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(54) **BIOMARKERS FOR BREAST CANCER**

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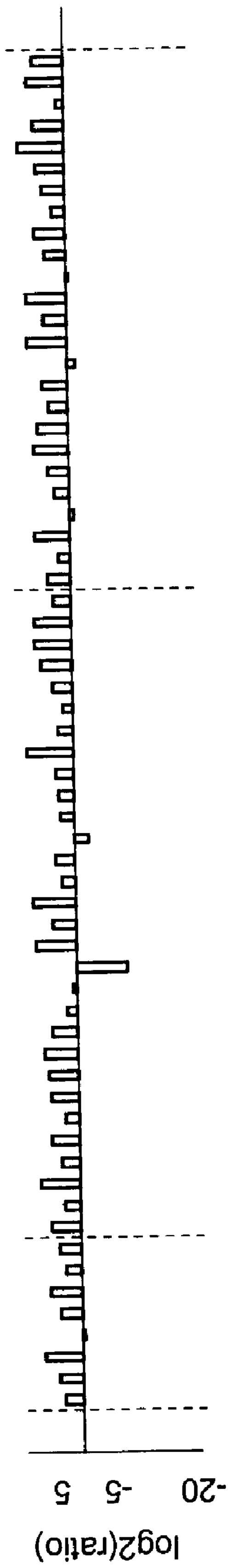
(57) **ABSTRACT**

Methods and compositions are provided for the detection of breast cancer based upon the identification of three biomarkers for non-normal breast cells. The biomarkers were identified based upon multiple sampling of reference breast tissue samples from independent cases of breast cancer. Two biomarkers display increased expression in non-normal cells while the third biomarker displays decreased expression in non-normal cells.

Fig. 1



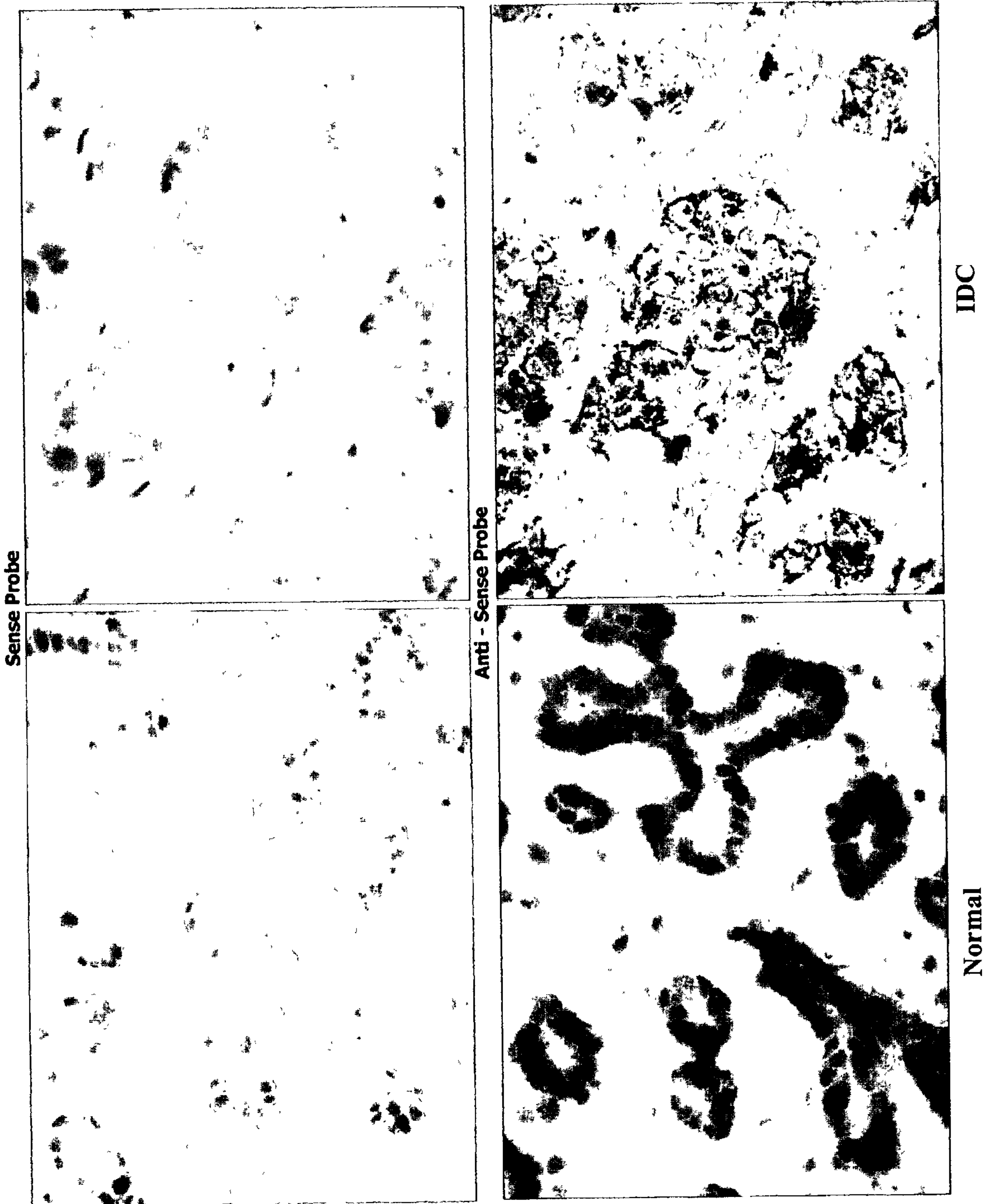


Fig. 2

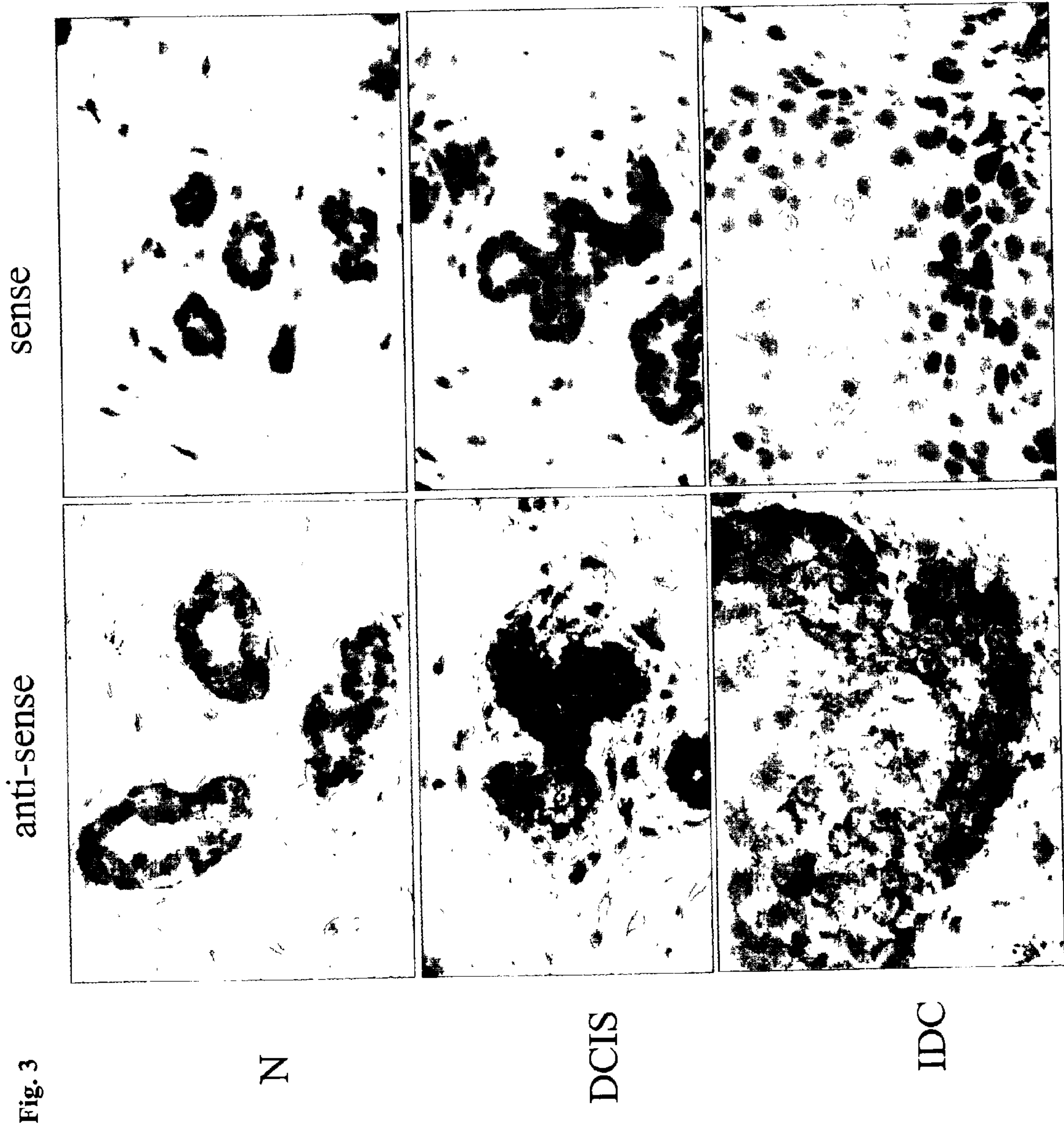


Fig. 3

BIOMARKERS FOR BREAST CANCER

RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. patent applications Ser. Nos. 10/028,018, filed Dec. 21, 2001, and 10/211,015, filed Aug. 1, 2002, which are hereby incorporated in their entireties as if fully set forth.

FIELD OF THE INVENTION

[0002] The invention relates to the identification and use of gene sequences which are differentially expressed in breast cancer. In particular, the invention provides the identities of three sets of sequences that may be used to identify the presence of breast cancer in tissue and cell samples. The expression of these sequences, whether embodied in nucleic acid expression, protein expression, nucleic acid amplification and/or activation, or other formats, are used in the diagnosis and/or treatment of breast cancer as well as for the study and/or determination of prognosis of a patient. When used for diagnosis, the expression levels of these sequences are used to identify the presence of breast cancer and provide guidance as to the treatment thereof.

BACKGROUND OF THE INVENTION

[0003] Breast cancer is by far the most common cancer among women. Each year, more than 180,000 and 1 million women in the U.S. and worldwide, respectively, are diagnosed with breast cancer. Breast cancer is the leading cause of death for women between ages 50-55, and is the most common non-preventable malignancy in women in the Western Hemisphere. An estimated 2,167,000 women in the United States are currently living with the disease (National Cancer Institute, Surveillance Epidemiology and End Results (NCI SEER) program, *Cancer Statistics Review* (*CSR*), www-seer.ims.nci.nih.gov/Publications/CSR1973 (1998)). Based on cancer rates from 1995 through 1997, a report from the National Cancer Institute (NCI) estimates that about 1 in 8 women in the United States (approximately 12.8 percent) will develop breast cancer during her lifetime (NCI's Surveillance, Epidemiology, and End Results Program (SEER) publication *SEER Cancer Statistics Review* 1973-1997). Breast cancer is the second most common form of cancer, after skin cancer, among women in the United States. An estimated 250,100 new cases of breast cancer are expected to be diagnosed in the United States in 2001. Of these, 192,200 new cases of more advanced (invasive) breast cancer are expected to occur among women (an increase of 5% over last year), 46,400 new cases of early stage (in situ) breast cancer are expected to occur among women (up 9% from last year), and about 1,500 new cases of breast cancer are expected to be diagnosed in men (Cancer Facts & Figures 2001 American Cancer Society). An estimated 40,600 deaths (40,300 women, 400 men) from breast cancer are expected in 2001. Breast cancer ranks second only to lung cancer among causes of cancer deaths in women. Nearly 86% of women who are diagnosed with breast cancer are likely to still be alive five years later, though 24% of them will die of breast cancer after 10 years, and nearly half (47%) will die of breast cancer after 20 years.

[0004] Every woman is at risk for breast cancer. Over 70 percent of breast cancers occur in women who have no identifiable risk factors other than age (U.S. General Accounting Office. Breast Cancer, 1971-1991: Prevention, Treatment and Research. GAO/PEMD-92-12; 1991). Only 5 to 10% of breast cancers are linked to a family history of breast cancer (Henderson IC, Breast Cancer. In: Murphy G P, Lawrence W L, Lenhard R E (eds). *Clinical Oncology*. Atlanta, Ga.: American Cancer Society; 1995:198-219).

[0005] The relationship between the expression of cellular factors and breast cancer has been an area of interest. STK15, a centrosomal protein kinase, has been observed as frequently amplified in breast cancer, and its quantitative expression levels positively correlate with tumor grade (Zhou, H. et al. Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. *Nat Genet* 20, 189-93, (1998)). RAD51 has recently been shown to interact with the tumor suppressor BRCA1 (Chen, J. J., et al. BRCA1, BRCA2, and Rad51 operate in a common DNA damage response pathway. *Cancer Res* 59, 1752s-1756s (1999)), and its expression also positively correlates with tumor grade in breast cancer (Maacke, H. et al. Over-expression of wild-type Rad51 correlates with histological grading of invasive ductal breast cancer. *Int J Cancer* 88, 907-13 (2000)).

[0006] Citation of documents herein is not intended as an admission that any is pertinent prior art. All statements as to the date or representation as to the contents of documents is based on the information available to the applicant and does not constitute any admission as to the correctness of the dates or contents of the documents.

SUMMARY OF THE INVENTION

[0007] The present invention relates to the identification and use of gene sequences identified as differentially expressed in breast cancer. The sequences of two of the genes display increased expression in non-normal (or abnormal) breast cells, such as those that would be identified as atypical ductal hyperplasia (ADH), ductal carcinoma in situ (DCIS), and invasive ductal carcinoma (IDC) by standard pathology techniques based upon cytological criteria. The sequences of the third gene display decreased expression the same non-normal cells.

[0008] The first set of sequences found to be more highly expressed in non-normal breast cells are those of human cysteine-rich intestinal protein 1 (CRIP1 or hCRIP1, also known as human cysteine-rich heart protein or HCRHP). CRIP1 has been mapped to human chromosomal segment 7q11.23 (see Garcia-Barcelo et al. Genomics, 47(3):419-422, 1998).

[0009] The second set of sequences found to be more highly expressed in non-normal breast cells are those of the "hematological and neurological expressed sequence 1" (HN1 or Hn1). Murine HN1 has been identified as being expressed in mouse hemopoietic and brain tissues (see Tang et al. Mamm. Genome, 8:695-696, 1997).

[0010] The set of sequences found to be expressed at lower levels in non-normal breast cells are those expressed with a "second epithelium restricted Ets transcription factor" termed ESE-2 (see Oettgen et al., J. Biol. Chem., 274(41):29439-52, 1999). The coding region sequence of ESE-2b is identical to that of the E74 like factor 5, termed ELF5 (see Zhou et al., Oncogene, 17(21):2719-32, 1998).

[0011] The identified sequences may thus be used in methods of detecting the presence of non-normal breast cells

in a tissue or cell containing sample from a subject. The presence of non-normal breast cells may also be used in methods of diagnosing the presence of breast cancer in a tissue or cell containing sample from a subject. A subject, from which a sample is taken, may be one afflicted with, or suspected of having, breast cancer.

[**0012**] The present invention provides a non-subjective means for detecting the presence of non-normal breast cells. This provides advantages over the use of histomorphological or cytological criteria in standard pathology techniques, which requires some level of interpretation by a pathologist trained in assessing the presence and/or progression of breast cancer. The expression levels of these sequences may also be used as a means to assay small, node negative tumors that are not readily assessed by conventional means.

[**0013**] The expression levels of the identified sequences may be used alone or in combination with other sequences capable of identifying the presence of non-normal cells or of various stages and/or grades of breast cancer. Preferably, the sequences of the invention are used alone or in combination with each other.

[**0014**] The present invention provides means for correlating a molecular expression phenotype with a physiological (cellular) stage or state of a non-normal or abnormal breast cell. This correlation provides a way to molecularly diagnose and/or monitor a cell's status in comparison to normal breast cell phenotypes as disclosed herein. Additional uses of the sequences are in the classification of cells and tissues; determination of diagnosis and/or prognosis. Use of the sequences to identify cells of a sample as non-normal or abnormal may also be used to determine the choice, or alteration, of therapy used to treat such cells in the subject from which the sample originated.

[**0015**] The ability to identify non-normal and abnormal breast cells is provided by the recognition of the relevancy of the level of expression of the identified sequences and not by the form of the assay used to determine the actual level of expression. An assay may utilize a means related to the expression level of the sequences disclosed herein as long as the assay reflects, quantitatively or qualitatively, expression of the sequence. Preferably, however, a quantitative assay means is preferred. Identifying features of the sequences include, but are not limited to, unique nucleic acid sequences used to encode (DNA), or express (RNA), the disclosed sequences or epitopes specific to, or activities of, proteins encoded by the sequences. Alternative means include detection of nucleic acid amplification as indicative of increased expression levels (CRIP1 and HN1 sequences) and nucleic acid inactivation, deletion, or methylation, as indicative of decreased expression levels (ESE-2 and ELF5 sequences). Stated differently, the invention may be practiced by assaying one or more aspect of the DNA template(s) underlying the expression of the disclosed sequence(s), of the RNA used as an intermediate to express the sequence(s), or of the proteinaceous product expressed by the sequence(s). As such, the detection of the amount of, stability of, or degradation (including rate) of, such DNA, RNA and proteinaceous molecules may be used in the practice of the invention.

[**0016**] The practice of the present invention is unaffected by the presence of minor mismatches between the disclosed sequences and those expressed by cells of a subject's sample. A non-limiting example of the existence of such mismatches are seen in cases of sequence polymorphisms between individuals of a species, such as individual human patients within *Homo sapiens*. Knowledge that expression of the disclosed sequences (and sequences that vary due to minor mismatches) is correlated with the presence of non-normal or abnormal breast cells and breast cancer is sufficient for the practice of the invention with an appropriate cell containing sample via an assay for expression.

[**0017**] In one aspect, the invention provides for the identification of the expression levels of the disclosed sequences by analysis of their expression in a sample containing breast cells. In one preferred embodiment, the sample contains single cells or homogenous cell populations which have been dissected away from, or otherwise isolated or purified from, contaminating cells beyond that possible by a simple biopsy. Multiple means for such analysis are available, including detection of expression within an assay for global, or near global, gene expression in a sample (e.g. as part of a gene expression profiling analysis such as on a microarray) or by specific detection, such as quantitative PCR or real time quantitative PCR.

