(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 September 2008 (12.09.2008)

PCT

(10) International Publication Number
WO 2008/108803 A2

(51) International Patent Classification:
G01N 33/574 (2006.01)

(21) International Application Number:
PCT/US2007/015956

(22) International Filing Date: 13 July 2007 (13.07.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/830,531 13 July 2006 (13.07.2006) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

(54) Title: IMMUNE CELL BIOSENSORS AND METHODS OF USING SAME

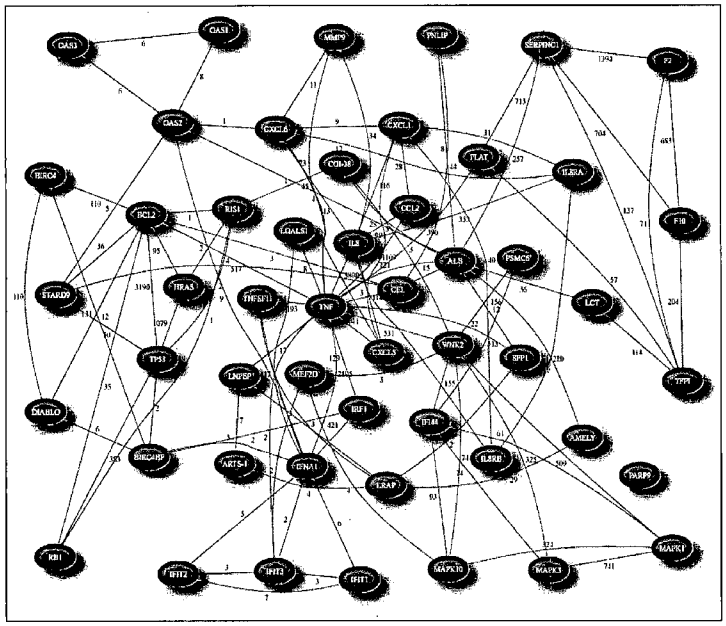

FIG. 8C

(57) Abstract: The present invention relates to immunological cells that are useful in detecting changes in physiological states, which provide for methods of diagnosing diseases or monitoring the course of patient therapy. Also provided are arrays of antigen presenting cell-specific markers for detecting changes in physiological states, and methods of detecting such changes.

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IMMUNE CELL BIOSENSORS AND METHODS OF USING SAME

BACKGROUND

Patients with diseases such as cancer, autoimmune diseases or cardiovascular disorders have cells that take on unique metabolic characteristics based on exposure to antigens. Particular cells, for example, display peptides on the cell surface that can not be found on the cell surface of healthy individuals. Inadequate antigen presentation in humans results in the failure of the human immune system to control and clear many pathogenic infections and malignant cell growth.

The ability of T-cells to recognize an antigen is dependent on association of the antigen with either major histocompatibility complex I (MHC-I) or major histocompatibility complex class II (MHC-II) proteins. Cytotoxic T-cells, for example, respond to an antigen in association with MHC-I proteins. A cytotoxic T-cell that kills a virus-infected cell will not kill a cell infected with the same virus if the cell does not also express the appropriate MHC-I protein. Helper T-cells recognize MHC-II proteins. Helper T-cell activity depends in general on both the recognition of the antigen on antigen presenting cells and the presence on these cells of "self" MHC-II proteins. This requirement to recognize an antigen in association with a self-MHC protein is called MHC restriction. MHC-I proteins are found on the surface of virtually all nucleated cells. MHC-II proteins are found on the surface of certain cells including macrophages, B-cells, and dendritic cells (DCs) of the spleen and Langerhans cells of the skin.

A crucial step in mounting an immune response in mammals is the activation of CD4+ helper T-cells that recognize MHC-II-restricted exogenous antigens. These antigens are captured and processed in the cellular endosomal pathway in antigen presenting cells, such as dendritic cells. In the endosome and lysosome, the antigen is processed into small antigenic peptides that are presented onto MHC-II in the Golgi compartment to form an antigen-MHC-II complex. This complex is expressed on the cell surface and induces the activation of CD4+ T-cells.

Other crucial events in the induction of an effective immune response in an animal involve the activation of CD8+ T-cells and B-cells. CD8+ cells are activated when the desired protein is routed through the cell in such a manner so as to be presented on the cell surface as processed proteins, which are complexed with MHC-I antigens. B-cells can interact with the antigen via their surface immunoglobulins (IgM and IgD) without the need for MHC proteins. The activation of the CD4+ T-cells, however, stimulates all arms of the immune system. Upon activation, CD4+ T-cells (helper T-cells) produce interleukins. These interleukins help activate the other arms of the immune system. Helper T-cells, for example, produce interleukin-4 (IL-4) and interleukin-5 (IL-5), which help B-cells produce antibodies; interleukin-2 (IL-2), which activates CD4+ and CD8+ T-cells; and gamma interferon, which activates macrophages. Since helper T-cells that recognize MHC-II-restricted antigens play a central role in the activation and clonal expansion of cytotoxic T-cells, macrophages, natural killer cells and B-cells, the initial event of activating the helper T-cells in response to an antigen is crucial for the induction of an effective immune response directed against that antigen.

Peptides and proteins expressed in diseased cells can be used as markers for the identification of such abnormal cells. Furthermore, the detection of antibodies in serum or other body fluids directed to these peptides or proteins can also be used as indicator of risk or as prognostic indicator. The concentrations of these disease-related peptides, however, are quite low, and isolating and identifying them is only efficacious when the disease predominates in the individual, which by that time usually precludes effective treatment. There remains a need in the art for a rapid and sensitive assay for detection of a pathological state in a mammal.

SUMMARY OF THE INVENTION

The present invention is based on the plasticity of antigen presenting cells, and the highly specific metabolic changes APCs, particularly DCs undergo after they encounter antigens. These changes can be quantitated and, when compared to reference positive (antigen-exposed) and negative (naïve) controls of APCs, provide information about the immune state and microenvironments of the mammal from which they are obtained.

On one embodiment, the invention is directed to a method for determining a subject's exposure to an agent, comprising a) determining a proteomic or gene expression signature from a sample obtained from the subject, wherein the sample comprises tissue sampling cells, and b) comparing the sample signature with a reference signature indicative of exposure to the agent, wherein congruity between the sample signature and the reference signature indicates the subject has been exposed to the agent. Ina particular embodiment, the subject is a mammal, *e.g.*, a human. In a particular embodiment, the agent is a chemical agent, a pathogenic agent or an autoimmune antigen. In one embodiment, the proteomic signature is determined using mass spectrometry. In another embodiment, the proteomic signature is determined using an immunochemical assay. In one embodiment, the gene expression signature is determined using a hybridization assay, *e.g.*, platform arrays or microarrays.

In another embodiment, the invention is directed toward a method for diagnosing a disease state in a mammal, comprising a) substantially isolating antigen presenting cells from a mammalian sample comprising a subpopulation of antigen presenting cells, b) deriving a genomic or proteomic mammalian sample signature for the isolated antigen presenting cells, and c) comparing the mammalian sample signature to a reference signature indicative of a disease state, wherein congruity between the mammalian sample signature and the reference signature indicates the presence of the disease state in the mammal. In a particular embodiment, the antigen presenting cells are dendritic cells. In one embodiment, the disease state is a cancer, cell proliferative disorder, pathogenic infection, viral infection or bacterial infection.

In another embodiment, the invention is directed to an array comprising a plurality of addresses, each address having affixed thereto a sample of nucleic acid corresponding to a gene expressed by an antigen presenting cell. In one embodiment, the array further comprises a plurality of secondary addresses, each secondary address having affixed thereto a sample of nucleic acid corresponding to genes expressed by an antigen presenting cell that has encountered an antigen. In another embodiment, the antigen presenting cell is a dendritic cell. In another embodiment, the antigen is a cancer antigen, a viral

antigen or a bacterial antigen.

In yet another embodiment, the invention is directed to a method for detecting a food-borne pathogen, comprising a) culturing naïve antigen presenting cells with a sample food product, b) obtaining a sample signature from the co cultured antigen presenting cells, and c) comparing the sample signature to a reference signature, wherein congruity between the sample signature and the reference signature indicates the presence of the food-borne pathogen in the food product. In a particular embodiment, the antigen presenting cell is a dendritic cell. The food-borne pathogen can be, for example, a bacterial pathogen or a viral pathogen.

In another embodiment, the invention is directed to a diagnostic method comprising a) substantially isolating the antigen presenting cells from a blood sample from a mammalian patient being treated for a disorder, b) deriving a genomic or proteomic patient sample signature for the isolated antigen presenting cells wherein the patient sample signature indicates the metabolic state of the antigen presenting cells in the subject, and c) comparing the patient signature to a reference signature, wherein the reference signature is derived from antigen presenting cells from a reference subject having the same disorder as the patient, wherein the congruity between the patient signature and the reference signature decreases during the treatment, thereby indicating the efficacy of the treatment in treating the disorder. The disorder can be a cell proliferative disease, and the treatment can be administration of an anti-neoplastic agent. In one embodiment the disorder is a cell proliferative disease, and the treatment provokes an immune response against the cell proliferative disease. In another embodiment, the disorder is a bacterial infection, and the treatment is administration of an antibacterial agent. In another embodiment, the disorder is a viral infection, and the treatment is administration of an antiviral agent.

In yet another embodiment, the invention is directed to a diagnostic method comprising a) substantially isolating the antigen presenting cells from a blood sample from a mammal afflicted with a disorder, b) identifying one or more marker polypeptides from the antigen presenting cells, where the marker polypeptide is expressed in the antigen presenting cell in response to antigen contact and where the antigen contacted is associated with or the causative agent of the disorder, c) obtaining an antibody to the marker polypeptide, and d) detecting in the antigen presenting cells of a subject, the presence or absence of a polypeptide that binds to the antibody, wherein the presence of the polypeptide confirms the presence of the disorder in the subject. In a particular embodiment, the antigen presenting cells are dendritic cells. In one embodiment, the disorder is a cancer, a cell proliferative disorder, a pathogenic infection, a viral infection, a bacterial infection, a prion infection or a fungal infection.

In another embodiment, the invention is directed to a method for determining whether a subject has been exposed to a foreign agent, comprising a) isolating RNA from a sample from the subject comprising a subpopulation of immune sampling cells, b) deriving a genomic or proteomic mammalian sample signature for the isolated cells wherein the sample signature indicates the metabolic state of the cells in the subject, and c) comparing the sample signature to a reference signature derived from cells that have responded to exposure from the agent.

wherein congruity between the sample signature and the reference signature indicates the subject has been

exposed to the agent.

In another embodiment, the invention is directed to a method for determining whether a subject has been exposed to a chemical agent, comprising a) determining a proteomic or gene expression sample signature of a sample obtained from a subject, and b) comparing the sample signature with a reference sample signature indicative of exposure to a chemical agent, wherein congruity between the sample signature and the reference signature is indicative of the subject's having been exposed to a chemical agent. In a particular embodiment, the chemical agent is 2-chloroethylethyl sulphide, azide or hypochlorous acid. In one embodiment, the reference sample signature comprises expression values or proteomic signatures of the genes or gene products from one or more genes listed in Tables 1, 2 or 3.

For the purposes of the invention, a mammalian subject is preferably a human, but can also be a veterinary subject such as a dog, cat, horse, pig, sheep, goat, or other mammal.

In another aspect, the invention provides for a antigen presenting cell, wherein the cell has been cultured in the presence of an antigen, and wherein the antigen expresses a plurality of genes that are specifically upregulated in response to antigenic challenge. In one embodiment, the antigen presenting cell is a dendritic cell. In yet another embodiment, the antigen is a cancer antigen. In still another embodiment, the antigen is a viral antigen. In even another embodiment, the antigen is a bacterial antigen. In still another embodiment, the antigen is a fungal antigen. In even another embodiment, the antigen is a prion antigen. In one aspect, the specific polypeptides that are produced in response to antigen contact (marker proteins) are isolated. These are used to raise antibodies, which are used in subsequent assays involving isolated APCs from patients, whereby expressed polypeptides in the patient isolated APCs are identified, *i.e.*, qualitatively and quantitatively by immunological assays, *e.g.*, ELISA, FACs, RIA and similar techniques.

In another aspect, the invention provides for determining the proteomic signature of an antigen presenting cell that has been exposed to an antigen. In one embodiment, the proteomic signature is obtained by subjecting the antigen presenting cell to SELDI mass spectroscopy. In another embodiment, the proteomic signature is obtained by subjecting the antigen presenting cell to MALDI-O-TOF and other forms of mass spectroscopy. In yet another aspect the invention provides for proteomic signatures obtained from antigen presenting cells that have been exposed to an antigen. In one embodiment, the antigen presenting cell is a dendritic cell. In yet another embodiment, the antigen is a cancer antigen. In still another embodiment, the antigen is a viral antigen. In even another embodiment, the antigen is a bacterial antigen. In still another embodiment, the antigen is a fungal antigen. In even another embodiment, the antigen is a prion antigen.

In another aspect, the invention includes a method of diagnosing exposure to an antigen comprising the steps of detecting the amount of protein/gene expression present in a sample of mammalian tissue or mammalian body fluids that has not been exposed to the antigen. Then the amount of protein/gene expression present in a sample of mammalian tissue or mammalian body fluids that has been exposed to the antigen is detected. A determination of the difference in the detected amount of protein/gene expression between the exposed and unexposed samples is made. A comparison of the

difference to a library of expected protein/gene expression for predetermined antigens is made. Finally, an evaluation is made whether the difference indicates the exposure to a particular antigen. The present invention is particularly useful because it can provide a diagnosis of whether a person has been exposed to an antigen before the onslaught of any symptoms. The present invention is also directed to a method of diagnosing exposure to an antigen comprising the steps of detecting the patterns of gene expression/proteins present in a sample of mammalian tissue or mammalian body fluids from persons that have been potentially exposed to the antigen, determining the relative amounts of expression of a panel of genes or proteins relative to house keeping genes and proteins expressed in those tissues from the potentially exposed individuals, comparing the relative amount differences to a library of expected gene expression/proteins for predetermined antigens; and evaluating whether the differences indicate that exposure has occurred to a known, catalogued, toxic agent, to a previously unknown antigen, or to a antigen mixed with potentiating agents. Housekeeping genes are genes that tend not to change upon exposure to antigens.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a gel view of four different samples of rat serum analyzed by mass spectrometry (MS).

FIG. 2 is a gel view of MS analysis showing differences in cytosolic protein expression between dendritic cells (DCs) and monocytes (MCs).

FIG. 3 is a gel view of MS analysis of DCs exposed to various chemical agents (control = unexposed, azide, ammonia, hypochlorous acid (HOCH) and 2-chloroethylethyl sulphide (CEES)). MS analysis of the cytosolic fraction of the DCs are shown.

FIG. 4 is a gel view of MS analysis of DCs exposed to various chemical agents (control = unexposed, azide, ammonia, HOCH and CEES). MS analysis of the nuclear fraction of the DCs are shown.

FIG. 5 is a gel view of MS analysis of DCs exposed to various chemical agents (control = unexposed, azide, ammonia, HOCH and CEES).

FIG. 6 is a Venn diagram indicating the number of response-specific genes useful in identifying exposure to particular chemical agents.

FIG. 7 is a bar graph showing the biological systems induced by various chemical agents.

FIGS 8A-8C are schematic illustrations of gene expression networks activated by various chemical agents. FIG. 8A: CEES. FIG. 8B: azide. FIG. 8C: HOCH.

FIG. 9 is a MS spectrogram of cytoplasmic proteins from myeloid cells (top), DCs (middle) and MOs (bottom), which had not been exposed to a foreign agent.

FIG. 10 is a MS spectrogram of cytoplasmic proteins from DCs that had been exposed to a viral agent (flu virus) or unexposed.

FIG. 11 is a MS spectrogram of cytoplasmic proteins from DCs that had been exposed to a flu virus (top) or a genetically-modified flu virus (bottom). The modified virus contains a bone

morphogenetic protein 2 (BMP2), which increases antigenicity. The spectrograms clearly indicate a signature that can distinguish between even very closely related viral agents.

FIGS 12A and 12B are MS spectrograms showing the signature for DCs infected with a control adenovirus (FIG. 12A) and a modified adenovirus (FIG. 12B). The modified adenovirus contains a copy of the BMP2 gene. Cytoplasmic proteins are shown.

FIGS. 13A and 13B are MS spectrograms showing the signature for myeloid cells infected with a control adenovirus (FIG. 13A) and a modified adenovirus (FIG. 13B). Cytoplasmic proteins are shown.

FIGS. 14A and 14B are MS spectrograms showing the signature for MOs infected with a control adenovirus (FIG. 14A) and a modified adenovirus (FIG. 14B). Cytoplasmic proteins are shown.

FIG. 15 is a series of spectrograms showing signatures for positively charged cytoplasmic proteins from DCs exposed to bacteria and bacterial toxin.

FIGS. 16A-D are MS data showing *Listeria monocytogenes* protein fraction signatures. Metal-binding fractions (FIG. 16A, spectrogram; FIG. 16B, gel view) and hydrophobic fractions (FIG. 16C, spectrogram; FIG. 16D, gel view) are shown.

FIGS. 17A-C are a series of MS spectrograms showing nuclear proteins from myeloid cells (FIG. 17A), DCs (FIG. 17B) and MOs (FIG. 17C) co-cultured in the presence of *Listeria monocytogenes*.

FIGS. 18A and 18B are gel views of MS analysis of DCs exposed to *Bacillus anthracis* and genetically modified *B. anthracis*. Shown are signatures for untreated DCs (top), wild-type-exposed DCs (middle) and genetically modified-exposed DCs (bottom) in two different *m/z* ranges (5000-10000, FIG. 18A; 10000-20000, FIG. 18B). As described below, "wild-type" refers to a pXO1 negative strain.

FIG. 19 is a Venn diagram showing the number of genes that are upregulated and downregulated in response to DC exposure to *B. anthracis* strains. This illustrates that even closely related bacterial strains (wt, wt^{++} and wt^{-}) can be distinguished based on their unique signatures.

FIGS 20A-C are MS analyses showing signatures of APCs exposed to *B. anthracis* and a *B. anthracis* anthrolysin O (ALO) knock-out strain. FIG. 20A is a spectrogram showing signatures for untreated (top), wild-type-exposed (12 hours, middle) and ALO-treated (12 hours, bottom) APCs. FIG. 20B is a gel view of the data. FIG. 20C is a gel view of data obtained after a 2 hour exposure to the agent. These figures show that closely related agents can be distinguished, but also that different stages of infection can be distinguished.

FIG. 21 is a series of spectrograms showing signatures of cells unexposed (top) and exposed to various classes of agents (*e.g.*, viral (vaccinia, middle) and bacterial (*Listeria*, bottom).

FIG. 22 is a schematic show gene expression of APCs induced by exposure to foreign agents. Expression data were obtained using a microarray comprising genes that had been identified as being differentially regulated in response to general bacterial infections, *Y. pestis* specifically and *B. anthracis* specifically. Analysis was performed using three sample of untreated cells, cells exposed to *B. anthracis*, cells exposed to ALO and cells exposed to *Yersinia pestis*. Expression data from three samples are shown.

FIGS. 23A-C are a series of MS signatures of cells exposed to skim and whole milk that has been untreated or treated with *Listeria monocytogenes*. FIG 23A is a spectrogram and FIG. 23B is a gel view of the signature. FIG. 23C is a gel view of the metal binding protein signature.

FIGS. 24A and 24B are MS data showing signatures specific for progression of sepsis in rats. FIG. 24A shows a series of spectrograms for DC- and MO-specific biomarkers identified during the progression of sepsis. FIG. 24B shows the Bruker spectra of the 24-hour sepsis time point. The arrows represent a particular 4.9 kDa protein marker of interest.

FIGS 25A-C are a series of gene expression profiles showing signature expression of informative genes whose expression is modulated by exposure to agents (ALO, *B. anthracis* (wild-type Sterne strain) and *Y. pestis*). FIG. 25A is a profile of genes specific to *Y. pestis* exposure; FIG. 25B is a gene expression profile comprising expression values of informative genes whose expression is specifically modulated in response to *B. anthracis*; and FIG. 25C is an expression profile for additional genes modulated by exposure to *Y. pestis*. System trees showing relatedness of systems that are modulated by exposure to particular agents are shown above the gene expression profiles.

FIG. 26 is a gene expression profile showing expression data from APCs for informative genes that can be used to distinguish *B. anthracis* (BAS) and ALO (BA-/-) exposure. Expression values were obtained for each of three samples for exposed cells.

FIGS 27A-C are a series of miRNA expression profiles showing signature expression of miRNA in cells having been exposed to an agent for 8 hours (*Y. pestis* (YP), *B. anthracis* (BAS) and untreated (Untx)). FIG. 27A is a profile of miRNAs specific to *B. anthracis* exposure; FIG. 27B is a profile of miRNAs for untreated cells; and FIG. 27C is a profile of miRNAs generally modulated during infection. System trees showing relatedness of systems that are modulated by exposure to particular agents are shown above the gene expression profiles.

FIGS. 28A and 28B show hierarchical clustering of agent-specific biomarkers. FIG. 28A shows the relatedness of biomarkers for four different *Y. pestis* strains and two *B. anthracis* relative to untreated cells. FIG. 28B shows the relatedness of biomarkers for *B. anthracis* and *Y. pestis* strains.

FIG. 29 is a plot showing the high degree of correlation between expression measurements from different gene expression analysis platforms.

FIG. 30 is a series of MS spectrograms showing signatures for unexposed (control), HIV-1 (BaL)-exposed, HIV-1 (I22)-exposed, lipopolysaccharide (LPS)-exposed and *Listeria*-exposed cells (positively charged nuclear proteins).

FIG. 31 is a series of MS spectrograms showing signatures for unexposed (control), HIV-1 (BaL)-exposed, lipopolysaccharide (LPS)-exposed and *Listeria*-exposed cells (positively charged cytoplasmic proteins).

FIG. 32 is a series of MS spectrograms showing signatures for unexposed (control), HIV-1 (BaL)-exposed, HIV-1 (I22)-exposed, lipopolysaccharide (LPS)-exposed and *Listeria*-exposed cells (copper-binding nuclear proteins).

FIG. 33 is a series of MS spectrograms showing signatures for unexposed (control), HIV-1 (BaL)-exposed, HIV-1 (I22)-exposed, lipopolysaccharide (LPS)-exposed and *Listeria*-exposed cells (copper-binding cytoplasmic proteins).

DETAILED DESCRIPTION

The present invention is based on the observed plasticity of tissue sampling cells, *e.g.*, antigen presenting cells (APCs) and peripheral blood mononuclear cells (PBMCs), and their use for the rapid detection of specific changes in gene and protein expression occurring in human dendritic cells (DCs) and monocytes (MOs) in response to exposure to pathogens, tumors, and hazardous agents. APCs, particularly DC macrophages and MOs, and to a lesser extent B-cells, constantly sample the various microenvironments found in the mammalian body. For example, DCs are found in an immature state in most tissues (CD1a+, $CD83^{Low}$), where they recognize and phagocytose pathogens and other antigens (Poindexter, *et al.*, *Breast Cancer Res.*, 6:408-415, 2004). Platelets, although not cells *per se*, can also bind and phagocytose infectious microorganisms and serum proteins, and can be considered as a reservoir for the detection of pathogens and cell fragments, such as tumor cells and apoptotic cell debris (Youssefian, *et al.*, *Blood*, 99:4021-9, 2002). An APC generally internalizes an agent and possesses the capacity to present antigens derived from the agent to other cells. Typically, if, for the example, the agent is viral or bacterial, the internalized is fragmented and a peptide antigen from the viral or bacterial agent is presented on the cell surface. Exemplary APCs include, but are not limited to, cells of lymphoid lineage such as T-cells, B-cells, lymphoid-related DCs and natural killer cells (NK), and cells of the myeloid lineage such as myeloid-related DCs, macrophages, MOs, megakaryocytes, platelets, granulocytes and neutrophils. Preferred are highly phagocytotic cells such as macrophages, monocytes and DCs.

Direct contact with agents that act as antigens leads to the maturation of APCs, which is characterized by an increase in antigen presentation, expression of co-stimulatory molecules, expression of cytokines, and subsequent stimulation of naïve T-cells in the lymphoid organs, as well as other cell specific markers such as surface CD83 expression in DCs. This maturation process is regulated by numerous corresponding changes in gene expression in these cells, which can be qualitatively and quantitatively measured. The series of gene expression changes that occur are highly specific, and they occur in specific response to the particular antigen to which the APC is exposed. APCs can differentiate between, for example, particular peptides, glycopeptides and glycolipids, and initiate responses that are similar but not identical, when exposed to various antigens. The particular changes in gene and protein expression of the APC in response to antigenic challenge represent very specific, measurable biological signatures, which can be used to identify an APC that has experienced an antigen, as well as the nature of the antigen itself, *e.g.*, its chemical composition and source. A "signature" is used herein to refer to a biochemical (metabolic) state of a cell that is specific to a particular microenvironment, *e.g.*, a biochemical state triggered by exposure to a particular signal, *e.g.*, an antigenic agent.

A sample signature is obtained, for example, from a subject (*e.g.*, a mammal, a human patient) and is compared to a reference signature indicative of exposure to an agent. A congruity, a statistical correlation, of the sample to the exposed reference signature indicates that the subject from whom the sample was derived had been exposed to the agent. For example, a congruity can be determined in terms of relative certain. When comparing a sample signature to a reference signature, the signatures are congruous if the sample is identified, *e.g.*, about 100% accurately, about 98% accurately, about 95% accurately, about 90% accurately or about 80% accurately. Optionally, a sample signature can be compared to a reference, unexposed signature. If the sample signature is not congruous with the exposed signature or is congruous with the unexposed signature, it can be concluded that the subject had not been exposed to the agent.

APCs serve as the body's natural immune biosensor. These cell types circulate through all tissues of the body and are responsible for surveying and identifying agents in most if not all tissues of the body by sampling the microenvironment. In doing so, they seek out areas of tissue that have a danger signal, *e.g.*, increased mitotic activity, activated innate and adaptive immune response, or viral/bacterial infections. Once this signal is detected, APCs initiate the early transcriptional changes that lead to cell surface antigen expression and inflammatory mediator release (Crawford *et al.*, *Blood*, 102:1745-1752, 2003). These cellular modifications are required for recruitment of other inflammatory cells to the site of involvement and improved immune cell-to-cell contact. APCs such as DCs possess pattern recognition receptors that allow them to bind to and discriminate between various pathogens (Chaussabel *et al.*, *Blood*, 102:672-8 1, 2003). Other receptors include Toll-like receptors, ICAMs such as ICAM-1, DCSIGN, and others.

According to the present invention, changes in APC response to antigenic challenge can be used to assay for persons in a pre-symptomatic (not ill) state, and can be used to monitor the progression of a disease or the efficacy of a therapeutic regimen in treating the disease (Bernardo *et al.*, *Antimicrob. Agents Chemother.*, 48: 546-444, 2004). This assay can detect a disease state prior to the disease state becoming symptomatic, or it can distinguish between disease states with similar symptoms. Similarly, changes in APC in response to antigenic challenge can be used to assay for persons who have been exposed to biological agent(s)- and can be used in early diagnosis of the high-risk exposed individual as well as for monitoring persons who are in the early stages of developing symptoms. For example, changes in APC in response to antigenic challenge from a viral or bacterial pathogen can provide for rapid identification of these pathogens, and can detect the prior to the pathogen's appearance in plasma by many hours or days. The method can also be used to monitor the effectiveness of a vaccination, for example, by assaying for DC interaction with one or more components of the vaccine. Similarly, changes in APC in response to antigenic challenge from tumors permit the detection of tumors before an individual becomes symptomatic, thereby permitting early and aggressive treatment. Changes in APC response to exposure to industrial chemicals, or biowarfare agents can also provide for identification of an unknown etiological agent to which an individual is exposed.

United States Patent 6,316,197 (Das *et al.*) describe a method of diagnosing exposure to toxic agents by measuring distinct pattern in the levels of expression of specific genes. Exposure of immature DCs to LPS stimulation contributes to their terminal differentiation into CD70+ DCs (Iwamoto *et al.*, *J. Leukoc. Biol.*, 78:383-392, 2005; Kumar *et al.*, *Leuk. Res.*, 19: 831-840, 1995; Hwang *et al.*, *Cell Res.*, 15(3):167-75, 2005; Shi *et al.*, *Cancer Sci.*, 96:127-33, 2005; Smith *et al.*, *J. Virol.*, 79:2807-13, 2005). In response to *Bacillus anthracis* lethal toxin (LeTx), 103 macrophage genes are upregulated. Similarly, Tucker *et al.* (*Cell Microbiol.*, 5: 523-532, 2003) describe LeTx cleavage of mitogen-activated protein kinase kinases (MAPKKs) in a variety of different APC cell types. Expression of genes regulated by MAPKK activity do not change significantly, yet a series of genes under glycogen synthase kinase-3-beta (GSK-3β) regulation changed expression following LeTx treatment (Green *et al.*, *Immunol. Lett.*, 43: 87-94, 1994; Hernychova *et al.*, *Immunol. Lett.*, 57:75-81, 1997; Clemens *et al.*, *Infect. Immun.*, 72:3204-3217, 2004; Ng *et al.*, *Adv. Exp. Med. Biol.*, 529:155-160, 2003). These genes include unknown ESTs, cytokines, enzyme of cytokine, receptors, ligands, transcriptional factors, inhibitor of transcriptional factor, proteins involved with the cytoskeleton, and seven genes that encode factors known to be associated with cell cycling and cell proliferation, with three of them playing a role in apoptosis (Saban *et al.*, *Physiol. Genomics*, 5: 147-160, 2001). Gene upregulation in response to LPS was observed in a cluster including the interleukin-6 (IL-6) receptor, alpha- and beta-nerve growth factor (α- and β-NGF), vascular endothelial growth factor receptor-1 (VEGF R1), C-C chemokine receptor, and P-selectin. Another tight cluster of genes with marked expression included the proto-oncogenes c-Fos, Fos-B, Fra-2, Jun-B, Jun-D, and Egr-1. Almost all interleukin genes were upregulated as early as one hour after stimulation with LPS. Nuclear factor-kappaB (NF-κB) pathway genes collected in a single cluster with a peak expression four hours after LPS stimulation. In contrast, most of the interleukin receptors and chemokine receptors presented a late peak of expression 24 hours after LPS exposure (Mendis *et al.*, *Genes Immun.*, 6: 84-94, 2005).

In certain embodiments, isolation of APCs permit the subsequent extraction and isolation of phagocytosed antigens or even whole pathogens from the APCs, which can be further characterized by mass spectrometry (MS) or similar tools. For example, DCs are known to internalize viral and bacterial pathogens without killing the pathogen (Sundquist *et al.*, *J. Immunol.*, 170:1635-9, 2004; Jantsch *et al.*, *Cell Microbiol.*, 5:933-945, 2003). Accordingly, one object of the present invention includes methods for harvesting parts and/or the entire pathogen or antigen, in addition to obtaining genomic/proteomic signatures of cells exposed to the pathogen or antigen. Particular embodiments of the invention involve determining a signature (*e.g.*, proteomic profile, gene expression profile of informative genes, micro-RNA (miRNA) profile) that is specific to a metabolic state induced in the APCs by an agent. The agent can be, for example, an exogenous agent, *e.g.*, a viral or bacterial pathogen, a toxic chemical, an environmental agent, or it can be an endogenous agent, *e.g.*, autoimmune antigens, cancer markers, etc. A proteomic signature can be obtained, for example, by MS or immunochemical techniques. Genomic and miRNA signatures can be obtained using, for example, microarrays or binding assays.

The invention provides for identification of "informative genes"- genes that are differentially regulated, *e.g.*, upregulated or downregulated, in response to an agent, that can be used to determine a cellular response signature. Expression of informative genes can be determined by analyzing the transcriptional or translational products of the informative gene. These genes and gene products are highly specific markers for antigen contact, and their expression is indicative that the APC has encountered a particular antigen. By isolating informative gene products (polypeptides and proteins), in whole or in part, antibodies can be raised, which can be used in subsequent assays to determine antigen contact.

The pathogenic polypeptides and other APC polypeptides provide for protein markers that are indicative of antigen contact. In one aspect, these polypeptide markers are isolated and used to raise antibodies. The anti-APC marker antibodies are then useful in assays that can be used to detect expression of APC marker polypeptides in cells obtained from patients suspected of antigen exposure. In one embodiment, the anti-APC marker antibodies are used in assays that employ immunological detection methods, such as fluorescent activated cell sorting (FACS), fluorescence resonance emission tomography (FRET), radioimmunoassay (RIA) and enzyme linked immunosorbant assays (ELISA). Other immunological detection assays are know to those of skill in the are and are suitable for the detection methods described herein.

As described above, APCs generate unique gene signatures in response to exposure to agents, *e.g.*, various pathogens. Studies of discordant gene expression in DCs and macrophages infected with bacteria, *Candida*, influenza, or different parasites using oligonucleotide arrays have suggested that of the approximately 6800 genes samples, about 1300 genes demonstrate significant modulation in expression patterns after exposure to antigens (Huang *et al.*, *Science*, 294:870-875, 2001). DCs express C-type lectins as pathogen recognition receptors, for example, the DC-specific ICAM-3 grabbing nonintegrin (SIGN)/CD209, which has been identified as the HIV-1 receptor on DCs, as well as for surface glycans for *Mycobacterium tuberculosis*, *Helicobacter pylori*, *Leishmania mexicana*, *Schistosoma mansoni* and other pathogens (Appelmelk *et al.*, *J. Immunol.*, 170:1635-9, 2003; Hofer *et al.*, *Immunol. Rev.*, 181:5-19, 2001; Pulendran *et al.*, *J. Immunol.*, 167:5067-76, 2001). These receptors and the cellular pathways they interact with provide unique markers for monitoring DC activation and response. More particularly, changes in DC response are measurable and provide pathogen-specific signatures evidencing DC interaction with particular disease agents (Machein, U. & Conca, W., *Adv. Exp. Med. Biol.*, 421:247-251, 1997).

Hazardous environmental agents are also detectable by the methods described herein, as they either can provoke an APC-specific immune cell response themselves, or will destroy cells and tissues causing an increase in inflammation, extravasation, and activation of APCs in response to cytokines and various cellular factors. These properties of human APCs make them suitable for the rapid detection of exposure to any pathogenic substance, for example an infectious pathogen, tumor, toxin or toxic industrial chemical (TIC), or weapon of mass destruction (WMD).

The APCs described, preferably MOs, and most preferably DCs, are useful to detect changes in the physiology of a subject, in response to, for example, diseases such as infectious diseases and cancers. As used herein, the term "antigen" is broadly used to refer to any composition that is generally foreign to a healthy mammal, or is native to the mammal but is mutated, aberrant or found in increased concentrations in the mammal having a pathological condition, or native to the animal but pathogenically provokes an immune response (*e.g.*, autoimmune disorders, graft versus host disease (GVHD)), wherein the antigen would normally stimulate an antigenic immune response. Examples of antigens include whole pathogens such as bacteria, viruses, fungi, protozoa, as well as one or more components of a pathogen, for example a bacterial antigen includes lipopolysaccharide (LPS), a viral antigen includes a viral coat protein such as gp120 of HIV or hemagglutinin of influenza, and a fungal antigen includes the cell-wall derived protein mannin. Prions are also antigenic, displaying specific peptide sequences associated with disease states. Antigens also include proteins and peptides associated with tumors, such as carcinoembryonic antigen (CEA) and aberrantly glycosylated mucin (MUC) as well as numerous other tumor specific antigens and proteins such as bcl-2, survivin, hepsin and the like. Accordingly, the common characteristic of an antigen, or antigenic agent, is the effect it has on an APC or other tissue sampling cell in that it causes specific biochemical changes in the APC such as the upregulation of antigen presentation proteins and co-receptors, as well as maturation and proliferation of APCs, tissue migration, and other properties that are indicative of exposure to an antigen. A detectable increase in APC activity is one where, for example, a two-fold or greater increase in the number of APCs are induced to develop or activate or mature in the mammal exposed to the antigen relative to those levels of APCs in the non-exposed or healthy mammal. Other detectable increases in APC activity includes, for example, a 1.1-, a 1.5-, a 3-, a 5- or a 10-fold increase in the number of APCs that are induced. Assay techniques for determining DCs and other blood cells are known in the art. Examples include, but the invention is not limited to FACS using mature DC cell markers CD2+ and CD83+, or immature marker CD1a+.

In one aspect, the invention provides immune cell-based methods for monitoring a patient's response to a disease state. Determining the pathogen or tumor-specific genomic and proteomic expression patterns, or signatures, provides an improved method of ongoing monitoring of the patient's immune response to the disease state. This information is used in conjunction with other relevant medical information such as decrease in tumor mass or tumor burden for a cancer patient, or a decrease in viral load for an HIV infected patient, or the clearance of mycobacteria in a tuberculosis patient, to allow monitoring of therapeutic efficacy, for example, in response to chemotherapy, anti-viral therapy, or administration of antibiotics.

APCs thus provide a useful diagnostic tool for identifying antigens and for monitoring the health of individuals, based upon changes in their cellular metabolism. Measurable changes occur in expression of numerous genes, proteins and secretory factors such as cytokines, and the antigens can also be detected in the cytoplasm of the APC (such as in the cytoplasm of platelets). As such, in one aspect the present invention provides for arrays of APC genes whose expression creates a characteristic signature, preferably MO or DC signatures. The array includes oligonucleotides, oligoribonucleotides or

polypeptides of a plurality of APC marker genes and proteins, *e.g.*, gene or protein products differentially expressed in APCs. More preferably, the array includes from about 500-1000 specific markers at individual addresses in a matrix. Even more preferably, the array includes about 5,000, about 10,000, about 20,000 or greater genes or gene products, represented on the array. Most preferably, the array is a genome-wide array, for example a mature DC cDNA array. Affymetrix and Illumina (both systems are complementary) arrays are exemplary. Individual genes or gene products can be duplicated on the array, for example, as controls or for quantitative analysis of gene expression. The manufacture and use of such arrays are described, for example, in United States Patents 6,741,344, 6,733,977, and 6,733,964. A method and apparatus for selectively applying a material onto a substrate for the synthesis of an array of, for example, oligonucleotides at selected regions or addresses on the substrate is further described by U.S. Patent number 6,667,394. The gene arrays produced are representative of the host reaction to the pathogen in great detail (typically 52,000 genes or more) and are not dependent the identification of one or a few genes (intrinsically biased), as is the case for identification by, *e.g.*, quantitative PCR (Q-PCR).

Numerous types of arrays are created to develop APC-based diagnostic arrays for a variety of purposes, but generally to obtain data sets for how APCs, particularly DCs, react upon exposure to different antigens. The use of a particular array depends on its chemical composition and will vary depending on whether the array has nucleic acids, peptides, both, or other chemical moieties such as lectins etc. By way of general illustration, arrays such as Affymetrix' GeneChip® use biotin-labeled cRNA prepared from cell extracts. About 5μg total RNA are an appropriate starting material. The cRNA produced from the RNA sample is exposed to the array and allowed to hybridize to the appropriate target. The array is washed and stained, *e.g.*, with streptavidin phycoerythrin, and visualized using Affymetrix's GeneChip® Scanner 3000 or an Agilent GeneArray® Scanner. This technique, as well as known immunological methods and other common methods of using proteomic and genomic arrays, is generally understood to those skilled in the art.

The arrays provide for the detection and identification of pathogens and pathogenic agents, as well as the detection and identification of transformed cells and tissues, using samples derived from subjects. Information about the disease state of a patient, that is, a patient data set, is obtained using one or more of the APC arrays described, by first obtaining a sample from a subject, *e.g.*, a blood sample, and then isolating the DCs from that sample. The DC signature from the subject, *e.g.*, a human patient, is compared to one or more control DC signatures, for example, using the hybridization arrays described. The control DC signatures on the array represent both the normal or healthy DC signatures and the abnormal or pathogenic DC signatures, for one or more disease states. Various other embodiments include additional control DC signatures that provide reference signatures for stages of various disease states, *e.g.*, cancer stages. The arrays and the data sets obtained are useful, for example, for discovering or diagnosing the existence of a genetic disease or chromosomal abnormality, or to provide information relating to identity, heredity or compatibility, diagnosing a predisposition to a disease or condition, diagnosing infection by a pathogenic organism, discovering or diagnosing neoplastic transformation of a

cell or tissue, determining exposure to and identification of biowarfare or chemical warfare samples, or toxic industrial chemicals.

In one aspect, APC arrays are developed that are designed to identify the presence or absence of particular pathogens as well as their immunological consequences during the progression of the disease state they are associated with. For example, arrays are created that provide for the detection and monitoring of a viral infection such as HIV. The arrays include consensus APC signatures from immature or naïve APCs, from APCs obtained from an HIV-exposed but asymptomatic person, APCs obtained from the exposed and early symptomatic person and from APCs in the later stage symptomatic person. Similar viral arrays are developed, for example ones useful for diagnosing and monitoring hepatitis, neoplastic viruses, or other chronic or pathogenic viral infections. Diagnostic arrays that can be used to monitor viral vectors used in gene therapy are also preferred, *e.g.*, those directed to vaccinia or poxviruses, and more particularly, those specific to the transformed vector, which should produce a different DC signature than the wild-type vector. In yet another aspect, the arrays include human APCs, particularly DCs and macrophage cells that are exposed to pathogens on the Center for Disease Control's priority list. These types of arrays facilitate rapid emergency diagnosis, etiologic studies, response and treatment of exposed or potentially exposed individuals. Arrays specific to homeland defense or military uses are also provided herein, as DC arrays specific to biological warfare pathogens provide for rapid detection and response to terrorist or enemy bioweapons attacks. Such arrays include smallpox arrays, *Bacillus anthracis* arrays, and other WMD pathogens. Arrays of human APCs, particularly DCs and macrophage cells that are exposed to toxic agents facilitate emergency diagnosis, response and treatment of exposed or potentially exposed individuals. In another embodiment, the array and patient data set obtained there from facilitate forensic or toxicology studies of an exposed individual.

