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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 December 2004 (16.12.2004)

PCT

(10) International Publication Number
WO 2004/108948 A2

(51) International Patent Classification[7]: C12Q

(21) International Application Number:
PCT/US2004/017613

(22) International Filing Date: 4 June 2004 (04.06.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/476,010 4 June 2003 (04.06.2003) US

(71) Applicant *(for all designated States except US)*: **PRESIDENT AND FELLOWS OF HARVARD COLLEGE** [US/US]; 17 Quincy Street, Cambridge, MA 02138 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **GYGI, Steven, P.** [US/US]; 4 Stockbridge Road, Foxboro, MA 02035 (US).

(74) Agents: **NEUNER, George, W.** et al.; Edwards & Angell, LLP, P.O. Box 55874, Boston, MA 02205 (US).

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

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: SYSTEMS, METHODS AND KITS FOR CHARACTERIZING PHOSPHOPROTEOMES

(57) Abstract: The invention provides systems, software, methods and kits for detecting and/or quantifying phosphorylatable polypeptides and/or acetylated polypeptides in complex mixtures, such as a lysate of a cell or cellular compartment (e.g., such as an organelle). The methods can be used in high throughput assays to profile phosphoproteomes and to correlate sites and amounts of phosphorylation with particular cell states.

WO 2004/108948 A2

EXPRESS MAIL LABEL NO.: EV 342587320 US
ATTORNEY DOCKET NO.: 58890 PCT (70207)

SYSTEMS, METHODS AND KITS FOR CHARACTERIZING PHOSPHOPROTEOMES

Cross-reference to Related Applications

This application claims priority from U.S. Serial No. 60/476,010 filed June 4, 2003.

Government Grants

This work was supported by NIH grants 5K22HG000041 and GM67945. The government may have certain rights in this invention.

Field of the Invention

This invention provides methods, systems, software and kits for characterizing phosphoproteomes. In particular, the invention provides methods, systems, software and kits for identifying differential protein phosphorylation, for quantifying phosphorylated proteins and for identifying modulators of phosphorylated proteins.

Background of the Invention

Determining the site of a regulatory phosphorylation event can often unlock the specific biology surrounding a disease, elucidate kinase-substrate relationships, and provide a handle to study the regulation of an essential pathway. Although the events leading up to and directly following protein phosphorylation are the subject of intense research efforts, the large-scale identification and characterization of phosphorylation sites is an unsolved problem.

Methods for evaluating gene expression patterns that capture data relating to the abundance of proteins in a cell typically fail to provide information regarding post-translational modifications of proteins. Such information may be critical in determining the activity of expressed proteins. For example, many proteins are

initially translated in an inactive form and upon modification, gain biological function. The addition of biochemical groups to translated polypeptides has effects on protein stability, oligomerization, protein secondary/tertiary structure, enzyme activity and more globally on signaling pathways in cells.

The activity of numerous proteins and association of proteins into functional complexes are frequently controlled by reversible protein phosphorylation (see, e.g., Graves, et al., *Pharmacol. Ther.* 82, 111-121, 1999; Koch, et al., *Science* 252, 668-674, 1991; Hunter, *Semin. Cell Biol.* 5, 367-376, 1994). Phosphorylation occurs by the addition of phosphate to polypeptides by specific enzymes known as protein kinases. Phosphate groups are added to, for example, tyrosine, serine, threonine, histidine, and/or lysine amino acid residues depending on the specificity of the kinase acting upon the target protein.

Reversible protein phosphorylation is a general event affecting countless cellular processes. The identification of phosphorylation sites is most commonly accomplished by mass spectrometry. Tandem mass spectrometry provides the ability to fragment the phosphopeptide to determine its sequence as well as pinpoint the specific serine, threonine, or tyrosine modified by a protein kinase. While protein sequence analysis by mass spectrometry is a mature technology with many papers reporting protein identifications in the thousands, the large-scale determination of phosphorylation sites is only just emerging. In fact, the two largest repositories of determined sites were both from yeast studies with 383 and 125 sites detected, respectively. Ficarro, S. B. et al., *Nat Biotechnol* 20, 301-5. (2002); Peng, J. et al., *Nat Biotechnol* 21, 921-6 (2003). In human cells, 64 sites were determined from a single sample. Ficarro, S. et al., *J Biol Chem* 278, 11579-89 (2003).

To date several disease states have been linked to the abnormal phosphorylation/dephosphorylation of specific proteins. For example, the polymerization of phosphorylated tau protein allows for the formation of paired helical filaments that are characteristic of Alzheimer's disease, and the hyperphosphorylation of retinoblastoma protein (pRB) has been reported to progress various tumors (see, e.g., Vanmechelen et al. Neurosci. Lett. 285:49-52, 2000, and Nakayama et al. Leuk. Res. 24:299-305, 2000).

The identification of phosphorylation sites on a protein is complicated by the facts that proteins are often only partially phosphorylated and that they are often present only at very low levels. Prior art methods for identifying phosphorylated proteins have included *in vivo* incorporation of radiolabeled phosphate and analysis of labeled proteins by electrophoresis and autoradiography, western blotting using antibodies specific for phosphorylated forms of target proteins, and the use of yeast systems to identify mutations in protein kinases and/or protein phosphatases. Generally, only highly expressed proteins are detectable using these techniques and it is difficult to readily identify the sequences of the modified proteins. Immunological methods can only detect phosphorylated proteins globally (e.g., an anti-phosphotyrosine antibody will detect all tyrosine-phosphorylated proteins).

The development of methods and instrumentation for mass spectrometry has significantly increased the sensitivity and speed of the identification of phosphorylated proteins. Several mass spectrometry based techniques have been employed for the mapping of phosphorylation sites. For example, Cao, et al, Rapid Commun. Mass Spectrom. 14: 1600-1606, 2000, report mapping phosphorylation sites of proteins using on-line immobilized metal affinity chromatography (IMAC)/capillary electrophoresis (CE)/electrospray ionization multiple stage tandem mass spectrometry (MS). The IMAC resin retains and preconcentrates phosphorylated proteins and peptides; CE separates the phosphopeptides of a mixture eluted from the IMAC resin, and MS provides information including the phosphorylation sites of each component.

Posewitz, et al., Anal. Chem. 71:2883-2892, 1999, reports using immobilized metal affinity chromatography in a microtip format to isolate phosphopeptides for direct analysis by matrix-assisted laser desorption/ionization time of flight and nanoelectrospray ionization mass spectrometry.

Enrichment analysis of phosphorylated proteins also has been used to probe the phosphoproteome (Chait et al., Nature Biotechnology 19: 379-382, 2001).

However, there are two major obstacles to phosphorylation site analysis, regardless of scale of the experiment. First, fragmentation of phosphopeptides by collision-induced dissociation in a tandem mass spectrometer commonly results in the

production of a single dominant peak corresponding to a neutral loss of phosphoric acid (H_3PO_4, 98 daltons) from the phosphopeptide. The lack of informative fragmentation at the peptide backbone severely reduces the precision of database searching algorithms to identify the phosphopeptide. In addition, when a phosphopeptide is identified, it is often not possible to define the site to a particular serine, threonine, or tyrosine residue due to the lack of informative fragmentation[2].

Another major obstacle to phosphorylation analysis is the often poor stoichiometry of the phosphorylated protein compared to the nonphosphorylated protein compounded by the already low expression levels of most phosphoproteins. For this reason, phosphopeptides are not readily detected from the direct analysis of complex proteolyzed protein mixtures even when multidimensional chromatography is used. It is essential to employ some type of enrichment strategy to overcome the tremendous complexity that a proteolyzed lysate represents. Efforts to isolate phosphopeptides in the past have utilized either i) chemical modification of phosphate groups, ii) phosphate-specific mass spectrometry-based methods, or iii) affinity-based methods (antibody or metal ion chromatography). Regardless of the enrichment procedure, amino acid sequence analysis and site determination were accomplished by tandem mass spectrometry. Each technique has been successful for the analysis of a few proteins (<30), but only IMAC has shown the potential for the identification of more than a few sites from complex mixtures.

Thus, new and better methods for analysis of proteins and determining the site of a regulatory phosphorylation event continue to be sought.

Summary Of The Invention

The ability to quickly screen for alterations in the phosphorylation state of proteins is important to characterize intra and inter cellular signaling events required for normal physiological responses. Identification and/or quantification of phosphorylatable proteins facilitates development of improved diagnostics for the detection of various disease states as well as providing candidate drug targets for developing treatment regimens.

The invention provides methods for screening for phosphorylatable polypeptides (e.g., including proteins and peptides) to determine sites of phosphorylation, numbers of phosphates present in a phosphorylated polypeptide, and/or the level of a phosphorylated or unphosphorylated form of a phosphorylatable polypeptide in a sample.

In one aspect, the method comprises separating a plurality of proteins according to at least one biological property, e.g., such as molecular weight, obtaining subsets of separated polypeptides, contacting the subsets with a protease activity to obtain peptides corresponding to each subset of separated polypeptides, and enriching for peptides comprising positive charges (e.g., from 1+ to 4+). Preferably, the enriched fraction so obtained is enriched for phosphorylated peptides.

In another aspect, the method comprises the identification of the N-terminal peptide of proteins after trypsin digestion. The trypsin digestion provides an acetylated N terminus of a peptide with a solution charge state of 1+ at pH 3.

In one aspect, separation according to the at least one biological property comprises separation according to molecular weight, such as by gel electrophoresis and subsets are obtained by cut a gel comprising electrophoresed proteins into sections and evaluating peptide digests of separated polypeptides within each gel section. In another aspect, separation according to the at least one biological property is based on binding affinity to a binding partner (e.g., such as by chromatography on an IMAC column). Separation also may be based on hydrophobicity, hydrophilicity, the presence of particular sequence domains and the like. However, in one aspect, separation of polypeptides is performed randomly, merely to reduce the complexity of the sample of polypeptides prior to further analysis.

In one particularly preferred aspect, enrichment is achieved by separating the peptides in each subset according to charge using strong cation exchange chromatography (SCX) at a pH of about 3 and selecting initial fractions eluted from the column. Preferably, data-dependent acquisition of MS^3 spectra for improved phosphopeptide identification also is utilized.

Phosphorylation sites within the phosphorylated peptides can be identified using methods known in the art or described herein. In one aspect, such a method comprises obtaining a peptide to be analyzed, generating a first series of precursor ions corresponding to the peptide, and a second series of fragment ions obtained by fragmentation of selected precursor ions, and, detecting, among the fragment ions, a fragment ion having the signature predicted for a modified amino acid. In another aspect, the mass of a fragment ion is compared to the mass of a reference ion characteristic of a phosphorylated amino acid, thereby identifying the phosphorylation state of the peptide being analyzed. As the initial fractions provide greater than 100,000 different peptides, expression profiles of modified peptides can be determined rapidly and efficiently for proteomes of cells and cell compartments.

In a further aspect, the invention provides a method for comparing the phosphorylation state of one or more proteins in a plurality of samples and for identifying and/or individually quantitating phosphorylated proteins.

The invention also provides a method for generating a peptide internal standard for detecting and quantifying phosphorylated proteins. The method comprises identifying a peptide digestion product of a target polypeptide comprising at least one phosphorylation site, determining the amino acid sequence of a peptide digestion product comprising a phosphorylation site and synthesizing a peptide having the amino acid sequence. The peptide is labeled with a mass-altering label (e.g., by incorporating labeled amino acid residues during the synthesis process) and fragmented (e.g., by multi-stage mass spectrometry). Preferably, the label is a stable isotope. A peptide signature diagnostic of the peptide is determined, after one or more rounds of fragmenting, and the signature is used to identify the presence and/or quantity of a peptide of identical amino acid sequence in a sample and to detect the presence or absence of the modification. In one aspect, panels of peptide internal standards are generated corresponding to (i.e., diagnostic of) different modified forms of the same protein (i.e., proteins which are phosphorylated at more than one site and/or which comprise other types of modifications (e.g., glycosylation, ubiquitination, acetylation, farnesylation, and the like).

Peptide internal standards corresponding to different peptide subsequences of a single target protein also can be generated to provide for redundant controls in a quantitative assay. In one aspect, different peptide internal standards corresponding to the same target protein are generated and differentially labeled (e.g., peptides are labeled at multiple sites to vary the amount of heavy label associated with a given peptide).

In a further aspect, a panel of peptide internal standards corresponding to amino acid subsequences of at least one phosphorylatable protein in a molecular pathway is generated. Preferably, internal standards corresponding to a plurality of phosphorylatable peptides are generated. In one aspect, the panel further comprises peptide internal standard(s) corresponding to one or more protein kinases or phosphatases.

Molecular pathways, include, but are not limited to signal transduction pathways, cell cycle pathways, metabolic pathways, blood clotting pathways, and the like. In one aspect, the panel includes peptide standards which correspond to different phosphorylated forms of one or more proteins in a pathway and the panel is used to determine the presence and/or quantity of the activated or inactivated form of a pathway protein.

In a further aspect, the invention provides a method for identifying a treatment that modulates phosphorylation of an amino acid in a target polypeptide, comprising: subjecting a sample containing the target polypeptide to a treatment, determining the level of phosphorlyation of one or more amino acids in the target polypeptide, both before and after the treatment; identifying a treatment that results in a change of the level of modification of the one or more amino acids after the treatment. The treatment may comprise exposure to an agent (e.g., such as a drug) or exposure to a condition (e.g., such as pH, temperature, etc.)

In one aspect, a labeled peptide internal standard and target peptide (i.e., a peptide being detected in a sample) are fragmented (e.g., using multistage mass spectrometry) and the ratio of labeled fragments to unlabeled fragments; is determined. The quantity of the target polypeptide can be calculated using both the

ratio and known quantity of the labeled internal standard. The mixtures of different polypeptides can include, but are not limited to, such complex mixtures as a crude fermenter solution, a cell-free culture fluid, a cell or tissue extract, blood sample, a plasma sample, a lymph sample, a cell or tissue lysate; a mixture comprising at least about 100 different polypeptides; at least about 1000 different polypeptides, at least about 100, 000 different polypeptides. or a mixture comprising substantially the entire complement of proteins in a cell or tissue. In one preferred aspect, the method is used to determine the presence of and/or quantity of one or more target polypeptides directly from one or more cell lysates, i.e., without separating proteins from other cellular components or eliminating other cellular components.

In a still further aspect of the invention, stable isotope labeling with amino acids in cell culture, or SILAC, is used. Cells representing two biological conditions are cultured in amino acid-deficient growth media supplemented with ^{12}C- or ^{13}C-labeled amino acids, e.g., Arg or Lys. The proteins in these two cell populations effectively become isotopically labeled as "light" or "heavy." The cells are isolated, mixed in equal ratios and processed. the method further includes co-eluting the proteins by chromatographic separation into the mass spectrometer, gathering relative quantitative information for each protein by calculating the ratio of intensities of the two peaks produced in the peptide mass spectrum (MS scan), and acquiring sequence data for these peptides by fragment analysis in the product ion mass spectrum (MS/MS scan), thereby providing accurate protein identification.

In one aspect, the presence and/or quantity of target polypeptide in a mixture are diagnostic of a cell state. In another aspect, the cell state is representative of an abnormal physiological response, for example, a physiological response which is diagnostic of a disease. In a further aspect, the cell state is a state of differentiation or represents a cell which has been exposed to a condition or agent (e.g., a drug, a therapeutic agent, a potential toxin). In one aspect, the method is used to diagnose the presence or risk of a disease. In another aspect, the method is used to identify a condition or agent which produces a selected cell state (e.g., to identify an agent which returns one or more diagnostic parameters of a cell state to normal).

In a further aspect, the method comprises determining the presence and/or quantity of target peptides in at least two mixtures. In another aspect, one mixture is from a cell having a first cell state and the second mixture is from a cell having a second cell state. In a further aspect, the first cell is a normal cell and the second cell is from a patient with a disease. In still a further aspect, the first cell is exposed to a condition and/or treated with an agent and the second cell is not exposed and/or treated. Preferably, first and second mixtures are evaluated in parallel. The methods can be used to identify regulators of phosphorylation, e.g., such as kinases and phosphatases. The agent may be a therapeutic agent for treating a disease associated with an improper state of phosphorylation (e.g., abnormal sites or amounts of phosphorylation). Suitable agents include, but are not limited to, drugs, polypeptides, peptides, antibodies, nucleic acids (genes, cDNAs, RNA's, antisense molecules, ribozymes, aptamers and the like), toxins, and combinations thereof.

Alternatively, the two mixtures can be from identical samples or cells. In one aspect, a labeled peptide internal standard is provided in different known amounts in each mixture. In another aspect, pairs of labeled peptide internal standards are provided each comprising mass-altering labels which differ in mass, e.g., by including different amounts of a heavy isotope in each peptide.

The invention also provides a method of determining the presence of and/or quantity of a phosphorylation in a target polypeptide. Preferably, the label in the internal standard is part of a peptide comprising a modified amino acid residue or to an amino acid residue which is predicted to be modified in a target polypeptide. In one aspect, the presence of the modification reflects the activity of a target polypeptide and the assay is used to detect the presence and/or quantity of an active polypeptide. The method is advantageous in enabling detection of small quantities of polypeptide (e.g., about 1 part per million (ppm) or less than about 0.001% of total cellular protein).

The presence and/or quantity of phosphorylated proteins can be used to profile the function of a pathway in a particular cell. In one aspect, the pathway is one or more of a signal transduction pathway, a cell cycle pathway, a metabolic pathway, a

blood clotting pathway and the like. The coordinate function of multiple pathways can be evaluated using a plurality of panels of standards.

The invention further provides reagents useful for performing the method described above. In one aspect, a reagent according to the invention comprises a peptide internal standard comprising a phosphorylation site labeled with a stable isotope. Preferably, the standard has a unique peptide fragmentation signature diagnostic of the phosphorylation state of the peptide. In one aspect, the peptide is phosphorylated. In another aspect, the peptide is unphosphorylated. In a further aspect, a pair of peptides is provided, a peptide internal standard corresponding to a phosphorylated peptide and a peptide internal standard corresponding to a peptide identical in sequence but not phosphorylated. In another aspect, the peptide is a subsequence of a known protein and can be used to identify the presence of and/or quantify the protein in sample, such as a cell lysate. In one aspect, the peptide internal standard comprises a label associated with a modified amino acid residue, such as a phosphorylated amino acid residue, a glycosylated amino acid residue, an acetylated amino acid residue, a farnesylated residue, a ribosylated residue, and the like.

In another aspect, panels of peptide internal standards corresponding to different amino acid subsequences of single polypeptide are provided, including peptides comprising phosphorylation sites and peptides lacking phosphorylation sites.

In a further aspect, panels of peptide internal standards are provided which correspond to different proteins in a molecular pathway (e.g., a signal transduction pathway, a cell cycle pathway, a metabolic pathway, a blood clotting pathway and the like). In still a further aspect, peptide internal standards corresponding to different modified forms of one or more proteins in a pathway are provided.

In still a further aspect, panels of peptide internal standards are provided which correspond to proteins diagnostic of different diseases, allowing a mixture of peptide internal standards to be used to test for the presence of multiple diseases in a single assay.

The invention additionally provides kits comprising one or more peptide internal standards labeled with a stable isotope. In one aspect, a kit comprises peptide internal standards comprising different peptide subsequences from a single known protein. In another aspect, the kit comprises peptide internal standards corresponding to different variant forms of the same amino acid subsequence of a target polypeptide. In still another aspect, the kit comprises peptide internal standards corresponding to different known or predicted modified forms of a polypeptide. In a further aspect, the kit comprises peptide internal standards corresponding to sets of related proteins, e.g., such as proteins involved in a molecular pathway (a signal transduction pathway, a cell cycle, etc) and/or to different modified forms of proteins in the pathway. In still a further aspect, a kit comprises a labeled peptide internal standard as described above and software for performing multistage mass spectrometry.

The kit may also include a means for obtaining access to a database comprising data files which include data relating to the mass spectra of fragmented peptide ions generated from peptide internal standards. The means for obtaining access can be provided in the form of a URL and/or identification number for accessing a database or in the form of a computer program product comprising the data files. In one aspect, the kit comprises a computer program product which is capable of instructing a processor to perform any of the methods described above.

The present invention also provides a system and software for facilitating the analysis of phosphoproteomes. The invention provides a system that comprises a relational database which stores mass spectral data relating to phoshorylation states for a plurality of proteins in a proteome. The system further comprises a data analysis system for correlating phosphorylation states to one or more characteristics relating to the source of the proteome, e.g., a cell or tissue extract, a patient group, etc.

Such characteristics include, but are not limited to: the activity of a kinase in the cell or tissue extract, the activity of a phosphatase in the cell or tissue extract, presence/absence of a disease in the source of the sample (i.e., a patient from whom the sample is obtained); stage of a disease; risk for a disease; likelihood of recurrence of disease; a shared genotype at one or more genetic loci; exposure to an agent (e.g., such as a toxic substance or a potentially toxic substance, a carcinogen, a teratogen,

an environmental pollutant, a therapeutic agent such as a candidate drug, a nucleic acid, protein, peptide, small molecule, etc.) or condition (temperature, pH, etc); a demographic characteristic (age, gender, weight; family history; history of preexisting conditions, etc.); resistance to agent, sensitivity to an agent (e.g., responsiveness to a drug) and the like.

In one aspect, the data management program comprises a data analysis program for identifying similarities of features of mass spectral signatures for one or more peptides in a plurality of peptides with mass spectral signatures for known peptides. In another aspect, the data analysis program identifies the amino acid sequences for one or more peptides in the plurality of peptides. In still another aspect, the plurality of peptides is a mixture of labeled peptides, a first set of peptides labeled with a first label and a second set of peptides labeled with a second label. In a further aspect, the first label has a first mass and the second label has a second, different mass. Preferably, the data analysis system comprises a component for determining the relative abundance of a first labeled peptide with a second labeled peptide.

In one aspect, the system is connectable to one or more external databases through a network server, such databases comprising genomic, proteomic, pharmacological data and the like.

The invention also provides a method for storing peptide data to a database. The method comprises acquiring mass spectrum signatures for one or more peptides in a plurality of peptides. The one or more peptides exist in a phosphorylated form in one or more cells having a cell state (e.g., a differentiation state, an association with a disease or response to an abnormal physiological condition, response to an agent, and the like). The signatures are stored in a database and correlated with the presence or absence of cell state. Preferably, pairs of signatures associated with both the phosphorylated and unphosphorylated states of the peptides are stored in the database. In one aspect, the mass spectrum signatures are obtained using mass analytical techniques, including, but not limited to: multistage mass spectroscopy, electron ionization mass analysis, fast atom/ion bombardment mass analysis, matrix-assisted laser desorption/ionization mass analysis and electrospray ionization mass analysis, and the like

Preferaby, mass spectral data is obtained by separating a peptide mixture according to mass and charge characteristics and subjecting separated peptides to one or more mass analyses where each peptide is fragmented and additional mass spectral signatures corresponding to fragmented peptides are produced.

The amino acid sequences of the peptides are determined using methods known in the art. See, e.g., U.S. Patent No. 6,017,693 and U.S. Patent No. 5,538,897. In one aspect, mass spectra from an experiment are input into a computer containing a database of sequence-associated spectrum. The computer then performs a search of the database and outputs results. Preferably, mass spectra are automatically queried against a database of spectral information to generate sequence information.

Differentially expressed phosphorylated peptides are correlated by the system with responses of a proteome to a stimulus, a condition, an agent (e.g., a therapeutic agent such as a drug, a toxic agent or potentially toxic agent, a carcinogen or potential carcinogen), a change in environment (e.g., nutrient level, temperature, passage of time), a disease state, malignancy, site-directed mutation, introduction of exogenous molecules (nucleic acids, polypeptides, small molecules, etc.) into a cell, tissue or organism from which the sample originated and other characteristics as described above.

Brief Description of the Figures

The objects and features of the invention can be better understood with reference to the following detailed description and accompanying drawings.

Figures 1A-C illustrate a method according to one aspect of the invention and illustrates how strong cation exchange chromatography separates peptides by solution charge. Figure 1A shows the separation of a complex peptide mixture by SCX chromatography with fraction collection every minute. Each fraction was analyzed by microcapillary LC-MS/MS techniques. Figure 1B shows the number of unique peptides identified in each fraction by the Sequest algorithm for each solution charge state. Figure 1C shows a mixed mode separation of polysulfoethyl-aspartamide based primarily on ionic charge but also on hydrophobicity.

Figure 2 shows a flowchart for large-scale analysis of nuclear protein. A nuclear preparation from HeLa cells (10 mg) was separated on a single SDS-PAGE preparative gel. Twenty regions (slices) were removed from the gel and subjected to in-gel tryptic digestion. The 20 complex peptide samples were separated further by strong cation exchange (SCX) chromatography with fraction collection every minute. Each fraction (n=1000) was then subjected to analysis by nano-scale microcapillary LC-MS/MS.

Figure 3 shows SCX chromatography separation of Slice 14 with respect to number of unique peptides identified per fraction. Upper panel shows the separation with UV detection at 214 nm. Fractions (200 microliters) were collected every minute. Each fraction was analyzed by LC-MS/MS with a 2-hr gradient. Peptides in each fraction were identified by Sequest (REF). Peptides identified having different solution charge states are shown in the lower panel.

Figure 4A shows mass spectral data for and the amino acid sequence of a peptide obtained using a method according to the invention. The peptide is a subsequence of the human polypeptide KP58_HUMAN. Figure 4B shows mass spectral data for and the amino acid sequence of a peptide obtained using a method according to the invention. The peptide is a subsequence of the polypeptide GP:AB033054. Figure 4C shows mass spectral data for and the amino acid sequence of a peptide obtained using a method according to the invention. The peptide is a subsequence of the polypeptide WEE1_HUMAN. Figure 4D shows mass spectral data for and the amino acid sequence of a peptide obtained using a method according to the invention. The peptide is a subsequence of the polypeptide PIR2:A38282. Figure 4E shows mass spectral data for and the amino acid sequence of a peptide obtained using a method according to the invention. The peptide is a subsequence of the polypeptide PYRG_HUMAN. Figure 4F shows mass spectral data for and the amino acid sequence of a peptide obtained using a method according to the invention. The peptide is a subsequence of the polypeptide GP:Y18004. Figure 4G shows mass spectral data for and the amino acid sequence of a peptide obtained using a method according to the invention. The peptide is a subsequence of the polypeptide

GP:AF161470. Figure 4H shows mass spectral data for and the amino acid sequence of a peptide obtained using a method according to the invention. The peptide is a subsequence of the polypeptide S3B2_HUMAN. Figure 4I shows mass spectral data for and the amino acid sequence of a peptide obtained using a method according to the invention. The peptide is a subsequence of the polypeptide GB:BC011630.

Figure 5A shows neutral loss of each fraction obtained by SCX from slice 14 as described in Example 1. Figure 5B shows control random loss of fractions, i.e., reflecting the level of variability or background in the analysis. Figure 5C shows numbers of neutral losses (y-axis) vs. fraction number.

Figures 6A-C shows a scheme for phosphopeptide enrichment by strong cation exchange (SCX) chromatography. Figure 6A shows, At pH 2.7, peptides produced by trypsin proteolysis generally have a solution charge state of 2^+ while phosphopeptides have a charge state of only 1^+. Figure 6B shows solution charge state distribution of peptides (5-40 amino acids in length) produced by a theoretical digestion of the human protein database with trypsin ($n=6.8 \times 10^8$ peptides). Sixty-eight percent of the predicted peptides have a net charge of 2^+. Any peptide in this category would shift to a 1^+ charge state upon phosphorylation. Figure 6C shows SCX chromatography separation at pH 2.7 for a complex peptide mixture of human proteins after trypsin digestion. The circled region is highly enriched for phosphopeptides.

Figures 7A-C show an analysis of human nuclear phosphorylation sites by LC/LC-MS/MS/MS. Figure 7A shows Eight mg of nuclear extract from asynchronous HeLa cells were separated by SDS-PAGE. The entire gel was excised into 10 regions and proteolyzed with trypsin followed by phosphopeptide enrichment by strong cation exchange (SCX) liquid chromatography (LC). Early eluting fractions were subjected to amino acid sequence analysis by reverse-phase LC-MS/MS with data-dependent MS^3 acquisition. 2,002 phosphorylation sites were identified by the Sequest algorithm, acquisition of MS^3 spectra, and manual validation. Figure 7B shows an example of a tandem mass (MS/MS) spectrum of a phosphopeptide showing a typical extensive neutral loss of phosphoric acid. Figure 7C shows the MS/MS/MS

(MS^3) spectrum of the neutral loss precursor ion from panel B. Abundant fragmentation now resulted at peptide bonds permitting the unambiguous identification of this peptide from the protein, cell division cycle 2-related protein kinase 7, with a phosphorylated serine residue marked by an asterisk.

Figures 8A-F show classification of identified phosphorylation sites and amino acid frequencies surrounding phosphorylated serine and threonine residues. Figure 8A shows a Venn Diagram representation of 1,833 precise sites of phosphorylation with respect to surrounding residues. Seventy seven percent of the detected phosphorylation sites could be assigned as either proline-directed or acidiphilic. Figure 8B shows phosphorylation sites grouped by protein localization and function. The largest class of proteins detected was "unknown" (uncharacterized or hypothetical). "Other" represents known proteins not in other categories (mostly well-characterized cytosolic proteins). Figure 8C is an intensity map showing the relative occurrence of residues flanking all phosphorylation sites. Figure 8D is an intensity map showing the relative occurrence of residues flanking proline-directed ({pSer/pThr} - Pro) phosphorylation sites. Figure 8E is an intensity map showing the relative occurrence of residues flanking acidiphilic ({pSer/pThr} – Xxx – Xxx – {Asp/Glu/pSer}) sites. Figure 8F is an intensity map showing the relative occurrence of residues flanking all other phosphorylation sites. To facilitate comparisons an intensity gradient of light to dark was used ranging from white (no occurrence) to black (high occurrence).

Detailed Description

The invention provides systems, software, methods and kits for detecting and/or quantifying phosphorylatable polypeptides and/or acetylated polypeptides in complex mixtures, such as a lysate of a cell or cellular compartment (e.g., such as an organelle). The methods can be used in high throughput assays to profile phosphoproteomes and to correlate sites and amounts of phosphorylation with particular cell states.

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton *et al.*, *Dictionary of Microbiology and Molecular Biology* (2nd ed. 1994); *The Cambridge Dictionary of Science and Technology* (Walker ed., 1988); *The Glossary of Genetics*, 5th Ed., R. Rieger *et al.* (eds.), Springer Verlag (1991); and Hale & Marham, *The Harper Collins Dictionary of Biology* (1991).

Definitions

The following definitions are provided for specific terms which are used in the following written description.

As used in the specification and claims, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof. The term "a protein" includes a plurality of proteins.

"Protein", as used herein, means any protein, including, but not limited to peptides, enzymes, glycoproteins, hormones, receptors, antigens, antibodies, growth factors, etc., without limitation. Presently preferred proteins include those comprised of at least 25 amino acid residues, more preferably at least 35 amino acid residues and still more preferably at least 50 amino acid residues.

As used herein, “a polypeptide” refers to a plurality of amino acids joined by peptide bonds. Amino acids can include D- , L- amino acids, and combinations thereof, as well as modified forms thereof. As used herein, a polypeptide is greater than about 20 amino acids. The term “polypeptide” generally is used interchangeably with the term “protein”; however, the term polypeptide also may be used to refer to a less than full-length protein (e.g., a protein fragment) which is greater than 20 amino acids.

As used herein, the term "peptide" refers to a compound of two or more subunit amino acids, and typically less than 20 amino acids. The subunits are linked by peptide bonds.

The terms "polypeptide", and "protein" are generally used interchangeably herein to refer to a polymer of amino acid residues. As used herein a peptide is generally about 100 amino acids or less.

As used herein, a "target protein" or a "target polypeptide" is a protein or polypeptide whose presence or amount is being determined in a protein sample. The protein/polypeptide may be a known protein (i.e., previously isolated and purified) or a putative protein (i.e., predicted to exist on the basis of an open reading frame in a nucleic acid sequence).

As used herein, a "protease activity" is an activity that cleaves amide bonds in a protein or polypeptide. The activity may be implemented by an enzyme such as a protease or by a chemical agent, such as CNBr.

As used herein, "a protease cleavage site" is an amide bond which is broken by the action of a protease activity.

As used herein, the term "phosphorylation site" or "phospho site" refers to an amino acid or amino acid sequence of a natural binding domain or a binding partner which is recognized by a kinase or phosphatase for the purpose of phosphorylation or dephosphorylation of the polypeptide or a portion thereof. A "site" additionally refers to the single amino acid which is phosphorylated or dephosphorylated. Generally, a phosphorylation site comprises as few as one but typically from about 1 to 10, about 1 to 50 amino acids, i.e., less than the total number of amino acids present in the polypeptide.

The term "agonist" as used herein, refers to a molecule that augments a particular activity, such as kinase-mediated phosphorylation or phosphatase-mediated dephosphorylation. The stimulation may be direct, or indirect, or by a competitive or non-competitive mechanism. The term "antagonist", as used herein, refers to a molecule that decreases the amount of or duration of a particular activity, such as

kinase-mediated phosphorylation or phosphatase-mediated dephosphorylation. The inhibition may be direct, or indirect, or by a competitive or non-competitive mechanism. Agonists and antagonists may include proteins, including antibodies, that compete for binding at a binding region of a member of the complex, nucleic acids including anti-sense molecules, carbohydrates, or any other molecules, including, for example, chemicals, metals, organometallic agents, etc.

The term "recombinant protein" refers to a protein which is produced by recombinant DNA techniques, wherein generally DNA encoding the expressed protein is inserted into a suitable expression vector which is in turn used to transform a host cell to produce the heterologous protein. Moreover, the phrase "derived from", with respect to a recombinant gene encoding the recombinant protein is meant to include within the meaning of "recombinant protein" those proteins having an amino acid sequence of a native protein, or an amino acid sequence similar thereto which is generated by mutations including substitutions and deletions of a naturally occurring protein.

The term "fractionated lysate", as used herein, refers to a cell lysate which has been treated so as to substantially remove at least one component of the whole cell lysate, or to substantially enrich at least one component of the whole cell lysate. "Substantially remove", as used herein, means to remove at least 10%, more preferably at least 50%, and still more preferably at least 80%, of the component of the whole cell lysate. "Substantially enrich", as used herein, means to enrich by at least 10%, more preferably by at least 30%, and still more preferably at least about 50%, at least one component of the whole cell lysate compared to another component of the whole cell lysate.

As used herein, an "isolated organelle" or "isolated cellular compartment" refers to a membrane bound intracellular structure which is substantially removed from a cell such that a sample comprising an isolated organelle or isolated cellular compartment comprises less than 50%, less than 20%, and preferably, less than 10% cellular proteins other than those which are part of (e.g., lie within or on the membrane of the membrane bound intracellular membrane structure).

"Small molecule" as used herein, is meant to refer to a composition, which has a molecular weight of less than about 5 kD and most preferably less than about 2.5 kD. Small molecules can be nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic (carbon containing) or inorganic molecules.

As used herein, a "labeled peptide internal standard" refers to a synthetic peptide which corresponds in sequence to the amino acid subsequence of a known protein or a putative protein predicted to exist on the basis of an open reading frame in a nucleic acid sequence and which is labeled by a mass-altering label such as a stable isotope. The boundaries of a labeled peptide internal standard are governed by protease cleavage sites in the protein (e.g., sites of protease digestion or sites of cleavage by a chemical agent such as CNBr). Protease cleavage sites may be predicted cleavage sites (determined based on the primary amino acid sequence of a protein and/or on the presence or absence of predicted protein modifications, using a software modeling program) or may be empirically determined (e.g., by digesting a protein and sequencing peptide fragments of the protein). In one aspect, a labeled peptide internal standard includes a modified amino acid residue.

"Percent identity" and "similarity" between two sequences can be determined using a mathematical algorithm (see, e.g., *Computational Molecular Biology*, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). For example, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch algorithm (J. Mol. Biol. (48): 444-453, 1970) which is part of the GAP program in the GCG software package (available at http://www.gcg.com), by the local homology algorithm of Smith & Waterman (Adv. Appl. Math. 2: 482, 1981), by the search for similarity methods of Pearson & Lipman (Proc. Natl. Acad. Sci. USA 85: 2444, 1988) and Altschul, et al. (Nucleic Acids Res. 25(17): 3389-3402, 1997), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and BLAST in the Wisconsin Genetics Software Package (available from, Genetics

Computer Group, 575 Science Dr., Madison, Wis.), or by manual alignment and visual inspection (see, e.g., Ausubel et al., *supra*). Gap parameters can be modified to suit a user's needs. For example, when employing the GCG software package, a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6 can be used. Examplary gap weights using a Blossom 62 matrix or a PAM250 matrix, are 16, 14, 12, 10, 8, 6, or 4, while exemplary length weights are 1, 2, 3, 4, 5, or 6. The percent identity between two amino acid or nucleotide sequences also can be determined using the algorithm of E. Myers and W. Miller (CABIOS 4: 11-17, 1989) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

As used herein, "a peptide fragmentation signature" refers to the distribution of mass-to-charge ratios of fragmented peptide ions obtained from fragmenting a peptide, for example, by collision induced disassociation, ECD, LID, PSD, IRNPD, SID, and other fragmentation methods. A peptide fragmentation signature which is "diagnostic" or a "diagnostic signature" of a target protein or target polypeptide is one which is reproducibly observed when a peptide digestion product of a target protein/polypeptide identical in sequence to the peptide portion of a peptide internal standard, is fragmented and which differs only from the fragmentation pattern of the peptide internal standard by the mass of the mass-altering label. Preferably, a diagnostic signature is unique to the target protein (i.e., the specificity of the assay is at least about 95%, at least about 99%, and preferably, approaches 100%).

As used herein, the interchangeable terms "biological specimen" and "biological sample" refer to a whole organism or a subset of its tissues, cells or component parts (e.g. body fluids, including but not limited to blood, mucus, lymphatic fluid, synovial fluid, cerebrospinal fluid, saliva, amniotic fluid, amniotic cord blood, urine, vaginal fluid and semen). "Biological sample" further refers to a homogenate, lysate or extract prepared from a whole organism or a subset of its tissues, cells or component parts, or a fraction or portion thereof. The biological sample can be in any form, including a solid material such as a tissue, cells, a cell pellet, a cell extract, a biopsy, a biological fluid such as urine, blood, saliva, spinal fluid, amniotic fluid, exudate from a region of infection or inflammation, or a

mouthwash containing buccal cells. In one aspect, a "biological sample" refers to a medium, such as a nutrient broth or gel in which an organism has been propagated, which contains cellular components, such as proteins or nucleic acid molecules.

As used herein, "modulation" refers to the capacity to either increase or decease a measurable functional property of biological activity or process (e.g., enzyme activity or receptor binding) by at least 10%, 15%, 20%, 25%, 50%, 100% or more; such increase or decrease may be contingent on the occurrence of a specific event, such as activation of a signal transduction pathway, and/or may be manifest only in particular cell types.

As used herein, the term "modulating the activity of a protein kinase or phosphatase" refers to enhancing or inhibiting the activity of a protein kinase or phosphatase. Such modulation may be direct (e.g. including, but not limited to, cleavage of- or competitive binding of another substance to the enzyme) or indirect (e.g. by blocking the initial production or activation of the kinase or phosphatase).

A "relational" database as used herein means a database in which different tables and categories of the database are related to one another through at least one common attribute and is used for organizing and retrieving data.

The term "external database" as used herein refers to publicly available databases that are not a relational part of the internal database, such as GenBank and Blocks.

As used herein, an "expression profile" refers to measurement of a plurality of cellular constituents that indicate aspects of the biological state of a cell. Such measurements may include, e.g., abundances or proteins or modified forms thereof.

As used herein, a “cell state profile” refers to values of measurements of levels of one or more proteins in the cell. Preferably, such values are obtained by determining the amount of peptides in a sample having the same peptide fragmentation signatures as that of peptide internal standards corresponding to the one or more proteins. A “diagnostic profile” refers to values that are diagnostic of a particular cell state, such that when substantially the same values are observed in a

cell, that cell may be determined to have the cell state. For example, in one aspect, a cell state profile comprises the value of a measurement of phosphorylated p53 in a cell. A diagnostic profile would be a value that is significantly higher than the value determined for a normal cell and such a profile would be diagnostic of a tumor cell. A "test cell state profile" is a profile that is unknown or being verified.

"Diagnostic" means identifying the presence or nature of a biological state, such as a pathologic condition, e.g., cancer. Diagnostic methods differ in their sensitivity and specificity. The "sensitivity" of a diagnostic assay is the percentage of samples which test positive for the state (percent of "true positives"). Samples not detected by the assay are "false negatives." Samples which are not from sources having the biological state and who test negative in the assay, are termed "true negatives." The "specificity" of a diagnostic assay is 1 minus the false positive rate, where the "false positive" rate is defined as the proportion samples which are from sources which do not have the state which test positive. While a particular diagnostic method may not provide a definitive diagnosis of a biological state, it suffices if the method provides a positive indication that aids in diagnosis. The methods of the present invention preferably provide a specificity of at least 80%, more preferably at least 85%. The methods of the present invention preferably provide a sensitivity of at least 70%, more preferably at least 75%, and most preferably at least 80%.

As used herein, a processor that "receives a diagnostic profile" receives data relating to the values diagnostic of a particular cell state. For example, the processor may receive the values by accessing a database where such values are stored through a server in communication with the processor.

As used herein, "a binding partner" refers to a first molecule which can form a stable, and specific, non-covalent association with a second molecule to be bound, enabling isolation of the second molecule from a population of molecules including the second molecule. "Stable" refers to an association which is strong enough to permit complexes to form which may be isolated.

