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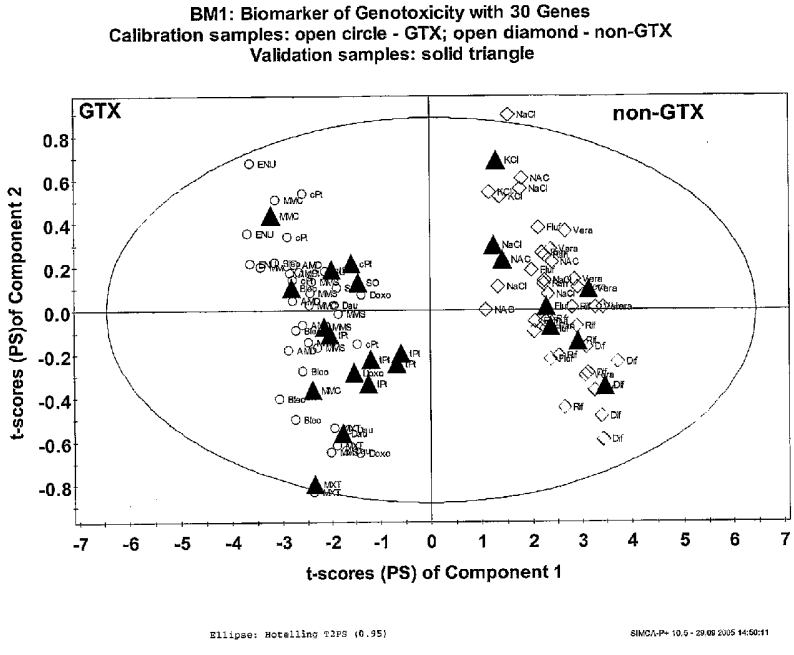
(54) **Title:** EVALUATION OF THE TOXICITY OF PHARMACEUTICAL AGENTS



(57) **Abstract:** The invention provides a rapid high throughput screening process to identify genotoxic compounds. This is accomplished by using a set of biomarker predictor genes that selectively screen for genotoxic or non-genotoxic compounds.

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EVALUATION OF THE TOXICITY OF PHARMACEUTICAL AGENTS

Background of the Invention

[0001] Performing toxicological studies for drug candidates is often time consuming and lengthy work. Prediction of endpoints such as carcinogenicity may take months or years to be completed and require a large number of laboratory animals. *In vitro* test systems (*e.g.*, the Ames test, or *in vitro* micronucleus assays) allow for a reduction in cost and time, and are routinely used in preclinical testing. The Ames test, based on genetic effects on a single bacterial gene, may however have minimal relevance to toxicological effects in interacting networks of genes in mammals, especially in humans. Thus, reliable *in vitro* test systems allowing for early detection of human safety concerns of lead candidates still need to be improved to prevent late loss of development compounds.

[0002] Toxicogenomics – the use of gene expression in toxicology – is a new tool to assist drug safety groups in determining undesirable side effects of newly developed candidate pharmaceutical agents. Toxicogenomics-based studies exploit the fact that gene expression changes can be seen within a few hours or days. Predictive Toxicogenomics may only use a small set of well-defined marker genes to predict and compare potential toxicity effects of compounds, thereby assisting the selection of early drug candidates for lead optimization. Predictive toxicogenomics requires the use of microarray experiments only initially, for the definition of marker gene sets. Predictive marker gene screens can then be implemented using cheaper and higher throughput gene expression analysis techniques.

[0003] There is a strong need in predictive toxicogenomics to develop robust methods of analysis that can be applied to the identification of appropriate marker genes, preferably as a set of marker genes or as a small number of sets of marker genes. There furthermore is a pressing need for identification of sets of marker genes that remain largely independent of the test system employed and of the nature of the subject drug candidate being tested. The present invention addresses these and related needs.

Summary of the Invention

[0004] The invention is based on the discovery that certain predictor genes can be used to screen for genotoxic or non-genotoxic compounds. The invention therefore provides a rapid high throughput screening process to identify genotoxic compounds that is time saving over conventional genotoxic compounds screening processes.

[0005] Accordingly, in one aspect, the invention pertains to a method of predicting genotoxicity of a compound using a predictor model. This is perfomed by identifying a plurality of biomarker genes that display an altered expression profile when exposed to a genotoxic compound or a non-genotoxic compound from a calibration set of samples. A sub-set of biomarker genes are identified from the calibration set that display an altered expression profile when exposed to a genotoxic compound or a non-genotoxic compound from a validation set of samples. The biomarker genes identified in the validation set of samples are classified as those that respond to a genotoxic compound or a non-genotoxic compound. The classified biomarker genes are then used to identify the genotoxicity of a test compound by exposing the test compound to cell sample and comparing the expression profile of the biomarker genes in the sample with those identified in the validation set of samples. Based on calibration samples, a predictive model was constructed to predict toxicity of test samples.

[0006] The classified biomarker genes can be selected from the group consisting of biomarker-1 (BM1) genes, biomarker-2 (BM2) genes and biomarker-3 (BM3) genes. Biomarker-1 genes include, but are not limited to, Xeroderma pigmentosum, complementation group C, ferredoxin reductase, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3C, hypothetical protein MGC5370, damage-specific DNA binding protein 2, 48kDa, transcribed locus, papilin, proteoglycan-like sulfated glycoprotein, fucosidase, alpha-L-1, tissue, carboxypeptidase M, tumor protein p53 inducible protein 3, cyclin-dependent kinase inhibitor 1A (p21, Cip1), phosphatidylinositol glycan, class F, interleukin 6 signal transducer (gp130, oncostatin M receptor), hypothetical protein FLJ10375, vacuolar protein sorting 54 (yeast), hv89d09, interleukin 6 signal transducer (gp130, oncostatin M receptor), phosphatidylserine receptor, alpha-cardiac actin, hypothetical protein FLJ11383, ras homolog gene family, member Q, thioredoxin interacting protein, hypothetical protein LOC339290, NCK-associated protein 1,

TBC1 domain family, member 17, ectodermal-neural cortex (with BTB-like domain), thioredoxin interacting protein, phosphatidylinositol glycan, class F, phosphatidylinositol glycan, class F, and solute carrier family 33 (acetyl-CoA transporter), member 1. In one embodiment, the Biomarker-1 genes are selected from the group consisting of Xeroderma pigmentosum, complementation group C, .Ferrodoxin reductase, apolipoprotein BmRNA editing enzyme, catalytic polypeptide – like 3C, hypothetical protein MGC5370, and damage-specific DNA binding protein 2,48 kDa.

[0007] Biomarker-2 genes include, but are not limited to, EST370545, H. sapiens adenosine deaminase (ADA), Homo sapiens chromosome 12 open reading frame 5 mRNA, polymerase (DNA directed), eta, isocitrate dehydrogenase 1 (NADP+), carboxypeptidase M, plexin B2, polymerase (DNA directed), eta, hypothetical protein FLJ12484, KIAA0907 protein, transcribed locus, ARP9, wb67g03, leucine-rich repeats and death domain containing potassium large conductance calcium-activated channel, subfamily M beta member 3, KAT11914, mitochondrial carrier triple repeat 1, tax1 (human T-cell leukemia virus type I) binding protein 3, sestrin 1, ret finger protein, SMAD, H. sapiens mitogen inducible gene mig-2, FLJ10378 protein, hypothetical protein MGC7036, ubiquitin-conjugating enzyme, KIAA0368, phosphatidylserine receptor, O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase), Mdm2, hypothetical protein LOC51061, NudE nuclear distribution gene E homolog like 1 (A. nidulans), HTPAP protein, and syndecan 1. In one embodiment, the Biomarker-2 genes are selected from the group consisting of EST370545, H. sapiens adenosine deaminase (ADA), Homo sapiens chromosome 12 open reading frame 5 mRNA, polymerase (DNA directed), eta, and isocitrate dehydrogenase 1 (NADP+).

[0008] Biomarker-3 genes include, but are not limited to, LAG1 longevity assurance homolog 5 (S. cerevisiae), hypothetical protein HSPC132, FKSG44 gene, adenosine deaminase, pleckstrin homology-like domain, ectodermal-neural cortex (with BTB-like domain), F-box protein 22, ribonucleotide reductase M2 B (TP53 inducible), guanidinoacetate N-methyltransferase, transmembrane 7 superfamily member 3, isocitrate dehydrogenase 1 (NADP+), phosphohistidine phosphatase 1, hypothetical protein FLJ20296, discoidin domain receptor family, member 1, transcribed locus, guanidinoacetate N-methyltransferase, human receptor tyrosine kinase DDR gene, transmembrane 7 superfamily member 3, 601565341F1 NIH_MGC_21 Homo sapiens

cDNA clone, F-box protein 22, cytosolic sialic acid 9-O-acetylesterase homolog, BTG family member 2, astrotactin 2, IKK interacting protein, surfeit 4, neutral sphingomyelinase (N-SMase) activation associated factor, ADP-ribosylation factor-like 1, golgi reassembly stacking protein 2, leucine-rich repeats and death domain containing mixed-lineage leukemia, hypothetical protein LOC253981, placenta-specific 8, glutathione peroxidase 1, KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2, syntaxin 7, lysosomal-associated multispanning membrane protein-5, and phosphoinositide-3-kinase catalytic alpha polypeptide. In one embodiment, the Biomarker-3 genes are selected from the group consisting of LAG1 longevity assurance homolog 5 (S. cerevisiae), hypothetical protein HSPC132, FKSG44 gene, and adenosine deaminase, pleckstrin homology-like domain.

[0009] In another aspect, the invention pertains to a method of predicting genotoxicity of a compound using a predictor model by exposing a test compound to a first set of a plurality of biomarker genes selected from the group consisting of biomarker-1 (BM1) genes, biomarker-2 (BM2) genes and biomarker-3 (BM3) genes. The distribution of the biomarker genes is compared against the distribution of gene expression of a known reference compound, and the test compound is separated into a class of compound based on the expression of the biomarker genes, wherein the class of compound is genotoxic compound or a non-genotoxic compound using the cascade of predictive models.

[0010] In yet another aspect, the invention pertains to a method of predicting genotoxicity of a compound using a predictor model by exposing a test compound to a plurality of biomarker-1 (BM1) genes selected from the group consisting of Xeroderma pigmentosum, complementation group C, ferredoxin reductase, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3C, hypothetical protein MGC5370, damage-specific DNA binding protein 2, 48kDa, transcribed locus, papilin, proteoglycan-like sulfated glycoprotein, fucosidase, alpha-L-1, tissue, carboxypeptidase M, tumor protein p53 inducible protein 3, cyclin-dependent kinase inhibitor 1A (p21, Cip1), phosphatidylinositol glycan, class F, interleukin 6 signal transducer (gp130, oncostatin M receptor), hypothetical protein FLJ10375, vacuolar protein sorting 54 (yeast), hv89d09, interleukin 6 signal transducer (gp130, oncostatin M receptor), phosphatidylserine receptor, alpha-cardiac actin, hypothetical protein FLJ11383, ras homolog gene family, member Q, thioredoxin interacting protein, hypothetical protein LOC339290, NCK-associated protein 1,

TBC1 domain family, member 17, ectodermal-neural cortex (with BTB-like domain), thioredoxin interacting protein, phosphatidylinositol glycan, class F, phosphatidylinositol glycan, class F, and solute carrier family 33 (acetyl-CoA transporter), member 1. The expression profile of the biomarker genes is compared against the distribution of gene expression of a known reference compound, and then the test compound is separated into a class of compound based on the expression of the biomarker genes, wherein the class of compound is genotoxic compound or a non-genotoxic compound.

[0011] In yet another aspect, the invention pertains to a method of predicting genotoxicity of a compound using a predictor model by exposing a test compound to a plurality of biomarker-2 (BM2) genes selected from the group consisting of EST370545, H. sapiens adenosine deaminase (ADA), Homo sapiens chromosome 12 open reading frame 5 mRNA, polymerase (DNA directed), eta, isocitrate dehydrogenase 1 (NADP+), carboxypeptidase M, plexin B2, polymerase (DNA directed), eta, hypothetical protein FLJ12484, KIAA0907 protein, transcribed locus, ARP9, wb67g03, leucine-rich repeats and death domain containing potassium large conductance calcium-activated channel, subfamily M beta member 3, KAT11914, mitochondrial carrier triple repeat 1, tax1 (human T-cell leukemia virus type I) binding protein 3, sestrin 1, ret finger protein, SMAD, H. sapiens mitogen inducible gene mig-2, FLJ10378 protein, hypothetical protein MGC7036, ubiquitin-conjugating enzyme, KIAA0368, phosphatidylserine receptor, O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase), Mdm2, hypothetical protein LOC51061, NudE nuclear distribution gene E homolog like 1 (A. nidulans), HTPAP protein, and syndecan 1. The distribution of biomarker genes is compared against a known reference compound. The test compound is separated into a class of compound based on the expression of the biomarker genes, wherein the class of compound is genotoxic compound or a non-genotoxic compound.

[0012] In yet another aspect, the invention pertains to a method of predicting genotoxicity of a compound using a predictor model by exposing a test compound to a plurality of biomarker-3 (BM3) genes selected from the group consisting of LAG1 longevity assurance homolog 5 (S. cerevisiae), hypothetical protein HSPC132, FKSG44 gene, adenosine deaminase, pleckstrin homology-like domain, ectodermal-neural cortex (with BTB-like domain), F-box protein 22, ribonucleotide reductase M2 B (TP53 inducible), guanidinoacetate N-methyltransferase,

transmembrane 7 superfamily member 3, isocitrate dehydrogenase 1 (NADP+), phosphohistidine phosphatase 1, hypothetical protein FLJ20296, discoidin domain receptor family, member 1, transcribed locus, guanidinoacetate N-methyltransferase, human receptor tyrosine kinase DDR gene, transmembrane 7 superfamily member 3, 601565341F1 NIH_MGC_21 Homo sapiens cDNA clone, F-box protein 22, cytosolic sialic acid 9-O-acetylesterase homolog, BTG family member 2, astrotactin 2, IKK interacting protein, surfeit 4, neutral sphingomyelinase (N-SMase) activation associated factor, ADP-ribosylation factor-like 1, golgi reassembly stacking protein 2, leucine-rich repeats and death domain containing mixed-lineage leukemia, hypothetical protein LOC253981, placenta-specific 8, glutathione peroxidase 1, KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2, syntaxin 7, lysosomal-associated multispanning membrane protein-5, and phosphoinositide-3-kinase catalytic alpha polypeptide. The distribution of biomarker genes is compared against a known reference compound. The test compound is separated into a class of compound based on the expression of the biomarker genes, wherein the class of compound is genotoxic compound or a non-genotoxic compound.

Brief Description of Figures

[0013] Figure 1. Graphical representation of the percentage of cells in G2 phase as a function of dilution of the indicated genotoxic and nongenotoxic compounds (points 1-9), with control samples at points 10-12. An original color image has been converted to grayscale by computer.

[0014] Figure 2. Graphical representation of the principal component analysis of gene expression of all 215 candidate genes extracted from expression data with 6 reference compounds, labelled by viable cell count. t[1] (the abscissa) represents the scores of principal component #1 explaining the highest proportion of variation and t[2] (the ordinate) represents the scores of principal component #2. Upper panel: original image with points in color; lower panel: image converted to grayscale by computer. As can be seen, cell count is randomly scattered and does not explain the genotoxic or non-genotoxic separation.

[0015] Figure 3. Graphical representation of the principal component analysis of gene expression of all 215 candidate genes labelled by Alamar Blue. t[1] (the abscissa) represents the scores of principal component #1 explaining the highest proportion of variation and t[2] (the ordinate) represents the scores of principal component #2. Upper panel: original image with points in

color; lower panel: image converted to grayscale by computer. As can be seen, Alamar Blue cell count is randomly scattered and does not explain genotoxic or non-genotoxic separation.

[0016] Figure 4. Scores of PC1 (principal component 1; t[1]) of Partial Least Squares-Discriminant Analysis (PLS-DA) conducted with all 215 genes. An original image in color has been converted to grayscale by computer.

[0017] Figure 5. Scores of PC1 (principal component 1; t[1]) of PLS-DA conducted with 23 best predictor genes based on 6 reference compounds. An original image in color has been converted to grayscale by computer.

[0018] Figure 6. Cluster analysis with 23 predictor genes after 6 reference compounds with cytotoxic and genotoxic compounds. The upper panel shows the original image with points in color, and the lower panel shows an image converted to grayscale by computer.

[0019] Figure 7. Cluster analysis with 6 predictor genes with cytotoxic and genotoxic compounds. The upper panel shows the original image with points in color, and the lower panel shows an image converted to grayscale by computer.

[0020] Figure 8. Scores of PC1 (principal component 1; t[1]) of PLS-DA conducted with all 6 predictor genes. An original image in color has been converted to grayscale by computer.

[0021] Figure 9. Validation of the predictive model by random response permutation. The x-axis presents the correlation of the original set of toxicity classes with the permuted ones; the y-axis represents the calculated R2 (goodness of fit) and Q2 (goodness of prediction) values. An original image in color has been converted to grayscale by computer.

[0022] Figure 10A is a scatter plot of the t-scores of calibration and validation samples of biomarker-1 (BM1) genotoxic samples cluster on the left-hand side and non-genotoxic on the right-hand side; the separation line is x=0. Apart from the trans-platinum samples all other validation samples were correctly predicted.

[0023] Figure 10B is a graph of the validation of BM1 by response permutation (n=100 times). For this type of validation the class membership of the samples is randomly shuffled and a predictive model constructed. The performance of these model with random data is assessed in

terms of the intercept R2 and Q2 and compared with the performance parameters of the model obtained with the correct class membership of (x=1).

[0024] Figure 11A is a scatter plot of the t-scores of calibration and validation samples of biomarker-2 (BM2). Genotoxic samples cluster on the left-hand side and non-genotoxic on the right-hand side; the separation line is x=0. Apart from the trans-platinum samples, all other validation samples were correctly predicted.

[0025] Figure 11B is a graph of the validation by response permutation (n=100 times)of BM2. The class membership of the samples is randomly shuffled and a predictive model constructed. The performance of these model with random data is assessed in terms of R2 and Q2 and compared with the performance parameters of the model obtained with the correct class membership.

[0026] Figure 12A is a scatter plot of the t-scores of calibration and validation samples of biomarker-3 (BM3). Genotoxic samples cluster on the left-hand side and non-genotoxic on the right-hand side; the separation line is x=0. Apart from the trans-platinum samples all other validation samples were correctly predicted.

[0027] Figure 12B is graph of the validation by response permutation (n=100 times) of BM3. The class membership of the samples is randomly shuffled and a predictive model constructed. The performance of these model with random data is assessed in terms of R2 and Q2 and compared with the performance parameters of the model obtained with the correct class membership.

Detailed Description of the Invention

[0028] Toxicity testing carried out early in the development program for a pharmaceutical agent is oftentimes done *in vitro*, and often represents testing that would not be considered acceptable by third party review agencies. Such tests may serve, nevertheless, to predict endpoints in toxicity testing later in a development program, such as *in vivo* organ toxicity. Prediction of late endpoints is a complex problem, and commonly does not correlate with single early markers. Therefore, an approach involving several early markers (*e.g.*, cellular markers like translocation,

micronuclei, or gene expression, proteins) should outperform other single endpoint systems. But such a "multi-endpoint approach" requires an even more sophisticated "prediction function" to identify appropriate testing elements. This is achieved in the present invention by training of the system.

[0029] In the present invention, toxicity is established using a class prediction or a class discrimination system in a predictor model for genotoxicity. As used herein, the term "predictor model" refers to a system that uses the expression profile of genes and computer algorithms to assess and classify compounds into genotoxic or non-genotoxic compounds based on the level of gene expression of a plurality of genes. The biomarker genes have been identified by a weighted voting system where the level of gene expression is given a weighing value. The predictive performance of the genes is further evaluated in cross-validation. This identifies certain genes that are predictive of genotoxicity. The resulting predictor model can then be used to identify compounds that are genotoxic or non-genotoxic based on the expression of the classified genes.

[0030] In embodiments of the invention described in detail in the Examples, two classes of compound, namely, genotoxic and nongenotoxic, were established. In general, more than two classes may be defined. Tools developed for diagnostic/predictive purposes are supervised, or knowledge-based methods (*e.g.*, Bayesian Networks, k-nearest neighbor (KNN), Partial Least Squares Discriminant Analysis (PLS-DA), or Support Vector Machines). In the embodiments of the Examples, certain supervised tools are designated for use in class prediction. Genes are identified that permit most effective prediction of the classes chosen. These methods include training of the classifier algorithm with reference data, such as the expression profiles obtained for the predictive genes using model class compounds. In summary, instead of seeking one single endpoint (*e.g.*, colony number), development of an optimized prediction or discrimination function is done using the expression of a set of selected marker genes.

[0031] In the general classification methods disclosed herein, in order to identify a set of marker genes a cell is exposed to a plurality of classes of compounds in culture. Preferably, for each compound, prior to the identifying procedure involving the exposure, a concentration of the compound is determined at which the cell exhibits a predetermined extent of cyto-toxicity. In commonly used procedures, the predetermined toxicity level is 50% cyto-toxicity. Nevertheless,

any intended level of toxicity may be predetermined, such as 20%, 25%, 30%, 40%, 60%, 70%, 75%, 80% toxicity of the compound with respect to the cell; in addition the predetermined level of toxicity may be other than a value listed here according to the needs or intention of a worker of skill in the field of the invention. An important aspect of this determination of toxicity level is that the same predetermined level of toxicity be chosen for all the compounds employed in the identification method. This will ensure that the response of the cell for each compound employed in the identifying procedure will be comparable for all compounds in the method.

[0032] In evaluating the predetermined level of toxicity, any method of establishing cell viability or, conversely, cell death (e.g., TK6 human lymphoblastoid cells), may be employed in evaluating the predetermined level of cyto-toxicity for the compound on the cell. Many dyes are known to workers of skill in fields related to the present invention that distinguish between living and dead cells. Among these are trypan blue dye and alamar blue, which are a chromophore and a fluorophore, respectively. Other viability reagents include Guava ViaCount™ (Guava Technologies, Hayward, CA), and the CellTiter-Glo® Luminescent Cell Viability Assay, based on bioluminescence (Promega, Madison, WI). Equivalent methods of establishing cell viability or death known to workers of skill in the field of the invention are within the scope of the present methods.

[0033] Once the concentrations of all compounds corresponding to the predetermined toxicity level are determined, a cell is exposed separately to each compound at that concentration. In advantageous embodiments the same cell is used in establishing the predetermined toxicity level and the assay of the effect of the compound on the cell. It is not necessary that the same cell be used in the two stages of the method, however. As noted, a variety of compounds is tested. The compounds are chosen to represent a plurality of classes.

[0034] Thus, at a minimum, the compounds are segregated into two classes, such as toxic and nontoxic, although it is advantageous to generate classifications with a greater degree of specialized attributes. Examples of specialization include, by way of nonlimiting example, genotoxic, nephrotoxic, hepatotoxic, neurotoxic, cytotoxic, and the like covering all known organ-specific, tissue-specific toxicities or other classes of toxicities or pathologies. In each case, a negative classification such as nongenotoxic, non-nephrotoxic, and so forth, *i.e.,* a class in

opposition to the first class, may be employed. Furthermore, within each category of toxicity specialization, sub-classes exist such as direct or indirect genotoxicity, and/or classes representing different pathologies responsible for a given organ toxicity. Any equivalent classification of compounds known to a worker of skill in the field of the invention may be employed, and falls within the scope of the present invention.

[0035] In the methods of the present invention the modality of evaluating the effect of the various compounds on the cell encompasses any consequence of incubating the cell with the compounds being tested. Thus, for example, cell morphology, cellular metabolism or physiology, any cellular phenotype, differential gene expression, differential protein expression, differential metabolic expression, and similar phenomena or attributes serve to identify a characteristic effect induced by the compound that is not evinced by a compound not falling in the same class as the compound in question. In embodiments presented in the Examples, differential gene expression provides the experimental output; differentially expressed genes are evaluated by hybridizing RNA obtained from the cell samples with probes that encompass a large proportion of the total genome of the species from which the cell originates. The experimental output from all the cells exposed to the various compounds in the plurality of classes used is evaluated by supervised statistical methods such as those identified above. Any equivalent set of statistical analyses that provide trainable evaluation methods, known to a worker of skill in fields related to the present invention, may be used to identify cellular characteristics that serve to distinguish the classes of compound from one another. In important embodiments of the present invention the cellular characteristics include those genes whose differential expression optimally distinguishes the classes of compound used. Those characteristics identified in this way become a predictor set of characteristics to be used in the present invention to classify candidate pharmaceutical agents.

[0036] Methods such as those described in the preceding paragraphs provide sets of cellular characteristics that are used to classify a new compound, such as a candidate pharmaceutical agent. In important embodiments of the invention, the classes that were used to identify the cellular characteristics have been classified as toxic versus nontoxic, and in certain exemplary cases the classes are genotoxic versus nongenotoxic, or genotoxic versus purely cytotoxic. In other important embodiments that are described in detail in the Examples, the cellular

characteristics employed to discern toxicity vs nontoxicity include coding sequences for genes that are identified by differential expression and application of supervised statistical analytical procedures.

[0037] The invention provides sets of isolated polynucleotides identified by methods such as those described herein that permit effective classification of a test compound as toxic or nontoxic, and in particular, as genotoxic or nongenotoxic, or as genotoxic or cytotoxic. These polynucleotide sets are further capable of permitting classification between subsets or sub-classes of given toxicity classifications, such as those described *supra*. The sets include two or more isolated polynucleotides or oligonucleotides (as explained below, these terms are used interchangeably in the present disclosure) to be employed in the methods of classifying the test compound. Commonly the polynucleotides are used as probes in differential gene expression assays, i.e., they serve as oligonucleotide probes. Sets of two or more, or three or more, or four or more, even larger numbers of oligonucleotides are identified for the first time in the present invention for use in the assay methods described herein. Importantly, whereas complete coding sequences are identified as the ones whose differential expression are to be used in classifying a test compound, typically, and although the complete coding sequence could constitute a particular probe polynucleotide, advantageously a probe oligonucleotide is a fragment of such a coding sequence. More comprehensively, a probe polynucleotide is either a) a complete coding sequence, such as sequence identified by an NCBI (National Center for Biotechnology Information) Accession Number (also termed a GenBank or Refseq Accession Number); b) a nucleotide sequence complementary to a coding sequence in item a); c) a nucleotide sequence that is at least 90% identical to a coding sequence identified in item a); d) a nucleotide sequence complementary to a nucleotide sequence identified in item c); or e) a nucleotide sequence that is a fragment of any of the nucleotide sequences of items a) through d).

[0038] As used herein the term "TEST", and related terms and phrases, relates to a compound or composition that is either a member of a population of compounds or compositions that will be identified as being useful in the classifying methods of the present invention, or the actual compounds or compositions so identified as a result of evaluating those compounds or compositions to be used in the methods. In important embodiments of the invention a TEST

compound is a TEST polynucleotide or a TEST protein or polypeptide. Thus TEST substances may be found in samples after treatment with model compounds or candidate compounds.

[0039] As used herein, the term "sample" and similar words, relate to any cell or component thereof, or any substance, composition or object that includes a cellular component such as a nucleic acid, polynucleotide or oligonucleotide, or a protein or polypeptide, a biochemical metabolite, a subcellular organelle, a lipid, a polysaccharide, or any other cellular component in a form identical to, or minimally altered from, the form of the nucleic acid, polynucleotide or oligonucleotide, or a protein or polypeptide, or a metabolite, or an organelle or other component in an intact cell. As used herein a sample has been treated with a model compound or a candidate pharmaceutical agent. Broadly, a sample can be a biological sample composed of intact cells. In this broad sense, DNA in a sample is genomic DNA, and RNA in a sample includes mRNA, tRNA, rRNA, and similar or other RNA such as, but not exclusively, microRNA. A sample may also contain DNA that is minimally altered from genomic DNA in view of steps such as isolating nuclei from a sample of cells, or disrupting nuclei contained in a sample of cells. In alternative meanings, a sample may be a subcellular fraction, or a subcellular component or organelle, or, when viewing an intact cell, the cell itself or a subcellular region of the cell.

[0040] As used herein, the term "reference" or "control" and similar words, relate to any substance, composition or object as defined above for "sample", with the exception that instead of being treated with a model compound or candidate compound, the reference is untreated or treated only with a carrier or medium which would otherwise contain the compound. More broadly, a reference is from a source that reliably can serve as a control, or as characterizing a nonexperimental status.

I. Detection and Labeling

[0041] A TEST substance such as a TEST polynucleotide or a TEST polypeptide or any TEST cellular component may be detected in many ways. Detecting may include any one or more processes that result in the ability to observe the presence and or the amount of a TEST polynucleotide or a TEST polypeptide. In one embodiment a sample nucleic acid containing a TEST polynucleotide may be detected prior to expansion, or amplification. In an alternative

embodiment a TEST polynucleotide in a sample may be expanded, or amplified, to provide an expanded TEST polynucleotide, and the expanded polynucleotide is detected or quantitated. Physical, chemical or biological methods may be used to detect and quantitate a TEST polynucleotide.

[0042] Physical methods include, by way of nonlimiting example, optical visualization including various microscopic techniques such as fluorescence microscopy, confocal microscopy, microscopic visualization of *in situ* hybridization, surface plasmon resonance (SPR) detection such as binding a probe to a surface and using SPR to detect binding of a TEST polynucleotide or a TEST polypeptide to the immobilized probe, or having a probe in a chromatographic medium and detecting binding of a TEST polynucleotide in the chromatographic medium. Physical methods further include a gel electrophoresis or capillary electrophoresis format in which TEST polynucleotides or TEST polypeptides are resolved from other polynucleotides or polypeptides, and the resolved TEST polynucleotides or TEST polypeptides are detected. Physical methods additionally include broadly any spectroscopic method of detecting or quantitating a substance, including without limitation absorption spectroscopy, fluorescence or phosphorescence spectroscopy, infrared spectroscopy, microwave spectroscopy, total internal reflectance spectroscopy, nuclear magnetic resonance spectroscopy and electron spin resonance spectroscopy.

[0043] Chemical methods include hybridization methods generally in which a TEST polynucleotide hybridizes to a probe. Chemical methods also include any diagnostic or enzymatic assay for detection of a cellular component such as a metabolite. Chemical methods for detecting polypeptides and certain other cellular components also include immunoassay methods. Such immunoassay methods include, but are not limited to, dot blotting, Western blotting, competitive and noncompetitive protein binding assays, enzyme-linked immunosorbant assays (ELISA), immunohistochemistry, fluorescence-activated cell sorting (FACS), and others commonly used and widely known to workers of skill in fields related to the present invention.

[0044] Biological methods include causing a TEST polynucleotide or a TEST polypeptide to exert a biological effect on a cell, and detecting the effect. The present invention discloses examples of biological effects which may be used as a biological assay. In many embodiments,

the polynucleotides may be labeled as described below to assist in detection and quantitation. For example, a sample nucleic acid may be labeled by chemical or enzymatic addition of a labeled moiety such as a labeled nucleotide or a labeled oligonucleotide linker. Many equivalent methods of detecting a TEST polynucleotide or a TEST polypeptide are known to workers of skill in fields related to the field of the invention, and are contemplated to be within the scope of the invention.

[0045] A nucleic acid of the invention can be expanded using cDNA, mRNA or any other type of RNA, or alternatively, genomic DNA, as a template together with appropriate oligonucleotide primers according to any of a wide range of PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to TEST nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

[0046] Expanded polynucleotides may be detected and/or quantitated directly. For example, an expanded polynucleotide may be subjected to electrophoresis in a gel that resolves by size, and stained with a dye that reveals its presence and amount. Alternatively an expanded TEST polynucleotide may be detected upon exposure to a probe nucleic acid under hybridizing conditions (see below) and binding by hybridization is detected and /or quantitated. Detection is accomplished in any way that permits determining that a TEST polynucleotide has bound to the probe. This can be achieved by detecting the change in a physical property of the probe brought about by hybridizing a fragment. A nonlimiting example of such a physical detection method is surface plasma resonance (SPR).

[0047] An alternative way of accomplishing detection is to use a labeled form of a TEST polynucleotide or a TEST polypeptide, and to detect the bound label. The polynucleotide may be labeled as an additional feature in the process of expanding the nucleic acid, or by other methods. A label may be incorporated into the fragments by use of modified nucleotides included in the compositions used to expand the fragment populations. A label may be a radioisotopic label, such as ^{125}I, ^{35}S, ^{32}P, ^{14}C, or ^{3}H, for example, that is detectable by its radioactivity. Alternatively, a label may be selected such that it can be detected using a spectroscopic method, for example. In one instance, a label may be a chromophore, absorbing

incident light. A preferred label is one detectable by luminescence. Luminescence includes fluorescence, phosphorescence, and chemiluminescence. Thus a label that fluoresces, or that phosphoresces, or that induces a chemiluminscent reaction, may be employed. Examples of suitable fluorescent labels, or fluorochromes, include a ^{152}Eu label, a fluorescein label, a rhodamine label, a phycoerythrin label, a phycocyanin label, Cy-3, Cy-5, an allophycocyanin label, an o-phthalaldehyde label, and a fluorescamine label. Luminescent labels afford detection with high sensitivity.

[0048] A label may furthermore be a magnetic resonance label, such as a stable free radical label detectable by electron paramagnetic resonance, or a nuclear label, detectable by nuclear magnetic resonance. A label may still further be a ligand in a specific ligand-receptor pair; the presence of the ligand is then detected by the secondary binding of the specific receptor, which commonly is itself labeled for detection. Nonlimiting examples of such ligand-receptor pairs include biotin and streptavidin or avidin, a hapten such as digoxigenin or antigen and its specific antibody, and so forth. A label still further may be a fusion sequence appended to a TEST polynucleotide or a TEST polypeptide. Such fusions permit isolation and/or detection and quantitation of the TEST polynucleotide or a TEST polypeptide. By way of nonlimiting example, a fusion sequence may be a FLAG sequence, a polyhistidine sequence, a fluorescent protein sequence such as a green fluorescent protein, a yellow fluorescent protein, an alkaline phosphatase, a glutathione transferase, and the like. In summary, labeling can be accomplished in a wide variety of ways known to workers of skill in fields related to the present disclosure. Any equivalent label that permits detecting and/or quantitation of a TEST polynucleotide or a TEST polypeptide is understood to fall within the scope of the invention.

[0049] Detecting, quantitating, including labeling, methods are known generally to workers of skill in fields related to the present invention, including, by way of nonlimiting example, workers of skill in spectroscopy, nucleic acid chemistry, biochemistry, molecular biology and cell biology. Quantitating permits determining the quantity, mass, or concentration of a nucleic acid or polynucleotide, or fragment thereof, that has bound to the probe. Quantitation includes determining the amount of change in a physical, chemical, or biological property as described in this and preceding paragraphs. For example the intensity of a signal originating from a label may be used to assess the quantity of the nucleic acid bound to the probe. Any equivalent

process yielding a way of detecting the presence and/or the quantity, mass, or concentration of a polynucleotide or fragment thereof that hybridizes to a probe nucleic acid is envisioned to be within the scope of the present invention.

