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(71) Applicant: **TECHNO-PATH (DISTRIBUTION)** [IE/IE];
Fort Henry Business Park, Co. Tipperary Ballina (IE).

(72) Inventors: **THOMPSON, Ben**; 2 Westbrook Bank, Sheffield South Yorkshire S118YJ (GB). **JAMES, David**; 47 Cortworth Road, Ecclesall, Sheffield South Yorkshire S119LN (GB). **DAVIES, Sarah Louise**; 41 Southwood Road, Great Moor, Stockport South Yorkshire SK27DJ (GB).

(74) Agent: **PURDY, Hugh Barry**; PURDYLUCEY Intellectual Property, 6-7 Harcourt Terrace, D2 Dublin (IE).

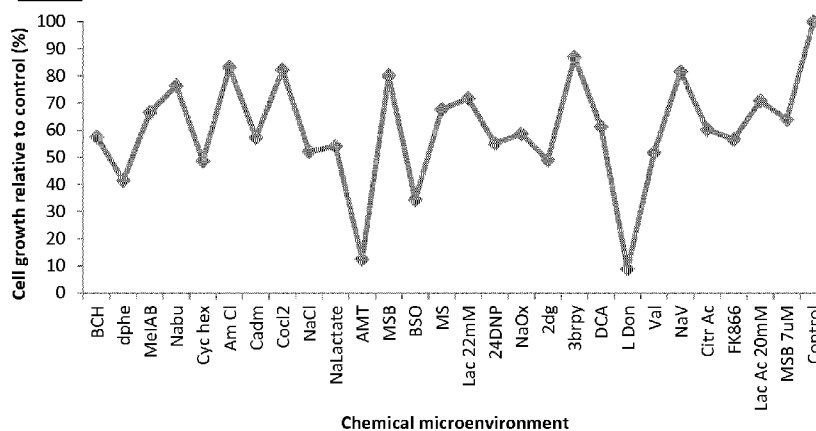
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(54) Title: A METHOD OF DETERMINING OR PREDICTING A CHARACTERISTIC OF A CELL

FIG. 2

(57) Abstract: A method of determining or predicting a characteristic of a query cell, for example the identity of the query cell, is described. The method comprises the steps of incubating the query cell with a plurality of chemical cell stressor molecules, determining the growth response of the query cell in the presence of each of the at least three chemical cell stressor molecules to generate a query cell-specific growth response fingerprint, and comparing the query cell-specific growth response fingerprint with one or more reference cell-specific growth response fingerprints corresponding to one or more cells having known characteristics. The characteristic of the cell (for example the identity of the cell) may be determined based on the level of correlation between the query cell-specific growth response fingerprint and the one or more reference cell-specific growth response fingerprints.

Title

A method of determining or predicting a characteristic of a cell.

Introduction

The invention relates to a method of determining or predicting a characteristic of a cell. In particular, the invention relates to a method of determining the identity of a cell or predicting the fed batch performance of a cell.

Background to the Invention

Many biopharmaceuticals are produced by genetically modified single cell organisms, known as producer cells. A well established system has been established for producing biopharmaceuticals using mammalian producer cells. The first step involves deriving a high-producing cell line that is suitable for large scale production, a process that involves first transfecting, cloning and then characterising a large number of cell lines. This is an expensive and labour and time intensive process, lasting up to 12 months. Once a gene of interest has been transfected and selection pressure applied using the expression system of choice, the next step involves selection and isolation of cells with acceptable growth and recombinant protein production rate. Cells are cultured and expanded to give clonal populations and once typically hundreds of clonal populations are established, cell line characterisation takes place. Use of so many clonally derived cells necessitates that each clone is stored separately and documented so that it can be easily identified prior to subsequent use. Currently storage containers for clones are marked ensuring that a clone can be correctly identified. However, if a problem occurs during cataloging the clones, and one or more clone containers is incorrectly labelled, it will not be possible to determine the identity of that clone. Other than sequencing (which is expensive and takes a substantial amount of time), there is no other way of validating the identity of a cryovial other than by trusting the paper work/label.

One step in the process of cell line characterisation is determining the fed batch performance of each clone, which involves monitoring each clone in culture over a period of generally greater than 10 days to determine some fed batch performance metric such as the product yield and IVCD50% for each clone in the panel being tested, which is defined as the integral of

viable cell density at the point in culture where the average cellular viability drops below 50%. This is a time and labour intensive process.

It is an object of the invention to overcome at least one of the above-referenced problems.

Statements of Invention

The invention is based on the finding that the growth response of a cell to a plurality of individual cell stressor molecules can act as a fingerprint or signature, i.e. a stress response fingerprint, that is unique to the cell and can be used as a means of determining or predicting a characteristic of the cell, for example the identity of the cell, the genetic or functional stability of a clonally derived cell, the predicted growth performance of the cell in fed batch culture (predicted fed batch performance), or other characteristics. The cell may be any type of cell, for example a clonally-derived cell or a cell from non-clonal source. In one application of the invention, a panel of cells (clonal or non-clonal) may be processed by incubating each cell with a plurality of cell stressor molecules, measuring the growth response of each cell to each cell stressor, and generating a unique signature or fingerprint for each cell comprising the plurality of growth responses. A panel of such signatures or fingerprints (hereafter “reference cell-specific growth response fingerprints”) may be stored and subsequently used as a means of identifying an unidentified cell from the panel, or to confirm the identity of a previously identified cell as an additional checkpoint in quality control. To identify, or confirm the identity, of a cell, it is incubated with a plurality of the same cell stressor molecules, and a query cell specific growth response fingerprint is obtained for the cell. The query fingerprint may then be compared with the panel of reference cell specific growth response fingerprints to identify, or confirm the identity of the cell. In another application, where the reference cell specific growth response fingerprints are obtained from cells with known characteristics, for example cells that are known to perform well or poorly in fed batch culture, comparison of the growth response fingerprint of the query cell with the reference growth response fingerprints allows the fed batch performance, both in terms of growth and productivity, of the query cell to be predicted.

In a first aspect, the invention provides a method of determining or predicting a characteristic of a cell comprising the steps of:

- incubating the cell with a plurality of individual chemical cell stressors;
- determining the growth response of the cell in the presence of each of the plurality of chemical cell stressors to generate a query cell-specific growth response fingerprint;
- comparing the query cell-specific growth response fingerprint with one or more reference cell-specific growth response fingerprints corresponding to one or more cells having known characteristics; and
- determining or predicting a characteristic of the cell based on the level of correlation between the query cell-specific growth response fingerprint and the one or more reference cell-specific growth response fingerprints.

Suitably, the characteristic of the cell is selected from cell identity, fed batch performance or genetic or functional stability. Thus, the method of the invention suitably relates to determining the identity of a cell, predicting fed batch performance of a cell, predicting relative fed batch performance of a panel of a panel of clonal cells, ideally a panel of clonal cells derived from a single parental host cell population, predicting or determining the genetic or functional stability of the cell.

In a more specific aspect, the invention provides a method of identifying a cell comprising the steps of:

- incubating the cell with a plurality of chemical cell stressors;
- determining the growth response of the cell in the presence of each of the plurality of chemical cell stressors to generate a query cell-specific growth response fingerprint;
- comparing the query cell-specific growth response fingerprint with one or more reference cell-specific growth response fingerprints corresponding to one or more known cells to identify the cell; and
- when the query cell-specific growth response fingerprint matches or correlates with a reference cell-specific growth response fingerprint, identifying the query cell.

In a preferred embodiment, the invention relates to a method for identifying a query clonal cell, the method comprising the steps of:

- incubating the query clonal cell with a plurality of chemical cell stressors;
- determining the growth response of the query clonal cell in the presence of each of the plurality of chemical cell stressors to generate a query clonal cell-specific growth response fingerprint;

- comparing the query clonal cell-specific growth response fingerprint with a plurality of reference cell-specific growth response fingerprints corresponding to a panel of clonal cells.

In a second aspect, the invention relates to a method of generating a reference growth response fingerprint corresponding to a specific cell, comprising the steps of:

- incubating the cell with a plurality of individual chemical cell stressors; and
- determining the growth response of the cell in the presence of each of the plurality of chemical cell stressors to generate a reference growth response fingerprint.

In a third aspect, the invention relates to a method of generating a panel of reference growth response fingerprints corresponding to a panel of distinct cells, comprising the steps of:

- generating a reference growth response fingerprint for each cell in the panel of cells; and
- storing the panel of reference growth response fingerprints,

wherein each reference growth response fingerprint is generated by the steps of:

- incubating one of the cells with a plurality of chemical cell stressors; and
- determining the growth response of the cell in the presence of each of the plurality of chemical cell stressors to generate a reference growth response fingerprint.

In a fourth aspect, the invention provides a system for identifying a cell, the system comprising:

- a device comprising a plurality of reaction chambers;
- optionally, a plurality of chemical cell stressors preferably disposed individually within the reaction chambers;
- a determination system for determining the growth response of the cell in the presence of each of the plurality of chemical cell stressors;
- optionally, a storage system for storing a cell-specific growth response fingerprint corresponding to the plurality of growth responses of the cell;
- a comparison system configured to compare the cell specific growth response fingerprint with one or more reference cell-specific growth response fingerprints; and
- optionally, an output system for displaying an output of the comparison step.

In another embodiment, the invention provides a computer implemented method of predicting relative fed batch performance of a panel of clonal cells derived from a single parental host cell population, the method comprising the steps of:

- assaying a level of growth of each clone in the presence and absence of a plurality of chemical cell stressors;
- comparing the level of growth of each clone in the presence and absence of each of the plurality of chemical cell stressors to provide a normalised growth response value for each clone in each of a plurality of stressed microenvironments;
- inputting a clone-specific growth response fingerprint comprising the normalised growth response value for each clone in each of the stressed microenvironments into a computational model, in which the computational model is generated from growth response fingerprints obtained from a calibration set of clones with known fed batch performance, wherein the computational model is configured to output the predicted fed batch performance value for each clone to predict the relative fed batch performance of the panel of clonal cells.

When a batch of new clones are generated from a parental cell line, rather than all or a select few being subjected to expensive and time-consuming growth studies in bioreactors, the method of the invention allows a large number of clones to be rapidly assayed for, and ranked according to, predicted fed batch performance in a rapid and high-throughput manner. This informs on which clones to take forward to scale-up/mini-bioreactor studies, for example only the good clones as illustrated in Fig. 3 below.

The method of this aspect of the invention employs data (cell specific growth response fingerprints) from a panel of pre-validated clones (clones of known fed batch performance), and a computational model, ideally a multiple linear computational model, based on this data, to allow prediction of relative fed batch performance of a new set of clones based on their rapidly obtained chemical fingerprints.

Preferably, the method comprises an additional step of ranking the clones according to predicted fed batch performance value.

The methods of the invention are typically carried out using a microtitre plate (multiwell plate), in which each assay is performed in a well of a microtitre plate. Suitably, the growth of each cell is assayed in the well of a multiwell plate.

Typically, the assay involves mixing a sample of a clone with a chemical cell stressor, and incubating the mixture from 1 to 4 days, and assaying the level of growth of the cells. Suitably,

the clone sample is provided at a concentration of from 0.1 to 1.0×10^6 cells per ml of mixture. Typically, the bioreactor-relevant chemical cell stressor is provided at a concentration of 0.5 to $2 \times \text{IC}_{50}$, ideally about $1 \times \text{IC}_{50}$.

Suitably, the growth of each clone is assayed after a period of incubation of less than 5 days. Ideally, the growth of each clone is assayed after a period of incubation of between 1-4 days, and ideally after 2 and 3 days.

Preferably, the growth of each clone is assayed simultaneously.

The chemical cell stressors are chemicals that cause a reduction in cell growth via one or more of multiple cellular pathways. Suitably, the plurality of chemical cell stressors comprise at least 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 21, 22, 23, 24, or 25 stressors. Typically, the plurality of chemical cell stressors are selected from the group consisting of amino acid transport inhibitors, cell cycle inhibitors, a source of carbon, a source of osmotic stress, a source of oxidative stress, an inducer of apoptosis, metabolic effectors, a pH modifier, an inhibitor of glycolysis, and a toxin. Typically, the plurality of chemical cell stressors include stressors selected from at least 4, 5, 6 or 7 of the groups consisting of amino acid transport inhibitors, cell cycle inhibitors, a source of carbon, a source of osmotic stress, a source of oxidative stress, an inducer of apoptosis, metabolic effectors, a pH modifier, an inhibitor of glycolysis, and a toxin.