As used herein, an "antibody" refers to monoclonal or polyclonal, single chain, double chain, chimeric, humanized, or recombinant antibody, or antigen-binding portion thereof (e.g., F(ab')2 fragments and Fab' fragments).

As used herein, "computer readable media" or a "computer memory" refers to any media that can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; digital video disc (DVDs), compact discs (CDs), hard disk drives (HDD), and magnetic tape and hybrids of these categories such as magnetic/optical storage media.

As used herein, the terms "processor" and "central processing unit" or "CPU" are used interchangeably and refers to a device that is able to read a program from a computer memory (e.g., ROM or other computer memory) and perform a set of steps according to the program.

As used herein, the term "in communication with" refers to the ability of a system or component of a system to receive input data from another system or component of a system and to provide an output response in response to the input data. "Output" may be in the form of data or may be in the form of an action taken by the system or component of the system.

As used herein, a "computer program product" refers to the expression of an organized set of instructions in the form of natural or programming language statements that is contained on a physical media of any nature (e.g., written, electronic, magnetic, optical or otherwise) and that may be used with a computer or other automated data processing system of any nature (but preferably based on digital technology). Such programming language statements, when executed by a computer or data processing system, cause the computer or data processing system to act in accordance with the particular content of the statements. Computer program products include without limitation: programs in source and object code and/or test or data libraries embedded in a computer readable medium. Furthermore, the computer program product that enables a computer system or data processing equipment device to act in preselected ways may be provided in a number of forms, including, but not limited to, original source code, assembly code, object code, machine language, encrypted or compressed versions of the foregoing and any and all equivalents.

Methods of Characterizing a Phosphoproteome

The invention provides methods for characterizing a phosphoproteome. The methods facilitate identification of phosphorylated proteins, identification of phosphorylation sites; quantitation of phosphorylation at one or more phosphorylation sites in a protein and determination of the biological function of phosphorylation. A phosphate group can modify serine, threonine, tyrosine, histidine, arginine, lysine, cysteine, glutamic acid and aspartic acid residues. The methods according to the invention are able to identify modifications at each of these groups and to distinguish between them. .

In one aspect, the method comprises providing a sample comprising a plurality of polypeptides and separating the polypeptides according to at least one physical property. Samples that can be analyzed by method of the invention include, but are not limited to, cell homogenates; cell fractions; biological fluids, including, but not limited to urine, blood, and cerebrospinal fluid; tissue homogenates; tears; feces; saliva; lavage fluids such as lung or peritoneal lavages; and generally, any mixture of biomolecules, e.g., such as mixtures including proteins and one or more of lipids, carbohydrates, and nucleic acids such as obtained partial or complete fractionation of cell or tissue homogenates.

Sub-tissue distribution, such as in particular cells, organelles, fractions and so on also can be examined. The tissue is treated to release the individual component cell or cells; the cells are treated to release the individual component organelles and so on. Those partitioned samples then can serve as the protein source. To provide a more particularized origin of protein, specific kinds of cells can be purified from a tissue using known materials and methods. To provide proteins specific for an organelle, the organelles can be partitioned, for example, by selective digestion of unwanted organelles, density gradient centrifugation or other forms of separation, and then the organelles are treated to release the proteins therein and thereof. The cells or subcellular components are lysed as described hereinabove. Other specific techniques for isolating single cells or specific cells are known such as Emmert-Buck et al., "Laser Capture Microdissection" *Science 274(5289)*: 998-1001 (1996).

Preferably, a proteome is analyzed. By a proteome is intended at least about 20% of total protein coming from a biological sample source, usually at least about 40%, more usually at least about 75%, and generally 90% or more, up to and including all of the protein obtainable from the source. Thus, the proteome may be present in an intact cell, a lysate, a microsomal fraction, an organelle, a partially extracted lysate, biological fluid, and the like. The proteome will be a mixture of proteins, generally having at least about 20 different proteins, usually at least about 50 different proteins and in most cases, about 100 different proteins, about 1000 different proteins, about 10,000 different proteins, about 100,000 different proteins, or more.

In one aspect, a proteome comprises substantially all of the proteins in a cell. In another preferred aspect, an organellar proteome is evaluated. For example, at least about at least about 50 different proteins and in most cases, about 100 different proteins, about 1000 different proteins, about 10,000 different proteins, about 100,000 different proteins, or more from an organelle such as a nucleus, mitochondria, chloroplast, golgi body, vacuole, or other intracellular compartment. In one preferred aspect, a complex mixture of cellular proteins is evaluated directly from a cell lysate, i.e., without any steps to separate and/or purify and/or eliminate cellular components or cellular debris. In another aspect, proteins are obtained from intracellular fractions corresponding comprising substantially purified preparations of intracellular organelles, e.g., such as cell nuclei, mitochondria, chloroplasts, golgi bodies, vacuoles, and the like.

Although the methods described herein are compatible with any biochemical, immunological or cell biological fractionation methods that reduce sample complexity and enrich for proteins of low abundance, it is a particular advantage of the method that it can be used to detect and quantitate peptides in complex mixtures of polypeptides, such as cell lysates. Unlike methods in the prior art, because the present invention detects diagnostic signatures that are highly selective for individual phosphorylatable peptides, the quantities of such peptides can be discerned even in a mixture of phosphorylated and unphosphorylated peptides of similar mass/charge ratios.

Generally, the sample will have at least about 0.01 mg of protein, at least about 0.05 mg, and usually at least about 1 mg of protein, at least about 10 mg of protein, at least about 20 mg of protein or more, typically at a concentration in the range of about 0.1-20 mg/ml. The sample may be adjusted to the appropriate buffer concentration and pH, if desired.

The physical property can include molecular weight, binding affinity for a ligand or receptor, hydrophobicity, hydrophilicity, and the like.

Preferred methods of separating polypeptides according to binding affinity include through the use of an array or substrate comprising a plurality of binding partners stably associated therewith (e.g., by attachment, deposition, etc.) for selectively binding to sample components. Suitable binding partners include, but are not limited to: cationic molecules; anionic molecules; metal chelates; antibodies; single- or double-stranded nucleic acids; proteins, peptides, amino acids; carbohydrates; lipopolysaccharides; sugar amino acid hybrids; molecules from phage display libraries; biotin; avidin; streptavidin; and combinations thereof. Generally, any molecule that has an affinity for desired sample components or which can selectively or specifically absorb a biological molecule can be used as a binding partner. Binding partners stably associated with the array may comprise a single type of molecule or functional group. In one aspect, the binding partner is a metal ion immobilized on an IMAC column.

In one preferred aspect, the plurality of polypeptides is separated at least according to molecular weight using liquid or gel-based separation on a 5-15% SDS polyacrylamide gel. For example, a cell lysate can be loaded onto a single lane gel and electrophoresed using methods known in the art to separate proteins.

In another aspect, polypeptides separated according to the at least one characteristic are divided into subsets. Inclusion in a particular subset may be based on a quality of the characteristic. For example, where the characteristic is molecular weight, polypeptides may be divided into subsets based on their molecular weights. Accordingly, polypeptides separated by gel electrophoresis may be divided into subsets by slicing the gel into fragments that are placed into separate containers (e.g., tubes) for subsequent analysis. The quality of the characteristic corresponding to each

subset is recorded for later correlation with other characteristics of one or more members of the subset (e.g., such as phosphorylation state). An aliquot of a sample may be run on a parallel gel which is stained to ensure the presence/quality of proteins in the sample.

In another aspect, the subset is selected at random, merely to reduce the complexity of polypeptides within the subset in further analyses.

Polypeptides within each subset are then contact with one or more proteases to digest the polypeptides into peptides. Generally, the type of protease is not limiting. Suitable proteases include, but are not limited to one or more of: serine proteases (e.g., such as trypsin, hepsin, SCCE, TADG12, TADG14); metallo proteases (e.g., such as PUMP-1); chymotrypsin; cathepsin; pepsin; elastase; pronase; Arg-C; Asp-N; Glu-C; Lys-C; carboxypeptidases A, B, and/or C; dispase; thermolysin; cysteine proteases such as gingipains, and the like.

In one aspect of the invention, peptide fragments ending with Lys or Arg residues are produced. While trypsin is an exemplary protease, many different enzymes can be used to perform the digestion to generate peptide fragments ending with Lys or Arg residues, including but not limited to, Thrombin [EC 3.4.21.5], Plasmin [EC 3.4.21.7], Kallilkrein [EC 3.4.21.8], Acrosin [EC 3.4.21.10], and Coagulation factor Xa [EC 3.4.21.6], and the like. See, e.g., Dixon, et al., In Enzymes (3rd edition, Academic Press, New York and San Francisco, 1979).

Other enzymes known to reliably and predictably perform digestions to generate the polypeptide fragments as described in the instant invention are also within the scope of the invention. Proteases may be isolated from cells or obtained through recombinant techniques.

Chemical agents with a protease activity also can be used (e.g., such as CNBr).

Protease digestion is allowed to proceed so that peptide fragments are produced comprising N-terminal peptides, C-terminal peptides and internal peptides.

The charge characteristics of the peptides will depend on the presence and nature of modifications of polypeptides from which the peptides derive.

Peptide products of this digestion are separated according to charge and enriched for phosphorylated peptides. In one aspect, peptides are also enriched for N-terminal and C-terminal peptides. N- and C-terminal peptides can be used to generate standards for quantitating phosphorylated peptides obtained from the same protein sequence from which an N- and or C-terminal peptide derives. Alternatively or additionally, N- and C-terminal peptides can be used to validate the start and stop points of ORF's identified from genomic sequence data.

In one preferred aspect, phosphorylated peptides are enriched for by separating the plurality of peptides in a subset of polypeptides using strong cation exchange techniques.

Cation ion exchange chromatography (CEX) is a separation technique which exploits the interaction between positively charged groups on a peptide and negatively charged groups on a substrate. Because pH determines the charges on peptides, the pH of the medium in which CEX is carried out determines separation performance. CEX substrates can be grouped into 2 major types; those which maintain a negative charge on the substrate over a wide pH range (strong CEX substrates) and those which maintain a negative charge on the substrate over a narrow pH range (weak CEX). Strong cation exchange (SCX) substrates usually incorporate sulphonic acids derivatives as functional groups (e.g. Sulphonates, S-type or Sulphopropyl groups, SP-types). Suitable strong cation exchangers include, but are not limited to sulfonated cellulose, phosphorylated cellulose, sulfonated dextran, phosphorylated dextran, sulfonated polyacrylamide and phosphorylated polyacrylamide. Examples of suitable strong CEX substrates include S-Sepharose FF, SP- Sepharose FF, SP-Sepharose Big Beads (all Amersham Pharmacia Biotechnology), Fractogel EMD-SO (3)650 (M) (E.Merck, Germany), polysulfoethyl aspartamide (The Nest Group, Southborough, MA). In one particularly preferred aspect of the invention, the cationic substrate is poly(2-sulfoethyl aspartamide)-silica. Cation exchangers may be in a granular state, film state or liquid state, although a granular state is generally most practical, facilitating absorption and elution of peptides, while permitting reuse of the granules

in a subsequent round of enrichment with a new subset of peptides. Methods of SCX are described in Peng, et al., *J. Proteome Res.* 2: 43-50, 2002.

Generally SCX columns comprise a methanol storage solvent for storage. The storage solvent should be flushed prior to use of the column to prevent salt precipitation. Preferably, the column is eluted with a strong buffer for at least one hour prior to its initial use. An exemplary buffer solution comprises 0.2 M monosodium phosphate and 0.3 M sodium acetate. Selectivity can be enhanced by varying the pH, ionic strength or organic solvent concentration in the mobile phase. For more strongly hydrophobic peptides, a non-ionic surfactant and/or acetonitrile comprise a suitable mobile phase modifier. Alternatively or additionally, the slope of a salt gradient used to elute peptides from the column can be modified.

At pH 3.0, amine functional groups of peptides almost exclusively contribute to the solution charge state. The nominal charge of any peptide can be determined by adding up the number of lysine, arginine, and histidine residues, with one additional charge contributed by the N-terminus of the peptide. Tryptic peptides generally have solution charge states of 2+ because they terminate in lysine or arginine and have a free N-terminus. A solution charge state of 3+ is seen for tryptic peptides containing one histidine residue. Tryptic peptides carrying a single charge in solution at pH 3.0 are highly specialized, representing either the C-terminal peptide from a polypeptide, an N-terminal peptide that is blocked (e.g., acetylated), or a phosphorylated peptide. Peptides which elute with solution charge states of 4+ or more also represent specialized peptides, e.g., such as disulfide-linked tryptic peptides, missed cleavages, etc. SCX can be used to distinguish among these various charged states.

SCX chromatography has the advantage of removing proteases and binding peptides in the presence of accessory molecules that carry no positive charge at pH 3.0, the pH at which peptide elution typically occurs. Thus, peptide binding and elution can occur in the presence of molecules typically used in cellular extraction processes, such as SDS, detergent, urea, DTT, and the like.

In order to maximize the performance of the SCX substrate, the pH of the medium in which the separation is carried out is usually below the isoelectric point of the peptide to be bound. It is a discovery of the instant invention that at a pH of about 3, phosphorylated proteins and acetylated proteins are enriched for in initial fractions obtained from a SCX column. Accordingly, in one aspect, the method comprises selecting initial fractions enriched for modified peptides, e.g., peptides which elute preferably within the first about 100 fractions, within the first about 90 fractions, within the first about 80 fractions, within the first about 70 fractions, within the first about 60 fractions, within the first about 50 fractions, within the first about 40 fractions, about 35 fractions, within the first about 30 fractions, within the first about 25 fractions, within the first about 20 fractions, within the first about 15 fractions, within the first about 10 fractions, within the first about 5 fractions, within the first about 2 fractions, within the first about 1 fraction after contacting the column with an elution substance such as a salt solution or volatile basic substance (e.g., , such as is ammonia, monomethylamine or dimethylamine). In one aspect, the initial fraction or a set of initial fractions (e.g., fractions 1-10, 1-15, 1-20, 1-25, 1-30, 1-35, 1-40, 1-45, 1-50, 1-60, 1-70, 1-80, 1-140, and any intervening increments thereof, comprise at least about 100,000 different peptides, at least about 160,000 different peptides, at least about 180,000 different peptides, at least about 190,000 different peptides, at least about 200,000 different peptides, at least about 220,000 different peptides, at least about 250, different peptides, at least about 260, 000 different peptides, at least about 280,000 different peptides, at least about 300,000 different peptides, at least about 320,000 different peptides, at least about 340,000 different peptides, at least about 360,000 different peptides, at least about 380,000 different peptides, at least about 400,000 different peptides, 420,000, at least about 440,000 different peptides, at least about 460,000 different peptides, or at least about 500,000 different peptides.

It was discovered further that, at pH 2.7, only lysines, arginines, histidines and the amino terminus of a peptide are charged. Trypsin proteolysis produces peptides with a C-terminal lysine or arginine. Thus, most tryptic peptides carry a net solution charge state of 2^+ as shown in Fig. 1a. Because a phosphate group maintains a negative charge at acidic pH values, the net charge state of a phosphopeptide is generally only 1^+. Interestingly, an exhaustive theoretical tryptic digest of the human

protein database from NCBI produced peptides with 68% predicted to have a net charge of 2^+ (Fig. 1b). Any of these peptides would have a net charge state of 1^+ after a single phosphorylation event. Strong cation exchange (SCX) chromatography separates peptides based primarily on ionic charge. The SCX separation of a complex peptide mixture at pH 2.7 generated by trypsin proteolysis is shown in Fig. 1c. Phosphopeptides with a charge state of 1^+ eluted earlier and were greatly enriched from the predominantly nonphosphorylated peptides.

The proteins eluted from the cation exchanger can be concentrated further for analysis by any suitable procedure. In one aspect, concentration is effected using reduced pressure or by heat concentration. Drying can be carried out, if necessary, after the concentration, by heat drying, spray drying or lyophilization.

Detection and Quantitation of Protein Modifications:
Identifying Protein Phosphorylation Sites

In one aspect, phosphorylated peptides are evaluated to determine their identifying characteristics, e.g., such as mass, mass-to-charge (m/z) ratio, sequence, etc. Suitable peptide analyzers include, but are not limited to, a mass spectrometer, mass spectrograph, single-focusing mass spectrometer, static field mass spectrometer, dynamic field mass spectrometer, electrostatic analyzer, magnetic analyzer, quadropole analyzer, time of flight analyzer (e.g., a MALDI Quadropole time-of-flight mass spectrometer), Wien analyzer, mass resonant analyzer, double-focusing analyzer, ion cyclotron resonance analyzer, ion trap analyzer, tandem mass spectrometer, liquid secondary ionization MS, and combinations thereof in any order (e.g., as in a multi-analyzer system). Such analyzers are known in the art and are described in, for example, *Mass Spectrometry for the Biological Sciences*, Burlingame and Carr eds., Human Press, Totowa, NJ).

In general, any analyzer can be used which can separate matter according to its anatomic and molecular mass. Preferably, the peptide analyzer is a tandem MS system (an MS/MS system) since the speed of an MS/MS system enables rapid analysis of low femtomole levels of peptide and can be used to maximize throughput.

In a preferred aspect, the peptide analyzer comprises an ionizing source for generating ions of a test peptide and a detector for detecting the ions generated. The peptide analyzer further comprises a data system for analyzing mass data relating to the ions and for deriving mass data relating to a phosphorylated peptide.

In one preferred aspect, peptides are analyzed by fragmenting the peptide. Fragmentation can be achieved by inducing ion/molecule collisions by a process known as collision-induced dissociation (CID) (also known as collision-activated dissociation (CAD)). Collision-induced dissociation is accomplished by selecting a peptide ion of interest with a mass analyzer and introducing that ion into a collision cell. The selected ion then collides with a collision gas (typically argon or helium) resulting in fragmentation. Generally, any method that is capable of fragmenting a peptide is encompassed within the scope of the present invention. In addition to CID, other fragmentation methods include, but are not limited to, surface induced dissociation (SID) (James and Wilkins, *Anal. Chem. 62*: 1295-1299, 1990; and Williams, et al., *J Amer. Soc. Mass Spectrom. 1*: 413-416, 1990), blackbody infrared radiative dissociation (BIRD); electron capture dissociation (ECD) (Zubarev, et al., *J. Am. Chem. Soc.* 120: 3265- 3266, 1998); post-source decay (PSD), LID, and the like.

The fragments are then analyzed to obtain a fragment ion spectrum. One suitable way to do this is by CID in multistage mass spectrometry (MS^n). Traditionally used to characterize the structure of a peptide and/or to obtain sequence information, it is a discovery of the present invention, that MS^n provides enhanced sensitivity in methods for quantitating absolute amounts of proteins.

Preferably, peptides are analyzed by at least two stages of mass spectrometry to determine the fragmentation pattern of the peptide. More preferably, the fragmentation pattern of phosphorylated and unphosphorylated forms of the peptide is determined. Most preferably, a peptide signature is obtained in which peptide fragments corresponding to phosphorylated and unphosphorylated forms have significant differences in m/z ratios to enable peaks corresponding to each fragment to be well separated. Still more preferably, signatures are unique, i.e., diagnostic of a peptide being identified and comprising minimal overlap with fragmentation patterns of peptides with different amino acid sequences. If a suitable fragment signature is

not obtained at the first stage, additional stages of mass spectrometry are performed until a unique signature is obtained.

The peptide analyzer additionally comprises a data system for recording and processing information collected by the detector. The data system can respond to instructions from processor in communication with the separation system and also can provide data to the processor. Preferably, the data system includes one or more of: a computer, an analog to digital conversion module; and control devices for data acquisition, recording, storage and manipulation. More preferably, the device further comprises a mechanism for data reduction, i.e., to transform the initial digital or analog representation of output from the analyzer into a form that is suitable for interpretation, such as a graphical display (e.g., a display of a graph, table of masses, report of abundances of ions, etc.).

The data system can perform various operations such as signal conditioning (e.g., providing instructions to the peptide analyzer to vary voltage, current, and other operating parameters of the peptide analyzer), signal processing, and the like. Data acquisition can be obtained in real time, e.g., at the same time mass data is being generated. However, data acquisition also can be performed after an experiment, e.g., when the mass spectrometer is off line.

The data system can be used to derive a spectrum graph in which relative intensity (i.e., reflecting the amount of protonation of the ion) is plotted against the mass to charge ratio (m/z ratio) of the ion or ion fragment. An average of peaks in a spectrum can be used to obtain the mass of the ion (e.g., peptide) (see, e.g., McLafferty and Turecek, 1993, *Interpretation of Mass Spectra*, University Science Books, CA).

Mass spectral peaks may be used to identify protein modifications. The decomposition of a precursor ion results in a product ion and a neutral loss. Neutral Loss is the loss of a fragment that is not charged and thus not detectable by a mass spectrometer. The mass of phosphate (80) is lost as a neutral loss from a peptide. When a phosphopeptide enters a mass spectrometer, the first thing lost is the phosphate (as a neutral loss), which gives a characteristic spectrum, particularly in an ion-trap mass spectrometer. Thus neutral loss of phosphate can act as a benchmark

for the presence of phosphopeptides. The control neutral loss is a random mass (in Figure 5B, 101), and is roughly flat as expected because it represents loss arising only from noise. As can be seen in Figures 5A-C, neutral loss events arise more frequently in the earliest fractions collected when performing SCX according to the methods described herein.

Mass spectra can be searched against a database of reference peptides of known mass and sequence to identify a reference peptide which matches a phosphorylated peptide (e.g., comprises a mass which is smaller by the amount of mass attributable to a phosphate group). The database of reference peptides can be generated experimentally, e.g., digesting non-phosphorylated peptides and analyzing these in the peptide analyzer. The database also can be generated after a virtual digestion process, in which the predicted mass of peptides is generated using a suite of programs such as PROWL (e.g., available from ProteoMetrics, LLC, New York; N.Y.). A number of database search programs exist which can be used to correlate mass spectra of test peptides with amino acid sequences from polypeptide and nucleotide databases (i.e., predicted polypeptide sequences), including, but not limited to: the SEQUEST program (Eng, et al., *J. Am. Soc. Mass Spectrom. 5*: 976-89; U.S. Patent No. 5,538,897; Yates, Jr., III, et al., 1996, *J. Anal. Chem. 68(17)*: 534-540A), available from Finnegan Corp., San Jose, *CA.*

Data obtained from fragmented peptides can be mapped to a larger peptide or polypeptide sequence by comparing overlapping fragments. Preferably, a phosphorylated peptide is mapped to the larger polypeptide from which it is derived to identify the phosphorylation site on the polypeptide. Sequence data relating to the larger polypeptide can be obtained from databases known in the art, such as the nonredundant protein database compiled at the Frederick Biomedical Supercomputing Center at Frederick, MD.

In one aspect, the amount and location of phosphorylation is compared to the presence, absence and/or quantity of other types of polypeptide modifications. For example, the presence, absence, and/or quantity of: ubiquitination, sulfation, glycosylation, and/or acetylation can be determined using methods routine in the art (see, e.g., Rossomando, et al., 1992, *Proc. Natl. Acad. Sci. USA 89*: 5779-578; Knight

et al., 1993, *Biochemistry 32*: 2031-2035; U.S. Patent No. 6,271,037 and PCT/US03/07527). The amount and locations of one or modifications can be correlated with the amount and locations of phosphorylation sites. Preferably, such a determination is made for multiple cell states.

Data-Dependent Acquisition Of MS^3 Spectra For Improved Phosphopeptide Identification

In the context of peptide mass spectrometry an MS^2 spectrum and MS^3 spectrum represent, respectively, the measurement of fragment ions derived from a single peptide, and fragment ions derived from a single peptide fragment. Thus, if an MS^2 spectrum of a phosphopeptide results in a dominant phosphate-specific fragment ion, an MS^3 spectrum from that dominant fragment ion can result in a more useful fragmentation pattern.

An MS^3 spectrum was collected when the following conditions were met. i) The MS^2 spectrum revealed a significant loss of phosphoric acid (49 or 98 Da) upon fragmentation. ii) The neutral loss event was the most intense peak in the MS^2 spectrum. Meeting these two criteria is common for phosphopeptides but extremely unlikely for nonphosphorylated peptides. In this way, MS^3 spectra were not acquired unless a phosphopeptide was suspected. An example of such a spectrum is shown in Fig. 2b. Upon fragmentation, this phosphopeptide produced mainly a single intense peak at 49 Da less than the precursor ion *m/z* ratio. This was recognized by software and an MS^3 scan was collected by isolating and fragmenting the neutral loss fragment ion from the MS^2 spectrum. The result was a much richer fragmentation spectrum from which the phosphopeptide sequence could be determined including the modified residue (a serine) because the loss of phosphoric acid converted the serine residue to a dehydroalanine.

The amount of time required to collect both the MS^2 and MS^3 spectra was less than 3 seconds.

Applications

The cell-division-cycle of the eukaryotic cell is primarily regulated by the state of phosphorylation of specific proteins, the functional state of which is determined by whether or not the protein is phosphorylated. This is determined by the relative activity of protein kinases which add phosphate and protein phosphatases which remove the phosphates from these proteins. Lack of function or improper function of either kinases or phosphatases may lead to abnormal physiological responses, such as uncontrolled cell division.

Additionally, many polypeptides such as growth factors, differentiation factors and hormones mediate their pleiotropic actions by binding to and activating cell surface receptors with an intrinsic protein tyrosine kinase activity. Changes in cell behavior induced by extracellular signaling molecules such as growth factors and cytokines require execution of a complex program of transcriptional events. To activate or repress transcription, transcription factors must be located in the nucleus, bind DNA, and interact with the basal transcription apparatus. Accordingly, extracellular signals that regulate transcription factor activity may affect one or more of these processes. Most commonly, regulation is achieved by reversible phosphorylation.

Accordingly, methods of identifying and quantifying phosphorylated proteins, polypeptides, and peptides according to the invention can be used to diagnose abnormal cellular responses including misregulated cell proliferation (e.g., cancer), to determine the activity of growth factors, differentiation factors, hormones, cytokines, transcription factors, signaling molecules and the like. Preferably, the methods are used to correlate activity with a cell state (such as a disease or a state which is responsive to an agent or condition to which a cell is exposed).

Phosphorylated proteins often comprises sequence motifs which when phosphorylated or dephosphorylated promote interaction with target proteins that modulate the activity (i.e., increase or decrease) of either the phosphorylated polypeptide or the target polypeptide. Non-limiting examples of such sequences include FLPVPEYINQSV, a sequence found in human ECF receptor, and AVGNPEYLNTVQ, a sequence found in human EGF receptor, both of which are

autophosphorylated growth factor receptors which stimulate the biochemical signaling pathways that control gene expression, cytoskeletal architecture and cell metabolism, and which interact with the Sen-5 adaptor protein; the p53 sequence EPPLSQEAFADLWKK that when phosphorylated prevents the interaction, and subsequent inactivation of p53 by MDM2. In one aspect, the methods of the invention are used to characterize the frequency of such sequence motifs in a phosphoproteome correlating with a particular cell state. In another aspect, the methods of the invention are used to identify and characterize novel sequence motifs and to further correlate the phosphorylation of such motifs with the activity of a known or novel kinase.

Knowledge of phosphorylation sites can be used to identify compounds that modulate particular phosphorylated polypeptides (either preventing or enhancing phosphorylation, as appropriate, to normalize the phosphorylation state of the polypeptide). Thus, in one aspect, the method described above may further comprise contacting a first cell with a compound and comparing phosphorylation sites/amounts identified in the first cell with phosphorylation sites/amounts in a second cell not contacted with the compound. Suitable cells that may be tested include, but are not limited to: neurons, cancer cells, immune cells (e.g., T cells), stem cells (embryonic and adult), undifferentiated cells, pluripotent cells, and the like. In one preferred aspect, patterns of phosphorylation are observed in cultured cells, capable of transformation to an oncogenic state.

The invention additionally provides a method of screening for a candidate modulator of enzymatic activity of a kinase or a phosphatase, the method comprising contacting a test sample comprising a kinase or phosphatase and a plurality of proteins including a protein comprising a peptide sequence identified as described above, contacting the plurality of proteins with an agent comprising a protease activity, thereby generating a plurality of peptide digestion products, and quantitating the amount of phosphorylated peptide in the sample. The level of phosphorylated peptide in the test sample is compared to levels in a control sample comprising known activities of the kinase/phosphatase to identify candidate modulators which either decrease or increase the activities relative to the baseline established by the control sample and/or which alters the site of phosphorylation in a polypeptide. In one

aspect, the method is used to identify an agonist of a kinase or phosphatase. In another aspect, the method is used to identify an antagonist of a phosphatase or kinase.

Compounds which can be evaluated include, but are not limited to: drugs; toxins; proteins; polypeptides; peptides; amino acids; antigens; cells, cell nuclei, organelles, portions of cell membranes; viruses; receptors; modulators of receptors (e.g., agonists, antagonists, and the like); enzymes; enzyme modulators (e.g., such as inhibitors, cofactors, and the like); enzyme substrates; hormones; nucleic acids (e.g., such as oligonucleotides; polynucleotides; genes, cDNAs; RNA; antisense molecules, ribozymes, aptamers), and combinations thereof. Compounds also can be obtained from synthetic libraries from drug companies and other commercially available sources known in the art (e.g., including, but not limited, to the LeadQuest® library) or can be generated through combinatorial synthesis using methods well known in the art.

Compounds identified as modulating agents are used in methods of treatment of pathologies associated with abnormal sites/levels of phosphorylation. For administration to a patient, one or more such compounds are generally formulated as a pharmaceutical composition. Preferably, a pharmaceutical composition is a sterile aqueous or non-aqueous solution, suspension or emulsion, which additionally comprises a physiologically acceptable carrier (i.e., a non-toxic material that does not interfere with the activity of the active ingredient). More preferably, the composition also is non-pyrogenic and free of viruses or other microorganisms. Any suitable carrier known to those of ordinary skill in the art may be used. Representative carriers include, but are not limited to: physiological saline solutions, gelatin, water, alcohols, natural or synthetic oils, saccharide solutions, glycols, injectable organic esters such as ethyl oleate or a combination of such materials. Optionally, a pharmaceutical composition may additionally contain preservatives and/or other additives such as, for example, antimicrobial agents, anti-oxidants, chelating agents and/or inert gases, and/or other active ingredients.

Routes and frequency of administration, as well doses, will vary from patient to patient. In general, the pharmaceutical compositions is administered intravenously,

intraperitoneally, intramuscularly, subcutaneously, intracavity or transdermally. Between 1 and 6 doses is administered daily. A suitable dose is an amount that is sufficient to show improvement in the symptoms of a patient afflicted with a disease associated an aberrant phosphorylation state. Such improvement may be detected by monitoring appropriate clinical or biochemical endpoints as is known in the art. In general, the amount of modulating agent present in a dose, or produced *in situ* by DNA present in a dose (e.g., where the modulating agent is a polypeptide or peptide encoded by the DNA), ranges from about 1 μg to about 100 mg per kg of host. Suitable dose sizes will vary with the size of the patient, but will typically range from about 10 mL to about 500 mL for 10-60 kg animal. A patient can be a mammal, such as a human, or a domestic animal.

In another aspect, the phosphorylation states (e.g., sites and amount of phosphorylation) of first and second cells are evaluated. In one aspect, the second cell differs from the first cell in expressing one or more recombinant DNA molecules, but is otherwise genetically identical to the first cell. Alternatively, or additionally, the second cell can comprise mutations or variant allelic forms of one or more genes. In one aspect, DNA molecules encoding regulators of a phosphorylatable protein can be introduced into the second cell (e.g., such as a kinase or a phosphatase) and alterations in the phosphorylation state in the second cell can be determined. DNA molecules can be introduced into the cell using methods routine in the art, including, but not limited to: transfection, transformation, electroporation, electrofusion, microinjection, and germline transfer.

Stable isotope labeling with amino acids in cell culture, or SILAC, also is a valuable proteomic technique. Ong, S.E., et al. (2002), *Methods* **29**, 124-130;. Ong, et al. (2003). *J. Proteome Res.* **2**, 173-181. Using SILAC in combination with the methods of the present invention can provide a powerful identification tool. Cells representing two biological conditions can be cultured in amino acid-deficient growth media supplemented with ^{12}C- or ^{13}C-labeled amino acids. The proteins in these two cell populations effectively become isotopically labeled as "light" or "heavy." Upon isolation of proteins from these cells, samples can then be mixed in equal ratios and processed using conventional techniques for tandem mass spectrometry. Because corresponding light and heavy peptides from the same protein will co-elute during

chromatographic separation into the mass spectrometer, relative quantitative information can be gathered for each protein by calculating the ratio of intensities of the two peaks produced in the peptide mass spectrum (MS scan). Furthermore, sequence data can be acquired for these peptides by fragment analysis in the product ion mass spectrum (MS/MS scan) and used for accurate protein identification. Finally, when more than one peptide is identified from the same protein, the quantification is redundant, providing increased confidence in both the identification and quantification of the protein.

System for Analysis of Phosphoproteomes

The present invention also provides a system and software for facilitating the analysis of phosphoproteomes. The invention provides a system that comprises a relational database which stores mass spectral data relating to phoshorylation states for a plurality of proteins in a proteome. The system further comprises a data management program for correlating phosphorylation states to the source of the proteome, e.g., a cell or tissue extract, a patient group, etc.

In one aspect, the data management program comprises a data analysis program for identifying similarities of features of mass spectral signatures for one or more peptides in a plurality of peptides with mass spectral signatures for known peptides. In another aspect, the data analysis program identifies the peptide sequences for one or more peptides in the plurality of peptides. In still another aspect, the plurality of peptides is a mixture of labeled peptides, a first set of peptides labeled with a first label and a second set of peptides labeled with a second label. In a further aspect, the first label has a first mass and the second label has a second, different mass. Preferably, the data analysis system comprises a component for determining the relative abundance of a first labeled peptide with a second labeled peptide. The system is connectable to one or more external databases through a network server.

The invention also provides a method for storing peptide data to a database. The method comprises acquiring mass spectral signatures for one or more peptides in a plurality of peptides. The one or more peptides exist in a phosphorylated form in

one or more cells having a cell state (e.g., a differentiation state, an association with a disease or response to an abnormal physiological condition, response to an agent, and the like). The signatures are stored in a database and correlated with the presence or absence of cell state. Preferably, pairs of signatures associated with both the phosphorylated and unphosphorylated states of the peptides are stored in the database. In one aspect, the mass spectrum signatures are obtained from mass analytical techniques, as described above.

The relational database may comprise a plurality of table or fields that may be interrelated via associations to facilitate searching the database. The database may comprise an object-oriented database, flat file database, data structures comprising linked lists, binary trees and the like. In one aspect, the database comprises a reference collection of mass spectral signatures corresponding to pairs of phosphorylated and unphosphorylated peptides comprising otherwise identical amino acid residues.

Preferably, the system further comprises a data management system. The data management system comprises a data analysis module which preferably interacts with instrumentation (e.g., such as a mass spectrometer) used to determine data features of the phosphorylated peptides obtained from strong cation exchange as described above. The data analysis system identifies peptide constituents from fractions obtained from SCX enriched for phosphorylated peptides and processes the data to obtain sequence information. Functions of the data analysis system include organizing data output, transforming or changing the format of data output, and performing statistical treatment of data. Preferably, the data analysis system interacts with the system database to organize, categorize and store data output comprising peptide signatures of phosphorylatable peptides.

In one aspect, the data analysis system preferably executes computer program code to identify peptides by comparison of mass spectral data with the database of mass spectral signatures. One such program for determining the identity of a peptide by matching tandem mass spectrum data with stored peptide spectra is the SEQUEST peptide identification program developed at the University of Washington

(http://www.washington.edu). Information on the SEQUEST program and system can be found on the Internet at http://thompson.mbt.washington.edu-.

Peptide-correlated output files containing the putative identities of the peptides determined from the spectral data analysis are then returned to the data analysis system for further processing such as correlation with a biological state relating to the proteome from which the peptides were derived (e.g., such as a disease state).

In one aspect, the data analysis system communicates with the system database by way of a communication medium, such as a network server. For example, the system comprises functionality for sending and receiving data through a suitable means, such as a TCP/IP based protocol. The communication medium may additionally provide accessibility to other external databases, e.g., such as genomic databases, pharmacological databases, patient databases, proteomic databases, and the like, such as GenBank, SwissProt, Entrez, PubMed, and the like, to acquire other information which may be associated with the peptides which may be added to the system database.

In another aspect, the data analysis system base identifies peaks or intensity curves corresponding to resolved peptides in a mass spectrum obtained from proteome analysis. The data analysis system further quantitates the amount of a phosphorylatable peptide associated with a particular mass spectral peak. Preferably, the system compares peak data corresponding to the same peptide in a plurality of different proteomes associated with different cell states. The results of such calculations are stored in the system database.

Data obtained from such analyses can be stored in fields of tables comprising the relational database and used to identify differences in the phosphoproteomes of two or more biological samples. In one aspect, for a cell state determined by the differential expression of at least one phosphorylatable protein, a data file corresponding to the cell state will minimally comprise data relating to the mass spectra observed after peptide fragmentation of a peptide internal standard diagnostic

of the protein. Preferably, the data file will include a data field for a value corresponding to the level of protein in a cell having the cell state.

For example, a tumor cell state is associated with the overexpression of p53 (see, e.g., Kern, et al., 2001, Int. *J. Oncol.* 21(2): 243-9). The data file will comprise mass spectral data observed after fragmentation of a labeled peptide internal standard corresponding to a subsequence of p53. Preferably, the data file also comprises a value relating to the level of p53 in a tumor cell. The value may be expressed as a relative value (e.g., a ratio of the level of p53 in the tumor cell to the level of p53 in a normal cell) or as an absolute value (e.g., expressed in nM or as a % of total cellular proteins). Most preferably, the data file comprises data relating to the phosphorylation state of the peptide (e.g., presence and amount of phosphorylation). Accordingly, in another aspect, one or more data fields may exist defining one or more phosphorylation sites for a protein, as well as data fields for defining an amount of protein in the sample phosphorylated at a given site.

These tables can be generated using database programming language known in the art, including, but not limited to, SQL or MySQL, in order to permit the fields and information stored in these Tables to be flexibly associated. Preferably, organization of data in the database permits search, query, and processing routines implemented by the data analysis system to associate mass spectrum peaks with one or more attributes of a protein such as amino acid sequence, phosphorylation state, mass, mass-to-charge ratio, amount of protein in a sample, and also preferably with one or more characteristics of a sample from which the mass spectrum peaks derive.

Such characteristics include characteristics relating to the sample source, including, but not limited to: presence of a disease; absence of a disease; progression of a disease; risk for a disease; stage of disease; likelihood of recurrence of disease; a genotype; a phenotype; exposure to an agent or condition; a demographic characteristic; resistance to agent, and sensitivity to an agent (e.g., responsiveness to a drug). In one aspect, the agent is selected from the group consisting of a toxic substance, a potentially toxic substance, an environmental pollutant, a candidate drug,

and a known drug. The demographic characteristic may be one or more of age, gender, weight; family history; and history of preexisting conditions.

The use of the relational database provides a means of interrelating data obtained from a plurality of different proteome evaluations. Preferably, database records are configured for automated searching and extraction of data in response to queries for proteins having similar data fields. In one aspect, data analysis includes determining a correlation coefficient or confidence score which is used to order the results based on the degree of confidence with which the peptide identification and/or comparison is made. Correlation coefficients may then be stored in the database. While correlation coefficients are usually scalar numbers between 0.0 and 1.0, correlation data may alternatively comprise correlation matrices, p-values, or other similarity metrics

Object-oriented databases, which are also within the scope of the invention. Such databases include the capabilities of relational databases but are capable of storing many different data types including images of mass spectral peaks. See, e.g., Cassidy, High Performance Oracle8 SQL Programming and Tuning, Coriolis Group (March 1998), and Loney and Koch, Oracle 8: The Complete Reference (Oracle Series), Oracle Press (September 1997), the contents of which are hereby incorporated by reference into the present disclosure.

Neural network analysis of a spectrum can be performed to aid in the identification of proteomic differences and to determine correlations between these differences and one or more sample characteristic. In a neural network processing program, information is analyzed by methods such as pattern recognition or data classification. The neural network is an adaptive system that "learns" or creates associations based on previously encountered data input. Preferably rules and output of neural network analysis are also stored within the database, permitting the database to grow dynamically as more and more phosphoproteomes are evaluated.

Classification models and other pattern recognition methods can be used to identify phosphorylatable proteins that are diagnostic of at least one characteristic of a

sample source. Classification models can be trained using the output from analysis of multiple samples to classify phosphorylated proteins into classes in which different phosphorylated proteins are weighted according to their ability to be diagnostic of a characteristic of a sample from which the proteins derive (e.g., such as the presence of a disease in a sample source). Classification methods may be either supervised or unsupervised. Supervised and unsupervised classification processes are known in the art and reviewed in Jain, *IEEE Transactions on Pattern Analysis and Machine Intelligence* 22 (1): 4-37, 2000, for example. Data mining systems utilizing such classification methods are known in the art.

Computer program code for data analysis may be written in programming language known in the art. Preferred languages include C/C++, and JAVA®. In one aspect, methods of this invention are programmed in software packages which allow symbolic entry of equations, high-level specification of processing, and statistical evaluations.

In one aspect, the system comprises an operating system in communication with each of the computer memory comprising the database and the computer memory comprising the data analysis system (the two may be the same or different). The operating system may be any system known in the art such as UNIX or WINDOWS. Preferably, the system further includes any hardware and software necessary for generating a graphical user interface on at least one user device connectable to the network using a communications protocol, such as a TCIP/IP protocol. In one aspect, the at least one user device is a wireless device.

The user device does not need to have computing power comparable to that of the database server and/or the data analysis server (the two may be the same or different servers); however, preferably, the user device is capable of displaying multiple graphical windows to a user.