II. Polynucleotides

[0050] As used herein the terms "nucleic acid" and "polynucleotide" and similar terms and phrases are considered synonymous with each other, and are used as conventionally understood by workers of skill in fields such as biochemistry, molecular biology, genomics, and similar fields related to the field of the invention. A polynucleotide employed in the invention may be single stranded or it may be a base paired double stranded structure, or even a triple stranded base paired structure. A polynucleotide may be a DNA, an RNA, or any mixture or combination of a DNA strand and an RNA strand, such as, by way of nonlimiting example, a DNA-RNA duplex structure. A polynucleotide and an "oligonucleotide" as used herein are identical in any and all attributes defined here for a polynucleotide except for the length of a strand. As used herein, a polynucleotide may be about 50 nucleotides or base pairs in length or longer, or may be of the length of, or longer than, about 60, or about 70, or about 80, or about 100, or about 150, or about 200, or about 300, or about 400, or about 500, or about 700, or about 1000, or about 1500, or about 2000 or about 2500, or about 3000, nucleotides or base pairs or even longer. An oligonucleotide may be at least 3 nucleotides or base pairs in length, and may be shorter than about 70, or about 60, or about 50, or about 40, or about 30, or about 20, or about 15, or about 10 nucleotides or base pairs in length. Both polynucleotides and oligonucleotides may be chemically synthesized. Oligonucleotides and polynucleotides may be used as probes.

[0051] As used herein "fragment" and similar words relate to portions of a nucleic acid, polynucleotide or oligonucleotide, or to portions of a protein or polypeptide, shorter than the full sequence of a reference. The sequence of bases, or the sequence of amino acid residues, in a fragment is unaltered from the sequence of the corresponding portion of the molecule from which it arose; there are no insertions or deletions in a fragment in comparison with the corresponding portion of the molecule from which it arose. As contemplated herein, a fragment of a nucleic acid or polynucleotide, such as an oligonucleotide, is 15 or more bases in length, or 16 or more, 17 or more, 18 or more, 21 or more, 24 or more, 27 or more, 30 or more, 50 or more,

75 or more, 100 or more bases in length, up to a length that is one base shorter than the full length sequence. Any fragment of a polynucleotide may be chemically synthesized and may be used as a probe.

[0052] As used herein and in the claims "nucleotide sequence", "oligonucleotide sequence" or "polynucleotide sequence", "polypeptide sequence", "amino acid sequence", "peptide sequence", "oligopeptide sequence", and similar terms, relate interchangeably both to the sequence of bases or amino acids that an oligonucleotide or polynucleotide, or polypeptide, peptide or oligopeptide has, as well as to the oligonucleotide or polynucleotide, or polypeptide, peptide or oligopeptide structure possessing the sequence. A nucleotide sequence or a polynucleotide sequence, or polypeptide sequence, peptide sequence or oligopeptide sequence furthermore relates to any natural or synthetic polynucleotide or oligonucleotide, or polypeptide, peptide or oligopeptide, in which the sequence of bases or amino acids is defined by description or recitation of a particular sequence of letters designating bases or amino acids as conventionally employed in the field.

[0053] Nucleotide residues occupy sequential positions in an oligonucleotide or a polynucleotide. Accordingly a modification or derivative of a nucleotide may occur at any sequential position in an oligonucleotide or a polynucleotide. All modified or derivatized oligonucleotides and polynucleotides are encompassed within the invention and fall within the scope of the claims. Modifications or derivatives can occur in the phosphate group, the monosaccharide or the base. Such modifications include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

[0054] As used herein and in the claims, a "nucleic acid" or "polynucleotide", and similar terms based on these, refer to polymers composed of naturally occurring nucleotides as well as to polymers composed of synthetic or modified nucleotides. Thus, as used herein, a polynucleotide that is an RNA, or a polynucleotide that is a DNA may include naturally occurring moieties such as the naturally occurring bases and ribose or deoxyribose rings, or they may be composed of synthetic or modified moieties as described in the following. The linkages between nucleotides

is commonly the 3'-5' phosphate linkage, which may be a natural phosphodiester linkage, a phosphothioester linkage, and still other synthetic linkages. Examples of modified backbones include, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates, 5'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, selenophosphates and boranophosphates. Additional linkages include phosphotriester, siloxane, carbonate, carboxymethylester, acetamidate, carbamate, thioether, bridged phosphoramidate, bridged methylene phosphonate, bridged phosphorothioate and sulfone internucleotide linkages. Other polymeric linkages include 2'-5' linked analogs of these. See United States Patents 6,503,754 and 6,506,735 and references cited therein, incorporated herein by reference. The monosaccharide may be modified by being, for example, a pentose or a hexose other than a ribose or a deoxyribose. The monosaccharide may also be modified by substituting hydryoxyl groups with hydro or amino groups, by esterifying additional hydroxyl groups, and so on.

[0055] The bases in oligonucleotides and polynucleotides may be "unmodified" or "natural" bases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). In addition they may be bases with modifications or substitutions. As used herein, modified bases include other synthetic and natural bases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-fluoro-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Further modified bases include tricyclic pyrimidines such as phenoxazine cytidine (1H-pyrimido[5,4-b][1,4]benzoxazin-2(3H)-one), phenothiazine cytidine (1-pyrimido[5,4-b][1,4]benzothiazin-2(3H)-one), G-clamps such as a substituted phenoxazine cytidine (*e.g.*, 9-(2-

aminoethoxy)-H-pyrimido[5,4-b][1,4]benzoxazin-2(3H)-one), carbazole cytidine (2H-pyrimido[4,5-b]indol-2-one), pyridoindole cytidine (H-pyrido[3', 2':4,5]pyrrolo[2,3-d]pyrimidin-2-one). Modified bases may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone.

[0056] Further bases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in The Concise Encyclopedia of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. I., ed. John Wiley & Sons, 1990, those disclosed by Englisch et al., Angewandte Chemie, International Edition (1991) 30, 613, and those disclosed by Sanghvi, Y. S., Chapter 15, Antisense Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B., ed., CRC Press, 1993. Certain of these bases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2° C. (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., eds., Antisense Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications. See United States Patents 6,503,754 and 6,506,735 and references cited therein, incorporated herein by reference.

[0057] Nucleotides may also be modified to harbor a label. Nucleotides bearing a fluorescent label or a biotin label, for example, are available from Sigma (St. Louis, MO).

[0058] As used herein an "isolated" nucleic acid molecule is one that is separated from at least one other nucleic acid molecule that is present in the natural source of the nucleic acid. Examples of isolated nucleic acid molecules include, but are not limited to, recombinant polynucleotide molecules, recombinant polynucleotide sequences contained in a vector, recombinant polynucleotide molecules maintained in a heterologous host cell, partially or substantially purified nucleic acid molecules, and synthetic DNA or RNA molecules. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism

from which the nucleic acid is derived. For example, in various embodiments, the isolated TEST nucleic acid molecule can contain less than about 50 kb, 25 kb, 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material or culture medium when produced by recombinant techniques, or of chemical precursors or other chemicals when chemically synthesized.

[0059] A nucleic acid molecule used in the present invention, e.g., a nucleic acid molecule having the nucleotide sequence identified herein by an NCBI GenBank or Refseq Accession Number, or a complement of any of these nucleotide sequence, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of any sequence identified herein by an NCBI Accession Number as a hybridization probe, TEST nucleic acid sequences can be isolated using standard hybridization and cloning techniques (e.g., as described in Sambrook et al., eds., MOLECULAR CLONING: A Laboratory Manual 3rd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001; and Brent et al., Current Protocols in Molecular Biology, Wiley Interscience Publishers, (2003)).

[0060] As used herein, the term "complementary" refers to Watson-Crick or Hoogsteen base pairing between nucleotides units of a nucleic acid molecule. As used herein and in the claims, the term "complementary" and similar words, relate to the ability of a first nucleic acid base in one strand of a nucleic acid, polynucleotide or oligonucleotide to interact specifically only with a particular second nucleic acid base in a second strand of a nucleic acid, polynucleotide or oligonucleotide. By way of nonlimiting example, if the naturally occurring bases are considered, A and T or U interact with each other, and G and C interact with each other. As employed in this invention and in the claims, "complementary" is intended to signify "fully complementary" within a region, namely, that when two polynucleotide strands are aligned with each other, at least in the region each base in a sequence of contiguous bases in one strand is complementary to an interacting base in a sequence of contiguous bases of the same length on the opposing strand.

[0061] As used herein, "hybridize", "hybridization" and similar words relate to a process of forming a nucleic acid, polynucleotide, or oligonucleotide duplex by causing strands with complementary sequences to interact with each other. The interaction occurs by virtue of complementary bases on each of the strands specifically interacting to form a pair. The ability of strands to hybridize to each other depends on a variety of conditions, as set forth below. Nucleic acid strands hybridize with each other when a sufficient number of corresponding positions in each strand are occupied by nucleotides that can interact with each other. It is understood by workers of skill in the field of the present invention, including by way of nonlimiting example molecular biologists and cell biologists, that the sequences of strands forming a duplex need not be 100% complementary to each other to be specifically hybridizable.

[0062] In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence in any sequence identified herein by an NCBI GenBank or Refseq Accession Number, or a portion of this nucleotide sequence. A nucleic acid molecule that is complementary to the nucleotide sequence identified herein by an NCBI GenBank or Refseq Accession Number is one that is sufficiently complementary to the nucleotide sequence identified herein by an NCBI GenBank or Refseq Accession Number that it can hydrogen bond with few or no mismatches to the nucleotide sequence identified herein by an NCBI GenBank or Refseq Accession Number, thereby forming a stable duplex.

[0063] A significant use of a nucleic acid, polynucleotide, or oligonucleotide is in an assay directed to identifying a target sequence to which a probe nucleic acid hybridizes. The selectivity of a probe for a target is affected by the stringency of the hybridizing conditions. "Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical evaluation dependent upon probe length, temperature, and buffer composition. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. Higher relative temperatures tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions and identifying hybridization conditions of varying stringency, see Brent et al., Current Protocols in Molecular Biology, Wiley Interscience Publishers, (2003), and Sambrook et al.,

Molecular Cloning: A Laboratory Manual, 3rd Ed., New York: Cold Spring Harbor Press, 2001. In addition, in high throughput or multiplexed assay systems, both the probe characteristics and the stringency may be optimized to permit achieving the objectives of the multiplexed assay under a single set of stringency conditions.

[0064] Nonlimiting examples of "stringent conditions" or "high stringency conditions", as defined herein, include those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1 % Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; (3) employ 50% formamide, 5xSSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2xSSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1xSSC containing EDTA at 55°C, or (4) employ 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$, 1 mM EDTA at 50°C with washing in 2X SSC, 0.1% SDS at 50°C.

[0065] "Moderately stringent conditions" include, by way of nonlimiting example, the use of washing solution and hybridization conditions (e.g., temperature, ionic strength and % SDS) less stringent that those described above. An example of moderately stringent conditions is overnight incubation at 37°C in a solution comprising: 20% formamide, 5xSSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured sheared salmon sperm DNA, followed by washing the filters in 1xSSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

III. Variant Test Polynucleotides

[0066] The invention further encompasses nucleic acid molecules that differ from the disclosed TEST nucleotide sequences. For example, a sequence may differ due to degeneracy of the genetic code. These nucleic acids thus encode the same TEST protein as that encoded by the

nucleotide sequence shown in a sequence identified herein by an NCBI GenBank or Refseq Accession Number. In such embodiments, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence identified herein by an NCBI or comparable GenBank or Refseq Accession Number.

[0067] In addition to the human TEST nucleotide sequences identified herein by an NCBI GenBank or Refseq Accession Number, it will be appreciated by those skilled in the art that DNA allelic sequence polymorphisms that lead to changes in the amino acid sequences of TEST protein may exist within a population (*e.g.*, the human population). Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the TEST gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in the TEST protein that are the result of natural allelic variation and that do not alter the functional activity of the TEST protein are intended to be within the scope of the invention.

[0068] Moreover, nucleic acid molecules encoding TEST orthologs from other species, and thus that have a nucleotide sequence that differs from the human sequence of any sequence identified herein by an NCBI GenBank or Refseq Accession Number, are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and orthologs of the TEST cDNAs of the invention can be isolated based on their homology to the human TEST nucleic acids disclosed herein using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

IV. Polypeptides

[0069] As used herein the term "protein", "polypeptide", or "oligopeptide", and similar words based on these, relate to polymers of alpha amino acids joined in peptide linkage. Alpha amino acids include those encoded by triplet codons of nucleic acids, polynucleotides and oligonucleotides. They may also include amino acids with side chains that differ from those encoded by the genetic code.

[0070] As used herein, a "mature" form of a polypeptide or protein disclosed in the present invention is the product of a naturally occurring polypeptide or precursor form or proprotein.

The naturally occurring polypeptide, precursor or proprotein includes, by way of nonlimiting example, the full length gene product, encoded by the corresponding gene. Alternatively, it may be defined as the polypeptide, precursor or proprotein encoded by an open reading frame described herein. The product "mature" form arises, again by way of nonlimiting example, as a result of one or more naturally occurring processing steps as they may take place within the cell, or host cell, in which the gene product arises. Examples of such processing steps leading to a "mature" form of a polypeptide or protein include the cleavage of the N-terminal methionine residue encoded by the initiation codon of an open reading frame, or the proteolytic cleavage of a signal peptide or leader sequence. Thus a mature form arising from a precursor polypeptide or protein that has residues 1 to N, where residue 1 is the N-terminal methionine, would have residues 2 through N remaining after removal of the N-terminal methionine. Alternatively, a mature form arising from a precursor polypeptide or protein having residues 1 to N, in which an N-terminal signal sequence from residue 1 to residue M is cleaved, would have the residues from residue M+1 to residue N remaining. Further as used herein, a "mature" form of a polypeptide or protein may arise from a step of post-translational modification other than a proteolytic cleavage event. Such additional processes include, by way of non-limiting example, glycosylation, myristoylation or phosphorylation. In general, a mature polypeptide or protein may result from the operation of only one of these processes, or a combination of any of them.

[0071] A TEST protein or polypeptide identified by the methods of the invention may be the product of alternative splicing processes. Thus protein homologues are considered that may have certain exons found in genomic DNA excluded from a particular mRNA, giving rise to a gene product lacking the sequence coded by the excluded exon.

[0072] As used herein an "amino acid" designates any one of the naturally occurring alpha-amino acids that are found in proteins. In addition, the term "amino acid" designates any nonnaturally occurring amino acids known to workers of skill in protein chemistry, biochemistry, and other fields related to the present invention. These include, by way of nonlimiting example, sarcosine, hydroxyproline, norleucine, alloisoleucine, cyclohexylalanine, phenylglycine, homocysteine, dihydroxyphenylalanine, ornithine, citrulline, D-amino acid isomers of naturally occurring L-amino acids, and others. In addition an amino acid may be modified or derivatized,

for example by coupling the side chain with a label. Any amino acid known to a worker of skill in the art may be incorporated into a polypeptide disclosed herein.

[0073] The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising a TEST polypeptide fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the polypeptide to which it is fused. The tag polypeptide preferably also is fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

[0074] As used herein, the terms "active" or "activity" and similar terms refer to form(s) of a polypeptide which retain a biological and/or an immunological activity of native or naturally-occurring TEST, wherein "biological" activity refers to a biological function (either inhibitory or stimulatory) caused by a native or naturally-occurring TEST other than the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring TEST and an "immunological" activity refers to the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring TEST.

V. Determining Similarity Between Two or More Sequences

[0075] To determine the percent similarity of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in either of the sequences being compared for optimal alignment between the sequences). As used herein amino acid or nucleotide "identity" is synonymous with amino acid or nucleotide "homology".

[0076] The term "sequence identity" refers to the degree to which two polynucleotide or polypeptide sequences are identical on a residue-by-residue basis over a particular region of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T or U, C, G, or I, in the case of nucleic acids) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.*, the window

size), and multiplying the result by 100 to yield the percentage of sequence identity. The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 80 percent sequence identity, preferably at least 85 percent identity and often 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison region. In polypeptides the "percentage of positive residues" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical and conservative amino acid substitutions, as defined above, occur in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.,* the window size), and multiplying the result by 100 to yield the percentage of positive residues.

[0077] "Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by, comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in (Computational Molecular Biology, Lesk. A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I. Griffin, A.M., and Griffin, H.G., eds. Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press. New York, 1991; and Carillo, H., and Lipman, D., SIAM J. Applied Math. (1988) 48: 1073. Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al. (1984) Nucleic Acids Research 12(1): 387), BLASTP, BLASTN, and FASTA (Atschul, S.F. et al. (1990)J. Molec. Biol. 215: 403-410. The BLAST X program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S., et al., NCBI NLM NIH Bethesda, MD. 20894; Altschul, S., et al.

(1990) J. Mol. Biol. 215: 403-410. The well known Smith Waterman algorithm may also be used to determine identity.

[0078] Additionally, the BLAST alignment tool is useful for detecting similarities and percent identity between two sequences. BLAST is available on the World Wide Web at the National Center for Biotechnology Information site. References describing BLAST analysis include Madden, T.L., Tatusov, R.L. & Zhang, J. (1996) Meth. Enzymol. 266:131-141; Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D.J. (1997) Nucleic Acids Res. 25:3389-3402; and Zhang, J. & Madden, T.L. (1997) Genome Res. 7:649-656.

VI. Test Proteins and Polypeptides

[0079] A protein employed in the invention includes an isolated TEST protein whose sequence is provided in any sequence identified herein by an NCBI or comparable GenBank or Refseq Accession Number. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue of a sequence identified herein by an NCBI or comparable GenBank or Refseq Accession Number, while still encoding a protein that maintains its TEST protein-like activities and physiological functions, or a functional fragment thereof. For example, the invention includes the polypeptides encoded by the variant TEST nucleic acids described above. In the mutant or variant protein, up to 20% or more of the residues may be so changed.

[0080] In general, a TEST protein-like variant that preserves TEST protein-like function includes any variant in which residues at a particular position in the sequence have been substituted by other amino acids, and further includes the possibility of inserting an additional residue or residues between two residues of the parent protein as well as the possibility of deleting one or more residues from the parent sequence. Any amino acid substitution, insertion, or deletion is encompassed by the invention. In favorable circumstances, the substitution is a non-essential or conservative substitution as defined above. Furthermore, without limiting the scope of the invention, positions of any sequence identified herein by an NCBI or comparable GenBank or Refseq Accession Number may be substituted such that a mutant or variant protein may include one or more substitutions.

[0081] The invention also includes use of isolated TEST proteins, and biologically active portions thereof, or derivatives, fragments, analogs or homologs thereof. Also provided are polypeptide fragments suitable for use as immunogens to raise anti-TEST protein antibodies. A fragment of a protein or polypeptide, such as a peptide or oligopeptide, may be 5 amino acid residues or more in length, or 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 15 or more, 20 or more, 25 or more, 30 or more, 50 or more, 100 or more residues in length, up to a length that is one residue shorter than the full length sequence. In one embodiment, native TEST proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, TEST proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a TEST protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques. Purification of proteins and polypeptides is described, for example, in texts such as "Protein Purification, 3rd Ed.", R.K. Scopes, Springer-Verlag, New York, 1994; "Protein Methods, 2nd Ed.," D.M. Bollag, M.D. Rozycki, and S.J. Edelstein, Wiley-Liss, New York, 1996; and "Guide to Protein Purification", M. Deutscher, Academic Press, New York, 2001.

VII. Variant Test Proteins

[0082] In addition to naturally-occurring allelic variants of the TEST sequence that may exist in the population, the skilled artisan will further appreciate that variants of the amino acid identified herein by an NCBI GenBank or Refseq Accession Number can be generated by a skilled artisan. Variant proteins may arise in a cell used in the present methods, or may serve as a standard for detecting protein expression in the present methods. Any amino acid change leading to a functional protein or retaining the ability to be detected is contemplated within the scope of the present invention. Accordingly, in another embodiment, the TEST protein is a protein that comprises an amino acid sequence at least about 45% similar, and more preferably about 55% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or even 99% or more similar to the amino acid sequence of any sequence identified herein by an NCBI or comparable GenBank or Refseq Accession Number.

VIII. Anti-Test Protein Antibodies

[0083] An important class of TEST protein is an antibody or antibody fragment that specifically binds a TEST protein gene product identified in the classification methods of the invention. Antibodies that bind identified TEST proteins or fragments or variants thereof are used in the detection of the TEST proteins. An anti-TEST antibody may be a polyclonal antibody, a monoclonal antibody, or specific-binding portion thereof that binds the antigen TEST protein, fragment or variant.

IX. Arrays

[0084] In important embodiments of the invention a set of isolated polynucleotides or a set of isolated polypeptides is affixed to a solid substrate to form an array. An important class of polypeptide affixed to an array includes anti-TEST antibody molecules. Each locus or spot in an array is addressable and is distinct from other loci or spots in the array. Each locus may be identified by the composition that is affixed thereto. Thus in principle each locus bears a unique composition that is identified by the address of the locus. By way of nonlimiting example, in an array made up of polynucleotide probes, for example, each locus of the array may have affixed thereto a probe polynucleotide that is either a) a complete coding sequence, such as sequence identified by an NCBI (National Center for Biotechnology Information) GenBank or Refseq Accession Number; b) a nucleotide sequence complementary to a coding sequence in item a); c) a nucleotide sequence that is at least 90% identical to a coding sequence identified in item a); d) a nucleotide sequence complementary to a nucleotide sequence identified in item c); or e) a nucleotide sequence that is a fragment of any of the nucleotide sequences of items a) through d). Other compositions, such as proteins or polypeptides, or specific binding agents that specifically bind particular proteins or polypeptides, may be affixed to the loci of an array, instead of polynucleotide probes.

[0085] Examples of solid supports for constructing arrays include, but are not limited to, membranes, filters, slides, paper, nylon, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, polymers, polyvinyl chloride dishes, etc. Any solid surface to which the oligonucleotides can be bound, either directly or indirectly, either covalently or non-covalently, can be used. A particularly preferred solid substrate is a high density microarray or GeneChip expression probe array (e.g., a GeneChip™ from Affymetrix Inc., Santa Clara, Calif.). These high density arrays

contain a particular oligonucleotide probe in a pre-selected location on the array. Each pre-selected location can contain more than one molecule of the particular probe. Because the oligonucleotides are at specified locations on the substrate, the hybridization patterns and intensities (which together result in a unique expression profile or pattern) can be interpreted in terms of expression levels of particular genes.

[0086] Arrays are prepared by any of a wide range of methods known in the art. Nonlimiting examples of sources describing the preparation of arrays of oligonucleotides and other compositions include Chetverin et al., "Oligonucleotide Arrays: New Concepts and Possibilities," Biotechnology, 12:1093-1099 (1994); Di Mauro et al., "DNA Technology in Chip Construction," Adv. Mater., 5(5):384-386 (1993); Dower et al., "The Search for Molecular Diversity (II): Recombinant and Synthetic Randomized Peptide Libraries," Ann. Rep. Med. Chem., 26:271-280 (1991); Diggelmann, "Investigating the VLSIPS synthesis process," Sep. 9, 1994; U. S. Patent No. 6,506,558; U. S. Patent No. 6,054,270; and U. S. Patent No. 5,830,645.

X. Methods of Classifying Candidate Compounds

[0087] The present invention is directed toward determining into which class of toxicity a candidate compound, such as a candidate pharmaceutical agent, falls. As noted above, important class distinctions of significance in the present invention include two-fold distinctions such as toxic and nontoxic, or genotoxic and nongenotoxic, as well as more complex classification schemes. In order to accelerate the process of identifying strong leads for compounds that may become pharmaceutical agents, it is advantageous to use high throughput assays such as *in vitro* assays for this purpose. *In vitro* cell based assays are included in this group. As described in detail above, any suitable cellular characteristic or group of cellular characteristics may be identified as providing the discrimination power to provide the classification result. These include, by way of nonlimiting example, cell morphology, cellular metabolism or physiology, any cellular phenotype, differential gene expression, differential protein expression, differential metabolic expression, and similar phenomena or attributes.

[0088] In order to classify a candidate compound, a concentration or range of concentrations at which the compound is expected to exert a beneficial pharmacological or therapeutic effect is determined. In the *in vitro* assays of the present method, a suitable cell that is considered to

provide results in assays that closely reflect those expected from *in vivo* tests is used. In several replicate samples, the cell is exposed to at least one concentration, and advantageously to several concentrations of the candidate compound under conditions, and for a length of time, that are considered sufficient for an effect, such as toxic effect, or a genotoxic effect, to be exerted on various classes of cellular component. In various embodiments of this procedure, nonlimiting examples of classes of cellular component that may be analyzed include nucleic acids such as DNA and various types of cellular RNA species, protein and polypeptide components of the cell, membrane-bound proteins and polypeptides, lipid components of a cell, metabolites characteristic of biochemical processes occurring within the cell, organelles and components thereof, and ionic components of the cell. After the passage of sufficient time, members of the cellular component of interest in the chosen method are isolated from the cell. One or more of members of the class has already been determined to respond to the application of compounds that permit classification to proceed.

[0089] As used herein the term "responsive" and similar terms and phrases relate to a cellular component whose presence, absence or concentration measurably differs when the cell from which the cellular component originates is incubated with a model compound or a candidate compound, compared to a control incubation lacking the compound. The measurable difference exceeds limits of detection or other criteria for significance imposed by a worker of skill in the field of the present invention when implementing the methods disclosed herein.

[0090] The responsive members of this class of cellular component are then subjected to analysis to evaluate their presence, absence or concentration. The ensemble of results for all the responsive members of the class are then characterized, using methods such as the supervised statistical analyses described in the Examples, to determine whether the characterization resembles a characterization obtained when a toxic model compound is used in similar experiments carried out simultaneously with the candidate compound, or prior to or after the experiments with the candidate compound are conducted. The results of the analysis and characterization provide a result that the candidate compound is classified as being toxic or nontoxic, or genotoxic or nongenotoxic, and so forth, depending on the classification system initially set up with the model compounds.

XI. Classifying Candidate Compounds Using Differential Gene Expression

[0091] In important embodiments of methods of classifying candidate compounds the cellular component subjected to analysis is the population of RNA molecules present in the cell in response to contacting the cell with the candidate compound. Prior to the characterization and classification of the candidate compound the cell has been used to identify a plurality of genes, using methods analyzing differential gene expression, that respond in statistically significant fashion to application of toxic as opposed to nontoxic compounds. In particularly significant embodiments the classification has been made according to genotoxicity or the lack thereof.

[0092] In this method of classifying a candidate compound, first a concentration or set of concentrations at which the compound exerts a predetermined toxic (genotoxic or cytotoxic) effect is identified. Next, a cell is exposed to the predetermined toxic concentration or set of concentrations of the compound. After the candidate compound has been allowed to exert an effect on the expression of RNA in the cell, the cellular RNA population is isolated; as noted, the presence, absence or concentration of at least some RNA species has been previously demonstrated to be responsive to the classes of compound being considered. The presence, absence or concentration of the responsive RNA species the RNA is determined, for example by hybridization to a plurality of probe nucleotide sequences that include at least fragments of the responsive gene sequences. Finally, the pattern of expression reflected in the hybridization procedure is used to determine whether the characterization resembles a characterization obtained when a toxic model compound is used, or a nontoxic model compound is used. The results of this analysis and determination thus classifies the candidate compound. Other classification schemes may be used, such as genotoxic versus nongenotoxic, or genotoxic versus cytotoxic, in establishing the classes of model compounds.

[0093] The Examples disclose use of an initial set of genotoxic compounds that may be considered to be an initial training set, as well as a set of cytotoxic but not genotoxic compounds, in the differential gene expression in a subject cell culture. In Examples 1-7, transcription profiles were obtained from TK6 human lymphoblastoid cells treated with control containing no experimental compound, three known genotoxic compounds (cis-Platinum, Methyl Methane

Sulfonate, and Mitomycin C), or three compounds known to be purely cytotoxic (NaCl, Rifampicin, and Trans-Platinum).

[0094] The experiments reported in the Examples 1-7 provided discriminant functions involving the expression pattern of two sets, believed to be novel, of predictor genes; one set containing 23 genes was identified using Partial Least Squares- Discriminant Analysis (PLS-DA), and a second set of 27 predictor genes was identified using KNN analysis. Six genes identified as being capable of separating samples treated with cytotoxic and genotoxic compounds without any misclassification were found to be in common to both predictor sets. Most of the 23 predictor genes derived from PLS-DA and most of the 27 predictor genes derived from KNN directly or indirectly represent correlates of molecular events that are involved in genotoxicity. Selected members of the gene sets are given in the following paragraphs.

[0095] In Example 8, additional reference compounds were included in the data set. These include five additional known genotoxic compounds (Ethyl nitroso urea, Doxorubicin HCl, Styrene oxide, Bleomycin sulfate, and Daunorubicin HCl), and five additional compounds known to be purely cytotoxic (KCl, N-Acetylcystein, Ranitidin HCl, Flufenamic acid, and Verapamil HCl).

[0096] The results from Example 8 further confirm the results from the initial experiments and provides evidence that certain biomarker genes can be used as predictors of genotoxicity of compounds in the predictor model. In one embodiment, the set of biomarker genes used to predict genotoxicty or non-genotoxicity of compounds are in the Biomarker-1 (BM1) group. These include, but are not limited to, Xeroderma pigmentosum, complementation group C, ferredoxin reductase, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3C, hypothetical protein MGC5370, damage-specific DNA binding protein 2, 48kDa, transcribed locus, papilin, proteoglycan-like sulfated glycoprotein, fucosidase, alpha-L-1, tissue, carboxypeptidase M, tumor protein p53 inducible protein 3, cyclin-dependent kinase inhibitor 1A (p21, Cip1), phosphatidylinositol glycan, class F, interleukin 6 signal transducer (gp130, oncostatin M receptor), hypothetical protein FLJ10375, vacuolar protein sorting 54 (yeast), hv89d09, interleukin 6 signal transducer (gp130, oncostatin M receptor), phosphatidylserine receptor, alpha-cardiac actin, hypothetical protein FLJ11383, ras homolog gene family, member

Q, thioredoxin interacting protein, hypothetical protein LOC339290, NCK-associated protein 1, TBC1 domain family, member 17, ectodermal-neural cortex (with BTB-like domain), thioredoxin interacting protein, phosphatidylinositol glycan, class F, phosphatidylinositol glycan, class F, and solute carrier family 33 (acetyl-CoA transporter), member 1. In one embodiment, the Biomarker-1 genes are selected from the group consisting of Xeroderma pigmentosum, complementation group C, .Ferrodoxin reductase, apolipoprotein BmRNA editing enzyme, catalytic polypeptide – like 3C, hypothetical protein MGC5370, and damage-specific DNA binding protein 2,48 kDa.

[0097] In one embodiment, the set of biomarker genes used to predict genotoxicty or non-genotoxicity of compounds are in the Biomarker-2 (BM2) group. These include, but are not limited to, EST370545, H. sapiens adenosine deaminase (ADA), Homo sapiens chromosome 12 open reading frame 5 mRNA, polymerase (DNA directed), eta, isocitrate dehydrogenase 1 (NADP+), carboxypeptidase M, plexin B2, polymerase (DNA directed), eta, hypothetical protein FLJ12484, KIAA0907 protein, transcribed locus, ARP9, wb67g03, leucine-rich repeats and death domain containing potassium large conductance calcium-activated channel, subfamily M beta member 3, KAT11914, mitochondrial carrier triple repeat 1, tax1 (human T-cell leukemia virus type I) binding protein 3, sestrin 1, ret finger protein, SMAD, H. sapiens mitogen inducible gene mig-2, FLJ10378 protein, hypothetical protein MGC7036, ubiquitin-conjugating enzyme, KIAA0368, phosphatidylserine receptor, O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase), Mdm2, hypothetical protein LOC51061, NudE nuclear distribution gene E homolog like 1 (A. nidulans), HTPAP protein, and syndecan 1. In one embodiment, the Biomarker-2 genes are selected from the group consisting of LAG1 longevity assurance homolog 5 (S. cerevisiae), hypothetical protein HSPC132, FKSG44 gene, adenosine deaminase, pleckstrin homology-like domain.

[0098] In one embodiment, the set of biomarker genes used to predict genotoxicty or non-genotoxicity of compounds are in the Biomarker-3 (BM3) group. These include, but are not limited to, LAG1 longevity assurance homolog 5 (S. cerevisiae), hypothetical protein HSPC132, FKSG44 gene, adenosine deaminase, pleckstrin homology-like domain, ectodermal-neural cortex (with BTB-like domain), F-box protein 22, ribonucleotide reductase M2 B (TP53 inducible), guanidinoacetate N-methyltransferase, transmembrane 7 superfamily member 3,

isocitrate dehydrogenase 1 (NADP+), phosphohistidine phosphatase 1, hypothetical protein FLJ20296, discoidin domain receptor family, member 1, transcribed locus, guanidinoacetate N-methyltransferase, human receptor tyrosine kinase DDR gene, transmembrane 7 superfamily member 3, 601565341F1 NIH_MGC_21 Homo sapiens cDNA clone, F-box protein 22, cytosolic sialic acid 9-O-acetylesterase homolog, BTG family member 2, astrotactin 2, IKK interacting protein, surfeit 4, neutral sphingomyelinase (N-SMase) activation associated factor, ADP-ribosylation factor-like 1, golgi reassembly stacking protein 2, leucine-rich repeats and death domain containing mixed-lineage leukemia, hypothetical protein LOC253981, placenta-specific 8, glutathione peroxidase 1, KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2, syntaxin 7, lysosomal-associated multispanning membrane protein-5, and phosphoinositide-3-kinase catalytic alpha polypeptide. In one embodiment, the Biomarker-3 genes are selected from the group consisting of LAG1 longevity assurance homolog 5 (S. cerevisiae), hypothetical protein HSPC132, FKSG44 gene, and adenosine deaminase, pleckstrin homology-like domain.

[0099] It will be appreciated by those skilled in the art that any one set of biomarker genes, (i.e., BM1, BM2 or BM3) can be used alone, or in combination with each other. For example, genes from the BM1 group can be used in combination with genes from the BM2 group or genes form the BM3 group to predict genotoxicity of the compound.

[0100] Also within the scope of the invention is adaptation of the predictor model in which genes identified from classical genotoxicity testing can be included in the dataset to predict genotoxicity of compounds.

[0101] From the experiments conducted herewith, a number of common predictor genes have been identified that play an important role in cell cycle and DNA repair processes. A representative few are as follows:

[0102] *Xeroderma Pigmentosum group C gene (XPC):* The nucleotide excision repair (NER) gene XPC is a DNA damage-inducible and p53-regulated gene and likely plays a role in the p53-dependent NER pathway. XPC defect reduces the cisplatin treatment-mediated p53 response, which suggests that the XPC protein plays an important role in the cisplatin treatment-mediated

cellular response. It may also suggest a possible mechanism of cancer cell drug resistance (Wang G, Dombkowski A, Chuan L; Xu XX: Cell Res. 2004 Aug;14(4):303-14).

[0103] *Ferredoxin Reductase (FDXR):* The ferredoxin reductase gene is regulated by the p53 family and sensitizes cells to oxidative stress-induced apoptosis. It increases the sensibility of H1299 and HCT116 cells to 5-fluorouracil-, doxorubicin- and H(2)O(2)- mediated apoptosis (Liu G, Chen X.: Oncogene. 2002 Oct 17;21(47):7195-204). FDXR contributes to p53-mediated apoptosis through the generation of oxidative stress in mitochondria.