In another aspect, the invention provides a microtitre plate suitable for generating a growth response fingerprint of a cell and comprising at least 24, 48 or 96 wells, and a plurality of chemical cell stressors disposed individually in at least some of the wells. Typically, the plate comprises at least 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 21, 22, 23, 24, or 25 chemical cell stressors. Suitably, each chemical cell stressor is disposed in at least two or three wells of the plate (i.e. duplicate or triplicate).

Preferably, the plurality of chemical cell stressors are selected from the group of 2-aminobicyclo-(2,2,1)heptane-carboxylic acid (BCH), D-phenylalanine, α -(methylamino)isobutyric acid (MeAIB), Sodium Butyrate (NaBu), cycloheximide, ammonium chloride, Cadmium acetate dihydrate, Cobalt chloride (CoCl_2), Sodium Chloride (NaCl), Sodium lactate (Na Lac), Aminotriazole (AMT), Menadione Sodium Bisulphite (MSB),

Buthionine Sulfoximine (BSO), Mercaptosuccinic Acid (MS), 2,4-Dinitrophenol (24DNP), Sodium Oxamate, 2-deoxyglucose (2dg), 3-bromopyruvate (3-BrPA), Dichloroacetate (DCA), 6-diazo-5-oxo-l-norleucine (l-don), Valproic acid (Val), Sodium Orthovandate (NaV), citric acid, FK866, lactic acid.

In another aspect, the invention provides a microtitre plate comprising at least 24, 48 or 96 wells, and a plurality of chemical cell stressors disposed individually in at least some of the wells, in which the plurality of chemical cell stressors comprise at least one chemical cell stressor selected from each of the groups consisting of amino acid transport inhibitors, cell cycle inhibitors, a source of carbon, a source of osmotic stress, a source of oxidative stress, an inducer of apoptosis, metabolic effectors, a pH modifier, an inhibitor of glycolysis, and a toxin.

The term “microtitre plate” or “multiwell plate” as used herein should be understood to mean plates have a plurality of reaction wells, generally at least 12, 24, 48 or 96 wells, each well generally having a volume of less than 5, 4, 3, 2 or 1 ml. The term should be understood to include rigid plates, and plates provided in the form of a flexible roll.

In one embodiment, the invention provides a microtitre plate comprising at least 96 wells, and at least 23 chemical cell stressors disposed individually in wells of the plate, in which the chemical cell stressors consist essentially of 2-aminobicyclo-(2,2,1)heptane-carboxylic acid (BCH), D-phenylalanine, α -(methylamino)isobutyric acid (MeAIB), Sodium Butyrate (NaBu), cycloheximide, ammonium chloride, Cadmium acetate dihydrate, Cobalt chloride (CoCl₂), Sodium Chloride (NaCl), Sodium lactate (Na Lac), Aminotriazole (AMT), Menadione Sodium Bisulphite (MSB), Buthionine Sulfoximine (BSO), Mercaptosuccinic Acid (MS), 2,4-Dinitrophenol (24DNP), Sodium Oxamate, 2-deoxyglucose (2dg), 3-bromopyruvate (3-BrPA), Dichloroacetate (DCA), 6-diazo-5-oxo-l-norleucine (l-don), Valproic acid (Val), Sodium Orthovandate (NaV), citric acid, FK866, lactic acid.

In another aspect, the invention provides a kit suitable for performing a method of the invention and comprising (a) a microtitre plate of the invention (b) a microtitre plate reader, and (c) a computer program comprising program instructions for (i) comparing the level of growth of each clone in the presence and absence of each of the plurality of chemical cell stressors to provide a normalised growth response value for each clone in each of a plurality of stressed microenvironments, (ii) inputting a clone-specific growth response fingerprint comprising the

normalised growth response value for each clone in each of the stressed microenvironments into a computational model, in which the computational model is generated from growth response fingerprints obtained from a calibration set of clones with known fed batch performance, wherein the computational model is configured to output the predicted fed batch performance value for each clone to predict the relative fed batch performance of the panel of clonal cells, and (iii) outputting the predicted fed batch performance value for each clone to predict the relative fed batch performance of the panel of clonal cells.

The invention also provides a computer implemented system for performing a method of predicting relative fed batch performance of a panel of clonal cells derived from a single cell line, the system comprising:

- a determination system for assaying a level of growth of each clone in the presence and absence of a plurality of individual chemical cell stressors to provide a normalised growth response value for each clone in a plurality of stressed microenvironments ;
- a computational model adapted to process a clone-specific growth response fingerprint comprising the normalised growth response value for each clone in each of the stressed microenvironments, in which the computational model is generated from growth response fingerprints obtained from a calibration set of clones with known fed batch performance, wherein the computational model is configured to output the predicted fed batch performance value for each clone to predict the relative fed batch performance of the panel of clonal cells, and
- means for outputting the predicted fed batch performance value for each clone to predict the relative fed batch performance of the panel of clonal cells.

Typically, the determination system comprises a plate reader, typically a plate reader configured to simultaneously detect changes in light emitted from the wells of a microtitre plate, and suitably correlate the emitted light with level of growth.

Suitably, the computational model is a multiple linear computational model. Ideally, the multiple linear computational model suitably employs a least squares solution to fit parameters (i.e. levels of cell specific growth responses) to the observed fed batch performance (preferably defined as IVCD50).

The invention also provides a computer program which when executed on a computer causes the computer to perform a method of predicting relative fed batch performance of a panel of clonal cells derived from a single cell line according to the invention.

The invention also relates to a computer program recording medium storing a computer program according to the invention.

In this specification, the term “cell” refers to any cell type, including prokaryotic or eukaryotic cells. Suitably, the cell is a eukaryotic cell, ideally a mammalian cell. Typically, the cell is a producer cell, preferably a mammalian producer cell. The cell may be clonally-derived or non-clonal. In a preferred embodiment, the cell is a clone from a panel of clonal cells, derived from a single parental cell population or derived from different transfected cells. The cell may also be from a distinct cell line, for example a genetically modified cell (i.e. cells with “knock-out” or “knock-in” mutations). The cell line may also be derived by directed evolution, by selection of cells which have adapted to a specific environment of interest. The term “panel of distinct cells” should be understood to mean a panel of cells that are different from each other. For example, the panel of cell may comprise a panel of clones that are all derived from a single parental cell population. Alternatively, the panel of cells may comprise cells of independent clonal origin, for example cells carrying different transgenes or different mutations, of the panel of cells may comprise non-clonal cells. The cell line may also be derived by directed evolution, by selection of cells which have adapted to a specific environment of interest. The panel of cells may also comprise cells of different cell type, for example different cell lines, different strains of bacteria or fungi, cells of the same cell type but at a different stage of development, and cells of the same cell type that differ genetically, for example cells of the same type or origin or cells derived from the same parental cell population that carry different transgenes.

In this specification, the term “panel of clonal cells” should be understood to mean a panel of clonal cell populations derived from a single cell line, and comprising from 2 to 500 or more clonal cell populations. Methods for generating panels of clonal cells are well known to a person skilled in the art, and described in *Production of recombinant protein therapeutics in cultivated mammalian cells* (2004) ,Wurm, Florian M, New York, NY, *Nature Biotechnology* 22 (2004), S. 1393-1398. Typically, the panel of clonal cell populations include from 10-500, 20-500, 30-500, 40-500, 50-500, 60-500, 70-500, 80-500, 90-500 or 100-500

clonal cell populations. Typically, the panel of clonal cell populations include from 100-500, 100-400, 150-400, 150-350 clonal cell populations.

In this specification, the term “identifying a cell” should be understood to mean identification of an unknown cell, or confirming the identity of a cell of known or suspected identity.

In this specification, the term “fed batch performance” should be understood to mean proliferation or production performance of the cell in fed batch culture. Preferably the term means IVCD50%, which is defined as the integral of viable cell density at the point in culture where the average cellular viability drops below 50%.

In this specification, the term “relative fed batch performance of a panel of clonal cells” should be understood to mean the fed batch performance of the clones in the panel relative to one another. Thus, in one embodiment, the clones are stratified according to their fed batch performance. In another embodiment, the clones are stratified to pick out the best performing clones, the worst performing clones, or both.

The invention involves incubating a cell with a plurality of cell stressor molecules. This means that the cell is incubated with each of the cell stressor molecules individually, to obtain a growth response of the cell in the presence of each cell stressor molecule. Incubation of the cell with the cell stressor molecule may be performed in any suitable reaction vessel, for example in the wells of a microtitre plate. Typically, the assay involves mixing a cell sample with a chemical cell stressor, and incubating the mixture from 1 to 4 days, and assaying the level of growth of the cells. Suitably, the cell sample is provided at a concentration of from 0.1 to 1.0×10^6 cells per ml of mixture. Typically, the cell stressor is provided at a concentration of 0.5 to $2 \times \text{IC}_{50}$, preferably about 0.8 to $1.2 \times \text{IC}_{50}$, and ideally about $1 \times \text{IC}_{50}$. Suitably, the growth of each clone is assayed after a period of incubation of less than 5 days. Ideally, the growth of each clone is assayed after a period of incubation of between 1-4 days, and ideally after 2 and 3 days. Preferably, the growth of each clone is assayed simultaneously.

The term “cell stressor molecule” or “cell stressor” should be understood to mean a molecule or compound that causes a reduction in cell growth via one or more of multiple cellular pathways, for example pathways involved in cell growth or metabolism. The stressor molecules are employed on the basis of their ability to simulate bioreactor stresses, for example one or

more of oxidative stress, cell cycle inhibition, inhibition of protein production, and inhibition of NAD. Typically, the plurality of chemical cell stressors are selected from the group consisting of amino acid transport inhibitors (i.e. BCH), cell cycle inhibitors (i.e. sodium butyrate), a source of carbon, an inducer of osmotic stress (i.e. sodium lactate), an inducer of oxidative stress (tert-BHP), an inducer of apoptosis (cycloheximide, TNF), a metabolic effector (i.e. a chemical that directly modulates metabolism), a pH modifier (i.e. citric acid), an inhibitor of glycolysis (i.e. 3-bromopyruvate), and a toxin (i.e. a compound or molecule that is toxic to a cell, for example cadmium). Preferably, the plurality of chemical cell stressors are selected from the group consisting of amino acid transport inhibitors, cell cycle inhibitors, an inducer of osmotic stress, an inducer of oxidative stress, an inducer of apoptosis, a metabolic effector, an inhibitor of glycolysis, and a toxin (i.e. a compound or molecule that is toxic to a cell). Typically, the plurality of chemical cell stressors include stressors selected from at least 4, 5, 6 or 7 of the groups consisting of amino acid transport inhibitors, cell cycle inhibitors, a source of carbon, an inducer of osmotic stress, an inducer of oxidative stress, an inducer of apoptosis, a metabolic effectors, a pH modifier, an inhibitor of glycolysis, and a toxin.

Examples of metabolic (catabolism) effectors are 1) chemicals which directly interfere with metabolic enzymes such as 2-deoxyglucose which competitively inhibits hexokinase (the first enzyme in the glycolysis pathway) and 2) chemicals that directly interfere with energy metabolism machinery such as 24DNP which causes pores in the mitochondrial membrane thus dissipating the proton gradient and lowering ATP production. Examples of metabolic (anabolism) effectors are FK866 which inhibits the synthesis of NAD. Additionally cycloheximide inhibits protein synthesis (and causes apoptosis).

In this specification, the term “plurality” as applied to cell stressor molecules should be understood to mean at least three different cell stressor molecules. Typically, the term refers to at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 different cell stressor molecules. Thus, the method of the invention may be performed by employing as few as three distinct cell stressor molecules to generate the cell-specific growth response fingerprint, for example: CoCl₂, NAV and MSB; Cadm, MSB and 2DG; and Cadm, dphe, MeiAB.