[**0018**] Preferably, the sample is isolated via non-invasive means. The expression of the disclosed sequence(s) in the sample may be determined and compared to the expression of said sequence(s) in reference data of non-normal breast cells. Alternatively, the expression level may be compared to expression levels in normal cells, preferably from the same sample or subject.

[**0019**] When individual breast cells are isolated in the practice of the invention, one benefit is that contaminating, non-breast cells (such as infiltrating lymphocytes or other immune system cells) are not present to possibly affect detection of expression of the disclosed sequence(s). Such contamination is present where a biopsy is used to generate gene expression profiles.

[**0020**] While the present invention has been described mainly in the context of human breast cancer, it may be practiced in the context of breast cancer of an animal known to be potentially afflicted by breast cancer by use of the corresponding sequences of the animal. Preferred animals for the application of the present invention are mammals, particularly those important to agricultural applications (such as, but not limited to, cattle, sheep, horses, and other "farm animals") and for human companionship (such as, but not limited to, dogs and cats).

BRIEF DESCRIPTION OF THE FIGURES

[**0021**] **FIG. 1** shows $\log_2$ plots of the ratio of expression in ADH, DCIS, and IDC cells to normal cells for CRIP1 and ELF5 sequences. The horizontal line is at "0" such that the ratio is "1" and all points above the line represent increases in expression relative to normal breast cells while all points below the line represent decreases in expression relative to normal breast cells.

[**0022**] **FIG. 2** shows the results of in situ hybridization with sense and anti-sense CRIP1 sequences to locate its expression at the cellular level in normal versus IDC cells of the same sample. CRIP1 signal localized to the epithelial cells, and its intensity was markedly increased in the IDC compartment of the same biopsy.

[0023] **FIG. 3** shows the results of in situ hybridization with sense and anti-sense CRIP1 sequences to locate its expression at the cellular level in normal versus DCIS and IDC cells. CRIP1 signal again localized to the epithelial cells, and its intensity was markedly increased in non-normal cells.

DETAILED DESCRIPTION OF THE SPECIFIC EMBODIMENTS

[0024] Definitions of terms as used herein:

[0025] A "sequence" or "gene sequence" as used herein is a nucleic acid molecule or polynucleotide composed of a discrete order of nucleotide bases. The term includes the ordering of bases that encodes a discrete product (i.e. "coding region"), whether RNA or proteinaceous in nature, as well as the ordered bases that precede or follow a "coding region". Non-limiting examples of the latter include 5' and 3' untranslated regions of a gene. It is appreciated that more than one polynucleotide may be capable of encoding a discrete product. It is also appreciated that alleles and polymorphisms of the disclosed sequences may exist and may be used in the practice of the invention to identify the expression level(s) of the disclosed sequences or the allele or polymorphism. Identification of an allele or polymorphism depends in part upon chromosomal location and ability to recombine during mitosis.

[0026] The terms "correlate" or "correlation" or equivalents thereof refer to an association between expression of one or more sequences and a physiologic state of a breast cell to the exclusion of one or more other states by use of the methods as described herein. The invention provides for the correlation between increases in CRIP1 and HN1 sequences and non-normal or abnormal breast cells. Similarly, the invention provides for the correlation between decreases in ESE-2/ELF5 sequences and non-normal or abnormal breast cells. Increases and decreases may be readily expressed in the form of a ratio between expression in a non-normal cell and a normal cell such that a ratio of one (1) indicates no difference while ratios of two (2) and one-half indicate twice as much, and half as much, expression in the non-normal cell versus the normal cell, respectively. Expression levels can be readily determined by quantitative methods as described below.

[0027] For example, increases in CRIP1 expression can be indicated by ratios of or about 1.1, of or about 1.2, of or about 1.3, of or about 1.4, of or about 1.5, of or about 1.6, of or about 1.7, of or about 1.8, of or about 1.9, of or about 2, of or about 2.5, of or about 3, of or about 3.5, of or about 4, of or about 4.5, of or about 5, of or about 5.5, of or about 6, of or about 6.5, of or about 7, of or about 7.5, of or about 8, of or about 8.5, of or about 9, of or about 9.5, of or about 10, of or about 15, of or about 20, of or about 30, of or about 40, of or about 50, of or about 60, of or about 70, of or about 80, of or about 90, of or about 100, of or about 150, of or about 200, of or about 300, of or about 400, of or about 500, of or about 600, of or about 700, of or about 800, of or about 900, or of or about 1000. A ratio of 2 is a 100% (or a two-fold) increase in expression. Similar ratios can be used with respect to increases in HN1 expression. Decreases in ESE-2/ELF5 expression can be indicaed by ratios of or about 0.9, of or about 0.8, of or about 0.7, of or about 0.6, of or about 0.5, of or about 0.4, of or about 0.3, of or about 0.2, of or about 0.1, of or about 0.05, of or about 0.01, of or about 0.005, of or about 0.001, of or about 0.0005, of or about 0.0001, of or about 0.00005, of or about 0.00001, of or about 0.000005, or of or about 0.000001. Non-limiting examples of such ratios are shown in **FIG. 1**.

[0028] A "polynucleotide" is a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides linked by phosphodiester bonds and encompasses the strand of a given sequence as disclosed herein as well as the complementary strand of a given sequence. The term refers only to the primary structure of the molecule. Thus, this term includes double- and single-stranded DNA and RNA as well as analogs thereof comprising a non-phosphodiester backbone. It also includes known types of modifications including labels known in the art, methylation, "caps", substitution of one or more of the naturally occurring nucleotides with an analog, and internucleotide modifications such as uncharged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), as well as unmodified forms of the polynucleotide.

[0029] The term "amplify" is used in the broad sense to mean creating an amplification product can be made enzymatically with DNA or RNA polymerases. "Amplification," as used herein, generally refers to the process of producing multiple copies of a desired sequence, particularly those of a sample. "Amplification" may also be used in the context of DNA amplification wherein copies of coding sequences within the cellular genome are increased. "Multiple copies" mean at least 2 copies. A "copy" does not necessarily mean perfect sequence complementarity or identity to the template sequence. Methods for amplifying mRNA are generally known in the art, and include reverse transcription PCR (RT-PCR) and those described in U.S. patent application Ser. No. 10/062,857 entitled "Nucleic Acid Amplification" filed on Oct. 25, 2001 as well as U.S. Provisional Patent Application Nos. 60/298,847 (filed Jun. 15, 2001) and 60/257,801 (filed Dec. 22, 2000), all of which are hereby incorporated by reference in their entireties as if fully set forth.

[0030] By corresponding is meant that a nucleic acid molecule shares a substantial amount of sequence identity with another nucleic acid molecule. Substantial amount means at least 95%, usually at least 98% and more usually at least 99%, and sequence identity is determined using the BLAST algorithm, as described in Altschul et al. (1990), J. Mol. Biol. 215:403-410 (using the published default setting, i.e. parameters w=4, t=17). Alternatively, RNA may be directly labeled as the corresponding cDNA by methods known in the art.

[0031] A "microarray" is a linear or two-dimensional array of preferably discrete regions, each having a defined area, formed on the surface of a solid support such as, but not limited to, glass, plastic, or synthetic membrane. The density

of the discrete regions on a microarray is determined by the total numbers of immobilized polynucleotides to be detected on the surface of a single solid phase support, preferably at least about 50/cm^2, more preferably at least about 100/cm^2, even more preferably at least about 500/cm^2, but preferably below about 1,000/cm^2. Preferably, the arrays contain less than about 500, about 1000, about 1500, about 2000, about 2500, or about 3000 immobilized polynucleotides in total. As used herein, a DNA microarray is an array of oligonucleotides or polynucleotides placed on a chip or other surfaces used to hybridize to amplified or cloned polynucleotides from a sample. Since the position of each particular group of primers in the array is known, the identities of a sample polynucleotides can be determined based on their binding to a particular position in the microarray.

[0032] Because the invention relies upon the identification of sequences that are over- or under-expressed, one embodiment of the invention involves determining expression by hybridization of mRNA, or an amplified or cloned version thereof, of a sample cell to a polynucleotide of a disclosed sequence. Preferred polynucleotides of this type contain at least about 20, at least about 22, at least about 24, at least about 26, at least about 28, at least about 30, at least about 32, at least about 34, at least about 36, at least about 38, at least about 40, at least about 42, at least about 44, or at least about 46 consecutive bases of a sequence that is not found in other human sequences. The term "about" as used in the previous sentence refers to an increase or decrease of 1 from the stated numerical value. Longer polynucleotides may of course contain minor mismatches (e.g. via the presence of mutations) which do not affect hybridization to the nucleic acids of a sample. Such polynucleotides may be label to assist in their detection; alternatively, the nucleic acids to which such polynucleotides will hybridize may be labeled. Such polynucleotides may also be immobilized, such as by attachment to a solid support.

[0033] Even more preferred are polynucleotides of at least or about 50, at least or about 100, at least about or 150, at least or about 200, at least or about 250, at least or about 300, at least or about 350, at least or about 400, at least or about 450, or at least or about 500 consecutive bases of a sequence that is not found in other sequences in the human genome. The term "about" as used in the preceding sentence refers to an increase or decrease of 10% from the stated numerical value. The polynucleotides may of course contain minor mismatches which do not affect hybridization to the nucleic acids of a sample.

[0034] In another embodiment of the invention, all or part of a disclosed sequence may be amplified and detected by methods such as the polymerase chain reaction (PCR) and variations thereof, such as, but not limited to, quantitative PCR (QPCR), reverse transcription PCR (RT-PCR), and real-time PCR, optionally real-time RT-PCR. Such methods would utilize one or two primers that are complementary to portions of a disclosed sequence, where the primers are used to prime nucleic acid synthesis. The newly synthesized nucleic acids are optionally labeled and may be detected directly or by hybridization to a polynucleotide of the invention. The newly synthesized nucleic acids may be contacted with polynucleotides (containing sequences) of the invention under conditions which allow for their hybridization.

[0035] Alternatively, and in another embodiment of the invention, expression of a sequence may be determined by analysis of expressed protein encoded by said sequence in a cell sample of interest by use of one or more antibodies specific for one or more epitopes of the individual products (proteins) in said cell sample. Such antibodies are preferably labeled to permit their easy detection after binding to the gene product. In the case of a protein that may be found in the blood, serum or other bodily fluid, the assay may modified to use such materials in place of a breast cell containing sample.

[0036] The term "label" refers to a composition capable of producing a detectable signal indicative of the presence of the labeled molecule. Suitable labels include radioisotopes, nucleotide chromophores, enzymes, substrates, fluorescent molecules, chemiluminescent moieties, magnetic particles, bioluminescent moieties, and the like. As such, a label is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means.

[0037] The term "support" refers to conventional supports such as beads, particles, dipsticks, fibers, filters, membranes and silane or silicate supports such as glass slides.

[0038] As used herein, a "breast tissue sample" or "breast cell sample" refers to a sample of breast tissue or fluid isolated from an individual, preferably suspected of being afflicted with, or at risk of developing, breast cancer. Such samples are primary isolates (in contrast to cultured cells) and may be collected by a non-invasive means, including, but not limited to, ductal lavage, fine needle aspiration, needle biopsy, the devices and methods described in U.S. Pat. No. 6,328,709, or another suitable means recognized in the art. Alternatively, the "sample" may be collected by an invasive method, including, but not limited to, surgical biopsy.

[0039] "Expression" and "gene expression" include transcription and/or translation of nucleic acid material, such as the sequences of the invention.

[0040] As used herein, the term "comprising" and its cognates are used in their inclusive sense; that is, equivalent to the term "including" and its corresponding cognates.

[0041] Conditions that "allow" an event to occur or conditions that are "suitable" for an event to occur, such as hybridization, strand extension, and the like, or "suitable" conditions are conditions that do not prevent such events from occurring. Thus, these conditions permit, enhance, facilitate, and/or are conducive to the event. Such conditions, known in the art and described herein, depend upon, for example, the nature of the nucleotide sequence, temperature, and buffer conditions. These conditions also

depend on what event is desired, such as hybridization, cleavage, strand extension or transcription.

[0042] Sequence "mutation," as used herein, refers to any sequence alteration in the sequence of a gene disclosed herein interest in comparison to a reference sequence. A sequence mutation includes single nucleotide changes, or alterations of more than one nucleotide in a sequence, due to mechanisms such as substitution, deletion or insertion. Single nucleotide polymorphism (SNP) is also a sequence mutation as used herein. Because the present invention is based on the relative level of sequence expression, mutations in non-coding regions of genes as disclosed herein may also be assayed in the practice of the invention. "Detection" includes any means of detecting, including direct and indirect detection of gene expression and changes therein. For example, "detectably less" products may be observed directly or indirectly, and the term indicates any reduction (including the absence of detectable signal). Similarly, "detectably more" product means any increase, whether observed directly or indirectly.

[0043] Increases and decreases in expression of the disclosed sequences are defined in the following terms based upon percent or fold changes over expression in normal cells. Increases may be of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, or 200% relative to expression levels in normal cells. Alternatively, fold increases may be of 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10 fold over expression levels in normal cells. Decreases may be of 10, 20, 30, 40, 50, 55, 60, 65, 70, 75, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 99 or 100% relative to expression levels in normal cells.

[0044] Unless defined otherwise all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs.

[0045] Specific Embodiments

[0046] The present invention relates to the identification and use of three sets of sequences for the detection of non-normal and cancerous breast cells. The differential expression of these sequences in non-normal or abnormal breast cells relative to normal breast cells is used to identify a breast cancer cell as being non-normal or otherwise abnormal. The invention is advantageously used to identify breast cells as being those of ADH, DCIS, and IDC as otherwise determinable standard pathological techniques. The invention may also be applied to the identification of breast cells as being those of atypical lobular hyperplasia (ALH), lobular carcinoma in situ (LCIS), and invasive lobular carcinoma (ILC) as otherwise determinable by standard pathological techniques.

[0047] Other non-limiting examples of non-normal or abnormal cells include malignant cells, atypical cells (including reactive and pre-neoplastic), neoplastic cells, tumor cells, and cancer or cancerous cells.

[0048] The sequences(s) identified by the present invention are expressed in correlation with non-normal breast cells, and thus negatively correlated with normal breast cells. For example, CRIP1, identified by I.M.A.G.E. Consortium CloneID 1323448 and cluster NM_001311 ("The I.M.A.G.E. Consortium: An Integrated Molecular Analysis of Genomes and their Expression," Lennon et al., 1996, Genomics 33:151-152; see also image.llnl.gov) has been found to be useful in discriminations between normal and ADH or DCIS or IDC breast cells.