In another aspect, the arrays are obtained from human APCs, particularly DCs and macrophage cells in patients having different tumors, including different stages of tumor growth. APC arrays are designed to identify the presence or absence of particular tumor antigenic markers, and the immunological consequence of the tumor on a patient during the progression of the patient's cancer. This type of array facilitates rapid diagnosis, tumor identification, and appropriate treatment of afflicted individuals. The following cancer types each result in specific APC responses, and are amenable to detection using the techniques described: Acute Lymphoblastic Leukemia, Adult; Acute Lymphoblastic Leukemia, Childhood; Acute Myeloid Leukemia, Adult; Acute Myeloid Leukemia, Childhood; Adrenocortical Carcinoma; Adrenocortical Carcinoma, Childhood; AIDS-Related Cancers; AIDS-Related Lymphoma; Anal Cancer; Astrocytoma, Childhood Cerebellar; Astrocytoma, Childhood Cerebral; Bile Duct Cancer, Extrahepatic; Bladder Cancer; Bladder Cancer, Childhood; Bone Cancer, Osteosarcoma/Malignant Fibrous Histiocytoma; Brain Stem Glioma, Childhood; Brain Tumor, Adult; Brain Tumor, Brain Stem Glioma, Childhood; Brain Tumor, Cerebellar Astrocytoma, Childhood; Brain Tumor, Cerebral Astrocytoma/Malignant Glioma, Childhood; Brain Tumor, Ependymoma, Childhood; Brain Tumor, Medulloblastoma, Childhood; Brain Tumor, Supratentorial Primitive Neuroectodermal Tumors, Childhood; Brain Tumor, Visual Pathway and Hypothalamic Glioma, Childhood; Brain Tumor,

Childhood (Other); Breast Cancer; Breast Cancer and Pregnancy; Breast Cancer, Childhood; Breast Cancer, Male; Bronchial Adenomas/Carcinoids, Childhood; Carcinoid Tumor, Childhood; Carcinoid Tumor, Gastrointestinal; Carcinoma, Adrenocortical; Carcinoma, Islet Cell; Carcinoma of Unknown Primary; Central Nervous System Lymphoma, Primary; Cerebellar Astrocytoma, Childhood; Cerebral Astrocytoma/Malignant Glioma, Childhood; Cervical Cancer; Childhood Cancers; Chronic Lymphocytic Leukemia; Chronic Myelogenous Leukemia; Chronic Myeloproliferative Disorders; Clear Cell Sarcoma of Tendon Sheaths; Colon Cancer; Colorectal Cancer, Childhood; Cutaneous T-Cell Lymphoma; Endometrial Cancer; Ependymoma, Childhood; Epithelial Cancer, Ovarian; Esophageal Cancer; Esophageal Cancer, Childhood; Ewing's Family of Tumors; Extracranial Germ Cell Tumor, Childhood; Extragonadal Germ Cell Tumor; Extrahepatic Bile Duct Cancer; Eye Cancer, Intraocular Melanoma; Eye Cancer, Retinoblastoma; Gallbladder Cancer; Gastric (Stomach) Cancer; Gastric (Stomach) Cancer, Childhood; Gastrointestinal Carcinoid Tumor; Germ Cell Tumor, Extracranial, Childhood; Germ Cell Tumor, Extragonadal; Germ Cell Tumor, Ovarian; Gestational Trophoblastic Tumor; Glioma, Childhood Brain Stem; Glioma, Childhood Visual Pathway and Hypothalamic; Hairy Cell Leukemia; Head and Neck Cancer; Hepatocellular (Liver) Cancer, Adult (Primary); Hepatocellular (Liver) Cancer, Childhood (Primary); Hodgkin's Lymphoma, Adult; Hodgkin's Lymphoma, Childhood; Hodgkin's Lymphoma During Pregnancy; Hypopharyngeal Cancer; Hypothalamic and Visual Pathway Glioma, Childhood; Intraocular Melanoma; Islet Cell Carcinoma (Endocrine Pancreas); Kaposi's Sarcoma; Kidney Cancer; Laryngeal Cancer; Laryngeal Cancer, Childhood; Leukemia, Acute Lymphoblastic, Adult; Leukemia, Acute Lymphoblastic, Childhood; Leukemia, Acute Myeloid, Adult; Leukemia, Acute Myeloid, Childhood; Leukemia, Chronic Lymphocytic; Leukemia, Chronic Myelogenous; Leukemia, Hairy Cell; Lip and Oral Cavity Cancer; Liver Cancer, Adult (Primary); Liver Cancer, Childhood (Primary); Lung Cancer, Non-Small Cell; Lung Cancer, Small Cell; Lymphoblastic Leukemia, Adult Acute; Lymphoblastic Leukemia, Childhood Acute; Lymphocytic Leukemia, Chronic; Lymphoma, AIDS-Related; Lymphoma, Central Nervous System (Primary); Lymphoma, Cutaneous T-Cell; Lymphoma, Hodgkin's, Adult; Lymphoma, Hodgkin's, Childhood; Lymphoma, Hodgkin's During Pregnancy; Lymphoma, Non-Hodgkin's, Adult; Lymphoma, Non-Hodgkin's, Childhood; Non-Hodgkin's During Pregnancy; Lymphoma, Primary Central Nervous System; Macroglobulinemia, Waldenström's; Male Breast Cancer; Malignant Mesothelioma, Adult; Malignant Mesothelioma, Childhood; Medulloblastoma, Childhood; Melanoma; Melanoma, Intraocular; Merkel Cell Carcinoma; Mesothelioma, Malignant; Metastatic Squamous Neck Cancer with Occult Primary; Multiple Endocrine Neoplasia Syndrome, Childhood; Multiple Myeloma/Plasma Cell Neoplasm; Mycosis Fungoides; Myelodysplastic Syndromes; Myelodysplastic/Myeloproliferative Diseases; Myelogenous Leukemia, Chronic; Myeloid Leukemia, Adult Acute; Myeloid Leukemia, Childhood Acute; Myeloma, Multiple; Myeloproliferative Disorders, Chronic; Nasal Cavity and Paranasal Sinus Cancer; Nasopharyngeal Cancer; Nasopharyngeal Cancer, Childhood; Neuroblastoma; Non-Hodgkin's Lymphoma, Adult; Non-Hodgkin's Lymphoma, Childhood; Non-Hodgkin's Lymphoma During Pregnancy; Non-Small Cell Lung Cancer; Oral Cancer, Childhood; Oral Cavity and Lip Cancer; Oropharyngeal Cancer; Osteosarcoma/Malignant Fibrous Histiocytoma of

Bone; Ovarian Cancer, Childhood; Ovarian Epithelial Cancer; Ovarian Germ Cell Tumor; Ovarian Low Malignant Potential Tumor; Pancreatic Cancer; Pancreatic Cancer, Childhood; Pancreatic Cancer, Islet Cell; Paranasal Sinus and Nasal Cavity Cancer; Parathyroid Cancer; Penile Cancer; Pheochromocytoma; Pineal and Supratentorial Primitive Neuroectodermal Tumors, Childhood; Pituitary Tumor; Plasma Cell Neoplasm/Multiple Myeloma; Pleuropulmonary Blastoma; Pregnancy and Breast Cancer; Pregnancy and Hodgkin's Lymphoma; Pregnancy and Non-Hodgkin's Lymphoma; Primary Central Nervous System Lymphoma; Primary Liver Cancer, Adult; Primary Liver Cancer, Childhood; Prostate Cancer; Rectal Cancer; Renal Cell (Kidney) Cancer; Renal Cell Cancer, Childhood; Renal Pelvis and Ureter, Transitional Cell Cancer; Retinoblastoma; Rhabdomyosarcoma, Childhood; Salivary Gland Cancer; Salivary Gland Cancer, Childhood; Sarcoma, Ewing's Family of Tumors; Sarcoma, Kaposi's; Sarcoma (Osteosarcoma)/Malignant Fibrous Histiocytoma of Bone; Sarcoma, Rhabdomyosarcoma, Childhood; Sarcoma, Soft Tissue, Adult; Sarcoma, Soft Tissue, Childhood; Sezary Syndrome; Skin Cancer; Skin Cancer, Childhood; Skin Cancer (Melanoma); Skin Carcinoma, Merkel Cell; Small Cell Lung Cancer; Small Intestine Cancer; Soft Tissue Sarcoma, Adult; Soft Tissue Sarcoma, Childhood; Squamous Neck Cancer with Occult Primary, Metastatic; Stomach (Gastric) Cancer; Stomach (Gastric) Cancer, Childhood; Supratentorial Primitive Neuroectodermal Tumors, Childhood; T-Cell Lymphoma, Cutaneous; Testicular Cancer; Thymoma, Childhood; Thymoma and Thymic Carcinoma Thyroid Cancer; Thyroid Cancer, Childhood; Transitional Cell Cancer of the Renal Pelvis and Ureter; Trophoblastic Tumor, Gestational; Unknown Primary Site, Carcinoma of, Adult; Unknown Primary Site, Cancer of, Childhood; Unusual Cancers of Childhood; Ureter and Renal Pelvis, Transitional Cell Cancer; Urethral Cancer; Uterine Cancer, Endometrial; Uterine Sarcoma; Vaginal Cancer; Visual Pathway and Hypothalamic Glioma, Childhood; Vulvar Cancer; Waldenström's Macroglobulinemia; and Wilms' Tumor.

Arrays are created that provide for the detection and monitoring of various cancers such as breast cancer, colon cancer, ovarian cancer, uterine cancer, prostate cancer, glioma, melanoma, small and large cell carcinoma, leukemia, and other neoplastic and precancerous disease states. Markers such as aberrantly glycosylated MUC-1, or expression of CEA or hepsin are examples of common tumor markers known to be associated with most of the above tumors. Comprehensive listings including tumor-specific markers are known in the medical literature. Exemplary arrays include consensus APC signatures from immature or naïve APCs, and from APCs obtained from persons having stage 0, 1, 2, 3 or 4 graded tumors. Histological profiles and other medical data can be used in connection with the APC arrays to provide additional information about the disease state. The plasticity and specificity of response of APCs to cancers allow very specific identification of the cancer type and the staging of the disease. They also permit a medical professional to monitor the course of a therapeutic regimen by monitoring changes in APC signatures during, for example, a chemotherapy regimen.

A patient with pancreatic cancer, for example, can be provided with gemcitabine, and before and during the course of gemcitabine therapy, DCs are extracted from the patient and used with a pancreatic cancer DC array. The array could indicate, for example, that the patient had grade 3 pancreatic cancer at

the outset of the treatment, and that after one month of gemcitabine treatment, the cancer has reverted to a grade 2 stage, thereby indicating continued gemcitabine therapy for the patient.

In yet another embodiment, arrays of APCs from individuals having a genetic disorder are created. Representative genetic disorders include for example, a disease state resulting from the presence of a gene, the expression product of the gene being a bioactive molecule that causes or contributes to the disease state, or the absence of a gene where the expression product of the gene in a healthy individual is a bioactive molecule that ameliorates or prevents the disease state. An example of the former is cystic fibrosis, wherein the disease state is caused by mutations in the CFTR protein. An example of the latter is PKU, where the disease state is caused by the lack of an enzyme permitting the metabolism of phenylalanine. Examples of genetic disorders appropriate for screening with the present assays and methods include, for example multiple sclerosis, endocrine disorders, Alzheimer's Disease, amyotrophic lateral sclerosis, lupus, angelman syndrome, Charcot-Marie-Tooth disease, epilepsy, essential tremor, fragile X syndrome, Friedreich's ataxia, Huntington's disease, Niemann-Pick disease, Parkinson's disease, Prader-Willi syndrome, Rett syndrome, spinocerebellar atrophy, Williams syndrome, Ellis-van Creveld syndrome, Marfan syndrome, myotonic dystrophy, leukodystrophy, atherosclerosis, Best disease, Gaucher disease, glucose galactose malabsorption, gyrate atrophy, juvenile onset diabetes, obesity, paroxysmal nocturnal hemoglobinuria, phenylketonuria, Refsum disease, and Tangier disease. Such arrays are useful in detecting a genetic disorder in a patient, and monitoring the patient having the genetic disorder during therapy. Similarly, the present assays provide for monitoring the course of gene therapy treatments, by monitoring the immunological state of the patient so treated, particularly for the appearance of the healthy gene product or for adverse reactions to the gene therapy vector.

In addition to genomic expression information obtained from tissue sampling cells, certain agents, *e.g.*, pathogens, introduce miRNA into a host cell to regulate the host cell's response to the pathogen. The mechanism by which miRNA regulates gene expression is hijacked by the pathogen, and specific metabolic pathways are downregulated by the presence of pathogen-specific miRNA. The pathogen-specific miRNA can be isolated from a host cell and provide the basis for a miRNA profile (signature) indicative of the host cell's having been exposed to a pathogen.

Proteomic data can be developed by a variety of techniques, for example but not limited to using surface plasmon resonance, or MS (for example, MALDI or SELDI, etc; see below). The combination of information obtained using genomic and proteomic approaches, in the format of a high throughput screen such as a DC gene array provides exceptionally specific diagnostic data, and thus a powerful tool for antigen identification or patient monitoring.

While the above discussion has focused on using APCs in diagnostics to determine biological changes in a sample, in another embodiment, direct analysis of the fluids of a subject, such as blood, sputum, urine, saliva, mucus, cerebrospinal fluid, lymphatic fluid and the like can be subjected to assay. These samples are analyzed using various medical techniques, *e.g.*, spinal tap to look for infection in cerebrospinal fluid, or laboratory techniques such as proteomic tools *e.g.*, mass spectroscopy, are generally known, and some will also be described below. Thus, the assays of the present invention can

involve the screening of APCs or PBMCs for changes in conjunction with direct analysis of the bodily fluids of a subject, which provides an even more sophisticated detection and monitoring method.

APCs provide a highly specific and rapid means for monitoring biological changes in an organism based on specific genomic and proteomic signatures that are typified by the DC in a particular state. The above discussion has centered on using APCs in assays that employ common techniques such as hybridization or immunological reactivity. Other proteomics tools are appropriate in determining changes in APC states. One method of obtaining a DC proteomic signature involves obtaining the mass spectra of the APC sample.

During the last decade, MS has become an important analytical tool in the analysis of biological macromolecules. MS provides a means of "weighing" individual molecules by ionizing the molecules *in vacuo* and making them "fly" by volatilization. Under the influence of combinations of electric and magnetic fields, the ions follow trajectories depending on their individual mass (m) and charge (z). To perform MS, the samples under study are subjected to Energy Desorption/Ionisation (EDI) from a surface by input of energy. Typically EDIs are thermal desorption/ionisation (TDI), plasma desorption/ionisation (PDI) and various kinds of irradiation desorption/ionisation (IDI) such as by fast atom bombardment (FAB), electron impact, etc. Where a laser is used to ionize the sample, the process is called laser desorption/ionisation (LDI), such as matrix-assisted laser desorption/ionisation (MALDI). Desorption can be assisted by presenting the MS analyte together with various helper substances or functional groups on the ionization surface, preferably such as surface-enhanced laser desorption/ionisation (SELDI). For molecules of low molecular weight, MS has long been part of the routine physical-organic repertoire for analysis and characterization of organic molecules by the determination of the mass of the parent molecular ion. Introduction of the so-called "soft ionization" methods, namely MALDI and ElectroSpray Ionization (ESI), permitted intact ionization, detection and exact mass determination of large molecules, *e.g.*, exceeding 300 kDa in mass, such as peptides and proteins (Fenn *et al.*, *Science*, 246, 64-71, 1989; Karas, M. & Hillenkamp, F., *Anal. Chem.*, 60, 2299-3001, 1988). In addition, by arranging collisions of the ionized parent molecule with other particles (*e.g.*, argon atoms), the ionized parent molecule is fragmented, forming secondary ions by collision induced dissociation (CID). The fragmentation pattern/pathway very often allows the derivation of more detailed information, for example structural information about the molecule.

MALDI-MS and ESI-MS have been used to analyze nucleic acids as well as proteins (see, Nordhoff *et al.*, *Mass Spectrom. Rev.*, 15: 67-138, 1997). However, since nucleic acids are very polar biomolecules, which are difficult to volatize, there has been an upper mass limit for clear and accurate resolution. A few reports on the MALDI-MS of large DNA molecules with lasers emitting in the ultraviolet (UV) have been reported (Ross P. & Belgrader, P., *Anal. Chem.*, 69:3966-3972, 1997; Tang *et al.*, *Rapid Commun. Mass Spectrum*, 8:727-730, 1994; Bai *et al.*, *Rapid Commun. Mass Spectrum*, 9:1172-1176, 1995; Liu *et al.*, *Anal. Chem.*, 67:3482-3490, 1995; and Siegert *et al.*, *Anal. Biochem.*, 243:55-65, 1997).

The analysis of nucleic acids by IR-MALDI with solid matrices (mostly succinic acid and, to a lesser extent, urea and nicotinic acid) has been described (Nordhoff *et al.*, *Rapid Commun. Mass Spectrom.*, 6:771-776, 1992; Nordhoff *et al.*, *Nucleic Acids Res.*, 21:3347-3357, 1993; Nordhoff *et al.*, *J. Mass Spec.*, 30:99-112, 1995). The 1992 Nordhoff *et al.* paper reports that a DNA 20-mer and a RNA 80-mer were about the uppermost limit for resolution. The 1993 Nordhoff *et al.* paper, however, provides a distinct spectra for a DNA 26-mer and a tRNA 104-mer. The 1995 Nordhoff *et al.* paper shows a substantially better spectra for the analysis of a 40-mer by UV-MALDI with the solid matrix, 3-hydroxy picolinic acid, than by IR-MALDI with succinic acid. In fact, the 1995 paper reports that IR-MALDI results in a substantial degree of prompt fragmentation.

In a Time-Of-Flight (TOF) mass spectrometer (TOF-MS), the mass-to-charge ratio m/z of ions can be determined from their time of flight. Although it is always the mass-to-charge ratio m/z that is measured in MS, with m being the mass and z being the number of elemental charges carried by the ion, in the following, for the sake of simplicity, only the mass m and its determination will be referred to. Since many types of ionization, such as MALDI, predominantly supply only single-charged ions ($z = 1$), the difference ceases to exist in practice for these types of ionization. In a TOF-MS that is equipped with an ion selector and a velocity-focusing reflector, it is possible to measure the daughter-ion or fragment-ion spectra of parent ions that are selected by the ion selector on the basis of their time of flight. The decay of parent ions into daughter or fragment ions can be induced by introducing excess energy during ionization (so-called PSD "Post Source Decay" spectra) or by applying other methods such as collisionally-induced fragmentation. The parent ions and the daughter ions resulting from their decay enter the reflector simultaneously with the same average velocity but with different mass-proportional energies, such that they will be dispersed according to their mass within the reflector by their different energies.

Mass spectroscopy as well as other tools that permit detection of, *e.g.*, the infrared and ultraviolet absorption spectra, nuclear magnetic resonance spectra, as well as analytical profiles such as biomolecular interaction analysis (*e.g.*, ELISA or surface plasmon resonance (SPR) profiles (Nedelkov, D. & Nelson, R., *Appl. Env. Microbiol.*, 69:5212-5215, 2003) and other techniques to measure the physical properties of a sample, provide methods for analyzing the samples. The information obtainable from methods using APCs described above, in connection with traditional laboratory methods, provides an integrated approach leading to the ability to resolve different properties of each sample under study. For example, where MS profiles for two samples display highly similar patterns, a second analysis such as an IR spectra, NMR spectra or SPR is used to provide additional comparative signatures and information. The result is an analytical signature profile, specific for each sample or sample under analysis, that provides for independent identification of the sample, (alone or in a mixture), and that can also provide, in certain embodiments, quantitative information about the sample such as concentration, as well as qualitative information, such as identification of other agents or materials in the sample mixture.

A preferred method utilizes mass spectroscopy to obtain proteomic signatures of APCs in healthy states and in response to challenge with antigens. Mass spectroscopy can also be used directly on

mixtures suspected of containing the antigens or other contaminants. Most preferred analytical methods for obtaining these signatures includes SELDI, such as Ciphergen's ProteinChip® System Series 4000, or MALDI O-TOF, based on an orthogonal platform coupling the MALDI to the MS, such as Perkin Elmer's prOTOF™ 2000 MALDI O-TOF Mass Spectrometer.

In one aspect, proteomic signatures of APCs, preferably DCs, are obtained after challenge from toxins and organisms on the National Institute for Allergy and Infectious Diseases Biodefense Priority Pathogens List. The DCs are cultured with the antigens or fragments thereof, as is described below, and proteomic signatures are obtained. The relevant antigens include *Bacillus anthracis* (anthrax), *Clostridium botulinum*, *Yersinia pestis*, *Variola major* (smallpox) and other pox viruses, *Francisella tularensis* (tularemia), and those causing viral hemorrhagic fevers, arenaviruses, such as, for example, lymphocytic choriomeningitis virus (LCMV), junin virus, machupo virus, guanarito virus, and those causing lassa fever, bunyaviruses and hantaviruses such as those causing rift valley fever, caliciviruses, hepatitis A, B and C), viral encephalitides such as west Nile virus, LaCrosse, California encephalitis, VEE, EEE, WEE, Japanese encephalitis virus, kyasanur forest virus, tickborne hemorrhagic fever viruses, Crimean-Congo hemorrhagic fever virus, tickborne encephalitis viruses, yellow fever, multi-drug resistant TB, influenza, other rickettsias and rabies, flaviruses, dengue, filoviruses, ebola, Marburg virus, *Burkholderia pseudomallei*, *Coxiella burnetii* (Q fever), *Brucella* species (brucellosis), *Burkholderia mallei* (glanders), ricin toxin (from *Ricinus communis*), epsilon toxin of *Clostridium perfringens*, *Staphylococcus* enterotoxin B, typhus fever (*Rickettsia prowazekii*), food and waterborne pathogens, bacteria such as diarrheagenic *E. coli*, pathogenic vibrios, *Shigella* species, *Salmonella*, *Listeria* monocytogenes, *Campylobacter jejuni*, *Yersinia enterocolitica*), and protozoa such as *Cryptosporidium parvum*, *Cyclospora cayatanensis*, *Giardia lamblia*, *Entamoeba histolytica*, toxoplasma and microsporidia.

These biosamples are amenable to detection and identification, based on criteria such as lipoprotein content, glycoprotein content, membrane composition, the presence and absence of viral envelopes, expression of particular proteins such as virulence factors, and other biochemical profiles (Dell, A. & Morris, H., *Science*, 291:2351-6, 2001; Rudd *et al.*, *Proc. Natl. Acad. Sci. USA*, 96:13044-9, 1999; Beerman *et al.*, *Biochem. Biophys. Res. Comm.*, 267:897-905, 2000).

Analytical signatures are obtained from samples of cells, fluids and tissues of a subject exposed to (or suspected of exposure to) one or more toxins and organisms on the National Institute for Allergy and Infectious Diseases Biodefense Priority Pathogens List. The signatures of DCs and fluids or tissues are compared to reference signatures to confirm exposure and to aid in monitoring treatment. A blood sample is obtained, for example, from a subject suspected of having been exposed to smallpox. The sample is split into two aliquots; the DCs recovered from one, and the plasma purified from the other. Both samples are subjected to mass spectroscopy. The DC signature is compared to reference signatures that provide positive and negative controls for exposed and naïve DCs, and the plasma is assayed for the presence of variola virus. The signatures can confirm infection, before the patient becomes viremic or symptomatic, thus facilitating their quarantine.

The SELDI or MALDI O-TOF mass spectrometer signatures can profile the nucleic acids, proteins, carbohydrates and lipids of a microbial sample, but can preferably profile and obtain a signature for the whole pathogen. The signatures distinguish between microbial species, and varieties within the species, *e.g., E. coli* O157; stages of microbial growth, *e.g.,* sporulative, vegetative or in active growth; relative age; and other characteristics such as pathogenicity of toxins, *e.g.,* pyrogenic exotoxin A production in group A streptococci, the cholera toxin in *Vibrio cholerae*, Shiga toxin-producing *E. coli* (STEC), or enterotoxin production in enterohemorrhagic (EHEC) strains of *E. coli*. For example, Lai *et al.* (*Syst. Appl. Microbiol.*, 27:186-191, 2004) discuss that sixty seven strains of *Carnobacterium*, atypical *Lactobacillus*, *Enterococcus durans*, *Lactobacillus maltaromicus* and *Vagacoccus salmoninarum* were examined by Fourier transform infrared (FT-IR) spectroscopy. The effects of culture age and reproducibility over a six month period were also investigated. The results were analyzed by multivariate statistics and compared with those from a previous numerical phenetic study, a pyrolysis mass spectrometry (PyMS) study and with investigations that used DNA-DNA and 16S rRNA sequencing homologies. Taxonomic correlations were observed between the FT-IR data and these studies. Culture age was observed to have little effect on the spectra obtained. The reproducibility study indicated that there is correlation between spectra produced on two occasions over the six month period. It was concluded that FTIR is a reliable method for investigating carnobacterial classification, and may have further potential as a rapid method for use in carnobacterium identification.

Similarly, Lee *et al.* (*Anal. Chem.*, 75:2746-52, 2003) discuss a bacterial analysis method coupling the flow field-flow fractionation (flow FFF) separation technique with detection by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The composition of carrier liquid used for flow FFF was selected based on retention of bacterial cells and compatibility with the MALDI process. The coupling of flow FFF and MALDI-TOF MS was demonstrated for *P. putida* and *E. coli*. Fractions of the whole cells were collected after separation by FFF and further analyzed by MALDI-MS. Each fraction, collected over different time intervals, corresponded to different sizes and different growth stages of bacteria.

Likewise, Lefmann *et al.* (*J. Clin. Microbiol.*, 42:339-46, 2004) discuss MALDI-TOF MS after base-specific cleavage of PCR-amplified and *in vitro*-transcribed 16S rRNA gene (rDNA), used for the identification of mycobacteria. Full-length 16S rDNA reference sequences of 12 type strains of *Mycobacterium spp.* frequently isolated from clinical specimens were determined by PCR, cloning, and sequencing. For MALDI-TOF MS-based comparative sequence analysis, mycobacterial 16S rDNA signature sequences (approximately 500 bp) of the 12 type strains and 24 clinical isolates were PCR-amplified using RNA promoter-tagged forward primers. T7 RNA polymerase-mediated transcription of forward strands in the presence of 5-methyl ribo-CTP maximized mass differences of fragments generated by base-specific cleavage. *In vitro* transcripts were subsequently treated with RNase T1, resulting in G-specific cleavage. Sample analysis by MALDI-TOF MS showed a specific mass signal pattern for each of the 12 type strains, allowing unambiguous identification. All 24 clinical isolates were identified unequivocally by comparing their detected mass signal pattern to the reference sequence-

derived *in silico* pattern of the type strains and to the *in silico* mass patterns of published 16S rDNA sequences. A 16S rDNA microheterogeneity of the *Mycobacterium xenopi* type strain (DSM 43995) was detected by MALDI-TOF MS and later confirmed by Sanger dideoxy sequencing. Analysis of 16S rDNA amplicons by MS after base-specific cleavage of RNA transcripts allowed fast and reliable identification of the *Mycobacterium tuberculosis* complex and ubiquitous mycobacteria (mycobacteria other than tuberculosis).

Thus, one embodiment of the present invention includes obtaining proteomic, genomic, lipid, carbohydrate, and whole organism signatures for bacterial pathogens. Preferably these are obtained using SELDI or MALDI O-TOF mass spectrometry, alone or in conjunction with other assays. Microbial identification is not limited to bacteria, and the analytic signatures of other pathogenic organisms thus include those of fungi, viruses, prions, and other infectious agents and pathogens.

The proteomic signatures derived from APCs and those obtained by direct assessment of the pathogens from the fluids of a patient are used in the diagnosis of disease as described, but are particularly useful for monitoring the course of therapy, *e.g.*, in response to antimicrobial compounds such as terbinafine, fluconizole, lamivudine, ciprofloxacin, vancomycin, penicillin, methicillin and other antibiotics. Signatures of tissues, fluids and cells of a subject therapeutically treated with antimicrobial compounds can also be analyzed for toxicity during such therapy. Detection of viral samples is described in, for example, Hong *et al.* (*J. Hepatol.*, 40:837-844, 2004), which assayed for mutations in hepatitis B virus (HBV) permitting lamivudine resistance that arise during prolonged treatment with that drug. Therapy with lamivudine frequently causes selection for HBV virions having amino acid substitutions in the YMDD motif of HBV DNA polymerase. MALDI-TOF MS genotyping detects HBV variants in a sensitive and specific manner. The assay was based on PCR amplification and mass measurement of oligonucleotides containing sites of mutation of the YMDD motif. The MALDI-TOF MS-based genotyping assay described therein is sufficiently sensitive to detect as few as 100 copies of HBV genome per milliliter of serum, with superior specificity for determining mixtures of wild-type and variant viruses. When sera from 40 patients were analyzed, the MALDI-TOF MS-based assay correctly identified known viral variants and additional viral quasi-species not detected by previous methods, as well as their relative abundance. It was concluded that the sensitivity, accuracy and amenability to high-throughput analysis makes the MALDI-TOF MS-based assay suitable for mass screening of HBV infected patients receiving lamivudine, and can help provide further understanding of disease progression and response to therapy.

One object of the present invention includes the proteomic, genomic, lipid, carbohydrate, and whole organism signatures for viral pathogens, and analytic signatures of DCs and other tissues, fluids and cells of a subject having a viral infection. Preferably these are obtained using SELDI or MALDI O-TOF mass spectrometry, alone or in conjunction with other assays.

Bonetto *et al.* (*J. Biol. Chem.*, 277:31327-34, 2002) discusses the elucidation of the structure and biological properties of the prion protein scrapie (PrP(Sc)) as fundamental to an understanding of the mechanism of conformational transition of cellular (PrP(C)) into disease-specific isoforms and the pathogenesis of prion diseases. They observed that a construct of 106 amino acids (termed PrP106 or

miniprion), derived from mouse PrP was highly toxic to primary neuronal cultures, and induced a remarkable increase in membrane microviscosity.

Accordingly, in still another aspect, the invention includes signatures of prion samples, and signatures of APC, and tissues, fluids and cells of a subject having a prion infection. Preferably these are obtained using SELDI or MALDI O-TOF mass spectrometry, alone or in conjunction with other assays.

It is yet another object of the invention to obtain the signatures, *e.g.,* SELDI or MALDI O-TOF MS and other analytic signatures, from healthy and from diseased subjects, *e.g.,* APCs, fluids and tissues, for example in diseases characterized by various stages of physical degeneration, such as, cardiac muscle, kidney, or neural tissues, in various stages of infection, such as viral or bacterial, or in various stages of transformation, malignancy or tumorogenicity. In particular, cancers and pre-malignant tissues all undergo significant biochemical changes relative to non-diseased cells and tissues, which can be readily detected by spectral and other types of analytical methods. One example of this is the change in glycosylation patterns seen in the tumor-associated antigen MUC-1 in many different cancers types, or the differential expression of chorioembrionic antigen (CEA), or tumor suppressor genes such as retinoblastoma (RB), p53, and cyclin dependent kinases CDKs. Numerous markers for cellular transformation and cancer are known in the medical literature, and all of these can be disease signatures for the purpose of the present invention. Likewise, these tissues exhibit changes to their metabolic states in response to treatment with chemotherapeutic samples and radiation. These changes are molecular signatures of a response to treatment, and are thus useful for the purposes described herein. The present methods can be used, for example, to identify the stage of a particular tumor type and monitor changes to the tumor over the course of therapy, such as chemotherapy or radiation. The methods described can also be used to monitor changes to healthy organs and tissues during such chemotherapy or radiation regimens, for example, to assess the systemic toxicity of the therapy for making adjustments to the course of treatment. In one embodiment, a toxicology profile for a chemotherapy regimen is provided. This profile comprises tissue-specific molecular analytical signatures of a plurality mammalian organs and tissues in an untreated state, *e.g.,* without exposure to a chemotherapy drug, as well as in response to a plurality of dosages of the drug. The profile can include a time dimension, *e.g.,* dose response signatures of the tissues over a period of time. The present invention thus includes analytical signatures useful in the detection and treatment of disease.

Chaurand *et al.* (*J. Proteome Res.*, 3:245-52, 2004) determined that analysis of thin tissue sections of organs results in over 500 individual protein signals in the mass range of 2 to 70 kDa that directly correlate with the protein composition within a specific region of the tissue sample. Such profiling, including imaging MS, has been applied to multiple diseased tissues, including human gliomas and non-small cell lung cancer. Interrogation of the resulting complex MS data sets has resulted in identification of both disease-state and patient-prognosis specific protein patterns.

Ahmed *et al.* (*Br. J. Cancer*, 91:129-140, 2004) discuss differentially expressed proteins in the serum of ovarian cancer patients that may be useful as biomarkers of this disease. A total of 24 serum proteins were differentially expressed in grade 1, 31 in grade 2, and 25 in grade 3 ovarian cancer patients.

Six of the protein spots that were significantly upregulated in all groups of ovarian cancer patients were identified by nano-electrospray quadrupole time-of-flight mass spectrometry (n-ESIQ(q)TOFMS) and matrix-assisted laser desorption ionization time-of-flight mass spectrometry as isoforms of haptoglobin-1 precursor (HAP1), a liver glycoprotein present in human serum. Further identification of the spots at different pathological grades was confirmed by Western blotting and immunohistochemical localization using monoclonal antibodies against a haptoglobin epitope contained within HAP1.

Bharti *et al.* (*Anticancer Res.*, 24:1031-8, 2004) discuss detection of serum tumor biomarkers at an earlier stage in order to improve the overall survival of cancer patients. Utilizing MALDI-TOF MS-based protein identification techniques, a SCLC-specific overexpressed protein was identified to be haptoglobin alpha-subunit, with its serum level correlating with the disease stage. The mean level of alpha-haptoglobin was increased in SCLC serum as compared to the normal controls. Serum HGF was also studied as potential tumor biomarker and was found to correlate with the disease status.

Several other tumor types are amenable to detection using the present methods. Iwadate *et al.* (*Cancer Res.*, 64:2496-501, 2004) discuss the detection and response to chemotherapeutic treatment of gliomas. The biological features of gliomas, which are characterized by highly heterogeneous biological aggressiveness even in the same histological category, are precisely described by global gene expression data at the protein level. Iwadate *et al.* investigated whether proteome analysis based on two-dimensional gel electrophoresis and matrix-assisted laser desorption/ionization TOF-MS could identify differences in protein expression between high- and low-grade glioma tissues. Proteome profiling patterns were compared in 85 tissue samples: 52 glioblastoma multiform, 13 anaplastic astrocytomas, 10 astrocytomas, and 10 normal brain tissues. The normal brain tissues were found to be completely distinguishable from glioma tissues by cluster analysis based on the proteome profiling patterns. Proteome-based clustering significantly correlated with the patient survival, and they could identify a biologically distinct subset of astrocytomas with aggressive nature. Discriminant analysis extracted a set of 37 proteins differentially expressed based on histological grading. Among them, many of the proteins that were increased in high-grade gliomas were categorized as signal transduction proteins, including small G-proteins. Immunohistochemical analysis confirmed the expression of identified proteins in glioma tissues.

Friedman *et al.* (*Proteomics*, 4:793-81, 2004) discuss two-dimensional difference gel electrophoresis (2-D DIGE) coupled with MS used to investigate tumor-specific changes in the proteome of human colorectal cancers and adjacent normal mucosa. Over 1500 protein spot-features were investigated in each paired normal/tumor comparison. Using DIGE technology with the mixed-sample internal standard, statistically significant quantitative comparisons were made for each protein abundance change across multiple samples simultaneously. MALDI-TOF MS provided sensitive and accurate mass spectral data for database interrogation, resulting in the identification of 52 unique proteins (including redundancies due to proteolysis and post-translationally-modified isoforms) that were changing in abundance across the cohort.

Hamler *et al.* (*Proteomics*, 4:562-77, 2004) discuss a two-dimensional liquid-phase separation scheme coupled with MS for proteomic analysis of cell lysates from normal and malignant breast

epithelial cell lines. Liquid-phase separations consist of isoelectric focusing as the first dimension and nonporous silica reverse-phase high-performance liquid chromatography (NPS-RP-HPLC) as the second dimension. Protein quantitation and mass measurement are performed using ESI-TOF MS. Proteins are identified by peptide mass fingerprinting using MALDI-TOF MS and MALDI-quadrupole time of flight (QTOF)-tandem mass spectrometry (MS/MS). Mass maps were created that allowed visualization of protein quantitation differences between normal and malignant breast epithelial cells. Of the approximately 110 unique proteins observed from mass mapping experiments over the limited pH range, 40 (36%) were positively identified by peptide mass fingerprinting and assigned to bands in the mass maps. Of these 40 proteins, 22 were more highly expressed in one or more of the malignant cell lines. These proteins represent potential breast cancer biomarkers that could aid in diagnosis, therapy, or drug development.

Veenstra *et al.* (*Curr. Opin. Mol. Ther.*, 5:584-93, 2003) discusses serum protein fingerprinting. Many proteomic studies have focused on the identification and subsequent comparative analysis of the thousands of proteins that populate complex biological systems such as serum and tissues.

Accordingly, these proteomic, carbohydrate, nucleic acid, and lipid spectroscopic profiles or patterns provide for signatures of numerous tissues in both healthy and disease states, and from a diagnostic perspective indicate the presence of disease and can be used to monitor changes in the organism having the disease. In one embodiment, serum or lymphatic samples are used for obtaining such signatures. In another embodiment, DCs are used. In other embodiments, APCs provide the analytical signatures. In yet other embodiments, blood cells, PBMCs, muscle tissues, nervous tissues, epithelial tissues and connective tissues are assessed.

Another object of the present invention includes determining the chemical signatures of toxic industrial chemicals, and the consequential proteomic, genomic, lipid, and carbohydrate signatures of APCs, tissues, fluids and cells of a subject that has been, or is suspected of being exposed to toxic industrial chemicals (TIC). Preferably these are obtained using SELDI or MALDI O-TOF mass spectrometry, alone or in conjunction with other analytical methods. The resultant signatures are stored in a database and made available for diagnostic and therapeutic applications. A toxic industrial chemical is generally understood as a material that has a toxicity (LC_{50} by inhalation) of less than 100,000 mg per min/M3 and an appreciable (undefined) vapor pressure at 20°C. The term TIC as used herein, includes Toxic Industrial Materials (TIM), generally regarded as any substance that in a given quantity produces a toxic effect in exposed personnel through inhalation, ingestion, or absorption. Examples of TICs and TIMs include fuels, oil, pesticides and herbicides, acids and bases, radiation sources, fertilizers, arsenic, chlorine, bromine, carbon disulfide, cyanide, metals (*e.g.*, cobalt, lead, mercury, cadmium and thallium), phosgene and other organic and heavy metal toxins. Many TICs and TIMs are known in industry, and the above referenced agents are not intended to be comprehensive or limiting.

In another aspect, the invention provide for signatures, *e.g.*, chemical, proteomic, genomic, lipid, carbohydrate, and whole organism signatures of agents of significance to national defense, such as biowarfare and chemical warfare agents (also known as WMD), and the proteomic, genomic, lipid, and

carbohydrate signatures of APCs, tissues, fluids and cells of a subject that has been, or is suspected of being exposed to such agents. Preferably these signatures are obtained with SELDI, MALDI O-TOF MS and other analytic methods. In particular, since spectral analysis provides a rapid and accurate detection means, it is possible to employ the present invention as part of a rapid or first response program, for field identification of biowarfare and chemical warfare agents in the samples.

The first step in the analytical process includes obtaining a sample of the agent (TIC, WMD) bacteria, virus, prion, cell, APC, fluid or tissue under study. The sample can be processed prior to examination, *e.g.,* dissolved in water or a solvent, or used intact. Simple analytical methods can be used to gain rudimentary information about the sample. Collection of a mass spectrum and analysis thereof follows. The sample is applied to an inlet port on the MS, and a mixture or a whole cell (or organism) can further contain one or more analytes, which can comprise lipid, carbohydrate, nucleic acid and/or peptide structure or any other inorganic or organic structure. Samples can undergo treatment prior to MS, where the sample can be transformed to one in which the MS-analyte is a derivative of the starting analyte, the amount(s) of non-analyte species have been changed compared to the starting sample, the relative occurrence of different MS-analytes in a sample is changed compared to the starting sample, the concentration of an MS-analyte is changed relative the corresponding starting analyte in the starting sample, or sample constituents, such as solvents, have been changed and/or the analyte has been changed from a dissolved form to a solid form, for instance in a co-crystallized form. Such treatments include, for example, digestion into fragments of various sizes and/or chemical derivatization of an analyte. Digestion can be purely chemical or enzymatic. Derivatization includes so-called mass-tagging of either the starting analyte or of a fragment or other derivative formed during a sample treatment protocol. Other treatments include purifying and/or concentrating the sample prior to analysis. Such treatments apply, for example, to analytes that are biopolymers comprising carbohydrate, lipid, nucleic acid and/or peptide structure. Alternatively, the sample can also pass through the microchannel structure without being changed.

The invention is directed to, for example, portable units capable of high-throughput (HT)screens. Mobile units could be used, for example, in cases of bioterrorism where large numbers of subject would be tested for exposure. A mobile unit could include HT gene expression detectors for determining signatures from a blood sample from a subject. Such HT expression detectors are known and could be, for example, mounted inside of a truck for rapid deployment in cases of emergency response.

EXEMPLIFICATION

Example 1.

The following example details the use of APCs as biosensors for disease. Reference standards of DC exposed to various pathogens are created, which are used in subsequent patient assays to determine exposure and to qualitate the immunological response to the pathogen.

Gene expression analysis: DCs and macrophages/monocytes from various donors are cultured in the presence and absence of pathogens to initiate a response to the pathogen, and are then harvested. Total

RNA is extracted from exposed and unexposed (infected and uninfected) cells, and used to create a reference array. Alternatively, the total RNA is converted to cDNA before being used in creating the reference array. Patient-derived samples of DCs are recovered, the nucleic acids extracted, and hybridized to the microarray and then scanned. During the microarray data processing, a filtering approach is used based on a fix change in average difference intensity values. For analysis of the raw data, GenNet, an extremely robust expression data analysis platform is used. Using such a platform meets high-throughput demands, and is scalable.

Pathogen Genotyping: Since DCs and macrophages serve as pathogen reservoirs, enrichment of these cells and the use of genotypic analysis for the presence of the pathogen provides a novel screening method. Conventional methods of viral and bacterial disease diagnosis require the detection of the pathogens themselves, *e.g.,* in blood cultures, or detection of pathogen-specific proteins or DNA/RNA, *e.g.,* by PCR, and correlation of these findings with clinical symptoms. To overcome the limitations associated with conventional screening methods, high-throughput genotyping assays have been developed, which are used to screen large numbers of pathogens. These assays are capable of detecting molecular variation in microbial strains thus allowing distinction of, for example, route of transmission, origin, and relationship of a particular bacterial, viral, fungal, or parasitic strain, etc.