The invention also provides a method for correlating a cell state associated with the expression profile of a phosphorylatable protein with the expression of a test protein using system as described above. The expression profile of the phosphorylatable protein comprises information relating to at least the

phosphorylation state of at least one phosphorylation site of the phosphorylatable protein in a sample. The profile further may comprise information relating to one or more of: levels of the phosphorylatable protein and information relating to a modification of at least one other modifiable site (e.g., such as information relating to phosphorylation at a second phosphorylation site). The method is implemented by a system processor in communication with a database and data analysis system as described above. Preferably, the system processor is further in communication with a graphical user interface allowing a user to selectively view information relating to a diagnostic fragmentation signature and to obtain information about a cell state. The interface may comprise links allowing a user to access different portions of the database by selecting the links (e.g. by moving a cursor to the link and clicking a mouse or by using a keystroke on a keypad). The interface may additionally display fields for entering information relating to a sample being evaluated.

Reagents and Kits

The invention additionally provides kits for rapid and quantitative analysis of phosphoproteins in a sample. In one aspect, a kit comprises pairs of peptides identical except for the presence of phosphorylation at one or more amino acid residues of the peptides. Preferably, one or both members of the pair comprises a label. In one aspect, the label comprises a stable isotope. Suitable isotopes include, but are not limited to, ^{2}H, ^{13}C, ^{15}N, ^{17}O, ^{18}O, or ^{34}S. In another aspect, pairs of peptide internal standards are provided, comprising identical peptide portions but distinguishable labels, e.g., peptides may be labeled at multiple sites to provide different heavy forms of the peptide. Pairs of peptide internal standards corresponding to phosphorylated and unphosphorylated peptides also can be provided.

In one aspect, a kit comprises peptide internal standards comprising different peptide subsequences from a single protein. In another aspect, the kit comprises peptide internal standards corresponding to sets of related proteins, e.g., such as proteins involved in a molecular pathway (a signal transduction pathway, a cell cycle, etc), or which are diagnostic of particular disease states, developmental stages, tissue types, genotypes, etc. Peptide internal standards corresponding to a set may be

provided in separate containers or as a mixture or "cocktail" of peptide internal standards.

In one aspect, a plurality of peptide internal standards representing a MAPK signal transduction pathway is provided. Preferably, the kit comprises at least two, at least about 5, at least about 10 or more, of peptide internal standards corresponding to any of MAPK, GRB2, mSOS, ras, raf, MEK, p85, KHS1, GCK1, HPK1, MEKK 1-5, ELK1, c-JUN, ATF-2, 3APK, MLK1-4, PAK, MKK, p38, a SAPK subunit, hsp27, and one or more inflammatory cytokines.

In another aspect, a set of peptide internal standards is provided which comprises at least about two, at least about 5 or more, of peptide internal standards which correspond to proteins selected from the group including, but not limited to, PLC isoenzymes, phosphatidylinositol 3-kinase (PI-3 kinase), an actin-binding protein, a phospholipase D isoform, (PLD), and receptor and nonreceptor PTKs.

In another aspect, a set of peptide internal standards is provided which comprises at least about 2, at least about 5, or more, of peptide internal standards which correspond to proteins involved in a JAK signaling pathway, e.g., such as one or more of JAK 1-3, a STAT protein, IL-2, TYK2, CD4, IL-4, CD45, a type I interferon (IFN) receptor complex protein, an IFN subunit, and the like.

In a further aspect, a set of peptide internal standards is provided which comprises at least about 2, at least about 5, or more of peptide internal standards which correspond to cytokines. Preferably, such a set comprises standards selected from the group including, but not limited to, pro-and anti-inflammatory cytokines (which may each comprise their own set or which may be provided as a mixed set of peptide internal standards).

In still another aspect, a set of peptide internal standards is provided which comprises a peptide diagnostic of a cellular differentiation antigen or CD. Such kits are useful for tissue typing.

Peptide internal standards may include peptides corresponding to one or more of the peptides listed in the tables herein.

In one aspect, the peptide internal standard comprises a label associated with a phosphorylated amino acid. In another aspect, a pair of reagents is provided, a peptide internal standard corresponding to a modified peptide and a peptide internal standard corresponding to a peptide, identical in sequence but not modified.

In another aspect, one or more control peptide internal standards are provided. For example, a positive control may be a peptide internal standard corresponding to a constitutively expressed protein, while a negative peptide internal standard may be provided corresponding to a protein known not to be expressed in a particular cell or species being evaluated. For example, in a kit comprising peptide internal standards for evaluating a cell state in a human being, a plant peptide internal standard may be provided.

In still another aspect , a kit comprises a labeled peptide internal standard as described above and software for analyzing mass spectra (e.g., such as SEQUEST).

Preferably, the kit also comprises a means for providing access to a computer memory comprising data files storing information relating to the diagnostic fragmentation signatures of one or more peptide internal standards. Access may be in the form of a computer readable program product comprising the memory, or in the form of a URL and/or password for accessing an internet site for connecting a user to such a memory. In another aspect, the kit comprises diagnostic fragmentation signatures (e.g., such as mass spectral data) in electronic or written form, and/or comprises data, in electronic or written form, relating to amounts of target proteins characteristic of one or more different cell states and corresponding to peptides which produce the fragmentation signatures.

The kit may further comprise expression analysis software on computer readable medium, which is capable of being encoded in a memory of a computer having a processor and capable of causing the processor to perform a method comprising: determining a test cell state profile from peptide fragmentation patterns in a test sample comprising a cell with an unknown cell state or a cell state being verified; receiving a diagnostic profile characteristic of a known cell state; and comparing the test cell state profile with the diagnostic profile.

In one aspect, the test cell state profile comprises values of levels of phosphorylated peptides in a test sample that correspond to one or more peptide internal standards provided in the kit. The diagnostic profile comprises measured levels of the one or more peptides in a sample having the known cell state (e.g., a cell state corresponding to a normal physiological response or to an abnormal physiological response, such as a disease).

Preferably, the software enables a processor to receive a plurality of diagnostic profiles and to select a diagnostic profile that most closely resembles or "matches" the profile obtained for the test cell state profile by matching values of levels of proteins determined in the test sample to values in a diagnostic profile, to identify substantially all of a diagnostic profile which matches the test cell state profile.

In another aspect, the kit comprises one or more antibodies which specifically react with one or more peptides listed in the tables herein. In one aspect, a kit is provided which comprises an antibody which recognizes the phosphorylated form of a peptide listed in Table 1 but which does not recognize the unphosphorylated form. Preferably, the antibody does not universally recognize phosphorylated proteins, i.e., the antibody also specifically recognizes the amino acid sequence of the peptide rather than recognizing all peptides comprising phosphotyrosine. In one aspect, pairs of antibodies are provided - an antibody which recognizes the phosphorylated form of a peptide and not the unphosphorylated form and an antibody which recognizes the unphosphorylated form. In another aspect, the invention provides an array of antibodies specific for different phosphorylation states of a plurality of proteins in a phosphoproteome. The array can be used to monitor kinase activity and/or phosphatase activity in a phosphoproteome and as a means of evaluating the activity of one or more proteins in a cellular pathway such as a signal transduction pathway. The presence of phosphorylated proteins and level of reactivity of the antibodies can be used to monitor the site specificity and amount of phosphorylation in a sample.

Panels of antibodies can be used simultaneously to perform the analysis (e.g., by using antibodies comprising distinguishable labels). Panels of antibodies also can be used in parallel or in sequential assays. Therefore, in one preferred aspect, a kit according to the invention comprises a panel of antibodies comprising antibodies

specific for phosphorylated peptides/polypeptides phosphorylated at one or more sites.

The presence, absence, level, and/or site-specificity of other types of modifications, such as ubiquitination, also can be determined along with the presence, absence, level and/or site specificity of phosphorylation.

Examples

The invention will now be further illustrated with reference to the following example. It will be appreciated that what follows is by way of example only and that modifications to detail may be made while still falling within the scope of the invention.

Example 1.

Tandem mass spectrometry (MS/MS) provides the means to determine the amino acid sequence identity of peptides directly from complex mixtures (Peng and Gygi, *J. Mass Spectrometry* 36: 1083-1091, 2001). In addition, the precise sites of modifications (e.g., acetylation, phosphorylation, etc.) to amino acid residues within the peptide sequence can be determined.

Organelle-specific proteomics provides the ability to i) more comprehensively determine the components by enriching for proteins of lower abundance, ii) study mature (functional) protein, and iii) evaluate proteomics within the boundaries of cellular compartmentalization. In the present example, the isolation, separation, and large-scale amino acid sequence analysis of the HeLa cell nucleus is described. Nuclear proteins were separated by preparative SDS-PAGE. Twenty gel slices were proteolyzed with trypsin and separated by off-line strong cation exchange (SCX) chromatography and fraction collection. Each fraction was subsequently analyzed via an automated vented column approach (Licklider, et al., *Anal. Chem.* 74: 3076-3083, 2001) by nano-scale microcapillary LC-MS/MS in a 2-hour gradient. The analysis of slices 9 and 14 is discussed further below.

SDS-PAGE Separation Of Nuclear Protein.

HeLa cells were harvested and nuclear protein obtained as described (McCraken, et. al., *Genes and Dev.* 11: 3306-3318, 1997). Ten mg of nuclear protein was separated on a 10% polyacrylamide preparative gel with a 4 cm stack. The gel was then lightly stained with Coomassie and cut into 20 slices for in-gel digestion

with trypsin as described. Following digestion, complex peptide extracts were dried in a speed-vac and stored at –80°C.

SCX Chromatography With Fraction Collection

For the SCX chromatography (Alpert and Andrews, *J. Chromatogr.* 443: 85-96, 1988), a commercially packed 2.1 mm x 150 mm polysulfoethyl aspartamide column (PolyLC, Columbia, MD) was used with an in-line guard column of the same material. Buffer A was 5 mM KH_2PO_4/25% acetonitrile (ACN), pH 2.7; Buffer B was the same as A with 350 mM KCl added. Following setup of the HPLC with the correct buffers and column, the flow rate was set to 200 µl/min, and a blank gradient was acquired followed by an analysis of standard peptides. A shallow gradient in the area from 5% to 35 % buffer B was implemented. The acidified peptide sample was loaded onto the column and 200 µl fractions were collected every minute. Eighty fractions were collected from the SCX analysis of both Slice 9 and 14. Following this stage of analysis, fractions were reduced in volume to ~50-100 µl by centrifugal evaporation in order to remove most of the acetonitrile permitting peptides to adsorb to the RP column.

RP Chromatography Of SCX Chromatography Fractions And Identification Of Protein

All fractions from slice 9 and 14 were analyzed in a completely automated fashion using a vented column approach (Licklider, et al., 2001, *supra*). Sample was loaded via an Endurance autosampler (Michrom BioResources, Inc) onto a 75 micron i.d. V-column. A gradient was developed by a Surveyor HPLC (ThermoFinnigan) with on-line elution into an ion trap mass spectrometer (LCQ-DECA, ThermoFinnigan) as described (Peng and Gygi, 2001, *supra*). Approximately 4000 MS/MS spectra were collected from each 2 hr analysis. All tandem mass spectra were searched against the human database (ftp://ftp.ncbi.nih.gov/blast/db/FASTA/) with the Sequest algorithm (Eng, et al., *J. Am. Soc. Mass Spectrometry* 5: 976-989, 1994).

Peptides were searched with no enzyme specificity and oxidized methionines and modified cysteines were considered. Peptide matches were filtered according to

the following criteria: a returned peptide must be 1) fully tryptic, 2) have an Xcorr of 2.0, 1.8, and 3.0 or greater for singly, doubly, and triply charged peptides respectively, and 3) have a delta-correlation of 0.08 or greater. Next, peptides meeting this criteria were examined for redundancy within the database using a new algorithm named Dredge. Dredge makes a second pass through the database in an attempt to untangle the relationship between peptide sequence and protein identity. In addition, Dredge calculates the minimum (and maximum) number of proteins from which the peptide set identified could have originated. The minimum number of proteins is the value reported here. Non-unique peptides (peptides belonging to one or more proteins) were assigned to the protein with the largest number of peptides. Finally, proteins identified by only a single peptide were manually verified (Peng, et al., 2003, A proteomics approach to understanding protein ubiquitination. *Nat. Biotech.* In press.; Peng, et al., J. *Proteome Res.* 2: 43-50, 2002).

Massive separation of nuclear proteins was obtained. More than 2000 proteins were identified from the analysis of two gel regions. Additionally, modified peptides (i.e., phosphorylated and acetylated proteins) were also found in abundance. The analysis of the remaining regions should provide nearly universal coverage of nuclear proteins.

Table 1. Summary Of The Analysis Of Slice 9 And Slice 14 From The SDS-PAGE Gel.

# fractions	60	80	140
# MS/MS	189,000	266,000	455,000
# Total peptides	10256	49591	59857
# Unique proteins	939	1963	2902
Average MW	97.3	49.7	N/A

Example 2.

In this experiment, the characterization of phosphoproteins from asynchronous HeLa cells was performed. Because of the complexity of the sample, the proteins present in a nuclear fraction were examined and a preparative SDS-PAGE separation was applied to allow milligram quantities of starting protein (FIG. 6A). The entire gel was excised into 10 regions and proteolyzed with trypsin followed by phosphopeptide enrichment by SCX chromatography. Early-eluting fractions were subjected to further analysis by reverse-phase liquid chromatography with on-line sequence analysis by tandem mass spectrometry (LC-MS/MS).

More than 12,000 MS^3 spectra were also acquired during the course of the experiment and used to help compliment database searches and manual interpretation of phosphorylation sites.

In total, 2,002 different phosphorylation sites were identified by the Sequest algorithm and each site was manually confirmed using in-house software by three different people. Matches were only deemed correct when they met exacting criteria such as the presence of intense proline-directed fragment ions, possession of the correct net solution charge state and good agreement in molecular weight of the parent protein and the region excised from the gel. The entire list of 2,002 sites is provided in Table 4.

METHODS

HeLa cell nuclear preparation, preparative SDS-PAGE separation and in-gel proteolysis

HeLa cell nuclear preparation was as described. Dignam, J. D., et al., *Nucleic Acids Res* **11**, 1475-89 (1983). Protein (8 mg) was separated by a preparative SDS-PAGE gradient (5-15%) gel. The gel was stopped when the buffer front reached 4 cm and stained with coomassie. The entire gel was then cut into ten regions, diced into small pieces (~1 mm^3), and placed in 15 ml falcon tubes. In-gel digestion with trypsin proceeded as described but with larger volumes. Shevchenko, A., et al., *Analytical*

Chemistry **68**, 850-8 (1996). Extracts were completely dried in a speed vac and stored at -20°C.

Strong Cation Exchange (SCX) Chromatography

Extracted peptides were redissolved in 500 µl SCX Solvent A immediately prior to analysis. Tryptic peptides were separated at pH 2.7 by SCX chromatography using a 3.0 mm x 20 cm column (Poly-LC) containing 5 µm polysulfoethyl aspartamide beads with a 200 Å pore size as described. Peng, J., et al., *J Proteome Res* **2**, 43-50 (2003). This column provided the best retention of singly-charged phosphopeptides. Fractions were collected every minute during a 60 minute gradient. Four fractions spanning the early-eluting peptides were desalted offline and completely dried. Rappsilber, J., et al., *Anal Chem* **75**, 663-70 (2003).

Mass Spectrometry

Early-eluting fractions were subsequently analyzed by reverse-phase LC-MS/MS using 75 µm inner diameter × 12 cm self-packed fused-silica C18 capillary columns as described. Peptides were eluted for each analysis using a 6-hr gradient in which the ions were detected, isolated and fragmented in a completely automated fashion on an LCQ DECA XP ion trap mass spectrometer (Thermo Finnigan, San Jose, CA). In addition, software to allow for the acquisition of a data-dependent MS^3 scan was produced and implemented through a collaboration with ThermoFinnigan. An MS^3 spectrum was automatically collected when the most intense peak from the MS^2 spectrum corresponded to a neutral loss event of 98 m/z, 49 m/z.

Database Correlation

All MS^2 and MS^3 spectra were searched against the non-redundant human database from NCBI (downloaded Aug. 2003) using the Sequest algorithm. Eng, J., et al., *J. Am. Soc. Mass Spectrom.* **5**, 976-989 (1994). Modifications were permitted to allow for the detection of oxidized methionine (+16), carboxyamidomethylated

cysteine (+57), and phosphorylated serine, threonine and tyrosine (+80). All peptides matches were filtered and then manually validated with the aid of in-house software.

Classification And Bioinformatic Analysis Of Phosphorylation Sites

The ability of a protein kinase to carry out the phosphorylation reaction of a protein is highly related to the primary amino acid sequence surrounding the site of interest. Protein kinases can be separated into serine/threonine and tyrosine kinases, although dual specificity kinases exist. The sites detected from our nuclear preparation were entirely serine and threonine with no tyrosine phosphorylation detected. Tyrosine phosphorylation is generally thought to represent <1% of all cellular phosphorylation, but it is not clear what fraction of nuclear proteins are targets of tyrosine phosphorylation.

Serine/threonine protein kinases can be further subdivided based on substrate specificity which has been determined for a number of kinases by phosphorylation of soluble peptide libraries. Obenauer, J. C., et al., *Nucleic Acids Res* **31**, 3635-41 (2003); O'Neill, T. et al., *J Biol Chem* **275**, 22719-27 (2000). Major groups include proline-directed (e.g., Erk1, Cdk5, Cyclin B/Cdc2, etc.), basophilic (PKA, PKC, Slk1, etc.) and acidiphilic (CK 1 delta, CK 1 gamma, CK II) kinases. Fig. 3a shows that proline-directed and acidiphilic sites accounted for 77% of all detected phosphorylation. In addition, the sites detected can be categorized by their biological function (FIG. 8B). Consistent with our preparation, most sites detected were nuclear in origin or from other organelles known to be present in nuclear preparations (mitochondria, endoplasmic reticulum). Finally, numerous protein kinases and transcription factors were identified demonstrating the sensitivity of the analysis. Table 2 shows 62 phosphorylation sites from 28 protein kinases detected in this study. Only six of these sites had been described previously.

Table 2

Phosphorylation Sites Determined From Protein Kinases Detected In This Study.

Protein Name	Gene name	Peptide [4]
Cell division cycle 2-like 1	AF067512 [1]	EYGS*PLKAYT*PVVVTLWYR
Tousled-like kinase 1	AF162666 [1]	ISDYFEYQGGNGSS*PVR
Tousled-like kinase 2	AF162667 [1]	ISDYFEFAGGSAPGTS*PGR
PAS-kinase	AF387103 [1]	GLSS*GWSSPLLPAPVCNPNK
Cell division cycle 2-like 5	AJ297709 [1]	GGDVS*PSPYSSSSWR
		S*PS*PAGGGSSPYSR
		S*PSYSR
		SLS*PLGGR
Unknown protein kinase	AK001247 [1]	EGDPVSLSTPLETEFGSPSELS*PR
		LSPDPVAGSAVSQELREGDPVSL...SELS*PR
		VFPEPTES*GDEGEELGLPLLSTR
Cdc2-related PITSLRE alpha 2-1	E54024 [2]	DLLSDLQDIS*DSER
Serine/threonine protein kinase	G01025 [2]	VPAS*PLPGLER
Mitogen-and stress-activated protein kinase-1	T13149 [2]	LFQGYS*FVAPSILFK
Serine-protein kinase ATM	ATM_HUMAN [3]	SLAFEEGS*QSTTISSLSEK
Cell division protein kinase 2	CDK2_HUMAN [3]	IGEGT*YGVVYK
Cell division cycle 2-related protein kinase 7	CRK7_HUMAN [3]	AIT*PPQQPYK
		GS*PVFLPR
		NSS*PAPPQPAPGK
		QDDSPSGASYGQDYDLS*PSR
		S*PGSTSR
		SPS*PYSR
		SVS*PYSR
		TVDS*PK
Protein kinase C, delta type	KPCD_HUMAN [3]	NLIDSMDQSAFAGFS*FVNPK
B-Raf proto-oncogene serine/threonine-protein kinase	RAB_HUMAN [3]	GDGGSTTGLSAT*PPASLPGSLTNVK
		SAS*EPSLNR
Megakaryocyte-associated tyrosine-protein kinase	MATK_HUMAN [3]	SAGAPASVSGQDADGSTS*PR
Dual specificity mitogen-activated protein kinase kinase 2	MPK2_HUMAN [3]	LNQPGT*PTR
3-phosphoinositide dependent protein kinase-1	PDPK_HUMAN [3]	ANS*FVGTAQYVSPELLTEK
Protein kinase C-like 1	PKL1_HUMAN [3]	TDVSNFDEEFTGEAPTLS*PPR
Protein kinase C-like 2	PKL2_HUMAN [3]	AS*SLGEIDESSELR
		TST*FCGTPEFLAPEVLTETSYTR
Serine/threonine-protein kinase PRP4 homolog	PR4B_HUMAN [3]	DAS*PINRWS*PTR
		EQPEMEDANS*EKS*INEENGEVSEDQSQNK
		S*LS*PKPR
		S*PIINESR
		S*PVDLR
		S*RS*PLLNDR
		SINEENGEVS*EDQS*QNK
		TLS*PGR
		TRS*PS*PDDILER
		YLAEDSNMSVPSEPSS*PQSSTR
DNA-dependent protein kinase catalytic subunit	PRKD_HUMAN [3]	LTPLPEDNS*MNVDQDGDPSDR
Serine/threonine protein kinase 10	STKA_HUMAN [3]	QVAEQGGDLS*PAANR
Wee1-like protein kinase	WEE1_HUMAN [3]	SPAAPYFLGSSFS*PVR
Mitogen-activated protein kinase kinase kinase kinase 1	M4K1_HUMAN [3]	DLRS*SS*PR
Mitogen-activated protein kinase kinase kinase kinase 4	M4K4_HUMAN [3]	AASSLNLS*NGETESVK
		TTS*RS*PVLSR
Mitogen-activated protein kinase kinase kinase kinase 6	M4K6_HUMAN [3]	LDSS*PVLSPGNK
Casein Kinase I, epsilon isoform	KC1E_HUMAN [3]	IQPAGNTS*PR
Phosphorylase B kinase, beta regulatory chain	KPBB_HUMAN [3]	QSST*PSAPELGQQPDVNISEWK

[1]Accession number derived from GenBank (NCBI). [2]Accession number derived from the Protein Information Resource (PIR). [3] Accession number derived from SwissProt human database. [4]Site of phosphorylation noted by asterisk (*).

The computer algorithm, Scansite (Obenauer, J. C., et al., *Nucleic Acids Res* 31, 3635-41 (2003)), makes use of soluble peptide library phosphorylation data to create matrices useful for the prediction of a linear amino acid sequence as a substrate for recognition by a specific kinase. Table 3 shows the results of correlating the linear sequences surrounding the sites identified by this study against the known matrices at the highest stringency level (0.002) and a lower stringency level (0.01).

Table 3

Scansite Prediction At Highest Stringency (0.2%) And Medium Stringency (1.0%) For Kinase Phosphorylation And Binding Motifs From This Dataset

Kinase	Type	Hits (0.2 %)	Hits (1.0 %)
Casein Kinase 2	Acidiphilic	65	172
GSK3	Proline-directed	64	206
CDC2	Proline-directed	55	262
AKT	Basophilic	53	122
Erk1	Proline-directed	51	235
CDK5	Proline-directed	49	260
P38 map kinase	Proline-directed	33	160
Protein Kinase A	Basophilic	17	48
Clk2	Basophilic	11	72
DNA-PK	Glutamine-directed	8	62
Cam Kinase 2	Basophilic	7	21
ATM	Glutamine-directed	6	23
PKC delta	Basophilic	2	9
PKC alpha/beta/gamma	Basophilic	1	7
Protein Kinase C epsilon	Basophilic	1	8
Casein Kinase 1	Other	0	23
Protein Kinase D	Basophilic	0	5
14-3-3 binding motif	Proline-directed	31	85
PDK1 binding motif	Proline-directed	2	3

At the highest stringency, Scansite predicted a significant number of phosphorylation sites within our dataset from each of the proline-directed kinases, the basophilic kinases (AKT, PKA, and Clk2), the acidiphilic kinase Casein kinase 2, and

the DNA damage activated kinases ATM and DNA-PK. It is also possible to use Scansite matrices to predict sites which require phosphorylation to become suitable binding domains. Our dataset included several known 14-3-3 binding sites, as well as two known PDK1 binding sites from protein kinase C delta and p90RSK. However, only a fraction of the total number of detected sites could be assigned with high confidence by Scansite suggesting that many more kinase motifs are present in our dataset.

With a dataset of this magnitude it is possible to begin to classify phosphorylation sites into specific motifs. To evaluate potential kinase motifs within such a large dataset, the relative occurrence of each amino acid (including pSer/pThr) flanking the site of phosphorylation was calculated and plotted using intensity maps. An examination of the entire dataset (FIG. 8C) revealed that a proline at the +1 position and/or a glutamic acid at position +3 were favored. To further elucidate significant flanking residues, the same maps were generating considering data which conformed to either pSer/pThr – Pro containing sites (FIG. 8D), pSer/pThr – Xxx – Xxx Glu/Asp/pSer containing sites (FIG. 8E), or the subset of all data which did not conform to either general classification (FIG. 8F).

Several further insights into kinase motifs can be made from the plots. For example, in FIG. 8E which shows the acidic residue at +3, it can be seen that an aspartic acid residue is highly favored at position +1 in this subset. Although this was not predicted by the soluble peptide libraries (Songyang, Z. et al., *Mol Cell Biol* 16, 6486-93 (1996)), a propensity for aspartic acid at the +1 position of Casein kinase 2 sites has been reported (Meggio, F., et al., *Faseb J* 17, 349-68 (2003)). In the proline-directed subset (FIG. 8D) additional prolines at the +2 and +3 position as well as serine at -3 and arginine at -2 are favored.

DISCUSSION

In eukaryotic cells, protein kinases add a phosphate moiety in an ATP-dependent manner to a serine, threonine, or tyrosine residue of a substrate protein. In addition to a critical role in normal cellular processes, malfunctions in protein

phosphorylation have been implicated in the causation of many diseases such as diabetes, cancer, and Alzheimer's disease. With more than 500 members and thousands of potential substrates, human protein kinases remain attractive drug targets, yet the therapeutic promise of intervention in protein phosphorylation systems remains almost entirely unrealized.

The method described here exploits a differential solution state charge of most tryptic phosphopeptides when compared with their nonphosphorylated counterparts. Because SCX chromatography separates peptides primarily based on charge, phosphopeptides containing a single basic group elute first and are highly enriched. The enriched phosphopeptides are then "sequenced" by reverse-phase LC-MS/MS with a new data-dependent acquisition of an MS^3 scan whenever a phosphopeptide is suspected. In this way, large numbers of phosphopeptides can be isolated, separated, and sequence-analyzed in an automated fashion. The identification of 2,002 phosphorylation sites from a HeLa cell nuclear preparation is provided to demonstrate the technique. This is the largest dataset of post-translational modifications ever determined.

Multidimensional chromatography often plays a key role in proteome analysis strategies. SCX chromatography is the most common primary separation tool prior to analysis by reverse-phase LC-MS/MS. The strategy reported here utilized off-line SCX chromatography with fraction collection. Because tryptic phosphopeptides eluted early (FIG. 6C), it is unlikely that these peptides would be amenable to analysis by on-line SCX chromatography utilizing "salt bumps".

This dataset provides new bioinformatic opportunities to study and predict kinase-substrate relationships. The intensity maps in Figure 8 provide some insight into sequence specific trends surrounding each phosphorylation site. Proline-directed and acidiphilic kinases make up a large fraction of our dataset.

The SCX isolation method has the caveat that some sites are not amenable to analysis. Specifically, a histidine-containing phosphopeptide would elute as a 2^+ peptide. Similarly a doubly-phosphorylated tryptic peptide with only two basic sites

would have a net charge state of zero. In essence, any phosphorylated peptide with a charge state other than 1^+ would not be detected by the method as implemented in this example. Importantly, the majority of phosphopeptides are predicted to be amenable to isolation via SCX chromatography (FIG. 6B).

The methodology of this invention significantly enhances the ability to routinely discover large numbers of phosphorylated species within complex protein mixtures by exploiting peptide solution charge states generated by tryptic digests. Enrichment by offline SCX chromatography increases the likelihood of selecting phosphorylated peptides for sequencing in the mass spectrometer, while data-dependent MS^3 software aids in confirming sequence and phosphorylation site location. Finally, the combination of stable isotope labeling with the methods described here would allow for a large-scale comparative phosphorylation analysis of different cell states where several hundred phosphorylation sites could be simultaneously profiled.

The methods of the present invention also are suitable for the identification of the N-terminal peptide of most proteins after trypsin digestion. This is because an acetylated N terminus will produce a peptide with a solution charge state of 1+ at pH 3 after trypsin digestion. These peptide are co-eluting with the phosphopeptides and can be detected in the same regions of the chromatogram. In the example below, the N-terminal peptide from more than 400 yeast proteins are sequenced. Because the N terminus is only acetylated about 50% of the time *in vivo*, the N termini were chemically modified by d3-acetylation. In this way, it can be determined i) whether or not the protein was present in a blocked (acetylated) state, and ii) whether or not the initiator methionine residue was cleaved. Tables 5A and 5B contain the list of proteins, their starting residues, and acetylation state.

Example 3.

Determining N-terminal Sequences And N-terminal Modifications Of Proteins From *Saccharomyces cerevisiae* On A Large Scale

S. cerevisiae strain S288C was grown on YPD-medium (Becton and Dickinson) at 30°C to midlog phase (OD_{600} of 1). Approximately $3x10^9$ cells were

harvested by centrifugation and the cell-pellet was resuspended in lysis buffer (50 mM Tris-HCl, pH 7.6, 0.1% SDS, 5mM EDTA, and a protease inhibitor cocktail: 2 µg/ml aprotinin; 10 µg/ml leupeptin, soybean trypsin inhibitor, and pepstatin; 175 µg/ml phenylmethylsulfonyl fluoride) and lysed using a French press. About 1 mg proteins from the obtained yeast whole cell lysate were separated on a 12 % SDS-PAGE gel. The gel was cut into 5 slices and the proteins were in-gel modified as described in the following: reduction with 10 mM DTT (pH 8.0) at 56°C, alkylation of Cys-residues with 55 mM iodoacetamide (pH 8.0) at RT in the dark, and d_3-acetylation of unblocked amino groups with 50 mM NH_4HCO_3 (pH 8.0)/MeOH/d_6-acetic anhydride (Sigma) 56:22:22 (v/v/v) at RT. Thevis, M. *et al.* (2003) *J. Proteome Res.* 2, 163-172.

The proteins were finally in-gel digested with modified trypsin (Promega), the peptides were extracted from the gel, and the peptides from each of the 5 gel slices were subjected individually to strong cation-exchange (SCX) chromatography on a 2.1 x 200 mm Polysulfoethyl A column (Poly LC) using a liquid phase from Buffer A (5 mM KH_2PO_4 pH 2.7, 33% ACN) and Buffer B (5 mM KH_2PO_4 pH 2.7, 33 % ACN, 350 mM KCl). A gradient of 5 to 60 % Buffer B in 50 min was applied and fractions were collected every 4 min. The fractions taken within the retention time range of 2 to 22 min were lyophilized, the residues were resuspended in H_2O/ACN/TFA 94.5:5:0.5 (v/v/v) and desalted using C_{18} solid-phase extraction (SPE) cartridges (BioSelect, Vydac).

The desalted samples were analyzed by reversed-phase nano-scale microcapillary high-performance liquid chromatography-tandem mass spectrometry (RP-LC-MS/MS) using a 150 µm x 10 cm capillary column self-packed with C_{18}-bonded silica (Magic C_{18} AQ, Michrom Bioresources), an Agilent 1100 binary pump (Buffer A, 2.5% ACN and 0.1% FA in water; Buffer B, 2.5% ACN and 0.1% FA in ACN; 60 min gradient from 5 to 35 % Buffer B in 60 min; flow rate, 300 nl/min), a Famos autosampler (LC Packings), and an LTQ FT mass spectrometer (Thermo Electron). The mass spectra were obtained in an automated fashion by acquiring 1 FTICR-MS scan followed by 10 data-dependent LTQ-MS/MS scans in a cycle time of approximately 4 sec. MS/MS spectra were searched against the known yeast ORF

database using the Sequest algorithm. Eng, J. *et al.* (1994) *J. Am. Soc. Mass. Spectrom.* 5, 976-989.

The Sequest results were filtered using in-house software. Minimum XCorr scores were set at 2, 2, and 3 for charge states 1+, 2+, and 3+, respectively. After searching using no enzyme specificity, only peptides that started with a Met or with a residue following a Met in the database entry, and ended with an Arg were considered for further manual validation. The resulting N-terminal peptides are listed in Table 5A and Table 5B.

Variations, modifications, and other implementations of what is described herein will occur to those of ordinary skill in the art without departing from the spirit and scope of the invention as described and claimed herein and such variations, modifications, and implementations are encompassed within the scope of the invention.

All of the references, patents and patent applications identified hereinabove are expressly incorporated herein by reference in their entireties.

Table 4

Hela Phosphorylation Peptides

Peptide	Protein
SS*DGEEAEVDEER	GP:AB000516_1
APS*LTDLVK	GP:AB002293_1
LSGEGDTDLGALSNDGSDDGPSVMDETS*NDAFDSLER	GP:AB002293_1
LVEPHS*PS*PSSK	GP:AB002293_1
TNS*MGSATGPLPGTK	GP:AB002293_1
TNS*PAYSDIS*DAGEDGEGKVDSVK	GP:AB002293_1
GSVSQPST*PS*PPKPTGIFQTSANSSFEPVK	GP:AB002308_1
VKS*PS*PK	GP:AB002330_1
DTGSEVPSGSGHGPCT*PPPAPANFEDVAPTGSGEPGATR	GP:AB002337_1
NGPLPIPSEGS*GFTK	GP:AB002366_1
LIDLES*PTPESQK	GP:AB007900_1
TLS*DESIYNSQR	GP:AB007900_1
EASAS*PDPAK	GP:AB007947_1
VPGPEEALVTQDQAWS*EAHAS*GEKR	GP:AB009265_1
GGPEGVAAQAVASAASAGPADAEMEEIFDDAS*PGKQK	GP:AB010882_1
RVS*PLNLSSVTP	GP:AB011472_1
SQLQALHIGLDSSS*IGS*GPGDAEADDGFPESR	GP:AB014519_1
GEQLRPWAPGDLS*VM	GP:AB014543_1
KAS*VVDPSTESSPAPQEGSEQPASPAS*PLSSR	GP:AB014576_1
TVFPGAVPVLPAS*PPPK	GP:AB015346_1
AAFGIS*DSYVDGSSFDPQR	GP:AB016092_1
AGMS*SNQSISSPVLDAVPR	GP:AB016092_1
AGMSSNQSISS*PVLDAVPR	GP:AB016092_1
APS*PSSR	GP:AB016092_1
AQSGS*DSSPEPK	GP:AB016092_1
AQSGSDSS*PEPK	GP:AB016092_1
AQT*PPGPSLSGSK	GP:AB016092_1
CLT*PQR	GP:AB016092_1
DGSGT*PSR	GP:AB016092_1
DQQSSS*SER	GP:AB016092_1
ELSNS*PLR	GP:AB016092_1
ENS*FGSPLEFR	GP:AB016092_1
FQSDSSS*YPTVDSNSLLGQSR	GP:AB016092_1
GEFSAS*PMLK	GP:AB016092_1
LPQSSSSESSPPS*PQPTK	GP:AB016092_1
MALPPQEDATAS*PPR	GP:AB016092_1
MAPALSGANLTS*PR	GP:AB016092_1
MGQAPSQSLLPPAQDQPRS*PVPSAFSDQSR	GP:AB016092_1
QGSITS*PQANEQSVTPQR	GP:AB016092_1
QGSITSPQANEQSVT*PQR	GP:AB016092_1
QS*HSES*PSLQSK	GP:AB016092_1
QS*HSGSIS*PYPK	GP:AB016092_1
S*DTSSPEVR	GP:AB016092_1
S*GAGSSPETK	GP:AB016092_1
S*GMSPEQSRFQS*DSSSYPTVDSNSLLGQSR	GP:AB016092_1
S*GSESSVDQK	GP:AB016092_1
S*GSSPEVDSK	GP:AB016092_1
S*GSSPGLR	GP:AB016092_1
S*GTPPRQGS*ITSPQANEQSVTPQR	GP:AB016092_1
S*PPAIR	GP:AB016092_1
S*PSSPELNNK	GP:AB016092_1
S*PVPSAFSDQSR	GP:AB016092_1
S*RS*PLAIR	GP:AB016092_1
S*RT*PPSAPSQSR	GP:AB016092_1
S*SPELTR	GP:AB016092_1
S*TSADSASSSDTSR	GP:AB016092_1
S*TTPAPK	GP:AB016092_1
S*VSPCSNVESR	GP:AB016092_1
SAT*PPATR	GP:AB016092_1
SATRPS*PS*PER	GP:AB016092_1
SDTSS*PEVR	GP:AB016092_1
SECDSS*PEPK	GP:AB016092_1
SES*DSSPDSK	GP:AB016092_1

Peptide	Protein
SGAGSS*PETK	GP:AB016092_1
SGMS*PEQSR	GP:AB016092_1
SGS*ESSVDQK	GP:AB016092_1
SGS*SPEVDSK	GP:AB016092_1
SGS*SPEVK	GP:AB016092_1
SGS*SPGLR	GP:AB016092_1
SGSESS*VDQK	GP:AB016092_1
SGSS*PEVDSK	GP:AB016092_1
SGSS*PEVK	GP:AB016092_1
SGSS*PGLR	GP:AB016092_1
SGT*PPRQGSITS*PQANEQSVTPQR	GP:AB016092_1
SLS*YSPVER	GP:AB016092_1
SLSYS*PVER	GP:AB016092_1
SPS*PASGR	GP:AB016092_1
SPS*SPELNNK	GP:AB016092_1
SPSS*PELNNK	GP:AB016092_1
SRS*GSS*PEVDSK	GP:AB016092_1
SRS*PSS*PELNNK	GP:AB016092_1
SRS*TT*PAPK	GP:AB016092_1
SRT*S*PVTR	GP:AB016092_1
SS*PELTR	GP:AB016092_1
SS*PEPK	GP:AB016092_1
SS*TPPRQS*PSR	GP:AB016092_1
SSS*ASSPEMK	GP:AB016092_1
SSS*PQPK	GP:AB016092_1
SSS*PVTELASR	GP:AB016092_1
SSS*PVTELASRS*PIR	GP:AB016092_1
SSSAS*SPEMK	GP:AB016092_1
SSSS*PPPK	GP:AB016092_1
SST*PPGESYFGVSSLQLK	GP:AB016092_1
SST*PPRQS*PSR	GP:AB016092_1
SSTGPEPPAPT*PLLAER	GP:AB016092_1
ST*TPAPK	GP:AB016092_1
STSADSASSSDT*SR	GP:AB016092_1
STT*PAPK	GP:AB016092_1
T*PLISR	GP:AB016092_1
T*PPVALNSSR	GP:AB016092_1
T*PPVTR	GP:AB016092_1
TPAAAAAMNLAS*PR	GP:AB016092_1
TPQAPAS*ANLVGPR	GP:AB016092_1
TS*PPLLDR	GP:AB016092_1
VKS*ST*PPR	GP:AB016092_1
VPS*PTPAPK	GP:AB016092_1
YSHSGSS*S*PDTK	GP:AB016092_1
ETESAPGS*PR	GP:AB018274_1
ST*PSLER	GP:AB018306_1
AITSLLGGGS*PK	GP:AB019494_1
NNTAAETEDDES*DGEDR	GP:AB019494_1
GPSQATS*PIR	GP:AB020626_1
EPVS*PMELTGPEDGAASSGAGR	GP:AB020683_1
S*PLSVVK	GP:AB020683_1
ANS*QENR	GP:AB020689_1
T*PTMPQEEAAEK	GP:AB020711_1
STGS*ATSLASQGER	GP:AB022657_1
STGSATSLAS*QGER	GP:AB022657_1
RPASPPAGLALAPRS*PSAS*PEPREGETLS*PSMQR	GP:AB023163_1
TNAVS*PK	GP:AB023227_1
ST*SIHYADSVK	GP:AB027443_1
YSVGSLS*PVSASVLK	GP:AB028069_1
SATTTPSGS*PR	GP:AB028971_1
SKS*ATTTPS*GSPR	GP:AB028971_1
VQTT*PPPAVQGQK	GP:AB028971_1
AAKPGPAEAPS*PTASPSGDAS*PPATAPYDPR	GP:AB028987_1
TGSGS*PFAGNSPAR	GP:AB028987_1
TGSGSPFAGNS*PAR	GP:AB028987_1
SNGELSES*PGAGK	GP:AB032251_1
IVPQSQVPNPES*PGK	GP:AB033023_1

