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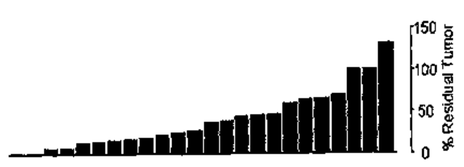
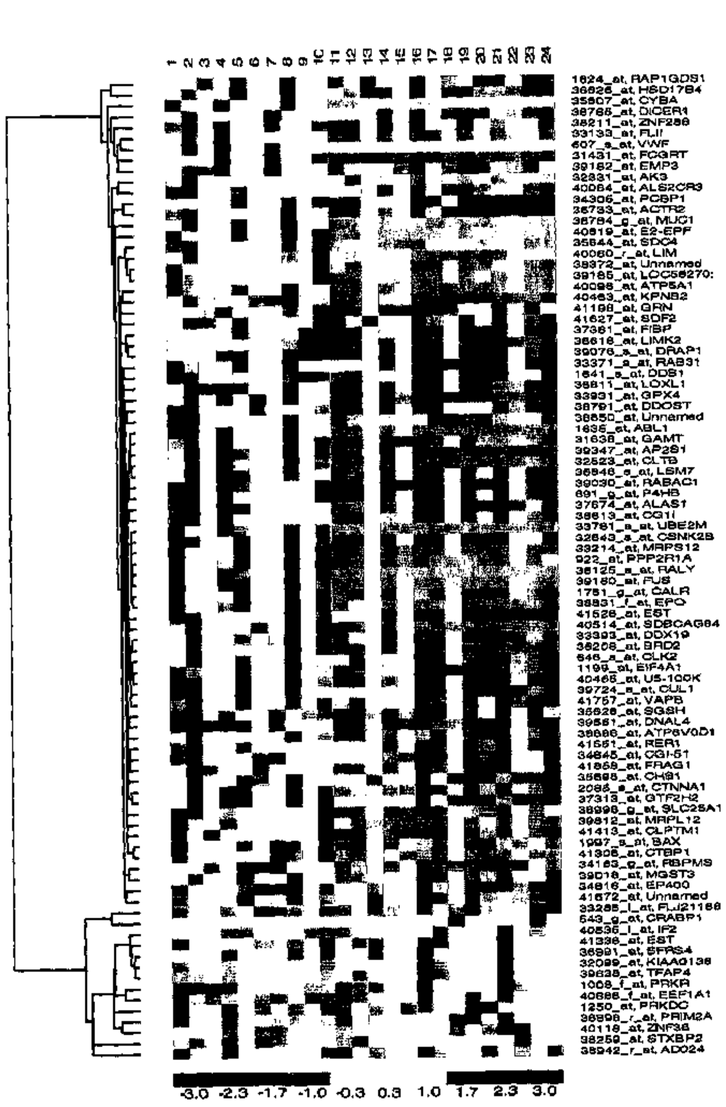
(54) Titre : CONFIGURATIONS DIFFERENTIELLES D'EXPRESSION GENETIQUE PERMETTANT LA PREVISION DE CHIMIOSENSIBILITE ET DE CHIMIORESISTANCE AU DOCETAXEL

(54) Title: DIFFERENTIAL PATTERNS OF GENE EXPRESSION THAT PREDICT FOR DOCETAXEL CHEMOSENSITIVITY AND CHEMORESISTANCE





(57) Abrégé/Abstract:
The invention pertains to differential gene expression profiles for docetaxel responsiveness. The invention identifies molecular profiles in primary breast cancers that appear to predict response or lack of response to docetaxel. This invention provides methods involving prediction of docetaxel responsiveness as well as arrays for use in determining docetaxel responsiveness.

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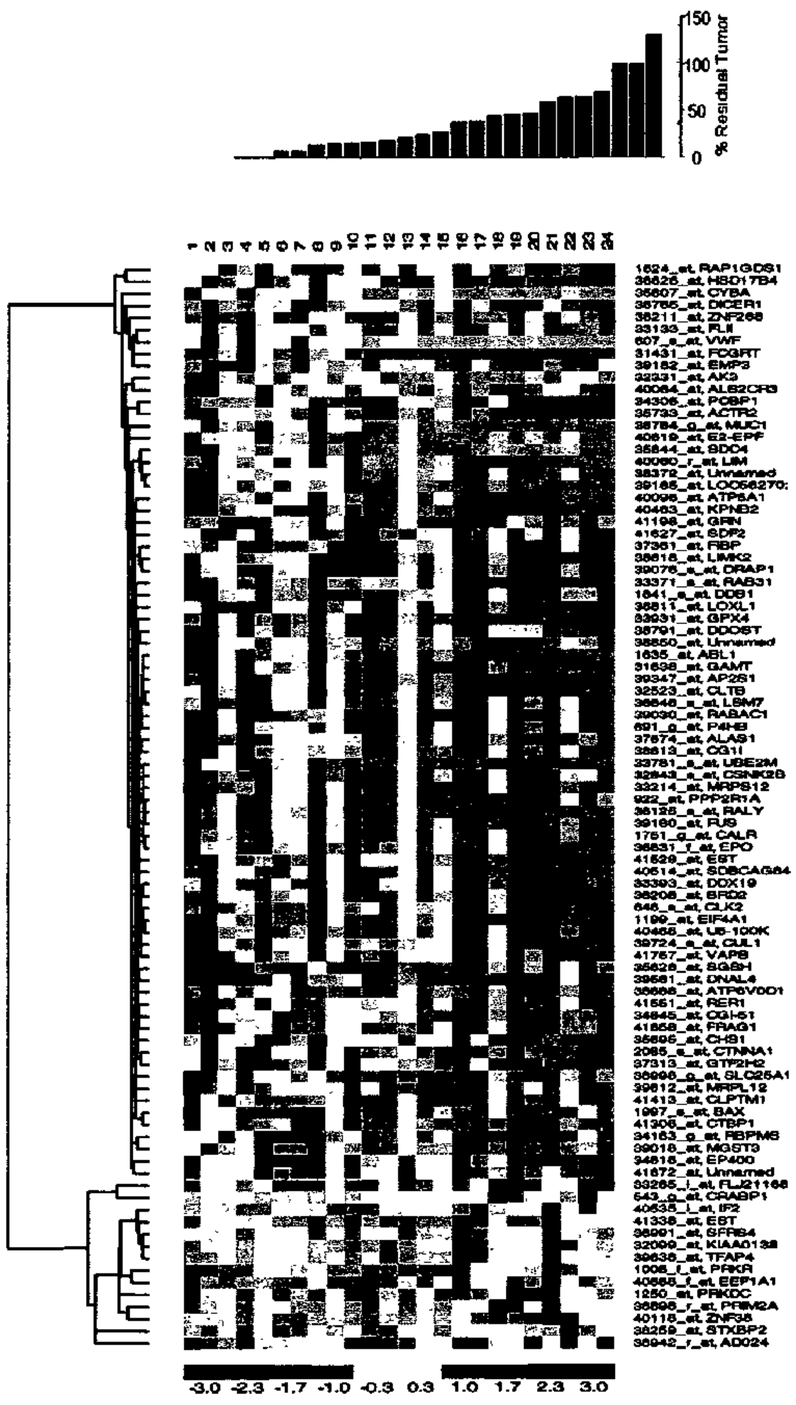
(54) Title: DIFFERENTIAL PATTERNS OF GENE EXPRESSION THAT PREDICT FOR DOCETAXEL CHEMOSENSITIVITY AND CHEMORESISTANCE



(57) Abstract: The invention pertains to differential gene expression profiles for docetaxel responsiveness. The invention identifies molecular profiles in primary breast cancers that appear to predict response or lack of response to docetaxel. This invention provides methods involving prediction of docetaxel responsiveness as well as arrays for use in determining docetaxel responsiveness.

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DIFFERENTIAL PATTERNS OF GENE EXPRESSION THAT PREDICT FOR DOCETAXEL CHEMOSENSITIVITY AND CHEMORESISTANCE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This applications claims the benefit of U.S. Provisional Application No. 60/381,141, filed May 17, 2002, which is hereby incorporated by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] The present invention was developed with funds from United States Army grant number BC000506. Therefore, the United States Government may have certain rights in the invention.

TECHNICAL FIELD

[0003] The field of the invention relates to gene expression profiles in breast cancer cells. The field of the invention also relates to docetaxel sensitivity or resistance in breast cancer cells.

BACKGROUND OF THE INVENTION

[0004] Optimal systemic treatment (adjuvant therapy) after breast cancer surgery is the most crucial factor in reducing mortality in women with breast cancer. Adjuvant chemotherapy and hormonal treatment both reduce the risk of death in breast cancer patients. However, while estrogen receptor status predicts for response to hormonal treatments, there are no clinically useful predictive markers for chemotherapy response. All eligible women are therefore treated in the same manner even though *de novo* drug resistance will result in treatment failures in many breast cancer patients. The taxanes, docetaxel (Taxotere™) and paclitaxel (Taxol™), are a new class of anti-microtubule

agents that are more effective than older drugs like the anthracyclines, although clinical trials with taxanes and anthracyclines in combination show that only a small subset of patients benefit from the addition of taxanes. Currently, there are no methods available to distinguish those patients who are likely to respond to taxanes from those who are not, and given the accepted practice of prescribing adjuvant treatment to most patients even if the average expected benefit is low, the *a priori* selection of appropriate patients most likely to benefit from adjuvant taxane therapy would represent a major advance in the clinical management of breast cancer today. A major impediment to study predictors of therapeutic efficacy in the adjuvant setting is the lack of surrogate markers for survival and, consequently, large numbers of patients with long-term follow-up are needed to conduct these studies.

[0005] There have been only a few publications on the utility of gene expression arrays in human breast cancers. Using printed oligonucleotide microarrays, van't Veer *et al.* found gene expression profiles to be more accurately prognostic of outcome in a small set of 78 young women with node-negative breast cancer, when compared to standard clinical and histologic criteria. The same authors subsequently validated this 70-gene classifier in a cohort of 295 patients, many of which were not in the original study. The poor prognostic signature included genes regulating cell cycle, invasion, metastasis, and angiogenesis. Using cDNA arrays, Perou *et al.* identified distinct patterns of gene expression that were termed "basal" or "luminal" type. These groups differed from each other with respect to clinical outcome. The object of the present invention is to provide gene expression patterns that *predict* response or lack of response to specific chemotherapy in primary breast cancer patients, as opposed to previous studies, which have dealt with patient *prognosis*.

[0006] U.S. Patent No. 6,107,034 describes the association of the expression of GATA-3 with estrogen receptor positive tumors that are responsive to docetaxel and other taxanes.

[0007] These gene expression patterns associated with docetaxel sensitivity and resistance are highly complex. In the past, studies utilizing single gene biomarkers to assess sensitivity and resistance to chemotherapy have seldom been conclusive. For example, in a recent breast cancer study, commonly measured predictive and prognostic markers (HER-2, p53, p27, or epidermal growth factor receptor) failed to find any correlation between these selected biomarkers and taxane sensitivity. The published literature in different cancer types has suggested that alterations in expression levels of β-tubulin isoforms may represent an important and complex mechanism of taxane resistance. Overexpression of some β-tubulin isoforms is associated with docetaxel resistance in some tumors, but not all. These results indicate that the patterns of gene expression for sensitivity and resistance involve multiple gene pathways, and that integration of many genes in these pathways leads to drug sensitivity and resistance. This supports the idea that assessment of expression of a few individual genes will not be powerful enough to untangle the heterogeneity of clinical breast cancer behavior, while patterns of expression of many genes may be more successful in distinguishing sensitive and resistant tumors.

[0008] In the present invention, gene expression patterns in primary breast cancer specimens that predict response to taxanes were identified. Neoadjuvant chemotherapy (treatment before primary surgery) allows for sampling of the primary tumor for gene expression analysis, and for direct assessment of response to chemotherapy by following changes in tumor size during the first few months of treatment. This clinical tumor response to neoadjuvant chemotherapy has been shown to be a valid surrogate marker of survival, with better outcome in those patients whose tumors regress

significantly after neoadjuvant chemotherapy compared to those with modest response or clinically obvious chemotherapy-resistant disease. With the advent of high-throughput quantitation of gene expression, it is now possible to assess thousands of genes simultaneously to identify expression patterns in different breast cancers that might correlate with and thereby predict excellent clinical response to treatment. These profiles have a great potential to penetrate the genetic heterogeneity of this disease and prioritize different treatment strategies based on their likelihood of success in individual patients. Hence, neoadjuvant chemotherapy provides an ideal platform to rapidly discover predictive markers of chemotherapy response. In the present study, core needle biopsies of the primary breast cancer were analyzed for gene expression profiling before patients received neoadjuvant docetaxel. The present invention demonstrates that 1) sufficient RNA is obtained from these core biopsies to assess gene expression, 2) there are groups of genes that are used to distinguish primary breast cancers that are responsive or resistant to docetaxel chemotherapy, and 3) certain gene pathways are important in the mechanism of resistance to docetaxel.

BRIEF SUMMARY OF THE INVENTION

[0009] An embodiment of the present invention is a method of screening a patient for response to docetaxel therapy comprising the steps of: obtaining a tumor sample from the patient; isolating RNA from the sample; determining relative expression of individual nucleic acids in the RNA of at least 10 of the nucleic acids selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID

NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, and SEQ ID NO:91; and subjecting the relative expression of the individual nucleic acids to a clustering algorithm, wherein the sample is docetaxel resistant if the results of the clustering algorithm indicate that the relative expression of the individual nucleic acids in the sample is characteristic of a docetaxel resistant tumor, and wherein the sample is docetaxel sensitive if the results of the clustering algorithm indicate that the relative expression of the individual nucleic acids in the sample is characteristic of a docetaxel sensitive tumor. In other embodiments, the expression levels of 50 of the nucleic acids selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34,

SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, and SEQ ID NO:91 are determined. In a specific embodiment, the expression levels of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76,

SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, and SEQ ID NO:91 are determined.

[0010] In a specific embodiment, the relative overexpression in the tumor sample of at least one nucleic acid selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:12, SEQ ID NO:18, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:43, SEQ ID NO:53, SEQ ID NO:63, SEQ ID NO:69, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:78, and SEQ ID NO:87 is associated with docetaxel resistance. In a further specific embodiment, the overexpression is at least 2.5-fold.

[0011] In another specific embodiment, the relative overexpression in the tumor tissue sample of at least one nucleic acid selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85,

SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, and SEQ ID NO:91 is associated with docetaxel sensitivity.