The term “growth response of the cell” refers to the growth of the cell in the presence of a cell stressor molecule. Typically, the growth response is a normalised growth response, which is determined by measuring the growth of in the presence and absence of the cell stressor

molecule and determining the difference in growth response due to the presence of the cell stressor molecule. Growth of cells may be determined using any technique known in the art. In one embodiment, a dye is added to the incubation mixture and a signal emitted by the dye is monitored over time and correlated with growth. For example, a fluorescent or phosphorescent dye may be employed. Examples of suitable dyes are redox dyes, such as Presto blue®. (Invitrogen, Paisley, UK)

The term “cell-specific growth response fingerprint” refers to a plurality of growth responses for a specific cell obtained by reacting the cell with a plurality of cell stressor molecules individually. The fingerprint may be embodied as a plurality of growth response values, or may be embodied in the form of a graph or any other type of visual presentation such as a pattern.

The term “query cell specific growth response fingerprint” refers to a cell specific growth response fingerprint corresponding to an unidentified cell or a cell whose identity is to be confirmed. The term “reference cell specific growth response fingerprint” refers to a growth response fingerprint for a cell the identity of which is known. Generally, the query and reference growth response fingerprints will be generated using the same set of cell stressor molecules.

The term “calibration set of clones” refers to a plurality of clones, each having a growth response fingerprint and a known fed batch performance. Typically, the calibration set of clones includes clones exhibiting a range of fed batch performance abilities from what the end user would consider ‘good’ performers to what the end user would consider ‘bad’ performers. Suitably, the calibration set of clones comprises at least 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, or 26 clones.

The term “stressed microenvironment” should be understood to mean an environment that includes a chemical cell stressor. Generally, this means that a clone is assayed for growth in a well of microtitre plate in the presence of the chemical cell stressor.

In this specification, the term “rapid” should be understood to mean a method of predicting fed batch performance that takes less than five days, and ideally less than four or three days.

In this specification, the term “high-throughput” should be understood to mean a method in which a large number of samples, for example 20-500, can be assayed simultaneously. In one embodiment, this involves assaying the growth of the clones in a multiwell plate, for example a 48, 96, or 192 well plate.

In this specification, the term “computational model” typically refers to a multiple linear computational model. Ideally, the multiple linear computational model employs a least squares solution to fit parameters (i.e. levels of cell specific growth responses) to the observed fed batch performance (preferably defined as IVCD50).

One aspect of the invention involves comparing a query cell specific growth response fingerprint with one or more reference cell specific growth response fingerprints to identify a match or level of correlation between the query and reference growth response fingerprint(s). Various methods may be employed to match a query fingerprint with one of the reference fingerprints including mathematical modelling, pattern recognition, or by visual inspection. For example, the growth responses for an unidentified cell may be plotted against cell stressors to provide a graph representing the growth response fingerprint for the cell, and the growth response fingerprint may be compared visually with the reference growth response fingerprints provided in the same format to identify a matching reference growth response fingerprint and thereby identify the cell. In a preferred embodiment, the comparison step may be performed by mathematical modelling using ‘Linear discriminant analysis’ and ‘nearest neighbour Euclidian distance minimisation’, using a subset of the chemical growth responses. Typically, the model employs a ‘within groups’ leave one out method. With this, ‘training data’ is optionally made by leaving one out for each group (i.e. 10 samples) and then building an LDA model, and then predicting the cell type for each of the 10 distinct cell types which were left out. Other methods of matching or correlating a query fingerprint with one or more reference fingerprint(s) involves simple Euclidian matching or hierarchical cluster analysis.

The invention also provides a system or kit for identifying a cell. The system or kit typically comprises a device having a plurality of reaction chambers. Preferably, the device is a microtitre plate, typically a microtitre plate having at least 12, 24, 48, or 96 wells.

The system or kit typically comprises a plurality of cell stressor molecules. Preferably, the cell stressor molecules are disposed individually within the wells of the microtitre plate, and are preferably adhered to the wells of the microtitre plate.

The system or kit comprises a determination system for determining the growth response of the cell in the presence of each of the plurality of chemical cell stressors. Typically, the determination system comprises a microtitre plate reader configured to detect growth of cells in the wells of the microtitre plate. Suitable microtitre plate readers are commercially available, and are sold by the company BMG. The determination system typically has computer executable instructions to provide e.g., growth response data in computer readable form.

The system or kit also comprises a storage system and a comparison system. These functional modules can be executed on one, or multiple, computers, or by using one, or multiple, computer networks. The determination system has computer executable instructions to provide e.g., sequence information in computer readable form.

The information determined in the determination system can be read by the storage system. As used herein the "storage system" is intended to include any suitable computing or processing apparatus or other device configured or adapted for storing data or information. Examples of an electronic apparatus suitable for use with the present invention include a stand-alone computing apparatus, data telecommunications networks, including local area networks (LAN), wide area networks (WAN), Internet, Intranet, and Extranet, and local and distributed computer processing systems. Storage devices also include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage media, magnetic tape, optical storage media such as CD-ROM, DVD, electronic storage media such as RAM, ROM, EPROM, EEPROM and the like, general hard disks and hybrids of these categories such as magnetic/optical storage media. The storage system is adapted or configured for having recorded thereon growth response information and growth response fingerprint information. Such information may be provided in digital form that can be transmitted and read electronically, e.g., via the Internet, on diskette, via USB (universal serial bus) or via any other suitable mode of communication.

The storage system may have reference growth response fingerprint information stored thereon. As used herein, "stored" refers to a process for encoding information on the storage

device. In one embodiment the reference data stored in the storage device to be read by the comparison module is compared, e.g., comparison of a query cell-specific growth response fingerprint with a set or panel of reference growth response fingerprints.

The "comparison system" can use a variety of available software programs and formats for the comparison operative to compare query growth response fingerprint with reference growth response fingerprints and identify a "match". In one embodiment, the comparison module is configured to use pattern recognition techniques to compare information from one or more entries to one or more reference data patterns. The comparison module may be configured using existing commercially-available or freely-available software for comparing patterns, and may be optimized for particular data comparisons that are conducted. The comparison module provides computer readable information related to the genotype of the sample. Preferably, the comparison system employs a computational model for comparison purposes.

The comparison module, or any other module of the invention, may include an operating system (e.g., UNIX) on which runs a relational database management system, a World Wide Web application, and a World Wide Web server. World Wide Web application includes the executable code necessary for generation of database language statements (e.g., Structured Query Language (SQL) statements). Generally, the executables will include embedded SQL statements. In addition, the World Wide Web application may include a configuration file which contains pointers and addresses to the various software entities that comprise the server as well as the various external and internal databases which must be accessed to service user requests. The Configuration file also directs requests for server resources to the appropriate hardware--as may be necessary should the server be distributed over two or more separate computers. In one embodiment, the World Wide Web server supports a TCP/IP protocol. Local networks such as this are sometimes referred to as "Intranets." An advantage of such Intranets is that they allow easy communication with public domain databases residing on the World Wide Web (e.g., the GenBank or Swiss Pro World Wide Web site). Thus, in a particular preferred embodiment of the present invention, users can directly access data (via Hypertext links for example) residing on Internet databases using a HTML interface provided by Web browsers and Web servers.

The comparison module typically provides a computer readable comparison result that can be processed in computer readable form by predefined criteria, or criteria defined by a user, to

provide a content based in part on the comparison result that may be stored and output as requested by a user using a display system.

In one embodiment of the invention, the content based on the comparison result is displayed on a computer monitor. In one embodiment of the invention, the content based on the comparison result is displayed through printable media. The display module can be any suitable device configured to receive from a computer and display computer readable information to a user. Non-limiting examples include, for example, general-purpose computers such as those based on Intel PENTIUM-type processor, Motorola PowerPC, Sun UltraSPARC, Hewlett-Packard PA-RISC processors, any of a variety of processors available from Advanced Micro Devices (AMD) of Sunnyvale, California, or any other type of processor, visual display devices such as flat panel displays, cathode ray tubes and the like, as well as computer printers of various types.

In one embodiment, a World Wide Web browser is used for providing a user interface for display of the content based on the comparison result. It should be understood that other modules of the invention can be adapted to have a web browser interface. Through the Web browser, a user may construct requests for retrieving data from the comparison module. Thus, the user will typically point and click to user interface elements such as buttons, pull down menus, scroll bars and the like conventionally employed in graphical user interfaces.

The invention also provides a method of identifying a cell comprising the steps of preparing a growth response fingerprint for the cell, in which the growth response fingerprint comprises the growth response of the cell to a plurality of chemical cell stressor molecules, comparing the growth response fingerprint for the cell with a plurality of growth response fingerprints corresponding to cells of known identity, and identifying the cell when the growth response fingerprint for the cell matches one of the growth response fingerprints corresponding to the known cells. Typically, the comparison step employs mathematical modelling. .

In a broader aspect, the invention provides a method of identifying a characteristic of a cell comprising the steps of:

- incubating the cell with a plurality of chemical cell stressors;
- determining the growth response of the cell in the presence of each of the plurality of chemical cell stressors to generate a query cell-specific growth response fingerprint;

- comparing the query cell-specific growth response fingerprint with one or more reference cell-specific growth response fingerprints corresponding to one or more cells having known characteristics; and
- identifying a characteristic of the cell based on the level of correlation between the query cell-specific growth response fingerprint and the one or more reference cell-specific growth response fingerprints.

Typically, the step of identifying a characteristic of the query cell comprises a step of matching the query cell-specific growth response fingerprint with a reference cell-specific growth response fingerprint.

The characteristic may be any characteristic of the cell, for example:

- the identity of the cell (where a query fingerprint is compared with reference fingerprints from cells of known identity),
- the growth characteristics of the cell (where a query fingerprint is compared with reference fingerprints from cells of known growth characteristics),
- the predicted fed batch performance of the cell (where a query fingerprint is compared with reference fingerprints from cells of known fed batch performance),
- the ability of a cell (i.e. a mammalian producer cell) to express recombinant protein (where a query fingerprint is compared with reference fingerprints from cells of known producing capacity),
- the ability of the cell to survive and/or grow in specific environments, (where a query fingerprint is compared with reference fingerprints from cells of that are known to survive and/or grow in specific environments),
- the stability (genetic and/or functional) of clonal cell lines (in which a query cell fingerprint is compared with fingerprints from cells that have demonstrated functional and/or genetic stability and/or functional and/or genetic instability over time, during storage, or throughout successive generations of growth). This aspect of the invention allows the identification of genetic or functional instability in clones, which is undesirable for cell lines used in manufacturing.

The level of correlation between the query cell-specific growth response fingerprint and the one or more reference growth response fingerprints may be determined by any means,

including visual comparison of data representing the fingerprints (for example, comparing graphs representing the fingerprints) or by means of mathematical modelling to identify, for example, a best fit or closest match. Typically, the mathematical modelling employs linear discriminant analysis (LDA), although other modelling approaches based on “matching things to groups” may be employed.

Brief Description of the Figures

Figure 1: A representation of the 4 ‘chemical fingerprints’ generated from each CHO cell line (A to J).

Figure 2: An example of a chemical growth fingerprint from a clone. Cells were grown for 72 hours in the presence or absence of a chemical of interest in a 96 well plate. Relative levels of cell growth were assayed using the “Presto-Blue” method as described above.

Figure 3: A dendrogram displaying the goodness of cell type clustering using a ‘best correlation’ method and “Ward’s Hierarchical Clustering”.

Figure 4: A two dimensional example of how a linear combination of chemical responses can be used to maximally separate two different groups.

Figure 5: A two dimensional example of how a linear combination of chemical responses can be used to classify a new data point (triangle point) into a group using the ‘maximum likelihood’ classification.

Figure 6: A two dimensional representation of how PCA can be used to reduce the dimensionality of a data set without losing substantial amounts of information.

Figure 7: Data could be expressed as 7 linear combinations of the chemical responses without while still being able to represent the vast majority of the variation in the data.

Figure 8: A representation of the ability of the LDA model to predict the CHO cell type of ‘unknown’ CHO fingerprints with 100% accuracy.

Figure 9 (2): An illustration of the fit of the multiple linear model to predict the relative performance ability (Y axis) from the model based on the clone chemical fingerprints.

Figure 10 (3): An example of how the multiple linear model can use clone chemical fingerprints to partition the set of clones into ‘good’ and ‘bad’ performers thus aiding the process of selecting which clones to take forward to the next step of clone screening.

Figure 11 (4): A demonstration, using bootstrap 3 fold cross validation techniques of how the multiple linear model is able to predict data not used to build the model (i.e. blindly predict the relative fed batch performance).