[0104] *Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3C* (APOBEC3C): APOBEC1 is the catalytic component of an RNA editing complex but shows homology to activation-induced cytidine deaminase (AID), a protein whose function is to potentiate diversification of immunoglobulin gene DNA. Here, we show that APOBEC1 and its homologs APOBEC3C and APOBEC3G exhibit potent DNA mutator activity in an E. coli assay. Indeed, like AID, these proteins appear to trigger DNA mutation through dC deamination. However, each protein exhibits a distinct local target sequence specificity. The results reveal the existence of a family of potential active dC/dG mutators, with possible implications for cancer (Harris RS, Petersen-Mahrt SK, Neuberger MS.: Mol Cell. 2002 Nov;10(5):1247-53.)

[0105] *Ribosomal Protein S27-like (RPS27L):* A recessive Arabidopsis mutant with elevated sensitivity to DNA damaging treatments was identified in one out of 800 families generated by T-DNA insertion mutagenesis. The T-DNA generated a chromosomal deletion of 1287 bp in the promoter of one of three S27 ribosomal protein genes (ARS27A) preventing its expression. Seedlings of ars27A developed normally under standard growth conditions, suggesting wild-type proficiency of translation. However, growth was strongly inhibited in media supplemented with methyl methane sulfate (MMS) at a concentration not affecting the wild type. This inhibition was accompanied by the formation of tumor-like structures instead of auxiliary roots. Wild-type seedlings treated with increasing concentrations of MMS up to a lethal dose never displayed such a trait, neither was this phenotype observed in ars27A plants in the absence of MMS or under other stress conditions. Thus, the hypersensitivity and tumorous growth are mutant-specific responses to the genotoxic MMS treatment. Another important feature of the mutant is its inability to perform rapid degradation of transcripts after UV treatment, as seen in wild-type

plants. Therefore, we propose that the ARS27A protein is dispensable for protein synthesis under standard conditions but is required for the elimination of possibly damaged mRNA after UV irradiation. (Revenkova E, Masson J, Koncz C, Afsar K, Jakovleva L, Paszkowski J.: Involvement of Arabidopsis thaliana ribosomal protein S27 in mRNA degradation triggered by genotoxic stress. EMBO J. 1999 Jan 15;18(2):490-9.)

[0106] *Damage-Specific DNA binding protein 2 (DDB2):* cDNA microarray analyses indicated that arsenic (AsIII) treatment decreased the expression of genes associated with DNA repair (e.g., p53 and Damage-specific DNA-binding protein 2) and increased the expression of genes indicative of the cellular response to oxidative stress (e.g., Superoxide dismutase 1, NAD(P)H quinone oxidoreductase, and Serine/threonine kinase 25). AsIII also modulated the expression of certain transcripts associated with increased cell proliferation (e.g., Cyclin G1, Protein kinase C delta), oncogenes, and genes associated with cellular transformation (e.g., Gro-1 and V-yes). These observations correlated with measurements of cell proliferation and mitotic measurements as AsIII treatment resulted in a dose-dependent increase in cellular mitoses at 24 h and an increase in cell proliferation at 48 h of exposure. (Hamadeh HK, Trouba KJ, Amin RP, Afshari CA, Germolec D.: Coordination of altered DNA repair and damage pathways in arsenite-exposed keratinocytes. Toxicol Sci. 2002 Oct;69(2):306-16.)

[0107] A newly identified patient with clinical xeroderma pigmentosum phenotype has a non-sense mutation in the DDB2 gene and incomplete repair in (6-4) photoproducts. (Itoh T, Mori T, Ohkubo H, Yamaizumi M. A newly identified patient with clinical xeroderma pigmentosum phenotype has a non-sense mutation in the DDB2 gene and incomplete repair in (6-4) photoproducts. J Invest Dermatol. 1999 Aug;113(2):251-7.).

[0108] Cells pretreated with UV light, mitomycin C, or aphidicolin, but not TPA or serum starvation, have higher levels of this damage-specific DNA binding (DDB) protein. These results suggest that the signal for induction of DDB protein can either be damage to the DNA or interference with cellular DNA replication. The induction of DDB protein varies among primate cells with different phenotypes: (1) virus-transformed repair-proficient cells have partially or fully lost the ability to induce DDB protein above constitutive levels; (2) primary cells from repair-deficient xeroderma pigmentosum (XP) group C, and transformed XP groups A and D,

show constitutive DDB protein, but do not show induced levels of this protein 48 h after UV; and (3) primary and transformed repair-deficient cells from one XP E patient are lacking both the constitutive and the induced DDB activity. The correlation between the induction of the DDB protein and the enhanced repair of UV-damaged expression vectors implies the involvement of the DDB protein in this inducible cellular response. (Protic M, Hirschfeld S, Tsang AP, Wagner M, Dixon K, Levine AS.: Induction of a novel damage-specific DNA binding protein correlates with enhanced DNA repair in primate cells. Mol Toxicol. 1989 Oct-Dec;2(4):255-70.)

[0109] *Polymerase (DNA directed), eta (POLH):* UV irradiation generates predominantly cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts in DNA. CPDs are thought to be responsible for most of the UV-induced mutations. Thymine-thymine CPDs, and probably also CPDs containing cytosine, are replicated *in vivo* in a largely accurate manner by a DNA polymerase eta (Pol eta) dependent process. Pol eta is encoded by the POLH (XPV) gene in humans. (Choi JH, Pfeifer GP.: The role of DNA polymerase eta in UV mutational spectra. DNA Repair (Amst). 2005 Feb 3;4(2):211-20.). Xeroderma pigmentosum V (XPV) is caused by molecular alterations in the POLH gene, located on chromosome 6p21.1-6p12. Affected individuals are homozygous or compound heterozygous for a spectrum of genetic lesions, including nonsense mutations, deletions or insertions, confirming the autosomal recessive nature of the condition. Identification of POLH as the XPV gene provides an important instrument for improving molecular diagnostics in XPV families. (Gratchev A, Strein P, Utikal J, Sergij G.: Molecular genetics of Xeroderma pigmentosum variant. Exp Dermatol. 2003 Oct;12(5):529-36.)

[0110] Systematic analysis of nucleotide excision repair mutants demonstrate the involvement of transcription-coupled nucleotide excision repair and a partial requirement for the lesion bypass DNA polymerase eta encoded by the human POLH gene. (Zheng H, Wang X, Warren AJ, Legerski RJ, Nairn RS, Hamilton JW, Li L.: Nucleotide excision repair- and polymerase eta-mediated error-prone removal of mitomycin C interstrand cross-links. Mol Cell Biol. 2003 Jan;23(2):754-61.)

[0111] *Leucine-rich and death domain containing (LRDD):* The protein encoded by this gene contains a leucine-rich repeat and a death domain. This protein has been shown to interact with other death domain proteins, such as Fas (TNFRSF6)-associated via death domain (FADD) and

MAP-kinase activating death domain-containing protein (MADD), and thus may function as an adaptor protein in cell death-related signaling processes. The expression of the mouse counterpart of this gene has been found to be positively regulated by the tumor suppressor p53 and to induce cell apoptosis in response to DNA damage, which suggests a role for this gene as an effector of p53-dependent apoptosis. Three alternatively spliced transcript variants encoding distinct isoforms have been reported.

[0112] *Protein phosphatase 1D magnesium-dependent, delta isoform (PPM1D):* The protein encoded by this gene is a member of the PP2C family of Ser/Thr protein phosphatases. PP2C family members are known to be negative regulators of cell stress response pathways. The expression of this gene is induced in a p53-dependent manner in response to various environmental stresses. While being induced by tumor suppressor protein TP53/p53, this phosphatase negatively regulates the activity of p38 MAP kinase, MAPK/p38, through which it reduces the phosphorylation of p53, and in turn suppresses p53-mediated transcription and apoptosis. This phosphatase thus mediates a feedback regulation of p38-p53 signaling that contributes to growth inhibition and the suppression of stress induced apoptosis. This gene is located in a chromosomal region known to be amplified in breast cancer. The amplification of this gene has been detected in both breast cancer cell line and primary breast tumors, which suggests a role of this gene in cancer development.

[0113] *Tax interaction protein 1 (TIP-1):* TIP-1 may represent a novel regulatory element in the Wnt/beta-catenin signaling pathway. Wnt signaling is essential during development while deregulation of this pathway frequently leads to the formation of various tumors including colorectal carcinomas. A key component of the pathway is beta-catenin that, in association with TCF-4, directly regulates the expression of Wnt-responsive genes. It was shown that overexpression of TIP-1 reduced the proliferation and anchorage-independent growth of colorectal cancer cells. [Kanamori M et al., 2003]

[0114] TBC1 domain family, member 5 (TBC1D5), hypothetical protein FLJ23311, and hypothetical protein MGC13024 have unknown function.

[0115] *Tumor necrosis factor receptor superfamily, member 1B (TNFRSF1B):* The protein encoded by this gene is a member of the TNF-receptor superfamily. This protein and TNF-

receptor 1 form a heterocomplex that mediates the recruitment of two anti-apoptotic proteins, c-IAP1 and c-IAP2, which possess E3 ubiquitin ligase activity. The function of IAPs in TNF-receptor signalling is unknown, however, c-IAP1 is thought to potentiate TNF-induced apoptosis by the ubiquitination and degradation of TNF-receptor-associated factor 2, which mediates anti-apoptotic signals. Knockout studies in mice also suggest a role of this protein in protecting neurons from apoptosis by stimulating antioxidative pathways.

[0116] *Discoidin domain receptor family, member 1(DDR1):* Receptor tyrosine kinases (RTKs) play a key role in the communication of cells with their microenvironment. These molecules are involved in the regulation of cell growth, differentiation and metabolism. The protein encoded by this gene is a RTK that is widely expressed in normal and transformed epithelial cells and is activated by various types of collagen. This protein belongs to a subfamily of tyrosine kinase receptors with a homology region to the Dictyostelium discoideum protein discoidin I in their extracellular domain. Its autophosphorylation is achieved by all collagens so far tested (type I to type VI). *In situ* studies and Northern-blot analysis showed that expression of this encoded protein is restricted to epithelial cells, particularly in the kidney, lung, gastrointestinal tract, and brain. In addition, this protein is significantly over-expressed in several human tumors from breast, ovarian, esophageal, and pediatric brain. This gene is located on chromosome 6p21.3 in proximity to several HLA class I genes. Three isoforms of this gene are generated by alternative splicing.

[0117] *Ketohexokinase (fructokinase) (KHK):* KHK encodes the gene ketohexokinase that catalyzes conversion of fructose to fructose-1-phosphate. The splice variant presented encodes the highly active form found in liver, renal cortex, and small intestine, while the alternate variant encodes the lower activity form found in most other tissues.

[0118] *Sirtuin (silent mating type information regulation 2, S.cerevisiae, homolog) 3 (SIRT3):* This gene encodes a member of the sirtuin family of proteins, homologs to the yeast Sir2 protein. Members of the sirtuin family are characterized by a sirtuin core domain and grouped into four classes. The functions of human sirtuins have not yet been determined; however, yeast sirtuin proteins are known to regulate epigenetic gene silencing and suppress recombination of rDNA. Studies suggest that the human sirtuins may function as intracellular regulatory proteins with

mono-ADP-ribosyltransferase activity. The protein encoded by this gene is included in class I of the sirtuin family.

[0119] *Transforming growth factor, beta 1 (TGFB1):* Transforming growth factor TGF beta1 is involved in a variety of important cellular functions,including cell growth and differentiation, angiogenesis, immune function and extracellular matrix formation. TGF beta(1) might be associated with tumor progression by modulating the angiogenesis in colorectal cancer and TGF beta(1) may be used as a possible biomarker. World J Gastroenterol. 2002 Jun;8(3):496-8.

[0120] *Protein tyrosine phosphatase, non-receptor type 22 (lymphoid) (PTPN22):* This gene encodes a protein tyrosine phosphatase which is expressed primarily in lymphoid tissues. This enzyme associates with the molecular adapter protein CBL and may be involved in regulating CBL function in the T-cell receptor signaling pathway. Alternative splicing of this gene results in two transcript variants encoding distinct isoforms.

[0121] *Actin, alpha 2, smooth muscle, aorta (ACTA2):* Actin alpha 2, the human aortic smooth muscle actin gene, is one of six different actin isoforms which have been identified. Actins are highly conserved proteins that are involved in cell motility, structure and integrity. Alpha actins are a major constituent of the contractile apparatus.

[0122] *Syndecan-1 (Sdc1):* Induction of syndecan-1 expression in stromal fibroblasts promotes proliferation of human breast cancer cells. Furthermore, high syndecan-1 expression in breast carcinoma is related to an aggressive phenotype and to poorer prognosis. Syndecan-1 expression in thyroid carcinoma: stromal expression followed by epithelial expression is significantly correlated with dedifferentiation.

EXAMPLES

Methods and Materials

(i) Chemicals, Media and Serums

[0123] All chemicals were of reagent grade (Sigma-Aldrich, St. Louis, MO; Fluka sold through Sigma Aldrich; Lancaster Synthesis, Lancashire, UK) and were purchased as "cell culture tested"

where possible. "RPMI 1640 Glutamax-I" medium, Penicillin/Streptomycin and Fetal Horse Serum were obtained from Gibco. RNeasy Mini Kits were from Qiagen.

(ii) Cell Culture

[0124] The human lymphoblastoid cell line TK6 (ATCC, Manassas, VA) was cultured in RPMI 1640 medium (with Glutamax and 10 % FHS) at a cell density of $0.2x10^5$ to $10x10^5$ cells/ml. Cells were routinely subcultured starting from frozen aliquots after passage number. For experiments, passage numbers between 3 to 15 were used.

(iii) Cytotoxicity Determination

[0125] Cytotoxic concentrations were determined either by measuring cell density on a Sysmex Cell Counter (Sysmex America, Inc., Mundelein, IL) or by metabolic cell activity using the Alamar Blue (Serotec Inc., Raleigh, NC) cytotoxicity assay. Alamar Blue indicator dye quantitatively measures proliferation in human and other cells. Alamar Blue is a sensitive fluorimetric and colorimetric reagent sensitive to the redox state of the growth medium. Cell density by Sysmex was measured after the 24 h treatment. Cytotoxicity by Alamar Blue was measured 3 hours prior to end of treatment, i.e., at 21 hours. 200 µl of cell suspension were mixed with 20 µl of Alamar Blue reagent in a 96-well plate and measured once/hour using a fluorescence plate reader with 544 nm excitation and 612 nm emission filters. Cell suspension samples from the cytotoxicity dilution series were analyzed for cytometry endpoints by Laser Scanning Cytometry .

(iv) Treatment Of Cell Cultures

[0126] TK6 human lymphoblastoid cells were exposed to following treatments (24 hours, $0.15x10^6$ cells/ml):

Table 1 Study design

Class	Compound	Abbreviation	Dose, µg/mL	# of Samples
Control	None			6
Cytotoxic	NaCl	NaCl	3,840	6
	trans-Platinum	tPt	33	6
	Rifampicin	Rif	167	6
Genotoxic	cis-Platinum	cPt	1.3	6
	Methyl Methane Sulfonate	MMS	6.25	6

Class	Compound	Abbreviation	Dose, µg/mL	# of Samples
	Mitomycin C	MMC	0.10	6

[0127] In Table 1, trans-Platinum is trans-diammineplatinum(II) dichloride and cis-Platinum is cis-diammineplatinum(II) dichloride. Dose-response determination to provide the doses given in column 4 of Table 1 was carried out with an initial cell density of $0.15x10^6$ cells/ml (see Example 1).

(v) RNA Isolation

[0128] Total RNA was isolated after 24 hours of treatment with the agents or control using Qiagen's (Hilden, Germany) RNeasy Mini Kits. Samples were made up of 10 ml TK6 cell suspensions with an approximate cell density of $0.3x10^6$ cells/ml. Column-purified RNA was eluted with 40 µl water and quality-checked by UV spectrometry and Agilent's "lab-on-a-chip" technology (RNA nano chip, Bioanalyzer 2100, Agilent Technologies, Santa Clara, CA). RNA extraction and purification is described by the manufacturer of the GeneChip system.

(vi) Microarray Hybridization

[0129] D1 examples 1 – 7, DNA microarray experiments were conducted for Examples 1-7 as recommended by the manufacturer of the GeneChip system (Affymetrix, Inc. 2002) and as previously described (Lockhart et al. 1996). Purified total human TK6 RNA was analyzed using the human specific Human Genome U133A 2.0 array (Affymetrix). The Human Genome U133A 2.0 array covers approximately 18,400 transcripts and variants, including 14,500 well-characterized human genes represented by more than 22,000 probe sets. Sequences used in the design of the array were selected from GenBank®, dbEST, and RefSeq. The sequence clusters were created from the UniGene database (Build 133, April 20, 2001) and then were refined by analysis and comparison with a number of other publicly available databases including the Washington University EST trace repository and the University of California, Santa Cruz Golden-Path human genome database (April 2001 release).

[0130] For experiments conducted in Example 8, the Human Genome U133 Plus 2.0 array was used. This array covers more than 47,000 transcripts in more than 54,000 probe sets. The sequences from which these probe sets were derived were selected from GenBank®, dbEST, and RefSeq. The sequence clusters were created from the UniGene database (Build 133, April 20,

2001) and then refined by analysis and comparison with a number of other publicly available databases, including the Washington University EST trace repository and the University of California, Santa Cruz Golden-Path human genome database (April 2001 release). In addition, it contains 9,921 probe sets representing approximately 6,500 genes based on sequences selected from GenBank, dbEST, and RefSeq. Sequence clusters were created from the UniGene database (Build 159, January 25, 2003) and refined by analysis and comparison with a number of other publicly available databases, including the Washington University EST trace repository and the NCBI human genome assembly (Build 31).

[0131] The resulting primary raw data, the image files (.dat files), were processed using the Microarray Analysis Suite 5 (MAS5) software (Affymetrix). Tab-delimited files were obtained containing data regarding signal intensity (Signal) and categorical expression level measurement (Absolute Call).

(vii) Microarray Data Analysis

[0132] MAS5-derived raw data was analyzed using Simca-P 10.5/GeneSpring 7.2.

Simca-P 10.5/GeneSpring 7.2

[0133] The "Simca-P 10.5/GeneSpring 7.2" approach combined the statistical tools of the SIMCA-P 10.5 software (Umetrics AB, S-Umea) with GeneSpring 7.2. The raw data obtained from the GeneChip by MAS5 were imported to GeneSpring 7.2 for analysis. Data were normalized per chip and per gene to the respective median. Genes were annotated according to LocusLink nomenclature (http://www.ncbi.nlm.nih.gov/LocusLink/).

[0134] For the development of a model being capable of differentiating the two classes of toxicity only samples treated with cytotoxic and genotoxic compounds were included in the analysis: control samples were excluded from the analysis. Filtering of the data was performed according to following criteria for each gene:

Fold-change > 1.4 OR Fold-change < 0.7; AND

Signal Mean(cytotoxic) > 50 OR Signal Mean(genotoxic) > 50; AND

Signal CV(cytotoxic) < 50% AND Signal CV(genotoxic) < 50%; where CV is the coefficient of variation.

[0135] Fold-change refers to the ratio of genotoxic versus cytotoxic. Since these studies seek robust predictor genes the limit ratios (1.4 and 0.7) were selected in order to excluded genes that show consistent but only small differences. Furthermore, the mean signal of at least one of the two classes should show a reliable signal with intensity greater than 50, and the coefficient of variation of gene expression signals within each class should be smaller than 50% in order to exclude highly variable genes. The filtering was performed by Microsoft Excel 2002 SP 2. 215 genes resulted from the filtering analysis.

[0136] After data filtering, two predictive modeling approaches were applied, the partial least squares – discriminant analysis and the k-nearest neighbor analysis.

[0137] Partial Least Squares – Discriminant Analysis (PLS-DA); all calculations were performed by the software package SIMCA-P version 10 (Umetrics AB, Umea, Sweden).

[0138] Raw gene expression intensities of the 215 genes were log-transformed, centered and scaled to uni-variance. Principal Component Analysis (PCA) was applied to the data to check their relative position in a low-dimensional space and to investigate the impact of cell count and Alamar Blue on their relative position.

[0139] PLS-DA was applied iteratively to the gene expression data with cyto- and genotoxicity as class variables. The evaluation of the differential gene pattern between the mean scores of either class identified the genes that contributed significantly to the separation. With each iteration the predictive model was cross-validated by a leave-one-out approach (LOO). The final model was validated by response permutation; i.e. the class membership of each sample was randomly attributed, evaluated by the model and contrasted to the solution of the model with the original class membership. 100 permutations were performed.

[0140] The second approach of predictive modeling was performed by k-nearest neighbor (KNN) analysis (GeneSpring 7.2). The same 215 candidate genes that were used for PLS-DA were used in this approach. Due to the limited sample size the composition of the calibration sample set and test sample set were permutated several times.

[0141] The intersection of predictor genes resulting from PLS-DA and the k-nearest neighbor approach were subjected to PLS-DA and a condition clustering (GeneSpring 7.2) in order to investigate the predictive power of the selected genes.

[0142] For the experiments conducted in Example 8, the following procedure was used:

Normalization: Per chip: normalization on sample median. Per gene: normalization on gene median of all samples (Genespring 7.2).

[0143] *Pre-Filtering of Genes*: Filter on flags: probe set needs to show present or marginal flags in at least 50% of samples and filter on intensities: probe set must have intensities > 50 in at least 50% of samples resulting in 18'512 probe sets (Genespring 7.2).

[0144] *Statistical Filtering*:Welch t-test (Genespring 7.2): All (98) Samples Default Interpretation - Genes from Present and signal GT 50 in 50% of samples with statistically significant differences when grouped by 'Class (non-genotoxic versus gtx)'; parametric test, variances not assumed equal (Welch t-test). p-value cutoff 0.001, multiple testing correction: Benjamini and Hochberg False Discovery Rate (FDR). This restriction tested 18'512 genes. About 0.1% of the identified genes would be expected to pass the restriction by chance. 4'911 probe sets passed this filter. Given a FDR of 0.1% 5 out of 4911 would be expected to be falsely positive.

Example 1. Determination of Cytotoxicity and G2 Phase Block

[0145] The six model compounds identified in Table 1 were applied in a dilution series to TK6 cells. The resulting dilution series for each of the six compounds provided individually optimized cytotoxic concentrations for 50% cell death (EC_{50}) as determined by cell density and the Alamar Blue assay. These data are shown in Figure 1 and Table 2. It is possible that cell density and the Alamar Blue assay may not give identical cytotoxicity profiles. Thus, concentrations of the compounds were optimized with the objective to have both cytotoxicity parameters within the range of 40-60% viability decrease. In addition, for representative dilution series several cytometry endpoints were analyzed (BrdU incorporation, KI-67 staining (Histogenex, Edegem, Belgium), propidium iodide staining), although these parameters were not used for concentration selection.

Table 2.

Class	Compounds or Drugs	Cell Density (± S.D.)	Alamar Blue (± S.D.)
Non-genotoxic	trans-Platinum	58 ± 7 %	36 ± 13 %

	Rifampicin	52 ± 9 %	61 ± 8 %
	NaCl	30 ± 4 %	65 ± 5 %
Genotoxic	cis-Platinum	53 ± 4 %	46 ± 5 %
	Mitomycin C	53 ± 3 %	48 ± 1 %
	Methyl methanesulfonate	44 ± 2 %	43 ± 8 %

[0146] The three non-genotoxic compounds tPt, Rif and NaCl belong to relatively diverse compound classes. Consequently, the most pronounced effects and finally the crucial mode-of-actions leading to strong cytotoxicity may be totally different. This situation is reflected by the obtained cytotoxicity profiles. The approximated EC_{50} values range between 33µM and 3.8 mM, similarly, the sensitivity of cell density and the redox endpoint (Alamar Blue) is compound dependent. Up to the ascertained EC_{50} values no significant shifts in cell cycle parameters were detected (Figure 1), concluding that none of the three compounds had specific impact on regulatory pathways of the cell cycle.

[0147] Compared with this the three genotoxic compounds had significantly lower EC_{50} values (ranging between 0.10 µM and 6.3 µM) and led to remarkable shifts in cell cycle parameters. The most outstanding effect is an obvious G_2 phase block with estimated maxima around the EC_{50} values, indicating DNA repair activity (Figure 1). This observation is in accordance with the fundamental hypothesis, that within this cellular model system an adaptive response as an answer to the exogenous genotoxic stress will occur. Fortunately, this is already visible on the cytometry level.

Example 2. Identification of Candidate Predictor Genes

[0148] For experiments leading to hybridization of RNA to human genomic probes, concentrations of the compounds as specified in Table 1 were used (Example 1). These concentrations are equicytotoxic (e.g., cPt: 1.3 µM, and tPt: 33 µM). Each compound was tested using six independent replicates on two or three different dates. After isolation of total RNA expression profiles were compiled using Affymetrix HGU133A PLUS 2 microarrays.

[0149] As noted in Materials and Methods, one vehicle, three cytotoxic reagents and three genotoxic reagents were used to treat TK6 cells. Each of the replicate samples was applied to an

Affymetrix HGU133A chip for hybridization and detection of the results. These data were filtered for fold-change, signal mean, and signal CV as described in Materials and Methods. Only 215 genes passed this rigorous filter process. These filtered genes are compiled in Table 3.

TABLE 3. 215 GENES FROM FILTERING

LOCUS-LINK	GENBANK	REFSEQ	UNIGENE	GENENAME
23498	BC029510, BC029510, NM_012205, Z29481	NM_012205.1	Hs.108441	3-hydroxyanthranilate 3,4-dioxygenase
27301	AB021260, AB021260, AB049211, AF119046, AJ011311, BC002959, BI837686	NM_014481.2	Hs.154149	APEX nuclease (apurinic/apyrimidinic endonuclease) 2
10058	AB039371, AB039371, AF070598, AF076775, AF308472, AJ289233, AK057026, BC000559, BC043423, NM_005689	NM_005689.1	Hs.107911	ATP-binding cassette, sub-family B (MDR/TAP), member 6
489	AF068220, AF068220, AF068221, AF458228, AF458229, BC035729, NM_005173, NM_174953, NM_174954, NM_174955, NM_174956, NM_174957, NM_174958, S68239, Y15724, Y15737, Y15738, Z69880, Z69881	NM_005173.2, NM_005173.2, NM_174955.1, NM_174953.1, NM_174954.1, NM_174956.1, NM_174958.1	Hs.5541	ATPase, Ca++ transporting, ubiquitous
694	BC009050, BC009050, BC016759, BC064953, NM_001731, X61123	NM_001731.1	Hs.255935	B-cell translocation gene 1, anti-proliferative
27113	AF332558, AF332558, AF354654, AF354655, AF354656, NM_014417	NM_014417.2	Hs.87246	BCL2 binding component 3
581	AF007826, AF007826, AF008195, AF008196, AF020360, AF247393, AF339054, AJ417988, BC014175, BE396495, BM706954, L22473, L22474, L22475, NM_004324, NM_138761, NM_138762, NM_138763, NM_138764, NM_138765, U19599	NM_004324.3, NM_004324.3, NM_138762.2, NM_138764.2, NM_138765.2, NM_138763.2	Hs.159428	BCL2-associated X protein
581	AF007826, AF007826, AF008195, AF008196, AF020360, AF247393, AF339054, AJ417988, BC014175, BE396495, BM706954, L22473, L22474, L22475, NM_004324, NM_138761, NM_138762, NM_138763, NM_138764, NM_138765, U19599	NM_004324.3, NM_004324.3, NM_138762.2, NM_138764.2, NM_138765.2, NM_138763.2	Hs.159428	BCL2-associated X protein
580	AF038034, AF038034, AF038035, AF038036, AF038037, AF038038, AF038039, AF038040, AF038041, AF038042, NM_000465, U76638	NM_000465.1	Hs.54089	BRCA1 associated RING domain 1
7832	AF361937, AF361937, NM_006763, U72649, Y09943	NM_006763.2	Hs.75462	BTG family, member 2
7832	AF361937, AF361937, NM_006763, U72649, Y09943	NM_006763.2	Hs.75462	BTG family, member 2

LOCUS-LINK	GENBANK	REFSEQ	UNIGENE	GENENAME
585	AF090947, AF090947, AF359281, AK075321, BC008923, BC027624, BI562463, BX647855, NM_033028	NM_033028.2	Hs.26471	Bardet-Biedl syndrome 4
641	BC034480, BC034480, NM_000057, U39817	NM_000057.1	Hs.383913	Bloom syndrome
9738	AB007879, AB007879, AC003108, BC030223, BC034140, BC036654, NM_014711	NM_014711.3	Hs.279912	CP110 protein
1663	AK021703, AK021703, BC001591, BC011264, BC012834, BC047317, BC050069, BC050522, NM_004399, NM_030653, NM_030655, U33833, U35241, U75967, U75968, U75969	NM_004399.1, NM_004399.1, NM_030655.2	Hs.443960	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11 (CHL1-like helicase homolog, S. cerevisiae)
1663	AK021703, AK021703, BC001591, BC011264, BC012834, BC047317, BC050069, BC050522, NM_004399, NM_030653, NM_030655, U33833, U35241, U75967, U75968, U75969	NM_004399.1, NM_004399.1, NM_030655.2	Hs.443960	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11 (CHL1-like helicase homolog, S. cerevisiae)
55247	AB079071, AB079071, AK001720, BC025954	NM_018248.1	Hs.405467	DNA glycosylase hFPG2
81620	AB053172, AB053172, AF070552, AF321125, BC000137, BC008676, BC008860, BC009410, BC014202, BC021126, BC049205	NM_030928.2	Hs.122908	DNA replication factor
1870	AF086395, AF086395, AK092799, BC007609, BC053676, L22846, NM_004091	NM_004091.2	Hs.231444	E2F transcription factor 2
23770	AC005387, AC005387, AY225339, BC009966, BX538124, BX647405, BX647720, L37033, NM_012181	NM_012181.2	Hs.173464	FK506 binding protein 8, 38kDa
79733	AK026964, AK026964, AK055206, BC028244, BU164108, BX504614, CB959621	NM_024680.2	Hs.94292	FLJ23311 protein
2491	BC005967, BC005967, BC012462, BG114761, BQ224168, M78295, NM_006733, X97249	NM_006733.2	Hs.348920	FSH primary response (LRPR1 homolog, rat) 1
2842	AK074729, AK074729, NM_006143, U55312, U64871	NM_006143.1	Hs.92458	G protein-coupled receptor 19
2760	AF173832, AF173832, BC009273, BX473154, L01439, M76477, NM_000405, X16087, X61094, X61095, X62078	NM_000405.3	Hs.387156	GM2 ganglioside activator protein
29893	AB030304, AB030304, AK126369, BC008792, NM_013290, NM_016556	NM_013290.3, NM_013290.3	Hs.279032	GT198, complete ORF
11147	AF126163, AF126163, AF126164, BC010922	NM_007071.1	Hs.142245	HERV-H LTR-associating 3
283638	AB006622, AB006622, AK025023, AK091980, BC047913	XM_208766.3	Hs.182536	KIAA0284
23354	AB020648, AB020648, BC013947	XM_049237.6	Hs.7426	KIAA0841

LOCUS-LINK	GENBANK	REFSEQ	UNIGENE	GENENAME
22889	AB020714, AB020714, AY112680, AY112681, AY112682, BC027182		Hs.24656	KIAA0907 protein
79682	AA761728, AA761728, AF469667, AK027121, BC031520, BG031878, BQ185248, BX355581	NM_024629.2	Hs.38178	KSHV latent nuclear antigen interacting protein 1
3985	AB016655, AB016655, AB016656, AC002073, AK093554, AL117466, BC013051, D45906, D85527, NM_005569, NM_016733	NM_005569.2, NM_005569.2	Hs.278027	LIM domain kinase 2
7805	D42042, D42042, NM_006762, U51240	NM_006762.1	Hs.436200	Lysosomal-associated multispanning membrane protein-5
7805	D42042, D42042, NM_006762, U51240	NM_006762.1	Hs.436200	Lysosomal-associated multispanning membrane protein-5
83463	AF114834, AF114834, AK057034, AL833959, BC000745, BC032586, BC041690	NM_031300.2	Hs.442993	MAX dimerization protein 3
4173	AK022899, AK022899, BC031061, BM781972, BQ058022, NM_005914, NM_182746, U63630, U90415, X74794	NM_005914.2, NM_005914.2	Hs.460184	MCM4 minichromosome maintenance deficient 4 (S. cerevisiae)
4276	AK094237, AK094237, AY204547, BC016929, L14848, NM_000247, U56940, U56941, U56942, U56943, U56944, U56946, U56947, U56948, U56950, U56951, U56952, U56953, U56954, X92841, Y16805, Y16806, Y16808, Y16810	NM_000247.1	Hs.90598	MHC class I polypeptide-related sequence A
123803	AF092440, AF092440, BC017336	NM_173474.2	Hs.351573	N-terminal asparagine amidase
79671	AB094095, AB094095, AK025131, AK056454, AK095247, AL049456, BC013199, BC023974, BK001111, BX647705, NM_024618	NM_024618.2, NM_024618.2	Hs.31097	NOD9 protein
4851	AF308602, AF308602, AK000012, BC013208, BC032414, M73980, NM_017617	NM_017617.2	Hs.311559	Notch homolog 1, translocation-associated (Drosophila)
8438	AA582917, AA582917, BM464345, NM_003579	NM_003579.2	Hs.66718	RAD54-like (S. cerevisiae)
5875	AL547259, AL547259, AU125148, BC003093, CB995791, CD370056, CD672493, NM_004581, NM_182836, Y08200	NM_004581.2, NM_004581.2	Hs.377992	Rab geranylgeranyltransferase, alpha subunit
6955	M12423, M12423, X01403, X02592			T cell receptor alpha locus
10312	AF025374, AF025374, AF033033, AW083897, BC018133, BC032465, NM_006019, NM_006053, U45285	NM_006019.2, NM_006019.2	Hs.46465	T-cell, immune regulator 1, ATPase, H+ transporting, lysosomal V0 protein a isoform 3
9779	AK097990, AK097990, BC013145, D86965		Hs.115740	TBC1 domain family, member 5