[0012] In yet another specific embodiment, the determining the relative expression of individual nucleic acids in the RNA comprises the steps of: providing a plurality of probes bound to a solid surface, at least 10, 50, or 91 of said plurality of probes being complementary to sequences selected from the group consisting of nucleic acids consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, and SEQ ID NO:91; contacting the probes with the RNA obtained from the tumor tissue sample, and detecting binding of the RNA to the probes; thereby identifying differences in relative expression of the nucleic acids. In a

specific embodiment, the solid surface is glass or nitrocellulose and the detecting of binding comprises detecting fluorescent or radioactive labels. The tumor tissue sample is a primary breast tumor, in a specific embodiment. In another embodiment of the present invention, the tumor tissue sample is a core biopsy, and the core biopsy is paraffin-embedded.

[0013] An embodiment of the present invention is method of monitoring a cancer patient receiving docetaxel therapy comprising the steps of: obtaining tumor tissue samples from the patient at various timepoints during the docetaxel therapy; isolating RNA from the samples; determining relative expression of individual nucleic acids in the RNA in the samples of at least 50 of the nucleic acids selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83,

SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, and SEQ ID NO:91; and subjecting the relative expression of the individual nucleic acids of the samples to a clustering algorithm, wherein the clustering algorithm is derived from an analysis of gene expression profiles of known docetaxel resistant and known docetaxel sensitive tumor samples, and wherein the sample is docetaxel resistant if the results of the clustering algorithm indicate that the relative expression of the individual nucleic acids in the sample is characteristic of a docetaxel resistant tumor, and wherein the sample is docetaxel sensitive if the results of the clustering algorithm indicate that the relative expression of the individual nucleic acids in the sample is characteristic of a docetaxel sensitive tumor. In a specific embodiment, if any individual sample exhibits a gene expression profile associated with docetaxel resistance, docetaxel therapy is interrupted.

[0014] An embodiment of the invention is an array for screening a patient for resistance to docetaxel comprising complementary nucleic acid probes attached to a solid surface for at least 50 of the nucleic acids selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56,

SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, and SEQ ID NO:91.

[0015] The foregoing has outlined rather broadly the features and technical advantages of the present invention in order that the detailed description of the invention that follows may be better understood. Additional features and advantages of the invention will be described hereinafter which form the subject of the claims of the invention. It should be appreciated by those skilled in the art that the conception and specific embodiment disclosed may be readily utilized as a basis for modifying or designing other structures for carrying out the same purposes of the present invention. It should also be realized by those skilled in the art that such equivalent constructions do not depart from the spirit and scope of the invention as set forth in the appended claims. The novel features which are believed to be characteristic of the invention, both as to its organization and method of operation, together with further objects and advantages will be better understood from the following description when considered in connection with the accompanying figures. It is to be expressly understood, however, that each of the figures is provided for the purpose of illustration and description only and is not intended as a definition of the limits of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee. For a more complete understanding of the present invention, reference is now made to the following descriptions taken in conjunction with the accompanying drawing, in which:

[0017] FIG. 1 depicts the algorithm of statistical analytical approach compared with methods used by van't Veer *et al.*, 2002. The prognostic analysis used by van't Veer *et al.* utilized oligonucleotide microarrays with 25,000 genes, from which 5,000 variably expressed genes were selected by filtering. Of these, 231 genes were found to be significantly associated with prognostic outcome ($|r|>0.3$). These 231 genes were then rank-ordered on the basis of the magnitude of the correlation coefficient and selected in groups of five to construct the smallest optimal classifier. Leave-one-out analysis was then conducted using the N=231 genes correlated with outcome to select a classification set of 70 genes. In contrast, in the analysis of the present invention, a subset of 1,628 genes was selected by filtering on signal intensity to eliminate genes with uniformly low expression or genes whose expression did not vary significantly across the samples. After log transformation, a t-test was used to select 91 discriminatory genes. Starting with 1,628 filtered genes, the entire gene selection and classifier construction process was repeated in an external leave-one-out cross-validation to estimate classifier performance, resulting in a classifier with an accuracy of 88%.

[0018] FIG. 2 is a hierarchical clustering of genes correlated with docetaxel response. Sensitive tumors (S) are defined as 25% residual disease or less (shown as blue bars), and resistant tumors (R) are defined as greater than 25% residual disease (shown as

red bars). The expression levels are shown in red (expression levels above the mean for the gene) and blue (levels below the mean for the gene). The color scale (see bottom of figure) ranges from 3 standard deviations (or more) below the mean (darkest blue) to 3 standard deviations above the mean (darkest red). Affymetrix probe set identifiers and corresponding gene symbols are shown on the right-hand side.

[0019] FIG. 3 is a Receiver Operating Characteristic (ROC) curve for predicting response to docetaxel using the 91-gene classifier, with positive and negative predictive values of 92% and 83% respectively. The area under the curve is 0.96.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

[0020] As used herein the specification, "a" or "an" may mean one or more. As used herein in the claim(s), when used in conjunction with the word "comprising", the words "a" or "an" may mean one or more than one. As used herein "another" may mean at least a second or more.

[0021] As used herein, the term "adjuvant" refers to a pharmacological agent that is provided to a patient as an additional therapy to the primary treatment of a disease or condition.

[0022] "Bind(s) substantially" refers to complementary hybridization between a probe nucleic acid and a target nucleic acid and embraces minor mismatches that can be accommodated by reducing the stringency of the hybridization media to achieve the desired detection of the target polynucleotide sequence.

[0023] The terms "background" or "background signal intensity" refer to hybridization signals resulting from non-specific binding, or other interactions, between the labeled target nucleic acids and components of the oligonucleotide array (e.g., the oligonucleotide probes, control probes, the array substrate, etc.). Background signals may also be produced by intrinsic fluorescence of the array components themselves. A single background signal can be calculated for the entire array, or a different background signal may be calculated for each target nucleic acid. In a preferred embodiment, background is calculated as the average hybridization signal intensity for the lowest 5% to 10% of the probes in the array, or, where a different background signal is calculated for each target gene, for the lowest 5% to 10% of the probes for each gene. Of course, one of skill in the art will appreciate that where the probes to a particular gene hybridize well and thus appear to be specifically binding to a target sequence, they should not be used in a background signal calculation. Alternatively, background may be calculated as the average hybridization signal intensity produced by hybridization to probes that are not complementary to any sequence found in the sample (e.g. probes directed to nucleic acids of the opposite sense or to genes not found in the sample such as bacterial genes where the sample is mammalian nucleic acids). Background can also be calculated as the average signal intensity produced by regions of the array that lack any probes at all. Depending on the analysis, one skilled in the art knows which background signal calculation to use.

[0024] As used herein, the expressions “cell”, “cell line”, and “cell culture” are used interchangeably and all such designations include progeny. Thus, the words “transformants” and “transformed cells” include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened

for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

[0025] The term "core biopsy" of the breast as used herein refers to either the small cylindrical sample of the breast tissue that is obtained from the core biopsy procedure, or to the procedure itself. Core biopsy of the breast is performed under local anaesthetic without need for sedation. The core biopsy needle is directed into the correct area of the breast and using a specially designed instrument and needle, several small cores of breast tissue are obtained from the affected area. The core biopsy needle is guided into the correct area of the breast using either ultrasound or stereotactic x-ray guidance. Generally, core biopsy is designed to provide a piece of breast tissue rather than just individual cells.

[0026] As used herein, an "expression profile" or "gene expression profile" comprises measurement of a plurality of mRNAs to indicate the relative expression or relative abundance of any particular transcript. The compilation of the expression levels of all of the mRNA transcripts sampled at any given time point in any given sample comprises the gene expression profile. Within eukaryotic cells, there are hundreds to thousands of signaling pathways that are interconnected. For this reason, changes in the levels or activity of proteins within a cell have numerous effects on other proteins and the transcription of other genes that are connected by primary, secondary, and sometimes tertiary pathways. This extensive interconnection between the function of various proteins means that the alteration of any one protein is likely to result in compensatory changes in a wide number of other proteins. In particular, the partial disruption of even a single protein within a cell, such as by exposure to a drug or by a disease state which modulates the gene copy number (e.g., a genetic mutation), results in characteristic compensatory changes in the transcription of enough other genes that these changes in transcripts can be used to

define a " characteristic expression profile" of particular transcript alterations which are related to the disruption of function. For example, a tumor sample which is docetaxel resistant will have a characteristic gene expression profile which is distinguishable from the characteristic gene expression profile of a docetaxel sensitive tumor sample.

[0027] The term "hybridizing specifically to", refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent conditions when that sequence is present in a complex mixture (e.g., total cellular) DNA or RNA. The term "stringent conditions" refers to conditions under which a probe will hybridize to its target subsequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. One skilled in the art knows how to select such conditions. Longer sequences hybridize specifically at higher temperatures. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. (As the target sequences are generally present in excess, at Tm, 50% of the probes are occupied at equilibrium). Typically, stringent conditions will be those in which the salt concentration is at least about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide.

[0028] The term "mismatch control" refers to a probe that has a sequence deliberately selected not to be perfectly complementary to a particular target sequence. The mismatch control typically has a corresponding test probe that is perfectly complementary to the same particular target sequence. The mismatch may comprise one or more bases.

While the mismatch(s) may be located anywhere in the mismatch probe, terminal mismatches are less desirable as a terminal mismatch is less likely to prevent hybridization of the target sequence. In a particularly preferred embodiment, the mismatch is located at or near the center of the probe such that the mismatch is most likely to destabilize the duplex with the target sequence under the test hybridization conditions.

[0029] The term "mRNA" refers to transcripts of a gene. Transcripts are RNA including, for example, mature messenger RNA ready for translation, products of various stages of transcript processing. Transcript processing may include splicing and degradation.

[0030] The terms "nucleic acid" or "nucleic acid molecule" refer to a deoxyribonucleotide or ribonucleotide polymer in either single-or double-stranded form, and unless otherwise limited, would encompass known analogs of natural nucleotides that can function in a similar manner as naturally occurring nucleotides.

[0031] An "oligonucleotide" is a single-stranded nucleic acid ranging in length from 2 to about 500 bases.

[0032] The term "overexpression" means that the relative expression for a particular gene is higher in one sample as compared to another sample. Parameters for overexpression may change as necessary for a particular algorithm. For example, it is contemplated that a gene may not be considered overexpressed unless its expression is at least 1.2, 1.5, 2, or 3 times higher than the control sample.

[0033] The term "polypeptide" as used herein is used interchangeably with the term "protein" and is defined as a molecule which comprises more than one amino acid subunit. The polypeptide may be an entire protein or it may be a fragment of a protein,

such as a peptide or an oligopeptide. The polypeptide may also comprise alterations to the amino acid subunits, such as methylation or acetylation.

[0034] As used herein a "probe" is defined as an oligonucleotide capable of binding to a target nucleic acid of complementary sequence through one or more types of chemical bonds, usually through complementary base pairing, usually through hydrogen bond formation. As used herein, an oligonucleotide probe may include natural (ie. A, G, C, or T) or modified bases (7-deazaguanosine, inosine, etc.). In addition, one skilled in the art recognizes that the bases in oligonucleotide probe may be joined by a linkage other than a phosphodiester bond, so long as it does not interfere with hybridization. Thus, oligonucleotide probes may be peptide nucleic acids in which the constituent bases are joined by peptide bonds rather than phosphodiester linkages.

[0035] The term "quantifying" when used in the context of quantifying transcription levels of a gene can refer to absolute or to relative quantification. Absolute quantification may be accomplished by inclusion of known concentration(s) of one or more target nucleic acids (e.g. control nucleic acids such as Bio B or with known amounts of the target nucleic acids themselves) and referencing the hybridization intensity of unknowns with the known target nucleic acids (e.g. through generation of a standard curve). Alternatively, relative quantification can be accomplished by comparison of hybridization signals between two or more genes, or between two or more treatments to quantify the changes in hybridization intensity and, by implication, transcription level.

[0036] As used herein, the term "relative gene expression" or "relative expression" in reference to a gene refers to the relative abundance of the same gene expression product, usually an mRNA, in different cells or tissue types. In a preferred embodiment, the expression of a gene in a tumor sample is compared to tumor samples

from the same patient taken at different time points, or it is compared to tumor samples from different patients. In another preferred embodiment, the tumor sample is a primary breast tumor and the relative gene expression is used to determine docetaxel sensitivity or resistance.

[0037] The term "sample" as used herein indicates a patient sample containing at least one cell. Tissue or cell samples can be removed from almost any part of the body. The most appropriate method for obtaining a sample depends on the type of cancer that is suspected or diagnosed. Biopsy methods include needle, endoscopic, and excisional.

[0038] "Subsequence" refers to a sequence of nucleic acids that comprise a part of a longer sequence of nucleic acids.

[0039] The term "target nucleic acid" refers to a nucleic acid (often derived from a biological sample), to which the oligonucleotide probe is designed to specifically hybridize. It is either the presence or absence of the target nucleic acid that is to be detected, or the amount of the target nucleic acid that is to be quantified. The target nucleic acid has a sequence that is complementary to the nucleic acid sequence of the corresponding probe directed to the target. The term target nucleic acid may refer to the specific subsequence of a larger nucleic acid to which the probe is directed or to the overall sequence (e.g., gene or mRNA) whose expression level it is desired to detect. The difference in usage will be apparent from context.

II. The Present Invention

[0040] In one preferred embodiment, the methods of this invention are used to monitor the expression (transcription) levels of nucleic acids whose expression is altered

in a disease state. For example, a breast cancer may be characterized by the overexpression of a particular marker. In another preferred embodiment, the methods of this invention are used to monitor expression of various genes associated with a certain clinical circumstance, such as docetaxel resistance or sensitivity. This is especially useful in drug research if the end point description is a complex one, not simply asking if one particular gene is overexpressed or underexpressed. Thus, where a disease state or the mode of action of a drug is not well characterized, the methods of this invention allow rapid determination of the particularly relevant genes.

[0041] The present invention identifies and confirms patterns of gene expression associated with docetaxel sensitivity or resistance. From human breast cancers, sufficient RNA was obtained from small core biopsies to assess gene expression patterns in individual tumors. The invention is identifies molecular profiles using gene expression patterns of human primary breast cancers to accurately predict response or lack of response to chemotherapy. The results indicate that molecular profiling as described herein can accurately predict docetaxel response in primary breast cancer patients.