Figure 12: An illustration of a computer system configured to perform one or more of the methods of the invention.

Detailed Description of the Invention

Experimental

Cell culture (cell identification):

10 types of clonally and non-clonally derived CHO cell lines were sourced. These were all industrially important cell lines from numerous industrial/commercial sources. These consisted of both clonally derived stable-producing clonal cell lines running on two distinct expression systems, clonally derived non-producing CHO cells, episomal replicating cells, DHFR-ve cells and non-clonally derived cells from different commercial CHO cell parentage. These are referred to as CHO-A, CHO-B, CHO-C, CHO-D, CHO-E, CHO-F, CHO-G CHO-H, CHO-I and CHO-J. These were all grown in their proprietary recommended culture media and culture conditions.

Cell culture (predicting IVCD50 of panel of clonal cells):

PM-CHO Cells were culture CD-CHO media (Invitrogen, Paisley, UK), 8mM L-Glutamine (Invitrogen, Paisley, UK), 1% HT supplement (Invitrogen, Paisley, UK) 12.5 µg/ml puromycin

(Invitrogen, Paisley, UK). Alternative (panel 2) CHO cells were cultured in CD-FORTI CHO (Invitrogen, Paisley, UK). Cells were routinely sub-cultured on an alternating 3 / 4 day regime. Using data from fed batch studies (with commercially available feed), IVCD50 (Integral of viable cell density at the point where culture viability drops below 50%) were used as the metric to judge the cell's ability to grow in fed batch culture. These IVCD50s were used to rank the cells. A ranking system was selected as this would be more robust to outliers and skewed data.

Small scale determination of cell growth:

Assaying cell growth in 96 well plates: "Presto Blue" (Invitrogen, Paisley, UK) was mixed 1:1 with CD-CHO media and 20 ul of this was added to each well. The plate was machine shaken and subsequently incubated for 30 minutes at 37C in a static humidified incubator. After incubation the fluorescence of the wells was measured (excitation 535nm emission 620 nm) using a fluoroskan ascent (Thermo-Fisher, Loughborough, UK) plate reader. It has been demonstrated prior that fluorescence is linearly correlated with viable cell density in the wells.

Preparation of the chemical test plate:

Method: The plurality of cell stressor molecules employed were:

2-aminobicyclo-(2,2,1)heptane-carboxylic acid (BCH)
D-phenylalanine -(D-Phe)
 α -(methylamino)isobutyric acid (MeAIB)
Sodium Butyrate (NaBu)
Cycloheximide
Ammonium chloride
Cadmium acetate hexhydrate
Cobalt chloride (CoCl₂)
Sodium Chloride (NaCl)
Sodium lactate (Na Lac)
Aminotriazole (AMT)
Menadione Sodium Bisulphite (MSB)
Buthionine Sulfoximine (BSO)
Mercaptosuccinic Acid (MS)
2,4,Dinitrophenol (24DNP)
Sodium Oxamate
2-deoxyglucose (2dg)
3-bromopyruvate (3-BrPA)
Dichloroacetate (DCA)
6-diazo-5-oxo-l-norleucine (l-don)
Valproic acid (Val)

Sodium Orthovanadate (NaV)
Citric acid
FK866
Lactic acid

Using an ‘in house’ parental cell line, inhibitory dose 50 (IC50) concentrations of the above chemicals were identified through growth studies. Here IC50 is defined as the concentration of the chemical, in question, which inhibits normal cell growth by 50% over a 3 day period. Once IC50s were established, 96 well plates were set up containing the above selection of chemicals at the IC50 concentration and including control wells which contained only cell growth media.

EXAMPLE 1: Identification of cell using stress response fingerprint

Duplicate vials for each cell line (CHO-A to CHO-J) were revived from cryostasis. These were cultured in their recommended growth media until the growth profile for each vial had become stable (around 4 successive subcultures post thawing). Duplicate vials were cultured separately throughout. Duplicate vials enabled an estimation of ‘vial to vial’ variation. Each cell line from a duplicate vial was used to seed a chemical plate on day 3 post subculture (Figure 1). Essentially each duplicate vial cell line, was grown in the plate containing the above mentioned chemicals for 3 days. Cells from each duplicate vial were used to seed duplicate plates, e.g. 4 plates per cell line.

After this period the level of cell growth in the plates (i.e. in the presence and absence of chemicals as mentioned above) was measured using the “Presto Blue” assay as described above. This allowed a cell specific growth fingerprint to be identified for each CHO cell line. Here a growth finger print is defined as the level of cell growth in each chemical microenvironment relative to the growth of the cells in the control well (i.e. the wells containing just media.). An example of this is illustrated in Figure 2.

Essentially, two different modelling approaches were employed to use aspects of the cellular chemical fingerprint to identify the “cell type”. These two methods, despite their substantially differing approaches, were both able to reliably identify cell type from fingerprint alone. These two approaches were “Euclidian Distance, Wards method Clustering” and “Linear discriminant analysis”

Details of “Euclidian Distance, Wards method, Clustering” modelling.

This approach seeks to find the best correlation of aspects of a fingerprint (chemical responses) to identify the cell type. Given a query fingerprint, it seeks to find the group of cell types which gives the best correlation and subsequently, allocate the query cell to this group.

The goodness of clustering was seen as thus: A query cell type is only classified as being in a specific group (cell type) if the lowest correlation to any other cell type within that group is higher than the highest correlation to any cell in a different group (other cell types). This gave stringent criteria for evaluation of “which subsets” of the 25 chemical responses gave the best separation of groups of cell types by “minimising Euclidian distance” i.e. correlation. This means maximising the within groups correlations compared to the outside groups correlations

All subsets of the chemicals were examined and it was discovered that numerous combinations of the 25 chemical growth responses could be used to successfully cluster all cell types together. The smallest set of these in this instance was 7 chemical responses, namely, d-phenylalanine, MeiAB, Cycloheximide, AmCl, Cadmium, lactate and 2-deoxyglucose.

A cluster dendogram illustrating the goodness of clustering is included in Figure 3.

Details of “Linear discriminant” modelling approach.

For each cell type, e.g. CHO A to J, duplicate fingerprints were obtained for each duplicate ‘vial origin’ of cell type (Fig 1). The 4 fingerprints generated from each CHO cell type was used to represent that CHO cell type as a ‘group’.

Using these fingerprints, linear discriminant analysis was used to generate a linear combination of distinct aspects of their chemical finger-print that best maximizes the ‘between’ CHO cell type variation to the ‘within’ CHO cell type variation (i.e. that between the 4 fingerprints generated for each CHO cell type). A two dimensional hypothetical example of this is illustrated in figure 4. Here, within CHO cell types variation can be seen as the variation of the fingerprints from the same CHO cell type i.e. variation due from the duplicate vials (vial to vial variation) and the duplicate plates (plate to plate variation).

Using information from this linear combinations of chemical responses, this allows the identification of new data points into appropriate groups. For example given the ‘chemical fingerprint’ of an unknown ‘query’ CHO cell type, the LDA model can be used to predict which CHO cell type the query is most likely to be and also to put a probability on which CHO cell type group (i.e. CHO A to J) the query belongs to. This is illustrated in figure 5.

Data dimension reduction to give more predictive LDA analysis

Due to the large size of the data set, there were many ‘multi-colinear’ variables (variable whose values can be strongly predicted from linear combinations of other variables). This can lead to poor cross validation, i.e. the ability of the LDA model to predict classify new data points using data which was not used to make to LDA model.

To address this, the size (dimensionality) of the data set was reduced using “principle component analysis”. This allows data to be mapped onto a lower dimensional space without losing substantial amounts of information. An illustration of this technique can be seen in Figure 6. This technique was used to reduce the dimensions of the data space from 25 dimensions to 7 dimensions. This is illustrated in Figure 7, which indicates that a vast amount of the variation in the data can be captured using 7 principle components of the data. Using these subsets of these dimensions, the best LDA analysis was performed. In this context, LDA is defined as the best linear combination of principle components which maximises the between groups variation relative to the within groups variation.

The best linear discriminant model found used 4 components of the principle component ‘breakdown’. This model was able to identify ‘which fingerprint belonged to which CHO cell type’ from the 40 different fingerprints generated with 100% accuracy.

Cross-Validation of the ability of the optimal LDA model to predict the CHO cell type using new, ‘unknown’ (i.e. unknown to the model) fingerprints.

Importantly, the optimal LDA model was ‘Cross-validated using a “leave one out” (LOO) cross-validation technique. Essentially for each of the 40 fingerprints, 1 would be ignored (seen as ‘test data’) and an LDA model would be built using the other 39 fingerprints (seen as ‘training data’). This model was then used to predict the CHO cell type of the ‘test data’ fingerprint.

This process is repeated using each of the 40 fingerprints as the ‘test’ data points, and the CHO cell type (class) of each ‘query’ was predicted using the LDA model built on the training data set.

Within groups “Leave one out” cross validation.

To provide a more stringent cross-validation of the data, a ‘within groups leave one out cross validation’ method was developed. Essentially, since there were 10 ‘groups’ (CHO cell types A-J), with 4 members in each groups, for a 1 member was ignored from each group (10 ignored in total). Subsequently, the remaining 30 data points (the “training data set”) were used to build a best LDA model. Using this, the groups of the ‘unknown’ (to the model) ignored data points (the “test data set”) were predicted. This was repeated until all data points had been used in the test and training data set.

In essence, using “new” data points (test data sets), the LDA model based on these components was able to correctly predict the cell type (CHO A-J) of a cell based on its fingerprint, with 100% accuracy (Figure 8).

The examples above demonstrate that using the plate based ‘metabolic fingerprint’ methods of the invention, it is possible to confidently identify a specific CHO cell type from a panel of closely related CHO cell types. The methods of the invention is also applicable for the identification of non-CHO types and even in the identification of non-mammalian cells.

EXAMPLE 2 – Prediction of fed batch performance (IVCD50) of a panel of clonal cells

A panel of size 20 of clonal cell lines with known fed batch performance metrics (i.e. IVCD50) was generated in house. Each clonal cell line was grown in the plate containing the above mentioned chemicals for 3 days. After this period the level of cell growth in the plates (i.e. in the presence and absence of chemicals as mentioned above) was measured using the “Presto Blue” assay as described above. This allowed a cell specific growth fingerprint to be identified for each clonal cell line. Here a growth finger print is defined as the level of cell growth in each microenvironment relative to the growth of the cells in the control well (i.e. the wells containing just media.)

An example of a typical growth fingerprint for a clone is shown in Fig. 2.

Using information from these chemical fingerprints, a multiple linear model was built using responses to individual chemicals as parameters.

A multiple linear model is defined as that satisfying the equation.

$$\hat{\beta} = (\mathbf{X}^T \mathbf{X})^{-1} \mathbf{X}^T \mathbf{y} = \left(\frac{1}{n} \sum \mathbf{x}_i \mathbf{x}_i^T \right)^{-1} \left(\frac{1}{n} \sum \mathbf{x}_i y_i \right).$$

Where X represents the matrix containing appropriate data from the explanatory variables and Y is the matrix of dependent (or response) variables. This is essentially finding a least squares solution to fit parameters (i.e. levels of cell specific growth responses) to the observed fed batch performance (here defined as IVCD50)

An example of a multiple linear model built from the plurality of clone microenvironments is

$$Y = -0.978(\text{Sodium Butyrate response}) + 0.763(\text{Cadmium response}) + 0.364(\text{Mercaptosuccinic acid response}) + 2$$

Where Y is the predicted standardized rank (which can easily be interpreted as rank). A graph illustrating the goodness of model fit using the above parameters is shown in Fig. 9. The R^2 value for this model is 0.7. This can be interpreted that 70% of the behaviour of the data can be explained by the model. Further use of this model can be illustrated as its ability to differentiate between the top and bottom 50% of clones in terms of their rank.

With the above mentioned modelling approach this can predict whether a clone is in the top 50% (good) or the bottom 50% (bad) with 85% accuracy as illustrated in Fig. 10.

By using bootstrap cross validation techniques it can be shown that a model built in the above way can be predictive of future data, i.e. data not used in the model. Fig. 11 illustrates the predictive ability of the modelling approach to build predictive models. The R^2 value for the bootstrap cross-validation is 0.66 which indicated the ability to predict new data.