Peptide	Protein
IVSGS*PISTPSPSPLPR	GP:AB033023_1
QPGQVIGATTPSTGS*PTNK	GP:AB033023_1
AGSSAAGASGWTSAGSLNSVPTNSAQQGHNS*PDS*PVTSAAK	GP:AB036090_1
ANFDEENAYFEDEEEDSSNVDLPYIPAENS*PTR	GP:AB036090_1
APDMSS*SEEFPSFGAQVAPK	GP:AB036090_1
NS*PSAASTSSNDSK	GP:AB036737_1
S*PTPALCDPPACSLPVASQPPQHLSEAGR	GP:AB036737_1
VAS*DTEEADR	GP:AB036737_1
ASDPQS*PPQVSR	GP:AB037782_1
QVPHSS*R	GP:AB037813_1
EFLPTSWS*PVGAGPTPSLYK	GP:AB037824_1
SLDSEPSVPSAAKPPS*PEK	GP:AB037911_1
GSS*PEAGAAAMAESIIIR	GP:AB040932_1
DQS*PPPS*PPPSYHPPPPPTK	GP:AB040955_1
GLAGPPAS*PGK	GP:AB040955_1
GS*PSGGSTAEASDTLSIR	GP:AB040955_1
S*PGASVSSSLTSLCSSSSDPAPSDR	GP:AB040955_1
TLS*PSSGYSSQSGTPTLPPK	GP:AB040955_1
EAS*PAPLAQGEPGR	GP:AB040975_1
SEVYDPSDPTGSDSSAPGSS*PER	GP:AB040975_1
GTEAS*PPQNNSGSSSPVFTFR	GP:AB040976_1
S*PGPGPSQSPR	GP:AB040976_1
YLLGNAPVS*PSSQK	GP:AB041557_1
NALTTLAGPLT*PPVK	GP:AB044549_1
SPTAPSVFS*PTGNR	GP:AB044549_1
LQQTVPADAS*PDSK	GP:AB045733_1
GPVGVCS*YTPTPVGRTMSLVSQNS*R	GP:AB046807_1
APS*PPPTASNSSNSQ	GP:AB046830_1
APSPPPTAS*NSSNSQ	GP:AB046830_1
DCSYGAVTS*PTSTLESR	GP:AB046856_1
LSS*LSSQTEPTSAGDQYDCSR	GP:AB051458_1
LTQAEISEQPTMATVVPQVPTS*PK	GP:AB051468_1
APS*PTGPALISGAS*PVHCAADGTVELK	GP:AB051472_1
FQAPS*PSTLLR	GP:AB051485_1
NSSLGSPSNLCGS*PPGSIR	GP:AB051540_1
RAS*QSS*LESSTGPPCIR	GP:AB051866_1
AFLASLS*PAMVVPEDQLTR	GP:AB053172_1
NEEPIDSEQDENIDT*R	GP:AB055056_1
SPS*PVQGK	GP:AB056107_1
GPS*PPGAK	GP:AB056152_1
S*PSVS*PSKQPVSTSSK	GP:AB058764_1
EVS*PSDVR	GP:AB059277_1
S*TPRSTPLASPSPS*PGR	GP:AB059277_1
LSLS*PLR	GP:AB062430_1
T*PS*PESHR	GP:AB062430_1
GS*PQPQQEPR	GP:AB063357_1
T*VPLPPS*SAM	GP:AB067519_1
AES*PEEVACR	GP:AB071605_1
AGSST*PGDAPPAVAEVQGR	GP:AB071605_1
DGGS*GNSTIIVSR	GP:AB071605_1
GSGTAS*DDEFENLR	GP:AB071605_1
SDGSGESAQPPEDSS*PPASSESSSTR	GP:AB071605_1
S*PSWMSK	GP:AB072355_1
QQEEEAVELQPPPPAPLS*PPPPAPTAPQPPGDPLMSR	GP:AB075829_1
QTSYEAS*PR	GP:AB082522_1
SQS*CSDTAQER	GP:AB082522_1
VLDTSSLTQSAPAS*PTNK	GP:AB082951_1
QT*VPTPVR	GP:AB086011_1
LSVPT*S*DEEDEVPAPKPR	GP:AB088096_1
AQPFGFIDS*DTDAEEER	GP:AB088099_1
DSDT*DVEEEELPVENR	GP:AB088099_1
GQASS*PTPEPGVGAGDLPGPTSAPVPSGS*QSGGRGSPVSPR	GP:AB088099_1
GQASS*PTPEPGVGAGDLPGPTSAPVPSGSQSGGRGS*PVSPR	GP:AB088099_1
LEPSTSTDQPVT*PEPTSQATR	GP:AB088099_1
LLLAEDS*EEEVDFLSER	GP:AB088099_1
SQTTTERDS*DT*DVEEEELPVENR	GP:AB088099_1
SSVKT*PETVVPTAPELQPSTSTDQPVTPEPTSQATR	GP:AB088099_1

Peptide	Protein
TPETVVPTAPELQPSTST*DQPVTPEPTSQATR	GP:AB088099_1
LGYLVS*PPQQIR	GP:AB112075_1
S*PPYPR	GP:AB112075_1
S*PQAFR	GP:AB112075_1
VTGTEGSSSTLVDYTSTSSTGGS*PVR	GP:AB112075_1
MEEEGTEDNGLEDDS*R	GP:AC004611_1
NTLETSS*LNFK	GP:AC004611_1
VTPDIEES*LLEPENEK	GP:AC004611_1
LGASNS*PGQPNSVK	GP:AC004858_3
FAELPEFRPEEVLPSPT*LQSLATS*PR	GP:AC006486_3
NSCQDS*EADEETSPGFDEQEDGSSSQTANKPSR	GP:AF005043_1
GVS*MPNMLEPK	GP:AF005654_1
STS*QGSINSPVYSR	GP:AF005654_1
TAS*LPGYGR	GP:AF005654_1
TLS*PTPSAEGYQDVR	GP:AF005654_1
QEQINTEPLEDTVLS*PTK	GP:AF017633_1
EVDGLLTSEPMGS*PVSSK	GP:AF034373_1
GPPQS*PVFEGVYNNSR	GP:AF034373_1
LQPSSS*PENSLDPFPPR	GP:AF034373_1
AWGPGLHGGIVGRS*ADFVVESIGSEVGSLGFAIEGPSQAK	GP:AF042166_1
SETDLSS*LTASIK	GP:AF042166_1
SRSQSPS*PS*PAR	GP:AF042800_1
TSSGAGSPAVAVPTHSQPSPT*PS*NESTDTASEIGSAFNSPLR	GP:AF045581_1
S*FDYNYR	GP:AF047448_1
AAS*PS*PQSVRR	GP:AF048977_1
APQTSSS*PPPVR	GP:AF048977_1
GTS*AEQDNR	GP:AF048977_1
KAAS*PS*PQSVR	GP:AF048977_1
KPPAPPS*PVQSQS*PSTNWSPAVPVK	GP:AF048977_1
KPPAPPS*PVQSQSPSTNWS*PAVPVK	GP:AF048977_1
LSPSAS*PPR	GP:AF048977_1
MAAADS*VQQR	GP:AF048977_1
QNQQSSSDSGSSS*SS*EDERPK	GP:AF048977_1
RAS*PS*PPPK	GP:AF048977_1
RLS*PSAS*PPR	GP:AF048977_1
RLSPS*AS*PPR	GP:AF048977_1
RS*PS*PAPPPR	GP:AF048977_1
RT*PS*PPPR	GP:AF048977_1
RYS*PS*PPPK	GP:AF048977_1
S*PQPNK	GP:AF048977_1
S*PS*PPPTRR	GP:AF048977_1
S*PSPAPPPR	GP:AF048977_1
S*PSPPPTR	GP:AF048977_1
SASPS*PR	GP:AF048977_1
SPS*PAPEK	GP:AF048977_1
SPS*PAPPPR	GP:AF048977_1
SPS*PPPTR	GP:AF048977_1
SRVS*VS*PGR	GP:AF048977_1
SVS*GSPEPAAK	GP:AF048977_1
SVSGS*PEPAAK	GP:AF048977_1
T*AS*PPPPPKR	GP:AF048977_1
T*PELPEPSVK	GP:AF048977_1
T*PT*PPPRR	GP:AF048977_1
T*PTPPPR	GP:AF048977_1
TAS*PPPPPK	GP:AF048977_1
TPS*PPPR	GP:AF048977_1
VSVS*PGRT*SGK	GP:AF048977_1
YSPS*PPPK	GP:AF048977_1
SFTSSSPSS*PSR	GP:AF049884_1
YQT*QPVTLGEVEQVQSGK	GP:AF051850_1
AGNALT*PELAPVQIK	GP:AF052052_1
KGS*DDDGGDS*PVQDIDTPEVDLYQLQVNTLR	GP:AF055993_1
LFDVCGS*QDFESDLDR	GP:AF057299_1
VFQT*EAELQEVISDLQSK	GP:AF057299_1
TTTPGPSLS*QGVSVDEK	GP:AF058696_1
TIS*PPTLGTLR	GP:AF060479_1
AYT*PVVVTLWYR	GP:AF067512_1

Peptide	Protein
EYGS*PLKAYT*PVVVTLWYR	GP:AF067512_1
AES*PGPGSR	GP:AF075587_1
GLS*VDSAQEVK	GP:AF076974_1
KPVTVSPTTPTS*PTEGEAS	GP:AF078849_1
LGSTAPQVLSTSS*PAQQAENEAK	GP:AF078856_1
ENS*PAAFPDR	GP:AF081287_1
EAASS*PAGEPLR	GP:AF083106_1
S*PGEPGGAAPER	GP:AF083106_1
YMAENPTAGVVQEEEEDNLEYDS*DGNPIAPTK	GP:AF083255_1
AILGSYDSELTPAEYS*PQLTR	GP:AF083811_1
DIS*PEKSELDLGEPGPPGVEPPPQLLDIQCK	GP:AF090114_1
FGQDIIS*PLLSVK	GP:AF092139_1
ETEEQDS*DSAEQGDPAGEGK	GP:AF096870_1
GGAPDPSPGATATPGAPAQPSS*PDAR	GP:AF097916_1
VRGGAPDPSPGAT*ATPGAPAQPSS*PDAR	GP:AF097916_1
QLLDS*DEEQEEDEGR	GP:AF098162_1
RT*VAAPS*KR	GP:AF103483_1
S*VTPPPPPR	GP:AF104413_1
AALGLQDS*DDEDAAVDIDEQIESMFNSK	GP:AF106680_1
ICS*DEEEDEEK	GP:AF108459_1
QQDS*QPEEVMDVLEMVENVK	GP:AF112222_1
TFS*ATVR	GP:AF115345_1
EDYFEPIS*PDR	GP:AF116724_1
DGEQS*PNVSLMQR	GP:AF116725_1
DSALQDTDDS*DDDPVLIPGAR	GP:AF116725_1
MEVGPFSTGQES*PTAENAR	GP:AF116730_1
QGS*PVAAGAPAK	GP:AF117106_1
EEQEILS*TR	GP:AF119230_1
IPS*PNILK	GP:AF121141_1
NKSSS*PEDPGAEV	GP:AF125568_1
LGAGGGS*PEKS*PSAQELK	GP:AF129085_1
LQVPTS*QVR	GP:AF133820_1
SDDES*PSTSSGSSDADQRDPAAPEPEEQEER	GP:AF136176_1
ILLVDS*PGMGNADDEQQEEGTSSK	GP:AF142328_1
EIPSATQS*PISK	GP:AF147709_1
DSGNWDTSGSELS*EGELEK	GP:AF151059_1
SDSPES*DAER	GP:AF151059_1
DWDKESDGPDDSRPESASDS*DT	GP:AF151873_1
GESAPTLSTSPSPSSPSPTSPS*PTLGR	GP:AF153415_1
WLDES*DAEMELR	GP:AF161470_1
SEGEGEAASADDGSLNTS*GAGPK	GP:AF161491_1
S*RIPSPLQPEMQGTPDDEPSEPEPS*PSTLIYR	GP:AF162447_1
ISDYFEYQGGNGSS*PVR	GP:AF162666_1
ISDYFEFAGGSAPGTS*PGR	GP:AF162667_1
QLS*LEGS*GLGVEDLKDNTPSGK	GP:AF169548_1
TYS*QDCSFK	GP:AF177387_1
GGNLPPVS*PNDSGAK	GP:AF180425_1
S*PEDQLGK	GP:AF180425_1
STDSEVSQS*PAK	GP:AF180474_1
GLNPDGTPALSTLGGFSPAS*KPSS*PR	GP:AF180920_1
LS*PTPSMQDGLDLPSETDLR	GP:AF180920_1
SPIS*INVK	GP:AF180920_1
EAYSGCSGPVDSECPPPPS*SPVHK	GP:AF188700_1
SGTSSPQS*PVFR	GP:AF188700_1
TGS*NAAQYK	GP:AF188700_1
QAEFFLS*QQASLLK	GP:AF191339_1
RSS*FSMEEES	GP:AF196779_1
AVGMPSPVS*PKLSPGNS*GNYSSGASSASASGSSVTIPQK	GP:AF197927_1
LS*PGNSGNYSSGASSASASGSSVTIPQK	GP:AF197927_1
NSYNNSQAPS*PGLGSK	GP:AF197927_1
HGGS*PQPLATTPLSQEPVNPPSEAS*PTR	GP:AF201422_1
HGGSPQPLATT*PLSQEPVNPPSEAS*PTR	GP:AF201422_1
HGGSPQPLATTPLS*QEPVNPPSEASPT*R	GP:AF201422_1
S*LSGSSPCPK	GP:AF201422_1
S*PSVSSPEPAEK	GP:AF201422_1
SASSS*PETR	GP:AF201422_1
SHS*GSSSPS*PSR	GP:AF201422_1

Peptide	Protein
SLS*GSSPCPK	GP:AF201422_1
SLSGS*SPCPK	GP:AF201422_1
SLSGSS*PCPK	GP:AF201422_1
SNS*SPEMK	GP:AF201422_1
SNSS*PEMK	GP:AF201422_1
SPS*VSSPEPAEK	GP:AF201422_1
SPSVS*SPEPAEK	GP:AF201422_1
SRS*VS*PCSNVESR	GP:AF201422_1
SRT*PPTS*R	GP:AF201422_1
SVS*PCSNVESR	GP:AF201422_1
LEPQELS*PLSATVFPK	GP:AF203474_1
ATGDGSS*PELPSLER	GP:AF205632_1
SLS*ESSVIMDR	GP:AF205632_1
KAEFPSSGSNSVLNT*PPTTPES*PSSVTVTEGSR	GP:AF214114_1
DGGPVTS*QESGQK	GP:AF230336_1
S*ESPSLTQER	GP:AF230336_1
SES*PSLTQER	GP:AF230336_1
SQNSQESTADES*EDDMSSQASK	GP:AF230336_1
MS*VTGGK	GP:AF230929_1
ALS*PAELR	GP:AF240677_1
LAEAPSPAPTPSPTPVEDLGPQTSTSPGRLS*PDFAEELR	GP:AF240677_1
AEGEPQEES*PLK	GP:AF249273_1
FNDS*EGDDTEETEDYR	GP:AF249273_1
IDIS*PSTLR	GP:AF249273_1
S*GSGSVGNGSSR	GP:AF249273_1
S*VSSQR	GP:AF249273_1
SGS*GSVGNGSSR	GP:AF249273_1
SGSGSVGNGS*SR	GP:AF249273_1
SSATSGDIWPGLS*AYDNSPR	GP:AF249273_1
SSATSGDIWPGLSAYDNS*PR	GP:AF249273_1
SSS*PYSKS*PVSK	GP:AF249273_1
SSSPYS*KS*PVSK	GP:AF249273_1
SSSSSASPSS*PSSR	GP:AF249273_1
SLS*VPVDLSR	GP:AF251040_1
TVNSGGSSEPS*PTEVDVSR	GP:AF251055_1
AAPPPPALT*PDSQTVDSSCK	GP:AF254411_1
GPSPAPASS*PK	GP:AF254411_1
QRS*PS*PAPAPAPAAAAGPPTR	GP:AF254411_1
VPST*PPPK	GP:AF254411_1
FADQDDIGNVS*FDR	GP:AF264779_1
IQQFDDGGS*DEEDIWEEK	GP:AF264779_1
ALVVPEPEPDSDS*NQER	GP:AF265230_1
VDEDSAEDTQS*NDGK	GP:AF273048_1
SCSPS*PVSPQVQPQAADTISDSVAVPASLLGMR	GP:AF273437_1
TPIS*PLK	GP:AF273437_1
TQS*LPVTEK	GP:AF273437_1
STEDLS*PQK	GP:AF276423_1
ESLPPAAEPS*PVSK	GP:AF283303_1
GIGLDESELDS*EAELMR	GP:AF286340_1
AAVGQES*PGGLEAGNAK	GP:AF294791_1
EQSSEAAETGVS*ENEENPVR	GP:AF294791_1
IISVT*PVK	GP:AF294791_1
AQPGS*PESSGQPK	GP:AF297872_1
LENEGS*DEDIETDVLYSPQMALK	GP:AF307332_1
ATVPVAAATAAEGEGS*PPAVAAVAGPPAAAEVGGGVGGSSR	GP:AF308285_1
S*PSPVQGK	GP:AF310246_1
GSESSDT*DDEELR	GP:AF314184_1
S*PIALPVK	GP:AF314184_1
S*PS*PVPQEEHS*DPEMTEEEKEYQMMLLTK	GP:AF314184_1
QAS*PTEVVER	GP:AF315591_1
DGSS*PPLLEK	GP:AF317391_1
LPEEDAS*SQSSK	GP:AF319995_1
LSSSGAPPADFPS*PR	GP:AF319995_1
TCGVNDDES*PSK	GP:AF319995_1
WQLSS*PDGVDTDDDLPK	GP:AF319995_1
T*DELNK	GP:AF322916_1
MNGVMFPGNS*PSYTER	GP:AF327345_1

Peptide	Protein
NHSDSSTSESEVSSVS*PLK	GP:AF327345_1
AGPSAQEPGSQT*PLK	GP:AF327452_1
SAS*QSS*LDKLDQELK	GP:AF327452_1
ATLSSTSGLDLMSESGEGEIS*PQR	GP:AF330045_1
EVAATEEDVTRLPSPT*SPFS*SLSQDQAATSK	GP:AF330045_1
ISINQT*PGK	GP:AF330045_1
LPS*PTSPFSSLSQDQAATSK	GP:AF330045_1
LPSPTS*PFSSLSQDQAATSK	GP:AF330045_1
TPNNVVSTPAPS*PDASQLASSLSSQK	GP:AF330045_1
VSAS*LPR	GP:AF330045_1
VTTEIQLPSQS*PVEEQSPASLSSLR	GP:AF330045_1
GS*PEPSALPPQR	GP:AF334584_1
SAS*DSGCDPASK	GP:AF338242_1
ATEDGEEDEVS*AGEK	GP:AF340183_1
ADQGDGPEGS*GR	GP:AF349313_1
DLNES*PVK	GP:AF349313_1
VPS*PGMEEAGCSR	GP:AF349313_1
ESGVVAVS*PEK	GP:AF356524_1
NVDAAVS*PR	GP:AF356524_1
RPQS*PGAS*PSQAER	GP:AF356524_1
TGGS*PSVR	GP:AF356524_1
ATPELGSSENSASS*PPR	GP:AF360549_1
AQS*VSPVQAPPPGGSAQLLPGK	GP:AF363689_1
KNS*TDLDSAPEDPTS*PK	GP:AF363689_1
EGNTTEDDFPSS*PGNGNK	GP:AF374416_1
SLS*NPDIASETLTLLS*FLR	GP:AF378754_1
FPGDQVVNGAGPELSTGPSPGS*PTLDIDQSIEQLNR	GP:AF378756_1
DPS*PESNK	GP:AF380154_1
MDRT*PPPPTLS*PAAITVGR	GP:AF380154_1
GLSS*GWSSPLLPAPVCNPNK	GP:AF387103_1
GRLT*PS*PDIIVLSDNEASSPR	GP:AF411836_1
GRLT*PSPDIIVLS*DNEASSPR	GP:AF411836_1
LTPSPDIIVLSDNEASS*PR	GP:AF411836_1
SAS*ADNLTLPR	GP:AF413522_1
VPAEDETQSIDS*EDSFVPGR	GP:AF434816_1
SDES*STEETDK	GP:AF441770_1
SES*PCESPYPNEK	GP:AF441770_1
TPATT*PEAR	GP:AF441770_1
LASVLLYSDYGIGEVPVEPLDVPLPSTIRPAS*PVAGSPK	GP:AF453478_1
AET*PPLPIPPPPPDIQPLER	GP:AF463523_1
KPS*PAQAAETPALELPLPSVPAPAPL	GP:AF464935_1
SKENGAS*V	GP:AF465616_1
VEEESTGDPFGFDS*DDESLPVSSK	GP:AF479418_1
GSEGSQS*PGSSVDDAEDDPSR	GP:AF488691_1
SDS*DSSTLSK	GP:AF506799_1
LQLS*DEESVFEEALMSPDTR	GP:AF506820_1
APSPPPT*ASNSSNSQSEKEDGTVSTANQNGVSSNGPGEILNK	GP:AF515446_1
YFDTNSEVEEES*EEDEDYIPSEDWK	GP:AF515446_1
DSS*GQEDETQSSN	GP:AF515447_1
NTPS*PDVTLGTNPGTEDIQFPIQK	GP:AF518874_1
T*PVPTVSLASR	GP:AF520569_1
S*AFPSFLVSFILF	GP:AF523356_1
ATS*LTLEGGR	GP:AF533230_1
QSSVTQVTEQS*PK	GP:AF534078_1
AGSNEDPILAPSGT*PPPTIPPDETFGGR	GP:AF547989_1
LEAAYS*PR	GP:AJ006778_1
SLSDNGQPGT*PDPADSGGTSAK	GP:AJ006778_1
IDGATQSS*PAEPK	GP:AJ223075_1
TEVPGS*PAGTEGNCQEATGPSTVDTQNEPLDMK	GP:AJ223075_1
DPGGITAGS*TDEPPMLTK	GP:AJ223980_1
GTEPSPGGT*PQPSRPVS*PAGPPEGVPEEAQPPR	GP:AJ223980_1
QEIES*DSESDGELQDRK	GP:AJ238403_1
SCDELSPVS*PTQGGYPSEPTR	GP:AJ278120_1
NFDFEGSLS*PVIAPK	GP:AJ278357_1
SLCLS*PSEASQMK	GP:AJ278357_1
EPDPFEFS*SGSESEGDIFTSPK	GP:AJ292190_1
IPPMLS*PVHVQDSTDLAPPS*PEPPMLAPVAK	GP:AJ292190_1

Peptide	Protein
IPPMLSPVHVQDS*TDLAPPS*PEPPMLAPVAK	GP:AJ292190_1
TAQSPAMVGS*PIR	GP:AJ292190_1
WIPLSSDAQAPLAQPES*PTASAGDEPR	GP:AJ293573_1
GGDVS*PSPYSSSSWR	GP:AJ297709_1
HSSIS*PST*LTLK	GP:AJ297709_1
S*PSPAGGGSSPYSR	GP:AJ297709_1
S*PSYSR	GP:AJ297709_1
SLS*PLGGR	GP:AJ297709_1
SPS*PAGGGSSPYSR	GP:AJ297709_1
FSGSKS*ANTAS*LTISGLR	GP:AJ399983_1
CSDNSS*YEEPLSPISASSSTSR	GP:AJ419231_1
ESCSS*PSTVGSSLTTR	GP:AJ430203_1
LTSPVTSIS*PIQASEK	GP:AJ430203_1
TITVPVSGS*PK	GP:AJ430203_1
TNS*SSSSPVVLK	GP:AJ430203_1
AVPMAPAPAS*PGSSNDSSAR	GP:AJ440784_1
TLS*NESEESVK	GP:AJ459424_1
TPTGS*PATEVSAK	GP:AJ459424_1
DGQDAIAQS*PEK	GP:AK000867_1
DSGS*DGEDDVNEQHSGS*DTGSVER	GP:AK000868_1
S*QSIEQESQEK	GP:AK001192_1
EGDPVSLSTPLETEFGSPSELS*PR	GP:AK001247_1
LSPDPVAGSAVSQELREGDPVSLSTPLETEFGSPSELS*PR	GP:AK001247_1
VFPEPTES*GDEGEELGLPLLSTR	GP:AK001247_1
VTS*PTTYVLDEDEPR	GP:AK001544_1
AVAS*PEATVSQTDENK	GP:AK001686_1
ALSSGGSITS*PPLSPALPK	GP:AK001739_1
KASS*PS*PLTIGTPESQR	GP:AK001969_1
TSDDGGDS*PEHDTDIPEVDLFQLQVNTLR	GP:AK021588_1
TGS*PTFVR	GP:AK021696_1
SILPYPVS*PK	GP:AK022696_1
DAEPQPGS*PAAESLEEPDAAAGLSSTK	GP:AK022759_1
DSALAEAPEGLS*PAPPAR	GP:AK022759_1
SEDPPGQEAGS*EEEGSSASGLAK	GP:AK023003_1
LAQTT*PVDSALGSSR	GP:AK023056_1
NLS*GSTLYPVSNIPR	GP:AK023056_1
AAGGAPS*PPPPVR	GP:AK023192_1
FLES*PSR	GP:AK023370_1
TVS*DNSLSNSR	GP:AK023681_1
GS*PEEELPLPAFEK	GP:AK024269_1
TPPT*PPSSIVAK	GP:AK024290_1
EGS*ASTEVLR	GP:AK024391_1
ES*DEDTEDASETDLAK	GP:AK024460_1
STETSDFENIES*PLNER	GP:AK027362_1
GDLS*DVEEEEEEEMDVDEATGAVK	GP:AK027559_1
AAVLS*DSEDEEK	GP:AK027561_1
DSDS*ESEER	GP:AK027561_1
GPASDS*ETEDASR	GP:AK027561_1
KAAVLS*DS*EDEEK	GP:AK027561_1
MSDS*ESEELPKPQVSDSES*EEPPR	GP:AK027561_1
SPAS*DSETEDALKPQIS*DSESEEPPR	GP:AK027561_1
TIAS*DS*EEEAGKELSDK	GP:AK027561_1
TIASDS*EEEAGK	GP:AK027561_1
VVSDADDSDS*DAVSDK	GP:AK027561_1
VVSDADDSDSDAVS*DK	GP:AK027561_1
AAS*PPASASDLIEQQQK	GP:AK027649_1
S*PGHHR	GP:AK027842_1
TVFS*PTLPAAR	GP:AK055851_1
SSPSLDSGDS*DSEELPTFAFLK	GP:AK055926_1
GLFQDEDS*CSDCSYR	GP:AK055931_1
DEASS*VTR	GP:AK056632_1
DPHS*PEDEEQPQGLS*DDDILR	GP:AK056632_1
SQDQDS*EVNELSR	GP:AK056632_1
SQDQDSEVNELS*R	GP:AK056632_1
TQS*PGGCSAEAVLAR	GP:AK056946_1
TSGAPGS*PQTPPER	GP:AK056946_1
GT*PPPVFTPPLPK	GP:AK074638_1

Peptide	Protein
GPEDYPEEGVEES*S*GEASKYTEEDPSGETLSSENK	GP:AK074719_1
WLIS*PVK	GP:AK074809_1
WVEENVPSSVTDVALPALLDS*DEER	GP:AK074870_1
GGS*PDLWK	GP:AK074894_1
GQESSS*DQEQVDVESIDFSK	GP:AK074894_1
LAPVPS*PEPQKPAPVS*PESVK	GP:AK074894_1
SPAGS*PELR	GP:AK074894_1
SSSVSPSSWKS*PPAS*PESWK	GP:AK074894_1
TAPPAS*PEAR	GP:AK074894_1
TTS*PEPR	GP:AK074894_1
HNGVGGS*PPK	GP:AK074903_1
YMNSDTTS*PELR	GP:AK074903_1
FPEFCSSPS*PPVEVK	GP:AK074979_1
GQSS*PPPAPPICLR	GP:AK090617_1
EEAS*DDDMEGDEAVVR	GP:AK090671_1
RS*PPS*PR	GP:AK091273_1
AVT*PVPTK	GP:AK091465_1
GLS*ASLPDLDSENWIEVK	GP:AK091465_1
GLSAS*LPDLDSENWIEVK	GP:AK091465_1
NTFTAWS*DEESDYEIDDR	GP:AK091465_1
SLPTTVPES*PNYR	GP:AK091465_1
STFVQSPADACTPPDTSSAS*EDEGS*LRR	GP:AK091597_1
NTS*PEENLR	GP:AK092570_1
AAALQALQAQAPTS*PPPPPPPLK	GP:AK092772_1
DGDLLS*PSLR	GP:AK092807_1
AFVEDS*EDEDGAGEGGSSLLQK	GP:AK093879_1
RS*TS*PIIGSPPVR	GP:AK094193_1
STS*PIIGSPPVR	GP:AK094193_1
SFNSDSPSIIGVPSETQTS*PVER	GP:AK096613_1
GSGVAQSPQQPPPQQQQQQPPQQPT*PPK	GP:AK096644_1
VNDAEPGS*PEAPQGK	GP:AK097078_1
TLDSDISCPLLESDLAYS*DDDVPSVYENGLSQK	GP:AK097133_1
MGGPRGSGGS*GGGGGR	GP:AK097337_1
SFS*ADNFIGIQR	GP:AK097751_1
GPVSQNS*EVGEEETSAGQGLSSR	GP:AK122582_1
SGIETFS*PPPPPPK	GP:AK122582_1
SSVASGPIS*PTNYR	GP:AL121829_7
EPSPTT*PK	GP:AL133553_2
NSAIS*PQK	GP:AL136109_1
SASSEEASES*PTAR	GP:AL136450_1
TS*PVPK	GP:AL136867_1
AEFTS*PPSLFK	GP:AL136910_1
AES*PESSAIESTQSTPQK	GP:AL137201_1
METVSNASSSSNPSS*PGR	GP:AL137201_1
AQQCVS*PSSSLCR	GP:AL713775_1
GPRT*PS*PPPPIPEDIALGK	GP:AL831833_1
TPS*PPPPIPEDIALGK	GP:AL831833_1
TSAVSS*PLLDQQR	GP:AL831833_1
TFLEGDWTS*PSK	GP:AL831838_1
CS*PTVAFVEFPSSPQLK	GP:AL831962_1
DDSFDSLDS*FGSR	GP:AL831962_1
QQS*LPPPK	GP:AL831962_1
QTPS*PDVVLR	GP:AL831962_1
S*PEPEATLTFPFLDK	GP:AL831962_1
SDSLS*PPR	GP:AL831962_1
DLSTS*PKPSPIPS*PVLGR	GP:AL833968_1
AAEAAPPT*QEAQGETEPTEQAPDALEQAADTSR	GP:AL834162_1
ISDS*ESEDPPR	GP:AL834178_1
NQAS*DS*ENEELPKPR	GP:AL834178_1
VS*DSESEGPQK	GP:AL834178_1
VSDS*ESEGPQK	GP:AL834178_1
TGWDTSESELS*EGELER	GP:AL834216_1
FSTYSQS*PPDTPSLR	GP:AL834312_1
AAEEQGDDQDS*EK	GP:AL834470_1
S*GDETPGSEVPGDK	GP:AL834470_1
SGDET*PGSEVPGDK	GP:AL834470_1
TVS*PSTIR	GP:AL834476_1

Peptide	Protein
SDS*GGSSSEPFDR	GP:AP000505_1
SSVKT*PETVVPAAPELQPPTSTDQPVTPEPTSR	GP:AP000512_4
HSVTAAT*PPPS*PTSGESGDLLSNLLQSPSSAK	GP:AY026388_1
HSVTAAT*PPPSPTSGES*GDLLSNLLQSPSSAK	GP:AY026388_1
ASSQVLSES*PSQDSLDAFMSEMK	GP:AY028435_1
NWEDEDFYDS*DDDTFLDR	GP:AY028435_1
FQSPQIQATIS*PPLQPK	GP:AY036974_1
EAEALLQSMGLTPESPIVPPPMS*PSSK	GP:AY037160_1
DSLGDFIEHYAQLGPSS*PEQLAAGAEEGGGPR	GP:AY039216_1
RGGGSGGGEES*EGEEVDED	GP:AY039216_1
ALS*PVTSR	GP:AY044869_1
LPASPSGSEDLSSVSSS*PTSSP	GP:AY050169_1
FLTDT*SHLLSAVR	GP:AY061759_1
MEISAELPQT*PQR	GP:AY061886_1
AFAAVPTSHPPEDAPAQPPTPGPAAS*PEQLSFR	GP:AY062238_1
MAESPCSPSGQQPPSPPS*PDELPANVK	GP:AY062238_1
NS*LESISSIDR	GP:AY062238_1
QSPAS*PPPLGGGAPVR	GP:AY062238_1
VQS*PEPPAPER	GP:AY062238_1
VS*PTGAAGR	GP:AY062238_1
AAVFIQS*K	GP:AY101367_1
QGGSQPSSFS*PGQSQVTPQDQEK	GP:AY130299_1
ATNES*EDEIPQLVPIGK	GP:AY154473_1
LSSPAAFLPACNS*PSK	GP:AY166851_1
ASS*LNVLNVGGK	GP:AY180166_1
RPPS*PDVIVLS*DNEQPSSPR	GP:AY186731_1
RPPS*PDVIVLSDNEQPSS*PR	GP:AY186731_1
TLS*SSAQEDIIR	GP:AY190323_1
VTETEDDS*DS*DS*DDDEDDVHVTIGDIK	GP:AY229892_1
GDSDIS*DEEAAQQSK	GP:AY283618_1
GNIETTSEDGQVFS*PK	GP:AY283618_1
S*KGDSDIS*DEEAAQQSK	GP:AY283618_1
S*LS*PSHLTEDR	GP:AY283618_1
SAS*PYPSHSLSS*PQR	GP:AY283618_1
TPS*PSYQR	GP:AY283618_1
GPQPPTVS*PIR	GP:BC000656_1
NNS*GEEFDCAFR	GP:BC001041_1
TPAPPEPGS*PAPGEGPSGR	GP:BC001728_1
GAFMLEPEGMSPMEPAGVS*PMPGTQK	GP:BC001937_1
SSS*ESYTQSFQSR	GP:BC003167_1
DLFSLDSEDPSPAS*PPLR	GP:BC003640_1
GFSQYGVSGS*PTK	GP:BC005883_1
WTVHTGEKS*FGCNEYGK	GP:BC006258_1
ATDSDLSS*PR	GP:BC006350_1
NSKYEYDPDIS*PPR	GP:BC006350_1
SSDSDLS*PPR	GP:BC006350_1
YEYDPDIS*PPR	GP:BC006350_1
LYSILQGDS*PTK	GP:BC006474_1
SAS*PDDDLGSSNWEAADLGNEER	GP:BC007103_1
AAS*PESASSTPESLQAR	GP:BC007642_1
NDQEPPPEALDFS*DDEKEK	GP:BC008207_1
SRIPS*PLQPEMQGTPDDEPSEPEPS*PSTLIYR	GP:BC009071_1
SPITSS*PPK	GP:BC009539_1
EEVGAGYNS*EDEYEAAAAR	GP:BC009917_1
SSYANVFGDGPYSTFLTSS*PIR	GP:BC010629_1
STLS*PPEASPGPPAAPR	GP:BC011630_1
ALS*IFVGLFNIEETNDNIQIVIK	GP:BC013576_1
S*PPYEGK	GP:BC014394_1
SVNEILGLAESS*PNEPK	GP:BC014658_1
IGELGAPEVWGLS*PK	GP:BC015354_1
FQSQADQDQQASGLQS*PPSR	GP:BC016029_1
VSSPLSPLS*PGIKS*PTIPR	GP:BC016029_1
SS*PQLDPLR	GP:BC016842_1
S*VSPSPVPLSSNYIAQISNGQQLMSQPQLHR	GP:BC017705_1
SNS*CSSISVASCISEWEQK	GP:BC017705_1
VENSPQVDGS*PPGLEGLLGGIGEK	GP:BC018184_1
FELEASLATLLLGLSNVTVIS*LAET*KDIPAAILHAFLR	GP:BC018426_1

Peptide	Protein
SGISTNHADYSSS*PAGS*PGAQVSLYNSPSVASPAR	GP:BC018775_1
LVGLNLS*PPMSPVQLPLR	GP:BC019232_1
NSNSPPS*PSSMNQR	GP:BC020516_1
QELGS*PEER	GP:BC020567_1
EPAFEDITLES*ER	GP:BC027178_1
ELSDQATAS*PIVAR	GP:BC028697_1
LTQTSST*EQLNVLETETEVLNK	GP:BC028697_1
SSS*PVQVEEEPVR	GP:BC029266_1
NDS*GEENVPLDLTR	GP:BC029608_1
ACAS*PSAQVEGSPVAGSDGSQPAVK	GP:BC030547_1
ACASPSAQVEGS*PVAGSDGSQPAVK	GP:BC030547_1
S*PGLCSDSLEK	GP:BC030687_1
LSS*EDEEEDEAEDDQSEASGKK	GP:BC030817_1
ETAVQCDVGDLQPPPAKPAS*PAQVQSSQDGGCPK	GP:BC032244_1
EVDFDS*DPMEECLR	GP:BC032244_1
ASALGLGDGEEEAPPSRS*DPDGGDS*PLPASGGPLTCK	GP:BC032463_1
ATDIPASAS*PPPVAGVPFFKQS*PGHQS*PLASPK	GP:BC032463_1
AVVLPGGTATS*PK	GP:BC032463_1
SDPDGGDS*PLPASGGPLTCK	GP:BC032463_1
TASISSS*PSEGTPTVGSYGCTPQSLPK	GP:BC033856_1
S*PEAVGPELEAEEK	GP:BC035076_1
VTPLQSPIDKPSDSLSIGNGDNSQQISNSDTPS*PPPGLSK	GP:BC035590_1
AKS*PTPS*PSPPRNS*DQEGGGK	GP:BC036187_1
AKS*PTPSPS*PPR	GP:BC036187_1
AKSPTPS*PS*PPR	GP:BC036187_1
EPSVQEAT*STSDILK	GP:BC036187_1
GASSS*PQR	GP:BC036187_1
GSS*PSRS*TR	GP:BC036187_1
SPTPSPS*PPRNS*DQEGGGK	GP:BC036187_1
SATDGNTSTT*PPTSAK	GP:BC036216_1
AVS*PLDPSK	GP:BC036831_1
ALEEGDGSVSGSS*PR	GP:BC037404_1
ATS*PESTSR	GP:BC037404_1
IDENS*DKEMEVEES*PEK	GP:BC037404_1
TGTDSNSTESSETST*GSLCK	GP:BC037404_1
ALSAAVADSLTNS*PR	GP:BC037556_1
YSPDEMNNS*PNFEEK	GP:BC037556_1
LLS*PLSSAR	GP:BC037565_1
TVLPTVPES*PEEEVK	GP:BC038513_1
VESSENVPSPTHPPVVINAADDDEDDDDQFS*EEGDETK	GP:BC038513_1
TNLTSQSSTTNLPGSPGSPGSPGS*PGSPGSVPK	GP:BC038932_1
VEVTPT*VPR	GP:BC039295_1
AAS*DDGSLK	GP:BC039612_1
GWAFGSNS*LPIAGSVGMGVAR	GP:BC039652_1
SRS*PES*QVIGENTK	GP:BC039814_1
SYSSSSSS*PER	GP:BC039814_1
DS*ENTPVK	GP:BC039843_1
EMDESLANLS*EDEYYSEEER	GP:BC040194_1
EMDESLANLSEDEYYS*EEER	GP:BC040194_1
ARPQPSGPAPSS*	GP:BC041166_1
AEAPSS*PDVAPAGK	GP:BC041631_1
TAVQYIESS*DSEEIETSELPQK	GP:BC044659_1
ASIGQS*PGLPSTTFK	GP:BC045623_1
DVEDMELS*DVEDDGSK	GP:BC045623_1
IIS*PGSSTPSSTR	GP:BC045623_1
LESESTS*PSLEMK	GP:BC045623_1
SAT*PEPVTDNR	GP:BC045623_1
SFNYS*PNSSTSEVSSTSASK	GP:BC045623_1
SDS*APPTPVNR	GP:BC047482_1
TSDDEVGS*PK	GP:BC047529_1
LPPPPPQAPPEEENES*EPEEPSGVEGAAFQSR	GP:BC048134_1
AS*DLEDEESAAR	GP:BC050463_1
DSGS*DQDLDGAGVR	GP:BC050463_1
DSGS*DQDLDGAGVRAS*DLEDEESAAR	GP:BC050463_1
GPTSS*PCEEEGDEGEEDRT*SDLR	GP:BC050463_1
KLGVS*VS*PSR	GP:BC050463_1
KLGVS*VSPS*R	GP:BC050463_1