Mass Spectrometry (MS): To improve the success and productivity of peptide identification the SELDI and MALDI time of flight (TOF) mass spectrometers are used. These are the most commonly used mass spectrometry methods for detecting peptide mass fingerprinting. This MALDI-O-TOF system uses orthogonal injection to introduce sample ions from the MALDI sources into a reflection TOF mass spectrometer. The MALDI sources of a conventional axial MALDI-TOF systems (linear or reflection mode) and is directly linked to the TOFMS. This direct linkage affects the instrument's accuracy, resolution, and sensitivity because any discrepancies associated with the sample target are transferred to the detector. In contrast, using orthogonal geometry, the MALDI source is separated from the TOF, thus eliminating discrepancies, increasing performance and simplifying method development. The protein signatures found in untreated cultured compared to treated cultures are established.

Biomarker detection: Commercially available pattern recognition and discovery software from Eclipse Diagnostics, or similar software to detect bio-markers is used. This software allows for the rapid detection of genomic and proteonomic bio-markers and other complex biological relationships. These biomarkers are part of the database and are used as a pathogen-specific reference.

Advantages: Myeloid cells concentrate pathogens within the cell thus improving sensitivity of detection. Additionally, cDNA microarray technology is a high resolution technology capable of analyzing 52,000 or greater genes per sample and is independent of culturing the pathogen from blood. The method of microarray construction involves a longer 70 mer probe design and 30-fold internal redundancy per gene. Also, the methods herein allow for diagnosis before symptoms occurs. In the case of bioengineered pathogens, traditional microbiology, ELISA- or PCR-based technology has to be created to detect new the

new pathogen. By evaluating key cellular pathways (*e.g.*, apoptotic, inflammatory, NF-κB, and inflammatory mediators), early events in exposure can be detected.

Herein is described a method of rapid enrichment (1.5 h) of DCs and macrophages from the blood, which does not require prolonged culturing or use of costly cytokines to force differentiation. In contrast, contemporary methods require culturing DC precursors for 7 days. DCs in culture with prolonged exposure to cytokines is known to induce specific alteration in cellular pathways, and have been demonstrated to have modified pathogen infectivity, gene activation, and protein synthesis. High-throughput (HT) gene arrays allow the analysis of greater than 1,000 samples in per day. The number samples processed per day is equipment dependent (this is also the case for the proteonomic technology (*e.g.*, 10,000 samples/day)). The integration of the HT system with the genomic and proteonomic database improves detection efficiency and also allows the real-time monitoring of progression of disease. Collectively, the combination serves to provide highly complex genomic- and proteonomic-based arrays and information databases for hospitals and laboratories that can be shared in real time over networks.

The present invention provides for methods of rapidly identifying pathogens in the body and in the environment. The following pathogens are amenable to detection and characterization using APC and the techniques described herein: bacteria, viruses, fungi, prions and protozoa.

Representative bacteria that are presented to APC to create pathogen exposed APC signatures include, Gram-positive and Gram-negative bacteria such as, for example, *Staphylococcus*, such as, for example, *S. epidermis* and *S. aureus*; *Micrococcus*; *Streptococcus*, such as, for example, *S. pyogenes*, *S. equis*, *S. zooepidemicus*, *S. equisimilis*, *S. pneumoniae* and *S. agalactiae*; *Corynebacterium*, such as, for example, *C. pyogenes* and *C. pseudotuberculosis*; *Erysipelothrix* such as, for example, *E. rhusiopathiae*; *Listeria*, such as, for example, *L. monocytogenes*; *Bacillus*, such as, for example, *B. anthracis*; *Clostridium*, such as, for example, *C. perfringens*; and *Mycobacterium*, such as, for example, *M. tuberculosis* and *M. leprae*. Gram negative bacterial species are exemplified by, but not limited to genera including: *Escherichia*, such as, for example, *E. coli* 0157:H7; *Salmonella*, such as, for example, *S. typhi* and *S. gallinarum*; *Shigella*, such as, for example, *S. dysenteriae*; *Vibrio*, such as, for example, *V. cholerae*; *Yersinia*, such as, for example, *Y. pestis* and *Y. enterocolitica*; *Proteus*, such as, for example, *P. mirabilis*; *Bordetella*, such as, for example, *B. bronchiseptica*; *Pseudomonas*, such as, for example, *P. aeruginosa*; *Klebsiella*, such as, for example, *K. pneumoniae*; *Pasteurella*, such as, for example, *P. multocida*; *Moraxella*, such as, for example, *M. bovis*; *Serratia*, such as, for example, *S. marcescens*; *Hemophilus*, such as, for example, *H. influenza*; and *Campylobacter* species. Other species suitable for assays of the present invention include spirochetes such as, for example, those causing Lyme disease, Enterococcus, Neisseria, Mycoplasma, Chlamidia, Francisella, Pasteurella, Brucella, and Enterobacteriaceae. Also detectable are CDC biological pathogens A, B, and C biological pathogens. Further examples of pathogenic bacterial species that are detectable according to the invention are obtained by reference to standard taxonomic and descriptive works such as, for example, Bergey's Manual of Determinative Bacteriology, 9th Ed., 1994, Williams and Wilkins, Baltimore, Md.

Representative viruses that are presented to APCs to create pathogen-exposed APC signatures include, for example, adenovirus (such as can be found in infantile gastroenteritis, acute hemorrhagic cystitis, non-bacterial pneumonia, and viral conjunctivitis), herpesvirus (such as herpes simplex type I and type II, varicella zoster (the etiological agent of chicken pox), cytomegalovirus, and mononucleosis (the etiological agent Epstein-Barr virus)), poxvirus (the etiological agent for such disorders as smallpox (variola major and variola minor)), Hepatitis A, B, and C, vaccinia virus, hantavirus and molluscum contagiosum, picornavirus (such as rhinovirus (the common cold, also caused by coronavirus)), poliovirus (poliomyelitus), an orthomyxovirus or paramyxovirus (such as influenza, and respiratory syncytial virus (RS)), parainfluenza virus (including such diseases as mumps), and rubeola (measles), rhabdovirus (rabies), vesicular stomatitis (VSV), togavirus such as rubella- the etiological agent causing German measles, and togaviridae causing encephalitis (EEE, WEE, and VEE), flavivirus such as the etiological agent causing dengue fever, west Nile fever, yellow fever, and encephalitis, bunyavirus and arenavirus, reovirus, coronavirus such as the agent causing SARS, hepatitis, a papovavirus infection such as papilloma virus, a retroviral infection such as HIV, HTLV-1, and HTLV-II.

Representative fungi that are presented to APCs to create pathogen exposed APC signatures include, for example, *Candida*, such as, for example, *C. albicans*; *Cryptococcus*, such as, for example, *C. neoformans*; *Malassezia* (Pityrosporum); *Histoplasma*, such as, for example, *H. capsulatum*; *Coccidioides*, such as, for example, *C. immitis*; *Hyphomyces*, such as, for example, *H. destruens*; *Blastomyces*, such as, for example, *B. dermatiditis*; *Aspergillus*, such as, for example, *A. fumigatus*; *Penicillium*, such as, for example, *P. marneffei*; *Pseudallescheria*; *Fusarium*; *Paecilomyces*; *Mucor/Rhizopus*; and *Pneumocystis*, such as, for example, *P. carinii*. Subcutaneous fungi, such as, for example, species of *Rhinosporidium* and *Sporothrix*, and dermatophytes, such as, for example, *Microsporum* and *Trichophyton* species, are amenable to prevention and treatment by embodiments of the invention herein. Other disease-causing fungi that can be detected include *Trichophyton*, *Microsporum*; *Epidermophyton*; *Basidiobolus*; *Conidiobolus*; *Rhizopus*; *Cunninghamelia*; *Rhizomucor*; *Paracoccidioides*; *Pseudallescheria*; *Rhinosporidium* and *Sporothrix*.

Representative protozoa that are presented to APC to create pathogen exposed APC signatures include the one or more single-celled, usually microscopic, eukaryotic organisms, such as amoebas, ciliates, flagellates, and sporozoans, for example, Plasmodium, Trypanosoma or Cryptosporidium.

Example 2.

The generation of APC-specific signatures is a two step process. The first step involves obtaining a population of immune cells. The isolated cells or fractions derived from isolated cells, *e.g.*, nuclear, membrane and cytosolic fractions are suitable for obtaining a signature. Preferred cells are of the myeloid lineage, but peripheral blood mononuclear cells (PBMCs) are suitable. Methods of enriching for myeloid cell populations, including DCs, are described in US patents 6,589,526 and 6,194,204. Myeloid cells include monocytes and dendritic cells, in roughly 90% to 10% proportions. Antigenic markers for monocytes include CD14+, HLA-DR or MHC-II, CD80+ CD86+. Antigenic markers for DCs include

CD2+, CD5+, CD14+ CD83+ and CD90+. These are obtained by positive or negative selection methods. Preferred cell types are myeloid, which express antigenic markers consistent with both DC and monocyte cells. It is preferred to use freshly isolated, *e.g.*, blood-purified myeloid cells instead of cultured myeloid cells. Differences in MS spectra between DCs and MOs are shown in FIGS. 1 and 2.

The following is a procedure for isolation of MOs from PBMCs: Buffy coats were isolated from healthy volunteers (Transfusion Therapy, Children's Hospital, Boston, MA) and washed and concentrated with PBS. The buffy concentrate can be incubated with a modified monocyte enrichment means, such as the RosetteSep Kit, commercially available from StemCell Technologies, for example. This rosette cocktail contains anti-CD3, anti-CD19, anti-CD54, and anti-CD62 monoclonal antibodies, which bind to T-cells, B-cells, NK cells and granulocytes. After 30 minute of incubation, this population was layered over ficoll gradient and centrifuged (Sorvall RT 6000, DuPont, Wilmington, DE) at 2500 rpm for 30 min to separate the low density DC and Mo from the high density (T-cells, B-cells, granulocytes and NK cells) density fractions. The low density cell population was >95% $CD14^{high}$ by flow cytometry. These cells were incubated with a 1:100 dilution of mouse mAb (in ascitic fluid) to human CD2 for 30 min at 4°C, washed, and incubated with goat anti-mouse IgG magnetic beads (Miltenyi Biotech). Following incubation, the preparation was passed through a magnetic column according to the manufacturer's instructions. The magnetic column retained the CD2+ cells, which were >96% pure, while the CD2- cells were >95% pure by flow cytometry with anti-CD2 and anti-CD14. A blocking buffer containing 10% v/v heat-inactivated pooled human serum (PHS) (Nabi, Boca Raton, FL) and human IgG (50 mg/ml; Immuno AG, Vienna, Austria) in HBSS without magnesium and calcium (Cellgro; Fisher Scientific, Pittsburgh, PA) was used to prevent nonspecific mAb binding during each stage of isolation or flow cytometric analysis. Culture medium (CM) containing RPMI 1640 (Cellgro) supplemented with 10% heat-inactivated PHS, 20 μg/ml gentamicin, 100 U/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Gaithersburg, MD) was used for morphologic and functional studies of freshly isolated, noncytokine-incubated CD2+ and CD2- Mo.

The second step involves obtaining mass spectra from the DCs, for example using QSTAR (ABI), SELDI TOF from Ciphergen or proTOF from Perkin Elmer. Spectra are taken for naïve APCs and those exposed to agents, *e.g.*, pathogens, tumors, or other antigens. This example describes the process for obtaining individual data sets (signatures) from pathogen-APC or pathogen-food samples (*e.g.*, *Listeria*-APC, *Listeria*-milk) to create a profile for bacterially contaminated and uncontaminated milk (see, for example, FIGS. 23A-C). The signature of a *Listeria*-infected individual, obtained from sampling of APCs from that individual, is also provided.

Each profile includes a proteomic signature of, for example but not limited to, the cell membrane, cytoplasmic proteins, and nuclear protein characteristics, protein charges (*e.g.*, positive and negative), Cu^{2+} chelating properties, cleavage patterns of native or denatured proteins with various endopeptidase, and the like, of the agents under study, measured by such properties as *m/z* size (kD), *m/z* intensity (μAmps), and standard deviation quantities. The frequency of occurrence of identifying features in a

signature is corroborated by obtaining spectra of replicate samples, preferably 3-4 samples, thereby providing a consensus signature.

Several commonly used methods for isolating, fractionating or enriching sample proteins are as follows, others are known in the art. PARIS Kit - this is a kit commercial available from Ambion, and allows for the rapid isolation of RNA and proteins from samples. This kit can be used for those studies involving the isolation of APC proteins from APC-viruses or bacteria co-cultures. For more sensitive detection and characterization of samples, it is advantageous to use cellular membrane fractions, which are obtained by common molecular biology techniques. The combination of the membrane, cytoplasm, and nuclear proteins enhances the sensitivity of APC-based detection methods.

Bacterial lysis by sonication can be used for both Gram-negative and Gram-positive bacteria. Sonication optionally involves the use of detergents, such as Tween, Triton X-100, digitonin, CHAPS, SDS, Nonidet and others, which are recommended for profiles of Gram-negative bacteria or microorganisms with a thick or tough cell membrane. This protocol was used in the milk studies. Bacteria were harvested from agar plate into 5 ml of TEN Buffer (10 mM Tris-HCl pH = 7.4, 1 mM EDTA, 100 mM NaCl). The harvested bacteria were pelleted at 5,000 x g for 10 minutes and subjected to 3 rounds of freeze/thawing in a dry ice/ethanol bath, thawing at 37°C. After resuspension into a small volume (0.1-0.5 ml) of ice-cold MTBS buffer (16 mM Na_2HPO_4, 4 mM NaH_2P0_4, 150 mM NaCl, 1% Triton X-100, 1 mM PMSF), the cells were sonicated with 15 seconds burst followed by 30 seconds incubation on ice (4 rounds of sonication). The bacterial debris was pelleted at 14,000 rpm and the supernatant was removed to a fresh tube. The protein concentration was measured by Bradford protein assay, standard curve, UV absorption or other method, and the lysed sample was stored at -70°C until ready for SELDI analysis.

Bacterial lysis by French Press can be used for Gram-negative bacteria and is not recommended for Gram-positive bacteria. To perform this technique, resuspend pellets of bacteria in 20 mM HEPES pH 7.4, 50 mM NaCl, 1% Triton-X 100, 1 mM PMSF are disrupted using a French Press at 750 psi. Cell debris is removed by centrifugation at 20,000 x g for 20 min at 4°C. Protein concentration is measured by Bradford protein assay or similar assay, and the sample is stored in aliquots at -70°C until performing the SELDI technique.

BugBuster Extraction kit- this is a commercially available kit sold by Novagen and allows for gentle disruption of the cell wall of *E. coli* to release active proteins. This is a simple, rapid, low-cost alternative to French Press or sonication for releasing expressed target protein in a cell preparation. Alternatively, a buffer such as 10 mM Tris-HCl pH 7.4, 8 M Urea, 2% (w/v) CHAPS, 1 mM PMSF can also be used as lysis buffer.

The SELDI experimental protocol described below uses the IMAC ProteinChip Array (PCA). The IMAC Arrays are coated with an NTA functional group to entrap transitional metals for subsequent metal affinity binding proteins. In these profiling studies, arrays are charged with copper prior to applying sample to the surface. Selectivity is determined by concentration of imidazole in the binding buffer. Increasing concentrations of imidazole in the binding/washing buffer, reduces the binding of

protein with weaker affinities for metal, thereby reducing background signals. The protocol for IMAC PCA is described in detail below and is similar to the other Ciphergen PCA protocols.

Using the 8 spot arrays: Assemble the PCAs in the bioprocessor and add 50 µl of IMAC charging solution to each well. Vortex for 5 minutes at RT. Remove the buffer from the wells. Rinse with water. Add 50 µl of IMAC neutralization buffer to each well. Vortex for 5 minutes RT. Remove the buffer from well and rinse. Add 150 µl of the IMAC binding buffer to each well and vortex at RT for 5 minutes. Remove buffer. Repeat binding buffer wash steps twice. Add 90 µl of IMAC binding buffer and 10 µl of sample and vortex for 30 minutes at RT. The ratio of IMAC binding buffer to sample concentration can vary depending of the desired protein concentration. Remove the sample and wash with IMAC binding buffer three-times, each wash requires a 5 minute agitation step. Once completed, rinse with de-ionized water, drain wells of the bioprocessors, and let air dry. Apply 1.0 µl EAM (matrix) solution to each spot and let air dry. PCA are analyzed on the Ciphergen Chip reader.

The Perkin-Elmer proTOF experimental protocol applies the isolated-protein sample directly to the MALDI surface. In this process, the MALDI surface binds to everything in the sample (*e.g.*, proteins and nonproteins). The manufacturer suggests cleaning the samples, for example microscale protein purification using Millipore ZIP TIPS®, filtered ion exchange pipette tips capable of removing certain proteins, thereby reducing total protein levels and reducing the complexity of the sample mixture. This technique is sensitive enough such that the tip performance, variable from tip to tip, can impact the resultant signature.

The next step involves derivation of marker proteins from the APC interaction with the pathogen. Once the marker proteins have been isolated, the proteins provide templates for the generation of diagnostic antibodies. These antibodies to the derived APC proteins can be used for immunological assays, *e.g.*, attached to a multiplexer or fluorescent readers for diagnostic purposes.

Example 3.

This experiment investigated the reproducibility of the APC-derived signatures for DC exposed to bacterial and viral pathogens. *Listeria monocytogenes* were processed by the bacterial lysis and sonication methods described above and evaluated on metal-binding or hydrophobic surfaces. Samples were processed in parallel and analyzed on different PCA chips on different chip analyzers. The results demonstrate excellent experimental reproducibility. Data is displayed in spectral or gel views.

Shown are MS data depicting the complexity of rat serum, as four different samples exhibit complex spectra (FIG. 1). The invention illustrates the cytoplasmic protein profiles (signatures) from untreated/uninfected myeloid cells from two sample (FIG. 2).

The invention illustrates the cytoplasmic protein profiles (signatures) of Mx, Mo, and DC (FIG. 9) cultured in the presence of a control adenovirus or in the presence of adenovirus with a single gene substitution (FIGS. 12A, 12B, 13A, 13B, 14A and 14B). The observed signatures show that APCs infected with wild-type and modified adenovirus can be identified and distinguished using the present

invention. Unique protein signatures can be obtained that can differentiate between viruses having one gene substitution/modification.

Signatures are also shown for response to flu virus (FIG 10) and a genetically modified flu virus (FIG. 11). These signatures further support the unexpected finding that viral infection can be detected, and, moreover, the specific viral pathogen can be identified, even if the pathogen is very closely related to another pathogen.

The invention illustrates the protein profiles (signatures) obtained from nuclear protein extracts of Mx, DC and Mo co-cultured in the presence of *Listeria monocytogenes*. APCs cultured in the presence of other Gram positive and Gram negative bacteria also generate unique signatures that can be used to identify the microorganism co-cultured with the APCs (FIG. 15). The proteomic signature of *Listeria* alone (FIGS. 16A-D), for example, not cultured with APC is distinct from the three co-culture signatures (FIGS. 17A-C), suggesting that *Listeria* overgrowth did not occur thereby contaminating the APC co-cultures.

The invention illustrates the spectroscopic profiles (signatures) of either skim or whole milk with *Listeria* contamination (FIGS. 23A-C). The results demonstrate the ability of the present invention to detect the presence of unique pathogens in milk and other food stuffs, independent of APC detection methods.

Example 4.

In addition to pathological agents, the diagnostic methods include the detection, diagnosis and staging of various cancers and genetic disorders. Cancers are detectable by numerous markers. Malignant cells often express antigens that are not found in normal cells; some of these antigens are found at the surface of the cell, for example CEA (chorioembryonic antigen) and differentially glycosylated (hypoglycosylated) MUC-1, are two well-known tumor associated antigens. MUC-1/DF-3 is overexpressed in the majority of human carcinomas, multiple myeloma, acute myelogenous leukemia, acute lymphoblastic leukemia, and follicular lymphoma among others. The antigen can initiate an HLA-restricted T-cell response following presentation of the antigen by DC (see, Brossart *et al.*, *Cancer Res.*, 61:6846-50, 2001). Other proteins upregulated in cancer cells include vascular endothelial growth factor (VEGF), Her-2/neu and hepsin. Intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1) and E-Selectin (ELAM-1) play an important role in the complex series of events associated with inflammatory responses associated with cancer and tumor suppression.

In addition to pathological agents, the diagnostic methods include the detection, diagnosis and staging of various tumors and neoplasms. The invention can detect any of various malignant neoplasms characterized by the proliferation of anaplastic cells that tend to invade surrounding tissue and metastasize to new body sites. For example, APCs are cultured with the following cancers to produce APC signatures of cancer-exposed cells: astrocytomas, gliomas, ependymomas, osteosarcoma, Ewing's sarcoma, retinoblastoma, bladder cancer, small and non-small cell lung cancer, oat cell lung cancer, pancreatic cancer, colorectal cancer, cervical cancer, endometrial cancer, vaginal cancer, ovarian cancer,

cancers of the liver, acute lymphocytic leukemia, acute myelogenous leukemia, lymphoma, myeloma, basal cell carcinoma, melanoma, thyroid follicular cancer, bladder carcinoma, glioma, myelodysplastic syndrome, testicular cancer, stomach cancer, esophageal cancer, laryngeal cancer, squamous cell carcinoma, adenocarcinoma, leiomyosarcoma, urothelial carcinoma, breast cancer or prostate cancer.

APC, particularly CD2+ DCs are cultured with primary or metastatic tumor cells obtained by biopsy, preferably cells taken from representative stages of tumor growth. Alternatively, DCs are cultured with purified preparations of CEA (chorioembryonic antigen) or differentially glycosylated (hypoglycosylated) MUC-1, or other tumor associated antigens. The DC lysates are used to prepare cDNA that is then used to create arrays of DC reference standards for high throughput screens. Arrays are prepared for each cancer type listed above, and preferably include each stage of the particular cancer type.

Example 5.

Kits are developed for isolation of samples from subjects and from the environment. For patient diagnostic uses, the kits include reagents and materials for obtaining and isolating blood samples from patients, as well as reagents and materials for enriching cells such as APCs, PBMCs and more preferably DCs, and for processing the cells into cytoplasmic, nuclear, or membrane fractions and optionally for processing larger proteins into smaller peptides.

Kits can further include chips or plates and reagents, appropriate for use with mass spectroscopy, such as those produced by Ciphergen for SELDI and Perkin Elmer for MALDI-O-TOF. The kits also include suitable instructions for use. In certain embodiments, the kits include one or more of the APC arrays mentioned above, *e.g.*, for use in diagnosing and staging cancers, or for determining the agent of infection and progression of the infection, or for forensic analysis. Other kit components include controls such as reference proteins, used to calibrate the mass spectrometer. In still other embodiments, the kit includes albumin or high molecular weight proteins, and is used for enhancing the resolution of low molecular with proteins in the signatures (the albumin bump technique). Kits for use with patients are suitable for human and veterinary uses.

The following kits are provided herein: biodefense kits for identifying pathogens associated with bioweapons in the environment and in exposed subjects; agricultural kits for sampling contamination of food dairy products and livestock; endocrine and metabolic kits for assessing endocrine and metabolic function in a subject; neurological kits for assessing degenerative changes; infectious disease kits for identifying pathogens in an exposed subject; prenatal kits for assessing fetal health; cancer kits for diagnosing and staging cancer progression in a subject and for monitoring chemotherapy regimens and disease progression; cardiovascular kits for detecting early signs of cardiac damage and ischemia and vessel occlusion; renal kits for detecting damage in the subject from, *e.g.*, contrast agents and chemotherapy drugs.

Example 6.

The potential threat to national security posed by terror attacks involving biological, chemical, nuclear, and radiological weapons is a serious international concern. One of the challenges facing public health officials responding to such an attack is a previously limited ability to diagnose individuals who have been exposed to these agents and do not show illness. Medical professionals and governmental officials all recognize that disease outbreaks- such as SARS in Asia and Canada, avian influenza in East Asia, and Ebola and Marburg virus in Africa-demonstrate that the speed of diagnosis and implementation of public health measures can mean the difference between an isolated outbreak and a global pandemic. The potential of terrorist attacks against agricultural targets (agro-terrorism) is increasingly recognized as a national security threat. Agriculture has several characteristics that pose unique problems for managing the threat due to the fact that agricultural production is geographically disbursed and in unsecured environments. Livestock are frequently concentrated in confined locations, and then transported and commingled with other herds. Foot and mouth disease (FMD) outbreaks in Europe, and the recent detection of a second case of BSE in the United States underscore the importance of detecting disease in livestock in a rapid and accurate manner. Foods such as milk are stored in accessible areas that can be purposefully contaminated. Pest and disease outbreaks can quickly halt economically important exports. Effective detection depends on a heightened sense of awareness, and on the ability to rapidly determine the level of threat by the ability to screen livestock, as well as foods rapidly. Lessons from past disease outbreaks, show that the speed of detection and diagnosis can determine the difference between an isolated incident and wide spread disease.

The present invention provides comprehensive genomic, bioinformatics, functional genomics, and immune cell (APC-based) proteomic approaches for the detection and monitoring of bioterrorism agents, and the qualitative and quantitative assessment of infectious agents and their effects on the immune system of a subject. These approaches also provide a critical resource for the scientific community that could lead to the discovery and identification of novel targets for the next generation of drugs, vaccines, diagnostics and immunotherapeutics. Most importantly, these approaches are rapid and accurate.

In one aspect, a Proteomic Pathogen Reference Library (PPRL), that is electronically searchable, is constructed. The PPRL includes records of immune surveillance cells (APC) that have been contacted with the individual and combinations of the toxins and pathogens on the CDC Bioterrorism Agents and Diseases List. The records provide reference signatures for positive exposure of APCs to these agents. The PPRL is useful in detecting exposure of biological/chemical pathogens in human subjects, and can also be used to detect exposure of other mammals such as livestock. The PPRL also provides a reference for screening food(s) for bacterial and chemical pathogens that could be potentially introduced accidentally or deliberately into the food supply.

Signatures of APCs are obtained using cells that have been contacted with the toxins and pathogens, for example, those on the CDC Bioterrorism Agents and Diseases List. These include: Anthrax toxins (*Bacillus anthracis*); Arenaviruses; *B. anthracis* (anthrax); *Clostridium botulinum* toxin);

Brucella species; *Burkholderia mallei*; *Burkholderia pseudomallei* (melioidosis); *Chlamydia psittaci*; *Cholera* toxin; *Clostridium botulinum* toxin (botulism); *Clostridium perfringens*; Ebola virus (hemorrhagic fever); Emerging infectious diseases such as Nipah virus and hantavirus; Epsilon toxin of *Clostridium perfringens*; *Escherichia coli* O157:H7 (*E. coli*); Food safety threats (*e.g., Salmonella* species, *Escherichia coli* O157:H7, *Shigella*); *Francisella tularensis* (tularemia); Glanders (*Burkholderia mallei*); Lassa fever; Marburg virus hemorrhagic fever; Melioidosis (*Burkholderia pseudomallei*); Psittacosis (*Chlamydia psittaci*); Q fever (*Coxiella burnetii*); Ricin toxin from *Ricinus communis* (castor beans); *Rickettsia prowazekii* (typhus fever); *Salmonella* species (salmonellosis); *Salmonella typhi* (typhoid fever); Smallpox (variola major); Staphylococcal enterotoxin B; *Vibrio cholerae* (cholera); Viral encephalitis (alphaviruses [*e.g.,* Venezuelan equine encephalitis, eastern equine encephalitis, western equine encephalitis]); Viral hemorrhagic fevers (filoviruses [*e.g.,* Ebola, Marburg] and arenaviruses [*e.g.,* Lassa, Machupo]); Water safety threats (*e.g., Vibrio cholerae*, *Cryptosporidium parvum*); and *Yersinia pestis* (plague).

Signatures of the exposed APC are digitized and recorded on computer readable media as an individual data record. In one aspect, proteomic signatures of antigen exposed intact APC (each cell subtype) are obtained using, for example SELDI, MALDI-O-TOF, and MALDI-TOF. Metadata is provided with the data record, including APC cell type, toxin or pathogen, buffer composition and materials/methods for obtaining the particular APC signature, donor information, and other pertinent medical and etiological information. This is a reference data set. The reference data set is obtained for each APC cell type and each toxin or pathogen, and collectively comprise the reference data records in a Proteomic Pathogen Reference Library. The PPRL includes an expert system for parsing the reference data records, excluding and including individual data sets based on user defined criteria, and a pattern recognition routine to match data sets, and particularly the signatures of the exposed APC, to the signature of the unknown data set having an input subject derived sample, based on similarity of proteomic signatures.

To use the PPRL, a medical professional obtains a sample of blood from a subject suspected of exposure to a CDC Bioterrorism Agent. APCs are isolated from the blood sample and are separated by APC cell subtype, *e.g.,* by FACS for antigenic markers such as CD2+ DC, or CD4+ T-cells, etc. Each APC subtype is used to obtain whole cell mass spec proteomic signatures (or, alternatively, genomic informative gene expression profile signatures or protein signatures from cellular fractions). The signatures are digitized and uploaded to a computer program having algorithms that compile the proteomic signature and allow the medical professional to add metadata relevant to the sample, thereby producing an unknown data set. The computer program allows the medical professional to further compile the data set with user defined information into a search query, and communicates the search query to the PPRL, preferably over a network. The expert system parses the reference data records with the unknown data set as described. Potential matches, defined as reference data sets having agreement with the unknown data set to a particular defined confidence interval, are returned to the medical

professional over the network. The medical professional is thus able to confirm or exclude exposure of the subject to the various CDC Bioterrorism Agents.

In one aspect, the PPRL is networked with one or more of the following CDC programs: the Active Bacterial Core Surveillance (ABCS); the Gonococcal Isolate Surveillance Project (GISP); the National Antimicrobial Resistance Monitoring System: Enteric Bacteria (NARMS:EB); the National Electronic Disease Surveillance System (NEDSS); the Health Information and Surveillance Systems Board (HISSB); the National Nosocomial Infections Surveillance System (NNIS); the Intensive Care Antimicrobial Resistance Epidemiology (ICARE); and the Surveillance of Emerging Antimicrobial Resistance Connected to Healthcare (SEARCH) program. In one embodiment, a query resulting in a positive identification of a CDC Bioterrorism Agent in a subject sample, alerts one or more of these program groups to the positive identification. In another embodiment, an alert is sent to the Department of Homeland Security. In yet another embodiment, an alert is sent to hospitals within a specified geographic area, *e.g.*, proximal to the site of detection.

Example 7.

The PPRL includes records of immune surveillance cells (*e.g.*, APCs) that have been contacted with bacterial pathogens, including the common disease producing pathogens, nosocomial pathogens and drug resistant pathogens. The records provide reference signatures for positive exposure of APCs to these bacterial pathogens. The PPRL is useful in detecting exposure of bacterial pathogens in human subjects, and can also be used to detect exposure of other mammals such as livestock.

Signatures of APCs are obtained using cells that have been contacted with the following pathogenic bacteria. These include: *Bacillus*; *Bordetella*; *Borrelia*; *Campylobacter*; *Clostridium*; *Corynebacterium*; *Enterococcus*; *Escherichia*; *Francisella*; *Haemophilus*; *Helicobacter*; *Legionella*; *Listeria*; *Mycobacterium*; *Neisseria*; *Pseudomonas*; *Salmonella*; *Shigella*; *Staphylococcus*; *Streptococcus*; *Treponema*; *Vibrio*; *Yersinia*; *Neisseria* resistant to penicillins, tetracyclines, spectinomycin, and fluoroquinolones; Methicillin-resistant *Staphylococcus aureus* (MRSA); drug-resistant *Streptococcus pneumoniae*; fluoroquinolone and other drug resistant *Salmonella typhi*; Vancomycin-Intermediate/Resistant *Staphylococcus aureus*; and Vancomycin-resistant *Enterococci*, as well as other multi-drug resistant strains of bacteria.

Signatures of the pathogen exposed APC are digitized and recorded on computer readable media as an individual data record. In one aspect, proteomic signatures of antigen exposed intact APC (each cell subtype) are obtained using, for example SELDI, MALDI-O-TOF, and MALDI-TOF. Metadata is provided with the data record, including APC cell type, toxin or pathogen, buffer composition and materials/methods for obtaining the particular APC signature, donor information, and other pertinent medical and etiological information. In another aspect, APC series are obtained from reference subjects having a bacterial infection, that have been in various stages of infection, *e.g.*, initial localized infection, disseminated infection, moderate sepsis, severe sepsis, SIRS, septic shock, and multiple organ dysfunction syndrome (MODS). This is a reference data set. The reference data set is obtained for each

APC cell type and each bacterial pathogen and infection stage, and collectively comprise the reference data records in a Proteomic Pathogen Reference Library. The PPRL includes an expert system for parsing the reference data records, excluding and including individual data sets based on user defined criteria, and a pattern recognition routine to match data sets, and particularly the signatures of the exposed APC, to the signature of the unknown data set having an input subject derived sample, based on similarity of proteomic signatures.

To use the PPRL, a medical professional obtains a sample of blood from a subject suspected of exposure to a bacterial pathogen. The APC are isolated from the blood sample and are separated by APC cell subtype, *e.g.*, by FACS for antigenic markers such as CD2+ DC, or CD4+ T-cells, etc. Each APC subtype is used to obtain whole cell mass spec proteomic signatures. The signatures are digitized and uploaded to a computer program having algorithms that compile the proteomic signature and allow the medical professional to add metadata relevant to the sample, thereby producing an unknown data set. The computer program allows the medical professional to further compile the data set with user defined information into a search query, and communicates the search query to the PPRL, preferably over a network. The expert system parses the reference data records with the unknown data set as described. Potential matches, defined as reference data sets having agreement with the unknown data set to a particular defined confidence interval, are returned to the medical professional over the network. The medical professional is thus able to confirm or exclude exposure of the subject to the various bacterial pathogens, and discern the stage of infection. Further assays such as amplification of antibiotic resistance genes by PCR can confirm or exclude bacteria that may be resistant to specific drugs, thus aiding the course of therapy.

Example 8.

A cancer proteomic reference library is created that includes records of immune surveillance cells (APCs) that have been obtained from patients having different types of cancer, and at various disease stages of these cancers. The records provide reference signatures for positive exposure of APCs to various cancers at different stages of disease progression. The library is useful in diagnosing the presence of cancer in a test subject, as well as identifying the cancer type and stage.

The following cancer types each result in specific APC responses, and are amenable to detection using the techniques described: Acute Lymphoblastic Leukemia, Adult; Acute Lymphoblastic Leukemia, Childhood; Acute Myeloid Leukemia, Adult; Acute Myeloid Leukemia, Childhood; Adrenocortical Carcinoma; Adrenocortical Carcinoma, Childhood; AIDS-Related Cancers; AIDS-Related Lymphoma; Anal Cancer; Astrocytoma, Childhood Cerebellar; Astrocytoma, Childhood Cerebral; Bile Duct Cancer, Extrahepatic; Bladder Cancer; Bladder Cancer, Childhood; Bone Cancer, Osteosarcoma/Malignant Fibrous Histiocytoma; Brain Stem Glioma, Childhood; Brain Tumor, Adult; Brain Tumor, Brain Stem Glioma, Childhood; Brain Tumor, Cerebellar Astrocytoma, Childhood; Brain Tumor, Cerebral Astrocytoma/Malignant Glioma, Childhood; Brain Tumor, Ependymoma, Childhood; Brain Tumor, Medulloblastoma, Childhood; Brain Tumor, Supratentorial Primitive Neuroectodermal Tumors,

Childhood; Brain Tumor, Visual Pathway and Hypothalamic Glioma, Childhood; Brain Tumor, Childhood (Other); Breast Cancer; Breast Cancer and Pregnancy; Breast Cancer, Childhood; Breast Cancer, Male; Bronchial Adenomas/Carcinoids, Childhood; Carcinoid Tumor, Childhood; Carcinoid Tumor, Gastrointestinal; Carcinoma, Adrenocortical; Carcinoma, Islet Cell; Carcinoma of Unknown Primary; Central Nervous System Lymphoma, Primary; Cerebellar Astrocytoma, Childhood; Cerebral Astrocytoma/Malignant Glioma, Childhood; Cervical Cancer; Childhood Cancers; Chronic Lymphocytic Leukemia; Chronic Myelogenous Leukemia; Chronic Myeloproliferative Disorders; Clear Cell Sarcoma of Tendon Sheaths; Colon Cancer; Colorectal Cancer, Childhood; Cutaneous T-Cell Lymphoma; Endometrial Cancer; Ependymoma, Childhood; Epithelial Cancer, Ovarian; Esophageal Cancer; Esophageal Cancer, Childhood; Ewing's Family of Tumors; Extracranial Germ Cell Tumor, Childhood; Extragonadal Germ Cell Tumor; Extrahepatic Bile Duct Cancer; Eye Cancer, Intraocular Melanoma; Eye Cancer, Retinoblastoma; Gallbladder Cancer; Gastric (Stomach) Cancer; Gastric (Stomach) Cancer, Childhood; Gastrointestinal Carcinoid Tumor; Germ Cell Tumor, Extracranial, Childhood; Germ Cell Tumor, Extragonadal; Germ Cell Tumor, Ovarian; Gestational Trophoblastic Tumor; Glioma, Childhood Brain Stem; Glioma, Childhood Visual Pathway and Hypothalamic; Hairy Cell Leukemia; Head and Neck Cancer; Hepatocellular (Liver) Cancer, Adult (Primary); Hepatocellular (Liver) Cancer, Childhood (Primary); Hodgkin's Lymphoma, Adult; Hodgkin's Lymphoma, Childhood; Hodgkin's Lymphoma During Pregnancy; Hypopharyngeal Cancer; Hypothalamic and Visual Pathway Glioma, Childhood; Intraocular Melanoma; Islet Cell Carcinoma (Endocrine Pancreas); Kaposi's Sarcoma; Kidney Cancer; Laryngeal Cancer; Laryngeal Cancer, Childhood; Leukemia, Acute Lymphoblastic, Adult; Leukemia, Acute Lymphoblastic, Childhood; Leukemia, Acute Myeloid, Adult; Leukemia, Acute Myeloid, Childhood; Leukemia, Chronic Lymphocytic; Leukemia, Chronic Myelogenous; Leukemia, Hairy Cell; Lip and Oral Cavity Cancer; Liver Cancer, Adult (Primary); Liver Cancer, Childhood (Primary); Lung Cancer, Non-Small Cell; Lung Cancer, Small Cell; Lymphoblastic Leukemia, Adult Acute; Lymphoblastic Leukemia, Childhood Acute; Lymphocytic Leukemia, Chronic; Lymphoma, AIDS-Related; Lymphoma, Central Nervous System (Primary); Lymphoma, Cutaneous T-Cell; Lymphoma, Hodgkin's, Adult; Lymphoma, Hodgkin's, Childhood; Lymphoma, Hodgkin's During Pregnancy; Lymphoma, Non-Hodgkin's, Adult; Lymphoma, Non-Hodgkin's, Childhood; Non-Hodgkin's During Pregnancy; Lymphoma, Primary Central Nervous System; Macroglobulinemia, Waldenström's; Male Breast Cancer; Malignant Mesothelioma, Adult; Malignant Mesothelioma, Childhood; Medulloblastoma, Childhood; Melanoma; Melanoma, Intraocular; Merkel Cell Carcinoma; Mesothelioma, Malignant; Metastatic Squamous Neck Cancer with Occult Primary; Multiple Endocrine Neoplasia Syndrome, Childhood; Multiple Myeloma/Plasma Cell Neoplasm; Mycosis Fungoides; Myelodysplastic Syndromes; Myelodysplastic/Myeloproliferative Diseases; Myelogenous Leukemia, Chronic; Myeloid Leukemia, Adult Acute; Myeloid Leukemia, Childhood Acute; Myeloma, Multiple; Myeloproliferative Disorders, Chronic; Nasal Cavity and Paranasal Sinus Cancer; Nasopharyngeal Cancer; Nasopharyngeal Cancer, Childhood; Neuroblastoma; Non-Hodgkin's Lymphoma, Adult; Non-Hodgkin's Lymphoma, Childhood; Non-Hodgkin's Lymphoma During Pregnancy; Non-Small Cell Lung Cancer; Oral Cancer, Childhood;

Oral Cavity and Lip Cancer; Oropharyngeal Cancer; Osteosarcoma/Malignant Fibrous Histiocytoma of Bone; Ovarian Cancer, Childhood; Ovarian Epithelial Cancer; Ovarian Germ Cell Tumor; Ovarian Low Malignant Potential Tumor; Pancreatic Cancer; Pancreatic Cancer, Childhood; Pancreatic Cancer, Islet Cell; Paranasal Sinus and Nasal Cavity Cancer; Parathyroid Cancer; Penile Cancer; Pheochromocytoma; Pineal and Supratentorial Primitive Neuroectodermal Tumors, Childhood; Pituitary Tumor; Plasma Cell Neoplasm/Multiple Myeloma; Pleuropulmonary Blastoma; Pregnancy and Breast Cancer; Pregnancy and Hodgkin's Lymphoma; Pregnancy and Non-Hodgkin's Lymphoma; Primary Central Nervous System Lymphoma; Primary Liver Cancer, Adult; Primary Liver Cancer, Childhood; Prostate Cancer; Rectal Cancer; Renal Cell (Kidney) Cancer; Renal Cell Cancer, Childhood; Renal Pelvis and Ureter, Transitional Cell Cancer; Retinoblastoma; Rhabdomyosarcoma, Childhood; Salivary Gland Cancer; Salivary Gland Cancer, Childhood; Sarcoma, Ewing's Family of Tumors; Sarcoma, Kaposi's; Sarcoma (Osteosarcoma)/Malignant Fibrous Histiocytoma of Bone; Sarcoma, Rhabdomyosarcoma, Childhood; Sarcoma, Soft Tissue, Adult; Sarcoma, Soft Tissue, Childhood; Sezary Syndrome; Skin Cancer; Skin Cancer, Childhood; Skin Cancer (Melanoma); Skin Carcinoma, Merkel Cell; Small Cell Lung Cancer; Small Intestine Cancer; Soft Tissue Sarcoma, Adult; Soft Tissue Sarcoma, Childhood; Squamous Neck Cancer with Occult Primary, Metastatic; Stomach (Gastric) Cancer; Stomach (Gastric) Cancer, Childhood; Supratentorial Primitive Neuroectodermal Tumors, Childhood; T-Cell Lymphoma, Cutaneous; Testicular Cancer; Thymoma, Childhood; Thymoma and Thymic Carcinoma Thyroid Cancer; Thyroid Cancer, Childhood; Transitional Cell Cancer of the Renal Pelvis and Ureter; Trophoblastic Tumor, Gestational; Unknown Primary Site, Carcinoma of, Adult; Unknown Primary Site, Cancer of, Childhood; Unusual Cancers of Childhood; Ureter and Renal Pelvis, Transitional Cell Cancer; Urethral Cancer; Uterine Cancer, Endometrial; Uterine Sarcoma; Vaginal Cancer; Visual Pathway and Hypothalamic Glioma, Childhood; Vulvar Cancer; Waldenström's Macroglobulinemia; and Wilms' Tumor. Signatures of the exposed APCs are digitized and recorded on computer readable media as an individual data record.

