu Arg Leu Pro Gln Leu Val Asp
355                 360                 365

Met Ala Ala Gln Ile Ala Ser Gly Met Ala Tyr Val Glu Arg Met Asn
370                 375                 380

Tyr Val His Arg Asp Leu Arg Ala Ala Asn Ile Leu Val Gly Glu Asn
385                 390                 395                 400

Leu Val Cys Lys Val Ala Asp Phe Gly Leu Ala Arg Leu Ile Glu Asp
405                 410                 415

Asn Glu Tyr Thr Ala Arg Gln Gly Ala Lys Phe Pro Ile Lys Trp Thr
420                 425                 430

Ala Pro Glu Ala Ala Leu Tyr Gly Arg Phe Thr Ile Lys Ser Asp Val
435                 440                 445

Trp Ser Phe Gly Ile Leu Leu Thr Glu Leu Thr Thr Lys Gly Arg Val
450                 455                 460

Pro Tyr Pro Gly Met Val Asn Arg Glu Val Leu Asp Gln Val Glu Arg
465                 470                 475                 480

Gly Tyr Arg Met Pro Cys Pro Pro Glu Cys Pro Glu Ser Leu His Asp
485                 490                 495

Leu Met Cys Gln Cys Trp Arg Lys Glu Pro Glu Glu Arg Pro Thr Phe
500                 505                 510

Glu Tyr Leu Gln Ala Phe Leu Glu Asp Tyr Phe Thr Ser Thr Glu Pro
515                 520                 525

Gln Tyr Gln Pro Gly Glu Asn Leu
530                 535


<210> SEQ ID NO 25
<211> LENGTH: 1333
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (101)...(1030)

<400> SEQUENCE: 25

ggggaggccg cctggttttc ctccctcctt ctgcacgtct gctggggtct cttcctctcc     60

aggccttgcc gtccccctgg cctctcttcc cagctcacac atg aag atg cac ttg      115
Met Lys Met His Leu
1               5
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caa agg gct ctg gtg gtc ctg gcc ctg ctg aac ttt gcc acg gtc agc      163
Gln Arg Ala Leu Val Val Leu Ala Leu Leu Asn Phe Ala Thr Val Ser
10                  15                  20

ctc tct ctg tcc act tgc acc acc ttg gac ttc ggc cac atc aag aag      211
Leu Ser Leu Ser Thr Cys Thr Thr Leu Asp Phe Gly His Ile Lys Lys
25                  30                  35

aag agg gtg gaa gcc att agg gga cag atc ttg agc aag ctc agg ctc      259
Lys Arg Val Glu Ala Ile Arg Gly Gln Ile Leu Ser Lys Leu Arg Leu
40                  45                  50

acc agc ccc cct gag cca acg gtg atg acc cac gtc ccc tat cag gtc      307
Thr Ser Pro Pro Glu Pro Thr Val Met Thr His Val Pro Tyr Gln Val
55                  60                  65

ctg gcc ctt tac aac agc acc cgg gag ctg ctg gag gag atg cat ggg      355
Leu Ala Leu Tyr Asn Ser Thr Arg Glu Leu Leu Glu Glu Met His Gly
70                  75                  80                  85

gag agg gag gaa ggc tgc acc cag gaa aac acc gag tcg gaa tac tat      403
Glu Arg Glu Glu Gly Cys Thr Gln Glu Asn Thr Glu Ser Glu Tyr Tyr
90                  95                 100

gcc aaa gaa atc cat aaa ttc gac atg atc cag ggg ctg gcg gag cac      451
Ala Lys Glu Ile His Lys Phe Asp Met Ile Gln Gly Leu Ala Glu His
105                 110                 115

aac gaa ctg gct gtc tgc cct aaa gga att acc tcc aag gtt ttc cgc      499
Asn Glu Leu Ala Val Cys Pro Lys Gly Ile Thr Ser Lys Val Phe Arg
120                 125                 130

ttc aat gtg tcc tca gtg gag aaa aat aga acc aac cta ttc cga gca      547
Phe Asn Val Ser Ser Val Glu Lys Asn Arg Thr Asn Leu Phe Arg Ala
135                 140                 145

gaa ttc cgg gtc ttg cgg gtg ccc aac ccc agc tct aag cgg aat gag      595
Glu Phe Arg Val Leu Arg Val Pro Asn Pro Ser Ser Lys Arg Asn Glu
150                 155                 160                 165

cag agg atc gag ctc ttc cag atc ctt cgg cca gat gag cac att gcc      643
Gln Arg Ile Glu Leu Phe Gln Ile Leu Arg Pro Asp Glu His Ile Ala
170                 175                 180

aaa cag cgc tat atc ggt ggc aag aat ctg ccc aca cgg ggc act gcc      691
Lys Gln Arg Tyr Ile Gly Gly Lys Asn Leu Pro Thr Arg Gly Thr Ala
185                 190                 195

gag tgg ctg tcc ttt gat gtc act gac act gtg cgt gag tgg ctg ttg      739
Glu Trp Leu Ser Phe Asp Val Thr Asp Thr Val Arg Glu Trp Leu Leu
200                 205                 210

aga aga gag tcc aac tta ggt cta gaa atc agc att cac tgt cca tgt      787
Arg Arg Glu Ser Asn Leu Gly Leu Glu Ile Ser Ile His Cys Pro Cys
215                 220                 225

cac acc ttt cag ccc aat gga gat atc ctg gaa aac att cac gag gtg      835
His Thr Phe Gln Pro Asn Gly Asp Ile Leu Glu Asn Ile His Glu Val
230                 235                 240                 245

atg gaa atc aaa ttc aaa ggc gtg gac aat gag gat gac cat ggc cgt      883
Met Glu Ile Lys Phe Lys Gly Val Asp Asn Glu Asp Asp His Gly Arg
250                 255                 260

gga gat ctg ggg cgc ctc aag aag cag aag gat cac cac aac cct cat      931
Gly Asp Leu Gly Arg Leu Lys Lys Gln Lys Asp His His Asn Pro His
265                 270                 275

cta atc ctc atg atg att ccc cca cac cgg ctc gac aac ccg ggc cag      979
Leu Ile Leu Met Met Ile Pro Pro His Arg Leu Asp Asn Pro Gly Gln
280                 285                 290

ggg ggt cag agg aag aag cgg gct ttg gac acc aat tac tgc ttc cgg     1027
Gly Gly Gln Arg Lys Lys Arg Ala Leu Asp Thr Asn Tyr Cys Phe Arg
295                 300                 305
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tga gactgggccc acatgggaac caacatctac tgcctgccta ctgcccaatg 1080
*

gctaggtcag gccccagagc caagccacac tcaacagagg gtccctgata ctattcacaa 1140

acatctccag gaagaagact gaaaatctct cacagagatt ttctctgtga aatctctttc 1200

tgttttcctg ggagtcccac tgtttttcca taggctaact ctggaaggag ctggctgaag 1260

taaatgagga aaactctgtg aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1320

aaaaaaaaaa aaa 1333

<210> SEQ ID NO 26
<211> LENGTH: 309
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 26