[0042] The present invention was to focuses on genes that could be reliably measured and to exclude those that were unlikely to be expressed in any sample. This study was not designed to discover specific genes for docetaxel response/resistance, but rather to detect a plurality of genes wherein the patterns of expression of many genes are used as a clinical predictive test for breast cancer patients. As a result, some biologically interesting genes like *AURORA-A* will be excluded because of low overall expression.

[0043] Although breast cancers are highly heterogeneous, the classifying gene list gives some clues to the mechanisms of sensitivity and resistance in some tumors. In general, the resistant tumors overexpressed genes associated with protein translation,

cell cycle, and RNA transcription functions, while sensitive tumors overexpressed genes involved in stress/apoptosis, cytoskeleton/adhesion, protein transport, signal transduction, and RNA splicing/transport. Consistent with an apoptosis-induction mode of action for taxanes, sensitive tumors had higher RNA expression of apoptosis-related proteins (e.g., BAX, UBE2M, UBCH10, CUL1). DNA damage-related gene expression in docetaxel-sensitive tumors (e.g., over expression of CSNK2B, DDB1, ABL, and underexpression of PRKDC) also appears to contribute to docetaxel sensitivity.

[0044] In addition, in sensitive tumors, overexpression of genes involved in stress-related pathways was also found, in particular heat shock proteins (HSPs). Overexpression of heat shock protein 27 (HSP27) has been shown to be associated with Adriamycin resistance in the MDA-MB-231 breast cancer cell line. In contrast, the same investigators have demonstrated that HSP27-overexpressing cell lines remain sensitive to docetaxel, suggesting that different non cross-resistant agents may have different gene patterns of sensitivity and resistance. Thus, specific patterns of gene expression can be utilized as tools to prioritize between these commonly used drugs.

[0045] In a leave-one-out cross-validation procedure, the classifier based on genes selected at the nominal value of $p \leq 0.001$ correctly classified tumors as sensitive or resistant in nearly 90% of the cancers. In addition, the predictive value of this classifier compares very favorably with estrogen receptor (ER), virtually the only validated predictive factor in breast cancer. ER has a positive predictive value for response to hormone therapy of about 60%, and a negative predictive value of about 90%. Given that about 70% of breast cancers are ER+, sensitivity and specificity for hormone responsive and non-responsive tumors are about 93% and 50%, respectively, and the area under the ROC curve for ER is only about 0.72. The docetaxel classifier was found to have positive and negative predictive values of 92% and 83% respectively, and the area under the ROC

curve of 0.96 (Fig 3). This indicates that gene expression-based classifiers compare favorably with other clinically validated predictive markers.

[0046] The present invention demonstrates that expression array technology can effectively and reproducibly classify tumors according to response or resistance to docetaxel chemotherapy.

III. Gene expression analysis

[0047] In general, gene expression data may be gathered in any way that is available to one of skill in the art. Although many methods provided herein are powerful tools for the analysis of data obtained by highly parallel data collection systems, many such methods are equally useful for the analysis of data gathered by more traditional methods. Commonly, gene expression data is obtained by employing an array of probes that hybridize to several, and even thousands or more different transcripts. Such arrays are often classified as microarrays or macroarrays, and this classification depends on the size of each position on the array.

[0048] In one embodiment, the present invention also provides a method wherein nucleic acid probes are immobilized on or in a solid or semisolid support in an organized array. Oligonucleotides can be bound to a support by a variety of processes, including lithography, and where the support is solid, it is common in the art to refer to such an array as a "chip", although this parlance is not intended to indicate that the support is silicon or has any useful conductive properties.

[0049] One embodiment of the invention involves monitoring gene expression by (1) providing a pool of target nucleic acids comprising RNA transcript(s) of one or more target gene(s), or nucleic acids derived from the RNA transcript(s); (2)

hybridizing the nucleic acid sample to a array of probes (including control probes); and (3) detecting the hybridized nucleic acids and calculating a relative expression (transcription) level.

A. Providing a nucleic acid sample.

[0050] One of skill in the art will appreciate that in order to measure the transcription level (and thereby the expression level) of a gene or genes, it is desirable to provide a nucleic acid sample comprising mRNA transcript(s) of the gene or genes, or nucleic acids derived from the mRNA transcript(s). As used herein, a nucleic acid derived from an mRNA transcript refers to a nucleic acid for whose synthesis the mRNA transcript or a subsequence thereof has ultimately served as a template. Thus, a cDNA reverse transcribed from an mRNA, an RNA transcribed from that cDNA, a DNA amplified from the cDNA, an RNA transcribed from the amplified DNA, etc., are all derived from the mRNA transcript and detection of such derived products is indicative of the presence and/or abundance of the original transcript in a sample. Thus, suitable samples include, but are not limited to, mRNA transcripts of the gene or genes, cDNA reverse transcribed from the mRNA, cRNA transcribed from the cDNA, DNA amplified from the genes, RNA transcribed from amplified DNA, and the like.

[0051] In a particularly preferred embodiment, where it is desired to quantify the transcription level (and thereby expression) of a one or more genes in a sample, the nucleic acid sample is one in which the concentration of the mRNA transcript(s) of the gene or genes, or the concentration of the nucleic acids derived from the mRNA transcript(s), is proportional to the transcription level (and therefore expression level) of that gene. Similarly, it is preferred that the hybridization signal intensity be proportional to the amount of hybridized nucleic acid. While it is preferred that the proportionality be relatively strict (e.g., a doubling in transcription rate results in a doubling in mRNA

transcript in the sample nucleic acid pool and a doubling in hybridization signal), one of skill will appreciate that the proportionality can be more relaxed and even non-linear. Thus, for example, an assay where a 5 fold difference in concentration of the target mRNA results in a 3 to 6 fold difference in hybridization intensity is sufficient for most purposes. Where more precise quantification is required appropriate controls can be run to correct for variations introduced in sample preparation and hybridization as described herein. In addition, serial dilutions of "standard" target mRNAs can be used to prepare calibration curves according to methods well known to those of skill in the art. Of course, where simple detection of the presence or absence of a transcript is desired, no elaborate control or calibration is required.

[0052] In the simplest embodiment, such a nucleic acid sample is the total mRNA isolated from a biological sample. The term "biological sample", as used herein, refers to a sample obtained from an organism or from components (e.g., cells) of an organism. The sample may be of any biological tissue or fluid. Frequently the sample will be a "clinical sample" which is a sample derived from a patient. Such samples include, but are not limited to, sputum, blood, blood cells (e.g., white cells), tissue or fine needle biopsy samples, urine, peritoneal fluid, and pleural fluid, or cells therefrom. Biological samples may also include sections of tissues such as frozen sections taken for histological purposes.

[0053] The nucleic acid (either genomic DNA or mRNA) may be isolated from the sample according to any of a number of methods well known to those of skill in the art. One of skill will appreciate that where alterations in the copy number of a gene are to be detected genomic DNA is preferably isolated. Conversely, where expression levels of a gene or genes are to be detected, preferably RNA (mRNA) is isolated.

[0054] Methods of isolating total mRNA are well known to those of skill in the art. For example, methods of isolation and purification of nucleic acids are described in detail in Chapter 3 of Laboratory Techniques in Biochemistry and Molecular Biology: Hybridization With Nucleic Acid Probes, Part I. Theory and Nucleic Acid Preparation, P. Tijssen, ed. Elsevier, N.Y. (1993) and Chapter 3 of Laboratory Techniques in Biochemistry and Molecular Biology: Hybridization with Nucleic Acid Probes, Part I. Theory and Nucleic Acid Preparation, P. Tijssen, ed. Elsevier, N.Y. (1993)).

[0055] In a preferred embodiment, the total nucleic acid is isolated from a given sample using, for example, an acid guanidinium-phenol-chloroform extraction method and polyA mRNA is isolated by oligo dT column chromatography or by using (dT)n magnetic beads (see, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual (2nd ed.), Vols. 1-3, Cold Spring Harbor Laboratory, (1989), or Current Protocols in Molecular Biology, F. Ausubel et al., ed. Greene Publishing and Wiley-Interscience, New York (1987)).

[0056] Frequently, it is desirable to amplify the nucleic acid sample prior to hybridization. One of skill in the art will appreciate that whatever amplification method is used, if a quantitative result is desired, care must be taken to use a method that maintains or controls for the relative frequencies of the amplified nucleic acids.

[0057] Methods of "quantitative" amplification are well known to those of skill in the art. For example, quantitative PCR involves simultaneously co-amplifying a known quantity of a control sequence using the same primers. This provides an internal standard that may be used to calibrate the PCR reaction. The array may then include probes specific to the internal standard for quantification of the amplified nucleic acid.

[0058] One preferred internal standard is a synthetic AW106 cRNA. The AW106 cRNA is combined with RNA isolated from the sample according to standard techniques known to those of skill in the art. The RNA is then reverse transcribed using a reverse transcriptase to provide copy DNA. The cDNA sequences are then amplified (e.g., by PCR) using labeled primers. The amplification products are separated, typically by electrophoresis, and the amount of radioactivity (proportional to the amount of amplified product) is determined. The amount of mRNA in the sample is then calculated by comparison with the signal produced by the known AW106 RNA standard. Detailed protocols for quantitative PCR are provided in PCR Protocols, A Guide to Methods and Applications, Innis et al., Academic Press, Inc. N.Y., (1990).

[0059] Other suitable amplification methods include, but are not limited to polymerase chain reaction (PCR) (Innis, et al., PCR Protocols. A guide to Methods and Application. Academic Press, Inc. San Diego, (1990)), ligase chain reaction (LCR) (see Wu and Wallace, Genomics, 4: 560 (1989), Landegren, et al., Science, 241: 1077 (1988) and Barringer, et al., Gene, 89: 117 (1990), transcription amplification (Kwoh, et al., Proc. Natl. Acad. Sci. USA, 86: 1173 (1989)), and self-sustained sequence replication (Guatelli, et al., Proc. Nat. Acad. Sci. USA, 87: 1874 (1990)).

[0060] In a particularly preferred embodiment, the sample mRNA is reverse transcribed with a reverse transcriptase and a primer consisting of oligo dT and a sequence encoding the phage T7 promoter to provide single stranded DNA template. The second DNA strand is polymerized using a DNA polymerase. After synthesis of double-stranded cDNA, T7 RNA polymerase is added and RNA is transcribed from the cDNA template. Successive rounds of transcription from each single cDNA template results in amplified RNA. Methods of in vitro polymerization are well known to those of skill in the art (see, e.g., Sambrook, supra.) and this particular method is described in detail by Van Gelder, et

al., Proc. Natl. Acad. Sci. USA, 87: 1663-1667 (1990) who demonstrate that in vitro amplification according to this method preserves the relative frequencies of the various RNA transcripts. Moreover, Eberwine et al. Proc. Natl. Acad. Sci. USA, 89: 3010-3014 provide a protocol that uses two rounds of amplification via in vitro transcription to achieve greater than 10^6 fold amplification of the original starting material thereby permitting expression monitoring even where biological samples are limited.

[0061] It will be appreciated by one of skill in the art that the direct transcription method described above provides an antisense (aRNA) pool. Where antisense RNA is used as the target nucleic acid, the oligonucleotide probes provided in the array are chosen to be complementary to subsequences of the antisense nucleic acids. Conversely, where the target nucleic acid pool is a pool of sense nucleic acids, the oligonucleotide probes are selected to be complementary to subsequences of the sense nucleic acids. Finally, where the nucleic acid pool is double stranded, the probes may be of either sense as the target nucleic acids include both sense and antisense strands.

[0062] The protocols cited above include methods of generating pools of either sense or antisense nucleic acids. Indeed, one approach can be used to generate either sense or antisense nucleic acids as desired. For example, the cDNA can be directionally cloned into a vector (e.g., Stratagene's p Bluscript II KS (+) phagemid) such that it is flanked by the T3 and T7 promoters. In vitro transcription with the T3 polymerase will produce RNA of one sense (the sense depending on the orientation of the insert), while in vitro transcription with the T7 polymerase will produce RNA having the opposite sense. Other suitable cloning systems include phage lamda vectors designed for Cre-loxP plasmid subcloning (see e.g., Palazzolo et al., Gene, 88: 25-36 (1990)).

[0063] In a particularly preferred embodiment, a high activity RNA polymerase (e.g. about 2500 units/μL for T7, available from Epicentre Technologies) is used.

B. Labeling nucleic acids.

[0064] In a preferred embodiment, the hybridized nucleic acids are detected by detecting one or more labels attached to the sample nucleic acids. The labels may be incorporated by any of a number of means well known to those of skill in the art. However, in a preferred embodiment, the label is simultaneously incorporated during the amplification step in the preparation of the sample nucleic acids. Thus, for example, polymerase chain reaction (PCR) with labeled primers or labeled nucleotides will provide a labeled amplification product. In a preferred embodiment, transcription amplification, as described above, using a labeled nucleotide (e.g. fluorescein-labeled UTP and/or CTP) incorporates a label into the transcribed nucleic acids.

[0065] Alternatively, a label may be added directly to the original nucleic acid sample (e.g., mRNA, polyA mRNA, cDNA, etc.) or to the amplification product after the amplification is completed. Means of attaching labels to nucleic acids are well known to those of skill in the art and include, for example nick translation or end-labeling (e.g. with a labeled RNA) by kinasing of the nucleic acid and subsequent attachment (ligation) of a nucleic acid linker joining the sample nucleic acid to a label (e.g., a fluorophore).

[0066] Detectable labels suitable for use in the present invention include any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention include biotin for staining with labeled streptavidin conjugate, magnetic beads (e.g., DynabeadsTM), fluorescent dyes (e.g., fluorescein, texas red, rhodamine, green fluorescent protein, and the

like), radiolabels (e.g., .sup.3 H, .sup.125 I, .sup.35 S, .sup.14 C, or .sup.32 P), enzymes (e.g., horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic (e.g., polystyrene, polypropylene, latex, etc.) beads. Patents teaching the use of such labels include U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241.

[0067] Means of detecting such labels are well known to those of skill in the art. Thus, for example, radiolabels may be detected using photographic film or scintillation counters, fluorescent markers may be detected using a photodetector to detect emitted light. Enzymatic labels are typically detected by providing the enzyme with a substrate and detecting the reaction product produced by the action of the enzyme on the substrate, and colorimetric labels are detected by simply visualizing the colored label.