[0049] In preferred embodiments of the invention, any sequence, or unique portion thereof, of the CRIP1 sequences identified by the cluster, as well as UniGene Homo sapiens cluster Hs.17409, maybe used. The consensus sequence of the I.M.A.G.E. Consortium cluster is as follows, with the assigned coding region (ending with a termination codon) underlined and preceded by the 5' untranslated and/or non-coding region and followed by the 3' untranslated and/or non-coding region:

```
(consensus sequence for CRIP1)
GGCACGAGGGCCCGTGCCGCCCCAGCCGCTGCCGCCTGCACCGGACCCGGAGCCGCC      SEQ ID NO:1

ATGCCCAAGTGTCCCAAGTGCAACAAGGAGGTGTACTTCGCCGAGAGGGTGACCTCTCTGGG

CAAGGACTGGCATCGGCCCTGCCTGAAGTGCGAGAAATGTGGGAAGACGCTGACCTCTGGGG

GCCACGCTGAGCACGAAGGCAAACCCTACTGCAACCACCCCTGCTACGCAGCCATGTTTGGG

CCTAAAGGCTTTGGGCGGGGCGGAGCCGAGAGCCACACTTTCAAGTAA

ACCAGGTGGTGGAGACCCCATCCTTGGCTGCTTGCAGGGCCACTGTCCAGGCAAATGCCAGG

CCTTGTCCCCAGATGCCCAGGGCTCCCTTGTTGCCCCTAATGCTCTCAGTAAACCTGAACAC

TTGGAAAAAAAAAAAAAAAA
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[0050] The sequences identified as belonging to the I.M.A.G.E. Consortium and UniGene clusters, with the assigned coding region underlined, follow below. The 5' and 3' untranslated and/or non-coding regions are by reference to the assigned coding region, which is presented as the complementary strand in the 3' to 5' direction for some of the sequences.

(CloneID 5103334)
GGGGATTGGAGATGTTCCCCTCATGGAGGGTGCTGAGGACCTTAGGGTGGGCTGCCAGGCTG SEQ ID NO:2

GGCGGATGCGGGCTAAGTGCACAGGGCCTTGGGCAGAGCTGGCTGCAAGAGGCGGGTACGCC

AGTGGTGGGTAGGCGCCCGCG

TCCTGCAGCGTCTCACCGGGGCCTGTCTGTGCCTCTGCAGCCGAGAGGGTGACCTCTCTGGG

CAAGGACTGGCATCGGCCCTGCCTGAAGTGCGAGAAATGTGGGAAGACGCTGACCTCTGGGG

GCCACGCTGAGCACGAAGGCAAACCCTACTGCAACCACCCCTGCTACGCAGCCATGTTTGGG

CCTAAGGCTTTGGGCGGGGCGGAGCCGAGAGCCACACTTTCAAGTAA

ACCAGGTGGTGGAGAGCCCATCCTTGGCTGCTTGCAGGGCCACTGTCCAGGCAAATGCCAGG

CCTTGTCCCCAGATGCCCAGGGCTCCCTTGTTGCCCCTAATGCTCTCAGTAAACCTGAACAC

TTGGAAAAAAAAAAAA

(CloneID 5777677)
GCACGAGCGCTGGGCTAGGGGCGCGGCTTGAACTCGCCTAAAGAGCTGCGCCCTCTCATCTC SEQ ID NO:3

GCGCCTGCAGCCCGTGCCGCCCCAGCCGCTGCCGCCTGCACCGGACCCGGAGCCGCC

ATGCCCAAGTGTCCCAAGTGCAACAAGGAGGTGTACTTCGCCCAGAGGGTGACCTCTCTGGG

CAAGGACTGGCATCGGCCCTGCCTGAAGTGCGAGAAATGTGGGAAGACGCTGACCTCTCGGG

GCCACGCTGAGCACGAAGGCAAACCCTACTGCAACCACCCCTGCTACGCAGCCATGTTTGGG

CCTAAAGGCTTTGGGCGGGGCGGAGCCGAGAGCCACACTTTCAAGTAA

ACCAGGTGGTGGAGACCCCATCCTTGGCTCCTTGCAGGGCCACTGTCCAGGCAAATGCCAGG

CCTTGTCCCCAGATGCCCAGGGCTCCCTTGTTGCCCCTAATGCTCTCAGTAAACCTGAACAC

TTGG

(CloneID 563289)
GCACGAGCGCTGGGCTACGGGCGCGGCTTGAACTCGCCTAAAGAGCTGCGCCCTCTCATCTC SEQ ID NO:4

GCGCCTGCACCCCGTGCCGCCCCAGCCGCTGCCCCCTGCACCGGACCCGGAGCCGCC

ATCCCCAAGTGTCCCAAGTGCAACAAGGAGGTGTACTTCGCCGAGAGCGTGACCTCTCTGGG

CAAGGACTGGCATCGGCCCTGCCTGAAGTGCCAGAAATGTGGGAAGACGCTGACCTCTGGGG

GCCACGCTGACCACGAACGCAAACCCTACTGCAACCACCCCTGCTACGCAGCCATGTTTGGG

CCTAAAGGCTTTGGGCGGGGCGGAGCCGAGAGCCACACTTTCAAGTAA

ACCAGGTGGTGGAGACCCCATCCTTGGCTGCTTGCAGGGCCACTGTCCAGGCAAATGCCAGG

CCTTGTCCCCAGATGCCCAGGGCTCCCTTCTTGCCCCTAATGCTCTCAGTAAACCTGAACAC

T

(CloneID 1627147 in 3' to 5' orientation)
TGGTTTTCCAAGTGTTCAGGTTTACTGAGAGCATTAGGGGCAACAAGGGAGCCCTGCGCATC SEQ ID NO:5

TGGGGACAAGGCCTGGCATTTGCCTGGACAGTGGCCCTGCAAGCAGCCAAGGATGGGGTCTC

CACCACCTGGT

TTACTTGAAAGTGTGGCTCTCGGCTCCGCCCCGCCCAAAGCCTTTAGGCCCAAACATGGCTA

CGTAGCAGGGGTGGTTGCAGTAGGGTTTGCCTTCGTGCTCAGCGTGCCCCCCAGAGGTCAGC

GTCTTCCCACATTTCTCGCACTTCAGGCAGGGCCGATGCCAGTCCTTGCCCAGAGAGGTCAC

CCTCTCGGCGAAGTACACCTCCTTGTTGCACTTGGGACACTTGGGCAT

GGCGGCTCCGGGTCCGGTGCAGGCGGCAGCGGCTGGGCCGGCACGGGCTGCAGGCGCGACAC

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TGGGTGGATCCGGCCTGGTCCGCGCCTTTCAGGGACCCCGGGACCCCGCCCCTTGGAGACAC

CGCCCCTC

(CloneID 1627139 in 3' to 5' orientation)
TGGTTTTCCAAGTGTTCAGGTTTACTGAGACCATTAGGGGCAACAAGGGAGCCCTGGGCATC SEQ ID NO:6

TGGGGACAAGGCCTGCCATTTGCCTGGACAGTGGCCCTGCAAGCAGCCAAGGATGGGGTCTC

CACCACCTGGT

TTACTTGAAAGTGTGGCTCTCGGCTCCCCCCCGCCCAAAGCCTTTAGGCCCAAACATGGCTA

CGTAGCAGGGGTGGTTGCAGTAGGGTTTGCCTTCGTGCTCAGCGTGGCCCCCAGAGGTCAGC

GTCTTCCCACATTTCTCGCACTTCAGGCAGGGCCGATGCCAGTCCTTGCCCAGAGAGGTCAC

CCTCTCGGCCAAGTACACCTCCTTGTTGCACTTGGGACACTTGGGCAT

GGCGGCTCCGGGTCCGCTGCAGGCGGCAGCGGCTGGGGCGGCACGGGCTGCAGGCGCGAGAC

TGGGTGGATCCGGCCTGGTCCGCGCCTTTCA

(CloneID 3296101 in 3' to 5' orientation)
TTGGTTTTCCAAGTGTTCAGGTTTACTGAGAGCATTAGGGGCAACAAGGCAGCCCTGCGCAT SEQ ID NO:7

CTGGGGACAAGGCCTGGCATTTGCCTGGACAGTGGCCCTGCAAGCAGCCAAGGATGGGGTCT

CCACCACCTGGT

TTACTTGAAAGTGTGGCTCTCGGCTCCGCCCCGCCCAAAGCCTTTAGGCCCAAACATGGCTG

CGTAGCAGGGCTGGTTGCAGTAGGGTTTGCCTTCGTGCTCAGCGTGGCCCCCAGAGGTCAGC

GTCTTCCCACATTTCTCGCACTTCAGGCAGGGCCGATGCCAGTCCTTGCCCAGAGAGGTCAC

CCTCTCGGCGAAGTACACCTCCTTGTTGCACTTGGGACACTTGGGCAT

GGCGGCTCCGGGTCCGGTGCAGCCGGCAGCGGCTGGGGCGGCACGCGCTGCATGCGCGAGAC

TGGGTGGATCCGGCCTGGTCCGCGCCTTTCAGGGACCCCGGGACCCCG

(CloneID 3631097)
GCCGCCTGCACCGGACCGGAGCCGCC SEQ ID NO:8

ATGCCCAAGTGTCCCAAGTGCAACAAGGAGGTGTACTTCGCCGAGAGGGTGACCTCTCTGGG

CAAAGGACTGGCATCGGCCCTGCCTGAAGTGCGAGAAATGTGGGAAGACGCTGACCTCTGTG

GGGCCACGCTGAGCACGAAGGCAAACCCTACTGCAACCACCCCTGCTACGCAGCCATGTTTG

GGCCTAAAGGCTTTGGGCGGGGCGGAGCCGAGGAGCCACACTTTCAAGTAA

AACCAGGGTGGTGGAGACCCCATCCTTGGCTGCTTGCAGGGCCCACTTGTCCAGGGCAAAAT

TGCCAGGCCTTTGTCCCCCACAATGCCCAGGGGCTCCCTTTGTTTGGCCCCCTTAATTGCTC

TCAGTAAACCCTTGAACACTTGGA

(CloneID 2782288)
CAGCCCGTGCCGCCCCAGCCGCTGCCGCCTGCACCGGACCCGGAGCCGCC SEQ ID NO:9

ATGCCCAAGTGTCCCAAGTGCAACAAGGAGGTGTACTTCGCCAAGAGGGTGACCTCTCTGGG

CAAGGACTGGCATCGGCCCTGCCTGAAGTGCGAGAAATGTGGGAAGACGCTGACCTCTGGGG

GCCACGCTGAGCACGAAGGCAAACCCTACTGCAACCACCCCTGCTACGCACCCATGTTTGGG

CCTAAAGGCTTTGGGCGGGGCGGAGCCGAGAGCCACACTTTCAAGTAA

ACCAGGTGGTGGAGACCCCATCCTTGGCTGCTTCCAGGGCCACTGTCCAGGCAATGCCAGG

CCTTGTCCCCAGATGCCCAGGGCTCCCTTGTTGCCCCTAATGCTCTCAGT

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(CloneID 3903337)
CACCGGACCCGGACCCGGCC                                          SEQ ID NO:10

ATGCCCAAGTGTCCCAAGTGCAACAAGGAGGTGTACTTCGCCGAGAGGGTGACCTCTCTGGG

CAAGGACTGGCATCGGCCCTGCCTGAAGTGCGAGAAATGTGGGAAGACGCTGACCTCTGGGG

GCCACGCTGAGCACGAAGGCAAACCCTACTGCAACCACCCCTGCTACGCAGCCATGTTTGGG

CCTAAAGGCTTTCGGCGGGGCGGAGCCGAGAGCCACACTTTCAAGTAA

ACCAGGTGGTGGAGACCCCATCCTTGGCTGCTTGCAGGGCCACTGTCCAGGCAAATGCCAGG

CCTTGTCCCCAGATGCCCAGGGCTCCCCTTGTTGCCCCCTAAATGCTCTCCAGGTAAAACCT

GAAACACTTGGAAAAAAAAAACAAAAAAAAAAA

(CloneID 2063820 in 3' to 5' orientation)
TCCAAGTGTTCAGGTTTACTGAGAGCATTAGGGGCAACAAGGGACCCCTGGGCATCTGGGGA  SEQ ID NO:11

AAAGGCCTGGCATTTGCCTGGACAGTGGCCCTGCAAGCAGCCAACGATGGGGTCTCCACCAC

CTGG

T

TTACTTGAAAGTGTGGCTCTCGGCTCCGCCCCGCCCAAAGCCTTTAGGCCCAAACATCGCTA

CGTAGCAGGGGTGGTTGCAGTAGGGTTTGCCTTCGTGCTCAGCGTGGCCCCCACAGGTCAGC

GTCTTCCCACATTTCTCGCACTTCAGGCAGGGCCGATGCCAGTCCTTGCCCAGAGAGGTCAC

CCTCTCGGCTGCAGAGGCACAGACAGGCCCCGGTGAGACGCTCCAGGA

CGCCGCGCCTACCCACCACTGGCGTACCCGCCTCTTGCAGCCAGCTCTGCCCAAGGCCCTGT

GCACTTAGCCCGCATC

(CloneID 2568304 in 3' to 5' orientation)
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTCCAAGGGTTCAGGTTTACTGAAAGCATTAGCGG  SEQ ID NO:12

CAACAAGGGAGCCCTGGGCATTTGGGGACAAGGCCTGGCATTTCCCTGGACAGGGGCCCTGC

AAGCAGCCAAGGATGGGGTCTCCACCACCTGGT

TTACTTGAAAGTGTGGCTCTCGGCTCCGCCCCGCCCAAAGCCTTTAGGCCCAAACATCGCTG

CGTAACAGGGGGGGGTTGCANTACGGTTTGCCTTCGGGCTCAGCGTGGCCCCCAAAGGTCAG

CGTCTTCCCACATTTTTCGCACTTCAGGCAGGGCCGATGCCAGTCCTTGCCCAAAAACGTCA

CCCTCTCGGCAAAGTACACCTCCTTGTTGCACTTGGGACACTTGGGCAT

GGCGGCTCCGGGTCCGGTGCAGGCGCCAACGGCTGGGGCGGCA

(CloneID 2329226 in 3' to 5' orientation)
CCAAGTGTTCAGGTTTACTGAGAGCATTAGGGGCAACAAGGGAGCCCTGGGCATCTGGGGAC  SEQ ID NO:13

AAGGCCTGGCATTTGCCTGGACAGTGGCCCTGCAAGCACCCAAGGATGGGGTCTCCACCACC

TGGT

TTACTTGAAAGTGTGGCTCTCGGCTCCCCCCCGCCCAAAGCCTTTAGGCCCAAACATGCCTA

CGTAGCAGGGGTGGTTGCAGTAGGGTTTGCCTTCGTGCTCACCGTGGCCCCCAGAGGTCAGC

GTCTTCCCACATTTCTCGCACTTCAGGCAGGGCCGATGCCAGTCCTTGCCCAGAGACGTCAC

CCTCTCGGCGAAGTACACCTCCTTGTTGCACTTGGGACACTTGGGCAT

GGCGGCTCCGGGTCCGGTGCAGGCGGCACCGGCTGGGGCCGCACGGGCTGCAGGCGCGAGA

(CloneID 5433206)
GGCCCCTGCCGCCCCAGCCGCTGCCGCCTGCACCGGACCCGGAGCCGCC             SEQ ID NO:14

ATGCCCAAGTGTCCCAAGTGCAACAAGGAGGTGTACTTCGCCGAGACGGTGACCTCTCTGGG
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CAAGGACTGGCATCGGCCCTGCCTGAAGTGCGAGAAATGTGGGAAGACGCTGACCTCTGGGG

GCCACGCTGAGCACGAAGGCAAACCCTACTGCAACCACCCCTGCTACGCAGCCATGTTTGGG

CCTAAAGGCTTTGGGCGGGGCGGAGCCGAGAGCCACACTTTCAAGTAA

ACCAGGTGGTGGAGACCCCATCCTTGGCTGCTTGCAGGGCCACTGTCCAGGCAAATGCCAGG

CCTTGTCCCCAGATGCCCAGGGCTCCCTTGTTGCCCCTAATGCTCTCAGTAAACCTGAACAC

TTGGAAACAAACACACAAAAAAAAAAACAAAACAAAAAAAACACAAACCAAAAAACACGAGA

AAAGCACAAAAAAAGAGGCAAAGAGAAAAACGCGCGCGGGATATCAGAGGCAGGAGGGGCGA

AAAGGGGGGAGACAGAGGAGGAACAGCGACAACCGGCACCGCGCGC

(CloneID 5220536)
GCCGTGCCGCCCAGCCGCTGCCGCTGCACCGGACCCGGAGCCGC SEQ ID NO:15

ATGCCCAAGTGTCCAAGTGCAACAAGGAGGTGTACTTCGCCGAGAGGGTGACCTCTCTGGGC

AAGGACTGGCATCGGCCCTGCCTGAAGTGCGAGAAATGTGGGAAGACGCTGACCTCTGGGGG

CCACGCTGAGCACGAAGGCAAACCCTACTGCAACCACCCCTGCTACGCAGCCATGTTTGGGC

CTAAAGGCTTTGGGCGGGGCGGAGCCGAGAGCCACACTTTCAAGTAAA

CCAGGTGGTGGAGACCCCATCCTTGGCTGCTTGCAGGGGCCACTGTCCAGGCAAATGCCAGG

CCTTGTCCCCCAGATGCCCAGGGCTTCCCTTTGTTGCCCCTAATGGCTCTCAGTAAACCTTG

AACAACTTGGAAACACCACACCAAAAACCAACACACAGGG

(CloneID 2387987 in 3' to 5' orientation)
CAAGTGTTCAGGTTTACTGAGAGCATTAGGGGCAACAAGGGAGCCCTGGGCATCTGGGGACA SEQ ID NO:16

AGGCCTGGCATTTGCCTGGACAGTGGCCCTGCAAGCAGCCAAGGATGGGGTCTCCACCACCT

GGT

TTACTTGAAAGTGTGGCTCTCGGCTCCGCCCCGCCCAAAGCCTTTAGGCCCAAACATGGCTG

CGTAGCAGGGGTGGTTGCAGTAGGGTTTGCCTTCGTGCTCAGCGTGGCCCCCAGAGGTCAGC

GTCTTCCCACATTTCTCGCACTTTAGGCAGGGCCGATGCCAGTCCTTGCCCAGAGAGGTCAC

CCTCTCGGCGAAGTACACCTCCTTGTTGCACTTGGGACACTTGGGCAT

GGCGGCTCCGGGTCCGGTGCAGGCGGCAGCGGCTGGGGCGGCACGGGCTGCAGGCGCGAGAT

GAGAGGGCGCAGCTCTTTAGGCGAGTTCAAGCCGCGCCCCTAGCCCAGCG

(CloneID 5186252)
TGCAGCCGTCCGCCCAGCCTGGTGCGCTGCACGGACCCGGAGCCGCAT SEQ ID NO:17

GCGACGAGTGTCCAATGTGCAACAAGGAGGTGTACTTCGCCGAGAGGGTGACCTCTCTGGGC

AAAGACTGGCATCTGGCCCTGCCTGAAGTGCGAGAAATGTGGGAAGACGCTGACCTCTGGGG

GCCACGCTGAGCACGAAGGCAAACCCTACTGCAACCACCCCTGCTACGCAGCCATGTTTGGG

CCTAAGGCTTTGGGCGGGGCGGAGCCGAGAGCCACACTTTCAAGTAA

ACCAGGTGGTGGAGACCCCATCCTTGGCTGCTTGCAGGGCCACTGTCCAGGCAAATTGCCAG

GGCCTTGTCCCCCAGATTGCCCAGGGCTCCCTCTTGTTGCCCCCTAAATTGCTCTCAGGTTA

GAACCTTGAAACAGCTTTGCGAACAAGCAAAAGAAGAGAAGGAGGTGCTGATAATAGCAGAC

GAAGTGAGAAGAGAACACCAATCACATGAACACGAATAGGCAGGCCATACAACGCAGGAGAT

ATCGAACCCGGCGATAGTGCCCGGT

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(CloneID 3609948)
CGGACCCGGAGCCGCC SEQ ID NO:18

ATGCCCAAGTGTCCCAAGTGCAACAAGGAGGTGTACTTCGCCGAGAGGGTGACCTCTCTGGG

CAAGGACTGGCATCGGCCCTGCCTGAAGTGCGAGAAATGTGGGAAGACGCTGACCTCTGGGG

GCCACGCTGAGCCAAGGCAAACCCTACTGCAACCACCCCTGCTACGCAGCCATGTTTGGGCC

TAAGCCTTTGGGCGGGGCGGAGCCGAGAGCCACACTTTCAAGTAA

ACCAGGTGGTGGAGACCCCATCCTTGGCTGCTTGCAGGGCCACTGTCCAGGCAAATGCCAGG

CCTTGTCCCCAGATGCCCAGGGCTCCCTTGTTGCCCCTAATGCTCTCAGTAAACCTGAACAC

TTGGA

(CloneID 1584777 in 3' to 5' orientation)
CCAAGTGTTCAGGTTTACTGAGAGCATTAGGGGCAACAAGGGAGCCCTGGGCATCTGGGGAC SEQ ID NO:19

AAGGCCTGGCATTTGCCTGGACAGTGGCCCTGCAAGCAGCCAAGGATGGGGTCTCCACCACC

TGGT

TTACTTGAAAGTGTGGCTCTCGGCTCCGCCCGCCCAAAGCCTTTAGGCCAAACATGGCTGCG

TAAGAGAGTGGTTGAAGTAGGGTTTGCCTTCGTGCTCAGCGTGGCCCCCAGAGGTGAGCGTC

TTCCCACATTTCTCGCAGTTCAGGCAGGGCAGAGTGCAGTCCTTGCCCAGAGAGGGGACCTC

TCGGCGAAGAGAACTCCTTGTTGCACTTGGGACACTTGGGCAT

GGCGGCTCCGGGTCCGGTGCAGGCGGC

(CloneID 2130246 in 3' to 5' orientation)
AGCGGCCGCCCTTTTTTTTTTTTTTTTTCCAAGTGTTCAGGTTTACTGAGAGCATTAGGGG SEQ ID NO:20

CAACAAGGCAGCCCTGGGCATCTGGGGACAAGGCCTGGCATTTGCCTGGACAGTGGCCCTGC

AAGCAGCCAAGGATGGGGTCTCCACCACCTGGT

TTACTTGAAAGTGTGGCTCTCGGCTCCGCCCCGCCCAAAGCCTTTAGGCCCAAACATGGCTG

CGTAGCAGGGGTCGTTGCAGTACGGTTTGCCTTCGTGCTCAGCGTGGCCCCCAGAGGTCAGC

GTCTTCCCACATTTCTCGCACTTCAGGCAGGGCCGATGCCAGTCCTTGCCCAGAGAGGTCAC

CCTCTCGCCGAAGTACACCTCCTTGTTGCACTTGGGACAC

(CloneID 1908782 in 3' to 5' orientation)
CCAAGTGTTCAGGTTTACTGAGACCATTAGGGGCAACAAGGGAGCCCTGGGCATCTGGGGAC SEQ ID NO:21

AAGGCCTGGCATTTGCCTGGACAGTGGCCCTGCAAGCAGCCAAGGATGGGGTCTCCACCACC

TGGT

TTACTTGAAAGTGTGGCTCTCGGCTCCGCCCCGCCCAAAGCCTTTAGGCCCAAACATGGCTG

CGTAGCAGGGGTGGTTGCAGTAGGGTTTGCCTTCGTCCTCAGCGTGGCCCCCACAGGTCAGC

GTCTTCCCACATTTCTCGCACTTCAGGCAGGGCCGATGCCAGTCCTTGCCCAGAGAGGTCAC

TTCTCTCGGCGAAGTCCACCCTCCTTGTTGCACCTTGGGACACCTT

(CloneID 1323448 in 3' to 5' orientation)
GAGCATTAGGGGCAACAAGGGAGCCCTGGGCATCTGGGGACAAGGCCTGGCATTTGCCTGGA SEQ ID NO:22

CAGTGGCCCTGCAAGCAGCCAAGGATGGGGTCTCCACCACCTGGT

TTACTTGAAAGTGTGGCTCTCGGCTCCGCCCCCCCAAAGCCTTTAGCCCAAAACATGGCTGC

GTACGAGGGTGGTTGCAGTAGGGTTTGCCTTCGTGCTCAGCGTGGCCCCCAGAGGTCAGCGT

CTTCCCACATTTCTCGCACTTCAGCCAGGGCGANTGCCAGTCCTTGCCCAGAGAGGTCACCC

TCTCGGCGAAGTACACCTCCTTGTTGC

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(CloneID 1837171 in 3' to 5' orientation)
TTTGGTTTTCCAAGTGTTCAGGTTTACTGAGAGCATTAGGGGCAACAAGGCACCCCTGGGCA SEQ ID NO:23

TCTGGGGACAAGCCCTGGCATTTGCCTGGACAGTGGCCCTGCAAGCAGCCAAGGATGGGGTC

TCCACCACCTGGT

TTACTTCAAACTGTGGCTCTCGGCTCCGCCCCGCCCAAAGCCTTTAGGCCCAAACATGGCTG

CGTAGCAGGGGTGGTTGCAGTAGGGTTTGCCTTCGTGCTCAGCGTGGCCCCCAGAGGTCAGC

GTCTTCCCACATTTCTCGCACTTCAGGCAGCGCCGATGCCAGTCCTTGCCCAGAGAGGTCAC

CCTCTCGGCGAAGTACACCTCCTTGTTGCACTTGGGACACTTGGG

(CloneID 2802267)
GCGTCGACCCCCGAGAGGGTCACCTCTCTGGGCAAGGACTGGCATCGGCCCTGCCTGAAGTG SEQ ID NO:24

CGAGAAATGTGGGAAGACGCTGACCTCTGGGGGCCACGCTGAGCACGAAGGCAAACCCTACT

GCAACCACCCCTGCTACGCAGCCATGTTTCGGCCTAAAGGCTTTGGGCGGGGCCGAGCCGAG

AGCCACACTTTCAAGTAA

ACCAGGTGGTGGAGACCCCATCCTTGGCTGCTTGCAGGGCCGCTGTCCAGGCAAATGCCAGG

CCTTGTCCCCAGATGCCCAGGGCTCCCTTGTTGCCCCTAATGCTCT

(CloneID 2847309)
AAGGTAGCGAAGCGAGAGGGTGACCTCTCTGGGCAAGGACTGGCATCGGCCCTGCCTGAAGT SEQ ID NO:25

GCGAGAAATGTGGGAAGACGCTGACCTCTGGGGGCCACGCTGACCACGAAGGCAAACCCTAC

TGCAACCACCCCTGCTACGCAGCCATGTTTGGGCCTAAAGGCTTTGGGCGGGGCGGAGCCGA

GAGCCACACTTTCAAGTAA

ACCAGGTGGTGGACACCCCATCCTTGGCTGCTTGCAGGGCCACTGTCCAGGCAAATGCCAGG

CCTTGTCCCCAGATCCCCAGGGCTCCCTTGTTGCCCCTAATGCTCTCAGTAAACCTGAACAC

TTGGAAAACCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

AAAATTTCN

(CloneID 5018693)
GCCCAAGTGTCCCAAGTGCAACAAGGAGGTGTACTTCGCCGAGAGGGTGACCTCTCTGGGCA SEQ ID NO:26

AGGACTGGCATCGGCCCTGCCTGAAGTGCGAGAAATGTGGGAAGACGCTGACCTCTGGGGGC

CACGCTGAGCACGAAGGCAAACCCTACTGCAACCACCCCTGCTACGCAGCCATGTTTGGGCC

TAAAGGCTTTGGGCGGGGCGGAGCCGAGAGCCACACTTTCAAGTAA

ACCAGGTGGTGGAGACCCCATCCTTGGCTGCTTGCAGGGCCACTGTCCAGGCAAATGCCAGG

CCTTGTCCCCAGATGCCCAGGGCTCCCTTGTTGCCCCTAATGCTCTCAGTAAACCTGAACAC

TTGGAAAAGCAAAAAAAAAAAAAAAAAA

(CloneID 155219 in 3' to 5' orientation wherein the coding
and 3' untranslated and/or non-coding regions are present or