Met Lys Met His Leu Gln Arg Ala Leu Val Val Leu Ala Leu Leu Asn
1 5 10 15

Phe Ala Thr Val Ser Leu Ser Leu Ser Thr Cys Thr Thr Leu Asp Phe
20 25 30

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

Ser Lys Leu Arg Leu Thr Ser Pro Pro Glu Pro Thr Val Met Thr His
50 55 60

Val Pro Tyr Gln Val Leu Ala Leu Tyr Asn Ser Thr Arg Glu Leu Leu
65 70 75 80

Glu Glu Met His Gly Glu Arg Glu Glu Gly Cys Thr Gln Glu Asn Thr
85 90 95

Glu Ser Glu Tyr Tyr Ala Lys Glu Ile His Lys Phe Asp Met Ile Gln
100 105 110

Gly Leu Ala Glu His Asn Glu Leu Ala Val Cys Pro Lys Gly Ile Thr
115 120 125

Ser Lys Val Phe Arg Phe Asn Val Ser Ser Val Glu Lys Asn Arg Thr
130 135 140

Asn Leu Phe Arg Ala Glu Phe Arg Val Leu Arg Val Pro Asn Pro Ser
145 150 155 160

Ser Lys Arg Asn Glu Gln Arg Ile Glu Leu Phe Gln Ile Leu Arg Pro
165 170 175

Asp Glu His Ile Ala Lys Gln Arg Tyr Ile Gly Gly Lys Asn Leu Pro
180 185 190

Thr Arg Gly Thr Ala Glu Trp Leu Ser Phe Asp Val Thr Asp Thr Val
195 200 205

Arg Glu Trp Leu Leu Arg Arg Glu Ser Asn Leu Gly Leu Glu Ile Ser
210 215 220

Ile His Cys Pro Cys His Thr Phe Gln Pro Asn Gly Asp Ile Leu Glu
225 230 235 240

Asn Ile His Glu Val Met Glu Ile Lys Phe Lys Gly Val Asp Asn Glu
245 250 255

Asp Asp His Gly Arg Gly Asp Leu Gly Arg Leu Lys Lys Gln Lys Asp
260 265 270

His His Asn Pro His Leu Ile Leu Met Met Ile Pro Pro His Arg Leu
275 280 285

Asp Asn Pro Gly Gln Gly Gly Gln Arg Lys Lys Arg Ala Leu Asp Thr

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290                 295                 300

Asn Tyr Cys Phe Arg
305


<210> SEQ ID NO 27
<211> LENGTH: 6378
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (140)...(3409)

<400> SEQUENCE: 27

cccattactg ttggagctac agggagagaa acaggaggag actgcaagag atcatttggg     60

aaggccgtgg gcacgctctt tactccatgt gtgggacatt cattgcggaa taacatcgga    120

ggagaagttt cccagagct atg ggg act tcc cat ccg gcg ttc ctg gtc tta     172
Met Gly Thr Ser His Pro Ala Phe Leu Val Leu
1               5                   10

ggc tgt ctt ctc aca ggg ctg agc cta atc ctc tgc cag ctt tca tta      220
Gly Cys Leu Leu Thr Gly Leu Ser Leu Ile Leu Cys Gln Leu Ser Leu
15                  20                  25

ccc tct atc ctt cca aat gaa aat gaa aag gtt gtg cag ctg aat tca      268
Pro Ser Ile Leu Pro Asn Glu Asn Glu Lys Val Val Gln Leu Asn Ser
30                  35                  40

tcc ttt tct ctg aga tgc ttt ggg gag agt gaa gtg agc tgg cag tac      316
Ser Phe Ser Leu Arg Cys Phe Gly Glu Ser Glu Val Ser Trp Gln Tyr
45                  50                  55

ccc atg tct gaa gaa gag agc tcc gat gtg gaa atc aga aat gaa gaa      364
Pro Met Ser Glu Glu Glu Ser Ser Asp Val Glu Ile Arg Asn Glu Glu
60                  65                  70                  75

aac aac agc ggc ctt ttt gtg acg gtc ttg gaa gtg agc agt gcc tcg      412
Asn Asn Ser Gly Leu Phe Val Thr Val Leu Glu Val Ser Ser Ala Ser
80                  85                  90

gcg gcc cac aca ggg ttg tac act tgc tat tac aac cac act cag aca      460
Ala Ala His Thr Gly Leu Tyr Thr Cys Tyr Tyr Asn His Thr Gln Thr
95                 100                 105

gaa gag aat gag ctt gaa ggc agg cac att tac atc tat gtg cca gac      508
Glu Glu Asn Glu Leu Glu Gly Arg His Ile Tyr Ile Tyr Val Pro Asp
110                 115                 120

cca gat gta gcc ttt gta cct cta gga atg acg gat tat tta gtc atc      556
Pro Asp Val Ala Phe Val Pro Leu Gly Met Thr Asp Tyr Leu Val Ile
125                 130                 135

gtg gag gat gat gat tct gcc att ata cct tgt cgc aca act gat ccc      604
Val Glu Asp Asp Asp Ser Ala Ile Ile Pro Cys Arg Thr Thr Asp Pro
140                 145                 150                 155

gag act cct gta acc tta cac aac agt gag ggg gtg gta cct gcc tcc      652
Glu Thr Pro Val Thr Leu His Asn Ser Glu Gly Val Val Pro Ala Ser
160                 165                 170

tac gac agc aga cag ggc ttt aat ggg acc ttc act gta ggg ccc tat      700
Tyr Asp Ser Arg Gln Gly Phe Asn Gly Thr Phe Thr Val Gly Pro Tyr
175                 180                 185

atc tgt gag gcc acc gtc aaa gga aag aag ttc cag acc atc cca ttt      748
Ile Cys Glu Ala Thr Val Lys Gly Lys Lys Phe Gln Thr Ile Pro Phe
190                 195                 200

aat gtt tat gct tta aaa gca aca tca gag ctg gat cta gaa atg gaa      796
Asn Val Tyr Ala Leu Lys Ala Thr Ser Glu Leu Asp Leu Glu Met Glu
205                 210                 215
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gct ctt aaa acc gtg tat aag tca ggg gaa acg att gtg gtc acc tgt      844
Ala Leu Lys Thr Val Tyr Lys Ser Gly Glu Thr Ile Val Val Thr Cys
220                 225                 230                 235

gct gtt ttt aac aat gag gtg gtt gac ctt caa tgg act tac cct gga      892
Ala Val Phe Asn Asn Glu Val Val Asp Leu Gln Trp Thr Tyr Pro Gly
240                 245                 250

gaa gtg aaa ggc aaa ggc atc aca atg ctg gaa gaa atc aaa gtc cca      940
Glu Val Lys Gly Lys Gly Ile Thr Met Leu Glu Glu Ile Lys Val Pro
255                 260                 265

tcc atc aaa ttg gtg tac act ttg acg gtc ccc gag gcc acg gtg aaa      988
Ser Ile Lys Leu Val Tyr Thr Leu Thr Val Pro Glu Ala Thr Val Lys
270                 275                 280

gac agt gga gat tac gaa tgt gct gcc cgc cag gct acc agg gag gtc     1036
Asp Ser Gly Asp Tyr Glu Cys Ala Ala Arg Gln Ala Thr Arg Glu Val
285                 290                 295

aaa gaa atg aag aaa gtc act att tct gtc cat gag aaa ggt ttc att     1084
Lys Glu Met Lys Lys Val Thr Ile Ser Val His Glu Lys Gly Phe Ile
300                 305                 310                 315

gaa atc aaa ccc acc ttc agc cag ttg gaa gct gtc aac ctg cat gaa     1132
Glu Ile Lys Pro Thr Phe Ser Gln Leu Glu Ala Val Asn Leu His Glu
320                 325                 330

gtc aaa cat ttt gtt gta gag gtg cgg gcc tac cca cct ccc agg ata     1180
Val Lys His Phe Val Val Glu Val Arg Ala Tyr Pro Pro Pro Arg Ile
335                 340                 345

tcc tgg ctg aaa aac aat ctg act ctg att gaa aat ctc act gag atc     1228
Ser Trp Leu Lys Asn Asn Leu Thr Leu Ile Glu Asn Leu Thr Glu Ile
350                 355                 360

acc act gat gtg gaa aag att cag gaa ata agg tat cga agc aaa tta     1276
Thr Thr Asp Val Glu Lys Ile Gln Glu Ile Arg Tyr Arg Ser Lys Leu
365                 370                 375

aag ctg atc cgt gct aag gaa gaa gac agt ggc cat tat act att gta     1324
Lys Leu Ile Arg Ala Lys Glu Glu Asp Ser Gly His Tyr Thr Ile Val
380                 385                 390                 395

gct caa aat gaa gat gct gtg aag agc tat act ttt gaa ctg tta act     1372
Ala Gln Asn Glu Asp Ala Val Lys Ser Tyr Thr Phe Glu Leu Leu Thr
400                 405                 410