[0068] The label may be added to the target (sample) nucleic acid(s) prior to, or after the hybridization. So called "direct labels" are detectable labels that are directly attached to or incorporated into the target (sample) nucleic acid prior to hybridization. In contrast, so called "indirect labels" are joined to the hybrid duplex after hybridization. Often, the indirect label is attached to a binding moiety that has been attached to the target nucleic acid prior to the hybridization. Thus, for example, the target nucleic acid may be biotinylated before the hybridization. After hybridization, an avidin-conjugated fluorophore will bind the biotin bearing hybrid duplexes providing a label that is easily detected. For a detailed review of methods of labeling nucleic acids and detecting labeled hybridized nucleic acids see Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 24: Hybridization With Nucleic Acid Probes, P. Tijssen, ed. Elsevier, N.Y., (1993)).

[0069] Fluorescent labels are preferred and easily added during an in vitro transcription reaction. In a preferred embodiment, fluorescein labeled UTP and CTP are incorporated into the RNA produced in an in vitro transcription reaction as described above.

C. Modifying sample to improve signal/noise ratio.

[0070] The nucleic acid sample may be modified prior to hybridization to the high density probe array in order to reduce sample complexity thereby decreasing background signal and improving sensitivity of the measurement. In one embodiment, complexity reduction is achieved by selective degradation of background mRNA. This is accomplished by hybridizing the sample mRNA (e.g., polyA RNA) with a pool of DNA oligonucleotides that hybridize specifically with the regions to which the probes in the array specifically hybridize. In a preferred embodiment, the pool of oligonucleotides consists of the same probe oligonucleotides as found on the array.

[0071] The pool of oligonucleotides hybridizes to the sample mRNA forming a number of double stranded (hybrid duplex) nucleic acids. The hybridized sample is then treated with RNase A, a nuclease that specifically digests single stranded RNA. The RNase A is then inhibited, using a protease and/or commercially available RNase inhibitors, and the double stranded nucleic acids are then separated from the digested single stranded RNA. This separation may be accomplished in a number of ways well known to those of skill in the art including, but not limited to, electrophoresis and gradient centrifugation. However, in a preferred embodiment, the pool of DNA oligonucleotides is provided attached to beads forming thereby a nucleic acid affinity column. After digestion with the RNase A, the hybridized DNA is removed simply by denaturing (e.g., by adding heat or increasing salt) the hybrid duplexes and washing the previously hybridized mRNA off in an elution buffer.

[0072] The undigested mRNA fragments which will be hybridized to the probes in the array are then preferably end-labeled with a fluorophore attached to an RNA linker using an RNA ligase. This procedure produces a labeled sample RNA pool in which the nucleic acids that do not correspond to probes in the array are eliminated and thus unavailable to contribute to a background signal.

[0073] Another method of reducing sample complexity involves hybridizing the mRNA with deoxyoligonucleotides that hybridize to regions that border on either side of the regions to which the array probes are directed. Treatment with RNAse H selectively digests the double stranded (hybrid duplexes) leaving a pool of single-stranded mRNA corresponding to the short regions (e.g., 20 mer) that were formerly bounded by the deoxyolignucleotide probes and which correspond to the targets of the array probes and longer mRNA sequences that correspond to regions between the targets of the probes of the array. The short RNA fragments are then separated from the long fragments (e.g., by electrophoresis), labeled if necessary as described above, and then are ready for hybridization with the high density probe array.

[0074] In a third approach, sample complexity reduction involves the selective removal of particular (preselected) mRNA messages. In particular, highly expressed mRNA messages that are not specifically probed by the probes in the array are preferably removed. This approach involves hybridizing the polyA mRNA with an oligonucleotide probe that specifically hybridizes to the preselected message close to the 3' (poly A) end. The probe may be selected to provide high specificity and low cross reactivity. Treatment of the hybridized message/probe complex with RNase H digests the double stranded region effectively removing the polyA tail from the rest of the message. The sample is then treated with methods that specifically retain or amplify polyA RNA (e.g., an oligo dT column or (dT)n magnetic beads). Such methods will not retain or

amplify the selected message(s) as they are no longer associated with a polyA.sup.+ tail.

These highly expressed messages are effectively removed from the sample providing a sample that has reduced background mRNA.

IV. Hybridization Array Design

A. Probe composition

[0075] One of skill in the art will appreciate that an enormous number of array designs are suitable for the practice of this invention. The array will typically include a number of probes that specifically hybridize to the nucleic acid expression which is to be detected. In a preferred embodiment, the array will include one or more control probes.

1) Test probes

[0076] In its simplest embodiment, the array includes "test probes". These are oligonucleotides that range from about 5 to about 50 nucleotides, more preferably from about 10 to about 40 nucleotides and most preferably from about 15 to about 40 nucleotides in length. These oligonucleotide probes have sequences complementary to particular subsequences of the genes whose expression they are designed to detect. Thus, the test probes are capable of specifically hybridizing to the target nucleic acid they are to detect.

[0077] In addition to test probes that bind the target nucleic acid(s) of interest, the array can contain a number of control probes. The control probes fall into three categories referred to herein as a) Normalization controls; b) Expression level controls; and c) Mismatch controls.

a) Normalization controls.

[0078] Normalization controls are oligonucleotide probes that are perfectly complementary to labeled reference oligonucleotides that are added to the nucleic acid sample. The signals obtained from the normalization controls after hybridization provide a control for variations in hybridization conditions, label intensity, "reading" efficiency and other factors that may cause the signal of a perfect hybridization to vary between arrays. In a preferred embodiment, signals (e.g., fluorescence intensity) read from all other probes in the array are divided by the signal (e.g., fluorescence intensity) from the control probes thereby normalizing the measurements.

[0079] Virtually any probe may serve as a normalization control. However, it is recognized that hybridization efficiency varies with base composition and probe length. Preferred normalization probes are selected to reflect the average length of the other probes present in the array, however, they can be selected to cover a range of lengths. The normalization control(s) can also be selected to reflect the (average) base composition of the other probes in the array, however in a preferred embodiment, only one or a few normalization probes are used and they are selected such that they hybridize well (i.e. no secondary structure) and do not match any target-specific probes.

[0080] Normalization probes can be localized at any position in the array or at multiple positions throughout the array to control for spatial variation in hybridization efficiently. In a preferred embodiment, the normalization controls are located at the corners or edges of the array as well as in the middle.

b) Expression level controls.

[0081] Expression level controls are probes that hybridize specifically with constitutively expressed genes in the biological sample. Expression level controls are designed to control for the overall health and metabolic activity of a cell. Examination of the covariance of an expression level control with the expression level of the target nucleic acid indicates whether measured changes or variations in expression level of a gene is due to changes in transcription rate of that gene or to general variations in health of the cell. Thus, for example, when a cell is in poor health or lacking a critical metabolite the expression levels of both an active target gene and a constitutively expressed gene are expected to decrease. The converse is also true. Thus where the expression levels of both an expression level control and the target gene appear to both decrease or to both increase, the change may be attributed to changes in the metabolic activity of the cell as a whole, not to differential expression of the target gene in question. Conversely, where the expression levels of the target gene and the expression level control do not covary, the variation in the expression level of the target gene is attributed to differences in regulation of that gene and not to overall variations in the metabolic activity of the cell.

[0082] Virtually any constitutively expressed gene provides a suitable target for expression level controls. Typically expression level control probes have sequences complementary to subsequences of constitutively expressed "housekeeping genes" including, but not limited to the β-actin gene, the transferrin receptor gene, the GAPDH gene, and the like.

c) Mismatch controls.

[0083] Mismatch controls may also be provided for the probes to the target genes, for expression level controls or for normalization controls. Mismatch controls are oligonucleotide probes identical to their corresponding test or control probes except for the

presence of one or more mismatched bases. A mismatched base is a base selected so that it is not complementary to the corresponding base in the target sequence to which the probe would otherwise specifically hybridize. One or more mismatches are selected such that under appropriate hybridization conditions (e.g. stringent conditions) the test or control probe would be expected to hybridize with its target sequence, but the mismatch probe would not hybridize (or would hybridize to a significantly lesser extent). Preferred mismatch probes contain a central mismatch. Thus, for example, where a probe is a 20 mer, a corresponding mismatch probe will have the identical sequence except for a single base mismatch (e.g., substituting a G, a C or a T for an A) at any of positions 6 through 14 (the central mismatch).

[0084] Mismatch probes thus provide a control for non-specific binding or cross-hybridization to a nucleic acid in the sample other than the target to which the probe is directed. Mismatch probes thus indicate whether a hybridization is specific or not. For example, if the target is present the perfect match probes should be consistently brighter than the mismatch probes. In addition, if all central mismatches are present, the mismatch probes can be used to detect a mutation. Finally, it was also a discovery of the present invention that the difference in intensity between the perfect match and the mismatch probe (I(PM)-I(MM)) provides a good measure of the concentration of the hybridized material.

2) Sample preparation/amplification controls

[0085] The array may also include sample preparation/amplification control probes. These are probes that are complementary to subsequences of control genes selected because they do not normally occur in the nucleic acids of the particular biological sample being assayed. Suitable sample preparation/amplification control probes include, for

example, probes to bacterial genes (e.g., Bio B) where the sample in question is a biological from a eukaryote.

[0086] The RNA sample is then spiked with a known amount of the nucleic acid to which the sample preparation/amplification control probe is directed before processing. Quantification of the hybridization of the sample preparation/amplification control probe then provides a measure of alteration in the abundance of the nucleic acids caused by processing steps (e.g. PCR, reverse transcription, in vitro transcription, etc.).

B. "Test Probe" Selection and Optimization.

[0087] In a preferred embodiment, oligonucleotide probes in the array are selected to bind specifically to the nucleic acid target to which they are directed with minimal non-specific binding or cross-hybridization under the particular hybridization conditions utilized.

[0088] There, however, may exist 20 mer subsequences that are not unique to a particular mRNA. Probes directed to these subsequences are expected to cross hybridize with occurrences of their complementary sequence in other regions of the sample genome. Similarly, other probes simply may not hybridize effectively under the hybridization conditions (e.g., due to secondary structure, or interactions with the substrate or other probes). Thus, in a preferred embodiment, the probes that show such poor specificity or hybridization efficiency are identified and may not be included either in the array itself (e.g., during fabrication of the array) or in the post-hybridization data analysis.

[0089] Thus, in one embodiment, this invention provides for a method of optimizing a probe set for detection of a particular gene. Generally, this method involves providing a array containing a multiplicity of probes of one or more particular length(s)

that are complementary to subsequences of the mRNA transcribed by the target gene. In one embodiment the array may contain every probe of a particular length that is complementary to a particular mRNA. The probes of the array are then hybridized with their target nucleic acid alone and then hybridized with a high complexity, high concentration nucleic acid sample that does not contain the targets complementary to the probes. Thus, for example, where the target nucleic acid is an RNA, the probes are first hybridized with their target nucleic acid alone and then hybridized with RNA made from a cDNA library (e.g., reverse transcribed polyA mRNA) where the sense of the hybridized RNA is opposite that of the target nucleic acid (to insure that the high complexity sample does not contain targets for the probes). Those probes that show a strong hybridization signal with their target and little or no cross-hybridization with the high complexity sample are preferred probes for use in the arrays of this invention.

[0090] The array may additionally contain mismatch controls for each of the probes to be tested. In a preferred embodiment, the mismatch controls contain a central mismatch. Where both the mismatch control and the target probe show high levels of hybridization (e.g., the hybridization to the mismatch is nearly equal to or greater than the hybridization to the corresponding test probe), the test probe is preferably not used in the array.

[0091] In a particularly preferred embodiment, an array is provided containing a multiplicity of oligonucleotide probes complementary to subsequences of the target nucleic acid. The oligonucleotide probes may be of a single length or may span a variety of lengths ranging from 5 to 50 nucleotides. The array may contain every probe of a particular length that is complementary to a particular mRNA or may contain probes selected from various regions of particular mRNAs. For each target-specific probe the array also contains a mismatch control probe; preferably a central mismatch control probe.

[0092] The oligonucleotide array is hybridized to a sample containing target nucleic acids having subsequences complementary to the oligonucleotide probes and the difference in hybridization intensity between each probe and its mismatch control is determined. Only those probes where the difference between the probe and its mismatch control exceeds a threshold hybridization intensity (e.g. preferably greater than 10% of the background signal intensity, more preferably greater than 20% of the background signal intensity and most preferably greater than 50% of the background signal intensity) are selected. Thus, only probes that show a strong signal compared to their mismatch control are selected.

[0093] The probe optimization procedure can optionally include a second round of selection. In this selection, the oligonucleotide probe array is hybridized with a nucleic acid sample that is not expected to contain sequences complementary to the probes. Thus, for example, where the probes are complementary to the RNA sense strand a sample of antisense RNA is provided. Of course, other samples could be provided such as samples from organisms or cell lines known to be lacking a particular gene, or known for not expressing a particular gene.

[0094] Only those probes where both the probe and its mismatch control show hybridization intensities below a threshold value (e.g. less than about 5 times the background signal intensity, preferably equal to or less than about 2 times the background signal intensity, more preferably equal to or less than about 1 times the background signal intensity, and most preferably equal or less than about half background signal intensity) are selected. In this way probes that show minimal non-specific binding are selected. Finally, in a preferred embodiment, the n probes (where n is the number of probes desired for each target gene) that pass both selection criteria and have the highest hybridization intensity for each target gene are selected for incorporation into the array, or where already present in

the array, for subsequent data analysis. Of course, one of skill in the art, will appreciate that either selection criterion could be used alone for selection of probes.

[0095] One set of hybridization rules for 20 mer probes in this manner is the following: a) Number of As is less than 9; b) Number of Ts is less than 10 and greater than 0; c) Maximum run of As, Gs, or Ts is less than 4 bases in a row; d) Maximum run of any 2 bases is less than 11 bases; e) Palindrome score is less than 6; f) Clumping score is less than 6; g) Number of As + Number of Ts is less than 14; h) Number of As+number of Gs is less than 15. With respect to rule d, requiring the maximum run of any two bases to be less than 11 bases guarantees that at least three different bases occur within any 12 consecutive nucleotide. A palindrome score is the maximum number of complementary bases if the oligonucleotide is folded over at a point that maximizes self complementarity. Thus, for example a 20 mer that is perfectly self-complementary would have a palindrome score of 10. A clumping score is the maximum number of three-mers of identical bases in a given sequence. Thus, for example, a run of 5 identical bases will produce a clumping score of 3 (bases 1-3, bases 2-4, and bases 3-5). If any probe fails one of these criteria (a-h), the probe is not a member of the subset of probes placed on the chip. For example, if a hypothetical probe was 5'-AGCTTTTTTCATGCATCTAT-3' the probe would not be synthesized on the chip because it has a run of four or more bases (i.e., a run of six). The cross hybridization rules developed for 20 mers were as follows: a) Number of Cs is less than 8; b) Number of Cs in any window of 8 bases is less than 4. Thus, if any probe fails any of either the hybridization ruses (a-h) or the cross-hybridization rules (a-b), the probe is not a member of the subset of probes placed on the chip. These rules eliminate many of the probes that cross hybridize strongly or exhibit low hybridization.