In one aspect, proteomic signatures of cancer exposed intact APC (each cell subtype) are obtained using, for example SELDI, MALDI-O-TOF, and MALDI-TOF. Metadata is provided with the data record, including APC cell type, histological information about the cancer, buffer composition and materials/methods for obtaining the particular APC signature, donor information, and other pertinent medical and etiological information. In another aspect, APC series are obtained from reference subjects having a cellular proliferative disease, that have been in various stages of the disease, *i.e.*, stage 1, stage 2, stage 3 or stage 4, etc. This is a reference data set. The reference data set is obtained for each APC cell type and each cancer type and disease stage, and collectively comprise the reference data records in a Proteomic Cancer Reference Library. The PCRL includes an expert system for parsing the reference data records, excluding and including individual data sets based on user defined criteria, and a pattern recognition routine to match data sets, and particularly the signatures of the exposed APC, to the signature of the unknown data set having an input subject derived sample, based on similarity of proteomic signatures.

To use the PCRL, a medical professional obtains a sample of blood from a subject under study. The subject may not have cancer and the procedure is simply a screen for disease, or the subject may be suspected of having a cancer due to genetic predisposition or preliminary medical examination, or the subject may have a confirmed cancer and the procedure is designed to monitor changes in the disease. APCs are isolated from the blood sample and are separated by APC cell subtype, *e.g.*, by FACS for antigenic markers such as CD2+ DC, or CD4+ T-cells, etc. Each APC subtype is used to obtain whole cell mass spec proteomic signatures. The signatures are digitized and uploaded to a computer program having algorithms that compile the proteomic signature and allow the medical professional to add metadata relevant to the sample, thereby producing an unknown data set. Software allows the medical professional to further compile the data set with user defined information into a search query, and communicates the search query to the PCRL, preferably over a network. The expert system parses the reference data records with the unknown data set as described. Potential matches, defined as reference data sets having agreement with the unknown data set to a particular defined confidence interval, are returned to the medical professional over the network. The medical professional is thus able to ascertain if the subject has an APC signature that indicates the presence of various cancers, and discern the stage of disease.

Example 9.

The methods described herein can be used to identify informative genes, *e.g.*, genes that are differentially expressed in cells that are exposed or unexposed to an agent. The agent can be, for example, a chemical agent such as, for example, 2-chloroethylethyl sulphide (CEES), azide, ammonia and hypochlorous acid (HOCH). Tables 1-3 show informative genes, described by their common names and GenBank accession numbers, and the relative change in expression for APCs that are exposed to a particular chemical agent. The different agents shown, whether CEES, HOCH or azide, each have a different set of informative genes that are indicative of exposure. From the tables, it is clear that expression values for the specific informative genes are useful in determining exposure to a foreign agent, and, at a more refined level, to which foreign agent a cell or individual has been exposed.

Table 1.

CEES		
GenBank	**Common Name**	**Fold Change**
NM_001511	CXCL1	81.95
NM_002993	CXCL6	42.91
AA488987	---------	39.75
A1738416	BAL	15
NM_02235	LRAP	14.43

Table 2.

HOCH		
GenBank	**Common Name**	**Fold Change**
AF277897	EGFR	25.85
BF059208	---------	16.75
A1569792	FANCA	14.59
AL832404	PIGL	8.72
NM_000221	KHK	8.1

Table 3.

AZIDE		
GenBank	**Common Name**	**Fold Change**
H981131	---------	10.82
NM_018638	EKl1	8.96
AB003476	AKAP12	7.35
A1147556	DKFZ	6.547
NM_02235	GOLGIN-67	6.454

Proteomic signatures of cytosolic fractions of DCs are shown in FIG. 3 and nuclear fractions in FIG. 4. A gel view of the proteomic signatures is shown in FIG. 5. FIG. 6 shows the overlap of informative genes useful in identifying specific exposure (Venn diagram). The 698 common informative genes could be used to diagnose a general chemical exposure, whereas informative genes specific to a particular agent would be used to identify the specific exposure a subject has had. FIG. 7 show some for the biological pathways that are regulated after exposure to a particular agent. FIGS. 8A-C show the network of genes involved in response to a particular agent.

Example 10.

Signature can be determined for exposure to bacterial pathogens. In methods described above, a proteomic signature was found that is specific to wild-type *Bacillus anthracis* (FIGS. 18A and B, top panel) and a genetically-modified *B. anthracis* (FIGS. 18A and B, bottom panel). Venn diagrams showing the common and specific informative genes that are upregulated and downregulated in response to various *B. anthracis* strains are shown in FIG. 19. A spectrogram and gel view of a strain with an ALO knockout is shown in FIGS. 20A and 20B, respectively.

The discrimination between agents, whether chemical or pathogenic (*e.g.*, viral and bacterial) is readily and unexpectedly demonstrated. FIG 21 shows signature differences between unexposed cells, virus-exposed cells (vaccinia) and bacteria-exposed cells (*Listeria*).

FIG. 22 shows a gene expression profile that readily distinguishes specific bacterial exposures. *Y. pestis* and two strains of *B. anthracis* are distinguished from each other and clearly distinguished from unexposed cells. Virulent strains of *B. anthracis* contain two large plasmids, pX01 and pX02. Both plasmids are required for full pathogenicity, and strains that contain only one of these plasmids are avirulent. The plasmid pX01 encodes all three components of the anthrax toxin, and pX02 encodes the

poly-D-glutamic acid capsule. The avirulent Sterne vaccine strain, which is pX01+/pX02-, produces toxin but no capsule and is used effectively as a live veterinary vaccine.

The agent that induces a response can lead to sepsis. Signatures are provided that indicate sepsis can be detected, and, moreover, signatures are provided that indicate the stage of septic shock a subject is in (FIGS. 24A and 24B). FIG. 24A shows that the progression of a disease state can be monitored using the methods and signatures described herein.

Proteomic signatures are provided showing distinguishing signature characteristics for untreated, HIV-1(BaL)-exposed, HIV-1 (I22)-exposed, LPS-exposed and *Listeria*-exposed cells (FIGS. 30, 31, 32 and 33).

A general comparison of informative genes can be found in FIGS. 25A-C, 26, 27, 28A-B and 29. Gene expression profiles are used to both provide power to distinguish exposure (and to provide specific exposure information related to the agent). The informative genes are clustered and specific biological pathways are identified as being activated or shut down in response to exposure.

EQUIVALENTS

From the foregoing detailed description of the specific embodiments of the invention, it should be apparent that unique detection methodologies have been described. Although particular embodiments have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the appended claims that follow. In particular, it is contemplated by the inventors that various substitutions, alterations, and modifications can be made to the invention without departing from the spirit and scope of the invention as defined by the claims. For instance, the choice of spectrum, or the APC used in the detection process is believed to be matter of routine for a person of ordinary skill in the art with knowledge of the embodiments described herein. All U.S. Patents and other references cited herein are hereby incorporated herein by reference in their entirety.

We claim:

1. A method for determining a subject's exposure to an agent, comprising:
   a. determining a proteomic or gene expression signature from a sample obtained from the subject, wherein the sample comprises tissue sampling cells, and
   b. comparing the sample signature with a reference signature indicative of exposure to the agent,

   wherein congruity between the sample signature and the reference signature indicates the subject has been exposed to the agent.
2. The method of claim 1, wherein the subject is a mammal.
3. The method of claim 2, wherein the mammal is human.
4. The method of claim 1, wherein the agent is a chemical agent or a pathogenic agent.
5. The method of claim 1, wherein the agent is an autoimmune antigen.
6. The method of claim 1, wherein the proteomic signature is determined using mass spectrometry.
7. The method of claim 1, wherein the proteomic signature is determined using an immunochemical assay.
8. The method of claim 1, wherein the gene expression signature is determined using a hybridization assay.
9. The method of claim 8, wherein the hybridization assay is performed using microarrays.
10. A method for diagnosing a disease state in a mammal, comprising:
    a. substantially isolating antigen presenting cells from a mammalian sample comprising a subpopulation of antigen presenting cells,
    b. deriving a genomic or proteomic mammalian sample signature for the isolated antigen presenting cells, and
    c. comparing the mammalian sample signature to a reference signature indicative of a disease state,

    wherein congruity between the mammalian sample signature and the reference signature indicates the presence of the disease state in the mammal.
11. The method of claim 10, wherein the antigen presenting cells are dendritic cells.
12. The method of claim 10, wherein the disease state is a cancer or cell proliferative disorder.
13. The method of claim 10, wherein the disease state is a pathogenic infection.
14. The method of claim 13, wherein the pathogenic infection is a viral infection.
15. The method of claim 13, wherein the pathogenic infection is a bacterial infection.
16. An array comprising a plurality of addresses, each address having affixed thereto a sample of nucleic acid corresponding to a gene expressed by an antigen presenting cell.
17. The array of claim 16, further comprising a plurality of secondary addresses, each secondary address having affixed thereto a sample of nucleic acid corresponding to genes expressed by an antigen presenting cell that has encountered an antigen.
18. The array of claim 17, wherein the antigen presenting cell is a dendritic cell.

19. The array of claim 18, wherein the antigen is a cancer antigen.

20. The array of claim 18, wherein the antigen is a viral antigen.

21. The array of claim 18, wherein the antigen is a bacterial antigen.

22. A method for detecting a food-borne pathogen, comprising:

a. culturing naïve antigen presenting cells with a sample food product,

b. obtaining a sample signature from the co cultured antigen presenting cells, and

c. comparing the sample signature to a reference signature,

wherein congruity between the sample signature and the reference signature indicates the presence of the food-borne pathogen in the food product.

23. The method of claim 22, wherein the antigen presenting cell is a dendritic cell.

24. The method of claim 22, wherein the food-borne pathogen is a bacterial pathogen.

25. The method of claim 22, wherein the food-borne pathogen is a viral pathogen.

26. A diagnostic method comprising:

a. substantially isolating the antigen presenting cells from a blood sample from a mammalian patient being treated for a disorder,

b. deriving a genomic or proteomic patient sample signature for the isolated antigen presenting cells wherein the patient sample signature indicates the metabolic state of the antigen presenting cells in the subject, and

c. comparing the patient signature to a reference signature, wherein the reference signature is derived from antigen presenting cells from a reference subject having the same disorder as the patient,

wherein the congruity between the patient signature and the reference signature decreases during the treatment, thereby indicating the efficacy of the treatment in treating the disorder.

27. The method of claim 26, wherein the disorder is a cell proliferative disease, and the treatment is administration of an anti-neoplastic agent.

28. The method of claim 26, wherein the disorder is a cell proliferative disease, and the treatment provokes an immune response against the cell proliferative disease.

29. The method of claim 26, wherein the disorder is a bacterial infection, and the treatment is administration of an antibacterial agent.

30. The method of claim 26, wherein the disorder is a viral infection, and the treatment is administration of an antiviral agent.

31. A diagnostic method comprising:

a. substantially isolating the antigen presenting cells from a blood sample from a mammal afflicted with a disorder,

b. identifying one or more marker polypeptides from the antigen presenting cells, where the marker polypeptide is expressed in the antigen presenting cell in response to antigen contact and where the antigen contacted is associated with or the causative agent of the disorder,

c. obtaining an antibody to the marker polypeptide, and

d. detecting in the antigen presenting cells of a subject, the presence or absence of a polypeptide that binds to the antibody, wherein the presence of the polypeptide confirms the presence of the disorder in the subject.

32. The method of claim 31, wherein the antigen presenting cells are dendritic cells.

33. The method of claim 31, wherein the disorder is a cancer or cell proliferative disorder.

34. The method of claim 31, wherein the disorder is a pathogenic infection.

35. The method of claim 31, wherein the disorder is a viral infection.

36. The method of claim 31, wherein the disorder is a bacterial infection.

37. The method of claim 31, wherein the disorder is a prion infection.

38. The method of claim 31, wherein the disorder is a fungal infection.

39. A method for determining whether a subject has been exposed to a foreign agent, comprising:

a. isolating RNA from a sample from the subject comprising a subpopulation of immune sampling cells,

b. deriving a genomic or proteomic mammalian sample signature for the isolated cells wherein the sample signature indicates the metabolic state of the cells in the subject, and

c. comparing the sample signature to a reference signature derived from cells that have responded to exposure from the agent,

wherein congruity between the sample signature and the reference signature indicates the subject has been exposed to the agent.

40. A method for determining whether a subject has been exposed to a chemical agent, comprising:

a. determining a proteomic or gene expression sample signature of a sample obtained from a subject, and

b. comparing the sample signature with a reference sample signature indicative of exposure to a chemical agent,

wherein congruity between the sample signature and the reference signature is indicative of the subject's having been exposed to a chemical agent.

41. The method of Claim 40, wherein the chemical agent is 2-chloroethylethyl sulphide.

42. The method of Claim 41, wherein the reference sample signature comprises expression values or proteomic signatures of the genes or gene products from one or more genes listed in Table 1.

43. The method of Claim 40, wherein the chemical agent is azide.

44. The method of Claim 43, wherein the reference sample signature comprises expression values or proteomic signatures of the genes or gene products from one or more genes listed in Table 2.

45. The method of Claim 40, wherein the chemical agent is hypochlorous acid.

46. The method of Claim 45, wherein the reference sample signature comprises expression values or proteomic signatures of the genes or gene products from one or more genes listed in Table 3.

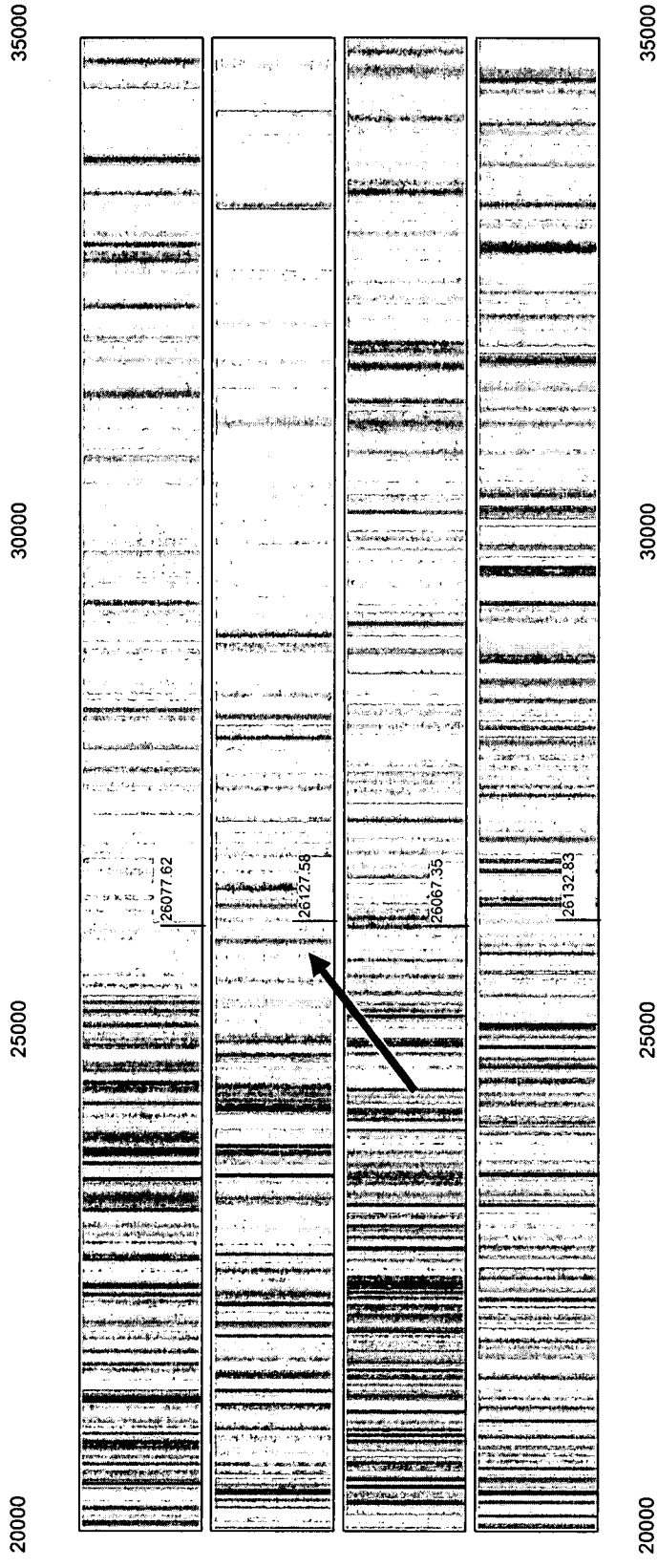


FIG. 1

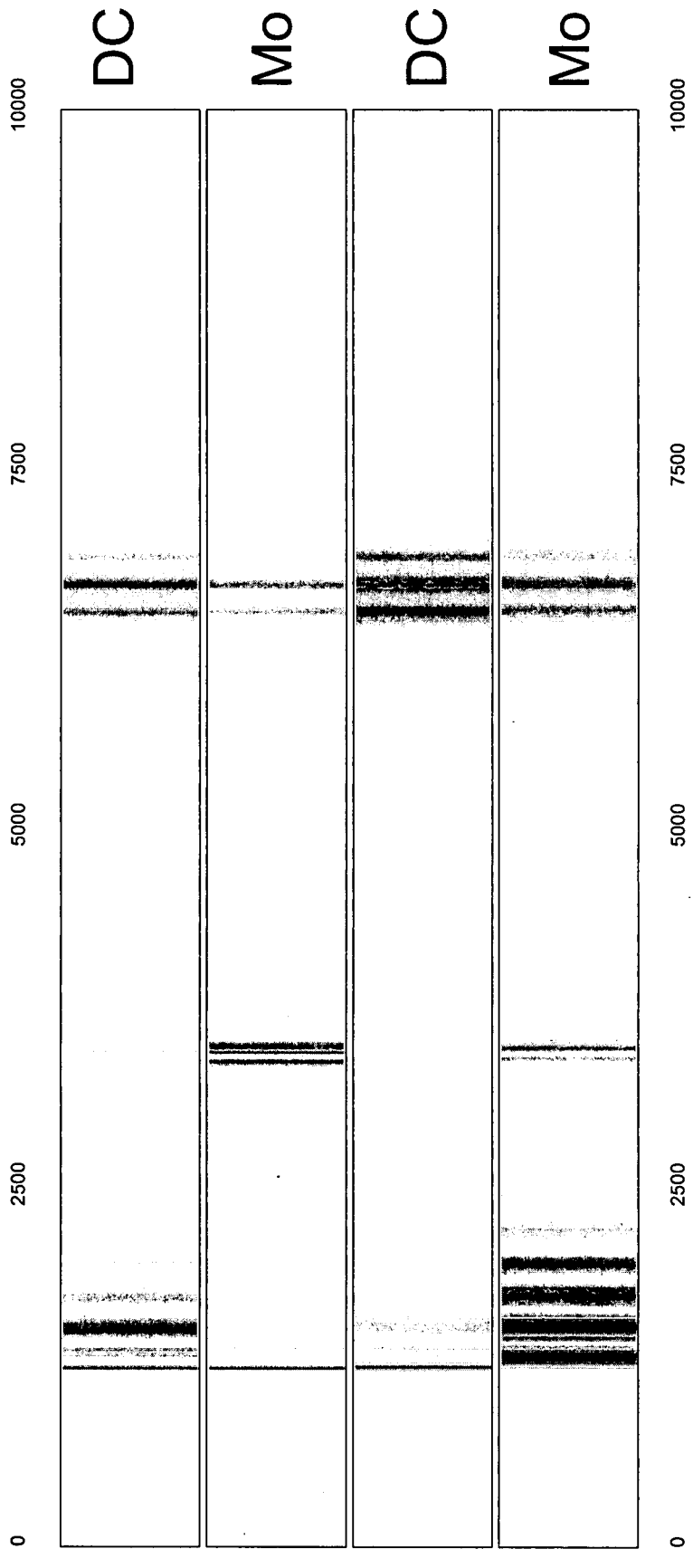
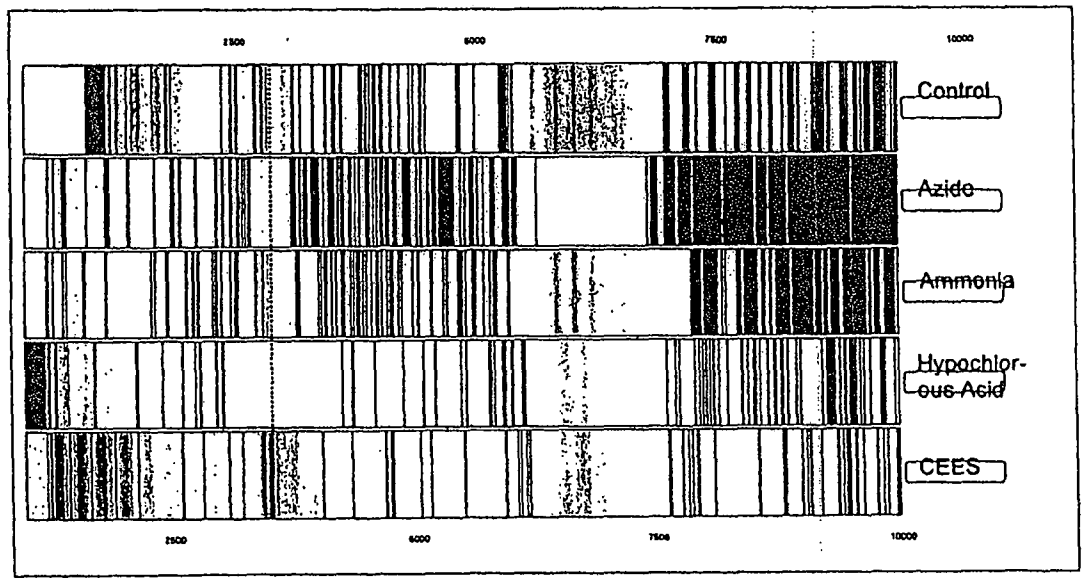


FIG. 2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CEES
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7500
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FIG. 3

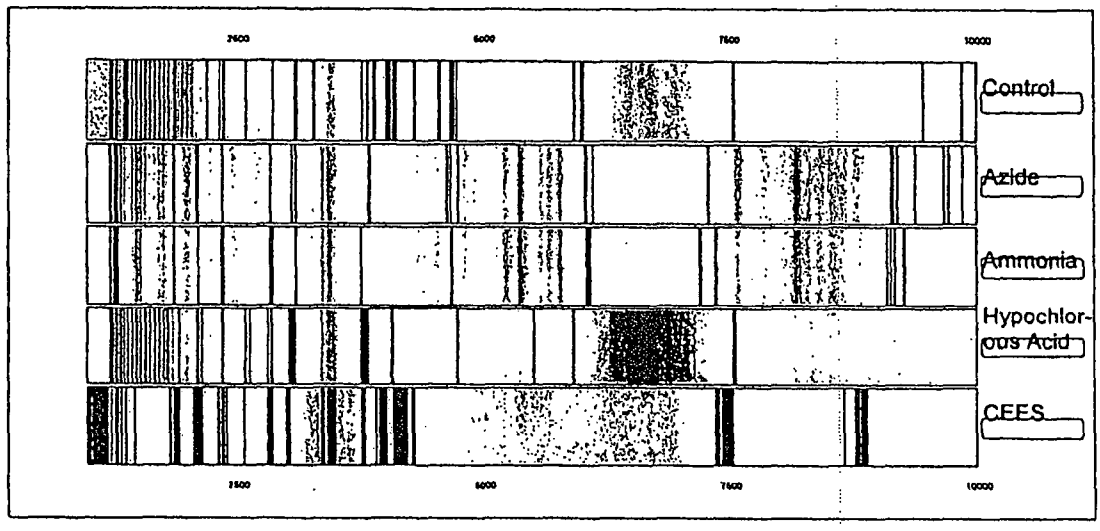
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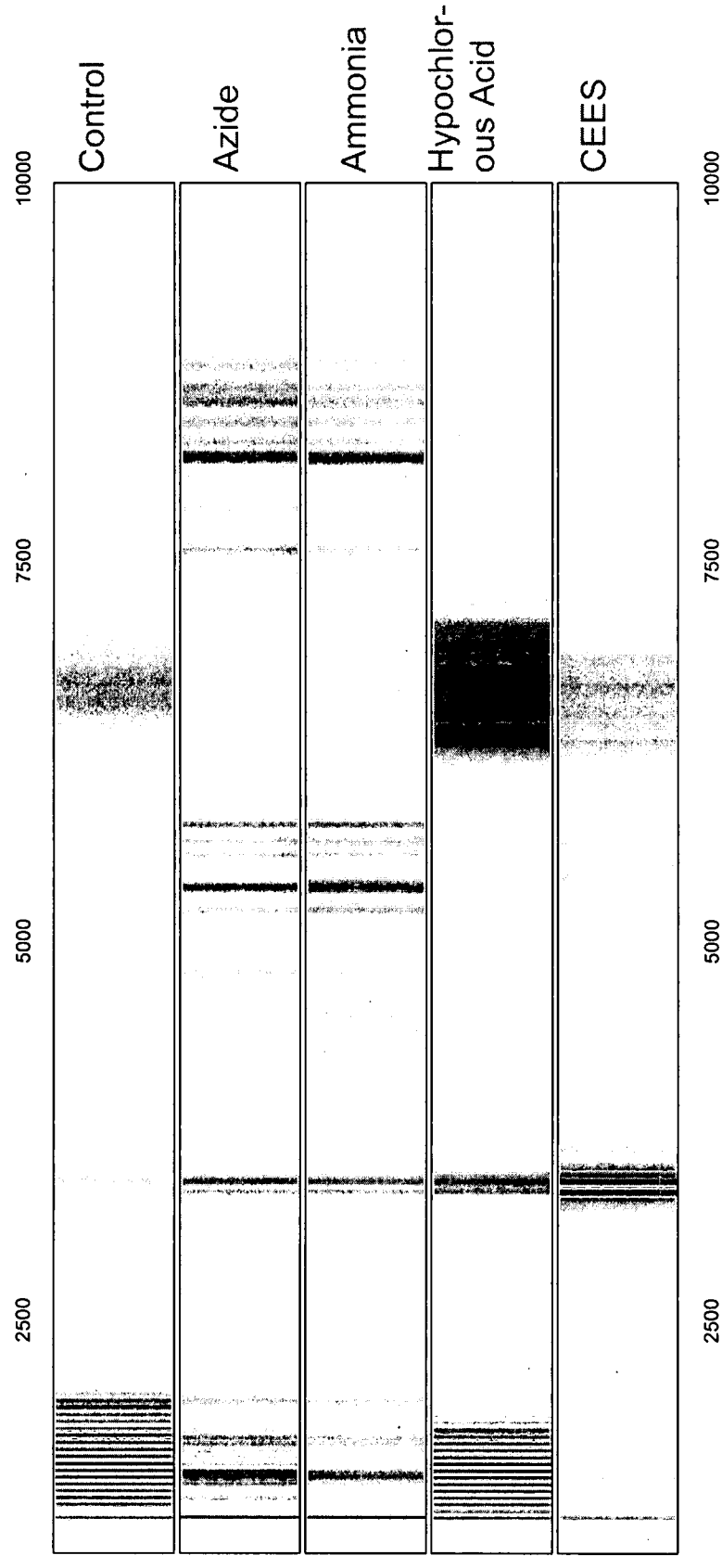
FIG. 4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5000
2500
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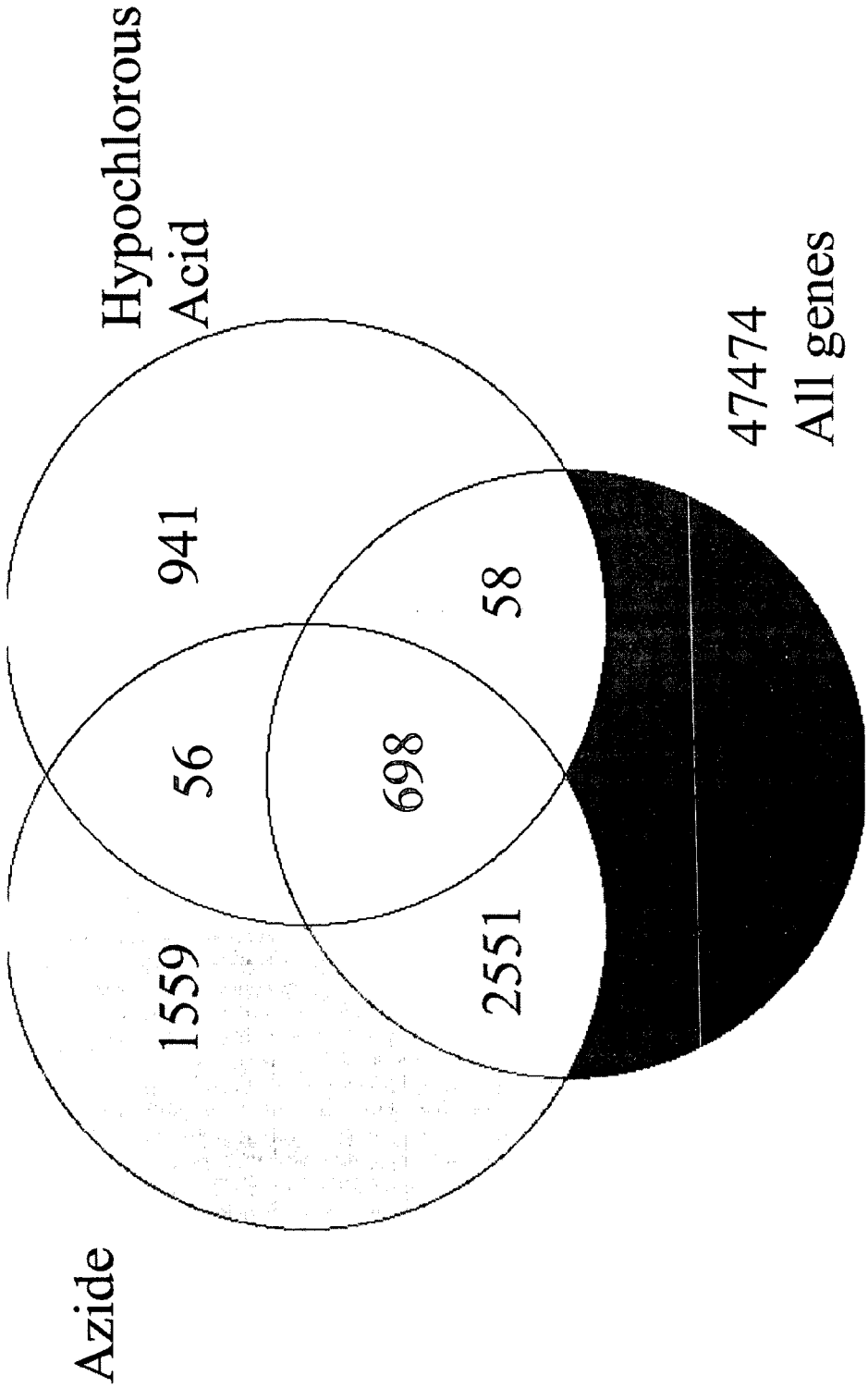
7500
5000
2500

FIG. 5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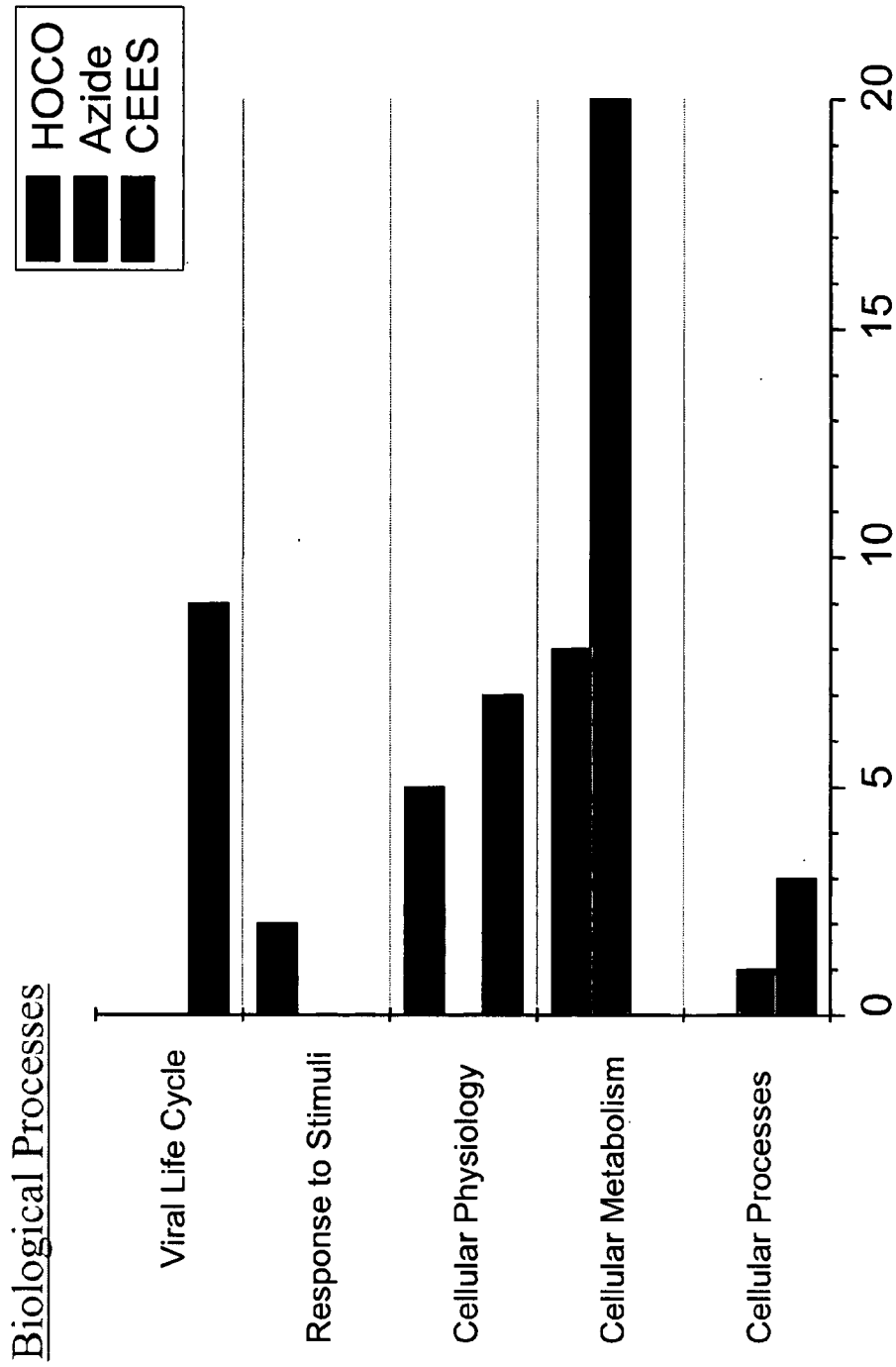
941
58
56
698
1559
2551
Azide
CEES
47474
All genes

FIG. 6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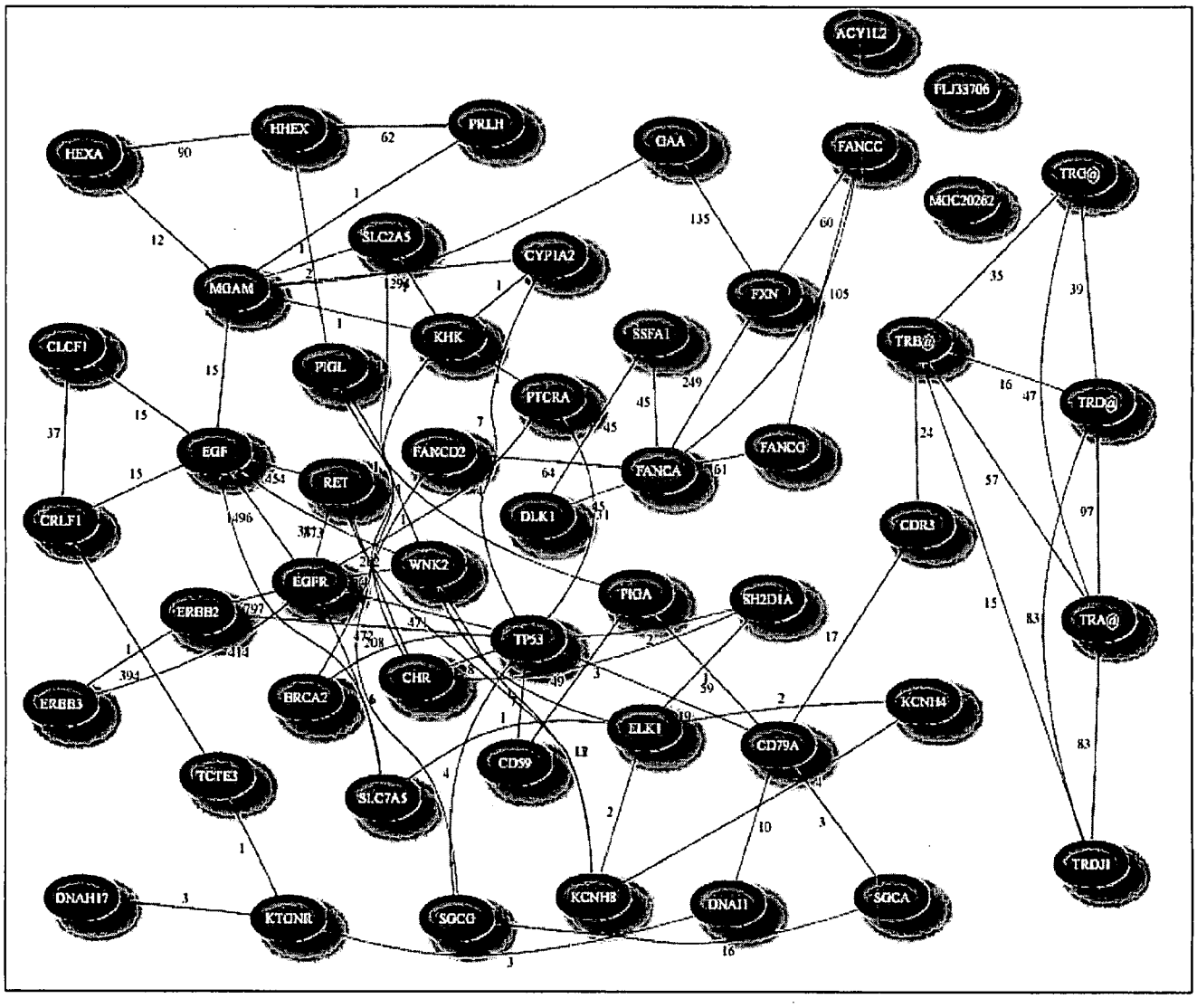
Azide
CEES
Biological Processes
Viral Life Cycle
Response to Stimuli
Cellular Physiology
Cellular Metabolism
Cellular Processes
0
5
10
15
20
Number of Genes ≥ 5-fold Gene Expression
Viral Life Cycle (DNA injury response)
Response to stimuli (complement activation)
Cellular Physiology (neutrophil activation)
Cellular Metabolism (glucose-6-phosphate)
Cellular Processes (BMP-2 signaling pathways)