caa gtt cct tca tcc att ctg gac ttg gtc gat gat cac cat ggc tca     1420
Gln Val Pro Ser Ser Ile Leu Asp Leu Val Asp Asp His His Gly Ser
415                 420                 425

act ggg gga cag acg gtg agg tgc aca gct gaa ggc acg ccg ctt cct     1468
Thr Gly Gly Gln Thr Val Arg Cys Thr Ala Glu Gly Thr Pro Leu Pro
430                 435                 440

gat att gag tgg atg ata tgc aaa gat att aag aaa tgt aat aat gaa     1516
Asp Ile Glu Trp Met Ile Cys Lys Asp Ile Lys Lys Cys Asn Asn Glu
445                 450                 455

act tcc tgg act att ttg gcc aac aat gtc tca aac atc atc acg gag     1564
Thr Ser Trp Thr Ile Leu Ala Asn Asn Val Ser Asn Ile Ile Thr Glu
460                 465                 470                 475

atc cac tcc cga gac agg agt acc gtg gag ggc cgt gtg act ttc gcc     1612
Ile His Ser Arg Asp Arg Ser Thr Val Glu Gly Arg Val Thr Phe Ala
480                 485                 490

aaa gtg gag gag acc atc gcc gtg cga tgc ctg gct aag aat ctc ctt     1660
Lys Val Glu Glu Thr Ile Ala Val Arg Cys Leu Ala Lys Asn Leu Leu
495                 500                 505

gga gct gag aac cga gag ctg aag ctg gtg gct ccc acc ctg cgt tct     1708
Gly Ala Glu Asn Arg Glu Leu Lys Leu Val Ala Pro Thr Leu Arg Ser
510                 515                 520
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gaa ctc acg gtg gct gct gca gtc ctg gtg ctg ttg gtg att gtg atc     1756
Glu Leu Thr Val Ala Ala Ala Val Leu Val Leu Leu Val Ile Val Ile
525                 530                 535

atc tca ctt att gtc ctg gtt gtc att tgg aaa cag aaa ccg agg tat     1804
Ile Ser Leu Ile Val Leu Val Val Ile Trp Lys Gln Lys Pro Arg Tyr
540                 545                 550                 555

gaa att cgc tgg agg gtc att gaa tca atc agc ccg gat gga cat gaa     1852
Glu Ile Arg Trp Arg Val Ile Glu Ser Ile Ser Pro Asp Gly His Glu
560                 565                 570

tat att tat gtg gac ccg atg cag ctg cct tat gac tca aga tgg gag     1900
Tyr Ile Tyr Val Asp Pro Met Gln Leu Pro Tyr Asp Ser Arg Trp Glu
575                 580                 585

ttt cca aga gat gga cta gtg ctt ggt cgg gtc ttg ggg tct gga gcg     1948
Phe Pro Arg Asp Gly Leu Val Leu Gly Arg Val Leu Gly Ser Gly Ala
590                 595                 600

ttt ggg aag gtg gtt gaa gga aca gcc tat gga tta agc cgg tcc caa     1996
Phe Gly Lys Val Val Glu Gly Thr Ala Tyr Gly Leu Ser Arg Ser Gln
605                 610                 615

cct gtc atg aaa gtt gca gtg aag atg cta aaa ccc acg gcc aga tcc     2044
Pro Val Met Lys Val Ala Val Lys Met Leu Lys Pro Thr Ala Arg Ser
620                 625                 630                 635

agt gaa aaa caa gct ctc atg tct gaa ctg aag ata atg act cac ctg     2092
Ser Glu Lys Gln Ala Leu Met Ser Glu Leu Lys Ile Met Thr His Leu
640                 645                 650

ggg cca cat ttg aac att gta aac ttg ctg gga gcc tgc acc aag tca     2140
Gly Pro His Leu Asn Ile Val Asn Leu Leu Gly Ala Cys Thr Lys Ser
655                 660                 665

ggc ccc att tac atc atc aca gag tat tgc ttc tat gga gat ttg gtc     2188
Gly Pro Ile Tyr Ile Ile Thr Glu Tyr Cys Phe Tyr Gly Asp Leu Val
670                 675                 680

aac tat ttg cat aag aat agg gat agc ttc ctg agc cac cac cca gag     2236
Asn Tyr Leu His Lys Asn Arg Asp Ser Phe Leu Ser His His Pro Glu
685                 690                 695

aag cca aag aaa gag ctg gat atc ttt gga ttg aac cct gct gat gaa     2284
Lys Pro Lys Lys Glu Leu Asp Ile Phe Gly Leu Asn Pro Ala Asp Glu
700                 705                 710                 715

agc aca cgg agc tat gtt att tta tct ttt gaa aac aat ggt gac tac     2332
Ser Thr Arg Ser Tyr Val Ile Leu Ser Phe Glu Asn Asn Gly Asp Tyr
720                 725                 730

atg gac atg aag cag gct gat act aca cag tat gtc ccc atg cta gaa     2380
Met Asp Met Lys Gln Ala Asp Thr Thr Gln Tyr Val Pro Met Leu Glu
735                 740                 745

agg aaa gag gtt tct aaa tat tcc gac atc cag aga tca ctc tat gat     2428
Arg Lys Glu Val Ser Lys Tyr Ser Asp Ile Gln Arg Ser Leu Tyr Asp
750                 755                 760

cgt cca gcc tca tat aag aag aaa tct atg tta gac tca gaa gtc aaa     2476
Arg Pro Ala Ser Tyr Lys Lys Lys Ser Met Leu Asp Ser Glu Val Lys
765                 770                 775

aac ctc ctt tca gat gat aac tca gaa ggc ctt act tta ttg gat ttg     2524
Asn Leu Leu Ser Asp Asp Asn Ser Glu Gly Leu Thr Leu Leu Asp Leu
780                 785                 790                 795

ttg agc ttc acc tat caa gtt gcc cga gga atg gag ttt ttg gct tca     2572
Leu Ser Phe Thr Tyr Gln Val Ala Arg Gly Met Glu Phe Leu Ala Ser
800                 805                 810

aaa aat tgt gtc cac cgt gat ctg gct gct cgc aac gtc ctc ctg gca     2620
Lys Asn Cys Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Leu Ala
815                 820                 825
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caa gga aaa att gtg aag atc tgt gac ttt ggc ctg gcc aga gac atc     2668
Gln Gly Lys Ile Val Lys Ile Cys Asp Phe Gly Leu Ala Arg Asp Ile
830                 835                 840

atg cat gat tcg aac tat gtg tcg aaa ggc agt acc ttt ctg ccc gtg     2716
Met His Asp Ser Asn Tyr Val Ser Lys Gly Ser Thr Phe Leu Pro Val
845                 850                 855

aag tgg atg gct cct gag agc atc ttt gac aac ctc tac acc aca ctg     2764
Lys Trp Met Ala Pro Glu Ser Ile Phe Asp Asn Leu Tyr Thr Thr Leu
860                 865                 870                 875

agt gat gtc tgg tct tat ggc att ctg ctc tgg gag atc ttt tcc ctt     2812
Ser Asp Val Trp Ser Tyr Gly Ile Leu Leu Trp Glu Ile Phe Ser Leu
880                 885                 890

ggt ggc acc cct tac ccc ggc atg atg gtg gat tct act ttc tac aat     2860
Gly Gly Thr Pro Tyr Pro Gly Met Met Val Asp Ser Thr Phe Tyr Asn
895                 900                 905

aag atc aag agt ggg tac cgg atg gcc aag cct gac cac gct acc agt     2908
Lys Ile Lys Ser Gly Tyr Arg Met Ala Lys Pro Asp His Ala Thr Ser
910                 915                 920

gaa gtc tac gag atc atg gtg aaa tgc tgg aac agt gag ccg gag aag     2956
Glu Val Tyr Glu Ile Met Val Lys Cys Trp Asn Ser Glu Pro Glu Lys
925                 930                 935