C. Attaching Nucleic Acids to the Solid Surface

[0096] The nucleic acid or analogue are attached to a solid support, which may be made from glass, plastic (e.g., polypropylene, nylon), polyacrylamide, nitrocellulose, or other materials. A preferred method for attaching the nucleic acids to a surface is by printing on glass plates, as is described generally by Schena *et al.*, 1995 (Quantitative monitoring of gene expression patterns with a complementary DNA microarray, Science 270:467-470). This method is especially useful for preparing microarrays of cDNA. See also DeRisi *et al.*, 1996 (Use of a cDNA microarray to analyze gene expression patterns in human cancer, Nature Genetics 14:457-460; Shalon *et al.*, 1996, A DNA microarray system for analyzing complex DNA samples using two-color fluorescent probe hybridization, Genome Res. 6:639-645; and Schena et al., 1995, Parallel human genome analysis; microarray-based expression of 1000 genes, Proc. Natl. Acad. Sci. USA 93:10614-10619). Each of the aforementioned articles is incorporated by reference in its entirety for all purposes.

[0097] A second preferred method for making microarrays is by making high-density oligonucleotide arrays. Techniques are known for producing arrays containing thousands of oligonucleotides complementary to defined sequences, at defined locations on a surface using photolithographic techniques for synthesis in situ (see, Fodor et al., 1991, Light-directed spatially addressable parallel chemical synthesis, Science 251:767-773; Pease et al., 1994, Light-directed oligonucleotide arrays for rapid DNA sequence analysis, Proc. Natl. Acad. Sci. USA 91:5022-5026; Lockhart et al., 1996, Expression monitoring by hybridization to high-density oligonucleotide arrays, Nature Biotech 14:1675; U.S. Pat. Nos. 5,578,832; 5,556,752; and 5,510,270, each of which is incorporated by reference in its entirety for all purposes) or other methods for rapid synthesis and deposition of defined oligonucleotides (Blanchard et al., 1996, High-Density Oligonucleotide arrays, Biosensors

& Bioelectronics 11: 687-90). When these methods are used, oligonucleotides (e.g., 20-mers) of known sequence are synthesized directly on a surface such as a derivatized glass slide. Usually, the array produced is redundant, with several oligonucleotide molecules per RNA. Oligonucleotide probes can be chosen to detect alternatively spliced mRNAs. Another preferred method of making microarrays is by use of an inkjet printing process to synthesize oligonucleotides directly on a solid phase.

[0098] Other methods for making microarrays, e.g., by masking (Maskos and Southern, 1992, Nuc. Acids Res. 20:1679-1684), may also be used. In principal, any type of array, for example, dot blots on a nylon hybridization membrane (see Sambrook et al., Molecular Cloning--A Laboratory Manual (2nd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989, which is incorporated in its entirety for all purposes), could be used, although, as will be recognized by those of skill in the art, very small arrays will be preferred because hybridization volumes will be smaller.

V. Microarray Data Analysis

[0099] Although microarray analysis determines the expression levels of thousands of genes in an RNA sample, only a few of these genes will be differentially expressed upon introduction of a particular variable. In the case of the present invention, breast tissues are either docetaxel sensitive or resistant. The identification of the genes which are necessary for classification in order to predict a clinical outcome is an object of the present invention.

Geneset Classification by Cluster Analysis

[0100] For many applications of the present invention, it is desirable to find basis gene sets that are co-regulated over a wide variety of conditions. This allows the

method of invention to work well for a large class of profiles whose expected properties are not well circumscribed. A preferred embodiment for identifying such basis gene sets involves clustering algorithms, which are well known to one with skill in the art. (for reviews of clustering algorithms, see, e.g., Fukunaga, 1990, Statistical Pattern Recognition, 2nd Ed., Academic Press, San Diego; Everitt, 1974, Cluster Analysis, London: Heinemann Educ. Books; Hartigan, 1975, Clustering Algorithms, New York: Wiley; Sneath and Sokal, 1973, Numerical Taxonomy, Freeman; Anderberg, 1973, Cluster Analysis for Applications, Academic Press: New York).

[0101] In order to obtain basis genesets that contain genes which co-vary over a wide variety of conditions, a plurality of genes are analyzed. In a preferred embodiment, at least 10 or more, preferably at least 50 genes are analyzed. On other embodiments, at least 91 genes are analyzed. Cluster analysis operates on a table of data which has the dimension m×k wherein m is the total number of groups that cluster (in the present invention, two groups are contemplated, docetaxel resistant and docetaxel sensitive) and k is the number of genes measured.

[0102] A number of clustering algorithms are useful for clustering analysis. Clustering algorithms use dissimilarities or distances between objects when forming clusters. In some embodiments, the distance used is Euclidean distance, which is known to one with skill in the art, in multidimensional space where I(x,y) is the distance between gene X and gene Y; X_i and Y_i are gene expression response under perturbation i. The Euclidean distance may be squared to place progressively greater weight on objects that are further apart. Alternatively, the distance measure may be the Manhattan distance, which is known to a skilled artisan, e.g., between gene X and Y. Again, X_i and Y_i are gene expression responses under perturbation i. Some other definitions of distances are Chebychev distance, power distance, and percent disagreement. Another useful distance

definition, which is particularly useful in the context of cellular response, is I=1-r, where r is the correlation coefficient between the response vectors X, Y, also called the normalized dot product XY/|X||Y|.

[0103] Various cluster linkage rules are useful for the methods of the invention. Single linkage, a nearest neighbor method, determines the distance between the two closest objects. By contrast, complete linkage methods determine distance by the greatest distance between any two objects in the different clusters. This method is particularly useful in cases when genes or other cellular constituents form naturally distinct "clumps." Alternatively, the unweighted pair-group average defines distance as the average distance between all pairs of objects in two different clusters. This method is also very useful for clustering genes or other cellular constituents to form naturally distinct "clumps." Finally, the weighted pair-group average method may also be used. This method is the same as the unweighted pair-group average method except that the size of the respective clusters is used as a weight. This method is particularly useful for embodiments where the cluster size is suspected to be greatly varied (Sneath and Sokal, 1973, Numerical taxonomy, San Francisco. W. H. Freeman & Co.). Other cluster linkage rules, such as the unweighted and weighted pair-group centroid and Ward's method are also useful for some embodiments of the invention. See., e g, Ward, 1963, J. Am. Stat Assn. 58:236, Hartigan, 1975, Clustering algorithms, New York: Wiley.

[0104] The cluster analysis may be performed using the hclust routine (see, e.g., `hclust`routine from the software package S-Plus, MathSoft, Inc., Cambridge, Mass.). Genesets may be defined based on the many smaller branches in the tree, or a small number of larger branches by cutting across the tree at different levels--see the example dashed line in FIG. 6. The choice of cut level may be made to match the number of distinct response pathways expected. If little or no prior information is available about the number

of pathways, then the tree should be divided into as many branches as are truly distinct. `Truly distinct` may be defined by a minimum distance value between the individual branches. Preferably, `truly distinct` may be defined with an objective test of statistical significance for each bifurcation in the tree. In one aspect of the invention, the Monte Carlo randomization of the experiment index for each cellular constituent's responses across the set of experiments is used to define an objective test.

[0105] Analysis of thousands of data points after performing a microarray experiment in order to identify those key genes which contribute significantly to tissue classification may be accomplished in a variety of ways. One approach may be unsupervised clustering techniques, such as hierarchical clustering, which identifies sets of correlated genes with similar behavior across the experiments, but yields thousands of clusters in a tree-like structure. Self-organizing-maps, or SOM, require a prespecified number and an initial spatial structure of clusters.

[0106] In a preferred embodiment of the invention, the microarray data from the breast tissue samples is analyzed by a supervised clustering algorithm. Any number of suitable algorithms may be used. For example, see Dettling *et al.*, 2002. Such algorithms may be user-designed or may be previously packaged in a microarray data analysis software system.

[0107] R-SVM is a supported vector machine (SVM)-based method for doing supervised pattern recognition(classification) with microarray gene expression data. The method is useful in classification and for selecting a subset of relevant genes according to their relative contribution in the classification. This process is recursive and the accuracy of the classification can be evaluated either on an independent test data set or by cross

validation on the same data set. R-SVM also includes an option for permutation experiments to assess the significance of the performance.

VI. Gene Descriptions

[0108] The genes described in the present invention are those whose expression varies by a predetermined amount between breast tumors that are sensitive to docetaxel versus those that are resistance to docetaxel. The following provides detailed descriptions of the genes of interest in the present invention. It is noted that homologs and polymorphic variants of the genes are also contemplated. As described above, the relative expression contributions of these genes may be measured through microarray analysis. However, other methods of determining expression of the genes are also contemplated. It is also noted that probes for the following genes may be designed using any appropriate fragment of the full lengths of the genes.

Table 1

GenBank ID	LocusLink ID	Official Symbol	Gene name	SEQ ID NO:
U50648	5610	PRKR	protein kinase, interferon-inducible double stranded RNA dependent	1
D13748	1973	EIF4A1	eukaryotic translation initiation factor 4A, isoform 1	2
U47077	5591	PRKDC	protein kinase, DNA-activated, catalytic polypeptide	3
X63465	5910	RAP1GDS1	RAP1, GTP-GDP dissociation stimulator 1	4
U07563	25	ABL1	v-abl Abelson murine leukemia viral oncogene homolog 1	5
U32986	1642	DDB1	damage-specific DNA binding protein 1 (127kD)	6
AD000092	811	CALR	calreticulin	7
U19599	581	BAX	BCL2-associated X protein	8
D14705	1495	CTNNA1	catenin (cadherin-associated protein), alpha 1 (102kD)	9
U12255	2217	FCGRT	Fc fragment of IgG, receptor, transporter, alpha	10
AC005329	2593	GAMT	guanidinoacetate N-methyltransferase	11
D50928	9667	KIAA0138	KIAA0138 gene product	12
X60673	205	AK3	adenylate kinase 3	13
M20470	1212	CLTB	clathrin, light polypeptide (Lcb)	14
M30448	1460	CSNK2B	casein kinase 2, beta	15

			polypeptide	
U80184	2314	FLII	flightless I homolog (Drosophila)	16
Y11681	6183	MRPS12	mitochondrial ribosomal protein S12	17
W26762	80143	FLJ21168	hypothetical protein FLJ21168	18
U59877	11031	RAB31	RAB31, member RAS oncogene family	19
AJ237946	11269	DDX19	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 19 (DBP5 homolog, yeast)	20
AF075599	9040	UBE2M	ubiquitin-conjugating enzyme E2M (UBC12 homolog, yeast)	21
X71973	2879	GPX4	glutathione peroxidase 4 (phospholipid hydroperoxidase)	22
D84111	11030	RBPMS	RNA-binding protein gene with multiple splicing	23
Z29505	5093	PCBP1	poly(rC) binding protein 1	24
AI143868	57634	EP400	trinucleotide repeat containing 12	25
AL035398	25813	CGI-51	CGI-51 protein	26
U30894	6448	SGSH	N-sulfoglucosamine sulfohydrolase (sulfamidase)	27
U67615	1130	CHS1	Chediak-Higashi syndrome 1	28
AF006082	10097	ACTR2	ARP2 actin-related protein 2 homolog (yeast)	29
M21186	1535	CYBA	cytochrome b-245, alpha polypeptide	30
D79206	6385	SDC4	syndecan 4 (amphiglycan, ryudocan)	31
L38696	22913	RALY	RNA-binding protein	32

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			(autoantigenic)	
D42040	6046	BRD2	bromodomain-containing 2	33
X87176	3295	HSD17B4	hydroxysteroid (17-beta) dehydrogenase 4	34
U24389	4016	LOXL1	lysyl oxidase-like 1	35
AA121509	51690	LSM7	U6 snRNA-associated Sm-like protein LSm7	36
X74331	5558	PRIM2A	primase, polypeptide 2A (58kD)	37
L14076	6429	SFRS4	splicing factor, arginine/serine-rich 4	38
U80017	2966	GTF2H2	general transcription factor IIH, polypeptide 2 (44kD subunit)	39
AF010187	9158	FIBP	fibroblast growth factor (acidic) intracellular binding protein	40
Y00451	211	ALAS1	aminolevulinate, delta-, synthase 1	41
AL050276	26137	ZNF288	zinc finger protein 288	42
AB002559	6813	STXBP2	syntaxin binding protein 2	43
U66042		Unnamed	Cluster Incl. U66042:Human clone 191B7 placenta expressed mRNA from chromosome X /cds=UNKNOWN /gb=U66042 /gi=15	44
U61837	10467	CG1I	putative cyclin G1 interacting protein	45
AC002073	3985	LIMK2	LIM domain kinase 2	46
X71490	9114	ATP6V0D1	ATPase, H+ transporting, lysosomal (vacuolar proton	47

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			pump), member D	
AB028449	23405	DICER1	ATPase, H+ transporting, lysosomal (vacuolar proton pump), member D	48
J05581	4582	MUC1	mucin 1, transmembrane	49
D29643	1650	DDOST	dolichyl-diphosphooligosaccharide-protein glycosyltransferase	50
AF053356	2056	EPO	erythropoietin	51
M11119		Unnamed	Cluster Incl. M11119:Human endogenous retrovirus envelope region mRNA (PL1) /cds=UNKNOWN /gb=M11119 /gi=182205	52
W28610	57405	AD024	AD024 protein	53
X96924	6576	SLC25A1	solute carrier family 25 (mitochondrial carrier; citrate transporter), member 1	54
AF026977	4259	MGST3	microsomal glutathione S-transferase 3	55
AJ133534	10567	RABAC1	Rab acceptor 1 (prenylated)	56
AI991040	10589	DRAP1	DR1-associated protein 1 (negative cofactor 2 alpha)	57
S62140	2521	FUS	fusion, derived from t(12;16) malignant liposarcoma	58
U87947	2014	EMP3	epithelial membrane protein 3	59
AF091083	56270	LOC56270:	hypothetical protein 628	60
X97074	1175	AP2S1	adaptor-related protein complex 2, sigma 1 subunit	61
AL008583	10126	DNAL4	dynein, axonemal, light polypeptide 4	62
S73885	7023	TFAP4	transcription factor AP-4	63