Peptide	Protein
LGVSVS*PSR	GP:BC050463_1
S*PAPAQTR	GP:BC050463_1
S*PQPPSR	GP:BC050463_1
TLSGSGSGSGSSYSGSSS*R	GP:BC050463_1
TSAS*SASASNSSR	GP:BC050463_1
TSASSASAS*NSSR	GP:BC050463_1
LFPS*PGLPTR	GP:BC050553_1
SDS*DSSTLAK	GP:BC053873_1
TLSLTSLGLS*MPADPCEGGAR	GP:BX248266_1
SFLVASVLPGPDGNINS*PTR	GP:BX537838_1
VTENGGS*PQGIK	GP:D49835_1
CASSESDS*DENQNK	GP:D63875_1
GGEFDEFVNDDT*DDDLPISK	GP:D63875_1
GS*DNEGSGQGSGNESEPEGSNNEASDR	GP:D63875_1
GS*GSEQEGEDEEGGER	GP:D63875_1
GSDNEGSGQGS*GNESEPEGSNNEASDR	GP:D63875_1
GSDNEGSGQGSGNESEPEGS*NNEASDR	GP:D63875_1
KGS*GS*EQEGEDEEGGER	GP:D63875_1
NS*NSNSDSDEDEQR	GP:D63875_1
NSNS*NSDSDEDEQR	GP:D63875_1
NSNSNSDS*DEDEQR	GP:D63875_1
SGSEAGS*PR	GP:D63875_1
GAPSS*PATGVLPSPQGK	GP:D79991_1
AVIVSS*PK	GP:D83032_1
SES*LSNCSIGK	GP:D86982_1
VVIDSDTEDSGS*DENLDQELLSLAK	GP:D87440_1
T*GGGGSGGGGSGGGGSDVK	GP:L43067_1
GEGGILLSS*PGGPTTDK	GP:S74786_1
S*AEDELAMR	GP:U07561_1
CETS*PPSSPR	GP:U22815_1
GVELCFPENET*PPEGK	GP:U49844_1
IGGDAATT*GNNSTPDFGFGGQK	GP:U69126_1
S*APTTPK	GP:U70136_1
ADS*LLAVVK	GP:U72355_1
NFWVSGLSST*TR	GP:U72355_1
S*VVSFDK	GP:U72355_1
SVVS*FDK	GP:U72355_1
DLDEEGS*EK	GP:U76992_1
LFDDS*DER	GP:U76992_1
LFDEEEDS*S*EKLFDDSDER	GP:U76992_1
LFEDDDS*NEK	GP:U76992_1
LFEES*DDKEDEDADGK	GP:U76992_1
VFDDES*DEKEDEEYADEK	GP:U76992_1
VLDEEGS*ER	GP:U76992_1
VLDEEGS*EREFDEDS*DEKEEEEDTYEK	GP:U76992_1
S*ISESSR	GP:U77718_1
VQIS*PDSGGLPER	GP:U94832_1
TPS*PSQPK	GP:U95825_1
RS*PQQTVPYVVPLS*PK	GP:Y18004_1
SPQQTVPYVVPLS*PK	GP:Y18004_1
QLEDIINTYGSAAS*TAGKEGS*AR	GPN:AB085905_1
IES*DEEEDFENVGK	GPN:AF227948_1
CSSSSGGGSS*GDEDGLELDGAPGGGK	GPN:AJ421269_1
LEDLDTCMMT*PK	GPN:AK000055_1
AVET*PPLSSVNLLEGLSR	GPN:AK000126_1
LPSS*EPDAPRLLRS*PVTCTPK	GPN:AK000538_1
TPSSS*PPITPPASETK	GPN:AK000742_1
ISSSFFFFLRQS*LTLSPR	GPN:AK025116_1
STDSSSYPSPCASPS*PPSSGK	GPN:AK025593_1
VDGIPNDSSDS*EMEDK	GPN:AK025593_1
LQQGAGLESPQGQPEPGAAS*PQR	GPN:AK025974_1
QEVVST*AGPR	GPN:AK026010_1
S*PGYESESSR	GPN:AK027089_1
SPGLVPPS*PEFAPR	GPN:AK027089_1
SPVQEASSATDTDTNS*QEDPADTASVSSLSLS*TGHTK	GPN:AK074370_1
AIS*PSIK	GPN:AK093809_1
LSST*PPLSALGR	GPN:AK093809_1

Peptide	Protein
S*LSSPTVTLSAPLEGAK	GPN:AY312514_1
SS*PEQPIGQGR	GPN:AY358482_1
GS*GGS*SGDELREDDEPVK	GPN:AY358600_1
VEEEQEADEEDVS*EEEAESK	GPN:AY358640_1
VPVLMES*R	GPN:AY358941_1
GQPGNAYDGAGQPSAAYLSMSQGAVANANST*PPPYER	GPN:BC000488_1
QPT*PPFFGR	GPN:BC000488_1
AGEPNS*PDAEEANS*PDVTAGCDPAGVHPPR	GPN:BC001041_1
ESTQLS*PADLTEGKPTDPSK	GPN:BC001041_1
VDIPS*PPPR	GPN:BC001044_1
SAS*SDTSEELNSQDSPPK	GPN:BC001443_1
YLFNQLFGEEDADQEVS*PDR	GPN:BC003153_1
ALPSLNTGSSS*PR	GPN:BC003553_1
LDSQPQETS*PELPR	GPN:BC003553_1
TLEEVVMAEEEDEGTDRPGS*PA	GPN:BC007448_1
GDSES*EEDEQDSEEVR	GPN:BC007664_1
QLEEPGAGTPS*PVR	GPN:BC008084_1
TEDGGWEWS*DDEFDEESEEGK	GPN:BC008726_1
AQPGAAPGIYQQSAEASSS*QGTAANSQSYTIMSPAVLK	GPN:BC008733_1
AQVPGPLT*PEMEAR	GPN:BC008948_1
LAAQLGAPTS*PIPDSAIVNTR	GPN:BC008948_1
QS*PPIVK	GPN:BC009039_1
ILDEDSWS*DGEQEPITVDQTWR	GPN:BC009746_1
ESLPPAAAAEPS*PVSK	GPN:BC010907_1
DTSATSQSVNGS*PQAEQPSLESTSK	GPN:BC011551_1
VFVGGLS*PDTSEEQIK	GPN:BC011714_1
S*GSLGSAR	PIR2:T00257
SAPSS*PAPR	PIR2:T00257
EPPS*PADVPEK	PIR2:T00262
AGNS*DSEEDDANGR	PIR2:T00347
AGNSDS*EEDDANGR	PIR2:T00347
QLVLETLYALTSS*TKIIK	PIR2:T00361
LSLTSDPEEGDPLALGPES*PGEPQPPQLK	PIR2:T00363
SS*LSGDEEDELFK	PIR2:T00363
SSLS*GDEEDELFK	PIR2:T00363
LSVQSNPS*PQLR	PIR2:T00368
DGGAAS*PATEGR	PIR2:T00387
S*PTGSTTSR	PIR2:T00387
SDIDVNAAAS*AK	PIR2:T00387
SIS*LGDSEGPIVATLAQPLR	PIR2:T01437
QEPQS*PSR	PIR2:T02672
ALS*PVIPLIPR	PIR2:T03454
EGAASPAPETPQPTS*PETSPK	PIR2:T08760
TTHLAGALS*PGEAWPFESV	PIR2:T08760
AETASQSQRS*PISDNSGCDAPGNSNPSLSVPSSAESEK	PIR2:T09073
LESS*EGEIIQTVDR	PIR2:T09073
QDQISGLS*QSEVK	PIR2:T09073
S*PISDNSGCDAPGNSNPSLSVPSSAESEK	PIR2:T09073
SSS*NDSVDEETAESDTSPVLEK	PIR2:T09073
SSSNDS*VDEETAESDTSPVLEK	PIR2:T09073
SSSNDSVDEETAES*DTSPVLEK	PIR2:T09073
SSSNDSVDEETAESDTS*PVLEK	PIR2:T09073
SSVAAPEKSS*S*NDSVDEETAESDTSPVLEK	PIR2:T09073
VGSSSS*ESCAQDLPVLVGEEGEVK	PIR2:T09073
GGAGAWLGGPAASLS*PPK	PIR2:T09219
GTPGS*PSGTQEPR	PIR2:T09219
SLS*PDEER	PIR2:T12518
LFQGYS*FVAPSILFK	PIR2:T13149
APQQQPPPQQPPPPQPPPQQPPPPPSYS*PAR	PIR2:T13159
NYILDQTNVYGS*AQR	PIR2:T13159
SFLSEPSS*PGR	PIR2:T17232
RAAAS*PPS*R	PIR2:T41998
CS*ATPSAQVKPIVSAS*PPSR	PIR2:T46375
ETEAAPTS*PPIVPLK	PIR2:T46385
TGDLGIPPNPEDRS*PS*PEPIYNSEGK	PIR2:G02919
ASWAS*ENGETDAEGTQMTPAK	PIR2:I38414
GYYS*PGIVSTR	PIR2:I38414

Peptide	Protein
KNS*STDQGS*DEEGSLQK	PIR2:I38414
NSSTDQGS*DEEGSLQK	PIR2:I38414
TSQPPVPQGEAEEDS*QGK	PIR2:I38414
GPGQVPTATSALSLELQEVEPLGLPQAS*PSR	PIR2:I52882
TRS*PDVISSASTALSQDIPEIASEALSR	PIR2:I52882
S*PS*PKPTK	PIR2:JC4525
SSSSSSSSGSPS*PSR	PIR2:JC4525
EEAGETS*PADESGAPK	PIR2:JC7079
STTPCMVLASEQDPDLELISDLDEGPPVLT*PVENTR	PIR2:JC7079
QSNASS*DVEVEEK	PIR2:JC7168
SLS*PQEDALTGSR	PIR2:JC7680
QPPGVPNGPSS*PTNESAPELPQR	PIR2:JC7807
RGSS*S*DEEGGPK	PIR2:JW0057
AVSTVVVTTAPS*PK	PIR2:S52863
S*PSPAVPLR	PIR2:S52863
SEAEDLAEPLSSTEGVAPLSQAPS*PLAIPAIK	PIR2:S52863
SPS*PAVPLR	PIR2:S52863
SMSSIPPYPASSLASSS*PPGSGR	PIR2:S55553
AT*PPPSPLLSELLK	PIR2:S68142
GSLLPTS*PR	PIR2:S68142
S*PVGSGAPQAAAPAPAAHVAGNPGGDAAPAATGTAAAASLATAAGS EDAEK	PIR2:S69501
LASEYLT*PEEMVTFK	PIR2:T00034
SANGGS*ESDGEENIGWSTVNLDEEK	PIR2:T00034
CGGVEQASSS*PR	PIR2:T00059
GPLEPS*EPAVVAAAR	DNA-3-methyladenine glycosylase
SLS*PGK	ATP-binding cassette, sub-family B, member 9 precursor
TDEVPAGGS*RS*EAEDEDDEDYVPYVPLR	DEAD-box protein abstrakt homolog
ELS*QNTDESGLNDEAIAK	Activator 1 140 kDa subunit
IIYDS*DS*ESEETLQVK	Activator 1 140 kDa subunit
QDPVTYIS*ETDEEDDFMCK	Activator 1 140 kDa subunit
ASLVALPEQTASEEET*PPPLLTK	Apoptotic chromatin condensation inducer in the nucleus
DPSSGQEVAT*PPVPQLQVCEPK	Apoptotic chromatin condensation inducer in the nucleus
DS*STSYTETKDPSSGQEVATPPVPQLQVCEPK	Apoptotic chromatin condensation inducer in the nucleus
DSSTSYTETKDPSS*GQEVATPPVPQLQVCEPK	Apoptotic chromatin condensation inducer in the nucleus
DSSTSYTETKDPSSGQEVAT*PPVPQLQVCEPK	Apoptotic chromatin condensation inducer in the nucleus
LS*EGSQPAEEEEDQETPSR	Apoptotic chromatin condensation inducer in the nucleus
LSEGS*QPAEEEEDQETPSR	Apoptotic chromatin condensation inducer in the nucleus
SKS*PS*PPR	Apoptotic chromatin condensation inducer in the nucleus
SLS*PGVSR	Apoptotic chromatin condensation inducer in the nucleus
SLSPGVS*R	Apoptotic chromatin condensation inducer in the nucleus
SPS*PPR	Apoptotic chromatin condensation inducer in the nucleus
TAQVPS*PPR	Apoptotic chromatin condensation inducer in the nucleus
TTS*PLEEEER	Apoptotic chromatin condensation inducer in the nucleus
TAS*FSESR	ATP-citrate synthase
GDEASEEGQNGSS*PK	Alpha adducin
SPGS*PVGEGTGSPPK	Alpha adducin
IEEVLSPEGSPS*KS*PSK	Gamma adducin
ELSPLISLPS*PVPPLSPIHS*NQQTLPR	AF-4 protein
IT*LDLLSR	AF-4 protein
RPGS*VSST*DQER	AF-4 protein
S*PAQQEPPQR	AF-4 protein
ITSVS*TGNLCTEEQTPPPRPEAYPIPTQTYTR	AF-6 protein
SSPNVANQPPS*PGGK	AF-6 protein
AS*LGSLEGEAEAEASSPK	Neuroblast differentiation associated protein AHNAK
ASLGS*LEGEAEAEASSPK	Neuroblast differentiation associated protein AHNAK
GGVTGS*PEASISGSK	Neuroblast differentiation associated protein AHNAK
IS*APNVDFNLEGPK	Neuroblast differentiation associated protein AHNAK
ISMQDVDLSLGS*PK	Neuroblast differentiation associated protein AHNAK
LGS*PSGK	Neuroblast differentiation associated protein AHNAK
SNS*FSDER	Neuroblast differentiation associated protein AHNAK
VKGS*LGATGEIKGPTVGGGLPGIGVQGLEGNLQMPGIK	Neuroblast differentiation associated protein AHNAK
VDSEGDFS*ENDDAAGDFR	A-kinase anchor protein 8
AIT*PPLPESTVPFSNGVLK	A kinase anchor protein 1, mitochondrial precursor
SNILSDNPDFS*DEADIIK	Acidic nucleoplasmic DNA-binding protein 1
LAS*PELER	Transcription factor AP-1
EWSLESSPAQNWT*PPQPR	ADP-ribosylation factor GTPase activating protein 1

Peptide	Protein
MS*GFIYQGK	Rho guanine nucleotide exchange factor 6
TQLWASEPGT*PPLPTSLPSQNPILK	Arsenite-resistance protein 2
SSGNSSSSGSGSGSTSAGSSS*PGAR	Aspartyl/asparaginyl beta-hydroxylase
EFDELNPS*AQR	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2
MPLDLS*PLATPIIR	Cyclic-AMP-dependent transcription factor ATF-2
SLAFEEGS*QSTTISSLSEK	Serine-protein kinase ATM
TSS*PPR	Transcriptional regulator ATRX
CS*PSSSSINNS*SSKPT*K	Ataxin-7
LAEDEGDS*EPEAVGQSR	Bromodomain adjacent to zinc finger domain protein 1B
SDVQEES*EGS*DTDDNKDSAAFEDNEVQDEFLEK	Bromodomain adjacent to zinc finger domain protein 1B
AS*PVTSPAAAFPTASPANK	Bromodomain adjacent to zinc finger domain 2A
AS*PPLQDSASQTYESMCLEK	Transcription regulator protein BACH1
ISES*PEPGQR	Transcription regulator protein BACH1
SQS*PAASDCSSSSSSASLPSSGR	BAG-family molecular chaperone regulator-3
SSVQGASS*REGS*PAR	BAG-family molecular chaperone regulator-3
VPPAPVPCPPPS*PGPSAVPSSPK	BAG-family molecular chaperone regulator-3
VPPAPVPCPPPSPGPSAVPSS*PK	BAG-family molecular chaperone regulator-3
EGPEPPEEVPPPTT*PPVPK	Large proline-rich protein BAT2
GNS*PNSEPPTPK	Large proline-rich protein BAT2
LIPGPLS*PVAR	Large proline-rich protein BAT2
AS*PEPQRENAS*PAPGTTAEEAMSR	Large proline-rich protein BAT3
ENAS*PAPGTTAEEAMSR	Large proline-rich protein BAT3
LQEDPNYS*PQR	Large proline-rich protein BAT3
T*PTAVQVK	BCE-1 protein
AVT*PVSQGSNSSSADPK	B-cell lymphoma 9 protein
IPVEGPLS*PSR	B-cell lymphoma 9 protein
LSVSSNDT*QESGNSSGPSPGAK	Brefeldin A-inhibited guanine nucleotide-exchange protein 1
LDS*T*QVGDFLGDSAR	Brefeldin A-inhibited guanine nucleotide-exchange protein 2
GNKS*PS*PPDGSPAATPEIR	Myc box dependent interacting protein 1
GNKS*PSPPDGS*PAATPEIR	Myc box dependent interacting protein 1
SPS*PPDGSPAATPEIR	Myc box dependent interacting protein 1
YSEWTSPAEDSS*PGISLSSSR	Bloom's syndrome protein
ADTTTPTPTAILAPGS*PASPPGSLEPK	Bromodomain-containing protein 2
KADTTTPTPTAILAPGS*PAS*PPGSLEPK	Bromodomain-containing protein 2
QASASYDS*EEEEEGLPMSYDEK	Bromodomain-containing protein 3
SES*PPPLSDPK	Bromodomain-containing protein 3
MPDEPEEPVVAVSS*PAVPPPTK	Bromodomain-containing protein 4
TEGVS*PIPQEIFEYLMDR	Peregrin
VAVEYLDPS*PEVQK	Mitotic checkpoint protein BUB3
YNAS*SFAK	Cadherin-17 precursor
LNSEAS*PSR	Chromatin assembly factor 1 subunit A
S*CPELTSGPR	Chromatin assembly factor 1 subunit A
TDTPPSSVPTSVISTPSTEEIQSETPGDAQGS*PPELK	Chromatin assembly factor 1 subunit B
TQDPSS*PGTTPPQAR	Chromatin assembly factor 1 subunit B
S*PPSLR	Signal transduction protein CBL-C
SIS*PSALQDLLR	CREB-binding protein
QGQSQAASSSSVTS*PIK	Cyclin T2
ES*EHDSDESS*DDDS*DSEEPSK	Leukocyte common antigen precursor
IGEGT*YGVVYK	Cell division protein kinase 2
VSNGS*PSLER	Cyclin-dependent kinase inhibitor 1B
KSS*PSTGS*LDSGNESK	Centaurin beta 2
ATPATAPGTS*PR	Centaurin gamma 3
VQEHEDS*GDS*EVENEAK	WD-repeat protein CGI-48
EVQAEQPSSSS*PR	Hypothetical protein CGI-79
ELQGDGPPSS*PTNDPTVK	Chromodomain helicase-DNA-binding protein 3
METEADAPS*PAPSLGER	Chromodomain helicase-DNA-binding protein 3
MSQPGS*PSPK	Chromodomain helicase-DNA-binding protein 4
MSQPGSPS*PK	Chromodomain helicase-DNA-binding protein 4
S*DSEGSDYTPGK	Chromodomain helicase-DNA-binding protein 4
STAPETAIECTQAPAPAS*EDEKVVVEPPEGEEK	Chromodomain helicase-DNA-binding protein 4
NIPS*PGQLDPDTR	Probable chromodomain-helicase-DNA-binding protein KIAA1416
T*PDTIR	Clathrin heavy chain 1
TSIDAYDNFDNIS*LAQR	Clathrin heavy chain 1
RFS*DS*EGEETVPEPR	CLN3 protein
SPSDLT*NPER	cAMP-specific 3',5'-cyclic phosphodiesterase 4C
FIIGSVSEDNS*EDEISNLVK	Acetyl-CoA carboxylase 1

Peptide	Protein
DADS*QNPDAPEGK	Coatomer alpha subunit
NLS*PGAVESDVR	Coatomer alpha subunit
GS*FPVAEKVNK	Cytochrome P450 2C18
SGPEAEGLGSETSPT*VDDEEEMLYGDSGSLFSPSK	Cleavage and polyadenylation specificity factor, 160 kDa subunit
VDTGVILEEGELKDDGEDS*EMQVEAPSDSSVIAQQK	Cleavage and polyadenylation specificity factor, 100 kDa subunit
AIT*PPQQPYK	Cell division cycle 2-related protein kinase 7
DGSGGASGTLQPSSGGGSSNS*R	Cell division cycle 2-related protein kinase 7
GS*PVFLPR	Cell division cycle 2-related protein kinase 7
NSS*PAPPQPAPGK	Cell division cycle 2-related protein kinase 7
QDDSPSGASYGQDYDLS*PSR	Cell division cycle 2-related protein kinase 7
S*PGSTSR	Cell division cycle 2-related protein kinase 7
SPS*PYSR	Cell division cycle 2-related protein kinase 7
SVS*PYSR	Cell division cycle 2-related protein kinase 7
TVDS*PK	Cell division cycle 2-related protein kinase 7
SVNEDDNPPS*PIGGDMMDSLISQLQPPPQQQPFPK	Cofactor required for Sp1 transcriptional activation subunit 2
FYDLS*DSDSNLSGEDSK	Hypothetical protein C20orf6
IEIPVTPTGQSVPSS*PSIPGTPTLK	Protein C20orf67
TFQQIQEEEDDDYPGSYS*PQDPSAGPLLTEELIK	Protein C20orf77
TTPES*PPYSSGSYDSIK	Hypothetical protein C20orf112
TPEELDDS*DFETEDFDVR	Alpha-1 catenin
MQGQS*PPAPTR	CH-TOG protein
MLQALS*PK	Cholinephosphate cytidylyltransferase B
FLPS*PVVIK	Cullin homolog 3
TPQS*PTLPPAK	Coxsackievirus and adenovirus receptor precursor
DAEPPS*PTPAGPPR	Adenylate cyclase, type VI
KPS*PQPSS*PR	Cyclin K
YT*RNLVDQGNGK	Cysteine dioxygenase type I
IS*ATSAEER	Cytohesin 4
ILQEKLDQPVS*APPS*PR	H4 protein
LDQPVSAPPS*PR	H4 protein
SGVDQMDLFGDMST*PPDLNSPTESK	Disabled homolog 2
SGVDQMDLFGDMSTPPDLNS*PTESK	Disabled homolog 2
SSPNPFVGS*PPK	Disabled homolog 2
ICTLPSPPS*PLASLAPVADSSTR	Death domain-associated protein 6
LLEDS*EESSEETVSR	Putative pre-mRNA splicing factor RNA helicase
ISLEQPPNGSDT*PNPEK	Probable ATP-dependent RNA helicase DDX20
YQES*PGIQMK	Probable ATP-dependent RNA helicase DDX20
NGFPHPEPDCNPSEAASEES*NSEIEQEIPVEQK	Nucleolar RNA helicase II
AQAVS*EEEEEEEGK	ATP-dependent RNA helicase DDX24
SPGKAEAESDALPDDT*VIESEALPSDIAAEAR	ATP-dependent RNA helicase DDX24
SEEVPAFGVAS*PPPLTDTPDTTANAEGDLPTTMGGPLPPHLALK	ATP-dependent RNA helicase A
GPAAPLTPGPQS*PPTPLAPGQEK	Deformed epidermal autoregulatory factor 1 homolog
GAGSIAGASAS*PK	Desmoplakin
GGGGYTCQS*GSGWDEFTK	Desmoplakin
GLPS*PYNMSSAPGSR	Desmoplakin
GLPSPYNMSSAPGS*R	Desmoplakin
SMS*FQGIR	Desmoplakin
SSSFS*DTLEESSPIAAIFDTENLEK	Desmoplakin
SSDQPLTVPVS*PK	Restricted expression proliferation associated protein 100
AGLESGAEPGDGDS*DTTK	Dyskerin
AKEVELVS*E	Dyskerin
HVTS*NAS*DSESSYR	Presynaptic protein SAP97
YHS*LGNISR	Dystrophia myotonica-containing WD repeat motif protein
AET*PTESVSEPEVATK	DNA ligase I
KQSQIQNQQGEDS*GSDPEDTY	DNA ligase I
TIQEVLEEQS*EDEDR	DNA ligase I
VLGS*EGEEEDEALSPAK	DNA ligase I
VLGSEGEEEDEALS*PAK	DNA ligase I
EADDDEEVDDNIPEMPS*PK	DNA (cytosine-5)-methyltransferase 1
LSS*PVK	DNA (cytosine-5)-methyltransferase 1
AIST*PETPLTK	DNA polymerase alpha 70 kDa subunit
S*PHQLLSPSSFS*PSATPSQK	DNA polymerase alpha 70 kDa subunit
IAS*PVSR	DNA polymerase alpha catalytic subunit
LS*S*PVLHR	Drebrin
AAAAGLGHPASPGGS*EDGPPGS*EEEDAAR	Dead ringer like-1 protein

Peptide	Protein
APS*PGAYK	Atrophin-1
AS*PGGVSTSSSDGK	Atrophin-1
QEPAEEYETPESPVPPARS*PS*PPPK	Atrophin-1
S*LNDDGSSDPR	Atrophin-1
SEEIS*ESESEETNAPK	Atrophin-1
SLNDDGSS*DPR	Atrophin-1
TAS*PPGPPPYGK	Atrophin-1
TAT*PPGYKPGS*PPSFR	Atrophin-1
TEQELPRPQS*PSDLDS*LDGR	Atrophin-1
TGT*PPGYR	Atrophin-1
DFQDYMEPEEGCQGS*PQR	Dynein light intermediate chain 2, cytosolic
RS*PTSSPT*PQR	Dynamin-1
EALNIIGDISTSTVSTPVPPPVDDTWLQSASSHSPT*PQR	Dynamin 2
GGS*PQMDDIK	Translation initiation factor eIF-2B epsilon subunit
EVAENQQNQSS*DPEEEK	Band 4.1-like protein 2
LVS*PEQPPK	Band 4.1-like protein 2
S*LDGAPIGVMDQSLMK	Band 4.1-like protein 2
AAEDDSAS*PPGAASDAEPGDEERPGLQVDCVVCGDK	Orphan nuclear receptor EAR-2
S*STPVPS*K	ECT2 protein
YGPADVEDTTGSGATDSKDDDDIDLFGS*DDEEESEEAK	Elongation factor 1-beta
FSVS*PVVR	Elongation factor 2
ELVEPLT*PSGEAPNQALLR	Epidermal growth factor receptor precursor
GPDEAMEDGEEGS*DDEAEWVVTK	EH-domain containing protein 2
TVDLLAGLGAERPETANTAQS*PYK	Epilepsy holoprosencephaly candidate-1 protein
YADSPGASS*PEQPK	ETS-related transcription factor Elf-1
SPS*LSPK	ETS-domain protein Elk-3
APVSSTESVIQSNTPT*PPPSQPLNETAEEESR	Echinoderm microtubule-associated protein-like 4
SS*PELLPSGVTDENEVTTAVTEK	Epidermal growth factor receptor substrate 15
ASSLSESS*PPK	Epithelial protein lost in neoplasm
NSPDECS*VAK	Transcriptional regulator ERG
AEPASPDS*PKGSS*ETETEPPVALAPGPAPTR	Steroid hormone receptor ERR1
S*NS*VEKPVSSILSR	Ena/vasodilator stimulated phosphoprotein-like protein
SAS*PTVPR	Envoplakin
ESSIIAPAPAEDVDT*PPR	Enhancer of zeste homolog 2
S*PILEEK	Fetal Alzheimer antigen
ADEASELACPT*PK	Fatty acid synthase
SGTNS*PPPPFSDWGR	F-box only protein 4
S*LEGGGCPAR	FH1/FH2 domains-containing protein
NNEES*PTATVAEQGEDITSK	FK506-binding protein 5
NAEAVLQS*PGLSGK	Flightless-I protein homolog
AFGPGLQGGSAGS*PAR	Filamin A
CSGPGLS*PGMVR	Filamin A
QEPLEEDS*PSSSSAGLDK	Fos-related antigen 2
S*PPAPGLQPMR	Fos-related antigen 2
HTLGDS*DNES	Ferritin heavy chain
MGAPESGLAEYLFDKHTLGDS*DNES	Ferritin heavy chain
LLSSEPLDLISVPFGNSSPSDIDVPKPGS*PEPQVSGLAANR	Forkhead box protein M1
LEPAS*PPEDTSAEVSR	General transcription factor II-I repeat domain-containing protein 1
SSS*PAPADIAQTVQEDLR	Ras-GTPase-activating protein binding protein 1
AASSSSPGS*PVASSPSR	Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1
VLSGNCNHQEGTS*S*DDELPSAEMIDFQK	GC-rich sequence DNA-binding factor
APGGESLLGPGPS*PPSALTPGLGAEAGGGFPGGAEPGNGLKPR	GC-rich sequence DNA-binding factor homolog
MADHLEGLS*S*DDEETSTDITNFNLEK	GC-rich sequence DNA-binding factor homolog
ISVIFS*LEELK	Gamma-tubulin complex component 6
SQSDLDDQHDYDSVAS*DEDTDQEPLR	ARF GTPase-activating protein GIT1
VPS*VESLFR	Golgi autoantigen, golgin subfamily A member 4
ALQS*PK	General transcription factor II-I
S*PGSNSK	General transcription factor II-I
SPS*WYGIPR	General transcription factor II-I
VPQALNFS*PEESDSTFSK	G2 and S phase expressed protein 1
AGGSAALS*PSK	Histone H1x
QNPQS*PPQDSSVTSK	Histone deacetylase 6
AGDLLEDS*PK	Hepatoma-derived growth factor
GNAEGS*S*DEEGKLVIDEPAK	Hepatoma-derived growth factor
NST*PSEPGSGR	Hepatoma-derived growth factor

Peptide	Protein
NSTPS*EPGSGR	Hepatoma-derived growth factor
S*PSPVQR	Potential helicase with zinc-finger domain
EDLPAENGETKTEES*PASDEAGEK	Nonhistone chromosomal protein HMG-14
QAEVANQET*KEDLPAENGETKTEESPAS*DEAGEK	Nonhistone chromosomal protein HMG-14
EESEES*EAEPVQR	HIRA-interacting protein 3
ESEQES*EEEILAQK	HIRA-interacting protein 3
EVS*DSEAGGGPQGER	HIRA-interacting protein 3
FNSESES*GSEASSPDYFGPPAK	HIRA-interacting protein 3
NGVAAEVS*PAKEENPR	HIRA-interacting protein 3
SLKES*EQES*EEEILAQK	HIRA-interacting protein 3
KLEKEEEEGIS*QES*S*EEEQ	High mobility group protein HMG-I/HMG-Y
KSLDS*DES*EDEEDDYQQK	28 kDa heat- and acid-stable phosphoprotein
SLDS*DESEDEEDDYQQK	28 kDa heat- and acid-stable phosphoprotein
ALSSAVQASPTS*PGGSPSSPSSGQR	Zinc finger protein HRX
NSSTPGLQVPVS*PTVPIQNQK	Zinc finger protein HRX
NTPSMQALGES*PESSSSELLNLGEGLGLDSNR	Zinc finger protein HRX
SPT*VPSQNPSR	Zinc finger protein HRX
TPSYS*PTQR	Zinc finger protein HRX
QLSS*GVSEIR	Heat shock 27 kDa protein
FELTGIPPAPRGVPQIEVT*FDIDANGILNVSAVDK	Heat shock cognate 71 kDa protein
IEDVGS*DEEDDS*GKDK	Heat shock protein HSP 90-beta
VKEEPPS*PPQS*PR	Heat shock factor protein 1
EGITGPPADSSKPIGPDDAIDALSSDFTCGS*PTAAGK	Calpain inhibitor
VSEEQTQPPS*PAGAGMSTAMGR	Gamma-interferon-inducible protein Ifi-16
IEPIPGES*PK	Translation initiation factor IF-2
INS*SGESGDESDEFLQSR	Translation initiation factor IF-2
INSSGES*GDESDEFLQSR	Translation initiation factor IF-2
INSSGESGDES*DEFLQSR	Translation initiation factor IF-2
QS*FDDNDS*EELEDKDSK	Translation initiation factor IF-2
VEMYS*GSDDDDDFNK	Translation initiation factor IF-2
WDGS*EEDEDNSK	Translation initiation factor IF-2
GIPLATGDTS*PEPELLPGAPLPPPKEVINGNIK	Eukaryotic translation initiation factor 3 subunit 4
QLT*PPEGSSK	Eukaryotic translation initiation factor 3 subunit 8
QNPEQS*ADEDAEK	Eukaryotic translation initiation factor 3 subunit 8
AQAVS*EDAGGNEGR	Eukaryotic translation initiation factor 3 subunit 9
TEPAAEAEAASGPSES*PS*PPAAEELPGSHAEPPVPAQGEAPGEQAR	Eukaryotic translation initiation factor 3 subunit 9
TEPAAEAEAASGPSESPS*PPAAEELPGSHAEPPVPAQGEAPGEQAR	Eukaryotic translation initiation factor 3 subunit 9
S*PPYTAFLGNLPYDVTEESIK	Eukaryotic translation initiation factor 4B
SQSS*DTEQQSPTSGGGK	Eukaryotic translation initiation factor 4B
SQSSDTEQQS*PTSGGGK	Eukaryotic translation initiation factor 4B
AAS*LTEDR	Eukaryotic translation initiation factor 4 gamma
EAALPPVS*PLK	Eukaryotic translation initiation factor 4 gamma
DSSKGEDS*AEETEAKPAVVAPAPVVEAVSTPSAAFPSDATAEQGPILTK	Interleukin enhancer-binding factor 3
GSSEQAES*DNMDVPPEDDSK	Interleukin enhancer-binding factor 3
LFPDT*PLALDANK	Interleukin enhancer-binding factor 3
IQEQESS*GEEDSDLSPEER	Protein phosphatase inhibitor 2
ALQS*PALGLR	Ras GTPase-activating-like protein IQGAP1
S*PGEYINIDFGEPGAR	Insulin receptor substrate-2
SNT*PESIAETPPAR	Insulin receptor substrate-2
SSEGGVGVGPGGGDEPPTS*PR	Insulin receptor substrate-2
VAS*PTSGVK	Insulin receptor substrate-2
S*PGPLPGAR	Insulin gene enhancer protein ISL-2
SAFTPATATGSSPS*PVLGQGEK	Intersectin 1
LFSSSSS*PPPAK	C-jun-amino-terminal kinase interacting protein 3
DATPPVS*PINMEDQER	Transcription factor jun-B
LAALKDEPQTVPDVPSFGES*PPLSPIDMDTQER	Transcription factor jun-D
SYTS*GPGSR	Keratin, type II cytoskeletal 8
ASYDVSDSGQLEHVQPWS*V	6-phosphofructokinase, type C
S*PPLPAVIR	Protein KIAA0852
SVAVS*DEEEVEEEAER	Protein KIAA0852
VYYS*PPVAR	Protein KIAA0889
IQPAGNTS*PR	Casein kinase I, epsilon isoform
MSDTGS*PGMQR	Kinesin-like protein KIF1B
SGLS*LEELR	Kinesin-like protein KIF1B
SVS*PSPVPLLFQPDQNAPPIR	Kinesin-like protein KIF23

Peptide	Protein
IQAAAST*PTNATAASDANTGDR	Glycogen synthase kinase-3 beta
EDSGSSS*PPGVFLEK	Protein KIAA1688
AQSLVIS*PPAPSPR	Antigen KI-67
IPCES*PPLEVVDTTASTK	Antigen KI-67
MPCESS*PPESADTPTSTR	Antigen KI-67
TPVQYSQQQNS*PQK	Antigen KI-67
ASS*LNFLNK	Kinesin light chain 2
QSST*PSAPELGQQPDVNISEWK	Phosphorylase B kinase beta regulatory chain
NLIDSMDQSAFAGFS*FVNPK	Protein kinase C, delta type
GDGGSTTGLSAT*PPASLPGSLTNVK	B-Raf proto-oncogene serine/threonine-protein kinase
SAS*EPSLNR	B-Raf proto-oncogene serine/threonine-protein kinase
TEGDEEAEEEQEENLEAS*GDYK	ATP-dependent DNA helicase II, 70 kDa subunit
LRLS*PS*PTSQR	Lamin A/C
SGAQASSTPLS*PTR	Lamin A/C
SYLLGNSS*PR	Lamin A/C
SADGS*APAGEGEGVTLQR	Large neutral amino acids transporter small subunit 1
LQAGEYVS*LGK	Long-chain-fatty-acid--CoA ligase 3
SS*PPSIAPLALDSADLS*EEK	Ligatin
S*PPPR	LIM-only protein 6
DGVLTLANNVT*PAK	Microtubule-associated protein 4
DMES*PTK	Microtubule-associated protein 4
DMS*PLSETEMALGKDVT*PPPETEVVLIK	Microtubule-associated protein 4
DVT*PPPETEVVLIK	Microtubule-associated protein 4
S*QESGYYDR	Matrin 3
S*YSPDGK	Matrin 3
SYS*PDGK	Matrin 3
SYS*PDGKES*PSDK	Matrin 3
SAGAPASVSGQDADGSTS*PR	Megakaryocyte-associated tyrosine-protein kinase
AIPELDAYEAEGLALDDEDVEELT*ASQR	DNA replication licensing factor MCM2
GNDPLTSS*PGR	DNA replication licensing factor MCM2
RTDALTS*S*PGR	DNA replication licensing factor MCM2
TDALTSS*PGR	DNA replication licensing factor MCM2
DGDSYDPYDFSDT*EEEMPQVHT*PK	DNA replication licensing factor MCM3
IAEPS*VCGR	DNA replication licensing factor MCM4
AEENTDQAS*PQEDYAGFER	Midasin
NGGEDT*DNEEGEEENPLEIK	Midasin
AETSEGSGSAPAVPEASAS*PK	Methyl-CpG-binding protein 2
NSVSPGLPQRPASAGAMLGGDLNS*ANGACPSPVGNGYVSAR	Myocyte-specific enhancer factor 2D
IVEPEVVGES*DS*EVEGDAWR	Microfibrillar-associated protein 1
IVEPEVVGESDS*EVEGDAWR	Microfibrillar-associated protein 1
MEREDS*S*EEEEEEIDDEEIER	Microfibrillar-associated protein 1
SLAALDALNT*DDENDEEEYEAWK	Microfibrillar-associated protein 1
AQETEAAPSQAPADEPEPES*AAAQSQENQDTRPK	Melanoma-associated antigen D2
LQSS*QEPEAPPPR	Melanoma-associated antigen D2
GAGATSGS*PPAGRN	Methylated-DNA--protein-cysteine methyltransferase
SPLVTGS*PK	Probable tumor suppressor protein MN1
LNQPGT*PTR	Dual specificity mitogen-activated protein kinase kinase 2
GVDFES*S*EDDDDDPFMNTSSLR	Double-strand break repair protein MRE11A
GVDFES*SEDDDDDPFMNTSSLR	Double-strand break repair protein MRE11A
TLHT*CLELLR	Double-strand break repair protein MRE11A
IHNVGS*PLK	DNA mismatch repair protein MSH6
SEEDNEIES*EEEVQPK	DNA mismatch repair protein MSH6
VIS*DS*ES*DIGGSDVEFKPDTK	DNA mismatch repair protein MSH6
VIS*DSESDIGGS*DVEFKPDTK	DNA mismatch repair protein MSH6
VAPVINNGS*PTILGK	Metastasis-associated protein MTA1
AES*FMFRT*WGADVINMTTVPEVVLAK	5'-methylthioadenosine phosphorylase
MDS*ALTARDR	Myosin Ic
GELIPIS*PSTEVGGSGIGTPPSVLK	Myb-related protein B
KFELLPT*PPLS*PSR	N-myc proto-oncogene protein
GPVGTVS*EAQLAR	Myoferlin
FSS*PIVK	Nuclear pore complex protein Nup153
S*PGSTPTTPTSSQAPQK	Nuclear pore complex protein Nup214
SPGSTPTT*PTSSQAPQK	Nuclear pore complex protein Nup214
QGGS*PDEPDSK	Neighbor of A-kinase anchoring protein 95
DGAVNGPSVVGDQT*PIEPQTSIER	Nuclear autoantigenic sperm protein
LVPS*QEETK	Nuclear autoantigenic sperm protein
AVS*LDSPVSVGSSPPVK	Nuclear receptor coactivator 3

Peptide	Protein
QSNSGAT*K	Nuclear receptor coactivator 6
HEAPSS*PISGQPCGDDQNAS*PSK	Nuclear receptor co-repressor 1
S*PGSISYLPSFFTK	Nuclear receptor co-repressor 1
VS*PENLVDK	Nuclear receptor co-repressor 1
YETPSDAIEVIS*PASSPAPPQEK	Nuclear receptor co-repressor 1
S*PGNTSQPPAFFSK	Nuclear receptor co-repressor 2
SGLEPASS*PSK	Nuclear receptor co-repressor 2
SRT*AS*GSSVTSLDGTR	NDRG1 protein
TAS*GSSVTSLDGTR	NDRG1 protein
TASGSSVTS*LDGTR	NDRG1 protein
YFVQGMGYMPSAS*MTR	NDRG1 protein
GSEGYLAATYPTVGQTS*PR	Neurofibromin
SNSGLATYS*PPMGPVSER	Neurofibromin
SVEDEMDS*PGEEPFYTGQGR	Nuclear factor 1 A-type
DAEQSGS*PR	Nuclear factor 1 C-type
SGSMEEDVDTSPGGDYYTSPSS*PTSSSR	Nuclear factor 1 C-type
SPFNSPS*PQDSPR	Nuclear factor 1 C-type
TEMDKS*PFNSPS*PQDSPR	Nuclear factor 1 C-type
AAPEASS*PPAS*PLQHLLPGK	Niban-like protein
GLLAQGLRPES*PPPAGPLLNGAPAGESPQPK	Niban-like protein
GGLS*PANDTGAK	Glycylpeptide N-tetradecanoyltransferase 1
EAAAGIQWSEEETEDEEEEKEVT*PESGPPK	Proliferating-cell nucleolar antigen p120
GGSISVQVNSIKFDS*E	Nucleolar phosphoprotein p130
GSS*PSR	Orphan nuclear receptor NR1D1
LLDEYNVTPS*PPGTVLTSALSPVICGPNR	Neurogenic locus notch homolog protein 2 precursor
TPSLALT*PPQAEQEVDVLDVNVR	Neurogenic locus notch homolog protein 2 precursor
DSENLAS*PSEYPENGER	Nuclear pore complex protein Nup98-Nup96 precursor
EVEEDS*EDEEMSEDEEDDSSGEEVVIPQKK	Nucleolin
KEDS*DEEEDDDSEEDEEDDEDEDEDEDEIEPAAM	Nucleolin
KEDSDEEEDDDS*EEDEEDDEDEDEDEDEIEPAAM	Nucleolin
VVVS*PTK	Nucleolin
ATVT*PS*PVKGK	Nuclear ubiquitous casein and cyclin-dependent kinases substrate
DSGSDEDFLMEDDDDS*DYGSSK	Nuclear ubiquitous casein and cyclin-dependent kinases substrate
NSQEDS*EDS*EDKDVK	Nuclear ubiquitous casein and cyclin-dependent kinases substrate
TPS*PKEEDEEPES*PPEK	Nuclear ubiquitous casein and cyclin-dependent kinases substrate
TS*TSPPPEKSGDEGSEDEAPSGED	Nuclear ubiquitous casein and cyclin-dependent kinases substrate
TSTS*PPPEK	Nuclear ubiquitous casein and cyclin-dependent kinases substrate
TSTSPPPEKS*GDEGSEDEAPSGED	Nuclear ubiquitous casein and cyclin-dependent kinases substrate
VVDYSQFQES*DDADEDYGR	Nuclear ubiquitous casein and cyclin-dependent kinases substrate
YGMGTS*VER	Pyruvate dehydrogenase E1 component alpha subunit, somatic form, mitochondrial precursor
SFSLASSSNS*PISQR	Oxysterol binding protein-related protein 11
MLAES*DES*GDEESVSQTDKTELQNTLR	Oxysterol-binding protein 1
SKELVSSSSSGSDS*DS*EVDK	Activated RNA polymerase II transcriptional coactivator p15
EQLSAQELMESGLQIQKS*PEPEVLSTQEDLFDQSNK	Tumor suppressor p53-binding protein 1
IDEDGENTQIEDTEPMS*PVLNSK	Tumor suppressor p53-binding protein 1
LMLSTSEYSQS*PK	Tumor suppressor p53-binding protein 1
MVIQGPSS*PQGEAMVTDVLEDQK	Tumor suppressor p53-binding protein 1
NGSTAVAESVAS*PQK	Tumor suppressor p53-binding protein 1
NS*PEDLGLSLTGDSCK	Tumor suppressor p53-binding protein 1
S*PEPEVLSTQEDLFDQSNK	Tumor suppressor p53-binding protein 1
SEDPPTT*PIR	Tumor suppressor p53-binding protein 1
SGTAETEPVEQDSS*QPSLPLVR	Tumor suppressor p53-binding protein 1
STPFIVPSS*PTEQEGR	Tumor suppressor p53-binding protein 1
TVSS*DGCSTPSR	Tumor suppressor p53-binding protein 1
VDVSCEPLEGVEKCS*DSQSWEDIAPEIEPCAENR	Coilin
LGFSLT*PSK	Cytosolic phospholipase A2
CSVS*LSNVEAR	Poly(A) polymerase alpha
TSPLNSSGSS*QGR	Poly(A) polymerase gamma
HYGITSPISLAS*PEEIDHIYTQK	