This model, build on a set of clones with known fed batch performance can use the ‘fingerprint’ of new clones and be able to predict their relative fed batch performance. Initially a model is generated using a panel of clones with known fed batch performance (in the above example 20 were used but there is no limitation to how many can be used, and as few as three may be

employed – it is preferable to use at least 20 clones of known performance to build the model). Subsequently when a large panel of clones is derived in the process of cell line development, these will be “screened” in the multiple microenvironment plates, and using the model generated from clones with known performance characteristics, it is possible to rank these clones or at least give information as to the likelihood of their future fed batch performance capabilities therefore aiding the process of selecting which clones are taken forward to the next stage of clones screening (i.e. small scale fed batch studies).

Additionally, referring now also to Figure 12, at least a portion of the systems, methodologies and techniques described with respect to the exemplary embodiments of present disclosure can incorporate a machine, such as, but not limited to, computer system 600, or any other computing device within which a set of instructions, when executed, may cause the machine to perform any one or more of the methodologies or functions discussed above. The machine may be configured to facilitate various operations conducted by the systems disclosed herein. For example, the machine may be configured to, but is not limited to, assist the systems by providing processing power to assist with processing for the computer implemented system configured to predict a relative fed batch performance of a panel of clonal cells derived from a single parental host cell population in a well, by providing storage capacity for storing instructions or data traversing the systems, or by assisting with any other operations conducted by or within the systems.

In some embodiments, the machine operates as a standalone device. In some embodiments, the machine may be connected (e.g., communications network 635) to and assist with operations performed by other machines. The machine may be connected with any component in the system, for example a microplate reader configured to generate a growth response profile for a cell. In a networked deployment, the machine may operate in the capacity of a server or a client user machine in server-client user network environment, or as a peer machine in a peer-to-peer (or distributed) network environment. The machine may comprise a server computer, a client user computer, a personal computer (PC), a tablet PC, a laptop computer, a desktop computer, a control system, a network router, switch or bridge, or any machine capable of executing a set of instructions (sequential or otherwise) that specify actions to be taken by that machine. Further, while a single machine is illustrated, the term “machine” shall also be taken

to include any collection of machines that individually or jointly execute a set (or multiple sets) of instructions to perform any one or more of the methodologies discussed herein.

The computer system 600 may include a processor 602 (e.g., a central processing unit (CPU), a graphics processing unit (GPU, or both), a main memory 604 and a static memory 604, which communicate with each other via a bus 608. The computer system 600 may further include a video display unit 610 (e.g., a liquid crystal display (LCD), a flat panel, a solid state display, or a cathode ray tube (CRT)). The computer system 600 may include an input device 612 (e.g., a keyboard), a cursor control device 614 (e.g., a mouse), a disk drive unit 616, a signal generation device 618 (e.g., a speaker or remote control) and a network interface device 620.

The disk drive unit 616 may include a machine-readable medium 622 on which is stored one or more sets of instructions 624 (e.g., software) embodying any one or more of the methodologies or functions described herein, including those methods illustrated above. The instructions 624 may also reside, completely or at least partially, within the main memory 604, the static memory 606, or within the processor 602, or a combination thereof, during execution thereof by the computer system 600. The main memory 604 and the processor 602 also may constitute machine-readable media.

Dedicated hardware implementations including, but not limited to, application specific integrated circuits, programmable logic arrays and other hardware devices can likewise be constructed to implement the methods described herein. Applications that may include the apparatus and systems of various embodiments broadly include a variety of electronic and computer systems. Some embodiments implement functions in two or more specific interconnected hardware modules or devices with related control and data signals communicated between and through the modules, or as portions of an application-specific integrated circuit. Thus, the example system is applicable to software, firmware, and hardware implementations.

In accordance with various embodiments of the present disclosure, the methods described herein are intended for operation as software programs running on a computer processor. Furthermore, software implementations can include, but not limited to, distributed processing or component/object distributed processing, parallel processing, or virtual machine processing can also be constructed to implement the methods described herein.

The present disclosure contemplates a machine readable medium 622 containing instructions 624 so that a device connected to the communications network 635, other network, or both, can send or receive voice, video or data, and to communicate over the communications network 635, other network, or both, using the instructions. The instructions 624 may further be transmitted or received over the communications network 635, other network, or both, via the network interface device 620.

While the machine-readable medium 422 is shown in an example embodiment to be a single medium, the term "machine-readable medium" should be taken to include a single medium or multiple media (e.g., a centralized or distributed database, and/or associated caches and servers) that store the one or more sets of instructions. The term "machine-readable medium" shall also be taken to include any medium that is capable of storing, encoding or carrying a set of instructions for execution by the machine and that cause the machine to perform any one or more of the methodologies of the present disclosure.

The terms "machine-readable medium" or "machine-readable device" shall accordingly be taken to include, but not be limited to: memory devices, solid-state memories such as a memory card or other package that houses one or more read-only (non-volatile) memories, random access memories, or other re-writable (volatile) memories; magneto-optical or optical medium such as a disk or tape; or other self-contained information archive or set of archives is considered a distribution medium equivalent to a tangible storage medium. The "machine-readable medium" or "machine-readable device" may be non-transitory in nature. Accordingly, the disclosure is considered to include any one or more of a machine-readable medium or a distribution medium, as listed herein and including art-recognized equivalents and successor media, in which the software implementations herein are stored.

In the specification the terms "comprise, comprises, comprised and comprising" or any variation thereof and the terms include, includes, included and including" or any variation thereof are considered to be totally interchangeable and they should all be afforded the widest possible interpretation and vice versa.

The invention is not limited to the embodiment hereinbefore described which may be varied in construction and detail without departing from the spirit of the invention.

Claims

1. A method of determining or predicting a characteristic of a query cell comprising the steps of:
 - incubating the query cell with at least three chemical cell stressor molecules;
 - determining the growth response of the query cell in the presence of each of the at least three chemical cell stressor molecules to generate a query cell-specific growth response fingerprint;
 - comparing the query cell-specific growth response fingerprint with one or more reference cell-specific growth response fingerprints corresponding to one or more cells having known characteristics; and
 - determining or predicting a characteristic of the cell based on the level of correlation between the query cell-specific growth response fingerprint and the one or more reference cell-specific growth response fingerprints.
2. A method according to Claim 1 which is a method of determining the identity of a cell.
3. A method according to Claim 1 which is a method of predicting fed batch performance of a cell.
4. A method as claimed in any preceding Claim in which the at least three chemical cell stressor molecules are each selected from different classes of chemical cell stressor molecules selected from amino acid transport inhibitors, cell cycle inhibitors, a source of carbon, a source of osmotic stress, a source of oxidative stress, an inducer of apoptosis, metabolic effectors, a pH modifier, an inhibitor of glycolysis, and a toxin.
5. A method as claimed in any preceding Claim in which the query cell is incubated with at least four chemical cell stressor molecules selected from different classes of chemical cell stressor molecules selected from amino acid transport inhibitors, cell cycle inhibitors, a source of carbon, a source of osmotic stress, a source of oxidative stress, an inducer of apoptosis, metabolic effectors, a pH modifier, an inhibitor of glycolysis, and a toxin.
6. A method as claimed in any preceding Claim in which the query cell is incubated with at least five selected from different classes of chemical cell stressor molecules selected from amino acid transport inhibitors, cell cycle inhibitors, a source of carbon, a source of osmotic

stress, a source of oxidative stress, an inducer of apoptosis, metabolic effectors, a pH modifier, an inhibitor of glycolysis, and a toxin.

7. A method as claimed in any preceding Claim in which the cell is a mammalian producer cell.
8. A method according to Claim 1 which is a method of identifying a query cell, the method comprising the steps of:
 - incubating the query cell with at least three individual chemical cell stressor molecules;
 - determining the growth response of the query cell in the presence of each of the three individual chemical cell stressor molecules to generate a query cell-specific growth response fingerprint;
 - comparing the query cell-specific growth response fingerprint with one or more reference cell-specific growth response fingerprints corresponding to one or more known cells to identify the cell; and
 - when the query cell-specific growth response fingerprint matches or correlates with a reference cell-specific growth response fingerprint, identifying the query cell.
9. A method as claimed in Claim 8 in which the known cells are a panel of clonal cells derived from a single parental cell population or derived from different parental cell populations.
10. A method as claimed in Claim 9 in which the query cell is a clonally derived cell, and in which the query cell-specific growth response fingerprint is compared with a plurality of reference cell-specific growth response fingerprints corresponding to a plurality of clonal cells derived from the same or different parental cell populations.,.
11. A method as claimed in any preceding Claim in which the step of comparing the query cell-specific growth response fingerprint with reference cell-specific growth response fingerprints comprises mathematical modelling, visual comparison, or pattern recognition.
12. A method as claimed in any preceding Claim, in which the cell is incubated with at least ten different chemical cell stressor molecules.
13. A method as claimed in any preceding Claim, for identifying a query clonal cell obtained from a panel of clonal cells, the method comprising the steps of:

- incubating the query clonal cell with a plurality of chemical cell stressors;
- determining the growth response of the query clonal cell in the presence of each of the plurality of chemical cell stressors to generate a query clonal cell-specific growth response fingerprint;
- comparing the query clonal cell-specific growth response fingerprint with a plurality of reference cell-specific growth response fingerprints corresponding to the panel of clonal cells; and
- when the query clonal cell-specific growth response fingerprint matches a reference cell-specific growth response fingerprint, identifying the query clonal cell.

14. A system suitable for identifying a cell according to a method of any of Claims 8 to 13, the system comprising:
- a device comprising a plurality of reaction chambers;
 - at least three individual chemical cell stressor molecules disposed individually within the reaction chambers;
 - a determination system for determining the growth response of the cell in the presence of each of the three individual chemical cell stressor molecules;
 - a storage system for storing a cell-specific growth response fingerprint corresponding to the at least three growth responses of the cell;
 - a comparison system configured to compare the cell specific growth response fingerprint with one or more reference cell-specific growth response fingerprints; and
 - a display system for displaying an output of the comparison step.
15. A system as claimed in Claim 14 in which the device is a microtitre plate.
16. A system as claimed in Claim 14 or 15 in which the determination system comprises a microtitre plate reader configured to detect growth of cells in the wells of the microtitre plate.
17. A system as claimed in any of Claims 14 to 16 in which the comparison system comprises a computational model configured to input a growth response fingerprint from a query cell, compare the growth response fingerprint with one or more reference growth response fingerprints, and output a content based in part of the comparison result.

18. A system as claimed in any of Claims 14 to 17 in which the one or more reference cell specific growth response fingerprints are stored locally on the storage system.
19. A system as claimed in any of Claims 14 to 17 in which the one or more reference cell specific growth response fingerprints are stored on a remote server and accessible through the internet.
20. A method of generating a reference growth response fingerprint corresponding to a specific cell, comprising the steps of:
- incubating the cell with at least three individual chemical cell stressor molecules; and
 - determining the growth response of the cell in the presence of each of the at least three chemical cell stressor molecules to generate a reference growth response fingerprint.
21. A method of generating a panel of reference growth response fingerprints corresponding to a panel of distinct cells, comprising the steps of:
- generating a reference growth response fingerprint for each cell in the panel of cells; and
 - storing the panel of reference growth response fingerprints,
- wherein each reference growth response fingerprint is generated by the steps of:
- incubating one of the cells with at least three individual chemical cell stressor molecules; and
 - determining the growth response of the cell in the presence of each of the three chemical cell stressor molecules to generate a reference growth response fingerprint.
22. A method as claimed in Claim 20 or 21 in which the at least three individual chemical cell stressor molecules are each selected from different classes of chemical cell stressor molecules selected from amino acid transport inhibitors, cell cycle inhibitors, a source of carbon, a source of osmotic stress, a source of oxidative stress, an inducer of apoptosis, metabolic effectors, a pH modifier, an inhibitor of glycolysis, and a toxin.
23. A method as claimed in any of Claims 20 to 22 in which the cell is incubated with at least four individual chemical cell stressor molecules.
24. A method as claimed in any of Claims 20 to 22 in which the cell is incubated with at least five individual chemical cell stressor molecules.