aga ccc tcc ttt tac cac ctg agt gag att gtg gag aat ctg ctg cct     3004
Arg Pro Ser Phe Tyr His Leu Ser Glu Ile Val Glu Asn Leu Leu Pro
940                 945                 950                 955

gga caa tat aaa aag agt tat gaa aaa att cac ctg gac ttc ctg aag     3052
Gly Gln Tyr Lys Lys Ser Tyr Glu Lys Ile His Leu Asp Phe Leu Lys
960                 965                 970

agt gac cat cct gct gtg gca cgc atg cgt gtg gac tca gac aat gca     3100
Ser Asp His Pro Ala Val Ala Arg Met Arg Val Asp Ser Asp Asn Ala
975                 980                 985

tac att ggt gtc acc tac aaa aac gag gaa gac aag ctg aag gac tgg     3148
Tyr Ile Gly Val Thr Tyr Lys Asn Glu Glu Asp Lys Leu Lys Asp Trp
990                 995                1000

gag ggt ggt ctg gat gag cag aga ctg agc gct gac agt ggc tac atc     3196
Glu Gly Gly Leu Asp Glu Gln Arg Leu Ser Ala Asp Ser Gly Tyr Ile
1005                1010                1015

att cct ctg cct gac att gac cct gtc cct gag gag gag gac ctg ggc     3244
Ile Pro Leu Pro Asp Ile Asp Pro Val Pro Glu Glu Glu Asp Leu Gly
1020                1025                1030                1035

aag agg aac aga cac agc tcg cag acc tct gaa gag agt gcc att gag     3292
Lys Arg Asn Arg His Ser Ser Gln Thr Ser Glu Glu Ser Ala Ile Glu
1040                1045                1050

acg ggt tcc agc agt tcc acc ttc atc aag aga gag gac gag acc att     3340
Thr Gly Ser Ser Ser Ser Thr Phe Ile Lys Arg Glu Asp Glu Thr Ile
1055                1060                1065

gaa gac atc gac atg atg gac gac atc ggc ata gac tct tca gac ctg     3388
Glu Asp Ile Asp Met Met Asp Asp Ile Gly Ile Asp Ser Ser Asp Leu
1070                1075                1080

gtg gaa gac agc ttc ctg taa ctggcggatt cgaggggttc cttccacttc        3439
Val Glu Asp Ser Phe Leu  *
1085

tggggccacc tctggatccc gttcagaaaa ccactttatt gcaatgcgga ggttgagagg   3499

aggacttggt tgatgtttaa agagaagttc ccagccaagg gcctcgggga gcgttctaaa   3559

tatgaatgaa tgggatattt tgaaatgaac tttgtcagtg ttgcctctcg caatgcctca   3619

gtagcatctc agtggtgtgt gaagtttgga gatagatgga taagggaata ataggccaca   3679
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gaaggtgaac tttgtgcttc aaggacattg gtgagagtcc aacagacaca atttatactg 3739

cgacagaact tcagcattgt aattatgtaa ataactctaa ccaaggctgt gtttagattg 3799

tattaactat cttctttgga cttctgaaga gaccactcaa tccatccatg tacttccctc 3859

ttgaaacctg atgtcagctg ctgttgaact ttttaaagaa gtgcatgaaa aaccattttt 3919

gaaccttaaa aggtactggt actatagcat tttgctatct tttttagtgt taagagataa 3979

agaataataa ttaaccaacc ttgtttaata gatttgggtc atttagaagc ctgacaactc 4039

attttcatat tgtaatctat gtttataata ctactactgt tatcagtaat gctaaatgtg 4099

taataatgta acatgatttc cctccagaga aagcacaatt taaaacaatc cttactaagt 4159

aggtgatgag tttgacagtt tttgacattt atattaaata acatgtttct ctataaagta 4219

tggtaatagc tttagtgaat taaatttagt tgagcataga gaacaaagta aaagtagtgt 4279

tgtccaggaa gtcagaattt ttaactgtac tgaataggtt ccccaatcca tcgtattaaa 4339

aaacaattaa ctgccctctg aaataatggg attagaaaca aacaaaactc ttaagtccta 4399

aaagttctca atgtagaggc ataaacctgt gctgaacata acttctcatg tatattaccc 4459

aatggaaaat ataatgatca gcaaaaagac tggatttgca gaagtttttt tttttttct 4519

tcatgcctga tgaaagcttt ggcaacccca atatatgtat tttttgaatc tatgaacctg 4579

aaaagggtca gaaggatgcc cagacatcag cctccttctt tcaccccctta ccccaaagag 4639

aaagagtttg aaactcgaga ccataaagat attctttagt ggaggctgga tgtgcattag 4699

cctggatcct cagttctcaa atgtgtgtgg cagccaggat gactagatcc tgggtttcca 4759

tccttgagat tctgaagtat gaagtctgag ggaaaccaga gtctgtattt ttctaaactc 4819

cctggctgtt ctgatcggcc agttttcgga aacactgact taggtttcag gaagttgcca 4879

tgggaaacaa ataatttgaa ctttggaaca gggttggaat tcaaccacgc aggaagccta 4939

ctatttaaat ccttggcttc aggttagtga catttaatgc catctagcta gcaattgcga 4999

ccttaattta actttccagt cttagctgag gctgagaaag ctaaagtttg gttttgacag 5059

gttttccaaa agtaaagatg ctacttccca ctgtatgggg gagattgaac tttccccgtc 5119

tcccgtcttc tgcctcccac tccatacccc gccaaggaaa ggcatgtaca aaaattatgc 5179

aattcagtgt tccaagtctc tgtgtaacca gctcagtgtt ttggtggaaa aaacatttta 5239

agttttactg ataatttgag gttagatggg aggatgaatt gtcacatcta tccacactgt 5299

caaacaggtt ggtgtgggtt cattggcatt ctttgcaata ctgcttaatt gctgatacca 5359

tatgaatgaa acatgggctg tgattactgc aatcactgtg ctatcggcag atgatgcttt 5419

ggaagatgca gaagcaataa taaagtactt gactacctac tggtgtaatc tcaatgcaag 5479

ccccaacttt cttatccaac tttttcatag taagtgcgaa gactgagcca gattggccaa 5539

ttaaaaacga aaacctgact aggttctgta gagccaatta gacttgaaat acgtttgtgt 5599

ttctagaatc acagctcaag cattctgttt atcgctcact ctcccttgta cagccttatt 5659

ttgttggtgc tttgcatttt gatattgctg tgagccttgc atgacatcat gaggccggat 5719

gaaacttctc agtccagcag tttccagtcc taacaaatgc tcccacctga atttgtatat 5779

gactgcattt gtgggtgtgt gtgtgttttc agcaaattcc agatttgttt ccttttggcc 5839

tcctgcaaag tctccagaag aaaatttgcc aatctttcct actttctatt tttatgatga 5899

caatcaaagc cggcctgaga aacactattt gtgacttttt aaacgattag tgatgtcctt 5959

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aaaatgtggt ctgccaatct gtacaaaatg gtcctatttt tgtgaagagg gacataagat 6019

aaaatgatgt tatacatcaa tatgtatata tgtatttcta tatagacttg gagaatactg 6079

ccaaaacatt tatgacaagc tgtatcactg ccttcgttta tatttttta actgtgataa 6139

tccccacagg cacattaact gttgcacttt tgaatgtcca aaatttatat tttagaaata 6199

ataaaaagaa agatacttac atgttcccaa aacaatggtg tggtgaatgt gtgagaaaaa 6259

ctaacttgat agggtctacc aatacaaaat gtattacgaa tgcccctgtt catgtttttg 6319

ttttaaaacg tgtaaatgaa gatctttata tttcaataaa tgatatataa tttaaagtt 6378

<210> SEQ ID NO 28
<211> LENGTH: 1089
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 28