FIG. 7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FANCD2
FANCA
FANCC
RET
CRLF1
DLK1
CDR3
EGFR
WNK2
PIGA
SH2D1A
ERBB2
TP53
TRA@
ERBB3
BRCA2
CHR
KCNH4
ELK1
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SOCG
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DNAI1
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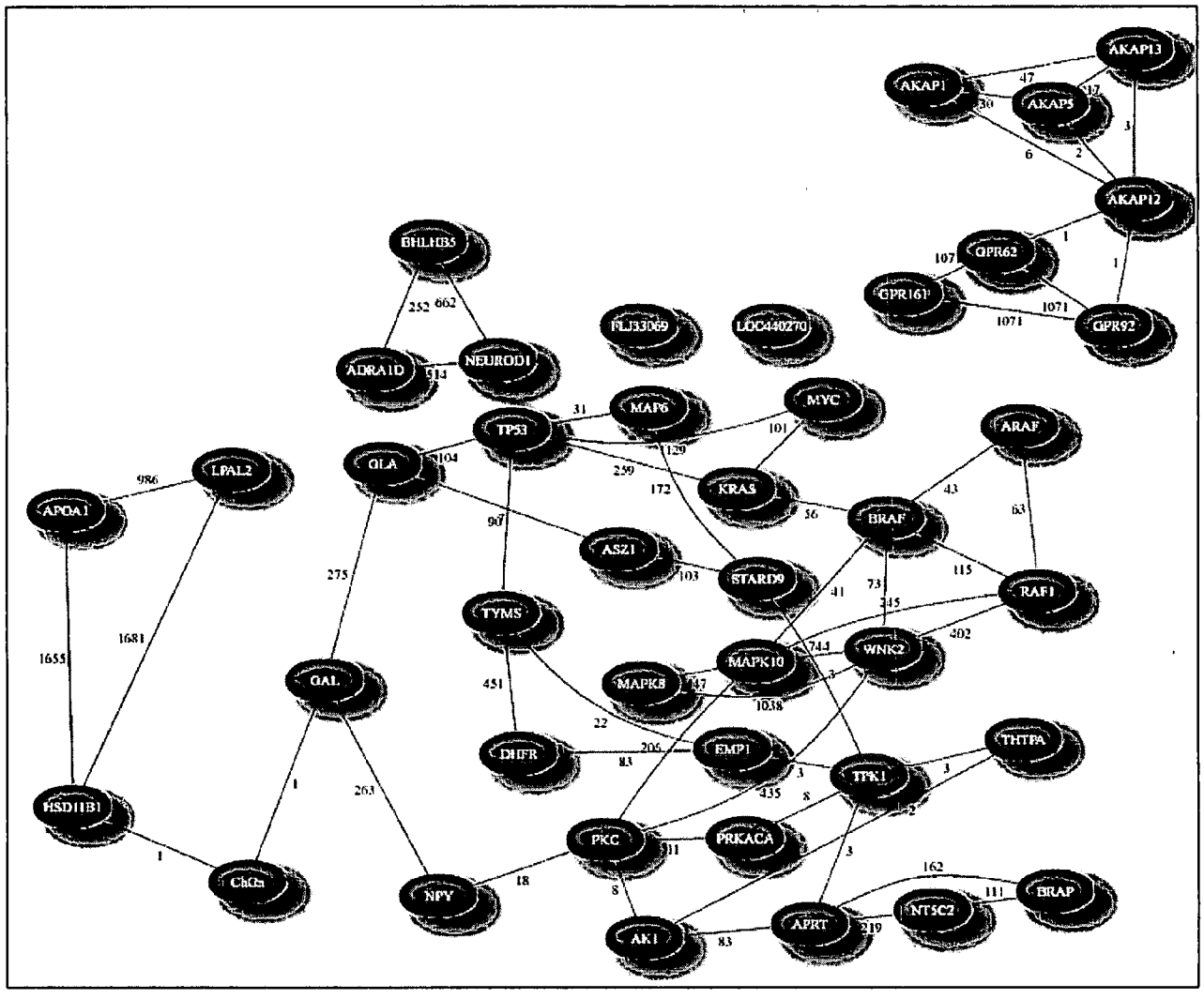
FIG. 8A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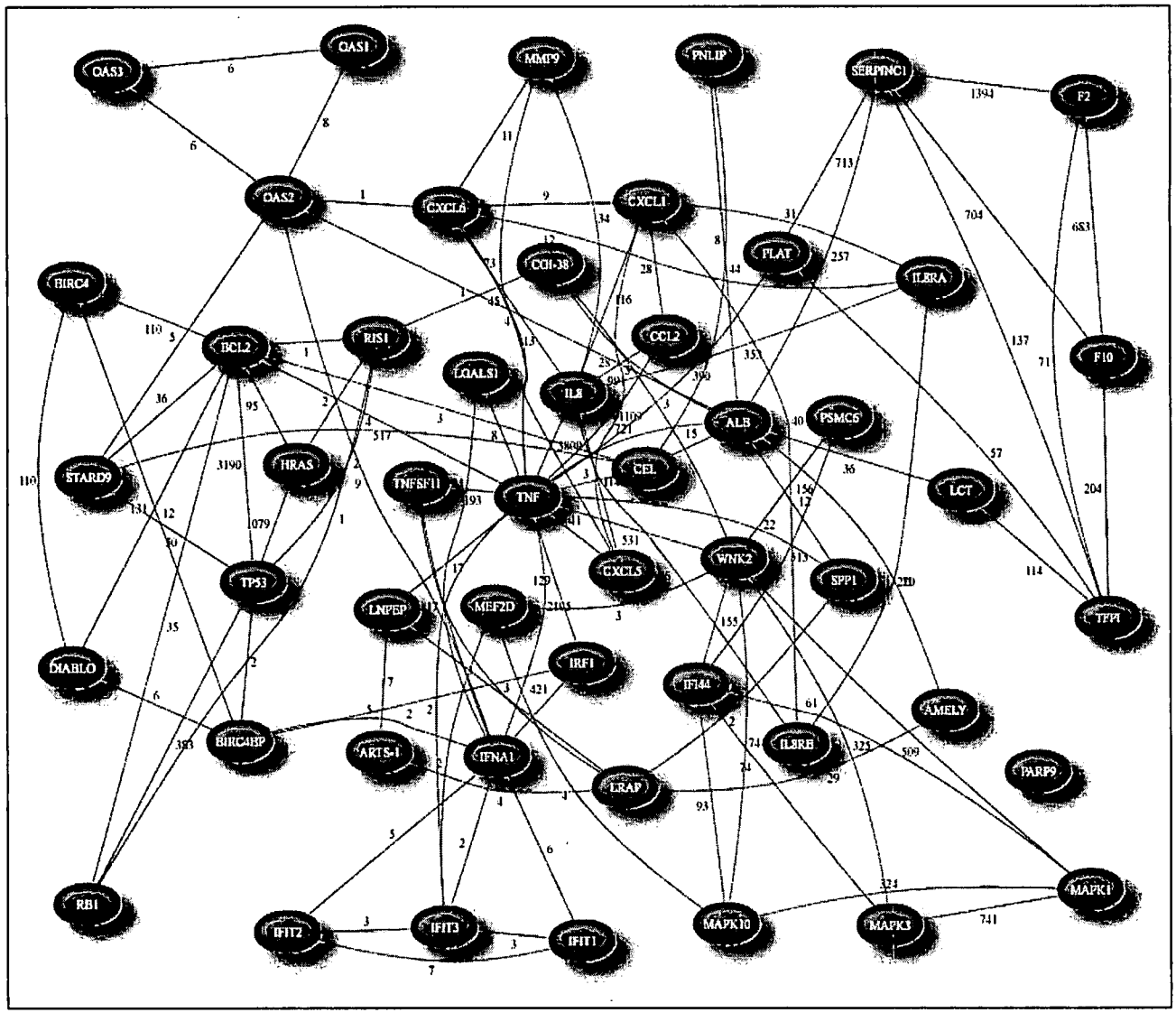
NEUROD1
MAP6
MYC
TP53
ARAF
GLA
LPAL2
APOA1
KRAS
BRAF
ASZ1
STARD9
RAF1
TYMS
MAPK10
WNK2
GAL
MAPK8
DHFR
EMP1
TTK1
THTPA
HSD11B1
PKC
PRKACA
ChCh
NPY
AK1
APRT
NT5C2
BRAP

FIG. 8B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BCL2
RIS1
CCL2
F10
LOALS1
IL8
ALB
PSMC5
STARD9
HRAS
CEL
LCT
TNFSF11
TNF
TP53
CXCL5
WNK2
SPP1
TFPI
LNPEP
MEF2D
DIABLO
IRF1
IF144
AMELY
BIRC4BP
ARTS-1
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MAPK10
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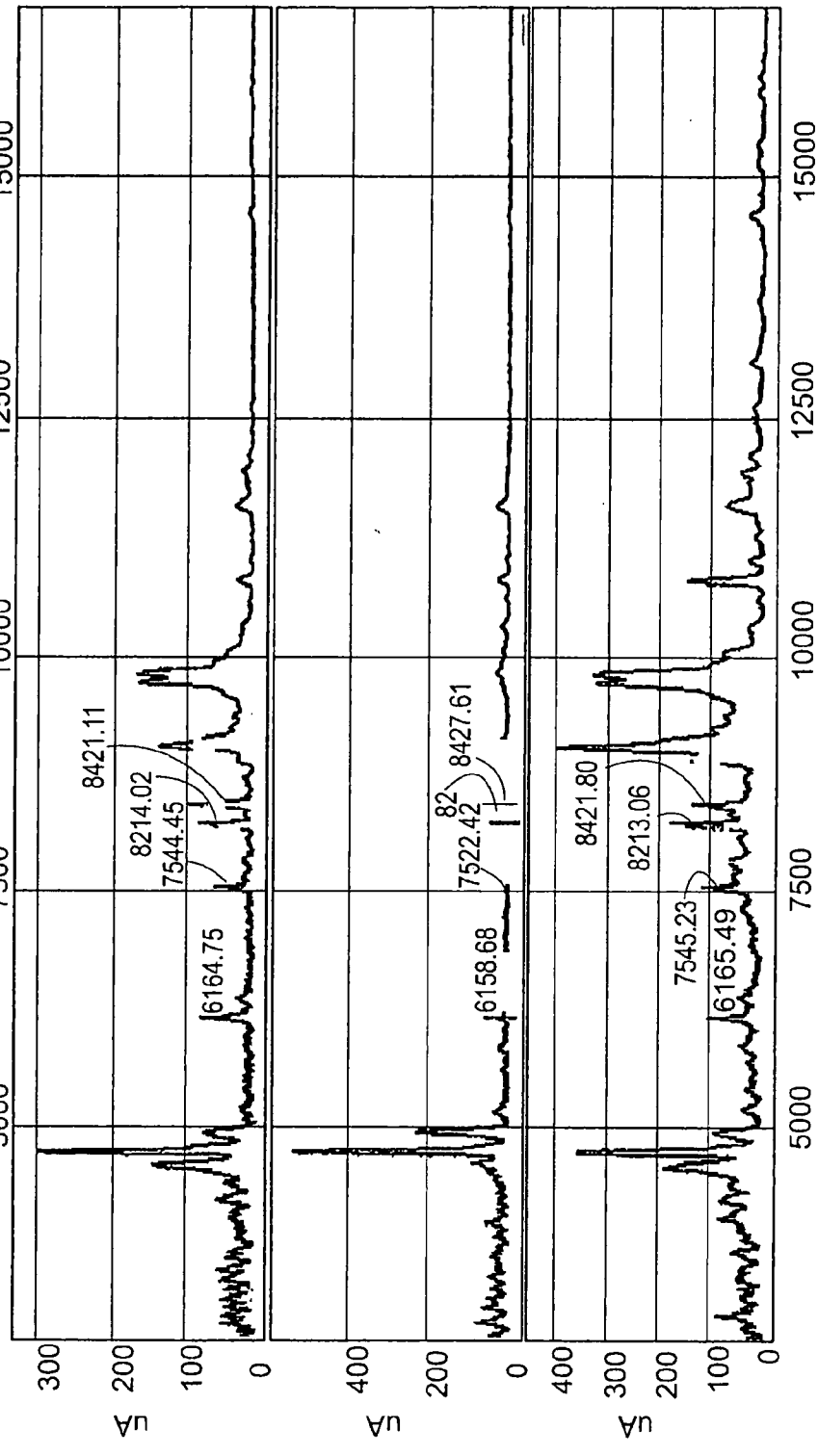
MAPK1

FIG. 8C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82
8427.61
8421.80
8213.06
7545.23
6165.49

FIG. 9

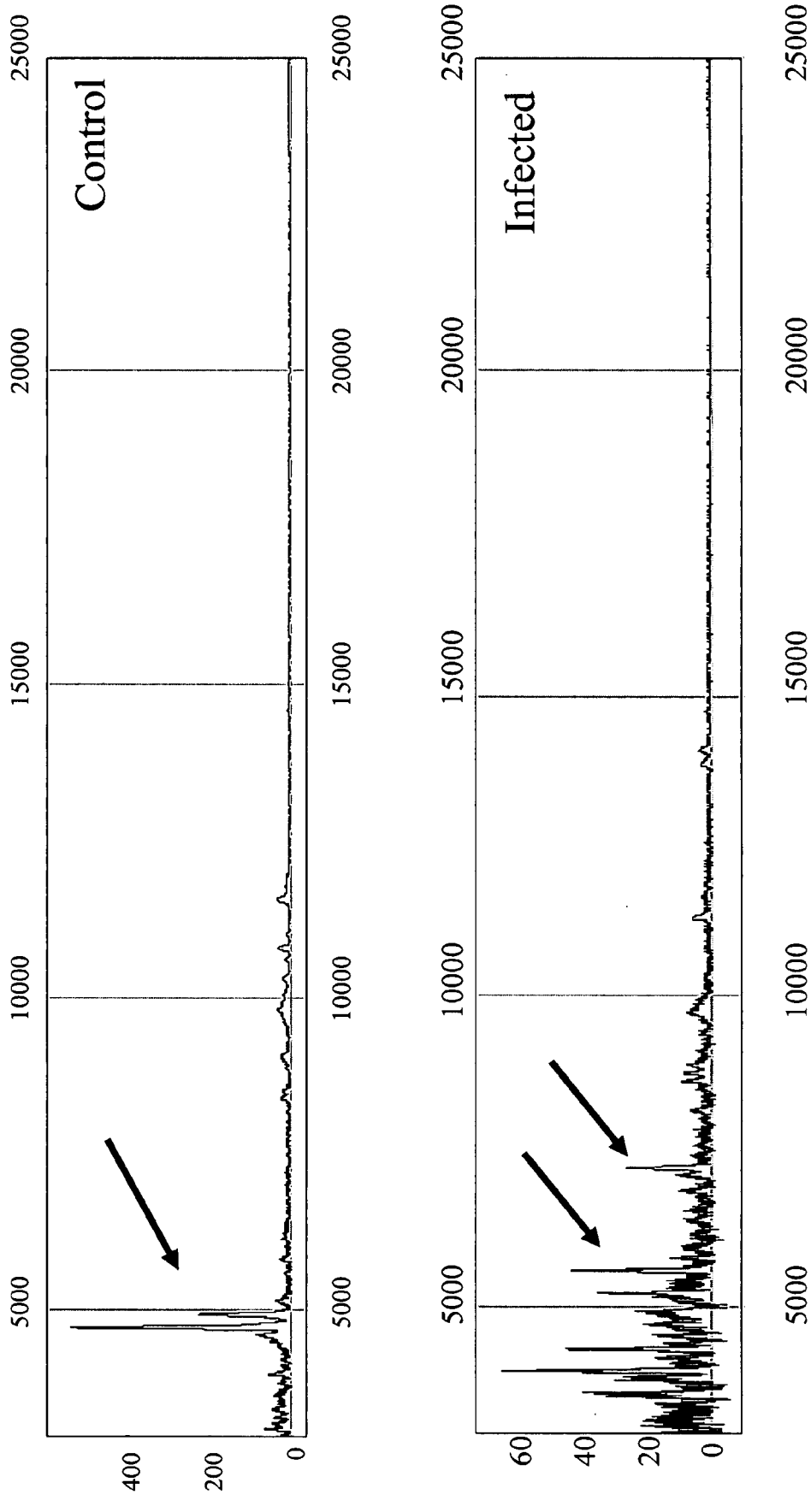

Control
Infected
5000
10000
15000
20000
25000
0
200
400
0
20
40
60

FIG. 10

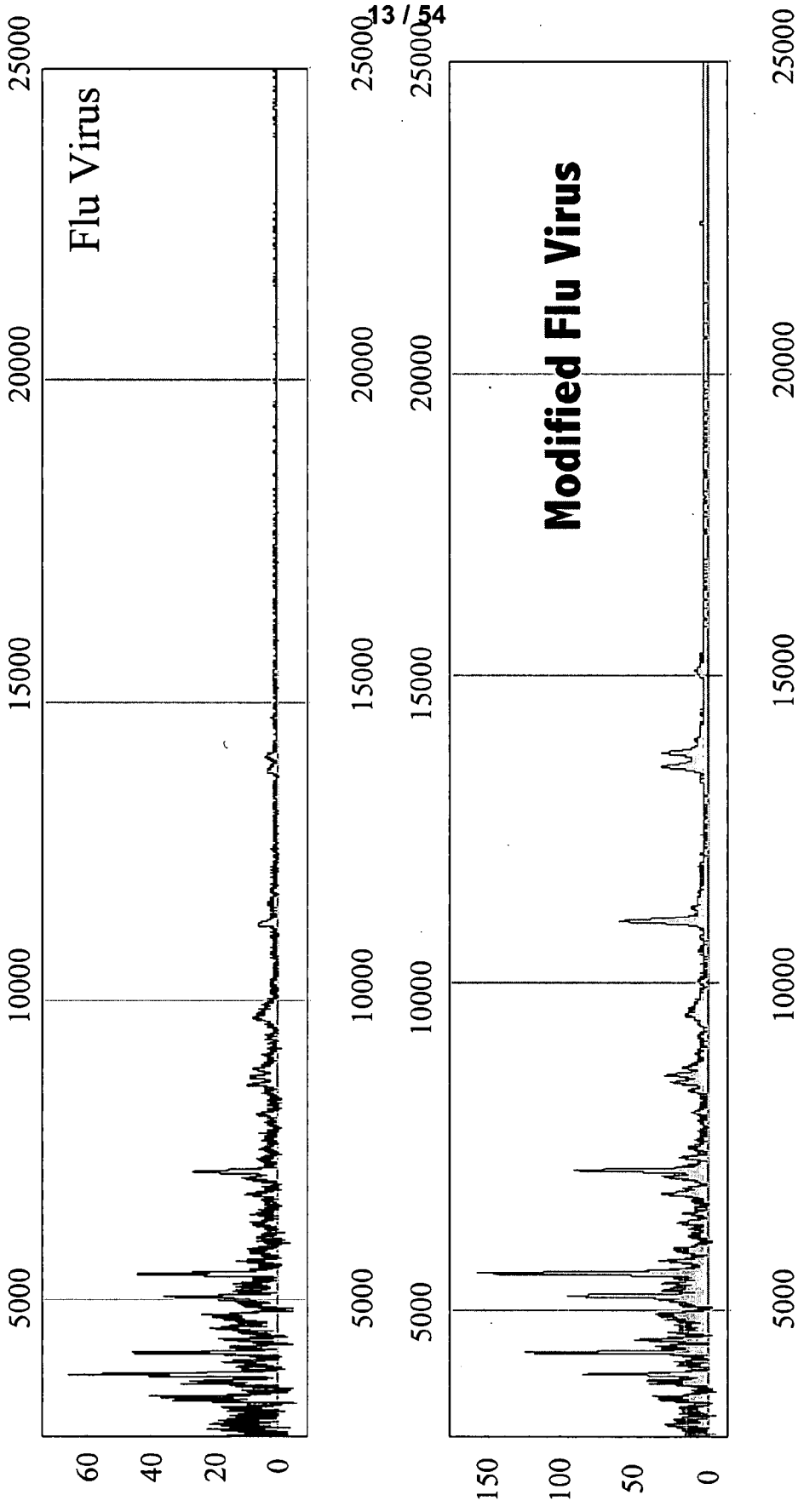

Flu Virus
Modified Flu Virus
5000
10000
15000
20000
25000
0
20
40
60
50
100
150

FIG. 11

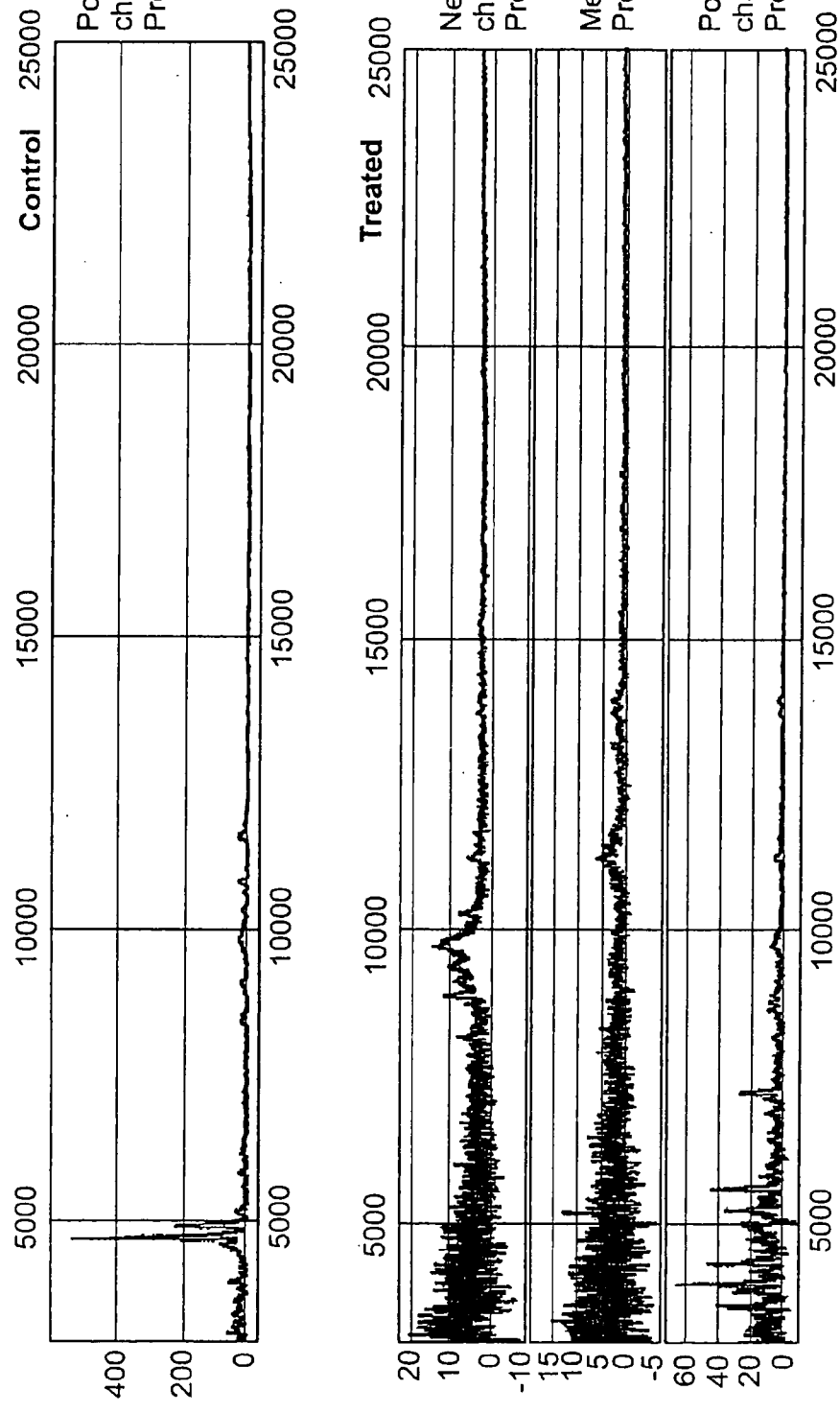

Control
Treated
Positively-charged Proteins
Negatively-charged Proteins
Metal-binding Proteins
Positively-charged Proteins
5000
10000
15000
20000
25000
400
200
0
20
10
0
-10
15
10
5
0
-5
60
40
20
0

FIG. 12A

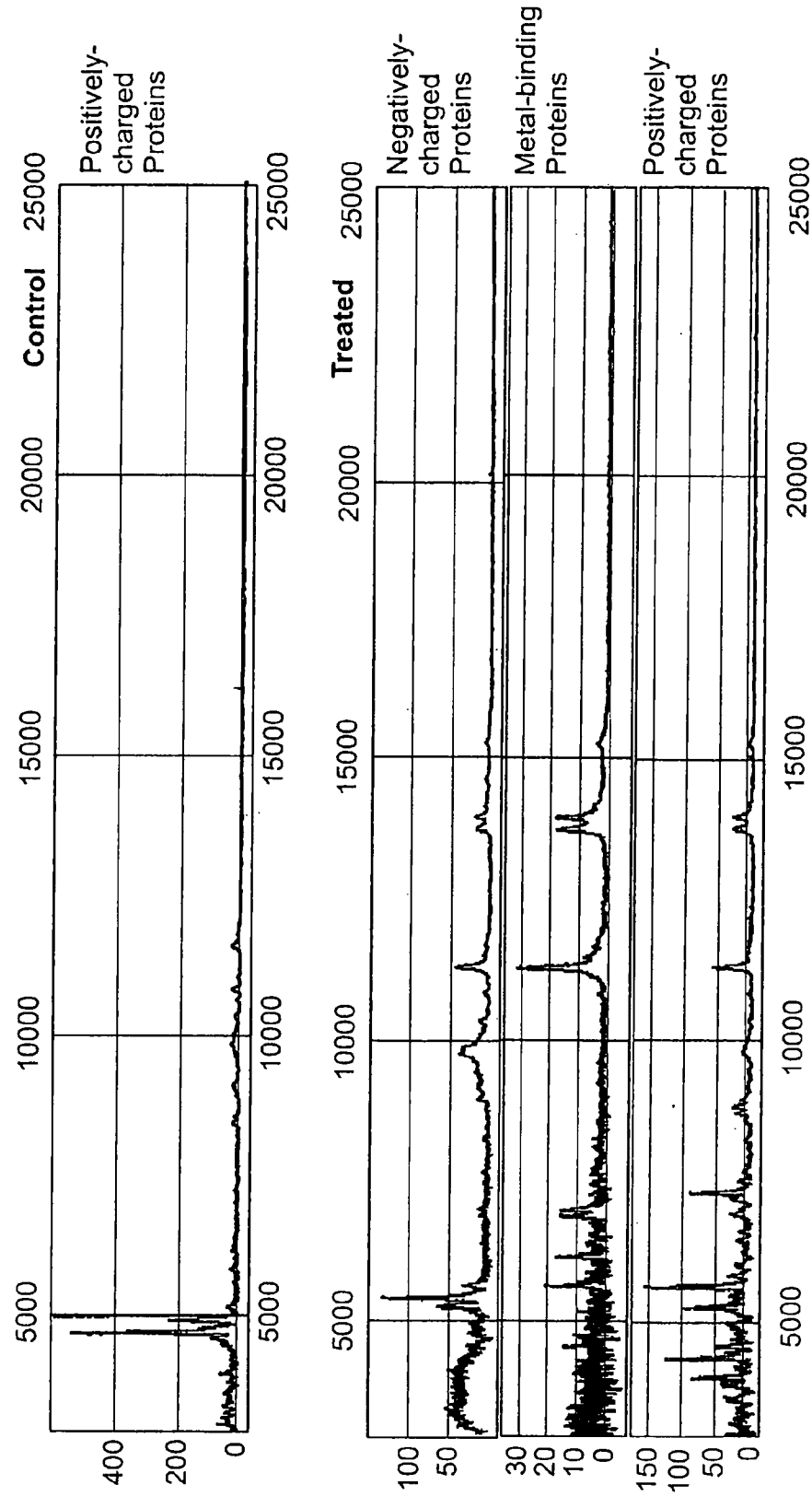

Control
Positively-charged Proteins
Treated
Negatively-charged Proteins
Metal-binding Proteins
Positively-charged Proteins
5000
10000
15000
20000
25000
0
200
400
50
100
0
10
20
30
50
100
150

FIG. 12B

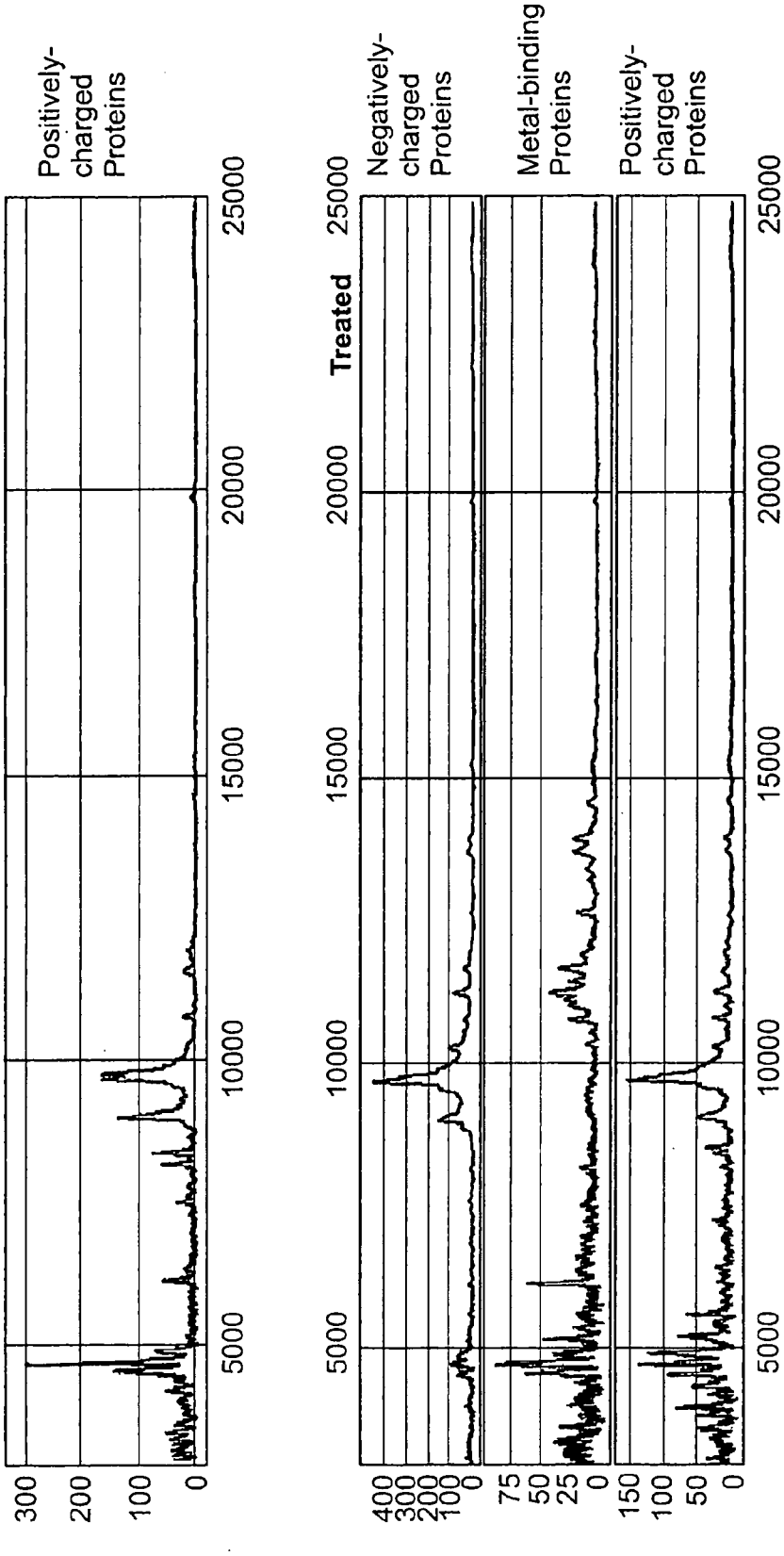

Control
Positively-charged Proteins
Treated
Negatively-charged Proteins
Metal-binding Proteins
Positively-charged Proteins
5000
10000
15000
20000
25000
0
100
200
300
0
100
200
300
400
0
25
50
75
0
50
100
150

FIG. 13A

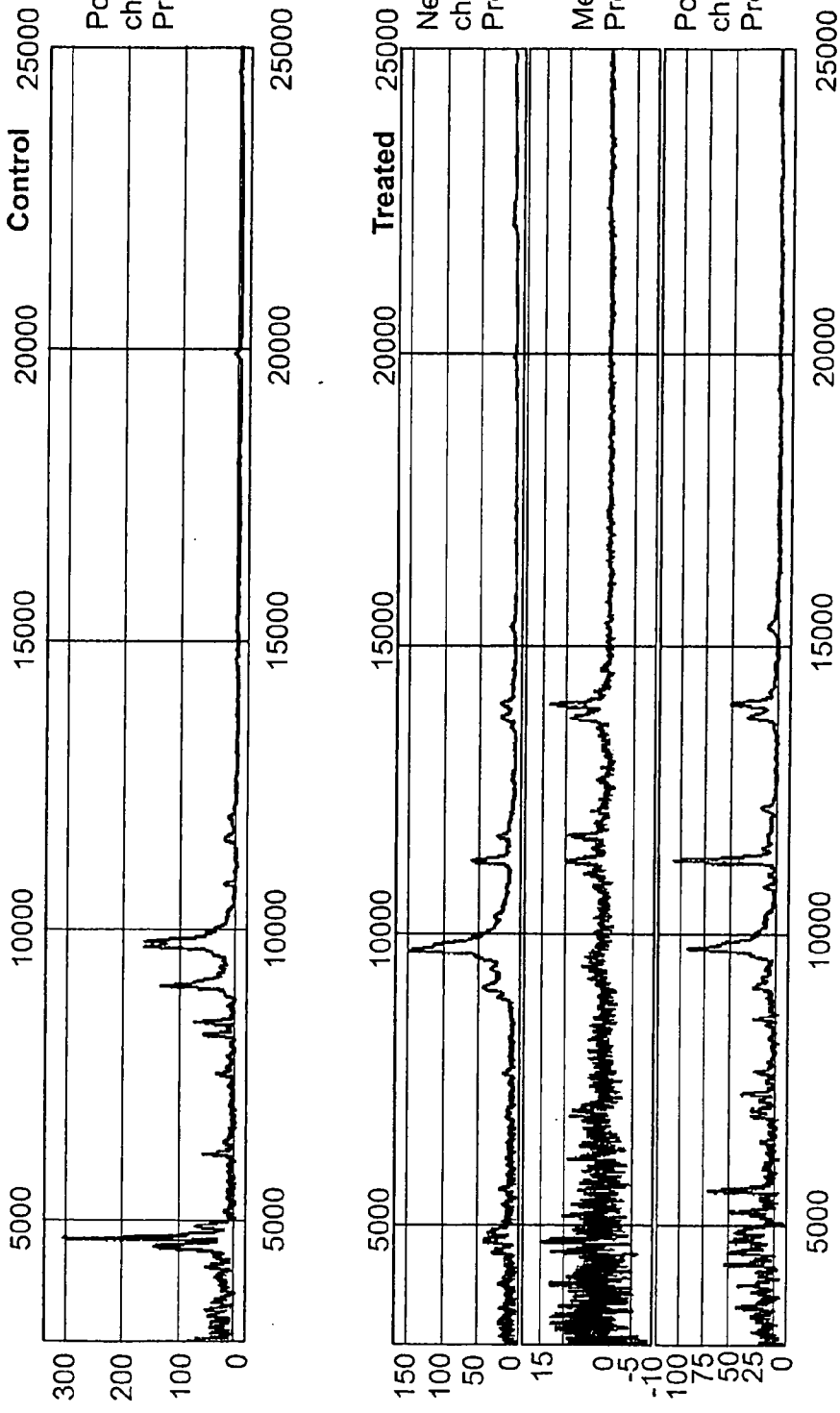

Control
Positively-charged Proteins
Treated
Negatively-charged Proteins
Metal-binding Proteins
Positively-charged Proteins
5000
10000
15000
20000
25000
300
200
100
0
150
100
50
0
15
0
-5
-10
100
75
50
25
0

FIG. 13B

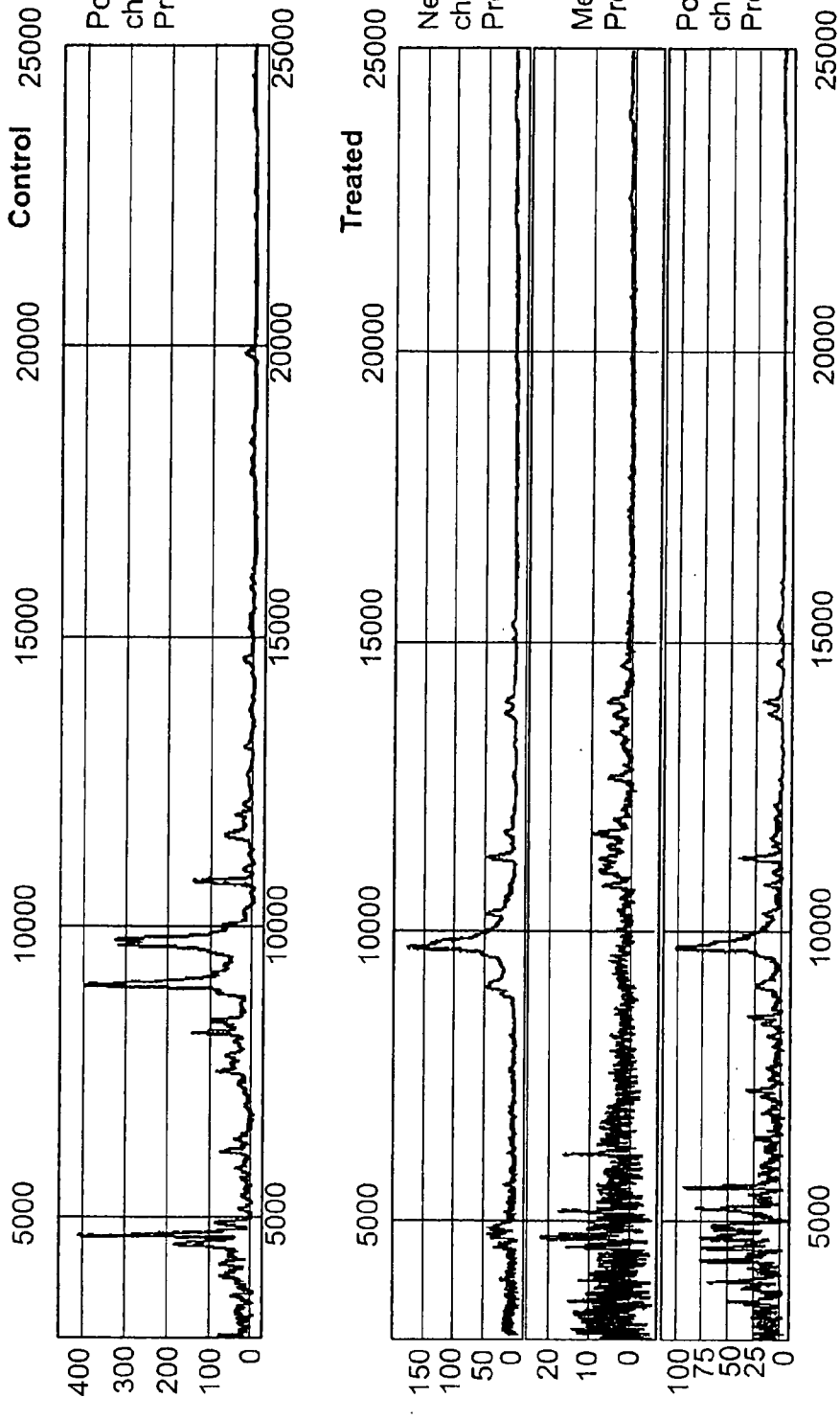

Control
Positively-charged Proteins
Treated
Negatively-charged Proteins
Metal-binding Proteins
Positively-charged Proteins
5000
10000
15000
20000
25000
0
100
200
300
400
50
150
10
20
25
75

FIG. 14A

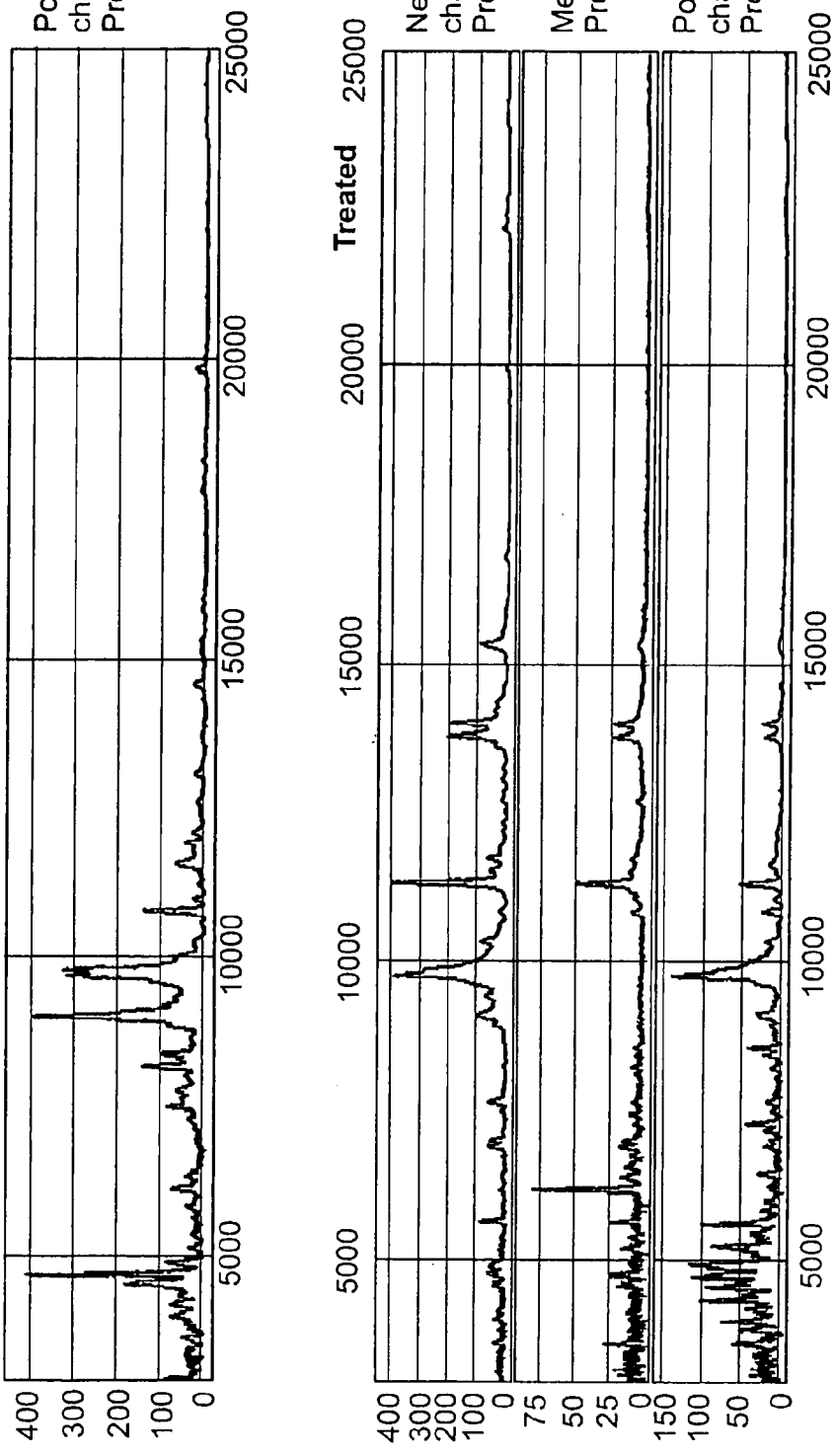

Control
Positively-charged Proteins
Treated
Negatively-charged Proteins
Metal-binding Proteins
Positively-charged Proteins
5000
10000
15000
20000
25000
400
300
200
100
0
75
50
25
150