LOCUS-LINK	GENBANK	REFSEQ	UNIGENE	GENENAME
7071	AF050110, AF050110, BC011538, BT006634, NM_005655, S81439, U21847	NM_005655.1	Hs.82173	TGFB inducible early growth response
9618	AF082185, AF082185, BC001769, BC026726, BC047358, NM_004295, NM_145751, X80200	NM_004295.2, NM_004295.2	Hs.8375	TNF receptor-associated factor 4
9618	AF082185, AF082185, BC001769, BC026726, BC047358, NM_004295, NM_145751, X80200	NM_004295.2, NM_004295.2	Hs.8375	TNF receptor-associated factor 4
11257	AB007455, AB007455, AB007456, AB007457, BC002709	NM_007233.1	Hs.274329	TP53 activated protein 1
11257	AB007455, AB007455, AB007456, AB007457, BC002709	NM_007233.1	Hs.274329	TP53 activated protein 1
30851	AF028823, AF028823, AF168787, AF234997, AF277318, AK001327, BC023980, NM_014604	NM_014604.1	Hs.12956	Tax interaction protein 1
30851	AF028823, AF028823, AF168787, AF234997, AF277318, AK001327, BC023980, NM_014604	NM_014604.1	Hs.12956	Tax interaction protein 1
7454	AF115548, AF115548, AF115549, AF196970, BC002961, BC012738, NM_000377, U12707, U18935, U19927	NM_000377.1	Hs.2157	Wiskott-Aldrich syndrome (eczema-thrombocytopenia)
59	BC017554, BC017554, D00618, J05192, K01741, K01742, K01743, K01744, K01745, K01746, K01747, M33216, NM_001613, X13839	NM_001613.1	Hs.208641	actin, alpha 2, smooth muscle, aorta
100	AL832305, AL832305, BC007678, BC040226, K00509, K02567, M13792, NM_000022, X02994, Z97053	NM_000022.1	Hs.407135	adenosine deaminase
375790	AF016903, AF016903, AK021586, AK125197, AK128761, BC004220, BC007649, BC034009, BC063620, S44195		Hs.273330	agrin
286	BC007930, BC007930, BC014467, BC030957, M28880, NM_000037, NM_020475, NM_020476, NM_020477, NM_020478, NM_020479, NM_020480, NM_020481, S82671, U49691, U50092, U50133, X16609	NM_000037.2, NM_000037.2, NM_020478.1, NM_020480.1, NM_020481.1, NM_020479.1, NM_020477.1, NM_020475.1	Hs.443711	ankyrin 1, erythrocytic
286	BC007930, BC007930, BC014467, BC030957, M28880, NM_000037, NM_020475, NM_020476, NM_020477, NM_020478, NM_020479, NM_020480, NM_020481, S82671, U49691, U50092, U50133, X16609	NM_000037.2, NM_000037.2, NM_020478.1, NM_020480.1, NM_020481.1, NM_020479.1, NM_020477.1, NM_020475.1	Hs.443711	ankyrin 1, erythrocytic

LOCUS-LINK	GENBANK	REFSEQ	UNIGENE	GENENAME
25959	AB040951, AB040951, AK000011, AK002094, AK023332, AL117489, AL701379, BC030030, BC032745, BC049201, BQ631109, NM_015493	NM_015493.3, NM_015493.3	Hs.284208	ankyrin repeat domain 25
9582	AK024854, AK024854, BC031803, BC053859, NM_004900, U61083, U61084	NM_004900.3	Hs.226307	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B
27350	AF165520, AF165520, BC011739, BC021080, NM_014508	NM_014508.2	Hs.441124	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3C
60489	AF182420, AF182420, AK022802, AK092614, AK093635, BC009683, BC024268	NM_021822.1	Hs.286849	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G
29108	AB023416, AB023416, AF184072, AF184073, AF255794, AF310103, AF384665, AK000211, BC004470, BC013569, NM_013258, NM_145182, NM_145183	NM_013258.3, NM_013258.3, NM_145182.1	Hs.197875	apoptosis-associated speck-like protein containing a CARD
23621	AB032975, AB032975, AB050436, AB050437, AB050438, AF161367, AF190725, AF200193, AF200343, AF201468, AF204943, AF338816, AF338817, AL833810, BC036084, BC065492, BM996673, NM_012104, NM_138971, NM_138972, NM_138973	NM_012104.2, NM_012104.2, NM_138971.1, NM_138972.1	Hs.49349	beta-site APP-cleaving enzyme
649	BC002593, BC002593, BC009305, BC032105, BC044626, L35278, L35279, M22488, NM_001199, NM_006128, NM_006129, NM_006130, NM_006131, NM_006132, U50330, Y08723, Y08724, Y08725	NM_001199.1, NM_001199.1, NM_006132.1, NM_006131.1, NM_006128.1, NM_006130.1	Hs.1274	bone morphogenetic protein 1
675	NM_000059, NM_000059, U43746, X95152, Z73359, Z74739	NM_000059.1	Hs.34012	breast cancer 2, early onset
634	AC004785, AC004785, AL833584, BC014473, BC024164, D12502, D90311, D90312, D90313, J03858, M69176, M72238, M76742, NM_001712, S71326, X14831, X16354, X16356	NM_001712.2	Hs.512682	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)
634	AC004785, AC004785, AL833584, BC014473, BC024164, D12502, D90311, D90312, D90313, J03858, M69176, M72238, M76742, NM_001712, S71326, X14831, X16354, X16356	NM_001712.2	Hs.512682	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)

LOCUS-LINK	GENBANK	REFSEQ	UNIGENE	GENENAME
834	BC041689, BC041689, BC062327, M87507, NM_001223, NM_033292, NM_033293, NM_033294, NM_033295, U13697, U13698, U13699, U13700, X65019	NM_001223.2, NM_001223.2, NM_033293.1, NM_033294.1, NM_033295.1	Hs.2490	caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)
56998	AB021262, AB021262	NM_020248.1	Hs.108222	catenin, beta interacting protein 1
9744	BC018543, BC018543, BT009788, D30758, NM_014716	NM_014716.2	Hs.337242	centaurin, beta 1
56997	AB073905, AB073905, AJ278126, AK074693, AK090494, AK092784, AK126200, AK126466, BC005171, BX648860, NM_020247	NM_020247.3	Hs.273186	chaperone, ABC1 activity of bc1 complex like (S. pombe)
8973	AB079246, AB079246, AB079247, AB079248, AB079249, AB079250, AB079251, BC014456, NM_004198, U62435, Y16282	NM_004198.2	Hs.103128	cholinergic receptor, nicotinic, alpha polypeptide 6
57103	AJ272206, AJ272206, AY425618, BC012340	NM_020375.1	Hs.24792	chromosome 12 open reading frame 5
79144	AK024699, AK024699, AL121829, BC002531, BC056416, NM_024299	NM_024299.2	Hs.79625	chromosome 20 open reading frame 149
54535	AB029331, AB029331, AB029343, AB112474, AB112475, AC004195, AF216493, AK000204, AK000217, AK000533, AY029160	NM_019052.2	Hs.110746	chromosome 6 open reading frame 18
55602	AF246705, AF246705, AK000043, AK096180, BC022270, BX538162	NM_017632.1	Hs.32922	collaborates/cooperates with ARF (alternate reading frame) protein
1435	BC021117, BC021117, M11038, M11295, M11296, M27087, M37435, M64592, M76453, NM_000757, NM_172210, NM_172211, NM_172212, U22386, X05825	NM_000757.3, NM_000757.3, NM_172212.1, NM_172211.1	Hs.173894	colony stimulating factor 1 (macrophage)
727	AV682721, AV682721, BC022299, BG533927, CB250401, M57729, M65134, NM_001735, T82068	NM_001735.2	Hs.1281	complement component 5
9134	AA830205, AA830205, AF091433, AF102778, AF106690, AF112857, BC007015, BC020729, BG720611, NM_004702, NM_057735, NM_057749	NM_004702.2, NM_004702.2, NM_057735.1	Hs.408658	cyclin E2
26999	AB032994, AB032994, AF132197, AF160973, AL136549, AL161999, BC011762, BC021008, BC026892, L47738, NM_014376	NM_014376.1, NM_014376.1	Hs.211201	cytoplasmic FMR1 interacting protein 2
26999	AB032994, AB032994, AF132197, AF160973, AL136549, AL161999, BC011762, BC021008, BC026892, L47738, NM_014376	NM_014376.1, NM_014376.1	Hs.211201	cytoplasmic FMR1 interacting protein 2

LOCUS-LINK	GENBANK	REFSEQ	UNIGENE	GENENAME
26999	AB032994, AB032994, AF132197, AF160973, AL136549, AL161999, BC011762, BC021008, BC026892, L47738, NM_014376	NM_014376.1, NM_014376.1	Hs.301824	cytoplasmic FMR1 interacting protein 2
26999	AB032994, AB032994, AF132197, AF160973, AL136549, AL161999, BC011762, BC021008, BC026892, L47738, NM_014376	NM_014376.1, NM_014376.1	Hs.301824	cytoplasmic FMR1 interacting protein 2
55526	AA009773, AA009773, AA393480, AL359587, BC002477, BC007955, BG469693, BI333272, BQ420710, BU187564, BU855972, BU927608, CB134342, CF593518, NM_018706	NM_018706.4	Hs.501565	dehydrogenase E1 and transketolase domain containing 1
1831	AB025432, AB025432, AF153603, AF183393, AF228339, AK092645, AK092669, AK127938, AL110191, AY007119, BC018148, BM047061, BX647854, NM_004089, NM_198057, Z50781	NM_004089.2, NM_004089.2	Hs.420569	delta sleep inducing peptide, immunoreactor
1719	BC000192, BC000192, BC003584, BC009634, J00139, J00140, NM_000791, V00507, X00855	NM_000791.2	Hs.464813	dihydrofolate reductase
1719	BC000192, BC000192, BC003584, BC009634, J00139, J00140, NM_000791, V00507, X00855	NM_000791.2	Hs.83765	dihydrofolate reductase
780	AK130776, AK130776, BC008716, BC013400, L11315, L20817, L57508, NM_001954, NM_013993, NM_013994, U48705, X74979, X98208, X99031, Z29093	NM_001954.3, NM_001954.3, NM_013993.1	Hs.423573	discoidin domain receptor family, member 1
780	AK130776, AK130776, BC008716, BC013400, L11315, L20817, L57508, NM_001954, NM_013993, NM_013994, U48705, X74979, X98208, X99031, Z29093	NM_001954.3, NM_001954.3, NM_013993.1	Hs.423573	discoidin domain receptor family, member 1
780	AK130776, AK130776, BC008716, BC013400, L11315, L20817, L57508, NM_001954, NM_013993, NM_013994, U48705, X74979, X98208, X99031, Z29093	NM_001954.3, NM_001954.3, NM_013993.1	Hs.423573	discoidin domain receptor family, member 1
780	AK130776, AK130776, BC008716, BC013400, L11315, L20817, L57508, NM_001954, NM_013993, NM_013994, U48705, X74979, X98208, X99031, Z29093	NM_001954.3, NM_001954.3, NM_013993.1	Hs.423573	discoidin domain receptor family, member 1
11072	AF038844, AF038844, AF120032, AK027210, BC000370, BC001894, BC004448, NM_007026	NM_007026.1	Hs.91448	dual specificity phosphatase 14
9538	AF010313, AF010313, BC002390, BC029333, NM_004879	NM_004879.2	Hs.343911	etoposide induced 2.4 mRNA

LOCUS-LINK	GENBANK	REFSEQ	UNIGENE	GENENAME
9156	AC004783, AC004783, AF042282, AF060479, AF084974, AF091740, AF091742, AF091754, AL080139, BC007491, BM465399, CD644038, NM_003686, NM_006027	NM_003686.3, NM_003686.3, NM_006027.3	Hs.47504	exonuclease 1
81691	AC004381, AC004381, AF332193, AK057254, AL136763, AL162035, BC007646	NM_030941.1	Hs.177926	exonuclease NEF-sp
81691	AC004381, AC004381, AF332193, AK057254, AL136763, AL162035, BC007646	NM_030941.1	Hs.177926	exonuclease NEF-sp
2678	AC000051, AC000051, AJ006806, AJ006854, AJ007378, AJ007379, AJ007380, AJ007493, AJ230125, AL832738, BC025927, BC035341, J04131, J05235, L20490, L20493, M24087, M24903, NM_005265, NM_013421, NM_013430, X60069	NM_005265.1, NM_005265.1, NM_013421.1	Hs.352119	gamma-glutamyltransferase 1
2678	AC000051, AC000051, AJ006806, AJ006854, AJ007378, AJ007379, AJ007380, AJ007493, AJ230125, AL832738, BC025927, BC035341, J04131, J05235, L20490, L20493, M24087, M24903, NM_005265, NM_013421, NM_013430, X60069	NM_005265.1, NM_005265.1, NM_013421.1	Hs.352119	gamma-glutamyltransferase 1
2678	AC000051, AC000051, AJ006806, AJ006854, AJ007378, AJ007379, AJ007380, AJ007493, AJ230125, AL832738, BC025927, BC035341, J04131, J05235, L20490, L20493, M24087, M24903, NM_005265, NM_013421, NM_013430, X60069	NM_005265.1, NM_005265.1, NM_013421.1	Hs.352119	gamma-glutamyltransferase 1
2678	AC000051, AC000051, AJ006806, AJ006854, AJ007378, AJ007379, AJ007380, AJ007493, AJ230125, AL832738, BC025927, BC035341, J04131, J05235, L20490, L20493, M24087, M24903, NM_005265, NM_013421, NM_013430, X60069	NM_005265.1, NM_005265.1, NM_013421.1	Hs.352119	gamma-glutamyltransferase 1
92086	AL133466, AL133466, BC040904, L20491, L20492, NM_080920, NM_178311, NM_178312	NM_080920.2, NM_080920.2, NM_178311.1	Hs.355394	gamma-glutamyltransferase-like activity 4
10243	AB037806, AB037806, AF272663, AJ272033, AJ272343, AK025169, BC030016, NM_020806	NM_020806.3	Hs.13405	gephyrin
2629	AF023268, AF023268, BC000349, BC003356, BC030240, BX648487, D13286, D13287, J03059, K02920, M16328, M19285, NM_000157	NM_000157.1	Hs.282997	glucosidase, beta; acid (includes glucosylceramidase)
2629	AF023268, AF023268, BC000349, BC003356, BC030240, BX648487, D13286, D13287, J03059, K02920, M16328, M19285, NM_000157	NM_000157.1	Hs.511984	glucosidase, beta; acid (includes glucosylceramidase)

LOCUS-LINK	GENBANK	REFSEQ	UNIGENE	GENENAME
2937	AL133324, AL133324, BC007927, NM_000178, U34683	NM_000178.2	Hs.82327	glutathione synthetase
1647	BC011757, BC011757, L24498, M60974, NM_001924	NM_001924.2	Hs.80409	growth arrest and DNA-damage-inducible, alpha
2593	AC005329, AC005329, AF010246, AF010247, AF010248, AF086508, AF188893, BC016760, BC017936, BI914772, NM_000156, NM_138924, Z49878	NM_000156.4, NM_000156.4	Hs.81131	guanidinoacetate N-methyltransferase
10973	AJ223948, AJ223948, AL834463, AY013288, BC039857	NM_006828.1	Hs.143917	helicase, ATP binding 1
55055	AK000898, AK000898, AK023175, AK027468, BC036900, BX640701	NM_017975.2	Hs.21331	hypothetical protein FLJ10036
55215	AB058697, AB058697, AK001581, AK027564, AK055176, BC004277, BC021859, NM_018193	NM_018193.1, NM_018193.1	Hs.334828	hypothetical protein FLJ10719
64782	AF327352, AF327352, AK022546, AK022624, BC005164, BC014407, BC020988	NM_022767.2	Hs.436102	hypothetical protein FLJ12484
80152	AK023173, AK023173, AK055237, AK056097, BC007642, BC007864, BC015202, BC042204, BX648617	NM_025082.1	Hs.288382	hypothetical protein FLJ13111
80178	AK023971, AK023971, AK093788, AK128408, BC008882, BC018719	NM_025108.1	Hs.288672	hypothetical protein FLJ13909
54884	AK000303, AK000303, AK075261, AL833237, AY358568, BC011418	NM_017750.2	Hs.440401	hypothetical protein FLJ20296
54923	AK000413, AK000413, AK054722, BC017016	NM_017806.1	Hs.149227	hypothetical protein FLJ20406
79891	AK027159, AK027159, AK091421, BC025728	NM_024833.1	Hs.180402	hypothetical protein FLJ23506
127544	AK074486, AK074486, BC020595, BC062374	NM_153341.1	Hs.511807	hypothetical protein FLJ90005
127544	AK074486, AK074486, BC020595, BC062374	NM_153341.1	Hs.511807	hypothetical protein FLJ90005
51499	AF161481, AF161481, BC002638, BC055313, CB141335, NM_016399, U75688	NM_016399.2	Hs.69499	hypothetical protein HSPC132
51257	AF151074, AF151074, AK130163, BC015910, BC032624	NM_016496.3	Hs.331308	hypothetical protein LOC51257
60492	AF182412, AF182412, AF182424, AF271782, AK055972, AL136791, BC014573, BC017771, BC020783, BC032701, BC048795	NM_021825.3	Hs.368866	hypothetical protein MDS025
84263	AK090940, AK090940, AL833735, AY093428, BC004331, BC036620, BC047074	NM_032303.2	Hs.388160	hypothetical protein MGC10940
84263	AK090940, AK090940, AL833735, AY093428, BC004331, BC036620, BC047074	NM_032303.2	Hs.388160	hypothetical protein MGC10940

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93129	BC006126, BC006126, BC015555, BC016150, BC022786	NM_152288.1	Hs.333488	hypothetical protein MGC13024
84296	BC005995, BC005995, BC027454	NM_032336.1	Hs.333166	hypothetical protein MGC14799
79154	AK026196, AK026196, AY358712, BC002731	NM_024308.2	Hs.435826	hypothetical protein MGC4172
3399	BC003107, BC003107, D28449, NM_002167, X66924, X69111, X73428	NM_002167.2	Hs.76884	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein
10437	AB049659, AB049659, AC007192, AF401212, BC021136, BC031020, BE515053, NM_006332	NM_006332.3	Hs.14623	interferon, gamma-inducible protein 30
3430	BC001356, BC001356, NM_005533, U72882	NM_005533.2	Hs.50842	interferon-induced protein 35
3594	AJ297688, AJ297688, AJ297689, AJ297690, AJ297691, AJ297692, AJ297693, AJ297694, AJ297695, AJ297696, AJ297697, AJ297698, AJ297699, AJ297700, AJ297701, BC029121, BX647221, NM_005535, NM_153701, U03187	NM_005535.1, NM_005535.1	Hs.223894	interleukin 12 receptor, beta 1
10300	AF052432, AF052432, BC001353, BC014141, BT007022	NM_005886.1	Hs.275675	katanin p80 (WD repeat containing) subunit B 1
3795	AK130033, AK130033, BC006233, BX648873, NM_000221, NM_006488, X78677, X78678, Y09336, Y09340, Y09341	NM_000221.1, NM_000221.1	Hs.412228	ketohexokinase (fructokinase)
4000	AF381029, AF381029, AK026584, AK056143, AK056191, AK057997, AK097801, AK098128, AK122732, AK130179, AY357727, BC000511, BC003162, BC014507, BC018863, BC033088, L12399, M13451, M13452, NM_005572, NM_170707, NM_170708, X03444, X03445	NM_005572.2, NM_005572.2, NM_170707.1	Hs.436441	lamin A/C
3965	AB005894, AB005894, AB006782, AK097892, AK126017, NM_002308, NM_009587, Z49107	NM_002308.2, NM_002308.2	Hs.81337	lectin, galactoside-binding, soluble, 9 (galectin 9)
55367	AF229178, AF229178, AF274972, AK074893, AL833849, BC014904, NM_018494, NM_145886	NM_018494.2, NM_018494.2, NM_145886.1	Hs.438986	leucine-rich and death domain containing
55367	AF229178, AF229178, AF274972, AK074893, AL833849, BC014904, NM_018494, NM_145886	NM_018494.2, NM_018494.2, NM_145886.1	Hs.438986	leucine-rich and death domain containing
3978	M36067, M36067, NM_000234	NM_000234.1	Hs.1770	ligase I, DNA, ATP-dependent
4066	AC005546, AC005546, BC002796, M22637, M22638, NM_005583	NM_005583.3	Hs.46446	lymphoblastic leukemia derived sequence 1
3140	AF010446, AF010446, AF010447, AF031469, AF073485, AJ249778, BC012485, NM_001531	NM_001531.1	Hs.101840	major histocompatibility complex, class I-related

LOCUS-LINK	GENBANK	REFSEQ	UNIGENE	GENENAME
3140	AF010446, AF010446, AF010447, AF031469, AF073485, AJ249778, BC012485, NM_001531	NM_001531.1	Hs.101840	major histocompatibility complex, class I-related
3140	AF010446, AF010446, AF010447, AF031469, AF073485, AJ249778, BC012485, NM_001531	NM_001531.1	Hs.101840	major histocompatibility complex, class I-related
3140	AF010446, AF010446, AF010447, AF031469, AF073485, AJ249778, BC012485, NM_001531	NM_001531.1	Hs.101840	major histocompatibility complex, class I-related
10916	AF126181, AF126181, AF128527, AF128528, AF148815, AF320907, AJ293618, AK091003, AK092463, AK098645, BC000304, BM043994, BM803170, BQ423605, BX647995, NM_006787, NM_014599, NM_177433, NM_201222, U92544, Z98046	NM_006787, NM_006787, NM_014599.4, NM_177433.1	Hs.376719	melanoma antigen, family D, 2
9088	AC004233, AC004233, AC004235, AF549406, AK097642, AK098452, BG530406, BQ017689, NM_004203, NM_182687	NM_004203.3, NM_004203.3	Hs.77783	membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase
4580	AF023268, AF023268, BC001906, BC035616, BE394487, BG717732, BQ003402, BU552401, CF529296, NM_002455, NM_198883, U46920	NM_002455.2, NM_002455.2	Hs.247551	metaxin 1
7786	AK094195, AK094195, BC037585, BC050050, NM_006301, U07358	NM_006301.2	Hs.211601	mitogen-activated protein kinase kinase kinase 12
51754	AF070572, AF070572, AF188239, AK074844, BC041377, BC043384	NM_016446.2	Hs.440953	nasopharyngeal carcinoma related protein
4900	BC002835, BC002835, NM_006176, U89165, X99075, X99076, Y09689, Y15059	NM_006176.1	Hs.232004	neurogranin (protein kinase C substrate, RC3)
4687	AF330627, AF330627, AK127905, BC002816, BC065731, M25665, M55067, NM_000265, U25793, U57835	NM_000265.1	Hs.1583	neutrophil cytosolic factor 1 (47kDa, chronic granulomatous disease, autosomal 1)
4687	AF330627, AF330627, AK127905, BC002816, BC065731, M25665, M55067, NM_000265, U25793, U57835	NM_000265.1	Hs.458275	neutrophil cytosolic factor 1 (47kDa, chronic granulomatous disease, autosomal 1)
4863	BC040356, BC040356, BC050561, D83243, NM_002519, U58852	NM_002519.1	Hs.89385	nuclear protein, ataxia-telangiectasia locus
23225	AB020713, AB020713, AK026042, AK074101, AK075545, AL117527, BC020573	NM_024923.2	Hs.292119	nucleoporin 210

LOCUS-LINK	GENBANK	REFSEQ	UNIGENE	GENENAME
4521	AB025233, AB025233, AB025234, AB025235, AB025236, AB025237, AB025238, AB025239, AB025240, AB025241, AB025242, AK026631, BC014618, BC022818, BC040144, BC051375, BC065367, D16581, D38591, D38592, D38593, D38594, NM_002452, NM_198948, NM_198949, NM_198950, NM_198952, NM_198953, NM_198954	NM_002452.3, NM_002452.3, NM_198948.1, NM_198949.1, NM_198950.1, NM_198952.1, NM_198953.1	Hs.413078	nudix (nucleoside diphosphate linked moiety X)-type motif 1
55270	AK001818, AK001818, BC064607	NM_018283.1	Hs.144407	nudix (nucleoside diphosphate linked moiety X)-type motif 15
64393	AF355465, AF355465, AK022358, AK122768, AY037945, BC002896, NM_022470	NM_022470.2, NM_022470.2	Hs.386299	p53 target zinc finger protein
23113	AB014608, AB014608, AJ318215, AY145132, BC002879, BC017747, BC028159	NM_015089.1	Hs.412832	p53-associated parkin-like cytoplasmic protein
56288	AB073671, AB073671, AF177228, AF196185, AF196186, AF252293, AF332592, AF332593, AF454057, AF454058, AF454059, AF467002, AF467003, AF467004, AF467005, AF467006, AK000761, AK025892, AK027735, BC011711, NM_019619	NM_019619.2	Hs.72249	par-3 partitioning defective 3 homolog (C. elegans)
23646	BC000553, BC000553, BC036327, NM_012268	NM_012268.1	Hs.257008	phospholipase D3
51316	AF208846, AF208846, AJ422147, AK000140, BC012205	NM_016619.1	Hs.371003	placenta-specific 8
23612	AF151100, AF151100, AK075179, BC014390	NM_012396.1	Hs.268557	pleckstrin homology-like domain, family A, member 3
23654	AB002313, AB002313, AK025415, AK025701, AK056543, AK074932, AK123131, AK126394, AL022328, BC004542, BT006887, S76730	XM_371474.1	Hs.278311	plexin B2
23654	AB002313, AB002313, AK025415, AK025701, AK056543, AK074932, AK123131, AK126394, AL022328, BC004542, BT006887, S76730	XM_371474.1	Hs.3989	plexin B2
1263	AA421212, AA421212, AJ293866, BC004135, BC004198, BC013899, BC013960, NM_004073, U56998	NM_004073.2	Hs.153640	polo-like kinase 3 (Drosophila)
57060	AF092441, AF092441, AF141340, AF176330, AF257770, AF257771, AF257772, AK001244, AK023993, BC003008, BC004153, BC017098, BX647811, NM_020418, NM_033008, NM_033009, NM_033010	NM_020418.2, NM_020418.2, NM_033008.1, NM_033009.1	Hs.20930	poly(rC) binding protein 4
5424	BC008800, BC008800, M80397,	NM_002691.1	Hs.279413	polymerase (DNA

LOCUS-LINK	GENBANK	REFSEQ	UNIGENE	GENENAME
	M81735, NM_002691			directed), delta 1, catalytic subunit 125kDa
10714	BC020587, BC020587, BC032636, BC041703, D26018, NM_006591, XM_166243	NM_006591.1, NM_006591.1	Hs.82502	polymerase (DNA-directed), delta 3, accessory subunit
29802	AF163825, AF163825, AP000348, BC020666, NM_013378	NM_013378.1	Hs.136713	pre-B lymphocyte gene 3
92335	AF308302, AF308302, AK074771, AK075005, AL832407, AY290821, BC043641, BK001542	NM_153335.3	Hs.279731	protein kinase LYK5
5564	AF022116, AF022116, AJ224515, AK127820, BC001007, BC001056, BC001823, BC017671, BC018818, BU539177, BX537486, NM_006253, U83994, U87276, Y12556	NM_006253.4	Hs.6061	protein kinase, AMP-activated, beta 1 non-catalytic subunit
5564	AF022116, AF022116, AJ224515, AK127820, BC001007, BC001056, BC001823, BC017671, BC018818, BU539177, BX537486, NM_006253, U83994, U87276, Y12556	NM_006253.4	Hs.6061	protein kinase, AMP-activated, beta 1 non-catalytic subunit
5613	BC041073, BC041073, NM_005044, X85545	NM_005044.1	Hs.147996	protein kinase, X-linked
8493	AA326266, AA326266, AU280469, BC016480, BC032826, BC033893, BC042418, BC060877, BT009780, NM_003620, U78305	NM_003620.2	Hs.286073	protein phosphatase 1D magnesium-dependent, delta isoform
29901	BC007448, BC007448, NM_013299	NM_013299.1	Hs.23642	protein predicted by clone 23627
7803	AF051160, AF051160, AJ420505, BC023975, BC040303, BI222469, NM_003463, U48296	NM_003463.2	Hs.227777	protein tyrosine phosphatase type IVA, member 1
26191	AF001846, AF001846, AF077031, AF150732, AL137856, BC017785, NM_012411, NM_015967, U69700	NM_012411.2, NM_012411.2	Hs.87860	protein tyrosine phosphatase, non-receptor type 22 (lymphoid)
26191	AF001846, AF001846, AF077031, AF150732, AL137856, BC017785, NM_012411, NM_015967, U69700	NM_012411.2, NM_012411.2	Hs.87860	protein tyrosine phosphatase, non-receptor type 22 (lymphoid)
5900	AB037729, AB037729, AF295773, AK000242, AK056462, AK074114, AK090450, AK127524, BC021581, BC033198, BC059362, NM_006266, U14417	NM_006266.1	Hs.106185	ral guanine nucleotide dissociation stimulator
5900	AB037729, AB037729, AF295773, AK000242, AK056462, AK074114, AK090450, AK127524, BC021581, BC033198, BC059362, NM_006266, U14417	NM_006266.1	Hs.106185	ral guanine nucleotide dissociation stimulator
5920	AF060228, AF060228, AF092922, NM_004585	NM_004585.2	Hs.17466	retinoic acid receptor responder (tazarotene induced) 3
6240	AF107045, AF107045, AK122695, BC006498, L10342, NM_001033,	NM_001033.2	Hs.383396	ribonucleotide reductase M1 polypeptide

LOCUS-LINK	GENBANK	REFSEQ	UNIGENE	GENENAME
	X59543, X59617, X65708			
6241	AK092671, AK092671, AK123010, AY032750, BC001886, BC028932, BC030154, NM_001034, X59618	NM_001034.1	Hs.226390	ribonucleotide reductase M2 polypeptide
51065	AF070668, AF070668, AK024591, BC003667, BC031307, BC047648	NM_015920.2	Hs.108957	ribosomal protein S27-like
6195	AK092955, AK092955, BC014966, BC039069, L07597, NM_002953	NM_002953.2	Hs.149957	ribosomal protein S6 kinase, 90kDa, polypeptide 1
9252	AF074393, AF074393, AF080000, AF090421, AL050099, BC017187, BF593074, BG699153, NM_004755, NM_182398	NM_004755.2, NM_004755.2	Hs.109058	ribosomal protein S6 kinase, 90kDa, polypeptide 5
6678	AK096969, AK096969, BC004974, BC008011, J03040, NM_003118, Y00755	NM_003118.1	Hs.111779	secreted protein, acidic, cysteine-rich (osteonectin)
27244	AF033120, AF033120, AF033121, AF033122, AK001886, NM_014454	NM_014454.1	Hs.14125	sestrin 1
6774	AF029311, AF029311, AF332508, AJ012463, BC000627, BC014482, BC029783, BI461226, L29277, NM_003150, NM_139276	NM_003150.2, NM_003150.2	Hs.421342	signal transducer and activator of transcription 3 (acute-phase response factor)
6494	AB005666, AB005666, AF029789, AF052232, AF052233, AF052237, AF052238, BC010492, BM677738, NM_006747, NM_153253	NM_006747.2, NM_006747.2	Hs.7019	signal-induced proliferation-associated gene 1
23410	AF083108, AF083108, AL137276, BC001042, NM_012239, U73637	NM_012239.3	Hs.511950	sirtuin (silent mating type information regulation 2 homolog) 3 (S. cerevisiae)
6518	BC001692, BC001692, BC001820, BC035878, M55531, NM_003039, U05344, U11843	NM_003039.1	Hs.33084	solute carrier family 2 (facilitated glucose/fructose transporter), member 5
55508	AF148713, AF148713, AY358943, BC008412, BC030504	NM_018656.1	Hs.445043	solute carrier family 35, member E2
6303	AL050290, AL050290, BC002503, BC008424, M55580, M77693, NM_002970, U40369, Z14136	NM_002970.1	Hs.28491	spermidine/spermine N1-acetyltransferase
2040	AL040491, AL040491, AU137947, BC010703, BI763647, BM451470, BM925356, CA447945, M81635, NM_004099, NM_198194, X60067, X85116	NM_004099.4, NM_004099.4	Hs.439776	stomatin
6382	AJ551176, AJ551176, BC008765, J05392, NM_002997, X60306, Z48199	NM_002997.3	Hs.82109	syndecan 1
6382	AJ551176, AJ551176, BC008765, J05392, NM_002997, X60306, Z48199	NM_002997.3	Hs.82109	syndecan 1
10628	BX537824, BX537824, NM_006472	NM_006472.1	Hs.179526	thioredoxin interacting protein

LOCUS-LINK	GENBANK	REFSEQ	UNIGENE	GENENAME
10628	BX537824, BX537824, NM_006472	NM_006472.1	Hs.179526	thioredoxin interacting protein
10628	BX537824, BX537824, NM_006472	NM_006472.1	Hs.179526	thioredoxin interacting protein
7083	BC006484, BC006484, BC007872, BC007986, K02581, M15205, NM_003258	NM_003258.1	Hs.164457	thymidine kinase 1, soluble
7205	AF000974, AF000974, AF025437, AF312032, AJ001902, AK056773, BC002680, BC004249, BC004999, BC021540, BC028985, L40374, NM_003302	NM_003302.1	Hs.380230	thyroid hormone receptor interactor 6
7153	AF064590, AF064590, AF069522, AF071738, AF071739, AF071740, AF071741, AF071742, AF071743, AF071744, AF071745, AF071746, AF071747, AF285157, AF285158, AF285159, AJ011741, AK024080, BC013429, J04088, NM_001067	NM_001067.2	Hs.156346	topoisomerase (DNA) II alpha 170kDa
6919	AK027824, AK027824, AW474513, BC014211, BC018896, BC031877, BC050623, BC050624, BC056407, BI668232, BI756937, CB961240, D50495, NM_003195, NM_198723	NM_003195.4, NM_003195.4	Hs.224397	transcription elongation factor A (SII), 2
6924	BC002883, BC002883, BC019949, BC020448, L47345	NM_003198.1	Hs.15535	transcription elongation factor B (SIII), polypeptide 3 (110kDa, elongin A)
7040	BC000125, BC000125, BC001180, BC022242, BT007245, M38449, NM_000660, X02812, X05839	NM_000660.1	Hs.1103	transforming growth factor, beta 1 (Camurati-Engelmann disease)
7108	AF023676, AF023676, AF048704, AF096304, BC009052, BC012857, BC038353, NM_003273	NM_003273.1	Hs.31130	transmembrane 7 superfamily member 2
51768	AB032470, AB032470, AK002031, AK023085, BC005176, NM_016551	NM_016551.1	Hs.10071	transmembrane 7 superfamily member 3
10346	AL360134, AL360134, AL360187, AL360190, BC022281, BC035582, NM_006074	NM_006074.2	Hs.318501	tripartite motif-containing 22
8743	AF178756, AF178756, BC009795, BC020220, BC032722, NM_003810, U37518, U57059	NM_003810.2	Hs.387871	tumor necrosis factor (ligand) superfamily, member 10
7133	AB030949, AB030949, AB030950, AB030951, AB030952, AY148473, BC011844, BC042167, BC052977, M32315, M35857, M55994, NM_001066, S63368, U52165	NM_001066.2	Hs.256278	tumor necrosis factor receptor superfamily, member 1B
9924	AB014610, AB014610, AB107585, AK001232, BC024043, BX648106	NM_014871.2	Hs.273397	ubiquitin specific protease 52
3265	AF493916, AF493916, AJ437024, BC006499, J00277, NM_176795	NM_176795.1	Hs.37003	v-Ha-ras Harvey rat sarcoma viral oncogene homolog

LOCUS-LINK	GENBANK	REFSEQ	UNIGENE	GENENAME
7508	BC016620, BC016620, D21089, NM_004628, X65024	NM_004628.2	Hs.320	xeroderma pigmentosum, complementation group C
7748	AF003540, AF003540, AK095720, AL833722, NM_007152	NM_007152.1	Hs.104382	zinc finger protein 195
10793	AK090648, AK090648, AL832810, BC063818, NM_021148, X78932	NM_021148.1	Hs.386264	zinc finger protein 273
25799	AF060503, AF060503, AK023989, AK092341, BC007717, NM_014347	NM_014347.1	Hs.296365	zinc finger protein 324
25799	AF060503, AF060503, AK023989, AK092341, BC007717, NM_014347	NM_014347.1	Hs.515660	zinc finger protein 324
7633	AK054606, AK054606, BC062309, NM_007135, X65232	NM_007135.1	Hs.512719	zinc finger protein 79 (pT7)
7633	AK054606, AK054606, BC062309, NM_007135, X65232	NM_007135.1	Hs.512719	zinc finger protein 79 (pT7)
29066	AF161540, AF161540, AK000325, AK001869, AK026827, AK026956, AK091803, AY163807, BC012575, BC036857, BC046363	NM_014153.2	Hs.371856	zinc-finger protein AY163807

[0150] The selected genes may be categorized e.g. by using the GeneOntology tool (http://www.geneontology.org), as providing a wide range of biological functions: regulation of transcription, cell death, cell growth and proliferation, cell cycle related, enzymes, polymerase and proteases, immune system related protein, signal transduction, transporters, cell adhesion, development related, and many unknowns (see Table 4).