25. A microtitre plate suitable for generating a growth response fingerprint for a cell and comprising at least 24 wells, and at least three individual chemical cell stressor molecules disposed individually in at least some of the wells, wherein the at least three individual chemical cell stressor molecules are each selected from different classes of chemical cell stressor molecules selected from amino acid transport inhibitors, cell cycle inhibitors, a source of carbon, a source of osmotic stress, a source of oxidative stress, an inducer of apoptosis, metabolic effectors, a pH modifier, an inhibitor of glycolysis, and a toxin.
26. A microtitre plate as claimed in Claim 25 including at least four individual chemical cell stressor molecules disposed individually in at least some of the wells, wherein the at least four individual chemical cell stressor molecules are each selected from different classes of chemical cell stressor molecules selected from amino acid transport inhibitors, cell cycle inhibitors, a source of carbon, a source of osmotic stress, a source of oxidative stress, an inducer of apoptosis, metabolic effectors, a pH modifier, an inhibitor of glycolysis, and a toxin.
27. A microtitre plate as claimed in Claim 25 including at least five individual chemical cell stressor molecules disposed individually in at least some of the wells, wherein the at least five individual chemical cell stressor molecules are each selected from different classes of chemical cell stressor molecules selected from amino acid transport inhibitors, cell cycle inhibitors, a source of carbon, a source of osmotic stress, a source of oxidative stress, an inducer of apoptosis, metabolic effectors, a pH modifier, an inhibitor of glycolysis, and a toxin.
28. A microtitre plate as claimed in any of Claims 25 to 27 and including at least ten individual chemical cell stressor molecules disposed individually in at least some of the wells of the plate.
29. A microtitre plate as claimed in any of Claims 25 to 28 in which the individual chemical cell stressor molecules are adhered to the wells of the microtitre plate.
30. A computer implemented method of predicting relative fed batch performance of a panel of clonal cells derived from a single parental host cell population, the method comprising the steps of:

- assaying a level of growth of each clone in the presence and absence of at least three chemical cell stressor molecules;
- comparing the level of growth of each clone in the presence and absence of each of the three chemical cell stressor molecules to provide a normalised growth response value for each clone in each of the three stressed microenvironments;
- inputting a clone-specific growth response fingerprint comprising the normalised growth response value for each clone in each of the stressed microenvironments into a computational model, in which the computational model is generated from growth response fingerprints obtained from a calibration set of clones with known fed batch performance, wherein the computational model is configured to output the predicted fed batch performance value for each clone to predict the relative fed batch performance of the panel of clonal cells.

31. A computer implemented method according to Claim 30 in which the computational model is multiple linear computational model.
32. A computer implemented method according to Claim 30 or 31 in which the growth of each clonal cell is assayed in the well of a multiwell plate.
33. A computer implemented method according to any of Claims 30 to 32 in which the growth of each clone is assayed during a period of less than 5 days.
34. A computer implemented method according to any of Claims 30 to 33, in which the level of growth of each clone is assayed in the presence and absence of at least six chemical cell stressor molecules.
35. A computer implemented method according to any of Claims 30 to 34 in which the at least three chemical cell stressor molecules include at least one chemical cell stressor from each of the groups consisting of amino acid transport inhibitors, cell cycle inhibitors, a source of carbon, a source of osmotic stress, a source of oxidative stress, an inducer of apoptosis, metabolic effectors, a pH modifier, an inhibitor of glycolysis, and a toxin.
36. A computer implemented method according to any of Claims 30 to 35, in which the plurality of chemical cell stressors are selected from the group of 2-aminobicyclo-(2,2,1)heptane-carboxylic acid (BCH), D-phenylalanine, α -(methylamino)isobutyric acid (MeAIB), Sodium

Butyrate (NaBu), cyclohexamide, ammonium chloride, Cadmium acetate hexhydrate, Cobalt chloride (CoCl₂), Sodium Chloride (NaCl), Sodium lactate (Na Lac), Aminotriazole (AMT), Menadione Sodium Bisulphite (MSB), Buthionine Sulfoximine (BSO), Mercaptosuccinic Acid (MS), 2,4-Dinitrophenol (24DNP), Sodium Oxamate, 2-deoxyglucose (2dg), 3-bromopyruvate (3-BrPA), Dichloroacetate (DCA), 6-diazo-5-oxo-L-norleucine (l-don), Valproic acid (Val), Sodium Orthovanadate (NaV), citric acid, FK866, lactic acid.

37. A computer implemented method according to any of Claims 30 to 36, in which the multiple linear computational model employs a least squares solution to fit growth response values to predicted fed batch performance.
38. A computer implemented system configured to predict a relative fed batch performance of a panel of clonal cells derived from a single parental host cell population, the system comprising:
- a determination system to assay a level of growth of each clone in the presence and absence of a plurality of individual chemical cell stressors to provide a normalised growth response value for each clone in a plurality of stressed microenvironments ; and
 - a processor to process a clone-specific growth response fingerprint comprising the normalised growth response value for each clone in each of the stressed microenvironments, in which a computational model is generated from growth response fingerprints obtained from a calibration set of clones with known fed batch performance, wherein the computational model is configured to output the predicted fed batch performance value for each clone to predict the relative fed batch performance of the panel of clonal cells, and
39. A computer implemented system as claimed in Claim 38, in which the determination system comprises a multiplate reader.
40. A computer implemented system as claimed in Claim 38 or 39 in which the computational model is a multiple linear computational model

41. A computer implemented system as claimed in Claim 41 in which the multiple linear computational model employs a least squares solution to fit parameters growth response values to predicted fed batch performance.

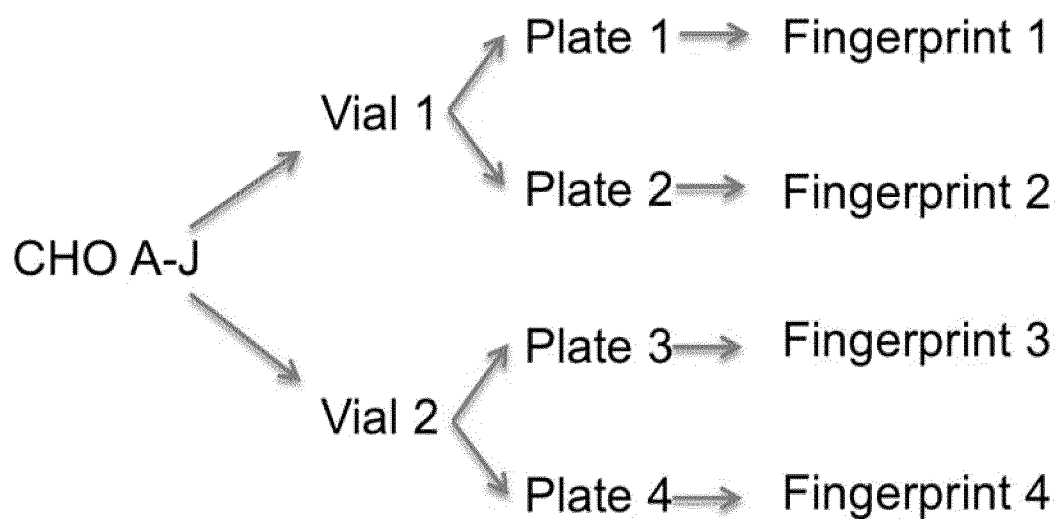
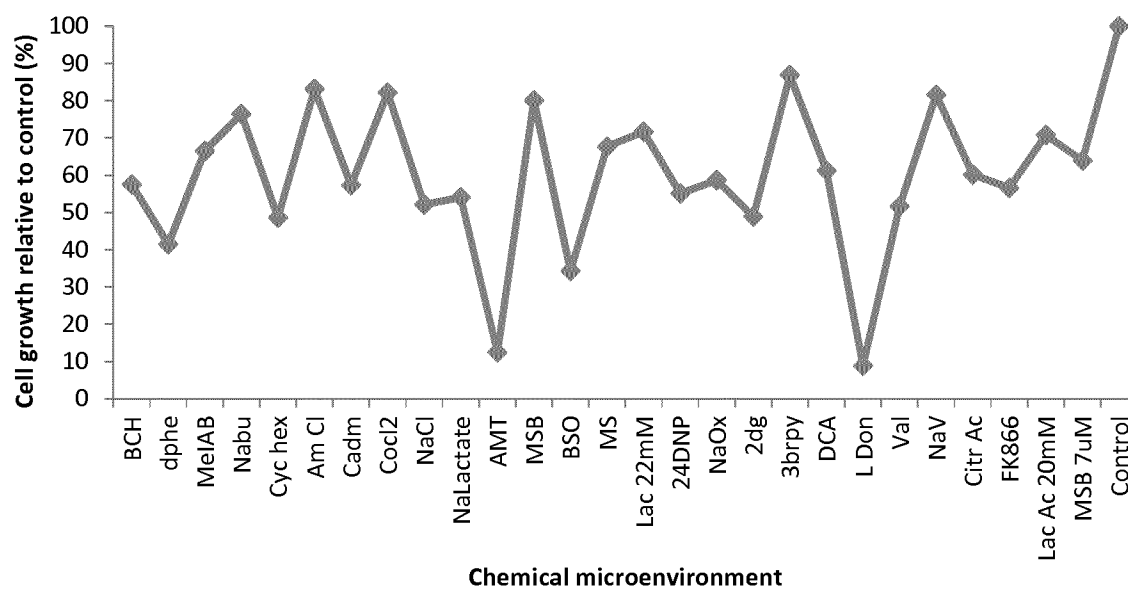
FIG. 1**FIG. 2**

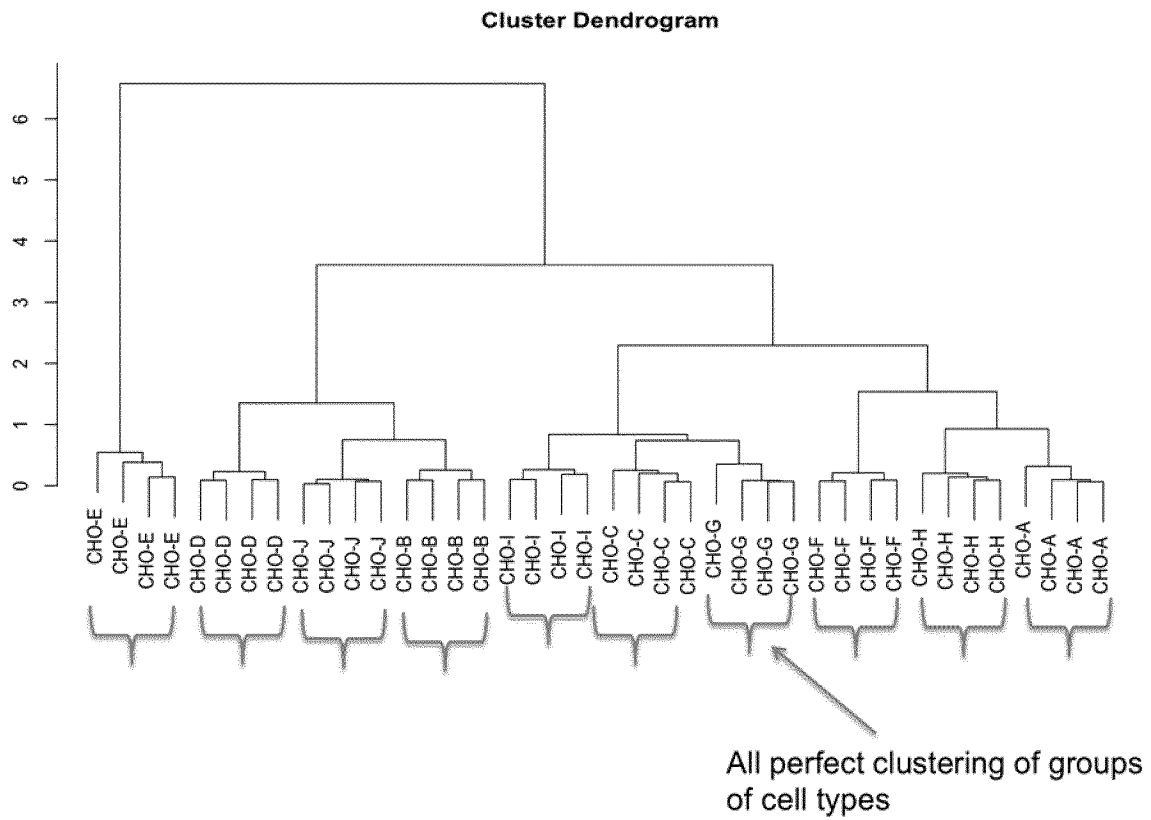
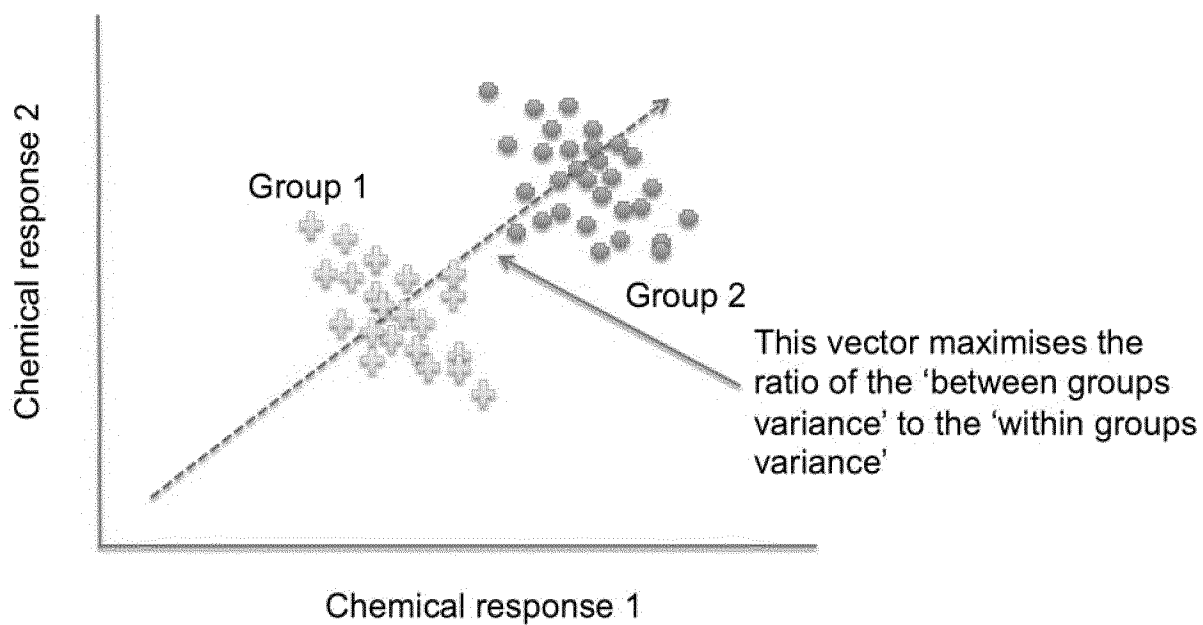
Fig. 3