Met Gly Thr Ser His Pro Ala Phe Leu Val Leu Gly Cys Leu Leu Thr
1 5 10 15

Gly Leu Ser Leu Ile Leu Cys Gln Leu Ser Leu Pro Ser Ile Leu Pro
20 25 30

Asn Glu Asn Glu Lys Val Val Gln Leu Asn Ser Ser Phe Ser Leu Arg
35 40 45

Cys Phe Gly Glu Ser Glu Val Ser Trp Gln Tyr Pro Met Ser Glu Glu
50 55 60

Glu Ser Ser Asp Val Glu Ile Arg Asn Glu Glu Asn Asn Ser Gly Leu
65 70 75 80

Phe Val Thr Val Leu Glu Val Ser Ser Ala Ser Ala Ala His Thr Gly
85 90 95

Leu Tyr Thr Cys Tyr Tyr Asn His Thr Gln Thr Glu Glu Asn Glu Leu
100 105 110

Glu Gly Arg His Ile Tyr Ile Tyr Val Pro Asp Pro Asp Val Ala Phe
115 120 125

Val Pro Leu Gly Met Thr Asp Tyr Leu Val Ile Val Glu Asp Asp Asp
130 135 140

Ser Ala Ile Ile Pro Cys Arg Thr Thr Asp Pro Glu Thr Pro Val Thr
145 150 155 160

Leu His Asn Ser Glu Gly Val Val Pro Ala Ser Tyr Asp Ser Arg Gln
165 170 175

Gly Phe Asn Gly Thr Phe Thr Val Gly Pro Tyr Ile Cys Glu Ala Thr
180 185 190

Val Lys Gly Lys Lys Phe Gln Thr Ile Pro Phe Asn Val Tyr Ala Leu
195 200 205

Lys Ala Thr Ser Glu Leu Asp Leu Glu Met Glu Ala Leu Lys Thr Val
210 215 220

Tyr Lys Ser Gly Glu Thr Ile Val Val Thr Cys Ala Val Phe Asn Asn
225 230 235 240

Glu Val Val Asp Leu Gln Trp Thr Tyr Pro Gly Glu Val Lys Gly Lys
245 250 255

Gly Ile Thr Met Leu Glu Glu Ile Lys Val Pro Ser Ile Lys Leu Val
260 265 270

Tyr Thr Leu Thr Val Pro Glu Ala Thr Val Lys Asp Ser Gly Asp Tyr
275 280 285

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Glu Cys Ala Ala Arg Gln Ala Thr Arg Glu Val Lys Glu Met Lys Lys
290                 295                 300

Val Thr Ile Ser Val His Glu Lys Gly Phe Ile Glu Ile Lys Pro Thr
305                 310                 315                 320

Phe Ser Gln Leu Glu Ala Val Asn Leu His Glu Val Lys His Phe Val
325                 330                 335

Val Glu Val Arg Ala Tyr Pro Pro Pro Arg Ile Ser Trp Leu Lys Asn
340                 345                 350

Asn Leu Thr Leu Ile Glu Asn Leu Thr Glu Ile Thr Thr Asp Val Glu
355                 360                 365

Lys Ile Gln Glu Ile Arg Tyr Arg Ser Lys Leu Lys Leu Ile Arg Ala
370                 375                 380

Lys Glu Glu Asp Ser Gly His Tyr Thr Ile Val Ala Gln Asn Glu Asp
385                 390                 395                 400

Ala Val Lys Ser Tyr Thr Phe Glu Leu Leu Thr Gln Val Pro Ser Ser
405                 410                 415

Ile Leu Asp Leu Val Asp Asp His His Gly Ser Thr Gly Gly Gln Thr
420                 425                 430

Val Arg Cys Thr Ala Glu Gly Thr Pro Leu Pro Asp Ile Glu Trp Met
435                 440                 445

Ile Cys Lys Asp Ile Lys Lys Cys Asn Asn Glu Thr Ser Trp Thr Ile
450                 455                 460

Leu Ala Asn Asn Val Ser Asn Ile Ile Thr Glu Ile His Ser Arg Asp
465                 470                 475                 480

Arg Ser Thr Val Glu Gly Arg Val Thr Phe Ala Lys Val Glu Glu Thr
485                 490                 495

Ile Ala Val Arg Cys Leu Ala Lys Asn Leu Leu Gly Ala Glu Asn Arg
500                 505                 510

Glu Leu Lys Leu Val Ala Pro Thr Leu Arg Ser Glu Leu Thr Val Ala
515                 520                 525

Ala Ala Val Leu Val Leu Leu Val Ile Val Ile Ile Ser Leu Ile Val
530                 535                 540

Leu Val Val Ile Trp Lys Gln Lys Pro Arg Tyr Glu Ile Arg Trp Arg
545                 550                 555                 560

Val Ile Glu Ser Ile Ser Pro Asp Gly His Glu Tyr Ile Tyr Val Asp
565                 570                 575

Pro Met Gln Leu Pro Tyr Asp Ser Arg Trp Glu Phe Pro Arg Asp Gly
580                 585                 590

Leu Val Leu Gly Arg Val Leu Gly Ser Gly Ala Phe Gly Lys Val Val
595                 600                 605

Glu Gly Thr Ala Tyr Gly Leu Ser Arg Ser Gln Pro Val Met Lys Val
610                 615                 620

Ala Val Lys Met Leu Lys Pro Thr Ala Arg Ser Ser Glu Lys Gln Ala
625                 630                 635                 640

Leu Met Ser Glu Leu Lys Ile Met Thr His Leu Gly Pro His Leu Asn
645                 650                 655

Ile Val Asn Leu Leu Gly Ala Cys Thr Lys Ser Gly Pro Ile Tyr Ile
660                 665                 670

Ile Thr Glu Tyr Cys Phe Tyr Gly Asp Leu Val Asn Tyr Leu His Lys
675                 680                 685

Asn Arg Asp Ser Phe Leu Ser His His Pro Glu Lys Pro Lys Lys Glu
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690 695 700

Leu Asp Ile Phe Gly Leu Asn Pro Ala Asp Glu Ser Thr Arg Ser Tyr
705 710 715 720

Val Ile Leu Ser Phe Glu Asn Asn Gly Asp Tyr Met Asp Met Lys Gln
725 730 735

Ala Asp Thr Thr Gln Tyr Val Pro Met Leu Glu Arg Lys Glu Val Ser
740 745 750

Lys Tyr Ser Asp Ile Gln Arg Ser Leu Tyr Asp Arg Pro Ala Ser Tyr
755 760 765

Lys Lys Lys Ser Met Leu Asp Ser Glu Val Lys Asn Leu Leu Ser Asp
770 775 780

Asp Asn Ser Glu Gly Leu Thr Leu Leu Asp Leu Leu Ser Phe Thr Tyr
785 790 795 800

Gln Val Ala Arg Gly Met Glu Phe Leu Ala Ser Lys Asn Cys Val His
805 810 815

Arg Asp Leu Ala Ala Arg Asn Val Leu Leu Ala Gln Gly Lys Ile Val
820 825 830

Lys Ile Cys Asp Phe Gly Leu Ala Arg Asp Ile Met His Asp Ser Asn
835 840 845

Tyr Val Ser Lys Gly Ser Thr Phe Leu Pro Val Lys Trp Met Ala Pro
850 855 860

Glu Ser Ile Phe Asp Asn Leu Tyr Thr Thr Leu Ser Asp Val Trp Ser
865 870 875 880

Tyr Gly Ile Leu Leu Trp Glu Ile Phe Ser Leu Gly Gly Thr Pro Tyr
885 890 895

Pro Gly Met Met Val Asp Ser Thr Phe Tyr Asn Lys Ile Lys Ser Gly
900 905 910

Tyr Arg Met Ala Lys Pro Asp His Ala Thr Ser Glu Val Tyr Glu Ile
915 920 925

Met Val Lys Cys Trp Asn Ser Glu Pro Glu Lys Arg Pro Ser Phe Tyr
930 935 940

His Leu Ser Glu Ile Val Glu Asn Leu Leu Pro Gly Gln Tyr Lys Lys
945 950 955 960

Ser Tyr Glu Lys Ile His Leu Asp Phe Leu Lys Ser Asp His Pro Ala
965 970 975

Val Ala Arg Met Arg Val Asp Ser Asp Asn Ala Tyr Ile Gly Val Thr
980 985 990

Tyr Lys Asn Glu Glu Asp Lys Leu Lys Asp Trp Glu Gly Gly Leu Asp
995 1000 1005

Glu Gln Arg Leu Ser Ala Asp Ser Gly Tyr Ile Ile Pro Leu Pro Asp
1010 1015 1020

Ile Asp Pro Val Pro Glu Glu Glu Asp Leu Gly Lys Arg Asn Arg His
1025 1030 1035 1040