FIG. 14B

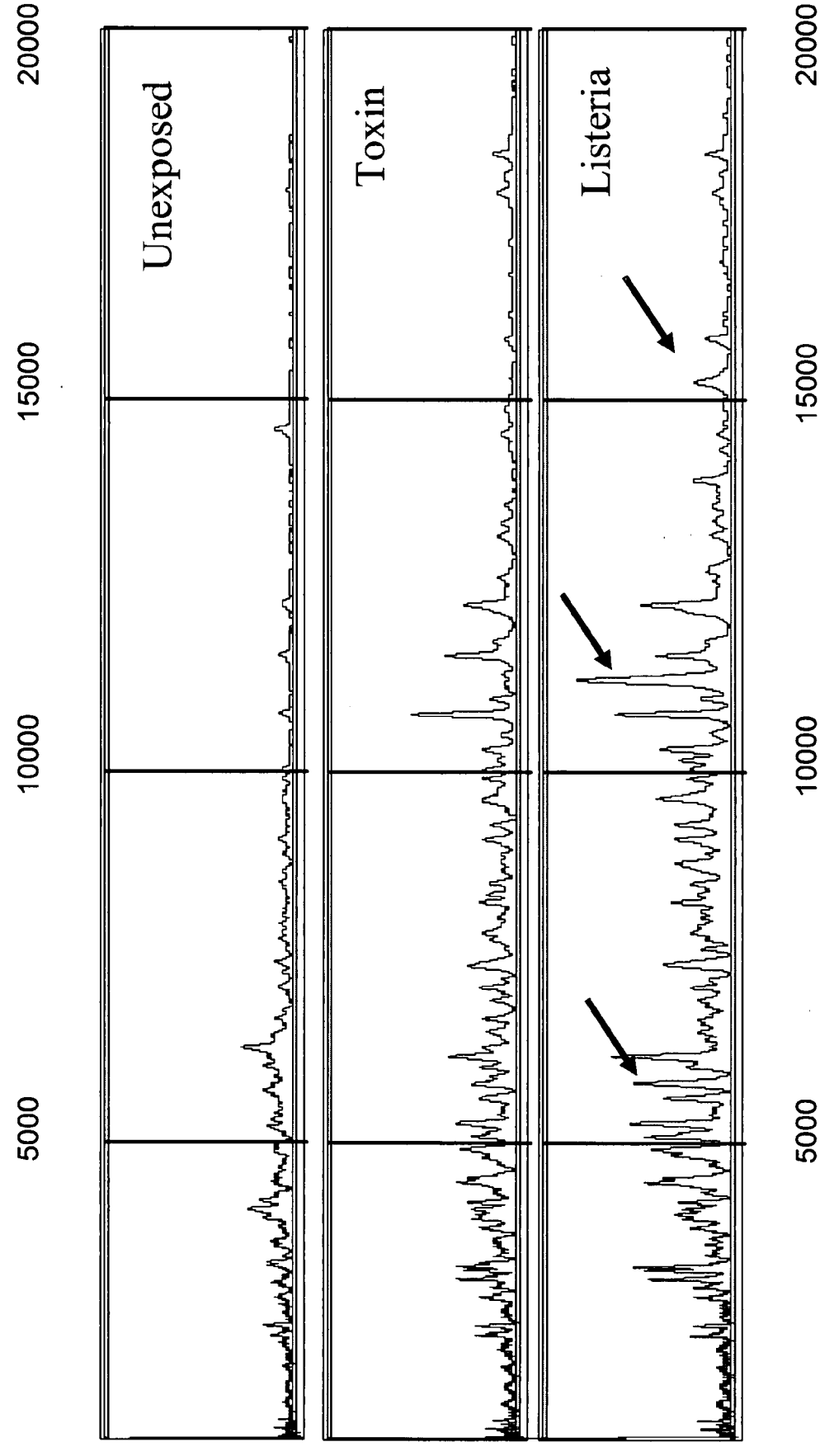


FIG. 15

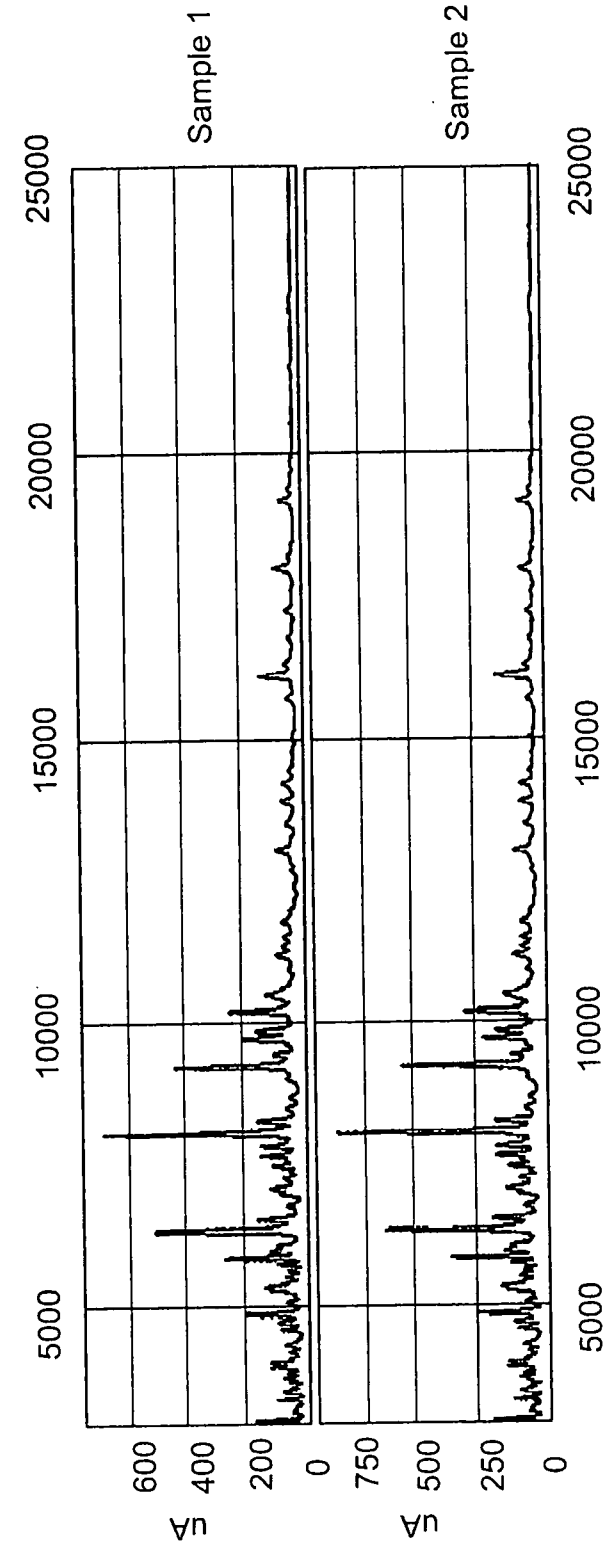

Sample 1
Sample 2
5000
10000
15000
20000
25000
uA
600
400
200
0
750
500
250
0

FIG. 16A

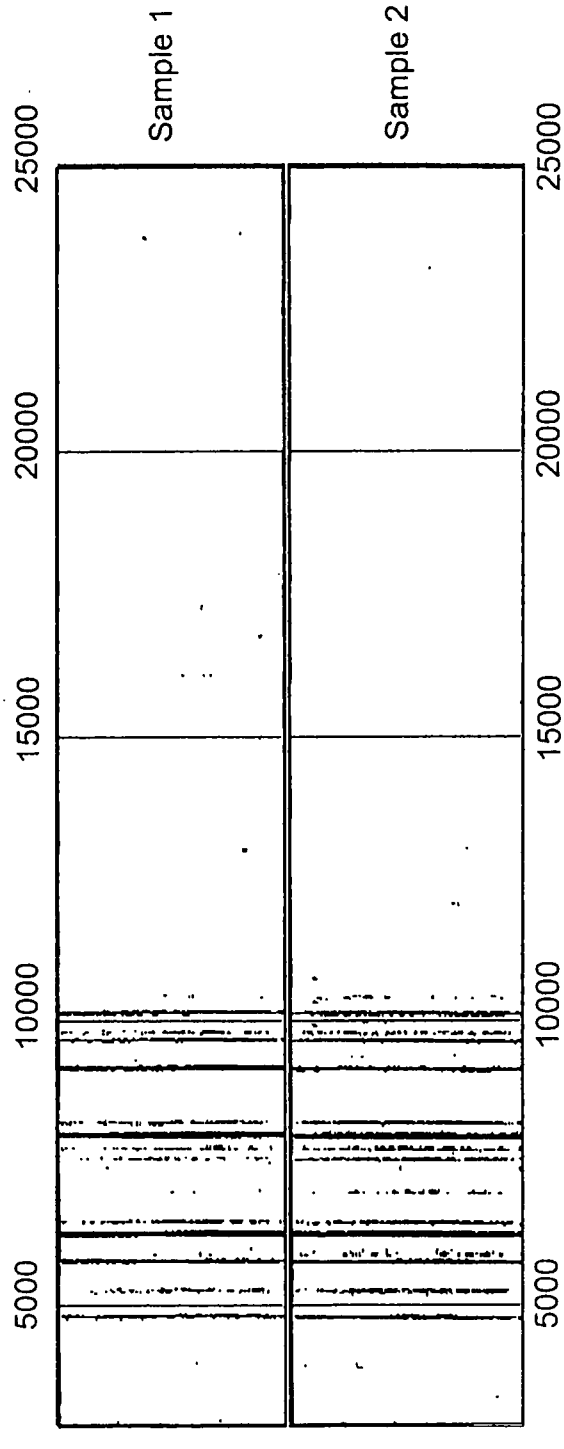


FIG. 16B

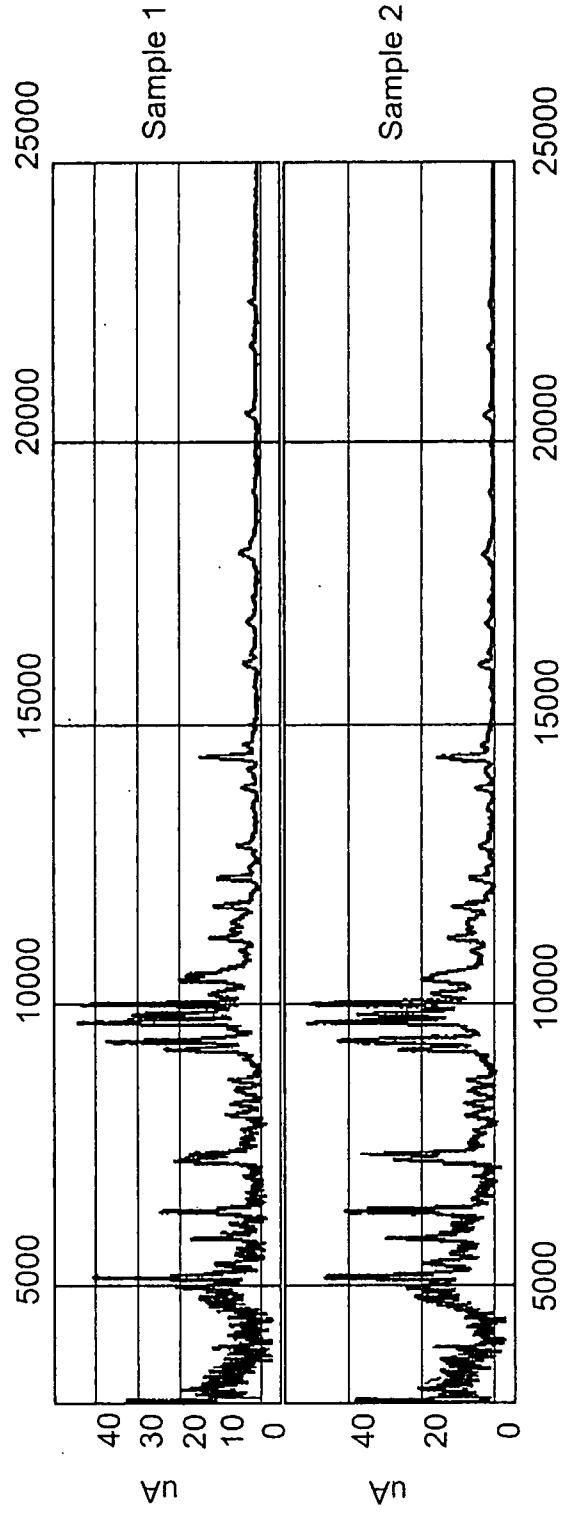


FIG. 16C

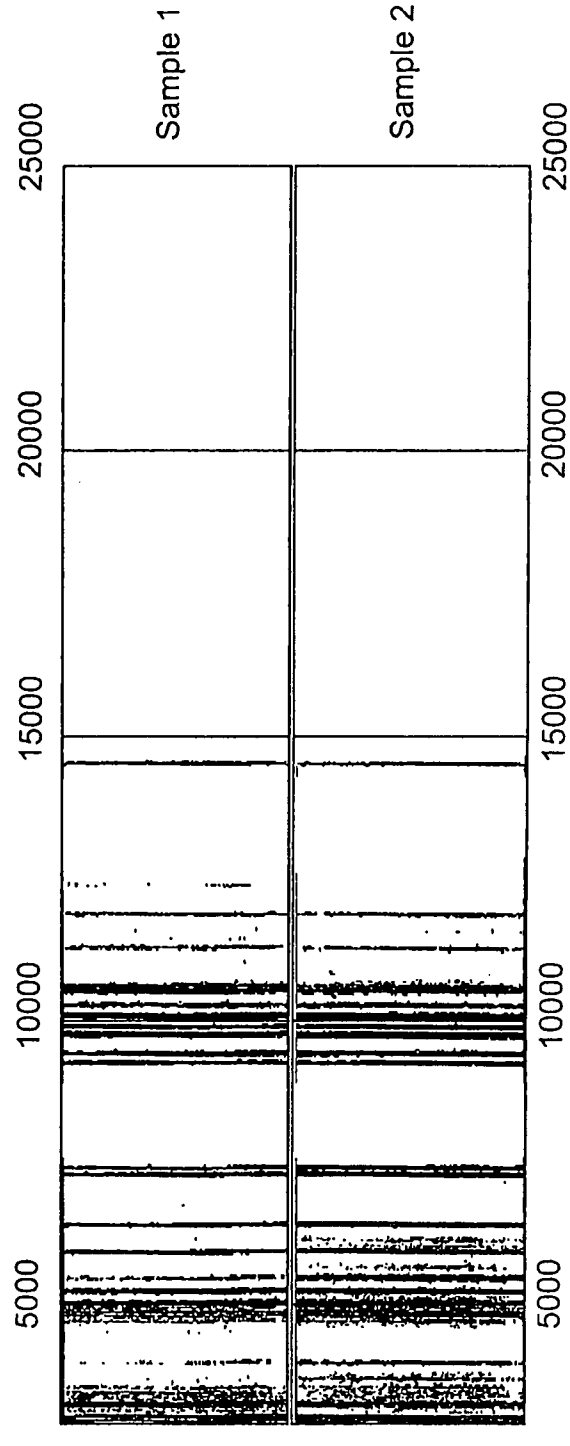


FIG. 16D

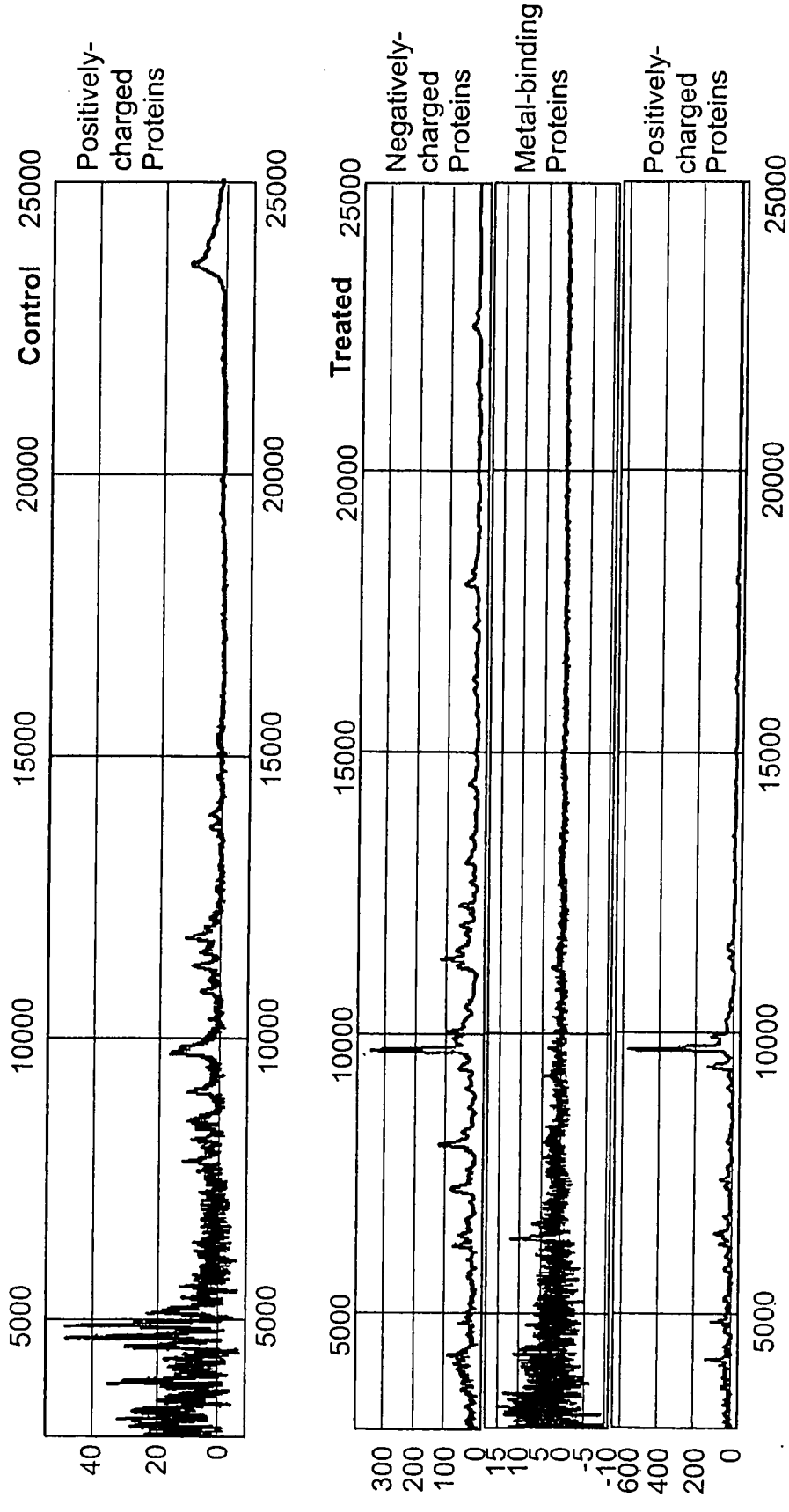

Control
Positively-charged Proteins
Treated
Negatively-charged Proteins
Metal-binding Proteins
Positively-charged Proteins
5000
10000
15000
20000
25000
40
20
0
300
200
100
0
15
10
5
0
-5
-10
600
400
200
0

FIG. 17A

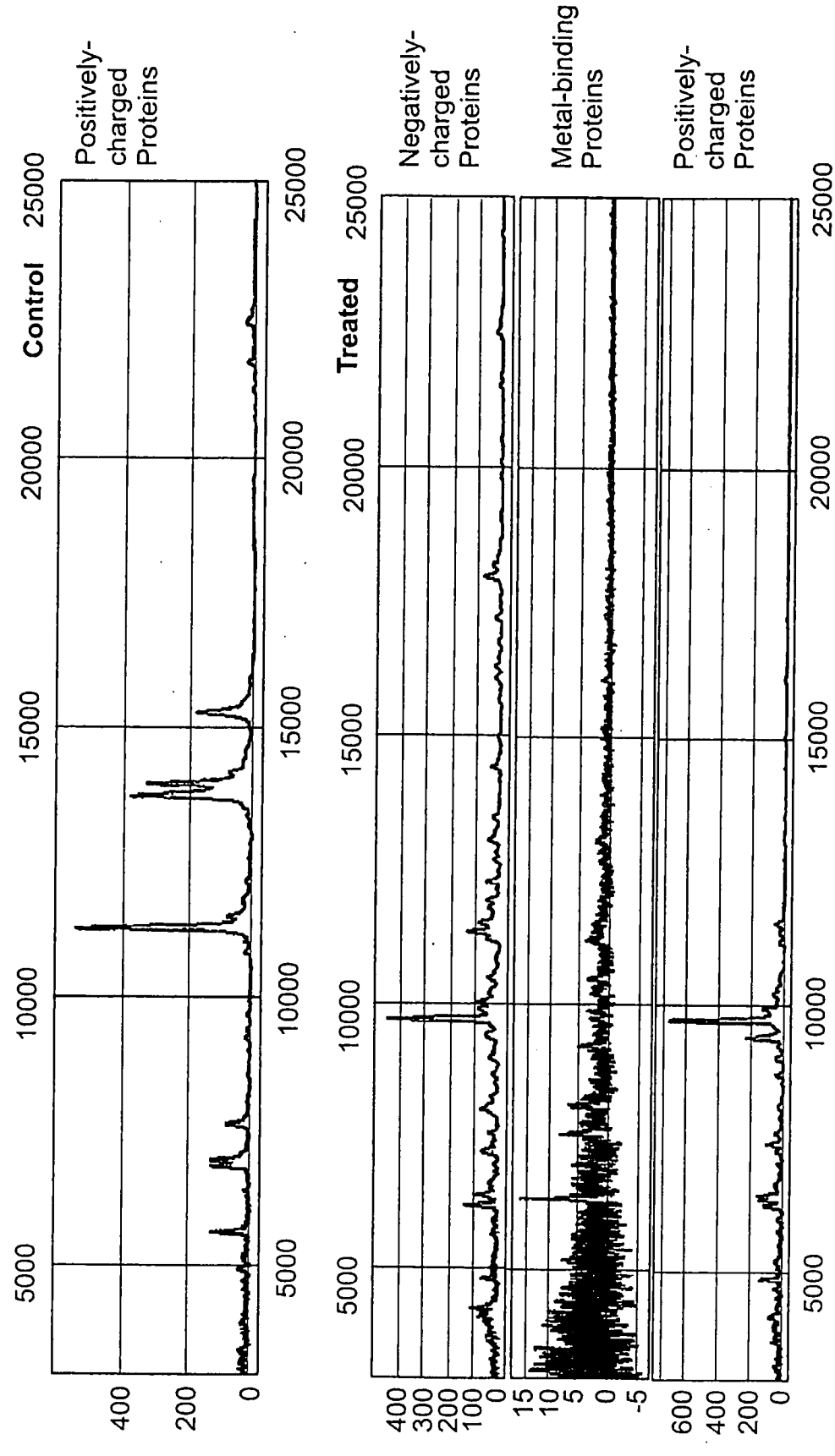

Positively-charged Proteins
Control
Negatively-charged Proteins
Metal-binding Proteins
Positively-charged Proteins
Treated
5000
10000
15000
20000
25000
0
200
400
100
300
-5
5
10
15
600

FIG. 17B

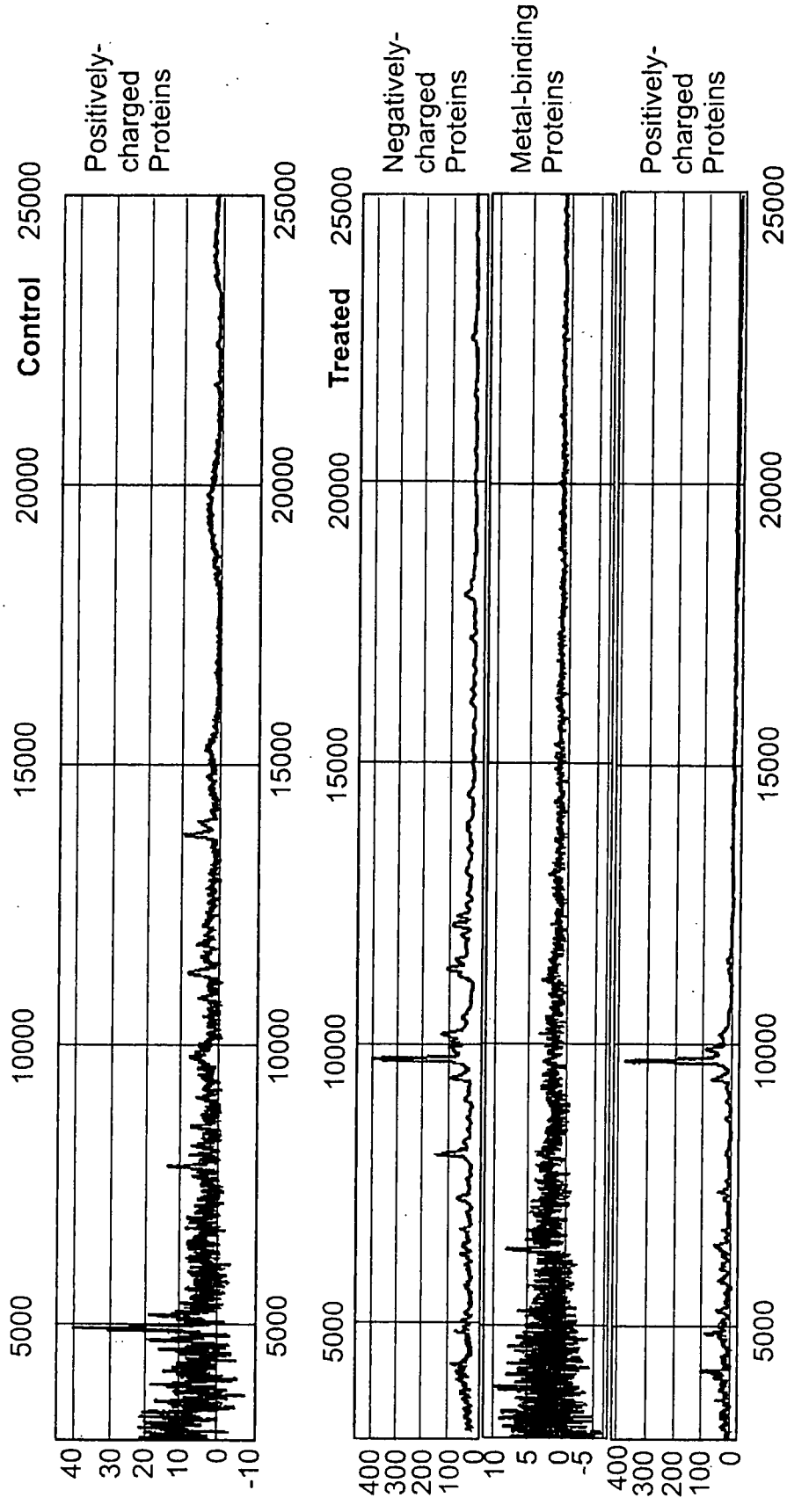

Positively-charged Proteins
Control
Negatively-charged Proteins
Metal-binding Proteins
Positively-charged Proteins
Treated
5000
10000
15000
20000
25000
40
30
20
10
0
-10
400
300
200
100
0
10
5
0
-5

FIG. 17C

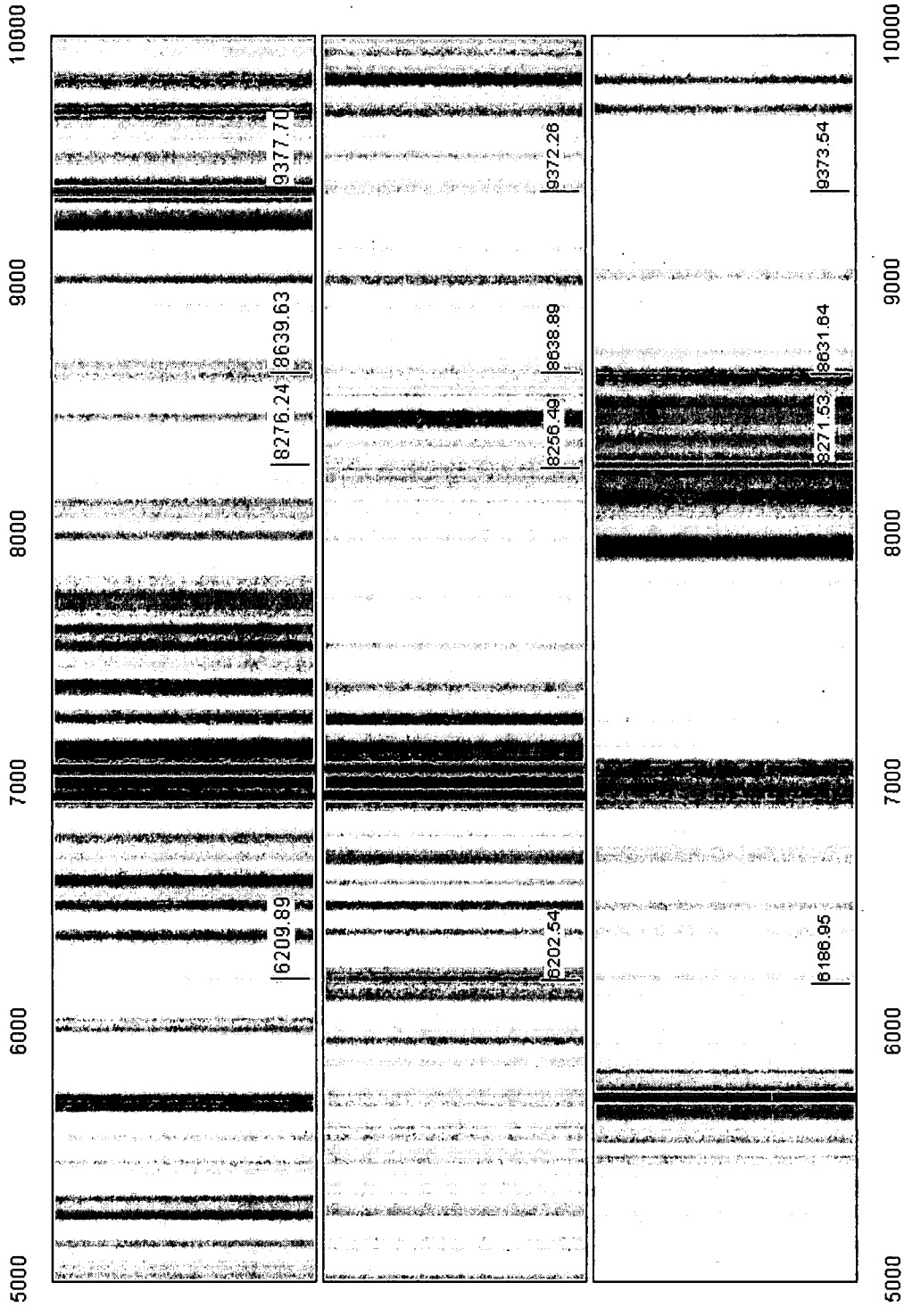

10000
9000
8000
7000
6000
5000
9377.70
8639.63
8276.24
6209.89
9372.26
8638.89
8256.49
6202.54
9373.54
8631.64
8271.53
6186.95

FIG. 18A

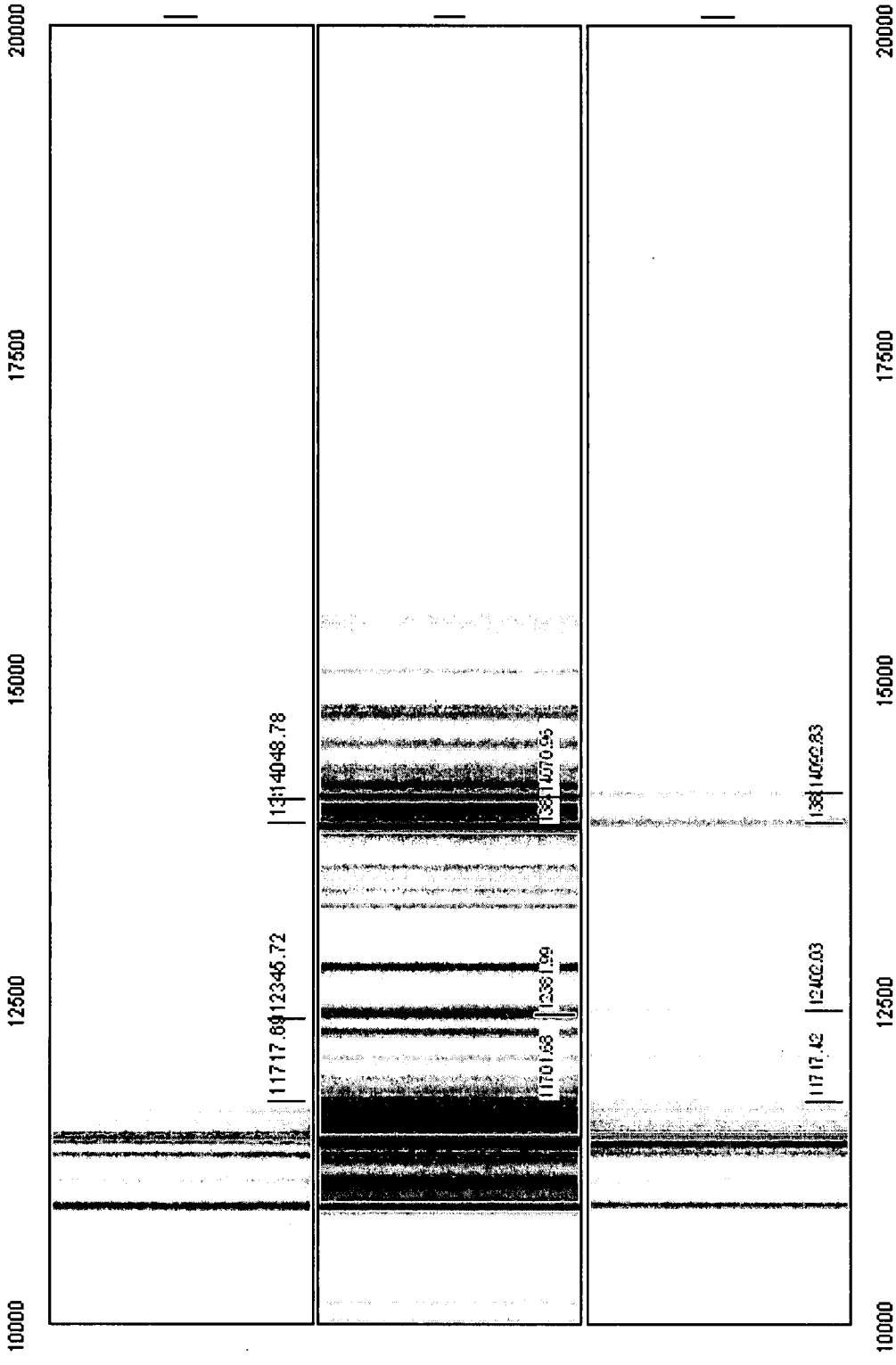

20000
17500
15000
12500
10000
14048.78
12345.72
11717.69
14062.83
12402.03
11717.42

FIG. 18B

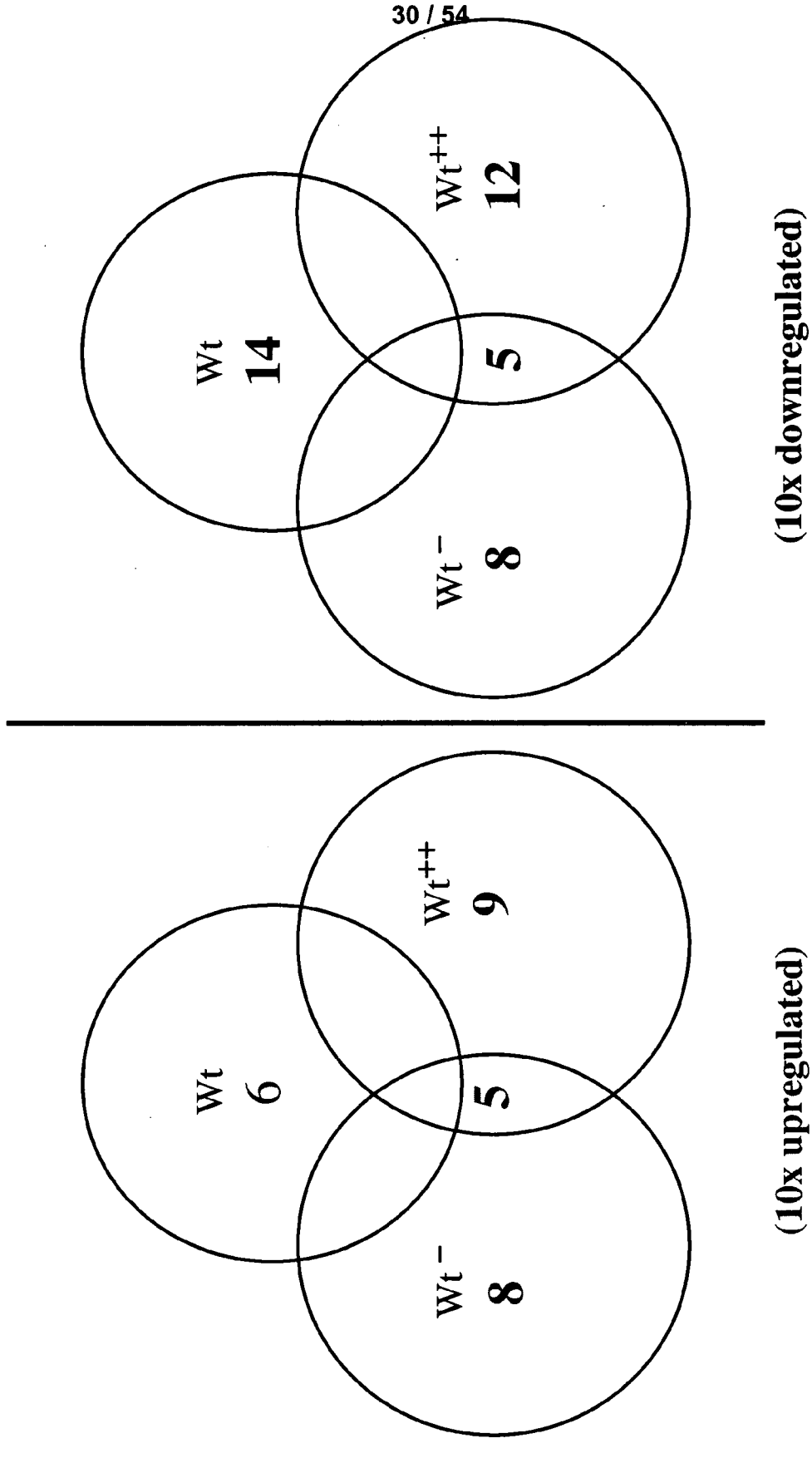

Wt
14
Wt++
12
5
Wt−
8
(10x downregulated)
Wt
6
Wt++
9
5
Wt−
8
(10x upregulated)

FIG. 19

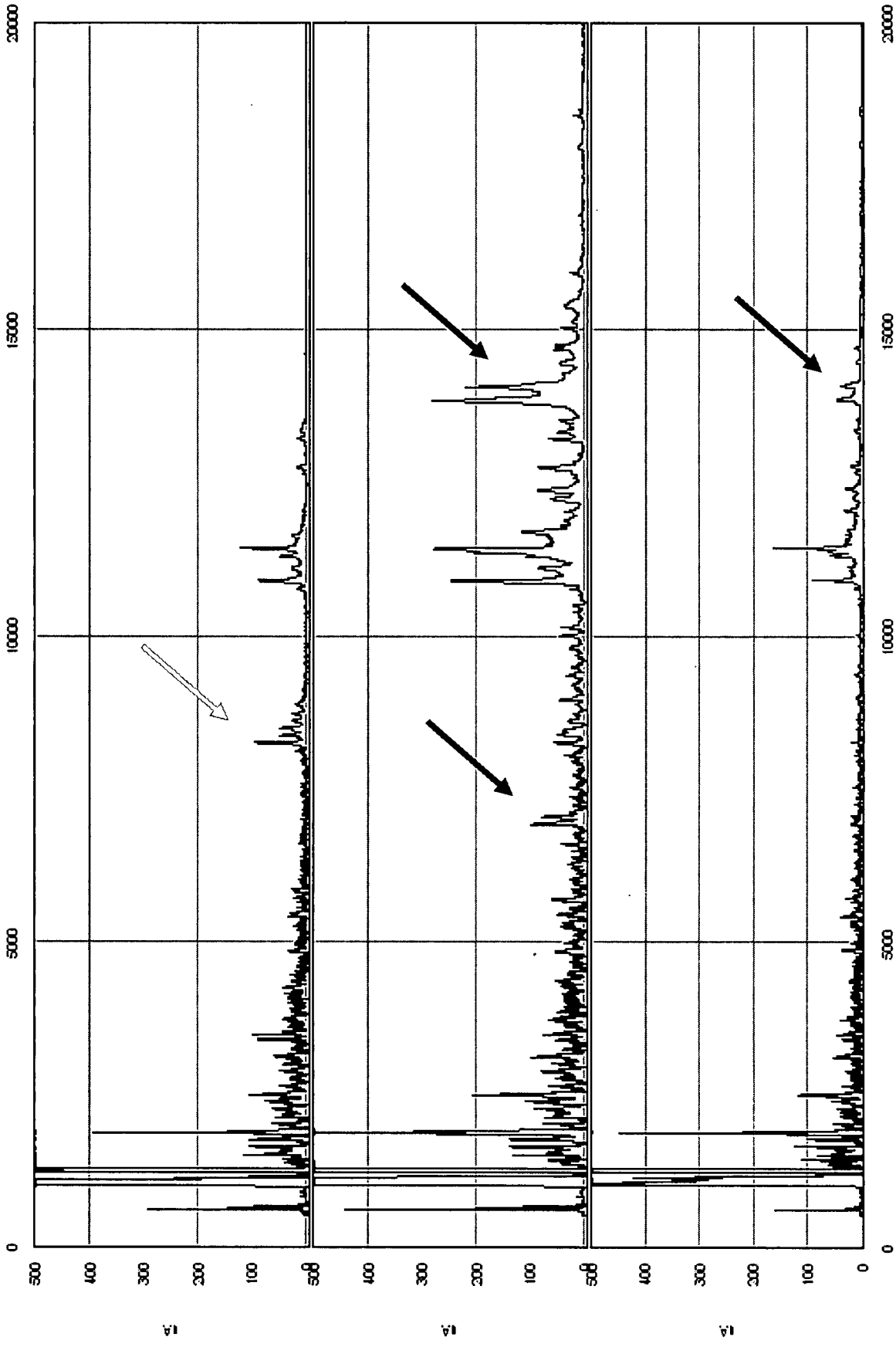


FIG. 20A

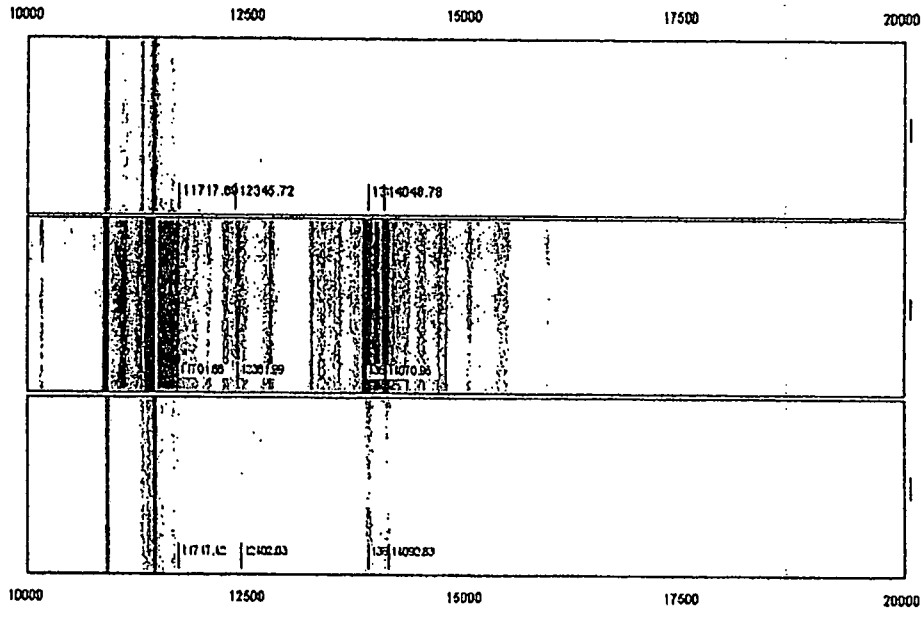

10000
12500
15000
17500
20000
11717.65
12345.72
13140.48
10000
12500
15000
17500
20000