FIG. 4**FIG. 5**

Distance along vector can be approximated by Gaussian distributions and thus the group probability of a new point can be calculated from the distance along the separation vector

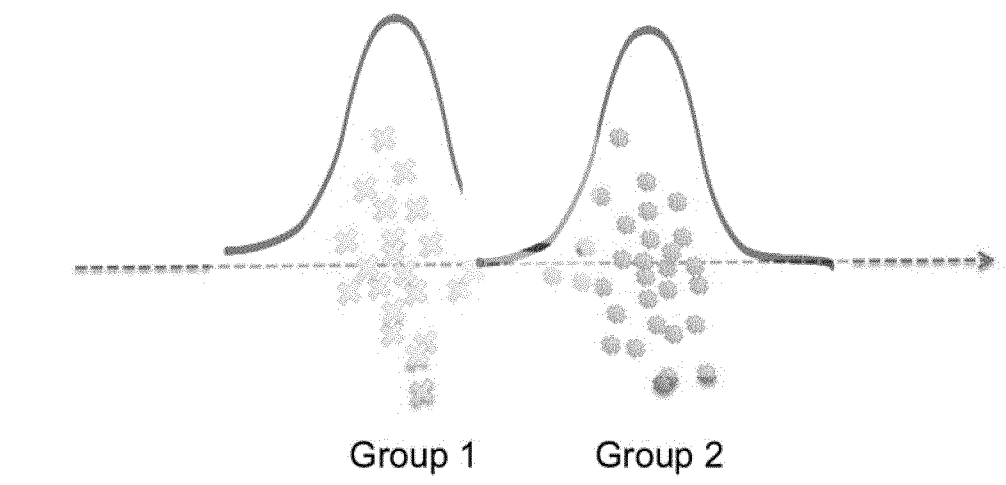


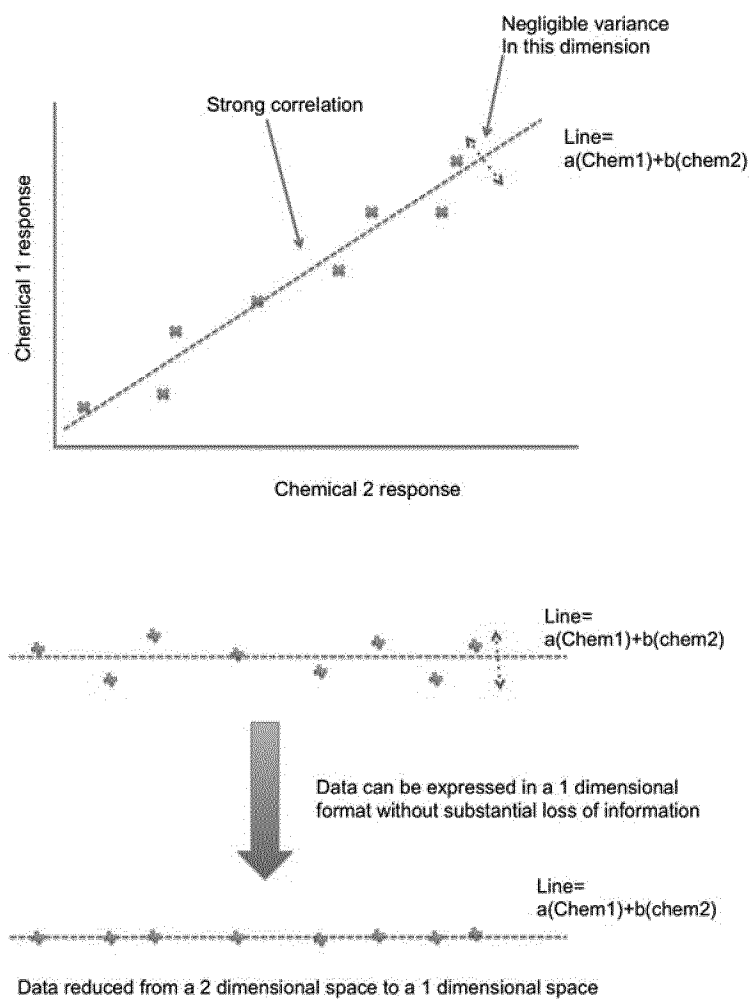
FIG. 6

FIG. 7

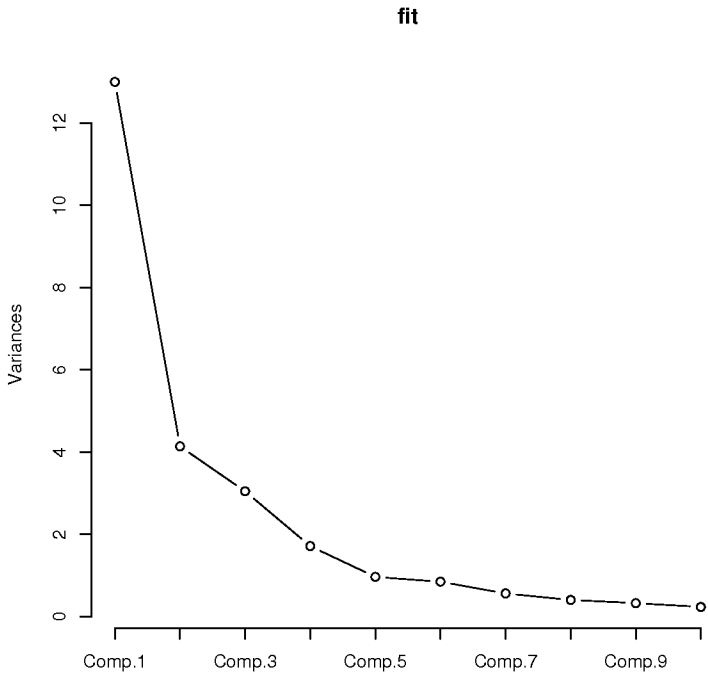


FIG. 8

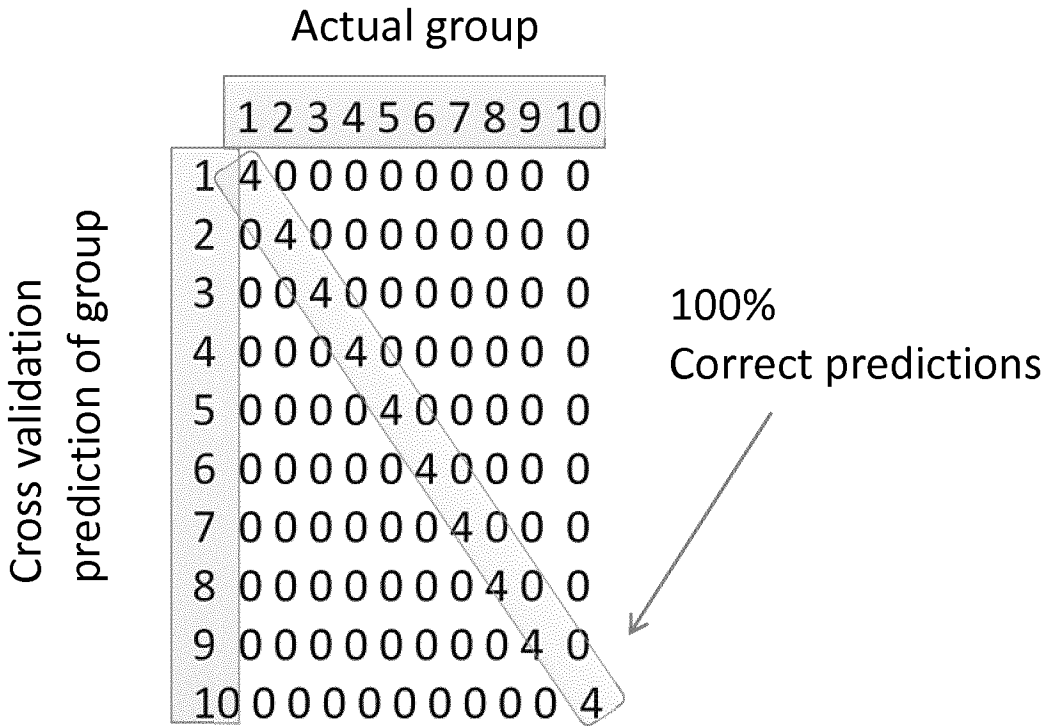


FIG. 9

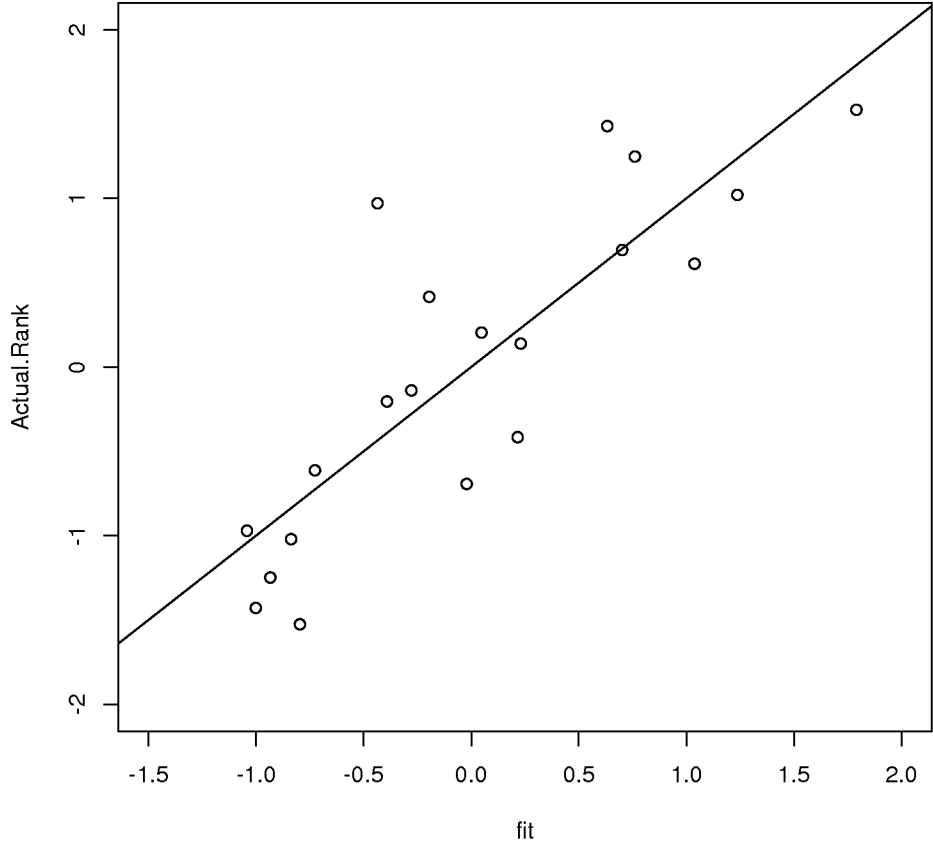


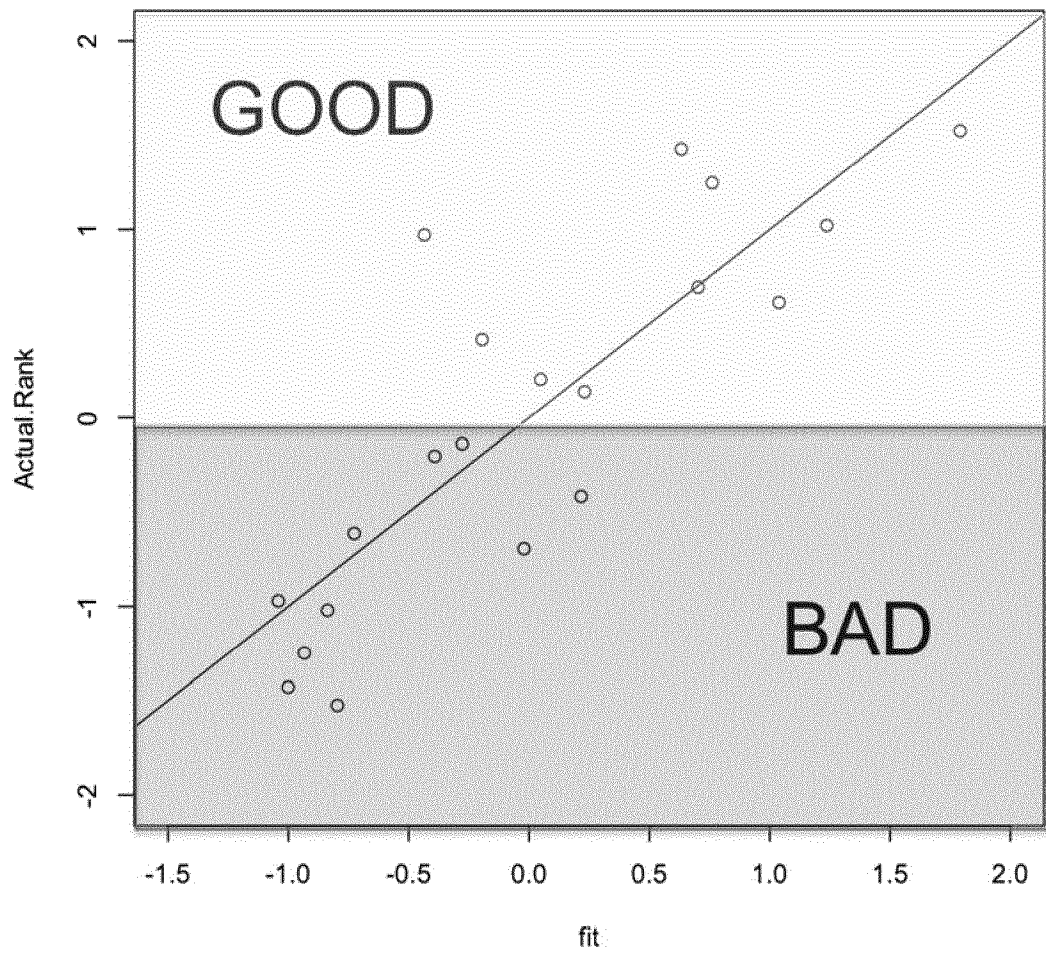
FIG. 10

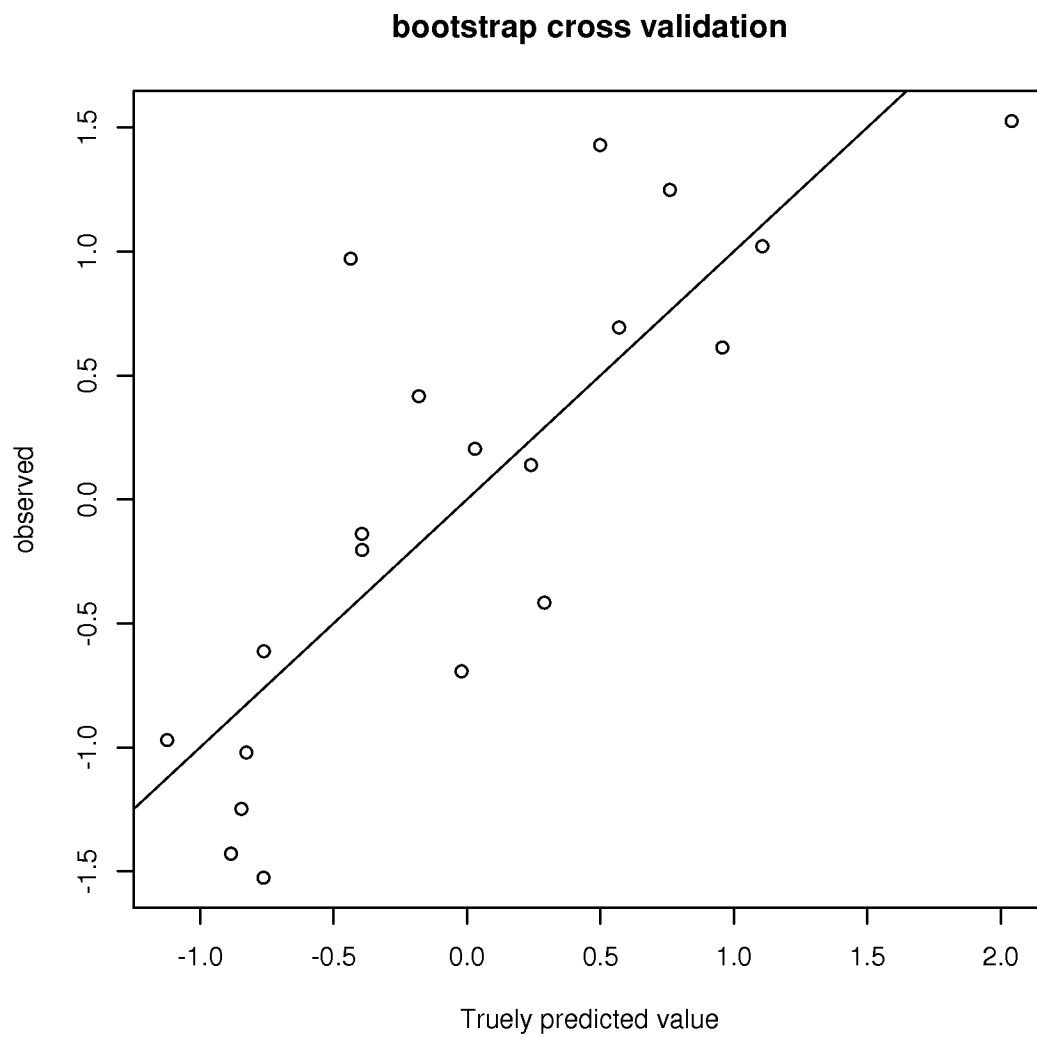
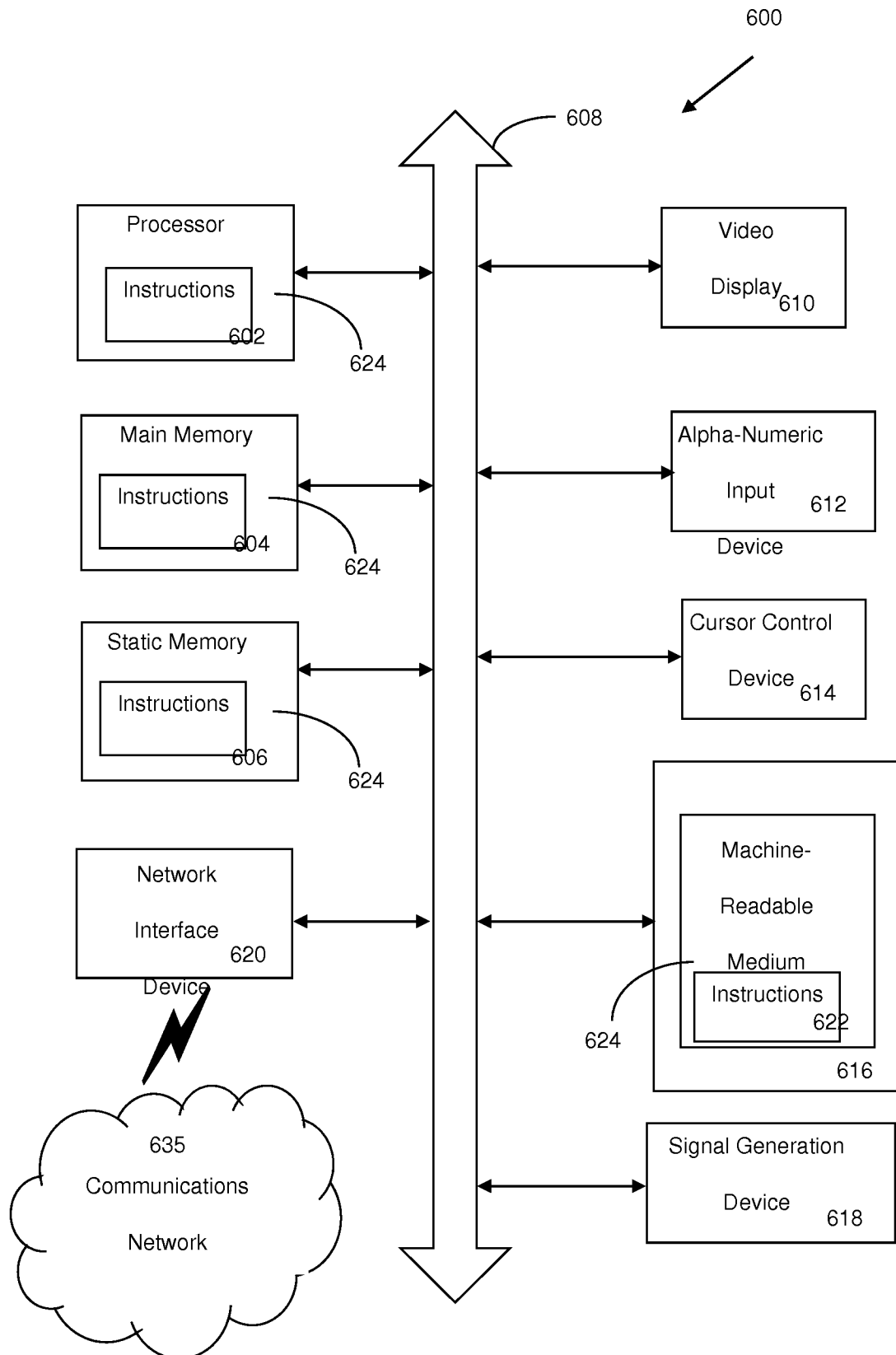
FIG. 11

FIG. 12

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/063625

A. CLASSIFICATION OF SUBJECT MATTER INV. G01N33/50 G05B17/02 G05B13/04 C12M1/36 C12M1/34 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) G05B C12M G01N		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, EMBASE, PAJ		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 8 October 2014		Date of mailing of the international search report 24/10/2014
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Boiangiu, Clara

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