Ser Ser Gln Thr Ser Glu Glu Ser Ala Ile Glu Thr Gly Ser Ser Ser
1045 1050 1055

Ser Thr Phe Ile Lys Arg Glu Asp Glu Thr Ile Glu Asp Ile Asp Met
1060 1065 1070

Met Asp Asp Ile Gly Ile Asp Ser Ser Asp Leu Val Glu Asp Ser Phe
1075 1080 1085

Leu

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<210> SEQ ID NO 29
<211> LENGTH: 2121
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (559)...(1878)

<400> SEQUENCE: 29

ctgctcgcgg ccgccaccgc cgggccccgg ccgtccctgg ctcccctcct gcctcgagaa 60

gggcagggct tctcagaggc ttggcgggaa aaaagaacgg agggagggat cgcgctgagt 120

ataaaagccg gttttcgggg ctttatctaa ctcgctgtag taattccagc gagaggcaga 180

gggagcgagc gggcggccgg ctagggtgga agagccgggc gagcagagct gcgctgcggg 240

cgtcctggga agggagatcc ggagcgaata gggggcttcg cctctggccc agccctcccg 300

cttgatcccc caggccagcg gtccgcaacc cttgccgcat ccacgaaact ttgcccatag 360

cagcgggcgg gcactttgca ctggaactta caacacccga gcaaggacgc gactctcccg 420

acgcggggag gctattctgc ccatttgggg acacttcccc gccgctgcca ggacccgctt 480

ctctgaaagg ctctccttgc agctgcttag acgctggatt tttttcgggt agtggaaaac 540

cagcagcctc ccgcgacg atg ccc ctc aac gtt agc ttc acc aac agg aac 591
Met Pro Leu Asn Val Ser Phe Thr Asn Arg Asn
1 5 10

tat gac ctc gac tac gac tcg gtg cag ccg tat ttc tac tgc gac gag 639
Tyr Asp Leu Asp Tyr Asp Ser Val Gln Pro Tyr Phe Tyr Cys Asp Glu
15 20 25

gag gag aac ttc tac cag cag cag cag cag agc gag ctg cag ccc ccg 687
Glu Glu Asn Phe Tyr Gln Gln Gln Gln Gln Ser Glu Leu Gln Pro Pro
30 35 40

gcg ccc agc gag gat atc tgg aag aaa ttc gag ctg ctg ccc acc ccg 735
Ala Pro Ser Glu Asp Ile Trp Lys Lys Phe Glu Leu Leu Pro Thr Pro
45 50 55

ccc ctg tcc cct agc cgc cgc tcc ggg ctc tgc tcg ccc tcc tac gtt 783
Pro Leu Ser Pro Ser Arg Arg Ser Gly Leu Cys Ser Pro Ser Tyr Val
60 65 70 75

gcg gtc aca ccc ttc tcc ctt cgg gga gac aac gac ggc ggt ggc ggg 831
Ala Val Thr Pro Phe Ser Leu Arg Gly Asp Asn Asp Gly Gly Gly Gly
80 85 90

agc ttc tcc acg gcc gac cag ctg gag atg gtg acc gag ctg ctg gga 879
Ser Phe Ser Thr Ala Asp Gln Leu Glu Met Val Thr Glu Leu Leu Gly
95 100 105

gga gac atg gtg aac cag agt ttc atc tgc gac ccg gac gac gag acc 927
Gly Asp Met Val Asn Gln Ser Phe Ile Cys Asp Pro Asp Asp Glu Thr
110 115 120

ttc atc aaa aac atc atc atc cag gac tgt atg tgg agc ggc ttc tcg 975
Phe Ile Lys Asn Ile Ile Ile Gln Asp Cys Met Trp Ser Gly Phe Ser
125 130 135

gcc gcc gcc aag ctc gtc tca gag aag ctg gcc tcc tac cag gct gcg 1023
Ala Ala Ala Lys Leu Val Ser Glu Lys Leu Ala Ser Tyr Gln Ala Ala
140 145 150 155

cgc aaa gac agc ggc agc ccg aac ccc gcc cgc ggc cac agc gtc tgc 1071
Arg Lys Asp Ser Gly Ser Pro Asn Pro Ala Arg Gly His Ser Val Cys
160 165 170

tcc acc tcc agc ttg tac ctg cag gat ctg agc gcc gcc gcc tca gag 1119
Ser Thr Ser Ser Leu Tyr Leu Gln Asp Leu Ser Ala Ala Ala Ser Glu
175 180 185

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tgc atc gac ccc tcg gtg gtc ttc ccc tac cct ctc aac gac agc agc     1167
Cys Ile Asp Pro Ser Val Val Phe Pro Tyr Pro Leu Asn Asp Ser Ser
190                 195                 200

tcg ccc aag tcc tgc gcc tcg caa gac tcc agc gcc ttc tct ccg tcc     1215
Ser Pro Lys Ser Cys Ala Ser Gln Asp Ser Ser Ala Phe Ser Pro Ser
205                 210                 215

tcg gat tct ctg ctc tcc tcg acg gag tcc tcc ccg cag ggc agc ccc     1263
Ser Asp Ser Leu Leu Ser Ser Thr Glu Ser Ser Pro Gln Gly Ser Pro
220                 225                 230                 235

gag ccc ctg gtg ctc cat gag gag aca ccg ccc acc acc agc agc gac     1311
Glu Pro Leu Val Leu His Glu Glu Thr Pro Pro Thr Thr Ser Ser Asp
240                 245                 250

tct gag gag gaa caa gaa gat gag gaa gaa atc gat gtt gtt tct gtg     1359
Ser Glu Glu Glu Gln Glu Asp Glu Glu Glu Ile Asp Val Val Ser Val
255                 260                 265

gaa aag agg cag gct cct ggc aaa agg tca gag tct gga tca cct tct     1407
Glu Lys Arg Gln Ala Pro Gly Lys Arg Ser Glu Ser Gly Ser Pro Ser
270                 275                 280

gct gga ggc cac agc aaa cct cct cac agc cca ctg gtc ctc aag agg     1455
Ala Gly Gly His Ser Lys Pro Pro His Ser Pro Leu Val Leu Lys Arg
285                 290                 295

tgc cac gtc tcc aca cat cag cac aac tac gca gcg cct ccc tcc act     1503
Cys His Val Ser Thr His Gln His Asn Tyr Ala Ala Pro Pro Ser Thr
300                 305                 310                 315

cgg aag gac tat cct gct gcc aag agg gtc aag ttg gac agt gtc aga     1551
Arg Lys Asp Tyr Pro Ala Ala Lys Arg Val Lys Leu Asp Ser Val Arg
320                 325                 330

gtc ctg aga cag atc agc aac aac cga aaa tgc acc agc ccc agg tcc     1599
Val Leu Arg Gln Ile Ser Asn Asn Arg Lys Cys Thr Ser Pro Arg Ser
335                 340                 345

tcg gac acc gag gag aat gtc aag agg cga aca cac aac gtc ttg gag     1647
Ser Asp Thr Glu Glu Asn Val Lys Arg Arg Thr His Asn Val Leu Glu
350                 355                 360

cgc cag agg agg aac gag cta aaa cgg agc ttt ttt gcc ctg cgt gac     1695
Arg Gln Arg Arg Asn Glu Leu Lys Arg Ser Phe Phe Ala Leu Arg Asp
365                 370                 375

cag atc ccg gag ttg gaa aac aat gaa aag gcc ccc aag gta gtt atc     1743
Gln Ile Pro Glu Leu Glu Asn Asn Glu Lys Ala Pro Lys Val Val Ile
380                 385                 390                 395