similar to that in CloneID 563289)
GCATTAGGGGCAACAAGGGAGCCCTGGGCATCTGGGGACAAGGCCTGGCATTTGCCNGGACA SEQ ID NO:27

GNGGCCCTGCAAGCAGCCAAGGATGGGGNCTCCACCACCTGGN

TNACTTGAAAGTGTGGCTCTCGGCTCCGCCCGCCCAAAGCCTTNAGGNCCAAACATGGCTGC

GNANAGGNGNTGGTTGCAGNAGGGTTTGCCTTCGTGCTCAGCGTGGCCCCCAGAGGTCAGCG

TCTTCCCACATTTCTCGCACTTCAGGCAGGGCCANTGCCANTCCTTNCCCAGAGAGGTCACC

CTCTCGGCGAAGTACACC

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(CloneID 307716 in 3' to 5' orientation wherein the coding
and 3' untranslated and/or non-coding regions are present or
similar to that in CloneID 563289) 307716 (3')
TTTTTTTTTTTTTTCCAAGTGTTCAGGTTTACTGAGAGCATTAGGGGCAACAAGGGAGCCCT SEQ ID NO:28

GGNATCTGGGGACAAGGCCTGGCATTTCCTGGACAGTGGCCCTGCAAGCAGCCAAGGATGGG

GTCTCCACCACCTGGT

TTACTTGAAAGTGTGGCTCTCGCCTCCGCCCGCCCAAAGCCTTTAGNCNCAAACATGGATAT

GGCTACGTAGCAGGGGTGGTTGCANTAGGGTTTGCCTTCGTGCTCAGCGTGGCCCCCAGAGG

TCAGCGTCTTCCCACATTTCTCGCACTTCAGGCAGGGCNAATGCCANTCCTTGCCCAGAGAG

GTCACCCTCTCGGG

[0051] In another set of preferred embodiments of the invention, any sequence, or unique portion thereof, of the HN1 sequences identified by the I.M.A.G.E. Consortium CloneID 471568 and cluster NM_016185, as well as UniGene Homo sapiens cluster Hs.109706, may be used. HN1 sequences are useful in discriminations between normal and DCIS or IDC (of grades I to III) breast cells. Its expression is also increased in grade III relative to grade I breast cancer cells.

[0052] The consensus sequence of the I.M.A.G.E. Consortium cluster is as follows, with the assigned coding region (ending with a termination codon) underlined and preceded by the 5' untranslated and/or non-coding region and followed by the 3' untranslated and/or non-coding region:

(consensus sequence for HN1)
TGCAGCGGTGGTCGGCTGTTGGCTCTGGAGTTTCCCAGCGCCCCTCGGGTCCGACCCTTTGA SEQ ID NO:29

GCGTTCTCCTCCGGCGCCAGCCTACCTCGCTCCTCGGCGCC

ATGACCACAACCACCACCTTCAAGGGAGTCGACCCCAACAGCAGGAATAGCTCCCGAGTTTT

GCGGCCTCCAGGTGGTGGATCCAATTTTTCATTAGGTTTTGATGAACCAACAGAACAACCTG

TGAGGAAGAACAAAATGGCCTCTAATATCTTTGGGACACCTGAAGAAAATCAAGCTTCTTGG

GCCAAGTCAGCAGGTGCCAAGTCTAGTGGTGGCAGGGAAGACTTGGAGTCATCTGGACTGCA

GAGAAGGAACTCCTCTGAAGCAAGCTCCGGAGACTTCTTAGATCTGAAGGGAGAAGGTGATA

TTCATGAAAATGTGGACACAGACTTCCCAGGCAGCCTGCGGCAGAGTGAAGAGAAGCCCGTG

CCTGCTGCGCCTGTGCCCAGCCCGGTGGCCCCGGCCCCAGTGCCATCCAGAAGAAATCCCCC

TGGCGGCAAGTCCAGCCTCCTCTTGGGTTAG

CTCTGACTGTCCTGAACGCTGTCCTTCTGTCTGTTTCCTCCATGCTTGAGAACTGCACAACT

TGAGCCTGACTGTACATCTTCTTGGATTTGTTTCATTAAAAAGAAGCACTTTATGTAAAAAA

AAAAAAAAAAAA

[0053] The sequences identified as belonging to the I.M.A.G.E. Consortium and UniGene clusters, with the assigned coding region underlined, follow below. The 5' and 3' untranslated and/or non-coding regions are by reference to the assigned coding region, which is presented as the complementary strand in the 3' to 5' direction for some of the sequences.

(Clone ID 4795778)
AGCGGGGCGGCTCCTGCAGCGGTGGTCGGCTGTTGGGTCTGGAGTTTCCCAGCGCCCCTCGG SEQ ID NO:30

GTCCGACCCTTTGAGCGTTCTGCTCCGGCGCCAGCCTACCTCGCTCCTCGGCGCC

ATGACCACAACCACCACCTTCAAGGGAGTCGACCCCAACAGCAGGAATAGCTCCCGAGTTTT

-continued

GCGGCCTCGAGGTGGTGGATCCAATTTTTCATTAGGTTTTGATGAACCAACAGAACAACCTG

TGAGGAAGAACAAAATGGCCTCTAATATCTTTGGGACACCTGAAGAAAATCAAGCTTCTTGG

GCCAAGTCAGCAGGTGCCAAGTCTAGTGGTGGCAGGGAAGACTTGGAGTCATCTGGACTGCA

GAGAAGGAACTCCTCTGAAGCAAGCTCCGGAGACTTCTTAGATCTGAAGGGAGAAGGTGATA

TTCATGAAAATGTGGACACAGACTTGCCAGGCAGCCTGGGGCAGAGTGAAGAGAAGCCCGTG

CCTGCTGCGCCTGTGCCCAGGCCCCAACCCACAGGGAGTCTCTCCGCAGAGCCTTCTTGGTG

TTGCCCTAACTTGCCAGTGGCCTTTGCTCAG

AGCCTCCTCCTGTGACATGTGAACAATGAAGAGGCCTGCGCCTCCTGCCTTGCCGCCTGCAA

AGCAAAGAAACTGCCTTTTATTTTTTAACCTTAACAAGTAGCCAGATAGTAACAAGACTGGC

TGGCTGATGAGCAAAGCCTTTGCTCTCACGCAGAGGACGGCTTGGATGTACAATGAAACTGG

CTGGAACTAAAGCAGTGAAGCAGGGGAGGCCATCACACTGAAGCGGGTCTTCCTCCAGGAAC

GGGTCCACAAGGGTGTGTCTGAATTACCTGATGCTGTGTGCTGATGCTGGCTCTTGACCATG

GACGGCAAGTTCATCTTAACCGTGCTGTCCTCACACCTGACTGTGCTCCCTTAACATTTCCC

(Clone ID 5239921)
CTCCTGCAGCCGTGGTCGGCTGTTGGGTGTGGAGTTTCCCAGCGCCCCTCGGGTCCGACCCT SEQ ID NO:31

TTGAGCGTTCTGCTCCGGCGCCAGCCTACCTCGCTCCTCGGCGCC

ATGACCACAACCACCACCTTCAAGGGAGTCGACCCCAACAGCAGGAATAGCTCCCGAGTTTT

GCGGCCTCCAGGTGGTGGATCCAATTTTTCATTAGGTTTTGATGAACCAACAGAACAACCTG

TGAGGAAGAACAAAATGGCCTCTAATATCTTTGGGACACCTGAAGAAAATCAAGCTTCTTGG

GCCAAGTCAGCAGGTGCCAAGTCTAGTGGTGGCAGGGAAGACTTGGAGTCATCTGGACTGCA

GAGAAGGAACTCCTCTGAAGCAAGCTCCGGAGACTTCTTAGATCTGAAGGGAGAAGGTGATA

TTCATGAAAATGTGGACACAGACTTGCCAGGCAGCCTGGGGCAGAGTGAAGAGAAGCCCGTG

CCTGCTGCGCCTGTGCCCAGCCCGGTGGCCCCGCCCCCAGTGCCATCCAGAAGAAATCCCCC

TGGCGGCAAGTCCAGCCTCGTCTTGGGTTAG

CTCTGACTGTCCTGAACGCTGTCGTTCTGTCTGTTTCCTCCATGCTTGTGAACTGCACAACT

TGAGCCTGACTGTACATCTCTTGGATTTGTTTCATTAAAAAGAAGCACTTTATGTACTGCTG

TCTTTTGTTATCCCTTTGGAAGAACAGGTTTCTCTCTGTCCTTGACTCTTGGGTCTGTGGGC

CATGGCATGAGTGTTCCTAGTAGTAGATTGGAGGGAAAAGTTTGTGACCCTTAGTACGGGGT

TTTAAGACGAAATAATTGGGTTCCA

(CloneID 4623018)
GGGGTGTGGAGTTTCCCAGCGCCCCTCGGGTCCGACCCTTTGAGCGTTCTGCTCCGGCGCCA SEQ ID NO:32

GCCTACCTCGCTCCTCGGCGCC

ATGACCACAACCACCACCTTCAAGGGAGTCGACCCCAACAGCAGGAATAGCTCCCGAGTTTT

GCGGCCTCCAGGTGGTGGATCCAATTTTTCATTAGGTTTTGATGAACCAACAGAACAACCTG

TGAGGAAGAACAAAATGGCCTCTAATATCTTTGGGACACCTGAAGAAAATCAAGCTTCTTGG

GCCAAGTCAGCAGGTGCCAAGTCTAGTGGTGGCAGGGAAGACTTGGAGTCATCTGGACTGCA

GAGAAGGAACTCCTCTGAAGCAAGCTCCGGAGACTTCTTAGATCTGAAGGGAGAAGGTGATA

TTCATGAAAATGTGGACACAGACTTGCCAGGCAGCCTGGGGCAGAGTGAAGAGAAGCCCGTG

CCTGCTGCGCCTGTGCCCAGCCCGGTGGCCCCGGCCCCAGTGCCATCCAGAAGAAATCCCCC

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TGGCGGCAAGTCCAGCCTCGTCTTGGGTTAG

CTCTGACTGTCCTGAACGCTGTCGTTCTGTCTGTTTCCTCCATGCTTGTGAACTGCACAACT

TGAGCCTGACTGTACATCTCTTGGATTTGTTTCATTAAAAAGAAGCACTTTATGTACTGCTG

TCTTTATTTTTTGCTTTGTGAAGACCAGGTTTCTCTCTGTCCTTGACCTCTGTGGGTCTGTG

GGCCATGGCATGAGTGTTTCCTAGTAGTAGATGGGAGGGAAAGCTTGTGACCCCTTAGTACT

GTGTTTTTACAC

(CloneID 5729213)
CGGTCCGGAATTCTCCGGATGCTGTTGGGTGTGGAGTTTCTACCGCCCCTCGGGTCCGACCC SEQ ID NO:33

TTTGAGCGTTCTGCTCCGGCGCCAGCCTACCTCGCTCCTCGGCGCC

ATGACCACAACCACCACCTTCAAGGGAGTCGACCCCAACAGCAGGAATAGCTCCCGAGTTTT

GCGGCCTCCAGGTGGTGGATCCAATTTTTCATTAGGTTTTGATGAACCAACAGAACAACCTG

TGAGGAAGAACAAAATGGCCTCTAATATCTTTGGGACACCTGAAGAAAATCAAGCTTCTTGG

GCCAAGTCAGCAGGTGCCAAGTCTAGTGGTGGCAGGGAAGACTTGGAGTCATCTGGACTGCA

GAGAAGGAACTCCTCTGAAGCAAGCTCCGGAGACTTCTTAGATCTGAAGGGAGAAGGTGATA

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(CloneID 5194208)

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(CloneID 5459503)
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(CloneID 4649355)
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(CloneID 4126162)
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(CloneID 2783676 in 3' to 5' orientation)
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AGTTGTGCAGTTCACAAGCATGGAGGAAACAGACAGAACGACAGCGTTCAGGACAGTCAGAG

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GGT

(CloneID 2783676)
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(CloneID 2822776 in 3' to 5' orientation)
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(CloneID 2822776)
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(CloneID 2781579 in 3' to 5' orientation)
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(CloneID 5419867)
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(CloneID 4328715)
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(CloneID 4138051)
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(CloneID 4766330)
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(CloneID 3958097)
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(CloneID 346318 in 3' to 5' orientation)
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CGGAGCTTGCTTCAGAGGAGTTCCTTCTCTCCAGTCCAGATGACTCCAAGTCTTCCCTGCCA

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(CloneID 346318)
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GATATTCATG

(CloneID 487018 in 3' to 5' orientation)
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(CloneID 487018)
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(CloneID 3509038)
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(CloneID 5094728)
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(CloneID 5590830)
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(CloneID 4550156)
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(CloneID 5258724)
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(CloneID 3458066)
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(CloneID 3940211)
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(CloneID 489924 in 3' to 5' orientation)

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(CloneID 3351305)
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(CloneID 2819763)
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(CloneID 5164014)
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(CloneID 2783324)
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(CloneID 471568 in 3' to 5' orientation)
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CTAACCCAAGACCAGGCTGGACTTGCCGCCAGGGGATTTCTTCTGCATGGCACTCGGGCCGG

GGCACCGGGCTTGGGCACAGCCGCANAGGCACGGGCTTCTCTTCACTCTGCCCCAGGCTGCC

TGGCAAGTCTGTGTCCACATTTTCATGAATATCACCTTCTCCCTTCAGATCTAAGAAAGTCT

CCGGAGCTTGCTTCAGAGGAGTTCCTTCTCTTGCAGTNCAGATTGACTCCAAGTCTTCCCTG

CCACCANTAGACTTTGGCANCTGCNGACTTTGGNCCAACAAAGTTGAATTTCTTCAAGNGGT

CCCAAAGATTT

(CloneID 471568)
CTGCAGCGGTGGTCGGCTGTTGGGTGTGGAGTTTCCCAGCGCCCCTCGGGTCTCGACCCTTT SEQ ID NO:183

GAGCGTTCTGCTCCGGCGCCACTACCTCGCTCCTCGGCGCC

ATCACCACAACCACCACCTTCAAGGGAGTCGACCCCAACAGCAGGAATAGCTCCCGAGTTTT

GCGGCCTCCAGGTGGTGGATCCAATTTTTCATTAGGTTTTGATGAACCAACAGAACAACCTG

TTG

(CloneID 4080434)
GACCCCAACAGCAGGAATAGCTCCCGAGTTTTGCGGCCTCCAGGTGGTGGATCCAATTTTTC SEQ ID NO:184

ATTAGGTTTTGATGAACCAACAGAACAACCTGTGAGGAAGAACAAAATGGCCTCTAATATCT

TTGGGACACCTGAAGAAAATCAAGCTTCTTGGGCCAAGTCAGCAGGTGCCAAGTCTAGTGGT

GGCAGGGAAGACTTGGAGTCATCTGGACTGCAGAGAAGGAACTCCTCTGAAGCAAGCTCCGG

AGACTTCTTAGATCTGAAGGGAGAAGGTGATATTCATGAAAATGTGNNNNNNNNNNNNNNNN

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N

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CCATGGCATGACTGTTTCTAGTAGTAATGGGAGGGAAAGCTTGGTGACACTTAGTACTGTGT

TTTTAAGACGAAATAATTGGGTTTCCAGATGTGTCACAGGATCTTCGTACTGAGGTTTAACA

CTTTACTGGGGTTTACCAAGCCTCAATGGAAGACATAACAGTCACAGGCCCGTCTGCAGGCC

AACCAAGGGAGTTCTCCGAGACCTCTGTTCTGCCTAATGCCATGGCTTGTCAAAGCCCTCTG

GCTGGC

(CloneID 4748522)
CGAGTTTTGCGGCTCCAGGTGGTGGATCCAATTTTTCATTAGGTTTTGATGAACCAACAGAA SEQ ID NO:185

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GCCCGTGCCTGCTGCGCCTGTGCCCAGCCCGGTGGCCCCGGCCCCAGTGCCATCCAGAAGAA

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TCTTTTTTTTTATTCCTTTGGAAGAACAGGTTTCTCTCTGTCCTTGACTCTGTGGGTCTGTG

GGCCATGGCATGAGTGTTTCCTAGTAGTAGATGGGAGGGAAACCTTGGTGACACTTAGTACT

GTGTTTTCAAGAAGAAATAATTGGGTCCCAGATGTGTTAGAGGATCTTGGTACTGAGGTTTT

TAACACTTTACTGTGGCTTTACCAAGCCTCAACTGGACAGACCATAAA

(CloneID 5187032)
CTAATATCTTTGGGACACCTGAAGAAAATCAAGCTTCTTGGGCCAAGTCAGCAGGTGCCAAG SEQ ID NO:186

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CTTGGGTTAG

CTCTGACTGTCCTCAACGCTGTCCTTCTGTCTGTTTCCTCCATGCTTGTGAACTGCACAACT

TGAGCCTGACTGTACATCTCTTGGATTTGTTTCATTAAAAAGAAGCACTTTATGTACTGCTC

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GCCCAGGGCATCAGTGTTCCATAGTAGTAGATGGGAGGGAAAGCTTGGCGACACTTAGTACG

GGGTTTTCAACAAGAAATAATCGGGTTCCAGAGTGTGTAAGAGGACTCTTGGGTACGCAGGT

TAAAACACTTAACTGGGGTATACCAAGCCTCAACGCGACAGACCATAAACGGTCCACAGGCA

CCGTTCCGTGCAGGGCCCAAGCCAAAGGGAGTCTCTCCGAAGAGCCTTCTGGGAGTAGCCCT

AACTTTGCCAGGTGGCCTTGGGTCAAGAGCCTCCTCCTGTGACCTGGTCA

(CloneID 3895688)
AATATCTTGGGACACCTGAAGAAAATCAAGCTTCTTGCGCCAAGTCAGCAGGTGCCAAGTCT SEQ ID NO:187

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TGCCAGGCAGCCTGGGGCAGAGTGAAGAGAAGCCCGTGCCTGCTCCGCCTGTGCCCAGCCCG

GTGGCCCCGGCCCCAGTGCCATCCAGAAGAAATCCCCCTGGCGGCAAGTCCAGCCTCGTCTT

GGGTTAG

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TCTTTTTTTTTTTCCTTTGGAAGAACAGCTTTCTCTCTGTCCTTGACTCTGGGGTCTGTGGG

CCAGGGCATGAGTGTCTCTAGTAGTAGATCGGAGGGAAAGCTTGGTGACACACTTTACTGGG

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ACCCAAGGGAGTCTCTCCGAGAGCCTTCTGGGTGTGGCCTAACTTGCCAGGGGCCTGGCTCA

GAGCTCCTCTGTGACTGTGACACTGAGCAG

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GGGAGAAGGTGATATTCATGAAAATGTGGACACAGACTTGCCAGGCAGCCTGGGGCAGAGTG

AAGAGAAGCCCGTGCCTCCTGCGCCTGTGCCCAGCCCGGTGGCCCCGGCCCCAGTGCCATCC

AGAACAAATCCCCCTGGCGGCAAGTCCAGCCTCGTCTTGGGTTAG

CTCTGACTGTCCTGAACGCTGTCGTTCTGTCTGTTTCCTCCATGCTTGTGAACTGCACAACT

TGAGCCTGACTGTACATCTCTTGGATTTGTTTCATTAAAAAGAAGCACTTTATGTACTGCTG

TCTTTTTTTTTTTCTTTTGAAGAACAGGTTTCTCTCTCCTTGACTCTGTGGGTCTGTGGG

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ACACTTTACTGTGGGTTTACCAAGCCTCAATGGACAGACCATAAACACTCCACGAGGCACCG

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(CloneID 4345766)

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(CloneID 3889866)

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CATCCAGAAGAAATCCCCCTGGCGGCAAGTCCAGCCTCGTCTTGGGTTAG

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TGAGCCTGACTGTACATCTCTTGGATTTGTTTCATTAAAAAGAAGCACTTTATGTACTGCTG

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GCAGTGGCTGGTCAGGAGCCCTCCCCTGTGACCTGTGACCCATGAAAGGCTGGGCTCTGCTG

CCGCTGAAGAAAAACGCTCTTATTTTACCTAAAAGTCCATTTACCGACGCGCGGTGACCCAC

TGTCCCCCAGAGGCGGGGTCACAACGCCCGACACACCGGCACGGGGCCCGAGGCGCCCAGGC

CCGTCGAAACATGTGCGCCGAAACACCGGCGCAGGCCA

(CloneID 4450834)
TCTTAGATCTGAAGGGAGAAGGTGATATTCATGAAAATGTGGACACAGACTTGCCAGGCAGC SEQ ID NO:191

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CCCAGTGCCATCCAGAAGAAATCCCCCTGGCGGCAAGTCCAGCCTCGTCTTGGGTTAG

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TGAGCCTGACTGTACATCTCTTGGATTTGTTTCATTAAAAAGAAGCACTTTATGTACTGCTG

TCTTTTTTTTTTTTCTTTTGAAGAACAGGTTTCTCTCTGTCCTTGACTCTGGGGTCTGTGGG

CCAGTGGCATGAGTGTTTTCTAGTAGTAGATGGGAGGGAAACCTTGGTCACACTTAGTACTG

TGTTTTTAAGAAGAAATAATTGGGTTCCAGATGTGTTAGAGGATCTTTGGTACTGAGGTTTT

TAACACTTTACTGGCGTTTACCAAGCCTCAACTGGACAGACCATAAACAGTCCACAGGCACC

GTTCCTGCCAGGCCCAACCCACAGGGAGTCTCTCCGAGAGCCTTCTGTGGTGTGGCCTAAAT

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CTTGGCGGCTGAAAGGAAAGAAACTGGCTTTATTTTTTAACCTTTAAAAGGTTGCGGATAGT

AACAAGATTGGGTGGGTGATGAGAAGGCTTGGTCTCAGCAACAGAGGTGGTTCAATGAAATG

CTGGATAAAAGCGTGACAG

(CloneID 5521426)
TTACCAACAGAACAACCTGTGAGGAAGAACAAAATGGCCTCTAATATCTTNTGGGACACNCT SEQ ID NO:192