Peptide	Protein
VMTIPYQPMPASS*PVICAGGQDR	Poly(rC)-binding protein 1
KVMDS*DEDDDY	Programmed cell death protein 5
IDT*PPACTEESIATPSEIK	Pre-mRNA cleavage complex II protein Pcf11
S*PSLSSK	Protocadherin 7 precursor
DGELPVEDDIDLS*DVELDDLGKDEL	Protein disulfide isomerase A6 precursor
ANS*FVGTAQYVSPELLTEK	3-phosphoinositide dependent protein kinase-1
AFT*PFSGPK	Xaa-Pro dipeptidase
AS*QEEQIAR	Periplakin
EGEEPTVYS*DEEEPKDESAR	Membrane associated progesterone receptor component 1
GDQPAASGDS*DDDEPPPLPR	Membrane associated progesterone receptor component 1
S*LGDEGLNR	1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta 3
ELSESVQQQSTPVPLIS*PK	Protein kinase C binding protein 1
STS*PASEK	Protein kinase C binding protein 1
TGQAGS*LSGS*PKPFSPQLSAPITTK	Protein kinase C binding protein 1
TDVSNFDEEFTGEAPTLS*PPR	Protein kinase C-like 1
AS*SLGEIDESSELR	Protein kinase C-like 2
TST*FCGTPEFLAPEVLTETSYTR	Protein kinase C-like 2
AGGLDWPEATEVS*PSR	Plakophilin 3
AQLEPVAS*PAK	Plectin 1
GYYS*PYSVSGSGSTAGSR	Plectin 1
GYYSPYSVSGSGST*AGSR	Plectin 1
SDEGQLS*PATR	Plectin 1
SSS*VGSSSSYPISPAVSR	Plectin 1
T*QLASWSDPTEETGPVAGILDTETLEK	Plectin 1
INPPSSGGTSSS*PIK	POU domain, class 2, transcription factor 1
SAVCIADPLPTPS*QEK	Ribonucleases P/MRP protein subunit POP1
VQAYEEPSVASS*PNGK	Ribonucleases P/MRP protein subunit POP1
LTFDSSFS*PNTGK	Voltage-dependent anion-selective channel protein 1
GLCIKS*REIFLS*QPILLELEAPLK	Serine/threonine protein phosphatase PP1-beta catalytic subunit
QVPDS*AATATAYLCGVK	Alkaline phosphatase, intestinal precursor
LPST*SDDCPAIGTPLR	Peroxisome proliferator-activated receptor binding protein
LPSTSDDCPAIGT*PLR	Peroxisome proliferator-activated receptor binding protein
MSS*LLER	Peroxisome proliferator-activated receptor binding protein
NSSQSGGKPGSS*PITK	Peroxisome proliferator-activated receptor binding protein
SQT*PPGVATPPIPK	Peroxisome proliferator-activated receptor binding protein
DAS*PINRWS*PTR	Serine/threonine-protein kinase PRP4 homolog
EQPEMEDANS*EKS*INEENGEVSEDQSQNK	Serine/threonine-protein kinase PRP4 homolog
S*LS*PKPR	Serine/threonine-protein kinase PRP4 homolog
S*PIINESR	Serine/threonine-protein kinase PRP4 homolog
S*PVDLR	Serine/threonine-protein kinase PRP4 homolog
S*RS*PLLNDR	Serine/threonine-protein kinase PRP4 homolog
SINEENGEVS*EDQSQNK	Serine/threonine-protein kinase PRP4 homolog
SINEENGEVSEDQS*QNK	Serine/threonine-protein kinase PRP4 homolog
SPS*PDDILER	Serine/threonine-protein kinase PRP4 homolog
TLS*PGR	Serine/threonine-protein kinase PRP4 homolog
TRS*PS*PDDILER	Serine/threonine-protein kinase PRP4 homolog
YLAEDSNMSVPSEPSS*PQSSTR	Serine/threonine-protein kinase PRP4 homolog
YLAEDSNMSVPSEPSSPQSST*R	PR-domain zinc finger protein 2
TAS*PPALPK	PR-domain zinc finger protein 14
T*SQLLPCSPSK	DNA-dependent protein kinase catalytic subunit
LTPLPEDNS*MNVDQDGDPSDR	Proteasome subunit alpha type 3
ESLKEEDES*DDDNM	Proteasome subunit alpha type 5
ITS*PLMEPSSIEK	26S proteasome non-ATPase regulatory subunit 1
TPEAS*PEPK	26S proteasome non-ATPase regulatory subunit 1
TSSAFVGKT*PEAS*PEPK	26S proteasome non-ATPase regulatory subunit 1
TVGT*PIASVPGSTNTGTVPGSEK	Periodic tryptophan protein 1 homolog
EKLQEEGGGS*DEEETGS*PSEDGMQSAR	CTP synthase
SGS*S*SPDSEITELKFPSINHD	CTP synthase
SGSSS*PDSEITELK	Postreplication repair protein RAD18
NDLQDTEIS*PR	Postreplication repair protein RAD18
NHLLQFALES*PAK	RNA-binding protein 8A
GFGSEEGS*R	Retinoblastoma-binding protein 1
NGTGQSS*DSEDLPVLDNSSK	Retinoblastoma-binding protein 2
QGPVS*PGPAPPPSFIMSYK	Retinoblastoma-binding protein 2
VVSSVSSS*PR	

Peptide	Protein
VSS*PVFGATSSIK	Retinoblastoma-binding protein 8
VVNPLIGLLGEYGGDSDYEEEEEEEQT*PPPQPR	RNA-binding protein 6
SFS*SPENFQR	Putative RNA-binding protein 7
GLVAAYSGES*DSEEEQER	RNA-binding protein 10
GLVAAYSGESDS*EEEQER	RNA-binding protein 10
LGGSGGSNGS*SSGK	Putative RNA-binding protein 15
LHS*YSS*PSTK	Putative RNA-binding protein 15
SLS*PGGAALGYR	Putative RNA-binding protein 15
AVVS*PPK	Ran-binding protein 2
LNQSGTS*VGTDEESDVTQEEER	Ran-binding protein 2
SALS*PSKS*PAK	Ran-binding protein 2
T*SPENVQDR	Ran-binding protein 2
YIASVQGSTPS*PR	Ran-binding protein 2
YSLS*PSK	Ran-binding protein 2
S*PPADAIPK	Regulator of chromosome condensation
SIS*ADDDLQESSR	RD protein
NLDNVS*PK	Double-stranded RNA-specific editase 1
VDDDS*LGEFPVTNSR	Zinc-finger protein ubi-d4
ATS*PLCTSTASMVSSS*PSTPSNIPQKPSQPAAK	Restin
TASESISNLSEAGS*IK	Restin
ESVS*PEDSEK	Activator 1 140 kDa subunit
ASETVSEAS*PGSTASQTGVPTQVVQQVQGTQQR	MHC class II regulatory factor RFX1
ILDPNTGEPAPVLSSPPPADVST*FLAFPSPEKLLR	Ran GTPase-activating protein 1
KILDPNTGEPAPVLSS*PPPADVSTFLAFPS*PEK	Ran GTPase-activating protein 1
VEAKEESEES*DEDMGFGLFD	60S acidic ribosomal protein P0
NMGGPYGGGNYGPGGSGGS*GGYGGR	Heterogeneous nuclear ribonucleoproteins A2/B1
DDEKEAEEGEDDRDS*ANGEDDS	Heterogeneous nuclear ribonucleoproteins C1/C2
EAEEGEDDRDS*ANGEDDS	Heterogeneous nuclear ribonucleoproteins C1/C2
MESEGGADDS*AEEGDLLDDDDNEDRGDDQLELIK	Heterogeneous nuclear ribonucleoproteins C1/C2
NEEDEGHSNSS*PR	Heterogeneous nuclear ribonucleoprotein D0
ATENDIYNFFS*PLNPVR	Heterogeneous nuclear ribonucleoprotein F
GFAFVTFES*PADAK	Heterogeneous nuclear ribonucleoprotein G
GLPWSCS*ADEVQR	Heterogeneous nuclear ribonucleoprotein H
DYDDMS*PR	Heterogeneous nuclear ribonucleoprotein K
IIPTLEEGLQLPS*PTATSQLPLESDAVECLNYQHYK	Heterogeneous nuclear ribonucleoprotein K
MET*EQPEETFPNTETNGEFGK	Heterogeneous nuclear ribonucleoprotein K
IFVGGLS*PDTPEEK	Heterogeneous nuclear ribonucleoprotein UP2
IFVGGLSPDT*PEEK	Heterogeneous nuclear ribonucleoprotein UP2
YSPTSPTYS*PTSPVYTPTSPK	DNA-directed RNA polymerase II largest subunit
YSPTSPTYSPTS*PK	DNA-directed RNA polymerase II largest subunit
AEGS*PNQGK	Ribosome-binding protein 1
NTDVAQS*PEAPK	Ribosome-binding protein 1
ANS*GGVDLDSSGEFASIEK	RAS-responsive element binding protein 1
DEILPTT*PISEQK	40S ribosomal protein S3
RFT*PPSTALS*PGK	Runt-related transcription factor 1
ISS*PTETER	S100 calcium-binding protein A14
LIHEQEQQSSS*	Putative S100 calcium-binding protein MGC17528
ASPGTPLS*PGSLR	Solute carrier family 21 member 12
NCAS*PSSAGQLILPECMK	Protein transport protein Sec24C
AEEPPSQLDQDTQVQDMDEGS*DDEEEGQK	Splicing factor 3 subunit 1
GGDSIGETPT*PGASK	Splicing factor 3B subunit 1
WDETPAS*QMGGSTPVLT*PGK	Splicing factor 3B subunit 1
WDETPASQMGGST*PVLTPGK	Splicing factor 3B subunit 1
SS*LGQSASETEEDTVSVSK	Splicing factor 3B subunit 2
SSLGQS*ASETEEDTVSVSK	Splicing factor 3B subunit 2
SSLGQSAS*ETEEDTVSVSK	Splicing factor 3B subunit 2
AKS*PT*PDGSER	Putative splicing factor YT521
GIS*PIVFDR	Putative splicing factor YT521
SEASDSGS*ESVSFTDGSVR	Putative splicing factor YT521
SGS*SASESYAGSEK	Putative splicing factor YT521
SGSSAS*ESYAGSEK	Putative splicing factor YT521
SGSSASESYAGS*EK	Putative splicing factor YT521
SPT*PDGSER	Putative splicing factor YT521
GSS*FQSGR	Exocyst complex component Sec5
ESIS*PQPADSACSSPAPSTGK	Sentrin-specific protease 6
LNYSDES*PEAGK	Sentrin-specific protease 6
S*RS*PPPVSK	Splicing factor, arginine/serine-rich 2

Peptide	Protein
SPPKS*PEEEGAVSS	Splicing factor, arginine/serine-rich 2
TS*PDTLR	Splicing factor, arginine/serine-rich 2
SPAS*VDR	Splicing factor, arginine/serine-rich 5
SVS*RS*PVPEK	Splicing factor, arginine/serine-rich 5
ARS*VS*PPPK	Splicing factor, arginine/serine-rich 6
S*NSPLPVPPSK	Splicing factor, arginine/serine-rich 6
S*VS*PPPKR	Splicing factor, arginine/serine-rich 6
SVS*PPPK	Splicing factor, arginine/serine-rich 6
S*RSPSGS*PR	Splicing factor, arginine/serine-rich 7
SAS*PERMD	Splicing factor, arginine/serine-rich 7
SPS*GSPR	Splicing factor, arginine/serine-rich 7
SPS*PK	Splicing factor, arginine/serine-rich 7
YFQS*PSR	Splicing factor, arginine/serine-rich 7
ARS*QSVS*PSK	Splicing factor, arginine/serine-rich 8
S*PGASR	Splicing factor, arginine/serine-rich 8
SQSVS*PSK	Splicing factor, arginine/serine-rich 8
STS*YGYSR	Splicing factor, arginine/serine-rich 9
SRT*PSASNDDQQE	Small glutamine-rich tetratricopeptide repeat-containing protein
ASS*LEDLVLK	Helicase SKI2W
GDTVSAS*PCSAPLAR	Helicase SKI2W
KACYS*K	Semaphorin 5A precursor
VQGLLENGDSVTS*PEK	SmcX protein
GPS*PSPVGSPASVAQSR	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin subfamily F member 1
NPQMPQYSSPQPGSALS*PR	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin subfamily F member 1
VSS*PAPMEGGEEEEELLGPK	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin subfamily F member 1
TTS*PEPQESPTLPSTEGQVVNK	Smoothelin
AEENAEGGESALGPDGEPIDESSQMS*DLPVK	Possible global transcription activator SNF2L2
EVDYSDS*LTEK	Possible global transcription activator SNF2L4
IPDPDS*DDVSEVDAR	Possible global transcription activator SNF2L4
VAELTSLS*DEDSGK	Zinc finger protein SNAI1
AVNTQALS*GAGILR	Sorting nexin 2
ESDQTLAALLS*PK	SON protein
S*AASPVVSSMPER	SON protein
S*FSISPVR	SON protein
S*PDPYR	SON protein
SAAS*PVVSSMPER	SON protein
SFSIS*PVR	SON protein
SVESTS*PEPSK	SON protein
YDVDLSLTTQDTEHDMVISTSPSGGS*EADIEGPLPAK	SON protein
IPESETESTASAPNS*PR	Son of sevenless protein homolog 1
TSISDPPES*PPLLPPR	Son of sevenless protein homolog 1
SSSTGSSSSTGGGGQESQPS*PLALLAATCSR	Transcription factor Sp1
ENNVSQPASSSSSSSSSNNGSASPT*K	Transcription factor Sp4
SGS*DAGEARPPTPAS*PR	Signal-induced proliferation-associated protein 1
CTELNQAWSS*LGK	Spectrin alpha chain, brain
GEQVS*QNGLPAEQGSPR	Spectrin beta chain, brain 1
GEQVSQNGLPAEQGS*PR	Spectrin beta chain, brain 1
TSSKESS*PIPS*PTSDR	Spectrin beta chain, brain 1
S*PQTLAPVGEDAMK	Symplekin
IEIIQPLLDMAAGTSNAAPVAENVTNNEGS*PPPPVK	CTD-binding SR-like protein RA4
TT*PTQPSEQK	CTD-binding SR-like protein RA4
AKTQT*PPVS*PAPQPTEER	Src substrate cortactin
LPSS*PVYEDAASFK	Src substrate cortactin
TQT*PPVSPAPQPTEER	Src substrate cortactin
VGGS*DEEASGIPSR	Suppressor of SWI4 1 homolog
EGMNPSYDEYADS*DEDQHDAYLER	Structure-specific recognition protein 1
SKEFVSS*DESSS*GENK	Structure-specific recognition protein 1
GTDAT*NPPEGPQDR	Stanniocalcin 2 precursor
QVAEQGGDLS*PAANR	Serine/threonine protein kinase 10
NLEQILNGGES*PK	Striatin 3
EYIPGQPPLSQSS*DSS*PTRNSEPAGLETPEAK	Bifunctional aminoacyl-tRNA synthetase
NQGGGLSSS*GAGEGQGPK	Bifunctional aminoacyl-tRNA synthetase
NSEPAGLET*PEAK	Bifunctional aminoacyl-tRNA synthetase
LLS*SNEDDANILSSPTDR	Thyroid hormone receptor-associated protein complex 100

Peptide	Protein
LLSS*NEDDANILSSPTDR	kDa component Thyroid hormone receptor-associated protein complex 100 kDa component
AS*AVSELSPR	Thyroid hormone receptor-associated protein complex 150 kDa component
ASAVSELS*PR	Thyroid hormone receptor-associated protein complex 150 kDa component
AVQEKSS*S*PPPR	Thyroid hormone receptor-associated protein complex 150 kDa component
EQTFSGGTS*QDTK	Thyroid hormone receptor-associated protein complex 150 kDa component
FSGEEGEIEDDES*GTENR	Thyroid hormone receptor-associated protein complex 150 kDa component
GSFS*DTGLGDGK	Thyroid hormone receptor-associated protein complex 150 kDa component
IDIS*PSTFR	Thyroid hormone receptor-associated protein complex 150 kDa component
S*PPSTGSTYGSSQK	Thyroid hormone receptor-associated protein complex 150 kDa component
SPPST*GSTYGSSQK	Thyroid hormone receptor-associated protein complex 150 kDa component
SSS*PPPR	Thyroid hormone receptor-associated protein complex 150 kDa component
SSSS*SS*QSSHSYK	Thyroid hormone receptor-associated protein complex 150 kDa component
SNDS*TDGEPEEK GAGGPAS*AQGSVK	TBP-associated factor 172 Thyroid hormone receptor-associated protein complex 240 kDa component
LLEPPVLTLDPNDENLILEIPDEKEEATSNS*PSK	Transcription initiation factor TFIID 250 kDa subunit
QEAGDS*PPPAPGTPK	Transcription initiation factor TFIID 70 kDa subunit
AS*PEPPGPESSSR	182 kDa tankyrase 1-binding protein
HNGS*LS*PGLEAR	182 kDa tankyrase 1-binding protein
VPSS*DEEVVEEPQSR	182 kDa tankyrase 1-binding protein
VSGAGFS*PSSK	182 kDa tankyrase 1-binding protein
WLDDLLAS*PPPSGGGAR	182 kDa tankyrase 1-binding protein
YESQEPLAGQES*PLPLATR	182 kDa tankyrase 1-binding protein
SGCSEAQPPES*PETR	Transforming acidic coiled-coil-containing protein 3
FIQELSGSS*PK	Transcription factor AP-4
SGYSSPGS*PGTPGSR	Microtubule-associated protein tau
SPVVSGDTS*PR	Microtubule-associated protein tau
RAVSEGCAS*EDEVEGEA	TBC1 domain family member 2
TSSTCS*NESLSVGGTSVTPR	TBC1 domain family member 4
EPAITSQNS*PEAR	Transcription elongation factor A protein 1
NNDQPQSANANEPQDSTVNLQS*PLK	Transcription factor 8
DSES*PSQK	Treacle protein
LDSS*PSVSSTLAAK	Treacle protein
LGAGEGGEAS*VSPEK	Treacle protein
LGAGEGGEASVS*PEK	Treacle protein
S*PAGPAATPAQAQAASTPR	Treacle protein
SSSS*ESEDEDVIPATQCLTPGIR	Treacle protein
TQPSSGVDSAVGTLPATS*PQSTSVQAK	Treacle protein
S*PSSVTGNALWK	Telomeric repeat binding factor 2 interacting protein 1
S*GEGEVSGLMR	Transcription intermediary factor 1-beta
AGSS*PAQGAQNEPPR	Transcription factor 20
LNAS*PAAREEATS*PGAK	Transcription factor 20
QLS*GQSTSSDTTYK	Transcription factor 20
SLT*PPPSSTESK	Transcription factor 20
GPPDFS*S*DEEREPTPVLGSGAAAAGR	Thymopoietin, isoform alpha
SSTPLPTISSS*AENTR	Thymopoietin, isoform alpha
VPEASSEPFDTSS*PQAGR	Triple homeobox 1 protein
ILAT*PPQEDAPSVDIANIR	Transketolase
DAPTS*PASVASSSSTPSSK	Transducin-like enhancer protein 3
ESSANNSVS*PSESLR	Transducin-like enhancer protein 3
VS*PAHS*PPENGLDK	Transducin-like enhancer protein 3
YDS*DGDKSDDLVVDVSNEDPATPR	Transducin-like enhancer protein 3
YDSDGDKS*DDLVVDVSNEDPATPR	Transducin-like enhancer protein 3
LDEGT*PPEPK	Talin 2
TTQSMQDFPVVDS*EEEAEEEFQK	Tuftelin-interacting protein 11

Peptide	Protein
FTMDLDS*DEDFSDFDEKT*DDEDFVPSDASPPK	DNA topoisomerase II, alpha isozyme
GSVPLS*SS*PPATHFPDETEITNPVPK	DNA topoisomerase II, alpha isozyme
KPS*TSDDS*DSNFEK	DNA topoisomerase II, alpha isozyme
NENTEGS*PQEDGVELEGLK	DNA topoisomerase II, alpha isozyme
SVVS*DLEADDVK	DNA topoisomerase II, alpha isozyme
TDDEDFVPSDAS*PPK	DNA topoisomerase II, alpha isozyme
TQMAEVLPS*PR	DNA topoisomerase II, alpha isozyme
VPDEEENEES*DNEK	DNA topoisomerase II, alpha isozyme
AS*GSENEGDYNPGR	DNA topoisomerase II, beta isozyme
FDS*NEEDSASVFSPSFGLK	DNA topoisomerase II, beta isozyme
VVEAVNS*DSDSEFGIPK	DNA topoisomerase II, beta isozyme
T*IDDLEDELYAQK	Tropomyosin alpha 3 chain
AADSQNS*GEGNTGAAESSFSQEVSR	Nucleoprotein TPR
RS*PS*PYYSR	Arginine/serine-rich splicing factor 10
SPS*PYYSR	Arginine/serine-rich splicing factor 10
DLVLPTQALPAS*PALK	Telomeric repeat binding factor 2
TS*PLVSQNNEQGSTLR	Thyroid receptor interacting protein 8
SES*PPAELPSLR	Thyroid receptor interacting protein 12
TT*PLPPPR	Myeloid/lymphoid or mixed-lineage leukemia protein 4
DIDHETVVEEQIIGENS*PPDYSEYMTGK	Transcriptional repressor protein YY1
YYPTAEEVYGPEVETIVQEEDT*QPLTEPIIKPVK	116 kDa U5 small nuclear ribonucleoprotein component
SQS*MDIDGVSCEK	Ubiquitin conjugation factor E4 B
NGS*EADIDEGLYSR	Ubiquitin-activating enzyme E1
AGEQQLS*EPEDMEMEAGDTDDPPR	Ubiquitin carboxyl-terminal hydrolase 7
NHSVNEEEQEEQGEGS*EDEWEQVGPR	Ubiquitin carboxyl-terminal hydrolase 10
TCNS*PQNSTDSVSDIVPDSPFPGALGSDTR	Ubiquitin carboxyl-terminal hydrolase 10
NINMDNDLEVLTSS*PTR	Ubiquitin carboxyl-terminal hydrolase 16
AVPPGNDPVS*PAMVR	Ubiquitin carboxyl-terminal hydrolase 19
SVDQGGGGS*PR	Ubiquitin carboxyl-terminal hydrolase 24
T*ISAQDTLAYATALLNEK	Ubiquitin carboxyl-terminal hydrolase 24
VSDQNS*PVLPK	Ubiquitin carboxyl-terminal hydrolase 24
APAGQEEPGT*PPSSPLSAEQLDR	Uracil-DNA glycosylase
TDNSVASS*PSSAISTATPSPK	Ubiquitously transcribed X chromosome tetratricopeptide repeat protein
DCDPGS*PR	Vigilin
VATLNS*EEESDPPTYK	Vigilin
LCDDGPQLPTS*PR	Vinexin
SPADPTDLGGQTS*PR	Vinexin
SS*SLQGMDMASLPPR	WD-repeat protein WDC146
SPAAPYFLGSSFS*PVR	Wee1-like protein kinase
SEAAAPHTDAGGGLS*S*DEEEGTSSQAEAAR	DNA-repair protein complementing XP-C cells
ELTPAS*PTCTNSVSK	DNA-repair protein complementing XP-G cells
FDSSLLSS*DDETK	DNA-repair protein complementing XP-G cells
INSSTENS*DEGLK	DNA-repair protein complementing XP-G cells
NAPAAVDEGSIS*PR	DNA-repair protein complementing XP-G cells
TEKEPDAT*PPS*PR	DNA-repair protein complementing XP-G cells
TLLAMQAALLGS*S*S*EEELESENRR	DNA-repair protein complementing XP-G cells
NEMGIPQQTTS*PENAGPQNTK	Hypothetical protein KIAA0008
SEPSGEINIDSS*GETVGSGER	Hypothetical protein KIAA0056
SLGVLPFTLNSGS*PEK	Hypothetical protein KIAA0056
SPAVATSTAAPPPPSS*PLPSK	Hypothetical protein KIAA0144
STSAPQMS*PGSSDNQSSSPQPAQQK	Hypothetical protein KIAA0144
YPSSISSS*PQK	Hypothetical protein KIAA0144
ASDSSS*PSCSSGPR	Hypothetical zinc finger protein KIAA0211
GSPSVAASS*PPAIPK	Hypothetical zinc finger protein KIAA0211
MSDYS*PNSTGSVQNTSR	Putative deoxyribonuclease KIAA0218
ASEGLDACAS*PTK	Hypothetical zinc finger protein KIAA0222
ADSGPTQPPLSLS*PAPETK	Hypothetical protein KIAA0310
QEPGGS*HGSET*EDTGR	Hypothetical protein KIAA0553
QAS*T*DAGTAGALTPQHVR	65 kDa Yes-associated protein
GGLLTSEEDSGFSTS*PK	Zinc finger protein 148
QPLEQNQTIS*PLSTYEESK	Zinc finger protein 148
LSS*FSHK	Zinc finger protein 198
MTGSAPPPS*PTPNK	Zinc finger protein 198
AGAES*PTMSVDGR	Zinc finger protein 217
DVTGS*PPAK	Zinc finger protein 217
QS*PPGPGK	Zinc finger protein 217

Peptide	Protein
TSVS*PAPDK	Zinc finger protein 217
S*ALNVHHK	Zinc finger protein 255
SAPTAPT*PPPPPPPATPR	Zinc finger protein 261
LDEDEDEDDADLSKYNLDAS*EEEDSNK	Zinc finger protein 265
YNLDAS*EEEDSNK	Zinc finger protein 265
EGAS*PVTEVR	Zinc finger protein 295
ESEVCPVPTNSPS*PPPLPPPPPLPK	Zinc finger protein 295
IQPLEPDS*PTGLSENPTPATEK	Zinc finger protein 295
SFS*ASQSTDR	Zinc finger protein 295
SLS*MDSQVPVYSPSIDLK	Zinc finger protein 295
TEPSS*PLSDPSDIIR	Zinc finger protein 295
DGPEPPS*PAK	Zinc finger protein 335
GPASQFYITPSTSLS*PR	Nuclear protein ZAP3
SVGDDEELQQNESGTS*PK	Zinc finger protein 40
ADPGEDDLGGTVDIVES*EPENDHGVELLDQNSSIR	Zinc finger X-chromosomal protein
AYS*PEYR	Tight junction protein ZO-2
GSYGS*DAEEEEYR	Tight junction protein ZO-2
SPS*PEPR	Tight junction protein ZO-2
GPPASS*PAPAPKFS*PVTPK	Zyxin
S*PGAPGPLTLK	Zyxin
S*PILLPK	Cytoskeleton-like bicaudal D protein homolog 2
KTSS*DDES*EEDEDDLLQR	WD-repeat protein CGI-48
NSSS*PVSPASVPGQR	Protein C14orf4
RNS*SS*PVSPASVPGQR	Protein C14orf4
RNS*SSPVS*PASVPGQR	Protein C14orf4
QEAIPDLEDSPPVS*DSEEQQESAR	Death associated transcription factor 1
S*PPEGDTTLFLSR	Death associated transcription factor 1
TAAPS*PSLLYK	Death associated transcription factor 1
SLSNS*NPDISGTPTSPDDEVR	Dedicator of cytokinesis protein 7
SLSNSNPDISGTPTS*PDDEVR	Dedicator of cytokinesis protein 7
LGAS*QER	Transcription elongation factor B polypeptide 3
GS*DGEDSASGGK	Separin
SSSLGS*YDDEQEDLTPAQLTR	Protein FAM13A1
SASEHSSS*AES*ER	Formin binding protein 3
ENSGPVENGVS*DQEGEEQAR	Gem-associated protein 5
AQSNGSGNGS*DSEMDTSSLER	Glucocorticoid receptor DNA binding factor 1
TSFSVGS*DDELGPIR	Glucocorticoid receptor DNA binding factor 1
AQS*SPAAPASLSAPEPASQAR	Histone deacetylase 7a
AQSS*PAAPASLSAPEPASQAR	Histone deacetylase 7a
TQT*PPLGQTPQLGLK	Eukaryotic translation initiation factor 4 gamma 2
ASMSEFLES*EDGEVEQQR	Polycomb protein SUZ12
SSS*PIPLTPSK	Male-specific lethal 3-like 1
DLRS*SS*PR	Mitogen-activated protein kinase kinase kinase kinase 1
AASSLNLS*NGETESVK	Mitogen-activated protein kinase kinase kinase kinase 4
TTS*RS*PVLSR	Mitogen-activated protein kinase kinase kinase kinase 4
EETEYEYS*GS*EEEDDSHGEEGEPSSIMNVPGESTLR	Mitogen-activated protein kinase kinase kinase kinase 6
LDSS*PVLSPGNK	Mitogen-activated protein kinase kinase kinase kinase 6
SPVPSPGSSS*PQLQVK	Molecule interacting with Rab13
VEQMPQAS*PGLAPR	Molecule interacting with Rab13
VPAMPGS*PVEVK	Protein CBFA2T2
FS*PDSQYIDNR	Partitioning-defective 3 homolog
GLIVYCVTS*PK	PDZ domain containing guanine nucleotide exchange factor 2
MAPPVDDLS*PK	PHD finger protein 3
QLQEDQENNLQDNQTSNSS*PCR	PHD finger protein 3
NSADDEELTNDS*LTLSQSK	PHD finger protein 14
GVQVPAS*PDTVPQPSLR	PHD finger protein 16
ETVQTTQS*PTPVEK	Putative RNA-binding protein 16
NSLLAGGDDDTMSVIS*GISSR	Cohesin subunit SA-2
NSLLAGGDDDTMSVISGISS*R	Cohesin subunit SA-2
LFQLGPPS*PVK	Securin
AAEKPEEEESAAEEESNS*DEDEVIPDIDVEVDVDELNQEQVADLNK	Splicing factor, arginine/serine-rich 16
ITFITSFGGS*DEEAAAAAAAAAASGVTTGKPPAPPQPGGPAPGR	Splicing factor, arginine/serine-rich 16
SQS*PSPS*PAREK	Splicing factor, arginine/serine-rich 16
SQSPS*PSPAR	Splicing factor, arginine/serine-rich 16
SRS*PT*PGR	Splicing factor, arginine/serine-rich 16
GTMDDISQEEGSS*QGEDSVSGSQR	Structural maintenance of chromosome 1-like 1 protein

Peptide	Protein
GTMDDISQEEGSSQGEDS*VSGSQR	Structural maintenance of chromosome 1-like 1 protein
MEEESQS*QGR	Structural maintenance of chromosome 1-like 1 protein
GDVEGSQSQDEGEGS*GESER	Structural maintenance of chromosome 3
GSGS*QSSVPSVDQFTGVGIR	Structural maintenance of chromosome 3
KGDVEGS*QS*QDEGEGSGESER	Structural maintenance of chromosome 3
EEGPPPPS*PDGASSDAEPEPPSGR	Structural maintenance of chromosomes 4-like 1 protein
REEGPPPPS*PDGASS*DAEPEPPSGR	Structural maintenance of chromosomes 4-like 1 protein
TES*PATAAETASEELDNR	Structural maintenance of chromosomes 4-like 1 protein
ANT*PDS*DITEKTEDSSVPETPDNER	SWI/SNF-related, actin-dependent regulator of chromatin subfamily A containing DEAD/H box 1
IEEAPEATPQPSQPGPSS*PISLSAEEENAEGEVSR	SWI/SNF-related, actin-dependent regulator of chromatin subfamily A containing DEAD/H box 1
NKIEEAPEATPQPSQPGPSS*PIS*LS*AEEENAEGEVSR	SWI/SNF-related, actin-dependent regulator of chromatin subfamily A containing DEAD/H box 1
TEDSS*VPETPDNER	SWI/SNF-related, actin-dependent regulator of chromatin subfamily A containing DEAD/H box 1
T*PPVVIK	Synapse associated protein 1
KAEDS*DS*EPEPEDNVR	5'-3' exoribonuclease 2
NS*PGSQVASNPR	5'-3' exoribonuclease 2
EES*DEEEEDDEESGR	GPN:BC011923_1
GDSIEEILADS*EDEEDNEEEER	GPN:BC012745_1
EPTPSIASDIS*LPIATQELR	GPN:BC013957_1
SSFYSGGWQEGSSS*PR	GPN:BC015239_1
YNAVLGFGALTPTS*PQSSHPDS*PENEK	GPN:BC015714_1
LLSS*ESEDEEEFIPLAQR	GPN:BC016470_1
MAGNEALS*PTSPFR	GPN:BC017269_1
DSDSGSDSDS*DQENAASGSNASGSESDQDERGDSGQPSNK	GPN:BC018147_1
GS*DSEDEVLR	GPN:BC018147_1
GSDS*EDEVLR	GPN:BC018147_1
KNAIAS*DSEADS*DTEVPK	GPN:BC018147_1
LTS*DEEGEPSGK	GPN:BC018147_1
NAIAS*DSEADSDTEVPK	GPN:BC018147_1
NAIASDSEADS*DTEVPK	GPN:BC018147_1
LEDSEVRS*VAS*NQSEMEFSSLQDMPK	GPN:BC018269_1
S*VASNQSEMEFSSLQDMPK	GPN:BC018269_1
SVAS*NQSEMEFSSLQDMPK	GPN:BC018269_1
YLPLNTALYEPPLDPELPALDS*DGDS*DDGEDGRGDEK	GPN:BC020954_1
S*FEVEEVETPNSTPPR	GPN:BC021192_1
FLNILLLIPTLQS*EGHIR	GPN:BC021969_1
ISNLS*PEEEQGLWK	GPN:BC026013_1
DMDEPS*PVPNVEEVTLPK	GPN:BC026222_1
S*PSPSPTPEAK	GPN:BC026222_1
SPS*PSPTPEAK	GPN:BC026222_1
TLTDEVNS*PDSDR	GPN:BC026222_1
VNQSALEAVTPS*PSFQQR	GPN:BC028599_1
ASVLSQS*PR	GPN:BC031107_1
QMS*VPGIFNPHEIPEEMCD	GPN:BC032847_1
AEQGS*EEEGEGEEEEEEGGESK	GPN:BC034488_1
KSS*VTEE	GPN:BC036379_1
EALGLGPPAAQLT*PPPAPVGLR	GPN:BC037428_1
AGVNSDS*PNNCSGK	GPN:BC038297_1
SS*ENNGTLVSK	GPN:BC038297_1
LTAS*PSDPK	GPN:BC042999_1
LYGS*PTQIGPSYR	GPN:BC042999_1
EGSCIFPEELS*PK	GPN:BC044254_1
ASS*PPDR	GPN:BC050434_1
SSDEENGPPSS*PDLDR	GPN:BC051844_1
SQS*LPTTLLSPVR	GPN:BC052581_1
APS*PPS*RR	GPN:BC052950_1
SPS*GAGEGASCSDGPR	GPN:BC052950_1
SPS*PAPAPAPAAAAGPPTR	GPN:BC053992_1
TSPGTSSAYTSDS*PGSYHNEEDEEEDGGEEGMDEQYR	GPN:BC055396_1
EESS*EDENEVSNILR	GPN:BC057242_1
TAADVVS*PGANSVDSR	GPN:BC057242_1
S*DLLANQSQEVLEER	GPN:BC058039_1
S*GTPTQDEMMDKPTSSSVDTMSLLSK	GPN:BX641025_1
LVT*STTAPNPVR	PIR1:A49724
LVS*PDLQLDAS*VR	PIR1:I38344

Peptide	Protein
NVSES*PNR	PIR1:JC5314
S*ET*PPHWR	PIR1:JC5314
SASS*ES*EAENLEAQPQSTVRPEEIPPIPENR	PIR1:JC5314
ATSS*TQSLAR	PIR2:A42184
TQPDGTSVPGEPAS*PISQR	PIR2:A42184
QQAAYYAQTS*PQGMPQHPPAPQGQ	PIR2:A53184
SCMLTGT*PESVQSAK	PIR2:A53184
TGEDEDEEDNDALLKENES*PDVR	PIR2:A53545
VTNDIS*PESSPGVGR	PIR2:A54103
ESVSTEDLSPPS*PPLPK	PIR2:A56138
RISAS*LSCDSPK	PIR2:A61382
GEDS*AEETEAKPAVVAPAPVVEAVSTPSAAFPSDATAENVK	PIR2:B54857
DLLSDLQDIS*DSER	PIR2:E54024
VPAS*PLPGLER	PIR2:G01025
S*DLPGSDK	PIR2:G01158
TQQSPISNGS*PELGIK	PIR2:G02318

Table 5A

N-Terminal Peptides - Saccharomyces cerevisiae

N-Terminal a-Amino Group Unblocked

Protein	Peptide
GP:Z75238_1	MDYERTVLKKRSR
PIR1:S69731	VVVGKSEVR
PIR2:S48569	VFGFTKR
PIR2:S50385	PALLKR
PIR2:S52504	PITIKSR
PIR2:S52698	VAISEVKENPGVNSSNSGAVTR
PIR2:S57377	MQLVPLELNR
PIR2:S59436	PDNNTEQLQGSPSSDQR
PIR2:S59832	GIQEKTLGIR
PIR2:S61156	VQAIKLNDLKNR
PIR2:S61160	AGENPKKEGVDAR
PIR2:S61668	VVNTIYIAR
PIR2:S64842	VNKVVDEVQR
PIR2:S65155	MLVKTISR
PIR2:S65218	MKGTGGVVVGTQNPVR
PIR2:S66925	AKRPLGLGKQSR
PIR2:S66937	TNKSSLKNNR
PIR2:S67033	VAPTALKKATVTPVSGQDGGSSR
PIR2:S67052	VPAESNAVQAKLAKTLQR
PIR2:S67059	VVQKKLR
PIR2:S67185	TKEVPYYCDNDDNNIIR
PIR2:S67655	VGGALICKYLPR
PIR2:S67696	AGSQLKNLKAALKAR
PIR2:S67704	PELTEFQKKR
PIR2:S67772	GSEEDKKLTKKQLKAQQFR
PIR2:S78735	MIEVVVNDR
SW:ACH1_YEAST	TISNLLKQR
SW:AGM1_YEAST	MKVDYEQLCKLYDDTCR