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			(activating enhancer binding protein 4)	
U58087	8454	CUL1	cullin 1	64
X79865	6182	MRPL12	mitochondrial ribosomal protein L12	65
AF061258	10611	LIM	LIM protein (similar to rat protein kinase C-binding enigma)	66
AB011121	66008	ALS2CR3	amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 3	67
D14710	498	ATP5A1	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1, cardiac muscle	68
X07290	7589	ZNF38	zinc finger protein 38 (KOX 25)	69
U70322	3842	KPNB2	karyopherin (importin) beta 2	70
AF026402	9416	U5-100K	prp28, U5 snRNP 100 kd protein	71
AF091085	51614	SDBCAG84	serologically defined breast cancer antigen 84	72
AI254524	9669	IF2	translation initiation factor IF2	73
M91670	27338	E2-EPF	ubiquitin carrier protein	74
W28170	1915	EEF1A1	eukaryotic translation elongation factor 1 alpha 1	75
AF055008	2896	GRN	granulin	76
U37408	1487	CTBP1	C-terminal binding protein 1	77
AI951946		EST	Cluster Incl. AI951946:wx39f10.x1 Homo sapiens cDNA, 3 end /clone=IMAGE-2546059	78

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			/clone_end=3 /gb=AI951946 /gi	
AF037339	1209	CLPTM1	cleft lip and palate associated transmembrane protein 1	79
W72239		EST	Cluster Incl. W72239:zd62h08.s1 Homo sapiens cDNA, 3 end /clone=IMAGE-345279 /clone_end=3 /gb=W72239 /gi=1382	80
AW044624	11079	RER1	similar to S. cerevisiae RER1	81
AW044624	11079	RER1	similar to S. cerevisiae RER1	82
D50645	6388	SDF2	stromal cell-derived factor 2	83
AF007128		Unnamed	Cluster Incl. AF007128:Homo sapiens clone 23870 mRNA sequence /cds=UNKNOWN /gb=AF007128 /gi=2852601 /ug=Hs.1246	84
W25933	9217	VAPB	VAMP (vesicle-associated membrane protein)-associated protein B and C	85
AL049261	27315	FRAG1	FGF receptor activating protein 1	86
S74445	1381	CRABP1	cellular retinoic acid binding protein 1	87
M10321	7450	VWF	von Willebrand factor	88
L29218	1196	CLK2	CDC-like kinase 2	89
J02783	5034	P4HB	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), beta polypeptide (protein disulfide	90
J02902	5518	PPP2R1A	protein phosphatase 2	91

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			(formerly 2A), regulatory subunit A (PR 65), alpha isoform	

EXAMPLES

[0109] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those skilled in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents that are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

Example 1

Study Design

[0110] From September 1999 to June 2001, patients with locally advanced breast cancer (primary cancers greater than 4 cm, or with clinically evident axillary metastases) were considered for a phase II study with neoadjuvant docetaxel. The inclusion criteria were 1) age greater than 18 years and a diagnosis of breast cancer confirmed by core needle biopsy, 2) premenopausal status accompanied by appropriate contraception, 3) adequate performance status, and 4) adequate liver and kidney function tests (all within 1.5 times the upper limit of normal). Exclusion criteria included 1) severe underlying chronic illness or disease, and 2) treatment with other chemotherapeutic drugs while on study.

[0111] Clinical staging and size of primary tumor was recorded at the start of treatment, at each cycle, and after completion of 4 cycles of chemotherapy. Tumor size (product of the two largest perpendicular diameters) measured before and after 4 cycles of neoadjuvant chemotherapy was used to compute the percentage of residual disease. The median residual disease was then calculated, and this degree of response was then used to divide the cancers into 2 groups of sensitive and resistant categories of approximately equal numbers before gene expression analysis.

[0112] Core biopsies of the primary cancers were undertaken before administration of single agent docetaxel as neoadjuvant treatment. Docetaxel at 100 mg/m2 was given every three weeks for a total of 4 cycles, and clinical response assessed after the fourth cycle, at 12 weeks. As the standard of care, patients were continued on neoadjuvant chemotherapy through the full 4 cycles unless there was clear documentation of progressive disease, defined as increase in tumor size of more than 25%. Primary surgery and standard adjuvant therapy was then administered following completion of neoadjuvant docetaxel. In order to maximize the likelihood of obtaining sufficient tissue, approximately six core biopsies using a Bard MaxCore Biopsy Instrument (#MC1410) were taken. Biopsies were performed under local anesthesia, using the same entry point, but reorienting the needle. Two to three core biopsy specimens were immediately transferred for snap freezing at -80^{0}C for cDNA array analysis. The remaining specimens were fixed in formalin for diagnostic and possible immunohistochemical analysis.

Example 2

RNA Extraction and Amplification

[0113] Total RNA was isolated from the frozen core biopsy specimens according to protocols recommended by Affymetrix (Santa Clara, CA) for GeneChip™

experiments. Total RNA was isolated using TRIzol reagent (Invitrogen Corporation, Carlsbad, CA). Samples were subsequently passed over a Qiagen RNeasy column (Qiagen, Valencia, CA) for control of small fragments that have been shown to affect RT-reaction and hybridization quality (ECW, unpublished data). Each core biopsy yielded 3 to 6 micrograms of total RNA. After RNA recovery, double-stranded cDNA was then synthesized by a chimeric oligonucleotide with an oligo-dT and a T7 RNA polymerase promoter at a concentration of 100pm/μL. Reverse transcription was carried out according to protocols recommended by Affymetrix (Santa Clara, CA) using commercially available buffers and proteins (Invitrogen Corporation, Carlsbad, CA). Biotin labeling and approximately 250-fold linear amplification followed phenol-chloroform cleanup of the reverse-transcription reaction product and was carried out by *in vitro* transcription (Enzo Biochem, New York, NY) over a reaction time of 8 hours. From each biopsy 15 micrograms of labeled cRNA was then hybridized onto the Affymetrix U95Av2 GeneChip™ following the recommended procedures for prehybridization, hybridization, washing, and staining with streptavidin-phycoerythrin (SAPE). Antibody amplification was accomplished using a biotin-linked anti-streptavidin antibody (Vector Laboratories, Burlingame, CA) with a goat-IgG (Sigma, St. Louis, MO) blocking antibody. A second application of the SAPE dye was employed subsequent to additional wash steps. Following automated staining and wash protocols (Affymetrix protocol EukGE-2v4), the arrays were scanned by the Affymetrix GeneChip Scanner (Agilent, Palo Alto, CA) and quantitated using MicroArray Suite V5.0 (Affymetrix, Santa Clara, CA). The Affymetrix U95Av2 GeneChip™ comprises about 12,625 probe sets, each containing approximately 16 perfect match and corresponding mismatch 25-mer oligonucleotide probes, representing sequences (genes) most of which have been characterized in terms of function or disease association. The raw, un-normalized probe level data were then analyzed by dChip for final

normalization and modeling. Median intensity was used for the normalization of the 24 arrays and the perfect match/mismatch (PM/MM) modeling algorithm was employed.

Example 3

Semi-Quantitative RT-PCR

[0114] Semi-quantitative RT-PCR (QRT-PCR) measurement of gene expression levels was conducted using the same amplified cRNA hybridized to the GeneChip. Twenty genes were selected for analysis based on their high variation in expression levels. Primers were designed for these loci using the freely available sequences and the Primer3 algorithm for primer design. Product sizes were kept short (<150bp) to maximize their ability to work under varying conditions relative to cRNA quality. Primers were optimized using a reverse-transcribed mixture of six samples. Fifteen duplicate reactions were prepared and samples were obtained at alternating cycle numbers between 15 and 33 to ensure that the sqRT-PCR reaction products were in a linear range of accumulation. These samples were then arranged in ascending order, diluted with 10μL loading buffer, and 3μL of each sample was loaded onto 6% denaturing acrylamide gels. Electrophoresis at 60W was conducted for 2 hours, or until sufficient separation of the xylene cyanol and bromophenol blue dyes was achieved. Gels were then fixed, removed from the rear-plate, transferred to filter paper, and dried. These dry gels were initially assessed by autoradiography (~8hr exposure, no intensification), and analyzable gels were then exposed to phosphorimaging screens. Primers failing to produce a single, clear band were re-attempted at varying annealing temperatures.

[0115] Fifteen of the twenty primers chosen proved suitable to this methodology and gave clean, single bands for analysis. The remaining five failed to

optimize properly and were not included in any further analysis. While high-cycle samples inevitably achieved pixel-saturation, care was taken to minimize exposure times so as to keep intensity within the informative range on a majority of the cycle-totals within each set. Linear range of the fifteen primers was determined using Excel-based graphing functions of the absolute intensities collected. Phosphorimager quantitation analysis (Bio-Rad Laboratories, Hercules, CA) was then carried out, and the RT-PCR product band intensities were quantitatively compared to normalized, model-based estimates of expression from the Affymetrix GeneChip data.

Example 4

Statistical Analysis

[0116] The analytical approach used in this study (Fig. 1) was similar to methods known to a skilled artisan. After scanning and low-level quantitation using MicroArray Suite (Affymetrix, Santa Clara, CA), the DNA-Chip Analyzer was used to normalize the arrays to a common baseline and to estimate expression using the PM-MM model of Li et al. Genes not "present" in at least 30% of samples were eliminated, and exported expression data for the remaining 6,849 genes to BRB Arraytools for further filtering and analysis. In the Pm-MM model, 14 to 20 probe pairs are used to interrogate each gene, each probe pair has a Perfect Match (PM) and Mismatch (MM) signal, and the average of the PM-MM differences for all probe pairs in a probe set (called "average difference") is used as an expression index for the target gene. The model allows one to account for individual probe-specific effects, and automatic detection of outliers and image artifacts. After transforming all data by taking logarithms, genes were ranked by variability over all 24 samples, and genes significantly more variable than the median variance were retained (N=1,628).

[0117] Analysis proceeded in several steps. It was first determined whether the number of differentially expressed genes exceeded what might be expected by chance. Differentially expressed genes were selected from the filtered gene list using the two-sample t-test. A global permutation test was used for an overall, multiple comparison-free assessment of the likelihood that the observed number of significant genes arose by chance. In this test the observed number of significantly differentially expressed genes was compared to the distribution of numbers of differentially expressed genes generated by repeatedly permuting the labels of the samples and recomputing t-test at the specified level of significance.

[0118] Next a classifier was developed to predict response. Given a list of discriminatory genes and their associated t-values, the Compound Covariate Predictor method of Radmacher et al. was used to construct a linear classifier. Resubstitution estimates of classification success, where the classifier is applied to the same samples used to create it, are invariably biased. Therefore, an external cross-validation procedure generated a more unbiased estimate of classification success. Starting with 1,628 genes that were more significantly variable than the median variance, which were filtered without any regard to class membership, the entire gene selection and classifier construction process was repeated in a leave-one-out cross-validation to estimate classifier performance. Finally, to estimate the likelihood that the observed degree of successful classification could have arisen by chance the entire cross-validation procedure was repeated N=2000 times, permuting the sample labels each time. The observed cross-validated classification success rate was then compared to the distribution of classification success in the permutation analysis. Cross-validated performance was summarized by observed sensitivity and specificity, and associated exact binomial confidence intervals.

Resubstitution classifier values were also used to generate a receiver operating characteristic curve (ROC curve) and to estimate the area under the curve.

[0119] The classifier was partially validated on an independent consecutive set of 6 patients treated on the same clinical trial. RNA was obtained from pre-treatment biopsies and hybridized to Affymetrix HgU95av2 GeneChips exactly as described above for the training sample. Probe level data were normalized to the same baseline array as the training set, and gene expression values were computed using previously estimated probe sensitivity values computed from the training sample. The 91-gene classifier was than applied to predict response in each new sample.

Example 5

Assessment of Clinical Response

[0120] The clinical characteristics of the 24 patients enrolled in this phase II neoadjuvant study are included in Table 1. Before treatment, the median tumor size was 8 cm (range 4 to 30 cm). Prior to gene expression analysis, the sensitivity and resistance was defined based on the percentage of residual disease after treatment. It was determined that the median residual disease after chemotherapy was 30%. Then, it was arbitrarily defined that sensitive tumors were those with 25% residual disease or less and resistant tumors were those with greater than 25% residual disease, as this cut-off divides the numbers of patients almost equally into two groups for statistical comparison. In addition, the presenting tumors were large in this study of locally advanced breast cancer, and tumor regressions of at least 75% following chemotherapy would almost certainly represent clinically responsive disease. Large tumor regressions following neoadjuvant

chemotherapy have been shown to directly correlate with the probability of long-term survival.

[0121] Of these 24 patients, 11 were sensitive (46%) to docetaxel and 13 were resistant (54%). Of the sensitive tumors, 5 patients (5/11, 45%) had minimal residual disease (<10% residual tumor), while of the resistant tumors, 7 patients had residual tumors ≥60% (7/13, 58%), and 3 of these women (3/13, 23%) had residual tumors that were 100% or greater of baseline.

Example 6

Core Biopsies and RNA yield

[0122] Prior to treatment, 6 core biopsies were obtained from each primary breast cancer. Two to three core biopsy specimens were immediately snap frozen at -80^{0}C for cDNA array analysis, and the remaining cores were processed for pathological evaluation. Each core biopsy measured approximately 1 cm by 1 mm. As these biopsies were too small for microdissection, tumor cellularity was ascertained of the pretreatment core biopsies. In general, the core biopsies showed good tumor cellularity, with median tumor cellularity of 75% (range 40% to 100%).

[0123] Each frozen core biopsy yielded 3 to 6μg of total RNA, which was more than sufficient to generate approximately 20μg of labeled cRNA needed for hybridization with the Affymetrix HgU95Av2 Gene Chip, using the manufacturer's standard protocol.