[0151] The selected genes may be categorized as providing a wide range of biological functions: regulation of transcription, cell death, cell growth and proliferation, cell cycle related, enzymes, polymerase and proteases, immune system related protein, signal transduction, transporters, cell adhesion, development related, and many unknowns (see Table 4).

Table 4: Categories of genes among the 215 candidate predictor genes

CATEGORY OF GENE	NUMBER FOUND
regulation of transcription	19
transcription, DNA-dependent	18
immune response	17
DNA repair	13
mitotic cell cycle	12
regulation of cell cycle	12
Apoptosis	11
DNA replication and chromosome cycle	10
negative regulation of cell proliferation	7

CATEGORY OF GENE	NUMBER FOUND
Phosphorylation	7
protein amino acid phosphorylation	7
regulation of apoptosis	7
DNA recombination	6
amino acid metabolism	6
enzyme linked receptor protein signaling pathway	6
positive regulation of programmed cell death	6
coenzyme biosynthesis	5
Dephosphorylation	5
glutathione biosynthesis	5
glutathione metabolism	5
protein amino acid dephosphorylation	5
M phase	4
humoral immune response	4
positive regulation of cell proliferation	4
protein catabolism	4
proteolysis and peptidolysis	4
antimicrobial humoral response	3
cellular defense response	3
humoral defense mechanism (sensu Vertebrata)	3
organelle organization and biogenesis	3
protein biosynthesis	3
protein kinase cascade	3
Unclassified	125

[0152] The results of principal component analysis (PCA) of these 215 genes are displayed in Figures 2 and 3. Figure 2 gives numerical values for cell count next to each point. Figure 3 gives numerical values for Alamar Blue next to each point. In Figures 2 and 3, points from NaCl and some points from tPt are in the upper left quadrant; points from Rif are in the lower left quadrant. Most points from MMS are in the lower right quadrant; half the points from MMC are in the upper right quadrant and half are in the lower right quadrant. One third of the points for cPt are in the upper right quadrant and two-thirds are in the lower right quadrant. The remaining points for tPt are close to t[1] = 0. From these results it is seen that a clear distinction between cytotoxic and genotoxic compounds is discernible; this is however expected due to the filtering used to select the genes. Rifampicin and NaCl treated samples form homogenous clusters which are clearly separated from the rest of the samples.

Example3. Identification of highly predictive genes using PLS-DA

[0153] Partial least squares discriminant analysis (PLS-DA) was applied to the set of 215 candidate genes identified in Example 2. This analysis provides the discriminant function that best separates the cytotoxic and the genotoxic compounds. The score plot of the first component t[1] based on these 215 genes is displayed in Figure 4 which shows a good separation between the two classes of compounds, with each sample for NaCl, Rif, and tPt above or at t[1] = 0, and all samples for cPt, MMC, and MMS below t[1] =0. However, two samples of the cis-Platinum group are located quite closely to the trans-Platinum samples. The investigation of the differential gene pattern by PLS-DA revealed 23 genes that contribute most strongly to the distinction between the cytotoxic and genotoxic samples. These genes are compiled in Tables 5A and 5B together with their means, coefficient of variation, fold-change and p-value of students t-test.

Table 5A. 23 predictor genes resulting from PLS-DA

LOCUS-LINK	REFSEQ	Mean Cyto	CV (%) Cyto	Mean Geno	CV(%) Geno	Ratio Geno:Cyto	t-test p-value
79733	NM_024680.2	111	42	282	31	2.5	7.87E-08
11147	NM_007071.1	269	39	514	17	1.9	1.01E-08
23354	XM_049237.6	120	24	198	15	1.7	2.68E-09
123803	NM_173474.2	178	13	259	16	1.5	5.42E-08
9779		394	39	665	20	1.7	2.24E-06
11257	NM_007233.1	115	24	164	19	1.4	1.92E-05
30851	NM_014604.1	150	32	336	25	2.2	1.09E-08
30851	NM_014604.1	144	24	232	19	1.6	1.93E-07
59	NM_001613.1	281	21	503	21	1.8	2.71E-08
780	NM_001954.3, NM_001954.3, NM_013993.1	137	36	229	24	1.7	6.97E-06
84263	NM_032303.2	289	27	494	15	1.7	1.96E-09
93129	NM_152288.1	120	22	191	13	1.6	1.06E-09
3795	NM_000221.1, NM_000221.1	53	33	74	17	1.4	2.12E-04
55367	NM_018494.2, NM_018494.2, NM_145886.1	228	22	380	19	1.7	4.54E-08
8493	NM_003620.2	54	30	94	19	1.7	6.59E-08
29901	NM_013299.1	38	34	57	16	1.5	8.34E-06
26191	NM_012411.2, NM_012411.2	68	36	109	14	1.6	1.50E-06
9252	NM_004755.2, NM_004755.2	136	23	254	15	1.9	2.09E-11

LOCUS-LINK	REFSEQ	Mean Cyto	CV (%) Cyto	Mean Geno	CV(%) Geno	Ratio Geno:Cyto	t-test p-value
23410	NM_012239.3	215	21	307	16	1.4	1.36E-06
6382	NM_002997.3	116	13	217	29	1.9	2.46E-06
7040	NM_000660.1	65	23	93	19	1.4	1.63E-05
7133	NM_001066.2	42	28	69	17	1.6	7.09E-08
29066	NM_014153.2	324	20	554	16	1.7	6.90E-10

Table 5B. 23 predictor genes resulting from PLS-DA

LOCUS-LINK	GENBANK	REFSEQ	UNIGENE	GENENAME
79733	AK026964, AK026964, AK055206, BC028244, BU164108, BX504614, CB959621	NM_024680.2	Hs.94292	FLJ23311 protein
11147	AF126163, AF126163, AF126164, BC010922	NM_007071.1	Hs.142245	HERV-H LTR-associating 3
23354	AB020648, AB020648, BC013947	XM_049237.6	Hs.7426	KIAA0841
123803	AF092440, AF092440, BC017336	NM_173474.2	Hs.351573	N-terminal asparagine amidase
9779	AK097990, AK097990, BC013145, D86965		Hs.115740	TBC1 domain family, member 5
11257	AB007455, AB007455, AB007456, AB007457, BC002709	NM_007233.1	Hs.274329	TP53 activated protein 1
30851	AF028823, AF028823, AF168787, AF234997, AF277318, AK001327, BC023980, NM_014604	NM_014604.1	Hs.12956	Tax interaction protein 1
30851	AF028823, AF028823, AF168787, AF234997, AF277318, AK001327, BC023980, NM_014604	NM_014604.1	Hs.12956	Tax interaction protein 1
59	BC017554, BC017554, D00618, J05192, K01741, K01742, K01743, K01744, K01745, K01746, K01747, M33216, NM_001613, X13839	NM_001613.1	Hs.208641	actin, alpha 2, smooth muscle, aorta
780	AK130776, AK130776, BC008716, BC013400, L11315, L20817, L57508, NM_001954, NM_013993, NM_013994, U48705, X74979, X98208, X99031, Z29093	NM_001954.3, NM_001954.3, NM_013993.1	Hs.423573	discoidin domain receptor family, member 1
84263	AK090940, AK090940, AL833735, AY093428, BC004331, BC036620, BC047074	NM_032303.2	Hs.388160	hypothetical protein MGC10940
93129	BC006126, BC006126, BC015555, BC016150, BC022786	NM_152288.1	Hs.333488	hypothetical protein MGC13024
3795	AK130033, AK130033, BC006233, BX648873, NM_000221, NM_006488, X78677, X78678, Y09336, Y09340, Y09341	NM_000221.1, NM_000221.1	Hs.412228	ketohexokinase (fructokinase)
55367	AF229178, AF229178, AF274972, AK074893, AL833849, BC014904, NM_018494, NM_145886	NM_018494.2, NM_018494.2, NM_145886.1	Hs.438986	leucine-rich and death domain containing

LOCUS-LINK	GENBANK	REFSEQ	UNIGENE	GENENAME
8493	AA326266, AA326266, AU280469, BC016480, BC032826, BC033893, BC042418, BC060877, BT009780, NM_003620, U78305	NM_003620.2	Hs.286073	protein phosphatase 1D magnesium-dependent, delta isoform
29901	BC007448, BC007448, NM_013299	NM_013299.1	Hs.23642	protein predicted by clone 23627
26191	AF001846, AF001846, AF077031, AF150732, AL137856, BC017785, NM_012411, NM_015967, U69700	NM_012411.2, NM_012411.2	Hs.87860	protein tyrosine phosphatase, non-receptor type 22 (lymphoid)
9252	AF074393, AF074393, AF080000, AF090421, AL050099, BC017187, BF593074, BG699153, NM_004755, NM_182398	NM_004755.2, NM_004755.2	Hs.109058	ribosomal protein S6 kinase, 90kDa, polypeptide 5
23410	AF083108, AF083108, AL137276, BC001042, NM_012239, U73637	NM_012239.3	Hs.511950	sirtuin (silent mating type information regulation 2 homolog) 3 (S. cerevisiae)
6382	AJ551176, AJ551176, BC008765, J05392, NM_002997, X60306, Z48199	NM_002997.3	Hs.82109	syndecan 1
7040	BC000125, BC000125, BC001180, BC022242, BT007245, M38449, NM_000660, X02812, X05839	NM_000660.1	Hs.1103	transforming growth factor, beta 1 (Camurati-Engelmann disease)
7133	AB030949, AB030949, AB030950, AB030951, AB030952, AY148473, BC011844, BC042167, BC052977, M32315, M35857, M55994, NM_001066, S63368, U52165	NM_001066.2	Hs.256278	tumor necrosis factor receptor superfamily, member 1B
29066	AF161540, AF161540, AK000325, AK001869, AK026827, AK026956, AK091803, AY163807, BC012575, BC036857, BC046363	NM_014153.2	Hs.371856	zinc-finger protein AY163807

[0154] The score plots of the PLS-DA model including these 23 genes is shown in Figure 5. Separation of the two classes is comparable to that of the model with 215 genes (Figure 4). The similarity between Figures 4 and 5 suggests strongly that the 23 genes identified include genes that are most responsible for the discriminant function between cytotoxicity and genotoxicity.

[0155] Figure 6 shows a cluster diagram using the results from these 23 genes. It is seen at the top that two major clusters are clearly delineated; indeed these clusters separate the samples into the expected cytotoxic and genotoxic classes (see the captions on the lowest line in Figure 6).

Example 4. Identification of highly predictive genes using k-Nearest Neighbor Analysis

[0156] The 215 candidate genes identified by the filter screen (Example 2) were analyzed by the GeneSpring predictor tool based on k nearest neighbor analysis (KNN). Due to the different normalization of the data between SIMCA-P and GeneSpring it was expected that the predictor genes identified by KNN might differ from those found by PLS-DA

[0157] The list of 26 of the 27 genes that carry the highest predictive strength as determined by KNN are listed in Table 6. The 27th gene (probe set) on the GeneChip has no identifying information associated with it. These genes were able to classify all samples correctly according to their genotoxicity or cytotoxicity, respectively.

Table 6. 26 predictor genes resulting from GeneSpring KNN

LOCUS-LINK	GENBANK	REFSEQ	UNIGENE	GENENAME
79733	AK026964, AK026964, AK055206, BC028244, BU164108, BX504614, CB959621	NM_024680.2	Hs.94292	FLJ23311 protein
30851	AF028823, AF028823, AF168787, AF234997, AF277318, AK001327, BC023980, NM_014604	NM_014604.1	Hs.12956	Tax interaction protein 1
30851	AF028823, AF028823, AF168787, AF234997, AF277318, AK001327, BC023980, NM_014604	NM_014604.1	Hs.12956	Tax interaction protein 1
634	AC004785, AC004785, AL833584, BC014473, BC024164, D12502, D90311, D90312, D90313, J03858, M69176, M72238, M76742, NM_001712, S71326, X14831, X16354, X16356	NM_001712.2	Hs.512682	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)
634	AC004785, AC004785, AL833584, BC014473, BC024164, D12502, D90311, D90312, D90313, J03858, M69176, M72238, M76742, NM_001712, S71326, X14831, X16354, X16356	NM_001712.2	Hs.512682	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)
780	AK130776, AK130776, BC008716, BC013400, L11315, L20817, L57508, NM_001954, NM_013993, NM_013994, U48705, X74979, X98208, X99031, Z29093	NM_001954.3, NM_001954.3, NM_013993.1	Hs.423573	discoidin domain receptor family, member 1
780	AK130776, AK130776, BC008716, BC013400, L11315, L20817, L57508, NM_001954, NM_013993, NM_013994, U48705, X74979, X98208, X99031, Z29093	NM_001954.3, NM_001954.3, NM_013993.1	Hs.423573	discoidin domain receptor family, member 1
780	AK130776, AK130776, BC008716, BC013400, L11315, L20817, L57508, NM_001954, NM_013993, NM_013994, U48705, X74979, X98208, X99031, Z29093	NM_001954.3, NM_001954.3, NM_013993.1	Hs.423573	discoidin domain receptor family, member 1
9156	AC004783, AC004783, AF042282, AF060479,AF084974, AF091740, AF091742, AF091754, AL080139, BC007491, BM465399, CD644038, NM_003686, NM_006027	NM_003686.3, NM_003686.3, NM_006027.3	Hs.47504	exonuclease 1
55215	AB058697, AB058697, AK001581, AK027564, AK055176, BC004277, BC021859, NM_018193	NM_018193.1, NM_018193.1	Hs.334828	hypothetical protein FLJ10719
80152	AK023173, AK023173, AK055237, AK056097, BC007642, BC007864, BC015202, BC042204, BX648617	NM_025082.1	Hs.288382	hypothetical protein FLJ13111

LOCUS-LINK	GENBANK	REFSEQ	UNIGENE	GENENAME
54884	AK000303, AK000303, AK075261, AL833237, AY358568, BC011418	NM_017750.2	Hs.440401	hypothetical protein FLJ20296
127544	AK074486, AK074486, BC020595, BC062374	NM_153341.1	Hs.511807	hypothetical protein FLJ90005
93129	BC006126, BC006126, BC015555, BC016150, BC022786	NM_152288.1	Hs.333488	hypothetical protein MGC13024
3594	AJ297688, AJ297688, AJ297689, AJ297690, AJ297691, AJ297692, AJ297693, AJ297694, AJ297695, AJ297696, AJ297697, AJ297698, AJ297699, AJ297700, AJ297701, BC029121, BX647221, NM_005535, NM_153701, U03187	NM_005535.1, NM_005535.1	Hs.223894	interleukin 12 receptor, beta 1
55367	AF229178, AF229178, AF274972, AK074893, AL833849, BC014904, NM_018494, NM_145886	NM_018494.2, NM_018494.2, NM_145886.1	Hs.438986	leucine-rich and death domain containing
55367	AF229178, AF229178, AF274972, AK074893, AL833849, BC014904, NM_018494, NM_145886	NM_018494.2, NM_018494.2, NM_145886.1	Hs.438986	leucine-rich and death domain containing
3978	M36067, M36067, NM_000234	NM_000234.1	Hs.1770	ligase I, DNA, ATP-dependent
23612	AF151100, AF151100, AK075179, BC014390	NM_012396.1	Hs.268557	pleckstrin homology-like domain, family A, member 3
10714	BC020587, BC020587, BC032636, BC041703, D26018, NM_006591, XM_166243	NM_006591.1, NM_006591.1	Hs.82502	polymerase (DNA-directed), delta 3, accessory subunit
92335	AF308302, AF308302, AK074771, AK075005, AL832407, AY290821, BC043641, BK001542	NM_153335.3	Hs.279731	protein kinase LYK5
8493	AA326266, AA326266, AU280469, BC016480, BC032826, BC033893, BC042418, BC060877, BT009780, NM_003620, U78305	NM_003620.2	Hs.286073	protein phosphatase 1D magnesium-dependent, delta isoform
9252	AF074393, AF074393, AF080000, AF090421, AL050099, BC017187, BF593074, BG699153, NM_004755, NM_182398	NM_004755.2, NM_004755.2	Hs.109058	ribosomal protein S6 kinase, 90kDa, polypeptide 5
6382	AJ551176, AJ551176, BC008765, J05392, NM_002997, X60306, Z48199	NM_002997.3	Hs.82109	syndecan 1
10628	BX537824, BX537824, NM_006472	NM_006472.1	Hs.179526	thioredoxin interacting protein
7508	BC016620, BC016620, D21089, NM_004628, X65024	NM_004628.2	Hs.320	xeroderma pigmentosum, complementation group C

Example 5. Predictor Genes Independent of Method of Analysis

[0158] Six genes were found to be common to the predictor gene sets derived from both PLS-DA and KNN; these are identified in Table 7. Figure 6 includes six arrows on the left that identify the six genes in the cluster diagram originating from the PLS-DA analysis. In order to demonstrate the effectiveness of this reduced gene set, predictive models using PLS-DA and KNN analyses were built containing these six only. This reduced gene set was able to

discriminate between the two classes of toxicity without any misclassification. Figure 7 shows the results of condition clustering (GeneSpring) which shows that the samples are segregated into two principal classes (see dendrogram on the left), which are precisely the genotoxic and cytotoxic samples (Figure 7, right). This result demonstrates clearly the separability of the two classes of toxicity. The same result is confirmed by PLS-DA as shown in Figure 8. The separation of the two classes of samples is comparable to that found with all 215 genes (Figure 4) as well as with the 23 genes in Tables 5A and 5B (Figure 5).

Table 7. 6 genes common to PLS-DA and KNN predictor lists

AFFYMETRIX ID	LOCUS-LINK	REFSEQ	DESCRIPTION	CLASSIFICATION
221640_s_at	55367	NM_018494.2, NM_018494.2, NM_145886.1	Leucine-rich and death domain containing	Cell Death
204566_at	8493	NM_003620.2	Protein phosphatase 1D magnesium-dependent, delta isoform	Enzymes
215464_s_at	30851	NM_014604.1	Tax interaction protein 1	Structural Protein
201813_s_at			TBC1 domain family, member 5	Unknown function
219990_at	79733	NM_024680.2	hypothetical protein FLJ23311	Unknown function
221864_at	93129	NM_152288.1	hypothetical protein MGC13024	Unknown function

[0159] The significance of the predictive power of the six genes model based on PLS-DA can be confirmed by random permutation which compares the results obtained with the true class membership with the results obtained after shuffling the class membership of the samples randomly; this was done one hundred times. The validation results are displayed in Figure 9. The original data are located at x=1, y=0.8; the data with randomly shuffled toxicity class membership are displayed at several values of $x<0.45$ indicating that the permutated data were not very similar to the original ones. R2 is a measure of "goodness of fit" and Q2 is a measure of "goodness of prediction". Both values are significantly higher for the original data compared to random response permutations. Negative intercept values of R2 and Q2 (-0.0612 and -0.162)

are significant. The intercept of the regression lines is an indicator of the power of the model. It was -0.0612 for R^2 and -0.162 for Q^2 which points towards a high predictive power being far away from random.

[0160] The results presented in Examples 3-5 show that two independent methods of statistical analysis resulted in two sets of 23 and 27 predictor genes, respectively. Significantly, the set of six genes common to both sets was also able to uniquely separate the two classes of model compounds according to their toxicity without any loss of predictive power, in spite of the relatively small size of the classes.

[0161] In addition, the present methods are sensitive enough to discriminate between ambiguous training samples, such as tPt and cPt. Trans-platinum has long been considered non-genotoxic, because in contrast to cis-platinum it does not show any anti-tumor activity. However, some older publications have noted that while trans-platinum is not a typical genotoxin, it may lead to some weakly positive effects at higher concentrations. Thus in spite of the widely disparate concentrations used in the Examples (trans-platinum: 33 μM, cis-platinum: 1.3 μM), the present methods succeeded in resolving them into their model classes without ambiguity. Alternatively, since cis- and trans-platinum are isomers which are only about 99% pure, it is possible that a slight impurity in trans-platinum consisting of cis-platinum, applied at the higher concentration of the former, might explain why both trans-platinum and cis-platinum are located close to the separation line.

Example 6. Use of extended sets of compounds to identify predictor gene sets.

[0162] Experiments such as those described in the Materials and Methods are carried out. In addition to the original set of three cytotoxic compounds and three genotoxic compounds used in Examples 1-5, or instead of those compounds, the genotoxic and nongenotoxic compounds shown in Table 8 are used. The genotoxic compounds generally have the characteristic of being direct-acting mutagens or clastogens.

Table 8.

Class	Compound or Drug
Control	
Genotoxic	Ethyl nitroso urea Methyl nitroso urea 4-nitroquinoline n-oxyde N-methyl-N'-nitro-N-nitrosoguanidine Dimethyl sulfate Styrene oxide Diepoxy butane Bleomycin Doxorubicin/Adriamycin. Daunorubicin Actinomycin D 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone Benzo[a]pyrene diole epoxide Mitoxantron
Non-genotoxic	Diflunisal Flufenamic acid Oxazepam Dexamethasone Benazepril Ranitidine Verapamil N-Acetylcysteine Tacrolimus

[0163] For each genotoxic and nongenotoxic compound, the concentration corresponding to 50% effectiveness in toxicity is obtained from the literature or evaluated experimentally. Human cells, such as TK6 cells, are cultured as described in Materials and Methods with the 50%-toxic dose of each compound. RNA is isolated from each sample and hybridized to an appropriate human gene probe set arrayed on a substrate. As described above, an Affymetrix HG-U133A PLUS 2 gene chip may be used; alternatively any equivalent array displaying probes originating from a significant portion the human genome may be used, as may or any other method that allows specific quantification of transcripts such as PCR. Hybridization results are scanned and evaluated by the procedures described in Materials and Methods, and in Examples 1-3. Predictor (discriminatory) gene sets of varying sizes and containing a variety of component genes are identified.

Example 7. Determination of Genotoxicity of a Candidate Compound.

[0164] A candidate compound is identified by appropriate research and development activities. The effective dosage for 50% toxicity is evaluated by dilution experiments (Example 1) applied to a human cell line, such as TK6 cells, in several replicates. The cells are cultured for an appropriate period of time (e.g., 24 hours) as described in Materials and Methods, and the total RNA is extracted from each sample. Control cells are also cultured and control RNA isolated. Each sample of RNA is hybridized to a suitable human gene array that includes at least probes from a predictor gene set identified herein (see Examples 2-6); in addition an internal standard probe such as that for beta actin or glyceraldehydes phosphate dehydrogenase may be included on the array employed in this Example. More generally an array such as described in Materials and Methods or equivalent as described in Example 6 may be used. The hybridization results are evaluated by a statistical method described in Materials and Methods and Examples 2-4. The results for the RNA samples obtained from cells treated with the candidate compound are classified by comparison to patterns found from the known model compounds. If the results from the candidate compound resemble those obtained with nongenotoxic compounds, it is concluded that the candidate compound is likely not genotoxic. If the results from the candidate resemble those obtained with genotoxic compounds, it is concluded that the candidate compound is likely genotoxic.

Example 8. Development of a Predictive Model of Genotoxicity

[0165] This example further characterizes the method of establishing a predictive model for genotoxicity. The experimental protocol was the same as that described above. However, additional compounds known to be genotoxic or non-genotoxic were used as reference compounds. The complete set of known genotoxic or non-genotoxic compounds is shown in Table 9 below.

Table 9: Reference compounds of known toxicity being used for biomarker identification

non-genotoxic (non-gtx)		
Number	Agent	Code
7	Diflunisal	Dif
8	Flufenamic acid	Fluf
3	KCl	KCl
4	N-Acetylcysteine	NAC
6	NaCl	NaCl
4	Ranitidine	Ran
6	Rifampicin	Rif
6	trans-Platin	tPt
8	Verapamil	Vera
52	total	9

genotoxic (gtx)		
X Number	Agent	Code
5	Actinomycin-D	AMD
6	Bleomycin	Bleo
6	cis-Platin	cPt
4	Daunorubicin	Dau
3	Doxorubicin	Doxo
3	ENU/Ethyl nitroso urea	ENU
6	Methylmethane sulfonate	MMS
6	Mitomycin C	MMC
4	Mitoxantrone	MXT
3	Styrene oxide	SO
46	total	10

[0166] MAS 5 processed data were statistically analyzed as described in the section entitled "Methods and Materials". Briefly, normalization involved per chip: normalization on sample median and Per gene: normalization on gene median of all samples (GeneSpring 7.2).

[0167] Pre-filtering of genes involved a filter on flags: probe set needs to show present or marginal flags in at least 50% of samples and a filter on intensities: probe set must have intensities > 50 in at least 50% of samples. This resulted in 18'512 probe sets (Genespring 7.2). Statistical filtering was performed using the Welch-t-test (Genespring 7.2).

Results

Predictive Modeling by PLS-DA

[0168] Generally, normalized values as described above were used for modelling. The normalized values were log-transformed (base 10) and Pareto scaled.

Pre-Test with all 98 samples

Table 10: Testing the predictive power of the data (all 98 samples)

Model	Components	R^2X	R^2Y	Q^2	# Probe Sets
M1	2	0.336	0.867	0.833	18'512
M2	2	0.628	0.952	0.942	455
M3	2	0.741	0.952	0.943	117
M4	2	0.801	0.958	0.951	39
M8	2	0.838	0.962	0.958	24
M9	2	0.865	0.958	0.955	18
M5	2	0.884	0.954	0.950	12
M6	2	0.920	0.936	0.934	6
M7	2	0.983	0.869	0.867	3

R^2X: fraction of sum of squares (SS) of all the X's explained by all components
R^2Y: fraction sum of squares (SS) of all the Y's explained by all components
Q^2: fraction of total variation of the Y's that can be predicted according to cross-validation

[0169] According to Table 10, the maximum of predictive power (Q2) is reached with about 24 probe sets. The predictive genes in models M1 – M9 correlate highly with the top ranking genes of the above mentioned Welch t-test.

Modeling with Calibration Samples only

[0170] The set of 98 samples were split randomly into a calibration set of 74 samples and a validation set consisting of 24 samples. Samples treated with trans-platinum were not included in the calibration samples because of a possible contamination; the gene expression pattern of most of the trans-platinum samples indicated genotoxicity rather than pure cytotoxicity as one would expect according to literature. However, all trans-platinum samples were member of the validation samples. The 100 top-ranking probe sets according to Welch t-test were used as a starting set of features for predictive modelling.

[0171] In total, three biomarker (BM1 –BM3) with almost equal predictive power could be constructed from these 100 candidate genes (See Table 11). Each biomarker consists of a set of independent genes and there is no overlap of genes (probe sets) among the different biomarkers.

Table 11: Predictive power of the three biomarkers

Model	Components	R^2X	R^2Y	Q^2	# Probe Sets
BM1	2	0.777	0.961	0.944	30
BM2	2	0.773	0.938	0.926	33
BM3	2	0.776	0.916	0.902	37

R^2X: fraction of sum of squares (SS) of all the X's explained by all components
R^2Y: fraction sum of squares (SS) of all the Y's explained by all components
Q^2: fraction of total variation of the Y's that can be predicted according to cross-validation

[0172] In terms of Q2 the performance of BM1 is better than BM2, and performance of BM2 is better than BM3 which is as expected. However, the difference is only marginal and of no practical importance. Validation by response permutation confirmed also a similar performance of the three biomarkers (see Figures 10A – 12B).

[0173] All genes are listed in Table 12 including the biomarker they belong to, genbank accession number, Affymetrix probe set number, gene symbol and description, as well as median gene expression intensities of non-genotoxic and genotoxic samples, fold-change, and Welch t-test p-value. Performance parameters of the three biomarkers are summarize in Table 12:

[0174] The classification of biomarker gene responses for BM1-BM3 to a genotoxic or a non-genotoxic compounds are shown in Figures 10A-12B and Tables 12 .

Table 12 : Predictive probe sets (genes) of three biomarkers of Genotoxicity (BM1 – BM3).

Model	VIP	Probe Set	Symbol	Gene Name	Genebank	Median		FC	Welch t-test
						non-GTX	GTX		p-value
BM1	0.94	209375_at	XPC	Xeroderma pigmentosum, complementation group C	D21089	415	1110	2.7	3.84E-25
BM1	1.15	207813_s_at	FDXR	Ferredoxin reductase	NM_004110	398	1674	4.2	3.11E-24
BM1	0.95	209584_x_at	APOBEC3C	Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3C	AF165520	702	1823	2.6	3.58E-23
BM1	1.05	229711_s_at	MGC5370	Hypothetical protein MGC5370	AA902480	780	2595	3.3	1.16E-21
BM1	1.04	203409_at	DDB2	Damage-specific DNA binding protein 2, 48kDa	NM_000107	716	2206	3.1	2.84E-21
BM1	1.19	238733_at		Transcribed locus	AI422414	74	323	4.4	2.91E-20
BM1	0.99	226435_at	PAPLN	Papilin, proteoglycan-like sulfated glycoprotein	AU145309	45	119	2.6	6.42E-20
BM1	1.59	202838_at	FUCA1	Fucosidase, alpha-L- 1, tissue	NM_000147	15	185	12.7	1.63E-19
BM1	1.14	217542_at	CPM	Carboxypeptidase M	BE930512	124	428	3.4	5.77E-19
BM1	1.33	210609_s_at	TP53I3	Tumor protein p53 inducible protein 3	BC000474	218	1709	7.8	1.05E-18
BM1	1.02	202284_s_at	CDKN1A	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	NM_000389	1011	3933	3.9	1.98E-18
BM1	0.80	212120_at	PIGF	Phosphatidylinositol glycan, class F	BE897886	1072	491	0.5	2.53E-17
BM1	0.79	212196_at	IL6ST	Interleukin 6 signal transducer (gp130, oncostatin M receptor)	AW242916	263	155	0.6	2.94E-17
BM1	0.83	218910_at	FLJ10375	Hypothetical protein FLJ10375	NM_018075	531	295	0.6	2.94E-17

Model	VIP	Probe Set	Symbol	Gene Name	Genebank	Median		FC	Welch t-test
						non-GTX	GTX		p-value
BM1	0.74	233656_s_at	VPS54	Vacuolar protein sorting 54 (yeast)	AL359939	659	410	0.6	4.05E-16
BM1	0.86	203164_at	SLC33A1	hv89d09.x1 NCI_CGAP_Lu24 Homo sapiens, cDNA clone IMAGE:3180593 3', mRNA sequence.	BE464756	538	265	0.5	5.21E-16
BM1	0.85	212195_at	IL6ST	Interleukin 6 signal transducer (gp130, oncostatin M receptor)	AL049265	878	502	0.6	5.30E-16
BM1	0.73	212723_at	PTDSR	Phosphatidylserine receptor	AK021780	558	342	0.6	1.42E-15
BM1	1.16	200974_at	TXNIP	synonym: ACTSA; alpha-cardiac actin; go_component: actin filament [goid 0005884] [evidence IEA]; go_component: striated muscle thin filament [goid 0005865] [evidence NAS]; go_function: motor activity [goid 0003774] [evidence IEA]; go_function: structural constituent of muscle [goid 0008307] [evidence NAS]; go_function: structural constituent of cytoskeleton [goid 0005200] [evidence IEA]; go_process: muscle development [goid 0007517] [evidence NAS]; Homo sapiens actin, alpha 2, smooth muscle, aorta (ACTA2), mRNA.	AA812232	268	1021	3.8	1.86E-15
BM1	0.83	1554256_a_at	IDH1	hypothetical protein FLJ11383	BC012846	1881	902	0.5	2.60E-15
BM1	0.85	214449_s_at	RHOQ	Ras homolog gene family, member Q	NM_012249	504	208	0.4	2.81E-15
BM1	0.79	201009_s_at	IDH1	Thioredoxin interacting protein	NM_005896	2132	1176	0.6	3.84E-15
BM1	0.83	215283_at	LOC339290	Hypothetical protein LOC339290	U79248	102	47	0.5	4.00E-15
BM1	0.79	207738_s_at	NCKAP1	NCK-associated protein 1	NM_013436	725	384	0.5	8.30E-15
BM1	0.84	218466_at	TBC1D17	TBC1 domain family, member 17	NM_024682	194	100	0.5	1.14E-14
BM1	1.08	201340_s_at	ENC1	Ectodermal-neural cortex (with BTB-like domain)	NM_003633	353	1096	3.1	1.80E-14
BM1	1.05	201008_s_at	TXNIP	Thioredoxin interacting protein	AI439556	281	859	3.1	2.19E-14
BM1	0.81	212117_at	PIGF	Phosphatidylinositol glycan, class F	BF978689	875	355	0.4	2.25E-14
BM1	0.79	212122_at	PIGF	Phosphatidylinositol glycan, class F	AW771590	129	57	0.4	4.31E-14
BM1	1.50	1554148_a_at	FLJ11383	solute carrier family 33 (acetyl-CoA transporter), member 1	BC008300	11	100	9.0	5.88E-14
BM2	1.18	238935_at	RPS27L	EST370545 MAGE resequences, MAGE Homo sapiens cDNA, mRNA sequence.	AW958475	305	710	2.3	2.35E-21
BM2	1.08	216705_s_at	ADA	H.sapiens adenosine deaminase (ADA) gene 5' flanking region and exon 1 (and joined CDS).	X02189	809	1869	2.3	2.84E-21
BM2	1.06	219099_at	C12orf5	go_function: catalytic activity [goid 0003824] [evidence IEA]; go_process: metabolism [goid 0008152] [evidence IEA]; Homo sapiens chromosome 12 open reading frame 5 (C12orf5), mRNA.	NM_020375	818	1679	2.1	3.10E-20
BM2	1.17	222879_s_at	POLH	Polymerase (DNA directed), eta	AF158185	84	242	2.9	4.96E-20
BM2	0.86	1555037_a_at	KDELR2	isocitrate dehydrogenase 1 (NADP+), soluble	BE962456	578	371	0.6	6.42E-20
BM2	1.19	225160_x_at	CPM	Carboxypeptidase M	AI952357	1016	2744	2.7	8.99E-20
BM2	1.04	208890_s_at	PLXNB2	Plexin B2	BC004542	677	1504	2.2	1.66E-19
BM2	1.06	233852_at	POLH	Polymerase (DNA directed), eta	AK025631	151	344	2.3	1.64E-18
BM2	1.06	219361_s_at	FLJ12484	Hypothetical protein FLJ12484	NM_022767	320	700	2.2	1.66E-18
BM2	1.09	214995_s_at	KIAA0907	KIAA0907 protein	BF508948	234	519	2.2	2.37E-17
BM2	1.13	235534_at		Transcribed locus	AI624156	80	190	2.4	3.63E-17