SW:AKR1_YEAST	VNELENVPR
SW:ALF_YEAST	GVEQILKR
SW:APG8_YEAST	MKSTFKSEYPFEKR
SW:ARO8_YEAST	TLPESKDFSYLFSDETNAR
SW:ASN1_YEAST	CGIFAAFR
SW:ATC6_YEAST	TKKSFVSSPIVR
SW:C1TC_YEAST	AGQVLDGKACAQQFR
SW:CAJ1_YEAST	VKETEYYDILGIKPEATPTEIKKAYR
SW:CAP_YEAST	PDSKYTMQGYNLVKLLKR
SW:CB34_YEAST	VTSNVVLVSGEGER
SW:CBS_YEAST	TKSEQQADSR
SW:CHD1_YEAST	AAKDISTEVLQNPELYGLR
SW:COPA_YEAST	MKMLTKFESKSTR
SW:COPP_YEAST	MKLDIKKTFSNR
SW:CYC1_YEAST	TEFKAGSAKKGATLFKTR
SW:CYC7_YEAST	AKESTGFKPGSAKKGATLFKTR
SW:CYP6_YEAST	TRPKTFFDISIGGKPQGR
SW:DBP3_YEAST	TKEEIADKKR
SW:DCUP_YEAST	GNFPAPKNDLILR
SW:DHAS_YEAST	AGKKIAGVLGATGSVGQR
SW:DHE2_YEAST	MLFDNKNR
SW:E2BE_YEAST	AGKKGQKKSGLGNHGKNSDMDVEDR
SW:EF2_YEAST	VAFTVDQMR
SW:EGD1_YEAST	PIDQEKLAKLQKLSANNKVGGTR
SW:ELO1_YEAST	VSDWKNFCLEKASR
SW:ENO1_YEAST	AVSKVYAR
SW:ERV2_YEAST	MKQIVKR
SW:FHP_YEAST	MLAEKTR
SW:GLO2_YEAST	MQVKSIKMR
SW:GLO3_YEAST	SNDEGETFATEQTTQQVFQKLGSNMENR
SW:GLY1_YEAST	TEFELPPKYITAANDLR
SW:HIS7_YEAST	TEQKALVKR
SW:HIS8_YEAST	VFDLKR
SW:HMD1_YEAST	PPLFKGLKQMAKPIAYVSR
SW:HOSC_YEAST	TAAKPNPYAAKPGDYLSNVNNFQLIDSTLR
SW:IF1A_YEAST	GKKNTKGGKKGR
SW:ILV3_YEAST	GLLTKVATSR
SW:KEL3_YEAST	AKKNKKDKEAKKAR
SW:KIN2_YEAST	PNPNTADYLVNPNFR
SW:KRE2_YEAST	ALFLSKR
SW:LA17_YEAST	GLLNSSDKEIIKR
SW:LAG1_YEAST	TSATDKSIDR
SW:LEO1_YEAST	SSESPQDQPQKEQISNNVGVTTNSTSNEETSR
SW:METE_YEAST	VQSAVLGFPR
SW:MFT1_YEAST	PLSQKQIDQVR
SW:MPG1_YEAST	MKGLILVGGYGTR
SW:MYS3_YEAST	AVIKKGAR
SW:NCE2_YEAST	MLALADNILR
SW:NHPB_YEAST	AATKEAKQPKEPKKR
SW:NOG1_YEAST	MQLSWKDIPTVAPANDLLDIVLNR

SW:OM22_YEAST	VELTEIKDDVVQLDEPQFSR
SW:OM70_YEAST	MKSFITR
SW:ORM1_YEAST	TELDYQGTAEAASTSYSR
SW:PCNA_YEAST	MLEAKFEEASLFKR
SW:PDR3_YEAST	MKVKKSTR
SW:PH81_YEAST	MKFGKYLEAR
SW:PH88_YEAST	MNPQVSNIIIMLVMMQLSR
SW:PMG1_YEAST	PKLVLVR
SW:POR1_YEAST	SPPVYSDISR
SW:PUF6_YEAST	APLTKKTNGKR
SW:PUR2_YEAST	MLNILVLGNGAR
SW:PUR8_YEAST	PDYDNYTTPLSSR
SW:PWP1_YEAST	MISATNWVPR
SW:PWP2_YEAST	MKSDFKFSNLLGTVYR
SW:R142_YEAST	ANDLVQAR
SW:R15A_YEAST	GAYKYLEELQR
SW:R15B_YEAST	GAYKYLEELER
SW:R24A_YEAST	MKVEIDSFSGAKIYPGR
SW:R24B_YEAST	MKVEVDSFSGAKIYPGR
SW:R261_YEAST	AKQSLDVSSDR
SW:R37A_YEAST	GKGTPSFGKR
SW:RAS2_YEAST	PLNKSNIR
SW:RIB4_YEAST	AVKGLGKPDQVYDGSKIR
SW:RL25_YEAST	APSAKATAAKKAVVKGTNGKKALKVR
SW:RL27_YEAST	AKFLKAGKVAVVVR
SW:RL31_YEAST	AGLKDVVTR
SW:RL35_YEAST	AGVKAYELR
SW:RL39_YEAST	AAQKSFR
SW:RL44_YEAST	VNVPKTR
SW:RL5_YEAST	AFQKDAKSSAYSSR
SW:RL6A_YEAST	SAQKAPKWYPSEDVAALKKTR
SW:RL6B_YEAST	TAQQAPKWYPSEDVAAPKKTR
SW:RL7A_YEAST	AAEKILTPESQLKKSKAQQKTAEQVAAER
SW:RL7B_YEAST	STEKILTPESQLKKTKAQQKTAEQIAAER
SW:RL8A_YEAST	APGKKVAPAPFGAKSTKSNKTR
SW:RL9A_YEAST	MKYIQTEQQIEVPEGVTVSIKSR
SW:RNT1_YEAST	GSKVAGKKKTQNDNKLDNENGSQQR
SW:RPB1_YEAST	VGQQYSSAPLR
SW:RPC1_YEAST	MKEVVVSETPKR
SW:RPD3_YEAST	VYEATPFDPITVKPSDKR
SW:RPF1_YEAST	ALGNEINITNKLKR
SW:RPN7_YEAST	VDVEEKSQEVEYVDPTVNR
SW:RS1B_YEAST	MLMPKQER
SW:RS3_YEAST	VALISKKR
SW:RS3A_YEAST	AVGKNKR
SW:SDS3_YEAST	AIQKVSNKDLSR
SW:SIS1_YEAST	VKETKLYDLLGVSPSANEQELKKGYR
SW:SLA1_YEAST	TVFLGIYR
SW:SMD1_YEAST	MKLVNFLKKLR
SW:SOF1_YEAST	MKIKTIKR

SW:SOK2_YEAST	PIGNPINTNDIKSNR
SW:SPB1_YEAST	GKTQKKNSKGR
SW:SPC3_YEAST	MFSFVQR
SW:SR54_YEAST	VLADLGKR
SW:SR68_YEAST	VAYSPIIATYGNR
SW:SRB2_YEAST	GKSAVIFVER
SW:ST12_YEAST	MKVQITNSR
SW:STL1_YEAST	MKDLKLSNFKGKFISR
SW:SWI6_YEAST	ALEEVVR
SW:SYAC_YEAST	TIGDKQKWTATNVR
SW:SYSC_YEAST	MLDINQFIEDKGGNPELIR
SW:T2FC_YEAST	VATVKR
SW:TCPG_YEAST	MQAPVVFMNASQER
SW:THRC_YEAST	PNASQVYR
SW:TKT1_YEAST	TQFTDIDKLAVSTIR
SW:TRF4_YEAST	GAKSVTASSSKKIKNR
SW:TRM8_YEAST	MKAKPLSQDPGSKR
SW:TTP1_YEAST	MLLTKR
SW:TYSY_YEAST	TMDGKNKEEEQYLDLCKR
SW:UFD2_YEAST	TAIEDILQITTDPSDTR
SW:UGA2_YEAST	TLSKYSKPTLNDPNLFR
SW:VAN1_YEAST	GMFFNLR
SW:VATB_YEAST	VLSDKELFAINKKAVEQGFNVKPR
SW:VP35_YEAST	AYADSPENAIAVIKQR
SW:YAD1_YEAST	VDVQKR
SW:YB01_YEAST	AFLNIFKQKR
SW:YB09_YEAST	TFMQQLQEAGER
SW:YBV2_YEAST	VEFSLKKAR
SW:YBY7_YEAST	VVLDKKLLER
SW:YCY4_YEAST	VSLFKR
SW:YEJ4_YEAST	MNGLVLGATGLCGGGFLR
SW:YEM6_YEAST	PPVSASKAKR
SW:YEV6_YEAST	PQNDYIER
SW:YFA7_YEAST	TANNDDDIKSPIPITNKTLSQLKR
SW:YG1I_YEAST	AKTIKVIR
SW:YG38_YEAST	PSLSQPFR
SW:YG3A_YEAST	MLFNINR
SW:YG3C_YEAST	TKKKAATNYAER
SW:YG3J_YEAST	VLKSTSANDVSVYQVSGTNVSR
SW:YGC9_YEAST	VNETGESQKAAKGTPVSGKVWKAEKTPLR
SW:YGF0_YEAST	AAQNAFEQKKR
SW:YGK1_YEAST	TAVNIWKPEDNIPR
SW:YGZ6_YEAST	GVSANLFVKQR
SW:YHD0_YEAST	SISSDEAKEKQLVEKAELR
SW:YIK8_YEAST	VGSKDIDLFNLR
SW:YIN0_YEAST	PEQAQQGEQSVKR
SW:YIV6_YEAST	GKVILITGASR
SW:YJ58_YEAST	MLKDLVR
SW:YJG8_YEAST	MKVVKEFSVCGGR
SW:YKV5_YEAST	MQKGNIR

SW:YL22_YEAST	PINQPSGQIKLTNVSLVR
SW:YMJ3_YEAST	AKKKSKSR
SW:YMY0_YEAST	SPMKVAVVGASGKVGR
SW:YN63_YEAST	VNFDLGQVGEVFR
SW:YN8U_YEAST	GTGKKEKSR
SW:YNK8_YEAST	AIENIYIAR
SW:YNM3_YEAST	TISLSNIKKR
SW:YNN2_YEAST	AKKAIDSR
SW:YNQ6_YEAST	GLDQDKIKKR
SW:YP46_YEAST	APTNLTKKPSQYKQSSR
SW:ZRC1_YEAST	MITGKELR

Table 5B

N-Terminal Peptides - Saccharomyces cerevisiae

N-Terminal a-Amino Group Acetylated

Protein	Peptide
GP:AB017593_1	SDWDTNTIIGSR
GP:L01880_1	SQGTLYLNR
PIR1:R3BY33	MDNKTPVTLAKVIKVLGR
PIR1:R5BY16	STKAQNPMR
PIR1:S53543	MFKKFTR
PIR2:S51406	SQLPTDFASLIKR
PIR2:S54047	SNLYKIGTETR
PIR2:S57985	SELEATIR
PIR2:S61039	ATFNPQNEMENQAR
PIR2:S61625	MDQSVEDLFGALR
PIR2:S65214	TSLYAPGAEDIR
PIR2:S65214	TSLYAPGAEDIR
PIR2:S67177	SELLAIPLKR
PIR2:S67177	SELLAIPLKR
PIR2:S70126	SESVKENVTPTR
SW:ACT_YEAST	MDSEVAALVIDNGSGMCKAGFAGDDAPR
SW:AIP1_YEAST	SSISLKEIIPPQPSTQR
SW:ALG3_YEAST	MEGEQSPQGEKSLQR
SW:AR20_YEAST	SQSLRPYLTAVR
SW:ARE2_YEAST	MDKKKDLLENEQFLR
SW:AROG_YEAST	SESPMFAANGMPKVNQGAEEDVR
SW:ATC1_YEAST	SDNPFNASLLDEDSNR
SW:ATP7_YEAST	SLAKSAANKLDWAKVISSLR
SW:BAS1_YEAST	SNISTKDIR
SW:BEM1_YEAST	MLKNFKLSKR
SW:CAPB_YEAST	SDAQFDAALDLLR
SW:CC11_YEAST	SGIIDASSALR
SW:CC12_YEAST	SAATATAAPVPPPVGISNLPNQR
SW:CC28_YEAST	SGELANYKR
SW:CDC3_YEAST	SLKEEQVSIKQDPEQEER

SW:CET1_YEAST	SYTDNPPQTKR
SW:CH10_YEAST	STLLKSAKSIVPLMDR
SW:CHMU_YEAST	MDFTKPETVLNLQNIR
SW:CISY_YEAST	SAILSTTSKSFLSR
SW:CK12_YEAST	SQVQSPLTATNSGLAVNNNTMNSQMPNR
SW:CLC1_YEAST	SEKFPPLEDQNIDFTPNDKKDDDTDFLKR
SW:COAC_YEAST	SFESLFESSPQKMEYFITNYSER
SW:CYAA_YEAST	SSKPDTGSEISGPQR
SW:CYPH_YEAST	SQVYFDVEADGQPIGR
SW:DCP1_YEAST	SEITLGKYLFER
SW:DEC1_YEAST	SDKIQEEILGLVSR
SW:DHH1_YEAST	GSINNNFNTNNNSNTDLDR
SW:DPD2_YEAST	MDALLTKFNEDR
SW:DPOA_YEAST	SSKSEKLEKLR
SW:E2BA_YEAST	SEFNITETYLR
SW:EF1G_YEAST	SQGTLYANFR
SW:EF1H_YEAST	SQGTLYINR
SW:EGD2_YEAST	SAIPENANVTVLNKNEKKAR
SW:ERF2_YEAST	SDSNQGNNQQNYQQYSQNGNQQQGNNR
SW:FAS1_YEAST	MDAYSTR
SW:FKBP_YEAST	SEVIEGNVKIDR
SW:FOLD_YEAST	AIELGLSR
SW:FPPS_YEAST	ASEKEIR
SW:GALY_YEAST	SAAPVQDKDTLSNAER
SW:GBLP_YEAST	ASNEVLVLR
SW:GC20_YEAST	ASIGSQVR
SW:GCN1_YEAST	TAILNWEDISPVLEKGTR
SW:GCS1_YEAST	SDWKVDPDTR
SW:GLNA_YEAST	AEASIEKTQILQKYLELDQR
SW:GLO3_YEAST	SNDEGETFATEQTTQQVFQKLGSNMENR
SW:GLY1_YEAST	TEFELPPKYITAANDLR
SW:GNA1_YEAST	SLPDGFYIR
SW:GSHR_YEAST	MLSATKQTFR
SW:GSP1_YEAST	SAPAANGEVPTFKLVLVGDGGTGKTTFVKR
SW:GUP1_YEAST	SLISILSPLITSEGLDSR
SW:H2A1_YEAST	SGGKGGKAGSAAKASQSR
SW:H2B2_YEAST	SSAAEKKPASKAPAEKKPAAKKTSTSVDGKKR
SW:HS77_YEAST	MLAAKNILNR
SW:HS78_YEAST	STPFGLDLGNNNSVLAVAR
SW:HXT2_YEAST	SEFATSR
SW:IF34_YEAST	SEVAPEEIIENADGSR
SW:IM09_YEAST	MDALNSKEQQEFQKVVEQKQMKDFMR
SW:IMA1_YEAST	MDNGTDSSTSKFVPEYR
SW:IMB1_YEAST	STAEFAQLLENSILSPDQNIR
SW:KM8S_YEAST	TTASSSASQLQQR
SW:LAG1_YEAST	TSATDKSIDR
SW:LAH1_YEAST	SEKPQQEEQEKPQSR
SW:LSM3_YEAST	METPLDLLKLNLDER
SW:LTV1_YEAST	SKKFSSKNSQR
SW:MAD2_YEAST	SQSISLKGSTR

SW:MP10_YEAST	SELFGVLKSNAGR
SW:MS16_YEAST	MLTSILIKGR
SW:MYS2_YEAST	SFEVGTR
SW:N157_YEAST	MYSTPLKKR
SW:NHPX_YEAST	SAPNPKAFPLADAALTQQILDVVQQAANLR
SW:NOP8_YEAST	MDSVIQKR
SW:NTF2_YEAST	SLDFNTLAQNFTQFYYNQFDTDR
SW:NU84_YEAST	MELSPTYQTER
SW:NUT1_YEAST	MEKESVYNLALKCAER
SW:OM06_YEAST	MDGMFAMPGAAAGAASPQQPKSR
SW:PAT1_YEAST	SFFGLENSGNAR
SW:PEXE_YEAST	SDVVSKDR
SW:PFD1_YEAST	SQIAQEMTVSLR
SW:PFD3_YEAST	MDTLFNSTEKNAR
SW:PGK_YEAST	SLSSKLSVQDLDLKDKR
SW:PGM1_YEAST	SLLIDSVPTVAYKDQKPGTSGLR
SW:PMT1_YEAST	SEEKTYKR
SW:PNPH_YEAST	SDILNVSQQR
SW:PP12_YEAST	MDSQPVDVDNIIDR
SW:PROA_YEAST	SSSQQIAKNAR
SW:PROF_YEAST	SWQAYTDNLIGTGKVDKAVIYSR
SW:PRP2_YEAST	SSITSETGKR
SW:PRP5_YEAST	METIDSKQNINR
SW:PSA3_YEAST	TSIGTGYDLSNSVFSPDGR
SW:PSA6_YEAST	SGAAAASAAGYDR
SW:PSB2_YEAST	MDIILGIR
SW:PUR4_YEAST	TDYILPGPKALSQFR
SW:PUR7_YEAST	SITKTELDGILPLVAR
SW:PUS1_YEAST	SEENLRPAYDDQVNEDVYKR
SW:PYR1_YEAST	ATIAPTAPITPPMESTGDR
SW:PYRF_YEAST	SKATYKER
SW:R10A_YEAST	SKITSSQVR
SW:R141_YEAST	SNVVQAR
SW:R142_YEAST	ANDLVQAR
SW:R14A_YEAST	STDSIVKASNWR
SW:R161_YEAST	SWEGFKKAINR
SW:R167_YEAST	SFKGFTKAVSR
SW:RCL1_YEAST	SSSAPKYTTFQGSQNFR
SW:REP2_YEAST	MDDIETAKNLTVKAR
SW:RFC2_YEAST	MFEGFGPNKKR
SW:RHO1_YEAST	SQQVGNSIR
SW:RHO3_YEAST	SFLCGSASTSNKPIER
SW:RIR1_YEAST	MYVYKR
SW:RIR4_YEAST	MEAHNQFLKTFQKER
SW:RL11_YEAST	SAKAQNPMR
SW:RL23_YEAST	SGNGAQGTKFR
SW:RL6A_YEAST	SAQKAPKWYPSEDVAALKKTR
SW:RL73_YEAST	SSTQDSKAQTLNSNPEILLR
SW:RL7A_YEAST	AAEKILTPESQLKKSKAQQKTAEQVAAER
SW:RL7B_YEAST	STEKILTPESQLKKTKAQQKTAEQIAAER

SW:RPA2_YEAST	SKVIKPPGQAR
SW:RPB3_YEAST	SEEGPQVKIR
SW:RPB8_YEAST	SNTLFDDIFQVSEVDPGR
SW:RPC5_YEAST	SNIVGIEYNR
SW:RPN2_YEAST	SLTTAAPLLALLR
SW:RPN6_YEAST	SLPGSKLEEAR
SW:RR44_YEAST	SVPAIAPR
SW:RRP1_YEAST	METSNFVKQLSSNNR
SW:RRP4_YEAST	SEVITITKR
SW:RRP6_YEAST	TSENPDVLLSR
SW:RS11_YEAST	STELTVQSER
SW:RS15_YEAST	SQAVNAKKR
SW:RS2_YEAST	SAPEAQQQKR
SW:RS20_YEAST	SDFQKEKVEEQEQQQQQIIKIR
SW:RS21_YEAST	MENDKGQLVELYVPR
SW:RS24_YEAST	SDAVTIR
SW:RS28_YEAST	MDSKTPVTLAKVIKVLGR
SW:SAHH_YEAST	SAPAQNYKIADISLAAFGR
SW:SC17_YEAST	SDPVELLKR
SW:SC23_YEAST	MDFETNEDINGVR
SW:SE33_YEAST	SYSAADNLQDSFQR
SW:SEC1_YEAST	SDLIELQR
SW:SEC2_YEAST	MDASEEAKR
SW:SEC8_YEAST	MDYLKPAQKGR
SW:SFT2_YEAST	SEEPPSDQVNSLR
SW:SMI1_YEAST	MDLFKR
SW:SNC2_YEAST	SSSVPYDPYVPPEESNSGANPNSQNKTAALR
SW:SPK1_YEAST	MENITQPTQQSTQATQR
SW:SPT6_YEAST	MEETGDSKLVPR
SW:SR21_YEAST	SVKPIDNYITNSVR
SW:SSB1_YEAST	SAEIEEATNAVNNLSINDSEQQPR
SW:STDH_YEAST	SIVYNKTPLLR
SW:SUM1_YEAST	SENTTAPSDNITNEQR
SW:SYG_YEAST	SVEDIKKAR
SW:SYLC_YEAST	SSGLVLENTAR
SW:TBF1_YEAST	MDSQVPNNNESLNR
SW:TCPA_YEAST	SQLFNNSR
SW:TCPB_YEAST	SVQIFGDQVTEER
SW:TCPD_YEAST	SAKVPSNATFKNKEKPQEVR
SW:TCPZ_YEAST	SLQLLNPKAESLR
SW:TFC5_YEAST	SSIVNKSGTR
SW:THI7_YEAST	SFGSKVSR
SW:THIL_YEAST	SQNVYIVSTAR
SW:TKT1_YEAST	TQFTDIDKLAVSTIR
SW:TPS2_YEAST	TTTAQDNSPKKR
SW:TREA_YEAST	SQVNTSQGPVAQGR
SW:UBA1_YEAST	SSNNSGLSAAGEIDESLYSR
SW:UBP6_YEAST	SGETFEFNIR
SW:VATA_YEAST	AGAIENAR
SW:VATE_YEAST	SSAITALTPNQVNDELNKMQAFIR

SW:VTC1_YEAST	SSAPLLQR
SW:YAD6_YEAST	STTVEKIKAIEDEMAR
SW:YBD6_YEAST	STGITYDEDR
SW:YBM6_YEAST	SANDYYGGTAGEKSQYSR
SW:YBN2_YEAST	SNITYVKGNILKPKSYAR
SW:YBV1_YEAST	MEKLLQWSIANSQGDKEAMAR
SW:YFL8_YEAST	SYKANQPSPGEMPKR
SW:YG1G_YEAST	ANSKFGYVR
SW:YG5U_YEAST	STATIQDEDIKFQR
SW:YGK1_YEAST	TAVNIWKPEDNIPR
SW:YHD1_YEAST	SSQPSFVTIR
SW:YHP9_YEAST	SLTEQIEQFASR
SW:YIE4_YEAST	STSVPVKKALSALLR
SW:YIK3_YEAST	SGSTESKKQPR
SW:YJA7_YEAST	CSRGGSNSR
SW:YJF4_YEAST	SSESGKPIAKPIR
SW:YJK9_YEAST	SSLSDQLAQVASNNATVALDR
SW:YK10_YEAST	SYLPTYSNDLPAGPQGQR
SW:YKA8_YEAST	STIKPSPSNNNLKVR
SW:YKL7_YEAST	SDKVINPQVAWAQR
SW:YL09_YEAST	SIDLKKR
SW:YL86_YEAST	MEKSIAKGLSDKLYEKR
SW:YM11_YEAST	MDAGLSTMATR
SW:YM28_YEAST	ADLQKQENSSR
SW:YM8W_YEAST	SQPTPIITTKSAAKPKPKIFNLFR
SW:YME8_YEAST	MEIYIR
SW:YML7_YEAST	SNSNSKKPVANYAYR
SW:YMS1_YEAST	SLISAVEDR
SW:YNJ9_YEAST	TSKVGEYEDVPEDESR
SW:YNU8_YEAST	SANEFYSSGQQGQYNQQNNQER
SW:YNZ8_YEAST	MESLFPNKGEIIR
SW:YP18_YEAST	SLEAIVFDR
SW:YRA1_YEAST	SANLDKSLDEIIGSNKAGSNR

What is claimed is:

1. A method for characterizing phosphorylated polypeptides in a sample comprising:

providing a biological sample comprising plurality of polypeptides;

digesting the polypeptides with a protease, thereby generating a plurality of test peptides;

collecting a fraction of test peptides which are enriched for positively charged peptides; and

determining an identifying characteristic of a positively charged peptide in the fraction.

2. The method according to claim 1, wherein collecting the fraction comprises exposing the plurality of test peptides to a strong cation exchanger.

3. The method according to claim 2, further comprising eluting peptides from the strong cation exchanger at pH 3 and collecting eluted peptides which are enriched for phosphorylated peptides.

4. The method according to claim 3, wherein the phosphorylated peptides comprise greater than about 50% of peptides in the initial fraction.

5. The method of claim 1, wherein the identifying characteristic is mass-to-charge ratio.

6. The method of claim 1, wherein the identifying characteristic is a peptide fragmentation pattern.

7. The method of claim 1 wherein the identifying characteristic is the amino acid sequence of the peptide.

8. The method of claim 1, further comprising sequencing substantially all of the positively charged peptides in the enriched subset.

9. The method of claim 1, further comprising determining the mass of substantially all of the positively charged peptides in the enriched subset.

10. The method of claim 1, further comprising separating the plurality of polypeptides prior to protease digestion according to at least one biological characteristic to obtain subsets of polypeptides.

11. The method of claim 10, wherein the at least one biological characteristic is molecular weight.

12. The method of claim 9, wherein separation is performed by gel electrophoresis and slicing a gel into a plurality of pieces each piece comprising a subset of polypeptides.

13. The method of claim 1, wherein the identifying characteristic is determined by performing multistage mass spectrometry.

14. A method comprising determining the presence, absence or level of one or more phosphorylated peptides identified using the method of claim 1 in a plurality of cells having a cell state and determining the degree of correlation between the presence, absence or level of the phosphorylated polypeptide with the cell state.

15. An isolated peptide of about 5-50 amino acids comprising an amino acid sequence which is a subsequence of a sequence according to any of the proteins listed in Table 4 and which comprise a phosphorylation site within said subsequence.

16. The isolated peptide of claim 15, wherein the peptide comprises an amino acid sequence selected from the group of amino acid sequences shown in Table 4.

17. The isolated peptide of claim 16, wherein the peptide comprises an amino acid sequence selected from the group of amino acid sequences shown in Table 4.

18. An isolated polypeptide selected from a polypeptide listed in Table 4 or a subsequence thereof and which is modified at a modification site as shown in the table.

19. The isolated polypeptide of claim 19 wherein the modification is acetylation or phosphorylation.

20. An isolated peptide comprising a mass spectral peak signature selected from the group of mass spectral peak signatures as shown in Figures 4A-I.

21. An isolated peptide comprising an amino acid sequence selected from the group of sequences shown in Figures 4A-I.

22. A method for identifying a treatment that modulates phosphorylation of an amino acid in a target polypeptide, comprising:

subjecting a sample comprising the target polypeptide to a treatment;

determining the level of phosphorylation of one or more amino acids in the target polypeptide before and after treatment;

identifying a treatment that results in a change of the level of modification of the one or more amino acids after treatment;

wherein the level of phosphorlyation is determined by digesting the target polypeptide with a protease and identifying the presence and/or level of a peptide identified according to the method of claim 1.

23. A method for generating a peptide standard comprising labeling a peptide obtained by the method of claim 1 with a mass altering label.

24. A pair of peptide standards comprising a peptide obtained by the method of claim 22, wherein the peptide is phosphorylated and a corresponding peptide comprising an identical amino acid sequence but which is not phosphorylated.

25. The method of claim 22, wherein the treatment comprises exposing the sample to a modulator of kinase activity.

26. The method of claim 22, wherein the treatment comprises exposing the sample to a modulator of phosphatase activity.

27. The method of claim 25, wherein the modulator is an agonist.

28. The method of claim 26, wherein the modulator is an agonist.

29. The method of claim 25, where the modulator is an antagonist.

30. The method of claim 26, where the modulator is an antagonist.

31. A system comprising a computer memory comprising data files storing information relating to the identifying characteristics of positively charged peptides identified in claim 1 and a data analysis module capable of executing instructions for organizing and/or searching the data files.

32. The system according to claim 29, wherein the information comprises the amino acid sequences of phosphorylated and acetylated proteins.

33. The system according to claim 29, wherein the information comprises the sites of phosphorylation of a plurality of polypeptides.

34. The system according to claim 30, wherein the information comprises the sites of phosphorylation of a plurality of polypeptides.

35. The system according to claim 29, wherein the information comprises the sites of phosphorylation of a plurality of polypeptides in a cell having a cell state.

36. The system according to claim 33, wherein the cell is from a patient having a disease.

37. The system according to claim 33, wherein the information comprises the sites of phosphorylation of a plurality of polypeptides in an organelle from a cell having a cell state.

38. The system according to claim 34, wherein the information comprises the sites of phosphorylation of a plurality of polypeptides in an organelle from a cell having a cell state.

39. The method according to claim 1, wherein the sample comprises one or more isolated organelles.

40. The method according to claim 1, wherein the sample comprises one or more isolated nuclei.

41. The method according to claim 1 wherein the plurality comprises at least bout 100,000 different peptides.

42. The method according to claim 1, wherein the identifying characteristic is determined for at least about 10 of the peptides.

43. The method according to claim 1, wherein the identifying characteristic is determined for at least about 100 of the peptides.

44. The method according to claim 1, wherein the identifying characteristic is determined for at least about 1000 of the peptides.

45. A computer program product comprising data relating to the identifying characteristics of positively charged peptides identified in claim 1 and comprising instructions for organizing and/or searching the data.

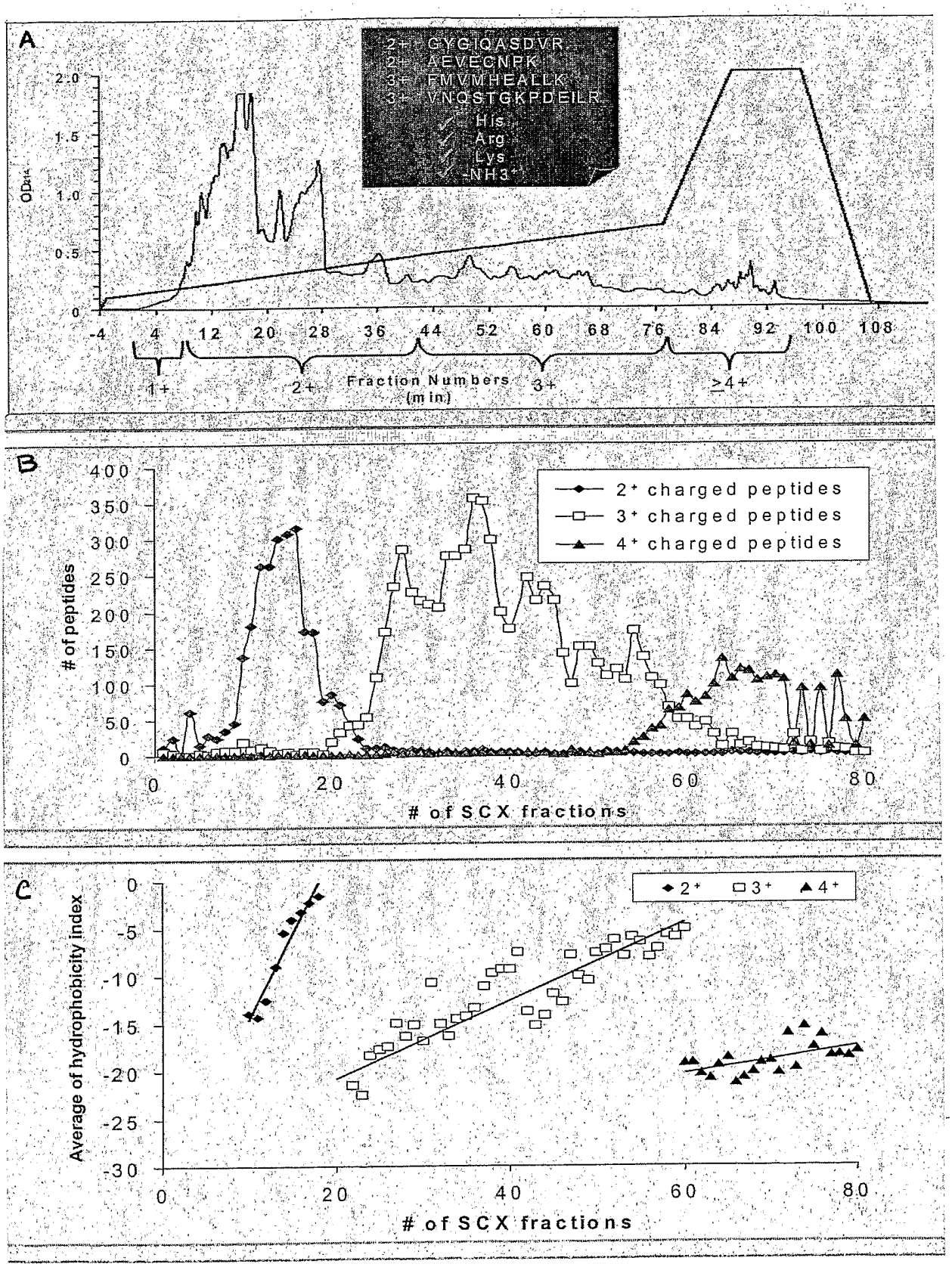
46. A method for identifying N-terminal peptides in a sample comprising:

providing a biological sample comprising plurality of proteins;

digesting the polypeptides with trypsin, thereby generating a plurality of peptides;

subjecting the peptides to SCX chromatography; and

collecting a fraction of test peptides which are enriched for positively charged peptides having a solution charge state of 1+.


A
2+ GYGIQASDVR
2+ AEVECNPK
3+ FMVMHEALLK
3+ VNQSTGKPDEILR
His
Arg
Lys
-NH3+
OD214
Fraction Numbers (min)
1+
2+
3+
≥4+
B
2+ charged peptides
3+ charged peptides
4+ charged peptides
# of peptides
# of SCX fractions
C
2+
3+
4+
Average of hydrophobicity index
# of SCX fractions

FIGURES 1A-C

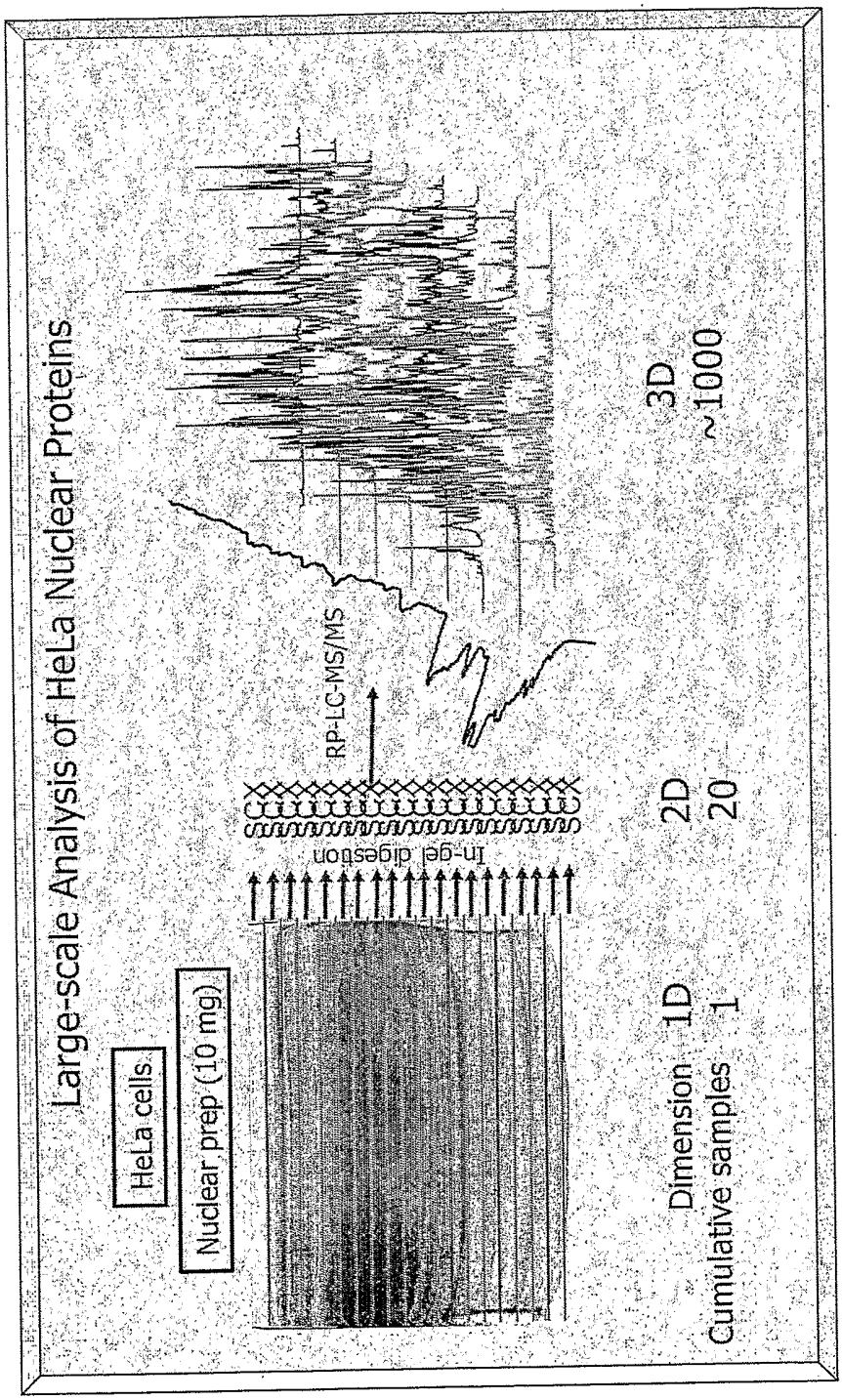

Large-scale Analysis of HeLa Nuclear Proteins
HeLa cells
Nuclear prep (10 mg)
In-gel digestion
SCX
RP-LC-MS/MS
Dimension 1D 2D 3D
Cumulative samples 1 20 ~1000