Example 7

Selection of Discriminatory Genes

[0124] The expression data in the sensitive and the resistant tumors were compared to identify genes significantly differentially expressed between the two groups (Fig. 2). First, a subset of candidate genes was selected by filtering on signal intensity to eliminate genes with uniformly low expression or genes whose expression did not vary significantly across the samples, retaining 1,628 genes. After log transformation, a t-test was used to select discriminatory genes. To evaluate the possibility of spurious results due to multiple comparisons, a global permutation test was performed, which evaluates the statistical probability of obtaining the observed number of differentially expressed genes (or more) by chance alone. T-tests with nominal P-values of 0.001, 0.01, and 0.05 selected respectively, 91, 300, and 551 genes as "differentially expressed". The probability that these numbers of genes would be selected by chance alone was estimated to be 0.0015, 0.001, and <0.001 respectively.

Example 8

Functional Classification of Discriminatory Genes

[0125] The 91 genes classed as most significantly "differentially expressed" at nominal P-value <0.001 are listed in Table 1. These genes showed 4.2-2.6 fold decreases or 2.5-15.7 fold increases in expression in resistant *versus* sensitive tumors. Functional classes of these differentially expressed genes included stress/apoptosis (21%), cell adhesion/cytoskeleton (16%), protein transport (13%), signal transduction (12%), RNA transcription (10%), RNA splicing/transport (9%), cell cycle (7%), and protein translation (3%); the remainder (9%) had unknown functions.

[0126] Only 14 of the 91 genes were overexpressed in the resistant cluster with major categories including unknown function, protein translation, cell cycle, and RNA

transcription, respectively. β-tubulin isoforms were associated with docetaxel resistance.

The genes described by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 12, SEQ ID NO: 18, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 43, SEQ ID NO: 53, SEQ ID NO: 63, SEQ ID NO: 69, SEQ ID NO: 73,,SEQ ID NO: 75, SEQ ID NO: 78, SEQ ID NO: 87 were overexpressed in the resistant cluster.

[0127] Of the 77 genes overexpressed in docetaxel-sensitive tumors, major categories were stress/apoptosis, adhesion/cytoskeleton (none were overexpressed in resistant tumors), protein transport, signal transduction, and RNA splicing/transport. In sensitive tumors, genes involved in apoptosis (e.g., overexpression of BAX, UBE2M, UBCH10, CUL1), and DNA damage-related gene expression (e.g., overexpression of CSNK2B, DDB1, and ABL, and underexpression of PRKDC) appear to contribute to docetaxel sensitivity.

[0128] This current analysis will exclude some differential genes with low expression. For example, it has been proposed that spindle checkpoint dysfunction is an important cause of aneuploidy in human cancers. The serine-threonine kinase gene *AURORA-A* may constitute a mechanism of spindle checkpoint dysregulation, and its amplification has been shown to predict resistance to taxanes. Nonetheless, this gene was not part of the 91-gene classifying list due to its overall low expression. This classifying list does not include all genes relevant to docetaxel sensitivity and resistance, but rather, identifies patterns of many genes that could be used as a predictive clinical test.

Example 9

Leave-one-out Cross-Validation

[0129] The feasibility of phenotype prediction with a linear classifier based on genes with a nominal P-value of 0.001 or better was tested with leave-one-out cross-validation. This analysis began with all 1,628 filtered genes (see above) to overcome selection bias. Each observation in turn was "left out", the remaining samples were used to select differentially expressed genes, and a compound covariate predictor was constructed and then used to classify the left-out sample. Ten of 11 sensitive tumors (specificity = 91%, exact binomial 95%CI 0.59-1.00) and 11 of 13 resistant tumors (sensitivity = 85%, 95% CI 0.55-0.98) were correctly classified, for an overall accuracy of 88% (95% CI = 68%-97%). Permutation testing indicates that such a high cross-validated classification accuracy is highly significant (P=0.008). The analogous predictor, constructed using 91 genes previously selected using all 24 samples, yielded identical classification success. Using this predictor, positive and negative predictive values for response to docetaxel were 92% and 83% respectively, and the area under the ordinary receiver operating characteristic (ROC) curve was 0.96 (Fig. 3).

Example 10

Confirmation of Expression Measurements

[0130] To confirm measurement of RNA levels, expression values derived from normalized Affymetrix data were correlated with values from semi-quantitative RT-PCR (QRT-PCR) for 15 variably expressed genes. Spearman rank correlations were positive for 13 genes and significantly positive for 6 of 15 genes.

Example 11

Validation in an Independent Cohort

[0131] The 6 additional patients enrolled in this prospective clinical study were studied to partially validate the 91-gene predictive classifier. In this small set all 6 patients had sensitive tumors (residual disease less than 25%) and were correctly classified by this classifier.

REFERENCES

[0132] All patents and publications mentioned in the specification are indicative of the level of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

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[0133] Although the present invention and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the invention as defined by the appended claims. Moreover, the scope of the present application is not intended to be limited to the particular embodiments of the process, machine, manufacture, composition of matter, means, methods and steps described in the specification. As one of ordinary skill in the art will readily appreciate from the disclosure of the present invention, processes, machines, manufacture, compositions of matter, means, methods, or steps, presently existing or later to be developed that perform substantially the same function or achieve substantially the same result as the corresponding embodiments described herein may be utilized according to the present invention. Accordingly, the appended claims are intended to include within their scope such processes, machines, manufacture, compositions of matter, means, methods, or steps.

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O'Connell, Peter

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ttcccccttt tcacagagaa ataaaggtct agaagtaaaa aaaaaaaaaa aaaaaaaaaa 2280

aaaaaaaaaa a 2291

CLAIMS

What is claimed is:

1. A method of screening a patient for response to docetaxel therapy comprising the steps of:

obtaining a tumor sample from the patient;

isolating RNA from the sample;

determining relative expression of individual nucleic acids in the RNA of at least 10 of the nucleic acids selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, and SEQ ID NO:91; and

subjecting the relative expression of the individual nucleic acids to a clustering algorithm, wherein the sample is docetaxel resistant if the results of the clustering algorithm indicate that the relative expression of the individual nucleic acids in the sample is characteristic of a docetaxel resistant tumor, and wherein the sample is docetaxel sensitive if the results of the clustering algorithm indicate that the relative expression of the individual nucleic acids in the sample is characteristic of a docetaxel sensitive tumor.

2. The method of claim 1, wherein relative expression of individual nucleic acids in the RNA of at least 50 of the nucleic acids selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79,

SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, and SEQ ID NO:91 is determined.

3. The method of claim 1, wherein relative expression of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, and SEQ ID NO:91 is determined.

4. The method of claim 1, wherein relative overexpression in the tumor sample of at least one nucleic acid selected from

the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:12, SEQ ID NO:18, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:43, SEQ ID NO:53, SEQ ID NO:63, SEQ ID NO:69, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:78, and SEQ ID NO:87 is associated with docetaxel resistance.

5. The method of claim 4, wherein the overexpression is at least 2.5-fold.

6. The method of claim 1, wherein relative overexpression in the tumor tissue sample of at least one nucleic acid selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, and SEQ ID NO:91 is associated with docetaxel sensitivity.

7. The method of claim 6, wherein the overexpression is at least 2.5 fold.

8. The method of claim 1, wherein the clustering algorithm is a supervised clustering algorithm.

9. The method of claim 1, wherein determining the relative expression of individual nucleic acids in the RNA comprises the steps of:

providing a plurality of probes bound to a solid surface, at least 10 of said plurality of probes being complementary to sequences selected from the group of nucleic acids consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, and SEQ ID NO:91;

contacting the probes with the RNA obtained from the tumor tissue sample, and

detecting binding of the RNA to the probes; thereby identifying differences in relative expression of the nucleic acids.

10. The method of claim 9, wherein at least 50 of said plurality of probes are complementary to sequences selected from the group of nucleic acids consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, and SEQ ID NO:91.

11. The method of claim 9, wherein at least 91 of said plurality of probes are complementary to sequences selected from the group of nucleic acids consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, and SEQ ID NO:91.

12. The method of claim 9, wherein the solid surface is glass or nitrocellulose.

13. The method of claim 9, wherein the detecting of binding comprises detecting fluorescent or radioactive labels.

14. The method of claim 1, wherein the tumor tissue sample is a primary breast tumor.

15. The method of claim 1, wherein the tumor tissue sample is a core biopsy.

16. The method of claim 15, wherein the core biopsy is paraffin-embedded.

17. A method of monitoring a cancer patient receiving docetaxel therapy comprising the steps of:

obtaining tumor tissue samples from the patient at various timepoints during the docetaxel therapy;

isolating RNA from the samples;

determining relative expression of individual nucleic acids in the RNA in the samples of at least 50 of the nucleic acids selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76,

SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, and SEQ ID NO:91; and

subjecting the relative expression of the individual nucleic acids of the samples to a clustering algorithm, wherein the sample is docetaxel resistant if the results of the clustering algorithm indicate that the relative expression of the individual nucleic acids in the sample is characteristic of a docetaxel resistant tumor.

18. The method of claim 18, wherein if any individual sample exhibits a gene expression profile associated with docetaxel resistance, docetaxel therapy is interrupted.

19. The method of claim 17, wherein relative overexpression in the tumor samples of at least one nucleic acid selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:12, SEQ ID NO:18, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:43, SEQ ID NO:53, SEQ ID NO:63, SEQ ID NO:69, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:78, and SEQ ID NO:87 is associated with docetaxel resistance.

20. The method of claim 15, wherein the overexpression is at least 2.5-fold.

21. The method of claim 14, wherein relative overexpression in the tumor tissue samples of at least one nucleic acid selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31,

SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, and SEQ ID NO:91 is associated with docetaxel sensitivity.

22. The method of claim 17, wherein the overexpression is at least 2.5 fold.

23. An array for screening a patient for resistance to docetaxel comprising complementary nucleic acid probes attached to a solid surface for at least 10 of the nucleic acids selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45,

SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, and SEQ ID NO:91.

24. The array of claim 23, wherein the array comprises at least 50 of the nucleic acids selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:58, SEQ ID NO:60, SEQ ID

NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, and SEQ ID NO:91.

25. The array of claim 23, wherein the array comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID

NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, and SEQ ID NO:91.

26. The array of claim 23, wherein the solid surface comprises glass or nitrocellulose.

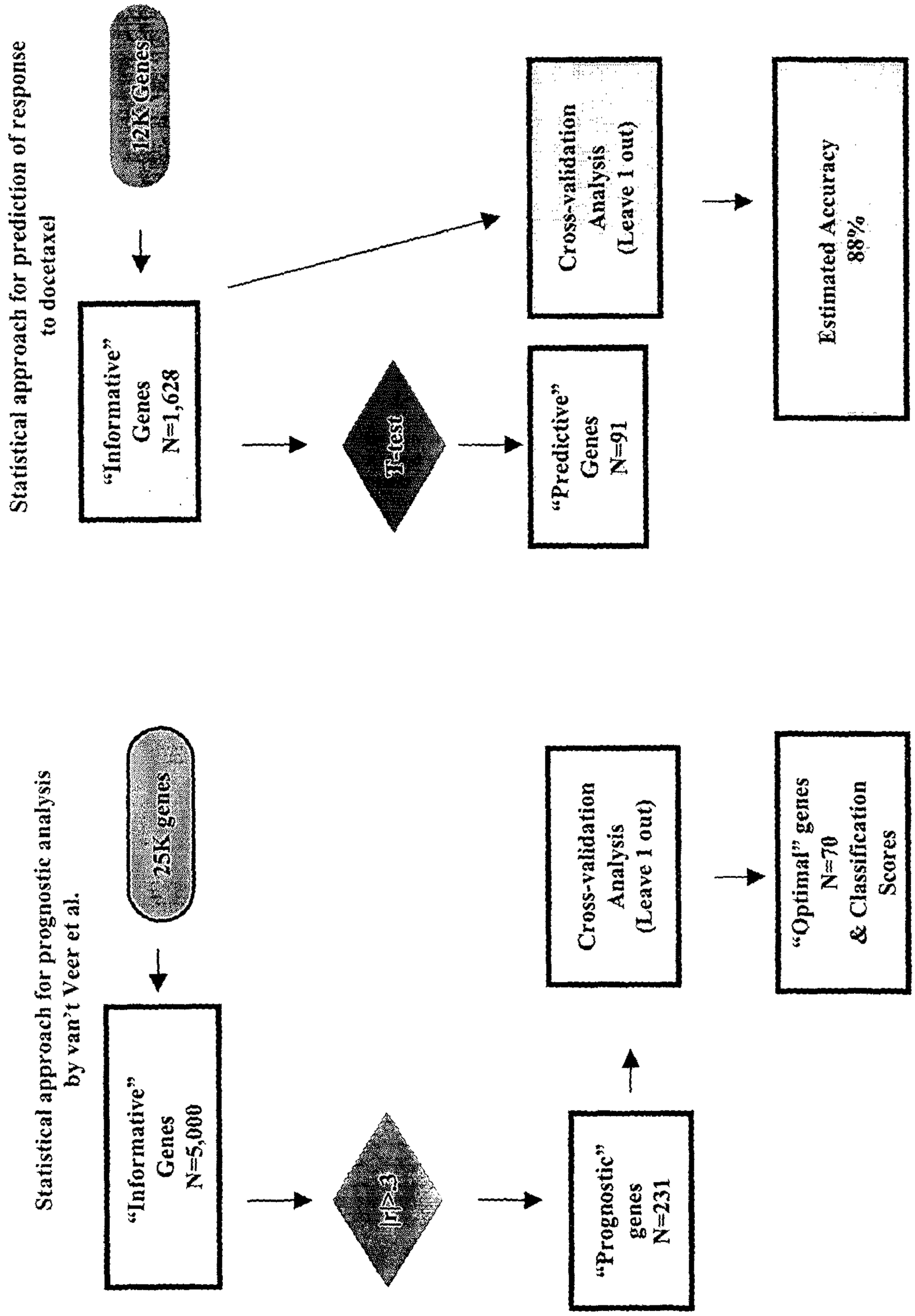

Statistical approach for prediction of response to docetaxel
12K Genes
"Informative" Genes N=1,628
T-test
"Predictive" Genes N=91
Cross-validation Analysis (Leave 1 out)
Estimated Accuracy 88%
Statistical approach for prognostic analysis by van't Veer et al.
25K genes
"Informative" Genes N=5,000
|r|>.3
"Prognostic" genes N=231
Cross-validation Analysis (Leave 1 out)
"Optimal" genes N=70 & Classification Scores