Model	VIP	Probe Set	Symbol	Gene Name	Genebank	Median		FC	Welch t-test
						non-GTX	GTX		p-value
BM2	1.09	204205_at	APOBEC3G; ARP9; CEM15; MDS019; FLJ12740; bK150C2.7; dJ494G10.1	synonyms: ARP9, CEM15, MDS019, FLJ12740, bK150C2.7, dJ494G10.1; phorbolin-like protein MDS019; go_component: nucleus [goid 0005634] [evidence IEA]; go_function: zinc ion binding [goid 0008270] [evidence IEA]; go_function: hydrolase activity [goid 0016787] [evidence IEA]; go_function: hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in cyclic amidines [goid 0016814] [evidence IEA]; Homo sapiens apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G (APOBEC3G), mRNA.	NM_021822	643	1596	2.5	4.85E-17
BM2	0.82	227534_at	C9orf21	wb67g03.x1 NCI_CGAP_GC6 Homo sapiens cDNA clone IMAGE:2310772 3', mRNA sequence.	AI655189	655	420	0.6	9.65E-17
BM2	1.23	221640_s_at	LRDD	Leucine-rich repeats and death domain containing	AF274972	144	444	3.1	1.79E-16
BM2	0.86	235980_at	KCNMB3	Potassium large conductance calcium-activated channel, subfamily M beta member 3	AA767763	131	83	0.6	3.68E-16
BM2	0.91	222978_at	SURF4; ERV29; FLJ22993	Homo sapiens cDNA: FLJ22993 fis, clone KAT11914.	AK026646	1748	1090	0.6	5.30E-16
BM2	0.86	227665_at	MCART1	Mitochondrial carrier triple repeat 1	BE968576	274	186	0.7	6.88E-16
BM2	1.08	209154_at	TAX1BP3	Tax1 (human T-cell leukemia virus type I) binding protein 3	AF234997	469	1456	3.1	1.15E-15
BM2	1.14	218346_s_at	SESN1	Sestrin 1	NM_014454	99	243	2.5	1.52E-15
BM2	0.76	212118_at	RFP	Ret finger protein	AL523814	374	261	0.7	1.70E-15
BM2	0.82	203077_s_at	SMAD2	SMAD, mothers against DPP homolog 2 (Drosophila)	NM_005901	362	245	0.7	2.81E-15
BM2	0.81	209210_s_at	PLEKHC1; MIG2; KIND2; mig-2; UNC112	H.sapiens mitogen inducible gene mig-2, complete CDS.	Z24725	769	559	0.7	2.81E-15
BM2	0.93	226750_at	FLJ10378	FLJ10378 protein	AI767732	393	229	0.6	3.57E-15
BM2	0.81	227983_at	MGC7036	Hypothetical protein MGC7036	AI810244	2588	1856	0.7	3.57E-15
BM2	0.78	217823_s_at	UBE2J1	Ubiquitin-conjugating enzyme E2, J1 (UBC6 homolog, yeast)	AL562528	1039	731	0.7	3.84E-15
BM2	0.87	212428_at	KIAA0368	KIAA0368	AW001101	840	542	0.6	4.00E-15
BM2	0.87	212722_s_at	PTDSR	Phosphatidylserine receptor	AK021780	371	236	0.6	4.86E-15
BM2	0.83	207564_x_at	OGT	O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase)	NM_003605	406	275	0.7	1.41E-14
BM2	1.35	217373_x_at	MDM2	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse)	AJ276888	84	307	3.6	1.64E-14
BM2	0.74	223325_at	LOC51061	Hypothetical protein LOC51061	AF131780	327	254	0.8	1.90E-14
BM2	0.82	208093_s_at	NDEL1	NudE nuclear distribution gene E homolog like 1 (A. nidulans)	NM_030808	647	453	0.7	1.93E-14
BM2	0.90	226150_at	HTPAP	HTPAP protein	BF111651	1392	829	0.6	2.44E-14
BM2	1.33	201287_s_at	ENC1	Syndecan 1	AF010314	58	201	3.4	3.63E-14
BM3	0.93	224951_at	LASS5	LAG1 longevity assurance homolog 5 (S. cerevisiae)	BE348305	360	642	1.8	1.71E-20
BM3	1.05	218403_at	HSPC132	Hypothetical protein HSPC132	NM_016399	1335	2403	1.8	3.20E-19
BM3	1.08	227964_at	FKSG44	FKSG44 gene	BF435621	573	1180	2.1	3.42E-19
BM3	1.00	204639_at	ADA	Adenosine deaminase	NM_000022	1252	2617	2.1	3.55E-19

Model	VIP	Probe Set	Symbol	Gene Name	Genebank	Median		FC	Welch t-test
						non-GTX	GTX		p-value
BM3	1.11	218634_at	PHLDA3; TIH1	synonym: TIH1; pleckstrin homology-like domain, family A, member 2; go_process: morphogenesis [goid 0009653] [evidence TAS] [pmid 10594239]; Homo sapiens pleckstrin homology-like domain, family A, member 3 (PHLDA3), mRNA.	NM_012396	241	514	2.1	7.13E-18
BM3	0.92	201341_at	ARL1	Ectodermal-neural cortex (with BTB-like domain)	BE890745	407	267	0.7	1.04E-17
BM3	0.88	225734_at	FBXO22	F-box protein 22	AW294765	503	822	1.6	1.17E-17
BM3	1.11	223342_at	RRM2B	Ribonucleotide reductase M2 B (TP53 inducible)	AB036063	286	554	1.9	1.35E-17
BM3	0.99	1552474_a_at	GAMT	Guanidinoacetate N-methyltransferase	NM_138924	378	751	2.0	2.53E-17
BM3	0.91	222477_s_at	TM7SF3	transmembrane 7 superfamily member 3	BC005176	1019	1855	1.8	2.94E-17
BM3	1.01	201193_at	BTG2	Isocitrate dehydrogenase 1 (NADP+), soluble	NM_006763	508	912	1.8	5.47E-17
BM3	0.99	223207_x_at	PHPT1	Phosphohistidine phosphatase 1	AF285119	1162	2256	1.9	5.97E-17
BM3	1.08	218124_at	FLJ20296	Hypothetical protein FLJ20296	NM_017750	223	402	1.8	1.03E-15
BM3	1.00	210749_x_at	DDR1	Discoidin domain receptor family, member 1	L11315	337	599	1.8	1.35E-15
BM3	0.88	230294_at		Transcribed locus	AV714462	78	128	1.6	2.60E-15
BM3	1.08	205354_at	GAMT	Guanidinoacetate N-methyltransferase	NM_000156	233	504	2.2	3.04E-15
BM3	0.98	1007_s_at		U48705 /FEATURE=mRNA /DEFINITION=HSU48705 Human receptor tyrosine kinase DDR gene, complete cds	U48705 mRNA	461	830	1.8	3.44E-15
BM3	1.03	217974_at	TM7SF3	Transmembrane 7 superfamily member 3	NM_016551	103	210	2.0	3.84E-15
BM3	1.09	244616_x_at	MGC5370	601565341F1 NIH_MGC_21 Homo sapiens cDNA clone IMAGE:3839914 5', mRNA sequence.	BE732830	110	251	2.3	3.84E-15
BM3	0.83	225737_s_at	FBXO22	F-box protein 22	BE966247	405	679	1.7	3.99E-15
BM3	0.97	224391_s_at	CSE-C	Cytosolic sialic acid 9-O-acetylesterase homolog	AF303378	142	256	1.8	4.08E-15
BM3	1.14	201236_s_at	SDC1	BTG family, member 2	NM_002997	164	446	2.7	4.64E-15
BM3	1.17	215407_s_at	ASTN2	Astrotactin 2	AK024064	76	196	2.6	5.27E-15
BM3	0.81	227295_at	IKIP	IKK interacting protein	AW182575	474	729	1.5	6.23E-15
BM3	0.93	222977_at	SURF4	Surfeit 4	AL518882	753	545	0.7	8.06E-15
BM3	1.00	203269_at	NSMAF	Neutral sphingomyelinase (N-SMase) activation associated factor	NM_003580	1178	895	0.8	9.28E-15
BM3	0.78	201657_at	LAPTM5	ADP-ribosylation factor-like 1	NM_006762	2414	3540	1.5	1.12E-14
BM3	0.95	207812_s_at	GORASP2	Golgi reassembly stacking protein 2, 55kDa	NM_015530	1473	1007	0.7	1.44E-14
BM3	1.13	219019_at	LRDD	Leucine-rich repeats and death domain containing	NM_018494	204	404	2.0	1.44E-14
BM3	1.00	230122_at	MLLT10	Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 10	BE219716	160	120	0.7	1.44E-14
BM3	1.03	226430_at	LOC253981	Hypothetical protein LOC253981	AI394438	87	183	2.1	1.60E-14
BM3	1.08	219014_at	PLAC8	Placenta-specific 8	NM_016619	1630	3287	2.0	1.67E-14
BM3	0.96	200736_s_at	ACTA2; ACTSA	Glutathione peroxidase 1	NM_001613	674	1293	1.9	1.93E-14
BM3	0.99	200699_at	GPX1	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2	NM_000581	2864	4986	1.7	2.08E-14
BM3	0.99	203457_at	STX7	Syntaxin 7	NM_003569	92	62	0.7	2.61E-14
BM3	1.04	201721_s_at	PRKAB1	Lysosomal-associated multispanning membrane protein-5	BC001007	379	724	1.9	4.08E-14
BM3	0.92	204369_at	PIK3CA	Phosphoinositide-3-kinase, catalytic, alpha polypeptide	NM_006218	637	438	0.7	5.47E-14

[0175] In conclusion, the data from the initial study (Examples 1-7) and the present study (Example 8) confirm the establishment of a rapid method for screening genotoxic and non-genotoxic compounds using a predictor model based on an alteration in gene expression of selected biomarker genes.

[0176] The initial biomarker of genotoxicity was based on 6 reference compounds of know toxicity, these being: rifampicin, NaCl, trans-platinum as non-genotoxic compounds, and methylmethan sulfonate, mitomycin C, and cis-platinum as known genotoxic compounds. 215 candidate genes were identified and subjected to supervised learning algorithms, such as Partial Least Squares – Discriminant Analysis (PLS-DA) and K-Nearest Neighbor (KNN) resulting in a predictive PLS-DA model of 23 genes and a predictive KNN model of 27 gene with six genes common to both models.

[0177] The three biomarkers of the present analysis are based on 9 non-genotoxic and 10 genotoxic compounds including the ones from the initial analysis. A statistical comparison (Welch t-test) of genotoxic versus non- genotoxic samples yielded 4911candidate genes with a FDR of 0.1%. 118 of the 215 candidate genes are also among the 4911 new candidate genes. The overlap between the 100 genes of biomarkers BM1-3 and the 27 KNN predictor genes is 9, and the overlap with the 23 PLS-DA predictor genes is 5. Table 13 summaries the data from Experiments 1-7 and Experiment 8.

[0178] In conclusion, it can be stated that the predictor genes of the initial biomarkers are still good predictor when applied to the extended data set which included a greater variety of genotoxic and non-genotoxic compounds. However, a feature extraction based on the extended data set provides a more powerful set of predictor genes for genotoxicity.

UNIGENE	Repeats	SYMBOL	GENENAME	Extended Analysis Biomarker	Initial Analysis Biomarker	non-GTX Median	GTX Median	FC	Welch t-test p-value
Hs.320	1	XPC	xeroderma pigmentosum, complementation group C	BM1	KNN	415	1110	2.68	5.98E-27
Hs.69745	1	FDXR	ferredoxin reductase	BM1		398	1674	4.21	4.84E-26
Hs.441124	3	APOBEC 3C	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3C	BM1; BM2		702	1823	2.60	5.57E-25
Hs.108957	2	RPS27L	ribosomal protein S27-like	BM2		305	710	2.33	3.66E-23
Hs.446564	1	DDB2	damage-specific DNA binding protein 2, 48kDa	BM1		716	2206	3.08	4.41E-23
Hs.458450	1	LASS5	LAG1 longevity assurance homolog 5 (S. cerevisiae)	BM3		360	642	1.78	2.67E-22
EST	1		EST	BM1		74	323	4.35	4.53E-22
Hs.24792	1	C12orf5	chromosome 12 open reading frame 5	BM2		818	1679	2.05	4.82E-22
Hs.155573	2	POLH	polymerase (DNA directed), eta	BM2		84	242	2.89	7.71E-22
Hs.11223	2	IDH1	isocitrate dehydrogenase 1 (NADP+), soluble	BM2; BM3		1881	902	-2.09	9.99E-22
Hs.458428	1	PAPLN	papilin, proteoglycan-like sulfated glycoprotein	BM1		45	119	2.62	9.99E-22
Hs.576	1	FUCA1	fucosidase, alpha-L- 1, tissue	BM1		15	185	12.68	2.54E-21
Hs.278311	2	PLXNB2	plexin B2	BM2		677	1504	2.22	2.58E-21
Hs.69499	1	HSPC132	hypothetical protein HSPC132	BM3		1335	2403	1.80	4.99E-21
Hs.362974	1	FKSG44	hypothetical protein FKSG44	BM3		573	1180	2.06	5.32E-21
Hs.407135	2	ADA	adenosine deaminase	BM2; BM3		1252	2617	2.09	5.52E-21
Hs.168732	4	MGC5370	hypothetical protein MGC5370	BM1; BM2; BM3		124	428	3.45	8.97E-21
Hs.50649	1	TP53I3	tumor protein p53 inducible protein 3	BM1		218	1709	7.83	1.63E-20
Hs.436102	1	FLJ12484	hypothetical protein FLJ12484	BM2		320	700	2.19	2.58E-20
Hs.370771	1	CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	BM1		1011	3933	3.89	3.08E-20
Hs.268557	1	PHLDA3	pleckstrin homology-like domain, family A, member 3	BM3		241	514	2.13	1.11E-19
Hs.438891	2	FBXO22	F-box only protein 22	BM3		503	822	1.63	1.82E-19
Hs.512592	1	RRM2B	ribonucleotide reductase M2 B (TP53 inducible)	BM3		286	554	1.94	2.10E-19
Hs.24656	1	KIAA0907	KIAA0907 protein	BM2		234	519	2.21	3.69E-19
Hs.81131	2	GAMT	guanidinoacetate N-methyltransferase	BM3		378	751	1.99	3.93E-19
Hs.426142	1	PIGF	phosphatidylinositol glycan, class F	BM1		1072	491	-2.18	3.93E-19
Hs.476055	1	FLJ10375	hypothetical protein FLJ10375	BM1		531	295	-1.80	4.57E-19
EST	1		EST	BM2		80	190	2.38	5.65E-19
Hs.409834	1	PHP14	phosphohistidine phosphatase	BM3		1162	2256	1.94	9.29E-19
Hs.44640	1	C9orf21	chromosome 9 open reading frame 21	BM2		655	420	-1.56	1.50E-18
Hs.120905	1	KCNMB3	potassium large conductance calcium-activated channel, subfamily M beta member 3	BM2		131	83	-1.58	5.73E-18

UNIGENE	Repeats	SYMBOL	GENENAME	Extended Analysis Biomarker	Initial Analysis Biomarker	non-GTX Median	GTX Median	FC	Welch t-test p-value
Hs.48499	1	VPS54	vacuolar protein sorting 54 (yeast)	BM1		659	410	-1.61	6.30E-18
Hs.285176	1	SLC33A1	solute carrier family 33 (acetyl-CoA transporter), member 1	BM1		538	265	-2.03	8.11E-18
Hs.71968	2	IL6ST	interleukin 6 signal transducer (gp130, oncostatin M receptor)	BM1		878	502	-1.75	8.25E-18
Hs.46791	1	MCART1	mitochondrial carrier triple repeat 1	BM2		274	186	-1.47	1.07E-17
Hs.440401	1	FLJ20296	hypothetical protein FLJ20296	BM3		223	402	1.80	1.61E-17
Hs.12956	2	TIP-1	Tax interaction protein 1	BM2	KNN; PLS-DA	469	1456	3.11	1.79E-17
Hs.14125	1	SESN1	sestrin 1	BM2		99	243	2.46	2.37E-17
Hs.440382	1	RFP	ret finger protein	BM2		374	261	-1.44	2.65E-17
Hs.208641	1	ACTA2	actin, alpha 2, smooth muscle, aorta	BM1	PLS-DA	674	1293	1.92	2.89E-17
Hs.436455	1	FLJ11383	hypothetical protein FLJ11383	BM1		11	100	9.03	4.05E-17
EST	1		EST	BM3		78	128	1.64	4.05E-17
Hs.110741	1	MADH2	MAD, mothers against decapentaplegic homolog 2 (Drosophila)	BM2		362	245	-1.48	4.37E-17
Hs.270411	1	PLEKHC1	pleckstrin homology domain containing, family C (with FERM domain) member 1	BM2		769	559	-1.38	4.37E-17
Hs.423573	4	DDR1	discoidin domain receptor family, member 1	BM3	KNN; PLS-DA	461	830	1.80	5.35E-17
Hs.151973	1	FLJ10378	FLJ10378 protein	BM2		393	229	-1.72	5.55E-17
Hs.488173	1	MGC7036	hypothetical protein MGC7036	BM2		2588	1856	-1.39	5.55E-17
Hs.512682	2	CEACAM 1	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)		KNN	23	93	4.05	5.80E-17
Hs.10071	2	TM7SF3	transmembrane 7 superfamily member 3	BM3		103	210	2.04	5.89E-17
Hs.184325	1	UBE2J1	ubiquitin-conjugating enzyme E2, J1 (UBC6 homolog, yeast)	BM2		1039	731	-1.42	5.89E-17
Hs.445255	1	KIAA0368	KIAA0368	BM2		840	542	-1.55	6.13E-17
AK056929, AK056929, BC027873, BC041875, BX648984, All Genbank Accessions	1	LOC339290	hypothetical protein LOC339290	BM1		102	47	-2.18	6.13E-17
Hs.10056	1	CSE-C	cytosolic sialic acid 9-O-acetylesterase homolog	BM3		142	256	1.80	6.26E-17
Hs.75462	2	BTG2	BTG family, member 2	BM3		508	912	1.80	7.12E-17
Hs.72660	2	PTDSR	phosphatidylserine receptor	BM1; BM2		371	236	-1.57	7.46E-17
Hs.30898	1	ASTN2	astrotactin 2	BM3		76	196	2.57	8.08E-17
Hs.406199	1	IKIP	IKK interacting protein	BM3		474	729	1.54	9.56E-17
Hs.284296	2	SURF4	surfeit 4	BM2; BM3		753	545	-1.38	1.24E-16
Hs.278411	1	NCKAP1	NCK-associated protein 1	BM1		725	384	-1.89	1.27E-16
Hs.372000	1	NSMAF	neutral sphingomyelinase (N-SMase) activation	BM3		1178	895	-1.32	1.43E-16

UNIGENE	Repeats	SYMBOL	GENENAME	Extended Analysis Biomarker	Initial Analysis Biomarker	non-GTX Median	GTX Median	FC	Welch t-test p-value
			associated factor						
Hs.372616	1	ARL1	ADP-ribosylation factor-like 1	BM3		407	267	-1.53	1.72E-16
Hs.325860	1	TBC1D17	TBC1 domain family, member 17	BM1		194	100	-1.95	1.74E-16
Hs.405410	1	OGT	O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase)	BM2		406	275	-1.47	2.16E-16
Hs.438986	2	LRDD	leucine-rich and death domain containing	BM2; BM3	KNN; PLS-DA	204	404	1.98	2.22E-16
Hs.6880	1	GORASP2	golgi reassembly stacking protein 2, 55kDa	BM3		1473	1007	-1.46	2.22E-16
Hs.446451	1	MLLT10	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 10	BM3		160	120	-1.34	2.22E-16
AK025431, All Genbank Accessions	1	LOC253981	hypothetical protein LOC253981	BM3		87	183	2.10	2.46E-16
Hs.212217	1	MDM2	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse)	BM2		84	307	3.65	2.52E-16
Hs.371003	1	PLAC8	placenta-specific 8	BM3		1630	3287	2.02	2.57E-16
Hs.104925	2	ENC1	ectodermal-neural cortex (with BTB-like domain)	BM1; BM3		58	201	3.44	2.77E-16
Hs.313847	1	LOC51061	hypothetical protein LOC51061	BM2		327	254	-1.29	2.92E-16
Hs.76686	1	GPX1	glutathione peroxidase 1	BM3		2864	4986	1.74	2.96E-16
Hs.3850	1	NDEL1	nudE nuclear distribution gene E homolog like 1 (A. nidulans)	BM2		647	453	-1.43	2.97E-16
Hs.446645	1	KDELR2	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2	BM3		578	371	-1.56	3.20E-16
Hs.179526	3	TXNIP	thioredoxin interacting protein	BM1		268	1021	3.82	3.37E-16
Hs.442989	3	ARHQ	ras homolog gene family, member Q	BM1		875	355	-2.46	3.46E-16
Hs.437179	1	HTPAP	HTPAP protein	BM2		1392	829	-1.68	3.75E-16
Hs.434916	1	STX7	syntaxin 7	BM3		92	62	-1.48	4.02E-16
Hs.82109	2	SDC1	syndecan 1	BM2	KNN; PLS-DA	164	446	2.71	5.53E-16
Hs.85701	1	PIK3CA	phosphoinositide-3-kinase, catalytic, alpha polypeptide	BM3		637	438	-1.46	8.34E-16
Hs.6061	2	PRKAB1	protein kinase, AMP-activated, beta 1 non-catalytic subunit			379	724	1.91	9.01E-16
Hs.371856	1	HSPC055	zinc-finger protein AY163807		PLS-DA	629	1011	1.61	1.19E-15
	1		EST			69	145	2.09	1.69E-15
Hs.318501	1	TRIM22	tripartite motif-containing 22			1486	2263	1.52	2.33E-15
Hs.286073	1	PPM1D	protein phosphatase 1D magnesium-dependent, delta isoform		KNN; PLS-DA	404	758	1.87	8.81E-15
Hs.273330	1	AGRN	agrin			201	377	1.88	7.36E-14
Hs.352119	4	GGT1	gamma-glutamyltransferase 1			68	157	2.32	1.70E-13

UNIGENE	Repeats	SYMBOL	GENENAME	Extended Analysis Biomarker	Initial Analysis Biomarker	non-GTX Median	GTX Median	FC	Welch t-test p-value
Hs.2490	1	CASP1	caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)			74	156	2.11	1.78E-13
Hs.279912	1	CP110	CP110 protein			396	622	1.57	1.78E-13
Hs.436200	2	LAPTM5	Lysosomal-associated multispanning membrane protein-5			1420	2545	1.79	2.10E-13
Hs.76884	1	ID3	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein			753	1577	2.09	2.30E-13
Hs.87860	2	PTPN22	protein tyrosine phosphatase, non-receptor type 22 (lymphoid)		PLS-DA	365	612	1.68	4.42E-13
Hs.211601	1	MAP3K12	mitogen-activated protein kinase kinase kinase 12			65	103	1.58	2.42E-12
Hs.512719	2	ZNF79	zinc finger protein 79 (pT7)			48	107	2.26	3.19E-12
Hs.331308	1	LOC51257	hypothetical protein LOC51257			101	169	1.67	4.77E-12
Hs.511807	1	FLJ90005	hypothetical protein FLJ90005			307	532	1.73	6.90E-12
Hs.436441	1	LMNA	lamin A/C			373	528	1.41	8.66E-12
Hs.1274	1	BMP1	bone morphogenetic protein 1			54	86	1.60	9.87E-12
Hs.511807	1	FLJ90005	hypothetical protein FLJ90005		KNN	258	404	1.57	1.43E-11
Hs.333488	1	MGC13024	hypothetical protein MGC13024		KNN; PLS-DA	559	901	1.61	1.85E-11
Hs.387871	1	TNFSF10	tumor necrosis factor (ligand) superfamily, member 10			51	122	2.38	1.92E-11
Hs.435826	1	MGC4172	hypothetical protein MGC4172			97	178	1.84	3.35E-11
Hs.380230	1	TRIP6	thyroid hormone receptor interactor 6			251	397	1.58	7.72E-11
Hs.1770	1	LIG1	ligase I, DNA, ATP-dependent		KNN	255	639	2.51	8.04E-11
Hs.279731	1	LYK5	protein kinase LYK5		KNN	300	433	1.44	8.04E-11
Hs.197875	1	ASC	apoptosis-associated speck-like protein containing a CARD			162	311	1.92	8.13E-11
Hs.149957	1	RPS6KA1	ribosomal protein S6 kinase, 90kDa, polypeptide 1			487	792	1.63	1.27E-10
Hs.111779	1	SPARC	secreted protein, acidic, cysteine-rich (osteonectin)			211	352	1.67	1.92E-10
Hs.81337	1	LGALS9	lectin, galactoside-binding, soluble, 9 (galectin 9)			42	109	2.57	2.60E-10
Hs.136713	1	VPREB3	pre-B lymphocyte gene 3			44	105	2.38	3.34E-10
Hs.386299	1	WIG1	p53 target zinc finger protein			545	854	1.57	3.81E-10
Hs.108441	1	HAAO	3-hydroxyanthranilate 3,4-dioxygenase			77	141	1.83	3.88E-10
Hs.355394	1	GGTLA4	gamma-glutamyltransferase-like activity 4			76	111	1.45	4.19E-10
Hs.153640	1	PLK3	polo-like kinase 3 (Drosophila)			124	185	1.49	4.91E-10
Hs.101840	4	MR1	major histocompatibility complex, class I-related			235	379	1.61	4.96E-10
Hs.147996	1	PRKX	protein kinase, X-linked			121	190	1.57	5.20E-10
Hs.182536	1	KIAA0284	KIAA0284			45	88	1.95	6.89E-10

UNIGENE	Repeats	SYMBOL	GENENAME	Extended Analysis Biomarker	Initial Analysis Biomarker	non-GTX Median	GTX Median	FC	Welch t-test p-value
Hs.311559	1	NOTCH1	Notch homolog 1, translocation-associated (Drosophila)			142	293	2.06	8.27E-10
Hs.227777	1	PTP4A1	protein tyrosine phosphatase type IVA, member 1			762	1098	1.44	9.26E-10
Hs.91448	1	DUSP14	dual specificity phosphatase 14			584	797	1.36	1.12E-09
Hs.368866	1	MDS025	hypothetical protein MDS025			1081	1599	1.48	1.80E-09
Hs.82327	1	GSS	glutathione synthetase			914	1270	1.39	5.02E-09
Hs.1103	1	TGFB1	transforming growth factor, beta 1 (Camurati-Engelmann disease)		PLS-DA	624	1115	1.79	6.82E-09
Hs.5541	1	ATP2A3	ATPase, Ca++ transporting, ubiquitous			106	185	1.74	1.05E-08
Hs.173894	1	CSF1	colony stimulating factor 1 (macrophage)			79	132	1.68	1.32E-08
Hs.104382	1	ZNF195	zinc finger protein 195			599	866	1.45	1.60E-08
Hs.103128	1	CHRNA6	cholinergic receptor, nicotinic, alpha polypeptide 6			27	53	2.00	1.73E-08
Hs.377992	1	RABGGTA	Rab geranylgeranyltransferase, alpha subunit			223	315	1.41	3.45E-08
M12423, M12423, X01403, X02592, All Genbank Accessions	1	TRA@	T cell receptor alpha locus			48	93	1.92	3.65E-08
Hs.273186	1	CABC1	chaperone, ABC1 activity of bc1 complex like (S. pombe)			178	236	1.33	5.25E-08
Hs.223894	1	IL12RB1	interleukin 12 receptor, beta 1		KNN	57	76	1.35	5.54E-08
Hs.37003	1	HRAS	v-Ha-ras Harvey rat sarcoma viral oncogene homolog			343	539	1.57	6.77E-08
Hs.351573	1	NTAN1	N-terminal asparagine amidase		PLS-DA	156	271	1.73	7.11E-08
Hs.274329	2	TP53AP1	TP53 activated protein 1		PLS-DA	136	179	1.32	9.42E-08
Hs.333166	1	MGC14799	hypothetical protein MGC14799			115	202	1.76	1.08E-07
Hs.388160	2	MGC10940	hypothetical protein MGC10940		PLS-DA	316	468	1.48	1.39E-07
Hs.257008	1	PLD3	phospholipase D3			313	422	1.35	2.38E-07
Hs.33084	1	SLC2A5	solute carrier family 2 (facilitated glucose/fructose transporter), member 5			130	200	1.54	2.74E-07
Hs.82173	1	TIEG	TGFB inducible early growth response			279	421	1.51	4.23E-07
Hs.144407	1	NUDT15	nudix (nucleoside diphosphate linked moiety X)-type motif 15			665	1141	1.72	5.67E-07
Hs.443711	2	ANK1	ankyrin 1, erythrocytic			35	61	1.73	9.09E-07
Hs.154149	1	APEX2	APEX nuclease (apurinic/apyrimidinic endonuclease) 2			209	335	1.60	1.33E-06
Hs.80409	1	GADD45A	growth arrest and DNA-damage-inducible, alpha			1609	2459	1.53	1.36E-06
Hs.255935	1	BTG1	B-cell translocation gene 1, anti-proliferative			2000	3113	1.56	1.44E-06

UNIGENE	Repeats	SYMBOL	GENENAME	Extended Analysis Biomarker	Initial Analysis Biomarker	non-GTX Median	GTX Median	FC	Welch t-test p-value
Hs.1583	2	NCF1	neutrophil cytosolic factor 1 (47kDa, chronic granulomatous disease, autosomal 1)			65	102	1.55	3.51E-06
Hs.7426	1	KIAA0841	KIAA0841		PLS-DA	91	176	1.93	7.78E-06
Hs.412832	1	PARC	p53-associated parkin-like cytoplasmic protein			108	133	1.24	8.63E-06
Hs.20930	1	PCBP4	poly(rC) binding protein 4			163	201	1.23	1.75E-05
Hs.164457	1	TK1	thymidine kinase 1, soluble			123	258	2.10	2.09E-05
Hs.443960	2	DDX11	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11 (CHL1-like helicase homolog, S. cerevisiae)			229	321	1.40	3.34E-05
Hs.8375	2	TRAF4	TNF receptor-associated factor 4			417	611	1.47	3.48E-05
Hs.343911	1	EI24	etoposide induced 2.4 mRNA			901	1357	1.51	3.48E-05
Hs.284208	1	ANKRD25	ankyrin repeat domain 25			299	634	2.12	5.70E-05
Hs.31097	1	NOD9	NOD9 protein			218	290	1.33	8.53E-05
Hs.278027	1	LIMK2	LIM domain kinase 2			87	128	1.47	9.88E-05
Hs.31130	1	TM7SF2	transmembrane 7 superfamily member 2			514	760	1.48	0.000105
Hs.232004	1	NRGN	neurogranin (protein kinase C substrate, RC3)			127	268	2.11	0.000143
Hs.50842	1	IFI35	interferon-induced protein 35			278	328	1.18	0.000206
Hs.23642	1	HSU79266	protein predicted by clone 23627		PLS-DA	640	1262	1.97	0.000219
Hs.224397	1	TCEA2	transcription elongation factor A (SII), 2			214	312	1.46	0.000226
Hs.110746	1	C6orf18	chromosome 6 open reading frame 18			146	183	1.25	0.000265
Hs.87246	1	BBC3	BCL2 binding component 3			159	206	1.29	0.000375
Hs.288382	1	FLJ13111	hypothetical protein FLJ13111		KNN	69	85	1.24	0.000424
Hs.256278	1	TNFRSF1B	tumor necrosis factor receptor superfamily, member 1B		PLS-DA	194	270	1.39	0.000476
Hs.413078	1	NUDT1	nudix (nucleoside diphosphate linked moiety X)-type motif 1			145	243	1.67	0.000544
Hs.439776	1	STOM	stomatin			206	229	1.11	0.000544
Hs.412228	1	KHK	ketohexokinase (fructokinase)		PLS-DA	68	118	1.73	0.000586
Hs.17466	1	RARRES3	retinoic acid receptor responder (tazarotene induced) 3			516	745	1.44	0.000716
	1		EST		KNN	194	242	1.25	0.000785
Hs.159428	2	BAX	BCL2-associated X protein			718	1119	1.56	0.000921
Hs.14623	1	IFI30	interferon, gamma-inducible protein 30			556	695	1.25	0.000931
Hs.7019	1	SIPA1	signal-induced proliferation-associated gene 1			53	88	1.66	0.000967
Hs.279413	1	POLD1	polymerase (DNA directed), delta 1, catalytic subunit 125kDa			279	651	2.33	0.00132
Hs.383396	1	RRM1	ribonucleotide reductase M1 polypeptide			1011	1579	1.56	0.00164
Hs.46465	1	TCIRG1	T-cell, immune regulator 1, ATPase, H+ transporting,			347	460	1.32	0.00188

UNIGENE	Repeats	SYMBOL	GENENAME	Extended Analysis Biomarker	Initial Analysis Biomarker	non-GTX Median	GTX Median	FC	Welch t-test p-value
			lysosomal V0 protein a isoform 3						
Hs.77783	1	PKMYT1	membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase			309	590	1.91	0.00195
Hs.26471	1	BBS4	Bardet-Biedl syndrome 4			129	149	1.16	0.00221
Hs.279032	1	HUMGT1 98A	GT198, complete ORF			172	289	1.68	0.00271
Hs.94292	1	FLJ23311	FLJ23311 protein		KNN; PLS-DA	410	589	1.43	0.00434
Hs.511950	1	SIRT3	sirtuin (silent mating type information regulation 2 homolog) 3 (S. cerevisiae)		PLS-DA	93	113	1.21	0.00529
Hs.32922	1	CARF	collaborates/cooperates with ARF (alternate reading frame) protein			331	397	1.20	0.00585
Hs.383913	1	BLM	Bloom syndrome			193	341	1.76	0.00614
Hs.387156	1	GM2A	GM2 ganglioside activator protein			149	223	1.49	0.00614
Hs.421342	1	STAT3	signal transducer and activator of transcription 3 (acute-phase response factor)			910	999	1.10	0.00697
Hs.460184	1	MCM4	MCM4 minichromosome maintenance deficient 4 (S. cerevisiae)			491	936	1.91	0.00721
Hs.386264	1	ZNF273	zinc finger protein 273			234	315	1.35	0.00829
Hs.108222	1	CTNNBIP 1	catenin, beta interacting protein 1			60	82	1.38	0.00863
Hs.143917	1	HELIC1	helicase, ATP binding 1			897	1349	1.50	0.00983
Hs.501565	1	DHTKD1	dehydrogenase E1 and transketolase domain containing 1			350	443	1.26	0.0101
Hs.122908	1	CDT1	DNA replication factor			234	495	2.11	0.0147
Hs.273397	1	USP52	ubiquitin specific protease 52			194	177	-1.10	0.0164
Hs.156346	1	TOP2A	topoisomerase (DNA) II alpha 170kDa			840	1603	1.91	0.0184
Hs.464813	2	DHFR	dihydrofolate reductase			1079	1896	1.76	0.022
Hs.1281	1	C5	complement component 5			80	134	1.67	0.022
Hs.2157	1	WAS	Wiskott-Aldrich syndrome (eczema-thrombocytopenia)			53	69	1.30	0.0228
Hs.47504	1	EXO1	exonuclease 1		KNN	209	404	1.93	0.0254
Hs.177926	2	LOC81691	exonuclease NEF-sp			170	221	1.30	0.0304
Hs.109058	1	RPS6KA5	ribosomal protein S6 kinase, 90kDa, polypeptide 5		KNN; PLS-DA	276	374	1.35	0.0337
Hs.149227	1	FLJ20406	hypothetical protein FLJ20406			68	90	1.33	0.0364
Hs.72249	1	PARD3	par-3 partitioning defective 3 homolog (C. elegans)			193	193	-1.00	0.0364
Hs.28491	1	SAT	spermidine/spermine N1-acetyltransferase			403	409	1.01	0.0487
Hs.211201	4	CYFIP2	cytoplasmic FMR1 interacting protein 2			683	880	1.29	0.0562
Hs.292119	1	NUP210	nucleoporin 210			133	200	1.50	0.0666
Hs.282997	2	GBA	glucosidase, beta; acid (includes glucosylceramidase)			287	349	1.22	0.0689

UNIGENE	Repeats	SYMBOL	GENENAME	Extended Analysis Biomarker	Initial Analysis Biomarker	non-GTX Median	GTX Median	FC	Welch t-test p-value
Hs.173464	1	FKBP8	FK506 binding protein 8, 38kDa			107	134	1.25	0.0737
Hs.21331	1	FLJ10036	hypothetical protein FLJ10036			479	656	1.37	0.0737
Hs.38178	1	KLIP1	KSHV latent nuclear antigen interacting protein 1			1185	2030	1.71	0.0745
Hs.288672	1	FLJ13909	hypothetical protein FLJ13909			68	159	2.33	0.0951
Hs.180402	1	FLJ23506	hypothetical protein FLJ23506			98	110	1.12	0.0972
Hs.34012	1	BRCA2	breast cancer 2, early onset			144	227	1.58	0.0981
Hs.89385	1	NPAT	nuclear protein, ataxia-telangiectasia locus			137	172	1.26	0.106
Hs.15535	1	TCEB3	transcription elongation factor B (SIII), polypeptide 3 (110kDa, elongin A)			117	117	-1.00	0.106
Hs.82502	1	POLD3	polymerase (DNA-directed), delta 3, accessory subunit		KNN	436	629	1.44	0.13
Hs.296365	2	ZNF324	zinc finger protein 324			122	113	-1.08	0.132
Hs.275675	1	KATNB1	katanin p80 (WD repeat containing) subunit B 1			62	77	1.26	0.134
Hs.66718	1	RAD54L	RAD54-like (S. cerevisiae)			206	340	1.65	0.135
Hs.334828	1	FLJ10719	hypothetical protein FLJ10719		KNN	458	680	1.48	0.174
Hs.226390	1	RRM2	ribonucleotide reductase M2 polypeptide			1629	3230	1.98	0.177
Hs.405467	1	FLJ10858	DNA glycosylase hFPG2			193	266	1.37	0.19
Hs.90598	1	MICA	MHC class I polypeptide-related sequence A			140	152	1.09	0.193
Hs.106185	2	RALGDS	ral guanine nucleotide dissociation stimulator			222	262	1.18	0.211
Hs.231444	1	E2F2	E2F transcription factor 2			67	106	1.58	0.224
Hs.337242	1	CENTB1	centaurin, beta 1			403	461	1.14	0.256
Hs.348920	1	FSHPRH1	FSH primary response (LRPR1 homolog, rat) 1			88	137	1.55	0.317
Hs.376719	1	MAGED2	melanoma antigen, family D, 2			428	434	1.01	0.318
Hs.142245	1	HHLA3	HERV-H LTR-associating 3		PLS-DA	219	228	1.04	0.319
Hs.46446	1	LYL1	lymphoblastic leukemia derived sequence 1			54	66	1.23	0.339
Hs.408658	1	CCNE2	cyclin E2			155	221	1.43	0.36
Hs.54089	1	BARD1	BRCA1 associated RING domain 1			392	582	1.48	0.379
Hs.79625	1	C20orf149	chromosome 20 open reading frame 149			767	840	1.10	0.405
Hs.442993	1	MXD3	MAX dimerization protein 3			92	127	1.39	0.423
Hs.107911	1	ABCB6	ATP-binding cassette, sub-family B (MDR/TAP), member 6			315	364	1.16	0.462
Hs.115740	1	TBC1D5	TBC1 domain family, member 5		PLS-DA	345	404	1.17	0.764

We claim:

1. A method of predicting genotoxicity of a compound using a predictor model, comprising:

identifying a plurality of biomarker genes that display an altered expression profile when exposed to a genotoxic compound or a non-genotoxic compound from a calibration set of samples;

identifying a sub-set of biomarker genes from the calibration set that display an altered expression profile when exposed to a genotoxic compound or a non-genotoxic compound from a validation set of samples;

classifying the biomarker genes identified in the validation set of samples as those that respond to a genotoxic compound or a non-genotoxic compound; and

using the classified biomarker genes to identify the genotoxicity of a test compound by exposing the test compound to cell sample and comparing the expression profile of the biomarker genes in the sample with those identified in the validation set of samples.