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CAGAGTGAAGAGAAGCCCGTGCCTGCTGCGCCTGTGCCCAGCCCGGTGGCCCCGCCCCCAGT

GCCATCCAGAAGAAATCCCCCTGGCGGCAAGTCCAGCCTCGTCTTGGGTTAC

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TGCGCCCTCCCTGGCTTTTCCCGGCTTGCAAAGAAAAGAAACTGGCCCTTTTTATTTTTTTA

ACCCTTTAAAAAGGAGGCCAGAATAAGTAACCAGAACTTGGCCTGGGCTTGATGTAAACCAA

AGCCCTTTGGTTCTCCCACCCCAAAACGAAAAGGCTTTTGGGT

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(CloneID 5267723)
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GGCCTCTAATATCTTTGGGACACCTGAAGAAAATCAAGCTTCTTGGCCCAAGTCAGCAGGTG

CCAAGTCTAGTGGTGGCAGCGAAGACTTGGAGTCATCTGGACTGCAGAGAAGGAACTCCTCT

CAAGCAAGCTCCGGAGACTTCTTAGATCTGAAGGGAGAAGGTGATATTCATGAAAATGTGGA

CACAGACTTGCCAGGCAGCCTGGGGCAGAGTGAAGAGAAGCCCGTGCCTGCTGCGCCTGTGC

CCAGCCCGGTGGCCCCGGCCCCAGTGCCATCCAGAAGAAATCCCCCTGGCGGCAAGTCCAGC

CTCGTCTTGGGTTAG

CTCTGACTGTCCTGAACGCTGTCGTTCTGTCTGTTTCCTCCATGCTTGTGAACTGCACAACT

TGAGCCTGACTGTACATCTCTTGGATTTGTTTCATTAACAAAGAAGCACTTTATGTACTGCT

GTCTTTTTTTTTTTCCTTTGGAAGAACAGGTTTCTCTCTGTCCTTGACTCTGTGGGTCTGTC

GGCCATGGCATGAGTGTTTCCTAGTAGTAGAT

(CloneID 4441495)
CAGGTGCCAAGTCTAGTGGTGGCAGCGAAGACTTGGAGTCATCTGGACTGCAGAGAAGGAAC SEQ ID NO:194

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GTTCCTTGCCAGGGCCCCAACCCACAGGGAGTCTCTCCCGGCAGAGCCTTTCTTGGTGTTTG

CCTTAAATTGGCAAGTCGGCCTTGGTCAAAGCCTCTTCCTGTGCATGTGAACACTGGAAAGA

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GCCCAAGTTAACAACGGGTGGTGTTGGAAACCTTGTTTCCCCAGAAGGGGGTGTGAAAAGGG

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(CloneID 5395070)
TGACCACAACCACCACCTTCAAGGGAGTCGACCCCAACAGCGAGGAATAGCTCCCGAGTTTT SEQ ID NO:195

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CCTGCTGCGCCTGTGCCCAGCCCGGTGGCCCCGGCCCCAGTGCCATCCAGAAGAAATCCCCC

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CCTGCCGCGTTCTTCACCGCCTTTTAACTGTGTGGTTATCCCAAGCCCTCCACTTGGACAGA

ACCATTAACCATGCTCCACAGCGCACCGTCACTTGCAGGCCCAACCCCCAGGGGGGTCTTCC

GAGAGACCTTTTGGTGTGCCTATTTGCCCAGGGCGTTGTCAACCCCCG

(CloneID 3445695)
GCAGAGTGAAGAGAAGCCCGTGCCTGCTGCGCCTGTGCCCAGCCCGGTCGCCCCGGCCCCTA SEQ ID NO:196

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CTCTGACTGTCCTGAACGCTGTCGTTCTGTCTGTTTCCTCCATGCTTGTGAACTGCACAACT

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CACTTTACTTGGGTTTACCAAGCCTCAACTGGACAGACCATAAACAGTCCACAGGCACCGTT

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GGAACGGGCCCCAAGGGTGTTGTTACACTACCCGCGGGGGTGCATAGCTGTGGCTGCCCTCA

CGAAGCTCCTAACAGGTTTGCTCACCGGCGGGGCCTGAAAATC

(CloneID 5519819)
TCAGCAGGTGCCAAGTCTAGTGGTGGCAGGGAAGACTTGGAGTCATCTGGACTGCAGAGAAG SEQ ID NO:197

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CAAGTCCAGCCTCGTCTTGGGTTAG

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TACTCTTTTATTTNCCCCGCGCGCGTGTTAAAAGCCGCAAAGGNNCCCCCACCGCACACACG

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TGTCCCCTCCGCCACTTCTTTTTACCCCCTCNGCGCTGTAGTTTTGCGGCGCCCCGACTACA

CTCACACCGATTATTTATTATCCCCCTTCANCCGACGCCGGNNGCCCCGAAAAAATGCCGCC

CCGGATCGATAACAGCTCACCCCCGCCCCCGCCAGGATGCCCCGCCGCCAGCTAAAAGGCGC

TCAACCA

(CloneID 2989794)

CACGAGCACTGATGCCCAAGAGTTAAATGGCCTCTAATATCTTTGGGACACCATGAAGAAAA SEQ ID NO:198

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GCCATCCAGAAGAAATCCCCACTGGCGGCAAGTCCAGCCTCGTCTTGGGTTAG

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ACAACTTGAGCCTGACTGTACATCTCTTGGAATTTGTTTCATTAAAAAAAAGCACTTATATG

TACTGCTGTCTTTATTATTAATATACTTTGTGCAAGAACAGGTAAACACATCTGACACATTG

ACCTCTGTGGACTCTGTGAGAGCCATAGGCATTGAGTTGTGAACTAGCTAGATACATGGCGA

GGGAACAGCTTTGTGAACACTAAGAACTCAGATCATAACGACAAAATACATGGGTCCAGATG

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GGGCCACCGGCCTGGGCACAGGCGCAGCAGGCACGGCCTTCTCTTCACTCTGCCCCAGGCTG

CCTGGCAAGTCTGTGTCCACATTTTCATCAATATCACCTTCTCCCTTCAGATCTAAGAAGTC

TCCGGAGCTTGCTTCAGAGGAGTTCCTTCTCTGCAGTCCAGATGACTCCAAGTCTTCCCTGC

CACCACTAGACTTGGCACCTGCTGACTTGGCCCAAGAAGCTTGATTTTCTTCAGGTGTCCCA

AAGATATTAGAGGCCATTTTGTTCTTCCTCACAGGTTGTTCTGTTGGTTCATCAAAACCTAA

TGAAAAATTGGATCCACCACCTGGAGGCCGCAAAACTCGGGAGCTATTCCTGCTGTTGGGGT

CGACTCCCTTGAAGGTGGT

(CloneID 1132042 in 3' to 5' orientation)

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CTAACCCAAGACGAGGCTGGACTTCCCGCCAGGGAGATTTCTTCTGGATGGCACTGGGGCCG

GGGCACCGGGCTGGGCACAGGCGCACGAGGCACGGGCTTCTCTTCACTCTGCCCCACGCTGC

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CTGGCAAGTCTGTGTCCACATTTTCATGAATATCACCTTCTCCCTTCAGATCTAAGAAGTCT

CCGGAGCTTGCTTCAGAGGAGTTCCTTCTCTGCAGTCCAGATGACTCCAAGTCT

[0054] In another set of preferred embodiments of the invention, the sequence, or a unique portion thereof, of the ESE-2/ELF5 sequences identified by the I.M.A.G.E. Consortium CloneIDs 1864302 and 4480123 as well as clusters C019657 and NM_001422 may be used. Similarly, sequences from the UniGene Homo sapiens cluster Hs.11713, may be used. ESE-2/ELF5 sequences are useful in discriminations between normal and ADH or DCIS or IDC breast cells based upon decreased expression in non-normal breast cells. Human ESE-2/ELF5 has been mapped to 11p13-p15.

[0055] ESE-2/ELF5 related sequences are as follows, with the assigned coding region (ending with a termination codon) underlined and preceded by the 5' untranslated and/or non-coding region and followed by the 3' untranslated and/or non-coding region. The 3' untranslated and/or non-coding regions are presented as the complementary strand in the 3' to 5' direction for some of the sequences.

[0056] SEQ ID NOS:204-209 are found in the 3' untranslated and/or non-coding regions of the disclosed ESE-2/ELF5 sequences.

(CloneID 4480123, ESE-2b/ELF5)

CACAAGGCTACAGGTGTCTTTATTTCCACTGCACGCTGGTGCTGGGAGCGCCTGCCTTCTCT SEQ ID NO:201

TGCCTTGAAAGCCTCCTCTTTGGACCTAGCCACCGCTGCCCTCACGGTA

ATGTTGGACTCGGTGACACACAGCACCTTCCTGCCTAATGCATCCTTCTGCGATCCCCTGAT

GTCGTGGACTGATCTGTTCAGCAATGAAGAGTACTACCCTGCCTTTGAGCATCAGACAGCCT

GTGACTCATACTGGACATCAGTCCACCCTGAATACTGGACTAAGCGCCATGTGTGGGAGTGG

CTCCAGTTCTGCTGCGACCAGTACAAGTTGGACACCAATTGCATCTCCTTCTGCAACTTCAA

CATCAGTGGCCTGCAGCTGTGCAGCATGACACAGGAGGAGTTCGTCGAGGCAGCTGGCCTCT

GCGGCGAGTACCTGTACTTCATCCTCCAGAACATCCGCACACAAGGTTACTCCTTTTTTAAT

GACGCTGAAGAAAGCAAGGCCACCATCAAAGACTATGCTGATTCCAACTGCTTCAAAACAAG

TGGCATCAAAAGTCAAGACTGTCACAGTCATAGTAGAACAAGCCTCCAAAGTTCTCATCTAT

GGGAATTTGTACGAGACCTGCTTCTATCTCCTGAAGAAAACTGTGGCATTCTGGAATGGGAA

GATAGGGAACAAGGAATTTTTCGGGTGGTTAAATCGGAAGCCCTGGCAAAGATGTGGGGACA

AAGGAAGAAAAATGACAGAATGACATATGAAAAGTTGAGCAGAGCCCTGAGATACTACTATA

AAACAGGAATTTTGGAGCGGGTTGACCGAAGGTTAGTGTACAAATTTGGAAAAAATGCACAC

GGGTGGCAGGAAGACAAGCTATGA

TCTGCTCCAGGCATCAAGCTCATTTTATGGATTTCTGTCTTTTAAAACAATCAGATTGCAAT

AGACATTCGAAAGGCTTCATTTTCTTCTCTTTTTTTTTTAACCTGCAAACATGCTGATAAAA

TTTCTCCACATCTCAGCTTACATTTGGATTCAGAGTTGTTGTCTACGGAGGGTGAGAGCAGA

AACTCTTAAGAAATCCTTTCTTCTCCCTAAGGGGATGAGGGGATGATCTTTTGTGGTGTCTT

GATCAAACTTTATTTTCCTAGAGTTGTGGAATGACAACAGCCCATGCCATTGATGCTGATCA

GAGAAAAACTATTCAATTCTGCCATTAGAGACACATCCAATGCTCCCATCCCAAAGGTTCAA

AAGTTTTCAAATAACTGTGGCAGCTCACCAAAGGTGGGGAAAGCATGATTAGTTTGCAGGT

TATGGTAGGAGAGGGTGAGATATAAGACATACATACTTTAGATTTTAAATTATTAAAGTCAA

AAATCCATAGAAAAGTATCCCTTTTTTTTTTTTTTGAGACGGGTTCTCACTATGTTGCCCAG

GGCTGGTCTTGAACTCCTATGCTCAAGTGATCCTCCCACCTCGGCCTCCCAAAGTACTGTGA

TTACAAGCGTGAGCCACGGCACCTGGGCAGAAAAGTATCTTAATTAATGAAAGAGCTAAGCC

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GTCAACACAATAAAATCGAAATTCATTGATTCCATCTCTGGTCCAGATGTTAAACGTTTATA

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TCTTTCTACCTGATGTGTATTCAAGTGCTATAACACGTATTTCCTTGACAAAAATAGTGACA

GTGAATTCACACTAATAAATGTTCATAGGTTAAAGTCTGCACTGACATTTTCTCATCAATCA

CTGGTATGTAAGTTATCAGTGACTGACAGCTAGGTGGACTGCCCCTAGGACTTCTGTTTCAC

CAGAGCAGGAATCAAGTGGTGAGGCACTGAATCGCTGTACAGGCTGAAGACCTCCTTATTAG

AGTTGAACTTCAAAGTAACTTGTTTTAAAAAATGTGAATTACTGTAAAATAATCTATTTTGG

ATTCATGTGTTTTCCAGGTGGATATAGTTTGTAAACAATGTGAATAAAGTATTTAACATGTA

AAAAAAAAAAAAAA

(ESE-2a)

GGCTGAGTGGTTTGCTCCTTCCCCTCTCTCTGGGAGGCTGAGCAGGGGTGCCTGGGTTGCTC SEQ ID NO:202

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GGCCTGTCCCTCCAACTCGGTGGACCCTCTCTGTGTGCATTTGGTGTGTGAGCCAGCTCTGA

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ATGCCATCTCTGCCTCACTCCCACAGGGTAATGTTGGACTCGGTGACACACAGCACCTTCCT

GCCTAATGCATCCTTCTGCGATCCCCTGATGTCGTGGACTGATCTGTTCAGCAATGAAGAGT

ACTACCCTGCCTTTGAGCATCAGACAGCCTGTGACTCATACTGGACATCAGTCCACCCTGAA

TACTGGACTAAGCGCCATGTGTGGGAGTGGCTCCAGTTCTGCTGCGACCAGTACAAGTTGGA

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AGGAGGAGTTCGTCGAGGCAGCTGGCCTCTGCGGCGAGTACCTGTACTTCATCCTCCAGAAC

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GTAGAACAAGCCTCCAAAGTTCTCATCTATGGGAATTTGTACGAGACCTGCTTCTATCTCCT

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AGACATTCGAAAGGCTTCATTTTCTTCTCTTTTTTTTTAACCTGCAAACATGCTGATAAAAT

TTCTCCACATCTCAGCTTACATTTGGATTCAGAGTTGTTGTCTACGGAGGGTGAGAGCAGAA

ACTCTTAAGAAATCCTTTCTTCTCCCTAAGGGGATGAGGGGATGATCTTTTGTGGTGTCTTG

ATCAAACTTTATTTTCCTAGAGTTGTGGAATGACAACAGCCCATGCCATTGATGCTGATCAG

AGAAAAACTATTCAATTCTGCCATTAGAGACACATCCAATGCTCCCATCCCAAAGGTTCAAA

AGTTTTCAAATAACTGTGGCAGCTCACCAAAGGTGGGGGAAAGCATGATTAGTTTGCAGGTT

ATGGTAGGAGAGGGTGAGATATAAGACATACATACTTTAGATTTTAAATTATTAAAGTCAAA

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GACCACTCAGCAAAACTTCCACAGACATCCTTCTGGACTTAAACACTTAACATTAACCACAT

TATTAATTGTTGCTGAGTTTATTCCCCCTTCTAACTGATGGCTGGCATCTGATATGCAGAGT

TAGTCAACAGACACTGGCATCAATTACAAAATCACTGCTGTTTCTGTGATTCAAGCTGTCAA

CACAATAAAATCGAAATTCATTGATTCCATCTCTGGTCCAGATGTTAAACGTTTATAAAACC

GGAAATGTCCTAACAACTCTGTAATGGCAAATTAAATTGTGTGTCTTTTTTGTTTTGTCTTT

CTACCTGATGTGTATTCAAGCGCTATAACACGTATTTCCTTGACAAAAATAGTGACAGTGAA

TTCACACTAATAAATGTTCATAGGTTAAAGTCTGCACTGACATTTTCTCATCAATCACTGGT

ATGTAAGTTATCAGTGACTGACAGCTAGGTGGACTGCCCCTAGGACTTCTGTTTCACCAGAG

CAGGAATCAAGTGGTGAGGCACTGAATCGCTGTACAGGCTGAAGACCTCCTTATTAGAGTTG

AACTTCAAAGTAACTTGTTTTAAAAAATGTGAATTACTGTAAAATAATCTATTTTGGATTCA

TGTGTTTTCCAGGTGGATATAGTTTGTAAACAATGTGAATAAAGTATTTAACATGCGAA

(alternative ELF5 encoding sequence)
CAAGGCTACAGGTGTCTTTATTTCCACTGCACGCTGGTGCTGGGAGCGCCTGCCTTCTCTTG SEQ ID NO:203

CCTTGAAAGCCTCCTCTTTGGACCTAGCCACCGCTGCCCTCACGGTA

ATGTTGGACTCGGTGACACACAGCACCTTCCTGCCTAATGCATCCTTCTGCGATCCCCTGAT

GTCGTGGACTGATCTGTTCAGCAATGAAGAGTACTACCCTGCCTTTGAGCATCAGACAGCCT

GTGACTCATACTGGACATCAGTCCACCCTGAATACTGGACTAAGCGCCATGTGTGGGAGTGG

CTCCAGTTCTGCTGCGACCAGTACAAGTTGGACACCAATTGCATCTCCTTCTGCAACTTCAA

CATCAGTGGCCTGCAGCTGTGCAGCATGACACAGGAGGAGTTCGTCGAGGCAGCTGGCCTCT

GCGGCGAGTACCTGTACTTCATCCTCCAGAACATCCGCACACAAGGTTACTCCTTTTTTAAT

GACGCTGAAGAAAGCAAGGCCACCATCAAAGACTATGCTGATTCCAACTGCTTGAAAACAAG

TGGCATCAAAAGTCAAGACTGTCACAGTCATAGTAGAACAAGCCTCCAAAGTTCTCATCTAT

GGGAATTTGTACGAGACCTGCTTCTATCTCCTGAAGAAAACTGTGGCATTCTGGAATGGGAA

GATAGGGAACAAGGAATTTTTCGGGTGGTTAAATCGGAAGCCCTGGCAAAGATGTGGGGACA

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GGGTGGCAGGAAGACAAGCTATGA

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AGACATTCGAAAGGCTTCATTTTCTTCTCTTTTTTTTTAACCTGCAAACATGCTGATAAAAT

TTCTCCACATCTCAGCTTACATTTGGATTCAGAGTTGTTGTCTACGGAGGGTGAGAGCAGAA

ACTCTTAAGAAATCCTTTCTTCTCCCTAAGGGGATGAGGGGATGATCTTTTGTGGTGTCTTG

ATCAAACTTTATTTTCCTAGAGTTGTGGAATGACAACAGCCCATGCCATTGATGCTGATCAG

AGAAAAACTATTCAATTCTGCCATTAGAGACACATCCAATGCTCCCATCCCAAAGGTTCAAA

AGTTTTCAAATAACTGTGGCAGCTCACCAAAGGTGGGGGAAAGCATGATTAGTTTGCAGGTT

ATGGTAGGAGAGGGTGAGATATAAGACATACATACTTTAGATTTTAAATTATTAAAGTCAAA

AATCCATAGAAAAGTATCCCTTTTTTTTTTGAGACGGGTTCTCACTATGTTGCCCAGGGCTG

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AGCGTGAGCCACGGCACCTGGGCAGAAAAGTATCTTAATTAATGAAAGAGCTAAGCCATCAA

GCTGGGACTTAATTGGATTTAACATAGGTTCACAGAAAGTTTCCTAACCAGAGCATCTTTTT

GACCACTCAGCAAAACTTCCACAGACATCCTTCTGGACTTAAACACTTAACATTAACCACAT

TATTAATTGTTGCTGAGTTTATTCCCCCTTCTAACTGATGGCTGGCATCTGATATGCAGAGT

TAGTCAACAGACACTGGCATCAATTACAAAATCACTGCTGTTTCTGTGATTCAAGCTGTCAA

CACAATAAAATCGAAATTCATTGATTCCATCTCTGGTCCAGATGTTAAACGTTTATAAAACC

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(CloneID 377520)
CAGACACTGGCATCAATTACAAAATCACTGCTGTTTCTGTGATTCAAGCTGTCAACACAATA SEQ ID NO:204

AAATCGAAATTCATTGATTCCATCTCTGGTCCAGATGTTAAACGTTTATAAAACCGGAAATG

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ATGTGTATTCAAGCGCTATAACACGTATTTCCTTGACAAAAATAGTGACAGTGAATTCACAC

TAATAAATGTTCATAAGGTTAAAGTCTGCACTGACATTTTCTCATCAATCACTGGTATGTAA

GTTATCAGTGACNGACAGCTAAGGTGNGACTGG

(CloneID 377520 in 3' to 5' orientation)
GAACATGTTAAATACTTTATTCACATTGTTTACAAACTATATCCACCTGGAAAACACATGAA SEQ ID NO:205

TCCAAAATAGATTATTTTACAGTAATTCACATTTTTTAAAACAAGTTACTTTGAAGTTCAAC

TCTAATAAGGAGGTCTTCAGCCTGTACAGCGATTCAGTGCCTCACCACTTGATTCCTGCTCT

GGTGAAACAGAAGTCCTAGGGGCAGTCCACCTAG

(CloneID 81671)
TGATTCAAGCTGTCAACACAATAAAATCGAAATTCATTGATTCCATCTCTGGTCCAGATGTT SEQ ID NO:206

AAACGTTTATAAAACCGGAAATGTCCTAACAACTCTGTAATGGCAAATTAAATTGTGTGTCT

TTTTTGTTTTGTCTTTCTACCTGATGTGTATTCAAGCGCTATAACAC

(CloneID 81671 in 3' to 5' orientation)
GCGGTGAAATACTTTATTCACATTGTTTACAAACTATATCCACCTGGAAAACACATGAATCA SEQ ID NO:207

AAATAGATTATTTTACAGTAATNACATTTTTTAAAACAAGTTACTTTGAAGTCAACTCTAAT

AAGGAGGTCTTCAGCCTGTACAGCGATTCAGTGCCTCACCACTTGATTCCTGCTCTGGTGAA

CAGAAGTCCTAGGGGCAATCCACCTAGCTGTCAGTCACTGATAACTTACATACCAGTGATTG

ATGAGAAAATGTCAGTGCAGACTTTAACCTATGAACATTTATTAGTGTGAATTCACTGTCAC

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ACGTTTACATCTGGGCCNGAGATGGGATCAATGAATTTCGNTTTTATTGTGTTGACCGCTTG

NATCACCTCGTGCCGATTTCCTGCGGCCCGGGGNTCCCCTAGTT

(CloneID 2956497 in 3' to 5' orientation)
AGACGGGTTCTCACTATGTTGCCCAGGGCTGGTCTTGAACTCCTATGCTCAAGTGATCCTCC SEQ ID NO:208

CACCTCGGCCTCCCAAAGTACTGTGATTACAAGCGTGAGCCACGGCACCTGGGCAGAAAAGT

ATCTTAATTAATGAAAGAGCTAAGCCATCAAGCTGGGACTTAATTGGATTTAACATAGGTTC

ACAGAAAGTTTCCTAACCAGAGCATCTTTTTGACCACTCAGCAAAACTTCCACAGACATCCT

TCTGGACTTAAACACTTAACATTAACCACATTATTAATTGTTGCTGAGTTTATTCCCCCTTC

TAACTGATGGCTGGCATCTGATATGCAGAGTTAGTCAACAGACACTGGCATCAATTACAAAA

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TCACTGCTGTTTCTGTGATTCAAGCTGTCAACACAATAAAATCGAAATTCATTGATTCCATC

TCTGGTCCAGATGTTAAACGTTTATAAAACCGG

(CloneID 1864302 in 3' to 5' orientation)

TGAACATGTTAAATACTTTATTCACATTGTTTACAAACTATATCCACCTGGAAAACACATGA SEQ ID NO:209

ATCCAAAATAGATTATTTTACAGTAATTCACATTTTTTAAAACAAGTTACTTTGAAGTTCAA

CTCTAATAAGGAGGTCTTCAGCCTGTACAGCGATTCAGTGCCTCACCACTTGATTCCTGCTC

TGGTGAAACAGAAGTCCTAGGGGCAGTCCACCTAGCTGTCAGTCACTGATAACTTACATACC

AGTGATTGATGAGAAAATGTCAGTGCAGACTTTAACCTATGAACATTTATTAGTGTGAATTC

ACTGTCACTATTTTTGTCAAGGAAATACGTGTTATAGCACTTGAATACACATCAGGTAGAAA

GACAAAACAAAAAAGACACACAATTTAATT