FIG. 20B

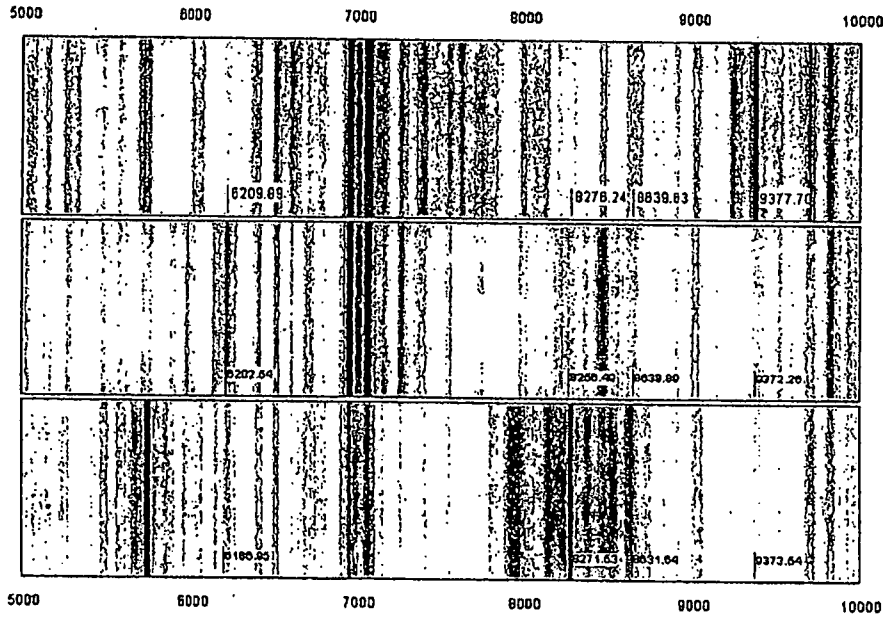

5000
6000
7000
8000
9000
10000
6209.69
8276.24
8839.83
9377.70
6202.54
9250.40
9839.80
9372.26
6180.65
9271.63
9831.64
9373.64
5000
6000
7000
8000
9000
10000

FIG. 20C

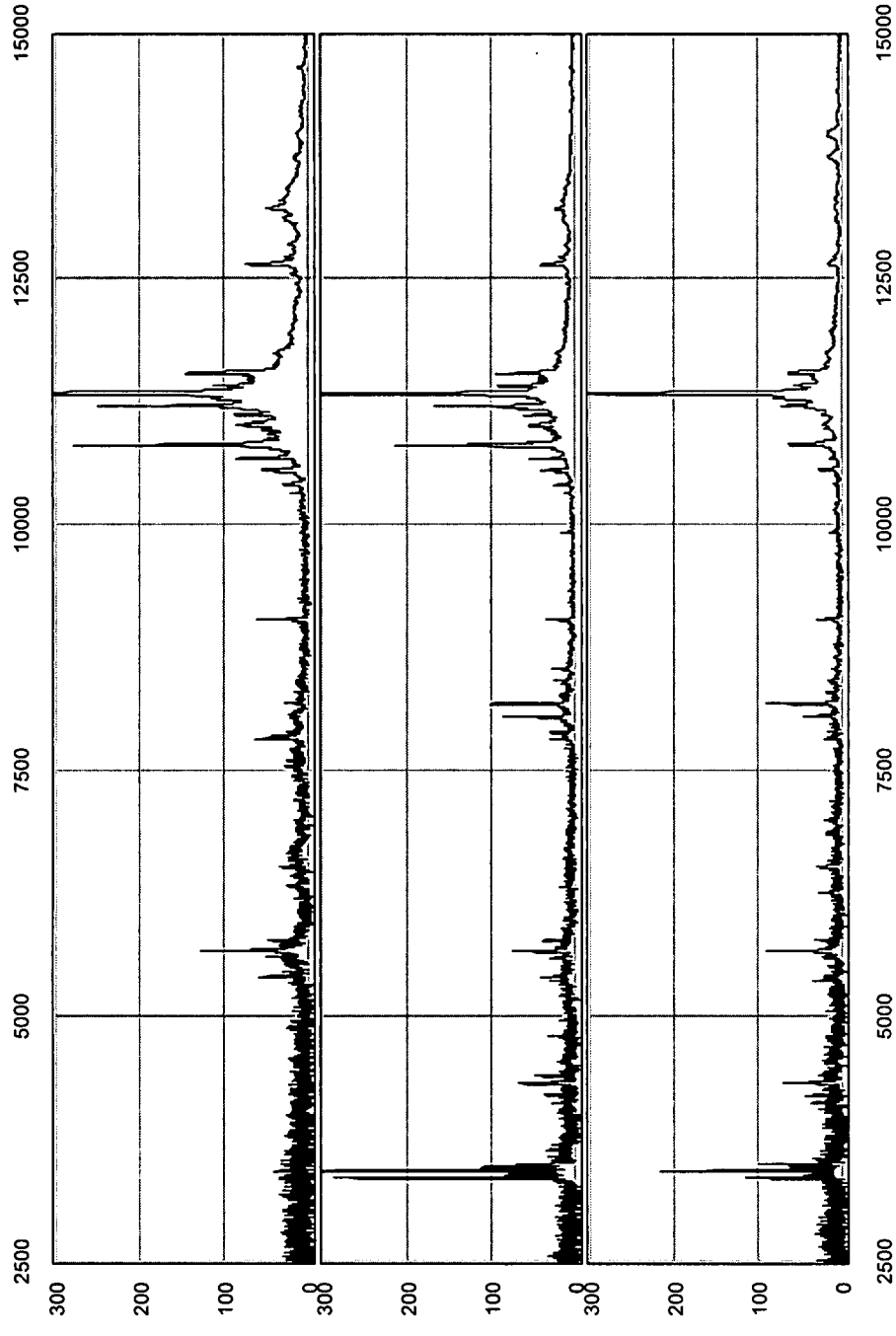

Intensity
300
200
100
0
2500
5000
7500
10000
12500
15000
Mass (Da)

FIG. 21

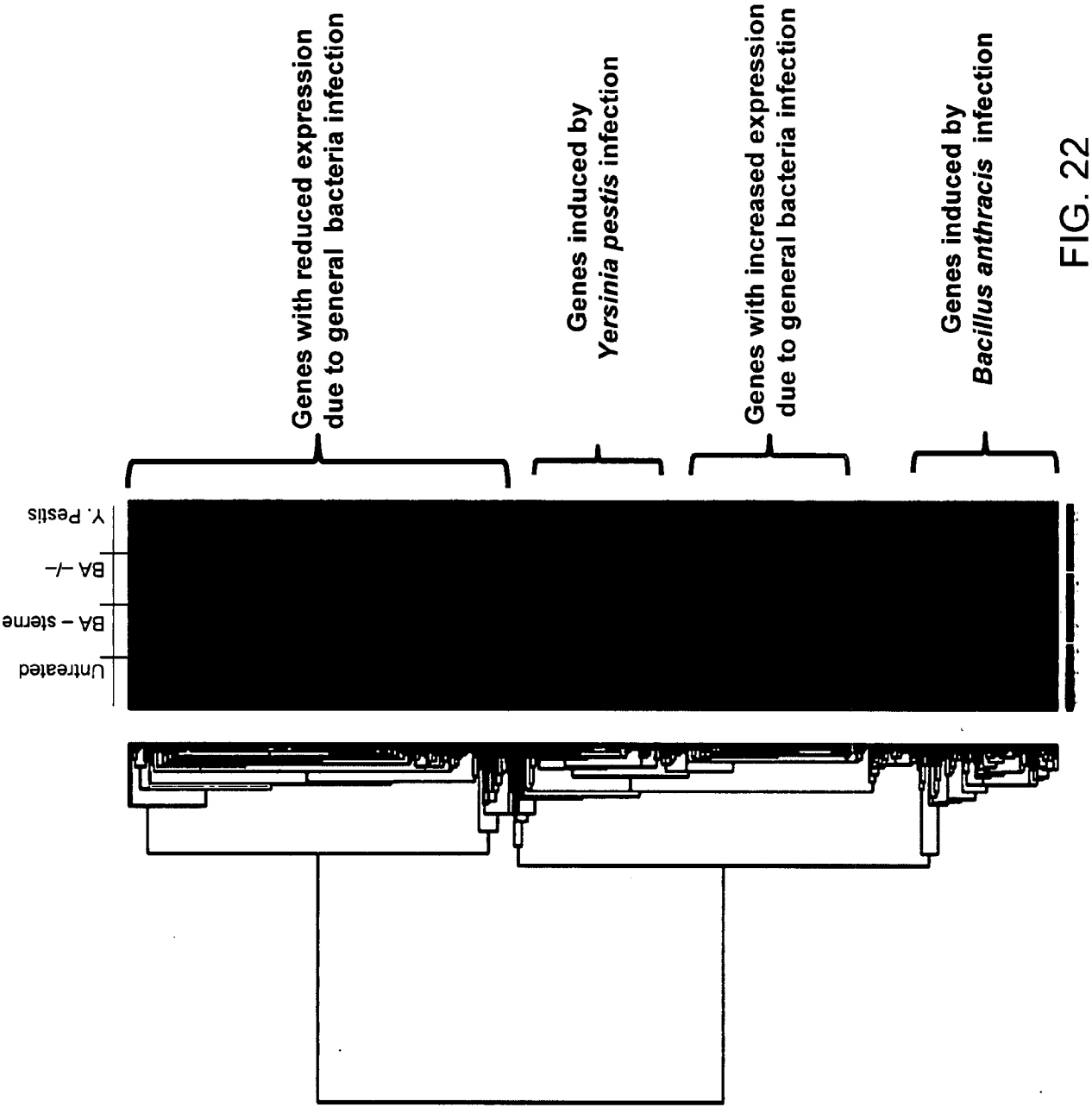

Genes with reduced expression due to general bacteria infection
Genes induced by Yersinia pestis infection
Genes with increased expression due to general bacteria infection
Genes induced by Bacillus anthracis infection
Untreated
BA – sterne
BA –/–
Y. Pestis

FIG. 22

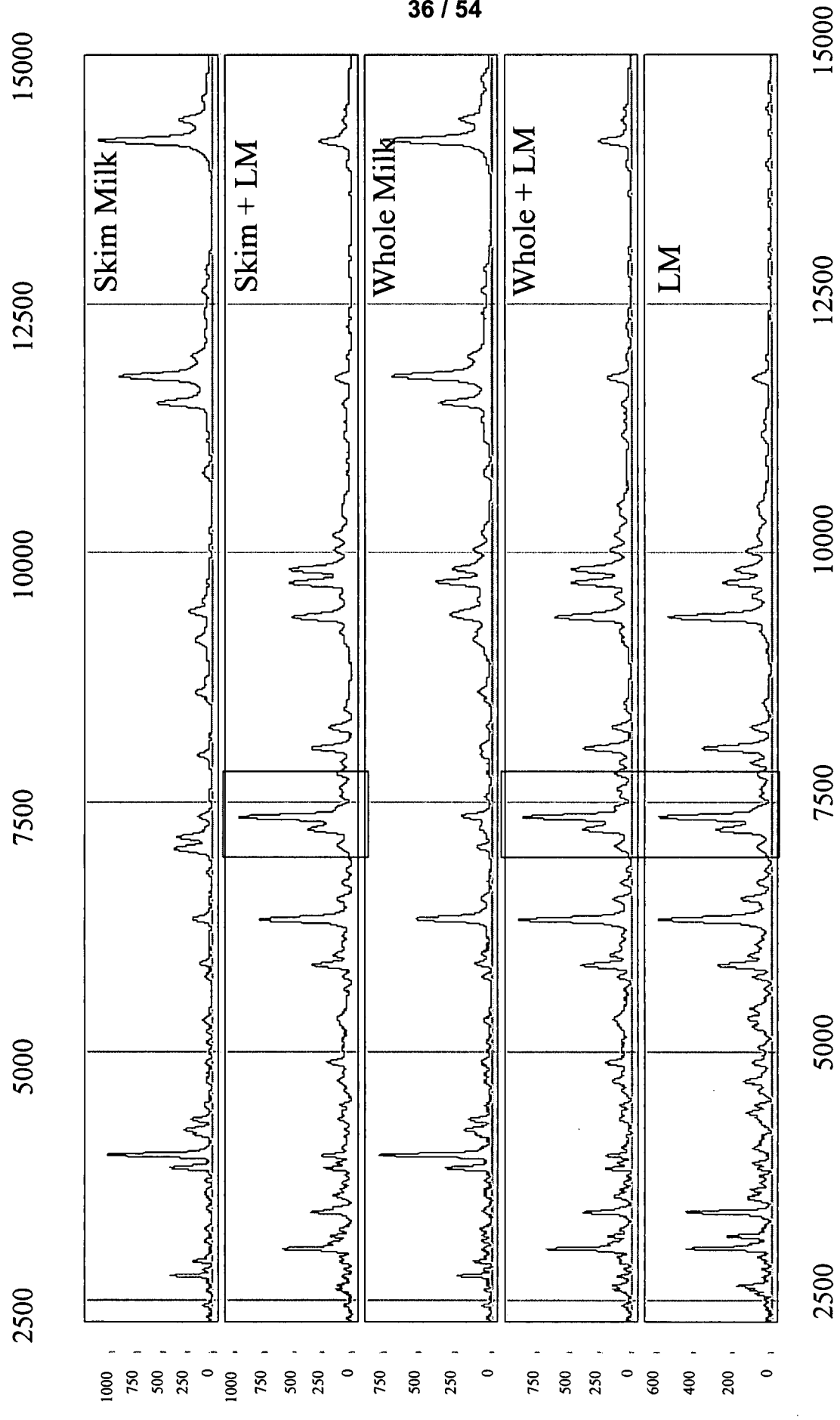

Skim Milk
Skim + LM
Whole Milk
Whole + LM
LM
2500
5000
7500
10000
12500
15000
1000
750
500
250
0
600
400
200

FIG. 23A

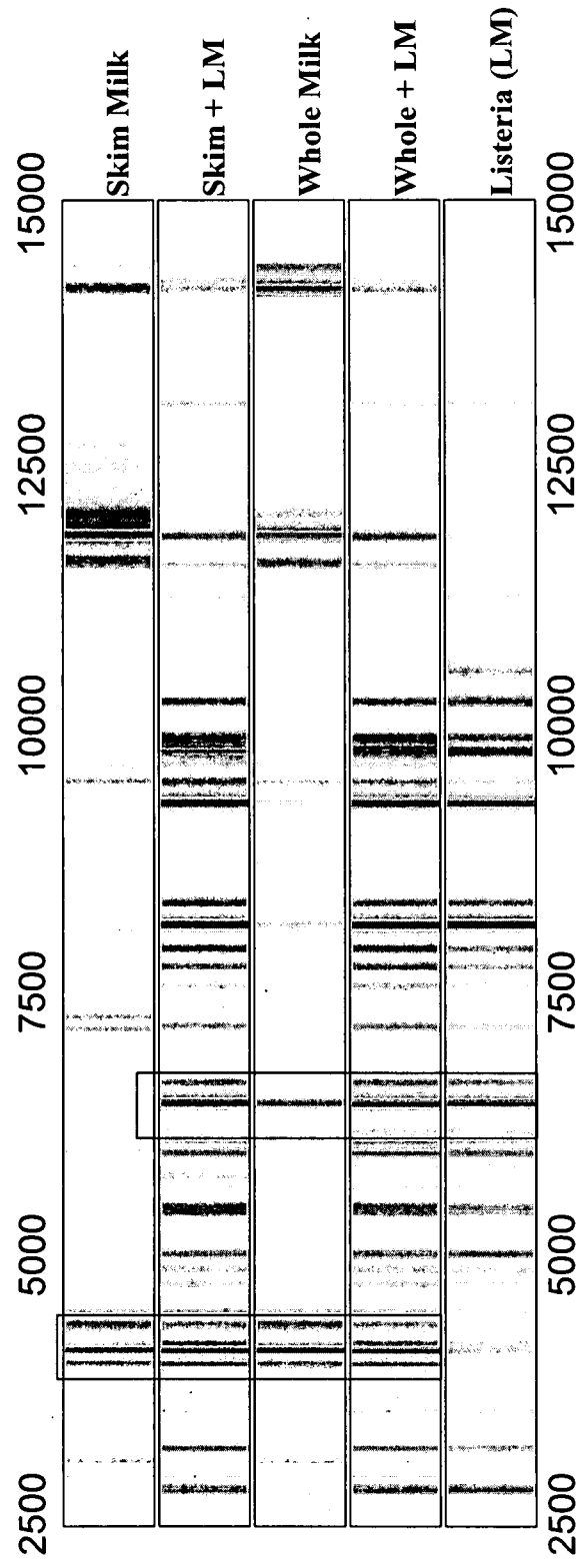

Skim Milk
Skim + LM
Whole Milk
Whole + LM
Listeria (LM)
15000
12500
10000
7500
5000
2500

FIG. 23B

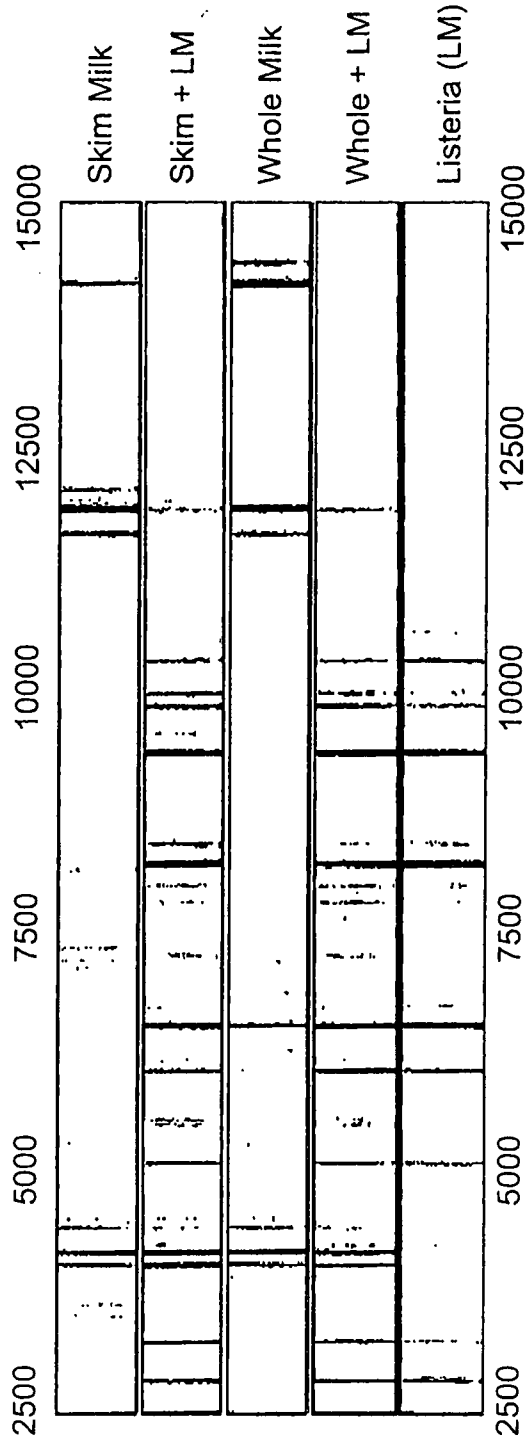

Skim Milk
Skim + LM
Whole Milk
Whole + LM
Listeria (LM)
15000
12500
10000
7500
5000
2500
*Metal binding proteins

FIG. 23C

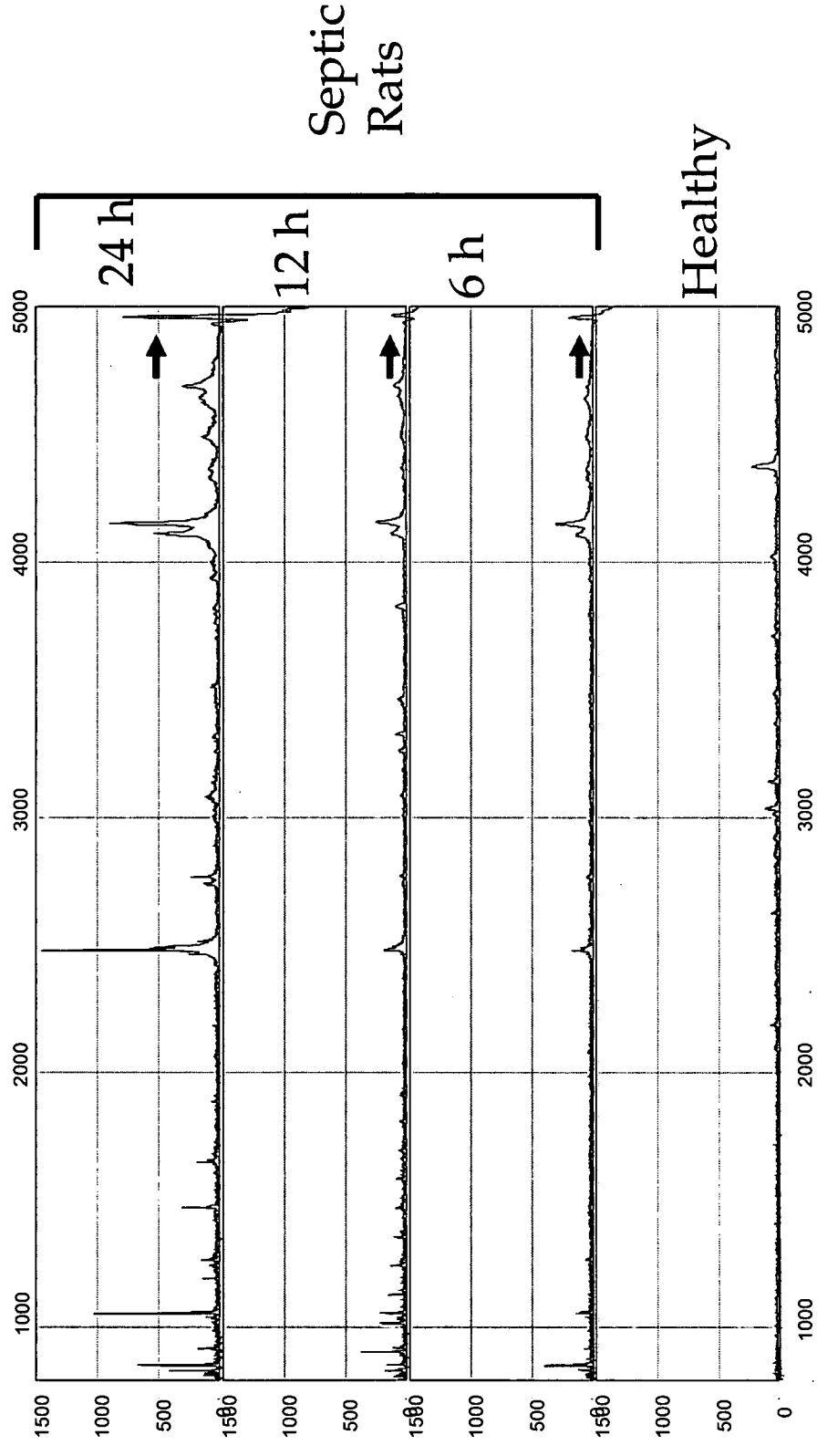

Septic Rats
24 h
12 h
6 h
Healthy
1000
2000
3000
4000
5000
1500
1000
500
0
Mass (Da)

FIG. 24A

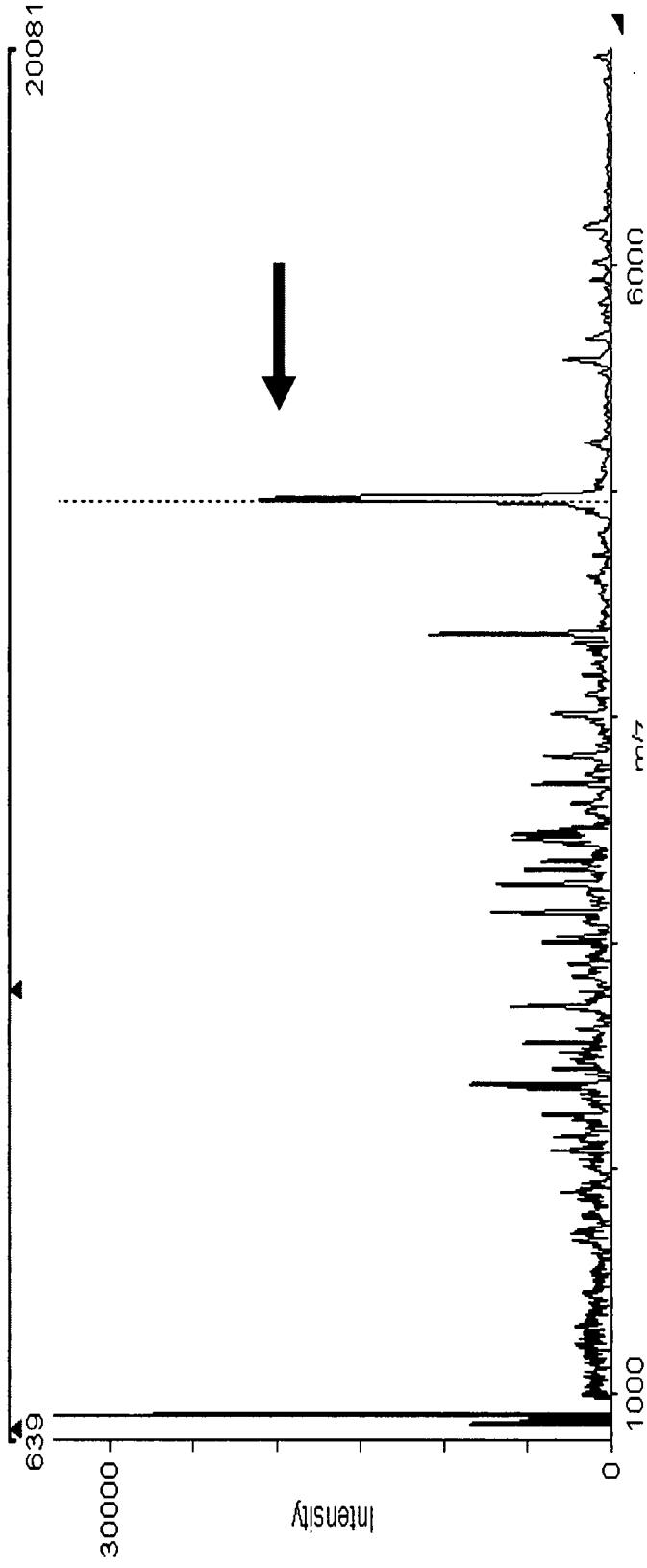


FIG. 24B

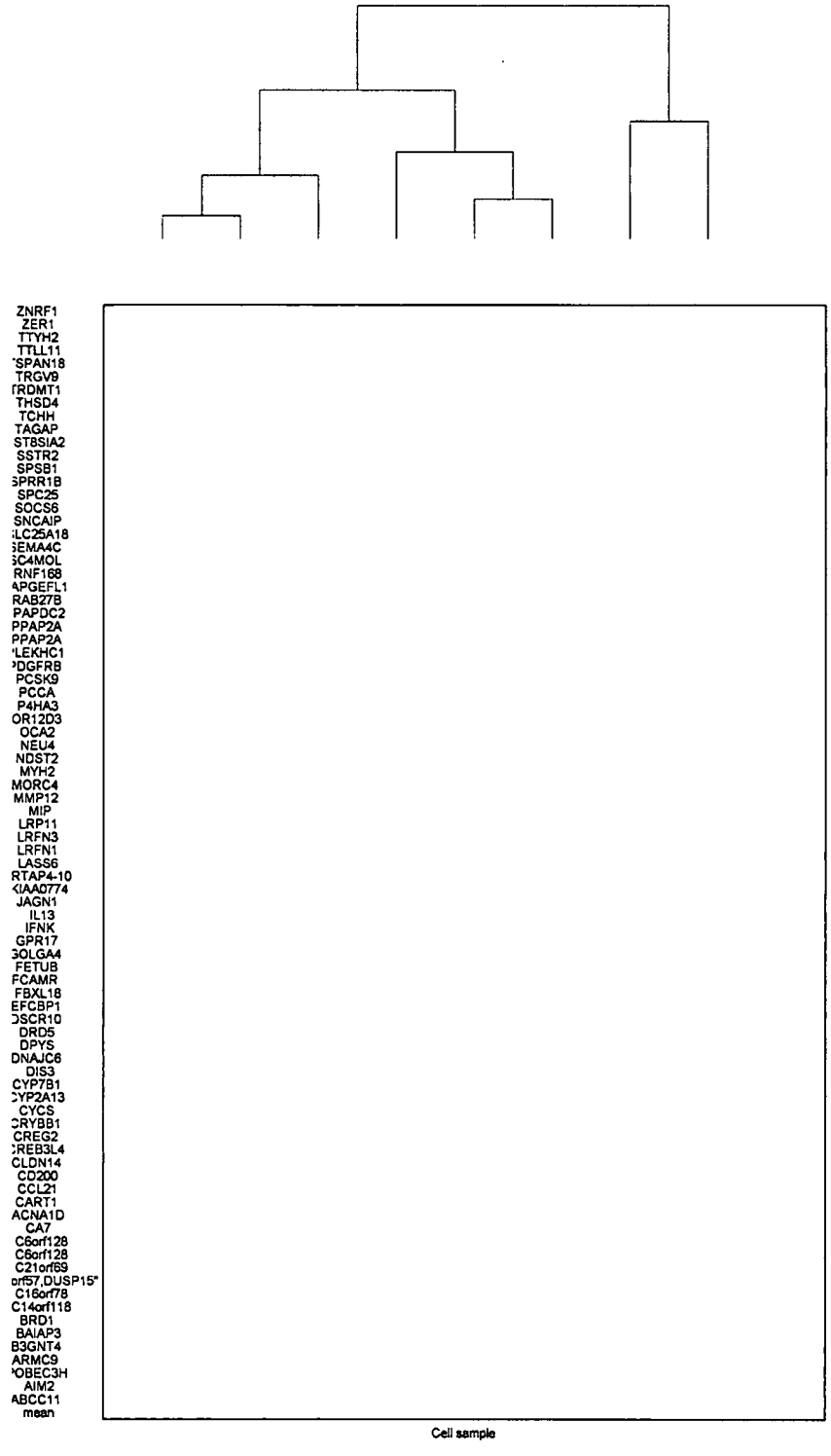


FIG. 25A BA-/- BA-S Ype

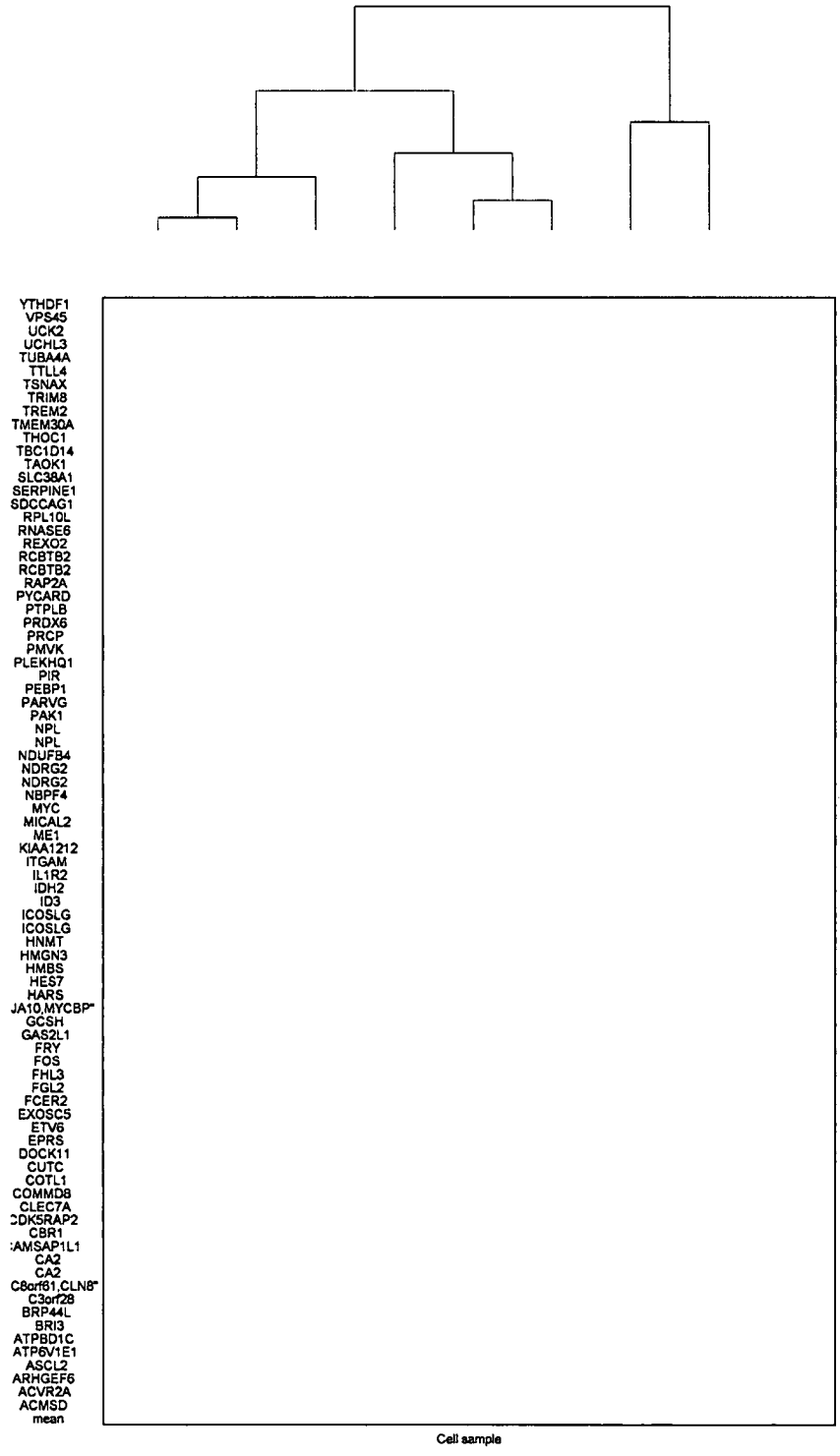
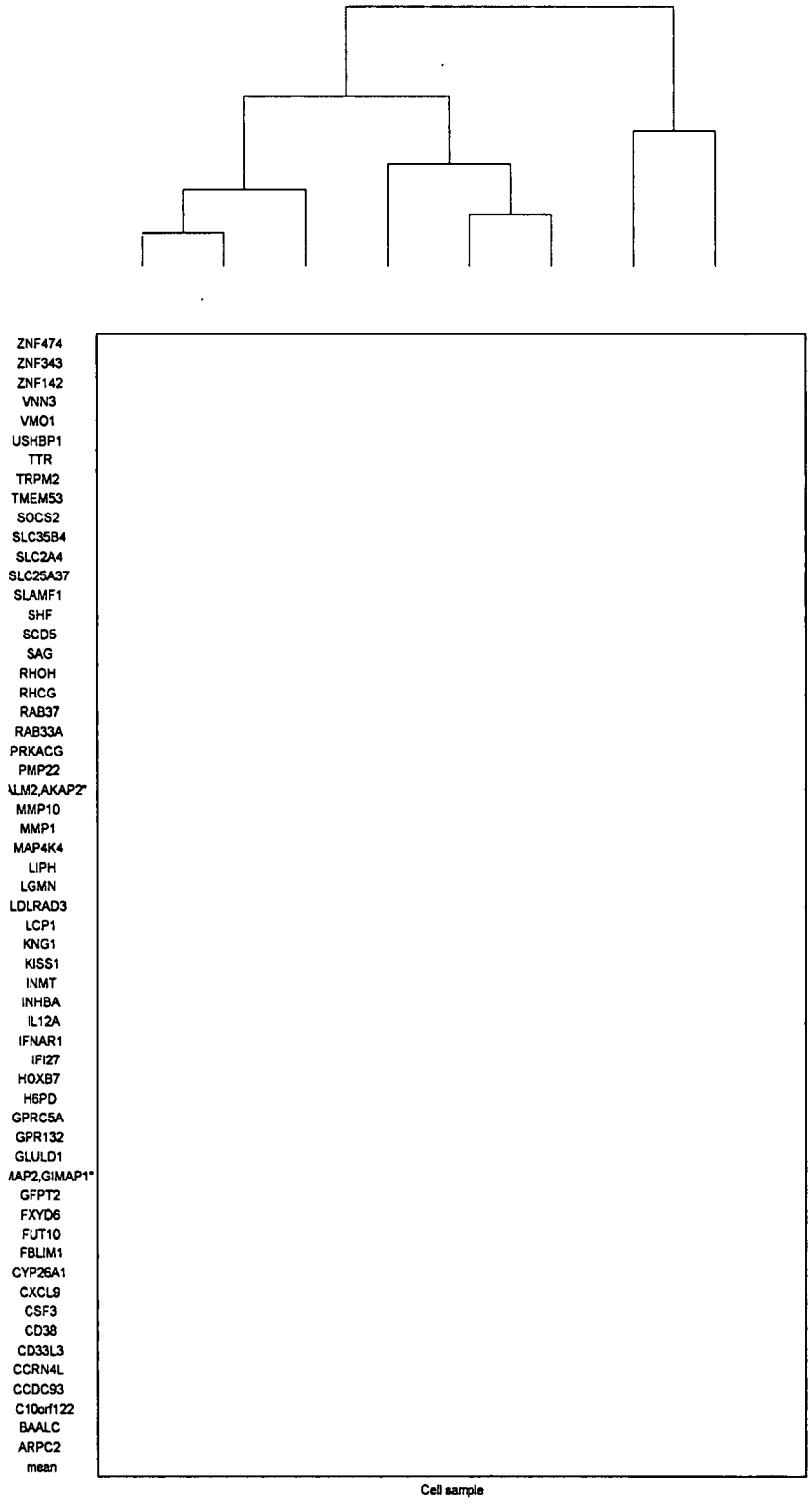


FIG. 25B

BA-/- BA-S Ype



BA-/- BA-S Ype

FIG. 25C

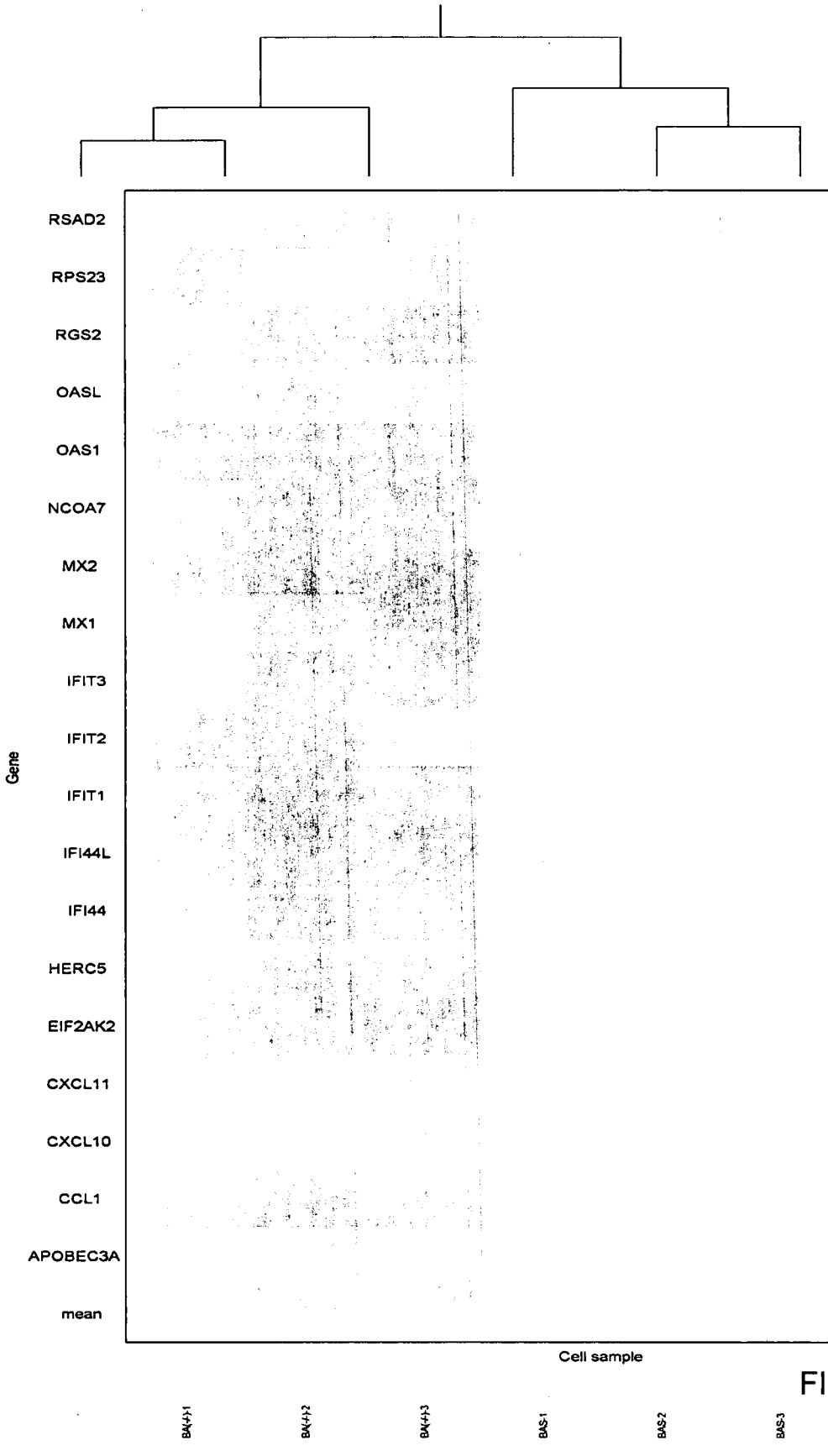

RSAD2
RPS23
RGS2
OASL
OAS1
NCOA7
MX2
MX1
IFIT3
IFIT2
IFIT1
IFI44L
IFI44
HERC5
EIF2AK2
CXCL11
CXCL10
CCL1
APOBEC3A
mean
Gene
Cell sample
BAK+/-1
BAK+/-2
BAK+/-3
BAS-1
BAS-2
BAS-3