2. The method of claim 1, wherein the classified biomarker genes are selected from the group consisting of biomarker-1 (BM1) genes, biomarker-2 (BM2) genes and biomarker-3 (BM3) genes.

3. The method of claim 2, wherein the biomarker-1 (BM1) genes are selected from the group consisting of Xeroderma pigmentosum, complementation group C, ferredoxin reductase, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3C, hypothetical protein MGC5370, damage-specific DNA binding protein 2, 48kDa, transcribed locus, papilin, proteoglycan-like sulfated glycoprotein, fucosidase, alpha-L-1, tissue, carboxypeptidase M, tumor protein p53 inducible protein 3, cyclin-dependent kinase inhibitor 1A (p21, Cip1), phosphatidylinositol glycan, class F, interleukin 6 signal transducer (gp130, oncostatin M receptor), hypothetical protein FLJ10375, vacuolar protein sorting 54 (yeast), hv89d09, interleukin 6 signal transducer (gp130, oncostatin M receptor), phosphatidylserine receptor, alpha-cardiac actin, hypothetical protein FLJ11383, ras homolog gene family, member Q, thioredoxin interacting protein, hypothetical protein LOC339290, NCK-associated protein 1, TBC1 domain family,

member 17, ectodermal-neural cortex (with BTB-like domain), thioredoxin interacting protein, phosphatidylinositol glycan, class F, phosphatidylinositol glycan, class F, and solute carrier family 33 (acetyl-CoA transporter), member 1.

4. The method of claim 3, wherein the biomarker-1 (BM1) genes are selected from the group consisting of Xeroderma pigmentosum, complementation group C, ferredoxin reductase, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3C, hypothetical protein MGC5370, and damage-specific DNA binding protein 2, 48kDa.

5. The method of claim 2, wherein the biomarker-2 (BM2) genes are selected from the group consisting of EST370545, H. sapiens adenosine deaminase (ADA), Homo sapiens chromosome 12 open reading frame 5 mRNA, polymerase (DNA directed), eta, isocitrate dehydrogenase 1 (NADP+), carboxypeptidase M, plexin B2, polymerase (DNA directed), eta, hypothetical protein FLJ12484, KIAA0907 protein, transcribed locus, ARP9, wb67g03, leucine-rich repeats and death domain containing, potassium large conductance calcium-activated channel, subfamily M beta member 3, KAT11914, mitochondrial carrier triple repeat 1, tax1 (human T-cell leukemia virus type I) binding protein 3, sestrin 1, ret finger protein, SMAD, H. sapiens mitogen inducible gene mig-2, FLJ10378 protein, hypothetical protein MGC7036, ubiquitin-conjugating enzyme, KIAA0368, phosphatidylserine receptor, O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase), Mdm2, hypothetical protein LOC51061, NudE nuclear distribution gene E homolog like 1 (A. nidulans), HTPAP protein, and syndecan 1.

6. The method of claim 5, wherein the biomarker-2 (BM2) genes are selected from the group consisting of EST370545, H. sapiens adenosine deaminase (ADA), Homo sapiens chromosome 12 open reading frame 5 mRNA, polymerase (DNA directed), eta, and isocitrate dehydrogenase 1 (NADP+).

7. The method of claim 2, wherein the biomarker-3 (BM3) genes are selected from the group consisting of LAG1 longevity assurance homolog 5 (S. cerevisiae), hypothetical protein HSPC132, FKSG44 gene, adenosine deaminase, pleckstrin homology-like domain, ectodermal-neural cortex (with BTB-like domain), F-box protein 22,

ribonucleotide reductase M2 B (TP53 inducible), guanidinoacetate N-methyltransferase, transmembrane 7 superfamily member 3, isocitrate dehydrogenase 1 (NADP+), phosphohistidine phosphatase 1, hypothetical protein FLJ20296, discoidin domain receptor family, member 1, transcribed locus, guanidinoacetate N-methyltransferase, human receptor tyrosine kinase DDR gene, transmembrane 7 superfamily member 3, 601565341F1 NIH_MGC_21 Homo sapiens cDNA clone, F-box protein 22, cytosolic sialic acid 9-O-acetylesterase homolog, BTG family member 2, astrotactin 2, IKK interacting protein, surfeit 4, neutral sphingomyelinase (N-SMase) activation associated factor, ADP-ribosylation factor-like 1, golgi reassembly stacking protein 2, leucine-rich repeats and death domain containing, mixed-lineage leukemia, hypothetical protein LOC253981, placenta-specific 8, glutathione peroxidase 1, KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2, syntaxin 7, lysosomal-associated multispanning membrane protein-5, and phosphoinositide-3-kinase catalytic alpha polypeptide.

8. The method of claim 7, wherein the biomarker-3 (BM3) genes are selected from the group consisting of LAG1 longevity assurance homolog 5 (S. cerevisiae), hypothetical protein HSPC132, FKSG44 gene, and adenosine deaminase.

9. A method of predicting genotoxicity of a compound using a predictor model, comprising:

exposing a test compound to a first set of a plurality of biomarker genes selected from the group consisting of biomarker-1 (BM1) genes, biomarker-2 (BM2) genes and biomarker-3 (BM3) genes;

comparing the distribution of biomarker genes against the distribution of gene expression of a known reference compound; and

separating the test compound into a class of compound based on the expression of the biomarker genes, wherein the class of compound is genotoxic compound or a non-genotoxic compound.

10. The method of claim 9, wherein the biomarker-1 (BM1) genes are selected from the group consisting of Xeroderma pigmentosum, complementation group C, ferredoxin reductase, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3C,

hypothetical protein MGC5370, damage-specific DNA binding protein 2, 48kDa, transcribed locus, papilin, proteoglycan-like sulfated glycoprotein, fucosidase, alpha-L-1, tissue, carboxypeptidase M, tumor protein p53 inducible protein 3, cyclin-dependent kinase inhibitor 1A (p21, Cip1), phosphatidylinositol glycan, class F, interleukin 6 signal transducer (gp130, oncostatin M receptor), hypothetical protein FLJ10375, vacuolar protein sorting 54 (yeast), hv89d09, interleukin 6 signal transducer (gp130, oncostatin M receptor), phosphatidylserine receptor, alpha-cardiac actin, hypothetical protein FLJ11383, ras homolog gene family, member Q, thioredoxin interacting protein, hypothetical protein LOC339290, NCK-associated protein 1, TBC1 domain family, member 17, ectodermal-neural cortex (with BTB-like domain), thioredoxin interacting protein, phosphatidylinositol glycan, class F, phosphatidylinositol glycan, class F, and solute carrier family 33 (acetyl-CoA transporter), member 1.

11. The method of claim 10, wherein the biomarker-1 (BM1) genes are selected from the group consisting of Xeroderma pigmentosum, complementation group C, ferredoxin reductase, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3C, hypothetical protein MGC5370, and damage-specific DNA binding protein 2, 48kDa.

12. The method of claim 9, wherein the biomarker-2 (BM2) genes are selected from the group consisting of EST370545, H. sapiens adenosine deaminase (ADA), Homo sapiens chromosome 12 open reading frame 5 mRNA, polymerase (DNA directed), eta, isocitrate dehydrogenase 1 (NADP+), carboxypeptidase M, plexin B2, polymerase (DNA directed), eta, hypothetical protein FLJ12484, KIAA0907 protein, transcribed locus, ARP9, wb67g03, leucine-rich repeats and death domain containing potassium large conductance calcium-activated channel, subfamily M beta member 3, KAT11914, mitochondrial carrier triple repeat 1, tax1 (human T-cell leukemia virus type I) binding protein 3, sestrin 1, ret finger protein, SMAD, H. sapiens mitogen inducible gene mig-2, FLJ10378 protein, hypothetical protein MGC7036, ubiquitin-conjugating enzyme, KIAA0368, phosphatidylserine receptor, O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase), Mdm2, hypothetical protein LOC51061, NudE nuclear distribution gene E homolog like 1 (A. nidulans), HTPAP protein, and syndecan 1.

13. The method of claim 12, wherein the biomarker-2 (BM2) genes are selected from the group consisting of EST370545, H. sapiens adenosine deaminase (ADA), Homo sapiens chromosome 12 open reading frame 5 mRNA, polymerase (DNA directed), eta, and isocitrate dehydrogenase 1 (NADP+).

14. The method of claim 9, wherein the biomarker-3 (BM3) genes are selected from the group consisting of LAG1 longevity assurance homolog 5 (S. cerevisiae), hypothetical protein HSPC132, FKSG44 gene, adenosine deaminase, pleckstrin homology-like domain, ectodermal-neural cortex (with BTB-like domain), F-box protein 22, ribonucleotide reductase M2 B (TP53 inducible), guanidinoacetate N-methyltransferase, transmembrane 7 superfamily member 3, isocitrate dehydrogenase 1 (NADP+), phosphohistidine phosphatase 1, hypothetical protein FLJ20296, discoidin domain receptor family, member 1, transcribed locus, guanidinoacetate N-methyltransferase, human receptor tyrosine kinase DDR gene, transmembrane 7 superfamily member 3, 601565341F1 NIH_MGC_21 Homo sapiens cDNA clone, F-box protein 22, cytosolic sialic acid 9-O-acetylesterase homolog, BTG family member 2, astrotactin 2, IKK interacting protein, surfeit 4, neutral sphingomyelinase (N-SMase) activation associated factor, ADP-ribosylation factor-like 1, golgi reassembly stacking protein 2, leucine-rich repeats and death domain containing mixed-lineage leukemia, hypothetical protein LOC253981, placenta-specific 8, glutathione peroxidase 1, KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2, syntaxin 7, lysosomal-associated multispanning membrane protein-5, and phosphoinositide-3-kinase catalytic alpha polypeptide.

15. The method of claim 14, wherein the biomarker-3 (BM3) genes are selected from the group consisting of LAG1 longevity assurance homolog 5 (S. cerevisiae), hypothetical protein HSPC132, FKSG44 gene, and adenosine deaminase.

16. The method of claim 9, wherein the reference compounds are selected from the group consisting of genotoxic reference compounds and non-genotoxic reference compounds.

17. The method of claim 9, wherein the genotoxic reference compounds are selected from the group consisting of actinomycin-D, bleomycin, cis-Platin, daunorubicin, doxorubicin, ENU/Ethyl nitroso urea, methylmethane sulfonate, mitomycin C, mitoxantrone, and styrene oxide.

18. The method of claim 9, wherein the non-genotoxic reference compounds are selected from the group consisting of diflunisal, flufenamic acid, potassium chloride, N-acetylcysteine, sodium chloride, ranitidine, rifampicin, trans-platin, and verapamil.

19. A method of predicting genotoxicity of a compound using a predictor model, comprising:

exposing a test compound to a plurality of biomarker-1 (BM1) genes selected from the group consisting of Xeroderma pigmentosum, complementation group C, ferredoxin reductase, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3C, hypothetical protein MGC5370, damage-specific DNA binding protein 2, 48kDa, transcribed locus, papilin, proteoglycan-like sulfated glycoprotein, fucosidase, alpha-L-1, tissue, carboxypeptidase M, tumor protein p53 inducible protein 3, cyclin-dependent kinase inhibitor 1A (p21, Cip1), phosphatidylinositol glycan, class F, interleukin 6 signal transducer (gp130, oncostatin M receptor), hypothetical protein FLJ10375, vacuolar protein sorting 54 (yeast), hv89d09, interleukin 6 signal transducer (gp130, oncostatin M receptor), phosphatidylserine receptor, alpha-cardiac actin, hypothetical protein FLJ11383, ras homolog gene family, member Q, thioredoxin interacting protein, hypothetical protein LOC339290, NCK-associated protein 1, TBC1 domain family, member 17, ectodermal-neural cortex (with BTB-like domain), thioredoxin interacting protein, phosphatidylinositol glycan, class F, phosphatidylinositol glycan, class F, and solute carrier family 33 (acetyl-CoA transporter), member 1;

comparing the distribution of biomarker genes against the distribution of gene expression of a known reference compound; and

separating the test compound into a class of compound based on the expression of the biomarker genes, wherein the class of compound is genotoxic compound or a non-genotoxic compound.

20. A method of predicting genotoxicity of a compound using a predictor model, comprising:

exposing a test compound to a plurality of biomarker-2 (BM2) genes selected from the group consisting of EST370545, H. sapiens adenosine deaminase (ADA), Homo sapiens chromosome 12 open reading frame 5 mRNA, polymerase (DNA directed), eta, isocitrate dehydrogenase 1 (NADP+), carboxypeptidase M, plexin B2, polymerase (DNA directed), eta, hypothetical protein FLJ12484, KIAA0907 protein, transcribed locus, ARP9, wb67g03, leucine-rich repeats and death domain containing potassium large conductance calcium-activated channel, subfamily M beta member 3, KAT11914, mitochondrial carrier triple repeat 1, tax1 (human T-cell leukemia virus type I) binding protein 3, sestrin 1, ret finger protein, SMAD, H. sapiens mitogen inducible gene mig-2, FLJ10378 protein, hypothetical protein MGC7036, ubiquitin-conjugating enzyme, KIAA0368, phosphatidylserine receptor, O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase), Mdm2, hypothetical protein LOC51061, NudE nuclear distribution gene E homolog like 1 (A. nidulans), HTPAP protein, and syndecan 1;

comparing the distribution of biomarker genes against the distribution of gene expression of a known reference compound; and

separating the test compound into a class of compound based on the expression of the biomarker genes, wherein the class of compound is genotoxic compound or a non-genotoxic compound.

21. A method of predicting genotoxicity of a compound using a predictor model, comprising:

exposing a test compound to a plurality of biomarker-3 (BM3) genes selected from the group consisting of LAG1 longevity assurance homolog 5 (S. cerevisiae), hypothetical protein HSPC132, FKSG44 gene, adenosine deaminase, pleckstrin homology-like domain, ectodermal-neural cortex (with BTB-like domain), F-box protein 22, ribonucleotide reductase M2 B (TP53 inducible), guanidinoacetate N-methyltransferase, transmembrane 7 superfamily member 3, isocitrate dehydrogenase 1 (NADP+), phosphohistidine phosphatase 1, hypothetical protein FLJ20296, discoidin domain receptor family, member 1, transcribed locus, guanidinoacetate N-methyltransferase, human receptor tyrosine kinase DDR gene, transmembrane 7 superfamily member 3, 601565341F1 NIH_MGC_21 Homo sapiens cDNA clone, F-box

protein 22, cytosolic sialic acid 9-O-acetylesterase homolog, BTG family member 2, astrotactin 2, IKK interacting protein, surfeit 4, neutral sphingomyelinase (N-SMase) activation associated factor, ADP-ribosylation factor-like 1, golgi reassembly stacking protein 2, leucine-rich repeats and death domain containing, mixed-lineage leukemia, hypothetical protein LOC253981, placenta-specific 8, glutathione peroxidase 1, KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2, syntaxin 7, lysosomal-associated multispanning membrane protein-5, and phosphoinositide-3-kinase catalytic alpha polypeptide;

comparing the distribution of biomarker genes against the distribution of gene expression of a known reference compound; and

separating the test compound into a class of compound based on the expression of the biomarker genes, wherein the class of compound is genotoxic compound or a non-genotoxic compound.

22. A method of identifying a discriminatory set of cellular components, wherein the discriminatory set is used to characterize a candidate agent, the method comprising the steps of:

a) providing at least one model toxic compound;

b) evaluating a concentration at which the compound exerts a predetermined extent of toxicity on a cell;

c) exposing the cell to the predetermined toxic concentration of the compound;

d) isolating a class of cellular component from the cell and separately evaluating the presence, absence or concentration of a plurality of members of the class; and

e) identifying those members of the class that contribute to characterization of the compound; thereby providing the discriminatory set.

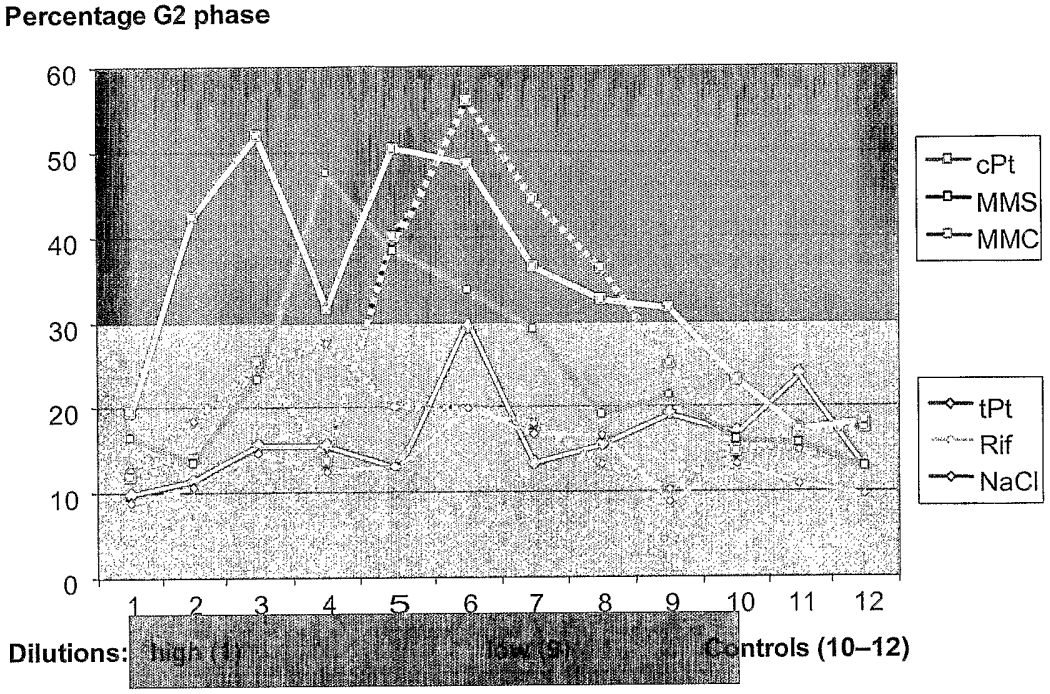
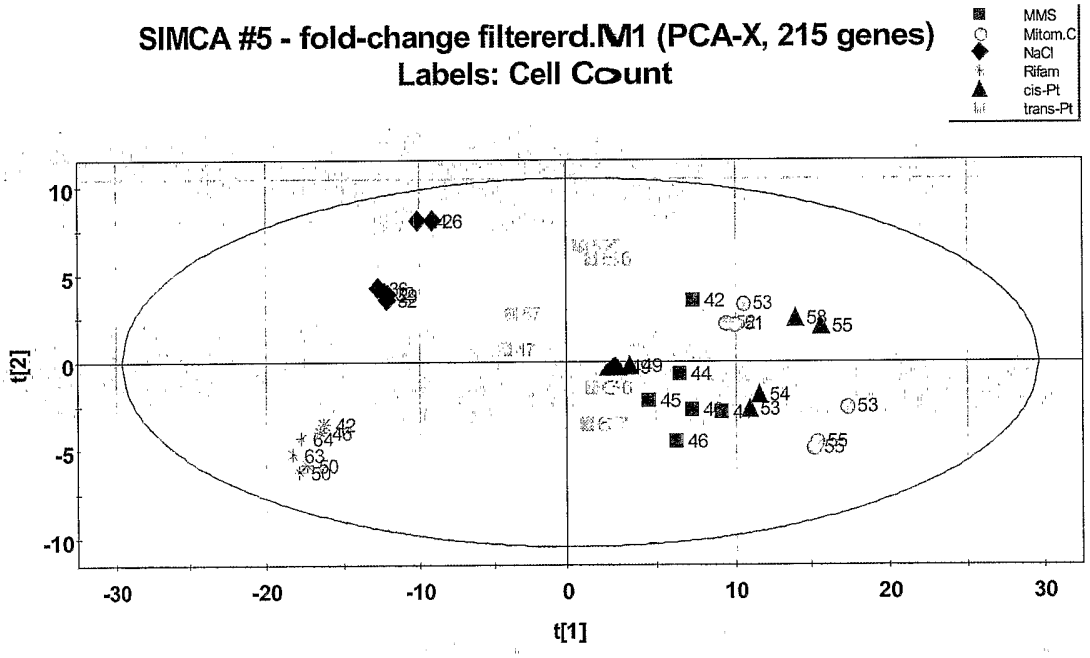

Percentage G2 phase
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
cPt
MMS
MMC
tPt
Rif
NaCl
Dilutions:
high (1)
low (9)
Controls (10–12)

Figure 1


SIMCA #5 - fold-change filtererd.M1 (PCA-X, 215 genes)
Labels: Cell Count
MMS
Mitom.C
NaCl
Rifam
cis-Pt
trans-Pt
t[1]
t[2]

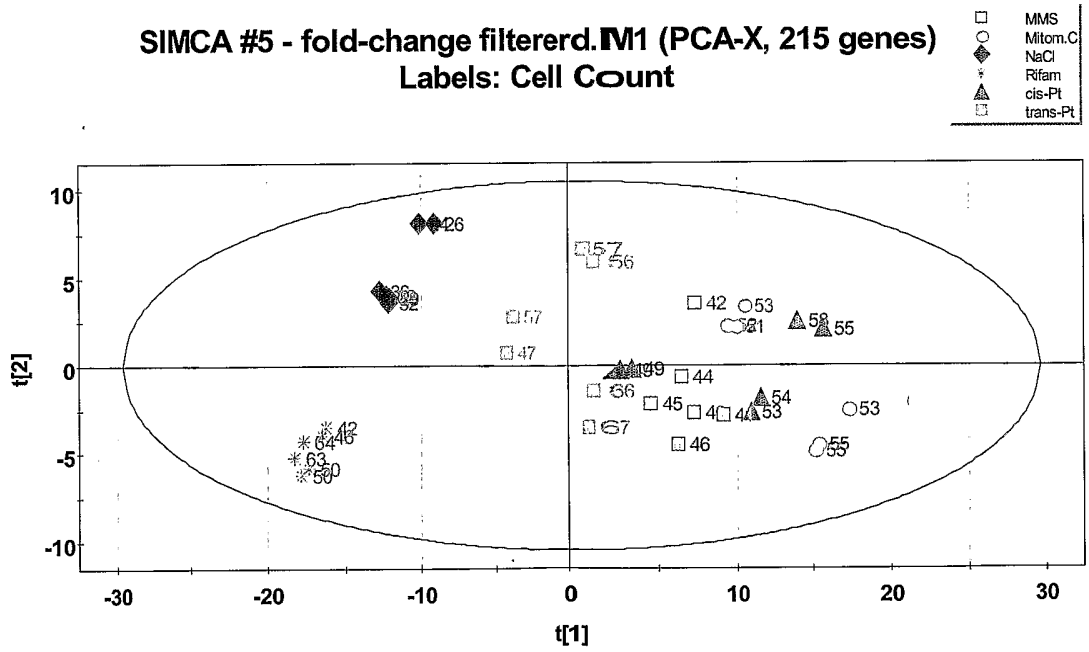
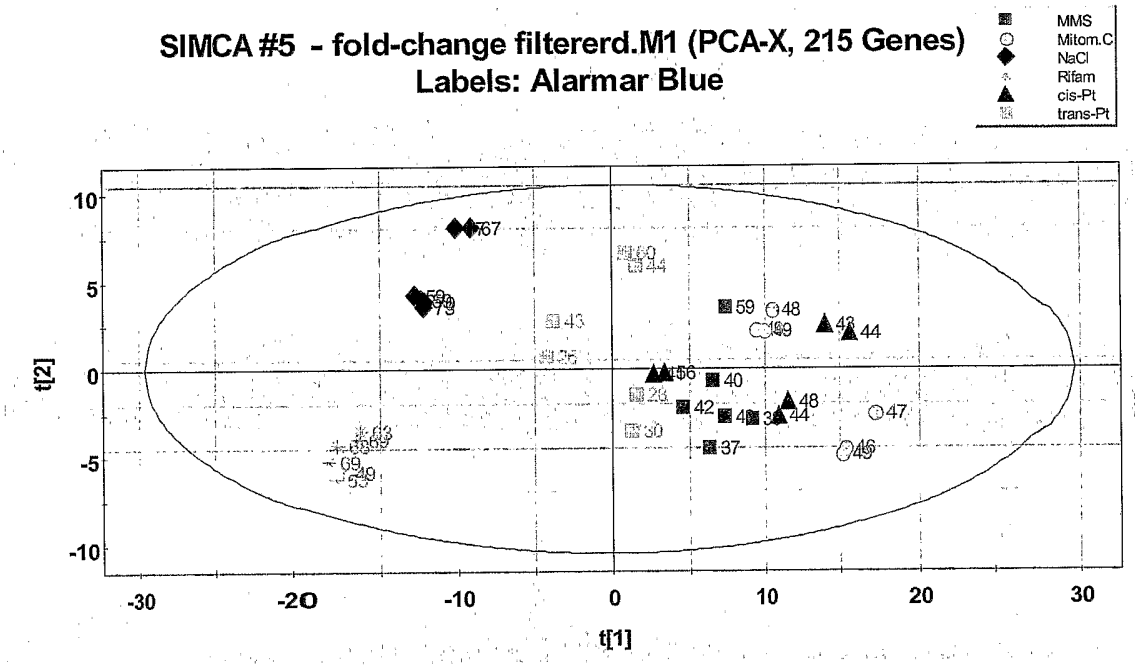

SIMCA #5 - fold-change filtererd.M1 (PCA-X, 215 genes)
Labels: Cell Count
MMS
Mitom.C
NaCl
Rifam
cis-Pt
trans-Pt
t[1]
t[2]

Figure 2


SIMCA #5 - fold-change filtererd.M1 (PCA-X, 215 Genes)
Labels: Alarmar Blue
MMS
Mitom.C
NaCl
Rifam
cis-Pt
trans-Pt
t[2]
t[1]
10
5
0
-5
-10
-30
-20
-10
0
10
20
30

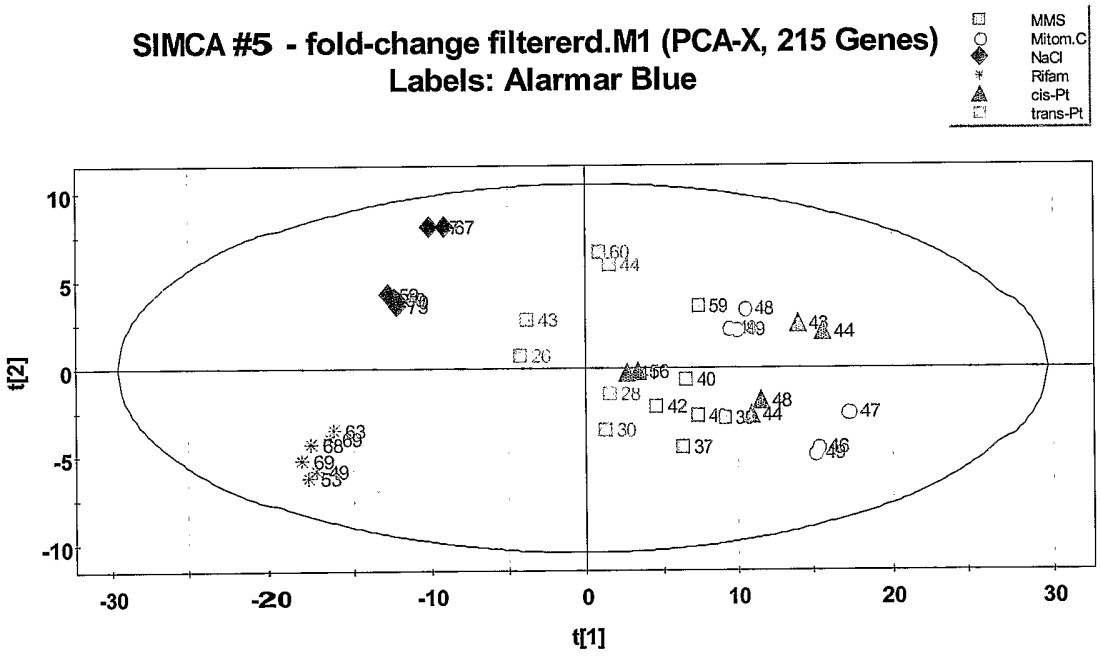

SIMCA #5 - fold-change filtererd.M1 (PCA-X, 215 Genes)
Labels: Alarmar Blue
MMS
Mitom.C
NaCl
Rifam
cis-Pt
trans-Pt
t[2]
t[1]
10
5
0
-5
-10
-30
-20
-10
0
10
20
30

Figure 3

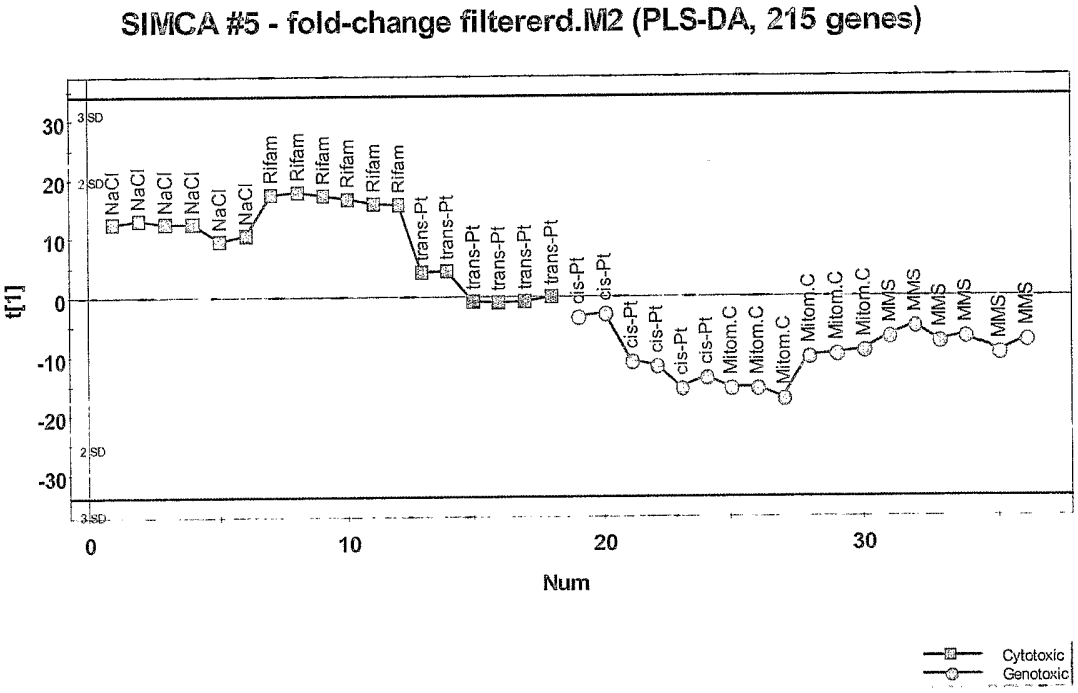

SIMCA #5 - fold-change filtererd.M2 (PLS-DA, 215 genes)
t[1]
Num
3 SD
2 SD
2 SD
3 SD
NaCl
Rifam
trans-Pt
cis-Pt
Mitom.C
MMS
Cytotoxic
Genotoxic