ctt aaa aaa gcc aca gca tac atc ctg tcc gtc caa gca gag gag caa     1791
Leu Lys Lys Ala Thr Ala Tyr Ile Leu Ser Val Gln Ala Glu Glu Gln
400                 405                 410

aag ctc att tct gaa gag gac ttg ttg cgg aaa cga cga gaa cag ttg     1839
Lys Leu Ile Ser Glu Glu Asp Leu Leu Arg Lys Arg Arg Glu Gln Leu
415                 420                 425

aaa cac aaa ctt gaa cag cta cgg aac tct tgt gcg taa ggaaaagtaa      1888
Lys His Lys Leu Glu Gln Leu Arg Asn Ser Cys Ala  *
430                 435

ggaaaacgat tccttctaac agaaatgtcc tgagcaatca cctatgaact tgtttcaaat   1948

gcatgatcaa atgcaacctc acaaccttgg ctgagtcttg agactgaaag atttagccat   2008

aatgtaaact gcctcaaatt ggactttggg cataaaagaa ctttttttatg cttaccatct   2068

ttttttttttc tttaacagat ttgtatttaa gaattgtttt taaaaaattt taa          2121
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<210> SEQ ID NO 30
<211> LENGTH: 439

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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 30

Met Pro Leu Asn Val Ser Phe Thr Asn Arg Asn Tyr Asp Leu Asp Tyr
1 5 10 15

Asp Ser Val Gln Pro Tyr Phe Tyr Cys Asp Glu Glu Glu Asn Phe Tyr
20 25 30

Gln Gln Gln Gln Gln Ser Glu Leu Gln Pro Pro Ala Pro Ser Glu Asp
35 40 45

Ile Trp Lys Lys Phe Glu Leu Leu Pro Thr Pro Pro Leu Ser Pro Ser
50 55 60

Arg Arg Ser Gly Leu Cys Ser Pro Ser Tyr Val Ala Val Thr Pro Phe
65 70 75 80

Ser Leu Arg Gly Asp Asn Asp Gly Gly Gly Gly Ser Phe Ser Thr Ala
85 90 95

Asp Gln Leu Glu Met Val Thr Glu Leu Leu Gly Gly Asp Met Val Asn
100 105 110

Gln Ser Phe Ile Cys Asp Pro Asp Asp Glu Thr Phe Ile Lys Asn Ile
115 120 125

Ile Ile Gln Asp Cys Met Trp Ser Gly Phe Ser Ala Ala Ala Lys Leu
130 135 140

Val Ser Glu Lys Leu Ala Ser Tyr Gln Ala Ala Arg Lys Asp Ser Gly
145 150 155 160

Ser Pro Asn Pro Ala Arg Gly His Ser Val Cys Ser Thr Ser Ser Leu
165 170 175

Tyr Leu Gln Asp Leu Ser Ala Ala Ala Ser Glu Cys Ile Asp Pro Ser
180 185 190

Val Val Phe Pro Tyr Pro Leu Asn Asp Ser Ser Ser Pro Lys Ser Cys
195 200 205

Ala Ser Gln Asp Ser Ser Ala Phe Ser Pro Ser Ser Asp Ser Leu Leu
210 215 220

Ser Ser Thr Glu Ser Ser Pro Gln Gly Ser Pro Glu Pro Leu Val Leu
225 230 235 240

His Glu Glu Thr Pro Pro Thr Thr Ser Ser Asp Ser Glu Glu Glu Gln
245 250 255

Glu Asp Glu Glu Glu Ile Asp Val Val Ser Val Glu Lys Arg Gln Ala
260 265 270

Pro Gly Lys Arg Ser Glu Ser Gly Ser Pro Ser Ala Gly Gly His Ser
275 280 285

Lys Pro Pro His Ser Pro Leu Val Leu Lys Arg Cys His Val Ser Thr
290 295 300

His Gln His Asn Tyr Ala Ala Pro Pro Ser Thr Arg Lys Asp Tyr Pro
305 310 315 320

Ala Ala Lys Arg Val Lys Leu Asp Ser Val Arg Val Leu Arg Gln Ile
325 330 335

Ser Asn Asn Arg Lys Cys Thr Ser Pro Arg Ser Ser Asp Thr Glu Glu
340 345 350

Asn Val Lys Arg Arg Thr His Asn Val Leu Glu Arg Gln Arg Arg Asn
355 360 365

Glu Leu Lys Arg Ser Phe Phe Ala Leu Arg Asp Gln Ile Pro Glu Leu
370 375 380

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Glu Asn Asn Glu Lys Ala Pro Lys Val Val Ile Leu Lys Lys Ala Thr
385                 390                 395                 400

Ala Tyr Ile Leu Ser Val Gln Ala Glu Glu Gln Lys Leu Ile Ser Glu
405                 410                 415

Glu Asp Leu Leu Arg Lys Arg Arg Glu Gln Leu Lys His Lys Leu Glu
420                 425                 430

Gln Leu Arg Asn Ser Cys Ala
435


<210> SEQ ID NO 31
<211> LENGTH: 1374
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (103)...(1047)

<400> SEQUENCE: 31

ggacagcttg gagatagggc ccggaattgc gggcgtcact ctgctcctgc gacctagcca     60

ggcgtgaggg agtgacagca gcgcattcgc gggacgagag cg atg agt gag aac       114
Met Ser Glu Asn
1

gcc gca cca ggt ctg atc tca gag ctg aag ctg gct gtg ccc tgg ggc      162
Ala Ala Pro Gly Leu Ile Ser Glu Leu Lys Leu Ala Val Pro Trp Gly
5                   10                  15                  20

cac atc gca gcc aaa gcc tgg ggc tcc ctg cag ggc cct cca gtt ctc      210
His Ile Ala Ala Lys Ala Trp Gly Ser Leu Gln Gly Pro Pro Val Leu
25                  30                  35

tgc ctg cac ggc tgg ctg gac aat gcc agc tcc ttc gac aga ctc atc      258
Cys Leu His Gly Trp Leu Asp Asn Ala Ser Ser Phe Asp Arg Leu Ile
40                  45                  50

cct ctt ctc ccg caa gac ttt tat tac gtt gcc atg gat ttc gga ggt      306
Pro Leu Leu Pro Gln Asp Phe Tyr Tyr Val Ala Met Asp Phe Gly Gly
55                  60                  65

cat ggg ctc tcg tcc cat tac agc cca ggt gtc cca tat tac ctc cag      354
His Gly Leu Ser Ser His Tyr Ser Pro Gly Val Pro Tyr Tyr Leu Gln
70                  75                  80

act ttt gtg agt gag atc cga aga gtt gtg gca gcc ttg aaa tgg aat      402
Thr Phe Val Ser Glu Ile Arg Arg Val Val Ala Ala Leu Lys Trp Asn
85                  90                  95                 100

cga ttc tcc att ctg ggc cac agc ttc ggt ggc gtc gtg ggc gga atg      450
Arg Phe Ser Ile Leu Gly His Ser Phe Gly Gly Val Val Gly Gly Met
105                 110                 115

ttt ttc tgt acc ttc ccc gag atg gtg gat aaa ctt atc ttg ctg gac      498
Phe Phe Cys Thr Phe Pro Glu Met Val Asp Lys Leu Ile Leu Leu Asp
120                 125                 130

acg ccg ctc ttt ctc ctg gaa tca gat gaa atg gag aac ttg ctg acc      546
Thr Pro Leu Phe Leu Leu Glu Ser Asp Glu Met Glu Asn Leu Leu Thr
135                 140                 145

tac aag cgg aga gcc ata gag cac gtg ctg cag gta gag gcc tcc cag      594
Tyr Lys Arg Arg Ala Ile Glu His Val Leu Gln Val Glu Ala Ser Gln
150                 155                 160

gag ccc tcg cac gtg ttc agc ctg aag cag ctg ctg cag agg tta ctg      642
Glu Pro Ser His Val Phe Ser Leu Lys Gln Leu Leu Gln Arg Leu Leu
165                 170                 175                 180