(ESE-2 promoter 1, positions 1—1046, and 5' untranslated region
of ESE-2b,
positions 1047 to >1106)

TTCAATCCCACTTCCTCCTTTTGCCACTGGGGAAAAATGAAGCCCAGAGAAGTCCAGGTTAC SEQ ID NO:210

CCAGCCAAGATAGGGGTCTAGGCAGTCACCTATCTTCCTTCAACAATTTTTCCATGATGACA

CCCCGAATCCTCTCACGAACTGCCTTTTGTTTCTGCAGACTATATGCGTATTTGTATTTGTG

TGTTTGTAACCATGCCCGGTGATCCCAAGAAAACATAACAAACCAGTCCTTGCTTTCGCTTT

AAAGCTTGGAGTCTGCCATTTGAATACAACATCTCGGCTGCCCAAGATGGCTAGAAGCAGAA

TGCAAAAAGGCACAAGGGTTATAAATACCTGTCTCATAGATGACCCGGGACACTTGTGCTTT

GCAGCCTAAATTAGGCAGAGTTTCTGTTGTCACGGAGAAGTACTAAAAAGCGGGCAGTTCTC

AGCGAGACACCTTGAGAGGCTGGCATCCACATGAGGAGAGGCCCCATCACTTACATTACACT

CATGAAGCCCAAGAGATGTTAAGCTACATTTTTCTAGGTAGCACAGCTAGGAAATGATGGAC

ACTGAATTTTGAATCTAAAGGCAACTAGCTGCAAAACCTGAGGTCTCAACACTGGGCCTATA

ATGCTCTTCCTTCTCACCACATGGGAAAAACGGAGAAAGACATGATCTTACAAAAGCACTGG

GCTCAGCCAGCCTGGGAGAGAGGCAGCGCAAACCGGCACCAGGGTGGAATCTCTGTGTTTCC

CCTCCAGCTTGCACAGAGGAAAATTCCTCACCAAACGCACGATCCCCGCACAGCTTCCCAGG

GACAAGAATTTTCTGCTTGTTTACTGAGTCCCCTGGGCTGGGAGTGGGGGGTTTGCAGGGCA

GGGGTGAGCTGCGCACAAAAGCAGGATAAAGGTAAACTTTCTGCATATGAGAACCATTTCCC

CCCCTCCATAGGAGCCGTGTCACACTGTATGTCACCGTCATCAAAGGGGCTGTGCGTAAACC

TGAAAAACCAAACGGACCTGTCTGTAGGTGTCACTTATATCACAAGGTACAGGTGTCTTTAT

TTCCACTGCACGCTGGTGCTGGGAGCGCCTGCCTTCTCTTGCCTTGAAAGCC

(ESE-2 promoter 2, positions 1—1879, and 5' untranslated region
of ESE-2a,
positions 1080 to >1975)

GTAAGCGCCTTTTCTTTGCTTTTTTGGATGAATCGTAAGGGGTGTCAGGGCTCAGAGGCTCG SEQ ID NO:211

GCCTGGGGCCTGGGTCTGCTCGTCCATCTCCAGCTCCAGGGCCGGCACCTGCGCTGGGTGCA

GTCCCAGGGCCTCCGGGAGCTGTTTGACACGGATGTGACGGCTTGCCTCAGAGAGACCTGCT

TGCCTCCGCAGGCCGTGTGGCCACCAGCCCTCCTCACAGCCACCACTCCCTCTGGATCCCTT

AGCTGAACCAAGGGGAATTAAGAGAACGCGCCCAGGCGCTCAGTTCTCTATCCTCCCTAAAG

CTTCTTTAGGAAGGGAAGCTGGATTTTACTCAGAAAAATATTCAGCTTCAAGGAAAGCCCCC

CACCTACCCTTCTCCCCTGACTGAATTCCTTTCCCCACCGGCCTGCCCACTGTCCCCCACCC

CCTCGATACAAAGAACTCCCTTATTATAGTTTGGGGCATTTTCAGGAATTTGTACAAAACAG

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TTTTAAAAGAAACCCATTTGCCCTCTGACATCTTCCCCAGAAGAGAATGCTTCCAGAAAAGC

AATGGTTAGCGTTTTTCCACTGTGTTCTCAGGCCTTGTCAGACTCTGCAGTGTGTGGGGTTT

CTGTGGGGTTGCCCCCTGCTGACAGCGACAGGTAGCACAAGCTCCCTCCTCAGGCTGAAGCC

GGGCCTGCGTGTGGATGCCTTTTGTACGCCCGAAAGTGTGTCAGACATCACTAGGTGCAATC

GCTTCATGTTACAGATGAGGAAACTGAGGCCAGAAAGGCTAGATGACTTAGGGAGGCAGCTC

TGTGGTTCAGTCTAGTCCAGAGACCCAGGGAAAGGGGTGGTTTGCCCATCCGAGTGAACGGC

TTGATTCTGTTGTCTTCGTGTTCTCTGCAATACTGTTTTTCATAATCTAAGGTATTCTCGGG

CCGGAGTTTTCAGTTTGGTCATCTCCCTTTTTCAGAAGTCACCAGAGTGGCAAATTTTTAGA

TTCATTGTTGATGGCTGATTTTTCCCTCTAAAACAGGATTGAATATATGTATTATGCCCCGT

CCTTTGCATATATCGGATTGCATGATTTAAAAAAAAAAAAAATCCCCATTTTAGAGATGAGGG

AAACCGTAGTTCGAAGAGGGGCATGAATATTTATATACCACATCTATATGAAAAAAAGCTTA

AGAATTTCAGCAGTCTGGGTAGTAAGAAATGTGCCCCACGTTCGCAGCAGCAGAGGTAGGAT

TTAACCCCTGATATATGTAATCCAAAACTGGTAAGCTTTGCATCGAAGCCTACAGCAAACTA

CATGGCATACCTTTTTGGATCTTCGCTGCACCTTCAGAAAAACAGCTACAAACAAACAAACA

CGCAAACTCCCTAAGCGAGCTAAAGAAAAATGAGGAATCACTTGACAAGAGGTCAGCAAATT

GGTTCTCACAGTTGCTAGGGTTCCTCTCAGAAGAACAAACCCTCAAGCACCCACCCTCAATC

TTTCTTTAGCAGTAAGTGTTGAAATACTCCACTGGGTTCTCCTCAGTGGGCCCCTTCTTAGG

GAGGTCCAAGGGCTGGGAAATTGACCCTCCCTTGCTCACAAGTCTTGGGACGGAGTGGACAT

TTGGGTATCACTGAGGCTTTAAGGAAGGAAAACCGATTTTCTTCCCAATGATACCACTCAGA

GGGTGGGGTTGAGGAAGCCCAATCTTGGCCACTTTTTTCTATATTTTGCACCCTATGGCCTA

GTTCTGCCCAGTGATGATTCGGCCCGTAAACAGCCAATGTGTAGATGCTTAATTGGGCCAAT

TTTTGGTCACATGCCCAGAGTGAAGTTGATGATCACCACCAGAGTCAGGAAGGAATTTTCCT

CCTCTGGCAAACTGGCCAAGGCTGAGTGGTTTGCTCCTTCCCCTCTCTCTGGGAGGCTGAGC

AGGGGTGCCGGGTTGCTCAGGCCATGGGAGCCACACCTGTTATTGCTGCCTCT

[0057] All sequences are provided using conventional representations of a DNA strand starting from the 5' phosphate linked end to the 3' hydroxyl linked end. The above assignment of coding regions is generally by comparison to available consensus sequence(s) and therefore may contain inconsistencies. These have no effect on the practice of the invention because the invention can be practiced by use of shorter segments (or combinations thereof) of sequences unique to each of the three sets described above and not affected by inconsistencies. As a non-limiting example, a segment of CRIP1 composed of a 3' untranslated region sequence and a sequence from the 3' end of the coding region may be used as a probe for the detection of CRIP1 expression without being affected by the presence of any inconsistency in the representations of the coding regions provided above. Similarly, the use of an antibody which specifically recognizes CRIP1 protein to detect its expression would not be affected by the presence of any inconsistency in the representation of the coding regions provided above.

[0058] As will be appreciated by those skilled in the art, some of the above sequences include 3' poly A (or poly T on the complementary strand) stretches that do not contribute to the uniqueness of the disclosed sequences. The invention may thus be practiced with sequences lacking the 3' poly A (or poly T) stretches. The uniqueness of the disclosed sequences refers to the portions or entireties of the sequences which are found only in CRIP1, HN1, and ESE-2/ELF5 nucleic acids. Preferred unique sequences for the practice of the invention are those which contribute to the consensus sequences for each of the three sets. These preferred unique sequences are of the lengths of polynucleotides of the invention as discussed herein.

[0059] To determine the (increased or decreased) expression levels of the above described sequences in the practice of the present invention, any method known in the art may be utilized. In one preferred embodiment of the invention, expression based on detection of RNA which hybridizes to polynucleotides containing the above described sequences is used. This is readily performed by any RNA detection or amplification+detection method known or recognized as equivalent in the art such as, but not limited to, reverse transcription-PCR (optionally real-time PCR), the methods disclosed in U.S. patent application Ser. No. 10/062,857 entitled "Nucleic Acid Amplification" filed on Oct. 25, 2001 as well as U.S. Provisional Patent Application Nos. 60/298,

847 (filed Jun. 15, 2001) and 60/257,801 (filed Dec. 22, 2000), the methods disclosed in U.S. Pat. No. 6,291,170, and quantitative PCR. Methods to identify increased RNA stability (resulting in an observation of increased expression) or decreased RNA stability (resulting in an observation of decreased expression) may also be used. These methods include the detection of sequences that increase or decrease the stability of mRNAs containing the CRIP1, HN1, and ESE-2/ELF5 sequences disclosed herein. These methods also include the detection of increased mRNA degradation.

[0060] In particularly preferred embodiments of the invention, polynucleotides having sequences present in the 3' untranslated and/or non-coding regions of the above disclosed sequences are used to detect expression or non-expression of CRIP1, HN1, and ESE-2/ELF5 sequences in breast cells in the practice of the invention. Such polynucleotides may optionally contain sequences found in the 3' portions of the coding regions of the above disclosed sequences. Polynucleotides containing a combination of sequences from the coding and 3' non-coding regions preferably have the sequences arranged contiguously, with no intervening heterologous sequence(s).

[0061] Alternatively, the invention may be practiced with polynucleotides having sequences present in the 5' untranslated and/or non-coding regions of the above CRIP1, HN1, and ESE-2/ELF5 sequences in breast cells to detect their levels of expression. Such polynucleotides may optionally contain sequences found in the 5' portions of the coding regions. Polynucleotides containing a combination of sequences from the coding and 5' non-coding regions preferably have the sequences arranged contiguously, with no intervening heterologous sequence(s).

[0062] Preferred polynucleotides contain sequences from 3' or 5' untranslated and/or non-coding regions of at least about 20, at least about 22, at least about 24, at least about 26, at least about 28, at least about 30, at least about 32, at least about 34, at least about 36, at least about 38, at least about 40, at least about 42, at least about 44, or at least about 46 consecutive nucleotides. The term "about" as used in the previous sentence refers to an increase or decrease of 1 from the stated numerical value. Even more preferred are polynucleotides containing sequences of at least or about 50, at least or about 100, at least about or 150, at least or about 200, at least or about 250, at least or about 300, at least or about 350, or at least or about 400 consecutive nucleotides. The term "about" as used in the preceding sentence refers to an increase or decrease of 10% from the stated numerical value.

[0063] Sequences from the 3' or 5' end of the above described coding regions as found in polynucleotides of the invention are of the same lengths as those described above, except that they would naturally be limited by the length of the coding region. The 3' end of a coding region may include sequences up to the 3' half of the coding region. Conversely, the 5' end of a coding region may include sequences up the 5' half of the coding region. Of course the above described sequences, or the coding regions and polynucleotides containing portions thereof, may be used in their entireties.

[0064] Polynucleotides combining the sequences from a 3' untranslated and/or non-coding region and the associated 3' end of the coding region are preferably at least or about 100, at least about or 150, at least or about 200, at least or about 250, at least or about 300, at least or about 350, or at least or about 400 consecutive nucleotides.

[0065] In another embodiment of the invention, polynucleotides containing deletions of nucleotides from the 5' and/or 3' end of the above disclosed sequences may be used. The deletions are preferably of 1-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-45, 45-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-125, 125-150, 150-175, or 175-200 nucleotides from the 5' and/or 3' end, although the extent of the deletions would naturally be limited by the length of the disclosed sequences and the need to be able to use the polynucleotides for the detection of expression levels.

[0066] In yet another embodiment of the invention, polynucleotides containing portions of the above disclosed sequences including the 3' end may be used in the practice of the invention. Such polynucleotides would contain at least or about 50, at least or about 100, at least about or 150, at least or about 200, at least or about 250, at least or about 300, at least or about 350, or at least or about 400 consecutive nucleotides from the 3' end of the disclosed sequences.

[0067] The invention thus also includes polynucleotides used to detect CRIP1, HN1, and ESE-2/ELF5 expression in breast cells. The polynucleotides may comprise a shorter polynucleotide consisting of sequences found in the above provided SEQ ID NOS in combination with heterologous sequences not naturally found in combination with CRIP1, HN1, and ESE-2/ELF5 sequences. As a non-limiting example, a polynucleotide of the invention may comprise a polynucleotide consisting of the sequence of SEQ ID NO: 29, with a deletion of one or more nucleotides from the 5' and/or 3' end, in combination with one or more non-HN1 sequences.

[0068] Other polynucleotides for use in the practice of the invention include those that have sufficient homology to those described above to detect expression by use of hybridization techniques. Such polynucleotides preferably have about or 95%, about or 96%, about or 97%, about or 98%, or about or 99% identity with CRIP1, HN1, or ESE-2/ELF5 sequences as described herein. Identity is determined using the BLAST algorithm, as described above. The other polynucleotides for use in the practice of the invention may also be described on the basis of the ability to hybridize to polynucleotides of the invention under stringent conditions of about 30% v/v to about 50% formamide and from about 0.01M to about 0.15M salt for hybridization and from about 0.01M to about 0.15M salt for wash conditions at about 55 to about 65° C. or higher, or conditions equivalent thereto.

[0069] In a further embodiment of the invention, a population of single stranded nucleic acid molecules comprising one or both strands of a human CRIP1 or HN1 sequence is provided as a probe such that at least a portion of said population may be hybridized to one or both strands of a nucleic acid molecule quantitatively amplified from RNA of a non-normal or abnormal breast cell. The population may be only the antisense strand of a human CRIP1 or HN1 sequence such that a sense strand of a molecule from, or amplified from, a non-normal or abnormal breast cell may be hybridized to a portion of said population. The population preferably comprises a sufficiently excess amount of said one or both strands of a human CRIP1 or HN1 sequence in comparison to the amount of expressed (or amplified) nucleic acid molecules containing a complementary CRIP1 or HN1 sequence from a normal breast cell. This condition

of excess permits the increased amount of nucleic acid expression in a non-normal or abnormal cell to be readily detectable as an increase.

[0070] Alternatively, the population of single stranded molecules is equal to or in excess of all of one or both strands of the nucleic acid molecules amplified from a non-normal or abnormal breast cell such that the population is sufficient to hybridize to all of one or both strands. Preferred non-normal cells are ADH, DCIS, or IDC cells. The single stranded molecules may of course be the denatured form of any CRIP1 and/or HN1 sequence containing double stranded nucleic acid molecule or polynucleotide as described herein.

[0071] The population may also be described as being hybridized to CRIP1 or HN1 sequence containing nucleic acid molecules at a level of at least twice as much as that by nucleic acid molecules of a normal breast cell. As in the embodiments described above, the nucleic acid molecules may be those quantitatively amplified from a breast cell such that they reflect the amount of expression in said cell.