Figure 4

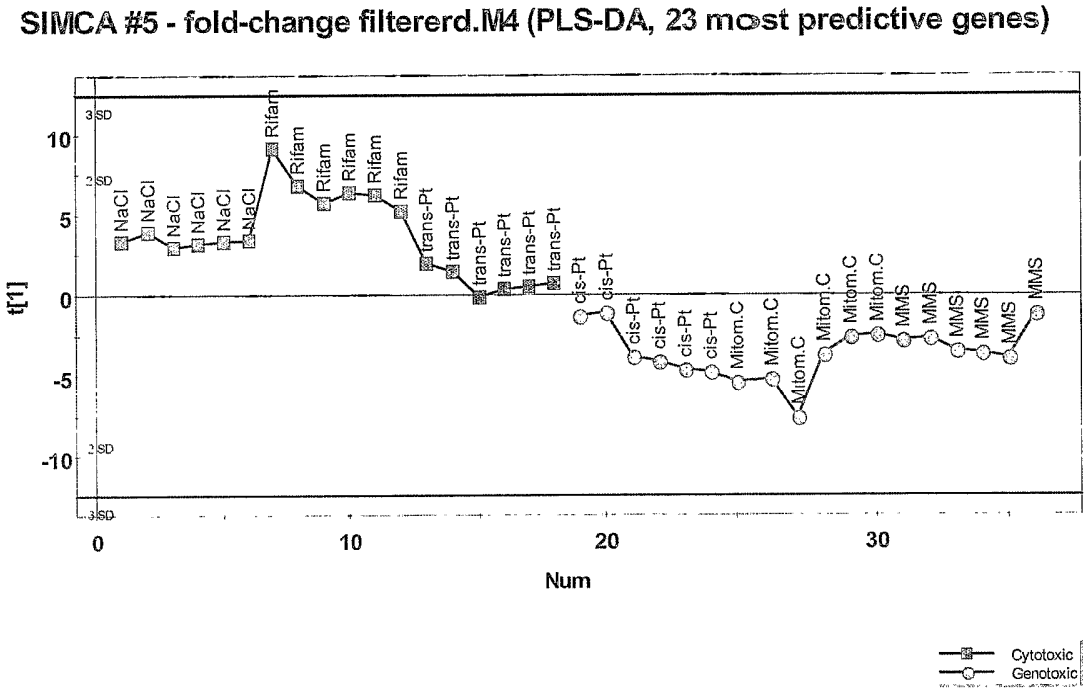

SIMCA #5 - fold-change filtererd.M4 (PLS-DA, 23 most predictive genes)
t[1]
Num
3 SD
2 SD
2 SD
3 SD
10
5
0
-5
-10
0
10
20
30
NaCl
Rifam
trans-Pt
cis-Pt
Mitom.C
MMS
Cytotoxic
Genotoxic

Figure 5

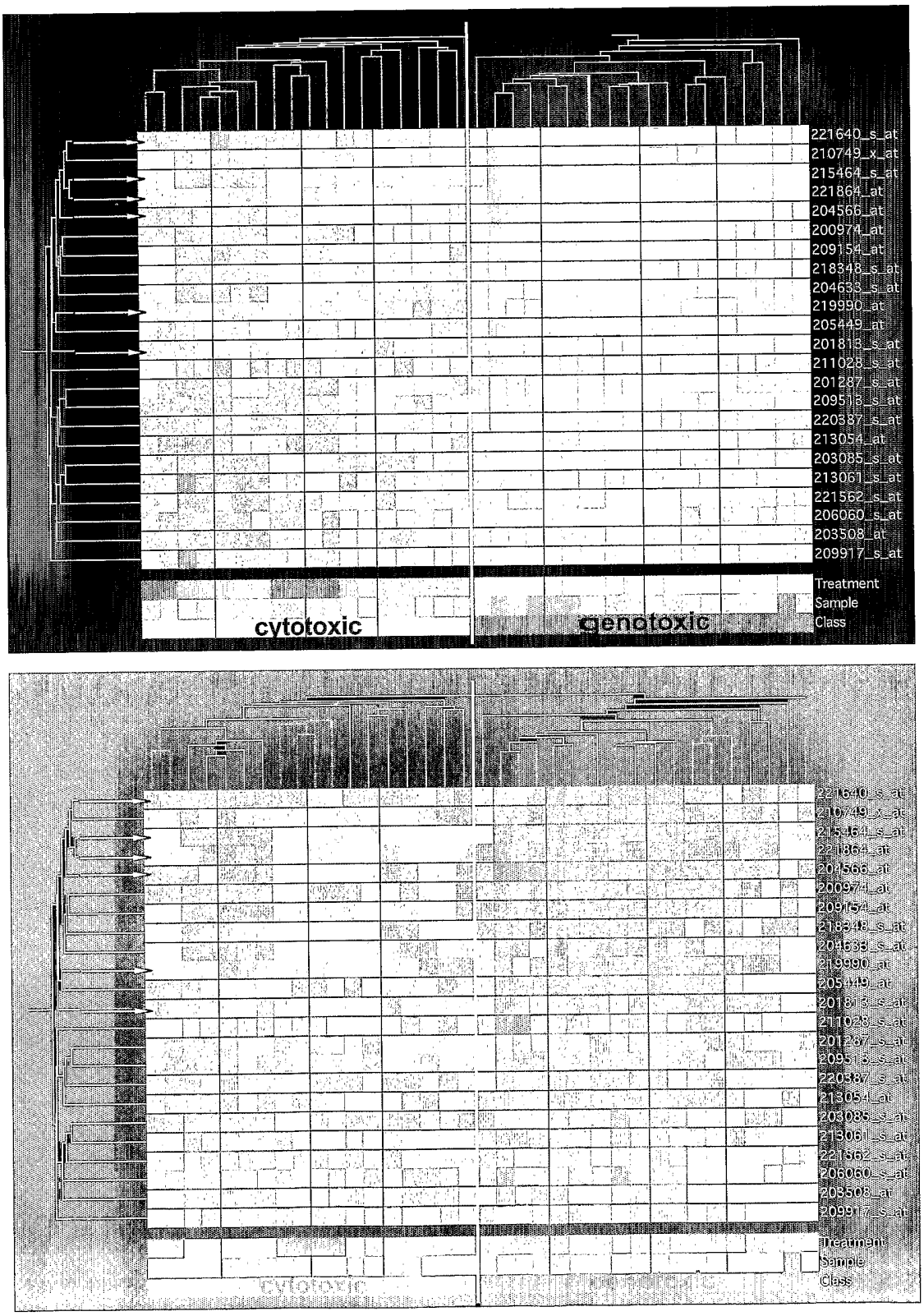

221640_s_at
210749_x_at
215464_s_at
221864_at
204566_at
200974_at
209154_at
218348_s_at
204633_s_at
219990_at
205449_at
201813_s_at
211028_s_at
201287_s_at
209518_s_at
220387_s_at
213054_at
203085_s_at
213061_s_at
221562_s_at
206060_s_at
203508_at
209917_s_at
Treatment
Sample
Class
cytotoxic
genotoxic

Figure 6

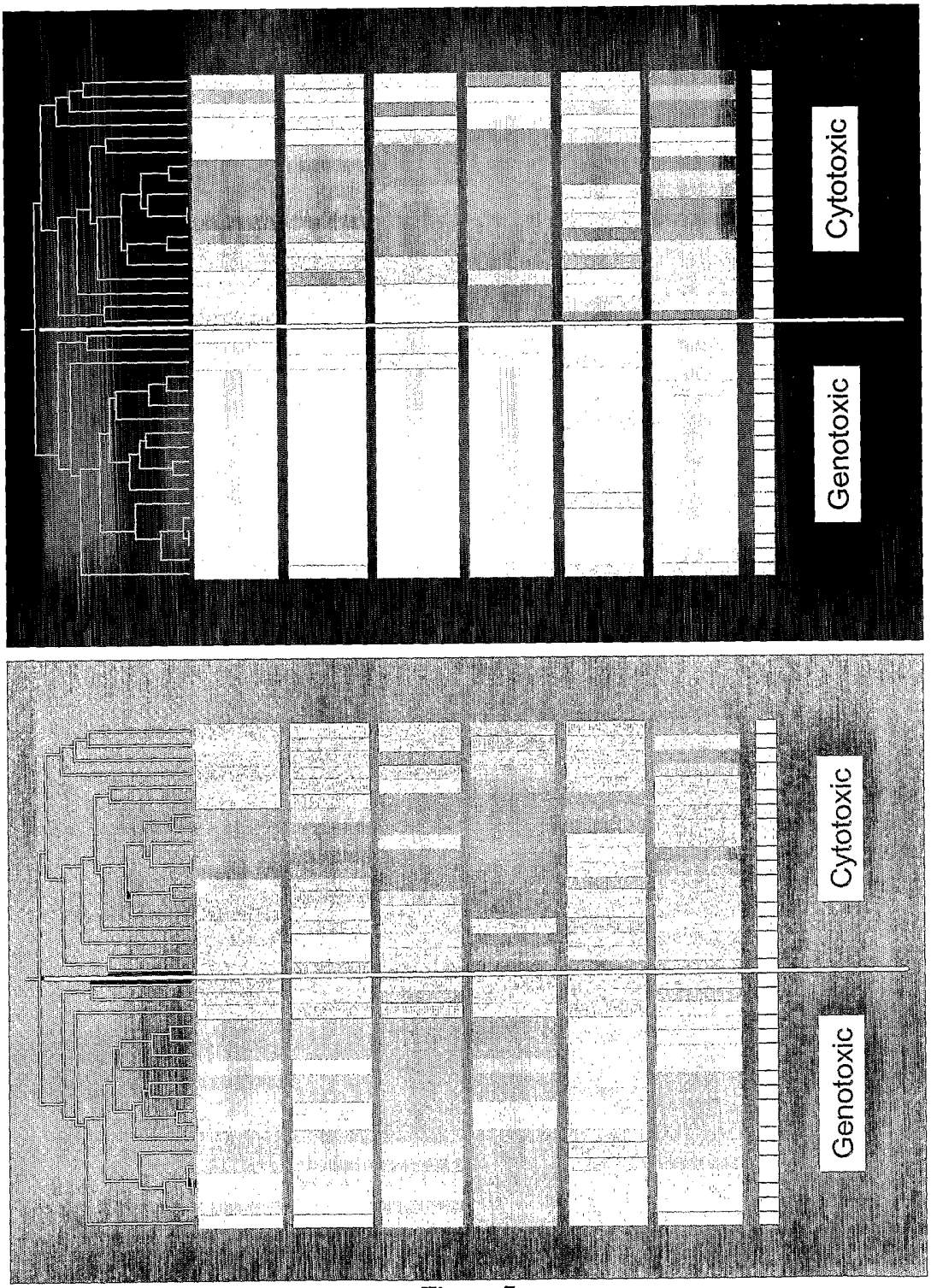


Figure 7

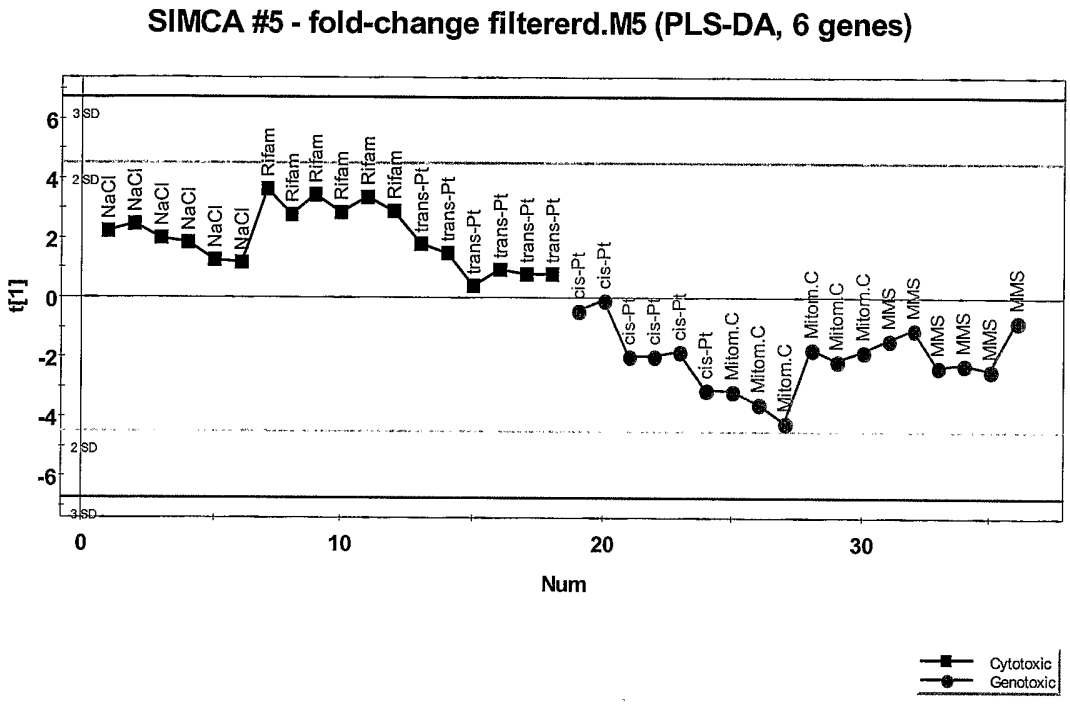

SIMCA #5 - fold-change filtererd.M5 (PLS-DA, 6 genes)
t[1]
Num
3 SD
2 SD
2 SD
3 SD
NaCl
Rifam
trans-Pt
cis-Pt
Mitom.C
MMS
Cytotoxic
Genotoxic

Figure 8

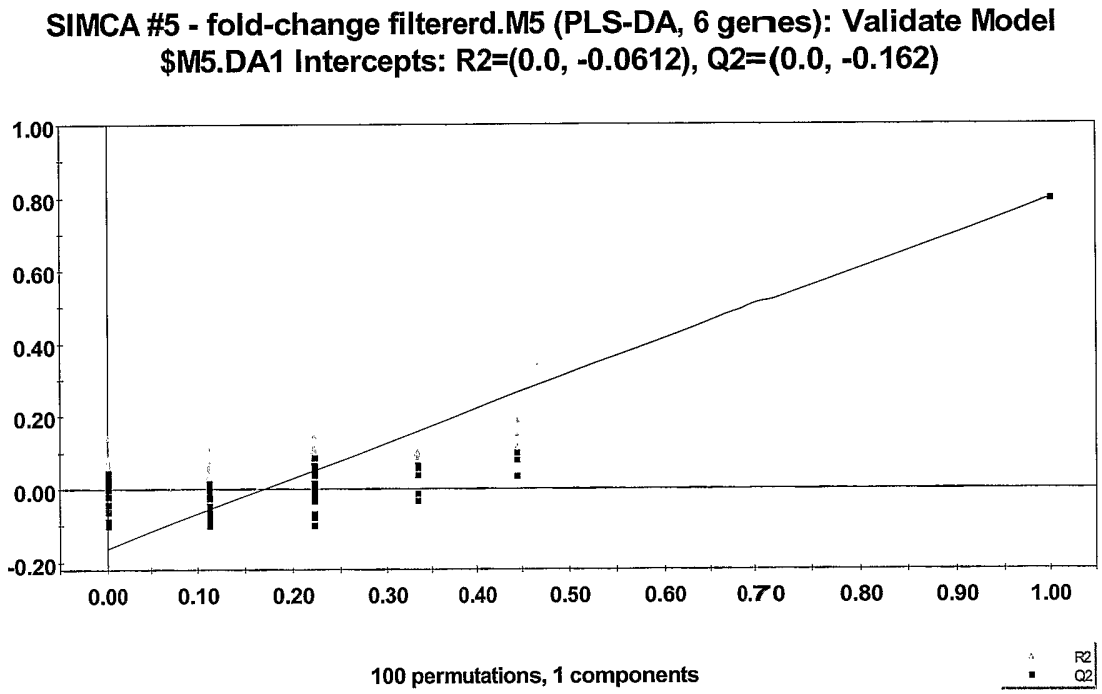

SIMCA #5 - fold-change filtererd.M5 (PLS-DA, 6 genes): Validate Model
$M5.DA1 Intercepts: R2=(0.0, -0.0612), Q2=(0.0, -0.162)
1.00
0.80
0.60
0.40
0.20
0.00
-0.20
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
1.00
100 permutations, 1 components
R2
Q2

Figure 9

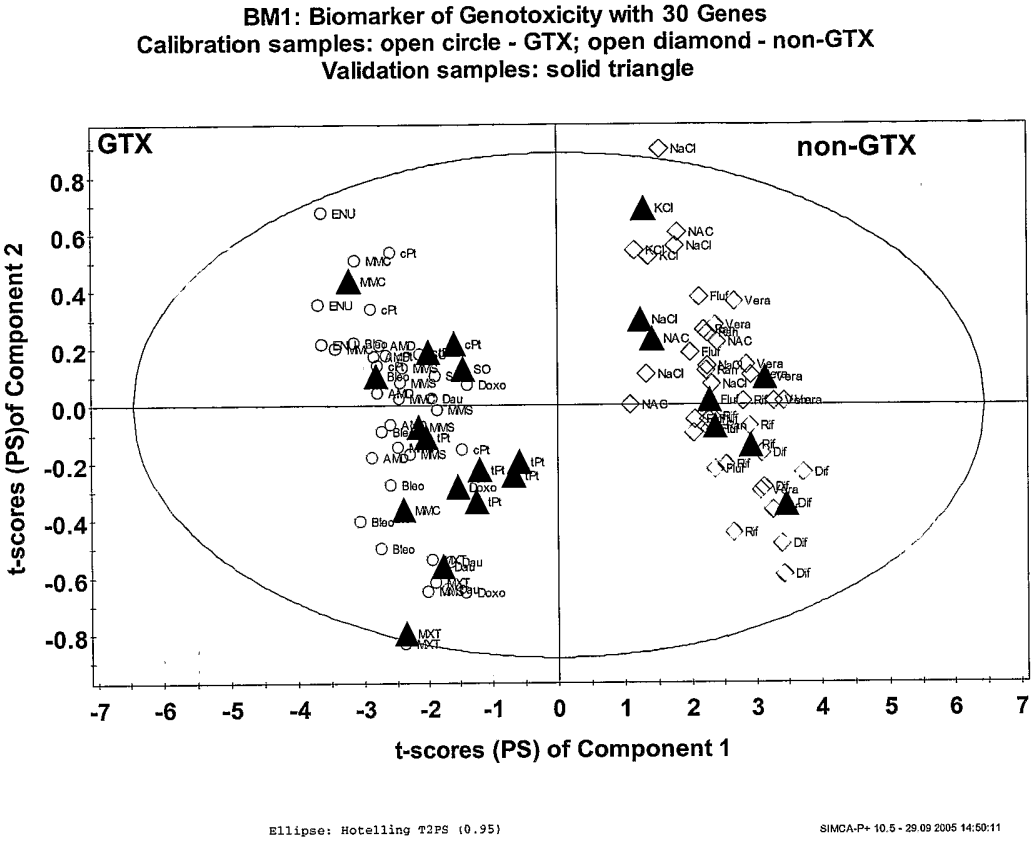

BM1: Biomarker of Genotoxicity with 30 Genes
Calibration samples: open circle - GTX; open diamond - non-GTX
Validation samples: solid triangle
GTX
non-GTX
t-scores (PS)of Component 2
t-scores (PS) of Component 1
Ellipse: Hotelling T2PS (0.95)
SIMCA-P+ 10.5 - 29.09.2005 14:50:11

Figure 10A

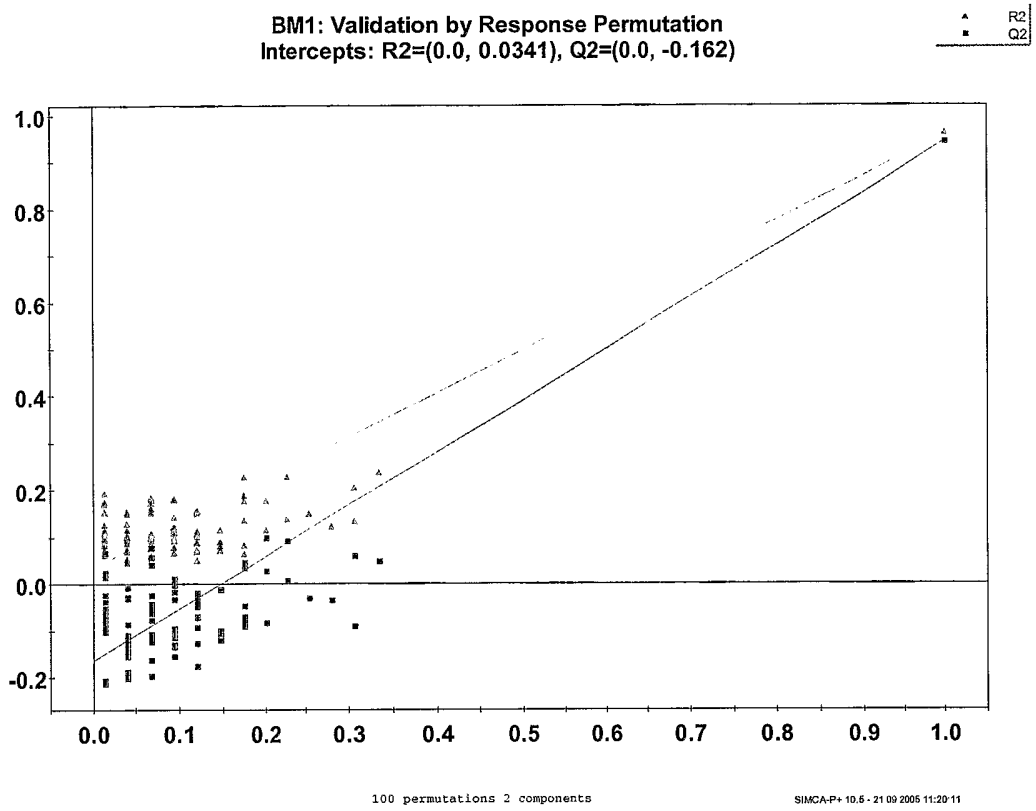

BM1: Validation by Response Permutation
Intercepts: R2=(0.0, 0.0341), Q2=(0.0, -0.162)
R2
Q2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
100 permutations 2 components
SIMCA-P+ 10.5 - 21 09 2005 11:20:11

Figure 10B

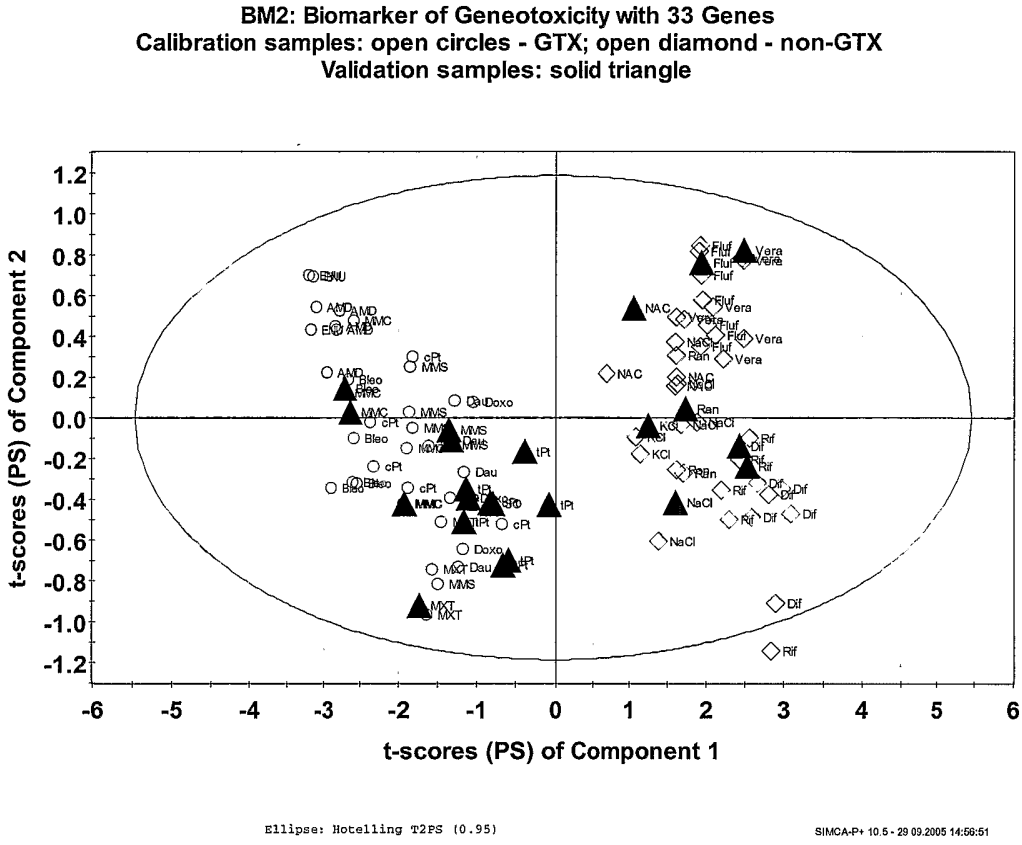

BM2: Biomarker of Geneotoxicity with 33 Genes
Calibration samples: open circles - GTX; open diamond - non-GTX
Validation samples: solid triangle
t-scores (PS) of Component 2
t-scores (PS) of Component 1
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-6
-5
-4
-3
-2
-1
0
1
2
3
4
5
6
Ellipse: Hotelling T2PS (0.95)
SIMCA-P+ 10.5 - 29.09.2005 14:56:51

Figure 11A

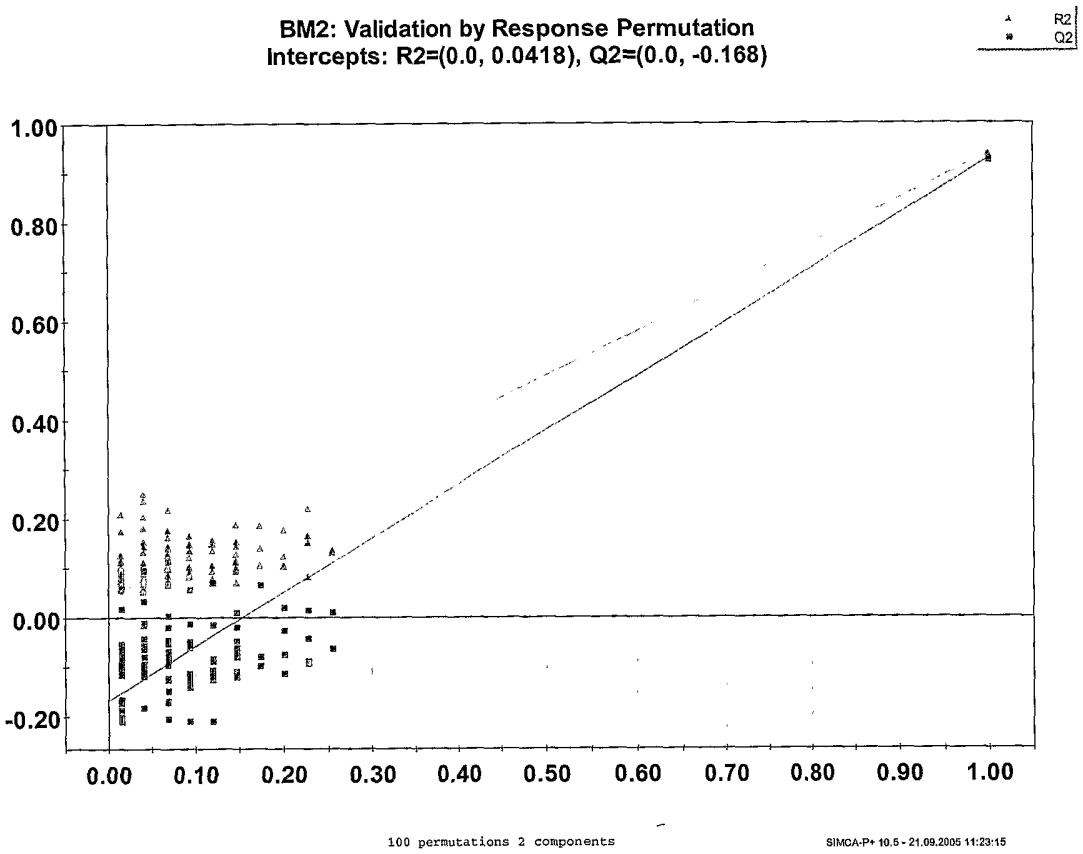

BM2: Validation by Response Permutation
Intercepts: R2=(0.0, 0.0418), Q2=(0.0, -0.168)
R2
Q2
1.00
0.80
0.60
0.40
0.20
0.00
-0.20
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
1.00
100 permutations 2 components
SIMCA-P+ 10.5 - 21.09.2005 11:23:15

Figure 11B

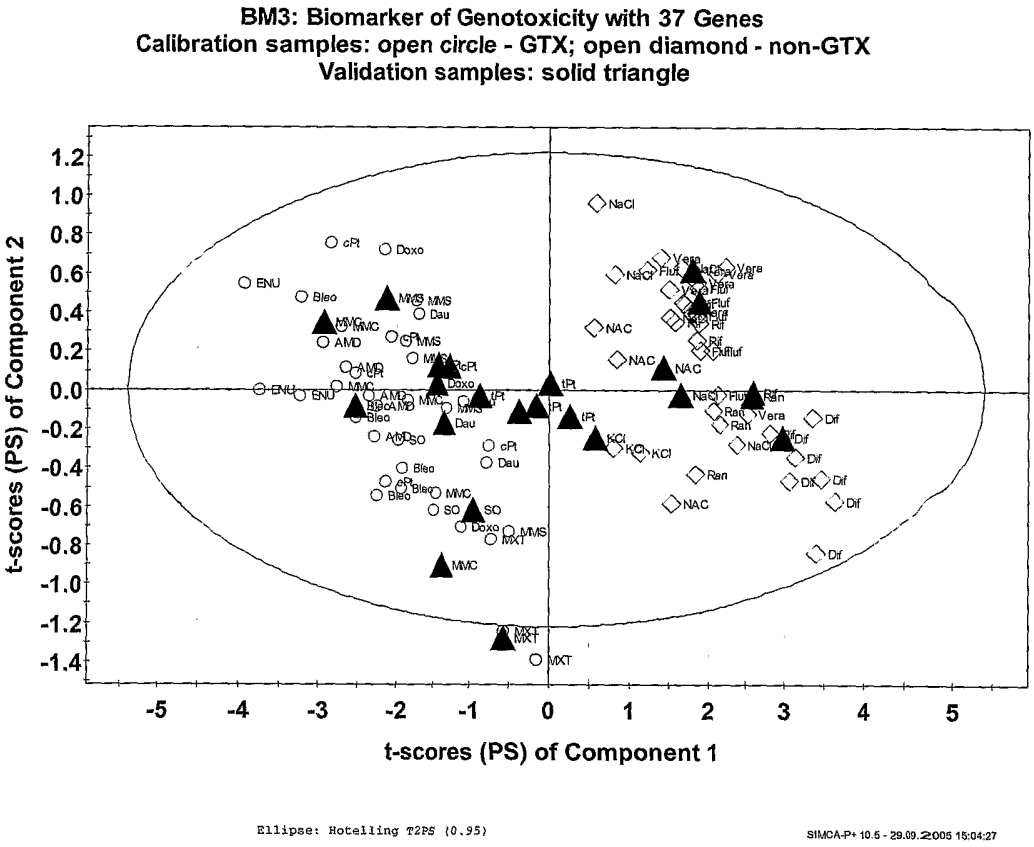

BM3: Biomarker of Genotoxicity with 37 Genes
Calibration samples: open circle - GTX; open diamond - non-GTX
Validation samples: solid triangle
t-scores (PS) of Component 2
t-scores (PS) of Component 1
Ellipse: Hotelling T2PS (0.95)
SIMCA-P+ 10.5 - 29.09.2005 15:04:27

Figure 12A

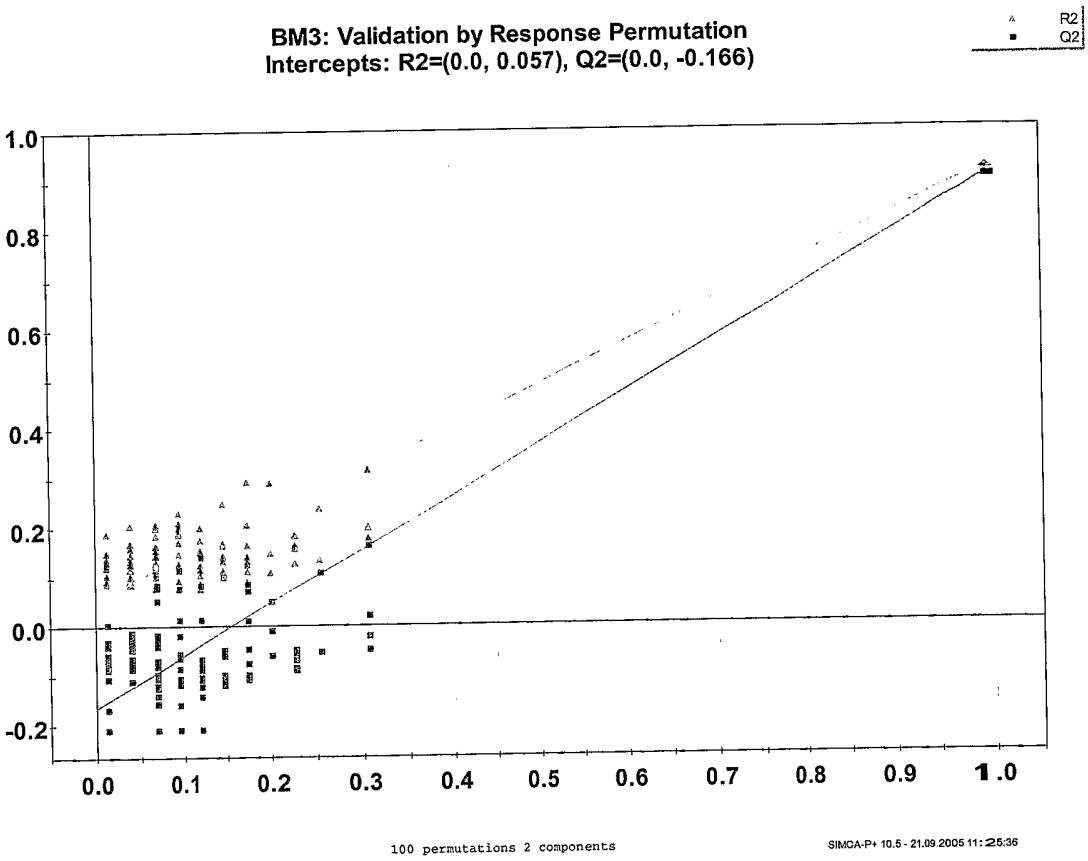

BM3: Validation by Response Permutation
Intercepts: R2=(0.0, 0.057), Q2=(0.0, -0.166)
R2
Q2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
100 permutations 2 components
SIMCA-P+ 10.5 - 21.09.2005 11:25:36

Figure 12B