FIG. 1


150
100
50
0
% Residual Tumor
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
1624_at, RAP1GDS1
36626_at, HSD17B4
35807_at, CYBA
38765_at, DICER1
38211_at, ZNF288
33133_at, FLII
607_s_at, VWF
31431_at, FCGRT
39182_at, EMP3
32331_at, AK3
40064_at, ALS2CR3
34305_at, PCBP1
35733_at, ACTR2
38784_g_at, MUC1
40619_at, E2-EPF
35844_at, SDC4
40060_r_at, LIM
38372_at, Unnamed
39185_at, LOC56270:
40096_at, ATP5A1
40463_at, KPNB2
41198_at, GRN
41627_at, SDF2
37361_at, FIBP
38618_at, LIMK2
39076_s_at, DRAP1
33371_s_at, RAB31
1641_s_at, DDB1
36811_at, LOXL1
33931_at, GPX4
38791_at, DDOST
38850_at, Unnamed
1635_at, ABL1
31638_at, GAMT
39347_at, AP2S1
32523_at, CLTB
36846_s_at, LSM7
39030_at, RABAC1
691_g_at, P4HB
37674_at, ALAS1
38613_at, CG1I
33781_s_at, UBE2M
32843_s_at, CSNK2B
33214_at, MRPS12
922_at, PPP2R1A
36125_s_at, RALY
39180_at, FUS
1751_g_at, CALR
38831_f_at, EPO
41528_at, EST
40514_at, SDBCAG84
33393_at, DDX19
36208_at, BRD2
646_s_at, CLK2
1199_at, EIF4A1
40465_at, U5-100K
39724_s_at, CUL1
41757_at, VAPB
35626_at, SGSH
39561_at, DNAL4
38686_at, ATP6V0D1
41551_at, RER1
34845_at, CGI-51
41858_at, FRAG1
35695_at, CHS1
2085_s_at, CTNNA1
37313_at, GTF2H2
38998_g_at, SLC25A1
39812_at, MRPL12
41413_at, CLPTM1
1997_s_at, BAX
41308_at, CTBP1
34163_g_at, RBPMS
39018_at, MGST3
34816_at, EP400
41672_at, Unnamed
33285_l_at, FLJ21168
543_g_at, CRABP1
40535_l_at, IF2
41338_at, EST
36991_at, SFRS4
32099_at, KIAA0138
39638_at, TFAP4
1008_f_at, PRKR
40888_f_at, EEF1A1
1250_at, PRKDC
36898_r_at, PRIM2A
40118_at, ZNF38
38259_at, STXBP2
38942_r_at, AD024
-3.0 -2.3 -1.7 -1.0 -0.3 0.3 1.0 1.7 2.3 3.0

FIG. 2

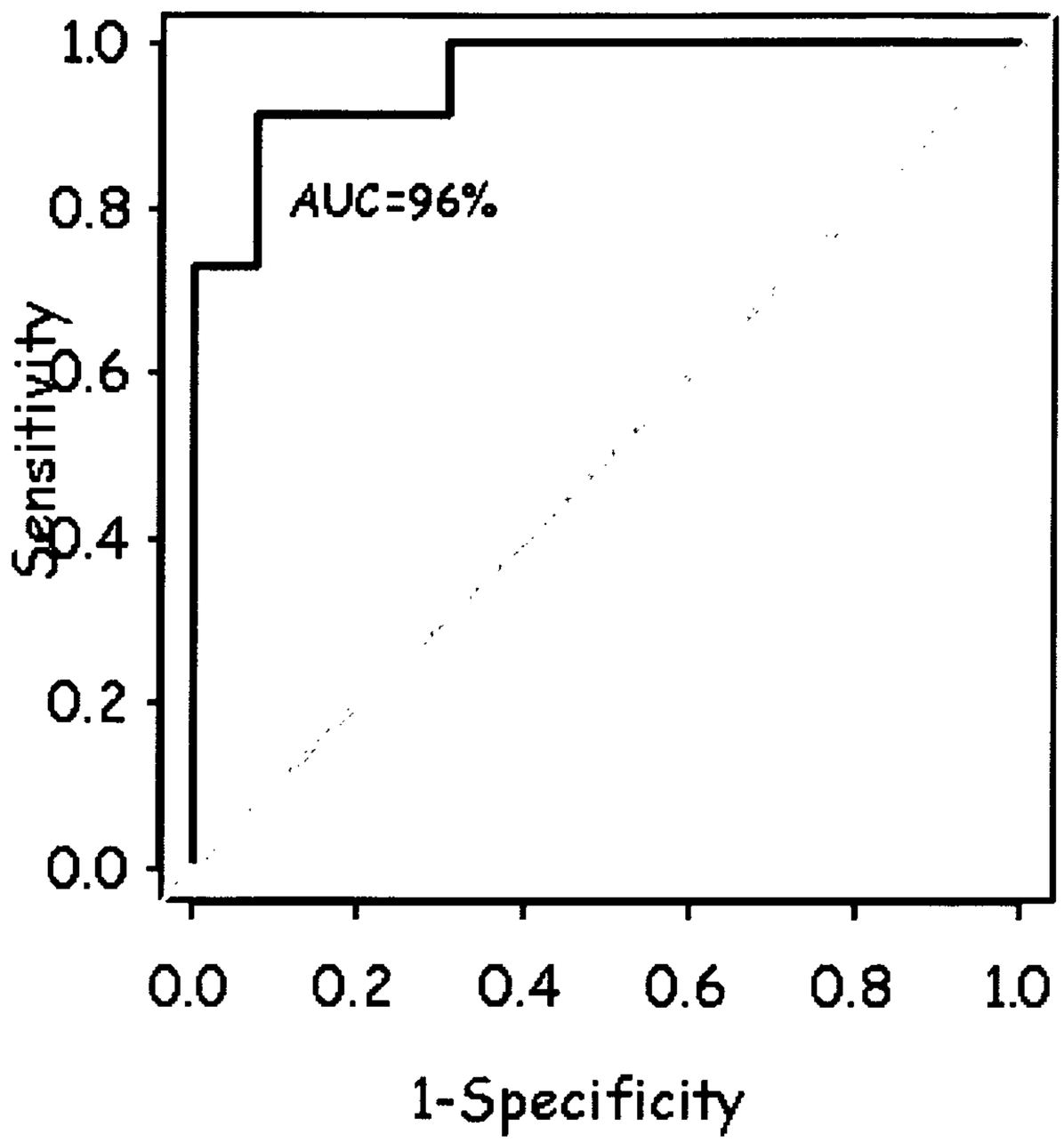
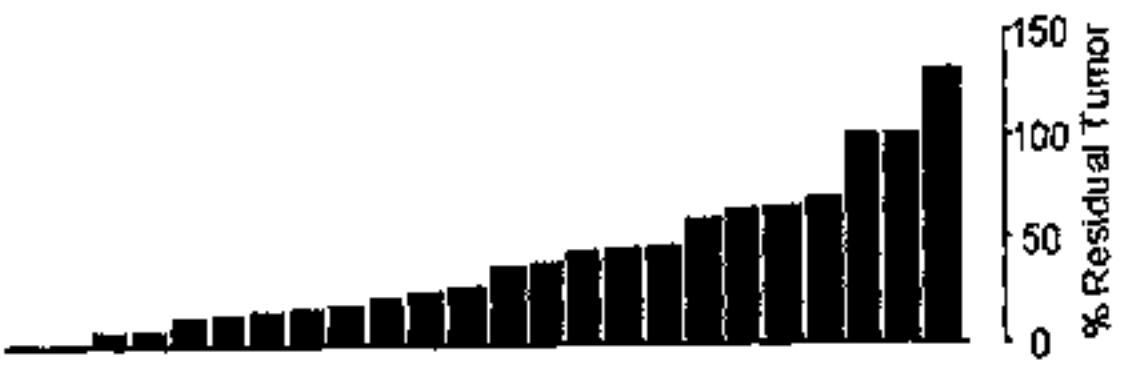
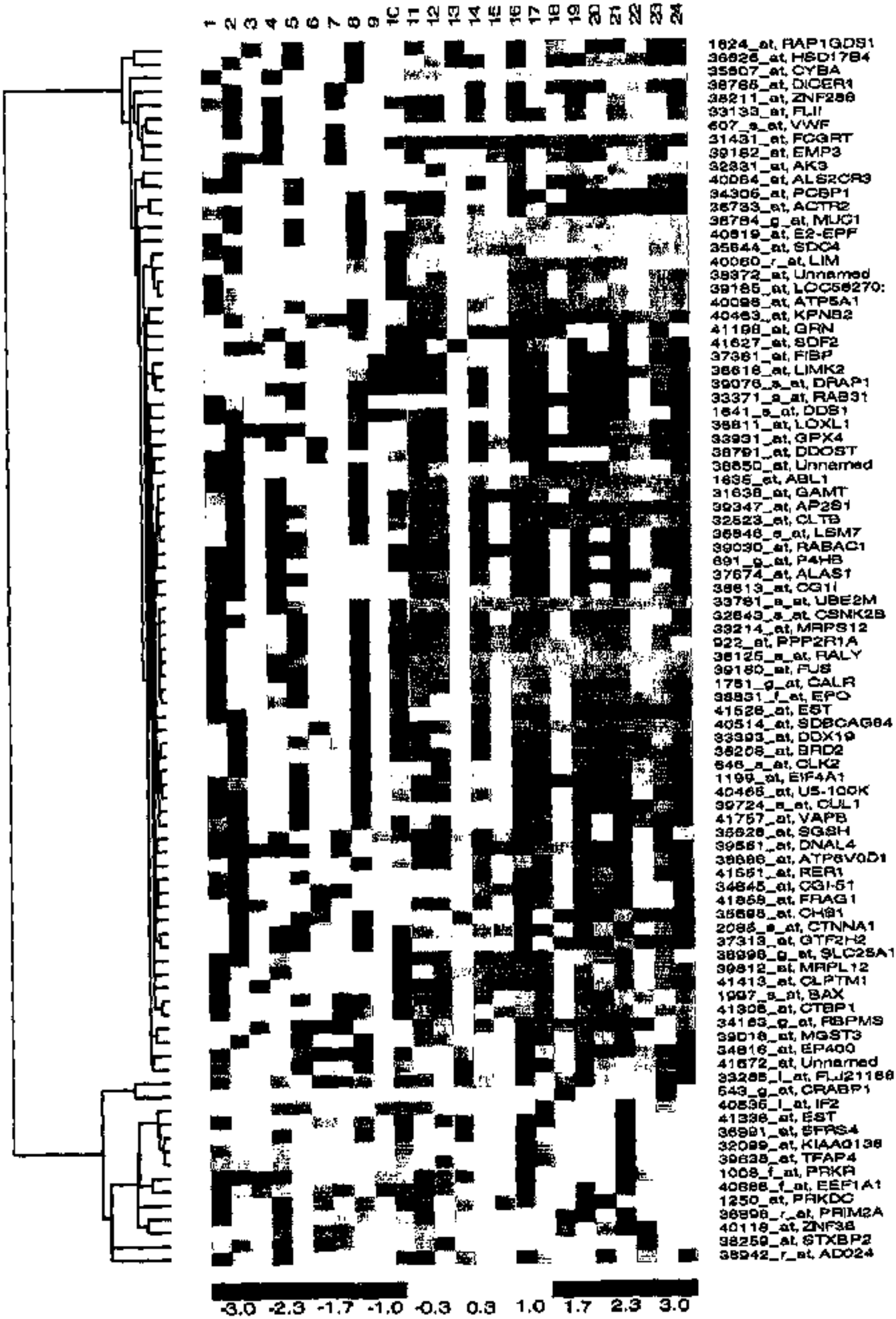

1.0
0.8
0.6
0.4
0.2
0.0
Sensitivity
AUC=96%
0.0
0.2
0.4
0.6
0.8
1.0
1-Specificity

FIG. 3


150
100
50
0
% Residual Tumor


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
1824_at, RAP1GDS1
36926_at, HSD17B4
35507_at, CYBA
38765_at, DICER1
38211_at, ZNF286
33133_at, FLI1
607_s_at, VWF
31431_at, FCGRT
39182_at, EMP3
32331_at, AK3
40084_at, ALS2CR3
34306_at, PCBP1
36733_at, ACTR2
38784_g_at, MUC1
40819_at, E2-EPF
35644_at, SDC4
40060_r_at, LIM
38372_at, Unnamed
39185_at, LOC56270:
40096_at, ATP5A1
40463_at, KPNB2
41198_at, GRN
41627_at, SOF2
37361_at, FIBP
36618_at, LIMK2
39076_s_at, DRAP1
33371_s_at, RAB31
1641_s_at, DDB1
38811_at, LOXL1
33931_at, GPX4
38791_at, DDOST
38850_at, Unnamed
1635_at, ABL1
31638_at, GAMT
39347_at, AP2S1
32523_at, CLTB
36846_s_at, LSM7
39030_at, RABAC1
691_g_at, P4HB
37674_at, ALAS1
38613_at, CG11
33781_s_at, UBE2M
32643_s_at, CSNK2B
33214_at, MRPS12
922_at, PPP2R1A
36125_s_at, RALY
39150_at, FUS
1751_g_at, CALR
38831_f_at, EPO
41528_at, EST
40514_at, SDBCAG84
33393_at, DDX19
38208_at, BRD2
646_s_at, CLK2
1199_at, EIF4A1
40466_at, U5-100K
39724_s_at, CUL1
41757_at, VAPB
35628_at, SGSH
39561_at, DNAL4
38886_at, ATP6V0D1
41551_at, RER1
34845_at, CGI-51
41859_at, FRAG1
35688_at, CHI3
2086_s_at, CTNNA1
37313_at, GTF2H2
38996_g_at, SLC2SA1
39812_at, MRPL12
41413_at, CLPTM1
1997_s_at, BAX
41306_at, CTBP1
34163_g_at, RBPMS
39018_at, MGST3
34816_at, EP400
41672_at, Unnamed
33285_i_at, FLJ21168
543_g_at, CRABP1
40536_i_at, IF2
41336_at, EST
36891_at, SFRS4
32099_at, KIAA0136
39638_at, TFAP4
1008_f_at, PRKR
40686_f_at, EEF1A1
1250_at, PRKDC
36896_r_at, PRIM2A
40118_at, ZNF36
38259_at, STXBP2
38942_r_at, AD024
-3.0 -2.3 -1.7 -1.0 -0.3 0.3 1.0 1.7 2.3 3.0