aag agc aat agc cac ttg agt gag gag tgc ggg gag ctt ctc ctg caa      690
Lys Ser Asn Ser His Leu Ser Glu Glu Cys Gly Glu Leu Leu Leu Gln
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185                 190                 195

aga gga acc acg aag gtg gcc aca ggt ctg gtt ctg aac aga gac cag      738
Arg Gly Thr Thr Lys Val Ala Thr Gly Leu Val Leu Asn Arg Asp Gln
200                 205                 210

agg ctc gcc tgg gca gag aac agc att gac ttc atc agc agg gag ctg      786
Arg Leu Ala Trp Ala Glu Asn Ser Ile Asp Phe Ile Ser Arg Glu Leu
215                 220                 225

tgt gcg cat tcc atc agg aag ctg cag gcc cat gtc ctg ttg atc aaa      834
Cys Ala His Ser Ile Arg Lys Leu Gln Ala His Val Leu Leu Ile Lys
230                 235                 240

gca gtc cac gga tat ttt gat tca aga cag aat tac tct gag aag gag      882
Ala Val His Gly Tyr Phe Asp Ser Arg Gln Asn Tyr Ser Glu Lys Glu
245                 250                 255                 260

tcc ctg tcg ttc atg ata gac acg atg aaa tcc acc ctc aaa gag cag      930
Ser Leu Ser Phe Met Ile Asp Thr Met Lys Ser Thr Leu Lys Glu Gln
265                 270                 275

ttc cag ttt gtg gaa gtc cca ggc aat cac tgt gtc cac atg agc gaa      978
Phe Gln Phe Val Glu Val Pro Gly Asn His Cys Val His Met Ser Glu
280                 285                 290

ccc cag cac gtg gcc agt atc atc agc tcc ttc tta cag tgc aca cac     1026
Pro Gln His Val Ala Ser Ile Ile Ser Ser Phe Leu Gln Cys Thr His
295                 300                 305

atg ctc cca gcc cag ctg tag ctctgggcct ggaactatga agacctagtg        1077
Met Leu Pro Ala Gln Leu  *
310

ctcccagact caacactggg actctgagtt cctgagcccc acaacaaggc cagggatggt   1137

ggggacaggc ctcactagtc ttgaggccca gcctaggatg gtagtcaggg gaaggagcga   1197

gattccaact tcaacatctg tgacctcaag ggggagacag agtctgggtt ccagggctgc   1257

tttctcctgg ctaataataa atatccagcc agctggagga aggaagggca ggctgggccc   1317

acctagcctt tccctgctgc ccaactggat ggaaaataaa aggttcttgt attctca      1374


<210> SEQ ID NO 32
<211> LENGTH: 314
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 32

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

Val Pro Trp Gly His Ile Ala Ala Lys Ala Trp Gly Ser Leu Gln Gly
20                  25                  30

Pro Pro Val Leu Cys Leu His Gly Trp Leu Asp Asn Ala Ser Ser Phe
35                  40                  45

Asp Arg Leu Ile Pro Leu Leu Pro Gln Asp Phe Tyr Tyr Val Ala Met
50                  55                  60

Asp Phe Gly Gly His Gly Leu Ser Ser His Tyr Ser Pro Gly Val Pro
65                  70                  75                  80

Tyr Tyr Leu Gln Thr Phe Val Ser Glu Ile Arg Arg Val Val Ala Ala
85                  90                  95

Leu Lys Trp Asn Arg Phe Ser Ile Leu Gly His Ser Phe Gly Gly Val
100                 105                 110

Val Gly Gly Met Phe Phe Cys Thr Phe Pro Glu Met Val Asp Lys Leu
115                 120                 125
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Ile Leu Leu Asp Thr Pro Leu Phe Leu Leu Glu Ser Asp Glu Met Glu
130                 135                 140

Asn Leu Leu Thr Tyr Lys Arg Arg Ala Ile Glu His Val Leu Gln Val
145                 150                 155                 160

Glu Ala Ser Gln Glu Pro Ser His Val Phe Ser Leu Lys Gln Leu Leu
165                 170                 175

Gln Arg Leu Leu Lys Ser Asn Ser His Leu Ser Glu Glu Cys Gly Glu
180                 185                 190

Leu Leu Leu Gln Arg Gly Thr Thr Lys Val Ala Thr Gly Leu Val Leu
195                 200                 205

Asn Arg Asp Gln Arg Leu Ala Trp Ala Glu Asn Ser Ile Asp Phe Ile
210                 215                 220

Ser Arg Glu Leu Cys Ala His Ser Ile Arg Lys Leu Gln Ala His Val
225                 230                 235                 240

Leu Leu Ile Lys Ala Val His Gly Tyr Phe Asp Ser Arg Gln Asn Tyr
245                 250                 255

Ser Glu Lys Glu Ser Leu Ser Phe Met Ile Asp Thr Met Lys Ser Thr
260                 265                 270

Leu Lys Glu Gln Phe Gln Phe Val Glu Val Pro Gly Asn His Cys Val
275                 280                 285

His Met Ser Glu Pro Gln His Val Ala Ser Ile Ile Ser Ser Phe Leu
290                 295                 300

Gln Cys Thr His Met Leu Pro Ala Gln Leu
305                 310


<210> SEQ ID NO 33
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: TaqMan primer

<400> SEQUENCE: 33

tacacaccac cttcgctgaa ag                                            22


<210> SEQ ID NO 34
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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That which is claimed:

1. A method for evaluating the prognosis of a breast cancer patient, said method comprising detecting overexpression of at least one biomarker in a sample from said patient, wherein said biomarker is selected from the group consisting of SLPI, p21ras, MUC-1, DARPP-32, phospho-p27, src, MGC 14832, myc, TGFβ-3, SERHL, E2F1, PDGFRα, NDRG-1, MCM2, PSMB9, and MCM6, wherein overexpression of said biomarker is indicative of prognosis, and thereby evaluating the prognosis of said breast cancer patient.

2. A method for evaluating the prognosis of a breast cancer patient, said method comprising:

a) obtaining a sample from said patient;

b) contacting said sample with at least one antibody, wherein said antibody specifically binds to a biomarker protein, wherein said biomarker protein is selected from the group consisting of SLPI, p21ras, MUC-1, DARPP-32, phospho-p27, src, MGC 14832, myc, TGFβ-3, SERHL, E2F1, PDGFRα, NDRG-1, MCM2, PSMB9, and MCM6;

c) detecting binding of said antibody to said biomarker protein;

d) determining if said biomarker protein is overexpressed in said sample, wherein overexpression of said biomarker protein is indicative of a poor prognosis; and,

e) thereby evaluating the prognosis of said breast cancer patient.

3. The method of claim **2**, wherein said biomarkers are selected from the group consisting of SLPI, p21ras, MUC-1, DARPP-32, phospho-p27, src, MGC 14832, myc, TGFβ-3, SERHL, E2F1, PDGFRα, NDRG-1, MCM2, PSMB9, and MCM6.

4. A kit comprising at least two antibodies, wherein each of said antibodies specifically binds to a distinct biomarker protein that is indicative of poor prognosis of a breast cancer patient, and wherein the biomarker proteins are selected from the group consisting of SLPI, p21ras, MUC-1, DARPP-32, phospho-p27, src, MGC 14832, myc, TGFβ-3, SERHL, E2F1, PDGFRα, NDRG-1, MCM2, PSMB9, and MCM6.

5. The kit of claim **4**, wherein said biomarker proteins are selected from the group consisting of E2F1, SLPI, MUC-1, src, p21ras, and PSMB9.

6. The kit of claim **4**, wherein said kit further comprises chemicals for the detection of antibody binding to said biomarker protein.

7. The kit of claim **4**, wherein said kit is used with a commercial antibody binding detection system.

8. The kit of claim **4**, wherein said kit further comprises a positive control sample.

9. The kit of claim **4**, wherein said kit further comprises instructions for use.

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