FIG. 26

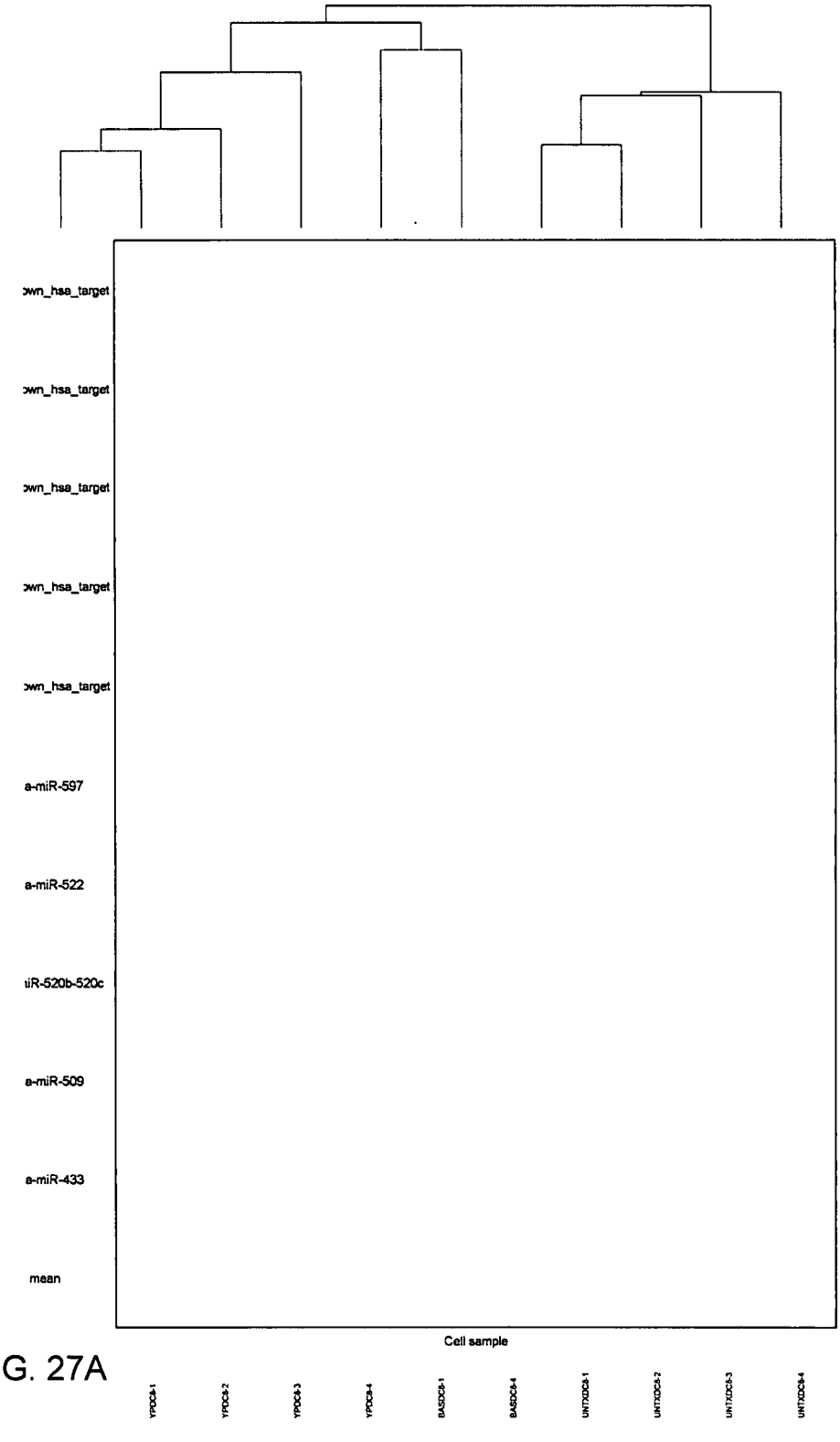

own_hsa_target
own_hsa_target
own_hsa_target
own_hsa_target
own_hsa_target
a-miR-597
a-miR-522
iR-520b-520c
a-miR-509
a-miR-433
mean
Cell sample
YPDC8-1
YPDC8-2
YPDC8-3
YPDC8-4
BASDC8-1
BASDC8-4
UNTXDC8-1
UNTXDC8-2
UNTXDC8-3
UNTXDC8-4

FIG. 27A

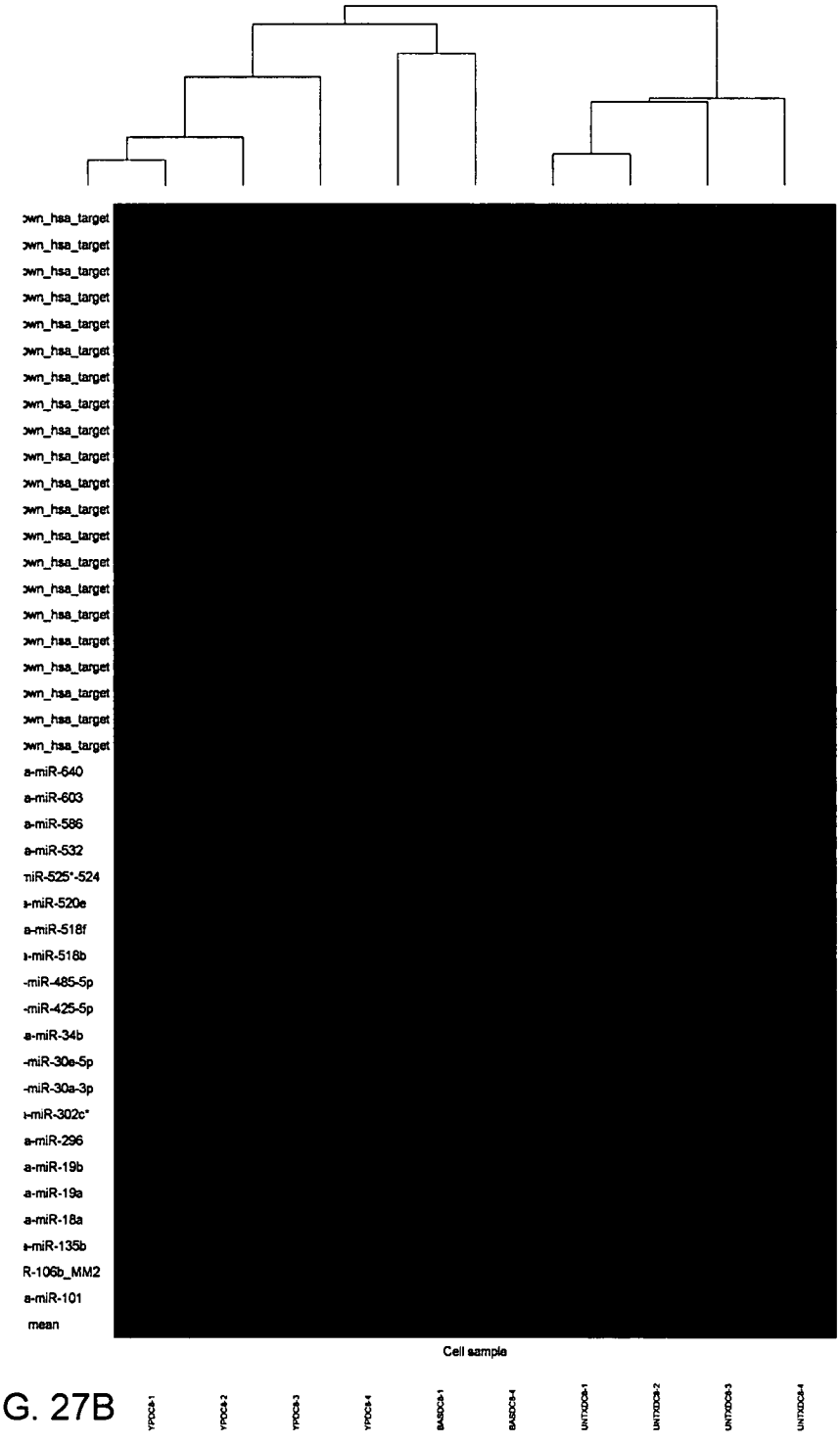

own_hsa_target
own_hsa_target
own_hsa_target
own_hsa_target
own_hsa_target
own_hsa_target
own_hsa_target
own_hsa_target
own_hsa_target
own_hsa_target
own_hsa_target
own_hsa_target
own_hsa_target
own_hsa_target
own_hsa_target
own_hsa_target
own_hsa_target
own_hsa_target
own_hsa_target
own_hsa_target
a-miR-640
a-miR-603
a-miR-586
a-miR-532
miR-525*-524
a-miR-520e
a-miR-518f
a-miR-518b
-miR-485-5p
-miR-425-5p
a-miR-34b
-miR-30e-5p
-miR-30a-3p
a-miR-302c*
a-miR-296
a-miR-19b
a-miR-19a
a-miR-18a
a-miR-135b
R-106b_MM2
a-miR-101
mean
Cell sample
YPDC8-1
YPDC8-2
YPDC8-3
YPDC8-4
BASDC8-1
BASDC8-4
UNTXDC8-1
UNTXDC8-2
UNTXDC8-3
UNTXDC8-4

FIG. 27B

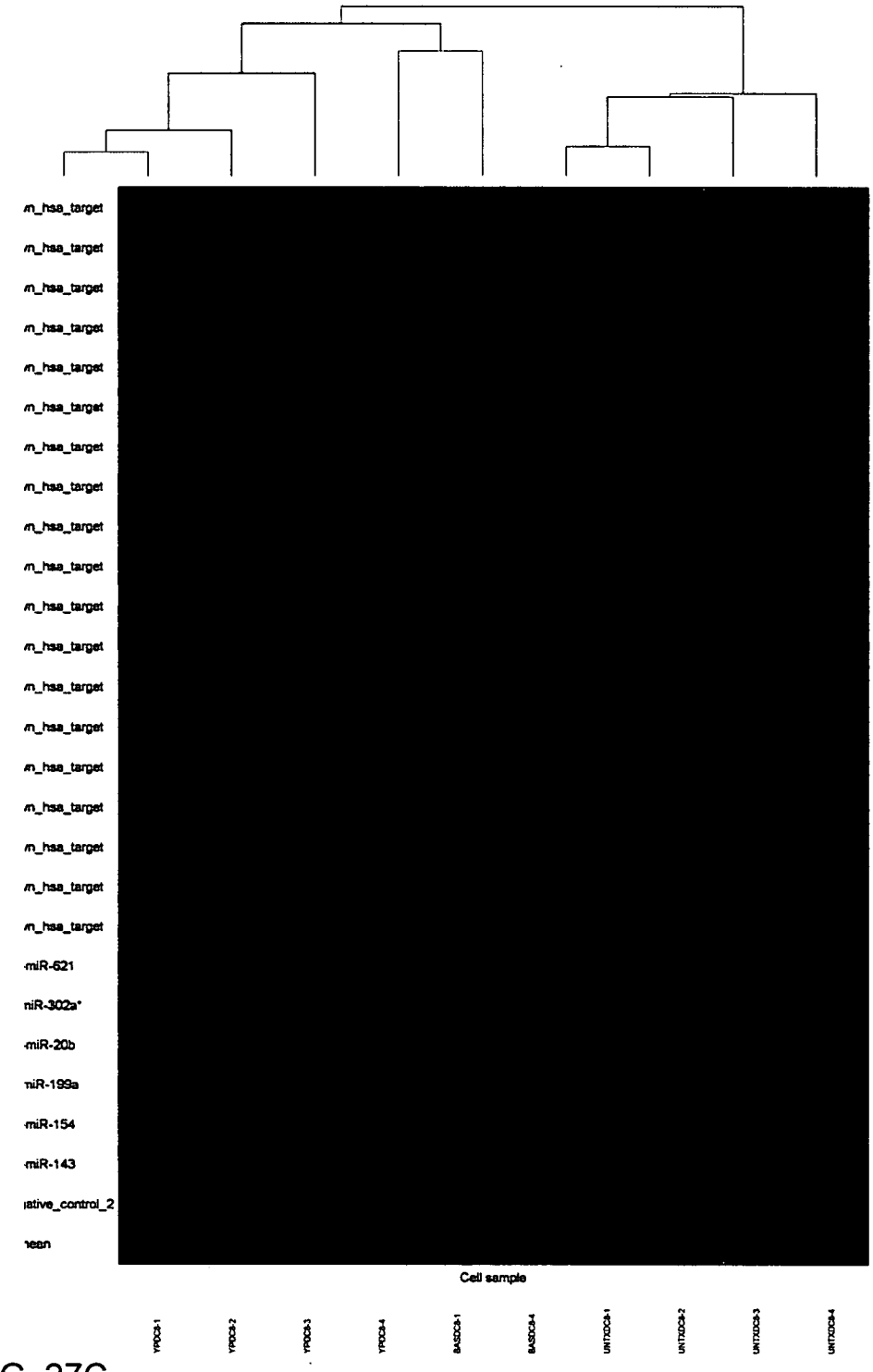

n_hsa_target
n_hsa_target
n_hsa_target
n_hsa_target
n_hsa_target
n_hsa_target
n_hsa_target
n_hsa_target
n_hsa_target
n_hsa_target
n_hsa_target
n_hsa_target
n_hsa_target
n_hsa_target
n_hsa_target
n_hsa_target
n_hsa_target
n_hsa_target
n_hsa_target
miR-621
niR-302a*
miR-20b
niR-199a
miR-154
miR-143
ative_control_2
ean
Cell sample
YPDD3-1
YPDD3-2
YPDD3-3
YPDD3-4
BASDD3-1
BASDD3-4
UNTDD3-1
UNTDD3-2
UNTDD3-3
UNTDD3-4

FIG. 27C

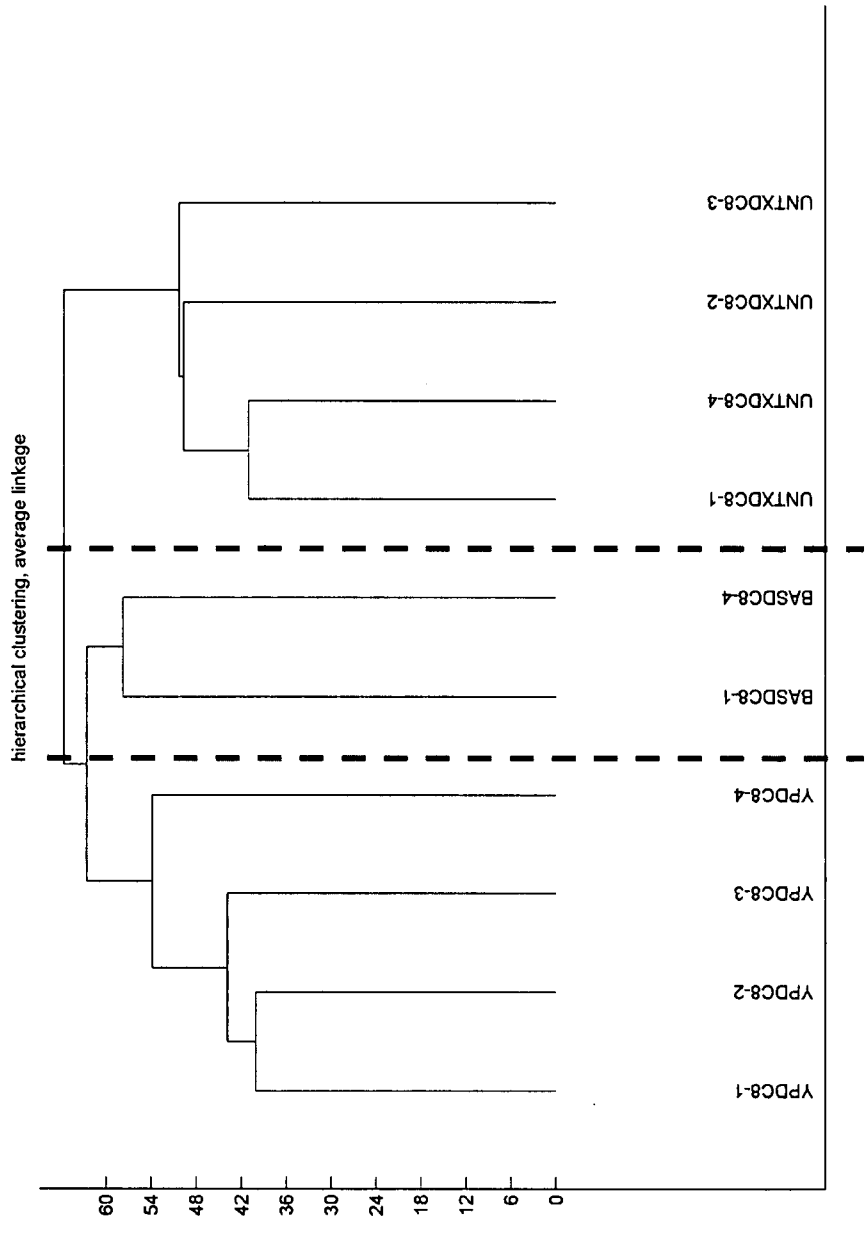

hierarchical clustering, average linkage
UNTXDC8-3
UNTXDC8-2
UNTXDC8-4
UNTXDC8-1
BASDC8-4
BASDC8-1
YPDC8-4
YPDC8-3
YPDC8-2
YPDC8-1
60
54
48
42
36
30
24
18
12
6
0

FIG. 28A

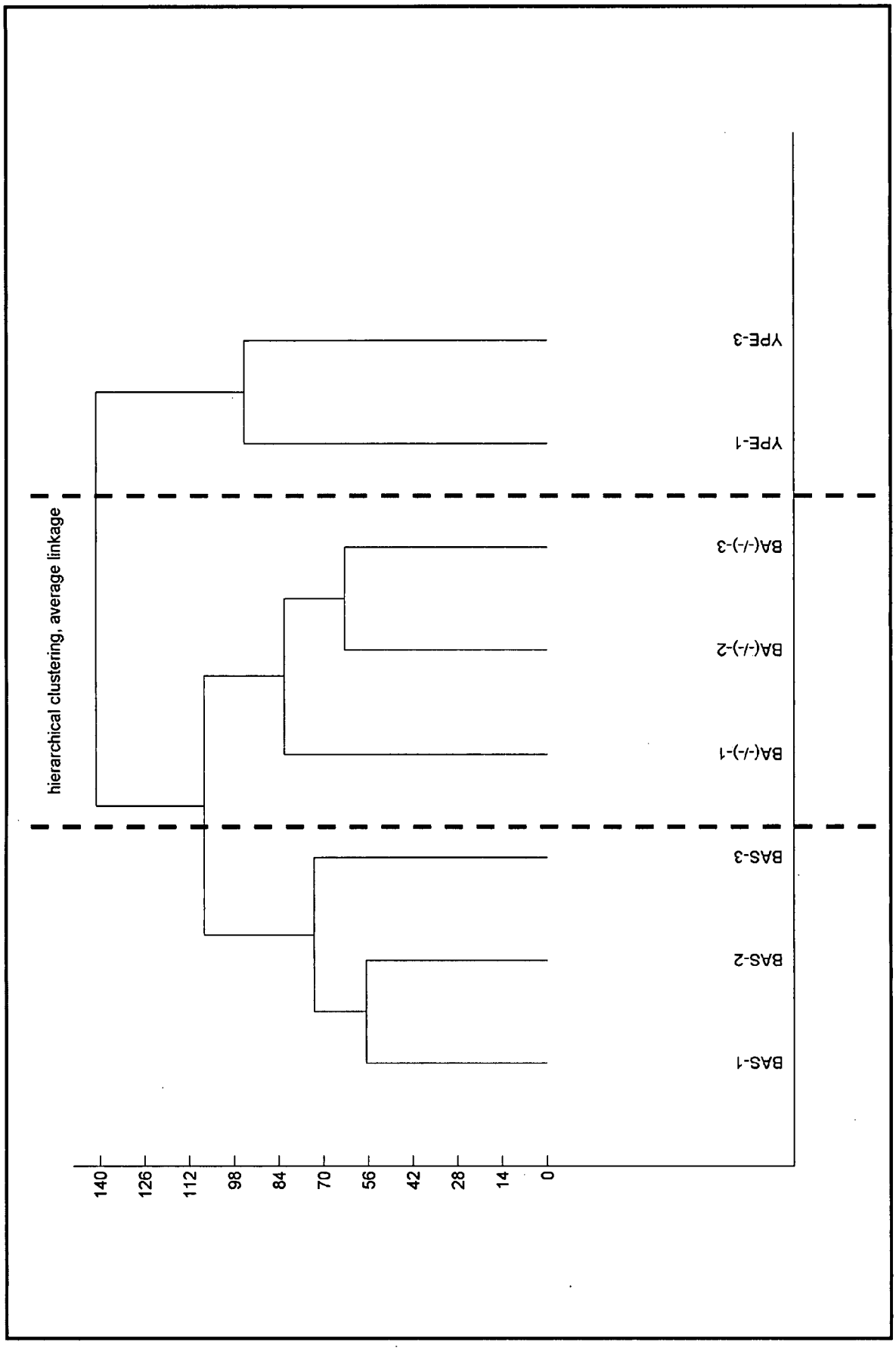

hierarchical clustering, average linkage
YPE-3
YPE-1
BA(-/-)-3
BA(-/-)-2
BA(-/-)-1
BAS-3
BAS-2
BAS-1
140
126
112
98
84
70
56
42
28
14
0

FIG. 28B

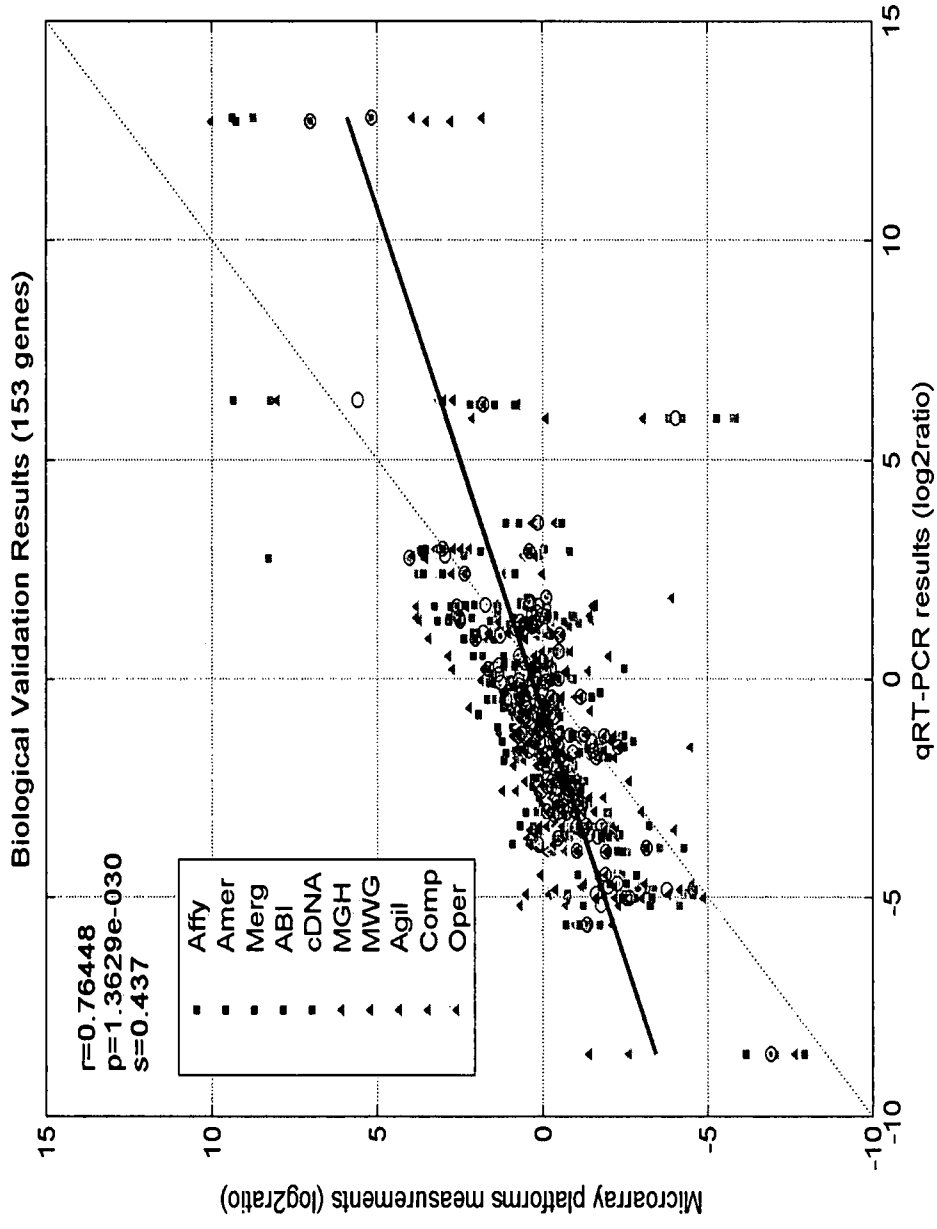

Biological Validation Results (153 genes)
r=0.76448
p=1.3629e-030
s=0.437
Affy
Amer
Merg
ABI
cDNA
MGH
MWG
Agil
Comp
Oper
Microarray platforms measurements (log2ratio)
qRT-PCR results (log2ratio)
15
10
5
0
-5
-10

FIG. 29

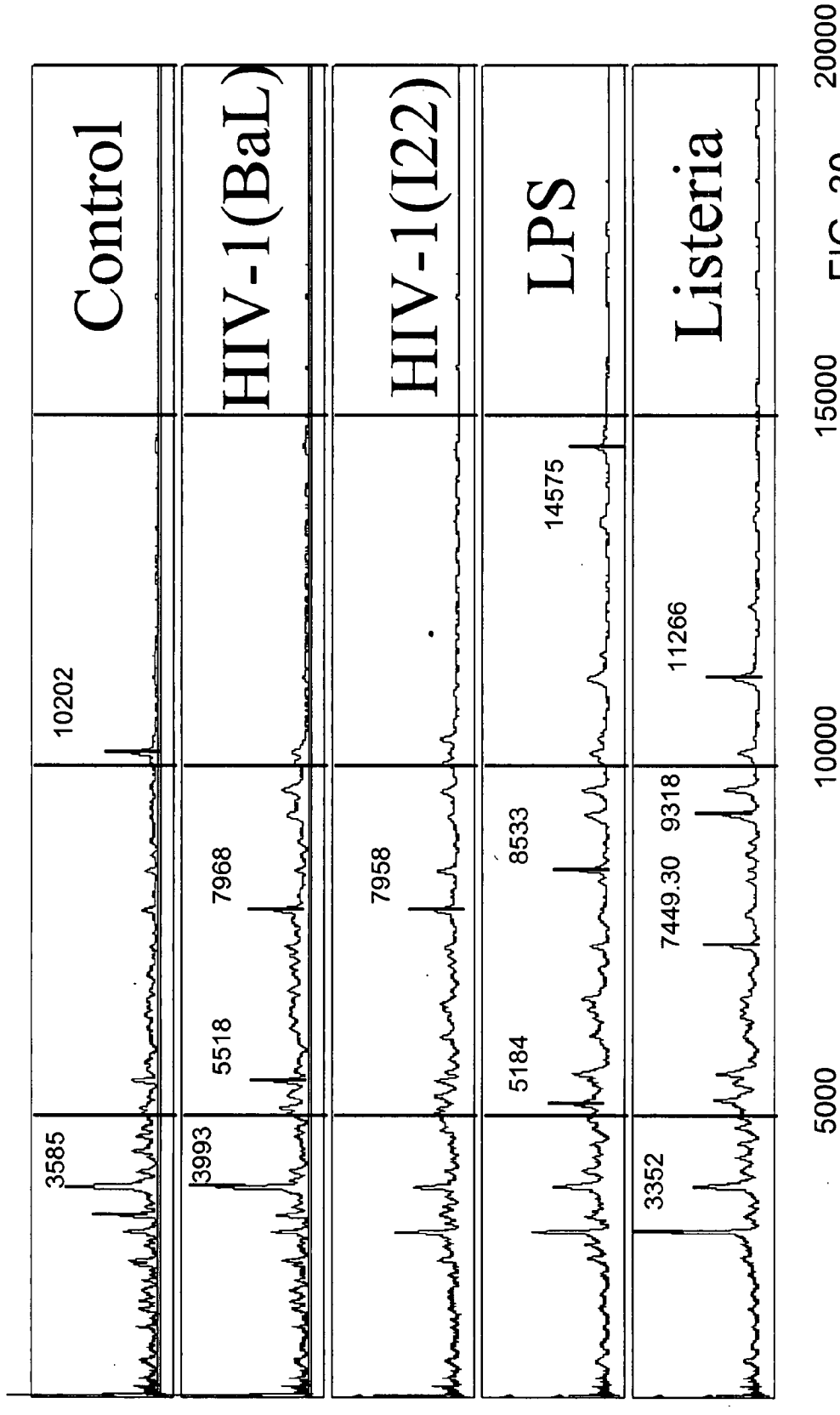

Control
HIV-1(BaL)
HIV-1(I22)
LPS
Listeria
5000
10000
15000
20000
3585
10202
3993
5518
7968
7958
5184
8533
14575
3352
7449.30
9318
11266

FIG. 30

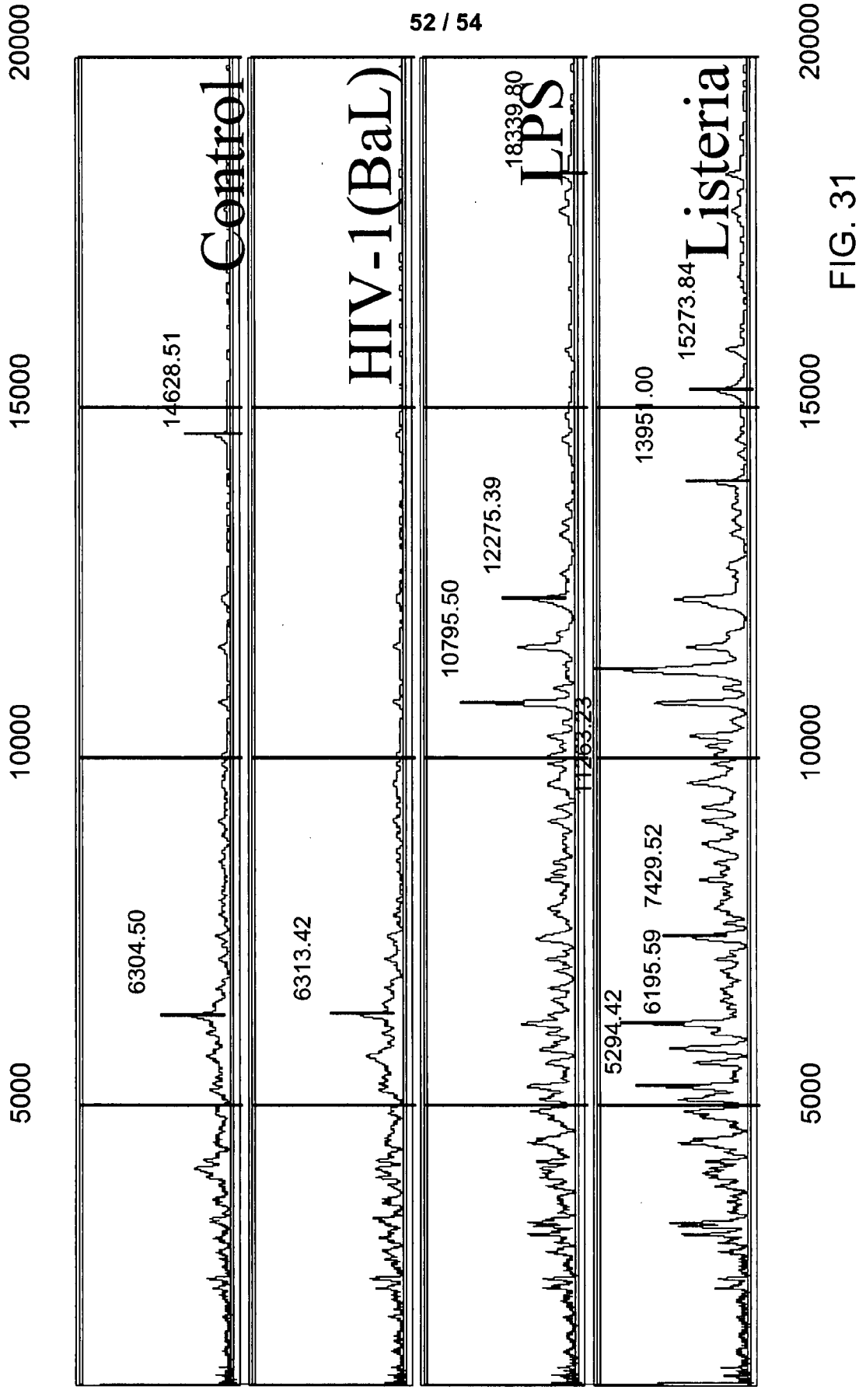

Control
HIV-1(BaL)
LPS
Listeria
6304.50
14628.51
6313.42
10795.50
12275.39
18339.80
5294.42
6195.59
7429.52
13951.00
15273.84
5000
10000
15000
20000

FIG. 31

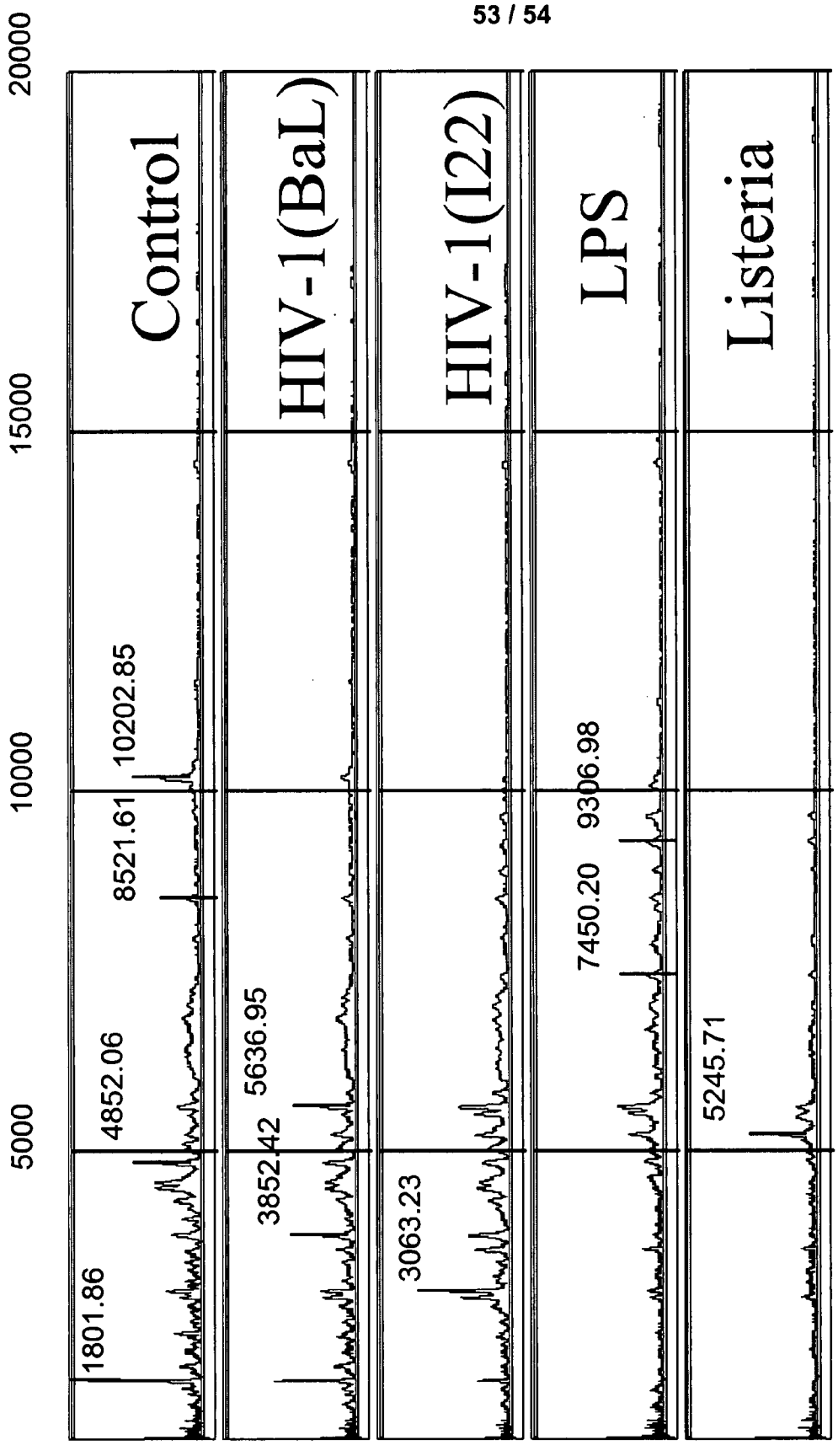

Control
HIV-1(BaL)
HIV-1(I22)
LPS
Listeria
1801.86
4852.06
8521.61
10202.85
3852.42
5636.95
3063.23
7450.20
9306.98
5245.71
5000
10000
15000
20000

FIG. 32

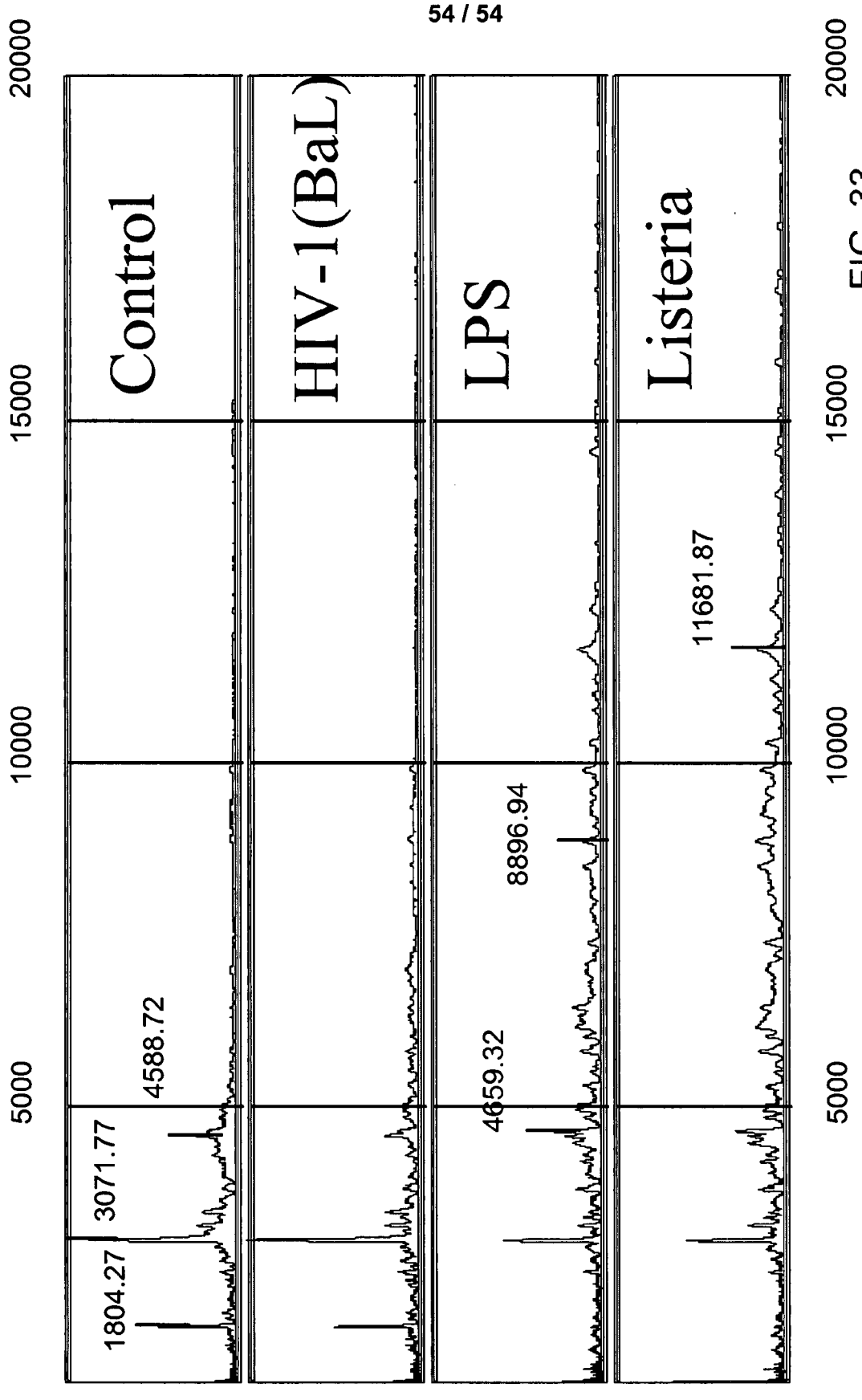

Control
HIV-1(BaL)
LPS
Listeria
1804.27
3071.77
4588.72
4659.32
8896.94
11681.87
5000
10000
15000
20000

FIG. 33