[0072] The population is preferably immobilized on a solid support, optionally in the form of a location on a microarray. A portion of the population is preferably hybridized to nucleic acid molecules quantitatively amplified from a non-normal or abnormal breast cell by real time PCR. The real time PCR may be practiced by use of amplified RNA from a breast cancer cell, as long as the amplification used was quantitative with respect to CRIP1 and/or HN1 containing sequences.

[0073] In another embodiment of the invention, expression based on detection of DNA status may be used. Detection of the ESE-2/ELF5 DNA as methylated, deleted or otherwise inactivated, may be used as an indication of decreased expression as found in non-normal breast cells. This may be readily performed by PCR based methods known in the art. The status of the promoter regions (SEQ ID NOS: 210 and 211) of the ESE-2/ELF5 may also be assayed as an indication of decreased expression of ESE-2/ELF5 sequences. A non-limiting example is the methylation status of sequences found in the promoter region.

[0074] Conversely, detection of the DNA of a sequence as amplified may be used for as an indication of increased expression as found in non-normal breast cells. This may be readily performed by PCR based, fluorescent in situ hybridization (FISH) and chromosome in situ hybridization (CISH) methods known in the art.

[0075] A preferred embodiment using a nucleic acid based assay to determine expression is by immobilization of one or more of the sequences identified herein on a solid support, including, but not limited to, a solid substrate as an array or to beads or bead based technology as known in the art. Alternatively, solution based expression assays known in the art may also be used. The immobilized sequence(s) may be in the form of polynucleotides as described herein such that the polynucleotide would be capable of hybridizing to a DNA or RNA corresponding to the sequence(s).

[0076] The immobilized polynucleotide(s) may be used to determine the state of nucleic acid samples prepared from sample breast cell(s) for which the pre-cancer or cancer status is not known or for confirmation of a status that is already assigned to the sample breast cell(s). Without limiting the invention, such a cell may be from a patient suspected of being afflicted with, or at risk of developing, breast cancer. The immobilized polynucleotide(s) need only be sufficient to specifically hybridize to the corresponding nucleic acid molecules derived from the sample.

[0077] In embodiments where only one or a few sequences are to be analyzed, the nucleic acid derived from the sample breast cancer cell(s) may be preferentially amplified by use of appropriate primers such that only the sequences to be analyzed are amplified to reduce contaminating background signals from other sequences present in the breast cell. Alternatively, and where the disclosed sequences are to be analyzed in combination with other sequences or where very few cells (or one cell) is used, the nucleic acid from the sample may be globally amplified before hybridization to the immobilized polynucleotides. Of course RNA, or the cDNA counterpart thereof may be directly labeled and used, without amplification, by methods known in the art.

[0078] Sequence expression based on detection of a presence, increase, or decrease in protein levels or activity may also be used. Detection may be performed by any immunohistochemistry (IHC) based, bodily fluid based (where a CRIP1, HN1, and/or ESE-2/ELF5 polypeptide is found in a bodily fluid, such as but not limited to blood), antibody (including autoantibodies against the protein where present) based, ex foliate cell (from the cancer) based, mass spectroscopy based, and image (including used of labeled ligand where available) based method known in the art and recognized as appropriate for the detection of the protein. Antibody and image based methods are additionally useful for the localization of tumors after determination of cancer by use of cells obtained by a non-invasive procedure (such as ductal lavage or fine needle aspiration), where the source of the cancerous cells is not known. A labeled antibody or ligand may be used to localize the carcinoma(s) within a patient.

[0079] Antibodies for use in such methods of detection include polyclonal antibodies, optionally isolated from naturally occurring sources where available, and monoclonal antibodies, including those prepared by use of CRIP1, HN1, and/or ESE-2/ELF5 polypeptides as antigens. Such antibodies, as well as fragments thereof (including but not limited to Fab fragments) function to detect or diagnose non-normal or cancerous breast cells by virtue of their ability to specifically bind CRIP1, HN1, or ESE-2/ELF5 polypeptides to the exclusion of other polypeptides to produce a detectable signal. Recombinant, synthetic, and hybrid antibodies with the same ability may also be used in the practice of the invention. Antibodies may be readily generated by immunization with a CRIP1, HN1, or ESE-2/ELF5 polypeptide, and polyclonal sera may also be used in the practice of the invention.

[0080] Antibody based detection methods are well known in the art and include sandwich and ELISA assays as well as Western blot and flow cytometry based assays as non-limiting examples. Samples for analysis in such methods include any that contain CRIP1, HN1, or ESE-2/ELF5 polypeptides. Non-limiting examples include those containing breast cells and cell contents as well as bodily fluids (including blood, serum, saliva, lymphatic fluid, as well as mucosal and other cellular secretions as non-limiting examples) that contain the polypeptides.

[0081] The above assay embodiments may be used in a number of different ways to identify or detect the presence of non-normal breast cells or breast cancer in a breast cancer cell sample from a patient. In some cases, this would reflect a secondary screen for the patient, who may have already undergone mammography or physical exam as a primary screen. If positive from the primary screen, the subsequent needle biopsy, ductal lavage, fine needle aspiration, or other analogous methods may provide the sample for use in the assay embodiments described herein. The present invention is particularly useful in combination with non-invasive protocols, such as ductal lavage or fine needle aspiration, to prepare a breast cell sample. The current analysis of ductal lavage samples is by cytological examination by a trained pathologist who classifies the samples in terms that are at least partly subjective: unsatisfactory (too few cells), benign (including fibrocystic change), atypical (or mild atypia), suspicious (or marked atypia), or malignant.

[0082] The present invention provides a more objective set of criteria, in the form of gene expression levels of discrete gene sequences, to discriminate (or delineate) between normal and non-normal breast cells.

[0083] In one embodiment of the invention, the isolation and analysis of a breast cancer cell sample may be performed as follows:

[0084] (1) Ductal lavage or other non-invasive procedure is performed on a patient to obtain a sample.

[0085] (2) Sample is prepared and coated onto a microscope slide. Note that ductal lavage results in clusters of cells that are cytologically examined as stated above.

[0086] (3) Pathologist or image analysis software scans the sample for the presence of atypical cells.

[0087] (4) If atypical cells are observed, those cells are harvested (e.g. by microdissection such as LCM).

[0088] (5) RNA is extracted from the harvested cells.

[0089] (6) RNA is assayed for the expression of CRIP1, HN1, and/or ESE-2/ELF5 sequences.

[0090] A specific example of the above method would be performing ductal lavage following a primary screen, observing and collecting non-normal cells (or cells suspected of being non-normal) for analysis. Alternatively, the sample may permit the collection of both normal and non-normal cells (or cells suspected of being non-normal) for analysis. The expression levels of CRIP1, HN1, and/or ESE-2/ELF5 sequences in each of these two populations may be compared to each other. This approach can be significantly more powerful than one using the non-normal cells only approach because it utilizes information from the normal cells and the differences between normal and non-normal cells to determine the status of the non-normal cells from the sample.

[0091] While many clinical settings focus on identification of the highest stage or grade of breast cancer, the detection of non-normal breast cells of any stage or grade is also important to identify with confidence the presence of, or susceptibility to, breast cancer as early as possible. With use of the present invention, skilled clinicians will be apprised of the presence of non-normal cells quickly and may begin treatment or additional testing based on such information.

[0092] The present invention may also be used with solid tissue biopsies. As a non-limiting example, a solid biopsy may be collected and prepared for visualization followed by determination of increased CRIP1 and/or HN1 expression to identify or diagnose the presence of non-normal cells. One preferred means is by use of in situ hybridization with polynucleotide or protein identifying probe(s) for assaying expression of said gene(s). An analogous method may be used to detect decreased expression of ESE-2/ELF5 sequences.

[0093] In an alternative method, the solid tissue biopsy may be used to extract molecules followed by analysis for expression of the disclosed sequence(s). This provides the possibility of leaving out the need for visualization and collection of only those cells suspected of being non-normal. This method may of course be modified such that only cells suspected of being non-normal are collected and used to extract molecules for analysis. This would require some form of selection as a prerequisite to gene expression analysis.

[0094] In a further modification of the above, both normal cells and cells suspected of being non-normal are collected and used to extract molecules for analysis of sequence expression. The approach, benefits and results are as described above using non-invasive sampling.

[0095] In a further alternative to all of the above, the sequence(s) identified herein may be used as part of a simple PCR or array based assay simply to determine the presence of non-normal cells in a sample from a non-invasive sampling procedure. If normal expression levels of the disclosed sequences are identified, no further examination may be necessary. If non-normal expression levels are detected, a more comprehensive analysis may follow.

[0096] The detection of sequence expression from samples may be by use of a single microarray able to assay expression of the disclosed sequences as well as other sequences, including sequences known not to vary in expression levels between normal and non-normal breast cells, for convenience and improved accuracy.

[0097] Other uses of the present invention include providing the ability to identify breast cancer cell samples as being non-normal for further research or study. This provides a particular advantage in many contexts requiring the identification of non-normal or cancerous cells based on objective genetic or molecular criteria rather than cytological observation.

[0098] The materials and methods of the present invention are ideally suited for preparation of kits produced in accordance with well known procedures. The invention thus provides kits comprising agents (like the polynucleotides and/or antibodies described herein as non-limiting examples) for the detection of expression of the disclosed sequences. Such kits, optionally comprising the agent with an identifying description or label or instructions relating to their use in the methods of the present invention, are provided. Such a kit may comprise containers, each with one or more of the various reagents (typically in concentrated form) utilized in the methods, including, for example, prefabricated microarrays, buffers, the appropriate nucleotide

triphosphates (e.g., dATP, dCTP, dGTP and dTTP; or rATP, rCTP, rGTP and UTP), reverse transcriptase, DNA polymerase, RNA polymerase, and one or more primer complexes of the present invention (e.g., appropriate length poly(T) or random primers linked to a promoter reactive with the RNA polymerase). A set of instructions will also typically be included.

[0099] The methods provided by the present invention may also be automated in whole or in part. All aspects of the present invention may also be practiced such that they consist essentially of a subset, or subregion, of the disclosed sequences to the exclusion of material irrelevant to the identification of non-normal or cancerous breast cells.

[0100] To identify changes in sequence expression in non-normal or cancerous breast cells, abnormal epithelium from ADH, DCIS and IDC and phenotypically normal epithelium (henceforth referred to as normal) from 36 breast cancer patients and 3 healthy mammoplasty reduction patients were isolated via laser capture microdissection (LCM). The resulting 300 independently microdissected samples were used to interrogate a microarray containing approximately 12,000 human genes.

[0101] One important advantage provided by LCM use is the ability to procure both normal and diseased cell populations from the same biopsy. Therefore, the expression level of each gene in a disease state (ADH or DCIS or IDC) is represented as the ratio to the patient-matched normal, which highlights differences due to disease state as opposed to the genetic background of a particular patient. Unsupervised hierarchical clustering revealed one sequence cluster demonstrating increased expression in a majority of the diseased samples. CRIP1 was included in the cluster and identified as displaying increased expression levels in ADH with persistence in DCIS and IDC samples. Its increased expression may thus be a potential biomarker for the detection of breast cancer including the pre-malignant stage of ADH.