FIG. 2

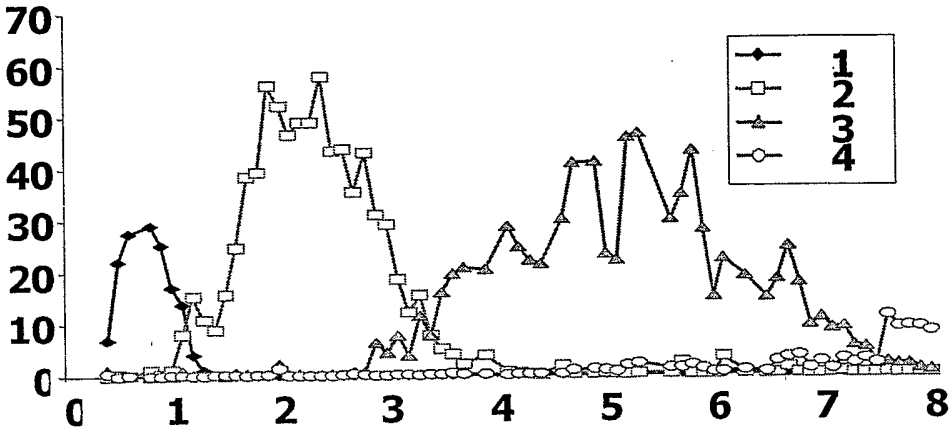


FIG. 3

KP58_HUMAN

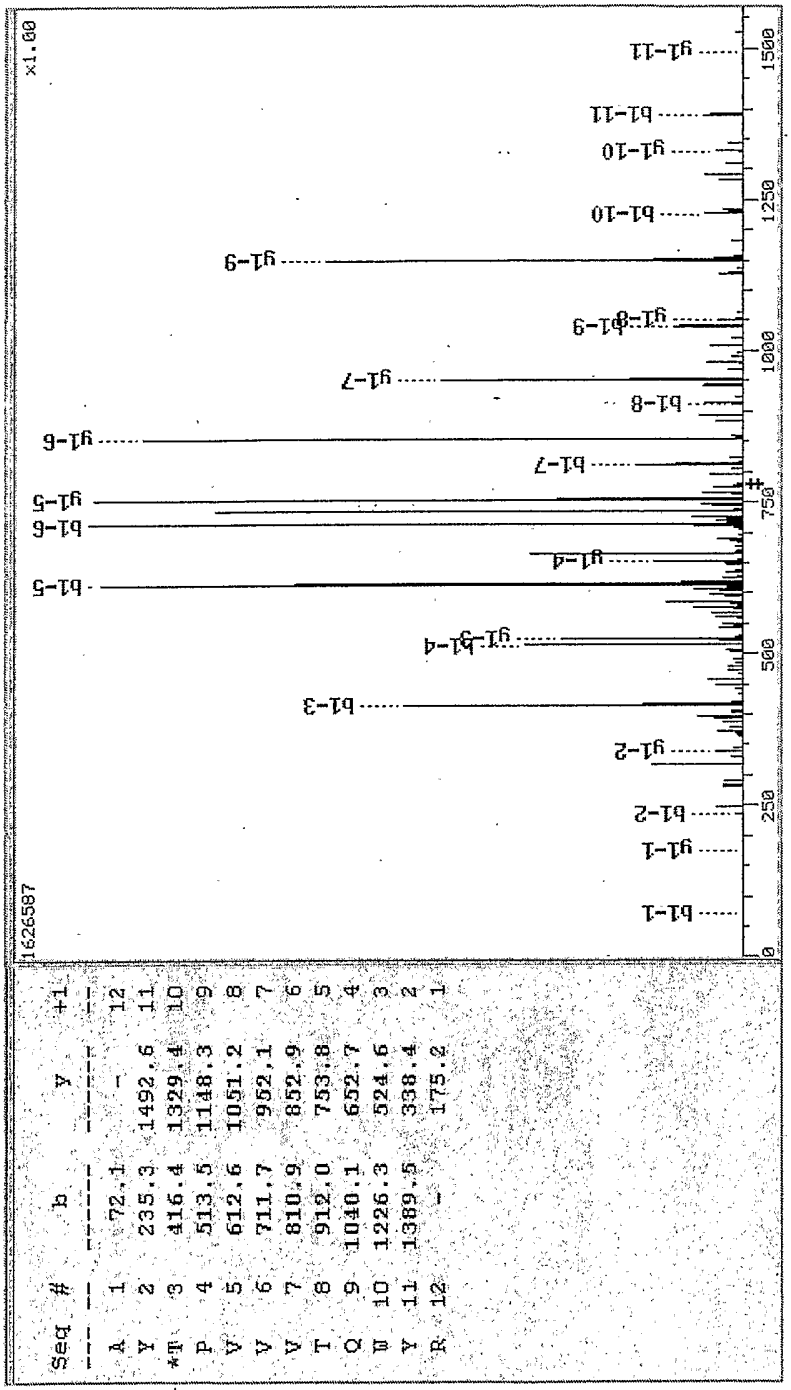


FIG. 4A

GP:AB033054

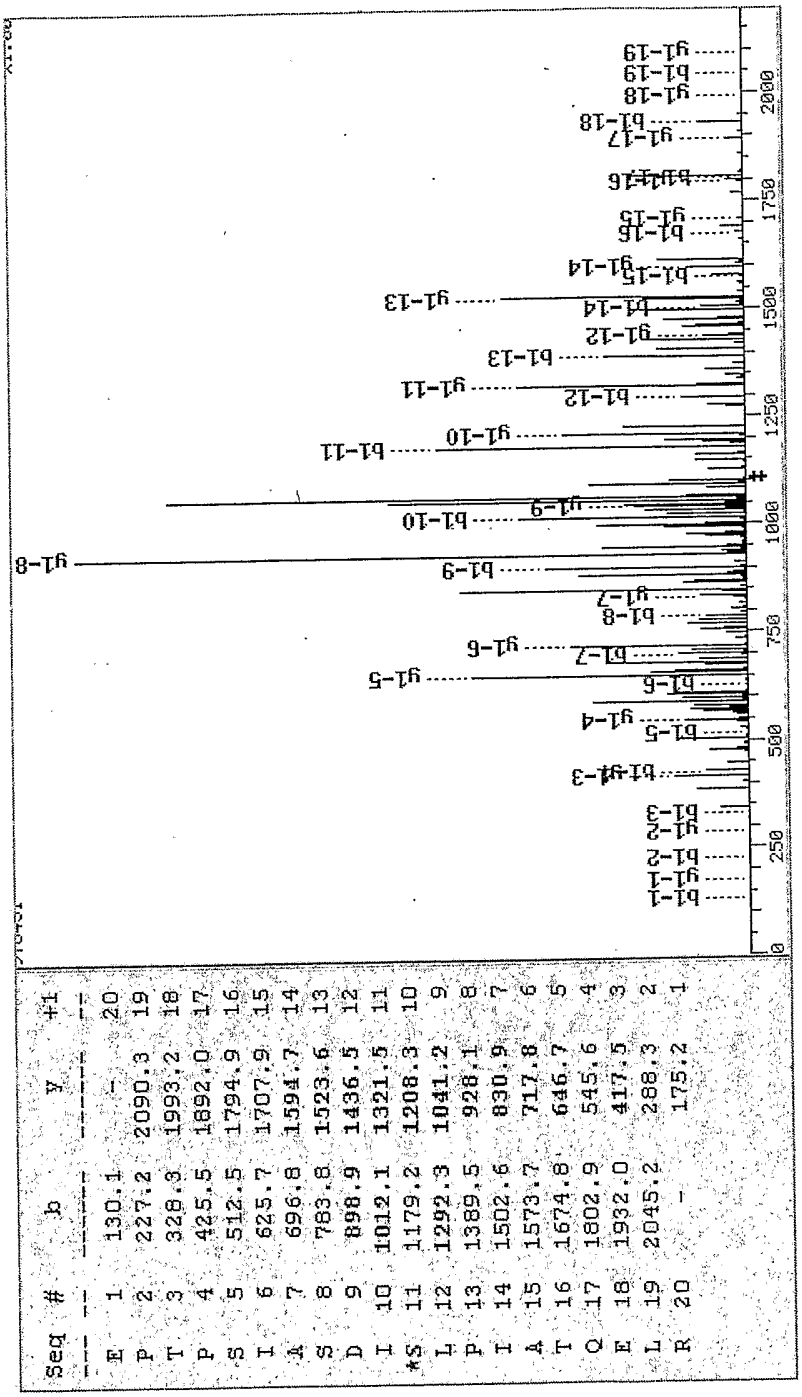


FIG. 4B

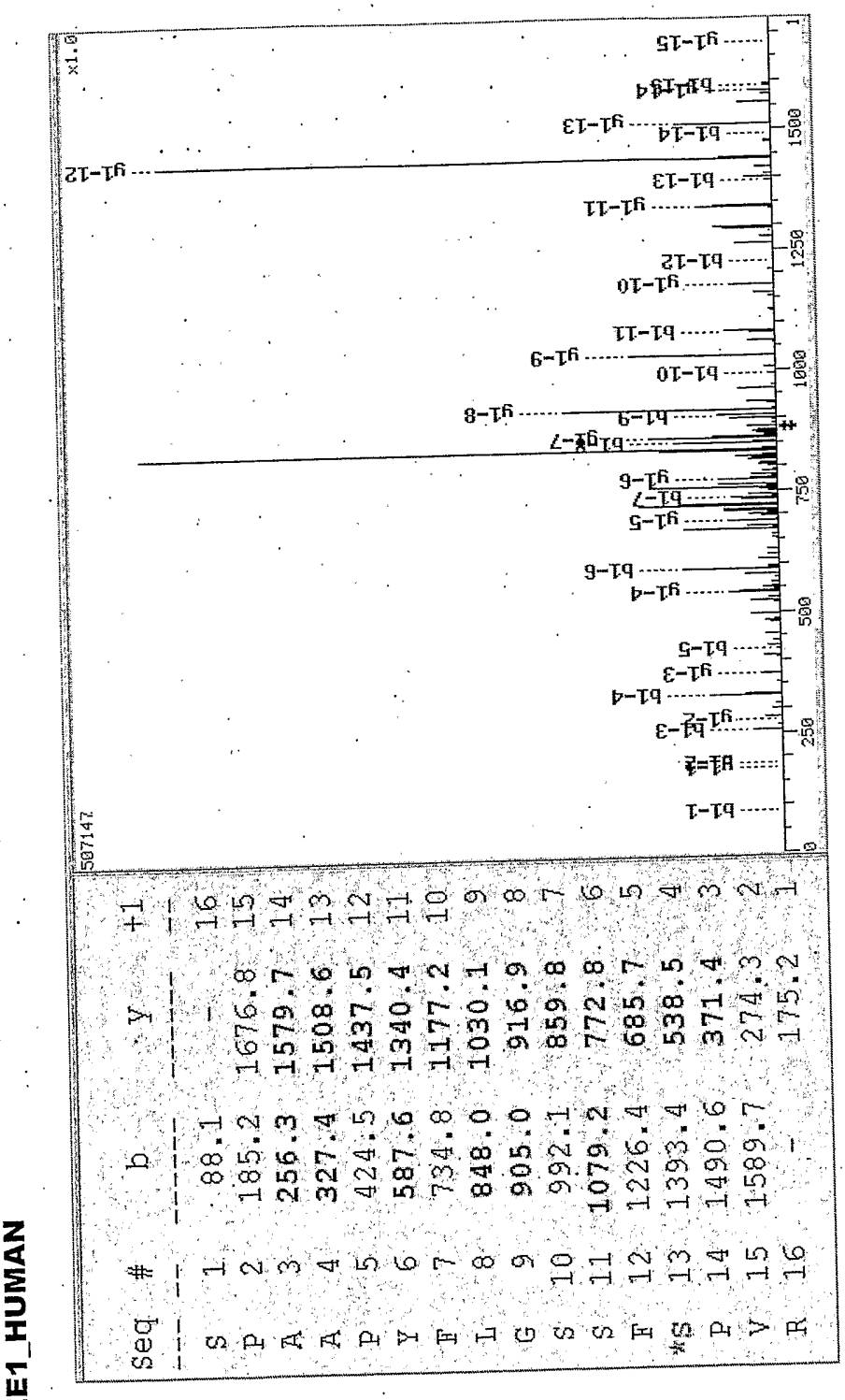

WEE1_HUMAN
Seq # b y +1
S 1 88.1 16
P 2 185.2 1676.8 15
A 3 256.3 1579.7 14
A 4 327.4 1508.6 13
P 5 424.5 1437.5 12
Y 6 587.6 1340.4 11
F 7 734.8 1177.2 10
L 8 848.0 1030.1 9
G 9 905.0 916.9 8
S 10 992.1 859.8 7
S 11 1079.2 772.8 6
F 12 1226.4 685.7 5
*S 13 1393.4 538.5 4
P 14 1490.6 371.4 3
V 15 1589.7 274.3 2
R 16 - 175.2 1
507147
x1.0
b1-1
b1-2
b1-3
y1-2
b1-4
y1-3
b1-5
y1-4
b1-6
y1-5
b1-7
y1-6
b1-9
y1-8
b1-10
y1-9
b1-11
y1-10
b1-12
y1-11
b1-13
y1-12
b1-14
y1-13
y1-15

FIG. 4C

PIR2:A38282

Seq	#	b	y	+1
---	--	------	------	--
A	1	72.1	-	12
Y	2	235.3	1477.6	11
*T	3	416.4	1314.5	10
P	4	513.5	1133.4	9
V	5	612.6	1036.2	8
V	6	711.7	937.1	7
V	7	810.9	838.0	6
T	8	912.0	738.8	5
L	9	1025.1	637.7	4
W	10	1211.4	524.6	3
Y	11	1374.5	338.4	2
R	12	-	175.2	1

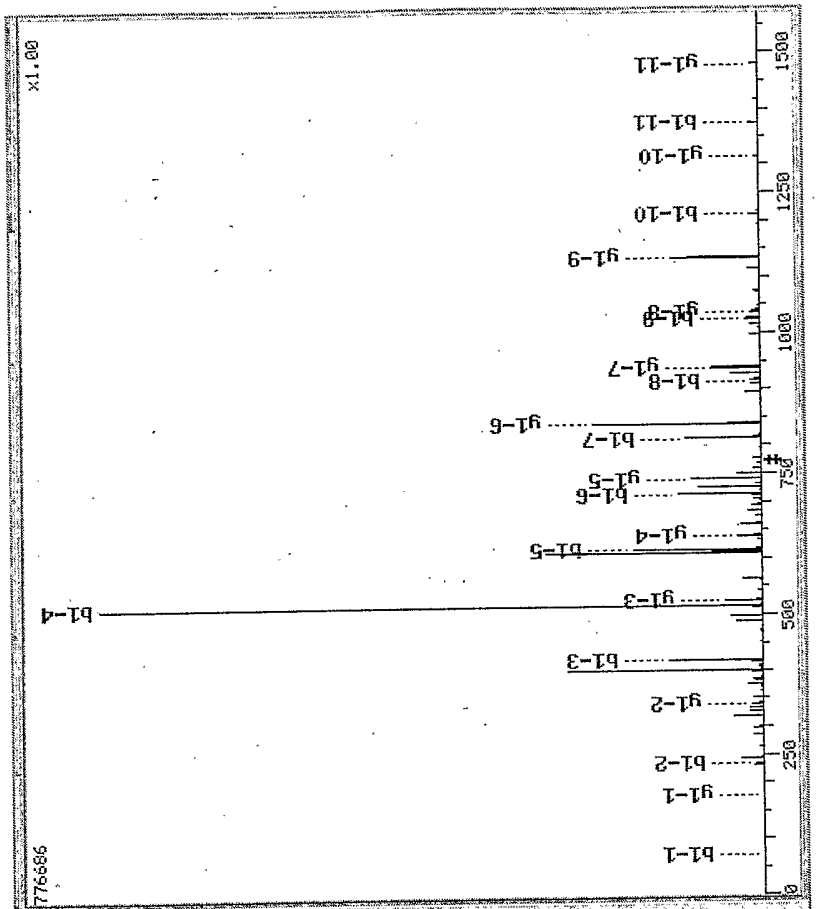


FIG. 4D

PYRG_HUMAN

Seq #		b	y	+1
		---	---	---
	S	1	88.1	- 14
	G	2	145.1	1430.4 13
	S	3	232.2	1373.3 12
	S	4	319.3	1286.3 11
	*S	5	486.4	1199.2 10
	P	6	583.5	1032.1 9
	D	7	698.6	935.0 8
	S	8	785.6	819.9 7
	E	9	914.8	732.8 6
	I	10	1027.9	603.7 5
	T	11	1129.0	490.6 4
	E	12	1258.1	389.4 3
	L	13	1371.3	260.3 2
	K	14	-	147.2 1

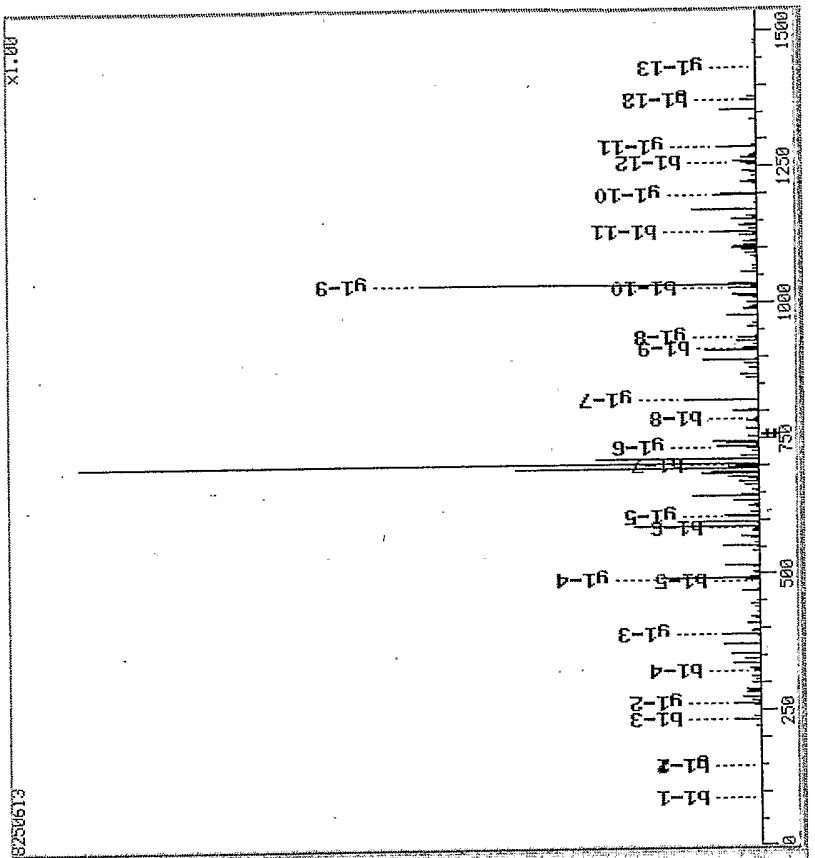


FIG. 4E

GP:Y18004

Seq	#	b	y	+1
R	1	157.2	-	16
*S	2	324.3	1800.9	15
P	3	421.4	1633.8	14
Q	4	549.5	1536.7	13
Q	5	677.6	1408.6	12
T	6	778.7	1280.4	11
V	7	877.9	1179.3	10
P	8	975.0	1080.2	9
Y	9	1138.2	983.1	8
V	10	1237.3	819.9	7
V	11	1336.4	720.8	6
P	12	1433.6	621.6	5
L	13	1546.7	524.5	4
*S	14	1713.8	411.4	3
P	15	1810.9	244.3	2
K	16	-	147.2	1

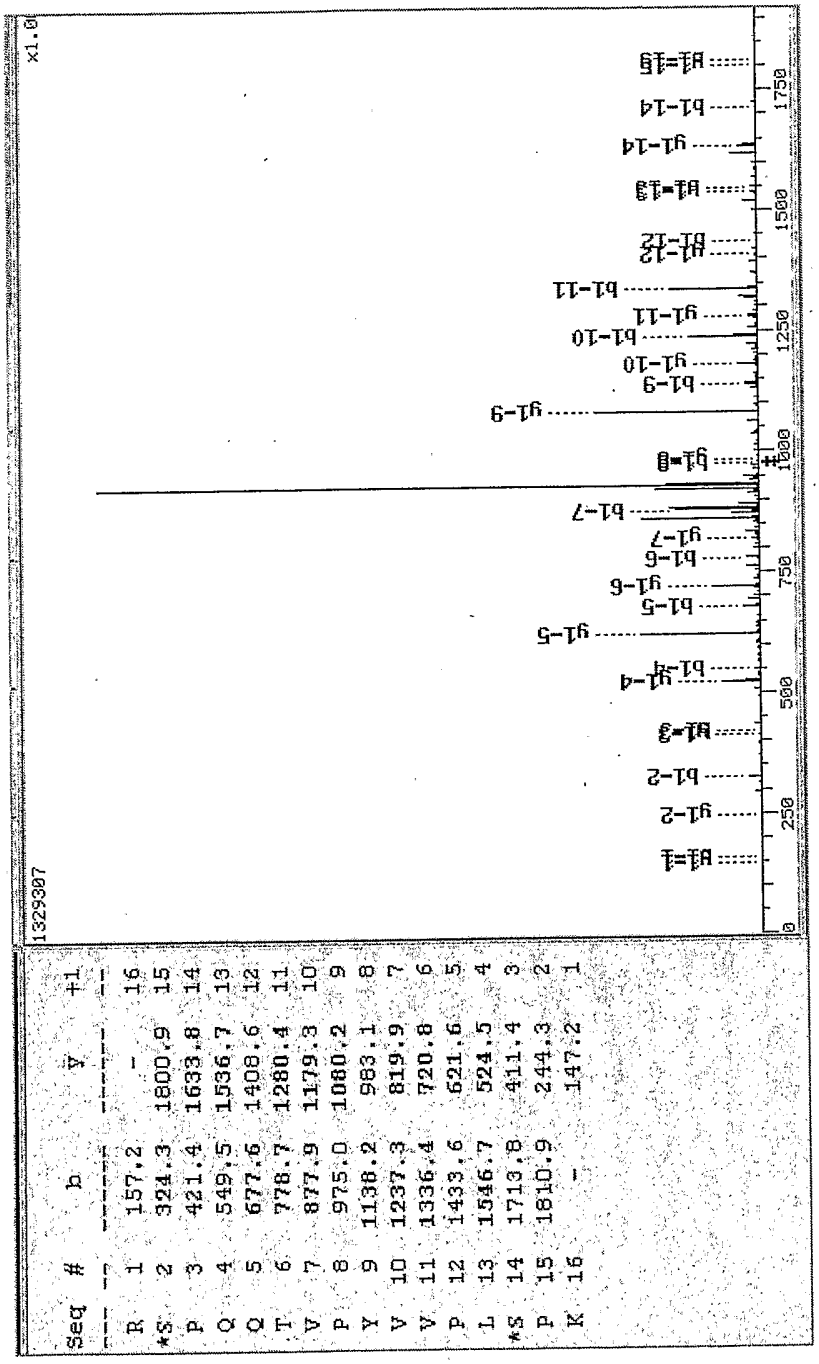


FIG. 4F

GP:AF161470

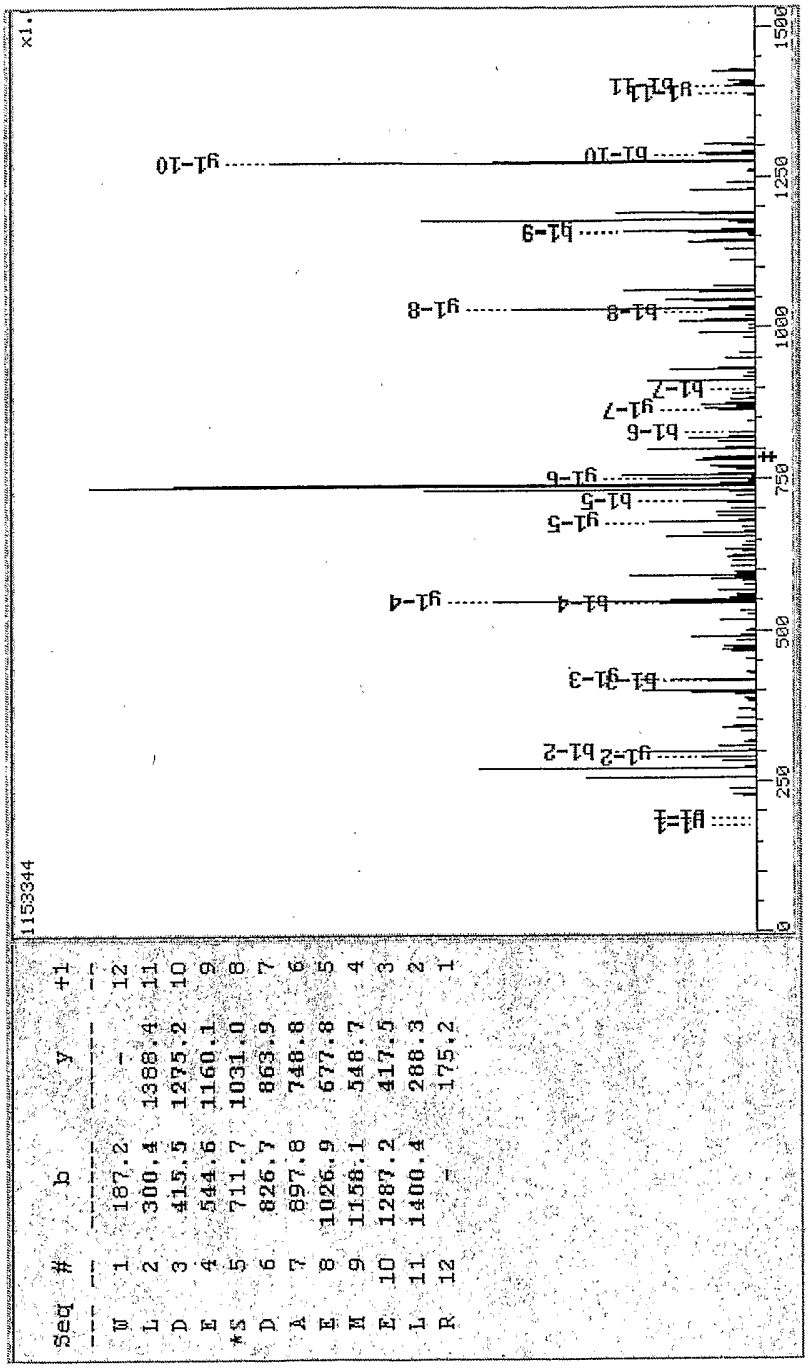


FIG. 4G

S3B2_HUMAN

Seq	#	b	y	+1
S	1	88.1	-	19
S	2	175.2	1934.9	18
L	3	288.3	1847.8	17
G	4	345.4	1734.7	16
Q	5	473.5	1677.6	15
S	6	560.6	**1549.5**	14
A	7	631.7	**1462.4**	13
*S	8	798.7	**1391.3**	12
E	9	927.8	1224.2	11
T	10	1029.0	**1095.1**	10
E	11	1158.1	**994.0**	9
E	12	1287.2	**864.9**	8
D	13	**1402.3**	**735.8**	7
T	14	**1503.4**	620.7	6
Y	15	**1602.5**	519.6	5
S	16	**1689.6**	**420.5**	4
V	17	**1788.7**	333.4	3
S	18	**1875.8**	234.3	2
K	19	-	147.2	1

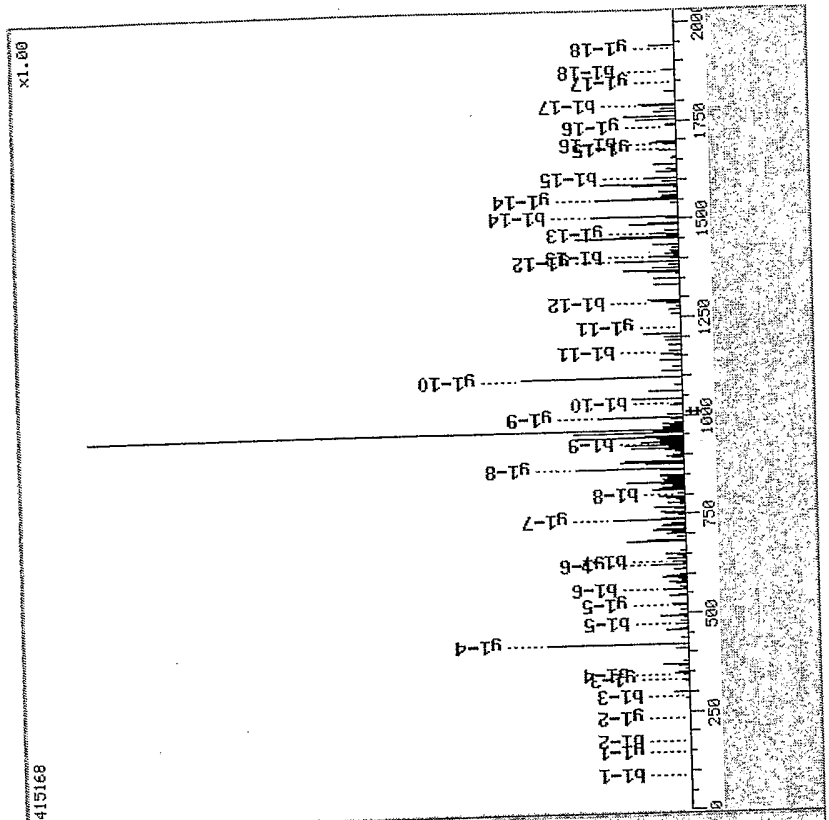


FIG. 4H

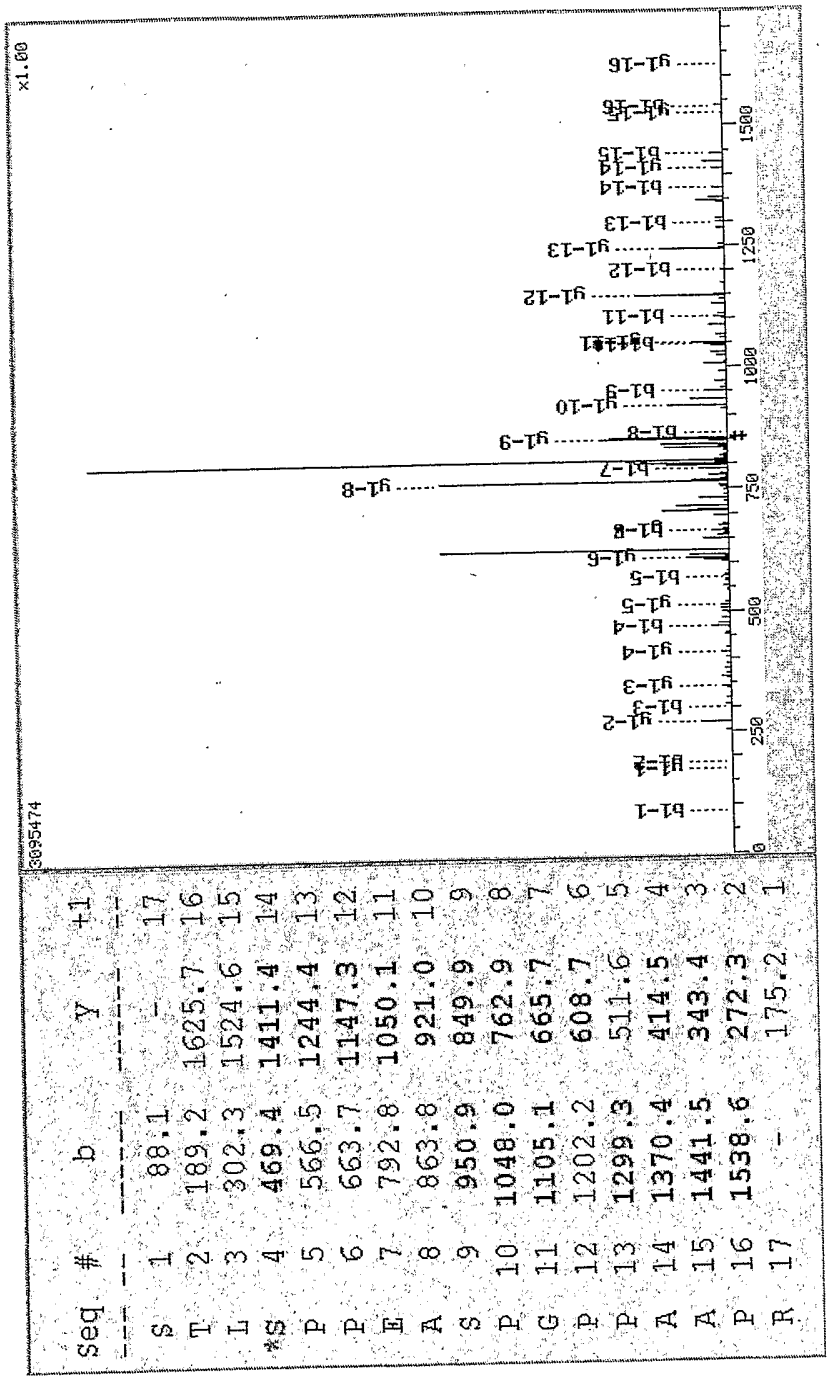


FIG. 4I

GB:BC011630

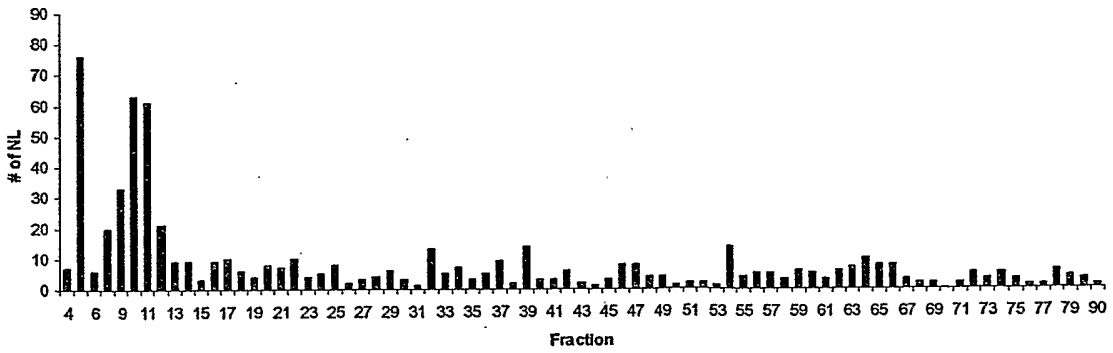


FIGURE 5A

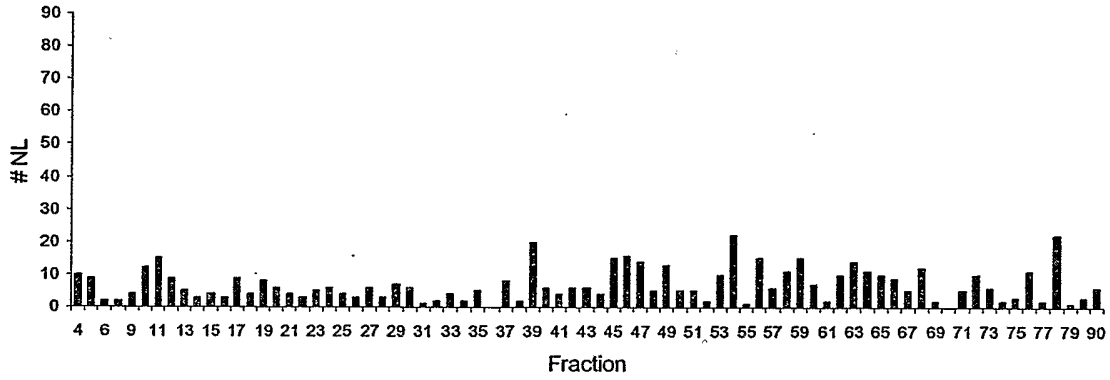


FIGURE 5B

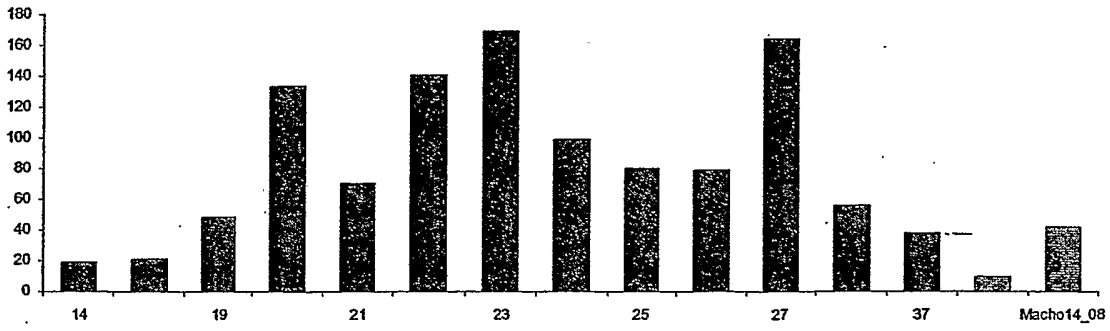


FIGURE 5C

FIG.6a

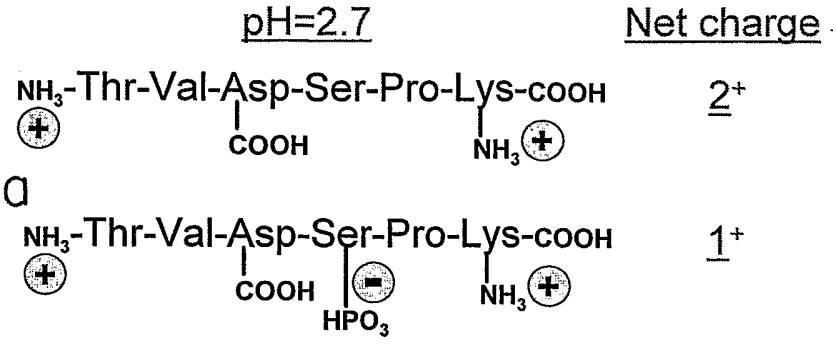


FIG.6b

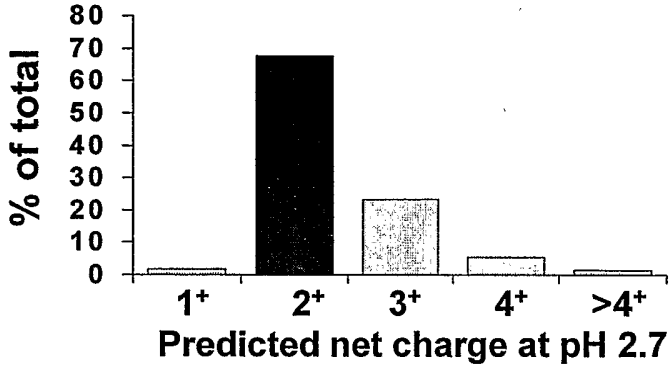
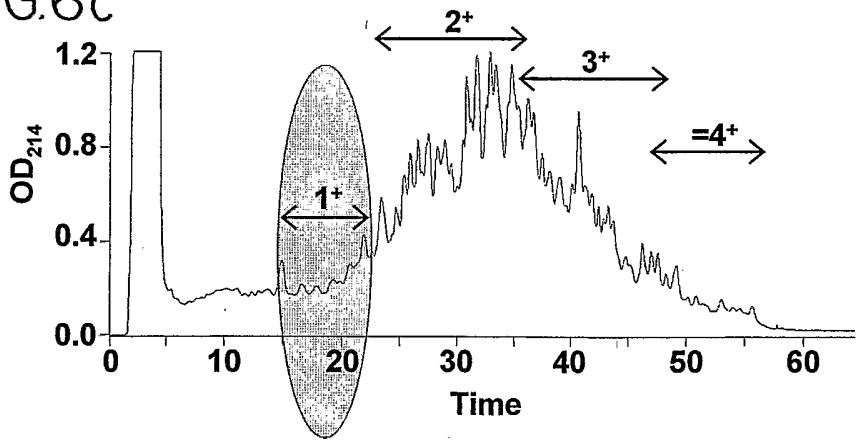


FIG.6c



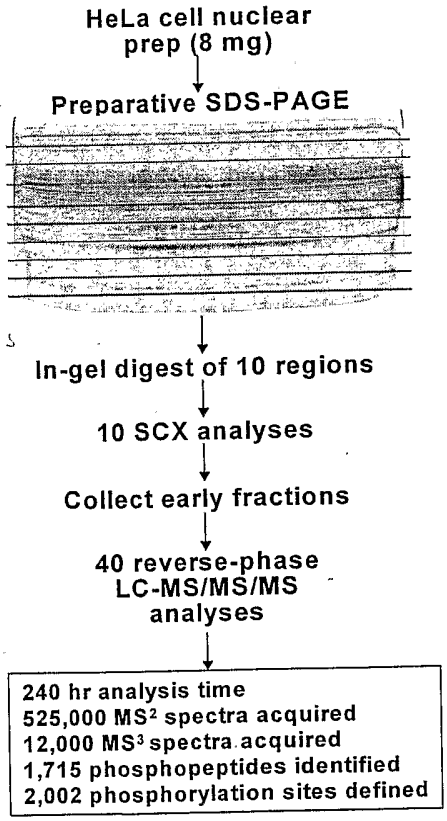
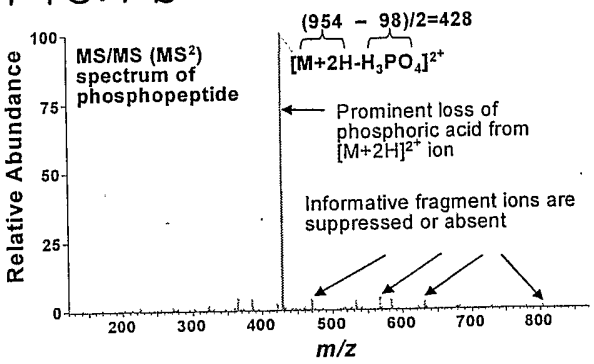


FIG.7a

FIG.7b



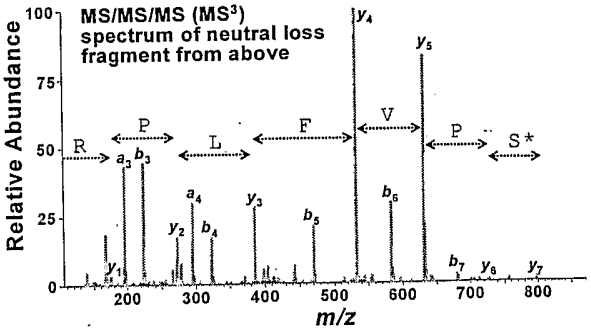


FIG. 7c

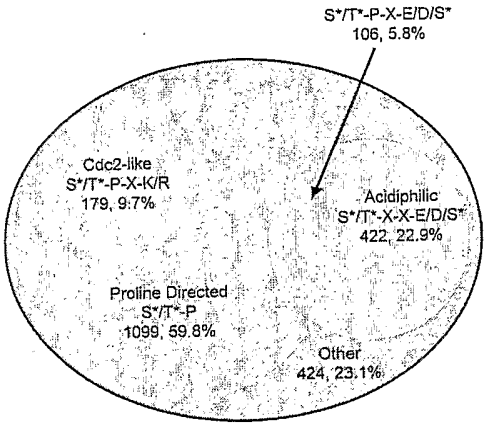


FIG. 8a

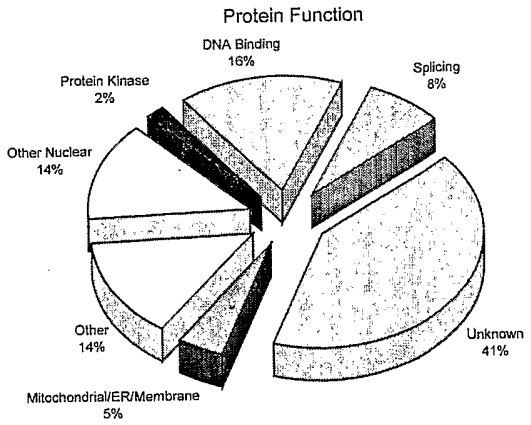


FIG. 8b

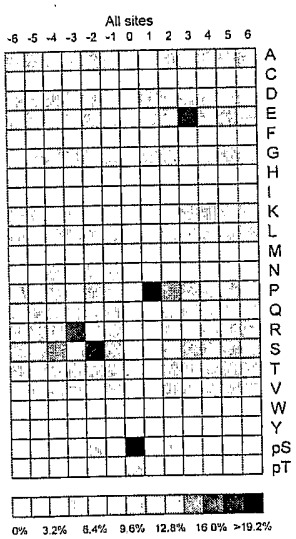


FIG. 8c

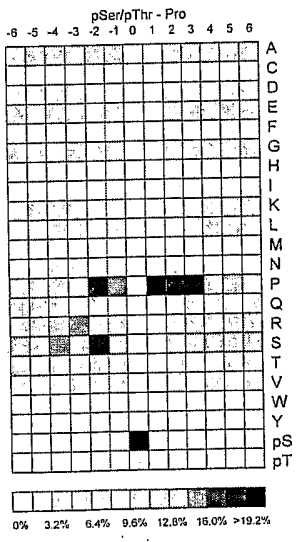


FIG. 8d

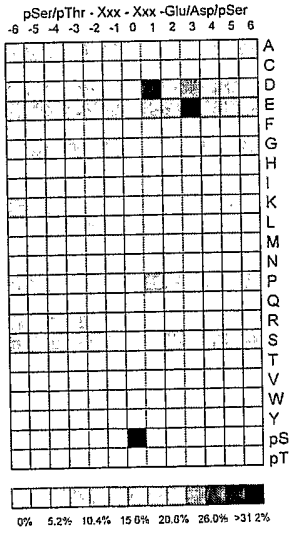


FIG. 8e

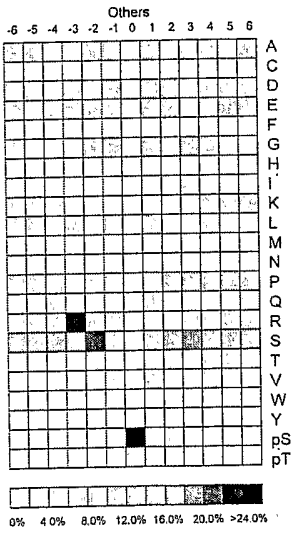


FIG. 8f