[0102] Sequences of another cluster demonstrated decreased expression in all three pathological stages. The epithelium-specific transcription factor ELF5 was included, and loss of expression of ELF5 in ADH may be an important first step in the initiation of breast malignancy.

[0103] Additional experiments resulted in the identification of increased expression of HN1 sequences in grade III DCIS and IDC cells.

[0104] Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

EXAMPLES

Example I

[0105] Materials and Methods

[0106] Clinical specimen collection and clinicopathological parameters. All breast specimens were obtained from the Massachusetts General Hospital between 1998 and 2001. Thirty-six breast cancer patients were selected, 31 of which were diagnosed with two or more pathological stages of breast cancer progression, and 5 of which were diagnosed with pre-invasive disease only. Three healthy women who underwent elective mammoplasty reduction were selected as disease-free normal controls. Tissue specimens that demonstrated one or more pathological lesions (ADH, DCIS and IDC) were selected for the study. Cases of ADH were selected as proliferative epithelial lesions that possessed some, but not all, of the features of carcinoma in situ (Page, D. L. et al. (1992)) and most closely resemble those lesions described as CAPSS (Oyama, T. et al. and Fraser, J. L. et al.). DCIS and IDC were classified (histological grade) according to the European classification (Holland, R. et al.) and by the Nottingham combined histological grade (Elston, C. W. et al.), respectively. ER and PR expression were determined by immunohistochemical staining (negative when none of the tumor cell nuclei showed staining), and Her-2 expression determined by immunohistochemistry or FISH. This study was approved the Massachusetts General Hospital human research committee in accordance with NIH human research study guidelines.

[0107] LCM and RNA isolation and amplification. Each component (Normal, ADH, DCIS or IDC) was laser capture microdissected in triplicate (from consecutive tissue sections) as described (Sgroi et al.) using a PixCell II LCM system (Arcturus Engineering Inc., Mountain View, Calif.). Total RNA was extracted from the captured cells using the Picopure™ RNA Isolation Kit (Arcturus). T7-based RNA amplification was carried out using the RiboAmp™ kit (Arcturus). Briefly, the RNA from each sample was primed with an oligo-dT primer containing a T7 promoter sequence, reverse transcribed and then converted to double stranded cDNA. The cDNA templates were then used in an in vitro transcription reaction using T7 RNA polymerase to generate amplified RNA (aRNA). To obtain enough aRNA for a microarray experiment, a second round of RNA amplification was performed on all samples. To serve as reference in microarray hybridizations, a human universal reference RNA from Stratagene (La Jolla, Calif.) was amplified identically.

[0108] Fabrication of microarrays. Sequence-verified human cDNA clones were obtained from Research Genetics (Huntsville, Ala.). cDNA clones (from the I.M.A.G.E. Consortium via Research Genetics) inserts were amplified by PCR, gel-purified, and spotted onto a 1×3-inch SuperAmine™ (TeleChem International, Sunnyvale, Calif.) glass microscope slide using an OmniGrid™ robotic arrayer (GeneMachines, San Carlos, Calif.). As used herein, the I.M.A.G.E. Consortium CloneID, or the IMAGE CloneID, lists the identifiers of the cDNA clones on the microarrays according to the I.M.A.G.E. Consortium and Research Genetics (www.resgen.com/). This provides a unique single identifier for each clone. Descriptive names of clones (or genes) use the UniGene symbols and titles (www.ncbi.nlm.nih.gov/UniGene/).

[0109] Probe labeling and hybridization. cDNA was transcribed from aRNA in the presence of 5-(3-aminoallyl)-2'-deoxyuridine 5'-triphosphate (aminoallyl dUTP) using Stratagene's FairPlay kit™ (La Jolla, Calif.). Cy3 or Cy5 monoreactive dye (Amersham, Piscataway, N.J.) was conjugated onto purified cDNA and the residual dye was removed using QiaQuick PCR Purification columns (Qiagen, Valencia, Calif.). Each Cy5-labeled cDNA was hybridized together with the Cy3-labeled reference probe to a microarray in 40

μL hybridization solution (5×SSC, 0.1 μg/μL COT I, 0.2%SDS, 50% formamide) at a concentration of 25 ng/μL per channel for 17 hrs at 42° C. in >60% relative humidity.

[0110] Washing, scanning and image analysis. After hybridization, slides were washed as follows: 1×SSC, 0.2% SDS at 42° for 5 min (two times), 1×SSC, 0.2% SDS at 55° C. for 5 min, 0.1×SSC, 0.2% SDS at 55° C. for 5 min and 0.1×SSC at RT for 2 min. Washed slides were scanned using ScanArray 5000 (PerkinElmer, Billerica, Mass.), and Cy5/Cy3-signals were quantitated using ImaGene 4.2 (BioDiscovery, Los Angeles, Calif.).

[0111] Data processing. Fluorescent intensities of Cy5 and Cy3 channels on each slide were subjected to spot filtering and normalization. Spots flagged by ImaGene were excluded from further analysis. Normalization was performed using a robust nonlinear local regression method (Yang, Y. H. et al.). The normalized ratios of Cy5/Cy3 were used to represent the relative gene expression levels in the experimental samples. Measurements from replicate samples were averaged after normalization.

[0112] Cluster and discriminant analysis. Hierarchical cluster analysis was performed in GeneMaths (v1.5, Applied-Maths, Austin, Tex.) using the cosine correlation coefficient as a measure of similarity between two genes or samples and complete linkage. Linear discriminant analysis with variance was performed within GeneMaths.

Example II

[0113] Analysis of Over and Under Expression

[0114] Quantitative real-time PCR analysis of CRIP1 and ELF5 was conducted to confirm their over and under expression in non-normal breast cells.

[0115] For the non-amplified RNA RT-PCR validation study, independently laser captured (~40,000) normal breast epithelial cells from case 215, and ~40,000 abnormal epithelial cells from DCIS (from cases 89, 178, 179) or IDC (from cases 97, 169, 170) were used. Total RNA was isolated and converted to double-stranded cDNA. For studies using amplified RNA (aRNA), 2 mgs of aRNA from each microdissected sample was converted into double-stranded cDNA. In all cases (cDNA derived from non-amplified and amplified RNA), the double-stranded cDNA was quantitated with PicoGreen (Molecular Probes) using a spectrofluorometer (Molecular Devices) and quantitative analysis of gene expression performed (RT-PCR) was performed with an ABI 7900HT (Applied Biosystems, Foster City, Calif.) as described (Sgroi et al., 1999).

[0116] Each reaction was performed in triplicate using 2.5 ng of double stranded cDNA from each sample as template. The relative standard curve method was used for linear regression analysis of unknown samples and data presented as fold change between samples. The sequences of the PCR primer pairs and fluorogenic probe (5' to 3'), respectively, that were used for each gene are as follows:

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CRIP1:
CCTGCTACGCAGCCATGTT,

GGATGGGTCTCCACCACCT,

VIC-CGGAGCCGAGAGCCACACTTTCAAGT-TAMRA;
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-continued

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ELF5:
TGATTCCTGCTCTGGTGAAACA,

ACATTTTCTCATCAATCA CTGGTATGT,

VIC-CAGTCCACCTAGCTGTCAGTCACTGATA-TAMRA;
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[0117] In agreement with microarray results used to initial identify sequences that were over and under expressed in non-normal breast cells, RT-PCR demonstrated over-expression of CRIP1 (>2-fold) in 7 of 8 ADH, 27 of 30 DCIS, and 23 of 25 IDC cases, and under-expression of ELF5 (>2-fold) in 7 of 8 ADH, 28 of 30 DCIS, and 25 of 25 IDC cases (**FIG. 1**).

[0118] In addition, we performed in-situ hybridization for CRIP1 to confirm its cellular specificity. As expected from the use of LCM, CRIP1 signal localized to the epithelial cells, and its intensity was markedly increased in the IDC compartment of the same biopsy (**FIGS. 2 and 3**), thus verifying the microarray-derived results at the level of cellular resolution.

[0119] All references cited herein, including patents, patent applications, and publications, are hereby incorporated by reference in their entireties, whether previously specifically incorporated or not.

[0120] Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without undue experimentation. This application is intended to cover any variations, uses, or adaptations of the invention, following in general the principles of the invention, that include such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth.

We claim:

1. A method to determine the presence of non-normal or abnormal breast cells in a sample from a human subject comprising assaying said sample for increased expression of one or more human CRIP1 or HN1 sequences.

2. The method of claim 1 wherein said assaying is for increased expression of human CRIP1 sequences.

3. The method of claim 1 wherein said assaying is for increased expression of human HN1 sequences.

4. The method of claim 1 wherein said sample is from a subject afflicted with, or suspected of having, breast cancer.

5. The method of claim 1 wherein said sample is obtained by solid tissue biopsy or a non-invasive procedure.

6. The method of claim 5 wherein said non-invasive procedure is selected from ductal lavage, fine needle aspiration, or a needle biopsy.

7. The method of claim 5 wherein microdissection is used to isolate breast cells from said sample before assaying for nucleic acid expression.

8. The method of claim 1 wherein said assaying is by hybridization to a polynucleotide comprising sequences of at least 24 nucleotides from the 3' untranslated region, the coding region, or the 5' untranslated region, of human CRIP1.

9. The method of claim 1 wherein said assaying is by hybridization to a polynucleotide comprising sequences of at least 24 nucleotides from the 3' untranslated region, the coding region, or the 5' untranslated region, of human HN1.

10. The method of claim 1 wherein said assaying is by PCR amplification of said sequences.

11. The method of claim 10 wherein said PCR is quantitative PCR.

12. The method of claim 1 wherein said non-normal cells are ADH, DCIS, or IDC cells.

13. A method to determine the presence of non-normal or abnormal breast cells in a sample from a human subject comprising assaying said sample for decreased expression of human ESE-2/ELF5 sequences.

14. The method of claim 13 wherein said sample is from a subject afflicted with, or suspected of having, breast cancer.

15. The method of claim 13 wherein said sample is obtained by solid tissue biopsy or a non-invasive procedure.

16. The method of claim 15 wherein said non-invasive procedure is selected from ductal lavage, fine needle aspiration, or a needle biopsy.

17. The method of claim 16 wherein microdissection is used to isolate breast cells from said sample before assaying for nucleic acid expression.

18. The method of claim 17 wherein said assaying is by hybridization to a polynucleotide comprising sequences of at least 24 nucleotides from the 3' untranslated region, the coding region, or the 5' untranslated region, of human ESE-2/ELF5.

19. The method of claim 13 wherein said assaying is by PCR amplification of said ESE-2/ELF5 sequence.

20. The method of claim 19 wherein said assaying is by quantitative PCR.

21. The method of claim 13 wherein said assaying is for inactivation or methylation of ESE-2/ELF5 sequences.

22. The method of claim 13 wherein said assaying comprises detection of increased mRNA degradation.

23. The method of claim 13 wherein said non-normal cells are ADH, DCIS, or IDC cells.

24. A polynucleotide comprising

a segment consisting of a fragment of an HN I sequence selected from SEQ ID NOS: 29-83 of between 24 and 500 nucleotides and

one or more non-HN1 nucleic acid molecules.

25. A polynucleotide consisting of a fragment of an HN1 sequence selected from SEQ ID NOS: 29-83 of between 24 and 500 nucleotides.

26. A population of singled stranded nucleic acid molecules comprising one or both strands of a human CRIP1 or HN1 sequence wherein at least a portion of said population is hybridized to one or both strands of a nucleic acid molecule quantitatively amplified from RNA of a non-normal or abnormal breast cell.

27. The population of claim 26 wherein the population is immobilized on a solid support.

28. The population of claim 27 wherein said solid support is a microarray.

29. The population of claim 26 wherein said nucleic acid molecules amplified from a non-normal or abnormal breast cell are amplified by quantitative real time PCR (RT-PCR).

30. The population of claim 29 wherein said quantitative RT-PCR is of amplified RNA of said breast cancer cell.

31. The population of claim 26 wherein said population of single stranded molecules is equal to or in excess of all of one or both strands of the nucleic acid molecules amplified from a non-normal or abnormal breast cell such that the population is sufficient to hybridize to all of one or both strands.

32. The population of claim 26 wherein said population of single stranded molecules comprising sequences of at least 24 nucleotides from the 3' untranslated region, the coding region, or the 5' untranslated region, of human CRIP1.

33. The population of claim 26 wherein said population of single stranded molecules comprising sequences of at least 24 nucleotides from the 3' untranslated region, the coding region, or the 5' untranslated region, of human HN1.

34. The population of claim 26 wherein said non-normal cells are ADH, DCIS, or IDC cells.

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